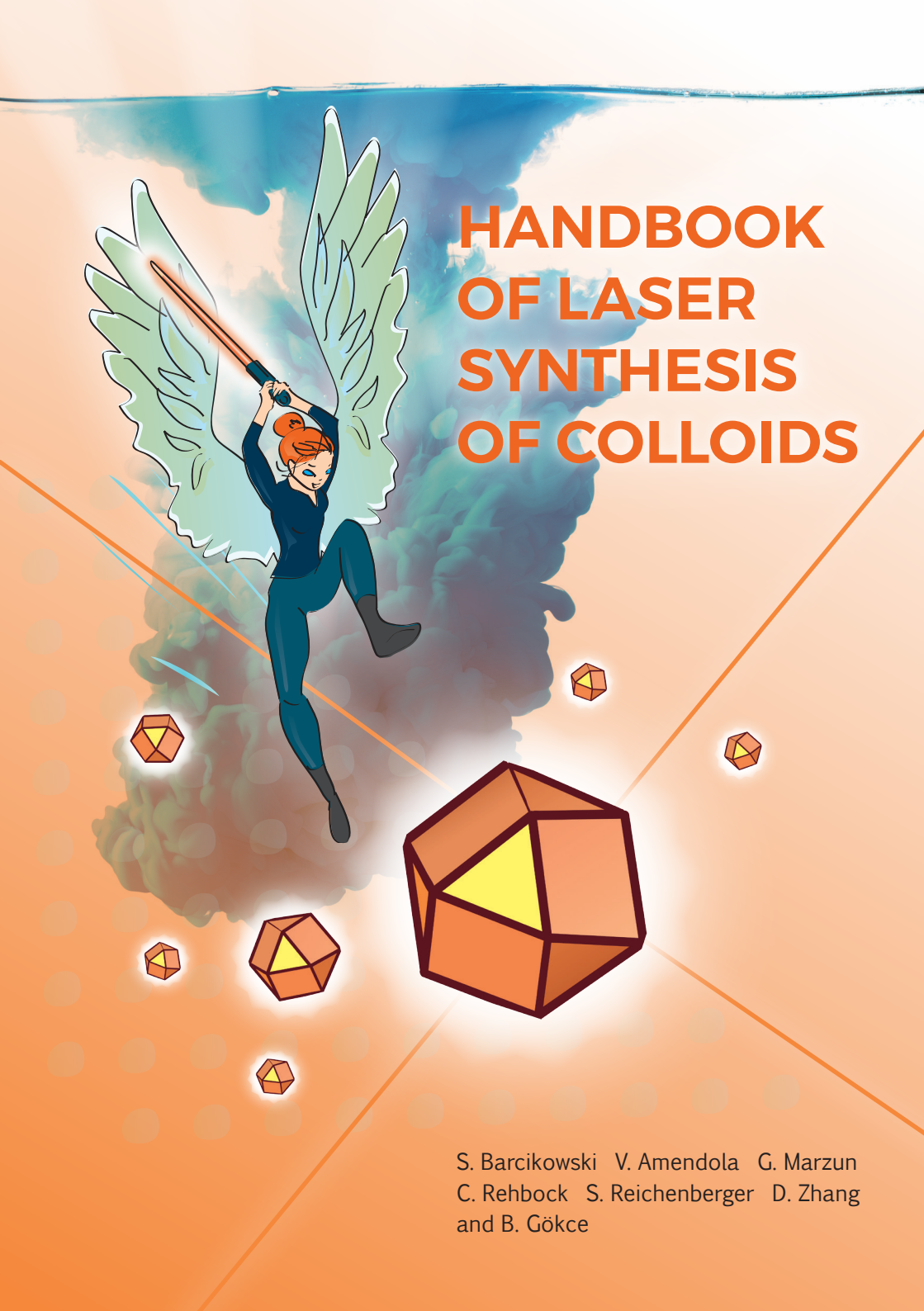

HANDBOOK OF LASER SYNTHESIS OF COLLOIDS

S. Barcikowski V. Amendola G. Marzun
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and B. Gökce

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CONTENT

PREFACE

”We aimed at a new type of nanostructures, but we really did not expect that the use of lasers could bring out something revolutionary”, Anton Fojtik wrote in his abstract for the 4th “Advanced Nanoparticle Generation and Excitation by Lasers in Liquids – ANGEL” conference, 23 years after his and Arnim Hengleińs pioneering work started this field in 1993.

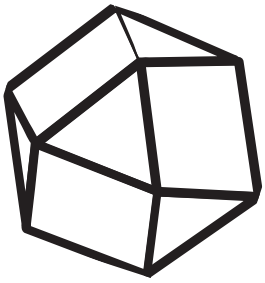
Today, the field of laser synthesis and processing of colloids (LSPC) has expanded to all over the world, so that whenever a researcher enters in this field, she/he has to fumble her/his way forward amidst a mass of scattered papers, focused reviews and Ph.D. theses all of which are not always educational. This is why it seemed to us that one coherent presentation in form of a handbook might help newbies (in the following referred to as “you”) with this predicament.

The main purpose of this book is to introduce you to this interdisciplinary topic, which contains elements from chemistry, physics, engineering, and sometimes even biology. While LSPC is pretty simple to apply, it is not as easy to understand, in part caused by its interdisciplinary nature. And to be honest even among the experts in the fields there are many disagreements on how LSPC really works. Although by now there is quite a collection of publications on LSPC, none of them treats it in a way suitable for beginners unravelling the secrets of experimental setups, reproducibility, colloidal stability, and yield.

Instead of aiming for a scientific and structural approach, which would first establish the theory and properties of LSPC and then try to view the consequences of these, we decided to arrange the book by phenomenological "steps" that you will need to take to be successful in our field. In order to visualize what really matters we tried to give instructional examples. Since there is no need for you to make the same mistakes that we did (yes, we can tell you!), we will also explain what you should avoid during your journey in the world where ultra-small particles meet giant laser intensities. Of course, it would be unreasonable to refrain from any theory if this really makes things simpler; hence we limited the fundamentals to a minimum and refer to the scientific reviews that are out there.

It was our intention to write all chapters of the book in a sufficiently leisurely style, while only minor preliminary knowledge is required to read it. After "getting started with setting up the experiments" in Chapter I and "getting more" colloidal nanoparticles in Chapter II for the extensive "characterization" in Chapter III, we explain how to "keep your colloids" stable in Chapter IV and how to "keep you alive" during your contact with lasers and chemicals in Chapter V. We hope that after reading the book you will not get lost in the zoo of materials, liquids and instruments. Last but not least, we would like to thank all of the 25 scientists who contributed to this work by words and quotes. We hope you enjoy reading this book as much as the authors enjoyed writing it!

Stephan Barcikowski and Bilal Gökce



INTRODUCTION

Two decades after the first colloidal nanoparticles were generated with a laser, more than 350 institutes all over the world are actively involved in the synthesis and processing of nanoparticles by laser ablation. If 3 students per institute would work in this field we would have a total of 1000 students that are eager to make their way into academia or industry using this laser-based method. Different terminologies such as “pulsed laser ablation/fragmentation/melting in liquids (PLAL/PLFL/PLML)” or “Laser Ablation Synthesis in Solution (LASiS)“ are used to describe the field that this book is about. If you want to know more about LASIK (Laser-assisted in situ keratomileusis or simply laser eye surgery), then you are wrong here and should consider reading another book. But if you want to know more about the practical aspects of the exciting field, which we call overall “laser synthesis of colloids” then you are at the right address.

The first chapter of this book starts as you would start your first days in the laboratory, you would first read about the basics and then about the specific experiment you plan to perform. Since the flexibility of this laser-based method is very high you have a huge set of parameters that you need to choose from and with each of these variations you will get a different result. If you don’t want to waste expensive gold or platinum targets for your first “walking steps” you might want to start with a cheaper and more abundant metal such as silver or iron. What do you do next? You need to choose the liquid that you want to use to collect your nanoparticles. However, for reactive metals such as iron the liquid medium isn’t just a collecting medium it also defines the nature of your synthesis product. If you use water you will get a mixture of iron oxide(s), if you use an alcohol you will additionally obtain iron carbides. You see that the choice is crucial. Let’s make a simple calculation to demonstrate the number of possible outcomes: the period table tells us that there are 91 metals, you can imagine that the number of liquids can’t even be numbered, estimates range from 10^{18} to $10^{200,}$ but the motto of this book is “keep it simple”, so let’s limit our consideration to solvents. Solvents can be categorized into inorganic solvents such as water or ammonia and organic solvents, which additionally are divided into, oxygenated solvents (e.g. ethanol), hydrocarbon solvents (e.g. hexane), and halogenated solvents (e.g. chloroform). If we now want to try five liquids from each of these categories for each metal in the periodic table, we will have almost 2000 possible combinations and possible outcomes. There is also the laser, which will not just have an impact on the physical parameters of your nanoparticles but might also change their chemistry. You see, you have to be smart about choosing the right target, liquid and laser.

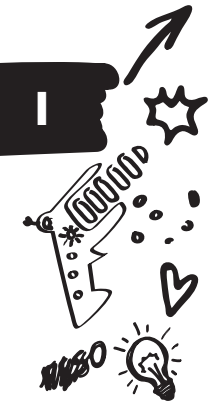
Let's say you chose to synthesize iron oxide nanoparticles in water. If you use a ns-laser and perform your ablation in a beaker for one hour you will get micrograms of nanoparticles. This might be sufficient for UV-vis spectroscopy, but if you want to analyze your nanoparticles properly, e.g. the crystal structure, or even apply them in a real-word application you have to perform a lot better. Chapter II will show you how you can boost your productivity by changing your ablation chamber, laser parameters, fluidics or your target shape. You don't necessarily need to buy the most expensive laser in order to get more nanoparticles, good news for your boss.

After you followed the steps in Chapter II and obtained, let's say, 100 mg of nanoparticles after one day of ablation you have to analyze your product(s). Just by looking at your colloid you won't be able to tell much, especially if you ablate iron. How big are your particles? What is it? What is on its surface? These are just some of the questions that immediately arise when you have a colloid of unknown properties. Chapter III summarizes all major methods that are out there to analyze nanoparticles or colloids. We will also mention practical aspects such as how much nanoparticles you need or how you prepare your particles for the analysis with each method.

You might not need to be a chemist in order to be successful in this field, but you definitely won't have an impact on the international community without basic knowledge of chemistry. Especially colloidal chemistry is essential for reasons such as to keep your colloids stable. You need stability for colloidal characterization but also for further processing of your particles. If you have aggregated particles on the bottom of your ablation chamber and the pure liquid above, you won't be happy with this outcome. So follow the steps in Chapter IV and be happy!

We not only want you to be happy but also to be safe during your journey that you've just started. The interdisciplinarity of laser synthesis of colloids is a big advantage but from a safety point of view there are additional dangers, you have to be concerned not only with chemistry but also with laser safety. If you don't want to perform unintended LASIK by your own – check out Chapter V. Many things can happen during laser ablation in chemicals, you have to be extremely careful. As you might imagine, flammable liquids don't go well with powerful lasers. We additionally included many "lab stories" from students, post-docs and even professors that are evenly distributed over the whole book to help you to realize what might get wrong during your experiments.

GET STARTED



You probably know what it is like to start something completely new. You are highly motivated and at the same time know that all beginnings are hard. Exactly here, this handbook comes into play. It will help you with tips and tricks and will let you avoid typical beginners' mistakes. You will not just learn how to dodge the mighty cavitation bubble and tune your experiment but also to study the exciting world of laser-generated nanoparticles. In order to start your first experiments on the synthesis of nanoparticles by laser ablation in liquids, you have to be well prepared. Thus not just experimental preparation is crucial but also theoretical knowledge is of fundamental importance for a successful work. At the beginning of this chapter the basics of lasers and laser ablation in liquids are summarized. Then, the most important equipment and setup you can choose will be described and you will learn how to manage your first steps of the practical work when starting with your laser ablation experiments.

STEP 1: LEARN THE BASICS

It all started with a joke: "A laser is a solution seeking for a problem", this is how the first working laser was described in 1960 by sceptics who wanted to make fun of Theodore Maiman's invention. Maiman made the world's first laser operate in his laboratory at Hughes in Malibu. The laser was based on an optical pumping of a ruby crystal using a flash lamp that generated pulsed laser radiation at 694 nm. As you know every beginning is difficult, the same happened to the father of optical lasers. Many researchers just did not realize the significance and the dimension of his invention. After his first report on the ruby laser was rejected by reviewers of the journal *Physical Review Letters*, Maiman turned to *Nature* where his paper was published on August 6th 1960. Even though the relevance of his discovery was not clear, Maiman believed in his device's potential. Nowadays lasers are everywhere and their applications have become an indispensable part of our daily life. Just have a closer look at your surroundings: applications can be found everywhere from research laboratories to steel industry, from dentistry to medical clinics, from discotheques to pointing devices. For example, lasers can be applied everywhere where an object has to be quickly identified without direct contact. Cashiers only need to bring a barcode close to the scanner without the need of typing the article number by hand. Isn't it great to have more time for shopping? At least for all women, this is a great invention. Lasers are used for online shopping when you use a computer mouse. In an optical computer mouse, a laser can identify the direction of the movement. In addition, all optical disc drivers like DVD or Blue-ray use laser diodes. Next to everyday products, pulsed lasers have gained huge importance in medical applications such as eye surgery or photothermal laser resection of

organs. Huge industries have found multiple ways to make money from laser technology including laser entertainment shows, cosmetics (laser tattoo and hair removal) and measuring large distances by lasers, to name just a few things. There is no car or airplane built without laser technology, either for steel welding or for drilling the gasoline nozzles. In addition, pulsed laser structuring is essential to build mobile phones and tablets. The skeptical colleagues of Maiman must have looked dumbfounded when they realized that indeed a lot of problems have been solved by the laser.

Laser Ablation in Liquids (LAL)

Lasers are powerful tools that can lead to an astonishing outcome if the laser beam is directed with high energy density onto a surface. You irradiate a piece of gold in water and obtain a red-colored liquid. As a result of irradiating a target, material detachment can take place, which can be exploited by scientists for particle synthesis. What a brilliant idea, isn't it? Particularly because the technique was originally used to structure the surface of the target and the particles were considered "just waste" for a long time. Focusing a laser beam in a liquid environment, enabled to catch the ablated materials in the solvent directly. This makes particle synthesis safer, because no fine dust or particulate matter is released in the surrounding workplace, avoiding health risks, in particular, respiratory diseases. But most important, colloids are easier and safer to process into valuable products than nanopowders filtered off the gas phase. The liquid is the key to quality and value of the synthesis. Fabrication of nanoparticles by laser ablation in liquids has become an interesting and important technique for many applications. Laser-generated particles are not only very useful for fundamental research, but also for their widespread use in medical devices, photonic materials, catalysts and more.

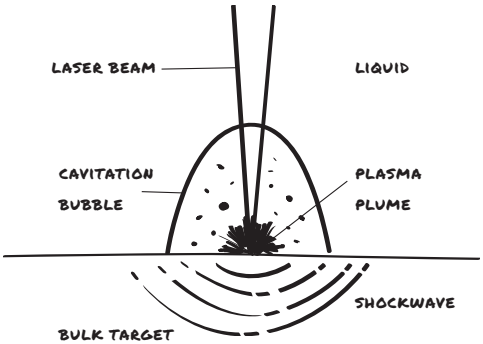


Figure 1: Sketch of what happens during LAL of a bulk target, just before the nanoparticles disperse in the liquid

Considering the principles of laser ablation in liquids, nanoparticle formation may take place by various mechanisms and is influenced by different laser parameters (such as pulse duration, wavelength, energy, repetition rate) and materials involved. When a laser beam irradiates a solid-state material in a liquid medium, the energy provided by the laser pulse can be absorbed by the target and leads to the formation of an expanding plasma plume containing the ablated material (Figure 1). This is accompanied by the emission of a shockwave that releases energy in the surrounding liquid. When the plasma cools down, it releases its heat to the liquid, which is transformed into hot vapor. This leads to the formation of an oscillating cavitation bubble containing both the ablated matter and the liquid vapor, where particle formation takes place. After the collapse of the cavitation bubble, another shockwave is generated and the particles are released into the solvent. In case of aqueous solutions and materials such as gold or zinc, electrostatic stabilization of the particle is then achieved due to partially or fully oxidized surfaces respectively, where ion adsorption takes place and forms an electrostatic double layer. By this method a variety of nanomaterials can be obtained purely in different solvents which opens a wide range of applications.

Of course to fully understand the basics you would need to go into more detail by reading one of the specific reviews that are out there.

Producing particles by LAL is quite simple. For the easiest setup, you just need a laser, a vessel filled with the solvent and a target, which is immersed into the liquid and appropriate focusing optics (Figure 2). The most expensive part of this setup is the laser itself which costs at least 30.000 € if you want a reasonable nanosecond laser. Maybe you share a laser with another lab, or look for used ones. All the other costs are almost negligibly small, but also depend on the target you use. Talking about laser ablation of gold in water the noble metal with about 40 €/g (purity of 99.99 %) mostly influences the price. The remaining equipment is quite cheap (a glass beaker, distilled water, lens). In total the costs for consumable materials amount to less than a hundred Euros for many weeks of fabricating colloidal nanoparticles. Search on the internet, you will find that buying only some 100 ml colloid is often more expensive. Note that for laser ablation not the whole target can be transformed to nanoparticles, but can be recycled to a new target. By laser ablation of a target, you multiply the value of your material. For example a liter of a colloid with 100 mg/L laser-generated gold nanoparticles in water is available at least for 1,000 €, which makes 10,000 €/g. In comparison, to obtain wet chemistry synthesized gold nanoparticles (where a gold precursor is used and reduced to nanoparticles), you will pay more and will get nanoparticles stabilized by citrate or other ligands. Depending on the application, you need to remove the ligands on the particles surface, which will again cost time and money.

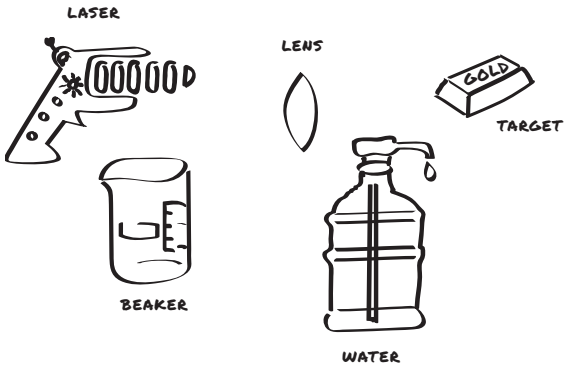


Figure 2: Basic elements needed for laser ablation in liquid

STEP 2: CHOOSE THE TARGET MATERIAL

Before starting with any experiments you should be well prepared. Take time to think about the material you want to ablate and about the suitable solvent for your research and application.

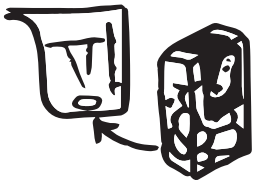
One of the main advantages of laser ablation in liquids is the possibility to choose a variety of materials. So if you realize that for instance gold is not the right material for your study, you just change the target and synthesize other nanoparticles, maybe put the gold in an oven with silver and go for ablation of alloys. Other chemists synthesizing nanoparticles would dream of it, because simplicity and robustness of LSPC experimental setup makes the research much more effective and more fun. Typically there are two different types of materials that are mostly used to generate nanoparticles by laser ablation in liquids: metals and oxides. But also other types of nanomaterials such as sulphides, nitrides or carbides were successfully fabricated by LAL. We recommend to go backward in material selection by the product you want, if its zinc oxide or titania, it's easier controllable to start with the oxide rather than going for metal ablation and hope for controlled oxidation in water. In the latter case it is likely that you will end up with a mixture of defect-rich crystal phases not always easy to reproduce. Laser ablation in liquids does not just allow the synthesis of pure materials such as metallic or oxide nanoparticles, but also alloy nanoparticles possessing solid solution or core-shell structures are accessible depending on the experimental parameter (target, solvent, solutes etc.) you use. For instance in the case you want to have alloy nanoparticles you can just ablate an alloy target. Try a series of AgAu, it gives beautiful colors, resembling the composition of the alloy nanoparticles. The composition of the targets determines the (starting) composition of your nanoparticles. However, you should carefully chose your solvent, which strongly influences the formation and thus the mixture

and structure of the particles. Acetone is always a good start. Another fancy approach for alloys is to use a self-made target. You can mix different powders and press them to form mixed powder targets. Depending on the compressibility of the materials and stability of the targets you can sinter the pellets by heating them up subsequently (this also avoids breaking of the consolidated powder targets). In case the diameter of your laser beam is larger than the grain size of your powder, you now will synthesize alloy nanoparticles. The same works with other composites, such as mixing silver powder with an oxide. The key to reproducibility is intense mixing of the powder in a mortar. Inspect color and gloss of the pressed target, is it the same on both sides or has the particle density and size difference caused inhomogeneities? This micropowder-based preparation method is a simple approach to achieve alloys or doped oxides on the nanoscale without much effort. It's up to you which composition you would like to take, because you prepare the educts as you want and it could be much cheaper compared to bulk alloy targets, which are usually only available in certain compositions.

Besides taking a solid bulk material for LAL, other shapes of a target can be used. If only powder is available in your lab or you are either just too lazy to press the powder or just don't have a press, you can use a suspension of the powder. This process is then called laser fragmentation in liquid (LFL). Main disadvantage here is that after irradiation of the powder you need to separate it from the colloidal nanoparticles. And also the determination of the particle concentration is long-winded compared to bulk materials, where you just need to weight the target before and after ablation.

Ina came desperately to me and said that the magnetic stirrer isn't working any more. After testing several others we found that the magnetic stirring bar isn't magnetic and is changing its appearance. After smelling a minty taste we realized that this is not a magnetic stirring bar but a tic tac. Imminently the question raised who was willing to sabotage our experiments by putting this tic tac into the box with stirring bars. After detailed investigations and having several suspects we found out that Galina lost a tic tac several days ago and Ina finding it was wrongly identifying it as a stirring bar, thus placing it in the box for the stirring bars. The lesson is clear: tic tac's should get a new shape!

Marcus Lau and Ina Haxhiaj, Essen



In case of powders you always need to separate them from the liquid and it's sometimes difficult to fully dry them before weighting. Sieve the powder before use, to remove everything larger than 50μm, facilitating dispersion and homogeneous ablation.

If you have already done some experiments by LAL with a solid target, you will quickly realize that sooner or later you need to change the target because you "burned" a hole into it. For a continuous ablation where you could synthesize a huge amount of nanoparticles without stopping the irradiation of the laser, a wire that is fed by a turning roll can be used. However handling wire ablation is much trickier and needs some experience. You will find further information on this in Step 6, where you choose your setup and Step 9 of Chapter II where target geometry effects are explained.

But there's one more thing to say. We always try to make our research convenient, and as characterization of the nanoparticles after synthesis consumes most of our time, we select the material always also by this criteria. It's a pity if you wait a long time for TEM or XRD data and it was the wrong colloid that was analyzed: It's easy to make 20 samples a day, but which to select for in-depth analysis? It is very convenient if the material you ablate leads to nanoparticles that have a color, since in this case you can immediately tell if you had success or not. Examples include the metal nanoparticles (red gold, bright yellow silver, brown copper) or the doped oxides, such as ruby. Colored, doped oxide targets you may find in gemstone shops. Also crystal defects may give colour, such as titania which turns nicely blue if titanium is ablated in water.

STEP 3: CHOOSE THE LIQUID

The liquid environment plays an important role in synthesizing nanomaterials by LAL since it influences the nature of the materials you will get. Basically you should have your application in mind. For instance if gold nanoparticles are needed for biomedical applications, ultra-pure water (and maybe addition of some albumin for stabilization in the saline biological media) is ideally qualified. As you know, less noble metals such as copper may lead to oxidation, hence alternatives are required if you want to obtain non-oxidized metallic particles. Materials, which are oxidation-sensitive, can be ablated in the presence of reducing agents or in solvents such as propanol, acetone, toluene etc. For example, acetone works well with copper. However, you have to keep the application in mind and you should be aware that some solvents (such as toluene, and even sometimes acetone) lead to a formation of a thin carbon layer on the particles' surface. Working with solvents, which have a low flash point and evaporate already at low temperatures, may cause flames if applying a laser beam on the solvent vapor. Then a dust particle crossing the laser beam in the vapor could be enough to ignite the mixture. In Chapter V, you'll learn how to deal with these powerful pulsed light sources. Mainly in case of non-noble

materials the pH value can influence the structure and the composition of your material. If you look at the Pourbaix diagrams, which show the redox-potential and pH-dependence of a certain material, you will note which oxides can be formed at a certain pH. Furthermore, the pH strongly influences the colloidal stability (as will be seen in Chapter IV). As a rule of thumb, stabilization of (noble) metals is achieved at high pH, and oxides often go well at neutral pH (not too low, to avoid dissolution). Some oxides that tend to dissolve at low pH can also be stabilized at very high pH, e.g. 11. Check the isoelectric points of the product in the web (already the value for the bulk gives a good hint), and keep the pH away from it. If you run into stability problems and notice aggregation of particles the next day even though you have tried different pH, you may like to read the chapter on particle stability. Just one word on that: add a trace of soap or protein. More precise, add a pinhead piece of curd soap or add albumin (works with almost everything). Of course, never use tab water, only distilled or deionized water.

As mentioned in the previous part, the liquid environment you use also influences the formation of alloy nanoparticles or core shell structures. This can get complicated. For instance laser ablation of an alloy target with an oxidation sensitive material such as iron and noble metal gold (equimolar Au:Fe) leads to a formation of an unoxidized elemental iron core with a gold shell in organic solvents. In contrast to this, LAL in water form a gold core surrounded by an oxidic iron shell. Just to name a few of other approaches for particle synthesis with a core-shell structure: a quite less investigated method is the ablation of a target which is already immersed in a colloidal solution with particles. But also a combination of LAL and chemical reduction is possible. In that case you can synthesize your particles and add a precursor solution or you directly ablate a target in the presence of a precursor, this is then called reactive laser ablation in liquid. Try ablation of a less noble element in a metal salt solution of a nobler element, the ablated species will reduce the precursor and form a composite nanoparticle. That's advanced colloidal nanoredoxphotochemistry done in 1 minute (and maybe understood after 1 year). No worries, in this handbook w e will keep it simple and most rules apply as well to the more complex material-liquid combinations.

STEP 4: CHOOSE THE LASER

The literature is full of papers dealing with laser ablation in liquids and often many different laser parameters are used, so that for beginners, it's not really clear why exactly that laser with that parameter was used. To be honest, mostly the laser, which is often the only one available in the lab, is used while the parameters are optimized for this given laser. But if you have an option to choose a certain laser you could pay attention to some general details.

It is mostly established to use the fundamental wavelength of 800-1064 nm for laser ablation in liquids, but also green light with 532 nm is often used

to ablate a material. However, if you want to prevent two different mechanisms that take place simultaneously, the fundamental wavelength is more appropriate to synthesize nanoparticles by laser ablation in liquids. For instance if you ablate a gold target with 532 nm in a vial, where the concentration of the particles increases and form an optically dense liquid, the particles will be re-irradiated. At this wavelength e.g. gold nanoparticles can absorb a high amount of the laser energy because of their intense plasmonic resonance band at about 520 nm (depending on particle size). Hence ablation of the target as well as re-irradiation of the particles take place simultaneously, affecting reproducibility. In addition, the colloidal solution efficiently swallows the laser energy and your productivity will quickly decrease (as will be addressed later in Chapter II). To avoid this, ablation synthesis at wavelengths where the particles show minimal absorption of the laser light is advisable for better control and understanding of the mechanism that takes place during ablation. In contrast, if you hunt for smaller particles, short wavelengths and long irradiation times may shift the process balance from ablation to colloid excitation, at the expense of productivity.

... everyone was quickly leaving the building, and heavily dressed fire workers seeking the fire source. Smoke sensors pointed at my laser lab, and the surprised firefighters found my guest scientist (a renowned guest professor) smoking. He later said he was blowing the cigarette smoke over in order to visualize the laser beam during its alignment on
the optical table.

Stephan Barcikowski, Essen

Before talking about laser power, energy and intensity it is useful to understand what these terms refer to. The power of a laser can be measured in Watts. This refers to the mean power output of the laser. The definition of the mean power output P_{mean} is simply calculated as the Energy E_{mean} released during the operation time t_{op} ($P_{mean}=E_{mean}/t_{op}$). For instance as shown in Figure 3 if a continuous wave-laser emits light with an energy of 3,000 J in 10 minutes, the laser has a mean power output of 5 Watts (3,000 J / (10 · 60 s)). When using pulsed lasers the situation is much different as there are two separate definitions of the laser power possible. Remember that a pulsed laser releases its energy in packages (laser pulses) during the operation time t_{op} with some repetition rate (e.g. 20000 pulses per second) having a given pulse duration t_{pulse}. If the pulsed laser fires 20,000 (= $2 \cdot 10^4$) pulses per second having the energy amount of e.g. 250 µJ (= $2.5 \cdot 10^{-4}$ J) per pulse, its mean output power simply equals to 5 W ($2 \cdot 10^4$ [pulse/s] · $2.5 \cdot 10^{-4}$ J [J/pulse]).

That́s the same average power output as the cw-laser before. But the cw one will not even make the target hot whereas the pulsed one is already quite productive in colloid synthesis. Accordingly, due the compression of the energy into pulses of a specific pulse duration t_{pulse} (e.g., 1 ns and 1 ps) the stream of single pulses is by far more efficient in removing matter from a solid surface. You wonder why? Imagine two situations: you are the target getting irradiated with a 250 µJ laser pulse of A: 1 ns pulse duration and B: 1 ps pulse duration. In case A you absorb 250 µJ of energy within 1 ns which means that the laser is irradiating you with a peak power of 250,000 W ($P_{peak} = E_{pulse}/t_{pulse} = 2.5 \cdot 10^{-4}$ J / ($1 \cdot 10^{-9}$ s) or in another unit 0.25 MW. This peak power P_{peak} is comparable to the added power of about 20 single-family houses. Now it's better to stop imagining being the target as we get to case B. In case of a 250 µJ pulse having a pulse duration of only 1 ps the peak power (or pulse power) impacting the target equals 250 MW which basically is the amount of energy a small power plant produces. Practically, nanosecond lasers often have similar peak powers than picosecond lasers, as ns lasers provide mJ pulse energies and ps laser pulses are in the µJ range. E.g., 200 mJ @ 10 ns or 200 µJ @ 10 ps will both shoot with 20 Megawatt peak power. But a typical femto laser with 100 µJ @ 100 fs will allow you to fire with 100 MW, allowing to disintegrate every solid (with the risk of vaporizing the liquid before reaching the target).

Of course this peak power only affects the target for a very short time however with a tremendous effect we call ablation. But do not underestimate the target's defense mechanisms, which mainly are light reflection and energy dissipation (for more details refer to Chapter II). In order to crack the defense mechanisms of the target you need to increase the penetration to use the full force of your single pulse. To do so you need to decrease the lateral extension of the pulse, which basically is the beam area. To achieve this, lenses are used to focus the laser beam. As depicted in Step 5 the other option is to use a telescope of which two telescope types namely the Kepler and the Galilei telescope are especially famous. The advantage in using telescopes is that the light wave still propagates in the same direction (parallel beam) while a focusing lens will result in a diverging beam. The telescopes are limited by the destruction limit of the lens material, which is why they can't be used in cases where very small spot areas and high pulse energy lasers are being used. Now, upon decreasing the beam area two properties of light called "intensity" and "fluence" will increase. Intensity is simply defined as the power (either mean power or, in our community, the peak power in Watt) per beam area (in cm^2). On the other hand, laser fluence is defined as the beam energy (in J) per area (cm^2) or speaking in formulas:

$$F = \frac{E_P}{A} = \frac{\bar{P}/\dot{N}}{A}$$

Hereby F depicts the desired laser fluence, A the laser beam area and represents the energy of a single laser pulse. The pulse energy E_P can be obtained by dividing the mean output laser power measured by the number of laser pulses per second.

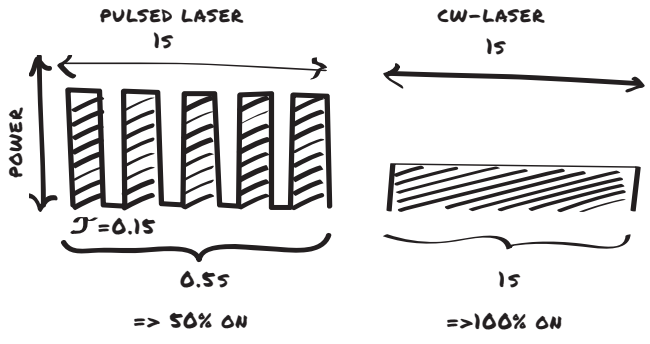
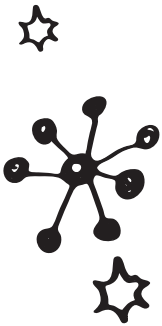


Figure 3: Comparison of laser power of a pulsed laser with a continuous wave laser beam. The average power of both are the same so that the peak power of the pulsed laser is far higher.

The definition of the laser fluence only applies to pulsed lasers as it is based on the pulse energy as shown above. The laser fluence is a key parameter for all laser processing of materials in liquids, for laser ablation, laser fragmentation, and laser melting. The fluence rules the productivity during laser ablation, sets the smallest size you can achieve by laser fragmentation, and is the main parameter to control particle size by pulsed laser melting in liquid. Hence, it is of utmost importance for the sake of reproducibility to precisely measure this parameter "laser fluence. And if you want to be kind to people that like to follow your footsteps, you will need to write down how you measured it. This however requires to measure the pulse energy and the spot area to be able to divide both values. The pulse energy is acquired by a power meter at the beam outlet, to be divided by the repetition rate. Better measurement position is a close to the ablation chamber as possible (e.g. behind the last mirror or just before the focusing optics), as energy losses accumulate at every optical element. Don't consider taking the value that is written on the laser or the last PhD thesis in the lab. Pumping lamps or diodes naturally degenerate, so that laser power decreases every year. Also, for most lasers laser power will be different in the morning than one hour after laser operation, because it needs to "warm up". We recommend measuring laser power 30 minutes after the laser resonator is on. Now you need to know the spot area on the target (for laser ablation) or the liquid entrance (for laser fragmentation/melting). Don't wonder if you will find very different values in literature regarding the laser fluence for the same setup (e.g. same laser, same focal length), because measuring the fluence is done differently. In principle, there are 3 ways of doing it.

1) The “theoretical fluence” you get by simple calculation based on focusing law (input parameters are raw beam diameter, wavelength, and focal length). We recommend then to measure at least the raw beam diameter before the lens as it often deviates from the value the system manufacturer provides. For this, you take a beam detector card and hold it into the beam (read chapter on safety before). If you don't have a beam card, (thermosensitive) telefax paper or thin black paper does a good job, too. Wetting the telefax paper before is better choice if you have high pulse energy to avoid non-representative big holes and smoke. 2) The same you can do to acquire the “measured fluence at target plane” at the distance from the lens where the target is going to be placed. Maybe you put the paper on top of the target. Test how many pulses you need to see a hole. But this will probably not work in liquid. 3) The most suitable way to determine the fluence is to measure it at the target in liquid, that's the closest to what you want to know. In order to obtain the spot diameter based on this setup, you will need to set a limited number pulses (e.g., start with 10 and 100) and measure the average crater diameter in the microscope. Very often, a larger heat affected zone surrounds the crater, but for calculating the fluence value, we look for the ablated crater diameter.



In case of continuous laser beams the impact of a laser can only be measured as a function of laser intensity, which was introduced above prior to the fluence. If the laser intensity is high enough, it is possible to ablate a target with continuous waves, however as already indicated while a 5 W pulsed laser can create peak powers of, e.g. 250 MW (in case of 1 ps pulses with 250µJ.) the continuous wave laser will still only operate at 5 W. That's why in cw laser manufacturing like laser welding or plate cutting, kilowatt laser output power is standard today, whereas for pulsed laser machining tens of watt are often enough. As hinted before the target has several “defense mechanisms” one being the energy dissipation. Against continuous wave lasers this works perfectly as the energy is continuously delivered it can be continuously dissipated resulting in slow heating and eventually melting of the target. The reason for this is that the different heat exchange mechanisms are influenced by the time scales when irradiating the target and thus it is affected by the pulse duration of the laser. The pulse duration mainly governs the heat affected zone (and with it the ablation mechanism), which is higher for nanoseconds (ns) pulses than for pulses much shorter than the heat diffusion time (fs to ps pulses). While longer pulse widths such as in nanosecond scale give ability to heat transfer into the liquid via the heated target surface, ultrashort pulses such as in pico (ps) or femtosecond (fs) scales are so fast that heating of the environment can be minimized. For instance, if you operate with ultrashort pulsed lasers, the ablation rate is so fast that the solvent even does not realize if the target is being hit by the laser (no heat transfer) and stays cool, which enables laser ablation in solvents with low boiling point. Furthermore, because of energy

losses to the environment in case of ns pulses, the ablation threshold is higher compared to ps pulses. The threshold fluence decreases with pulse duration (< 100 ps, better < 10 ps) because of negligible energy dissipation by thermal conduction, and thus leads to higher ablation efficiencies. That's the theory, but in practical application, also ps and fs laser ablation will heat up the target slightly for longer ablation time, caused by accumulation of minimal residual heat that every pulse leaves at the target. It is not much, but noticeable for high repetition rate lasers.

Even though the ablation threshold is smaller for ultrashort pulses, these may cause an optical breakdown, namely a strong local ionization of a medium (the liquid) due to energy absorption. For better insights look up in Chapter II, which describes the effect of the pulse duration in more detail and nicely illustrates these phenomena.

Recently at the TEM I could observe the presence of impurities in nearly all of my colloids consisting of an organic solvent. According to EDX measurement they were made of silicon compounds and were present in a comparable concentration to the nanoparticles. The impurities interacted with the electron beam of the microscope and covered up the particles, no possibility to get nice images. Different sources like air dust, dirty grids or production additives of the solvents stood in suspicion. Then a mass spectroscopy analysis of different organic solvents revealed high amounts of polysiloxanes in a p.a. grade acetone I normally used for synthesis.

Friedrich Waag, Essen

However ns lasers are usually available with a high pulse energy and high power at low or moderate repetition rates and could be more appropriate concerning high productivities because of another important point which influences the ablation efficiency, namely the cavitation bubble. The bubble, which is caused due to a laser pulse, can shield the following laser pulses and thus prevent that energy reaches to the target. As a result less material can be ablated. It should be noted that the lifetime and the size of the cavitation bubble depends on the pulse energy, which is usually smaller for ps and fs lasers. The bubble typically has a lifetime in the (hundreds of) microseconds to (single) milliseconds regime, while its size is in the (hundreds of) micrometers to (single) millimeter regime. Hence, the laser-cavitation bubble interaction takes place only at high (> kHz) repetition rates, where the temporal pulse distance reaches values of the bubble lifetime. To prevent that the cavitation bubble interferes with the laser beam a spatial or temporal pulse separation is needed in order to get higher productivities by bypassing the bubble. If you use a < 100 Hz laser, you definitely will not have to worry about any cavitation bubble shielding.

However in case you think about ablation of a pressed powder target, mild conditions are favorable to prevent a removing of the initial bulky particles. High pulse energies (typical for low repetition rate lasers) are more likely causing partial disruption of a pressed pellet. As you see, the laser parameters strongly depend on your interests and the materials or liquids you use.

Table 1: Pros and Cons of several laser pulses for pulsed laser ablation in liquids

Laser pulse duration	Pros	Cons
ns	· more and cheaper high power lasers available (i.e. high productivity) · high power at moderate repetition rates	· heat transfer from target to the liquid · less efficient
ps	· 'gentle ablation' · insignificant heat transfer to liquid · compromise between efficiency and productivity/costs · high power systems available	· optical breakdown at high pulse energies · high power at high repetition rates (bubble shielding) · high power more costly than ns
fs	· 'gentle ablation' · very efficient ablation per pulse · insignificant heat transfer to liquid	· high power at high repetition rates (bubble shielding) · optical breakdown · high power more costly

STEP 5: USE THE RIGHT OPTICS

Working with a laser requires at least basic knowledge about laser optics, since they need to be chosen carefully. The main items are lenses and mirrors. The area of optics is a perfect playground and you can tumble-around especially with the variety of lenses. Starting from the simplest lens, common lenses to focus or to spread the laser beam are shown in Figure 4.

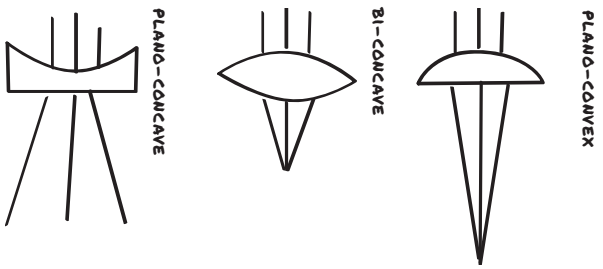


Figure 4: Types of lenses: Plano-concave, bi-convex and plano-convex

Two main orientations should be kept in mind, namely convex and concave. A convex lens is curved outwards and a concave lens inward. In order to memorize the geometric structures you can help yourself by thinking on flat surface, which is curved inward, to make a little cave (concave) or outward to make humps like a T-Rex (convex). To focus the laser beam and thus concentrate the entire laser power to a small spot a convex lens is used. You can decide whether you want to bundle the beam at long distances by using a plano-convex lens or at shorter distances if working with a biconvex lens, which is symmetrical on both sides (collecting lens). However focusing a laser beam with a plano-convex lens leads to reduced spherical abberations ("sharper" focusing) and is mostly used for laser ablation, whereby the curved surface of the lens is faced opposite to the direction of the laser beam (Figure 4). Concave lenses can be used to expand the laser beam or to increase the focal lens in an optical system (diffusing lens). For instance if you want to change the laser fluence (remember that this is energy per area and the entrance key to any laser synthesis castle!) you can change the spot diameter by using such a lens. Now you may ask, how can the beam be expanded to keep the beam parallel? For this a beam expansion system can be assembled by combining two lenses. You can build a beam telescope by displacing the concave and convex lens against each other. There are two main assemblies. A Galilei telescope consists of a collecting and a diffuse lens, whereas a Kepler system is build up with two collecting lenses (Figure 5). How much the beam is expanded depends on the focus of the lenses.

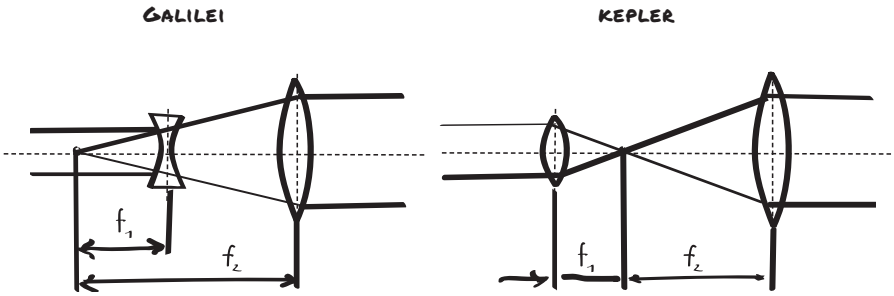


Figure 5: Systems for parallel beam expansion: Galilei (left) and Kepler (right)

As you can see in Figure 5 using a Kepler telescope a focal point is created between the two lenses. If the laser is strong enough even the air can be ionized and forms a plasma in that point, which can be clearly observed as a bright white-purple spark of light. To avoid losses of laser power, Galilean telescopes are more frequently used (Figure 5, left). Of course, you can use it in both directions, making a raw beam wider or smaller, keeping it parallel before and after the telescope. Now think that you want to increase the laser fluence. A plano-convenex lens will sharply focus the light to the highest fluence (e.g., to increase productivity during laser ablation, see Chapter II), and the fluence will be higher the larger your raw beam diameter is (better focusability), and the shorter the focal distance (the supplier sells focal lenses with fixed focal distances, very typical are 50, 60, 100, and 300 mm) the sharper the spot. But sharpness also goes along with divergence around the spot, that is high fluence gradient before and after the focal plane. This will increase the required precision of target positioning at a defined distance, and would make re-adjustments needed once the target gets thinner. Very often, we are quite happy with 60 or 100 mm for laser ablation synthesis of colloids. When you read papers on laser fragmentation or laser melting, often very long focal distances (e.g. 300 mm) are used in order to minimize fluence deviation (sharpness) along the beam path in the liquid. Hence, the thicker the liquid (e.g., 10 mm) you pass during laser fragmentation or laser melting of suspended particles in liquids, the longer the focal distance of the lens should be to allow similar condition everywhere along the irradiated volume. For this situation, the Galilei telescope is a good deal, as it allows to conveniently adjusting the fluence while keeping the beam non-divergent, so that the fluence is always constant no matter where you put the cuvette.

"A strange smoky smell comes from laser lab!", told me Fabrizio. Few seconds later I realized for the first time that a little aspiration chamber is not enough to avoid the ignition of solvents during laser ablation in alcohol! The holders of lenses and mirrors (made of plastic) were melt by a flame developed over the ablation flask. From that time I always performed laser ablation in sealed cells, in cases of flammable solvents...

Lucio Litti, Padova

Compared to a 'standard' lens as described above which focus a laser beam on a spherical area an f-theta lens allows focussing on a larger planar surface. This enables a steady focusing in one plane independent of the incoming position of the beam on the lens. For instance, in case of LAL a focal point will

be always on the flat target and the same energy density reaches the target. This is very useful if using a scanner system, where the beam is always scanned and thus moves in several positions on the lens. This leads to a minimized fluence deviation when scanning the beam by a galvanometric laser scanner, in particular at short (< 100 mm) focal distances and large (> cm²) target areas.

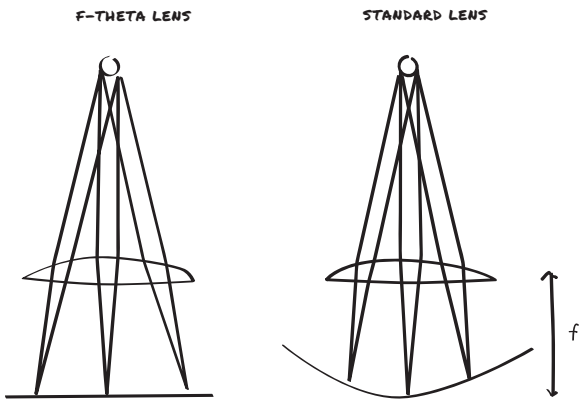


Figure 6: Comparison of the focal position using an f-theta (left) and a standard lens (right).

If you are working with lasers, you cannot avoid using mirrors. Mirrors are useful to reflect the laser beam in a desired direction without changing the position of the laser beam source. But, here you have to note a few things when working with mirrors for instance in combination with high-energy lasers. In all cases, you need to make sure that your lens and mirrors are suited for the working conditions you want to apply. Important questions you need to answer before using the optical device by checking the manufacturer specifications:

- Is the wavelength you are planning to work at suitable for the optical device?
- What is the damage threshold of the optical device at the wavelength and pulse duration you plan to work at?
- Does the intensity you plan to work with exceed the damage threshold?
- Are the optics suitable for CW or pulsed lasers?

The story behind these questions is that optical devices are sealed with an optical coating, which is optimized on a specific wavelength regime. Hence, it is important to check whether the devices are suited for the given wavelength to make sure they even perform properly. Secondly although the optical devices

are made of glass (e.g. BK7 or Quartz glass) a little amount of the laser energy (<0,5%) will be absorbed, depending on the wavelength and the type of glass. Therefore, there is a laser intensity (peak power per raw beam area irradiating the mirror or lens) at which the glass will get damaged which is specified by the manufacturer as the damage threshold. Making sure that the working conditions are in specification of the optical devices is essential for a successful ablation (ask provider for information on: suitable wavelength, maximum raw beam diameter, and threshold fluence).

You can differentiate between two main different types of coatings. Shiny metals such as gold, copper or bronze have been used as mirrors since ancient history. Nowadays glass is coated with a tiny layer of metals such as silver or alumina. The reflectivity of metallic coated mirrors is determined by the physical properties of the metal. Using mirrors with a metallic coating are quite cheap but less resistant to high laser energies, thus dielectric coatings are mostly used to synthesize particles by LAL, where strong laser beams are common.
By using dielectric coatings defined optical interference between the reflected and transmitted light can be set and the reflectivity can be precisely adjusted by the thickness of the coating layer. Each layer reflects the beam and weakens the intensity that reaches the next lower layer. You already know that absorbing laser energy lead to ablation of a substrate, the same can happen to glass.

Once we focused with a green Nd:YAG laser into a cylindric glass vial (one of the cheap ones with the snap-on lid) from the side for laser post-irradiation of a platinum colloid. Of course, we did not see the beam because we were wearing safety glasses, but we had a camera on and looked at the pictures later. There was a meter-sized laser light wing reflected into the half lab, on half body height. Luckily, the laser was not on full power that day and no one was too close to the vial.

Thus, the dielectric coating are used to prevent energy absorption due to many reflective layers. A good deal is to buy mirrors with “HR” = high reflectance coatings. Are you then on the safe side? Yes, but only when you avoid dust particles to settle on the optics. A dust particle is an absorbance center and will cause spikes at the optics that again act as even more effective collecting centers. When you shut the laser down, put coverings (simple plastic bags) over each mirror. When you notice that laser power is lost after a while, it’s very often the optics that need to be cleaned. Imagine a single mirror “consumes” only 5% and you have three of them aligned, what you get at the end is the cumulative loss, mathematically 0.95 x 0.95 x 0.95 = 0.85 energy yield. Small thing, big

effect, and easy to avoid. The thickness of the layer is in nanometer range and thus the coatings are sensitive to any scratches. You need to be careful not to destroy the layers if you want to clean the mirrors. For this purpose specific optical cleaning supplies are commercially available. These will be useful many years for the whole laser lab. For instance, extremely soft tissues can be wetted with very pure methanol and glided carefully over the lenses or mirrors without scrubbing it. If you just need to remove only the dust on your optics air duster that blow the dust away are suitable.

STEP 6: CHOOSE YOUR ABLATION SETUP

Besides the simplest setup described in Step 1, several experimental configurations are possible to perform particle synthesis by laser ablation in liquids. To ensure uniform mixing of the dispersed particles in the solvent a magnetic stirrer can be added. For a high utilization of the target, the vessel or a target holder can be moved during laser ablation. Some examples of the setups with different kind of sample holder are sketched in Figure 7.

Of course, it is also possible to penetrate a vessel with the laser beam and for instance to work with closed setups with flammable solvents. In that case you need to be careful not to damage the glass. You should carefully choose the distance of the laser focus, which is not to close to the glass so that high fluences are reached only in proximity of target and not on the glass window. For a higher stability it is recommended to use quartz glass (like a cuvette) or a self-designed chamber with a coated window that enables to minimize surface reflections and thus any loss at the optical surfaces due to reflections.

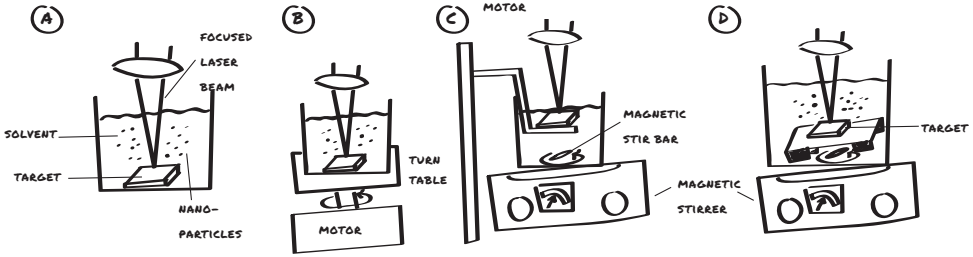


Figure 7: Pulsed laser ablation in liquids using different setups in a batch operation mode.

Thinking about long-term particle production, where a large amount of particles and solvent are required, a continuous ablation process can be quite useful. Sounds complicated, but in fact it makes multi-milligram scale synthesis easier and more robust. Here, the solvent flows over the target and carries the just formed particles (and the bubbles) away from the ablation zone and collects the product in a storage vessel (Figure 8). More detailed setups are show in Chapter II. As long as the target is not perforated you will have a continuous ablation process, however if you need to change a thin target often this can take longer than in a batch process. Most importantly, it enables a so-called steady-state, where concentration does not change with time in a given volume everywhere downstream. You may imagine that steady state synthesis conditions favor steady state particle growth conditions, improving reproducibility. A gold plate could feed a laser ablation process for many hours, and all you have to do manually is the taking the vessel at the end, it is like tapping nanoparticle colloids *(see: http://youtube.com/nanofunction)*. Since time is money, and being lazy is linked to creativity (in particular for sunshine-affine Italian guest scientists), researchers also study how to optimize the process by wire ablation. This technique enables a fully continuous synthesis of nanoparticles. For LAL the wire may be either just dipped and fed into an ablation chamber or run without a chamber being surrounded by a concurrent liquid jet. In the setup a motor enables to move the wire continuously through a cannula (a tip broken from a syringe) with a desired velocity (Figure 8). The setting of the feeding speed then should be adapted for the entire wire thickness is ablated. By this method, a higher productivity can be achieved due to different cavitation bubble dynamics compared to the processes involved during LAL of a bulk target. The mass that is ablated can be easily pre-calculated and pre-set by knowing the length of the wire that is ablated. Note that many metals are available in wire shape at no extra costs, and a roll of silver wire can run for a long time. A disadvantage of the ablation of a wire is that process stability is crucial. For example, large micro to millimeter nuggets can be removed from the wire if the feeding rate is too high, and thus not the whole material can be converted into a nanoparticle colloid. Hence using this setup is more convenient for experienced operators, because you have to optimize your experimental setup very precisely.

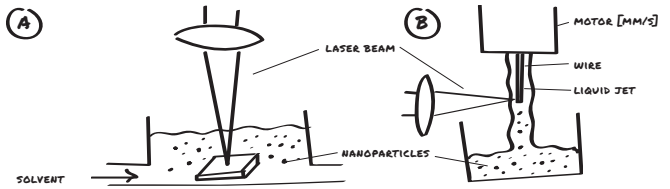


Figure 8: Pulsed laser ablation in liquids using a continuous flow system (A) with a wire (B).

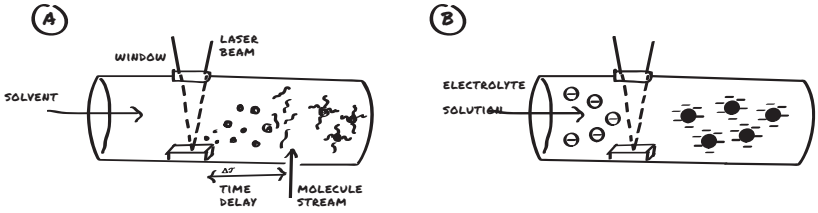


Figure 9: Continuous ablation with the possibility of particle property control by (A) delayed conjugation and (B) in the presence of electrolytes in a flow-through chamber.

For many applications such as biology or catalysis small nanoparticles are desired. In case you want to do laser ablation in a continuous operating mode and tuning the particles properties by adding molecules or specific ions you can construct a chamber with inlets. There you have a reservoir where you add, e.g. surfactant, albumin, or salts. When pristine particles are synthesized by LAL in liquid flow, they tend to grow on in the stream. In case a quencher molecule is added, this growth stops immediately and the particles become smaller. This can be utilized by using a flow-through reactor where quencher molecules are added after certain time delays. The time delay within the reactor can be controlled by the flow rate and by the distance between the point of particle generation and the point of quencher addition (for more detailed information please check Chapter II). Then the size can be controlled by the downstream position of the delayed injection (Figure 9 A) or in situ by adding electrolytes or stabilizers to the feed (Figure 9 B).

Overall, for experiments where you only need to do ablation for a couple of minutes or need to change the target often, the batch chamber is in most cases the best choice. It is always worth to add a mixing effect (magnetic stirrer, fast rotating target, ...). If you have to run laser ablation longer and have thicker targets, add a pump to create a continuous flow. Depending on concentration you want, the flow is easily switched to run recirculated or run in one path. One path flow creates steady state for particle formation, increasing reproducibility.

STEP 7: FIND THE FOCUS

You may imagine that irradiating something with a laser beam e.g. such as a laser pointer, does not automatically lead to ablation of a material. The criteria for doing so include an adequate intensity of a beam. Using a high-energy laser and focusing a beam on the target enables a high local fluence, due to the bundled energy in a tiny spot area. In this manner, the ablation threshold – the minimum fluence at which ablation takes place – can be overcome and the solid

can be vaporized. This threshold defines the minimal amount of energy per area needed in order to heat up the target strong enough, such that material gets vaporized and turned into plasma. For most materials this threshold typically will be found in a range of 1-10 $\frac{J}{cm^2}$ when using the very common nanosecond, fundamental Nd:YAG laser wavelength of 1064nm. To focus the beam onto the target a convex lens is required. The focal position can be found by moving the target or the lens, which has a certain focal length. But how does it work, to 'find' the focal position? In the field of laser materials processing many parameters are used to describe the laser beam, focal position etc.. Since we do not want to complicate the first steps, let's keep it simple.

You knew that you have to use your hands and your eyes for pulsed laser ablation in liquids, now you learn that you can use your ears as well! The probably simplest and fastest possibility to position the focus onto the target is by hearing the intensity of the sound, which a laser causes if ablating a material. If the laser beam hits the target with an appropriate intensity, it starts to ablate the material and makes noise. At the position where you hear the loudest noise, the highest ablation rate can be affirmed due to highest effective fluence at the focal position. However, for systematic research studies, this method is not accurate and the sound intensity depends on the bubble cavitation (the sound frequency you will hear in liquid is not the laser pulse frequency). So probably, your boss won't hear a sound, while you will hear more precisely high frequency noise just because you are younger than he or she is. If you don't believe it, just test how old your years are by hearing to several frequencies in the internet. You'll find a lot of different self-tests at YouTube, simply by typing 'how old are my ears'.

Figure 10: Sketch of someone, who is listening to "laser music"

To find the focus in a way independent of your ears, a microphone can be used. The magnitude of microphone signal linearly correlates with the laser fluence hitting the target. You don't need a special microphone system, if you want to

keep your approach simple. In case you want to make sure the productivity is high, you can easily do that by using a basic 'singstar' microphone e.g. coupled with a readout software. And look for high frequencies, maybe around several kHz.

I planned to do some UV-Vis measurement and I washed a cell. After that, I wanted to dry it as fast as possible so I used a nitrogen blower. Since I didn't think of the gas pressure and hence I underestimated it so that the cell blow up in my face and broke. It was really high pressure!

Mizutaru Tsukasa, Tsukuba

Back to other possibilities for an appropriate focal positioning, you can use an indirect but very result-oriented method to find the properly focal distance to the target. That is by mesuring the productivity. You can provide a series of ablation experiments, where the distance between the lens and the target is varied. The obtained colloids, which are synthesized at several positions of the lens to the target, can then be analyzed by UV-Vis spectroscopy (see Chapter III). Oftentimes (depending on the material that is ablated) the extinction of the spectra at a certain wavelength correlates with the particle concentration. E.g., in case of gold the extinction at 380 nm increases with a higher production rate. If there is no specific interband wavelength, use the intensity of the shortest wavelength in the spectrum where the solvent does not absorb. By varying the position of the target relative to the lens, you can find the maximum of the extinction and by this way the focal distance. Alternatively just weighting the target before and after ablation works as well, which is more appropriate for several materials. An important thing to note is, before doing any laser ablation tests, the laser spot should be located only on the target and not e.g. on the ablation chamber itself. For this, first position the laser beam with a low energy or use a positioning laser, which is directly integrated into some laser systems.

Once you know which materials you need and how to find the focal position, you should be able to synthesize nanoparticles by pulsed laser ablation in liquid. What do you think will happen if you perform laser ablation of the target for a while - let's say 5 minutes (note that the time depends on the repetition rate, pulse duration, fluence and so on)? You'll be able to efficiently drill a hole into your target, however this is not what you call efficient use of material and you will also ablate the material that is behind the target and get impurities in the colloid. In order to avoid a fast hole-formation and thus contaminations, two approaches are possible. On the one hand, the target can be mechanically moved e.g. by circular movement of the whole chamber. By this way, you may

exploit a larger surface of the target that makes it possible to use it for longer ablation time without the need of replacing the target.

A much more elegant but also much more expensive solution is the continuous shift of the laser beam on the target, either by using a motor stage moving the chamber or by using a rapid scanner system moving the beam. By using a scanner system (it's always faster than most axis systems) the cavitation bubble can be spatially bypassed and thus a higher ablation rate can be achieved, since the laser beam is not shielded from the bubble. A scanner system can create different ablation pattern. The simplest form of scanning patterns are stripes (Figure 11 B). However if one strip is scanned to the end, the mirrors cause a certain delay time by moving back to start the next strip. During this period, no pulse is coupled and thus the productivity is influenced. For example in case of a 50 mm long strip and a scan speed of 1 m/s, the scanner needs 0.05 s to move this distance back. By working with a repetition rate of 5 kHz about 250 pulses cannot be coupled in this time. Second, unless you have an advanced "on the fly" scanner, the software will accelerate and decelerate the beam guidance mirrors at the beginning and at the end of the line, respectively. This will cause deeper ablation there, and again the target is not ablated homogeneously in the whole area, making early target replacement necessary. Thus it is more efficient to move the laser beam continuously. For example by moving the beam 50 mm to the right, 50 mm downwards and then 49 mm to the left generates a rectangular spiral pattern (11 C). However in that case only one mirror is moving in each direction, which again cause a delay, before the other mirror reacts (and again, deeper ablation at these turning points, and it's the same with motor stages). Alternatively, scanning the laser beam in an Archimedean spiral pattern with 'rounded corner' has proved worth and preferable shape, which enables to exploit as much as possible of a target per time and assures maximal target use (11 D). Also here at the end of the spiral one delay occurs if the scanner had to move to its initial position. The highest utilization rate then would be by using a Fermat's spiral pattern, which is unified within itself and allows a continuous movement of the laser beam (11E). Anyway, for an optimal utilization of the target material, the target needs to have the form of the scanning pattern, e.g. in case of stripes a rectangular target is the best and a round target shape can be used with a Fermat's spiral. A set of annex spirals can mimic a rectangular shape.

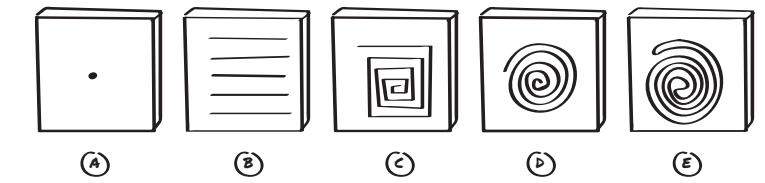


Figure 11: Scan pattern on the target for laser ablation in liquids: (A) Without scanning, (B) Stripes, (C) Rectangular spiral, (D) Archimedian spiral, (E) Fermat's spiral.

STEP 8: SPICE UP YOUR NANOPARTICLES

A huge advantage of laser-generated nanoparticles is the exclusion of toxic substances or by-products that adsorb onto the particles surface, since chemical precursors are not needed. Depending on the laser parameter, pulsed laser ablation in pure water very often leads to polydisperse particle sizes or even multimodal size distributions. To spice up your nanoparticles you can use (either micromolar saline solution for noble metals, or) several post-treatment methods. Centrifugation is such a method to play with the size distribution (compare Chapter III). By using a combination of Svedbergs' equation and Stokes-Einstein relation a pre-defined cut-off can be calculated. The cut off is the particle diameter value that defines the fractionation size level between supernatant and sediment. Using this formula the centrifugation time can be calculated if parameters such as particle size (intended cut-off), nanoparticle material density (take the value of the bulk), viscosity of the solvent, rotation speed of the centrifuge and dimension parameter of the centrifuge are known (rotor type that holds the vials). Due to the faster sedimentation of larger particles compared to smaller ones, different fractions can be separated to desired particles sizes by taking advantage of centrifugal forces. In this manner, after centrifugation the largest particles or agglomerates are located at the bottom (sediment or pellet) and small particles (supernatant) at the top of the centrifugation tube shown in Figure 12.

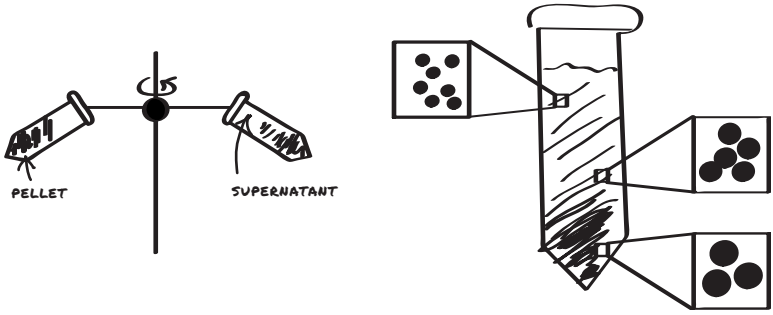


Figure 12: Particle size separation by centrifugation.

Even though size separation by centrifugation is a quite simple method, one main disadvantage is the loss of mass if only one fraction is needed (the pellet cannot always be redispersed). Typical loss of a bimodal colloid of laser-generated noble metal nanoparticle colloid in water, if the pre-set cut-off is in the range of 10-20 nm (to get rid of the large fraction by-product of LAL), is about 30-50% of the mass. It will take only 3-5 minutes with typical benchtop

centrifuges (far longer centrifugation times should be avoided in any way, unless you have a centrifuge or ultracentrifuge that cools down the colloid). Finally, you will end up with a monomodal, often monodisperse colloid, free of any additive.

The size of nanoparticles can be also tuned by re-irradiation of a colloid with pulsed lasers. At high laser fluence, post-irradiation of nanoparticles leads to fragmentation of nanoparticles and thus to smaller sizes and narrower size distributions. This Laser Fragmentation in Liquids (LFL) generally benefits from a wavelength at which the particles can absorb significant light, however laser fluence should be still efficient. As a rule of thumb: the product of absorption at the laser wavelength (measured in the UV-Vis spectrometer) and the pulse energy at that wavelength should be maximal.

For very small nanoparticles, surfactants can be used during laser fragmentation. But also re-irradiation of nanoparticles in totally ligand-free environments or micromolar saline solution facilitate size reduction by high laser fluence laser fragmentation. For noble metals in water, maybe adding 0.1-0.3 mM NaOH or carbonate buffer (there is CO_2 in water, anyway) could be a worth a try.

In contrary to laser fragmentation, which yields smaller nanoparticles, Laser Melting in Liquid (LML) can be applied for the production of spherical particles of several hundreds of nm in size by using low fluences applied to metal or dielectric nanoparticle (aggregate) dispersions. At moderate fluences (try 10-300 mJ/cm²) nanoparticles are stimulated to aggregate and the aggregates of nanoparticles then melt, fuse together and form submicron spheres. There large spheres are easily sedimented by gentle centrifugation and are often well redispersable.

Re-irradiation of a colloid can be followed in different setups. The simplest approach is laser fragmentation in a batch chamber, where a colloid is irradiated from above or from the side (Figure 13, left). Similar to this, post-irradiation can proceed in a continuous operation mode, in which a colloid is operated in a flow-through mode and is irradiated by a pulsed laser light (Figure 13, middle). A significant disadvantage of these setups is that focusing a laser beam in the colloid results in a deviance of fluence at several positions in the liquid, thus leading to different mechanisms in particle re-formation. In sharply focused situation with long beam path in liquid, you may cause LFL in the focal volume but LML before and behind it, creating a mixture you may not want. Also, if the total liquid volume is larger than then irradiated volume, educt-product mixing appears, and the process renders to be less efficient. The most precise post-irradiation can be performed in a liquid-jet reactor (Figure 13, right). Sounds extravagant, but it is just a funnel, more detailed a flask with the educt on top reservoir with a mm capillary outflow at the bottom, omitting the use of a pump. The geometry of a reactor with a liquid jet allows a tight focusing of the laser beam on a thinly volume, so that each particle is reached by the same laser

light fluence and losses of excitation light by scattering or absorption in front of the focus can be reduced. Furthermore, even at high light intensity, there is no risk to damage any optical element like windows or cuvettes, the beam entrance is the liquid itself. If the reservoir is empty, you have done one passage with very defined energy input (think of placing a laser powermeter behind the liquid jet to quantify the energy intake) and can see if the colloid meets your demands. If not, just refill and go for the second passage. The outflow speed slightly changes during the process because hydrostatic pressure changes when the reservoir gets empty. A workaround is quite simple: use the concept of a Marriotte's bottle (see Wikipedia) to assure constant volume and particle mass flow passing the beam.

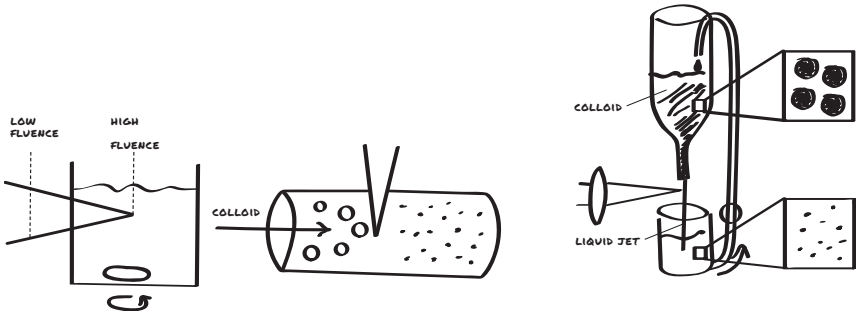


Figure 13: Set-ups for pulsed laser post-Irradiation of nanoparticles in liquids.

How to influence the particle size by using ions or other additives such as biomolecules will be explained in Chapter III.

STEP 9: NOW, JUST DO IT

You are entering the lab and see this 'magic box', which is more powerful than it may seem. You don't need to be afraid of powerful lasers, but you should treat them with respect. You need to take the security aspects into account, which are described in detail in Chapter V ("Stay alive"). By this, you'll have best precondition to safely operate with lasers.

The day should start with all preparation steps for the set-up. Sometimes experiments can fail, if only a screw is missing, thus it is worthy to make a list with the required preferably on the afternoon before you actually intend to use the laser (Figure 14). It may be satisfactory having everything waiting for you in the lab and get very productive already right after your morning coffee.

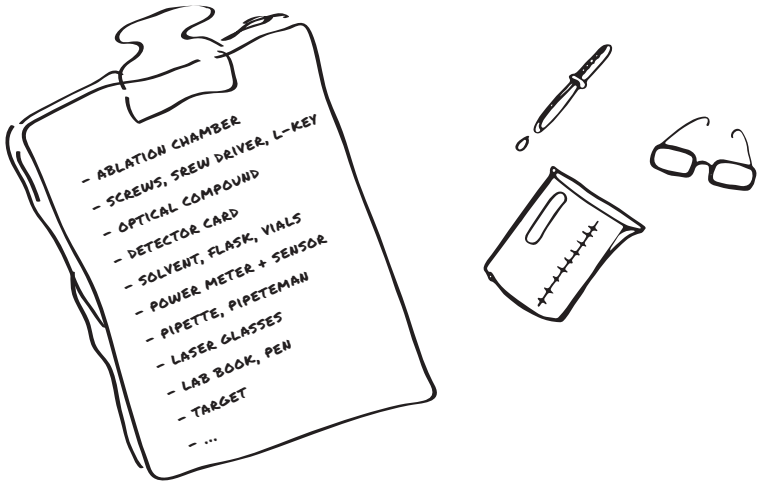


Figure 14: Check-list for preparing pulsed laser ablation in liquids in the afternoon before your day in the laser synthesis lab.

When you have all the required things together, you can start to prepare the setup. Before switching on the laser, don't forget to put on the laser glasses and let others know that laser is on!

You need to build up the optical components to align laser beam to a desired position. All optic compounds should be free of any defects and dust, to ensure a high yield of light reaching the target. To locate the laser beam a detector card can be used (as your laser is invisible through safely glasses), where a photosensitive region allows to view the light spot. Before use, such a card requires "charging" with visible light, and for optimal brightness of the beam one must move the card around because the emission from the card is not persistent. If you use a laser scanner, align the laser beam with a low intensity into a scanner optic and then onto a thermal laser power sensor, which is connected to a powermeter. Note, that you should measure the power only in the raw beam or out of the focal plane to avoid a destruction of the sensor. Point out that you have to check the fluence of the raw beam directly at the source and – most relevant for the synthesis - behind all mirrors (right before the focusing lens) before you start in order to avoid potential fluctuations in the laser fluence. Once you have directed the raw beam spot on the sensor, you can slowly increase the laser intensity and measure the power that you intend to use for laser ablation.

Wait a couple of seconds for thermal equilibrium of the sensor (and remember, many lasers need a warm up period of 20-30 minutes to reach steady state of output power).

Using the example of laser ablation in a flow-through chamber, the preparation steps are briefly described. First, the mass of the target should be determined, hence the ablated particle mass and with it the colloidal mass concentration can be concluded by weighting the dry target before and dried target after ablation. The ablation chamber should be prepared by embedding the target and screwing all component parts. After placing the chamber in front of the scanner in a distance of the focal length, very often, because of liquid refraction, the point of maximal productivity is behind the focal plane in air (or the focal distance the lens supplier has provided), e.g., at 1-2mm longer distance. Tubes should be connected with the chamber and the pump. A certain volume of the solvent should be filled in a flask and the tubes should be dipped into the solvent. Before starting ablation, the liquid should be passed through the chamber and recycled back to the flask until no bubbles can be observed to prevent ablation in air or bubble scattering. Be sure, that no solvent is leaking during the flow.

The target can be ablated in a continuous process, where the solvent is pumped through one flask to another collecting vessel. If you want a highly concentrated colloid, quasi-continuous ablation (so-called semi-batch) can be carried out by cycling the solvent during laser ablation in the same flask. It should be noted, that by this mode of concentrating operation particles can be re-irradiated more intensely by the laser beam. In most setups, you will have some post-irradiation, and no flow setup is fast enough to cope with kHz repetition rates. But flow brings everything in a controlled steady state.

Now you can align the laser beam through a scanner optic onto the target (or directly on a moving target) by directing a laser beam with a lowest possible energy. Some lasers have integrated an additional positioning laser, which helps to adjust the beam on a target object. Oftentimes your laser is equipped with a shutter, which can be controlled by a software or opened and closed manually. After you have found the optimal set up parameter, and you are sure that the beam hits only the target, you can start to pump the solvent and increase the laser energy.

Since you have synthesized a colloid, you may notice that the concentration of nanoparticles in the liquid is not as high or the particles even start to agglomerate after a short time. Now the time has come when you can use your knowledge on finding the focal position and continue to perform systematic studies on laser synthesis and stabilization of colloids.

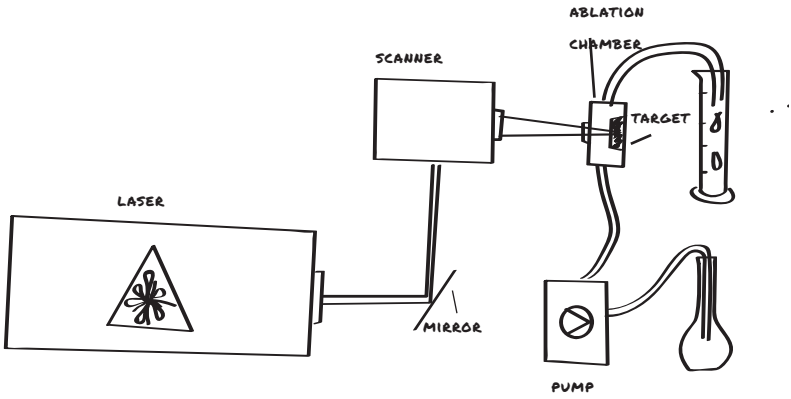


Figure 15: Optimized experimental set-up for pulsed laser ablation in liquid using a flow-through chamber with continuous operating mode.

At the beginning, many mistakes can be made and of course, you learn from all of them. However, there are some typical mistakes that can be avoided. The list of lessons learnt by us may help you to prevent unnecessary waste of time and difficulties:

- Forgetting to weight the target
- Working without laser goggles
- Leaving shutter closed, when trying to find the beam
- Aligning the beam with maximum laser energy
- Leaving shutter open, before pump was started
- Adjusting the beam out of focal position
- Ablating till hole formation at the target causing ablation of the chamber material
- Setting optics incorrectly (maligned optics or covered with dust)
- Sealing ablation set-up (chamber, pump) insufficient
- Irradiating in open flammable solvents or leaving combustible material near laser set-up

You can find all the things mentioned above in the following that can help you to synthesize your first particles by laser ablation in a liquid:

PREPARATION

- ✓ Do your checklist with all necessary objects for your set up
- ✓ Bring all the things together the day before you start synthesis
- ✓ Check your optics and clean it if necessary
- ✓ Weight your target
- ✓ Prepare the solvent

SET UP

- ✓ Build up your set up (optics + chamber)
- ✓ Remove all flammable items near the laser beam (solvents, paper, packaging, ...)
- ✓ Put your laser goggles on
- ✓ Check laser alignment, use detector card and low power
- ✓ Allow laser warm-up and measure the laser power (raw beam!) after all positions (behind the laser source, lenses and mirrors)
- ✓ Position the chamber and fill it with solvent

LASER ABLATION

- ✓ Align the beam on the target
- ✓ Find the focus
- ✓ Start to produce your particles
- ✓ Enjoy

Good luck and may the force be with you!

GET MORE: PRODUCTIVITY

II

In the previous chapter, we used the power of light to produce very pure and tiny nanoparticles by pulsed laser ablation of different materials in liquids. With this fancy setup, you will be able to delve down into the amazing playground of nano-sized objects. However, exploring this small universe doesn't necessarily mean being content with tiny quantities. In case we want to characterize or even apply our nanoparticles in some novel material combinations it is not enough to end up with small amounts. Therefore, let's grow the scales and make small things huge – not just in size but also in quantity.

STEP 1: HOW TO DEFINE THE NANOPARTICLE PRODUCTIVITY?

To start with our scale up procedure it is important to initially think about how we want to measure productivity and consequently find a proper definition for the expression "nanoparticle productivity". Does "yield", "efficiency" and "productivity" have the same meaning? Yield refers to the product, e.g. 80% after centrifugation or "100 ml with 1 mg monodisperse gold". Efficiency needs something to refer to, so that there is often the effort you put in for the synthesis in the denominator, e.g. mass you obtained per laser energy (mg/J). Productivity has the unit of time in the denominator, e.g. mg/h. The meaning of productivity seems obvious, but once we look closer in this topic there will be several possible ways to define the nanoparticle productivity.

Let's begin with the most apparent choice and define the productivity as the total amount of ablated target mass per unit time. It can be obtained by simply weighting the target before and after an ablation interval. The precision of this gravimetrical technique is only dependent on the chosen time of ablation and the scale that is being used. Evidently, the significance of your results will be enhanced, if an extended timespan is selected. In general the ablation time should be chosen such that a single ablation experiment will last at least 5 minutes while the minimum amount of ablated target mass should not fall below 3-5 mg. The latter limitation is necessary in order to keep the relative weighting error lower than 5% (following a typical error of about 0,1mg for a standard analytical balance). Additionally it should be kept in mind that most lasers do need about 20-30 minutes pumping to reach a stationary power output (new lasers maybe 2-5 minutes). Consequently, the laser beam should be aligned into a beam trap or onto a power meter before starting laser synthesis, until the stationary power output has been reached in order to maximize reproducibility.

Working with intense laser radiation is a task, which requires concentration due to the risks of burning ones skin and eyes. Still, there are easier ways to get hurt. When checking lenses and mirrors one should make sure not to bend over in a way that leads to hitting the edge of a safety screen and suffering of a bleeding ear afterwards.

Alexander Letzel, Essen

As easy and convenient as the gravimetrical approach may seem, it always includes interrupting the ablation process making time resolved productivity studies impossible. However, in case some knowledge about the temporal alteration of the productivity is required, a second definition of productivity may be introduced. In contrast to the "gravimetrical productivity", let's additionally define a "visible productivity" considering the amount of colloid being detectable e.g. by optical measuring techniques. You may be thinking – shouldn't those two definitions be the same? Bear in mind that nanoparticles may not be stable and precipitate or even dissolve in the liquid. It may also be required to exclude generated micrometer sized particles from the productivity definition as they often aren't the desired product. Therefore, it is obvious that both definitions may differ significantly depending on the chosen metal-liquid system and the required nanoparticle specifications.

In order to quantify the "visible productivity" the nanoparticle concentration may be monitored by UV-VIS absorbance spectroscopy over time. With this method the concentration of a colloid can usually be calculated considering the extinction of incident light over a given path length. More about this type of detection will be given in Chapter III. As the ablated mass always gets converted into particles with 100% yield, both the "gravimetrical" and the "detectable" definition are equal as long as the particles are sufficiently stable over some period of time. For the ablation of most noble metals this statement holds, however when ablating metal oxides the "detectable productivity" and the "gravimetrical productivity" approaches may significantly deviate as nano-/microparticle precipitation is often observed in those systems.

The final question about which of the two definitions is to be chosen, may arise. To answer this question: it depends on what you're interested in! The gravimetrical approach is easy to measure (and does not need material-specific calibration curves) but lacks temporal resolution and ignores precipitation and dissolution whereas the "visible productivity" definition gives rise to a well-defined time resolved productivity profile while requiring more effort

and knowledge about the used nanoparticle system. The latter will however become a very powerful tool when thinking about long term continuous ablation methods. However, it requires a calibration curve, to be done by simply diluting a colloid and find out which UV-Vis wavelength gives best correlation coefficient in the linear fit (don't force it to go through zero) of the diagram concentration vs intensity. By knowing the different ways to measure productivity we are now fit to generate our first nanoparticles.

II

STEP 2: CROSS THE ABLATION THRESHOLD

Well, maybe this chapter should be called crossing the information desert without some refreshments in you back-pack but unfortunately, our sleeves will still remain unwrapped until we haven't thought about the basic idea behind the laser synthesis of colloids. In fact, to generate some of our desired midgets it is required to reach the so called ablation threshold already introduced in Chapter I Step 7. This threshold depends on a manifold of different properties. Next to material properties, it is defined by laser parameters such as laser wavelength, laser fluence and the pulse duration. Keep in mind - Laser synthesis of colloids is like performing martial arts – so let's consider the aforementioned parameters from the angle of e.g. Muhammad Ali. Let's say the laser wavelength represents your striking precision, the laser fluence creates your jap virtue and the pulse duration is your quickness. Analyzing your opponent's weaknesses first, you want to precisely strike where it hurts most. For an ablation target, you'll find its weaknesses in its wavelength dependent light absorption. Choosing the right laser wavelength will significantly increase your impact. But maybe you can't choose a weak spot (i.e. fixed laser wavelength of your laser) on your own, it is obvious that you can always strike harder (increase laser fluence) to make up for that. Of course your striking power is limited so you have to make use of your quickness (pulse duration) as well. A fast strike with a laser pulse of very short duration will surprise the target making it "bleed" and loose nanoparticles.

I ENTERED THE LASER LAB AND FOUND THE SURFACE OF THE POWER METER SIGNIFICANTLY DAMAGED, WITH A DISTINCT ABLATION SPIRAL. OBVIOUSLY ONE OF THE STUDENTS TRIED TO MEASURE THE ENERGY OF THE LASER CLOSE TO THE FOCUS DURING OPERATING AND SCANNING THE ABLATION SPIRAL.

SEBASTIAN KOHSAKOWSKI, DUISBURG

The reason for the higher impact of a laser pulse with short pulse duration is that it strongly influence's the energy loss due to energy transport processes in the following way:

$F_{theshold}$: *femtosecond pulse* ≤ *picosecond pulse* < *picosecond pulse*

resulting in different time scales for the successively occurring heat exchange mechanisms. Basically, the target material will just be struck faster than it is able to react. This situation is roughly summarized in Figure 16. As you can see, there are mainly two different extreme cases: one when using a nanosecond pulse, and the other with a femtosecond pulse. The reason for the two extreme cases is a property of materials, which is the so-called electron-phonon-relaxation time. When the laser beam starts being absorbed the electrons get excited. It's the same as if Muhammed Ali struck his opponent: in the first second after striking his opponent, he doesn't feel any pain. However once the brain of his maltreated victim realizes there was a strike, his quarry collapses due to KO. The same happens in the material when the excited hot electrons start to interact with the cold lattice, heating up the material. The delay for this heating process is the aforementioned electron-phonon-interaction time. The usual timescale of these is in the picosecond regime for metals. Subsequent on this interaction delay the target temperature increases while the electron temperature decreases until both, lattice and electron temperature are roughly the same. Now if you consider the first extreme of, e.g., a 10 nanosecond laser pulse, the pulse duration is far longer than the time required for the electron-phonon relaxation Figure 16 (middle). Therefore electron- phonon relaxation (several picoseconds) is already occurring, thus heating the target in the irradiated area, while the laser pulse is still continuing. Due to the increasing temperature gradient between target and liquid as well as inside the target itself an additional heat transfer kicks in after a few nanoseconds, leading to heat conduction into the target and heat transfer (via the plasma plume) into the liquid while the laser pulse is still ongoing (Figure 16, middle). Now, from this argument you may already imagine that this condition results in deep heating, finally resulting in vaporization and plasma formation, which is why this case is called hot ablation. The laser is still on when the plasma arises into the liquid, hence the incoming light is shielded and consumed by the plasma, lowering ablation efficiency (ablated mass per pulse or ablated mass per joule pulse energy) compared to ultrashort pulsed (often called "ultrafast") picosecond and femtosecond lasers. Going back to boxing, this nanosecond technique is similar to Nikolai Walujew standing in the ring. Due to his slow punches he needs more force (energy) to knock his opponent out which is why we may get the impression nanosecond pulses are rather less desirable. Therefore, let's think about a very short laser pulse in the femtosecond regime. In this extreme case the laser beam duration is already finished before the electron-phonon-interaction is over with the result that the whole energy gets stored by the electrons and suddenly released into the lattice, creating a sudden expansion and explosion of the material.

As no heating occurs this ablation is often called cold ablation (in practice, there is a small portion of heat deposit in the target that is often not noticed when working in liquid unless you go for very high repetition rates). It is obvious that this technique creates much less heat loss which is why a higher fraction of energy is used for the real ablation process.

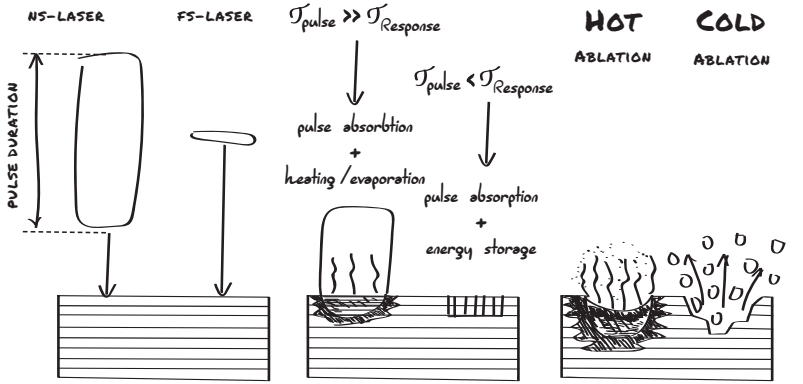


Figure 16: The effect of pulse duration on the ablation mechanism.

Hence, when using nanosecond pulses, a higher fluence is needed to cross the ablation threshold. Of course, from this behavior you may infer that femtosecond or picosecond pulses should be highly favored especially because the ablation is more energy efficient. Yet we're only at the beginning of our journey and we will see that high efficiency also comes with a cost, creating additional efforts and therefore rendering inefficient brute force is the easier option in some situations...

STEP 3: MAXIMIZE THE ABLATION EFFICIENCY

With the gained knowledge about the ablation threshold, especially about its dependence on the pulse duration, it is time to discuss how we can increase the ablation efficiency without varying the pulse duration of the laser beam. That will become your routine work, because pulse duration is fixed at most laser systems. You may already smell the rat – in order to increase the fluence

we have two options: either increase the pulse energy or decrease the beam area. Before working on practical approaches on how to achieve either of these alternatives, we may split our ideas into cost-intensive and non-cost intensive measures. It is obvious that you will always endorse yourself by using a non-cost intensive approach saving lots of money. Did you already figured out which of the two given approaches may embody the trail towards success and popularity amongst your boss? If your choice fell upon maximizing the pulse energy you probably chose the sledgehammer to crack a nut. It is almost certain that initially following this approach you will sooner or later end up buying a new expensive laser because the old one seems too feeble. Of course, the laser manufacturers will be pleased by this approach however your budget may not be amused. Also, very high pulse energies don't make you happy once you've seen how huge pulse energies damage your optical components or ablation chamber windows. To save some of your money, initially the non-cost intensive approach of simply changing the beam spot area should be chosen. In order to do so the laser beam can be focused using a collecting lens. This is one of the easiest actions available to significantly increase your ablation efficiency and should always be your first priority when thinking about scale up measures. With the collecting lens introduced into our setup your first scale up action is to optimize the distance between lens and target as shown in Figure 17. When ablating in gaseous media, the highest ablation rate will usually be found when the beam focus is aligned with the target surface as the fluence reaches a maximal value within the focal spot. Having a target immersed in a liquid – say water - interactions of the laser beam with the liquid have to be considered. One of those interactions is the refraction of the laser beam at the gas-liquid phase boundary, shifting the focal spot depending on the chosen liquid height. This shift can easily be calculated using linear optics for the known liquid height. Depending on setup, laser and liquid applied, you may however want to discard the calculation of the refraction (considering the manifold of possible disturbances to be addressed soon) and simply choose an experimental approach following Figure 17. Here, the displacement between lens and target is being varied until the highest productivity has been found. As a general rule of thumb, the initial distance between target and collecting lens should equal the working distance of the collecting lens given by the manufacturer of the lens (Figure 17, point A), after which you increase the distance.

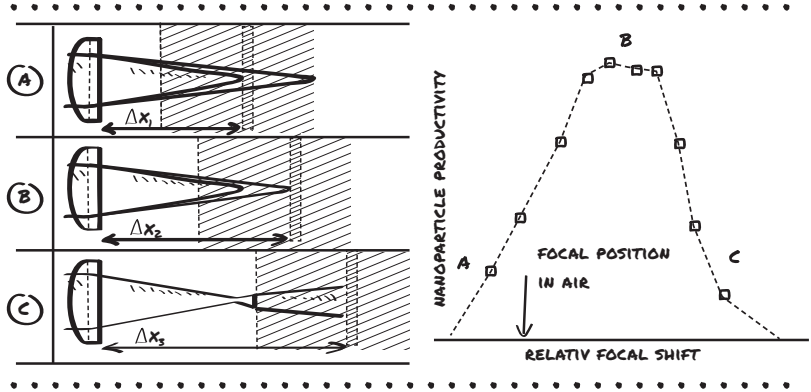


Figure 17: Optimization of productivity by changing the distance Δx_i between collecting lens and target beginning at point A where the Δx_i equals the focal distance of the collecting lens. The productivity optimum is reached at point B where the beam waist is located on the target surface. Shifting Δx_i further towards point C the productivity is again decreased.

The productivities shown in Figure 17 may be determined by the gravimetrical or the visible approach (or sound amplitude) discussed earlier. Once approaching point B, the productivity reaches a desired maximal value as the beam area at the target surface becomes minimal (fluence is maximized) with the focus located on the target surface. A further increase of the distances Δx_i should be avoided. Above the optimal distance Δx_i your laser focus will be located in the liquid, typically forming a plasma within the liquid leading to severe absorption, local boiling and bubble formation, significantly decreasing the LAL's nanoparticle productivity. In case the ablation is done in an ablation chamber where the fluid is confined by a quartz window (e.g. dashed line in Figure 17) a focus placed on the used window should be avoided. Otherwise damaging of the window and termination of the experiment (maybe even inflammation of the liquid, e.g. when using acetone or ethanol) due to a bursting window may be happening. Therefore, it is highly recommended to only conduct this optimization in between the range of A and B while starting at point A until B is reached. This routine is one of the main basics when trying to maximize productivity. It should always be repeated if the setup (e.g. liquid height, target thickness, different lens) or the liquids (refractive index) were changed in the meanwhile. You do not need to do this with the precious target material reserved for your flagship experiments series. Instead, you may take another material with same thickness. Avoid using high reflective materials for finding the focus (such as silver) if you have no idea where the focus is roughly, as it may back-reflect the partly focused laser light (from position A in Figure 17) and inscribe or break the window from the inner side.

However, it was already insinuated that there are numerous biasing effects that may still decrease the productivity or even avoid finding a proper productivity maximum. The next section will address those interferences and prevent you from spending too much of your precious lifetime while waiting for your nanoparticles being generated.

STEP 4: AVOID FLUID BREAKDOWN AND NONLINEAR EFFECTS

The previously described basic method will always be a starting point of your optimization. But as soon as the productivity starts rising, the previous simplicity becomes a rare luxury. When applying high laser fluencies and/or very short pulse durations a whole series of stumbling blocks may be evoked on your way towards higher productivities. Let's shed some light on how to evade those obstacles and make sure we boost our productivity to the maximum.

The first "banana peel" may possibly occur when focusing your laser beam into an open beaker filled with the target immersed in e.g. water. Let's consider the worst case scenario of high laser intensities, lenses with a long focal distance and a low liquid layer thicknesses being applied. Once exceeding a given fluence threshold in the liquid, you risk causing a significant fluid vaporization followed by severe bubble generation as shown in Figure 18 – Case A. This effect will drastically reduce your nanoparticle productivity, due to severe scattering of light at the bubble interface, rendering all effort of finding the best focal position inefficient and pointless. In order to avoid liquid vaporization when using lenses with long focal distance (small focusing angle α, see Case A and B) you will be forced to work at rather high liquid layer thicknesses (or smaller pulse energies). However increasing the liquid layer thickness (or decreasing the pulse energy) will again cause a substantial depletion of our desired productivity, as laser light might be scattered by possible gas bubbles (from solute gas) or absorbed of already produced nanoparticles.

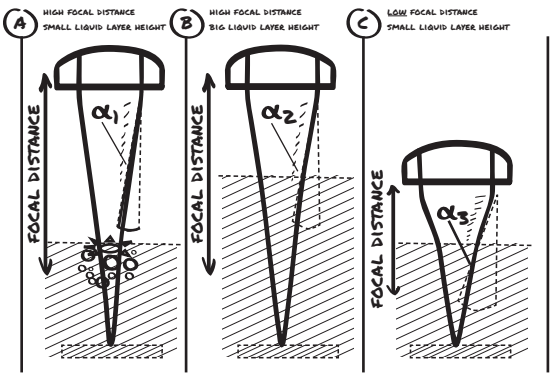


Figure 18: Influence of focal distance and liquid layer height on the generation of an optical breakdown at high pulse energies (high peak power). Case A: High focal distance and small liquid layer resulting in a small beam area (high fluence) at the phase boundary causing in optical breakdown. Case B and Case C: Fluence at phase boundary doesn't excess breakdown threshold → no optical breakdown occurs.

II

To avoid the aforementioned disadvantages it is feasible to refrain from using collecting lenses with a very long focal length. The application of shorter focal distances should be favored as those lenses possess higher focusing angles (short Rayleigh length) and therefore a bigger beam area at the liquid surface even at small layer height (Figure 18 – Case C). A smaller focal length will additionally result in a smaller beam waist at the focal spot leading to higher fluencies or, generally speaking, higher productivities. A big downside of lenses with short focal distance ($f << 100$ cm) is the limited action window. In this context, the action window refers to the spacing of the plateau at Point B in Figure 17 shown in Step 3. The shorter the focal distance the smaller this plateau, resulting in a stronger sensitivity of productivity on changes in the focal distance when using this type of lenses. One effect causing constant variation of the focal distance is caused by the ablation itself. Let's consider ablating a target, which implies removing material. It is obvious that a crater of increasing depth will be forming with ongoing ablation, continuously increasing the distance between the target surface and the focal spot position. Considering a lens with short focal length, you'll find a fast drop in productivity with increasing crater depth (due to the small action window / short focal distance) in case you're not constantly adjusting the distance between target and focusing lens. Therefore working with lenses of short focal distances increases short term productivity which on the contrary quickly drops if the distance is not constantly adjusted (e.g., every 15 minutes) increasing the working effort. On the other hand, a long focal distance ($f > 100$ cm) will increase the operating window but strongly limits the productivity due to the higher beam waist area. Hence, a suitable compromise between these opposing tendencies is necessary. A proper trade-off was usually found when using f-theta lenses with a focal distance of around 60 mm (and liquid layer thickness of around 4-6 mm).

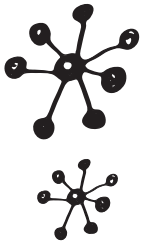
In my laser lab there is also a micro Raman spectrometer, that often needs long measurement sessions by my colleagues. For this reason, I'm sure to obtain some terrible curses by them when I tried laser ablation in iodo-benzene. Perhaps this solvent has not a scaring datasheet, but it has a persistent and really unlovable smell!

Of course I realized that a sort of "technological limit" stays in front of me: lasers cannot be positioned under laboratory extractor fan, and the least cannot be moved over the entire optical bench. So the solution was a sort of little portable extractor fan, linked with a flexible tube and equipped with some metal shields that allow to fit on my laser ablation set up. The next Raman users sincerely appreciated my set-up improvement!

Lucio Litti, Padova

Now, since we discussed what may happen when working with high laser fluencies, there is another extreme we didn't address until now – short pulse durations. As already discussed in Step 2, using short pulse durations in the femtosecond regime will result in nearly no heat losses and therefore high ablation efficiencies (based on energy input). In contrast to the mean power being released by the laser while switched on - the peak power is much closer to the apparent ablation process. From the example given in Chapter I Step 4 it is obvious that an ultrashort pulse duration will cause a very high peak power during the pulse, which can be similar to the power a power plant produces, causing a more efficient ablation (as discussed in Step 2). However, this peak power will also have a tremendous effect on the liquid in which the target is immersed. As soon as the peak power exceeds a threshold that depends on the liquid nature, events like self-focusing and optical breakdown will be the result.

In this sense self-focusing comprises a nonlinear effect usually occurring at peak powers in the order of GW. Mostly these high peak powers can only be reached with femtosecond pulses. The basis of self-focusing is the so-called Kerr effect which describes a dependence of the refractive index on the laser intensity. In normal applications, the refractive index of a liquid is only dependent on the wavelength of light. However, in case of such an intense electromagnetic field strength like in a laser pulse with a peak power of several GW, the positive Kerr effect results in an increase of the refractive index with increasing laser intensity. This results in a lensing effect where the medium acts like an additional collecting lens significantly shortening your focal distance. Therefore when working with femtosecond pulses in liquid, the focal position maybe found even above the geometrical focal plane. You will notice self-focusing in liquid by a white-light filament, like a straw of light. It causes some energy loss, but benefits from tight focusing.



The other effect called optical breakdown mainly happens when a laser beam of sufficient peak intensity enters a liquid out of air. And it will strongly diminish productivity. The intensity threshold for optical breakdown to occur was found to be $2{,}2 \cdot 10^{13} \frac{W}{cm^2}$ in case of water (800 nm and 120 fs) while the thresholds of organic liquids are often even lower. To get an idea about the scale of this value, let's just consider an example. In typical femtosecond ablations setup 100 fs and a beam waist diameter of 100 µm are common. If you consider the fluence threshold given above, optical breakdown will already occur when a pulse energy of about 170 µJ has been exceeded. However, this actually is a typical pulse energy when working with ultrafast lasers. Therefore, optical breakdown and reduced productivity are very likely when femtosecond lasers are being used at high pulse energies. Unfortunately, your only option to avoid optical breakdown of the liquid is to increase pulse duration or lower the fluence. Moreover, in many cases even this measure does not let you escape, because colloidal nanoparticles, in particular plasmonic nanoparticles are very effective "sensitizers" for filamentation. In practice, unless you have created

severe optical breakdown and splashing of the liquid, filamentation can still be handled. Many groups work with fs and ps lasers in liquids since decades and are quite happy with that. Maybe productivity is not always optimal for laser ablation synthesis, but there are no better laser tools for efficient fragmentation (downsizing) of colloids than ultrashort pulsed lasers.

Also keep in mind that during the "cold ablation" where the pulse duration is shorter than the electron-phonon-relaxation time (Figure 16), the fluence threshold for laser ablation and therefore the productivity (assuming the same laser fluence and wavelength) is less dependent on the pulse duration, rendering 100 fs - 10 ps laser ablation with similar efficiency. The shorter pulse duration is more efficient at the ground, but disturbed to a higher extend before reaching it. Increasing the beam area may be a proper alternative in these cases. However, when applying pulse durations significantly longer then the electron-phonon-relaxation time (that is, nanosecond lasers) the ablation efficiency forces you to bring out the earlier called sledge hammer to crack the productivity nut. In this context, the focusing conditions are crucial for nanosecond lasers.

Wrapping up the take home message of Step 4, if you try cutting target chunks using a scalpel (femtosecond laser) make sure to be gentle (limit pulse energy) and easygoing (prefer ps pulses) or you may spook the solvent (liquid break down) while carving out the desired target areas. In case you prefer using the machete (nanosecond laser) avoid wielding it like a claymore (using inappropriate lenses with $f > 300$ cm) while also not begin fencing it (going too short for $f < 50$ cm). Nanoseconds are the working horses of LAL, and with ultrafast lasers you are on the safer side when working in organic liquids. This in mind let's move on to the next step trying to outrun some bubbles.

STEP 5: DODGE THE CAVITATION BUBBLE

In Chapter I, the concept of cavitation bubbles occurring during laser ablation was already introduced and discussed. These cavitation bubbles are a significant issue for productivity as they operate as a lateral limited protection shield of the target deflecting the strikes of subsequent laser pulses during bubble lifetime scattering. Consequently, we need to think how to handle this shielding wall in order to stop it from limiting our desired productivity.

We actually suggest two different ways – the temporal and the lateral evasion of the cavitation bubble. The idea of temporal evasion technique is to successively reduce the repetition rate until the subsequent laser pulse hits the same ablation spot only after the cavitation bubble is gone. Hereby the ablation efficiency (mass per laser pulse) can be significantly increased once the temporal spacing between the pulses is longer than the lifetime of the cavitation bubble. A typical cavitation bubble lifetime is 200 µs which would equal a maximal "undisturbed" repetition rate of 5 kHz. Hence, this technique is only at

all feasible for lasers operating in the lower kHz regime. Not a big issue if you work with a laser from the 90ies that delivers only 10 Hz. But, if you are lucky to run a kHz laser with more power, you may already sense another flaw in this idea. When the repetition rate (number of pulses per second) is being decreased the mean laser power output may decrease as well depending on the type of laser and its characteristics. Even if the amount of ablated mass per pulse increases by the temporal bypassing, decreasing the number of pulses (due to a lower repetition rate) may still cause the overall productivity to stagnate or only slightly increase. Therefore, the temporal evasion is a robust strategy for increase of productivity, but has physical limits. Again, the bubble size and lifetime scales with the pulse energy. If you are allowed to select a laser from scratch, 5-10 kHz is enough at high (>> 10 mJ) pulse energies, maybe 100 kHz at low pulse energies (<< 1 mJ) may still get to the ground undisturbed by cavitation bubbles.

After conducting a series of measurements for different calibration curves at the UV/Vis spectrophotometer, nothing but noise could really be extracted from the data. After reassuring that the samples were prepared correctly, we expected a major defect of the instrument. However, upon opening the lid, it became apparent that only the cuvette changer was not calibrated correctly so that the light beam could neither reach the sample nor the detector. Lesson learned: check the instrument setup and question your results directly before continuing with 100 more samples and wasting your time.

Carmen Streich, Essen

Still following the idea of a peaceful solution, the second and probably most generally applicable idea is the lateral evasion of the cavitation bubble. In order to achieve this goal it is mandatory to use a scanner or displace the target during the ablation (e.g. by spinning the target). For simplicity, let's only consider using a scanner at this point. One of the most efficient patterns applied during ablation is the use of a Fermatian spiral (closed spiral) as described in Chapter I. In this case, the laser follows this spiral, homogeneously ablating the target. The curved nature of the path makes sure the mirrors in the scanner are constantly moving, whereas the adoption of a path with corners would result in sudden operation of one mirror and consequent introduction of mapping errors. Back to the cavitation bubble, in case you plan working at high repetition rates, e.g. >> 5 kHz there may be an interaction of subsequent laser pulse and cavitation bubble even when

the target displacement is applied. Yet if you manage to guide the second laser pulse towards a space next to the cavitation bubble fast enough, no interaction between pulse and bubble occurs. Consequently, the ablation efficiency and, as the repetition rate remains unchanged, the overall productivity will increase significantly. Note that this also makes sure that every shot gets the same energy to the ground, increasing reproducibility. To manage the previously said you simply need to increase the lateral interpulse distance by increasing the scanning speed. Measuring the productivity after stepwise increase of the scanning speed (interpulse distance) an optimal interpulse distance can be found. A good starting point for this optimization is to start at a rather low interpulse distance of e.g. 5 $\frac{\mu m}{Pulse}$ (which equals a scanning speed of 50 $\frac{mm}{s}$ at 10kHz). The idea of the technique is shown in Figure 19.

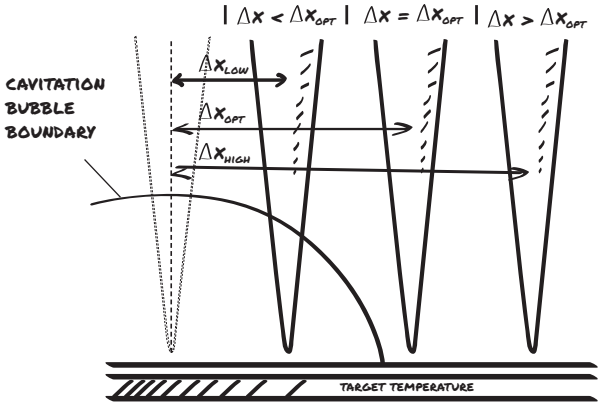


Figure 19: Variation of the interpulse distance Δx in order to avoid scattering of a subsequent laser pulse at the boundary of a cavitation bubble generated by the preceeding laser pulse. Therein Δx_{opt} represents the optimal interpulse distance. On the bottom of the picture, a scheme with dashed lines (close lines depict higher temperatures) depicts the temperature evolution in the target considering residual heat accumulation.

In case of too small interpulse distances, the laser pulse is likely to be scattered at the phase boundary of the cavitation bubble. In this case, your precious productivity will be low unless you increase the interpulse distance. Reaching the optimal interpulse distance Δx_{opt} you will just bypass the prior cavitation bubble increasing your overall productivity. When exceeding this optmal interpulse distance heat diffusion and preheating of the subsequently ablated area needs to be considered. Consequently, it is feasible to ablate this already preheated spot and limit the interpulse distance to an upper value. In case of e.g. 10 kHz the time between two pulses is 100 µs which is more than enough to have heat conduction into several hundreds of µm around the previously ablated spot (in particular for ns laser). A thorough examination of the optimal ablation distance should be made in this case. Typical galvanometric scanners easily provide

lateral scan speeds of 1 m/s, allowing to separate 10 kHz pulses laterally by 100 µm, maybe already enough if you work at moderate pulse energies (causing moderate bubble sizes).

STUDENT: THE DEVICE ISN'T WORKING, I'VE TRIED EVERYTHING.

ASSISTANT: IS IT PLUGGED IN?

STUDENT: OH

ALEX HEINEMANN, ESSEN

However, in case the scanner being used has high scanning speed of up to 100 m/s (e.g. a polygon scanner), the previously mentioned upper interpulse distance limit will be rather irrelevant when working in the MHz laser pulse repetition rate regime. The reason for this statement is the short interpulse delay of only several tens of nanoseconds being shorter than the heat diffusion timescale, rendering it impossible to use residual heat from a previous laser pulse. Simply speaking, the timescale for heat diffusion to take place between two subsequent laser pulses in MHz regime is just too short. Therefore, when you're working with MHz-repetition rates (as modern high-power pulsed lasers often provide), while having a sufficient scanner at hand, you don't need to worry about working at too high interpulse distances and rather run your scanner at maximum speed. However, in case you have a galvanometric scanner with $v_{scan} \ll 10$ m/s, even your highest scanning speed may not be sufficient to fully bypass the cavitation bubble in case you work with repetition rates of several hundreds kHz. This again is strongly depending on the cavitation bubble size and lifespan which again is based on applied pulse energy, pulse duration etc. As you may realize, due to the huge number of different possible parameters depending on the setup chosen, it is hard to find a general rule directly giving the optimal scanning speed (interpulse distance) for obtaining maximal productivity. Therefore, following the previously explained technique the optimal working conditions need to be determined exclusively for the used setup, target material and liquid you are working in. To keep it simple, when you work at 100 Hz or below, you do not have to worry about the bubble. Approaching the kHz regime may render faster movement of beam relative to the target a good choice to improve both productivity and reproducibility. Approaching the MHz regime, polygon scanners of latest generation are demanded to bring every pulse to an undisturbed site at the ground.

STEP 6: KEEP IT THIN

During the remarks made in Step 4 it was already mentioned that scattering and absorption of laser light by nanoparticles is a possible reason for the reduction of productivity. Actually, this is another big issue when working with a batch chamber. There are three ways to compensate this effect – either decrease the ablation time such that the nanoparticle concentration remains low or work with non-circulation liquid flow or decrease the liquid layer height so that the laser beam has a very short path through the liquid containing nanoparticles. All approaches aim at minimizing absorption and scattering losses due to nanoparticles or bubbles. Those vapor bubbles should not be underestimated. In order to maximize productivity and avoid disturbances of the laser beam during its path through the liquid it is therefore feasible to minimize the final concentration and additionally decrease the liquid layer height. When working in an open environment with the laser beam passing a liquid-air boundary it was already stated in Step 4 that there is a lower boundary for the liquid layer height to avoid liquid vaporization. Using the setup shown in Figure 20 the case is a little different. In this setup, the laser passes a glass-liquid phase boundary (like in a cuvette irradiated from the side or a glass-covered chamber).

Due to both the similar refractive index liquid and the (quartz) glass window and the high damage threshold of glasses (higher than liquid vaporization threshold) no direct ablation of the window is expected at its entrance side. However, window damage is often observed if the liquid layer is too thin. With the setup using a quartz-glass window the lower limit of liquid layer thickness is mainly determined by the dimensions of the cavitation bubble and the range of its mechanical ablation by outward jets ejected from the bubble during collapse. Usually lowering the distance between target and glass window below 2-3 mm will result in the abrasion and consequently in the destruction of the window.

We once designed an innovative setup for wire ablation in a flow-through chamber. However, it had one downside. For evaluating the wire position you had to put your head directly on the table and look through the optics. I had a student who worked with this setup for months. One day he felt a slight stinging on the back of his head while putting his head in. Apparently the laser was still on. From this we can learn 1) Accidents happen particularly when everything is routine 2) Do not put your head into the path of the beam, EVER, but put safety first during design of optical setups.

Christoph Rehbock, Essen

Therefore, it is recommended to not reduce the liquid layer height below 3mm when using glass windows (air/glass/water boundary) instead of an air/water phase boundary for the laser to enter the liquid. For maximal LAL productivity, of course, the liquid layer should be as thin as possible (4 – 6 mm). And liquid flow always helps.

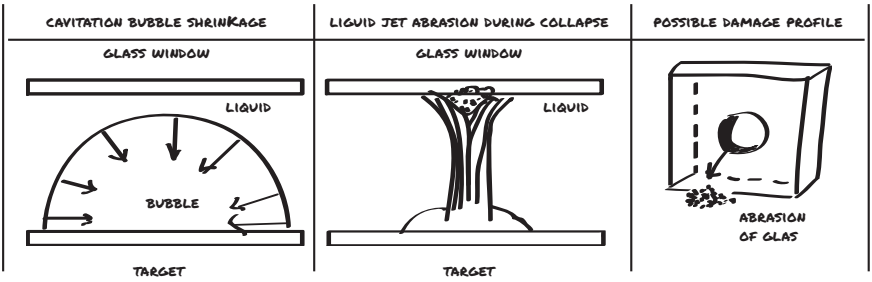


Figure 20: Typical glass damage scenario during ablation with a too low liquid layer height. To the right a typical picture of a glass cover is shown after abrasion occurred.

STEP 7: SCALING IT UP – FROM BATCH WISE TOWARDS CONTINUOUS PROCESSING

Since we already learned how to find the best operation conditions and geometry for an ablation setup it is now time to think about optimizing the whole setup concept. Of course, the widely used batch process is good for a “quickie”, but will not be very efficient. Due to the previously mentioned accumulation of nanoparticles during ablation, productivity and nanoparticle yield per pulse will decrease during the process. Additionally, when thinking by economical means, emptying the batch chamber after a specific concentration is reached, re-adjustment and setting it up for another run will consume crucial and “precious” working hours. In order to circumvent the problem about the nanoparticle accumulation and the high workload of a scientist, it is time to think about changing to a more compartible continuous nanoparticle production using a flow chamber. A simple and very effective setup to achieve a temporally continuous ablation process is given in Figure 21.

II

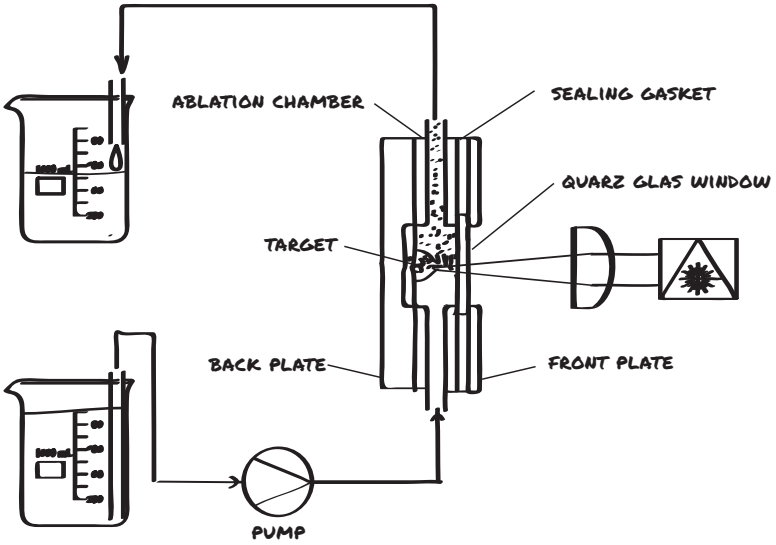


Figure 21: Scheme of a continuous flow processing including a flow chamber cross-section view.

The design is held simple and chambers can be built with basic workshop equipment. It is recommended to use aluminum plates as a basis material as it is light and easy to handle when mechanically working it. As basic shape a cube with 40mm x 40mm x 5mm dimension for each chamber part is convenient. The chamber is built of 3 basic parts:

- BACK PLATE (HOLDING THE TARGET)
- MIDDLE PLATE (ABLATION CHANNEL FOR LIQUID PERFUSION AND PARTICLE TRANSPORT)
- FRONT PLATE (FIXING THE QUARTZ WINDOW)

Additionally the middle piece should be equipped with suiting hose connection in order to connect tubes to your chamber.

In order to assemble the 3 chamber parts, four drilling holes in the corner of each part and slid bolds may be used as depicted in Figure 22. The different plates should be separated by some gaskets preventing leaks. In this context 0.5mm Teflon or Nylon slabs are recommended. In case of persistent leaks, Teflon paste applied onto the gaskets proofed to be a handy tool to prevent leaks occurring after assembly. The chamber may be fixed on a breadboard attached to a 1 axis or 2 axis translation stage using a suitable chamber holder, facilitating chamber positioning towards the focal plane. With this chamber, you are now able to easily perform a continuous nanoparticle production.

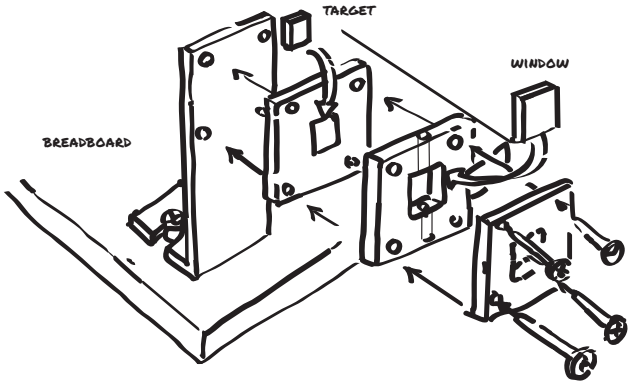


Figure 22: Assembly of a simple, but fine working flow chamber to achieve continuous ablation conditions. Liquid flow is directed upwards. Use Teflon sealings with additional Teflon paste between the parts.

Of course, the target needs to be changed once it is completely ablated. Yet, before starting your first experiments, it is highly recommended to anodize all chamber parts to make sure the surface is covered by an aluminum oxide layer. Without passivation, aluminum may dissolve into e.g. the water pumped through the chamber. The dissolved aluminum ions will significantly destabilize your produced colloids leading to agglomeration (yes, we suffered from this experience). More details on the stability of nanoparticles will be given in Chapter IV; however keep in mind that a healthy aluminum oxide layer is very important. Now, before we start looking into more detail about the performance of the new ablation process let's address some issues you should be considering in the first place:

- avoid pH < 4 and pH > 9 in order to keep the aluminum oxide layer intact. For corrosive liquids, use Telfon or polyamide
- do not use tubes and seals made of silicone and other materials that have considerable porosity, to minimize nanoparticle losses and contamination carryover due to deposition inside the pores
- check solvent compatibility table for all seals, tubes or plastics in contact with the solvent you plan to ablate your nanoparticles in. Recommended tube materials when using solvents like acetone are Polyamide (PA12) or Teflon or PVC.
- consider leaching of plasticizers from your tube (i.e. from cheap PVC) and seals material especially when ablating in organic liquids like acetone, in order to avoid impurities (one example siloxanes leached from silicones and seals immersed in acetone – siloxane impurities will adsorb onto nanoparticles, even after several cleaning steps!).

- make sure the pump is clean and specified for the liquids you plan to work with (e.g. solvent). Pump oil is transparent, not dark-brown.

According to the previously listed points, silicone should be avoided and Teflon based tubes and seals should be preferred. Those however may provide less efficient sealing properties and some tinkering to attach the tubes to the hose connector resulting in leaks during liquid perfusion. To avoid leaks consider using Teflon paste additionally to the already used gaskets and tubes. Teflon is thermoplastic, so it tightens best only during the first mounting.

With your finished sealed setup you're ready to perfuse liquid through the chamber. Prior to pumping make sure the inlet is attached to the lower hose connector following the sketch in Figure 21. In doing so you make sure that all gas bubbles either sucked in by the pump or generated during ablation are leaving the ablation chamber at the top without accumulating. In case you decide to connect the inlet to the top you may end up with a chamber full of gas bubbles.

Finally, you are ready to start your new continuous production line tapping nanoparticles. This setup helps in minimizing reassembling times as the interruption of the process is only necessary when the target is consumed. Considering the reassembly time as a timespan where no nanoparticles are produced the productivity of your process will be drastically increased when using this setup as a guide. And you will benefit from reproducibility. In order to examine the continuously produced nanoparticle, the setup shown in Figure 21 may be upgraded downstream with a fiber UV-VIS set up probing a flow cuvette. As every LAL process has a tune-in period of a few minutes, we recommend to waste the first 3-5 min of the colloid stream and then relax while your setup is doing the work. A batch process also suffers from this tune-in period, but here extraction of that fraction is impossible

STEP 8: LOWER THE RESIDENCE TIME USING 3D-PRINTING TECHNIQUES

In the previous step, a very flexible chamber and setup design was introduced. The chamber is easy to access and can be manufactured with a simple tool kit. However, this design is still not optimal when it comes to consider the flow profile inside the cell. Now before thinking about better chamber designs exhibiting a better flow profile, we may first think about the impact of liquid flow on the productivity of nanoparticles.

In previous sections, the issue of nanoparticle accumulation was already addressed several times, stating that enrichment of nanoparticles within the beam path way needs to be avoided in order to minimize absorption and scattering losses. Within the batch process, we found that our only degrees of freedom are the liquid layer height and nanoparticle concentration. Using continuous flow setup we created a new degree of freedom to handle "laser

shielding" minimizing absorption and scattering by already produced particles (and some drifting bubbles). But chamber volume geometry has a big effect, and needs not much effort to be optimized. Figure 23 displays unwanted geometries on the left and a laminar flow pattern on the right with minimized back-flow. As you can imagine, if some particles are moving into the flowing direction away from the ablation spot, while others are surfing on eddies into the opposite direction, right back into the ablation spot, large channel width (and a steep change in channel opening diameter) may not be the best solution. In case of the chamber design presented on the left in Figure 23 strong levels of re-mixing are to be expected due to the small liquid entrance / outlet and the quick widening of the channel. On the other hand, a more tube like shape with smooth geometry changes results in a more homogeneous flow pattern. In order to get an idea of the flow pattern inside the chamber you can simply use software tools like "open foam" or "ANSYS fluent" in order to create a rough CFD (computational fluid dynamics) simulation similar to the sketch in Figure 23. A good is to choose a design where the liquid is directly guided towards the drain avoiding obstacles like corners. A proper design to assure a laminar flow pattern would be that of a standard pipe having no surface roughness. However, a tube is far from feasible to act as an ablation chamber holding a planar target. Yet if we cut off two sides of the cylinder such that one side can be covered with the quartz glass and the other with the target, a laminar flow pattern without too much re-mixing is likely to appear. Note that laminar flows have the highest flow rate in its center, exactly the place where the laser ablation happens and nanoparticles need to be effectively drained off.

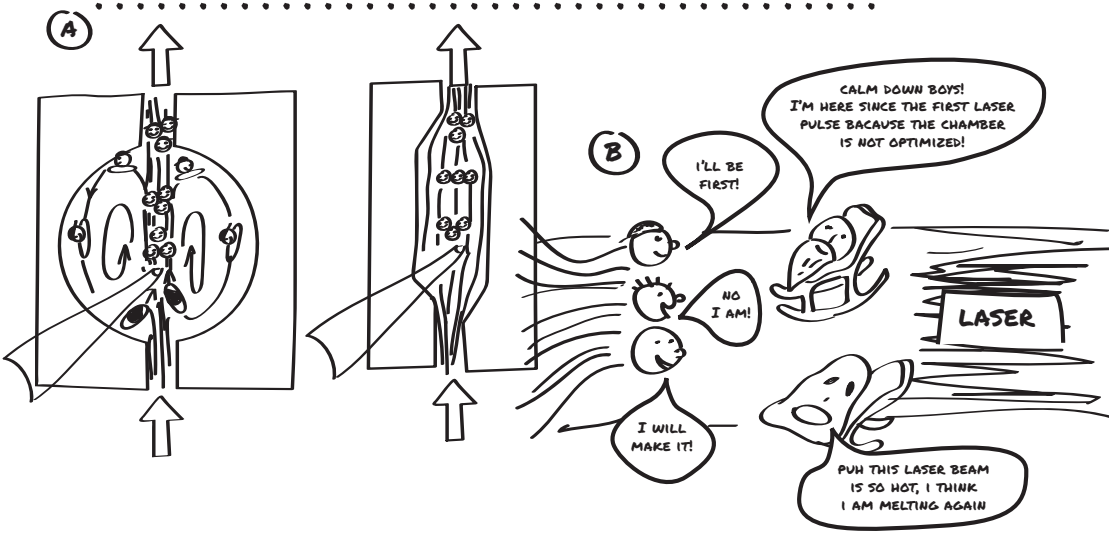


Figure 23 (A): Flow pattern inside a steep channel geometry change in the chamber volume (left) and the optimized chamber design (right). (B): Sketch of fresh particles arriving, while previously produced old ones are still present.

With this new concept in mind you should however avoid attending your workshop manager in case you want to leave the workshop without bruises. Actually, the optimized chamber concept, especially the gradual diameter reduction shown in Figure 23 (left) is not that simple to manufacture. Yet once you are able to consider yourself to be a proud owner of a 3D printer you're able to simply print this chamber within a couple of minutes. Obviously, the 3D printer won't complain whatever design you have in mind. In case you decide to use a 3D printer it is however crucial to choose the right material especially when working with organic solvents. A good choice for a printable polymer material with good acidic, basic and solvent stability would in this case be nylon. By following all previously given steps (focussing, scanning, liquid level) and using an ablation flow chamber with optimized flow properties like the one in Figure 23 (right) included into the flow concept presented in Figure 21 you will be able to maximize the nanoparticle productivity towards hundred mg nanoparticle per hour with tens of Watt laser power.

STEP 9: MOVE BETWEEN DIMENSIONS

Since you already became an expert regarding optimizing the laser ablation of a two dimensional target, it is time to finish this chapter on productivity maximization with some additional novel concepts regarding the laser ablation technique. Until now, the presented ablation concept concluded in a continuous process which significantly decreased assembly times and outages due to target or liquid change, compared to the initially presented batch process. However, to think big scale a fully continuous automatic setup is required. Therefore, the last obstacle to overcome is to get rid of time-consuming target replacement steps. Now how can we achieve something like that?

The answer to this question may be counter intuitive to our goal but as we are already in the predicament of going big scale with the small scale one additional bafflement shouldn't be too hard to swallow. So prepare yourself! It is time to cut off unnecessary target dimensions.

Let's begin with the 2nd dimension and get rid of this large area of metal but instead consider a very long metal strip of rather small thickness (e.g. several tens or hundreds of microns, as thin as the focused laser beam). Therefore let's decrease the dimensions move to a 1D shape which would be a simple target wire. Wire ablation has been studied in literature for a while and can be considered as one of the ablation techniques with the highest potential to be applied in fully automated, continuously driven ablation processes.

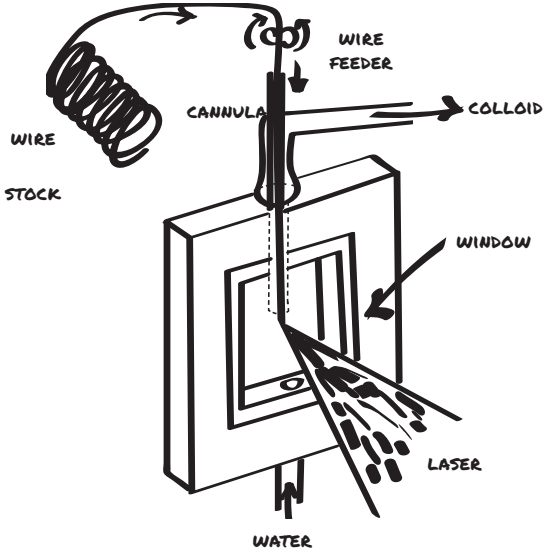


Figure 24: Sketch of a continuous wire ablation setup.

As depicted in Figure 24 wire ablation is done in the same ablation chamber presented in Figure 22 only with the exception that the back plate holding the target is exchanged by a second window and a second front plate such that the laser can pass through the chamber. The wire is to be fed into the ablation chamber using a cannula inserted at the top. Using a wire conveyor the wire may be pushed through the cannula while the laser spot position needs to be adjusted such that the wire tip is being ablated. By controlling the feeding speed and choosing a wire diameter that is slightly thinner than the focal spot diameter the wire can be constantly ablated over a long period of time. The ablation time is only limited by the chosen feeding speed and wire length which can be chosen to be a couple of 100 m if needed. When properly adjusted the wire is ablated with 100% material yield without any non-ablated metal waste. Using the feeding speed set for the wire conveyor, the productivity can directly be set. And isn't a continuously fed target a perfect match to continuous flow and continuously provided laser pulses?

Obviously wire ablation already is a very suitable concept and we've seen that with decreasing target dimensions more and more issues disappear. Maybe it is time to think about cutting off the last dimension as well and move towards a zero dimensional target. It may sound paradox – in the mathematical definition a zero dimensional target would be represented by a point in space with infinitely small diameter taken in absolute units. Naturally, this is not possible in reality but we may converge when considering decreasing target dimensions up to the point where the target particle diameter is considerably small compared to the laser spot diameter in the working area. This assumption is valid for most commercially available micro-powders. Using the same setup suggested for the wire setup, probably leaving the cannula, it is possible to form nanoparticles from irradiating micro-powders dispersed into a liquid (e.g. water) under continuous flow conditions (Figure 24).

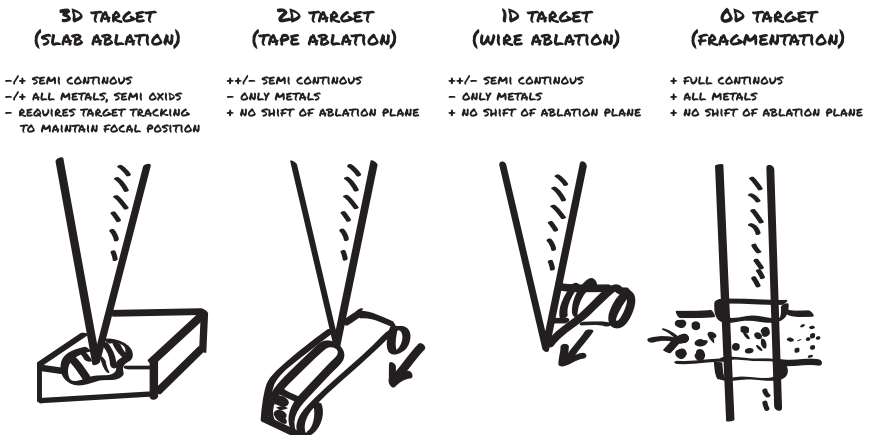


Figure 25: Comparison of different ablation geometries including an overview of the major up- and downsides.

Applied to micro- or nanoparticles this technique is commonly discussed as laser fragmentation in liquid (LFL) as already mentioned in Chapter I and mainly used to produce particles with downsized particle diameter depending on the applied laser fluence. Obviously by changing the target dimensions down to 1D or even 0D (Figure 25) we gracefully managed to pass the last obstacles and introduced a final concept for a 100% continuous process with 100% nanoparticle yield 0% waste and high productivity potential.

Concluding, much effort is necessary during the adventure of climbing the productivity mountain. The path may be cloudy and every herein discussed step needs to be taken with diligence and caution in order to make sure not to fall off the steep cliffs appearing on your way up to the top. However, by understanding and avoiding the obstacles and barriers presented you may soon be sitting on the peak of the mountain enjoying the view over the nanoparticle ocean.

One day I went into the lab to produce some gold nanoparticles immersed in acetone. I was a bit worried as acetone is flammable it was by far not my intention to burn down the lab. I decided to use the flow chamber – which is a good choice as it is sealed and ones filled with acetone, its interior is free of oxygen. I remembered a short lesson about safety issues stating that a burnable liquid can only burn if both oxygen and an ignition source are present. Now as the oxygen was ruled out in the sealed flow chamber I considered this experiment quite save. While I was ablating using a high power laser that I know likes to kill the glass cover of the ablation chamber I still was a little cautious about the whole process. But thinking – no risk no colloid! I started the acetone flow and the laser – surprisingly without any accidents ... well at least in the first place... I was nearly finished when I heard some strange deviations in the ablation sound which hasn't been there before. Now I thought – whatever – eyes shut and go for it ... just a little more ... but this sound ... where does it come from? So I looked a little closer not being aware of that leaky little hole in the glas cover. Suddenly the laser ignites this ejected jet of acetone forming a pretty big flame making my heart jump. Lucky for me – as soon as the flame appeared it was gone again as the acetone jet burned off instantly and couldn't continue burning as the acetone flow through the leak was too slow. To avoid further fire spectacles I directly turned off the pump followed by the laser. Except of a little shock not much had happened however nowadays my fingers do not leave the turn-off-button of pump and laser control in order to turn everything of directly when I hear something suspicious.

Sven Reichenberger, Duisburg

III

UNDERSTAND IT: OPTICAL AND SOLID MATTER CHARACTERISATION

Imagine you synthesized one-of-a-kind nanoparticles and spiced them up. What would you do next? Show them to the world by putting it on your website? Make a video of them and put it on YouTube (e.g., *http://youtube.com/nanofunction*)? Or maybe you would write a manuscript about your particles and submit it to "Nature"? You should first hold your horses and imagine the following: someone gave you a shiny diamond ring and disappeared. What would you do with this ring? Run to the love of your life and propose to her (sorry this story only works if "I" am a man and "you" are a woman)? You know that this might backfire if the ring is fake. The obvious step would be characterizing it or having it characterized. Is it a gold ring? Is the diamond real? What is its clarity, color grade, shape, Carat weight? All these questions need to be answered for a proper estimation of its value and if it's suitable for the love of your life. Same thing goes for your nanoparticles. You need an exhaustive characterization to fully identify and confirm the nature of your products.

The questions that usually arise in front of a sample of nanoparticles with unknown nature (no matter if obtained by laser ablation or by other methods) can be summarized as follows (Figure 26):

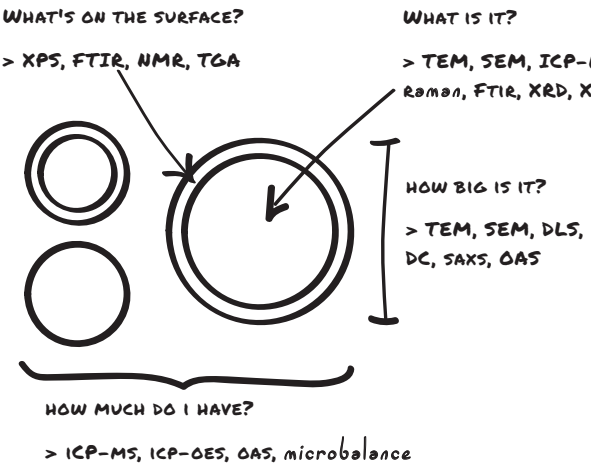


Figure 26: Illustration of nanoparticles and questions that arise while trying to understand them. Once this is done, function (e.g. optics, catalysis, biomedicine) can be attributed to structure. This structure-function-relationship is exactly what everyone is hunting for.

To answer each of these questions, you can use the different characterization methods in a step-by-step approach, as described in the following paragraphs and summarized in Figure 26. Of course, depending on the material and synthesis objective, the sequence of these questions can change from case to case. In fact, characterization of nanomaterials is often non trivial and Ancients would have said that we must proceed "cum grano salis" (latin for "with a grain of salt", i.e. use your brain before acting!) to avoid misinterpretation of results and exponential growth of the research work. In particular, for each technique, it is important to have very clear in mind the answer to the following questions:

- A. What do I see?
- B. How does it work?
- C. Which amount of sample is needed?
- D. How do I prepare the sample for analysis?
- E. Are there any tips/ warnings/hazards?
- F. Where to look if you want to learn more

Let's see how the answer to the above questions can help us to achieve a perfect knowledge of our laser-generated nanoparticles.

STEP 1: PROVE THAT YOU ACTUALLY HAVE "NANO" PARTICLES

Try to ask any Italian who was abroad: he/she will confirm that not enough to have pasta, tomatoes, salt and water to make a genuine Italian style pasta... The same goes for the synthesis of nanoparticles by laser ablation: it is not necessarily true that shooting the laser at an object immersed in a liquid is enough to collect a colloidal nanoparticle solution (or make a paper on such "shoot and run" experiment and ruin your reputation). Therefore, first of all we need direct evidence on the existence of nanoparticles in our sample. The "Queen" of characterization techniques for nanoparticles is, without any doubt, transmission electron microscopy (TEM), because of its unmatched spatial resolution and completeness of achievable data. Though with lower spatial resolution, scanning electron microscopy (SEM) permits in most cases the direct visualization of nanoparticles with size larger than ~ 5 - 10 nm (and larger particles often may be a mass-dominant side product). A great advantage of these electron microscopies is that they can analyse a single nanoparticle at time. A relevant consequence of this peculiar ability is that electron microscopies are the only methods allowing real and direct "number weighted" analysis (i.e. by counting nanoparticles one by one), contrary to all the other techniques that only give volume-weighted results (i.e. measuring signals which are proportional to the x-th power of the volume of each nanoparticle, with $x \geq 1$).

However, TEM and SEM are not easily or immediately available, and analysis of one particle by one may be time consuming without the support of appropriate software (e.g. the freeware ImageJ). Also, EM techniques provide bad statistics (< 1000 particles of millions in a drop) and tend to over-estimate small particles. In this case, the quickest and simplest way to check the existence of nanoparticles in a liquid medium is the optical absorption spectroscopy (OAS). In fact, visible (vis) and ultraviolet (UV) light interact in a very peculiar way with objects of nanometric size homogeneously dispersed in a transparent matrix. Besides, when dealing with nanoparticles dispersed in a liquid matrix, OAS does not need any sample preparation. A great advantage of OAS is that it can be performed also in real time during laser ablation or irradiation experiments, especially if the absorbance is monitored at a single wavelength, by coupling a photodiode with a laser beam crossing the sample chamber.

In a limited number of cases, depending on the properties of nanoparticles under investigation, fluorescence spectroscopy (FS) can be a viable technique for immediate characterization of nanoparticles which emit light. Indeed, emission bands are usually located in a smaller spectral range than absorption bands for the same compound (i.e. fluorescence bands are more specific and easier to recognize). Besides, position and intensity of fluorescence bands in semiconductors are distinctive of their size. Let's look at the features of these techniques and what we can learn from each of them.

During my research internship I was working with centrifugation of colloids to separate big particles from smaller one. To avoid unbalances I filled my tubes all equally to a certain volume and inserted them into the tube holder. For saving the time I chose the highest rotation number, which was 5000 RPM. Once I started the centrifugation, everything went so fast. During the acceleration the sound was slightly different than commonly and suddenly the centrifuge itself started to dance on the table. A moment of shock came into me and my heart beats strong and fast. Immediately after a short second of rigidity I pushed the button to stop the centrifuge. Fortunately the speed was just at about 100 RPM and the centrifugation stopped before something worse happened. I opened it and realized that one of the tube holder was interlocked and thus didn't have the possibility to float freely which caused the unbalances. The machine seemed so monstrous to me already only at very low speeds, so that after this happening I always checked everything at least twice with care. In the meantime we purchased a new centrifuge, where the holder are already in the right position and no interlocking is feasible. However this experience always makes me wait until the full speed is reached before dedicating to other work.

Galina Marzun, Duisburg

1.1. Optical absorption spectroscopy (OAS).

A. What do I see?

By OAS, we see if nanoparticles are present in the liquid medium. In the large majority of cases, OAS is performed with bench-top spectrometers, giving an averaged information on all the nanoparticles dispersed in the solution, clearly meaning that it is not a single particle technique.

In case of nanoparticles with well defined absorption bands, OAS gives the absolute certainty of their presence. If the absorption band is related in some way to nanoparticles size and shape, this information is also achievable, such as in case of plasmonic (Figure 27, left) and semiconductor nanoparticles, although the precise interpretation of the results requires accurate elaboration of the experimental spectrum with complex theoretical models.

Some polymer, oxide or wide band gap semiconductor nanoparticles may not have well defined absorption bands in the UV-visible range. In that case, OAS can still detect the presence of nanoparticles due to light scattering effect, whose intensity scales with the -4th power of wavelength ($\propto \lambda^{-4}$, the so-called Rayleigh scattering curve). Once you've seen this characteristic curve (Figure 27, right), you can be sure to have submicro or nano inside.

B. How does it work?

The principle of OAS is simple: a light beam with well defined wavelength (λ) is transmitted through the sample, typically contained in a cuvette or on a transparent substrate. A light intensity detector is placed behind the sample. The procedure is repeated in a range of wavelengths predetermined by the operator. At each wavelength, the ratio between transmitted (I) and incident light (I_0), called transmittance (T), is measured. More useful is the absorbance (Abs) defined as the

$$Abs = -log_{10}(T)$$

because it is proportional to the concentration (C) of absorbing objects according to the Lambert-Beer law:

$$Abs = \varepsilon bC$$

where b is the length of the path travelled by light in the sample ("optical path", it depends on the sample geometry), and ε is the coefficient of molar extinction (it depends on sample nature, being higher for highly absorbing objects).

It is worth to stress that two distinct phenomena contribute to the decrease of I/I_0 light "absorption" and "scattering". Absorption means that photons are annihilated and their energy transferred to the absorbing object, whereas scattering means that photons just change their travel direction, and will not reach the light intensity detector behind the sample, without releasing energy to the scattering object. In case of "real" absorption, it is often possible to recognize distinct peaks in the spectrum (Figure 27, left) In case of scattering, the spectrum shows a typical $\sim\lambda^{-4}$ trend, without a clearly identifiable absorption peak (Figure 27, right).

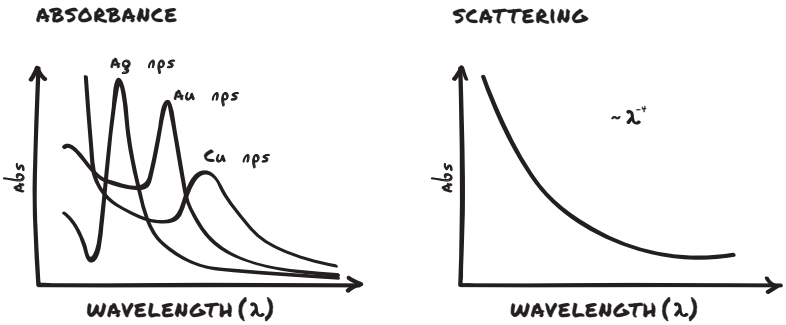


Figure 27: Typical OAS spectra of purely absorbing (left) or scattering (right) nanoparticles.

c. Amount of sample needed?

A great advantage of OAS is that, with the use of appropriate cuvettes, 100µL of solution may be enough, although typically 0.5 – 3 mL of liquid is required. However, the detection of samples depends on the combination of three factors in the Lambert-Beer law (b, ε and C). When nanoparticles are present at very low concentration in the liquid solution, the simplest way to improve the signal intensity is to increase the optical path (b) of the light beam passing through the sample. This can be done using cuvettes with 1 cm or even longer optical path. In ordinary spectrometers, the optimum interval of absorbance is between 0.5 and 2.5. Obviously, ε is a material property and cannot be modified. Most spectrometers are not linear anymore above absorbance of 2, dilution is a simple workaround.

d. How do I prepare the sample for analysis?

In the majority of cases, you just need to fill the cuvette with the solution. However, if nanoparticles are not stable in the liquid phase for the time required from the analysis (< 5 minutes), they can be included in a polymeric matrix (e.g. add PVP) and/or deposed on a transparent substrate (i.e. a microscope slide).

E. Tips / warnings / hazards?

Simplest way to stabilize an aqueous colloid is: add a pinhead-sized peace of soap (the cheap, solid one, not the coloured and perfumed). Of course, any organic additive will screen the UV part of the spectrum, same with plastic cuvettes compared to quartz cuvettes. In water, sometimes sodium hydroxide helps for stabilisation (adjust pH around 10 or 11), keeping the UV range free for analysis.

You must consider that absorption and scattering are phenomena strongly correlated to nanoparticles volume. In particular, absorption scales linearly with the volume of the nanoparticle, whereas scattering scales with the 2nd power of the volume. As a consequence, the information obtained by OAS are the volume (or volume^2) weighted average of the nanoparticles in the sample.

Store and handle it cold (fridge is enough, don't freeze) to minimize sedimentation.

F. To know more?

– Fundamentals of modern UV-visible Spectroscopy – Primer; Owen, Tony; 2000; Agilent Technologyes; free at *http://www.agilent.com/cs/library/primers/public/59801397_020660.pdf*

– UV-VIS Spectroscopy and Its Applications; Perkampus, Heinz-Helmut; Springer-Verlag; 1992.

1.2. Transmission electron microscopy (TEM) imaging.

A. What do I see?

TEM allows the direct visualization of a single nanoparticle with nanometric or subnanometric spatial resolution. In the high-resolution modality (HR-TEM), atomic planes and rows within the single nanoparticle are visible. This gives straightforward access to a series of information such as nanoparticle size, size distribution, structure homogeneity or, alternatively, phase segregation, formation of shells, crescents and heterostructures, crystalline order, lattice defects.

B. How does it work?

The principle is the same of an optical microscope, with the remarkable difference that a monochromatic electron beam is transmitted through the sample, instead of a photon beam. Electrons are scattered (sometimes also absorbed) by nanoparticles, with an efficiency which depends on the square of the atomic number (z) of elements composing the nanoparticle, and on their crystalline order and orientation. This generates a contrast between electron dense and not dense regions (i.e. an inorganic nanoparticle and a thin carbon

film acting as substrate), which is at the basis of image generation. As in the optical microscopy, spatial resolution depends on the wavelength of electrons, which is determined by their kinetic energy according to the De Broglie equation (corrected for relativistic effects due to the high speed of electrons in a TEM):

$$\lambda_e \approx \frac{h}{\sqrt{2m_0E\left(1+\frac{E}{2m_0c^2}\right)}}$$

where h is Planck constant, m_0 electron mass, c light speed and E the energy of the accelerated electron. The consequence of the De Broglie equation is that, usually, nanometric spatial resolution is possible with TEM operating at 100 kV of accelerating voltage, whereas subnanometric resolution is possible only with acceleration voltages of 200 kV or higher.

c. Amount of sample needed?

Being a technique with single nanoparticle capability, virtually 1 nanoparticle is enough. Obviously a statistic analysis of each sample is possible only by considering more than N~500 nanoparticles each time. In practice, 1 – 10 μL of solution are always enough.

d. How do I prepare the sample for analysis?

Nanoparticles must be deposed on TEM grids, which host a thin film with high transparency to the electron beam, such as nanometric thick carbon films or Si_3N_4 membranes. A drop of solution can be casted on the grid and left drying in air (TEM operates in vacuum to avoid interference of atmospheric gas with the electron beam, therefore samples must be dried prior to analysis). If there are non-volatile organic residues in the sample, prior plasma cleaning (standard TEM sample preparation equipment) may be required to improve the contrast.

e. Tips / warnings / hazards?

The electron beam depositeda considerable amount of energy on the nanoparticles, and this energy increases with increasing magnification (the energy density per unit area increases). Therefore, care must be used when observing materials which undergo modifications under electron beam illumination (especially organic nanoparticles and semiconductors). Even noble metals or refractory materials can undergo modification, melting, segregation, regrowth and etching when exposed to an electron beam. This is especially true for laser-generated nanoparticles, whose surface is clean, uncoated and very reactive, and whose composition can be metastable. Nanoparticle modification can usually be observed in real time during TEM imaging and can be reduced only by lowering the magnification (i.e. decreasing the energy density for unit area), the exposure time and the TEM operating voltage. Another workaround is dilution. If you see peanut-shaped gold particle twins, they often are artefacts from fusion caused by the electron beam.

Note that today's SEMs provide transmission mode as well, it's called "STEM" (ask operator if the instrument is equipped with this option, its also not too expensive to buy later for modern instruments, because it's essentially just a sample holder), and before said for TEM is true as well for this STEM technique.

f. To know more?

– Transmission Electron Microscopy: A Textbook for Materials Science; Williams, David B., Carter, C. Barry; 2009, Springer-Verlag.

He checked out his setup and examined all the optical parts on the table. He started the laser and frightened because of a machine gun–like regenerated noise. After he switched off the laser he examined what happened. It took him several minutes to find out the reason but he realized that his laser had drilled some holes in the coating of the laboratory door. Obviously a loose mirror had twisted independently.

Sebastian Kohsakowski, Duisburg

1.3. Scanning electron microscopy (SEM) imaging.

a. What do I see?

It gives the size and morphology down to the single nanoparticle level, for objects larger than about 5 – 10 nm. Due to the spatial resolution of ~1-10 nm, morphological information is reliably extracted only for larger objects (>10 nm).

b. How does it work?

A focused electron beam is scanned over the sample area, and a detector collects the electrons emitted from the sample. There are two types of electrons emitted from the sample under electron beam illumination, and both can be used for image formation in a SEM: backscattered and secondary electrons. Backscattered electrons are high-energy electrons of the incident electron beam, reflected from the specimen by elastic scattering interactions with sample atoms. Secondary electrons are extracted from atoms in the specimen by an inelastic scattering process promoted by the interaction with the electron beam. Each type of electrons is collected by a distinct detector, which works pretty much like a photocamera that collects the light reflected by a shiny three-dimensional object. Therefore, SEM images contain information on surface topography of the sampled area and show the typical "light and shade" effect due to the directional imaging.

Backscattering is more intense with high – z elements, providing complementary information to secondary electron imaging in samples where high – z compounds are deposed on low – z substrates. In this imaging modality, heavier atoms appear brighter.

c. Amount of sample needed?

Same as TEM, few μL of solution is enough.

d. How do I prepare the sample for analysis?

Same as TEM, a drop of solution can be casted on a substrate and left drying before analysis (also SEM operates in vacuum). The substrate should be as flat as possible (in this way, topographical information will enhance nanoparticles over the flat surface), conductive, and should allow z-contrast with your nanoparticles. For instance, carbon tape for electron microscopy is suitable for oxides, and doped Si is very well suited for metals and almost all types of nanoparticles with elements heavier than silicon.

e. Tips / warnings / hazards?

Specimen topography is greatly affected by any impurity or solute (i.e. salts, surfactants, chemicals, synthesis by-products) eventually present in the solution and forming a matrix embedding nanoparticles in the dried sample. The smaller the nanoparticles are, the higher must be the purity of the liquid.

You get nice pictures from your particles if you tilt the sample to the maximum possible: in this way you will get nice 3D appearance. Note that this modus it is not accurate to measure height, it's just to make it beautiful.

f. To know more?

- Scanning Electron Microscopy and X-ray Microanalysis; Goldstein, J., Newbury, D.E., Joy, D.C., Lyman, C.E., Echlin, P., Lifshin, E., Sawyer, L., Michael, J.R.; 2003, Springer.

1.4. Fluorescence spectroscopy (FS).

a. What do I see?

Only a limited number of materials shows appreciable fluorescence: in these lucky cases, FS easily allows the detection of these compounds in your sample. In case that fluorescence bands are size-dependent, direct proof of the presence of nanometric particles is achievable at the same time!

b. How does it work?

In FS, photons are emitted by the sample as a consequence of a light absorption process. Therefore, monochromatic light is extracted from a lamp and conveyed to the sample, where it is absorbed by the fluorescent compound (fluorophore), whose electronic structure passes from the ground state to an excited state. Fluorophores have the ability to release an appreciable fraction of the absorbed energy by emission of other photons, which have longer wavelength (lower energy) compared to the absorbed ones. The photons emitted by the sample

are dispersed by a diffracting optic and counted by a photodetector. The fluorescence spectrum reports fluorescence intensity (in arbitrary units) versus photon wavelengths. Absolute fluorescence efficiency (defined as the ratio of emitted to absorbed photons and called "quantum yield") is achievable only by comparison with a standard with known quantum yield in the same spectral range of the sample.

➞ c. Amount of sample needed?

Typically 2 – 3 mL of liquid solution with absorbance <0.15 in the excitation and emission spectral regions. The solution is placed in a 1 cm x 1 cm cuvette.

➞ d. How do I prepare the sample for analysis?

As in OAS, you just need to fill the cuvette with the solution. However, if nanoparticles are not stable in the liquid phase for the time required from the analysis (< 5 minutes), they can be included in a polymeric matrix and deposed on a transparent substrate (i.e. a microscope slide). The slide can be analyzed with a ~30° or ~60° geometry to the direction of excitation and of collection (which are at an angle of 90° one each other). Avoid placing the film at 45° to minimize reflection of excitation beam toward the detector.

➞ e. Tips / warnings / hazards?

In principle, fluorescence intensity is proportional to the concentration of fluorophores. However, self-absorption takes place already at very low absorbance. Therefore care must be used in the choice of the absorbance of the sample for analysis. Absorbance higher than ca. 0.15 in the absorption and emission spectral regions usually results in artefacts due to self-absorption. Run calibration curves to find a robust concentration range of linearity.

➞ f. To know more?

- Principles of Fluorescence Spectroscopy; Lakowicz, Joseph R.; 2006, Springer.

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The person prepared his sample and measurement device for the automatic pH dependent zeta potential measurement, to find out the isoelectric point of his sample. He left the device to go to lunch. After one hour he came back with his colleague, examines the measurement and immediately began to grumble on the device. The zeta potential was only measured for one pH-value. His colleague examined the device and started laughing. His college forgot to switch the pH electrode from buffer solution into the device.

Sebastian Kohsakowski, Duisburg

STEP 2: MEASURE THEIR SIZE

Nanomaterials take their name from their size, hence sizing the nanoparticles of our sample is an obvious step. Therefore, an obvious question arising about your colloid is "What is the size of your nanoparticles?". Pulsed laser ablation generally yields not one defined particle size but a whole "zoo" of particles composed of different sizes. So a more meaningful question in this context would be: "How many particles of which size can be found within your colloid ?". This correlation between the frequency of particles in a colloid and the individual particle sizes is called a particle size distribution. The main points raised in this paragraph will be how to interpret particle size distributions, how to measure them and finally how to alter them by simple methods during laser ablation in liquids.

Expressing particle size distributions

Particle size distributions can give lot of interesting information about your colloid. In this context, however, you need to keep in mind that there are at least three meaningful ways to express particle size distributions in colloidal science, which require weighting based on number, volume and surface.
In a number weighted size distribution you basically count the number of particles in your sample associated to a certain size class, e.g. all particles with a diameter of 20 nm and plot this number against the respective particle size. If you repeat this procedure for all particle sizes in your sample, you will end up with your number weighted size distribution as illustrated in Figure 28 A.
Number weighted size distributions are generally highly relevant as they are the direct result of size distributions obtained from electron microscopy. And this is the standard in literature. Unfortunately, for most "nano-functions", this statistic

is less meaningful (and sometimes without any meaning), as most functions are proportional to either the volume or mass (e.g., ion release, optical effects), or the surface (catalysis, bio-response, ...) of the particles. In a volume weighted size distribution, on the other hand, you basically plot which volume is filled by all particles of a certain size class, e.g. which is the combined volume of all particles with a diameter of 20 nm, and plot this against the particle size (Figure 28 C). Volume weighted size distributions are highly relevant as they are generally the data obtained from light scattering measurements. In a surface weighted particle size distribution (Figure 28 B), the particle surface area found in a certain size class is displayed. In case you know the geometry of your particles, conversion of the different size distributions is pretty straightforward. During laser ablation you are generally lucky as most nanoparticles can be considered spherical and $V_{sphere}=4/3\ \pi\ r^3$ and $A_{sphere}= 4\ \pi\ r^2$ are the only equations you will need. When you compare the particle size distributions depicted in Figure 28 you will notice that volume weighted size distributions generally over represent larger particles, while number weighted size distributions are shifted to smaller particles and surface weighted size distributions are located in the middle. This is a universal rule and it is important to consider this correlation when further interpreting particle size distributions.

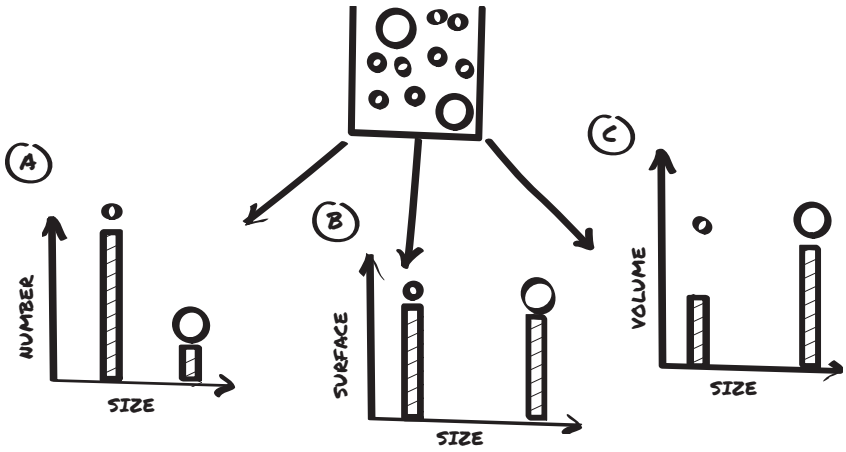

Figure 28: Illustration of particle size distributions for a typical example of laser-generated particles with 2 different particle sizes (bimodal): (A) Number weighted, (B) Surface weighted, (C) Volume weighted particle size distribution. It's the same sample, and all results are "true".

As you have now learned about the different types of particle size distributions you may now pose the question: "Which of these distributions do I use and when?". In general, all particle size distributions carry the same information and are equivalent. However, in some cases one or the other may be physically more meaningful. For example, when you want to support metal nanoparticles on metal oxide carriers (e.g., for heterogeneous catalysis), this is usually expressed as mass load and hence volume distributions should be used as volume is directly proportional to mass. On the other hand, in many relevant areas of colloidal science e.g. toxicity, binding of surface ligands and catalytic reaction rate, the surface area is the driving factor and surface weighted size distributions should be used in this case. So there is no simple answer to this question as it highly depends on the addressed application, this is something you can choose. The next thing you may want to learn is how to interpret particle size distributions.

Interpreting particle size distributions

In all cases, nanoparticles will not be identical to each other, and the average size and the size distribution can be used to describe our sample. By measuring the size of a reasonable large number of particles (N), generally larger than 300, a size histogram can be generated. When particle size distributions is fitted with an appropriate mathematical model, its interpretation is much more straightforward. Nanoparticles obtained by laser ablation usually show lognormal size distribution $f_{LN}(d)$:

$$f_{LN}(d) = \frac{1}{\sqrt{2\pi}(wd)} exp\left[-\frac{1}{2w^2}\left(log\left(\frac{d}{d_{max}} \right) \right)^2 \right]$$

where d_{max} is the point of maximum and w is the parameter determining the width of the distribution. Conversely, a Gaussian size distribution $f_G(d)$ is often observed in nanoparticles obtained by laser irradiation (e.g. after laserfragmentation):

$$f_G(d) = \frac{1}{\sqrt{2\pi}\sigma} exp\left[-\frac{(d - \langle d \rangle)^2}{2\sigma^2} \right]$$

where $\langle d \rangle$ is the mean size and σ its standard deviation.

In general, you have multiple options to present the most abundant (average) particle size of a size distribution fitted by a gaussian or Log-normal curve. The first and most obvious way would be to look at the peak maximum (mode) and to pick the value with the highest abundance and to blank out all other particles. Other common ways of presenting average values in particle size distributions are the mean and the median value. While the mean represents the sum of all particle sizes, divided by the number of all particles, the median or

D_{50} represents the value where exactly 50% of the particles are larger and 50% are smaller. Generally mean > median > mode is a rule of thumb. The difference between these values is illustrated in Figure 29.

Indeed, the most general parameters for identification of nanoparticle size (derived from Gaussian distribution) is the average size expressed as the arithmetic mean $\langle d \rangle$ and its standard deviation σ, expressed as

$$\langle d \rangle = \frac{1}{N} \sum_{i=1}^{N} d_i$$

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left(\langle d \rangle - d_i \right)^2}$$

The advantage of $\langle d \rangle$ and σ are twofold: 1) they are of immediate interpretation for the comparison of different samples with different size distributions, and 2) they can be always extracted from any dataset. The disadvantage is that only the size histogram tells you all the truth on the polydispersity and mono or multimodality of a sample. Therefore, additional useful information are the size range (i.e. minimum and maximum size observed) and the width of the size distribution, obtained by fitting the size histogram with lognormal or Gaussian curves.

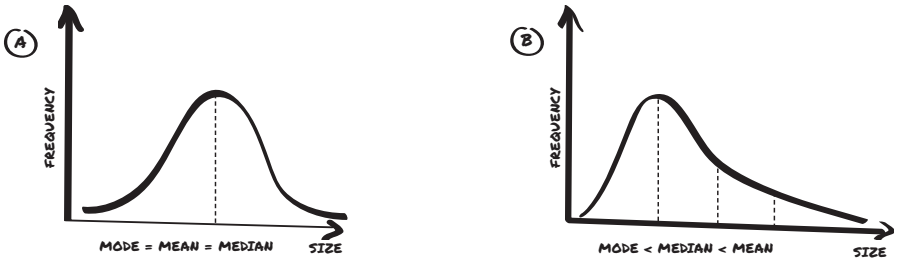

Figure 29: Illustration of mode, median and mean values during interpretation of (A) Gauss and (B) log-normal fitted particle size distributions.

Based on what you have learnt so far, you can definitely say that the presentation of an average particle size is far from trivial. You need to consider the type of particle size distribution as well as the mathematical model. Consequently you will have to be extremely careful when presenting and particularly when comparing average values from different studies. And if someone tells you about the size he made: ask how it was weighted and how the maximum was found. Meaningless: “size of 3 nm”. Precise: “Number-weighted, lognormal mode particle diameter measured by TEM” or “Surface-weighted Gaussian mean diameter of 3 nm +/- 1 nm, as measured by XY”.

The second point which needs to be addressed about particle size distributions is that you need to differentiate between the modality and the dispersity. Modality indicates whether there are two or more distinct populations of particles within a particle size distribution, which can be clearly differentiated. The clearest indication is a disruptive size distribution with multiple peaks. Based on this, a colloid can be characterized as monomodal (having one peak/ most abundant size) or bi-, tri-, multimodal (having more than one peak/ most abundant size). Interpretation of multimodal size distributions usually necessitates fitting of every mode with an individual fit function, an endeavor which may be tricky, particularly in case there is a certain overlap between the different modes.

III

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On the other hand, dispersity indicates how broad your particle size distribution is. Dispersity is generally used to characterize monomodal size distributions or individual modes within a multimodal distribution. The difference between dispersity and modality is illustrated in Figure 30. So within a multimodal size distribution, each mode can be characterized concerning its dispersity. Suitable ways to characterize this value are the standard deviation and the variance. Both can be taken directly from your fit function (mostly log-normal) and give you a rough idea about to what extent the different particle sizes scatter around the average value, generally the higher the variance and the standard deviation, the broader the size distribution. Based on these values one can define the polydispersity index (PDI), calculated via: PDI = variance/mean^2. In this formula the Gaussian "mean" may be substituted by the Lognormal mode (x_c). Note that variance is usually displayedin units of nm².

My student and I were performing a fragmentation experiment. I was just checking in on him when I scented a funny smell. After some time I realized the bright spot on my sleeve originating from some laser reflection. It was a mirror that some other student had muddle headedly put in the wrong box.

Sven Reichenberger, Duisburg

E.g. a diameter of "5 +/- 2 nm" tells: mean of 5 nm and standard deviation is 2. But 2 nm is not the variance! Depending on the software you use, you might not get the variance or standard deviation. For instance, OriginPro gives you the width (w) of your Gaussian fit, which equals to two times the standard deviation.

The variance (that you need for a PDI calculation) in turn is obtained by squaring the standard deviation (in case of Gaussian distribution). Hence, 5 nm +/- 2 nm is monodisperse (PDI = 0.16), but 5 nm +/- 3 nm is not (PDI = 0.36).

Based on their PDI, particle size distributions can be characterized as monodisperse (having a narrow size distribution and a PDI<0.3) or polydisperse (having a broad size distribution PDI>0.3). However, you need to keep in mind that these definitions are by no means fixed within the nanoscience community and can greatly differ based on what PDI values are achievable by the corresponding standard technique. For example, you may proudly call your laser-generated colloid monodisperse based on the above mentioned definition, however a chemist synthesizing oligoatomic clusters with atomic precision or a physicist requiring very narrow size distribution e.g. for optical application may tend to strongly disagree with your definition even laugh at you or even worse, reject your beloved manuscript during peer review.

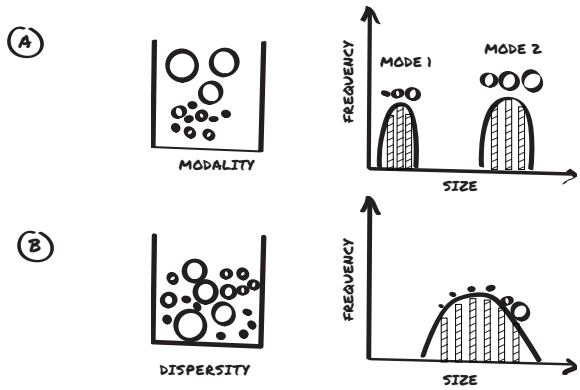


Figure 30: Modality and dispersity: (A) Illustration of a bimodal particle size distributions. (B) Illustration of a monomodal and polydisperse particle size distributions.

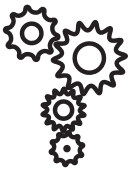
Note that it is easier to achieve monidisperity with number-weighted histograms than with volume-weighted histograms. If you hunt for very high quality, rate your own colloid by volume-weighted criteria. The other statistics will always be better, so that you're on the safe side.

So up to this point it can be concluded that asking for "nanoparticle size" is a huge simplification and needs to be expanded to the question of the particle size distributions within the colloid. Furthermore, you have learned how to express and term a particle size distribution weighted by number, surface or volume and how to analytically interpret them based on average value, modality and dispersity. Now you know about the interpretation of particle size distributions, a suitable follow-up should be how to measure them.

Altering particle size distributions

There are multiple strategies suitable for controlling particle size distributions in laser-fabricated colloids, while some of those were already mentioned in Chapter I. These strategies are basically: I) post processing methods like e.g. centrifugation or laser post irradiation where size distributions are changed after the laser process and II) In situ methods where particle size distributions are changed during the process. While the focus in Chapter I is on ex situ methods, here emphasis will be on in situ methods which are easy to implement during basic laser ablation synthesis.

III



I) Influence of ablation time: Particle size may be changed by altering the ablation time. Even though the effects are not very strong, usually changes of a few nm can be achieved, however this strategy is very easy to use. As a rule of thumb you can memorize that the particles become larger with increasing ablation time. The reason for this is pretty obvious. The final particle size is driven by ripening and growth processes in the solution. In case the concentration of particles are higher due to long ablation times, these growth processes are more pronounced, yielding larger particles (unless you use high repetition or high-intensity ultrasoft pulsed lasers that cause downsizing by laser fragmentation during laser ablation). If you want to avoid concentration supersaturation, simply use liquid flow and drain the colloid off the chamber.

II) Influence of the solvent: laser ablation experiments can be conducted in multiple solvents. The critical parameter controlling particle size, in this context, is the in situ stabilization of initial nanoparticles by solvent molecules. The stronger the interaction, the smaller the particles you obtain. For example, the ablation of gold in acetone yields smaller gold nanoparticles than in water, probably due to an efficient adsorption of solvent molecules on the particle surface.

III) Influence of pH, ionic strength and stabilizing ligands: as a general rule of thumb you can remember that whatever stabilizes your nanoparticles in solution usually also induces a size quenching effect. As described previously, stabilization can be efficiently done by adding ligands and low salinity electrolytes with highly polarizable anions. Furthermore, it may be concluded that a pH far from the isoelectric point is good for stability. So you can simply use the same approach to efficiently reduce the average size of your particle size distributions as well as the PDI. Typical additive concentrations are in the sub-millimeter range for size quenching.

Measuring particle size distribution.

Here you can basically differentiate between four types of methods: electron microscopy, dynamic light scattering, analytical disc centrifugation and small angle x-ray scattering.

According to what was stated in the previous paragraph, TEM (and SEM in case of particles larger than 5 – 10 nm) allows direct imaging of nanoparticles and consequent identification of their shape and size distribution. However, laser synthesis frequently gives a sol, i.e. a colloidal dispersion of solid particles in a liquid matrix. Although the sol can be stabilized by the presence of surface charges on particles surface, the formation of agglomerates of nanoparticles with final size larger than that of its single constituting "monomers" is very frequent in laser generated nanomaterials (as will be discussed in Chapter IV). Due to spontaneous soft agglomeration or hard aggregation of nanoparticles on a substrate during solvent evaporation, the size of these assemblies in the liquid phase is not accessible by SEM. It can be accessed by TEM only using cryo-microscopy (a type of microscopy carried out below the freezing temperature of the solvent, which is very time consuming and expensive!), or by embedding the nanoparticles in a polymeric matrix which can "artificially" freeze the aggregation state in solution (you will find literature reports about how to do it in detail).

STUDENT: I THINK THE LASER DOESN'T WORK ANYMORE. FOR THREE SAMPLES I DIDN'T ABLATE ANYTHING EVEN AFTER 15 MINUTES.

ASSISTENT: DID YOU REMOVE THE PROTECTION CAP OF THE SCANNER?

STUDENT: YES.

ASSISTENT: DID YOU PRESS „START" AT THE LASER? STUDENT: YES.

ASSISTENT: DID YOU TURN ON THE SCANNER?

THE STUDENT MADE A THUNDERSTRUCK FACE AND MOVED BACK TO THE LASER WITHOUT ANY FURTHER COMMENTS.

NINA MILLION, ESSEN

The size (d) measured by electron microscopy is the geometric size, also called Feret diameter (Figure 31), and it is defined as the distance between the two parallel lines tangential to the object, as if you use a caliper to measure particle diameter.

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In comparison to all other techniques, electron microscopy has a couple of striking advantages. The main point is that it will give you a direct insight on the shape of your nanoparticles and tell you the exact size of the metal core (Feret diameter). However, electron microscopy also suffers from some drawbacks when it comes to particle size distributions. First, unless you use cryomicroscopy or a trick such as inclusion in a polymeric matrix, it works on dried samples, which means you can obtain no information of your particles in the colloidal state. For example, information whether your particles are agglomerated in the colloid are very difficult to verify with electron microscopy as particle agglomerates you see in the images could also form during sample drying. The next drawback you have to face with electron microscopy is limited statistics. When you look at typical TEM images of nanoparticles, you usually find, depending on the resolution, up to a few hundred particles per image. Consequently, you usually characterize less than 10^3 particles per sample. Just for comparison, 1 mL of a gold colloid with a mass concentration of 50 mg/L (that's already reddish in color) and a particle size of 10 nm will contain about $5 \cdot 10^{12}$ particles, which means you will have characterized only ~10^{-8} % of your sample with TEM. As a result of this, proper TEM image interpretation necessitates that your sample is very homogeneous and thorough mixing prior to loading your sample on the carrier is absolutely vital. In addition, even this characterization is very labor intensive because you usually have to measure all particles by hand. Even though there are algorithms for automated analysis of TEM images, they usually fail for non-uniform particles or overlapping particles, which are unfortunately quite common in laser-generated colloids. So your will have to get used to spending hours staring at your computer screen.

Other techniques exist that can measure nanoparticles size directly in the colloidal solution, without the need for solvent evaporation. One of these is small angle X-ray scattering (SAXS), which is able to simultaneously extract the size of aggregates (secondary particles) and of monomers (secondary particles) contained therein, although at the price of complex data analysis.

STUDENT: IT TAKES AGES TO TRANSFER ALWAYS 1.5 ML WITH THE 1 ML PIPETTE. I HAVE ALWAYS TO CHANGE BETWEEN 0.5 AND 1.

ASSISTANT: JUST PUT IT ON 0.75 ML.

ALEX HEINEMANN, ESSEN

In addition to the Feret diameter, also the hydrodynamic size is important for nanoparticles in a liquid solution. The hydrodynamic size, also called Stokes-Einstein size, is defined as the size of a hard sphere that diffuses in the liquid medium at the same rate as that of the object (i.e. the size of the sphere with the same diffusion coefficient D of the object, Figure 31). There are two techniques which are sensitive to the hydrodynamic size: dynamic light scattering (DLS) and disc centrifuge (DC). Remarkably, DLS and DC also give information on the hydrodynamic size of nanoparticles coated by a layer of stabilizing molecules (the typical example is that of organic ligands grafted on the surface of noble metal nanoparticles). The shell of organic ligands has lower electronic density than the inorganic core, which makes its detection with electron microscopy or by SAXS uncertain. Besides, the ligand shell often has a "soft" structure, meaning that the size in the liquid phase is generally different than after drying of the sample for TEM/SEM imaging. It is worth to observe that also in case of charged ligand free nanoparticles, the hydrodynamic size may differ slightly from the Feret one, due to the presence of an electrochemical double layer slowing Brownian motion or sedimentation in centrifuge (mimicking smaller primary particles as would be expected).

Therefore, in comparison to electron microscopy, DLS and DC can characterize nanoparticles in their colloidal state. This has a few nice advantages: DLS and DC are generally much faster, do not need sophisticated sample preparation and possess better statistics as they are prone to characterize a huge collective of particles. Also, most colloids are tested for functionality in liquid state, to DLS and DC assess a size that is often more close to the colloid's *in operando* state. However, you need to be careful when interpreting the results because size distributions from electron microscopy and light scattering are fundamentally different:

I) Light scattering characterizes colloidal particles consisting of the metal/hard matter core as well as a shell of counter ions or potential organic ligands. This sum of core diameter and shell is called hydrodynamic diameter, which of course is larger than the Feret diameter obtained from electron microscopy. So as a rule of thumb you can memorize that particle size distributions from light scttering are always larger than those from electron microscopy. This difference is usually negligible when completely ligand-free particles are evaluated, however, differences by a factor of two or three can be found in case bulky ligands like proteins or polymers are used.

II) Light scattering can usually not differentiate between single particles and agglomerates/aggregates dispersed in solution. This can lead to huge differences between particle size distributions from electron microscopy and light scattering. Therefore, whenever you encounter discrepancies between electron microscopy and light scattering techniques, which are too big to be explained by a hydrodynamic shell (in most cases the hydration shell is even less than a nanometer, and ligands or sufactants have typical length of only a few nanometers), agglomeration/aggregation in the dispersion is the most probable cause.

While electron microscopy gives direct access to the particle size, light scattering yields indirect results which originate from an approximation by a mathematical model. In case you do not properly understand these underlying models, data can be easily misinterpreted. Hence it is mandatory that you critically evaluate all data obtained by light scattering.

It is now clear that the definition of nanoparticle size may be tricky, unless you don't have in mind that it is important to discriminate between:

i) the Feret diameter of the objects physically surrounded by the liquid environment in the sample (which often is an aggregate of "monomers"),

ii) the Feret diameter of constitutive units ("monomers") in the aggregates,

iii) the hydrodynamic size of the aggregates (or of monomers if no aggregation occurs), which also contains the contribution of the ligand shell if present.

Depending of which size we are interested in, we must use the appropriate investigation technique, and none of the techniques is generally better or worse for colloid analysis, but they bring complementary information. So it is very useful to verify your particle size distribution always (!) with at least two different methods. It is very unlikely that 2 methods give the same value. Instead, by the difference in diameters, you will learn a lot about your sample and may give impressively precise statements in your thesis or manuscript.

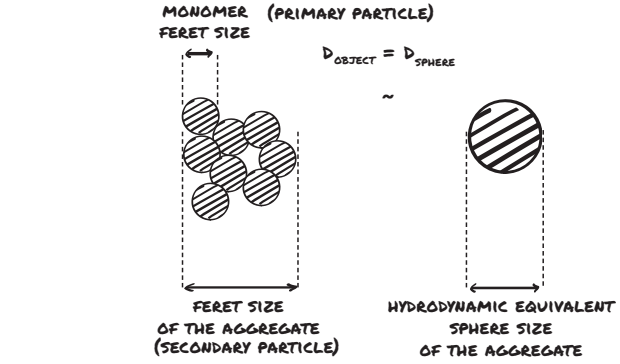

Figure 31: Understanding nanoparticle "size" coming out from different techniques.

2.1. Dynamic light scattering (DLS).

a. What do I see?

The total (core + shell) average hydrodynamic size of all the objects dispersed in the sol, and an estimation of their size distribution. Typically, size distribution can be weighted on the volume or on the number of nanoparticles. However DLS is not applicable to very small nanoparticles (i.e. below 5 nm).

B. How does it work?

The physical principle exploits here the fact that the diffusion coefficient of a particle is size dependent, i.e. small particles diffuse faster than large particles. DLS exploits a coherent monochromatic polarized laser beam (typically in the red) to collect the light scattered by the nanoparticles in the sample. The scattered beam forms a speckle pattern on the detector area, i.e. a pattern with inhomogeneous density of photons due to the occurrence of constructive or destructive interference in different parts of the detector area. In fact, the scattering objects have different position in the sample, and light scattered from each object will have a different phase on the detector surface. By repeating the measurement at different time intervals, time evolution of scattered patterns can be correlated to the Brownian motion of the scattering objects (i.e. to the random walk of particles suspended in a liquid, resulting from their collision with the liquid molecules or with other particles). It is intuitive that scattering signals coming from smaller particles change/fluctuate much more quickly than those stemming from larger particles. Based on this, the instrument acquires an autocorrelation function, indicating how quickly signal intensity changes and based on this calculates diffusion coefficients and particle sizes.

The use of complex mathematical functions can be applied to the time dependent scattered pattern to extract the diffusion coefficients D of the scattering objects, and their relative distribution. Finally, the size of the objects is extracted from D by using the Stokes – Einstein equation:

$$D = \frac{\kappa_B T}{6\pi\eta R_S}$$

with T the temperature, k_B the Boltzmann constant, η solvent viscosity and R_S the equvalent sphere radius.

However, no worries: the mathematical analysis is done automatically by the instrument, and the operator just needs to set parameters, place sample and launch analysis with the mouse. The underlying software for data interpretation is designed to work even for unskilled personal. The main advantage of DLS is that measurements are very fast and results can usually be obtained after a few minutes. In fact, DLS is the standard technique for the characterization of size distribution in colloids, and this type of instrument is frequently found in research labs.

However, the technique has one striking disadvantage which can be particularly critical when analyzing colloids from laser ablation experiments, where you may be faced with polydisperse or even polymodal size distributions: DLS measurements, the scattering intensity is generally proportional to the sixth power of the particle diameter! Even though the underlying algorithm considers this correlation, the light emitted from small particles in the presence of larger ones may be too weak to be detected (the detector is dazzled). Just to illustrate

this point, let us assume you intend to analyze a sample collective containing 5 nm and a 50 nm particle with DLS. In this case the light intensity from the 5 nm particles is a million times lower. Based on this it can be concluded that DLS often fails in characterizing small particles in the presence of large ones. Note that the widely used Malvern DLS instrument always puts out artificially symmetric Gaussian distribution around a calculated peak value, independent on how asymmetric your sample size distribution looks like.

➞ c. Amount of sample needed?

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Approximately 1 – 2 mL of solution are enough, at a concentration which allows scattered light to cross the sample without considerable re-absorption. Actually, the use of high concentration and high absorbance solutions is not compatible with DLS: in these cases dilution of the sample until convergence of DLS results is suggested. Obviously, excessive dilution will result in a weak scattering signal and noisy/not reproducible measurements: the presence of few dust grains in solution can completely alter the results in this case!

➞ d. How do I prepare the sample for analysis?

You just need to fill the DLS cuvette with the colloidal solution, as in OAS.

If you don't see the small particles, try again after dilution. Typical concentration threshold (e.g. for gold) is 20 mg/L, better 50 mg/L. Use a simple syringe dead-end-filter (e.g. with 400 nm pores, often called virus filter) to quickly remove the dust. Repeat measurement after 1-2 days.

➞ e. Tips / warnings / hazards?

In case of samples with a wide distribution of size, the largest size will dominate the scattering response, and the only reliable size distribution is the volume weighted one. In this case, DC can give more reliable information because the sample is sorted in size during the analysis.

For the same reason, the presence of large objects in the liquid, such as dust grains, must be avoided.

➞ f. To know more?

- Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy; Pecora, Robert; 1985, Springer-Verlag.

- Dynamic Light Scattering: An Introduction in 30 Minutes; Malvern Inc.; free at

http://www.malvern.com/en/support/resource-center/technical-notes/TN101104DynamicLightScatteringIntroduction.aspx

2.2. Disc Centrifuge (DC)

A. What do I see?

DC gives the full size distribution of the sample and is compatible to polydisperse and multimodal colloids. In case of high density nanoparticles, DC is not applicable for sizes below 5 nm, and this limit increases with decreasing particle density.

B. How does it work?

In a DC, particles are separated by size using centrifugal sedimentation in a liquid medium. The basic principle is the separation of particles of different sizes in a gravitational field, generated by a rotating disk. Naturally, larger particles will sediment more quickly than small ones. The sedimentation is promoted by the centrifugal force generated by the rotation of a discoid cell containing the sample (in the inner part) and a liquid phase with increasing viscosity from the center to the extremity of the disc (Figure 32). A sucrose initiated density gradient within the disk ensures that individual size fractions of particles uniformly move to the edge of the disk. The cell and the liquid phase are optically clear so that, when particles approach the outside edge of the rotating disc, where an optical detector is located, they are detected by absorption or scattering of a light beam passing through the disc at a fixed position. The change in light intensity recorded at a fixed wavelength over time is converted by the software into a particle size distribution, because the time required for nanoparticles to cross the light beam is a function of the hydrodynamic particle size and medium viscosity. In order to correlate sedimentation time and particle size, a calibration standard needs to be added prior to each measurement. Based on the known speed of the disk and an external calibration standard, the instrument “knows” which particle size fraction is meant to pass the detector and which time. The scattering intensities for each size fractions can then be recorded transformed into a particle size distribution. Due to this measuring principle, analytical disk centrifugation is particularly well suited to characterize polydisperse and polymodal particle size distributions, predominantly formed during laser ablation experiments. Main disadvantages, however, are that measurements can be highly time consuming, particularly for very small particles or particles with low density (measurment time ~ $(\text{diameter})^{-2}$).

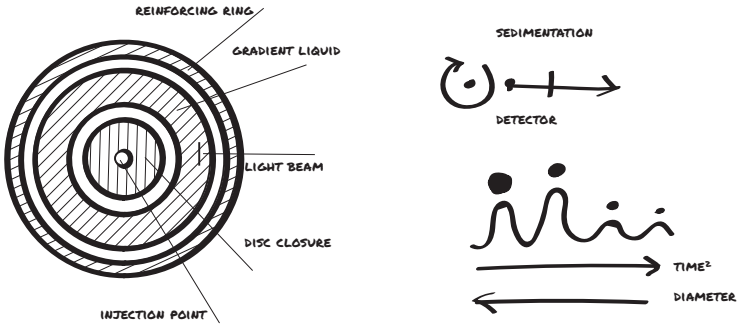


Figure 32: Sketch of the DC disc, sedimentation principle and chromatogram.

c. Amount of sample needed?

Only 0.1 mL of solution is required, at a concentration that allows detection by the light beam, corresponding to 10^{-8}-10^{-6} g of sample in the best cases.

d. How do I prepare the sample for analysis?

The sample just needs to be injected in the center of the DC discoid cell, after appropriate build up of the viscosity/density gradient.

e. Tips / warnings / hazards?

DC works under two main assumptions:

i) particles will have the same hydrodynamic size in the viscous medium as in the original solution.

ii) absorbance of nanoparticles with different size is well reproduced by the intrument software (the software uses a correlation function to consider absorbance of different sizes, that is why optical parameters of measured particle species need to be added).

Obviously, these assumptions should be checked before use with independent techniques. Concerning assumption *i)*, you must verify particles aggregation or disaggregation for instance by mixing the nanoparticles solution with the viscous solution and performing DLS, or by crossing DC analysis with TEM imaging, at least the first times the analysis is performed.

Assumption *ii)* is more complicated to be verified (think for instance to aggregates of plasmonic nanoparticles, whose absorption is strongly related to size and shape), and actually requires a solid a priori knowledge of the sample. But even for unknown absorption property, not the peak intensity but its hydrodynamic diameter will be analyzed correctly.

f. To know more?

– Centrifugal Separations in Biotechnology; Leung, Wallace Woon-Fong; 2007, Academic Press.

– Disc Centrifuge Particle Size Analyzers; CPS Instruments Europe; free at *http://www.cpsinstruments.eu/library.html*

We had a student who has been working on the laser for a couple of days. One morning he was working within the laser lab with a colleague being present. After several ablations and breaks he put on his laser safety goggles and started the laser for the next ablation. However, he didn't make sure that everyone else in the lab was wearing their laser safety goggles. The colleague noticed the sound of the ablation and was able to react right away. When this break in safety procedure was addressed the student tried to make excuses stating that the colleague was wearing his goggles some time before. However, it is the responsibility of the laser operator to ensure that everyone within the lab wears the personal equipment prior to turning on the laser every single time.

Elisabeth Maurer, Essen

General comment on DLS and DC:

The software of both methods, DLS and DC often prints out the "z-Average" or "intensity distribution". Both are useful to see if you have strong scatterers in the sample that may have screened the small ones, but are scientifically meaningless and need to be converted into volume or surface or number weighted statistics.

We have made round-robin tests and compared many instruments under different conditions. Although instrument suppliers may claim different, you cannot reproducibly measure particle diameters < 4 nm. We always measure down to ≥ 3 nm diameter and take everything serious until 3-4 nm. For the smaller fraction, you will need the help of TEM. Also, you will need to put the density or an optical parameter into the software before measurement. A big gold particle property will not be influenced largely by a monolayer of ligand in density or extinction, so you take the value of gold. But for lighter particles or bulky ligands this may have an effect. Choose the material parameter of the core and disclose this in the experimental part of your thesis or manuscript. Now you know why a hydrodynamic diameter measured by DC may be counter-intuitively even smaller than a diameter measured by TEM, and larger by DLS

(even if it does not aggregate). A ligand has two effects. It may reduce the particle's density, to make it appear smaller. But most importantly, it adds a drag force to the particle's mobility. Thereby it is diffusing slower mimicking a bigger particle in DLS, and sedimenting far slower, mimicking a smaller particle in DC. That's great to know, since it provides evidence for successful grafting a ligand on a colloidal particle.

2.3. Small angle X-ray scattering (SAXS)

III

→ A. What do I see?

Size and structure of particles in the 3 – 150 nm range can be measured.

→ B. How does it work?

SAXS is based on the elastic scattering of monochromatic X-rays by atoms in a sample. Collection of scattered X-ray intensity takes place in a very small angle interval (0.1° - 10°, Figure 33) from the beam propagation direction (forward scattering), where the scattering events are more probable and diffractive effects are nearly absent (see III.3.3). Although theory behind SAXS is not simple, the main concept is that X-ray scattering depends on the discontinuity of electron density in the sample, when it occurs on a length scale of 1 – 100 nm. Consequently, the scattering pattern at small angles contains information on the typical size and shape of the scattering objects with high electronic density immersed in the matrix with low electronic density. It can measure polydisperse samples, but intensities are dominated by high mass fraction of the sample (the large particles). And size differentiation of overlapping modes is almost impossible (e.g. differentiation of 6nm and 8nm particles).

The typical SAXS plot reports X-ray intensity versus angle, and it is fitted with mathematical models which includes the size and shape of the scattering object as fitting parameters. Contrary to DLS, these mathematical models must be applied by the operator, making SAXS data analysis a non trivial task. Besides, SAXS instrumentation is not available in most labs.

SAXS

On the other hand, the advantage of SAXS compared to DLS is that it can be used to characterize polydisperse samples and it may provide additional information on the particle shape. In addition, SAXS is the only method suitable for monitoring processes in optically opaque media e.g. inside the laser-induced cavitation bubble.

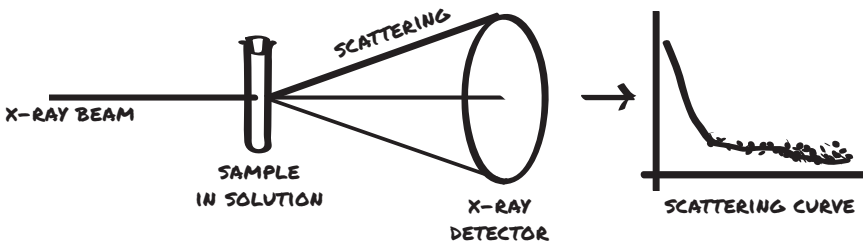


Figure 33: Sketch of the working principle of SAXS.

c. Amount of sample needed?

The technique requires ~1 mL of sample with concentration of the order of 0.1 – 0.5 mg/mL (depending on the z of the elements in the scattering object).

d. How do I prepare the sample for analysis?

Stable liquid dispersions of nanoparticles are fine.

e. Tips/ warnings/hazards?

If the colloid is not stable, viscosity of solution can be increased to grant stability for the duration of the analysis, which may require up to several days to achieve acceptable signal-to-noise. To this purpose, you can use glycerol or high concentration polymer solutions.

f. To know more?

– Structure Analysis by Small-Angle X-Ray and Neutron Scattering; Feigin, L.A. and Svergun, D.I.; 1987; free at http://www.saxier.org/forum/viewtopic.php?t=282

Table 1: Summary of properties, advantages and limitations of the principal methods for the characterization of particle size distribution.

	Electron Microscopy	**Dynamic Light Scattering (DLS)**	**Small Angle X-ray Scattering (SAXS)**	**Disk Centrifugation (DC)**
Type of particle size	Feret (core)	hydrodynamic	hydrodynamic	hydrodynamic
Acquisition of particle size	direct	modelling	modelling	modelling
Sample processing	very slow	fast	very slow	average
Skills required for data interpretation	low	low	very high	low
Raw data output (size distribution)	number	volume	volume	volume
Statistics (number of analyzed particles)	~10^{-8} %	up to 100%	up to 100%	up to 100%
Information on colloidal state	generally no (unless using specific tricks)	yes	yes	yes
Information on ligand conjugation	generally no (sometimes from interparticle distance)	yes	no	yes
Characterization of single particles	yes	no	no	no
Characterization of aggregates	generally no (unless using specific tricks)	yes	yes	yes
Polymodal and polydisperse particles	yes	limited	yes	yes
Particle morphology	yes	no	yes	no

STEP 3: IDENTIFY THEIR MORPHOLOGIES

Imagine you're in Italy, on a sunny day, but well arranged under a vine-covered pergola in the porch of a restaurant from which you can admire green hills planted with dozens of different types of fruit and vegetables. You're tasting a wonderful pasta with a red flavorful tomato sauce as you did not know that it existed. The question you put to the waiter at the first opportunity will be "what are the ingredients of this dish?" (or maybe it will be your second question, just after asking him "Can I have some more?"). With our beloved nanoparticles it is the same: the time comes when we have to exactly know about their ingredients. Therefore, once you are sure that nanoparticles are present in our sample, and you know their size distribution, the next step is to clearly identify their elemental composition and phase.

The quickest way to qualitatively identify elemental composition is the energy dispersive X-ray spectroscopy (EDS). Most TEM and all SEM are equipped with EDS, with the obvious difference that TEM allows elemental analysis with nanometric resolution, while SEM only allows resolution of the order of 10 nm or higher in ordinary conditions. Quantitative information on the elemental composition by EDS is also possible, but it suffers of matrix effects and becomes reliable only on isolated clusters of nanoparticles.

Alternative techniques such as inductively coupled plasma assisted (ICP) mass spectrometry (MS) or optical emission spectroscopy (OES) gives reliable quantitative information on particles ensemble, with sensitivity as low as part per trillions (ppt) in best cases.

Elemental composition still does not tell us the phase of the nanoparticles, as solid matter can be either amorphous or ordered, and every combination of elements can be achieved in a multitude of phases. Just to give an example, iron and oxygen can form magnetite, maghemite, hematite, wustite, goethite and amorphous iron hydroxide, and all of them are achievable by laser synthesis. Ordered phases are accessible by diffraction techniques, which for nanoparticles are X-ray diffraction (XRD) or electron diffraction (ED). XRD is accurate and easier to apply, and it can also give reliable quantitative information on the weight fraction of all phases constituting the sample, but these are averaged information over all particles in the specimen. ED is possible with TEM and can give information on a single nanoparticle at time, but its quantitative application is often not possible.

For some classes of materials such as oxides and semiconductors, qualitative identification of ordered phases is possible by vibrational spectroscopy, such as Raman and Fourier-transformed infra red (FTIR).

In the most complicated cases, where complex, disordered or unknown phases are obtained, the above investigation methods may be not enough to precisely identify the nature of the sample. In these "desperate" cases, you can refer to

a group of "magic" techniques which is applicable to any material: the group of X-ray absorption spectroscopies (XAS), including X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS). Although the XAS group gives average information on the sample, these information are complete and cover the composition, the chemical state of each element and the composition and arrangement of nearest neighbour (NN) and next nearest neighbour (NNN) atoms. This is very useful for multielement materials, such as alloys or doped oxides. Unfortunately, the payback is high because XAS analysis requires a synchrotron radiation facility and at least one dedicated expert for data collection and analysis (which is absolutely not trivial!).

III

3.1. Energy dispersive spectroscopy (EDS)

A. What do I see?

EDS allows the detection of elements with z larger than Be, although sensitivity increases with z and depends on matrix composition. Quantitative information can be obtained easily when matrix effects are negligible.

B. How does it work?

EDS is based on the fact that i) X-rays are produced by the interaction of an high energy electron beam with matter, and ii) each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum. In particular, the collision of the electron beam with sample atoms promotes the kick off of electrons from inner atomic shells, leaving an empty energy level which is filled by electrons of the outer shells. The energy difference is released as a photon with frequency typical of X-rays. Since the electronic structure is specific of each element, the spectroscopy of X-rays produced by the sample allows the identification of elements contained therein. X-rays are measured by an energy dispersive spectrometer exposed on the sample, and compared to a database for element identification. The typical EDS spectrum reports X-ray counts (in arbitrary units) versus their energy (in keV, Figure 34). Integration of peaks and comparison with a standard or a database allows the extraction of quantitative information.

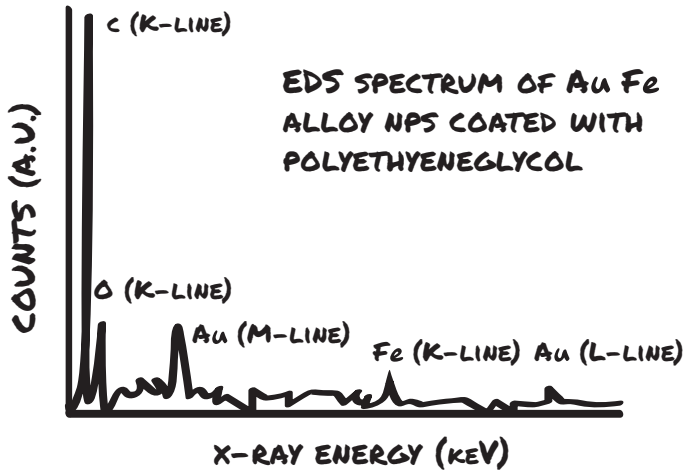


Figure 34: Typical EDS spectrum of AuFe alloy nanoparticles coated with polyethylenglycol.

EDS is based on the fact that i) X-rays are produced by the interaction of an high energy electron beam with matter, and ii) each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum. In particular, the collision of the electron beam with sample atoms promotes the kick off of electrons from inner atomic shells, leaving an empty energy level which is filled by electrons of the outer shells. The energy difference is released as a photon with frequency typical of X-rays. Since the electronic structure is specific of each element, the spectroscopy of X-rays produced by the sample allows the identification of elements contained therein. X-rays are measured by an energy dispersive spectrometer exposed on the sample, and compared to a database for element identification. The typical EDS spectrum reports X-ray counts (in arbitrary units) versus their energy (in keV, Figure 34). Integration of peaks and comparison with a standard or a database allows the extraction of quantitative information.

➞ C. AMOUNT OF SAMPLE NEEDED?

See in III.1.2.c (TEM) and III.1.3.c (SEM).

➞ D. HOW DO I PREPARE THE SAMPLE FOR ANALYSIS?

See in III.1.2.d (TEM) and III.1.3.d (SEM).

→ E. Tips / warnings / hazards?

Avoid thick or crowded agglomerates of nanoparticles to minimize matrix effects and to increase the reliability of quantitative results, which is best in isolated nanoparticles. Donŕt worry about carbon, itś always there, and sometimes you will find silicon (or silicates), which is an additive in seals and are found everywhere in the lab and sample holders, in particular where organic solvents were used for cleaning by you or your colleagues. Of course, you will not use any equipment made of metal (spatula etc.) during synthesis or sample handling.

→ F. To know more?

See III.1.2.f (TEM) and III.1.3.f (SEM)

3.2. Inductively coupled plasma assisted (ICP) mass spectrometry (MS) and optical emission spectroscopy (OES).

→ A. What do I see?

All the elements in the sample can be detected and precisely quantified, excluded hydrogen. It will also tell you the amount of dissolved elements (e.g. analyzing the supernatant after centrifugation)

→ B. How does it work?

Measurement takes place in two stages: atomization of the sample and analysis of atomic species. The inductively coupled plasma serves for the atomization of the sample. The ICP is alimented by an electromagnetic coil which has the function of inductively heating the plasma, which is composed of a highly ionized and electrically conductive argon gas. The liquid sample is introduced in the plasma with a nebulizer, immediately reaching the same temperature and ionization conditions.

In ICP-MS, detection takes place by conveying the atomized sample to a mass spectrometer, where charged particles are separated by a mass selector which exploits the Lorentz force, and counted by a charged particles counter, to obtain signal intensity versus mass for unit charge.

In ICP-OES, the plasma is coupled to a diffraction grating and a photodetector (typically a photomultiplier or a CCD), to measure the intensity of light emitted at the wavelength characteristic of highly ionised atomic species. Atomic species emit a set of very sharp bands, which clearly are related to the electronic structure of the emitting element.

Quantitative analysis requires calibration with a standard in both MS and OES methods.

c. Amount of sample needed?

Detection limits of ICP-MS are impressively low (usually below parts per billions, ppb), thus requiring few μL of solution. ICP-OES has higher detection limits, but fully compatible with a typical sample containing laser generated nanoparticles.

d. How do I prepare the sample for analysis?

For quantitative analysis, sample preparation requires the complete dissolution of the solid phase into ions (sample digestion), which typically requires the use of acids or bases at high temperature. This means that a colloidal solution is not suitable for analysis without appropriate sample preparation, if reliable quantitative results are desired.

Several days after my first experience with high intensity lasers, I began to feel more confident in handling these machines. However, once after a short break I went to the laser lab and continued my experiments, when I observed increasing smoke formation I realized that I forgot to remove the cap of the focus lens.

Sandra Jendrzej, Essen

e. Tips / warnings / hazards?

Sample digestion is critical for quantitative measurements: check literature for the most appropriate procedure for your sample (for instance aqua regia, which is a 1:3 mixture of HNO_3 and HCl, works very well in dissolving gold nanoparticles, but cannot be used with Ag nanoparticles because it can form the insoluble AgCl precipitate). For contamination with C, Si, metals, see (III.3.1.e).

f. To know more?

- AAS, XRF, and MS Methods in Chemical Biology of Metal Complexes; Ott, Ingo; Biot, Christophe; Hartinger, Christian; in Inorganic Chemical Biology: Principles, Techniques and Applications; Gasser, Gilles; 2014, John Wiley & Sons.

3.3. X-ray diffraction (XRD)

A. What do I see?

The ordered phases can be identified, and the volume-weighted average size of ordered domains in the sample can be evaluated. By applying the Rietveld analysis, quantitative information on the mass fraction of each crystalline phase can be achieved. Hence, crystal diameter can never be smaller than the particle diameter. But particles (e.g. larger metal particles) may consist of several crystals (e.g. twins) even if they are spherical, so that combination of XRD with TEM of DC/DLS may already tell a lot about the sample. XRD is "blind" for amorphous materials, whereas SAXS sees both, crystalline and amorphous scattering objects.

B. How does it work?

X-rays are scattered by atoms. When atoms form ordered arrays with well defined translation periodicity on a length scale comparable to X-ray wavelength (~0.1 nm), the X-rays are scattered only at well defined angles (Θ) typical of the crystalline structure. In this way, a diffraction pattern is obtained by the measure of the intensity of scattered X-rays versus the scattering angle. The XRD pattern (intensity and diffraction angle of all peaks) is related to the atomic position and interplanar distances in the crystal, as described by the Bragg's law (Figure 35):

$$n\lambda = 2l\sin\theta$$

where n is any integer, λ is X-ray wavelength, l is the distance between reflection sites (typically interplanar distances d), and Θ is the angle between the reflected beam and the plane containing the sample.

The comparison of the XRD pattern with a database allows the identification of the compound. The volume-weighted average size (τ) of ordered domains in the sample can be measured by using the Debye-Scherrer formula

$$\tau = \frac{K\lambda}{\beta\cos\theta}$$

where K is a constant (typically ~ 0.9), λ is the X-ray wavelength and β the full width at half maximum (FWHM) of the diffraction peak located at the diffraction angle Θ. Hence, XRD tells you the type of crystal and its size. Sharp, high peaks indicate large particles.

In case of multiple compounds, the Rietveld analysis is used to extract information on the relative mass abundance of all species involved. By this, lattice strains from doping, defects or alloying can be attributed. It's good to have Rietveld fits for a phospors or alloy nanoparticle series.

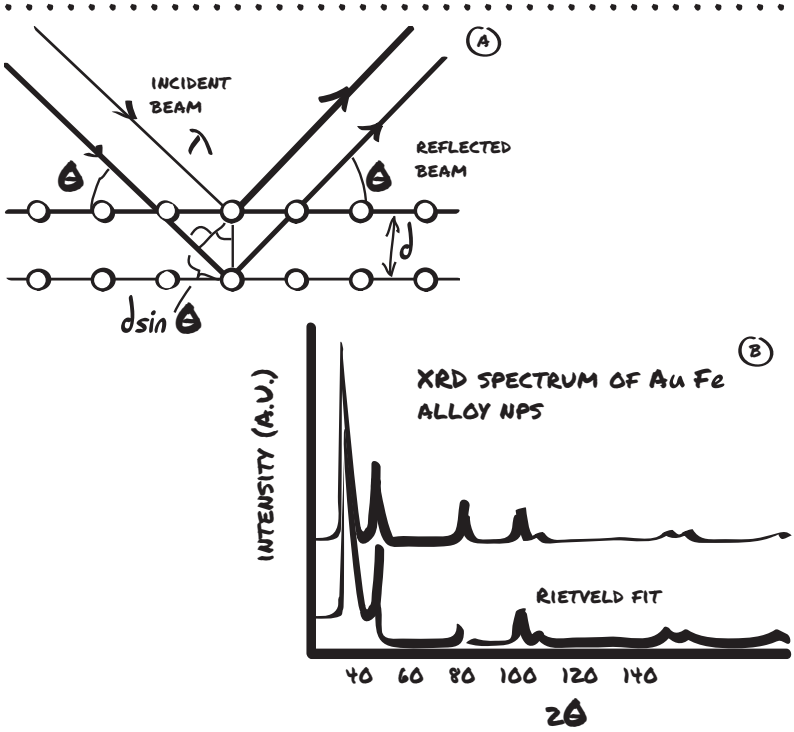


Figure 35: (A) The principle of Bragg's law and (B) a typical XRD spectrum of AuFe alloy nanoparticles.

➞ c. Amount of sample needed?

At least 2 – 10 mg of nanoparticles are required. Yes, you might need upscaling (Chapter II)

➞ d. How do I prepare the sample for analysis?

Nanoparticles are deposed on XRD substrates (for instance quartz plates).

➞ e. Tips / warnings / hazards?

It is important to avoid heating of sample during deposition, in all those cases where oxidation, segregation or phase transition may occur, such as with iron oxides or metastable alloy phases. Because of the volume-sensitivity of the method, minute amount of larger particles will screen the information (crystal structure and size) of the small fraction. First calculate crystal size by Scherrer equation, if it's far larger than expected (e.g. sharp, high peaks are not nanocrystals) maybe filter or centrifuge the sample and repeat.

f. To know more?

- Basic Concepts of X-Ray Diffraction; Zolotoyabko, Emil; 2014, John Wiley & Sons.

Everybody knows that one should wear laser protection glasses when the laser is running. So what to do, when you notice that your colleague does not wear glasses? For sure, do not slap him into the face while trying to protect his eyes by hand. It's better to tell him: Please wear your protection glasses.

Lisa Gamrad, Essen

3.4. Electron diffraction (ED)

a. What do I see?

Ordered phases can be detected and identified by ED, with the possibility to perform the analysis at the single nanoparticle level.

b. How does it work?

The principle is the same of XRD (III.3.3.b), with the sole difference that here the diffraction pattern is produced by scattering of a monochromatic high energy electron beam instead of an X-ray beam.

c. Amount of sample needed?

See III.1.2.c (TEM).

d. How do I prepare the sample for analysis?

See III.1.2.d (TEM).

e. Tips / warnings / hazards?

Measurement of scattering intensity is not simple with ED, because of its dependence on nanoparticle orientation and on the limited number of nanoparticles probed. Therefore, ED is more suited for qualitative identification of nanoparticle structure, while quantitative information on the ordered phases are preferably obtained by XRD.

f. To know more?

See III.1.2.f (TEM).

3.5. Vibrational spectroscopy (Raman, FTIR).

a. What do I see?

Several compounds (mostly oxides and semiconductors) can be identified based on the presence of peaks ascribable to well defined chemical bonds.

b. How does it work?

In both cases, the energy of vibrational levels is probed. In Raman spectroscopy, the sample is irradiated with a monochromatic visible laser beam, and the portion of backscattered light is collected. The backscattered light has a main component due to elastic scattering, which is discarded, and a low intensity inelastic scattering portion originated by Raman scattering. The Raman scattered light is dispersed on a detector to measure intensity versus photon energy. The energy difference between inelastic scattered photons and incident photons (the Raman shift) is the energy of vibrational levels in the sample. Raman spectra are reported as intensity (counts) versus wavenumber or Raman shift (expressed in cm^{-1}, Figure 36). The position of each peak is distinctive of specific functional groups in a compound. However, a certain level of crystalline order is required to observe appreciable Raman bands from crystalline solids, which typically happens only for sizes above ~5 nm.

In FTIR, vibrational levels are probed directly by measuring the transmittance of the sample as a function of infrared radiation energy. The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform of the transmitted beam is used to obtain the final spectrum (don't worry the instrument software will do it for you). FTIR spectra are reported in transmittance (T) versus wavenumber (in $cm.^{-1}$, Figure 36).

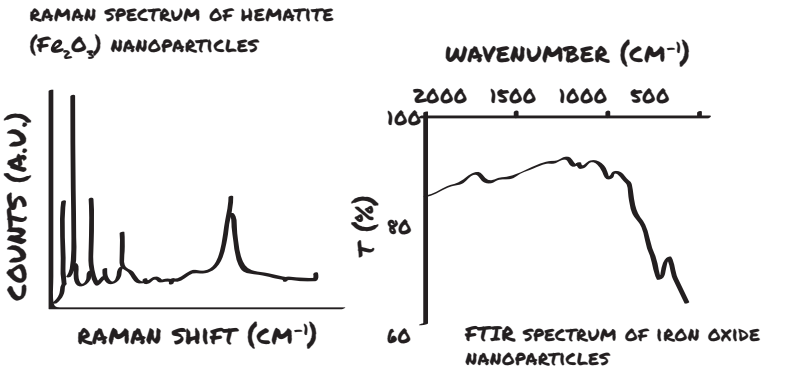


Figure 36: Typical Raman and FTIR spectrum.

c. Amount of sample needed?

Raman spectroscopy only requires few μg of material, when coupled to a microscope (micro-Raman). FTIR requires more than 0.5 – 1 mg of material.

d. How do I prepare the sample for analysis?

Solvent must be removed for the analysis, and nanoparticles must be deposited on a substrate with negligible background. For Raman spectroscopy, this can be a metal foil or a glass slide. For FTIR, specific crystals which are transparent to infrared radiation must be used (such as CaF_2 or KBr windows).

e. Tips / warnings / hazards?

Often Raman and FTIR gives complementary information about inorganic materials, hence the two techniques give their best when used together.

f. To know more?

- Raman Spectroscopy for Nanomaterials Characterization; Kumar, Challa S. S. R.; 2012, Springer.

- Infrared and Raman Spectroscopy Principles and Spectral Interpretation; Larkin, Peter; 2011, Elsevier

3.6. X-ray absorption spectroscopy (XAS).

A. What do I see?

Composition, chemical state, type of NN and NNN and their location in space.

B. How does it work?

XAS principle is pretty much like OAS, being based on the measurement of X-ray absorption bands from the sample. However, the physics behind absorption of X-rays is rich and strongly related to the chemical nature of the sample. Contrary to OAS, that typically shows absorption peaks, in XAS you find absorption edges. These edges can be divided in three portions: the pre-edge, the rising edge and the extended edge structure (Figure 37). The pre-edge contains the information on the element, being determined by the electronic structure of the atom absorbing X-rays. Absorption intensity is related to the abundance of the absorbing element by the same Lambert-Beer law exploited for OAS (see III.1.1.b).

X-ray absorption is followed by emission of a photoelectron (photoelectric effect). The great point of XAS is that photoelectrons undergo a scattering process with surrounding atoms, and this generates a modulation of the X-ray absorption intensity in the region of the rising edge and the extended edge. In particular, the rising edge reflects the interaction of the photoelectron with the NN, and it's the subject of XANES. The extended edge reflects the interactions with NNN and other atoms, and it's the subject of EXAFS. The XANES and EXAFS portions of the X-ray absorption edge can be fitted with complex mathematical models which account for the relative position and composition of NN, NNN and so on. In this way, you can find the average composition and atomic disposition around the absorbing element. In particular, XANES is more sensitive to the oxidation state and to the atomic site symmetry, while EXAFS can give the full atomic coordination number, the chemical nature of the surrounding atoms and the interatomic distances.

NNN
NNN
NNN
NNN

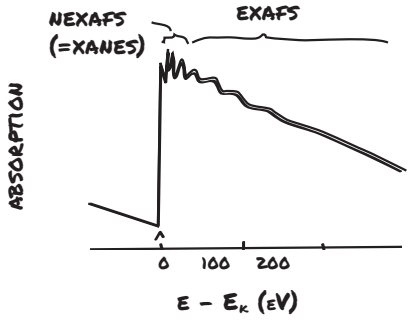


Figure 38: Sketch of a XAS edge and its three parts.

c. Amount of sample needed?

XAS is very sensitive, thanks to the high performance of synchrotron light: the amount of material needed is the same necessary to cover a small area of few mm^2 on the sample holder.

d. How do I prepare the sample for analysis?

Simply by drop casting on the XAS substrate, followed by solvent evaporation. It can also run wet samples

e. Tips / warnings / hazards?

Anyone working with nanoparticles sooner or later needs a friend expert of XAS. The tip is...be nice to any XAS expert!

f. To know more?

– X-Ray Absorption and X-Ray Emission Spectroscopy: Theory and Applications; van Bokhoven, Jeroen A. and Lamberti, Carlo; 2016; Wiley.

STEP 4: QUANTIFY THEM

In Chapter II you already obtained good information about how to evaluate the productivity of your set up. In addition, it is good to know that the best way for the quantification of matter in a sample is ICP-MS or ICP–OES, as discussed in section III.3. Sometimes, you may be tempted to use a microbalance to quantify the mass of a sample, but this procedure can be error prone for amounts lower than ~5 mg, and you must always account for solvent residuals and solutes when drying the colloid. On the other hand, the weight of the target can be altered by the formation of micrometric fragments which are not part of the colloid.

In case of absorbing nanoparticles, OAS can be used for quantification thanks to the Lambert-Beer law, which simply relates absorbance to nanoparticles concentration (see III.1.1.b). This method works well with metals and oxides whose size and optical properties are already known, and especially benefits of start-up calibration with an independent and more reliable technique such as, for instance, ICP-MS.

It is worth to stress that the most common unit for the quantification of a sample of nanoparticles is mass (e.g., mg) or mass per volume (e.g., mg/mL). However, the comparison of laser ablation yield among nanoparticles with different composition is possible only by considering the number of atoms in your sample, expressed as mol or mol/L (or M). The reason can be easily understood considering that a sample with concentration 0.001 mol/L correspond to 0.197 mg/mL if composed of Au, and to 0.063 mg/mL if composed of Cu (Figure 38).

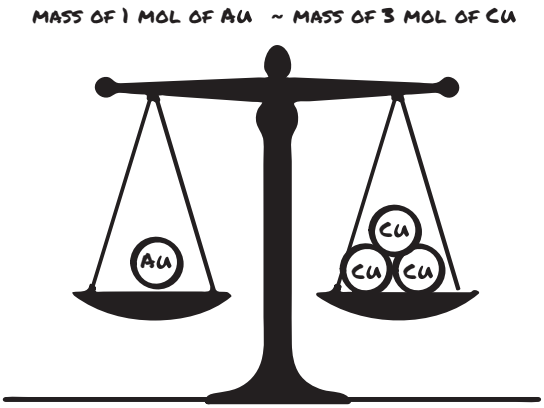


Figure 38: Comparison between the mass of 1 mol of gold and 1 mol of copper.

STEP 5: ANALYZE THEIR SURFACES

How many times you said "You can't judge a book by its cover"? Sorry, but this is not the case of nanoparticles: since the proportion of surface atoms to volume atoms increases while decreasing the size of condensed matter, the surface has a special importance in nanomaterials. For instance, approximately 1 atom every 10 is a surface atom in a 10 nm Au nanoparticle.

Surface atoms have different reactivity than bulk ones, due to incomplete saturation of their atomic bonding capability. In case of laser generated nanoparticles, which are often obtained in the absence of any stabilizer or coating agent with an highly reactive surface, this is even more true. For this reason, surface stoichiometry and oxidation state are typically different than in the nanoparticle core. The consequences for electronic, optical, catalytic or biomedical properties are huge, and these justify the need to obtain a thorough knowledge of nanoparticle surface, with the same dignity of the "body" of nanoparticles.

In addition, the surface of nanoparticles has a crucial importance when it is coated with specific functional molecules, such as in noble metal nanoparticles conjugated with biopolymers, dyes, DNA or antibodies.

The "queen" technique for surface studies is X-ray photoelectron spectroscopy (XPS), which gives complete information on the chemical nature of the sample. However, in case of nanoparticles coated with organic molecules, other techniques can be more informative such as FTIR, nuclear magnetic resonance (NMR) or thermogravimetric analysis (TGA).

5.1. X-ray photoelectron spectroscopy (XPS).

A. What do I see?

It tells you about the composition of the nanoparticle surface and its oxidation. Surface elements with $Z \geq 3$ (Li) are detected, and their oxidation state identified. Although in principle quantitative information on the surface abundance of each element is achievable, in case of nanoparticles these information are averaged between the surface and the inner layers, due to the complex matrix effects and geometry of the sample. Hence it is good for seeing oxidation trends and roughly knowing what composition you have created.

B. How does it work?

Monochromatic X-rays are used to extract electrons from the sample (photoelectrons). The energy difference between the kinetic energy of electrons and the energy of X-rays is related to the binding energy of the electrons in the atoms of provenience, being therefore characteristic of each element. Besides, the energy resolution of XPS is high enough to be sensitive to minimal energy modifications which take place when an atom establishes chemical bonds with other species, thus giving information on the oxidation state and chemical environment of each element.

Since photoelectrons of the nanoparticle surfacevery low mean free path in condensed matter, only the first 1 – 10 nm are probed (depending on the electronic density of the sample and the used angle between sample and detector).

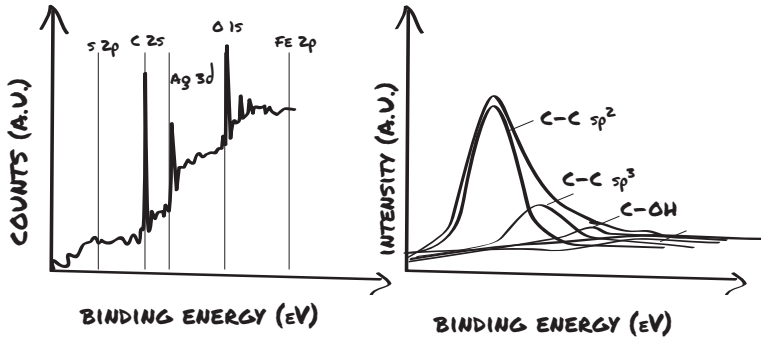


Figure 39: Typical XPS spectrum (left) and magnification of one of the peaks (C peak for example, right), showing that it can be deconvoluted in different components corresponding to different oxidation states and/or chemical environment.

A typical XPS spectrum reports the number of electrons detected (as counts) versus their binding energy (in eV, Figure 39), and each element produces a characteristic set of XPS peaks at characteristic binding energy values.

➞ c. Amount of sample needed?

XPS is only sensitive to the first nm of a sample, therefore a monolayer of nanoparticles spread over an area of ~1 cm^2 is enough.

➞ d. How do I prepare the sample for analysis?

Sample must be dried before use (XPS is performed in ultrahigh vacuum), and can be prepared by drop casting on a clean and flat substrate which has no interfering peaks with those of our sample.

➞ e. Tips / warnings / hazards?

Due to the high surface sensitivity of XPS, contaminants like salts or synthesis byproducts can shield the signals from the nanoparticles. In principle, the topmost layer can be removed by ion sputtering in the XPS chamber, but sometimes this can alter the stoichiometry of the surface. Sputtering also significantly changes the oxidation state of the remaining species making it hard to trace the oxidation state before sputtering.

In case the material of interest being shielded e.g. by a carbon layer – the oxidation state can be accessed if the sputtering time is adjusted so that the covering carbon layer is not fully removed. However some uncertainty about the oxidation state remains when sputtering is used. Note that the holder itself has an effect on the peak shifts, e.g. carbon needs different calibration than silicon.

Peak deconvolution (e.g., to extract the degree of oxidation) is very tricky, please ask a trained person to help you at least when you do it the first time, you will see that it makes a big difference how you deconvolute (deconvolution is "dissectioning" a shouldered peak into two or more Gaussian peaks, Figure 42).

➞ f. To know more?

- Auger- and X-Ray Photoelectron Spectroscopy in Materials Science: A User-Oriented Guide; Hofmann, Siegfried; 2013, Springer.

5.3. Nuclear magnetic resonance (NMR).

A. What do I see?

The typical NMR spectroscopy is performed on protons (^{1}H nuclei), and it is therefore sensitive to their presence and chemical environment. The NMR pattern tells us about which organic molecule is present in a sample, and its relative abundance.

B. How does it work?

III

Some nuclei (such as ^{1}H, ^{13}C or ^{14}N) have a non-zero nuclear spin. In the presence of an external magnetic field, the nuclear spin acts much like a small magnet, and can assume a collinear (lowest energy in ^{1}H) or anticollinear (highest energy in ^{1}H) orientation with respect to the direction of the external field. Electromagnetic radiation can promote the transition from one state to the other. Therefore, in NMR spectroscopy, an external magnetic field is exploited to produce a splitting of spin energy levels in ^{1}H nuclei, and the absorption of electromagnetic radiation by the sample is measured as a function of its wavelength (pretty much like in absorbance spectroscopy). Since the energy gap in ^{1}H nuclei is very small, radiofrequency radiation (10^{2} MHz) is used instead of visible light. The energy gap in ^{1}H nuclei is sensitive to the chemical environment, because electron density exerts a diamagnetic shielding effect on the external magnetic field, which depends on the distance and number of electrons in proximity of the hydrogen nuclei. Besides, the spin of ^{1}H nuclei interacts with each other (when sufficiently close), generating a splitting of the energy levels. For these reasons, NMR spectroscopy gives information on the structure of the molecule, and thus the NMR spectrum is a fingerprint characteristic of each molecule.

C. Amount of sample needed?

NMR typically requires 0.1 – 1 mg of organic matter. However, signal intensity is sensibly damped when short molecules are bound to nanoparticles, and in these cases up to 10 mg of organic matter can be needed.

D. How do I prepare the sample for analysis?

Samples for NMR are in liquid phase. The only requisite is matching the concentration required for appreciable results.

E. Tips/ warnings/hazards?

Since the signal of molecules bound to nanoparticles are greatly damped, sometimes it is simpler and quicker to remove the ligands from nanoparticles surface and analysing only the organic component. With noble metal cores, this is performed by addition of strongly interacting thiols (such as dithiothreitol), which replace the original ligands on a timescale of 1-2 days.

Then nanoparticles can be removed by centrifugation or the surnatant can be collected by dialysis for NMR analysis.

➞ F. To know more?

– The basics of NMR; Hornak, Joseph P.; 2014, free at https://www.cis.rit.edu/htbooks/nmr/

5.4. Thermogravimetric analysis (TGA).

➞ A. What do I see?

In case of organic ligands which undergo complete oxidation into volatile compounds (CO_2, H_2O, NO_x etc.), the mass fraction of the organic component can be easily and precisely quantified. This is possible only when the nanoparticle (inorganic) core is chemically inert at the combustion temperature of the organic component. TGA can also be run with inert gas. So you may find out the amount of ligand that is covering the nanoparticle.

➞ B. How does it work?

TGA basically consists of a microbalance and of a chamber with controlled temperature and atmosphere. While temperature is increased, the microbalance register any weight increase or decrease. In case of organic compounds, combustion in presence of oxygen is detected by weight decrease. The temperature ramp can be continuous or step by step, and weight can be reported as a function of temperature or time.

➞ C. Amount of sample needed?

In micro-TGA, few milligrams of oxygen-reactive matter are enough.

➞ D. How do I prepare the sample for analysis?

Solvent must be removed and the dried sample deposed on the TGA holder.

➞ E. Tips / warnings / hazards?

In the first run, take a slower heating program. To exclude chemical modification of the inorganic core, run TGA also on uncoated nanoparticles as a reference measurement to exclude background contributions, and on a known amount of ligands without nanoparticles. Plot the first derivative of the signal. Do you see a shift of the ligand's temperature? This indicates ligand binding (immobilization), in particular for polymers grafted on nanoparticles. TGA is very powerful if coupled with calorimetry (so-called, TGA-DSC), then you also know if mass loss is endotherm (e.g. degassing or drying) or exotherm (oxidation).

F. To know more?

Thermogravimetric Analysis (TGA) A Beginner's Guide; PerkinElmer, Inc.; free at *http://www.perkinelmer.com/CMSResources/Images/44-74556GDE_TGABeginnersGuide.pdf*

STEP 6: ANALYZE THEIR PROPERTIES

The reason for generating nanoparticles is that they have some properties of interest for scientific or technological applications. Obviously this opens up a range of different possibilities for completing the investigation of our product, and each nanomaterial requires a specific technique or a group of techniques. However, no matter what is the property of interest, it will be always related to the structure of the nanomaterial, and therefore all the previous steps are starting pillars for the functional study or the direct application of our laser-generated nanoparticles.

Conclusions

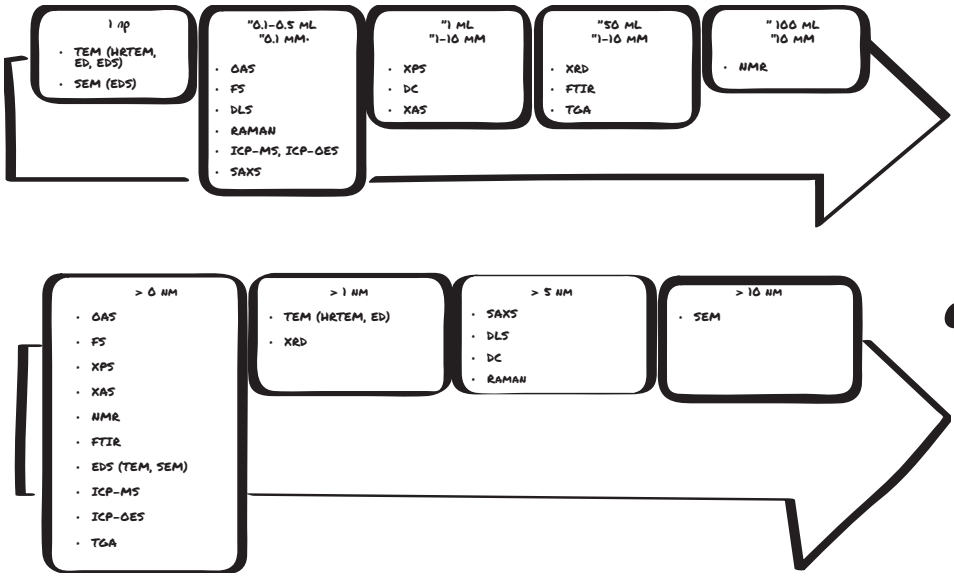
In summary, the range of techniques available for the characterization of our laser-generated colloids is wide, but they should be used "cum grano salis" to save resources and obtain reliable results. As a last step to guide the characterization of our samples, it is useful to conclude the chapter with a sketch where all the techniques previously described are classified on the basis of:

i) the amount of matter required for the investigation, starting from the single nanoparticle techniques up to the most demanding ;

ii) the smallest size detectable.

The power of colloid characterization comes from combining the methods. 2 methods may already be enough to extract nice insights. Examples :

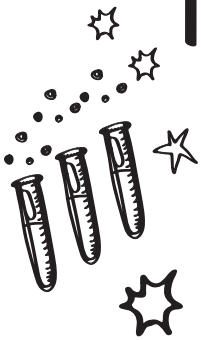
- XRD gives smaller sizes than other methods : you have multi-crystalline particles
- DC gives smaller particles than TEM or XRD : you have successfully conjugated the particle
- DLS gives volume-weigthed diameter of 50 nm, but TEM tells 6 nm. It's aggregated, you may want to add surface charge by anoins or pH, or add a surfactant or polymer for stabilisation
- You have ablated elemental Pt but find 40% of its surface is oxidized in XPS. Nice to know for catalysis. Maybe try Zeta potential measurement (see Cahpter IV) to see this surface charge in liquid as well?



Sketch 1: Described techniques classified by amount needed and smallest detecteble size.

- You ablated an alloy but the XPS or EDX composition gives a different molar ratio compared to the educt. Try ICP to see what has been dissolved in the liquid
- XRD crystal structure does not fit to elemental composition. Try SAXS or HR-TEM to learn about amorphous fraction.
- You know the above table but have no time and your supervisor needs results by tomorrow: start with UV-Vis and DLS/DC. Collect arguments (e.g., present different histogram statistics) to ask for more. Tell that always 2 independent analytical methods are needed to validate results ready for publication with her/his highly reputed name on it.
- You tried everything but are lost. Send an email to the authors of this book.

KEEP IT: STABILITY



IV

After your first days in the laser lab you will probably have produced your first samples of laser-generated colloids and you will be able to proudly present them to your boss with a full set of structural characterization data. At this stage, two questions will inevitably come to mind, if not to yours then definitely to your boss:

How stable are the nanoparticles? And how can stability be influenced during or after laser ablation?

These will be basic questions sticking with you up to the end of your life in the world of laser-generated colloids and nanoscience. Guided by these fundamental questions, this chapter will give you a basic understanding of how to define "stability" in the field of colloidal science, how to measure its value, how to interpret the results and how to alter the corresponding parameter using simple modifications of the laser ablation process.

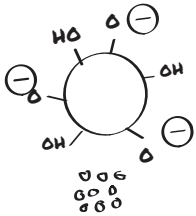
How stable are the nanoparticles ?

You will certainly have to experience the hard way that particle stability is a critical issue during synthesis and handling of laser-generated colloids. After you have spent an entire day in the laser lab generating beautiful colloidal samples, the next day you may be in for quite an unpleasant surprise. You could find them either containing large soft flocks or situated as an ugly, brown or black precipitate on the bottom of your vessel or almost irreversible fused with your container's wall. Another phenomenon you may encounter (and we often did) could be that some of your samples are perfectly stable while others precipitated, even though you are sure you treated them exactly the same. At this point you will certainly, depending on your level of stress and your personality, either think or cry out: "Stability Sucks". In order to avoid or at least minimize such pitfalls this paragraph will give you a step-by-step overview on how to define stability, how to control it during laser ablation in liquids and how to measure it.

STEP 1: DEFINE STABILITY

The definition of this term in colloidal science is not always universal, however a straightforward answer could be that stability actually defines to what extent your sample changes with time. When specifying the word stability you have to differentiate between chemical stability and colloidal stability. Chemical stability defines whether your colloid is subject to chemically-induced changes

caused by the environment, in most cases by the solvent. The most relevant processes are oxidation and dissolution of your solid material in the respective solvent. While chemical stability is a very specialized phenomenon strongly depending on the used material, colloidal stability is universally applicable to all colloidal systems. It is, furthermore, particularly critical in laser-generated colloids which are generally completely ligand free and hence only "meta-stable" without any external stabilizing agents. Consequently, this paragraph will focus on the aspect of colloidal stability. Colloidal stability indicates to what extent your nanoparticles are resistant to return from the dispersed (finely distributed within the solvent) state to the bulk state. From a thermodynamic point of view, all colloids are unstable since a reduction of their surface area is energetically favored. However, this process can be kinetically inhibited, which could lead to colloids stable in solution for centuries (as the gold colloid made by Faraday 150 years ago). So in order to understand the concept of colloidal stability you need to basically understand the nature of attractive and repulsive forces in a colloidal system. This knowledge can be provided by the DLVO theory named after its inventors Boris Derjaguin, Lev Landau, Evert Verwey and Theodoor Overbeek. Based on this theory, the interactions between colloidal particles are driven by 2 main forces 1) the van der Waals attraction and 2) the electrostatic repulsion. Van der Waals forces are caused by the formation of induced dipoles between uncharged but polarizable materials and are much stronger on a short distance. Electrostatic forces on the other hand are caused by the so-called electrical double layer around a charged particle, and are usually more pronounced at longer distances. Based on this, a potential curve can be constructed (Figure 40 A), which gives you the sum of all the potential energy in correlation with the distance between two particles in the colloids. Stability means keeping a large distance. Looking at this graph you can observe that there are two minima of potential energy.



Our laser lab has sheets covering the window. On a Monday I entered the room in the morning and instantly got the feeling that something was different. After some time I realized the sunlight coming in through holes in the sheets covering the windows. I also saw some straight lines in some covering boards leading towards the holes. Actually the last student that had worked with the laser on a Friday was dithery adjusting the laser beam while the laser was running on high power settings thereby unintendedly optimizing the sunlight incidence into the laser lab...

Sven Reichenberger, Duisburg

The first, the so-called primary minimum, is located at lower distance and indicates a state where two nanoparticles are irreversibly fused, this process is called aggregation and the resulting materials are hence named aggregates. In addition, there is a second minimum found at greater distance between the particles. Here the particles are more loosely bound, predominantly in a reversible fashion. This process is called agglomeration and the resulting materials are termed agglomerates. For the stability of the laser-generated colloids you can conclude that there are two main processes, which could interfere with the colloidal stability of the particles, being reversible "loose" agglomeration and irreversible "tight" aggregation. While agglomerates may generally be re-dispersed easily e.g. by intensive shaking or short ultrasonication, aggregates are generally permanent and you may need to adapt your synthesis parameters in order to avoid it. In the potential curve diagram (Figure 40 A), a stable colloid needs particles kept at a distance of Min2 or better even farer away from each other.

IV

To enhance the general understanding of colloidal stability you now need to take a closer look at the main stabilizing force (that is, the repulsive force) in ligand-free colloids, which are surface charge and the consequent formation of the electrical double layer. The first fundamental question relevant in this context should be why laser-generated colloids, e.g. composed of metal, possess a surface charge at all. A pure metal surface in a solvent should not be charged and hence the particles should be subject to immediate aggregation. E.g. elemental gold is hydrophopic, insoluble in water and loves to binds to other particles by van der Waals force, and once the solids are in contact, inter-particle atom diffusion diminishes particle boundaries causing irreversible fusion. However, the laser process generally yields a partial surface oxidation of the colloidal metal nanoparticles, and partial reduction of oxide nanoparticles. Hence, the surface of laser-generated nanoparticles carries defects (which are very valuable for both application and colloidal stability), and these defects attract surface adsorbates. These surface adsorbates are of course counter-charged. Coming back to an elemental metal nanoparticles with electronic defects at its surface (e.g. a few Au^{+} and Au^{3+} sites at the surface of a sphere dominated by Au^{0} atoms): These oxidized surface atoms can then attract hydroxyl from water and form a pH-dependent equilibrium between M-OH and $M\text{-}O^{-}$ (M=metal) groups, which generally yield a partially negative surface charge in laser-generated colloids. Hence, the particle became an acid, ready to exchange protons with water. Yes, it even has a defined value where the charge flips towards positive charge ($M\text{-}OH_2^{+}$) if more protons are taken up at low pH. E.g., for laser-made gold nanoparticles, the flipping point, the so-called isoelectric point is reached at a pH of around 2, which shows that ligand-free gold nanoparticles are very acidic.

In case these charged nanoparticles are dispersed in an electrolyte like water they attract counterions and form an electrical double layer. As the term

double layer suggests it consists of two components (Figure 40 B): i) An inner layer termed Stern or Helmholtz layer where ions are densely bound to the nanoparticle surface (e.g., M-OH-) and ii) a diffuse layer of loosely bound, more or less mobile counter ions (the beneath negative Stern layer attracts cations, which in turn bring anions, and so forth). The most critical criterion for colloidal stability is, whether the electrical double layer extends far enough to keep the nanoparticles at an appropriate distance and prevent aggregation or agglomeration. This can be described by the Debye screening length (κ^{-1}), which indicates the distance at which the electrostatic potential has dropped by 1/e. This means that the E_{pot} at the Debyés distance has dropped by about 67% of its original value.

For oxides, the same rules apply, just the charge sign is turned, with oxygen defects resulting in positive Stern layer, attracting negative counterions, etc. E.g. ZnO made by laser ablation in water is a base with oxygen defects (oxygen vacancies) and (slightly) positive surface charge at neutral pH.

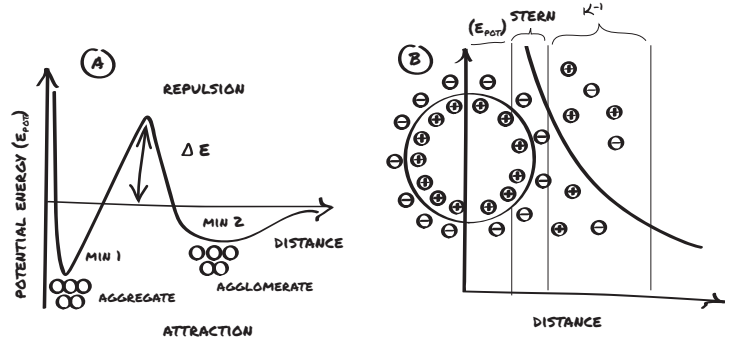


Figure 40: Stay away: DLVO theory and electrical double layer. (A) Potential energy-distance curve in a colloidal system indicating the primary minimum (Min1), secondary minimum (Min2) and activation energy (ΔE). (B) Illustration of the electrical double layer around a spherical particle. Indicated is the potential energy curve, the Stern layer and the diffused layer. κ^{-1} illustrates the extension of the Debye screening length.

Now you know how to define the term “colloidal stability” and you have learnt that electrostatic repulsion and van der Waals attraction are the main forces at work. Based on this you have learnt the physical origin of surface charge as well as the electrical double layer in a completely ligand-free laser-generated colloidal system. As a follow up it would be interesting to know how you can affect colloidal stability.

STEP 2: INCREASE COLLOIDAL STABILITY...

In this context, it is essential to know practically how to affect colloidal stability by external parameters like temperature, particle concentration, pH, ionic strength, impurities and surface ligands.

...BY TEMPERATURE:

The impact of temperature on colloidal stability is straightforward when you look at the potential energy curves derived from DLVO theory. From this graph, you can clearly see that agglomeration and aggregation processes are generally inhibited by a barrier of potential energy (ΔE in Figure 40). The only way for a certain particle to cross this barrier is when it collides with another particle: only when the kinetic energy of the two surmounts this energy barrier, the barrier will be surpassed. And it's our intention in a stable system to avoid that this happens, so that particles keep their distance.

It is well known that the mean kinetic energy which corresponds to the movement of particles (k_BT, with k_B being the Boltzmann constant and T the absolute temperature) increases with temperature. And this movement is the cause of collisions between two particles. This means that at higher temperature the number of particles exceeding the necessary energy barrier for agglomeration and aggregation is elevated. As a result, aggregation and agglomeration are more pronounced at higher temperatures. To clarify this point let's assume you have a collective of 6 particles with 1 particle possessing the necessary kinetic energy to induce agglomeration or aggregation, each of them exposed to one collision event within the observed timeframe (Figure 41 A). Let us further assume that elevated temperature doubles the number of particles with the necessary kinetic energy to 2. Consequently, the probability of agglomeration or aggregation due to a collision with a particle of sufficient energy would increase (Figure 41 B). Based on this, you should consider keeping the temperature of your colloids as low as possible to enhance stability. Concluding this thought, storage in the fridge is recommended, 8 degrees Celsius is fine. However, you should keep in mind that freezing a ligand-free system should be avoided.

STUDENT: THE CONTACT ANGLE MEASUREMENT IS NOT WORKING.

ASSISTANT: THE COVER IS STILL ON THE CAMERA.

SVEN KOENEN, ESSEN

There is another negative effect of high temperature: the liquid's viscosity decreases exponentially with the temperature. That is a pity, because the diffusion constant will in turn increase by the same factor. Knowing that diffusion is also proportional to kinetic energy (T_k, see above), means that the speed of a particle that approaches collision with another particle is proportional to $T \cdot e^T$. Hence, every degree of temperature is your foe in the lab. Note that your finger tips are permanent 36°C heaters, and a small 1 ml vial taken freshly from the 8°C fridge has nothing to defend itself while you carry it to the DLS machine measuring aggregation.

Cooling is the most simple, effective, and contaminant-free way to preserve a colloid's stability. Everybody in a cool lab is chilled, so are the samples.

... BY CONCENTRATION:

While the impact of temperature on colloidal stability is pretty obvious, the effect of particle concentration is less plain. You learnt that a lower mean kinetic energy of the particles is beneficial in order to not exceed the activation barrier of aggregation and agglomeration. In addition to this, not only the energy of the corresponding particles can enhance the probability of aggregation but also the frequency of collisions, which, of course, is elevated at higher particle number concentration. Lets take the example from the previous paragraph: a collective of 6 particles, 1 exceeding the activation barrier, one collision each. Let's now assume that an increase in particle concentration would double the number of collisions (Figure 41 C). Consequently, the probability of agglomeration or aggregation would also increase. Based on this it can be assumed that colloidal stability is generally favored in less concentrated colloids. So to make it simple: dilute your colloid in order to enhance colloidal stability. You will often need to dilute it for analysis, anyway. We have had good experience keeping gold at 100 mg/L stable for months stored in the fridge. But ligand-free 300 mg/L or even 500 mg/L gold stored in at room temperature is a lucky or bad shot, maybe depending on the degree sunshine on the day of synthesis.

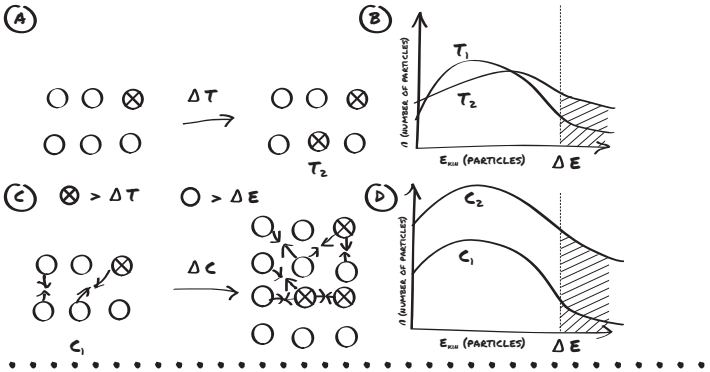


Figure 41: Impact of temperature and concentration on energy distribution of colloidal particles: (A) Cartoon illustrating an increasing number of particles with a kinetic energy (E_{kin}) > activation energy for aggregation (ΔE) and (B) energy distribution of the corresponding particles showing a higher number of particles with E_{kin} > ΔE at higher temperature. (C) Cartoon illustrating an increasing number of particles with increasing particle concentration as well as an elevated collision probability (indicated by a higher number of arrows). (D) Energy distribution of the corresponding particles showing an increasing number of particles with E_{kin} > ΔE at increasing particle concentration, due to the higher number of particles and due to elevated collision probability.

IV

... BY PH VALUE:

pH

Laser-generated metal colloids in aqueous medium are at least partially oxidized and carry a portion of M-OH_2^+/ M-OH/ M-O^- (M=metal) groups on their surface. Again, this is true for most oxides as well, resulting in cationic defects (oxygen vacancies), e.g., positively charged $ZnO_{(1-x)}$. Hence, noble metals are often acidic nanoparticles, and oxides are often alkaline.

It is obvious that alterations in the pH-value could lead to a protonation and deprotonation of these surface groups, which can critically influence surface charge and colloidal stability. Consequently, there is a certain pH-value where all surface groups, in sum, are in an uncharged state. This value where the charge flips sign is termed point of zero charge or isoelectric point (IEP). Naturally, this point highlights the regime of lowest colloidal stability and should be avoided during fabrication and storage of laser-generated colloids. A general rule for achieving maximum stability should be to adjust your medium pH to be as far as possible from the isoelectric point (but still avoiding dissolution), and with the medium being about one pH unit away from it you are often already on the safe side. This concept is illustrated in Figure 42 A, with the pH of the medium increasing from the left to the right, crossing the IEP in the middle picture. Knowledge on the isoelectric point can be either obtained from literature (already Wikipedia has a short list at the entry “isoelectric point”), in case you are working with a well established material, or determined by titration experiments. In the latter case you titrate your colloid by adding acid or base

and the point of zero charge is reached when your particles aggregate and precipitate. This procedure is ideally probed by zeta potential measurements (the concept of zeta potential is described later). But the aggregation point is often so easy to see that IEP can be found simply with the naked eye and a pipette of acid or base.

... BY IONIC STRENGTH:

The impact of ions on the colloidal stability of laser-generated colloids is rather ambiguous. But once you understood it you will never want to miss this mighty tool, in particular for laser synthesis in water. In our lab, the salinity-trick is used on a daily basis and basically entails adding low salt concentrations to increase stability. But let's first understand the basics. The first and foremost thing you need to be aware of in this context is that high ionic strengths critically reduce the Debye length (κ^{-1}) and a low Debye length is actually a stability death sentence. The underlying physical phenomenon is a screening of repulsive surface charges by an overabundance of counterions (Figure 42 B). To put it simply: With a lot of counterions around, the particles can no longer "feel" the repulsion from neighboring particles and hence they aggregate due to attractive forces. This correlation between the Debye screening length and the ionic strength (I) in water at 25 °C is given by the following equation:

$$\kappa[nm^{-1}] \approx 3.3 \cdot (I[M])^{1/2}$$

Based on this you could calculate a Debye screening length (κ^{-1}) of 30 nm in the presence of a 100 µM electrolyte, however this value is reduced to only 0.7 nm in a 200 mM electrolyte, completely annihilating colloidal stability and initiating aggregation under these circumstances. As a rule of thumb you can conclude that high ionic strengths > 2 mM should be avoided when synthesizing and handling laser-fabricated colloids. In addition, you need to keep in mind that the destabilizing effect is intensified in case multivalent ions $M^{Z+/-}$ like Ca^{2+} or SO_4^{2-} are present. So the utilization of these ions should be totally avoided during laser ablation in water. Whereas the concentration screens stability by $c^{0.5}$, the ions valence contribute additionally by $c^{Z\cdot0.5}$ to kill stability.

In contrast to the above mentioned well known fact of oversalting (or "salting-out-effect"), recent studies have concluded that the page is turned at a minute amount of salt! This is an effect that is very specific and can be very beneficial for ligand-free particles, as those are the once we make by laser ablation or laser fragmentation in water.

The *in situ* presence of certain anions at micromolar salinity during the particle synthesis process can be beneficial for colloidal stability. In situ means that the salt has to be dissolved in the liquid before you switch on the laser. In this way, it is most effective. This effect has been reported by the authors of this handbook to work best with highly polarizable anions (e.g. Cl^-, Br^-, I^-) and only on noble

metal nanoparticles with low oxidation tendencies (Au, Pt, Pd).
Using this method, the colloids are far more stable and size distribution is narrowed. That's two flies with one clap. But how does it work? These effects were attributed to a specific anion adsorption to the nanoparticle's surface during the particle formation process. Sodium chloride works well, also carbonate or phosphate works in our lab. Best friends are sodium bromide and sodium hydroxide at 100 – 300 mg/L. So, during your noble metal particle synthesis you may add low salinity electrolytes to enhance colloidal stability. Make your first try with dissolving 200 micromol table salt. Note that this is only 0.1 mg per liter, a bit less than a grain of salt.

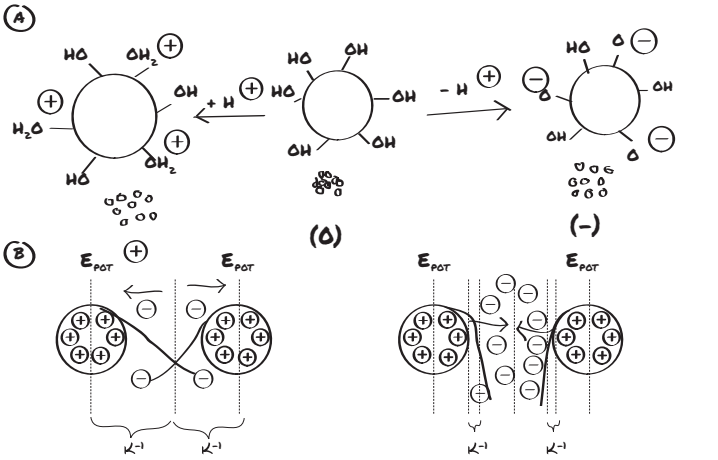
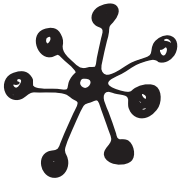


Figure 42: Neutral is worth nothing. Stay in charge. Add a grain of salt. Effect of pH and ionic strength on colloidal stability: (A) Cartoon illustrating the effects of stable protonation at acidic pH (left) and stable deprotonation at alkaline pH (right) of a particle at the colloidal instable isoelectric point. (B) Cartoon illustrating the potential energy (E_{pot}) of two particles in the presence of low counterion concentrations (left) and high counterion concentrations (right) as well as the extension of the Debye length (κ^{-1}) as a measure of repulsive stabilisation. Arrows indicate repulsive forces between the particles (left) and attractive forces (right).

...BY IMPURITIES:

During your laser ablation experiments, sometimes colloids aggregate, while others remain perfectly stable, even though your synthesis conditions seem completely the same. This lack of reproducibility can be highly frustrating and is in most cases because you have worked dirty, so in other words it is attributed to impurities in the sample. These can be simply dust particles from the surrounding atmosphere (often partly soluble in liquid), residual particles remaining in your chamber from previous ablation experiments as well as multivalent ions leaking from your glass vessel or metal chamber. Hence, if you want to precipitate a colloid (of your nasty colleague in order to keep him/her in good mood):

add calcium chloride, or make sure to deposit some salty fingerprints. Also metal containers, stainless steel spatula and chambers deliver multivalent ions even after using them for years. If you need to work with metal in contact with the liquid, use anodized metals (mechanical workshops do that easily, or use so-called Piranha liquid), or passivated metals such as aluminum chambers, or just Teflon.



Other impurities may come from the target material, in case it is not of highest purity. A noble metal even at 99.9% purity naturally contains silver and, even worse, copper, easily dissolving from the target or the particles after ablation and destabilizing the colloid via charge screening. So in conclusion, etch before use, and buy highest grade purity.

I once asked a student to perform laser ablation experiments in saline solutions, while I gave her a concentration regime of 5 – 500 millimolar. She discovered some interesting effects on size quenching, however, we later discovered that she made a mistake in her dilution series and she actually examined 5– 500 micromlar concentrations. This is how we discovered that salinity quenching occurs even at very low ionic strengths.

Christoph Rehbock, Essen

In addition, the liquid itself is a well known source of impurities. Organic solvents always contain unknown impurities, whose concentrations can be way higher than your colloid mass concentration. Let's assume a typical colloid mass concentration of 50 mg/L and technical grade acetone with 99.9 % purity. In this case the amount of undefined dirt (0.1 %) would still sum up to 800 mg. So in total you have a 16 times higher mass of dirt in your sample compared to the mass of your particles. As a consequence, the only way to avoid interference from impurities is to work in an environment as clean as possible. But still very high commercial solvent purities still will have enough contaminants inside to cover the surface of all particles you've fabricated. So you can't be lazy, you need to clean your solvents on your own by distillation and checking purity with gas chromatography is the only way out if 100% purity is what you want. But very often, in our lab, simply the solvents used for cleaning have been detected as source of contamination. Once the solvent bottle is unsealed, the solvent takes up softeners and additives from the seal, the tubings, your protective wear, etc. And this is retarded in the equipment and found as traces even during the aqueous synthesis post cleaning. Practical advice you should adhere to is:

I) Thoroughly clean all your glassware (or use disposables), your chamber as well as your target. Use the solvent you work with later for cleaning as well.

II) Get target materials at maximum purity

III) Purify your solvents e.g. by distillation

IV) Preferably do not use the same chamber for different materials, never use non-anodized metal chambers or spatula

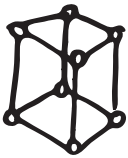
V) Use a chamber composed of a material that can be easily cleaned by the strongest possible oxidants like “aqua regia”. We like Teflon.

VI) In case of aqueous solutions: use plastic containers instead of glass vessels for long time storage, however in case of organic solvents, interactions between solvent and container may render glass more suitable.

VII) For the tubings, silicon is nicely flexible but the worst you can do because it retards everything in its pores. Better go for Telfon or something similar that is resistant to solvents and has a smooth surface.

... BY LIGANDS:

The most important characteristic of laser-generated colloids is their purity due to a completely ligand-free synthesis process. However, some applications e.g. utilization in a salty biological environment may necessitate the presence of stabilizing or biofunctional surface ligands. Once you have effectively grafted stabilizers to your surface, in particular bulky ones, your colloid will survive everything, even freezing and repeated freeze-drying. It’s wonderful to see a freeze-dried, golden pellet to eject a perfectly rubin-coloured liquid once you put a drop of water on it.



These stabilizers can either just be serum proteins, present in a biological environment anyway, chemical polymer ligands, as well as functional ligands used in bioactive nanobioconjugates. Basically, conjugation processes can either be conducted in situ or ex situ. During in situ conjugation, the ligands are present during the laser ablation or laser fragmentation process. The main advantages in this case are generally a more narrow size distribution as well as a very high surface coverage due to an instantaneous reaction (surface adsorption) between the just formed nanoparticle surface and the ligand. The downside of this procedure, however, is also pretty obvious. Firstly, organic ligands are present during the laser ablation process. This may go along with a significant degradation of conjugates by post-irradiation events by the laser beam, a problem which necessitates careful adjustment of the applied laser fluence during ablation (Chapter II). Another impairment originates from the fact that the nanoparticle-to-ligand-ratio cannot be properly controlled in situ.

While the ligand concentration in the ablation medium is fixed, the particle concentration increases during the laser synthesis process. As a consequence, the ligand-to-nanoparticle-ratio decreases with time and uniform conjugation is unlikely. Ex situ conjugation, on the other side, entails a two step process where the ligands are added to the previously generated colloids. This process allows for a precise adjustment of the ligand-to-nanoparticle-ratio and a well controlled conjugation process. This approach, however, requires alternate strategies for size control, e.g. salinity-induced size quenching (see above). As a rule of thumb, single-digit micromolar ligand concentrations already work (if ligand has high affinity).

The presence of surface ligands has a significant impact on the stability of colloids as surface charge density in this case will be dominated by the properties of the ligand as well as by those of the metal core. Based on the nature of the ligand, stabilizing effects can either be electrostatic, steric, or electrosteric (illustrated in Figure 43 A), and the steric is always the most robust.

Working with substances in the micromolar concentration range often includes the necessity of using volumes below 10 µL which is in fact not more than a droplet. In this case, never trust a physicist doing bioconjugation and telling you to dip the tip with your biomolecule into your colloid. You will never get nearly reproducible results, in particluar concerning the surface coverage.

Lisa Gamrad, Essen

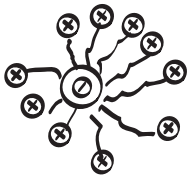
Electrostatic stabilization usually occurs when small highly charged ligands (e.g. citrate or short peptides) are used. When these ligands covalently or electrostatically attach to the particle surface, their net charge is added to the particle's surface charge. In case the charge of the particle and the charge of the ligand carry the same sign, e.g. negatively-charged citrate on negatively charged gold nanoparticles, this may lead to a significant stabilization of the colloid. However, in case the charge of the particle and the charge of the ligand carry opposite signs, an effect commonly referred to as charge balancing occurs (illustrated in Figure 43 B). As a consequence, colloidal stability in oppositely charged systems is generally reduced. On the other hand, steric stabilization is basically found when nanoparticles are coupled to bulky uncharged ligands e.g. hydrophobic polymers (bound by van der Waals force). The main stabilizing force at work here is Born repulsion, that is, the ligands prevent the particles

from reaching the necessary distance required for aggregation and hence keep the particles dispersed in the solvent. Amphiphilic polymers are the best for metal nanoparticles, since they may bind to the particle's hydrophobic surface, and at the same time provide hydrophilic groups keeping the particle dispersed in water. Such a polymer widely used in laser synthesis in literature is polyvinylpyrrolidine (PVP). If you want to go Bio, a typical serum protein like albumin is another amphiphilic macromolecule that provides steric stabilization, and is present in most biofluids and cell culture media, anyway.

A hybrid form of stabilization, termed electrosterical stabilization, is found in case particles are conjugated with bulky, charged surface ligands e.g. proteins or oligonucleotides or classical surfactants. How big shall the ligand be? As a rule of thumb, 12 carbon bond lengths are required to add enough steric character to the electrostatic stabilization. Maybe you heard about sodium dodecylsulfate? Same works in non-polar solvents, e.g. dodecane-thiol is a gold standard of capping agent in hexane. But if the ligand gets too bulky, its diffusion coefficient is lowered, decreasing the effectiveness of reaching the particle's surface during its growth (in situ) or before aggregation starts (ex situ).

IV

The final thing you want to optimize is the tightness of binding to the nanoparticle surface. The stronger it binds the more is the equilibrium shifted towards the nanoparticle, and you will need less excess of ligand for grafting a monolayer. In ideal cases, such as thiolated peptides pr thilated oligonucleotides, almost 100% conjugation efficiency is achieved. Decorating the surface of laser-generated nanoparticles with surface ligands usually requires a strong interaction between the ligand and a metal core, which is usually achieved by electron donor moieties like amines or thiols, which strongly interact with the metal surface atoms. It is an old rule of conjugation chemistry: the soft Lewis acid (noble metal nanoparticle) has highest affinity to soft Lewis base (disulfide > thiols > thioethers > carboxy, amines). For noble metals, thiols work very efficiently, so that this is the gold standard for fabrication gold nanoparticle bioconjugates. For the peptides it is most simple, just add a terminal cysteine amino acid. Same works with polymers, gold conjugated to mercapto-polyethyleneglycol (m-PEG) has survived the highest salinities among several stabilizing agents we have tested. And for metals that oxidize or oxides? Carboxy groups have been reported to work well to be anchored on oxides, and you will need a carbon chain pointing outwards to contribute to steric stabilisation. Without going into detail, you will need to have the right pH adapted to both the molecule (deprotonation) and particle (IEP). Maybe the molecule MEEAA (check the internet for that) or other complexing agents are a good start.



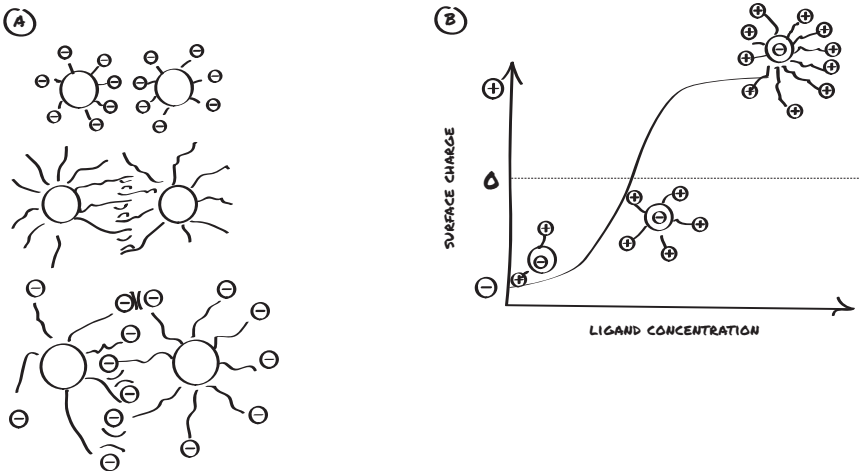


Figure 43: Decorate. Stability delivered by ligands: (A) Cartoon illustrating electrostatic (top), steric (middle), and electrosteric (bottom) stabilization of nanoparticles. The electrosteric stabilization by surfactants is often a double-layer with the charges pointing both inwards and outwards. (B) Scheme illustrating charge compensation while adding positively charged ligands to a negatively charged colloid.

In the previous part of this chapter you learned how to define the term colloidal stability and how it can be correlated with surface charge as well as properties of the electrical double layer. In addition, you learned how colloidal stability changes when temperature, pH, ionic strength are altered and when impurities as well as different surface ligands are present. The final step to a unified understanding of colloidal stability is how to measure it.

STEP 3: MEASURE COLLOIDAL STABILITY…

In order to measure colloidal stability you need to monitor to what extent the properties of your colloid change with time. In this context you need to keep in mind that visible aggregation, like discoloration of your colloid and formation of precipitates are only the tip of the iceberg, more subtle effects are far more frequent. Popular and very powerful tool to monitor colloidal stability are UV-Vis spectroscopy and zeta potential measurements, which are explained in the following subsections.

... BY UV-VIS ABSORPTION SPECTROSCOPY

UV-Vis spectroscopy is a fast and easy to use standard method available in most laboratories. The basic principle entails shining light through your sample while the attenuation (extinction) of your light intensity is observed in comparison to a standard, e.g. the pure solvent. When this is done for all wavelengths you acquire a spectrum were extinction is plotted against wavelength. More information on how UV-Vis spectroscopy works and what else it can be used for is included in Chapter III where all relevant characterization methods are listed. Almost all colloidal nanoparticles show a distinctive extinction spectrum, the most obvious example are plasmon resonant metal nanoparticles like Au, Ag or Cu. While the details of spectra interpretation are explained in Chapter III this paragraph focuses on how this technique can be used to evaluate colloidal stability. The simplest way to utilize this method is to measure whether or to what extent your spectrum changes with time. In this case the simplest rule of thumb is: I) The spectrum does not change with time = stable colloid II) The spectrum changes = unstable colloid. This approach is admittedly rather crude but gives you general information about your colloid and it is generally applicable for all colloids that possess any kind of extinction in the UV-Vis. A proper follow-up could be to use either the extinction at a certain wavelength or even better the sum (integral) of all extinction values in a certain wavelength regime to quantify your stability e.g. the loss with time (Figure 44 A). The decrease of extinction with time is the most straightforward phenomenon, as precipitation is meant to occur in case colloidal stability deteriorates. Maybe a good definition is that the integral of extinction does not change by more than 5% in 4 weeks.

Unfortunately, it is not always that easy. Sometimes you may encounter that your extinction even increases with time, would this mean that particles get more stable? Most certainly not! This observation would be a clear indication that there is more going on in your sample (it is still “aging”) like chemical changes e.g. the formation of reaction products with different optical parameters or change of particle concentration due to particle growth from precursors with time. In this case the aforementioned simple stability criterion fails and more detailed investigations e.g. of particle size need to be added (compare Chapter III). To give one example, after laser fragmentation (post-irradiation) of a platinum colloid is terminated, the extinction still drastically increases as there are still atom clusters that undergo coalescence and ripening to particles, increasing the colloid’s extinction. This is happening in real time, you can follow it in the UV-Vis directly after laser fragmentation. So better wait until it is calmed down, in order to probe and report reproducible properties.

However, UV-Vis spectroscopy can do more than just measure total change in extinction. In many materials it can be used to monitor the aggregation or agglomeration process as larger particles generally scatter light at a longer wavelength. As a consequence, an increase in infrared extinction can be an

indicator to monitor particle aggregation. A commonly used parameter is the ratio of primary particles to aggregates; the Primary Particle Index (PPI). For gold nanoparticles, this value is already well established and is defined by dividing the extinction at the gold interband extinction (@λ=380 nm) by the extinction of the aggregates or agglomerates in the NIR regime (@λ=800 nm). A high PPI value goes along with a low number of agglomerates and consequently with a good colloidal stability (Figure 44 B). A similar PPI value can also be used for semiconductor nanoparticles e.g. ZnO where the extinction at the bandgap energy in the UV can be divided by the NIR aggregate/agglomerate scattering. So concerning the PPI you can memorize that it is a suitable way to quantitatively evaluate aggregation and colloidal stability. As a rule of thumb you should keep in mind that for ligand-free gold colloids a PPI < 4 should be avoided in order to prevent precipitation of your colloid. A PPI of > 10 sounds stable.

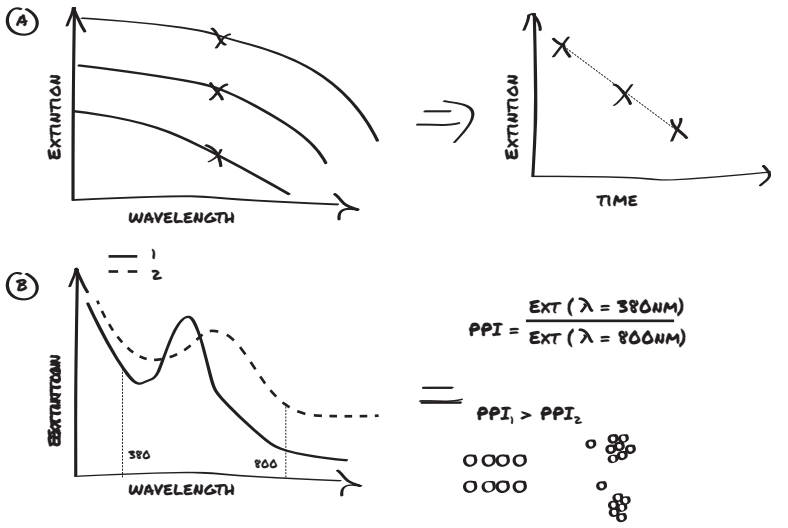


Figure 44: Stay with the color. UV-Vis spectroscopy to evaluate particle stability. (A) Illustration of how to use time-resolved changes in the UV-Vis extinction to evaluate the loss of colloidal stability. (B) Illustration of the primary particle index (PPI) in gold colloids evaluating colloid 1, with a high PPI, to be more stable than colloid 2 with a lower PPI.

... by Zeta Potential:

Many works use zeta potential measurements to evaluate colloidal stability, but information obtained from this parameter can tell you much more. Therefore, the first question which needs to be address is: “What is the zeta potential?”. The zeta potential (ζ) or electrokinetic potential is closely linked to the above mentioned surface charge and it is a powerful technique for the characterization

of the electrical double layer. In case charged colloidal particles are exposed to an external electric field these particles will move within the field; e.g. in case they have a negative surface charge, they will drift to the positively charged electrode. The tightly bound ions in the Stern layer will follow the movement of the particle, those situated in the diffuse layer cannot and will slip off when a certain potential is reached (because they cannot cope with the speed of the particle core and are peeled off by the drag force). This potential at the slipping plane is called the zeta potential and can be considered an indicator of the surface potential caused by surface charge. Naturally, it requires more energy (a higher potential) to remove ions from a highly charged particle in comparison to a particle with lower charge. However, you need to be really careful when interpreting zeta potential measurements. On the particle you will basically have 3 values of relevance: 1) The surface potential caused by charged surface atoms, 2) The Stern potential caused by tightly bound counter ions and 3) the zeta potential at the slipping plane (Figure 45 A). The zeta potential is the only one of these potentials which is experimentally accessible in liquid but during all your interpretations you always need to keep in mind that it is not necessarily equivalent to surface potential/ surface charge. The surface charge/oxidation state of the inorganic core can be measured by XPS in the dry state.

A WEAKNESS WHEN WORKING WITH PLAL IS THE GLASS OF THE CHAMBER. ONCE WHEN WORKING WITH ETHANOL IN A FLOW CHAMBER THE GLASS CRACKED AND ETHANOL WAS PUMPED OUT OF THE CHAMBER. THE ORGANIC SOLVENT WAS PUMPED WITH 250 ML/MIN RIGHT INTO THE LASER BEAM. AND SO THE INEVITABLE HAPPENED: THE ORGANIC SOLVENT WAS IGNITED BEFORE THE LASER AND THE PUMP COULD BE STOPPED. AFTER THE IGNITION SOURCE WAS ELIMINATED THE FLAMES WERE EASILY EXTINGUISHED, BUT ONLY DUE TO A TIMELY RESPONSE.

ELISABETH MAURER, ESSEN

The next question which needs to be addressed is: "How can you measure the zeta potential?". The zeta potential cannot be measured directly but it is usually calculated from the drift velocity of the charged particles in an AC electric field. Measurement of the particle's drift velocity (v) is usually conducted via light scattering techniques. The drift velocity itself, however, is not a suitable value as it is linearly dependent on the external electric field (E). In order to obtain a measured value which is independent of the external field, the electrophoretic mobility (μ) is usually used, which can be obtained via:

$$v = \mu \cdot E$$

The electrophoretic mobility (μ) is linearly correlated with the zeta potential and can be calculated by approximations made by Smoluchowski and Hückel. The most important rules of thumb you have to be aware of are:

I) Smoluchowski: Valid for high ionic strengths and larger (i.e. > 300 nm @ 1 mM) particles

II) Hückel: Valid for low ionic strengths and smaller (i.e.< 20 nm @ 1 mM) particles

III) Transformation: $\zeta_{Hückel} = 1.5 \cdot \zeta_{Smoluchowski}$

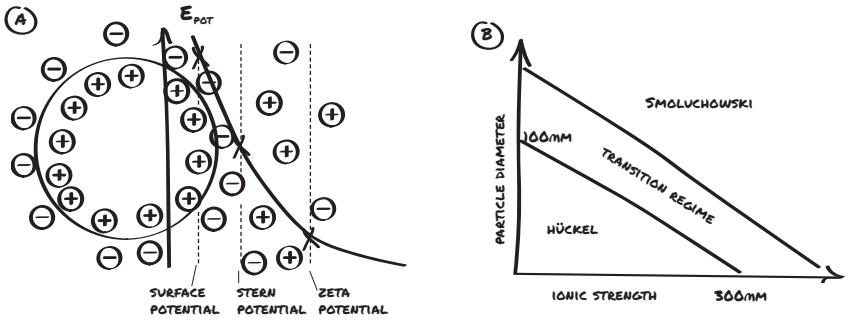


Figure 45: Measure the charge. (A) Illustration of surface potential, Stern potential and zeta potential in the electrical double layer of a ligand-free nanoparticle. Dashed lines represent (from left to right): the surface charge, the Stern layer and the slipping plane. Ions located outside the slipping plane are stripped off during electrophoretic mobility measurements. (B) Representation of the validity of the Hückel and the Smoluchowski regime for different ionic strengths and particle diameter.

Based on what you have learnt so far, you basically know what the zeta potential is and how to measure it. As a logical follow-up it has to be addressed what practical uses the zeta potential has for evaluating the properties of your colloid, or in other words: “What can you learn from the zeta potential ?”. The most straightforward answer is that the zeta potential can give you a general idea on the surface charge of your colloid. The sign of your zeta potential is a good representation on whether you find a positive or a negative surface charges. In addition, the value of the zeta potential gives you a rough indication on the electrostatic stability of your colloidal system, as a high value usually goes along with a high surface charge and hence a high stability.

This correlation may, in some cases, even be used to calculate the surface charge density. A general rule of thumb often found in literature is that colloidal stability can be found in case $\zeta > \pm$ 30 mV. However, you need to keep in mind that this value is not universal. It was calculated based on the potential energy required to surmount a kinetic energy k_BT found at room temperature (25°C). So obviously, there are some important points you need to consider when correlating the zeta potential with surface charge and colloidal stability:

I) Effects of temperature and concentration: As previously mentioned, high temperatures and high particle concentrations can impair colloidal stability. This is usually not represented by appropriate changes in zeta potential. Consequently you need to figure out individual stability criteria in case these values dramatically deviate from standard conditions. And only measure the zeta potential at the temperature that is relevant for the stability information you want. Most instruments have thermostats, but you need to wait at least 10 minutes for your sample to equilibrate to the temperature set in the instrument's software.

II) Effects of particle size distributions: The effect of particle size is generally considered while evaluating whether the Smoluchowski or the Hückel regime are used. Some difficulties may arise in case you try to determine zeta potentials of particles with a very broad size distributions (see chapter on Light scattering DLS). These problems, however, do not originate from the modelling but are rooted in drawbacks of the corresponding measurement of the electrophoretic mobility via light scattering. As the scattering signal from bigger particles is significantly larger than that of small particles, the signals from small particles may not be properly detected. As a consequence you only determine the zeta potential of your large particles while the smaller ones remain invisible (at least significantly underestimated) to your method.

III) Effects from specific ion adsorption: When laser ablation is carried out in the presence of micromolar salinity electrolytes, the particles are prone to specific anion adsorption. This can yield a significant electrostatic stabilizing effect, which does not necessarily show in the zeta potential as the adsorption may occur in the Stern layer behind the slipping plane. Hence these effects would not be detectable by zeta potential measurements (but by the PPI in the UV-VIS, and in the XPS).

IV) Effects from ligands: In the presence of ligands, particularly when they are bulky, zeta potential can no longer be considered a reliable indicator of surface charge and stability. This drawback basically originates from the measuring principle. During measurements of the electrophoretic mobility you determine the movement of charged particles in an electric field.

If these particles are now covered with surface ligands, these ligands will induce a drag force which slows down the particle. Consequently, the correlation between electrophoretic mobility and zeta potential based on simple models like Smoluchowski and Hückel are no longer valid and the displayed zeta potentials are too low. This reduction of the zeta potential was frequently observed in case nanoparticles were surrounded by a protein corona when exposed to a biological medium and has nothing to do with changes in surface charge. In addition, the correlation between colloidal stability and zeta potential also fails in ligand-capped systems as the underlying theory is based on electrostatic effects and does not consider steric stabilization effects. A polymer-stabilized particle often has a zeta potential around zero, although it is very stable.

In this chapter you have obtained basic insight into colloidal stability, you know how to define it, how to measure it and how to affect it via different physical and chemical parameters.

During fragmentation experiments of an oxide in ethanol smoke emerged from the flow chamber used. First it was thought that the organic solvent was a problem. After a while it was realized that the chamber itself was the reason. The chamber was custom-made by 3D-printing of nylon. Due to scattered radiation the chamber was heated up, melted and charred. Therefore it is important to choose the right chamber and to monitor

Elisabeth Maurer, Essen

STAY ALIVE: LASER SAFETY

After reading the former chapters, you must be very confident and excited to do some experiments on your own, maybe now you are planning which materials and liquids to use. Also, you must be astonished by the convenience and simplicity of this technique, just one-step for the nanoproducts. And laser synthesis of colloids is an emerging field, growing since decades. How amazing is that!

For experimental operation, in fact, you do not need a master's or doctor's degree. But is it indeed this simple? It depends on the precautions you take and the hazards you prevent and how smooth your experiments will go. You can compare laser ablation of a target to a "nanomine" you dig, where the laser is your mining tool and nanoparticles are the minerals you extract (Figure 46). In the newspapers, television news, broadcasts, etc. lots of reports cover mining accidents, some disastrous ones even causing peoples' death. The average lifetime of the miners who worked tens of years' ago was less than 40 years. From this fact, you should get a glance at the danger hiding behind traditional mining. Since the technique of laser ablation in liquid is an interdisciplinary field of chemistry, physics, material science, optics and engineering, the dangers during mining of nanomaterials by laser digging in liquids are summarized from different subject perspectives (e.g., chemistry, material and optics), in order to show you the appropriate precautions to prevent the dangers of being poisoned or injured. In detail, the contents of this chapter follows the sequence of Laser risk → Precaution → Experimental operation → Sample Labeling → Nanowaste disposal → Nanocolloids Storage. Note that the main purpose for this chapter is to provide some introductive information about experimental workplace safety specific to laser synthesis and processing of colloids. It is not intended to replace the mandatory chemical and laser safety training from institution. Perhaps you read this chapter to prepare yourself with questions for the safety training day?

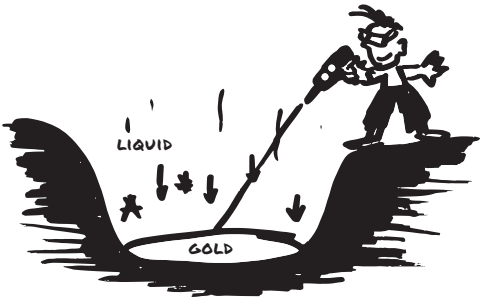


Figure 46: Nanomining in liquid using laser gun

STEP 1: LEARN THE RISKS

Maybe, till now you have never had a chance to use a laser but most of you are familiar with the scenes of lasers. They have become an important element in science fiction movies, laser guns in Terminator 2 and Star Trek or lightsabers in the Star Wars universe. Although your interaction with lasers won't be as lethal as in those movies, the consequences can still be so severe. In the following part, four sections related to laser risks are introduced, including laser classification (section 1), laser damage on human eyes (section 2) and skin (section 3) and some typical accidents that have already happened before.

1.1 Laser Classification

Lasers are generally classified into four groups (Class 1-4) according to U.S. Food and Drug Administration (FDA) laser regulations and international standard IEC 60825, as shown in Table 2. The laser beam of a Class 1 laser is safe under normal operation. Even direct exposure to eyes will not induce any damage. But the laser power threshold is very low and varies with laser wavelength. For example, the often mentioned Nd:YAG laser system at a fundamental wavelength of 1064 nm has a laser security threshold that is only 0.6 mW, at the second harmonic wavelength (532 nm), this threshold even drops down to 0.39 μW, and at fourth harmonic (266 nm), the threshold will be further lowered to 0.8 nW. You should be aware that a potential hazard still exists when the laser beam is enhanced or refocused by a telescope or microscope with large aperture. When a laser is in the range of Class 2-4, a label should be put on the door of your laser lab at the eye level to warn the potential risk of "laser exposure" as shown in Figure 47. Class 2 lasers are also considered to be safe thanks to the self-defense mechanism of human eyes, the so called "blink or aversion response" which limits the continuous exposure to less than 0.25 s when a bright light enters your eyes. Jet pilots have that kind of fast reflex. If you intend to suppress your eye blink under this condition, eye injury will still occur.

The student switched on the high power laser system to perform the proper adjustment of the laser. During the adjustment he noticed a burning smell and switched off the laser again. After examining the setup and lab, he realized some black spots in the windows curtain. An inadvertently movement of one of his mirror with his elbow was the trigger for this incident.

Sebastian Kohsakowski, Duisburg

Most laser pointers at 1 mW fall under this class. But note that these are only eyesafe for healthy, awake people. After 6 hours working in the lab or listening to 8 hours of chemistry classes, your reflex will be far slower and even these "supermarket lasers" are not eye-safe at all for you and your colleagues, anymore.

When the laser scales up to Class 3, especially 3B with medium laser power from 5-499 mW, eye hazard appears whereas skin hazard is not so apparent. The degree of eye damage depends on the laser power. The higher the power is, the less time needed to cause irreversible retinal damage. Therefore, eye protection by eyewear is indispensable and a mark of eye risk should be posted close to the label of "laser exposure" warning. The most dangerous lasers are Class 4 lasers because they can cause severe skin burn or permanent eye damage. Also, in this case, the laser power is high enough to ignite flammable/combustible materials/gases, such as paper, ethanol and hydrogen, as a result it can trigger an explosion when the flammable gases are in high density. If you want to make a grill outside the lab and you are short of lighter for the fire, lasers at class 4 are good candidates as fire-starters. Note that practically all lasers used for laser synthesis and processing of colloids – your lasers – are in that high class. It's the same class as lasers used to weld a car roof. You think the power of your laser is lower? No, it's only the average power that's lower, pulsed lasers often create pulse powers and intensities that are even higher than those used in steel industry. And a single nanosecond pulse may be enough to cause severe, irreversible damage to the eye. Note that by using special laser safety encapsulation you can also reduce the class of your laser system from class 4 to class 1. This has to be done by certified personnel and you know this by getting a certificate and a plate installed at the laser. But note that its only safe when the enclosing is shut, once you open the door to install a chamber or sample, it's a class 4 laser again, and in this situation it is enough that electrical power is on, no matter if the beam shutter is closed since these sometimes fail. Hence, before you remove the casing on a class 1 laser like this, everyone in the lab has to wear laser glasses and lab entrance has to signal laser operation.

Assistant I: You can't walk around here with your coat.

Assistant II: Sure – as if this coat ever saw chemicals

Alex Heinemann, Essen

To help researchers circumvent the potential risks from the class 4 laser lab, a brief summary titled as "workplace safety standards" about the parameters of the laser systems (e.g., maximal power, laser wavelength, pulse duration, repetition rate, etc.) as well as the correct operation procedures should be also posted near the laser warning label, as shown in Figure 47 For the sake of the researchers' security, a lamp to signify the laser current status (on or off) should be also assembled in a prominent place outside the laser lab. A lamp for the laser status is indispensable in order to rule out the possibility that people get injured without notification.

Figure 47: Warning label for laser in Class 2 and higher.

Table 2: We're in a high class. In laser synthesis, you will have class 4 laser setups according to official Laser Classification Table.

Class	US:FDA/CDRH	IEC60825 (Amendment 2)	Example
Class 1	· No known hazards during to eye or skin during normal operation · Note: Service Operation may require access to · hazardous embedded lasers		· laser printers · CD players · DVD devices · geological survey · equipment · laboratory
Class 1M	N/A	· No known hazards to eye or skin, unless collecting optics are used	

<table>
<tr><td>Class 2a</td><td>· Visible lasers not intended for viewing
· No known hazards to maximum exposure time of 1000 seconds</td><td>N/A</td><td rowspan="3">· visible continuous wave Helium-Neon lasers
· laser pointers
· laser scanners</td></tr>
<tr><td>Class 2</td><td colspan="2">· Visible lasers
· No known hazards with 0.25 seconds (aversion response)</td></tr>
<tr><td>Class 2M</td><td>N/A</td><td>· No known hazard with 0.25 seconds (aversion response) unless</td></tr>
<tr><td>Class 3a</td><td>· Similar to Class 2 with the exception that collecting optics cannot be used to directly view the beam
· Visible only</td><td>N/A</td><td rowspan="2">· HeNe lasers above 1 milliwatt but not exceeding 5 milliwatts radiant power</td></tr>
<tr><td>Class 3R</td><td>N/A</td><td>· Replaces Class 3a (with different limits)
· 5x Class 2 limit for visible
· 5x Class 1 limit for some invisible</td></tr>
<tr><td>Class 3B</td><td colspan="2">· Medium-powered (visible or invisible)
· Intrabeam and specular eye hazard
· Generally not a diffuse or scatter hazard</td><td>· Spectrometry
· stereolithography
· entertainment light shows</td></tr>
<tr><td>Class 4</td><td colspan="2">· High powered lasers (visible or invisible)
· Acute eye and skin hazard intrabeam, specular and scatter conditions
· Non-beam hazard (fire, toxic fumes, etc.)</td><td>· (all!) LAL lasers such as Nd:YAG or Ti:Sapphire</td></tr>
</table>

1.2 Eye Damage

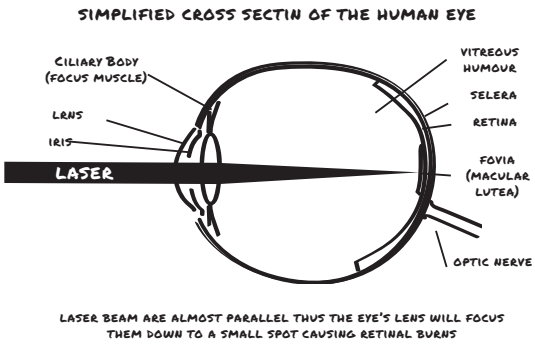


Figure 48: Laser focus when it enters the human eyes

The interior of an eye is shown in Figure 48. It mainly contains the cornea on the transparent front part being responsible for protecting eyes, the iris serving as light modulator for controlling the light volume inside the eyes, and the lens which focus the light on the retina. The retina consisting of many photoreceptors detect the light information and convert it to electrical signals which are then carried to brain through optic nerve. Your friends think one watt is nothing, less than a light bulb? To better understand the laser's effect, you can do a small experiment by yourself using sun light as a reference. When you directly view the sun (please do it only shortly when you are in Australia), the power that arrives at the retina is about 10 W/cm^2, in which case you can not bear long-period of exposure and may become dizzy afterwards. In comparison, 1 watt laser beam can be focused to already 100,000 W/cm^2 (!) at the retina, four orders of higher than sun exposure, therefore damage may immediately occur to the eye structures and cause permanent loss of vision. The pathological effects (e.g. cataract, corneal burn and photokeratitis) towards eyes (e.g., retina, eye lens and corneal) are laser wavelength dependent due to resulting different photon energies, as summarized in Table 3. The lasers with wavelengths from 400 – 1400 nm are much more dangerous than other wavelengths so that this zone is specially defined as a "Retinal hazard region". When the retina is damaged, the vision will be dramatically reduced. Such vision loss is normally permanent with little chance to be repaired, while laser with wavelength of 315 ~ 400 nm cause injury toward eye lens and trigger the photochemical cataract. Near infrared (near-IR) and infrared (IR) laser (i.e. those with the wavelength in the region of 0.78 μm – 1.4 μm and 1.4 μm – 1 mm, respectively); both lead to corneal burn.

After her accident, Anne H. said she would not have expected that she did not notice immediately that a part of her retina has got irreversibly damaged. A part of her vision angle was lost, and she only noticed it later the day when she drove a car and got headache. The doctor did the right diagnosis, but couldn't do anything anymore. She can still see with both eyes and drive car again (after some months her brain needed to compensate the vision angle on her upper right vision hemisphere that was lost), but always has to keep in mind having a "dead angle".

She was trying to remember how that could have happened. She was wearing a clock on her arm while adjusting an invisible 1 watt femtosesond laser with a beam viewer card, and she thinks the glass or housing of the arm clock might have reflected the beam towards her eye.

V

If this just happens to the corneal epidermis, the vision can recover in two or three days, but if it occurs to deeper part of the corneal and causes corneal scar, you will become incurably blind. Therefore, put your security first, you and all other persons in the lab, including your professor, have to wear laser goggles to lower the risk for your eyes to be exposed by laser beam.

Are you safe with the certified laser goggle? Yes, if you use it as protection and within its specification. This means that it has to be suitable for your laser source, if you have more than one laser source the goggles might not be certified for both lasers. They are made to stand some seconds of direct laser radiation, but are not build to allow directly looking into the beam for more than a minute. Unfortunately, this laser break though happens suddenly, due to so-called incubation effects.

In our lab, we only have fully closed safety goggles. Unfortunately, you will not look like Keanu Reeves or Lara Croft with that decoration, but is has several advantages: None will borrow or steel it for a party. You can share with friends wearing eye glasses. And dangerous side reflections (from chamber holders, misalignment, back reflection, walls, etc.) are captured.

Table 3: Pathological effect towards eyes and skin of laser exposure at different wavelength (as summarized by some institutes and universities for safety guidance *http://www.oseh.umich.edu/pdf/LaserEyeSkinHazards.pdf*).

Wavelength range	Pathological effect towards	Pathological effects towards skin
180–315 nm (UV-B, UV-C)	photokeratitis (inflammation of the cornea, equivalent to sunburn)	Erythema (sunburn), Skin cancer, Hyperpigmentation, accelerated skin aging
315–400 nm (UV-A)	photochemical cataract (clouding of the eye lens)	Hyperpigmentation, Erythema
400–780 nm (visible)	photochemical damage to the retina, retinal burn	Photosensitive reactions, Erythema
780–1400 nm (near-IR)	cataract, retinal burn	Erythema
1.4–3.0μm (IR)	aqueous flare (protein in the aqueous humour), cataract, corneal burn	Erythema

1.3 Skin Damage

Besides being harmful to eyes, laser beams are also detrimental to the skin after long time exposure originating from either photochemical reaction (mainly from UV laser) or thermal reaction (mainly from IR laser). The laser power threshold for skin injury is almost the same as eye damage except in the retinal hazard region (400–1400 nm). Due to large surface area of skin compared to eyes, it is highly vulnerable when the operators are not dressed properly. Similar to eye damage, skin damage is also laser wavelength dependent due to different accessible depth of skin tissues by lasers at different wavelengths.

Human skin is composed of epidermis, dermis and subcutaneous tissue. The epidermis layer with a thickness of 50-150 μm is the outermost living layer of the skin laying beneath the stratum corneum. A layer of dermis consisting of collagen, elastic tissue, and reticular fibers locate underneath the epidermis with varying thickness of 0.3-3.0 mm, providing the elasticity and supportive strength of skin. The subcutaneous tissue is a layer of fat and connective tissue to help regulate the skin temperature. In general, the harmful effect is associated

with the laser exposure period. When skins are exposed to laser for several nanoseconds, only superficial evaporation happens, while in the range from 100 μs to several seconds, thermal effects are predominant, longer exposure than 100 s can induce photochemical effect. The symptom of erythema (e.g., burn) normally originates from immediate effects and thermal effect. Similarly to the blink self-defense mechanism of eyes for visible radiation (not for the invisible radiation), skin also has a warning signal when it feels the danger of laser exposure from almost all lasers except for some high-power far-infrared lasers.

Also a professor is not impeccable: everybody who works with anti-reflex coated windows knows: „never touch the coating". But sometimes, when a hardworking student presents proudly his new chamber to his professor he of course touches the window and damages its coating.

René Streubel, Essen

After skin absorbs laser energy, the skin temperature increases and a sensation of warmth comes and then human subconsciously responds to get rid of further laser exposure. Any laser with power larger than 0.1 W/cm^2 can give a warmth warning. A temporary skin pain form short period of laser exposure will heal very soon and will not cause severe injury, but it may become more serious when skin are exposed to high power laser irradiation for a long period.

There are three degrees of skin burn according to the injury degree. The first degree only affects the epidermis like the sunburn and causes pain and erythema, but erythema often heals within a few days without a risk of leaving behind a skin scar. If all layers of the epidermis and part of the dermis are affected by laser irradiation, erythema scales up to the second degree. Furthermore, when the full thickness of both epidermis and dermis and part of subcutaneous tissue are affected, third-degree erythema forms and usually leads to a permanent scar. Lasers with wavelengths less than 200 nm or larger than 1 μm are mainly absorbed by the epidermis layer, UV-A can reach dermis layer, while visible and near IR (700-1200 nm) can reach subcutaneous layer. Compared to other laser wavelengths, UV lasers, particular UV-B lasers (280 nm - 315 nm) can only induce the second degree of erythema, however they are much more harmful because of their induced long-term carcinogenic effects leading to skin cancer. Besides carcinogenic effect, long-term

exposure to UV lasers are also able to accelerate skin aging and give rise to hyperpigmentation (pigment-darkening effect), erythema and blistering. Although large areas of skin exposure is unlikely to happen in normal laser work, precautions are still needed to be taken to prevent these adverse effects, especially in summer when people dress less, and do not work with chemicals (colloids) that are to be handled with a labcoat and gloves. Hence in a laser lab, even when working with gold and water only, protect your skin.

Ultrashort pulses may not even hurt in a classical sense, and photochemical damage may feel "just itchy" with red skin. As skin damage by lasers is quite complex to classify how severe it is, and it may even promote cancer or be underneath the skin: always seek the doctor's advice.

1.4 Accidents: Little Mistakes with Big Consequences

The danger of laser exposure towards both eyes and skins has been discussed above. In the following, we will introduce some accidents to show you what can happen if you make similar mistakes, giving you also a warning to be very careful to circumvent similar risks from the static "laser weapon" in you lab (Figure 49). It is worth pointing out that if you even have many years of laser experience, you still need to be very careful because you are the most endangered group who might neglect the rules and take your experiments for granted, even though nothing bad has happened in your lab.

In March 2004, a Berkeley graduate student was subject to a serious injury to his left eye from an Nd: YAG laser. At the beginning, he carefully wore eyewear during his experiment. But when he returned back after dinner he thought there was no danger for laser exposure any more since the system was aligned. Then an accident happened to him from a reflected laser when he leaned over the optical table and adjusted the power meter. This accident affected his professor as well. Since then, his funding was significantly cut. Although the student's vision was impaired a little several months later, he must have regretted about his misconduct and the corresponding bad effect on his professor (a typical laser accident for students and professors, *http://www.photonics.com/EDU/Handbook.aspx?AID=25167*). Wearing the eyewear would have completely avoided this accident. But similar accidents also occurred in other laser labs, even though some of the personnel had many years of laser experience. For example, in October 2001, an Argonne scientist with over 15 years laser experience suffered a serious eye injury from beam while manipulating the 800 nm beam from a Ti: sapphire laser (invisible, femtosecond) with a mirror. The consequence was disastrous, reducing his vision to near-blindness. A Chinese scientist burned his retina and lost some sight in his left eye because he removed his goggles to check the crystal he used. Secondary-reflection of laser light from a cylinder of liquid caused a central foveal lesion with vitreous hemorrhage of a Dutch scientist.

Above are some painful lessons from experiment users, from which the laser safety training is strongly reinforced in every country nowadays. Under the current condition that laser power is increased almost year by year to a new level, any mistake may be disastrous. But also 1 watt is too much for our eye it is a pulsed laser. Generally, in human's unconscious mind, they fear the things unknown or dangerous so that they will strictly follow the rules whatever they are taught, especially the young students. When they become experienced and see nothing bad has happened, they may relax and decline his/her vigilance level and deliberately challenge the rules, hence the danger comes and may be triggered by a small mistake. This scenario is very familiar to the most often reported drown swimmers who have good swimming skills. But they normally overestimated their strength and underestimated the potential danger of the torrent or river they challenged, they eventually went to heaven quietly without telling their parents and friends.

Figure 49: A scenario of eye damage by laser when you do not wear goggles.

Other non-beam potential hazards should also be considered. Laser systems are typical high voltage devices with several kV at high laser power. Most of them are equipped with a high pressure water cooling system which may also create a danger if there is something wrong with pressure control. "Laser accident database (http://www.rli.com/resources/accident.aspx)" lists many kinds of accidents on account of the researchers' misconducts or the laser malfunction. For example, the fiber of a fiber laser suddenly broke in half and shot the operator who was calibrating laser and the shutter failure made the laser leak out of the laser system and burned the labia of the operator. A faulty shutter accidently caused exposure from ruby laser to a French scientist and led to central scotoma and pigmented scar. These accidents should arouse your attention of regular maintenance of your laser system and the optical elements. Furthermore, during laser alignment, pay attention to applicable range of the

optics, which may also cause big consequences. For example, a laser hit a researcher's eye and resulted in a retinal burn when the beam exceeded prism's critical angle (USA) or burned a hand and the base of a thumb while doing adjustments to the laser (Germany). In some cases, the optical elements are not fixed well, it may deviate of desirable direction and hit an unprotected people working into lab or just passed by.

The layout of the laser lab is another important issue from the safety concern, especially the room with glass windows. Chemical labs often have a window for safety reasons, but laser labs shall not. In 1980, in a laser demonstration class in an American University, the beam went out of the class window and struck a student passing by, resulting in his shadow interference and blurred vision. So put a blackened alumina plate on the window, and place a warning sign outside. There are sometimes people who cannot read, or bosses who mainly follow on what they have written themselves. Best and cheapest way is a safety curtain behind the door (it's a heavy plastic curtain that can easily fixated and cut in any length needed), anyone who is entering is protected against direct laser light, and can communicate with the people in the lab. That's also the place where we the laser glasses are hung (outside installment is often not wanted, and inside they do not provide optimal use to entering people in case the laser is already on).

STEP 2: TAKE PRECAUTIONS

Since your nanoparticles are synthesized in liquids, both optical and chemical precautions are indispensable. Here are some precautions tips (Table 4) leading you to the shining way that at least can keep yourself healthy and alive if no natural disaster happens. Let's start how you should dress. The principle of clothing is to reduce the chance for you to be exposed by both chemicals and laser exposure. Your shoes should be resistant to the chemical solution permeation. No flip flops! Your pants should be as long as possible to cover most of your skin, in which case those with long-sleeve and no cuffs are preferred. Another choice is to use professionally protective clothing, one is for chemical protection containing garments, gloves, boots and coveralls. Experimental clothes cannot be repeatedly used in different experiments and cross used by different persons. Each individual should have his/her own protective clothing and one closet for clothing storage. Gloves, especially the chemical resistance gloves are generally selected according to the solutions/ solvents you want to use. Take DMSO for example, when it gets in touch with human skin, it may induce allergic reactions or headaches, even worse symptoms if it brings about the aggregation of contaminants and toxins on human skin. To prevent the penetration of DMSO to human skin, butyl rubber, fluoroelastomer gloves should be adopted rather than nitrile ones because they may be degraded by DMSO. Ventilation system is also suggested to be

established in the working places to reduce the concentration of volatile flammable gases (e.g. ethanol or acetone) or to remove poisonous substances generated during laser ablation.

Table 4: Different protective equipment during nanoparticle synthesis by laser ablation or processing.

Type	General precautions
Dressing	· closed-toed shoes made of a low permeability material · long pants without cuffs, a long-sleeved shirt · gauntlet-type gloves or nitrile gloves with extended sleeves · wear splash goggles · watches and jewelry (e.g., bracelet, ring, etc.) are not allowed
Laser	· Put on eyewear fitting your laser system · Do not wear the gloves outside the lab · Cover your hair under the clothing or bundle it if they are long · Check the wavelength and power fitting between optics/ scanner and laser beam · Check the stability of every optical elements along the laser path
Tools	· Check the cracks and deformities of tubes and bottles before use · Ensure the containers are dry and clean. · Never overfill the tubes (threshold of 3/4 volume).
Environment	· Remove nearby flammable/combustible materials to limit fuel in the case of fire
Others	· No food or drink inside

Both men and women dress nicely to give a good impression to others. What are favorite things for them? In my opinion, most men like cars/watches to show their wealth and taste, while women like all kinds of jewelries as more and shining as possible to put on their head, ears, wrist, etc. Most of these decorations are shining under the sunlight, making you feel comfortable when you touch them. Sometimes, they are the gifts from your friends and are very meaningful for you as a period of good memory is behind them. If you get married, you also have to wear rings to demonstrate your marital status. Do you know that some of your favorite watch/jewelry (e.g., bracelet, ring, etc.) may endanger you during your laser experiments, especially during the laser alignment? The surface of watches/jewelries are always smooth enough to reflect laser light, which may impose risks to your skin or eyes or other researchers or devices nearby causing skin/eye damage or device destroying. For instance, watches have shiny metallic housing and glass window, and you put your own hands on the optical bench: in principle, no laser beam is on while operating, but in reality it can easily happen that the laser is on and your clock reflects its beam in random directions. The necklaces are more subtle because you are used to suppose they are well adherent to the body, and instead they are ready to hang in unknown areas of the optical bench when you lean on it to manipulate optics. Although these can be bad news for girls, for boys it may actually be a good one: they are legitimated to adopt an elegant pocket watch because the wrist is not recommended to those who operate on optical tables!

Therefore, for your own security, take them off before you enter the laser lab. Additional attention should be paid to your long hair, which may catch fire by laser expose. Put your long hair under the lab clothing or fasten it on your head. For laser eyewear, goggle, it is often functional in specific wavelength and has a maximal threshold of laser power, check the applicability of your goggles, otherwise it will be useless to protect your eyes.

Another rule to minimize risks with laser light is NEVER to sit at the same height of the optical beam, especially when someone else is operating on it. If the optical table is equipped with a PC, move it (in particular the screen) higher than the optical bench, so that it has to be used while standing, making sure the operators head is higher than the optical bench (for example while sitting on a stool).

In last section, the danger from laser exposure has been discussed. Precautions should also be taken to other optical elements/scanners other than the laser system itself. First check all the optical elements and fasten them during the light alignment and make sure that the laser is stable all the time and has no chance to deviate its direction. Another suggestion for the optical elements is to make them arrange in a good order, not so crowded and crossed in another experimental set up. When two laser experiments are implemented simultaneously, the risk is squared. Meanwhile, these optical elements are better to be kept away from the edge of the laser stage to give your arms some free space to move and for your notebooks to be put on top to record the data.

Attention should also be paid to the wavelength/power fitting range of the optical elements/scanners. If they are not fitted well, the optics and scanners will be damaged, which means you will lose thousands of euros and have to postpone your experiments to wait for their substitutes to come back.

In order to make your experiments reproducible, all the tools you use must be very clean, including the ablation chamber, glassware, etc. This is because you are not sure whether your colloids will interact with the pollutants and whether the pollutants or the reaction products are poisonous. Meanwhile, as already addressed in Chapter IV, from the viewpoint of nanoparticles reproducibility, it is also strongly recommended that all of the tools you use are pure, if possible as pure as laser-generated NPs.

Great concern should be drawn to the environmental factors, including inflammable solutions, windows blocking and spilled colloids collection. Do not store large volume (only the volume for 11 day consumption) of inflammable solvent in the lab, such as ethanol, methanol, etc. because once there is a fire, these solutions are good fuels for fire expansion. In some cases, it may also lead to explosions. The experiments using these inflammable solvents need also to be very careful, in particular when the laser energy is high.

Once upon a time, a student (not named here) used UV laser to do some UV laser fragmentation using ethanol flowing out of a funnel. A fire started at the beginning of interaction between laser pulses and the falling down ethanol, it immediately expanded to the beaker which was used to collect the ethanol. Some of the splashed ethanol even dropped out and burned the laser table. Fortunately, the student responded very quickly. He stopped the laser system and moved his notebook away. After about one minute, the fire extinguished naturally without causing serious consequences. Just image if there is some flammable solution nearby, the fire may expand very quickly without control, and the whole lab may be destroyed, in particular the laser beam source which is very sensitive to aerosol contaminations.

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Most of you have heard of the “laser tag” game, some of you may have already played it before. It is a funny game where you can use laser guns to your opponents to collect points and become the hero of your team. In this game, once you are hit, you are deactivated and after tens of seconds you revive and can go on playing. As mentioned above, real lasers can shoot people passing by like in this game. But the big difference is that the one who is shot by laser in reality might not have a chance, and you will regret to have doing this to him/

her. Then how to prevent such “laser tag” game happen in your lab? One way is to block all the possible spaces for laser beam to go out of your lab. You can either cover the windows using high ablation threshold materials or install a metallic fence outside the window to absorb the laser energy. Since the laser arrived at these materials is not focused, the materials will not be ablated dramatically. To ensure the security of your colleagues in you lab, you can use some metallic resisting shields to block the laser from all possible reflected or transmitting directions (we installed simple metal sheets and tubes guiding the beam wherever often justage is not required. Of course the metal in anodized and blackened to minimize reflection, just in case).

Before experiments, get to know something about your materials and predict the products you could get. Take the corresponding precautions according to the potential risks you may face.

STEP 3: TURN ON THE LASER

When all the materials and the tools are ready for your experiments and you can take all precautions described in the previous section. The time has come for you to do experiments in liquid. When you step in front of the laser lab, even though the laser status lamp is off, still do not enter the lab without laser goggles. Wear them and check the condition in the lab yourself. When you go inside the lab, do not forget to close the curtain shield near the door to give your colleagues a safe space to dress goggles.

In this section, some hints that are easily neglected are summarized. First, before you start the laser system, check whether the lens cap is still on. If so, take them off. Sometimes, you start the laser but you can not find the beam. You may get confused or become worried whether the laser system is malfunctioning during your experiments. This small mistake often happens. The ablated cap fogging will condense at the lenses, and our laser experiments will have less yield. Lens cleaning is very time consuming.

Second, put all your things in a good order, do not put them randomly on the table, especially fragile glassware. A messy layout of your samples may distract you and might result in the spill of the chemicals when you behave improperly , in particular when you are in hurry. And scientists always are.

When you switch on the laser system, you have responsibility to inform other colleagues who are still working in the lab and give them enough time to put on the goggles. For those students who are curious about the goggles, sometimes they have a compulsion to check the ability of goggles by holding it bin the beam. Do never do this, trust their quality, otherwise you and your colleague have the risk to be exposed by high power laser beam (as the goggle stands the beam only for limited time, once, at one spot. It incubates the exposure but you don't see it). You should also know how you can turn off the laser in the fastest

possible way in case of an emergency, so check for emergency-buttons. When you finish your experiments, first remember to check whether your colleague have finished their experiments. Under this condition, saying is better than seeing, ask them: is laser on? If they went for lunch, shut it off and ask them to pay next lunch. Only when you are sure that other laser system is off, you can take off your goggles. During your experiments, maybe you are short of materials or liquid or just want to go to the restroom, during you absence, please remember to turn the laser power to 0% and close the shutter (not the laser system), otherwise, someone may enter the laser lab and get injured without be notified (I remember my Professor was demonstrating to a guest the laser lab while my student was out for a break and he almost burned his hand by the UV laser). During break, of course, leave gloves and protective clothing in the lab.

3.1 Focus finding

In Chapter I and II you have learned how to find the laser focus. For this purpose, some people like to use a piece of paper, in which case due to high energy near the laser focus, paper is easily burned, releasing some unpleasant smoke. Trying to stop the fire will distract your attention to forget the existence of the laser. And as a result, you can easily be burned yourself. Therefore, paper should be totally restricted for searching for the laser focus. The proper way to follow the beam is by using low laser power and a detector which is suitable for the wavelength you use, e.g. an IR detector for 1064nm (so-called beam viewer). Alternatively, a watered telefax paper can be used at low power. Precise finding of the focus is to weight the target before and after several minutes' ablation at different positions along the beam transmission direction and then choose the position where you achieve the biggest difference of the target weight. Note that targets reflect laser light. This method is a little time-consuming because the sample must be dried and weighed twice for each focus position. But it's the only method that is correlated to the real nanoparticle yield, as highest yield is often achieved mm away from the geometrical focal plane in air (see literature or email the authors abut that issue).

3.2 Long-period Ablation

In Chapter II you have learned how to obtain a large amount of nanoparticles. For this purpose, sometimes, long-term laser ablation is needed, which is always implemented by continuously flowing liquid with the aid of a pump. In this case, liquid may be spilled out due to the improperly sealed pipe and will arouse a disaster for the electrical lines or devices embedded underneath or positioned on the floor. Meanwhile, it will also rust your optical table, which will not be repairable any more.

When I first arrived at my current lab and did some experiments using flowing liquid, due to inappropriate connection of the chamber, some water flowed out and then fell down on the ground. I did not recognize this was a big problem, but afterwards when it became a pool, even enough for a frog to swim. I suddenly saw some electrical wires and a socket were floating on the water, "oh, how stupid I am and how dangerous it is!" Then I stopped the liquid and then start cleaning the floor, and luckily nothing happened at time. But the optical table now has rust spores.

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When you plan to do long-period laser ablation, liquid evaporation is another issue to be taken into account. Organic solutions are volatile, as the ablation proceeds, the liquid thickness gradually decrease. Once they are unable to immerse the target underneath, you are performing laser ablation in air, in which case the ejected particles will no longer be confined in liquid, but haunt in the air. As a result, it is easy for you to inhale them and receive toxic effect after inhalation. Another concern is the flammability of solvent vapor. Once the environment is rich of this flammable gas, the laser will then become the matchstick to ignite it and an explosion may also happen. By law, it is not allowed to combine an ignition source (a pulsed laser) with an inflammable liquid. So how to do laser ablation in ethanol or acetone, as widely reported in literature? To answer this questions expands the scope of this handbook. In general, you need to write a risk assessment scenario together with your safety officer, taking into account what may happen and which safety measures can be appropriate. In our lab, we focus a 500 watt picosecond laser in a flow chamber pumping large amounts of inflammable liquid. For that, we have built an active safety chamber around it with gas sensors and valves that automatically open and flood the chamber with nitrogen, and shuts the beam and the liquid pump. This is the upper end. For laser ablation of gold in a 20 ml ethanol batch the safety measure will be less extensive, but still you will have to take into account what may happen in the worst case. Consult your safety staff before working with organic liquids and lasers.

STEP 4: BE ORGANIZED

When you collect colloids, what you have to do is labelling your sample. Sample labelling is a good habit for you to know the information after many days. In China, there is famous saying “good memory is inferior to sodden ability to write”. Even if you have memory like a computer and can search for the information whenever you want, it is still better for you to categorize the

samples. Sample labelling should be done labelling at least to their composition, production date, and name (e.g., "gold-silver-alloy in acetone, 28.4.2014, S. Yusuf"). In the lab book, you write down processing parameters (e.g., laser power, repetition rate, scanning speed, wavelength, pulse duration, processing period; liquid volume, liquid thickness, liquid composition), etc. Do not forget to put the foreseen disposal date as well.

One of the most important things from the security point of view is to check whether your colloidal solutions have one or some of the following hazards (Figure 50), e.g., exploding bomb, corrosion, flame over circle, gas cylinder, environment, skull & crossbones, exclamation mark, health hazard and flame. Besides the exploding bomb and gas cylinder are very rare for the nanoproducts from laser ablation, all other marks are closely associated with your desirable products.

Figure 50: Labels for different kinds of chemicals.

The danger of corrosive substances (e.g., acids, alkalis, organic halides) towards other substances (e.g., metals, organic, living tissue) upon contact should be kept in mind during your pH adjustment of the liquid for laser ablation or container washing. If there is little chance to form corrosive substances after laser ablation, even if you are not sure, please label them using this tag. Oxidizing materials, like hydrogen peroxides (e.g., H_2O_2), that can supply oxygen or other oxidizing substances, or react chemically to oxidize combustible materials to increase the risk of a fire or explosion should be used very carefully. Attention to acute toxicity during synthesis of arsenic-based compounds should be paid since rapid occurrence of adverse effects often takes place within 14 days. "Flammable chemicals" means that they are very easy to catch fire. The most frequent flammable chemicals are organic solvents you use for

Since I am an engineer I only had a limited chemical background. Before I suffered from an accident I hardly knew anything about chemical and nanoparticle toxicity. One day, with the aim to do some experiments with cobalt oxide powders, I had to first mechanically press them into a pellet. But unfortunately, before I started experiments, I did not look up the toxicity information so that I forgot to wear gloves (That is a bad habit for me! Maybe also for you!). I touched the powders with my hand. Even though I washed my hand afterwards, I still had a severe skin allergy at that night (see Figure 51), I did not know the reason. The first thing that came to mind is that there should be something wrong with the food. But that day I just ate a döner which I have ate tens of times before. One hour later, it came to my mind that I had done some experiments with cobalt oxide. Then I quickly searched from the internet and found skin touching would cause skin allergy. From this event, I learned that some materials are really dangerous. Something happened later taught me that even laser ablation in liquid is one of the purest synthesis method but not totally safe for your health. After pressing these cobalt oxide palates, I did some experiments with gloves. But at that night, I still had a skin allergy and much severe than last time which should be related to the water evaporation during long period of laser ablation, containing cobalt oxide or cobalt ions inside. They were airborne and were inhaled inside my body during my breath. When I went to the hospital, even the doctor did not know the toxicity of cobalt oxide. You see, I became a knowledgeable person from my accident. If you want to try something else and test the ability of the doctors in your country, you can do your own experiments carelessly like me. But I can not promise you are so lucky enough to just have the symptom of skin allergy.

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Figure 51: Skin allergy induced by exposure of cobalt oxide colloids and powders.

particle generation by laser ablation, including ethanol, methanol, isopropanol, etc. Carcinogenic colloids (cobalt-, nickel- and copper-based nanoparticles) or solutions that can directly cause cancer are the most dangerous substances towards human. Reproductive toxicity is related to your offsprings because it influences your sexual function and fertility. Since oocyte cell of women can be endangered by DMF's exposure leading to birth defects, women should be very careful with DMF or replace it with other solvents.

STEP 5: DISPOSE OF YOUR WASTE

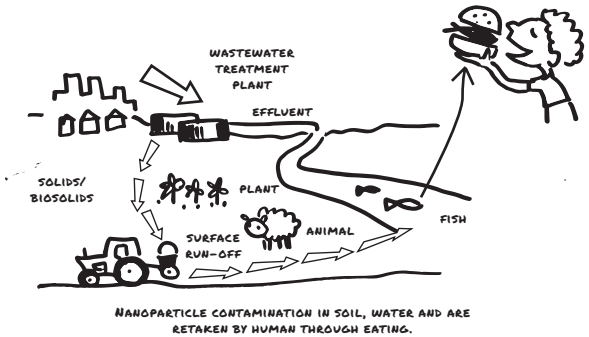

Figure 52: Nanoparticle contamination in soil, water and are retaken by human through eating.

Not every laser shot is successful in generating nanoparticles. Hence, some of the colloids become trash during your focus search or rechecking the reproducibility. Then, how to dispose of these colloids? The easiest way that might come to your mind is to dump them in the sewage, like you do with water after you boiled eggs in it. Its only mg of nanoparticles in standard liquid, isn't it? Yes, this is the most convenient way, but it is not a wise choice. Some solids are biopersistent and are taken up during plant growth, and then eaten by the animals. Once these animals or fishes or vegetables are eaten by human (Figure 52), you can image that what you have done to the environment has gone back to you. So do not throw the colloidal trash away in the drain! Store them in the specific waste containers for professional staff/company to collect. Organic and aqueous solutions are better separately stored as well as the glass and plastic trashes. Meanwhile, the colloidal solutions you want to keep for further characterization should best be stored in a fridge away from heat and light at 2-25 °C.

FINAL STEP

After reading this book you hopefully realized that the force is strong with you. Now, it's time for you to take your laser, spread your wings, dive into the liquid and start synthesizing nanoparticles that will make a difference in science and technology.

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DOI: 10.17185/duepublico/41087

S. Barcikowski, V. Amendola, G. Marzun, C. Rehbock, S. Reichenberger, D. Zhang, and B. Gökce. Handbook of Laser Synthesis of Colloids, DuEPublico, 2016.