Craic MicroSpectroPhotometer (MSP) Operating Procedure

I - Turning on the microscope and the Lambda Fire software

1) Remove the dust cover from the system.
2) Turn on the components you will need in the order in which they are numbered. **You cannot turn a light source on after the computer is running without risking serious damage to the system, so turn everything you think you’ll need on at the beginning!** See Appendix C for the spectral outputs from the Xe and Hg lamps.
3) Choose an objective to use, and load it if it’s not already in the lens turret (see Appendix A for objective choices). The turret has threaded slots that will accept the larger lenses. For the 10x & 40x lenses (thinner) a black reducer ring has to be fitted onto the lens before inserting onto the turret. You may need to move the stage down in order to more easily add or remove an objective. Note the working distance (WD) of the lenses. This will give you a general idea of how far your sample must be from the end of the objective to be in focus. Large WD objectives are safer to work with because your sample won’t be as close to the objective.
4) Choose an appropriate turret position. Above the objectives on the microscope base is the Imaging Mode turret. A grey plastic plate shows available modes corresponding to numbers on turret. Slots 1, 2 and 3 are fixed. Fluorescent cubes can be loaded as needed in the remaining slots (4-6). If you’ve never changed filter cubes before, please ask for assistance. See Appendix D for a full list of filter cubes.
5) Make sure the correct base plate is in place on the stage. The base plate with an opening is used for transmission experiments; all other experiments use the solid base plate.
6) Move the stage down away from the objectives, and the condenser down away from the stage. Load your sample. **Be very careful not to touch the condenser below the sample or run your sample into the objective.**
7) Choose a light source. Select the light path with the push buttons on the right side of microscope: RL for reflection (light comes in from the top) and TL for transmission (light comes in from bottom). For reflected light also select either the Xe or Hg source with the switch between these lamps behind the microscope.
8) Open the Lambda Fire software, or the Lambda Fire R software for Raman experiments.
9) Tell the software what objective you’re using by clicking on the camera icon, and choosing the correct objective in the Configurations tab. (If there are no configurations listed, choose the correct objective in the Video Modes tab and the correct image flip for the experiment you are doing in the Signal Processing tab: vertical for Lambda Fire and horizontal for Lambda Fire R.)
10) In the Settings tab, you may need to adjust the Camera Exposure and the Camera Gain in order to see an image.

<table>
<thead>
<tr>
<th>WD of Lenses</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x = 7.4 mm</td>
</tr>
<tr>
<td>20x = 2.2 mm</td>
</tr>
<tr>
<td>40x = 0.36 mm</td>
</tr>
<tr>
<td>50x = 0.22/9.4 mm</td>
</tr>
<tr>
<td>100x = 0.2/0.28 mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Imaging Modes</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 1: Transmission &amp; Raman</td>
</tr>
<tr>
<td>b. 2: Reflectance - Brightfield</td>
</tr>
<tr>
<td>c. 3: Reflectance - Darkfield</td>
</tr>
<tr>
<td>d. 4-6: Fluorescence filter cubes</td>
</tr>
</tbody>
</table>
II – Obtaining a Focused Image

11) Transmission mode:
   a. Try to adjust the condenser height by eye (medium black knob just below the stage) to produce the smallest spot of light on your sample. Typically the condenser will be ~1 cm from the top of your sample. We’ll fine tune this position later in step 11d.
   b. Focus on your sample. Be careful not to run your sample into the end of the objective! To be safe, get in the habit of moving the stage up while monitoring the distance between the objective and the sample by eye. Then either look through the eye pieces (with the silver knob next to the eye pieces pushed in) or at the camera image (with the silver knob next to the eye pieces pulled out) while moving the stage down. Following this rule will help prevent crashing the objective. Sometimes moving the sample stage in the x-y direction will help you find a feature to focus on.
   c. In transmission, there may be significantly more light than in reflection mode. You may need to adjust the camera settings (settings tab under the camera icon) in order to see an image on the camera.
   d. With a sample in focus, close down the appropriate Field (F) diaphragm until an octagon appears. Use the condenser height adjustment (medium black knob just below the stage) to focus the f-stop octagon, and use the condenser screws (two silver screws in the front of the condenser) to center the Field diaphragm in the image. Once you’ve finished this, you’ll probably want the f-stop open about ½ way.
   e. You can also adjust the amount of light making it to the sample by adjusting how open the condenser is with the silver slider on the front of the condenser.

12) Reflectance:
   a. Focus on your sample. Be careful not to run your sample into the end of the objective! To be safe, get in the habit of moving the stage up while monitoring the distance between the objective and the sample by eye. Then either look through the eye pieces (with the silver knob next to the eye pieces pushed in) or at the camera image (with the silver knob next to the eye pieces pulled out) while moving the stage down. You will not crash the objective if you follow this rule. Sometimes moving the sample stage in the x-y direction will help you find a feature to focus on.
   b. In reflectance mode, there may be significantly less light than in transmission. You may need to adjust the camera settings (settings tab under the camera icon) in order to see an image on the camera.
   c. In reflectance mode, the f-stop should be in focus when your sample is in focus. You can sometimes use this feature to help you find focus since it can be easier to focus on the f-stop octagon if there aren’t many features on your sample. You can also center the field diaphragm using an allen key and the adjustment set screws above and below the field diaphragm adjustment dial.
   d. You can also adjust the amount of light making it to the sample by adjusting the A-stop; the dial for this is just behind the f-stop.

III - Absorbance, Transmission, Reflection, Fluorescence Measurements (For Raman skip to step 27)
13) The Settings tab of the Parameter window will contain values for both spectrometers (Spectrometer 1: 146 – 1023 nm; Spectrometer 2: 900 – 2133 nm). From the settings tab, you may set the following items:
   a. Range of spectral acquisition (nm)
   b. Scans to Average
   c. Sampling time in milliseconds (integration time). This will be set by the Autoset Optimize feature, but you can manually change it here if you like.
   d. Resolution factor (boxcar averaging). The larger this number is, the smoother your spectra will be, but you’ll lose resolution. Typically we do not smooth (resolution factor of 0) unless absolutely necessary.

14) Choose the desired aperture size using the automatic aperture buttons (1 through 6 see Appendix B for aperture sizes).

15) Press My Folder to ensure the appropriate file path has been set.

16) Press Auto-Set Optimize for the best integration time (in milliseconds). In transmission mode, you’ll want to do this with the aperture over your blank area. In reflectance, you may want to do this with the aperture over an area where you expect relatively low light signals. If successful, a happy face message will appear. If unsuccessful, a frowning face message will appear, and you can follow the steps in a-c below.
   a. If the time in the window is 8 ms, this means there is too much light and the detector is likely saturated. You can check this by pressing the live counts button – the system saturates at ~65,000 counts. To reduce the counts in transmission, you can close the condenser and in reflectance, you can close the a-stop. In either mode you can reduce the aperture size. You can also manually decrease the integration time in the parameters window.
   b. If the time in the window is 1000 ms, you have low counts. For certain experiments like fluorescence this is common, and you may just have to manually increase your integration time in the parameters window, or just use the 1,000 ms default that it recommends. You can also try to increase your input light intensity by opening the condenser in transmission, opening the a-stop in reflectance, or choosing a larger aperture in either mode.
   c. After these adjustments, you may want to redo your Auto-Set Optimize to see if you get a happy face.

17) Press Collect Dark and Reference Scan while the aperture is positioned over a blank region in your sample. The system will sample the electronic (dark) noise of the system, as well as a background reference scan. Note that the reference scan will often be similar to that of the lamp output.

18) Move your sample so that the aperture is positioned over your sample area.

19) Press Collect Sample. An additional window will appear at this time to select the following:
   a. Type of measurement desired (absorbance, transmission, reflectance, fluorescence)
   b. Objective magnification
   c. Name of spectral file (it’s best to include a lot of detail in your file name, especially for parameters that are manually set. You may want to include: objective, F-stop and A-stop positions, turret position, light source, condenser setting (for transmission), and your sample. Repeat measurements with the same filename will automatically increment by +1.

20) Peak picking will automatically detect and notate peak/valley locations. Pressing the Peak Picking icon in the icon panel will activate/deactivate the notation on-screen. To change
peak picking settings, choose **Edit** from the top dropdown menu and choose **Peak Picking Settings**.

21) There are features that allow you to do spectral math, normalization, and smoothing of your data under the Mathematics tab. If you need assistance with these, ask a CHANL staff member.

22) If you need to export your spectra, go to File and then Convert MSP Files to CSV. This option will allow you to convert only the files you select (hold down the Ctrl key to select multiple files). The Batch Convert Folder to CSV will convert all of the .msp files in a folder to .csv files (including all of your darkscan and reference files which you may not need).

23) If you just need to save an image, press the disk icon at the top of the page. It will then ask you where to save your data.

24) Applications like Mapping Pro, Time Pro, and Film Pro are described in Appendix E.

25) Shut Down: Turn things off in the reverse order from which you turned them on (8->1).

26) Replace the dust cover over the microscope. *Do not cover the lamps in the back, as they are hot and will melt the cover.*

### IV - Raman Spectroscopy

27) Make sure that the laser power supply was turned on during start-up. If it wasn’t, you’ll need to turn off the computer, the stage, the microscope, and the MSP (steps 8->5) before you can turn on the laser (step 4 in the start-up procedure). There are laser safety glasses for you to wear when the laser is in use.

28) Open the LambdaFire Raman software. (Images of your sample will not display correctly in the normal LambdaFire software).

29) There are two large silver knobs on the right side of the microscope. Make sure that the top one is pulled out to direct the image to the Raman camera.

30) Choose an objective for your work. Raman objectives include 10x, 20x, 50x LD, and 100x Neo (typically ones with larger working distances). Also make sure that the Raman collar is on the objective so that you can protect yourself and other users from stray laser radiation.

31) Collect an image of your sample either with the reflectance Xe source or the blue LED imaging source (switch on the right side of the laser power supply box), as described in step 12.

32) In order to turn the laser on, the laser key must be in the horizontal position and both of the large silver knobs on the front right side of the microscope must be pulled out. At this point, you’ll see a blue light illuminated on the front of the instrument and on the front of the laser power supply box. In order to get the laser onto your sample, you also need to pull the ND filter out at least one stop.

33) Make sure you know where the laser will be hitting your sample by exposing your sample to the laser and seeing where the laser appears on the camera image. You can check for sample damage by taking an image of your sample before and after laser exposure.

34) The Settings tab of the Parameter window allows you to set the spectral range (Raman can go out to 3000 cm⁻¹, but if you get too close to 0, you’ll see light from the laser). From the settings tab, you may set the following items:
   a. Range of spectral acquisition (cm⁻¹)
   b. Scans to Average
c. Sampling time in milliseconds (integration time). You will manually set this here since there is no AutoSet Optimize feature in the Raman version of the software. Often long integration times (1000 ms or longer) are required to get good Raman signal.

d. Resolution factor (boxcar averaging). Typically this is set to 0 for Raman (no smoothing).

35) In the Raman software there is no choice to define an aperture size because you will just collect data from the area that the laser illuminates your sample. You may want to collect a reflectance image in which you can also see the laser spot in order to know where you’re taking data.

36) Press My Folder to ensure the appropriate file path has been set.

37) Before taking any Raman data, make sure that the light for sample illumination is off (press the RL button on the right side of the microscope if using the Xe lamp or turn the blue LED off on the Laser power supply unit).

38) Change the filter cube turret position to 1 (transmission) before collecting data.

39) Press Darkscan while the ND filter for the laser is all the way in. The system will sample the electronic (dark) noise of the system.

40) Press Collect Spectrum. An additional window will appear at this time to select the following:
   a. Objective
   b. Name of spectral file. (It’s best to include a lot of detail in your file name, especially for parameters that are manually set like objective, f-stop and a-stop position, turret position, your sample name, etc. Repeat measurements with the same filename will automatically increment by +1.)
   c. Right before you press Scan, make sure that the bottom large silver knob on the right side of the microscope is pulled out (blue laser light indicators should be on), and that you pull the ND filter out to the appropriate position.

41) To be safe, adopt good practices: When not collecting data, push the bottom large silver knob on the right side of the microscope in (blue laser light indicators should be off), and that you push the ND filter all the way in to block the laser.

42) Adjust your integration time and/or the neutral density filter position if necessary. It’s best to start with short integration times and large ND values to protect your sample. (The ND settings are 6.0, 2.0, 0.6, and 0.3 as you pull the ND filter out.) Then if needed you can increase the exposure time/intensity to get the data you need.

43) You may need to subtract a baseline in order to remove contributions from fluorescence. Go to the Mathematics tab and select Baseline Correction. You can adjust the smoothness and asymmetry values to adjust the fit. Under some conditions, the baseline will start to go up into the peak. If this happens, readjust your values to minimize this effect.

44) Shut Down: Turn things off in the reverse order from which you turned them on. 8->1

45) Replace the dust cover over the microscope. Do not cover the lamps in the back, as they are hot and will melt the cover.

Questions, comments, concerns, complaints?

Contact CHANL:
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Wallace Ambrose: wambrose@unc.edu; 919-966-5223
Appendix A. Objectives

- Ultrafluar: Objective for excitation in the UV wavelength range
- EC Epiplan-Neofluar: Best universal objectives, best field flattening, very good correction
- EC Epiplan-Apochromat: High-power, apochromatically corrected objectives for reflected light

<table>
<thead>
<tr>
<th>Objective Type</th>
<th>Mag (x)</th>
<th>Numerical Aperture</th>
<th>Working Distance (mm)</th>
<th>Correction Flatness</th>
<th>Color</th>
<th>Transmission UV VIS IR</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrafluar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brightfield, fluorescence imaging</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td>7.4</td>
<td>*</td>
<td>*</td>
<td>*****</td>
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<td>**** ****</td>
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<tr>
<td>40</td>
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<tr>
<td>100</td>
<td>0.9</td>
<td>0.2</td>
<td>*</td>
<td>*</td>
<td>*****</td>
<td>***</td>
<td>**** ****</td>
</tr>
<tr>
<td>EC Epiplan-Apochromat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High power, corrected in reflection</td>
</tr>
<tr>
<td>50</td>
<td>.95</td>
<td>0.22</td>
<td>*****</td>
<td>*****</td>
<td>*</td>
<td>*****</td>
<td>****</td>
</tr>
<tr>
<td>EC Epiplan-Neofluar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Best universal imaging and correction for transmitted and reflected light</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>2.2</td>
<td>****</td>
<td>****</td>
<td>**</td>
<td>***</td>
<td>**** ****</td>
</tr>
<tr>
<td>100</td>
<td>0.9</td>
<td>0.28</td>
<td>****</td>
<td>****</td>
<td>**</td>
<td>***</td>
<td>**** ****</td>
</tr>
<tr>
<td>EC Epiplan-Neofluar LD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>As above plus long working distance</td>
</tr>
<tr>
<td>50</td>
<td>0.55</td>
<td>9.4</td>
<td>****</td>
<td>****</td>
<td>**</td>
<td>***</td>
<td>**** ****</td>
</tr>
</tbody>
</table>

Appendix B. 20/30 PV™ Microspectrophotometer Aperture Sizes (in microns)

<table>
<thead>
<tr>
<th>Aperture Number</th>
<th>10x Lens Objective*</th>
<th>36X Reflecting Objective*</th>
<th>40X Lens Objective*</th>
<th>50X Lens Objective*</th>
<th>LD 50X Lens Objective*</th>
<th>100X Ultrafluar Objective*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>97.6 x 97.6</td>
<td>32.4 x 32.4</td>
<td>24.2 x 24.2</td>
<td>19.7 x 19.7</td>
<td>19.7 x 19.7</td>
<td>9.7 x 9.7</td>
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<tr>
<td>2</td>
<td>72.9 x 72.9</td>
<td>24.2 x 24.2</td>
<td>18.1 x 18.1</td>
<td>14.7 x 14.7</td>
<td>14.7 x 14.7</td>
<td>7.2 x 7.2</td>
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<tr>
<td>3</td>
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<td>15.7 x 15.7</td>
<td>11.8 x 11.8</td>
<td>9.6 x 9.6</td>
<td>9.6 x 9.6</td>
<td>4.7 x 4.7</td>
</tr>
<tr>
<td>4</td>
<td>25.1 x 25.1</td>
<td>8.3 x 8.3</td>
<td>6.2 x 6.2</td>
<td>5.1 x 5.1</td>
<td>5.1 x 5.1</td>
<td>2.5 x 2.5</td>
</tr>
<tr>
<td>5</td>
<td>11.1 x 11.1</td>
<td>3.7 x 3.7</td>
<td>2.8 x 2.8</td>
<td>2.2 x 2.2</td>
<td>2.2 x 2.2</td>
<td>1.1 x 1.1</td>
</tr>
<tr>
<td>6</td>
<td>6.2 x 6.2</td>
<td>2.0 x 2.0</td>
<td>1.5 x 1.5</td>
<td>1.2 x 1.2</td>
<td>1.2 x 1.2</td>
<td>0.6 x 0.6</td>
</tr>
<tr>
<td>7 Camera Field of View</td>
<td>1006.8 x 840.0</td>
<td>334.1 x 278.8</td>
<td>250.1 x 208.7</td>
<td>203.6 x 169.8</td>
<td>202.9 x 169.3</td>
<td>99.8 x 83.2</td>
</tr>
</tbody>
</table>

*Tables above denote measurements taken at factory.
Appendix C. Lamp characteristics

Spectral output of Xe lamp

Appendix D. Filter Cubes

<table>
<thead>
<tr>
<th>Filter Cube Label</th>
<th>Excitation</th>
<th>Dichroic</th>
<th>Emission</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>280</td>
<td>275-285 nm</td>
<td>325 nm</td>
<td>325 nm LP</td>
<td></td>
</tr>
<tr>
<td>365</td>
<td>325-380 nm</td>
<td>395 nm</td>
<td>400 nm LP</td>
<td></td>
</tr>
<tr>
<td>420</td>
<td>395-440 nm</td>
<td>460 nm</td>
<td>470 nm LP</td>
<td></td>
</tr>
<tr>
<td>546</td>
<td>540-552 nm</td>
<td>580 nm</td>
<td>590 nm LP</td>
<td></td>
</tr>
<tr>
<td>Custom 520</td>
<td>500-540 nm</td>
<td>560 nm</td>
<td>575 nm LP</td>
<td>Specially designed for detection out to 2,000 nm</td>
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<tr>
<td>Custom 980</td>
<td>970-990 nm</td>
<td>1050 nm</td>
<td>1020 nm LP</td>
<td>Specially designed for detection out to 2,000 nm</td>
</tr>
</tbody>
</table>

UV: 200 - 400 nm  Yellow: 570 - 585 nm
Violet: 400 - 420 nm  Orange: 585 – 620 nm
Indigo: 420 - 440 nm  Red: 620 – 780 nm
Blue: 440 - 490 nm  Near IR: 780 – 2000 nm
Green: 490 - 570 nm
Appendix E. Special Applications (Mapping Pro, Time Pro, Film Pro)

Mapping Pro: Allows you to define a matrix of points at which the system will take data.

1) Go to Applications and select CRIAC Mapping Pro. This will open a new window that will allow you to define your matrix of points and move certain distances known distances.

2) The Digital Readout section allows you to define where you’d like 0,0 to be. Drive the stage to that position (using the joystick), and then press the Zero Position button. As you drive to new locations, the new x, y position will be displayed here. The Goto Abs button will allow you to type in an x, y position relative to 0, 0 and it will drive the stage to that position.

3) You can use the Measure section to measure distances. Move the stage to the position where you’d like to start measuring, and press the Mark button. Then drive to the second location. This portion of the window will show the distance moved in x, the distance moved in y, and the total distance moved (diagonal).

4) The Stage Move section will allow you to step the stage by a set amount each time you press an arrow button. This distance can be set by going to Setup and choosing X (Y) Step Distance.

5) In order to set your matrix of points, you’ll need to press the Pattern button. This will bring up a new portion of the window that will allow you to choose the total area that you want to map, and the number of steps (not the number of positions) in both x and y.

6) For example, the matrix to the right consists of 2 steps in x and 2 steps in y (for 3 spots in x and 3 spots in y). The current stage position will be position 1 in the box to the right, and it will take data at the spots in the order they are numbered. Your current position is always the bottom right corner of your matrix. (While the system is taking this data, it will show you a matrix where green dots are spots where data has been taken and red dots are still to be analyzed. This matrix will start in the upper left corner, work its way down and then to the right. It does not correspond to the actual matrix of spots on your sample.)

7) When you’re ready to take your data, set your scan conditions under the Parameters menu, Do an AutoSet Optimize to determine an optimal integration time, and take dark and reference scans.
8) Press the Collect Spectrum button. Make sure to check the 3D Mapping button so that it knows to collect the map.
9) Spectra will be overlayed in the spectral window as they collect.

**Time Pro:** Allows you to take data in the same spot over time

1) Find the region on your sample where you’d like to take data over a period of time.
2) Go to Applications – TimePro.
3) Press the Tools pull down at the top and select Set Parameters. Under the Kinetic Settings Tab you can set the time sequence for collecting your data.
   a. Here you can choose the **Wait Time Between Scans** – this will be the time between the start of one scan and the start of the next scan (it’s not the time between when it finishes one scan and starts the next).
   b. You can also set the **Total Scan Time**.
4) Under the Spectrometers Tab of the Set Parameters window, you can set the spectral limits, scans to average, the sampling time, and the resolution factor.
5) Press the **Autoset Optimize** button to allow the system to determine an appropriate integration time.
6) Choose a wavelength(s) to monitor during the acquisition in the bottom left portion of the window.
7) Press My Folder to ensure the appropriate file path has been set.
8) Press **Collect Dark and Reference Scan** while the aperture is positioned over a blank region in your sample. The system will sample the electronic (dark) noise of the system, as well as a background reference scan.
9) Move your sample so that the aperture is positioned over your sample area.
10) Press Time Scan. An additional window will appear at this time to select the following:

   a. Sample Name. These files will be incremented with the time point at which the data was taken (10 sec, 20 sec, 30 sec, etc.)
   b. Type of measurement desired (i.e., transmission, absorbance, reflectance, fluorescence, etc.)
   c. Objective
   d. How you’d like to save your data – as individual or cumulative files.

**FilmPro:** Allows you to determine film thickness from either transmission or reflectance measurements

1) Take a reflectance or transmission spectrum of your sample. You should see some interference fringes.
2) Go to Applications - Film Pro.
3) Select the recipe that best fits your sample, your reflectance standard material, substrate, and your layers.
4) Enter estimates for substrate and film thickness. The fit will depend somewhat on these estimates, so the more information you can provide the better.

5) Press the Fit button. From the curves and the merit function determine the quality of the fit. The merit function is a quantitative measure of the difference between the theoretical fit and the measured spectrum. The lower the number the better the fit. Keep in mind, however, that there is no specific number threshold for the merit function that determines the fit is good or not. The merit function will vary depending on the sample, the wavelength range, and other factors.

6) If the fit is not satisfactory, you can fit the spectrum again by adjusting a few parameters. Try a new initial thickness estimate and fit again. The Gaussian half-width value may be adjusted as well to account for the coherence length of the light source. This is particularly important for thick films. A good starting value is 11nm. If the baseline intensity of the sample and fitted spectra are offset, try adjusting the baseline of the sample spectrum with the buttons on the right of the graph such that the spectra better match. Then try and fit again. The difference in baseline intensity between the model and spectrum may be caused by sample surface roughness or by a poor estimate of the refractive index of one or more of the films.

Polarizers

1) Polarizers can be used for either transmission or reflectance work. See the figure below for the input and analyzer ports for the analyzers. The polarizers are located in a wooden box marked Polarizers.
   a. The input polarizer must be in the unlocked position before inserting it into the microscope. You can use a hex key to make sure it’s unlocked before inserting it. Make sure to lock it after inserting.
   b. The analyzer polarizer is in place when you feel the spring loaded clip engage.

2) **DO NOT LEAVE THE UV-VIS-NIR POLARIZER ENGAGED FOR ANY LONGER THAN 90 SECONDS AT ONE TIME. YOU RISK THERMAL DAMAGE TO THE OPTIC WHEN ENGAGING FOR LONGER PERIODS OF TIME.** Allow one minute for polarizer to thermally stabilize between UV light engagements.

3) You can rotate the polarizers a full 360°. Please note that a full 360° rotation of the dial will correspond to ~180° of the polarizer (both the input and analyzer are noticeably off by ~20°!), and a rotation of the dial by 720° corresponds to ~360° rotation of the polarizer. The important positions are when the polarizers are crossed and when they are aligned. You can determine these positions when the polarizers are in the system. The polarizers are crossed at the point when the least amount of light reaches the detector, and the polarizers are aligned when the most light reaches the detector.