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Dark Field Microscopy for Analytical Laboratory Courses

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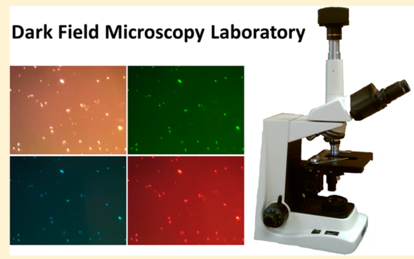
Supporting Information

ABSTRACT: An innovative and inexpensive optical microscopy experiment for a quantitative analysis or an instrumental analysis chemistry course is described. The students have hands-on experience with a dark field microscope and investigate the wavelength dependence of localized surface plasmon resonance in gold and silver nanoparticles. Students also observe and measure individual crystal growth during a replacement reaction between copper and silver nitrate. The experiment allows for quantitative, qualitative, and image data analyses for undergraduate students.



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It is important to give undergraduate chemistry students exposure to current research techniques being used in the field to prepare them for industry and academic research laboratory work.[1] Undergraduate laboratory experiments for conventional separation and spectroscopy techniques have been well documented and implemented. However, optical microscopy has received little attention in undergraduate curriculum,[1] even though it has received an increase in interest in analytical chemistry and related fields due to the rise of nanoparticle research.[2,3]

Currently, there are few student laboratory experiments using optical microscopy. In the published experiments, the optical microscope has only been used as a platform for studying chemical reactions. For example, Thiessen and Beste published an experiment in which students used a polarized light microscope to look at electrolysis reactions of alkali metals and explore the effects of temperature and electrode potential.[4] Winokur et al. employed a dissecting microscope so students could study reactions between magnesium and hydrochloric acid where magnesium particles are active with Brownian motion as hydrogen gas is formed; a second reaction between copper metal and silver nitrate allowed for the observation of crystal growth.[5]

Most recently, Flowers used optical microscopy by employing the microscope as a microscpectrophotometer. Students were able to explore the effects of path length, effective spectral aperture, transillumination, stray light, and various magnification settings.[6] Others have used low magnification light microscopes to study fusion reactions in which a flux was performed to detect cations and anions in solution,[7] and studied the reactivity of metals such as calcium, silver, and magnesium using replacement reactions.[8]

The experiment described here introduces students to the dark field microscope, an instrument commonly used in analytical and nanomaterials research. There are three parts to the experiment. First, students work with a dark field microscope to watch and measure crystal growth of a replacement reaction between copper wire and a silver nitrate solution. Second, students examine three types of nanoparticles with different bandpass filters. Third, students investigate what makes a "good" filter while simultaneously looking at nanospheres of different size (100−200 nm) and composition (gold, silver, and silica) in an attempt to compare the particles' light-scattering properties. The experiment was successfully studied with students who had completed quantitative analysis and instrumental analysis courses. Students took 2 h to complete the experiment while working individually.

■ BACKGROUND

Dark Field Microscopy

Dark field microscopy relies on the principles of light scattering. This is accomplished by illuminating the sample with light at oblique angles due to a light stop in the center of the condenser. The blocking of the direct light to the sample and the creation of the large light angles creates a dark background on which the sample appears bright. Anything that is able to scatter light located on the sample plane will scatter light, which is then collected by the objective; only light scattered in the direction of the objective is detected. Once the light is collected by the objective, it is directed toward a charge-coupled device (CCD) where it is then converted into a digital signal. The signal is then sent to a computer that can project the data as a visual image.[9,10] The working principles of a dark field microscope are shown in Figure 1.

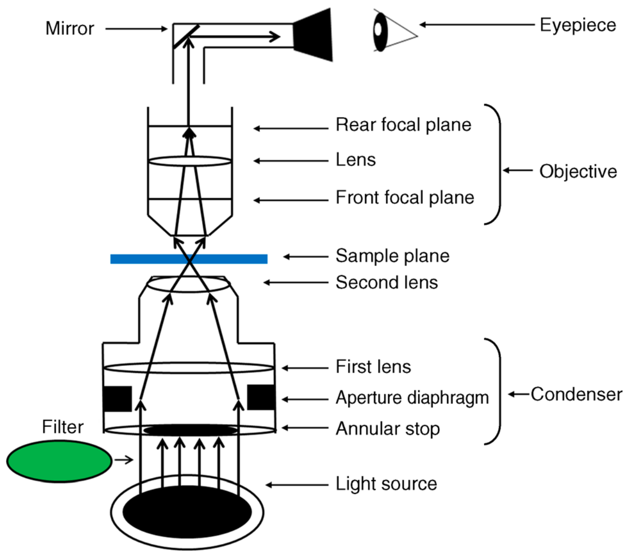

Figure 1. Schematic of a dark field microscope with the light path shown.

Nanoparticles and Plasmons

Nanoparticles are defined as any structure between 10 and 100 nm. Interestingly, the optical properties of nanoparticles are typically quite different than their bulk counterparts, due to their small size. Noble metal nanoparticles have a signature optical property known as localized surface plasmon resonance (LSPR). When a nanoparticle is irradiated with specific wavelengths of light, its electron cloud will oscillate. This enables the nanoparticle to scatter and absorb light. The LSPR wavelength and intensity is material dependent as well as reliant on the dielectric value of the surrounding medium. Gold spheres have an LSPR at approximately 550 nm, whereas silver spheres have an LSPR at approximately 500 nm.[11]

■ METHODS AND RESULTS

Materials and Chemicals

The OMAX dark field microscope (model no.: M834-A191) and digital CCD camera (model no.: A35140U) were purchased from microscopenet.com (Tustin, CA). The silver nitrate and copper wire (16 AWG) were purchased from Fisher Scientific (Pittsburgh, PA). All of the nanoparticles were purchased from Sigma-Aldrich (St. Louis, MO): gold nanospheres (3.8×10^9 particles/mL; 100 nm diameter; stabilized suspension in citrate buffer); silver nanospheres (3.8×10^9 particles/mL; 100 nm diameter; stabilized suspension in citrate buffer); and mesoporous silica nanospheres (200 nm diameter). The silica spheres were suspended in de-ionized water with a concentration of 0.01 mg/mL.

Filters were bandpass filters with 70% transmittance and variable wavelength ranges: 580−660, 390−540, 350−590, 300−600, and 510−590 nm. The glass microscope slides (3 in. × 1 in., 1.0 mm thick) were purchased from Electron Microscopy Science (Hatfield, PA). Coverslips (22 mm × 22 mm) were purchased from Daigger (Vernon Hills, IL). The clear nail polish (Sally Hansen; 4860-01 Invisible) was purchased from Wal-Mart.

Part 1: Growth of Silver Crystals

Students placed a 0.5 cm piece of copper wire on a glass slide that had been placed on the microscope stage. Students were instructed to check the alignment of the condenser and objective by way of setting the Köhler illumination. This ensured even illumination and optimized the spatial resolution. The copper wire was then brought into focus, and a few drops of a 0.0125 M silver nitrate solution were added to the copper wire. As the silver crystals grew, students brought the crystals into focus, rather than the copper wire, and took an image of the crystals every 30 s over the course of the reaction, approximately 2 min. The reaction was run a few times with variable gain and exposure time settings.

After the reaction had taken place, students were able to measure the length of individual silver crystals with the aid of ImageJ, a free software package available for download from the National Institute of Health. The length of individual crystals was then graphed over time. Student-collected data from this section of the experiment are shown in Figure 2.

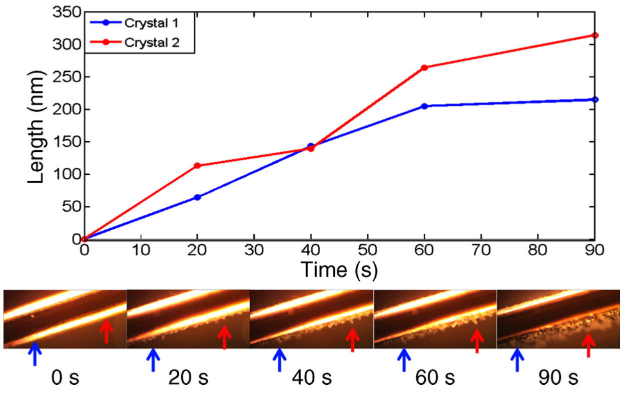

Figure 2. Copper wire and silver nitrate replacement reaction. As the silver crystals grew, students were able to image the growth and then graph the length of individual crystals. Crystals 1 and 2 are pointed out by the blue and red arrows, respectively.

Part 2: Nanospheres and Bandpass Filters

In the second section of the experiment, students made three samples of nanospheres (gold, silver, and silica) to view under the microscope. Each sample was prepared by casting 6 μL of a nanosphere solution onto the slide. The sphere solution was then covered with a glass coverslip, and the edges of the coverslip were sealed with clear nail polish. The nail polish was allowed to dry for approximately 30 min. After a slide was prepared, it was placed on the stage and the spheres were brought into focus; the Köhler illumination was checked again to ensure proper alignment of the condenser and objective.

Each sample was imaged without a filter and followed subsequently with the five filters. Each time a filter was placed in the light path between the light source and condenser, the quantity of light passing through the microscope was limited. Therefore, the students had to reset the gain and exposure time to obtain the cleanest image for each sample with each filter. This caused the students to investigate the relationship between gain and exposure time, the wavelength dependence of plasmons for each type of sphere, and spatial resolution. Student data from this section of the experiment are shown in Figure 3.

Part 3: Assessment of Bandpass Filters

After each nanosphere sample was imaged, students were asked to decide which filters have the cleanest image for each type of sphere. They were then asked to take a transmission spectrum

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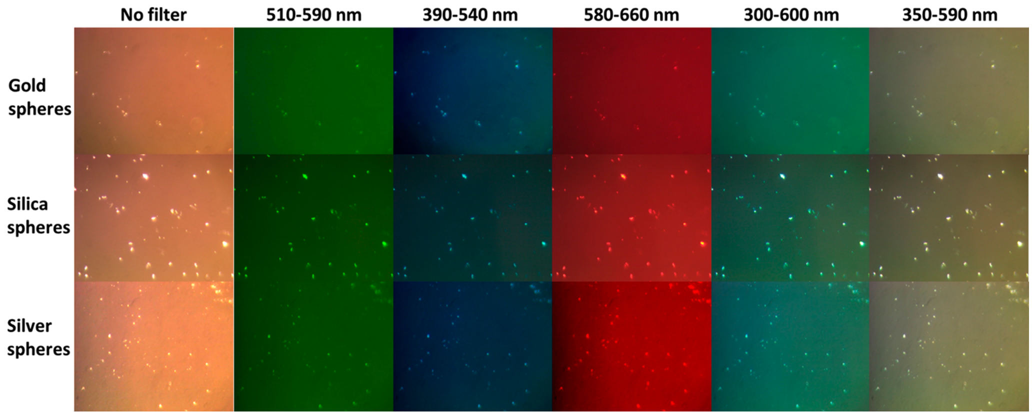

Figure 3. Gold, silica, and silver nanospheres imaged with and without bandpass filters. Students were able to see how different wavelengths of light excite the plasmons in the nanostructures. The composition of the nanostructure affects the spectral position of the LSPR.

of the three filters to determine, more accurately, the wavelength ranges of each filter they chose. The students were then given the absorption spectra of each sphere type and they were asked to find the relationship between the LSPR of each sphere sample and the corresponding filter.

Lab Report

After the students completed the lab experiment, they wrote a lab report, which consisted of the data the students obtained from the experiment, analysis questions for each section of the experiment, and post-lab questions. In part one, the students analyzed the images of the silver crystal growth on copper wire by measuring and graphing the length of the crystals over time; the crystals' length was measured with ImageJ. In the second and third sections, students were required to take images of each nanomaterial and UV−vis spectra of filters used to take images of nanomaterials. The students were required to turn in the spectra of the filter(s) that they thought gave the cleanest image of each material, with a written justification of why they chose that filter. To help them with this decision, they were given analysis questions. The analysis questions at the end of each part of the experiment helped the students reflect on what they did and to interpret their data.

HAZARDS

Silver nitrate can cause respiratory, eye, and skin irritation. Gold, silver, and silica nanoparticles can cause skin irritation if absorbed through skin; gloves should be worn to avoid contact. Eye and respiratory tract irritation can also occur if nanoparticles are inhaled. Nanoparticles may also be harmful if they are ingested.

CONCLUSION

In this laboratory experiment, students were given a dark field microscope, which allowed them to watch crystal growth during a replacement reaction between copper wire and a silver nitrate solution. Students also looked at nanospheres and their wavelength dependency.

This experiment gave students an opportunity to work with an instrument commonly used in analytical research laboratories, and it also provided students experience with experimental design that is currently being performed in analytical research. This dark field microscopy experiment is also an innovative and inexpensive experiment that can be implemented easily into any analytical or instrumental analysis laboratory curriculum.

ASSOCIATED CONTENT

Supporting Information

The student lab manual designed experiment for an instrumental analysis course. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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