

What is Analytical Ultracentrifugation?

Kristian Schilling
Nanolytics GmbH

Contents

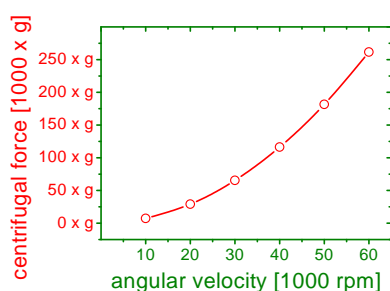
1	Analytical Ultracentrifugation (AUC)	2
2	AUC in Principle	3
3	AUC - a Versatile Instrument	5
4	Properties Accessible with AUC	6
4.1	The Primary Result: Sedimentation Velocity	7
4.2	Particle Size	7
4.3	Particle densities and density distributions	8
4.4	Molar Masses and Molar Mass Distributions	8
4.5	Particle Shape and Particle Swelling	9
4.6	Interactions	10
4.7	Diffusion	10
4.8	... and lots more	11
5	Linking the AUC to other Methods	12
6	AUC for Custom Measurements	14
7	Our Competence	15
8	Contact us	16

1 Analytical Ultracentrifugation (AUC)

Powerful tool for Colloid and Polymer Analysis

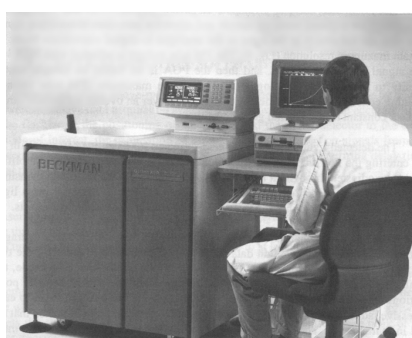
For more than 70 years, Analytical Ultracentrifugation (AUC) has been contributing valuable services in the fields of biochemical and colloidal sciences. The widespread applications of AUC in chemistry, biochemistry and pharmaceutical science involve - among others - determination of molar masses, molar mass distributions, particle size distributions and particle density distributions.

Particles, coils, micelles, molecules - any dispersed or solute particles can be subjected to analysis in the AUC, as long as they sediment or float in the sedimentation field.



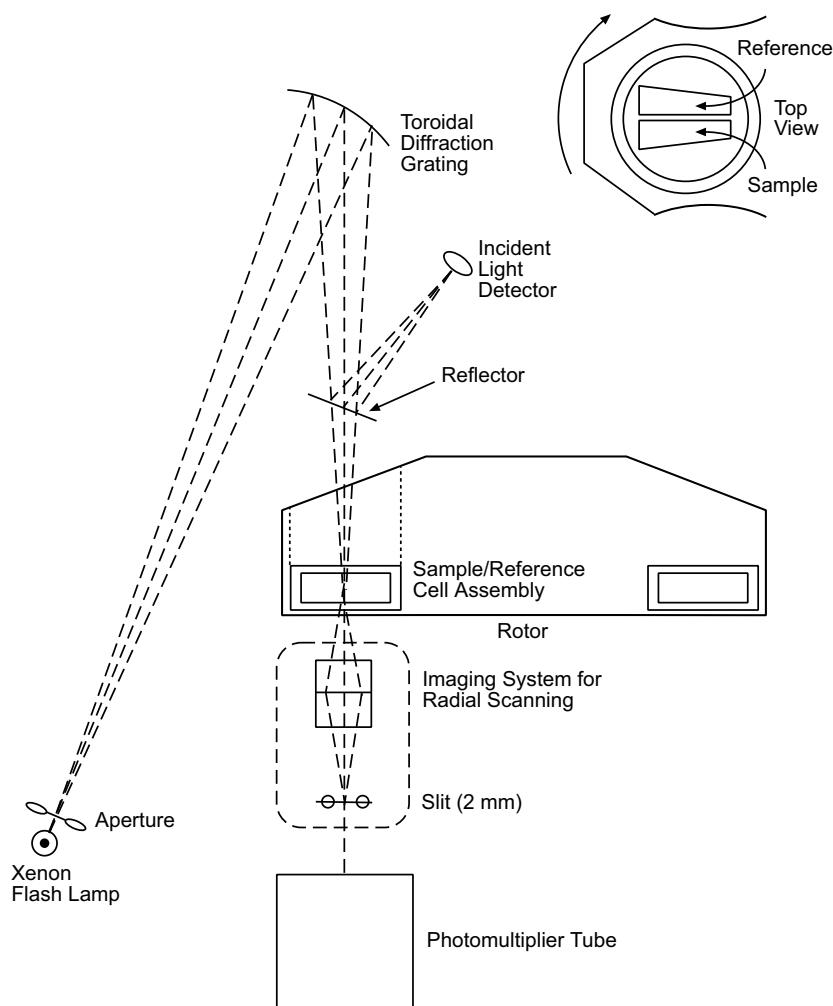
At a maximum angular velocity of 60000 rpm, an AUC will develop sedimentation fields equivalent to the Earth's gravity 260000 fold. Exposed to this field, almost any particles will be forced to sediment or float. This process is monitored with optical systems and then evaluated in respect to particle properties.

We use modern equipment, an Optima XL-A/XL-I, supplied by the leading manufacturer Beckman Coulter, which features two independent optical systems for detection and represents the state of the art for such devices. Radial resolution is 0.01 mm; absorbance optics applies a monochromator with a 1 nm step width. A thermostated rotor allows experiments to be carried out at temperatures between 0 and 40°C.



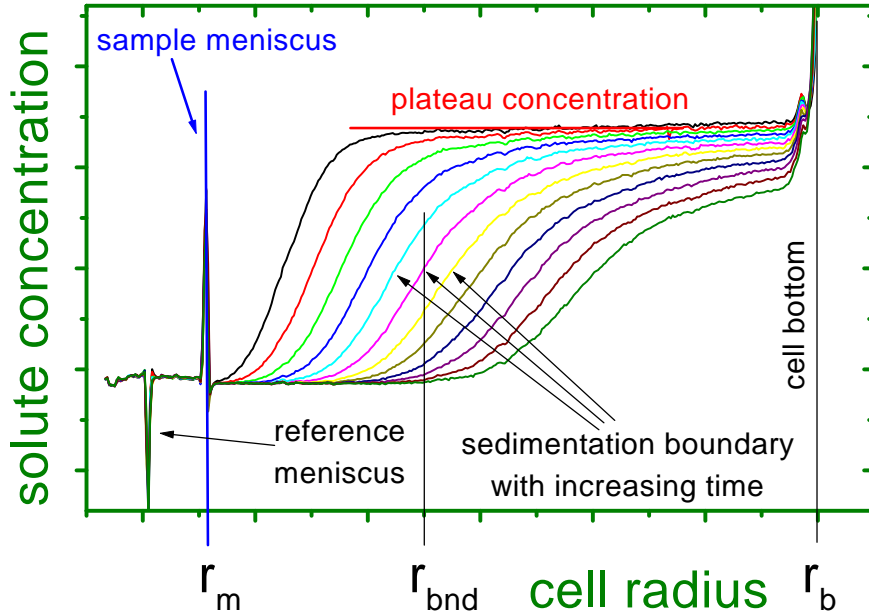
2 AUC in Principle

The AUC fractionates by *size*, *density* and *shape*. The principle of measurement is an everyday experience: such as a large object sinking to the bottom of a liquid faster than a small one, a steel object faster than one of aluminum, a compact sphere faster than a rod or a coil of the same mass. One fascination of AUC lies in its simplicity!



During sedimentation, the sample chamber is screened parallel to the rotational axis. Our centrifuge is equipped with both absorbance and Rayleigh interference optics to make a large scale of particles detectable. As a primary result, a concentration profile along the radial axis of the sample cell is obtained; repeated measurements during proceeding sedimentation yields time-resolved information. Different components will be separated in the

course of sedimentation. The concentration profiles are then evaluated in respect to the information desired.



Locally resolved concentration profiles as detected by the AUC are displayed. From the meniscus, an increasing depletion of particles is observed. The material is accumulated at the cell bottom. At the sedimentation boundary, particle concentration increases steeply.

From the velocity of sedimentation boundary movement towards the cell bottom, particle properties can be calculated. For multimodal systems, multiple sedimentation boundaries are observed.

Apart from sedimentation, the dispersed particles also experience diffusion. Back diffusion increases with accumulation of material at the cell bottom, to eventually reach an equilibrium between sedimentation and diffusion. The so called sedimentation diffusion equilibrium provides an independent access to particle properties, since transport processes are eliminated. Often enough, the process of sedimentation delivers enough information for analysis so that equilibrium need not be attained.

3 AUC - a Versatile Instrument

AUC is an absolute method; no calibration is required and no interactions with a stationary phase take place. Arbitrary solvents, even aggressive or highly viscous chemicals, can be applied. Little amounts of substance and solvent are required, which makes this method attractive especially for biochemical applications.

A list of basic experiments shows the remarkable versatility of the AUC:

- sedimentation velocity
- sedimentation equilibrium
- density gradient
- synthetic boundary

In addition, variations to all experiments can be designed for special needs and situations.

A large variety of particles are detectable due to the two optical systems the machine is equipped with. In considering particles, keep in mind that on a nanoscopic scale, the difference between solutions and dispersions becomes semantic. . . Molar masses of several thousands up to million can be characterized on the AUC.

Organic solvents, acidic or basic systems, or samples of high viscosity present no problem to the AUC. We have sample cells manufactured from titanium, the cell windows are made of sapphire. No interactions with a stationary phase influence the system within the screened range of the sample cell.

Nobody can guarantee that, for a specific system, the method may be inappropriate. To minimize the risk for our customers, we offer a trial run for a special rate to determine whether your particles are detectable by the optical systems. These costs are refunded in case the data acquired are used in a future evaluation.

4 Properties Accessible with AUC

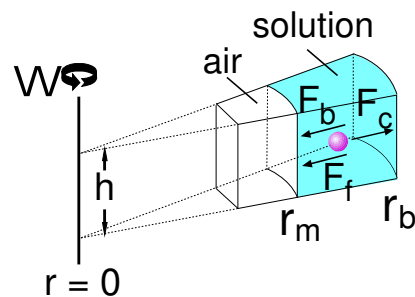
Basically, two transport processes take place in the Analytical Ultracentrifuge: sedimentation and diffusion. Sedimentation is governed by particle density and friction. Diffusion, however, is independent on particle density. Only the particle size has influence on particle diffusion. Thus, the particle properties in question may be reduced to questions of density, mass and the frictional ratio.

All processes taking place in the Analytical Ultracentrifuge are covered by the Lamm equation (1), a differential equation already developed in 1929.

$$\frac{\partial c}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left[r \cdot D \frac{\partial c}{\partial r} - s \omega^2 r^2 c \right] \quad (1)$$

Although there is no analytical solution to the Lamm equation, it is the basis of all evaluation methods. It contains the transport properties named above in the sedimentation constant s and the diffusion constant D . The term r is a local coordinate giving the distance from the axis of rotation and ω is the rotor's angular velocity.

A particle in a sedimentation field is exposed to three forces: a centrifugal force, a bouyant force and friction. Whereas the centrifugal force is independent of particle properties, bouyancy is governed by particle volume and density, and friction by particle size and shape. This is the set of variables that describe directed particle motion in a sedimentation field.



The Svedberg equation (2) combines the transport properties s and D with the particle properties mass M and density \bar{v} .

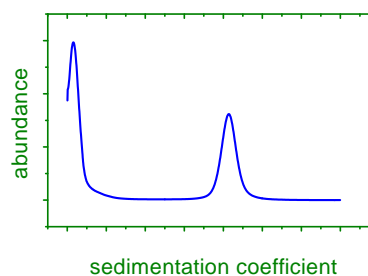
$$M = \frac{s R T}{D (1 - \bar{v} \rho)} \quad (2)$$

The frictional properties of the particle are expressed by the diffusion constant. The Svedberg equation is the foundation for most AUC experimental evaluations.

4.1 The Primary Result: Sedimentation Velocity

In a sedimentation velocity experiment, a concentration gradient within the sample cell is recorded. Without prior assumptions, this profile can be converted into the sedimentation coefficient distribution (s distribution).

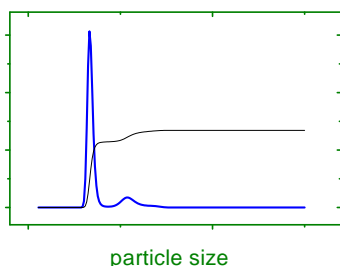
Sedimentation coefficients measure the velocity a particle needs to cover a certain distance if exposed to a standard sedimentation field. This is the primary experimental result; no information is given as to which particle properties result in this sedimentation velocity.



Useful information is already contained in this primary result: the sedimentation coefficient distribution gives the number and concentration of components, sample homogeneity, aggregates and polydispersity. Often enough, this experiment is already sufficient to solve the analytical problem in question.

4.2 Particle Size

One of the most important particle characteristics in colloidal science is the particle size distribution (PSD) that can be obtained directly from the s distribution.



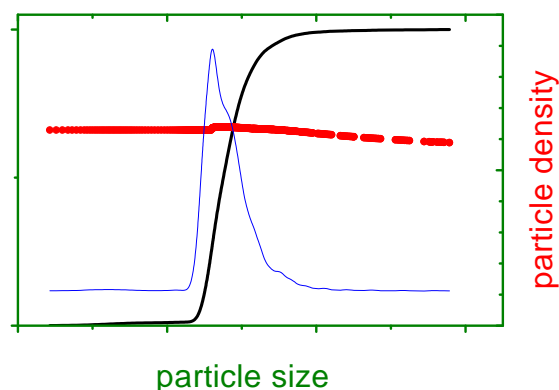
For this conversion, the particle density is required. If it is unknown, a density variation experiment on the AUC or an independent method can be used to obtain this parameter. For spherical, compact particles, direct calculation of the diameter is possible. In other cases, further assumptions or information may be required.

Even smallest colloid particles can be characterized in Ångström resolution.

4.3 Particle densities and density distributions

Particle size distributions can also be calculated for heterogenous or unknown particle densities. In this case, the procedure of density variation is applied where particles sediment in two solvents of different density. Comparing the resulting data yields a density distribution for all detected particles.

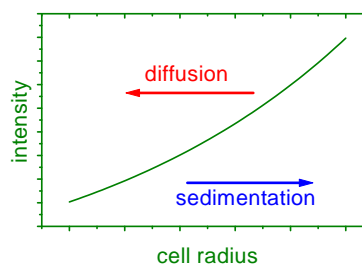
The density variation technique may be applied both for sedimentation velocity and equilibrium. In equilibrium, weight averages for the density and the molar mass are obtained; in sedimentation velocity, the experiment results in simultaneous determination of particle size and particle density distributions.



4.4 Molar Masses and Molar Mass Distributions

Measuring molar masses is a classical AUC application. Two methods are available: sedimentation equilibrium yields molar mass averages whereas sedimentation velocity results in molar mass distributions.

Sedimentation equilibrium is attained when sedimentation to the cell bottom and back diffusion reach a stationary state. No transport processes take place any more, and a molar mass will be derived *independently of sedimentation and diffusion constants*.

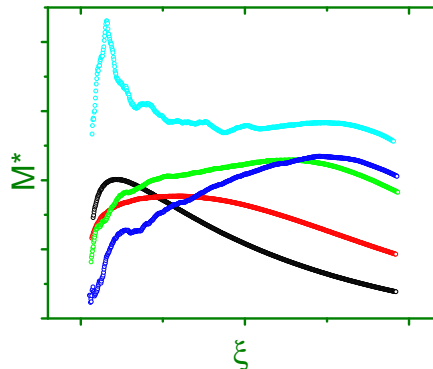


For this reason, error bars are below 3% which makes this method a very reliable one. Today, sedimentation velocity experiments can be conducted with comparable precision. However, sedimentation equilibrium is mostly better suited when investigating systems containing very small, thus rapidly diffusing particles. This complicates the evaluation of sedimentation velocity experiments whereas equilibrium is reached in reasonable time.

In sedimentation equilibrium, multiple species will sum up in one concentration profile, thus requiring assumptions, e. g. concerning number and relative concentration of involved species.

For this reason, we prefer the M^* function for evaluation. M^* is model independent and provides diagnostic information that helps select an appropriate model for the system without introducing assumptions at an early state of evaluation.

Due to particle interactions, it is necessary to carry out concentration series measurements in sedimentation equilibrium. The concentration dependency is then obtained in the Second Osmotic Virial Coefficient.



From *sedimentation velocity*, molar mass *distributions* are obtained. However, the particle densities and frictional ratios for all species are required for evaluation.

4.5 Particle Shape and Particle Swelling

A particle's sedimentation velocity contains information on its frictional properties, most commonly given by the frictional ratio f/f_0 . The frictional ratio can be calculated from AUC data using equation (3).

$$\frac{f}{f_0} = \frac{M(1 - \bar{v}\rho_0)}{N_A \cdot 6\pi\eta_0 s_{20,w}^2} \cdot \sqrt[3]{\frac{4\pi N_A}{3\bar{v}M}} \quad (3)$$

f/f_0 is unity for compact spheres. The frictional ratio is higher for nonspherical particles; the axis ratio for ellipsoids of revolution can be calculated from f/f_0 for a given molar mass. A full scale characterisation of a nonspherical particle can be achieved by combining sedimentation equilibrium (M) and sedimentation velocity (f/f_0) data.

Swollen particles also exhibit frictional ratios greater than 1, allowing hydration to be calculated from sedimentation velocity data. The more complex the particle, the more information is required for full characterization. Various AUC experiments as well as independent methods offer a large number

of options to obtain a complete set of variables and thus describe particles of high complexity.

4.6 Interactions

In AUC experiments, interparticle interactions are found

- in the concentration dependency of sedimentation coefficients (k_s , sedimentation velocity),
- in the concentration dependency of the apparent molar mass (A_2 , sedimentation equilibrium).

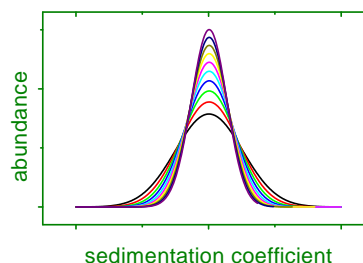
From concentration series, valuable information on particle interactions is derived. The corresponding parameters can be combined with related parameters from independent methods.

Dissociation constants of aggregating systems can also be obtained in this manner.

4.7 Diffusion

As can be seen from the Lamm equation (1), the transport processes of sedimentation and diffusion are always coupled. Often, diffusion complicates evaluation as it leads to broadening of the obtained distributions. The influence of diffusion can then be suppressed to some extent by appropriate experimental conditions and evaluation procedures.

With increasing runtime, the influence of sedimentation becomes more and more dominant over diffusion due to different time dependencies of both effects. Both phenomena can be separated by the means of appropriate evaluation procedures. Other evaluation methods perform global fitting where all particle properties, including diffusion coefficients, are involved.

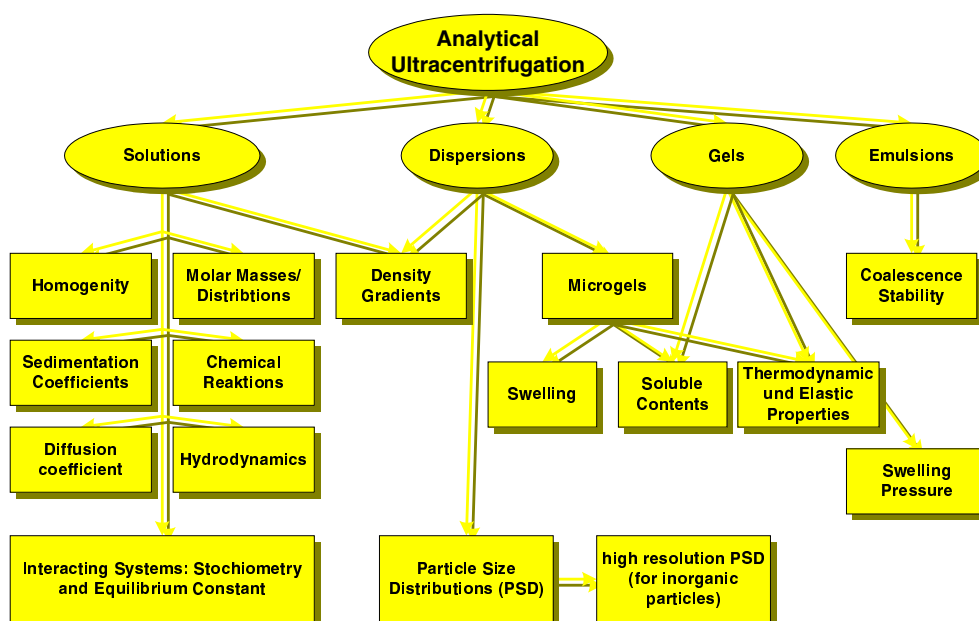


Although diffusion coefficients can be obtained from dynamic light scattering of field flow fractionation with less effort, there are cases where the AUC is still used for determination of D today.

4.8 ... and lots more

It is apparent that the versatility of the AUC permits far more applications than could be listed here. For instance, it can be used as a pressure generator to force separation of emulsions under high pressure and thus to determine and compare stabilities much faster than with routine methods. Another application is to deswell gels, permitting access to thermodynamic properties. The AUC's optical systems can be used in a versatile way as well. Different species with different absorbance or refractive properties can be registered independently in *one* experiment.

Furthermore, wavelength scans of fractionating species can be obtained. The AUC offers a broad platform for the user's creativity.



5 Linking the AUC to other Methods

Complex systems often require more than one method for full characterization. As others, AUC is not a master method that answers all questions. A combination with other methods will deliver more information about your system than the sum of their results. Be it necessary or supplemental: the information output is not added, but multiplied.

That's why we apply other methods as well, some of them on our own, some in cooperation with public research facilities:

- transmission electron microscopy
- viscometry
- densiometry
- rheometry
- field flow fractionation
- high-end dynamic light scattering

An especially fruitful combination is AUC and viscometry. Hydrodynamic shape functions permit determination of axis ratios for nonspherical particles. Some examples are the ν , P and R functions:

$$\nu = \frac{[\eta]}{v_s} \quad (4)$$

$$P = \frac{M(1 - \bar{v}\rho_0)}{N_A \cdot 6\pi\eta_0 s_{20,w}^0} \cdot \sqrt[3]{\frac{4\pi N_A}{3\bar{v}M}} \cdot \sqrt[3]{\frac{1}{\frac{w}{\bar{v}\rho} + 1}} \quad (5)$$

$$R = \frac{k_s}{[\eta]} \approx \frac{2(1 + P^3)}{\nu}, \quad (6)$$

These functions take on defined values for certain axis ratios. The variables contained are all accessible by AUC except for the intrinsic viscosity $[\eta]$ available from viscometry. For swollen particles, the partial specific volume v_s is different from \bar{v} , the partial specific volume of the solid material. v_s can

also be expressed by terms of w , the hydration parameter. When dealing with swollen particles of unknown solvent content, hydration independent functions can be used, e. g. the molar mass calculation according to Lavrenko (7) or the Π function (8).

$$M = N_A \cdot \sqrt{\left(\frac{6\pi\eta s}{1 - \bar{v}\rho}\right)^3} \cdot \sqrt{\frac{3k'[\eta]\bar{v}}{4\pi k_s} \left(\frac{k_s}{2\bar{v}} - \frac{k'[\eta]}{k_s}\right)}; \quad \frac{v_s}{\bar{v}} = \frac{k'[\eta]}{k_s}, \quad (7)$$

$$\Pi \approx \frac{2A_2M}{[\eta]}, \quad (8)$$

These functions use other particle properties in order to obtain a complete set of variables. The concentration dependence of sedimentation velocity k_s or the Second Osmotic Virial Coefficient A_2 can be measured with AUC experiments.

Evidently, a large number of options allows characterization of complex systems by means of AUC and viscometry. Of course, other additional methods may be invoked as well. The best strategy depends on existing and desired information on your system. Nanolytics will develop it for you and perform all evaluations up to a consistent model describing your system.

6 AUC for Custom Measurements

An Analytical Ultracentrifuge is an expensive setup. Only for high sample numbers is it worth the costs and effort to maintain an own machine. Nanolytics bundles the demand for the method and thus offers measurements for a reasonable price. You benefit from a powerful method for much lower costs than if you ran the method in your own business.

What's more: you profit from our experience and expertise in respect to experimental strategies. Data evaluation requires interpretation and extensive know-how that Nanolytics can contribute. AUC is no plug 'n play method with commercially available software yielding unambiguous results.

We supply results in comprehensive plots and on demand also as data files for further handling in respect to calculations or data presentation.

We can supply you with separation parameters if you want to use a centrifuge for preparative separation.

If our results don't meet your expectations: we believe in what we measure. We will not "smooth" our results to match what you expect to see. What we have, you will get. Your measurements and results are treated strictly confidential; we are independent and don't belong to one of your competitors.

7 Our Competence

Nanolytics is part of the Kompetenzzentrum Nanotechnologie, a German academic/industrial network. We maintain cooperations with other, mostly small, companies and public research facilities. Working with us, you will also profit from our network, if you wish.

The company's founder wrote his PhD thesis at the Max Planck Institute for Colloids and Interfaces. In his work, he has focused on AUC and has enhanced the methods of density variation and diffusion broadening.

Nanolytics puts continued efforts into fundamental research in order to further develop the AUC's potentials and extend the field of application. Problems our customers pose are not only our business. They also help us in bringing the AUC to its max. Just come over with it!

8 Contact us

Nanolytics

Gesellschaft für Kolloidanalytik mbH

Am Muehlenberg 11

14476 Potsdam

Tel. +49 331 5818360

Fax +49 3322 5818361

e-mail: info@nanolytics.de

www.nanolytics.de

This introduction is available as a pdf file on our website:

<http://nanolytics.de/pdf/en/auc-introduction.pdf>