

Viewing Microstructures of Materials using the Optical Microscope

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Abstract:

This module introduces the student to microscopy using the light microscope. Standard components of the microscope are detailed along with major points regarding microscopy operation for best resolution. Examples are shown from metallic samples using reflected light microscopy, but the principles also apply to transmitted light microscopy. The module is appropriate for students who may need to use the microscope in their career as an engineer or technician.

Module Objectives:

The demonstration or lab provides an introduction to light optical microscopy and the various ways to manipulate this characterization tool in order to optimize viewing of a sample. The module provides the basis for understanding how the light microscope works and to identify its various components through experimentation and/or demonstration.

Student Learning Objectives: Students will be able to

- Understand how to use the optical microscopes and how it works
- Determine the appropriate use the objective lens, eyepiece, focus and movement of the x/y stage in order to observe specific features of the sample
- Understand the influence of numerical aperture and index of refraction

MatEd Core Competencies Covered

- 0.B Prepare Tests and Analyze Data
- 0.D Demonstrate General Technical Competence
- 1.C Demonstrate Laboratory Skills
- 8.A Demonstrate the Planning and Execution of Materials Experiments
- 8.C Perform Visual and Nondestructive Testing Methods for Solids

Key Words: Optical microscope, microscopy, resolution, microstructures

Type of Module: PowerPoint presentation with in-class demonstration or in-class use depending on microscope availability

Pre-requisite Knowledge: Basic physics of light

Target grade level: Advanced High School, Introductory College/Technical School

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Equipment and Supplies Needed:

- PowerPoint projection system
- Optical microscope(s) for demonstration and use
- Demonstration microscope samples

Curriculum Overview and Notes for Instructor

Optical microscopy is an invaluable technique utilized by the materials engineer. Even though it can be quite expensive, sometimes costing up to \$100,000, this is a fraction of the cost compared to that of an electron microscope. This characterization technique allows for full color analysis at a fraction of the cost.

The main principal of light optical microscopy (LOM) is to shine a light through (transmitted light) or onto the surface (reflected light) of a specimen and examine it under magnification. The main components of the LOM are the objective lens, eyepiece, condenser, and light source. A cutaway section of a transmitted LOM and an illustration of the compound microscope are shown in the PowerPoint.

The basic components of a light microscope are:

- Objective lens - magnifies the object.
- Eyepiece - enlarges the image but does not increase the resolution.
- Condenser - focuses the light source onto the specimen for uniform illumination, eliminating stray light.
- Iris (which is associated with the condenser) - affects the resolution vs. the depth of field and contrast. Unfortunately, nature dictates that improvement in one results in the loss of another.

Standard light sources are often used in ordinary microscopes. The primary illumination sources found in modern LOMs are incandescent tungsten filament lamps. The tungsten filament emits light ranging from 300 to 1400 nm with a color temperature range of 2200 to 3400 K. The color temperature is the temperature of an ideal black-body radiator that radiates light of comparable

color to that of the light source. The best light sources are those in which the light intensity is controlled by a voltage adjustment.

The range of useful magnification for an objective/eyepiece combination is defined by the numerical aperture, or the measure of the microscope’s ability to gather light and resolve fine specimen detail. The numerical aperture is determined by the acceptance angle of the objective and the refractive index of the imaging medium, as is shown in Equation 1 below. This indicates that short focal lengths yield higher numerical apertures, which is significantly different than the common magnifying glass.

$$\text{Numerical Aperture (NA)} = n \cdot \sin(\mu) \quad \text{Eqn. 1}$$

The maximum acceptance angle is 90°, yielding a sin(μ) value of one. The maximum possible numerical aperture is then determined by the index of refraction, which is defined as the speed of light in a medium relative to the speed of light in a vacuum. (The higher the index the slower light travels through that medium.) A majority of microscopes use air as the imaging media, although immersion oils offer a significant improvement in the refractive index and the numerical aperture.

The refractive indices of several materials and media are given in Table 1.

Table 1: Refractive index of select materials and media

Material/Medium	Refractive Index
Air 1.0003	1.003
Water 1.33	1.33
Glycerol 1.47	1.47
Immersion Oils Up to 1.515	Up to 1.515
Glass 1.52	1.52
Flint 1.66	1.66
Zircon 1.92	1.92
Diamond 2.42	2.42
Lead Sulfide 3.91	3.91

The range of magnification for an LOM is generally set at 1000 times the numerical aperture, yielding about 1000x with air objectives and 1500x for oil immersion objectives. Exceeding these magnifications will not yield additional image details (resolution) and can lead to image degradation. The phenomenon in which the image is enlarged without additional resolution is called empty magnification, and it can easily result from the use of a high magnification eyepiece. An example of the phenomena is to increase the size of a digital image 100 times without adding extra pixels to fill the gaps.

Low magnification objectives can be used with high magnification eyepieces; however, high magnification objectives should only be used with 10 and 12.5x objectives in order to minimize the empty magnification effect.

The theoretical resolving power of an optical microscope resides in the 0.2 micron range; for most microscopes under non-ideal conditions, the resolution is significantly worse. The resolution of an LOM is also affected by the wavelength of the imaging light source. Short wavelengths yield vast improvements in resolution, as shown in Table 2.

Table 2: Resolution as a function of wavelength at a fixed numerical aperture of 0.95

Wavelength (nm)	Resolution (μm)
360	0.19
400	0.21
450	0.24
500	0.26
550	0.29
600	0.32
650	0.34
700	0.37

The basic concept of wavelength is further exploited by the electron microscope, in which the wavelengths of excited electrons are many orders of magnitude smaller than that of optical waves, yielding resolutions in the angstrom range ($1 \times 10^{-10} \text{m}$).

One should keep in mind that there are two artifacts caused by the interaction of light with glass lenses: spherical and chromatic aberrations. The spherical aberration is related to the distortion of the image due to the spherical nature of the lenses, and the chromatic aberration arises from the variation of refractive indices over the wide range of wavelengths found in visible light.

One drawback of the optical microscope is the relatively small depth of field (also known as the depth of focus) which is illustrated in Fig. 3. This is the distance above and below the geometric image plane within which the image remains in focus. As one goes to a higher magnification, the depth of field gets significantly smaller and it becomes difficult to keep the entire specimen in focus. Low-power microscopes have a greater depth of focus than high-power microscopes.

The light source is an important component of the optical microscope and one must understand the fundamental concepts of light to accurately discern the variables. Modern physics teaches the wave-particle duality of light. In a nutshell, this states that under some circumstances light is better described as a wave and under other circumstances light is better described as a particle. In LOM, it is most useful to utilize the wave model of light in interpreting the behavior of light and its effects on resolution and numerical aperture.

Module Procedure:

This module includes both PowerPoint presentations and the opportunity for hands-on microscopy operation.

1. Explain the importance of the microscope in understanding materials, and that the microscope can be used to find the microstructure of materials. Begin if you can with a microscope on the podium then show the PowerPoint presentation (comments on each slide are given below).
2. Pause in the presentations as appropriate to demonstrate use of the microscope on the podium.
 - a. (Slides 2,3) Materials science and technology relates to the relationships between structure, properties and processing of materials. Typical examination of metals relates to the observation of the microscopic structure, called "microstructure."

- b. (Slide 4) Observation of microstructures is usually undertaken using the optical microscope. This slide introduces the microscope and its parts. Most students will have used a simple microscope before, but few will have studied details of their operation. Here it is best to go over and name each main component. Details on each are given in an excellent Wikipedia discussion (see references).
 - c. (Slide 5) Magnification of microstructures allows us to see the single crystal grains. It shows changes in the grain size and elongation due to cold rolling. Ask students to describe the differences between the microstructures in the micrographs displayed.
 - d. (Slide 6) Magnification is the basic variable in a microscope, and is based on magnification of the objective lens. The eyepiece only magnifies the image—use of high magnification eyepieces does not add to resolution of the microscope. Resolution is a basic concern and some details on resolution are noted here and on the following slides.
 - e. (Slide 7) Resolution of the image is dependent on a variety of factors. Some details of resolution are noted here and on the following slides.
 - f. (Slide 8) Numerical aperture is discussed here related to index of refraction of the medium used (usually air). Prior to the advent of the electron microscope, oil immersion lenses were used to increase resolution.
 - g. (Slide 9) This slide deals with problems encountered when trying to get higher magnification with a light microscope as well as covering depth of focus (also called depth of field) which relates to magnification and to the wavelength of the light used.
 - h. (Slide 10) This slide will discuss wavelength and its importance in the development of the electron microscope.
 - i. (Slide 11) Slide lists the basic concepts to be taken from the module.
 - j. (Slide 12) This slide concludes the presentation and lists the references that may be used by the instructor or by the students for additional detailed information.
3. In the lab or in the classroom, demonstrate use of a microscope to observe materials samples.
 - a. Use demonstration samples to show typical microstructure of a metal, or

- b. Show typical biological samples
4. Depending on microscope availability, allow the students to experiment observing demonstration samples
5. If your microscope is so equipped, demonstrate the use of polarized light in sample examination, and/or the use of dark field microscopy (see reference by Rack), then have the students experiment with anisotropic materials such as mica or scotch tape.

Supporting Materials and References:

~Please see accompanying PowerPoint

Wikipedia: Optical Microscope, http://en.wikipedia.org/wiki/Optical_microscope

Optical Microscope by Philip D. Rack,

<http://web.utk.edu/~prack/MSE%20300/Lightmicroscopyhandout.pdf>

Optical Microscopy for Biomedical Applications, <http://www.olympusmicro.com/>

Sources for demonstration samples for microscopy:

~Instructors can prepare samples if adequate equipment is available~

Samples can also be obtained from local Universities

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Evaluation

Student evaluation questions (discussion or quiz):

1. How does wavelength affect the resolution in the light optical microscope?
2. Can you gain both resolution and depth of focus on a sample? Why or why not?
3. What is the objective lens used for?
4. Can you see individual grains in a metal sample? If so, are the grain structures in different metals the same?
5. What is the limit in magnification for the samples? Explain.

Instructor evaluation questions:

1. At what grade level was this module used?
2. Was the level and rigor of the module what you expected? If not, how can it be improved?
3. Did the module work as presented? Did it add to student learning? Please note any problems or suggestions.
4. Was the background material sufficient for your background? Sufficient for your discussion with the students? Comments?
5. Did the module generate interest among the students? Explain.
6. Can this module be improved? Please include comments or suggestions concerning the approach, focus and effectiveness of this activity in your context.

Course evaluation questions (for the students):

1. Was the module clear and understandable?
2. Was the instructor's explanation comprehensive and thorough?
3. Was the instructor interested in your questions?
4. Was the instructor able to answer your questions?
5. Was the importance of understanding microscope detail made clear?
6. What was the most interesting thing that you learned from the module?