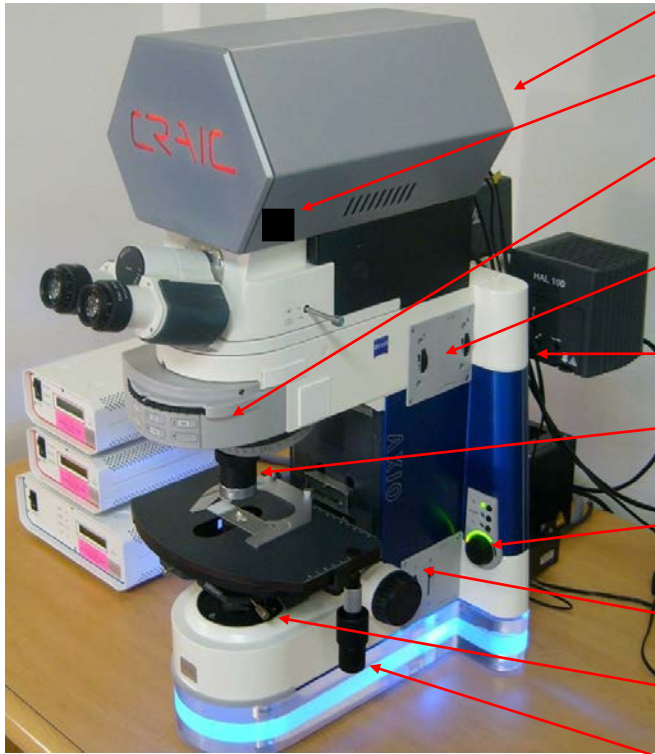


Quick Guide to Operating the CRAIC QDI 2010 UV-vis/NIR Microspectrophotometer

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- Power Switch (ON/OFF, spectrometer)
- Field stop (spectrometer)
- Rotating control for reflectance and fluorescence
- Aperture stop and Field stop for Reflectance
- Diffuser for reflectance and fluorescence
- 15x objective
- Electronic Shutters and Halogen Light Voltage Control
- Field stop and Diffuser for Transmission
- Condenser centering controls
- Manual stage control





Setting for:

1. Transmission (%T)
2. Reflectance (%R)
3. Fluorescence Excitation 365 nm
4. Fluorescence Excitation 420 nm
5. Fluorescence Excitation 546 nm






- Condenser focus
- Condenser centering
- Coarse and fine focus with shutter control
- Power switch (ON/OFF, Microscope)

Initial Start Up


- 1.) Log on via FOM.
- 2.) Open the N₂ gas valve.
- 3.) Turn ON the lamp power supply (**Transmission, Reflection/Fluorescence**, or both depending on your measurements)
- 4.) Turn ON the power switches of the microscope and the detector. Turn on the computer if necessary.
- 5.) Allow the lamp and scope to *warm up 30 minutes before use*.
- 6.) Click on the **CRAIC CCD Image** icon  to open the image software.
- 7.) Click on the **CRAIC MSP** icon  to open the data acquisition software.
- 8.) Align the microscope and ensure Kohler illumination
 - **At transmission mode (%T), adjust the field diaphragm (F-stop).** Close the field diaphragm until only a small (20-30%) of the field is illuminated when observed through the binoculars.
 - **Focus the condenser.** Adjust the condenser height until the illumination ring is sharply focused. If the image moves out of the field of view, center the image with the condenser centering screws.
 - **Open the field diaphragm (F-stop).** Open the diaphragm to a point where only the immediate field of view is illuminated, with the diaphragm at the edge of the field of view. If needed, center the illumination ring by adjusting the condenser centering controls.
 - **Adjust the condenser aperture diaphragm** to optimize contrast and resolution. From a fully open state, reduce the diaphragm until the image just begins to go dark or flare.

Collecting Absorbance and Transmission Spectra


1.) Set Up

- a. Load your sample onto the microscope stage.
- b. Move the turret to position 1 (labeled %T) if it is not already there.
- c. Move the objective of choice into position.
- d. Insert both *diffusion filters* to reduce the light level reaching the detectors.
- e. Move a clear area of the slide holding your sample to the illumination path.
- f. On the interface of **CRAIC MSP** program, click the **set parameters** icon,  to select the wavelength range, integration time, and number of scans for measurements.
- g. Select the data folder  in which your data will be saved.
- h. Press the “**Autoset Optimize**” button  on the **CRAIC MSP** software to adjust the optimization of the detectors. Both detectors should give a smiley face icon if optimization is successful.

2.) Collect the Dark Scan (Background)

- a. Block the detector optical path by closing transmission shutter and pushing in the eyepiece rod.
- b. Press “**Collect Darkscan**” 
- c. The spectrum displayed should have nothing but noise in it. This spectrum is used to correct for instrument response.
- d. Slide the binocular rod back out to allow light to reach the detector elements.

3.) Collect the Reference Scan

- a. Move a clear area of the slide to the illumination path.
- b. Press the “**Collect Reference**” button 

4.) Collect the Sample Scan

- a. Move the area that you want to sample from to the illumination path.

- b. Press the “**Collect Sample**” button Collect Sample and select either the Absorbance or Transmission option from the dialog menu.
- c. Press “**Scan**” to acquire the spectrum.

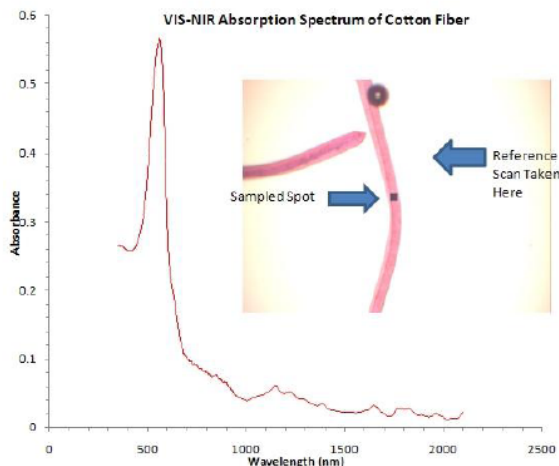


Figure 1: Absorbance spectrum of dyed cotton fiber collected using **CRAIC QDI 2010**. This figure also demonstrates the export of data in *.txt format for plotting using Excel or similar programs, image capture showing the point of spectroscopic analysis, and proper positioning for collection of Reference Scans (near the object being sampled, but far enough removed to not be influenced by its spectroscopic signature).

Collecting Reflectance Spectra

1.) Set Up

- a. Turn on the reflectance light source (the upper lamp).
- b. Rotate the filter turret to position 2
- c. Make sure the diffuser in the back of the scope is engaged (**up**).
- d. Place the sample on the stage and focus on the sample using both the course and fine focus controls.
- e. If necessary, block transmitted light using the electronic shutter.
- f. Move an area that serves as a reference or standard to the light path.
- g. Press “**Autoset Optimize**” button to adjust the sensitivity of the detectors.

2.) Collect Dark Scan (background)

- a. Block light to the detectors by inserting the binocular rod.
- b. Press “**Collect Darkscan**”.
- c. Remove the binocular rod to restore the light path to the detectors.

3.) Collect Reference Scan

- a. Click on the “**Reference Scan**” button to collect the Reference Scan.
- b. The reference scan should give a spectrum of the source emission (Xe lamp profile).

4.) Collect Sample Scan

- a. Move the sample to the illumination path.
- b. Press “**Collect Sample**” button and choose *Reflectance* from the dialog menu.
- c. Enter a filename for saving data if needed.
- d. Press “**Scan**” to collect the spectrum.

Collecting Fluorescence Spectra

1.) Set Up

- a. Make sure that the fluorescence lamp is on and warmed up (the upper lamp).
- b. Remove the diffuser from the optical train.
- c. Rotate the turret to select the appropriate excitation filter (see Notes for choices of filters). Select the appropriate objective.
- d. Place a slide with your sample on the stage and focus.
- e. Block the transmission source (lower lamp) with the electronic shutter.
- f. Press “**Set Parameters**” on the toolbar and reset the integration time *manually*.

2.) Collect Dark Scan (Background)

- a. Close reflectance shutter to block light from reaching the detector elements.
- b. Press “**Collect Darkscan**”.

3.) Collect Sample Scan

- a. Open the reflectance shutter to restore the light path.
- b. Press “**Collect Sample**” and select Fluorescence from the dialog menu.
- c. Enter the file name for saving your data if you have not already done so.
- d. Press “**Scan**” to acquire the spectrum.

4.) Tips

- a. For low fluorescence intensity samples, you may need to use quartz slides and coverslips. Regular slides, coverslips, and mounting medium may have too much intrinsic fluorescence that will swamp out the emission from your sample.
- b. Glycerin based mounting medium has low fluorescence background.
- c. You can improve your data by using longer integration times and/or by averaging multiple spectra.

Images

- 1.) Images are displayed live (real time) using the CCD image software. To activate, click the film camera icon on the Main Toolbar (toggles the camera on and off).
- 2.) To acquire an image, click on “**Save Image**” from the File menu.
- 3.) The image can be manipulated either by modifying the acquisition properties or post-acquisition using standard image analysis software. The most likely adjustments to make prior to acquisition are:
 - a. Exposure- select from “**Properties**” on the Device menu to adjust the exposure time
 - b. Whitebalance One Push- select from “**Properties**” on the Device menu to autocorrect the camera for red and blue brightness balance of each pixel.

Shut Down

- 1.) Close the image program and the spectrophotometer program.
- 2.) Turn off each of the power switches in the same order that they were turned on, saving the lamp(s) for last.
- 3.) Close the N₂ gas valve.
- 4.) Log off the instrument via FOM.

Notes on Using the CRAIC Microspectrophotometer

The spectral range of the microspectrophotometer is 200-2100 nm. To achieve this spectral range, two binned CCDs are used- one Si (200-950nm) and one InGaAs (850-2300nm)

Two objectives are available, both using mirror optics as opposed to the normal lens optics encountered with other microscope objectives. Note that these objectives are not parfocal, so some refocusing will be required when switching between them.

- o 15x mirror objective, NA 0.28
- o 36x mirror objective, NA 0.5 (with coverslip adjustment of 0.1-0.2 mm).

You may choose from between six pre-set aperture sizes as listed in Table 1 below. Change apertures by pulling out the aperture rod on the right hand side of the microscope, twisting to select the size (1-6, 1 is the largest, 6 the smallest), and pushing in to engage. Note that the travel or distance that you push in is not the same for each aperture.

Table 1: Effect of aperture and objective magnification of spectroscopic field of view when sampling with the **CRAIC QDI 2010**. Note that all apertures are square in geometry.

Aperture	15x Objective	36x Objective
1	75x75 μm	31x31 μm
2	56x56 μm	23x23 μm
3	37x37 μm	15x15 μm
4	19x19 μm	7.7x7.7 μm
5	9.4x9.4 μm	3.8x3.8 μm
6	4.7x4.7 μm	1.9x1.9 μm

Images may be saved as either bitmaps (*.bmp) or in jpg (*.jpg) format. For movies, data are saved in the .avi format.

Spectra may be saved in Galactic Industries Spectroscopy format (*.SPC) or in ASCII format as a text file (x,y, as .txt).