

- I. Instrument Name: LEO Scanning Electron Microscope/Oxford Energy Dispersive X-ray System (SEM/EDS)
- II. Suggested Uses: High resolution and magnification imaging with enhanced depth of field for trace evidence. Non-destructive elemental analysis of gunshot residue particles, paint, metal, powders, and other trace particulate material.

### III. Operating Procedures

- A. Start-up and calibration procedures (Consult manuals and Help Menu for Leo 1450 Variable Pressure SEM and Oxford Energy Dispersive X-ray System)

#### SEM

1. Engage the SEM Green “ON” button on the front of the plinth (DO NOT TOUCH the “OFF” button which is red in color).
2. After the visual screen for the SEM (TV monitor 2) appears, follow the Dialog Boxes for logging into the network by typing in your name/password as requested (You may have to use CTRL-ALT function and 2 in order to access screen 2).
3. After logging into the network successfully, you will need to open the LEO USER INTERFACE 2 icon which will ask for your name and another password. type in this information and wait for the interface to bring up the SEM screen. All commands for the SEM are presented/accessed through this screen. The mouse is used to activate or deactivate each command. For most commands, there are several places or options for performing a task. Only one option per task is given in these Procedures.
4. To change samples, be sure that the filament and EHT (extra high tension) are “OFF” (This is done through the EHT or Filament options on the Status Toolbar located at the bottom of the screen). Then mouse click on the Vacuum option on the Status Toolbar, followed by mouse clicking on Vent. Select “YES” for venting, unlatch both chamber door latches, and adjust nitrogen pressure to 3-4 psi.
5. After the chamber is fully vented, turn off the nitrogen supply, and gently pull out the chamber door with the attached stage (Be careful to clear cables near the door). To change a sample, you will need to remove the stage holder and place it on a clean surface (You need to either use gloves or restrict touching the holder to a minimum). Place samples in the holder (noting position of each sample), tighten the screws for each mount position, and place the holder back on the stage exactly as you removed it.

Precision on returning to the same stage positions depends on accurately seating the flat edge of the mount against the flat area of the stage. Once this is accomplished, gently roll the stage back into the chamber, securely latch the chamber door latches, and use the vacuum selection on the Status Toolbar to pump the chamber back to Vacuum Ready Condition.

6. Use the Status Toolbar to activate the EHT and Filament. The last saved working conditions will be automatically brought up for the filament. Once this is accomplished, check the emission mode to determine if the filament is properly aligned and saturated. The Gun Alignment option under BEAM on the User Toolbar will allow access to the emission image and filament adjustments. Use the electronic positioning adjustments to center the fully saturated emission image. Then adjust saturation of the filament as follows: For the best image, set saturation slightly below or at the second crossover. For lower quality image and typical probe work, a saturation point between the first and second crossover is usually recommended. The lower the saturation, the longer the filament life.
7. Adjust working parameters of the instrument to match the requirements of the sample analysis. Many of these parameters can be accessed through the “Go to Control Panel” under TOOLS on the User Toolbar. Once opened, the “Go To Control Panel” (or any other opened pull down menu) can be moved to TV Monitor 3 (far right monitor) through the click and drag technique. This alleviates clutter on Monitor 2.
8. Calibration of the SEM is performed by Service Engineer on routine service calls.

#### EDS

1. The EDS system is turned on through the Hewlett Packard computer located in lower right area of the EDS table. The Power button is the larger one on the far right of the computer. Depressing this button will bring up the Hewlett Packard EDS monitor which is considered Monitor 1 in our setup. You may need to use CTRL-ALT 1 command before using the CTRL-ALT DEL to log into the EDS system. The dialog box will then ask the user to log into the network. Type in your name/password for the EDS system. Once the EDS system is fully powered up, you may choose between two working platforms: the ISIS format or the INCA format. The ISIS platform is used for Automated Gunshot Residue Analysis.
2. Whenever quantitative results are needed or at least once a month, a quantitative calibration should be performed using a standard such as copper or manganese which are available in the SEM laboratory. The quantitative calibration function can be activated in ISIS through the drop down menu (In INCA, the quantitative calibration is constantly displayed

on the screen at all times). Acquisition Rate of 6 should be used for optimum resolution. Once the element selection is made, this program runs automatically. In addition to the quantitative calibration, once a month a strobe resolution test should be performed on the Oxford X-ray system. Mouse click on the Customer Validation icon, then the Button menu, and finally the Detector Diagnosis choice under the Button menu. Answer the first page of questions and select Menu format. The select Strobe Resolution Test. Using a sample such as the Manganese standard, adjust count rate between 500 and 1000 counts using the SEM spot size (Detailed instructions appear on the screen). After running the Resolution Strobe Test, close and open the File Menu, then Edit choice, then the File choice, and finally select Print to print a full report. Place the report in the Calibration notebook for the Oxford EDS system.

## B. Collection and Storage of Data

### SEM

1. Collection of information from the SEM will depend on the sample and analysis being performed. The operator must continually monitor the parameters of the instrument to determine if a different accelerating voltage, current setting, signal collector, or display variable would enhance the image on the display monitor.
2. Perform sizing of particles if needed. If the image is to be saved, label pertinent information on the monitor using Annotation drop down menu and save both the image and annotation to the hard drive.
3. The image can then be printed using the high resolution Codonics printer.

### EDS

1. Set collection time and acquisition rate using the Edit function in Isis. Use Acquisition Rate 6 for most analysis except Gunshot Residue Analysis (Rate 4) and mapping (Rate 1-2). Analysis time will vary with 200 seconds being an average time. Click on X-ray start button and collect enough counts to obtain about 30% dead time. Adjust dead time using SEM spot size. Stop x-ray counting as soon as dead time adjustments are

made.

2. Then click on start and collect an x-ray spectra for the preset time. During and after collection, you may expand different regions of the spectra and identify spectral peaks either automatically or manually.
3. Once spectral peaks are identified and confirmed, the x-ray spectra may be stored or saved to the hard drive. Hard copies of spectra can be printed on the Epson color printer.

#### C. Shut-down Procedures

##### SEM

1. The SEM vacuum always remain on so NEVER LEAVE THE SYSTEM FOR EXTENDED PERIODS EXCEPT AT HIGH VACUUM.
2. Shut-down procedure for the SEM involves closing the SEM operating window and answering the dialog questions as needed. Then close down the SEM user interface followed by shutting down the computer using the Start icon in the lower left corner of the monitor. Once closed, you may press yellow STANDBY button on the front of the column (or plinth). This will keep the electronics on but in a standby mode. If you press the red OFF button, you will remove all the voltage to the instrument and will lose stage settings. Therefore, never press the red OFF button except for major resetting of the instrument or for servicing of the system as described below:

**CAUTION:** If you are going to work on the electronics of the instrument, be sure to also press the red OFF button so that the 220V current to the instrument is removed. Failure to do this could be very dangerous to one's health.

##### EDS

1. If the x-ray detector is still counting, click on the STOP function to terminate counting by the detector. Save spectra if needed.
2. Close out all windows in ISIS or INCA.
3. Shut down the computer. Power is left to the monitor unless you cut it off separately. Generally, we leave it on because it automatically goes to a Power Save mode.

#### IV. Safety Concerns

Although we monitor the radiation from the EDS, there are few health risk concerns from

EDS x-rays. Liquid nitrogen used to cool the detector of the EDS can be dangerous to the skin and eyes if exposed for prolonged periods. It is recommended to use safety gloves and eye protection when filling the dewar of the EDS system manually. However, we have an automatic Liquid Nitrogen Fill System which should prevent having to manually fill the liquid nitrogen dewar.

## V. Other Information