1.0 Purpose and Scope

The purpose of this procedure is to establish a method for taking digital images using the Olympus BX60 Microscope.

This document is a supplement to manufacturer’s manuals for the operation of the Olympus BX60 Microscope, and the Olympus BX-FLA Reflected Light Fluorescence attachment.

The Apogee KX85 digital camera is controlled by software via the host computer. This document is a supplement to the manufacturer’s manuals for the operation of the Apogee KX85 digital camera and the Image-Pro Plus software controlling the digital camera.

This supplement is not intended to be formal instrument documentation, nor does it cover all features or use of the instrument, but rather a hands-on user’s guide to the typical operation of both the microscope and digital camera. Details of microscope operation, the Apogee KX85 digital camera operation via the host computer software can be found in the manufacturer’s manuals kept in the lab.

Acronyms and definitions for terms used in this procedure may be found in the Glossary located at the Sandia National Laboratories (SNL) WIPP Online Documents web site.

2.0 Implementation Actions

2.1 Responsibility

The Principal Investigator (PI) or designee is responsible for implementing the requirements of this procedure.

The Technical Staff is responsible for performing the measurements following the requirements of this procedure, and documenting all required information as described in this SP. Technical staff should familiarize themselves with the manufacturer’s manuals for the Olympus BX60 Microscope, and the Olympus BX-FLA Reflected Light Fluorescence attachment for an understanding of microscope assembly parts and nomenclature, the controls, operation, and observation methods. The digital camera is controlled by software via the host computer. Technical Staff should familiarize themselves

If the procedure cannot be worked as written, the user has the responsibility to stop work and resolve all concerns with the PI/designee or safety, as appropriate, prior to proceeding with the work.

### 2.2 Safety

The activities described in this SP shall conform with SNL Environmental Safety and Health programs (ES&H). All activities described in this SP are also subject to ES&H requirements governed by the WIPP Industrial Safety Program and the WIPP Industrial Hygiene Program when performed within the WIPP Land Withdrawal Area.

As addressed in the Olympus BX-FLA Reflected Light Fluorescence instruction manual, page 9, ensure UV protective shielding is in place before use.

### 2.3 Sample Preparation

A. Wet or oil based slides with non-immersion objective lenses need a cover slip.

B. Thin and thick sections should not have a cover slip. The objectives assume a cover glass thickness of 0. A departure from this may result in deterioration of the image.

C. The specimen should be labeled according to [NP 13-1](#), “Control of Samples and Standards”.

### 2.4 Instrument Procedures

Follow the instructions in the Olympus BX60 System Microscope, the Olympus BX-FLA Reflected Light Fluorescence, Olympus BXP Polarizing Microscope, and the Olympus Video/photo adapter’s instruction manuals. The instructions below are for typical operation. Details on how to perform these operations are contained in the above references. In the below text, there are references to knobs and buttons on the instrument control panel and/or methods for computer control. Most features have alternative control methods which are available and described in the instruction manuals.

#### 2.4.1 Instrument Start-up

The Olympus BX60 Microscope in the lab is a very powerful instrument with a wide variety of uses and tools. This procedure will focus on the use of the microscope for obtaining digital images.

Before you begin viewing a sample verify the following:

**Note:** Technical Staff should refer to section 2, Assembly, of the BX60 instruction manual and section 2, nomenclature, of the BX-FLA instruction manual on the location of microscope controls.

A. **Light Path Selector for Specimen Viewing:** The light path selector knob (BX60 pg 26) should be in the middle position, with 20% of the light for the binocular eyepieces, and 80% for the cameras. If a dark specimen is selected the selector should be pushed in to the first position, with 100% of the light traveling to the binocular eyepieces.

B. **Light Path Selector for Photography Equipment:** The light path selector knob (Video/Photo adapters pg 18) should be in to the second position (A B), with 50% of the light for the 35mm camera (port A) and 50% of the light for the digital camera (port B).
With the selector in this position one will be able to view the specimen through the binocular eyepieces and through the photo eyepiece. The view through the photo eyepiece is the image which will be captured by the digital camera.

C. Bertrand Lens (above the universal cube housing): Knob should be pulled out to the $\bigcirc$ position. The Bertrand Lens should be disengaged from the light path.

D. U-AN360 Polarizing Analyzer Lens: Analyzer should be pulled to its outermost position, its first click stop. The analyzer should be disengaged from the light path.

E. U-PO Lens: Filter slider should be pulled to its outermost position. The polarizer should be disengaged from the light path.

F. Light excluding shutter (on the Universal reflected light fluorescence vertical illuminator): Shutter knob should be pulled out to the $\bullet$ position. Light should not be entering the observation tube through this shutter. This shutter allows light from the External Mercury Burner to enter the observation tube.

G. External Mercury Burner Power Supply: Power supply should be off.

Note: Once the power supply has been turned on, it must run for at least 15 minutes before turning it off.

H. Filter Levers: Filter levers LBD, ND25 and ND6 should be disengaged by turning their levers so that the $\bigcirc$ mark on the lever is aligned with the $\bullet$ mark on the base.

LBD filter – Color Balancing Filter
ND25 filter – Natural Density Filter
ND6 filter – Natural Density Filter

2.4.2 Cubes Available for Observation

Depending on the sample you will be viewing you will need to select the appropriate cube. There are four selections available on the turret, BF, upside down BF, WB and DIC. Information on the cubes is listed below.

A. Brightfield (BF) Cube

Brightfield illumination is probably the most widely used observation mode in optical microscopy. The technique is best suited for observation of fixed, transparent and therefore stained specimens or other kinds of samples that naturally absorb significant amounts of visible light. Images produced with brightfield illumination appear dark and/or highly colored against a bright, often light grey or white, background.

B. Brightfield with 0.5% ND Filter (upside down BF) Cube

This cube is the same as the cube described above, with the addition of a neutral density filter of 0.5%.

C. Blue Wide Band (WB) Cube

This cube is used for fluorescence observation. Refer to the BX-FLA instruction manual page 16 for more information on cube U-MWB.

D. Differential Interference Contrast (DIC) Cube

An excellent mechanism for rendering contrast in transparent specimens, differential interference contrast (DIC) microscopy is a beam-shearing interference system in which the reference beam is sheared by a minuscule amount. The technique produces a monochromatic shadow-cast image that effectively displays the gradient of optical paths for both high and low spatial frequencies present in the specimen. Those regions of the
specimen where the optical paths increase along a reference direction appear brighter (or
darker), while regions where the path differences decrease appear in reverse contrast.
As the gradient of optical path difference grows steeper, image contrast is dramatically
increased.

2.4.3 Light Source Types Available for Observation

A. Transmitted Light:

The light from the illumination lamp is guided through the specimen to the objective. The
light transmitted through the specimen is observed through the objective. Transmitted
light is useful for observation of transparent specimens.

B. Reflected Light:

The light from the illumination lamp is vertically guided through the objective onto the
specimen. The light reflected from the specimen is observed through the objective.
Reflected light is useful for observation of opaque specimen.

2.4.4 Observation

A. Select a method for observation:

- Transmitted Light Brightfield Observation – refer to section 6-1 of the BX60
  instruction manual, page 32.
- Reflected Light Fluorescence Observation – refer to section 6, item 1 of the BX-FLA
  instruction manual, page 18. The WB cube should be engaged.
  Note: The Mercury Burner is normally used as the light source for fluorescence
  observation. Follow the instructions for its operation beginning on page 11 of the BX-
  FLA instruction manual.
- Reflected Light Brightfield Observation – refer to section 5-1 of the BX-FLA
  instruction manual, page 32.
- Reflected Light Nomarski Differential Interface Contrast Observation – refer to
  section 5-2 BX-FLA instruction manual, page 32.
- Reflected Light Simple Polarized Light Observation – refer to section 5-3 BX-FLA
  instruction manual, page 34.

B. Lower the stage to allow enough space to place the specimen on the stage without
touching the objective. Place the specimen on the stage.

C. Press the transmitted/reflected light selector switch to select the appropriate light mode,
and press the main switch to “I” (ON). Adjust the light intensity with the light intensity
lever for comfortable viewing.

D. Turn the revolving nosepiece to engage the 10X or 20X objective.

E. While looking through the eyepieces of the binocular observation tubes, adjust the
eyepieces closer or farther together to fuse the two circles of light into one circle.

F. With your right eye only slowly raise or lower the stage by use of the coarse adjustment
knob to bring the specimen into focus. Use the fine adjustment knob to perfect the focus.

Note: Take special care, while using the coarse focus adjustment knob, not to raise the
stage so high that the specimen and the objective touch. It is wise to raise the stage to
the highest position, while watching to make sure the objective does not come into
contact with the specimen, then, while observing through the eyepieces, move the stage
away from the objective using the coarse focus adjustment knob.
**Note:** The pre-focusing lever can be engaged to set an upper limit on the coarse adjustment movement. The pre-focusing lever is on the left-hand coarse adjustment knob. The lever can be set by pulling the lever toward the front of the stage. (See page 29 of the BX60 instruction manual).

G. Using your left eye only, rotate the knurled ring on the left eyepiece tube to bring the image into focus for your left eye.

H. If you wish to move to a higher power objective, it should take very little movement of the fine adjustment knob to bring the image into focus.

I. Identify the portion of the specimen for observation by rotating the stage, or using the fine adjustment knobs on the mechanical stage. The fine adjustment knobs will move the specimen right or left and up or down across the observation area.

J. Adjust illumination as necessary. If the BF cube has been selected adjust the field iris diaphragm and Aperture iris diaphragm as necessary to obtain adequate illumination for observation. (Refer to page 28 and 32 of the BX60 instruction manual.)

K. Engage filters as required. The filters can be engaged and disengaged, independently, using the levers on the right side of the base. The filters are engaged when the \( \bullet \) mark on the lever is aligned with the \( \bullet \) mark on the base. The filters are disengaged when the \( \bullet \) mark on the lever is aligned with the \( \circ \) mark on the base.

<table>
<thead>
<tr>
<th>Filters/Analyzer</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-LBD Color Balancing Filter</td>
<td>To convert the color temperature of the source to the color temperature of daylight. Used for comfortable observation and when taking color photographs.</td>
</tr>
<tr>
<td>U-ND25 Neutral Density Filter</td>
<td>To adjust illumination brightness. (Transmission ratio 25%)</td>
</tr>
<tr>
<td>U-ND6 Neutral Density Filter</td>
<td>To adjust illumination brightness. (Transmission ratio 6%)</td>
</tr>
</tbody>
</table>

L. Engage the Analyzer and Polarizer Filter as necessary. Both the analyzer and polarizer filter should be pushed in to the second click position to engage into the light path. Adjust the U-AN360 polarizer dial until complete extinction is obtained. See the BX-FLA instruction manual section 5-2, page 32 for more information.

**Note:** If the DIC cube was selected for observation it is not necessary to engage the U-AN360 analyzer and the U-PO polarizer.

### 2.4.5 Capturing Digital Images

A. Light Path Selector for Specimen Viewing: The light path selector knob (BX60 instruction manual pg 26) should be in the outermost position, with 0% of the light for the binocular eyepieces, and 100% for the cameras.

B. Light Path Selector for Photography Equipment: The light path selector knob (Video/Photo Adapters instruction manual pg 18) should be in to the outermost position (B), with 100% of the light for the digital camera (port B).

C. Start up the Image-Pro Plus software on the computer next to the BX60 microscope.

E. Once the image quality is satisfactory then save the image to the computer hard drive, with a unique image name (image id).

F. Record information contained in Appendix A in a scientific notebook or supplement. Final resolution and exposure time information will reported from the Acquire tab dialogue.

2.4.6 Instrument Shutdown

A. Shut down the Image-Pro Plus software.
B. Lower stage and remove specimen from the stage.
C. Turn off power to all light sources.

Note: If the Mercury Burner power supply has been turned on it must run for at least 15 minutes before turning it off.

2.5 References

Olympus, Instructions, BX60 System Microscope
Olympus, Instructions, BX-FLA Reflected Light Fluorescence Attachment
Olympus, Instructions, BXP Polarizing Microscope
Olympus, Instructions, Video/Photo Adapters

3.0 Records

Appendix A provides, in a checklist format, those pieces of information necessary to document the acquisition of digital images from a specimen. Appendix A was designed to be inserted into the scientific notebook used by Technical Staff, but is not required. If Technical Staff choose not to use Appendix A as an insert, they should be sure to include all the information from Appendix A in their scientific notebook. Digital images obtained will be printed, labeled with the image id, and stored in a scientific notebook or supplement.

The following records, generated through implementation of this procedure, shall be prepared and submitted to the WIPP Records Center in accordance with NP 17-1, "Records":

QA Record
- SN (Scientific Notebook)
- SNS (Scientific Notebook Supplement) media
- Image from camera

Non Quality (NQ) Record
- Electronic Media associated with the SNS

4.0 Appendices

Appendix A: Form SP 12-13-1, Digital Image Obtained Using the Olympus BX60 Microscope
### Appendix A

**Digital Image Obtained Using the Olympus BX60 Microscope**

Document inserted in Scientific Notebook on page __________

<table>
<thead>
<tr>
<th>ACTIVITY/PROJECT SPECIFIC PROCEDURE</th>
<th>Form Number:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP 12-13-1</td>
</tr>
</tbody>
</table>

| Date: ______________________ | Sample Id: ______________________ |
| Operator: __________________ | Sample Description: __________________ |
| Image Id: __________________ | __________________ |

Select the Cube Employed:
- [ ] BF – Bright Field Cube
- [ ] DIC – DIC Nomarski Cube
- [ ] WB – Blue Wide Band Cube (Fluorescence)
- [ ] BF (upside down) – BF Cube with 0.5% ND filter for XE/HG

Select the Light Source Employed:
- [ ] Transmitted Light
- [ ] Reflected Light
- [ ] Reflected Light – Mercury Burner

Select the Filter(s) Employed:
- [ ] U-LBD – Color Balancing Filter
- [ ] U-ND25 – Neutral Density Filter
- [ ] U-ND6 – Neutral Density Filter
- [ ] U-AN360 – Analyzer
- [ ] U-PO – Polarizer Filter

Select the Objective Employed:
- [ ] 5x/0.15, ∞/-, UMPlanFl
- [ ] 10x/0.30, ∞/-, UMPlanFl
- [ ] 20x/0.46, ∞/0, UMPlanFl
- [ ] 40x/0.75, ∞/0, UMPlanFl
- [ ] 100x/0.95, ∞/0, UMPlanFl

Image Information:
- Exposure Time: Hours: ________________
  Minutes: ________________
  Seconds: ________________
  Milliseconds: ________________
- Final Capture Resolution: ________________
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