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If any component of the Equipment does not function properly during the Warranty Period due to defects in material or workmanship, CytoViva will, at its option, either repair or replace the component without charge, subject to the conditions and limitations stated herein. Such repair service will include all labor as well as any necessary adjustments and/or replacement parts. If replacement components are used in making repairs, these components may be remanufactured, or may contain remanufactured materials. Repair or replacement without charge is CytoViva's only obligation under this warranty. This warranty is NOT transferable from the original purchaser of the Equipment.

Limitations

Other components of the product package, specifically including, but are not limited to, the light source(s), light source power transformer(s) and cord(s), liquid light guide(s), optical filters, spectrophotometer, camera(s), software, microscope part(s), and motorized stage are warranted based on the individual original manufacturer's warranties and policies. There is no warranty whatsoever on the contrast filters, bulbs or the coil (on the motorized stage) purchased as part of the Equipment.

This warranty does not cover circumstances beyond CytoViva's control, breakage, or a malfunction that has resulted from improper or unreasonable use or maintenance, accident, tampering, misuse, neglect, improper installation, modification, improper maintenance or service, cleaning procedures, shipping or repacking of Equipment, or service or parts to correct problems where such service or parts are performed or provided by anyone other than CytoViva or an authorized agent of CytoViva; service required as the result of unauthorized modifications or service misuse or abuse; failure to follow CytoViva's operating, maintenance or failure to use items supplied by CytoViva. This warranty is also void if the light source, light source power transformer and cord, or liquid light guide is not used in accordance with the original manufacturer's instructions, recommendations, or documentation.

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CytoViva[®] Quick Start Guide

Part 1: Starting CytoViva ENVI Software

A CytoViva HSI microscopy session is started by first running ENVI. The ENVI icon appears on the desktop. Double click the icon to start ENVI. The main ENVI toolbar will open. All of the operations for files, image display and data analysis are contained in the drop down menus of the tool bar.

1. Find the menu "CytoViva" located near the right of the tool bar and begin an imaging session by selecting the menu and clicking on the contents. Be sure that the stage controller has been turned on and that it is connected to the computer before proceeding further.

If the stage is not turned on at this time, an error message will be generated and stage movement may occur after the controller is turned on.

2. Enter the COM port number (see Figure 1) which has been assigned to the automated stage by your computer (usually 3). Click OK.

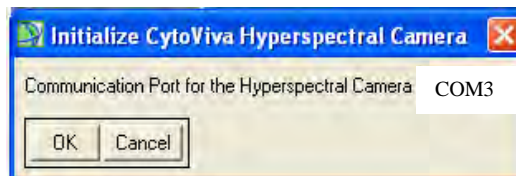


Figure 1. COM Port Window

3. After a few seconds the CytoViva Hyperspectral Camera Settings window appears as below in Figure 2. The dialogs in this box are used to set up the size and other attributes of the HSI image that will be acquired.

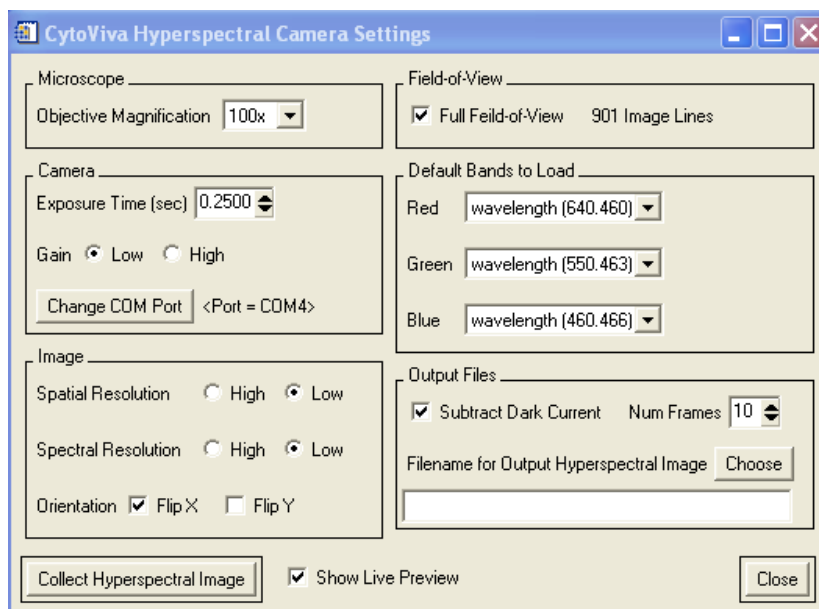


Figure 2. The control and image scanning control window

Part 2: Operating CytoViva ENVI Software

The Camera Settings dialog allows the HSI system to recognize your microscope configuration. The following controls appear in the dialog box:

Microscope:

Objective Magnification: This is set to the magnification of the microscope objective that will be used to collect the image (example 10X). The setting should always match the objective, otherwise images will look stretched or compressed from top to bottom.

Camera:

Exposure Time: Enter a camera exposure time. This value is the time that the camera will be exposed to the spectral image on each line of the HSI scan. For bright fields of view, exposures between 0.1 and 0.5 are appropriate. Longer exposures will be needed for low light such as obtained from fluorescent samples (0.5 - ∞). The time needed to complete the HSI image collection is proportional to the exposure time that you enter. Either use the scroll bar to change the value in 1 second increments, or enter the value directly in the text box, followed by Return.

Gain: The gain refers to the sensitivity setting on the camera. Usually the gain is set low. High gain can be used for low light imaging, however image quality is better if the gain is kept low and the camera exposure is lengthened. If photobleaching is a problem, use of high gain will allow a shorter camera exposure, and thus a faster scan that limits the amount of bleaching.

COM Port: You will not need to change the COM port so do not use this feature.

Image:

Spatial Resolution: Either "high" or "low" spatial resolution can be selected for the HSI image data. Low resolution is recommended since this mode results in smaller file sizes and increased image brightness. Usually brightness is preferred over resolution to get the most information from an HSI image. High resolution effectively doubles the spatial resolution, however the HSI file sizes are also significantly increased. The default resolution is "low".

Spectral Resolution: we recommend setting it to low. The spectral resolution when high is selected is 1.25nm and when low is selected it is 2.50nm.

Orientation: Make sure only the FlipX box is checked so that the image will be displayed as a direct view instead of a mirror image.

Field of View:

The number of scan lines that are needed to achieve a square image scan is calculated by ENVI. You may want to record from a smaller area of the field of view. To save time and reduce the size of the image file, the number of lines can be reduced. Change the number of lines by un-checking the box, enter a new number and press return. Remember that more lines will require a longer scan. If the area of interest is located only in the center of the field of view near the intersection of the eye piece cross hairs, 200-300 lines will be sufficient.

Default Bands to Load:

The spectral image bands that will be used to represent red, green and blue are selected using the drop down lists for each color. The default bands will give a true color representation of the data. When the HSI data does not contain intensities at a default wavelengths, other image bands can be selected to represent one of the colors. To choose a new band to represent a color, press the down button. A list of the HSI bands and associated wavelengths for each band appears. Scroll through the list and select a band. The dialog jumps automatically from red, to green, and to blue as new selections are made.

Output Files:

Dark values can automatically be subtracted from the image data during the scan. Check the box to enable this feature. Enter the number of frames to average for subtraction. The default value of ten is recommended.

The folder and name that will be given to the HSI image is selected next. Press "Choose".

Browse to the desired folder and enter a new file name. A new folder for the file can be created if desired. It is helpful, though not necessary, to add the extension ".img" to identify the file as an HSI image.

***** Important: If an old filename is used, for example by rescanning without changing the filename, the old file will be overwritten by the next scan and the old data will be lost. ****

Part 3: Starting the Scan

After entering the camera settings, the HSI scan is started by pressing "Collect Hyperspectral Image" at the lower left.

1. A Live Preview of the spectral image can be included before the scan to help in the selection of the camera exposure time. This information is used to:
 - 1) confirm the placement of objects in the field of the scan
 - 2) to aid in adjusting focus,
 - 3) to confirm that camera exposure is appropriate.To see the Preview dialog, check "Show Live Preview" before starting the scan.
2. Show "Live Preview" was selected, the HSI Microscopy Preview screen appears. This screen is used by first putting the cursor inside its borders. If the cursor is outside the window, some information will not be displayed. The upper panel of Preview shows a histogram containing the number of image pixels that have values between zero and the maximum value. Under the histogram, the panel displays a live spectral image that is being captured by the camera. In this image, the horizontal axis represents the X axis of the HSI image that will be recorded. The vertical axis represents the wavelengths of the spectrum captured in the HSI image. In the image below, the vertical streaks are spectra from objects that are in the view of the HSI camera. This image shows that two bright objects are in the view. Overlaid onto the spectral image is an intensity plot. Placing the cursor at any place on the image corresponding to a spectral wavelength causes the intensities of all the points across the image, at that wavelength, to be plotted.
3. **Placement of objects:** The features on the specimen that will be scanned are first moved into the field of view of the eyepiece and centered at the intersection of the cross hairs using the stage joystick. Note any places where the features are especially bright and move these so that they intersect the horizontal crosshair. The spectra of these same objects will appear in the Preview screen. Note that the live frames are flipped (reversed) about the X axis. The spectral images below are from two bright objects that appeared directly above the horizontal crosshair. With the specimen positioned thus, the stage can be moved forward and backward small amounts to center the desired area on the crosshairs.
4. **Focus:** Typically if the objects are in focus in the eyepiece of the microscope then they are in focus to the HSI camera. Often the thickness of the specimen makes it difficult to properly adjust for best focus. This can be done more easily with the Preview image. If focus is poor, the spectra will appear blurred, as they are shown in Figure 3. When focused correctly, the spectra become sharp as in the Figure 4. Focusing with the Preview window insures that the HSI image will be sharp.

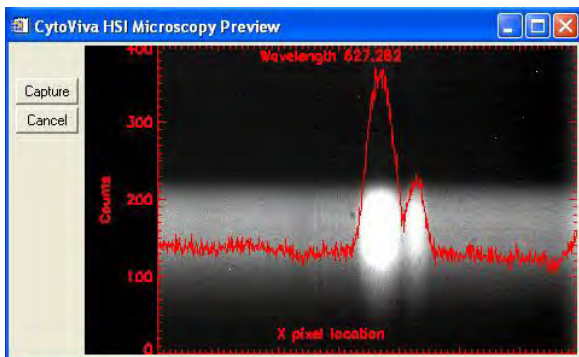


Figure 3. Frame out of focus

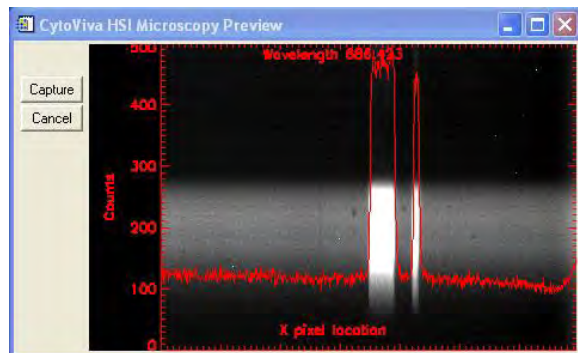


Figure 4. Frame in Focus

5. **Camera exposure:** The intensity plot can be used to adjust the camera exposure. Begin by moving the brightest image features to the crosshair. If the exposure is too long, the intensities will be seen to "flat top" on the plot (see Figure 5). If this should occur, the camera is overexposed to these areas and data will be missing in the HSI image. If the exposure is too short, the spectral data will be noisy. The best exposure will be obtained when the intensity of bright features is near 3000. Intensities of a count under 200 don't yield sufficient spectra data. Intensities will clip around 4000. The intensity values appear on the plot ordinate. The plot scale will adjust to more and less intense parts of the spectral image as you move the cursor. The most intense readings will occur at the spectral peaks, and thus the cursor should be placed at the brightest portion of the spectra to insure that the HSI image will not "flat top" any of the recorded spectra. Usually the spectral peak is near the center wavelength, so the cursor would be placed halfway between the lower and upper ends of the spectrum. Make changes to the camera exposure by canceling the Preview window and returning to the Camera Settings dialog. After making a change, go back to the Live Preview by pressing "Collect Hyperspectral Image" and review the new intensities. When the Preview shows good data, press "Capture" to start the scan.

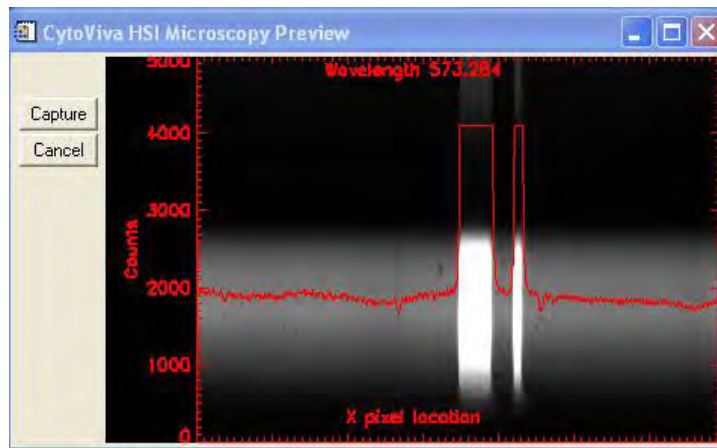


Figure 5. Intensity Plot showing "flat top"

6. If dark subtraction was selected, the following message appears (see Figure 6). Do not press OK yet.

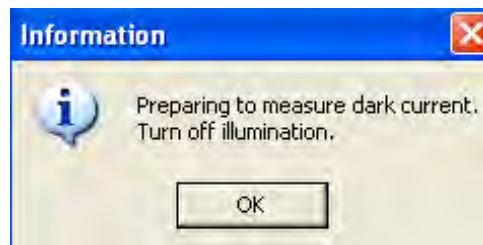


Figure 6

At this point, divert the light path from the HSI camera to the microscope eyepiece by pushing the slide bar in all the way on the trinocular head. Then press OK. Do not turn off or change the brightness of the illuminator. A recording of the dark image will be made from the requested number of dark frames. The dark image that will be used is the average of the recorded dark frames.

7. A message to begin the scan appears next (see figure 7):

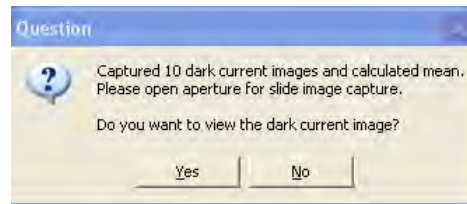


Figure 7

At this point divert the light path from the eye pieces to the HSI camera by pulling the slide bar all the way out on the trinocular head. In response to the question, select "No". It is not necessary to view the dark image.

8. The HSI scan will begin and the acquisition progress bar appears (see figure 8):

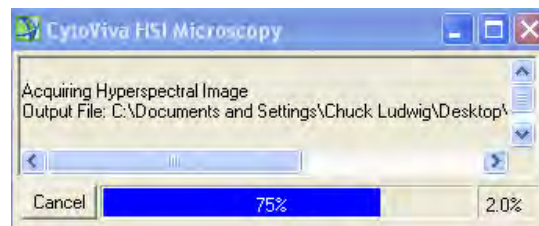


Figure 8

The stage moves to the beginning of the scanning area and then the individual lines of the HSI image are acquired, until the stage has moved to the end of the scan area.

During this process, the percentage of completion of the scan is reported on the progress bar. Ignore the percent value in the lower right. After the scan finishes, the HSI image automatically opens in ENVI.

***** Important: if an image was rescanned using the same filename as a previous scan, the old data is lost. However, the display still contains the old images. If this happens, remove the old data from the Available bands list by selecting close all files and open the newly scanned image into the List. *****

9. Highlight the old data in the List. Press "File" in the main image and click on "Close Selected Files". Click through the display warning. The old data will be removed.
10. Open the newly scanned File to place it in the List and obtain the new image. See "Open an Existing File" below for more details.

Part 4: Displaying HSI Images

The HSI image display includes three windows:

Image: This window is sized to include a user selected area of the full HSI image. The default is a square window of 400 x 400 pixels. The image can be resized by pulling at the lower right corner with the cursor.

Scroll: This window contains the full HSI image. The size and position of the Image window is marked with a red square. The red square can be moved to enter different parts of the full HSI image into the Image window.

Zoom: A sub region of the Image window appears in the Zoom window. The red square marks the shape and the position of the zoom area in the Image window. This window can also be resized and moved within the Image window. The zoom scale can be increased (+) or decreased (-) at the lower left corner of the window.

Here is the ENVI display of an HSI image of gold nanoparticles, using the color default (Figure 9).

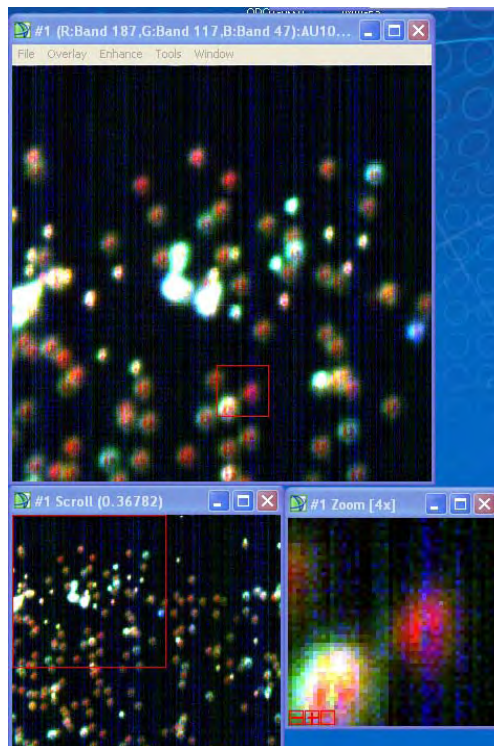


Figure 9. Color image set after scan includes Image, Scroll and Zoom displays

Part 5: Opening an Existing File

1. Using the Main Menu bar, previously recorded HSI images can be opened from the File/Open dialog. The HSI image is automatically loaded into three displays: Image, Scroll and Zoom windows.
2. Upon opening an image file, the file name appears in the "Available Bands List", shown in Figure 10. Under the name, the individual HSI image bands are listed, showing the associated wavelengths. Individual bands can be selected and displayed.

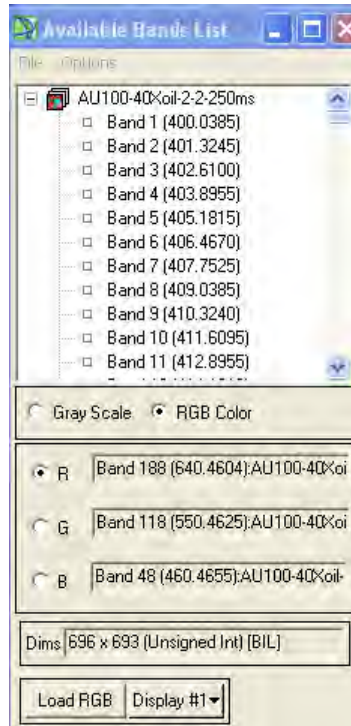


Figure 10. Available Bands List

Part 6: Selecting Display Bands

Images opened in ENVI can have different display properties. The images can be display in either Gray Scale or Color format. Color is the default format.

1. When the image is first loaded, the ENVI automatically picks which image bands to use for red, green and blue in the color display. The default bands give a true color rendering of the image. To be sure that true color is used, right click next to the file name at the top of the available bands list and select "Load True Color to Current". The newly displayed HSI image will revert to the default bands if they are not already being used.
2. Other bands can be selected for red, green and blue. One reason for selecting new bands is if the HSI image is known to contain no intensity at one or more of the default bands. Changing to a different combination of bands will result in false color rendering, however it may be desirable to do so in order to create image contrast based on the features of the recorded spectrum.
3. Choose new bands by clicking to the left of red, green or blue. Then scroll to the desired band in the window above and click on it. After changing the image band associated with one of the colors, ENVI automatically moves to the next color. When you are done selecting the bands, press the Load RGB button.

**** When Gray Scale is selected, only one band is used to display the image. ****

Part 7: Opening New Displays

Additional display windows can be opened using the Display# button on the Available Bands List.

1. Select "New Display". A blank display window appears. Open a new HSI image file. Press "Load" to update the blank display with the new image.
2. Multiple HSI images that have already been opened, and appear in the Available Bands List, can be displayed in separate windows. First, select a filename from the List. Start a new display to obtain the next numerical display number. Press the "Load" button to update the display and repeat for the other images. Displays are numbered on the header at the top of the image.

Part 8: Image Enhancements

The intensity scale of the image can be stretched or compressed by using several built-in options in the Enhance drop down menu on the Image window.

Stretch Option:

1. First select the window containing the HSI image. Select the Enhance menu. A drop down list shows the built in options (see Figure 12 below). Note that stretch options can be selected for any of the display windows: image, zoom or scroll. The stretch option will be applied to the selected window, and also to the other windows. However, only the selected window will fully adhere to the rules for the stretch option.

Linear: Causes the full range of the display to span between the lowest and highest intensity values of the image. This type of image shows the full intensity range without clipping.

Linear 0-255: Only displays values between 0 and 255. This display is used more for 8 bit images but it can also be used for faint 16 bit images to see fainter objects.

Linear 2% (default): displays between 0 and the 2% point on the input histogram. This display emphasizes very faint objects.

Gaussian, Equalization and Square Root: The stretch options "Gaussian", "Equalization" and "Square Root" perform a nonlinear transformation of the input histogram and the output histogram. "Square Root" is most useful for visualizing both faint and bright objects together, and is recommended for displaying cell and tissue samples.

2. Use the stretch options to obtain the best contrast of objects against the image background. Stretching can also reduce the appearance of vertical stripes that are caused by noise in the HSI images. Going clockwise in Figure 11, these four stretches show the gold nanoparticles at increasing contrast against background. The strength of the stripes also increases. The "Square Root" stretching makes both faint and bright objects more visible. **Stretching the display does not affect the image data.** "Linear 2%" and "Equalization" stretching show the faintest objects.

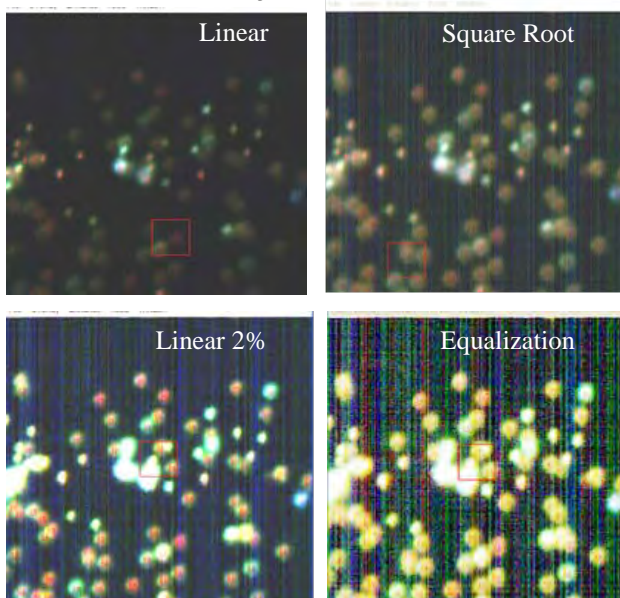


Figure 11. Examples of image with faint and bright features using different stretch options.

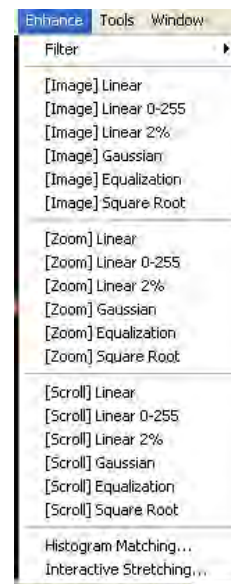


Figure 12. Enhance drop down menu

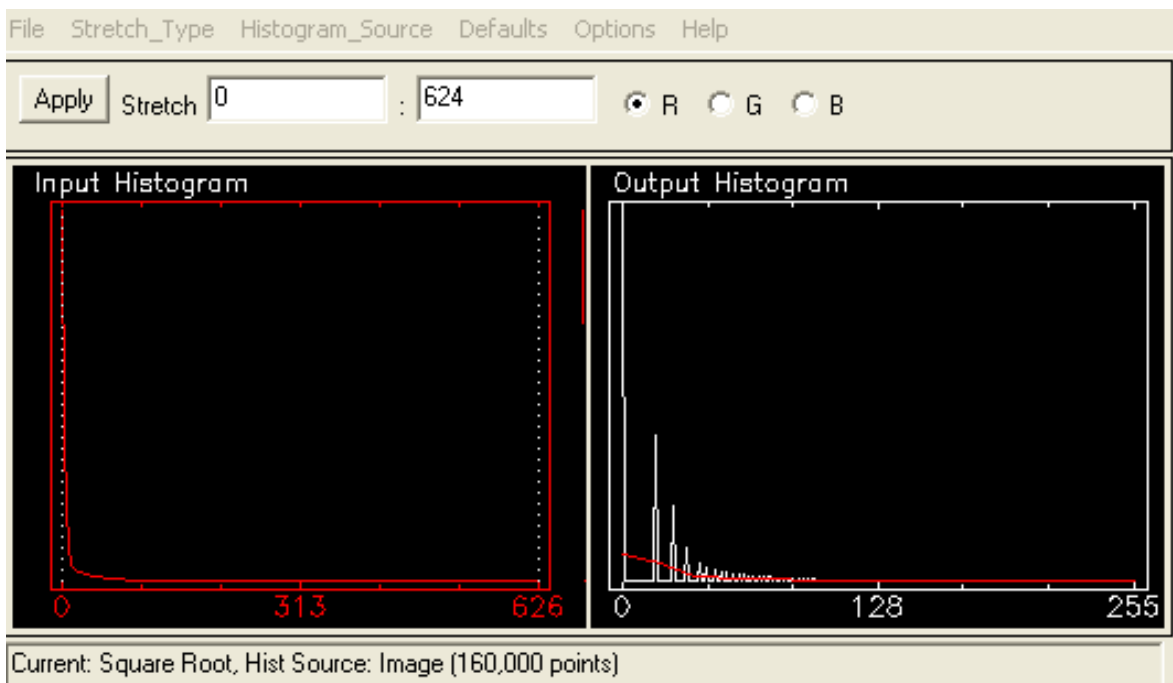
Interactive Stretching:

ENVI also allows the image contrast to be set interactively. This mode is selected at the bottom of the "Enhance" drop down menu: Interactive stretching.

Interactive Stretching with Input & Output Histograms:

The display can be adjusted by changing the relationship between the input and output (display) histograms. This option provides greater flexibility to adjust the image display.

1. The vertical dashed lines at the left and right edges of the input histogram can be moved to reset the bottom and top of the display range.
2. Alternatively, these boundaries can be set by typing the new values into the stretch boxes.
3. The new stretch is made effective by pressing "Apply".
4. Image noise is effectively removed from the display by setting the lower boundary value between 5 and 20.
5. For RGB images, the process is repeated for each color by clicking the R, G, or B.



↑
Input histogram of the red image band
for the red channel

↑
Output display histogram
for the red channel

Part 9: Plotting

Intensity profiles along the X, Y and Z (spectral) axis can be plotted using the profile tool at the top of the main image window.

1. Click on Tools, then high light Profiles. The drop down menus appear as in Figure 14.

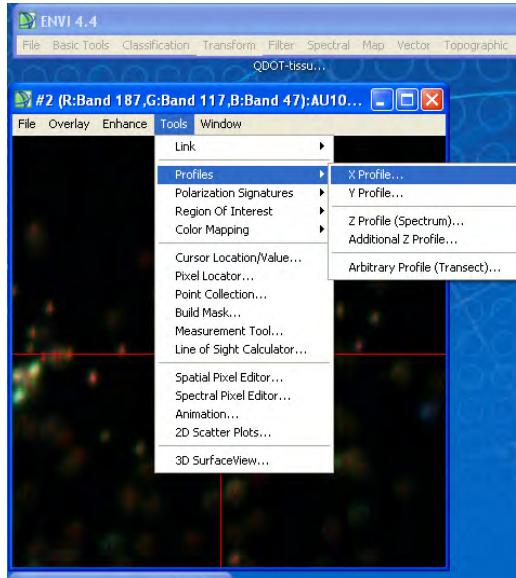


Figure 14. The Profile List is under Tools in the Image tool bar.

2. Select a profile (X, Y or Z). In this example from an image of gold nanoparticles, the X profile was selected and then the Z profiles was selected following the same procedure. When a Profile is selected, a cross hair appears on the Image and the intensity plot is displayed. The plot will contain the image intensities along the horizontal crosshair (X) or the vertical crosshair (Y), or the Spectral Profile that is contained in the HSI image pixels at the crosshair intersection. Examples of these plots are shown in Figure 15.

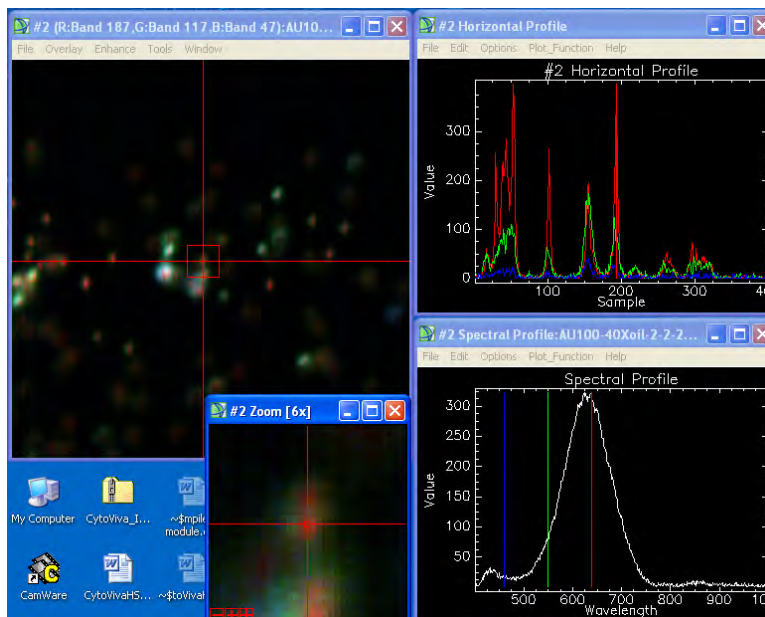


Figure 15. Plot Profiles

3. To view the Plot Key, right click in the Spectral Profile Window and click on Plot Key (see Figure 16)
4. To display spectra from multiple regions of the image, right click in the Spectral Profile window and select "Collect Spectra". A new curve is added to the plot each time a new area of the image is clicked. (see Figure 16) You can go back to the Zoom Window and select pixels to collect spectra from. If a spectra is collected and you would like to delete it you can by right clicking the spectra in the plot key and choosing remove. NOTE: the first spectra collected can't be removed.
5. In the Zoom window, clicking (+) increases the zoom while (-) decreases it. It is easier to center the cross hair on image features using a higher zoom setting,

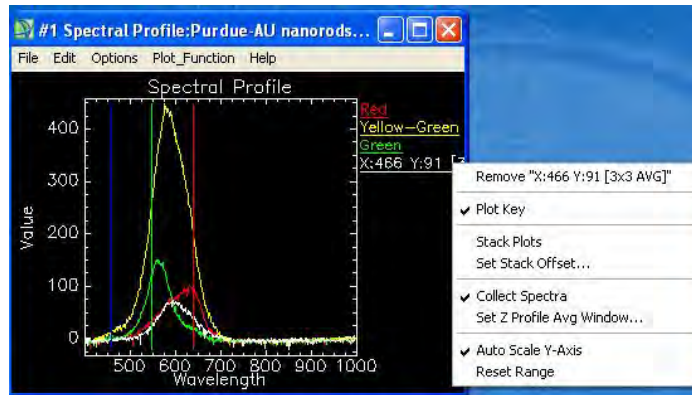


Figure 16. Window to collect spectra from multiple regions

Noise Reduction:

Noise Reduction can be applied to the Spectral Plot by averaging the pixels near the crosshair.

Averaging Pixels:

1. To reduce noise in the spectrum, right click in the Spectral profile
2. Select Z profile average window. (see Figure 17)
3. By increasing the average window size to 3x3 this will significantly reduce the noise on the spectrum. Larger window sizes can be used to further reduce noise if the spectrum of interest comes from a feature that is at least this size. Excessively large window sizes will begin to include spectra from areas unrelated to the feature, changing the spectrum.
4. The Z Profile is useful for quickly sampling the spectrum from any part of the image.

The spectral profile (lower right) is marked with red, green and blue lines that show the wavelengths of the RGB display bands. The HSI band and wavelength are shown below the plot when the user clicks on the line. The default bands are "true color" wavelengths: red = 640nm, green = 550 nm and blue = 460 nm. Images will appear with normal colors using the default setting. (see Figure 18)



Figure 17. Z Profile Average

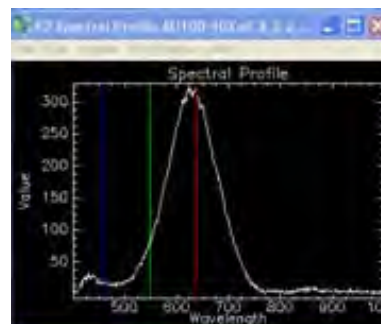


Figure 18. Spectral Profile, wavelength display

Part 10: Working with Regions of Interest (ROI)

The Region of Interest (ROI) tool is used to group pixels of the HSI image for further processing. This powerful feature allows preset shapes (rectangle, ellipse) and user defined areas (polygons, lines and pixels) to define a special region of the image.

**** ROI's allow groups of pixels to be selected for analysis and plotting. ROI's appear on the display as color overlays. ****

1. By right clicking in the image and selecting ROI Tool.
2. Expand the ROI Tools window to the right to see all ROI attributes above the box.
3. The Tool Selections in this window are used to change the type of ROI, add and delete ROIs from the active list, and selecting ROIs for plotting:

ROI Type (top tool bar): Selects different drawing tools.

Window: Selects the display in which the ROI is to be drawn. Select the off button when you are only moving the ROI and not wanting to create an ROI.

New Region: Adds a new ROI to the current list. You are able to change the color by right clicking the current color and picking the new color.

Delete: Removes the ROI item in the list.

Select All: Causes all ROI's to be processed or plotted together.

Stats: Plots the spectrum of each selected ROI.

Example of creating an ROI:

1. In the ROI Type menu, select the polygon drawing tool and select the zoom window display.
2. Outline the boundary of a feature by first clicking near the feature. Then drag the cursor around the feature, clicking at points where the edge of the ROI changes direction. Return to near the first point and right click once.
3. After the first click, a handle is shown that can be used to move the ROI. After the second click, the area encircled will fill in with a solid color, marking the ROI on the image. See the zoom window below in Figure 19 for examples created with the ellipse and the polygon drawing tools.

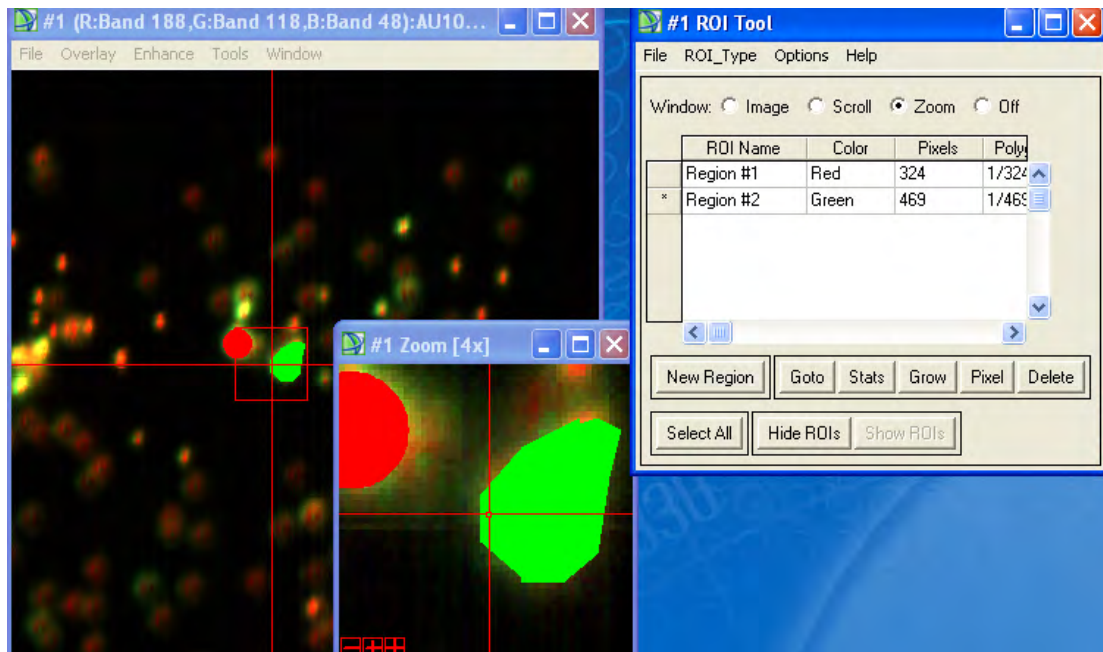


Figure 19. The ROI Tool and examples

Part 11: Plotting and labeling spectra from ROI's

Regions in the ROI list can be selected for plotting. To select all regions, press "Select All". Individual ROI's are selected by clicking to the left of the name in the list.

1. Select the ROI's whose spectra are to be plotted. Use the Stat button to create a plot window containing the spectra for each ROI. Below, the spectral curves from the red and green ROI's above are plotted (Figure 20, left plot).
2. In the plot on the right, a data legend was added by right clicking in the plot and selecting Plot key.
3. To change the title and axis labels right click, select edit, then select Plot Parameter. This dialog also is used to change scales of the axes, the background color, style of axis markers and plot margins. The Edit Plot Parameters dialog also allows the right margin of the plot to be made wide, so that longer names are displayed properly. Change the number in "Right Margin to a larger value.
4. The label at the right margin shows the coordinates of the pixel used to create the spectrum. The label can be changed to a name that refers to the spectral feature by right clicking the window, selecting Edit and then selecting the Edit Data Parameters dialog. Select labels appearing in the list and enter a new name in the box below, then click on Apply. In the plot at the right, the labels are changed to refer to different particles.

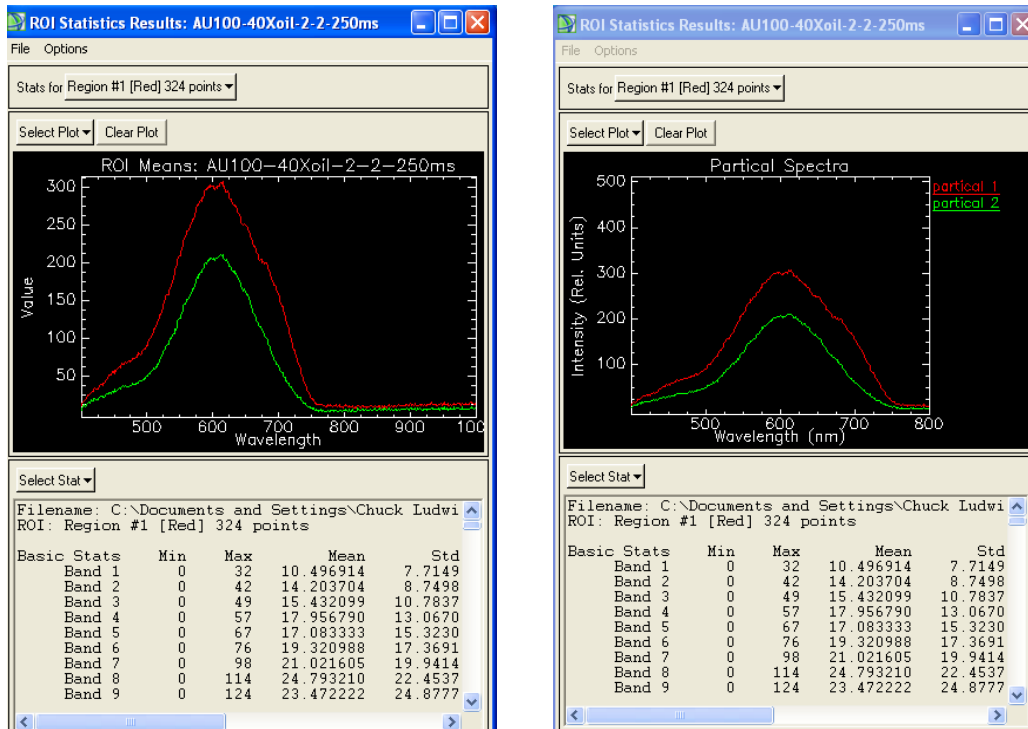


Figure 20. Spectral plots of ROI's.

Part 12: Saving Images

The hyperspectral image data are saved when the image is scanned, before an image is displayed. This is why the filename for the HSI file must be given before scanning. There is no need to save this data again. ENVI can open images from files and display them, or bring selected HSI bands into a gray scale or RGB image display. These display images can be saved in various formats. It is possible to save the main image and the zoom image.

1. In "File" on the tool bar over the main image select Save Image As, then select Image File. The Output Display to Image File dialog opens (see Figure 21).
2. If the image is color, use 24 bit BSQ resolution. For gray scale images use 8 bit.
3. A portion of the image can be saved using "Spatial Subset". The number of samples (NS) is equal to the number of image columns. The number of lines (NL) is equal to the number of lines scanned, and to the number of image rows.
4. Click the Image button to create the portion desired. You will see a red border around the image. Grab the corner and resize the border to the desired image size, then click OK.
5. Click OK in the Select Spatial Subset Window.
6. The Output File Type gives a list of image types supported by ENVI. Select one of your choosing.
7. To enter the filename and folder select Choose, then enter the File Name and click Open. Then click OK in the Output Display to Image File.

The Zoom window can be saved in exactly the same manner, choosing "Save Zoom Image" and selecting "Image File".

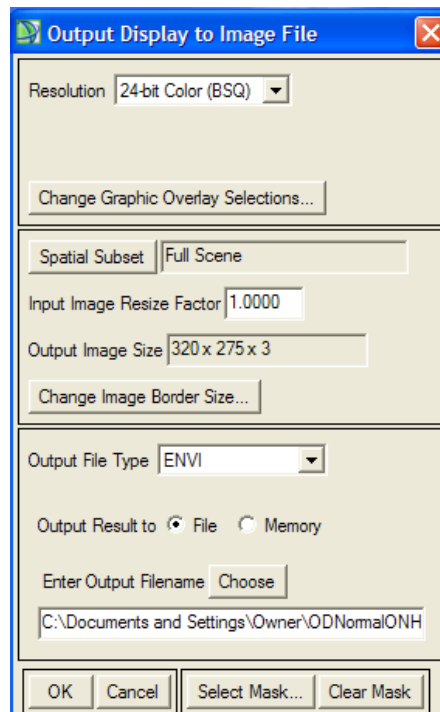




Figure 21. Output Display to Image File

Glossary

Image File:  This is the file of the original image scanned with the spectrophotometer.

Spectral Library File:  This file contains the different spectra that the user will collect from the scanned image.

Rule Image File:  This file contains the Rule image of the sample.

Classification (SAM) File:  This contains the classification (SAM) Image of the spectra that matched the endmembers.

Rule Image: The rule image is the displayed grayscale image showing the degree of matching between the known and unknown spectra from each image pixel. The Rule image is shown on a scale of light to dark the relative degree to which unknown spectra in each image pixel match the known spectra.

Spectral Angle Mapper Classification (SAM): an automated procedure for determining if a known material is present in the input image, and the pixels containing the material. SAM accomplishes these tasks by comparing unknown spectra in Hyperspectral imagery with known laboratory spectra for the material in question.

Classification (SAM) Image: this image shows the location of pixels whose spectra match that of the endmember.

Header File: A separate file created with the Hyperspectral image file, with the same name. This file must be copied with the image file when data is transferred. The header file includes information for CytoViva-ENVI to allow the image program to be opened and used.

Endmember: This is any spectra that have been chosen as important for identification purposes in a sample.

Output File: This is a file that you create to include several different output files such as: Spectral Library Files, Header Files, Spectral Angle Mapper Files, etc. This file will allow you to easily access this information when needed.

Z Profile Average Window: Setting the size windows will either decrease or increase the noise in the plotted spectrum.

Plot Key: This is the key that contains the labels of your spectral curves chosen.

X Axis (Default): The X-Axis default is the Wavelength. By moving the cursor over the point of the peak you can see the wavelength of the spectra in the selected location.

Y Axis (Default): The Y-Axis is the Value. This provides the intensity levels of the spectra.

Data Parameters: this can be found in the plot key by click on the image menu bar and selecting edit, then select Data Parameters. The Data Parameters will allow you to change the Key Plot names, line style (dotted, thickness) and color.

Plot Parameters: this can be found in the plot key by click on the image menu bar and selecting edit, then select Plot Parameters. The Plot Parameters will allow you to change the title, X & Y axis, background and foreground colors, and the font.

Spatial Resolution: The minimum spatial separation that can be detected in the image. The control of spatial and spectral resolutions work the same way.

Spectral Resolution: The minimum separation in wavelengths that can be detected. The low spectral resolution setting is equal to half that of the Hi setting. Therefore if the hi resolution were 1.5 nm, the low resolution would be 3 nm.

Trouble Shooting

1. What is the number for Technical Support?

1-888-737-3130

2. After the scan has finished the Image on the screen is Black?

One of the slide bar wasn't opened or closed at the proper time. Refer to the Manual, Chapter 2: Quick Start Guide, Part 3: Starting the Scan, numbers 6 & 7.

3. The scanned image is out of focus?

Before you scan your sample be sure to double check that the sample is in focus. Sometimes the slightest movement can make the sample move.

4. When I rescan an image the original image is still showing?

Go to the Available Bands list and choose close all files and then in the Available Bands list and open the new image you just scanned. You can refer to the Manual in Chapter 2: Quick Start Guide, Step 3: Starting the Scan, and number 8 which provides more detail.

5. There is a black area at the right or left end of my scanned image?

This is due to the slit not being pushed in all the way on the spectrophotometer. Call Technical Support.