Spectroscopy with a Light Optical Microscope

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Introduction

The microspectrophotometer can be described as a type of hyphenated instrument: it is a hybrid that combines the magnifying power of a light microscope with a UV-visible-NIR (ultraviolet–visible–near infrared) range spectrophotometer. These instruments are used to measure the molecular spectra from microscopic samples, from the deep ultraviolet to the near infrared region. Microspectrophotometers can be configured in many different ways and used to measure absorbance, reflectance, and even emission spectra, such as fluorescence, of sub-micron-sized sample areas. With the addition of specialized algorithms, the microspectrophotometer can also be used to measure the thickness of thin films or to act as a colorimeter for microscopic samples.

There are many reasons to use such tools. The most obvious is that spectra can be acquired from microscopic sample areas. Additionally, these instruments require only small amounts of samples in either solid or liquid form. Another advantage is that very little or no preparation is required for many samples. Also, color comparisons by spectroscopy tend to be more accurate with spectrophotometers because these instruments have a broader spectral range, can correct for lighting variations, and can measure the intensity of each wavelength band of light.

Before the advent of microspectroscopy, the only way to analyze many types of microscopic samples was to use micro-chemical testing and then some sort of visual examination. Unfortunately, this testing tends to be destructive, requires a considerable volume of sample, and suffers from the inaccuracies of the human visual system. UV-visible-NIR microspectroscopy avoids these issues and is much faster. Microspectrophotometers can also "see" beyond the range of the human eye and can detect variations that would not be apparent by eye. On a practical note, it is easier and more accurate to compare numerical data rather than just colors.

Microspectrophotometer Design

The microspectrophotometer integrates a light microscope with a spectrophotometer (see Figure 1). The microscope is an instrument designed to enlarge an image of small objects, which allows them to be studied easily. The spectrophotometer is a device that measures the intensity of various wavelengths of light. With the UV-visible-NIR microspectrophotometer, a scientist or engineer is able to acquire absorbance, reflectance, and emission spectra with sampling areas on the micrometer scale.

In order to cover such a broad spectral range while maintaining good image and spectral quality, a special microscope must be built and integrated with the spectrophotometer. Standard optical microscopes have a limited spectral range, covering only a portion of the visible region because of the materials used for the optics as well as the light sources. The modern UV-visible-NIR range microspectrophotometer uses a custom-built microscope with an optical design and light source optimized for the deep UV through the NIR.

The spectrophotometer itself must be designed for microspectroscopy in order to obtain good spectral results. It must have a high dynamic range because users commonly switch from transmission or reflectance spectroscopy to fluorescence spectroscopy when measuring the same sample. This means that the spectrophotometer must be highly sensitive while still maintaining an acceptable spectral resolution. Stability is also an issue because the microspectrophotometer is a single-beam instrument, so reference spectra are obtained prior to measuring the sample.

Integration of the spectrophotometer with the microscope is critically important. Although the microscope and spectrophotometer must both be optimized for microspectroscopy, the key to acceptable microspectrophotometer operation is the hardware that enables them to work together.

Figure 1: A microspectrophotometer integrated with a microscope can analyze the UR-visible-NIR region.

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The microscope-spectrophotometer interface has several basic requirements. Most importantly, it must funnel the electromagnetic energy collected by the microscope from the sample into the spectrophotometer. The user must be able to visualize the sample measurement region but also see the surrounding sample. This is done by having the entrance aperture of the spectrophotometer at the same focal plane as the sample image. The sample may then be moved until the image of the entrance aperture is over the area to be measured. In Figure 2, the black square in the center of the image is the entrance aperture of the spectrophotometer. All this is done in real time so that the spectroscopy of the sample is as quick and easy as the microscopy.

As shown in Figure 3, the microscope optics focus electromagnetic energy onto the sample. Those photons interact with molecules in the sample, and the light from the sample is collected by the microscope objective. From the objective, the light is focused onto the entrance aperture of the spectrophotometer, which has a mirrored surface. The majority of the light is reflected from the entrance aperture surface onto the camera. The spectrophotometer aperture is also imaged by the camera so that it appears as a black square on the sample (see Figure 2). This enables rapid alignment of the microspectrophotometer. The light that passes through the entrance aperture then passes into the spectrophotometer where a spectrum measured.

The microscope can be configured with different illumination schemes depending on the type of experiment to be performed. Incident illumination with white light allows for reflectance microspectroscopy from the deep UV to the near IR. Also, appropriate illumination can be employed for fluorescence or phosphorescence microspectroscopy. In addition, transmission microspectroscopy is possible with a white light focused onto the sample through the microscope condenser.

Applications of Microspectroscopy

The first microspectrophotometers were developed in the 1940s, and since then a host of different applications have been developed. With the ability to acquire spectra from microscopic sample areas, microspectrophotometers are used everywhere from university laboratories to production lines for quality control and failure analysis. The following is a brief summary of some of the more established applications.

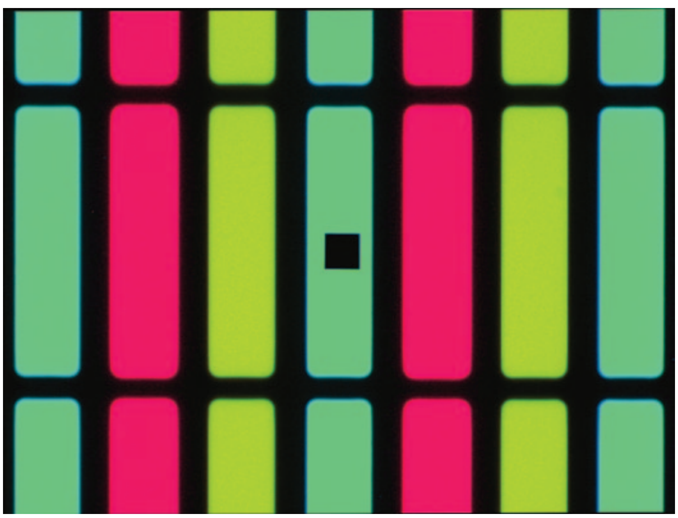

Figure 2: When imaging a sample with a microspectrophotometer, the black square in the center of the image is the entrance aperture for the spectrophotometer. The user simply moves the sample stage so the black square is superimposed over the area to be measured as with this organic light emitting diode (OLED) display.

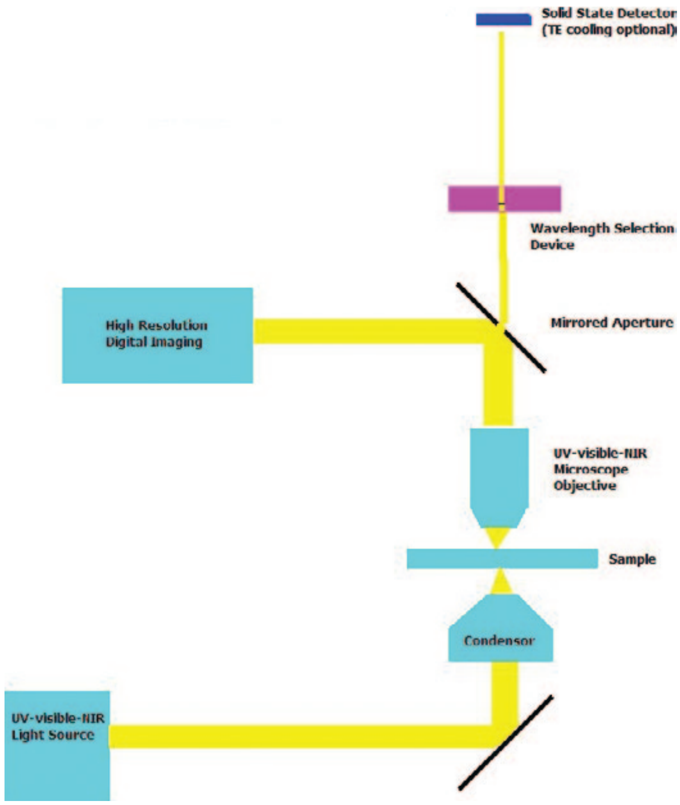

Figure 3: Schematic showing the optical path for a microspectrophotometer configured for absorbance microspectroscopy and imaging.

Forensic science. The analysis of forensic evidence has been one of the most important applications for microspectrophotometers since the early 1980s. The greatest effort was in the analysis of trace evidence, specifically textile fibers and paint chips [1, 2]. As their names suggest, these types of samples are usually microscopic and, being evidence, should not be damaged or destroyed by testing. With fibers, microspectrophotometers are used to measure the UV-visible-NIR absorbance and fluorescence spectra of individual fibers. Paint chips are usually cross-sectioned, and then the absorbance spectrum of each layer is measured so that known and evidence samples may be compared with a high degree of discrimination. Questioned documents are another area where forensic science uses microspectrophotometers. Here, both the inks and papers are analyzed in a number of ways. Traditionally, they are analyzed by reflectance microspectroscopy because most papers are too thick to pass much light. Additionally, the fluorescence spectra may be acquired. Microspectroscopy is important for document analysis because the ability to measure such small features as individual pen strokes, micro printing, and even security features is essential.

Small-spot thin-film measurement. Microspectrophotometers have been built specifically to measure the thickness of thin films [3] used in the manufacture of integrated circuits.

The advantage of using the microspectrophotometer is that it is quick, non-contacting, and non-destructive. It can also measure the thickness of multiple film layers of micrometer-scale sample areas, which can be especially important when trying to measure the film thickness of patterned circuits with today's microscopic critical dimensions.

Measuring thin-film thickness begins with the microspectrophotometer acquiring either a reflectance or a transmission spectrum—depending on whether the substrate is transparent or not—of a small sampling area. Interference from the films, similar to the effect seen with an oil film on water, is then measured. Special software is then used to calculate the thickness of the films deposited on the substrate. An important part of quality control testing in the semiconductor industry, small-spot film thickness measurements are now used in the development and manufacture of optics, flat panel displays, and many other devices.

Energy. Coal and petroleum source rock contain vitrinite and other macerals. Microspectrophotometers are used to grade the thermal maturity [4], and therefore the energy content, of coal and kerogen. Traditionally this is done by measuring the absolute reflectivity of vitrinite on a polished sample. Depending on the reflectivity, the thermal maturity of the sample may be determined.

Materials science. Microspectrophotometers have been used in many types of research in materials science [5, 6] because of their ability to measure transmission, reflectance, and emission spectra with the same instrument. For example, microspectrophotometers are used in the development and characterization of nanoparticles of gold and silver. The ability to measure the optical effects from surface plasmon resonance is important in the development of these materials with their novel optical properties (see Figure 4). Microspectrophotometers are critical in the development of new film-type materials, whether they be polymers or more exotic films such as diamond. These instruments are used not only for their ability to measure the thickness of the film, but because they can characterize the materials themselves from their emission spectra.

Microfluidics and biotechnology. The microspectrophotometer is essential in the development of new microfluidic devices, because they have the flexibility to make different types of measurements from small test points anywhere on the device. Microspectrophotometers are also used in vision research to monitor changes in the chemistry of the eye. This is done by measuring the spectral response of microscopic portions of the eye both before and after exposure to light. Other applications are even more exotic, such as the study of the optical effects from individual barbules of bird feathers to the mapping of butterfly wings. These last two examples have yielded a number of important insights not only into biology, but also into fundamental optics.

Conclusion

The microspectrophotometer is a device that melds the microscope with a spectrophotometer to acquire spectra of microscopic sample areas. Such instruments are capable of absorbance and reflectance spectra from the deep UV through the visible and into the NIR regions. The microspectrophotometer can also measure fluorescence and other types of emission spectra. These devices have found

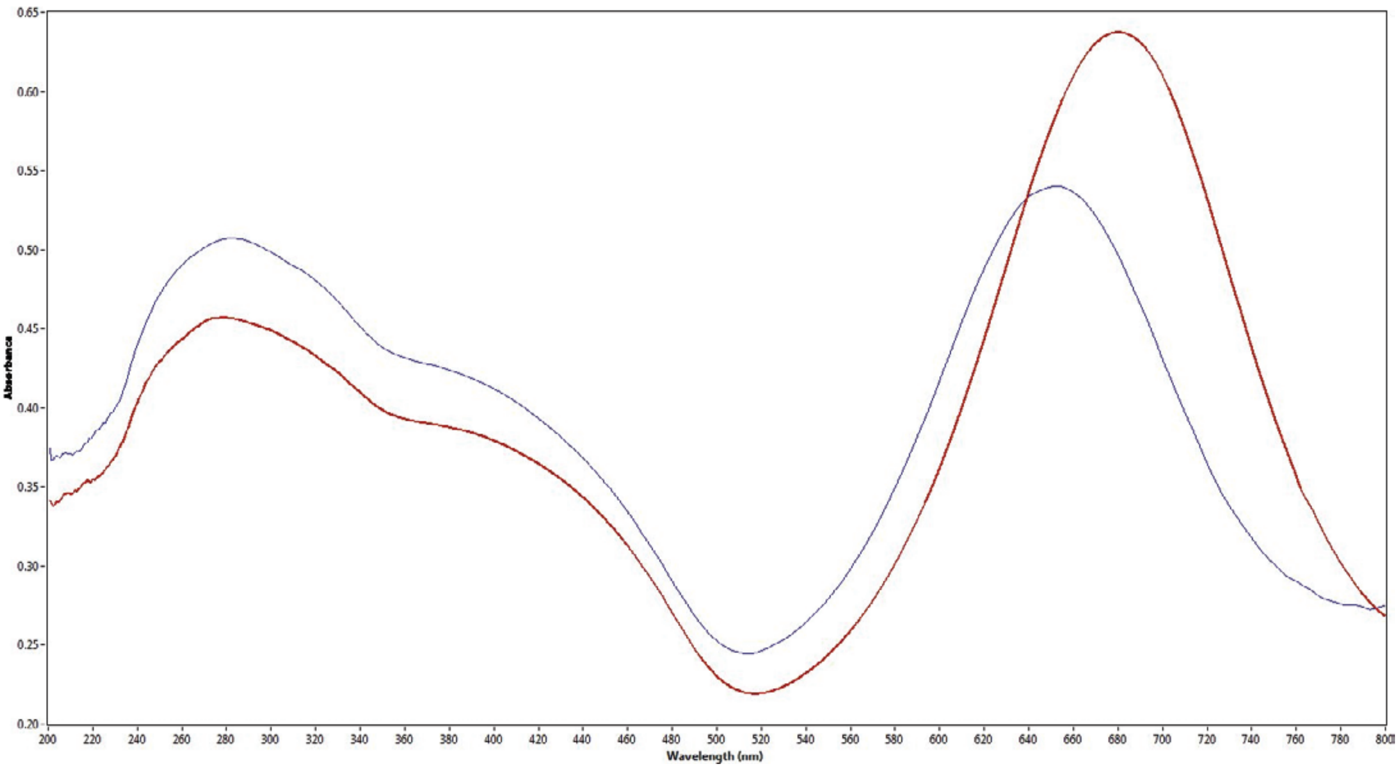

Figure 4: The UV-visible-NIR absorbance spectra of thin films of gold nanoparticles under development as moisture sensors. The red trace is from the dry surface of the film, and the blue trace is from the wetted surface of the film.

uses in many fields including forensic science, semiconductor and optical film thickness measurement, biotechnology, and materials science.

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