Microscopy

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The Invention of Immersion Ultramicroscopy in 1912—The Birth of Nanotechnology?**

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history of science · nanoparticles · nanotechnology · ultramicroscopy

1. Introduction

2012 marks the centenary of the invention of one of the most remarkable techniques in nanoscience, one which has opened a new window to the study of colloidal solutions. This invention—the immersion ultramicroscope—may be said to mark the moment when modern nanotechnology began.

Colored glass of the Roman times has fascinated historians for a long time. One of the most prominent examples is the Lycurgus Cup, which dates back to the 4th century A.D., and nowadays is on display at the British Museum.[1] While this vessel appears pale-green in reflected light, its glass changes color when illuminated by transmitted light and appears translucent and bright-red.[2] This effect is caused by nanoparticles of a colloidal silver–gold alloy embedded in the glass matrix.[3] No description has survived from Roman times to tell how the glass workshops were able to make this material containing colloidal metal. More than 1300 years after this cup had been crafted, numerous publications in the 17th century appeared describing the preparation of colloidal gold. The most frequently cited report, though not the first, was published by Andreas Cassius in 1685.[4,5] It would take another two centuries before Michael Faraday (1791–1867) speculated about the size of these finely distributed gold particles. In his "*Bakerian Lecture: Experimental Relations of Gold (and Other Metals) to Light*" in 1857 Faraday demonstrated the results of his work on what he called "gold sols".[6] He attributed the color of his gold solutions to the size of the metal particles. Faraday used a projection microscope to demonstrate how the reduction of gold in "*exceeding fine particles*" resulted in a ruby-red-colored fluid. Using a microscope, he demonstrated a transformation of the fluid's color to blue by mixing salt with his gold sol. While he could not explain the reason for the color alteration, he considered this effect to be an indication that "*a mere variation in the size of its particles gave rise to a variety of resultant colours*". Although he had no clue about the real size of the particles causing this coloring, he suspected the waves of light to be "*large compared to the dimensions of the particles*". Today, one of the microscope slides that he used for the experiments carried out during his lecture is kept at the Whipple Museum of the History of Science, University of Cambridge.[7]

It took nearly another half a century before the chemist Richard Zsigmondy (1865–1929) and the physicist Henry Siedentopf (1872–1940) determined the size of colloidal gold by introducing a novel microscopical method. Interesting enough, Zsigmondy did not know about Faraday's work until he had successfully created colloidal gold by the reduction of gold chloride with formaldehyde in a weakly alkaline solution. After Zsigmondy had access to Faraday's work, he followed the Englishman's approach on using phosphorus as a reducing agent. By applying his experimental experience with formaldehyde and gold but using phosphorus as a reducing agent, Zsigmondy generated even finer gold particles. These fine particles were later used by Theodor Svedberg for his diffusion experiments.[8] But how was Zsigmondy able to determine the size or mass of his colloids?

At the beginning of the 20th century, several approaches emerged to push the resolution limit of microscopes. Most of them were based on the theoretical work of Hermann von Helmholtz and Ernst Abbe's theories on resolution. Consequently, microscope objectives with (very) high numerical apertures were introduced, or alternatively shorter wavelengths, reaching into the deep ultraviolet, were used for microscopy. This resulted in the use of immersion oils with high refractive indices, such as 1-bromonaphthalene, for objectives having numerical apertures of 1.60,[9] as well as the introduction of monochromatic objectives for UV microscopy at a wavelength of 275 nm.[10] Zsigmondy and

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Siedentopf followed an alternative approach for resolution enhancement with a novel dark-field illumination. In order to highlight the capabilities of their invention, they named this scientific instrument the "ultramicroscope". While Zsigmondy himself was performing extensive studies with his ultramicroscope, observing single gold particles with diameters less than 4 nm in solid material,[11] Jean-Baptiste Perrin applied the ultramicroscope in 1908 to plot the movements of nanoparticles.[12] The instrument enabled Perrin to experimentally verify the existence of atoms by confirming the predictions of Albert Einstein and Marian von Smoluchowski on Brownian motion.[13–16] Thus, Zsigmondy's approach enabled the extensive study of colloids for the first time, and one may consider this point in time to be the start of modern nanotechnology.

Zsigmondy's inventions would lead directly to the award of three Nobel Prizes and facilitate work associated with several more. Richard Zsigmondy himself was honored in 1925 with the Nobel Prize for Chemistry "*for his demonstration of the heterogeneous nature of colloid solutions and for the methods he used, which have since become fundamental in modern colloid chemistry*".[17] In the following year, Perrin was awarded the Nobel Prize in Physics for the discontinuous structure of matter he had observed with the ultramicroscope. In 1926, Svedberg was awarded the Nobel Prize in Chemistry for his work on "*disperse systems*",[18] in particular for the ultracentrifuge, which he had named "*in analogy with the naming of the ultra-microscope*".[19]

Hardly any of these ultramicroscopes have survived in an operational state. We have repeated some of the historical optical experiments utilizing an original microscope as used by Zsigmondy, and in this Essay we compare these results with those obtained by state-of-the-art technology.

2. Extreme Dark-Field Microscopy

Zsigmondy studied chemistry in Vienna and then moved to Munich where he graduated with a PhD in 1889. After working in Berlin as a postdoctoral scientist, he moved to the University of Graz and was finally employed by the Schott Works in Jena, in 1897. While working with ruby glass in 1898, Zsigmondy proved that its color originates from fine gold suspensions created by the thermal reduction of gold salt using stannous chloride.[20] Having created a series of glasses containing gold particles which appeared blue, violet, and red in color, and knowing the gold concentration in each of them, Zsigmondy wanted to determine the sizes of the suspended gold particles. For this purpose, Zsigmondy pursued the development a dark-field method jointly with Siedentopf, who was an employee of Carl Zeiss in Jena at the time. As samples, they used pieces of glass with gold concentrations that were predefined during the production of the glass. Using a convergent beam of intense sunlight they illuminated a precisely defined volume of each glass sample. Light hitting the colloid gold particles scattered and formed Tyndall cones. These cones were observed and counted with a conventional microscope which had its optical axis oriented orthogonal to the illumination axis (Figure 1).[11] Now, a simple calculation was used to determine the mean particle size, as the gold concentration was known, the volume observed was defined, and the number of particles therein had been counted.

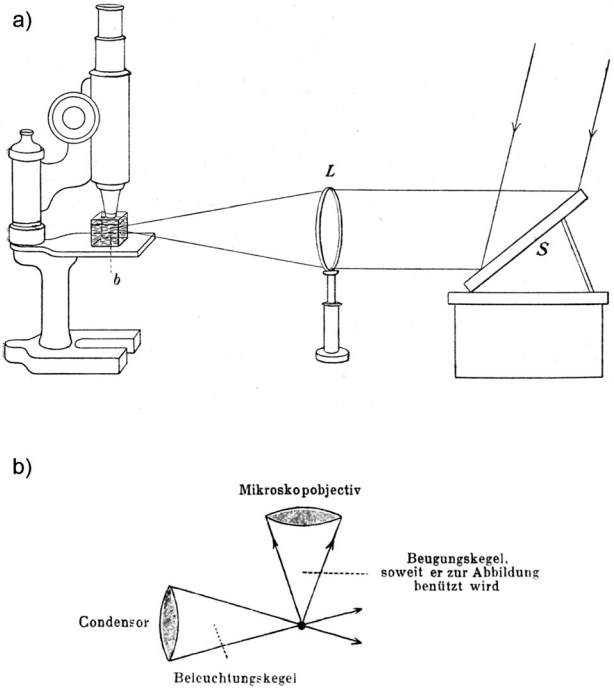

Figure 1. First setup for ultramicroscopical imaging. a) Illustration of the block of ruby glass (*b*) illuminated with direct sunlight passing through the lens (*L*).[11] b) Schematic of the condenser and the microscope objective used in the setup taken from the original publication.[11] The limited numerical aperture of the optics used is clearly visible.

For orthogonal-plane fluorescence optical sectioning (OPFOS) of biological samples, a similar configuration was re-invented in 1993.[21] High-resolution fluorescence microscopy, introduced in 2004, is based on another modification to the idea of Siedentopf and Zsigmondy: two independently operated lenses are used for illumination and detection to realize light sheet fluorescence microscopy (LSFM) and selective plane illumination microscopy (SPIM).[22,23]

Characterizing the solid-state samples motivated Zsigmondy to study gold hydrosols with the "slit-ultramicro-

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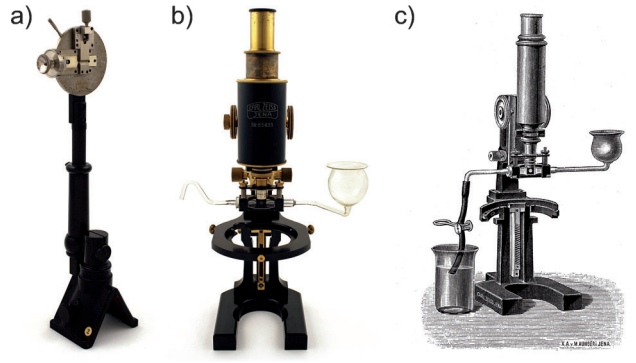

Figure 2. Slit ultramicroscope developed by Carl Zeiss Jena for observing nanoparticles in aqueous solutions. a) Adjustable slit for slit-ultramicroscopy on a rider for the optical bench, built in approximately 1910. b) Large research microscope equipped with a cuvette for ultramicroscopy. c) Engraving of a microscope with mounted cuvette from the company's leaflet in 1907.[24]

scope" (Figure 2)[24] mounted on an optical bench. The liquid to be examined was contained in a cuvette with two quartz glass windows. A slit (Figure 2a) was projected with a microscope objective through one window into the volume inside the cuvette (Figure 2b, Figure 2c), and a defined area was illuminated. A water-immersion microscope objective with a long working distance (40×, N.A. 0.75) was used to observe the Tyndall cones of the suspended nanoparticles (Figure 3a, Figure 3b). The concepts developed by Zsigmondy and Siedentopf's team were commercialized and could thus be rapidly and widely spread to the scientific community.[25]

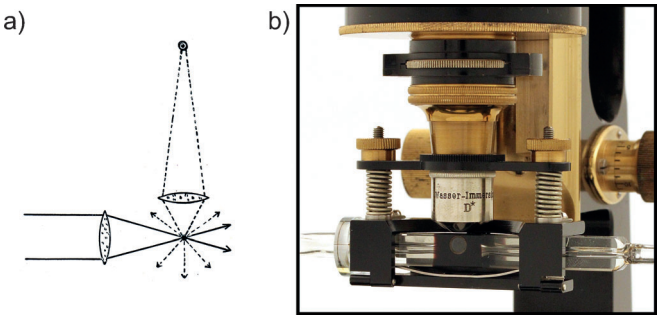

Figure 3. a) Sketch of the illumination from 1907.[24] b) Close-up photograph of the contemporary setup shown in Figure 2b: Water-immersion objective Zeiss D* (40×, N.A. 0.75) and mounted cuvette, the quartz window for illumination is clearly visible just orthogonal to the optical axis of the objective.

3. Nanoparticles in a Cuvette Formed by the Objectives

After Zsigmondy had been appointed to the chair of Inorganic Chemistry at the University of Göttingen in 1908, he contacted the local microscope manufacturer, Rudolf Winkel in Göttingen, to improve the ultramicroscopical equipment for studying smaller nanoparticles.[26] Over a three-year period, Zsigmondy developed the immersion ultramicroscope, jointly with Albert Winkel and Hermann

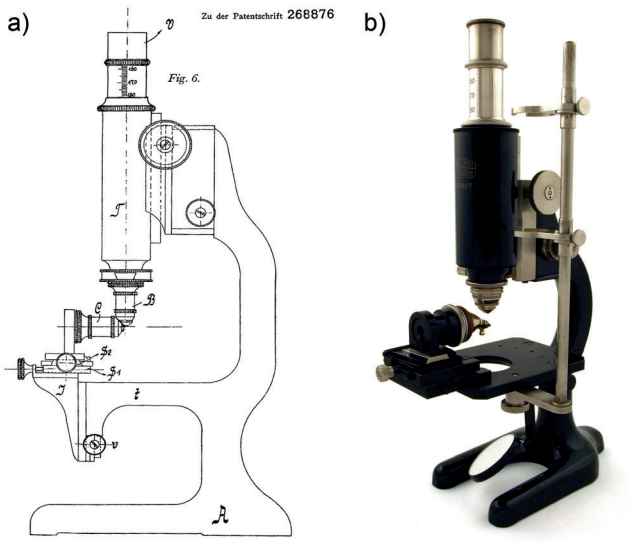

Figure 4. Immersion ultramicroscopes built by R. Winkel and Winkel-Zeiss Göttingen. a) Sketch of the original microscope stand as shown in the patent DRP 268876 from 1912.[28] b) Immersion ultramicroscope built in 1930 based on this patent, which we used for repeating the historical experiments.

Winkel, by designing an entirely new microscope stand (Figure 4a, Figure 4b) and two dedicated microscope objectives.[27] In 1912, a patent was granted for their invention, which was exclusively designed to examine nanoparticles in aqueous solutions.[28]

One of the main obstacles the inventors faced was the short working distance of immersion objectives with high numerical aperture. The mere mechanical mounting of standard lenses did not make it possible for one objective to focus on the image of the slit projected by the other objective, when both objectives were oriented along orthogonal axes. Thus, for each of the two introduced objectives, the quartz glass front lenses and mountings were partly removed by grinding and subsequent polishing on one side. Figure 5a shows the observation objective with the front lens partly cut at a 45° angle. When approaching the one objective, with its cut front lenses and mounting, relative to the other one (Figure 5b), just a shallow slit remained in between the two objectives. This slit could be used as an actual cuvette for the

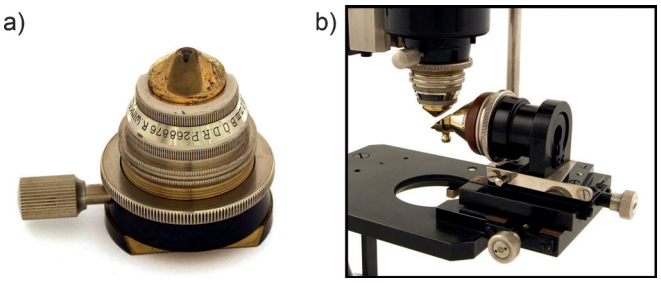

Figure 5. Immersion ultramicroscope by Winkel-Zeiss Göttingen. a) Water-immersion objective 6.2 mm, N.A. 1.05 to be used solely with this stand. The front lens and its mounting is partly cut at 45° and thus has an elongated shape. b) Close-up of the two objectives mounted on the microscope.

observation of "hanging droplets": the sample fluid served as the immersion liquid itself. Only one droplet was required for observation, enabling the characterization of samples that were rather limited in volume.

4. Historical Optical Experiments versus State-of-the-Art Technology

Because the scientific journals of Zsigmondy's time could not reproduce color photographs, nearly all descriptions of his findings were in written text only. As the first illustrations of his results were colored drawings not showing sufficient detail,[29,30] it is not possible to directly compare his findings with our own. However, we could make a qualitative comparison with one of his experiments, complementing his written descriptions on the optical examinations of the nanoparticles by 1) micrographs, 2) transmission electron microscope (TEM) image analysis, and 3) spectral analysis. Therefore we used the microscope illustrated in Figure 4b mounted on an optical bench. Because we could not obtain a contemporary carbon arc-lamp, nor the suggested clockwork heliostat (to use on a bright sunny day to simulate the same lighting conditions as in Zsigmondy's time), we used the halogen fiber optic illuminator Fiber-Lite MI 150 (Dolan-Jenner, Boxborough, MA, USA) as the light source instead. Additionally, an optical bench with spherical lenses was set up for beam shaping, and an adjustable slit was used for illumination. The optical illumination setup was based on the instruction manual for use of the microscope; it realized an image of the slit in the focal plane of the two objectives.[31,32] As samples, we used solutions of gold and silver nanoparticles of various shapes in the size range of 20–80 nm, suspended in water. We imaged their Tyndall cones directly with an EM-510 eyepiece camera (BigCatch, Torrance, CA, USA) instead of using the eyepiece and a plate camera.

In Figure 6 our comparison is depicted: In a colloidal silver solution prepared by the EDTA method,[33] we observed lively, colored particles mostly in green, but also some in blue and red, when imaged with the Immersion-Ultramicroscope (compare video in the Supporting Information). TEM characterization was used to determine the dimensions and shape of the silver particles. We found a mixture of particles mainly about 50 nm in size, containing spherical particles but also some in the shape of prisms. Since the 1990s there has been increasing interest in gold and silver nanoparticles, and numerous publications describe how the shape of the particles influences their surface plasmon resonance.[34] Single-particle spectroscopy can be used to correlate particle size and shape with their spectroscopic properties (color), even for rather heterogeneous solutions such as those studied by Zsigmondy.

Consistent with the historic publication, the color of the Tyndall cones depends on the shape of the actual nanoparticle. While Zsigmondy found no correlation between the colors of the Tyndall cones observed and the size of the nanoparticles (when considered to be cubes), he explained this effect by assuming that both the material and, in particular, shape would strongly influence the colors observed.[35] Zsigmondy described lively, moving nanoparticles in blue, violet, yellow, green, and red in Bredig's colloidal silver solution (3.8 mg Ag^{-1} 100 g^{-1} solution)[36]. He calculated the linear dimensions of the particles in this sample to be 50–77 nm, while assuming the nanoparticles would be cubic.[35]

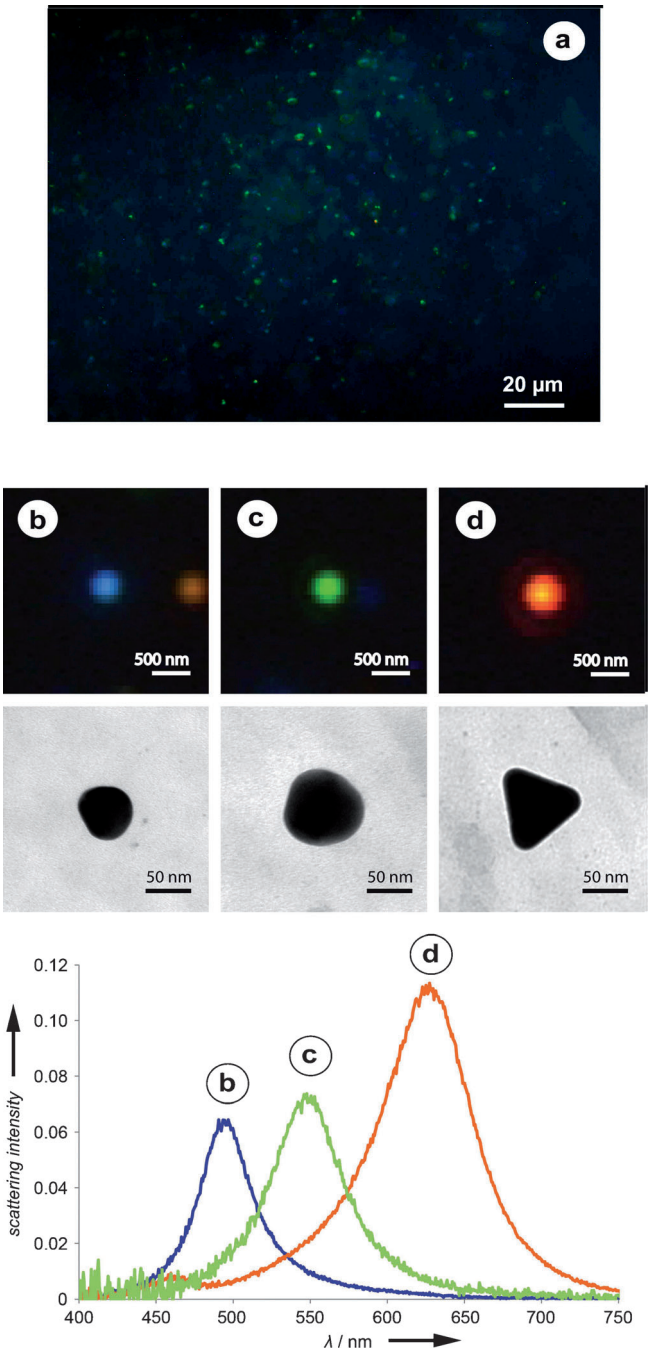

Figure 6. Colloidal solution examined. a) Ultramicroscopic image collected with the immersion ultramicroscope shown in Figure 4b, equipped with an eyepiece camera. b–d) Dark-field scattering micrographs (top row) and transmission electron micrographs (bottom row) of single silver nanoparticles together with the respective single-nanoparticle Vis spectra (bottom) for the spherical shape (b, c) and prismatic particles (d). For a video of the observations with the antique microscope see Supporting Information.

5. Summary

Zsigmondy could only describe the colors of the Tyndall cones of the moving nanoparticles in written text, and there was no technology available until many decades after his death to prove his assumptions to be correct. He was awarded the Nobel Prize for both his descriptions of the colloids and for the innovative methods he introduced and utilized to make the nanocosmos visible.

As a result of his inventions he was probably the first human being to observe nanoparticles moving by Brownian motion in solution, and to be able to control this process by changes in parameters such as concentration and particle coating. One might thus define this point in time as the foundation of the age of modern nanotechnology.

By applying original equipment of Zsigmondy's time and state-of-the-art technology for the characterization of a sample colloidal solution described by Zsigmondy,[35] we were able to compare directly the results obtained with the different methods on the same sample. We have confirmed both of his assumptions to be correct: 1) The size and shape of the nanoparticles are in the range he expected, 2) the spectra of the Tyndall cones match his verbal description. It is therefore remarkable that long before the fine structure of these infinitesimal particles had been elucidated in detail, Zsigmondy and his colleagues had correctly interpreted the results of their experiments, and in doing so laid the foundations for nanotechnology as we know it today.

After these inventions and the subsequent discoveries it took another two decades until Ernst Ruska (1906–1988) and Max Knoll (1897–1969) introduced the transmission electron microscope in 1932.[37] Ruska and colleagues soon were able to push the resolution limit of electron microscopy (EM) to 10 nm,[38] although no liquid media could be observed with this technology.

Zsigmondy and colleagues were studying nanoparticles in motion. Today's ultramicroscopy has reached the resolution of atomic-scale motions by the application of ultrafast EM as four-dimensional electron microscopy, recently reviewed in detail by Ahmed H. Zewail.[39] The light-sheet microscopy introduced by Zsigmondy and Siedentopf has evolved into OPFOS, LSFM, and SPIM, widely used in biological research.[21–23] Today, the reconstruction of images in far-field microscopy makes molecular-scale resolution feasible,[40–42] and microscopy has evolved to nanoscopy.[43]

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