Module 4B

Introduction to Forensic Microscopy

Forensic Science Teacher Professional Development

Part 2 Introduction to Forensic Microscopy

There are several steps in the process of recovery and examination of trace evidence:

1. Detection
2. Isolation
3. Identification
4. Comparison
5. Evaluation

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1) **Detection** of trace evidence is usually achieved by observations of crime scene investigators at a scene of crime or by a trace analyst in the crime laboratory.

Magnifiers, stereomicroscopes with an alternate light source, are always used to facilitate the discovery and detection of trace evidence from a crime scene or from an object.

Figure 3 A regular view of the keyboard of a laptop

Figure 4 A shaft of hair can be better discovered under this macro view of the keyboard.

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- In Figure 3 and Figure 4, a hair can be better observed under a macro view of the keyboard.
- Since trace evidence is often invisible to the naked eye, it can be better detected under a macro view.
- The crime scene investigator or trace analyst should bring his or her eyes closer to the object with bright lights (or alternate light sources) at the stage of trace evidence detection (or evidence search).

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Similar examples can be seen in Figure 5 and Figure 6. With a macro view, the observer can recognize more details of the object.

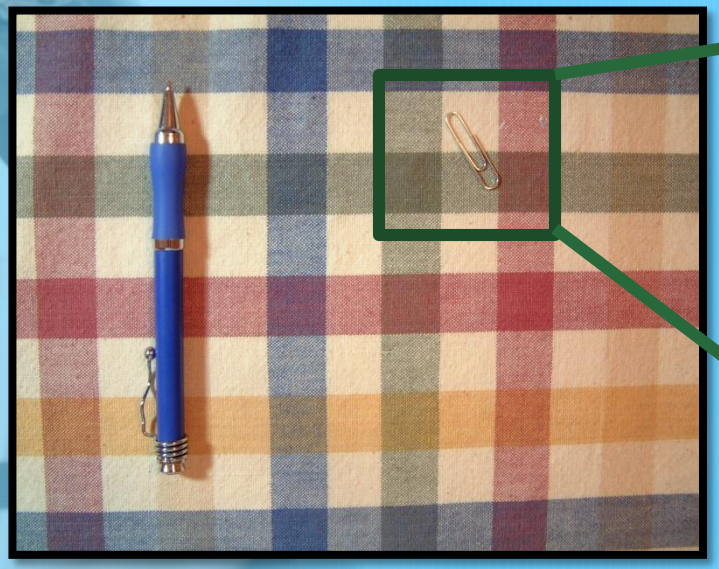

Figure 5 A regular view of the objects on a table

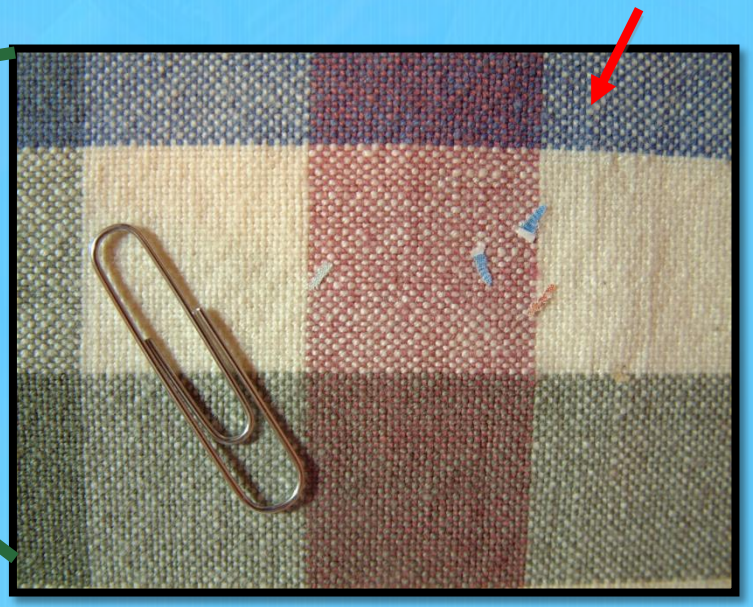

Figure 6 A macro view of the objects on a table

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2) Isolation (physical removal) of trace from a substrate can be carefully performed with tools like tweezers, scalpels, or needles under a stereomicroscope, once the trace is detected.

3) Identification occurs through physical and chemical methods, after targeted trace evidence is isolated from a substrate. Generally, least destructive analytical methods should be performed first before destructive methods.

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4) Comparisons between known samples and questioned samples can then be performed based on the results from the physical examination and the chemical analysis of the samples.

5) Evaluation occurs to determine the location of the trace evidence, the origin of the manufacturer, and its end-use. Unique information can be generated as an investigative lead.

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- Microscopic examination of trace evidence is the fast and affordable way to identify and compare trace evidence.
- A microscope is the most fundamental tool in a trace laboratory.
- Microscopic examination of trace evidence is usually performed with a stereomicroscope followed by a compound light microscope.
- Optical properties, such as refractive index and birefringence, can be readily determined by the use of a polarized light microscope (PLM).

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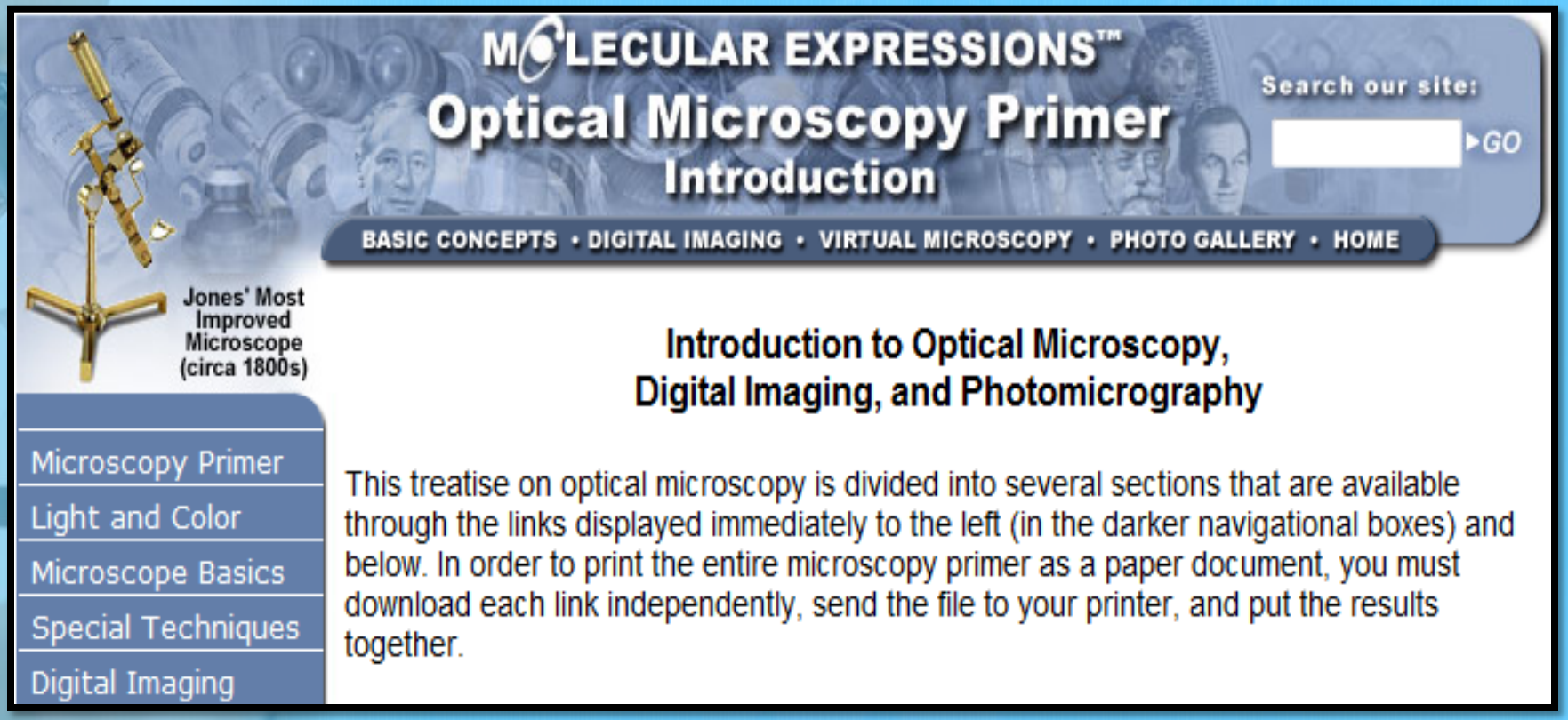
To learn the fundamentals of microscopy, a visit to the following website is recommended.



http://micro.magnet.fsu.edu/primer/index.html

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Fundamental properties of light and color include the following:

- Electromagnetic property of light
- Particle property of light
- Wave property of light
- Sources of visible light
- Fluorescence
- Speed of light
- Reflection of light
- Refraction of light
- Diffraction of light
- Polarization of light
- Interference of light
- Optical birefringence
- Color temperature
- Light filters
- Human vision and color perception
- Light and energy
- Lenses and geometrical optics
- Basic properties of mirrors
- Prisms and beam splitters
- Laser light sources

Part 2 Introduction to Forensic Microscopy

Review these links:

Introduction to Microscopy

Microscopes are instruments designed to produce magnified visual or photographic images of objects too small to be seen with the naked eye. The microscope must accomplish three tasks: produce a magnified image of the specimen, separate the details in the image, and render the details visible to the human eye or camera. This group of instruments includes not only multiple-lens (compound microscopes) designs with objectives and condensers, but also very simple single lens instruments that are often hand-held, such as a loupe or magnifying glass.

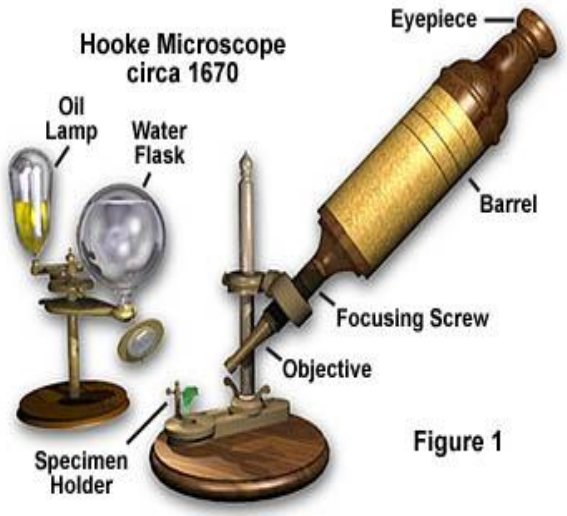

Figure 1

1. What is a microscope?

http://micro.magnet.fsu.edu/primer/anatomy/introduction.html

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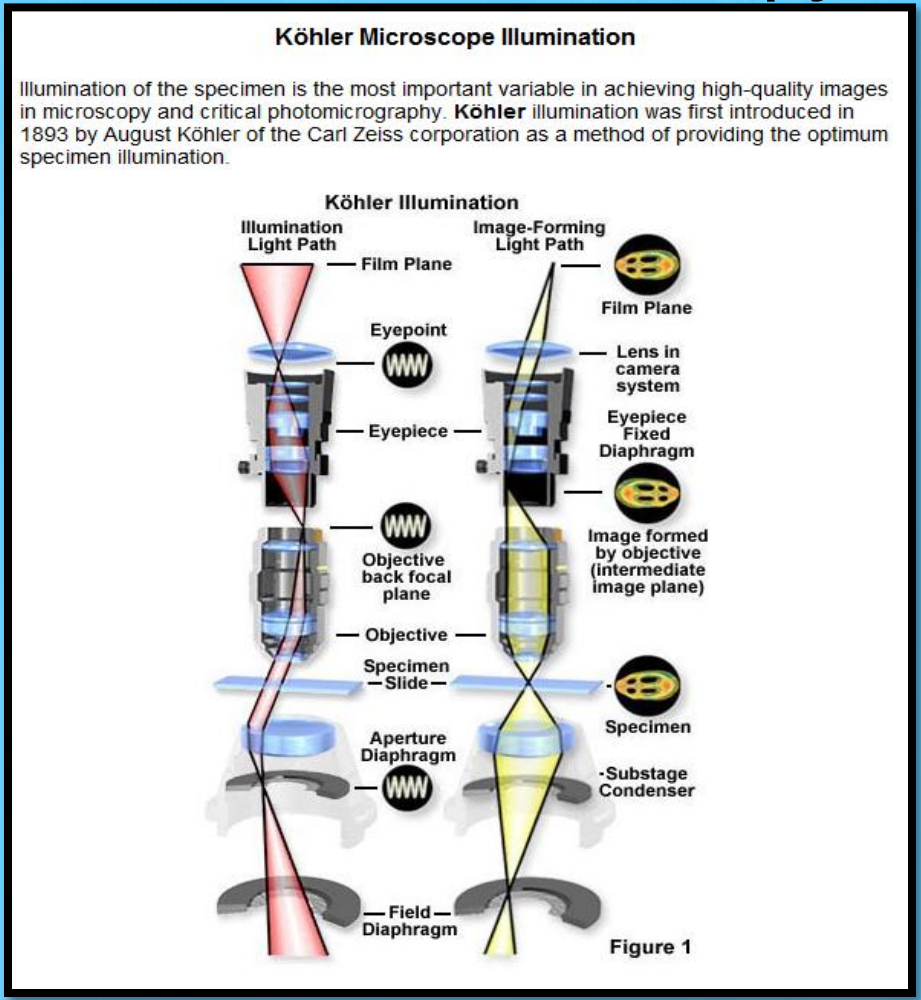
Review these links:



2. What is Köhler Illumination?

http://micro.magnet.fsu.edu/primer/anatomy/kohler.html

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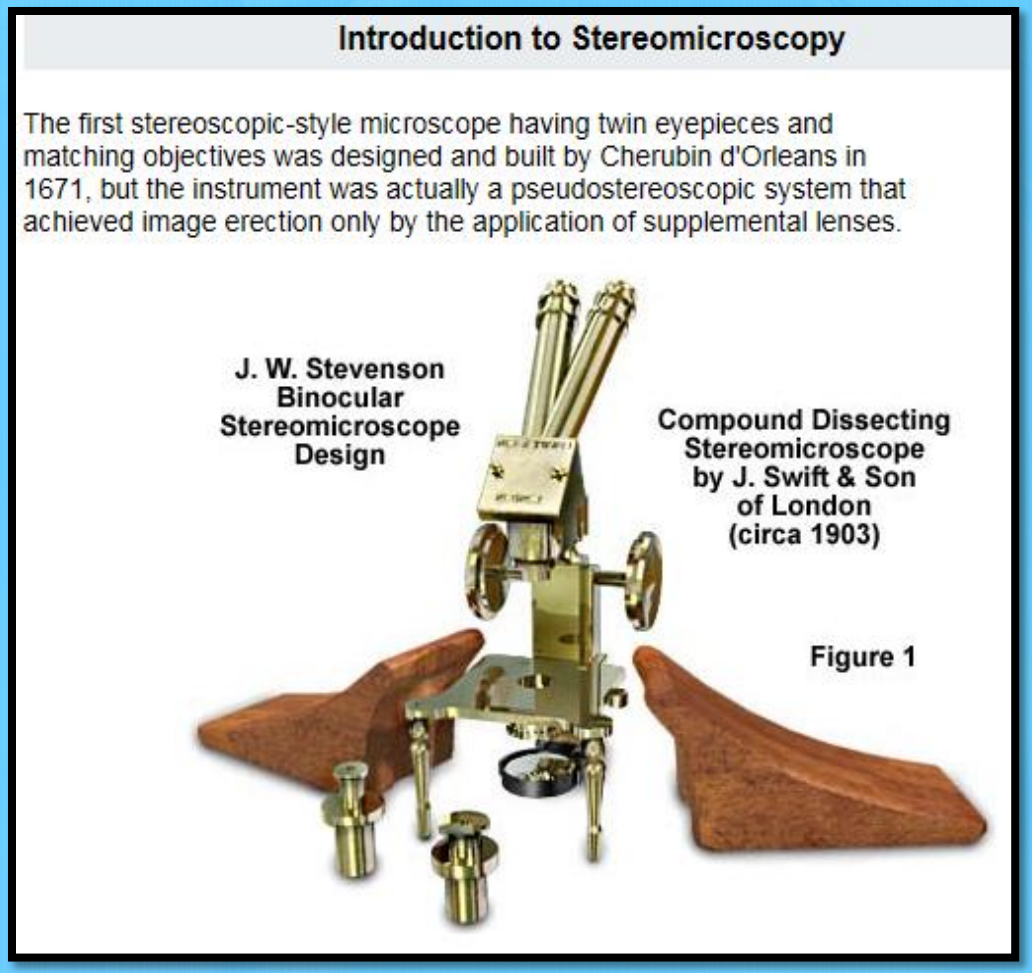
Review these links:



3. What is a stereomicroscope?

http://www.microscopyu.com/articles/stereomicroscopy/stereointro.html

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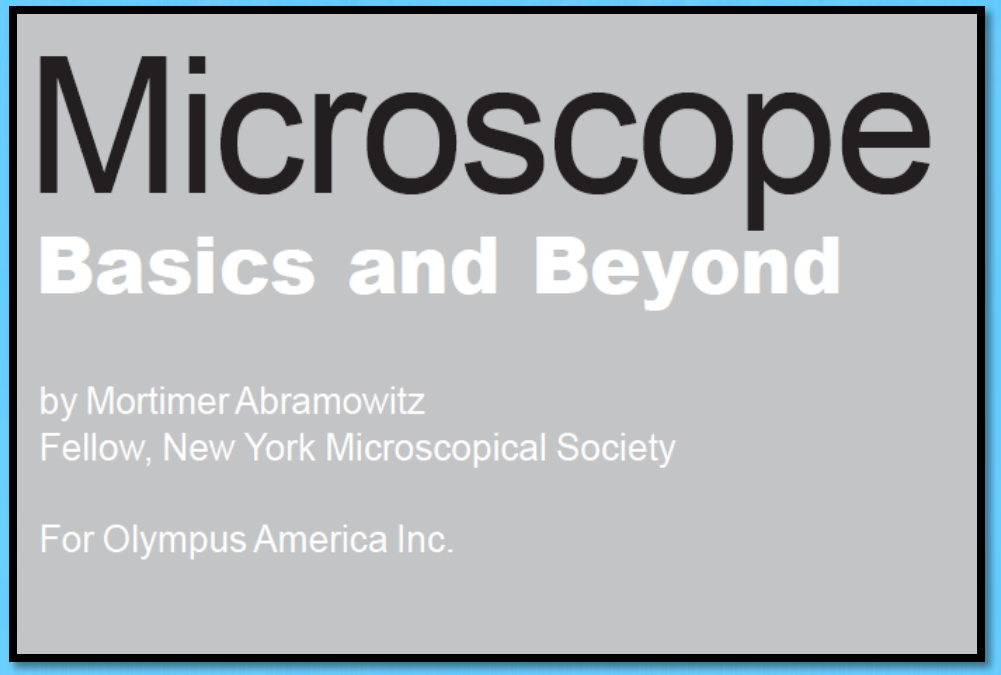
Review these links:



4. Microscope: Basics and Beyond (50 pages in PDF format; 20.7 MB)
http://micro.magnet.fsu.edu/primer/pdfs/basicsandbeyond.pdf

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Review these links:

50 Most Frequently Asked Questions About Optical Microscopy

Answered by Mortimer Abramowitz
Technical Consultant at Olympus America, Inc.

Question 1. What does the inscription 0.17 on the objective signify?

Answer: 0.17 refers to the thickness, in millimeters, of the cover glass that was assumed by the lens designer in computing the corrections for the objective. For objectives with a numerical aperture higher than 0.45, a departure in this assumed thickness (or no cover glass at all) may result in deterioration of the image.

5. 50 Most Frequently Asked Questions About Optical Microscopy

http://micro.magnet.fsu.edu/primer/faq.html

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Techniques of microscopy, such as

- Bright field
- Dark field
- Differential Interference Contrast (DIC)
- Fluorescence
- Phase contrast
- Polarized light

These can also be found on the listed websites and reading assignment.

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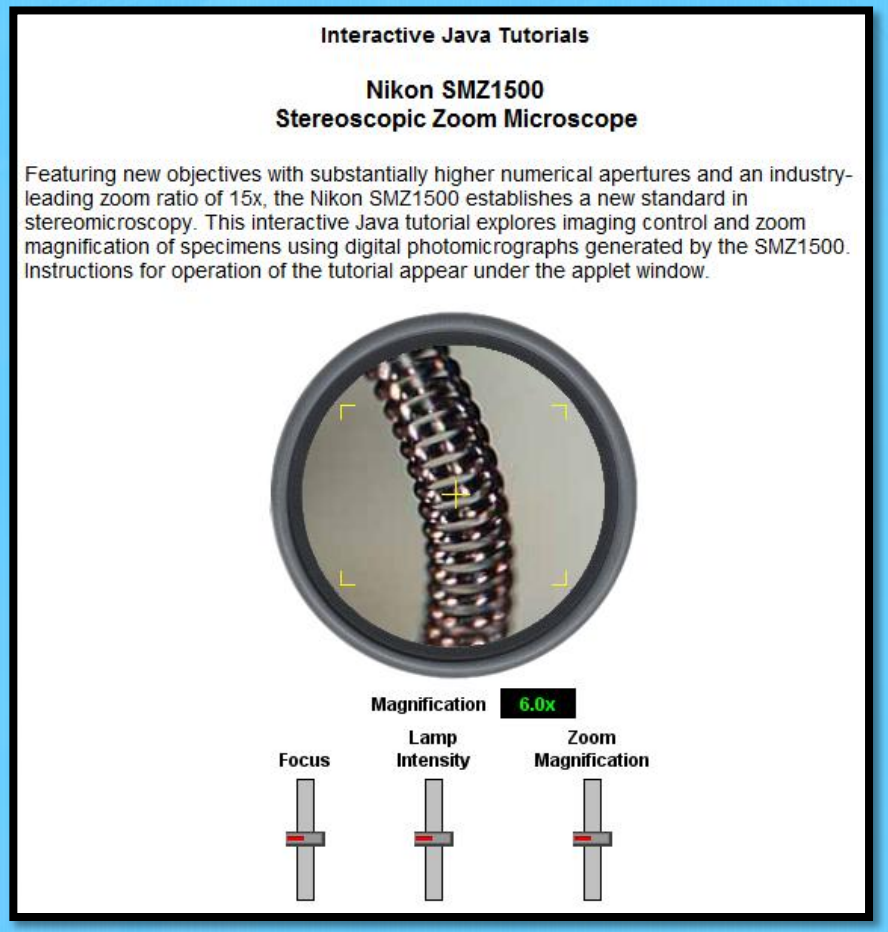
Exercise 1

Review this site:

Nikon SMZ1500 Stereoscopic Zoom Microscope



http://micro.magnet.fsu.edu/primer/virtual/smz1500zoom12/index.html

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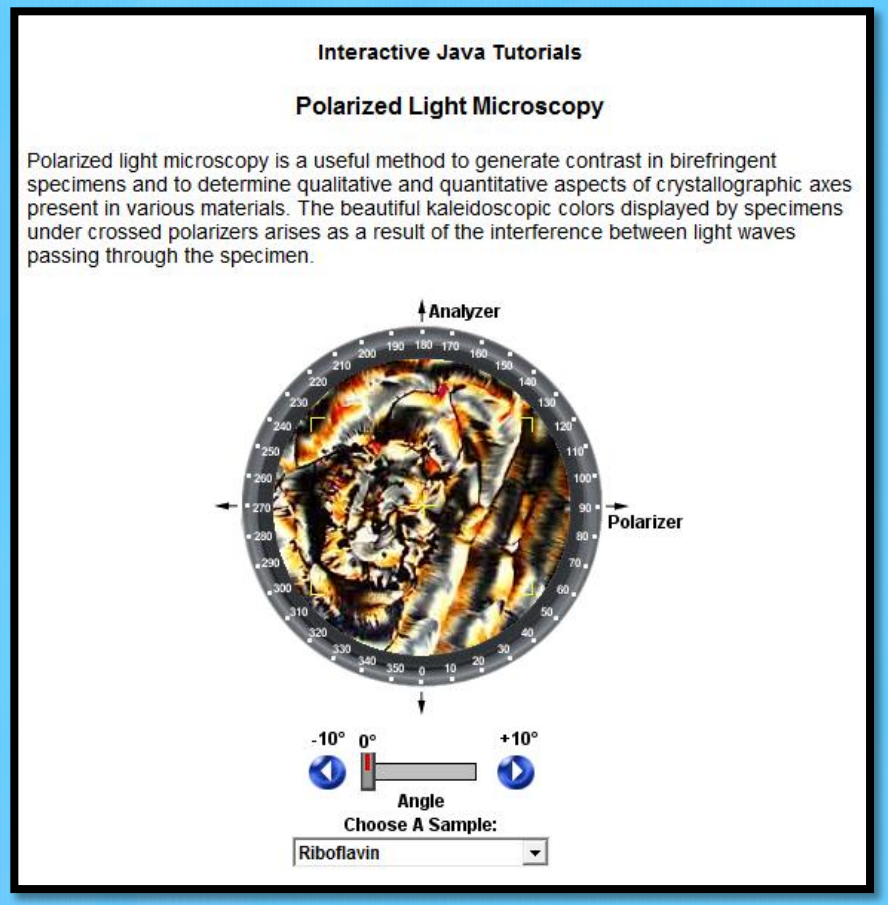
Exercise 2

Review this site:

Polarized light microscope



http://micro.magnet.fsu.edu/primer/virtual/polarizing/index.html

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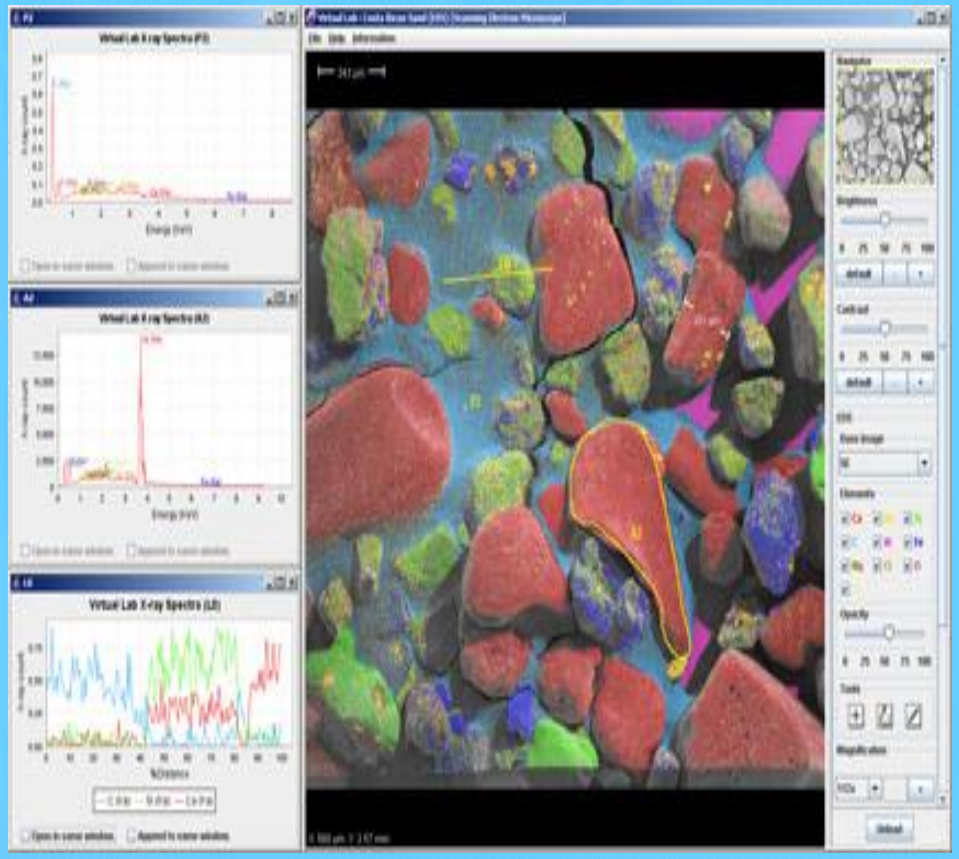
Exercise 3

Review this site:

Virtual Microscope



http://virtual.itg.uiuc.edu/

Part 2 Introduction to Forensic Microscopy

Important optical properties of material

Mounting Media (Mountant)

- Using a mounting medium not only preserves the sample against oxidation, but also provides a better contrast for the observation of sample morphologies.
- The magnitude of contrast to which a colorless transparent specimen can be seen when immersed in different mounting media is known as relief.
- The higher the difference of refractive index (RI) between the mounting medium and samples, the better the contrast (the higher the relief).
- The contrast oil, a mounting medium with a known refractive index, can be used to temporarily prepare samples for microscopic examinations.

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Important optical properties of material

Mounting Media (Mountant), continued

- For example, refractive index (matching) liquids from Cargille can be good mounting media for a temporary mount.
- Many trace evidences can be permanently mounted using Canadian balsam, which is a natural resin with excellent optical properties and a refractive index near that of glass.
- A semi-permanent mount such as Cargille Meltmount™ can be used for a variety of samples.
- It is a waxy solid at room temperature, but it liquefies under modest heat from a water bath or a hotplate for easy mounting and removal.
- Permount™ Resin dissolved in toluene can also be used for a semi-permanent mount for trace evidence.

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Becke line

- The refractive index (RI) of a microscopic material can be determined by the immersion method, in which a sample is immersed in different mounting media with known RIs.
- The difference between the RIs of the mounting medium and the sample can be determined by observing the movement of the Becke line when the working distance is increased.
- The Becke Line is a bright halo observed along the edges of a sample when the focus is raised after the sample is in sharp focus.

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Becke line

- The Becke line always moves toward the substance with the higher RI when the focus of a sharply focused sample is raised (resulting in increasing working distance).

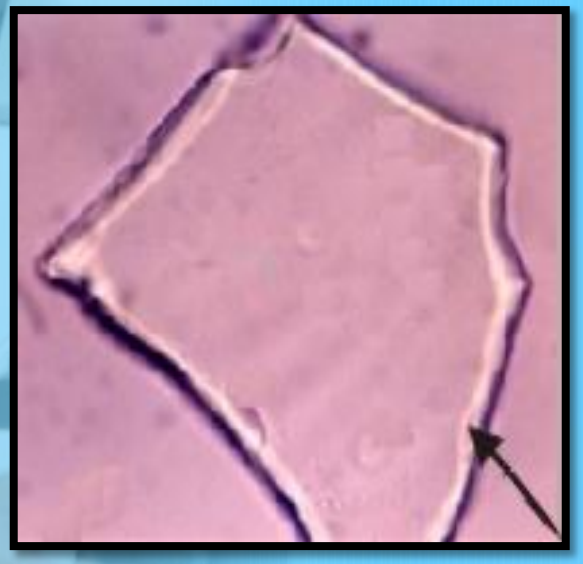

Figure 7 When glass has a higher refractive index, the Becke line is observed inside the glass sample as the working distance increases.

Figure 8 When glass has lower refractive index, the Becke line is observed outside the glass sample as the working distance increases.

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In a crime laboratory, the refractive index of trace glass evidence is usually measured by a GRIM ® (Glass Refractive Index Measurement) system, shown in Figure 8. The GRIM can measure the RI of a trace glass particle using an immersion method with a temperature-controllable hot stage. The GRIM determines the matching point temperature of the immersion oil at which the contrast of the glass image is at a minimum. The matching point temperature is then converted to a refractive index from a calibration data of the immersion oil.

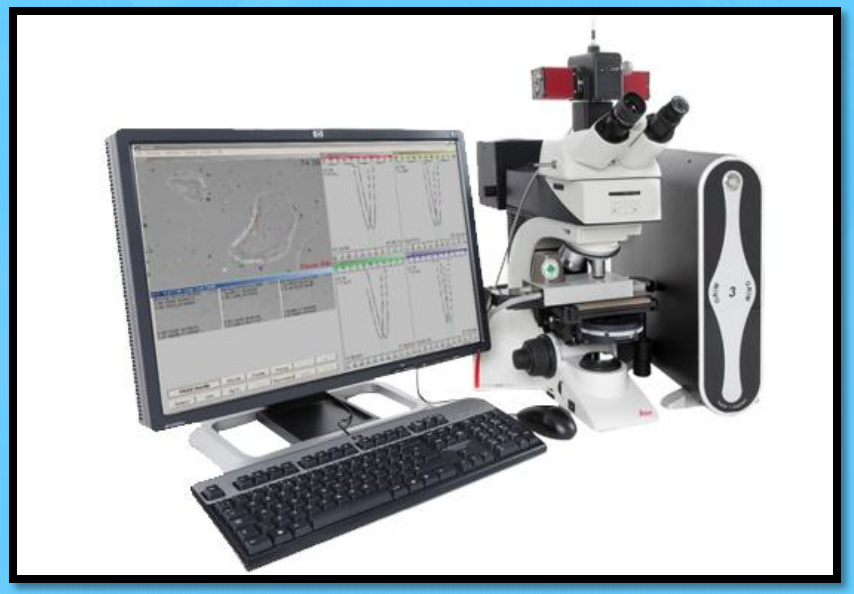

Figure 9 A GRIM ® (Glass Refractive Index Measurement) system can measure the refractive index of a microscopic glass sample with high precision.

http://www.fosterfreeman.com/tech-5/412-glass-ri.html

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Isotropic

- A material is classified as isotropic when there is only one refractive index observed from the material.
- The refractive index is uniform regardless of the light ray's angle of incidence.
- Examples are gases, liquids (excluding "liquid crystals"), and amorphous solids (glass, cubic crystals).

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Anisotropic

- A material is classified as anisotropic when there are two or more refractive indices observed from the material. In other words, when a light source attempts to pass through an anisotropic material, the light will experience two or more refractive indices in different directions within the materials. Birefringence is the difference of the maximum and minimum indices of refraction in an anisotropic material represented by the formula,

  birefringence = (RI max – RI min). *RI refers to Refractive Index

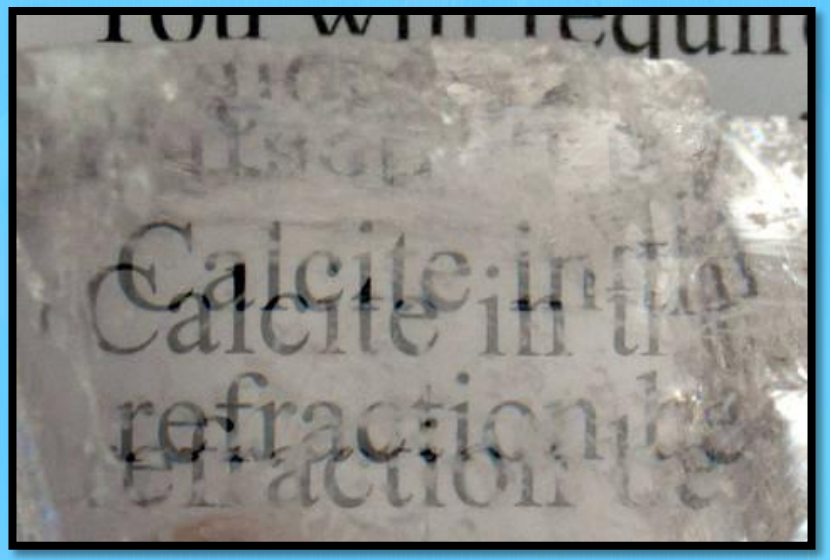

Figure 10 An example of birefringence of calcite

End of Module 4B

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