Sample Curriculum:
Maintenance of the Light Microscope

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A 3 day, hands-on Light Microscopy course designed especially for Maintenance Technicians.

A. Day 1- AM: *Koehler*

1. Principles and actions of the major lens systems in a microscope
2. Adjusting the microscope for optimal illumination and imaging
3. Choosing correct objectives and condenser for optimal images
4. Basic terminology
   a. Magnification
   b. Resolution
   c. Field number and Field of view
   d. Working distance
5. Differences between reflected and transmitted light
6. Hands-on exercises for establishing Koehler Illumination.

B. Day 1 – PM: Preventative maintenance and alignment workshop

1. Diagnostic use of otoscope, microscope ocular, and centering telescope or Bertrand lens for peering into the back focal plane of the objective

C. Day 2 – AM: Diffraction Theory

Numerical aperture of objective and condenser

1. Calculating resolution
2. Applying diffraction to image formation
3. Relationship between diffraction pattern and various contrast techniques

Contrast methods

1. Interaction of light and matter
2. Imaging of various sample characteristics using axial, oblique, and darkfield illumination, and DIC.

D. Day 2 – PM: Optical alignment workshop

1. Set-up, troubleshooting, and maintenance of contrast systems

E. Day 3 – AM: Fluorescence & Polarized Light

Fluorescence

1. Optical components
2. Comparison of light sources
3. Filter selection and troubleshooting

Introduction to polarized light

1. Methods for generating Polarized light;
2. Applications of polarized light
3. Origins and interpretation of Pol colors
4. Differentiating between isotropic and anisotropic materials
5. Michel-Levy chart – relationships between color, retardation, thickness and birefringence
6. Using the Michel-Levy chart to analyze addition, subtraction, compensation

F. Day 3 – PM: Microscope mechanics workshop
Focusing, zoom, and stage movement diagnostics

MME will supply all workbooks and necessary demonstrations.

The client will supply:

- classroom and laboratory space;
- one microscope for each two students with the necessary components (in this case, fluorescence illuminator and filter cubes), preferably fitted with video systems and, if possible, printers;
- an overhead projector
- 35mm projector
- and relevant samples for observation.

Please note that, for maximum impact and effectiveness, we prefer to move the microscopes into a conference room, away from the general lab. If this move is a problem, please let us know as early as possible. Also, if there is a problem with the number of microscopes available, please let us know as soon as possible. We will try to coordinate with your local dealer for loan of equipment.

A. Koehler Illumination

1. Locate the three major lenses in the microscope and describe the function of each.
2. In your own words, describe the steps for establishing Koehler illumination.
3. Align the microscope for Koehler illumination.
4. Discuss two criteria for matching the objective and condenser.
5. Define *magnification* in terms of the relative size of image and object.
7. Define field of view both verbally and mathematically.
8. Locate the field number.
9. Given the field number and the magnification of an objective, calculate the field of view.
10. Define *working distance*.
11. Describe the differences between a transmitted and a reflected light system.
12. Starting with the light source, show on a microscope the difference between a transmitted and reflected light system.
13. Locate each of the conjugate planes in the (a) imaging pathway and (b) illumination pathway.
14. Using conjugate planes, describe how to troubleshoot an imaging problem.
15. Optics trouble shooting:
    a. Use otoscope to locate dirt, alignment, and delamination problems
    b. Use the microscope ocular to observe and analyze surface problems on optical surfaces

B. Diffraction Theory

1. Define numerical aperture.
2. Locate the numerical aperture markings on the microscope.
3. Given a specific objective, discuss the impact of its numerical aperture on imaging.
4. Given a specific wavelength and NA, calculate the theoretical limit of resolution for an objective – condenser set.
5. Briefly discuss the differences in this calculation for transmitted versus reflected light systems.
6. Using a sample with a simple periodic structure (ex: a grating), produce a diffraction pattern. Identify each of the following:
    a. The diffraction spot responsible for the background.
    b. the part of the diffraction pattern responsible for spacing information.
    c. the part of the diffraction pattern responsible for orientation.
    d. the part of the diffraction pattern responsible for good edge information.
7. Briefly discuss how numerical aperture affects the microscope's ability to collect the

diffraction pattern.

8. If the equipment is available, evaluate that impact using several objectives of the same magnification but different NAs.

C. Contrast Techniques

1. For each of the contrast techniques, identify a good test sample.
   a. Axial
   b. Oblique
   c. Darkfield
   d. Color staining
   e. Polarized light
   f. DIC

2. Briefly discuss how to use the color wheel to choose a filter which will
   a. Enhance contrast
   b. Suppress contrast

3. Indicate what information each of the following contrast techniques brings out in the image:
   a. Axial illumination
   b. Oblique illumination
   c. Darkfield illumination

4. On the microscope,
   a. Show the components necessary for each of the following contrast techniques
   b. Demonstrate how to set up each technique

5. Diffraction pattern and contrast
   a. Explain then demonstrate how to manipulate the diffraction pattern to create darkfield.
   b. Explain then demonstrate how to manipulate the diffraction pattern to create axial illumination.
   c. Explain then demonstrate how to manipulate the diffraction pattern to create oblique illumination.

D. Optical alignment workshop:

On the microscope, demonstrate how to use information in the image and in the back focal plane of the objective to analyze if each system is working properly.

E. Fluorescence

1. On the microscope: starting with the light source, identify the components necessary for fluorescence.

2. Briefly describe what property must be present in a sample in order for it to respond to fluorescence.

3. Compare the efficiency and output for three typical illuminators: halogen, mercury arc and xenon arc.

4. Briefly explain the function of each of the filters in the fluorescence filter cube and how to confirm that the correct filter has been chosen for a specific sample.

5. Demonstrate how to evaluate the health of a filter.