Chapter 10
Richard Zsigmondy and Henry Siedentopf's Ultramicroscope

10.1 Introduction

The concept that led to the invention of the ultramicroscope, a new type of dark-field microscope designed for the investigation of colloids, in 1903 by Richard Adolf Zsigmondy (1865–1929) is not contested (Cahan, 1996). Zsigmondy is credited with the concept of the ultramicroscope and its application to the field of colloid chemistry; he tested the ultramicroscope with various colloids and devised the technique in which the ultramicroscope is used to measure the size of ultra-microscopic colloidal particles (Cahan, 1996). Zsigmondy sometimes worked independently and other times in collaboration with the physicist Henry Friedrich Wilhelm Siedentopf (1872–1940) of Zeiss Werke. What was Siedentopf's role? He constructed the prototype ultramicroscope and subsequent versions and optimized their optical performance (Cahan, 1996).

Their collaboration is significant for several reasons. First, this is a success story involving collaboration between two researchers with disparate expertises. This is an example of the enormous benefits that can follow from interdisciplinary collaboration. Second, the microscope was conceived, designed, and constructed to solve a specific problem: how to study submicroscopic colloids. Colloids were discovered in 1861 by Thomas Graham. A colloid, or a colloidal dispersion, consists of large molecules or submicroscopic particles of one type that are dispersed in a second substance. Examples include colored glass, clouds, smoke, milk, and gelatin. Individual colloidal particles have diameters between 1 and 1000 nm. Third, the ultramicroscope does not image individual colloidal particles; it makes possible their localization and visualization. Localization is different from imaging. With the ultramicroscope colloidal particles of dimensions below the Abbe and Helmholtz limits of resolution in a light microscope can still be observed (i.e., visualized but not imaged). Siedentopf used the words "rendering visible" in his publications (Siedentopf, 1903). Fourth, the invention of the ultramicroscope is the antecedent of the modern technique of light-sheet fluorescence microscopy that I

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describe in Chapter 11 (Huisken et al., 2004; Stelzer and Lindek, 1994; Voie, Burns, and Spelman, 1993).

I begin with a biographical introduction to the lives and works of Zsigmondy and Siedentopf (Cahan, 1996; Sönnichsen and Fritzsche, 2007; Zsigmondy, 1925). Zsigmondy was born in Vienna a year before Graham discovered colloids in 1865. The study of colloids was inextricably connected to Zsigmondy's interests and his professional life. His early interdisciplinary studies in chemistry and physics set the foundation for his future research in colloid chemistry. However, he majored in organic chemistry at the Technische Hochschule in Vienna and then at the Technische Hochschule in Munich. His doctorate in organic chemistry was awarded in 1889 by the University of Erlangen (Sönnichsen and Fritzsche, 2007) (Fig. 10.1).

A remarkable change in his interests occurred between 1891 and 1892 under his mentor Professor August Kundt at the University of Berlin when he became fascinated with inorganic chemistry and thus began his lifelong work on colloid chemistry. What was the connection between inorganic chemistry and colloid chemistry? The answer is glass; specifically, gold red glass. What interested Zsigmondy was the nature of the gold particles in gold red glass: were they colloidal particles? Other researchers claimed that the gold particles were in suspension. His journey from organic chemistry to inorganic chemistry and the study of gold red glass developed during his Habilitation work at the Technische

Fig. 10.1 Richard Adolf Zsigmondy

Hochschule Graz, which allowed him to become a lecturer in glass technology. His knowledge of glass and the production of colored glass led him to work at the Schott glass factory where he studied gold red glass for seven years. It was while Zsigmondy worked at the Schott glass factory that he met Siedentopf. After leaving the Schott glass factory Zsigmondy worked in Jena as an independent colloid chemist.

The then emerging field of colloid chemistry had important applications to various industries despite much controversy over the nature of colloidal particles and their roles in producing the wide variety of observable phenomena. As far as Zsigmondy was concerned chemical analysis of colloids had reached a dead-end; therefore, he reached out to physical techniques (in particular, optical techniques such as microscopy). Nevertheless, the standard bright-field light microscope could not resolve individual colloidal particles as they were below the resolution limits derived in 1873 by Abbe and in 1874 by Helmholtz.

What followed next is a lesson in the process of discovery in science. Success in research often depends on asking the right questions. This involves formulating questions in such a way that they can be experimentally addressed. Often this drives the invention and development of new instrumentation.

Zsigmondy cogently formulated several critical questions (Cahan, 1996). First, what is the size and composition of the gold particles? How many atoms compose one particle? Second, he asked a question about the scattered light that forms the Faraday–Tyndall light cone present in all colloidal solutions (van de Hulst, 1957). Zsigmondy asked whether the Faraday–Tyndall light cone is specific to gold particles in solution (van de Hulst, 1957). In that way he could experimentally answer the contentious question: are the gold particles in glass a colloidal solution or a suspension? His initial experiments using standard light microscopes failed; what was required was a microscope that could "render visible" the scattered light of the light cone from individual gold particles. From his preliminary analysis of the distance between individual gold particles he concluded that this distance was less than the wavelength of the incident light. Zsigmondy could "render visible" particles that exceeded the resolution limits given by both Abbe and Helmholtz. In the next section I describe the design, development, and applications that followed on from these inchoate investigations.

In 1905 Zsigmondy published his seminal book *Zur Erkenntnis der Kolloide. Über irreversible Hydrosole und Ultramikroskopie* (Zsigmondy, 1905). This work summarized his investigations into the nature of colloids and the ultramicroscope. Zsigmondy departed Jena in 1907 and settled in Göttingen as an associate professor at the University of Göttingen. The following year he became director of the Institute of Inorganic Chemistry.

In 1919 Zsigmondy became a full professor. The Nobel Prize in Chemistry 1925 was awarded to Zsigmondy (in 1926) "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" (Zsigmondy, 1926). Zsigmondy used his interdisciplinary knowledge of chemistry and physics to invent new instrumentation and to apply new techniques to advance colloid chemistry (Sönnichsen and Fritzsche, 2007).

Next, I present some information about Zsigmondy's collaborator Henry Friedrich Wilhelm Siedentopf (1872–1940). Siedentopf is known for his work in connection with the 1904 invention of the ultraviolet microscope by August Köhler and Moritz von Rohr at Zeiss Werke. Their motivation was to increase the resolution of the light microscope by reducing the wavelength of illumination. Shortly afterward Siedentopf made an amazing discovery: the ultraviolet light used for illumination in the ultraviolet microscope caused the object to fluoresce. But the fluorescence diminished the contrast of the image of the object! This observation led in 1908 to the development of a prototype fluorescence microscope by Köhler and Siedentopf at Zeiss Werke.

A short biographical introduction to Siedentopf is appropriate (Sönnichsen and Fritzsche, 2007). Siedentopf studied physics at Leipzig University and received his doctorate from the University of Göttingen. From 1899 to 1938 he worked at Zeiss Werke in Jena where he became director of the microscopy division in 1907. Both Siedentopf and Zsigmondy independently studied the Tyndall light cone from colloids (Sönnichsen and Fritzsche, 2007). Their collaborative research led to the development in 1902 of a new type of light microscope: the ultramicroscope. The ultramicroscope could "render visible" submicroscopic colloidal particles that were seen as points of light. Further developments led to the invention of their immersion ultramicroscope in 1913 and numerous advances and discoveries in the field of colloid science (Siedentopf, 1903; Siedentopf and Zsigmondy, 1902; Zsigmondy, 1905, 1907, 1909, 1913, 1920).

10.2 The Ultramicroscope: Design, Development, and Applications

The principle that is fundamental to the ultramicroscope is easily understood by analogy. A beam of sunlight enters a small hole in a dark room. The observer is positioned to observe the light beam from a direction that is perpendicular to the direction of propagation of the light beam. When small particles of dust in the air are transported across the light beam the observer will see very small spots of light that result from dust particles scattering the incident light.

In Zsigmondy's initial experiments the object was a cube of glass with gold colloidal particles dispersed within the glass. The source of illumination was the sun. A heliostat was used to track the relative movement of the sun and the earth. A lens was used to focus sunlight on a very small area within the glass cube. The glass cube was observed with a standard upright light microscope. The optical axis of the light microscope was perpendicular to the axis of illumination to maintain the critical constraint that no illumination light would directly enter the optical axis of the microscope. Zsigmondy was able to observe the light cone due to light scattered from individual colloidal particles (Zsigmondy, 1926).

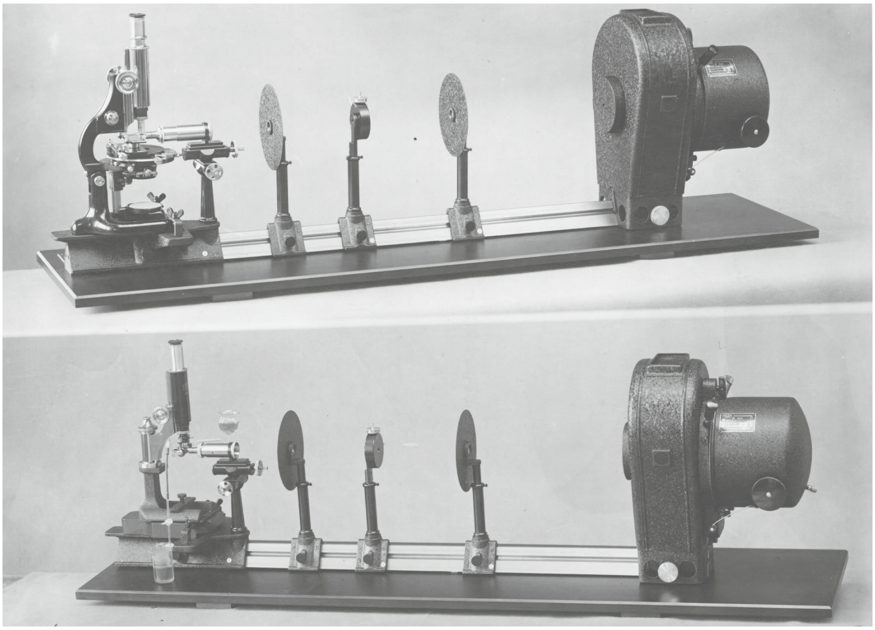

Fig. 10.2 Slit ultramicroscope after Siedentopf and Zsigmondy. Reproduced with permission from the ZEISS Archives

As Zsigmondy explained in his Nobel Lecture, it was the motivation to improve his first prototype of the ultramicroscope that led him to collaborate with Siedentopf at Zeiss Werke.

The next version of the ultramicroscope (shown in Fig. 10.2) was the slit ultramicroscope. It incorporated several changes that improved the capability of observing Tyndall light cones from individual colloidal particles. Optical components from the light source to the upright light microscope were mounted on an optical bench to provide stability. The light source was an arc lamp. A telescope objective focused the image of the light source onto an adjustable slit and a microscope objective acted as a condenser to focus the image of the slit onto the colloidal solution that was in a small dish. The optical axis of the upright microscope was perpendicular to the optical axis of the illumination system and the optical bench maintained mechanical stability.

Using the new slit ultramicroscope Zsigmondy determined the size of gold particles in both solution and in glass, the gold content of the solution was known (Zsigmondy, 1926). Furthermore, in his Nobel Lecture Zsigmondy explains that several improvements were made in the design of different versions of the ultramicroscope. He credits Siedentopf with the design of a paraboloid and a cardioid condenser, Reichert with the design of a mirror condenser, and Ignatowski and Jentzsch with the design of a ball condenser (Zsigmondy, 1926). In 1912 he invented the immersion ultramicroscope to investigate colloids in liquids (Mappes et al., 2012). The immersion ultramicroscope was the result of collaborative work between Zsigmondy and the Göttingen microscope company Rudolf Winkel (Mappes et al., 2012; Zsigmondy, 1926).

Further insight into the instrumentation and applications of the ultramicroscope are contained in two publications by the coworkers in the development of this new type of microscope (Siedentopf, 1903; Siedentopf and Zsigmondy, 1902). In their joint paper Siedentopf and Zsigmondy introduced a new technique to visualize particles smaller than the Abbe and the Helmholtz resolution limits for diffraction-limited light microscopes (1902). To implement their technique the optical system had the following requirement: the diffraction cone of the microscope objective oriented vertically along the optical axis of the microscope and perpendicular to that axis is the horizontally oriented illumination cone of the condenser (Siedentopf and Zsigmondy, 1902). In other words, the light from the condenser light cone is unable to enter the microscope objective and propagate to the eye of the observer. But light from the particle's diffraction light cone can enter the microscope objective and thus "render visible" submicroscopic colloidal particles.

In subsequent sections of their publication Zsigmondy described his technique to determine the diameters of colloidal particles using the slit ultramicroscope. Zsigmondy then applied his new technique to investigate the question: what is the relationship between the diameter of the gold particles and the color of gold red glass? There was no correlation in his preliminary studies.

The authors made a prescient claim in their paper: they proposed that their invention of the slit ultramicroscope was ideally suited to the investigation of Brownian motion, discovered in 1828 by Robert Brown, in liquids (Siedentopf and Zsigmondy, 1902). Einstein and Smoluchowski (independently) derived theoretical models of Brownian movement based on mean square displacements of particles (Einstein, 1905, 1906; Smoluchowski, 1906). Experimental validation of the independent theories of Einstein and Smoluchowski on Brownian motion is the work of Jean Perrin who also determined an accurate value for Avogadro's number (Perrin, 1909). A major effect of Perrin's experimental work on Brownian motion was that it gave strong experimental support to the claim that atoms existed.

In 1903 Siedentopf published his seminal paper "On the rendering visible of ultra-microscopic particles and of ultra-microscopic bacteria" (Siedentopf, 1903). Two things that have great pedagogical value struck me on reading the original paper. First, the degree of caution in the writing is exceptional. Second, Siedentopf's prescient suggestion that the ultramicroscope may be useful in imaging cells and tissues is impressive. The author clearly gives words of caution on the capabilities of the ultramicroscope. He states that the ultramicroscope does not "render visible" the correct shape and size of submicroscopic colloidal particles. Although the image of an ultramicroscopic particle is always a small diffraction disk, the author proposes that a perfected version of the ultramicroscope may be useful for bacteriologists to "render visible" bacteria that are so far unknown.

The invention of the ultramicroscope led to advances in the new science of colloidal chemistry, to validation of the theories of Brownian motion that gave strong support for the existence of atoms, and to the modern development of light-sheet microscopy.

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