
Technical Procedure for Microscopes

1.0 Purpose – This technical procedure shall be followed for the operation of the microscopes within the Trace Unit.

2.0 Scope – This procedure applies to all microscopes located within the Trace Unit.

3.0 Definitions – N/A

4.0 Equipment, Materials, and Reagents

- Stereomicroscopes
- Polarized light microscopes
- Reflected light microscopes
- Comparison microscopes
- Fluorescence microscopes
- Optical micrometer

5.0 Procedure

5.1 New Instrumentation

5.1.1 New microscopes shall be installed by a certified engineer according to the manufacturer's instructions.

5.2 Köhler Illumination

5.2.1 Setting up Köhler illumination is one of the most widely accepted techniques of ensuring that a microscope is properly aligned, exhibits high intensity and uniform illumination and is correctly focused. Köhler illumination adjustments shall be performed as needed.

5.2.2 Depending on a particular microscope and its design, setting up Köhler illumination may be possible in full or only in part. This procedure shall not apply to stereomicroscopes.

5.2.3 Procedure:

5.2.3.1 Using the highest magnification dry objective (fixed objective) and looking through only one ocular, focus on a specimen.

5.2.3.2 Now looking only through the other ocular, focus the specimen by adjusting the diopter ring on the ocular.

5.2.3.3 Completely open the aperture diaphragm.

5.2.3.4 Remove one ocular or insert the Bertrand lens and focus and center the filament in the back focal plane. Replace the ocular.

- 5.2.3.5 Close the field diaphragm.
- 5.2.3.6 Focus the substage condenser by bringing the leaves of the closed field diaphragm into focus.
- 5.2.3.7 Using the substage condenser centering screws, center the substage condenser.
- 5.2.3.8 Open the field diaphragm so that the leaves are just outside the field of view.
- 5.2.3.9 Using the stage centering screws, center the stage.
- 5.2.3.10 Remove an ocular or insert the Bertrand lens and adjust the aperture diaphragm so that it is open at least two-thirds of the diameter of the back focal plane. Replace the ocular.
- 5.2.3.11 Rotate the nosepiece to the next objective and center the objective using the centering screws on the side of each objective. Do not move the stage or the substage condenser.
- 5.2.3.12 Repeat 5.2.3.11 for each additional objective.

5.3 Calibrations – N/A

5.4 Instrument Maintenance

- 5.4.1 All of the microscopes used within the Trace Unit shall be cleaned and serviced annually by an outside vendor. A record of this cleaning and service shall be maintained.
- 5.4.2 Lenses (including condenser lenses, objective lenses and oculars) shall be cleaned during use as needed.

5.5 Standards and Controls

- 5.5.1 An optical micrometer shall be used to determine the spacing of the ocular measuring graticule.
- 5.5.2 No measurements made on the microscopes in the Trace Unit shall be considered critical measurements.

5.6 Sampling and Sample Selection – N/A

5.7 Calculations – N/A

5.8 Uncertainty of Measurement – N/A

6.0 Limitations – N/A

7.0 Safety

- 7.1 Avoid looking directly into the fluorescence source.
- 7.2 Do not spend long periods of time making observations at high light intensities.
- 7.3 When changing bulbs, ensure that the power cord is disconnected.

8.0 References

Abramowitz, M. *Microscope Basics and Beyond*. Lake Success: Olympus Corporation, 1985.

Birk, G. *Instrumentation and Techniques for Fluorescence Microscopy*. Sydney: Wild Leitz Pty Limited, 1984.

Delly, J.G. *Essentials of Polarized Light Microscopy*. Westmont, IL: College of Microscopy, 2009.

McCrone, W.C., L.B. McCrone and J.G. Delly. *Polarized Light Microscopy*. Chicago: McCrone Research Institute, 1984.

Saferstein, R. *Forensic Science Handbook*. Volume II. Englewood Cliffs: Prentice Hall, 1988.

9.0 Records

- Microscope annual cleaning/maintenance records.

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original ISO Document
10/18/2013	2	Added issuing authority to header
08/29/2014	3	Updated header to Physical Evidence Section – Trace Unit, issuing authority to Physical Evidence Section Forensic Scientist Manager. Updated all references in procedure from Trace Evidence Section to Trace Unit 4.0 - Added fluorescence microscope