

## Materials

- a) Standard 1mm glass microscope slide
- b) Glass cover slip
- c) Immersion oil
- d) Fine tip permanent marker

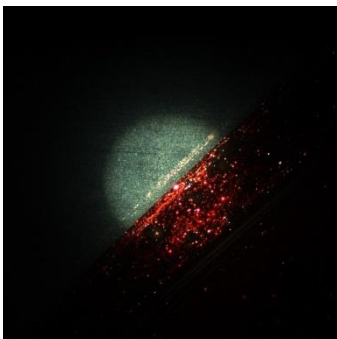
## Set-up

- 1) Begin by taking a permanent marker and placing an "X" near the center of the slide.
- 2) Allow a couple of minutes for the ink to dry.
- 3) Place a drop of immersion oil directly onto the "X" mark and then cover with a glass cover slip.  
*Note: The color of the marker is not critical as we are only using this mark for a focal point and no imaging or data will take place where the marking is present.*



## Focusing the Light Source

- 4) Place the slide on the CytoViva microscope system and set-up the system for enhanced darkfield transmitted light imaging.



- 5) Begin at 10x, focusing on the "X" mark on the slide. To ensure that you are focused on the "X", you can move the sample left or right until you visualize the ink (appears red in this illustration) as compared to the adjacent blank area of the slide.

- 6) Once the “X” is in focus at 10x and the CytoViva illuminator is aligned, move to 100x magnification.
- 7) After rechecking focus at 100x, move the stage left or right to an area of the slide where there is no visible mark or any other debris on the slide. Do not adjust the focus any further after this step.
- 8) Once a clean area of the slide is located, remove your eyes from the microscope oculars to avoid bright light in the following steps.

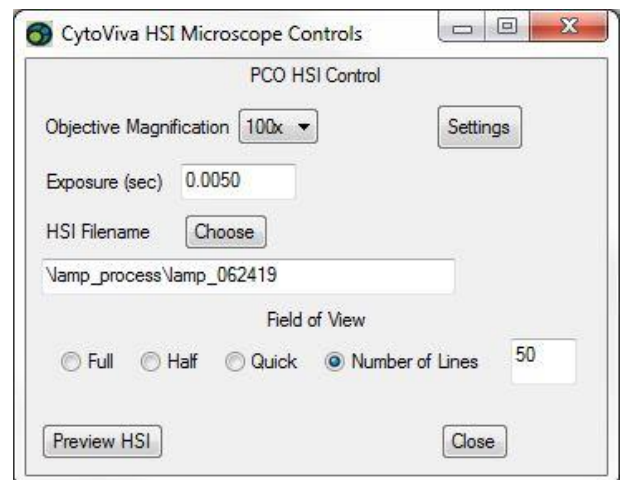


- 9) Again, without adjusting the focus or bumping the microscope stage, carefully reach up and turn the adjustable iris of the 100x objective counter-clockwise which opens the iris fully. If you carefully glance into the microscope oculars from a distance you will see nothing but bright light, which is expected.

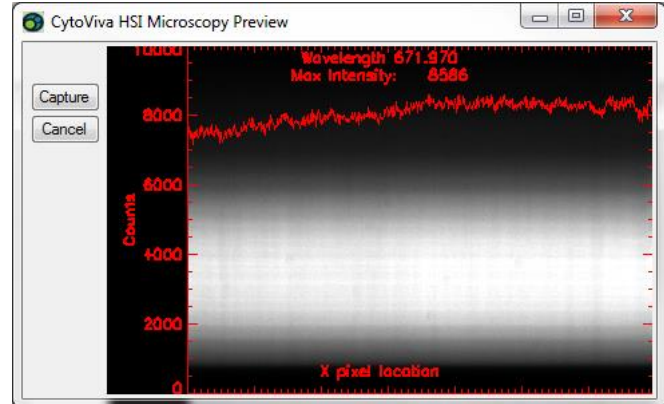


Adjustable iris. Twist counter-clockwise to open fully.

- 10) In ENVI, set up the image capture process. Note the following:
  - a. **Objective Magnification** – Ensure that this is 100x
  - b. **Exposure (sec)** – In this illustration this is set at 0.0050. Since all lamp intensities will vary, this will be set appropriately per your system to capture an intensity value of the lamp ranging from 4000 – 10,000.
  - c. **HSI Filename** – It is suggested that you use the date in the file name so it is clear the date of the image capture.
  - d. **Field of View** – Only a small spatial image is required. Here we have selected to capture 50 lines.

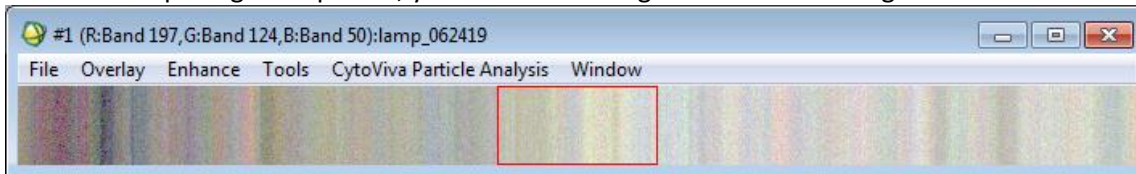


11) Here we are checking the intensity of the lamp, which is within the desired range. *If it is not, select **Cancel**, adjust the **Exposure (sec)** as needed until the intensity is within the desired range.*



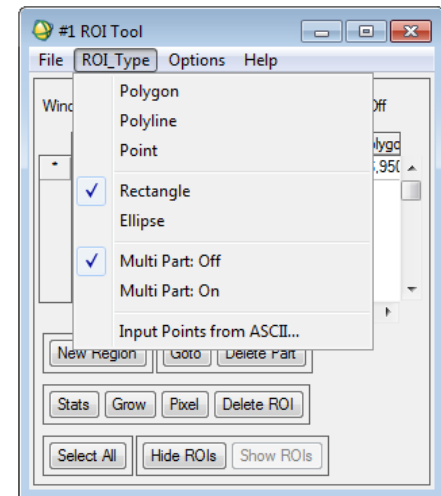
12) Select **Capture**, follow the standard capture process to capture the hyperspectral image of the “white light” of the lamp.

13) Once the lamp image is captured, you will see an image similar to the image below.

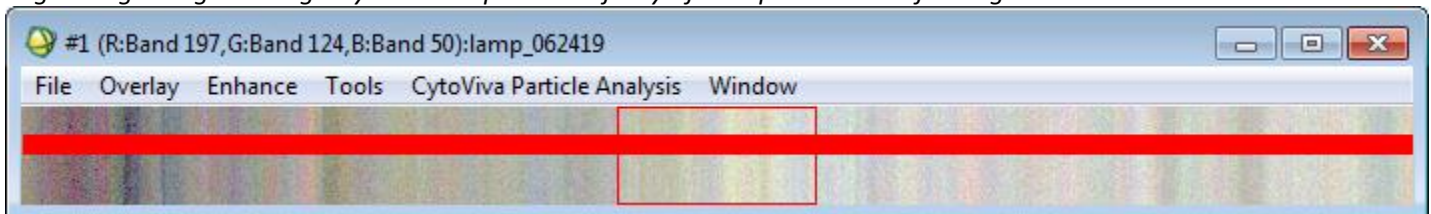


14) Next, we will capture a ROI (region of interest) to get an average of the spectral profile for the lamp. To start, right-click on the image and choose **ROI Tool**...

15) In **ROI Type**, select **Rectangle**.

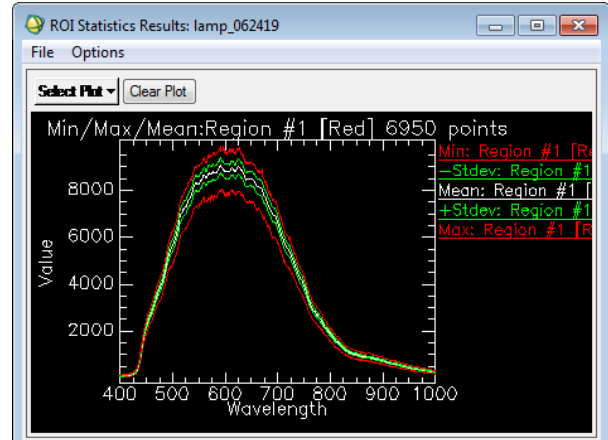


16) Draw a rectangle from left to right and select this area for analysis. *This area does not have to be exact from left edge to right edge so long as you encompass a majority of the spatial area left to right.*

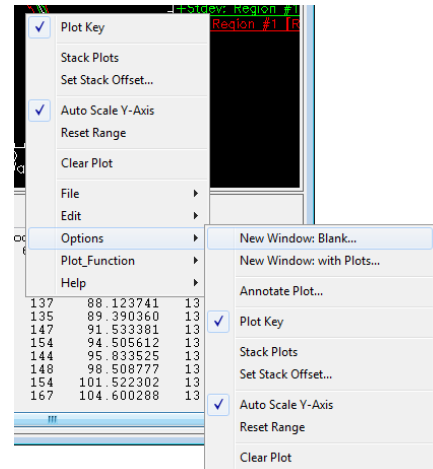


17) Select **Stats**.

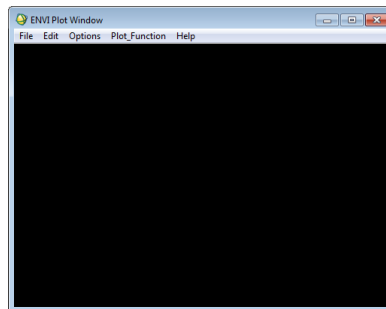
18) Once the data is processed, you will be presented with the **ROI Statistics Results** for the rea selected as seen below. Right-click on this plot and select **Plot Key** to display the key on the upper-right side of the plot.



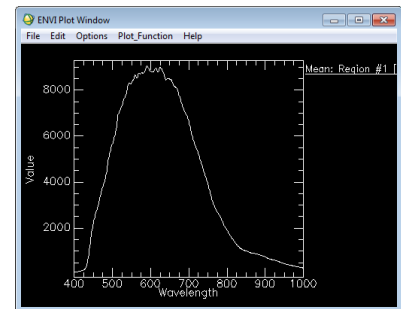
19) Now we need to bring the plot for the mean (*Mean: Region #1 [Red]*) to a separate plot by itself. First right-click on the plot, then choose **Options** >> **New Window Blank** and a new window will be displayed.



20) On the original plot, left-click on the mean plot key (*Mean: Region #1 [Red] 6950 Points*) and hold, drag to the blank window, then release the left click button to drop the mean (*Mean: Region #1 [Red] 6950 Points*) into the new window.

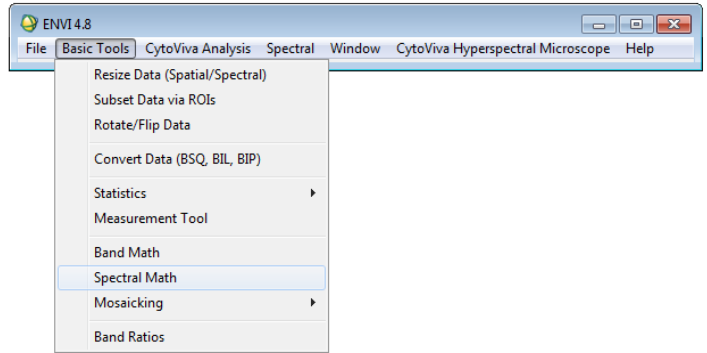


New Window

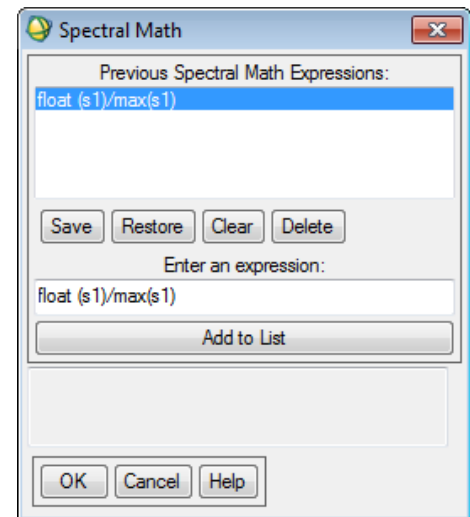


New Window with Mean Plot

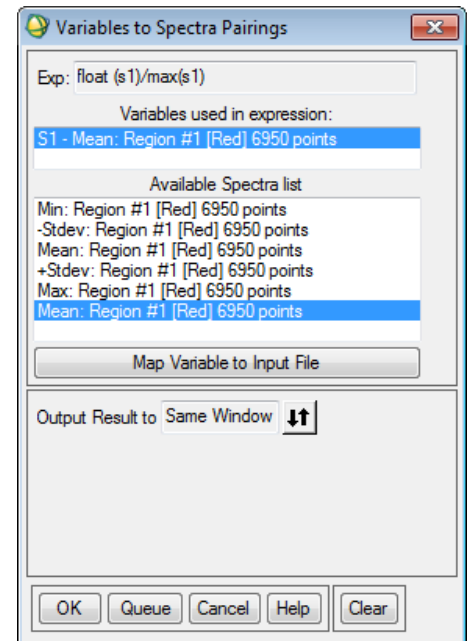
21) Next, we need to normalize this mean plot to a value of 1. With the new window with the mean plot open, go to the main menu and select **Basic Tools >> Spectral Math**.



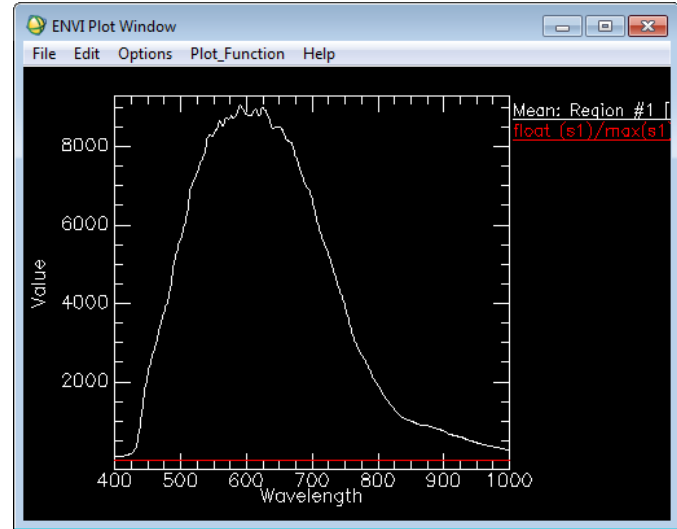
22) In the next window, if the math expression is not visible from previous use, enter **float (s1)/max(s1)** in the 'Enter an expression' text box, then select **OK**.



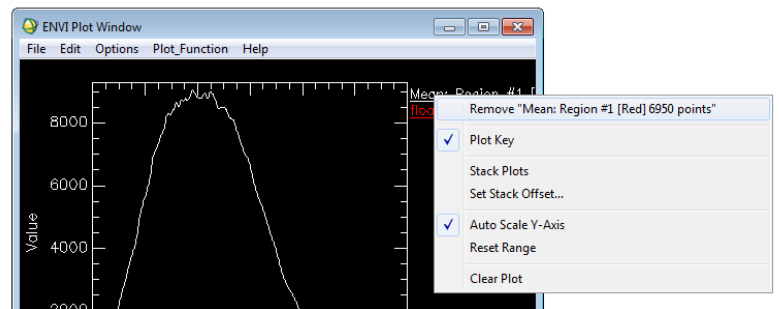
23) In the next window, you will need to elect the plot from the list that you will apply the math expression to. Since the mean plot that we moved to the new window by itself was the last plot that we worked with, it is the plot at the bottom of the list. Highlight the desired plot and then select **OK**. *This list is generated in chronological order, oldest plots at the top, newest plots at the bottom.*



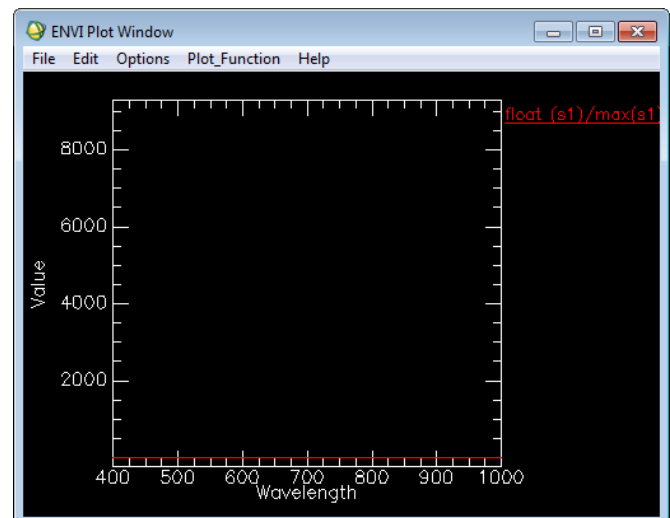
24) In the new window with the mean plot, you will see that a new plot has been added. In this example the plot is titled **float (s1)/max(s1)**, which is the math expression applied to this plot.



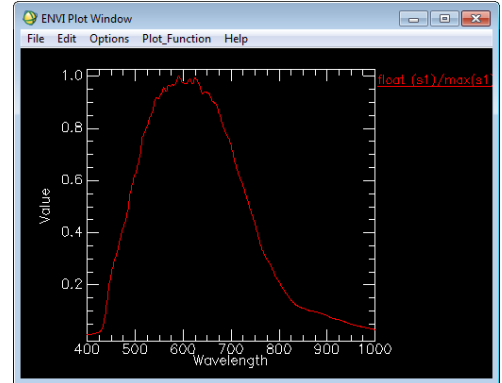
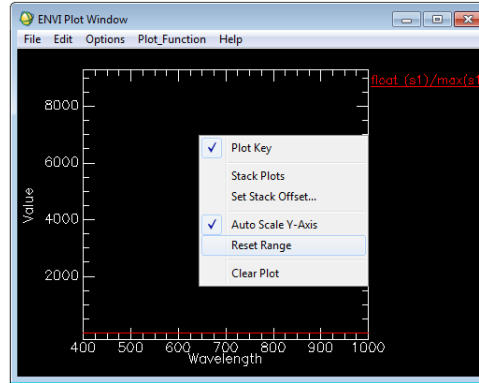
25) We need to remove the original plot, leaving only the plot **float (s1)/max(s1)**. To do so, right-click on the plot key **Mean: Region #1 [Red]** and select **Remove "Mean: Region #1 [Red] 6950 points"**.



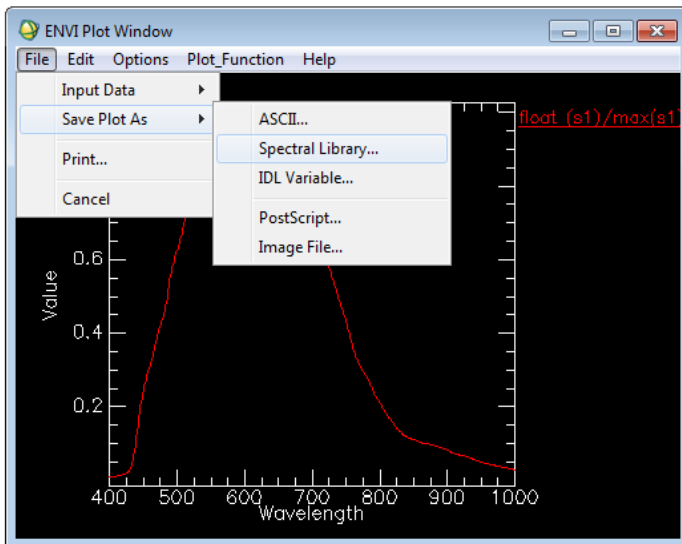
26) After removing the original plot, you are left with the **float (s1)/max(s1)** plot, which we previously applied the math expression to. It appears as a flat line because the math expression normalized the plot to a maximum intensity value of "1".



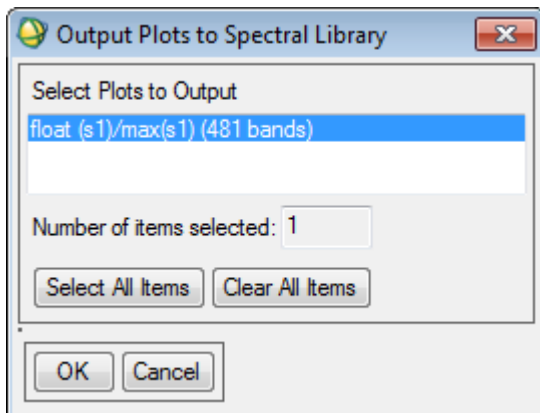
27) To correct the plot's Y scale where the `float (s1)/max(s1)` plot is visible, right-click on the plot and select **Reset Range**.



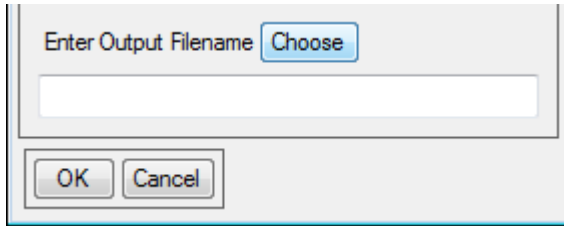
28) After resetting the range, the final step is to save this plot as a spectral library. Select **File >> Save Plot As >> Spectral Library...**



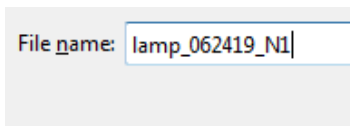
29) Select the plot, then **OK**.



30) Select **Choose** to set the file name.

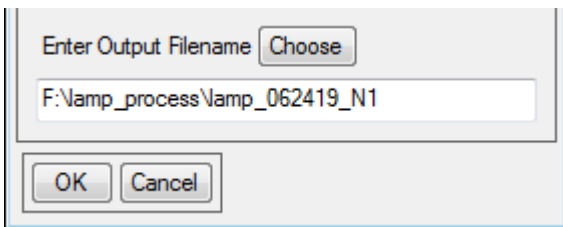


31) Set the file name, remembering to include the date in the file name. Also be sure to add nomenclature to the file name to know that this is the spectral library that has been normalized to an intensity value of 1. (e.g. N1)



32) Select **Open**.

33) Select **OK**.



34) The file has been saved as a spectral library and is now visible in the list of active files for your current session.

