

Spectronon Benchtop Quick Start Guide

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This quick start guide will take you through some essential steps to acquire scans and perform basic data analysis. It represents a small subset of the software's capabilities but will provide the tools to jumpstart productivity while getting you acquainted with the workflow.

A full description of Spectronon's capabilities can be found in the User Manual:

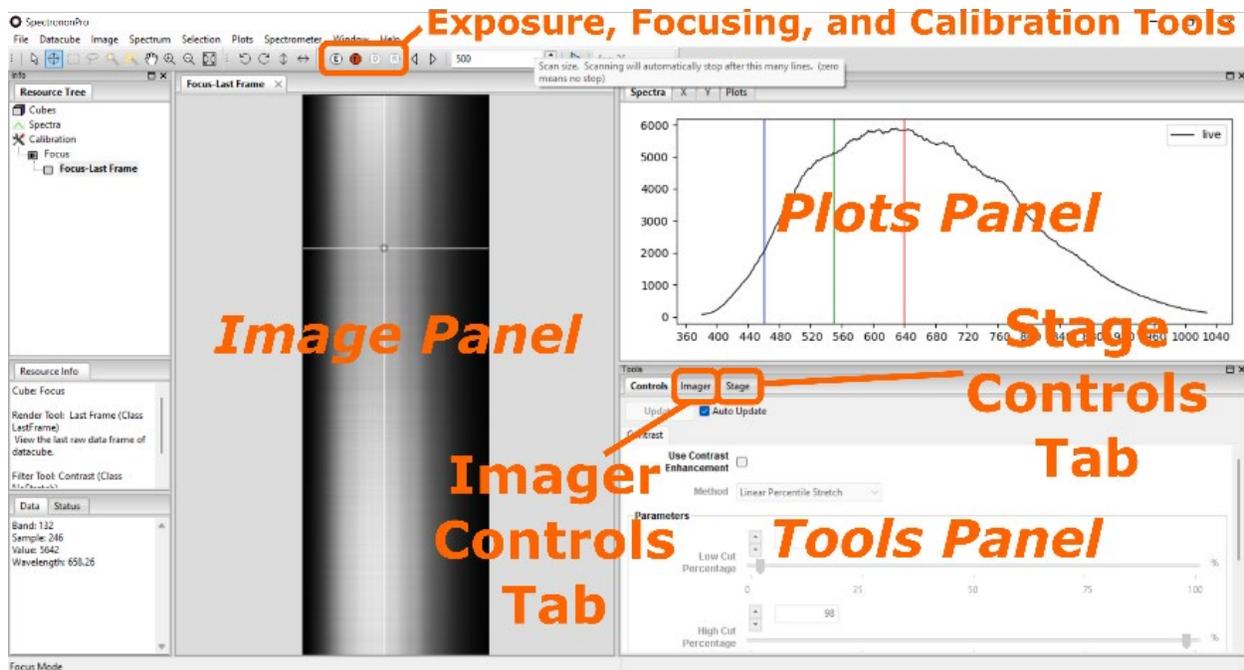
<https://docs.resonon.com/spectronon/SpectrononUserManual/SpectrononUserManual.pdf>

Assembly

This guide assumes that your benchtop system has been previously assembled. It focuses on the use of Spectronon, which is the data-acquisition and analysis software.

Preparing for scanning

1. Turn on the light source (preferably 15 minutes in advance for warm-up and stabilization).
2. Ensure that all data and power cables are properly connected. The IR imagers are powered via a dedicated power supply (12-pin round connector). They connect to the computer with an ethernet cable. All other cameras are connected *and* powered via a MICRO-B USB 3.0 cable to the computer. The stage has its own power supply *and* a communication cable that connects to one of the computer's USB ports.
3. Launch the Spectronon software.
4. Make sure that Spectronon has successfully recognized the imager and stage (the "Camera" and "Stage" tabs in the bottom right *Tools* panel are present, and *not* dimmed out). If not, check that all connections have been made according to the instructions above, and detect and/or reload the imager or scanning stage via the *Spectrometer* menu item.

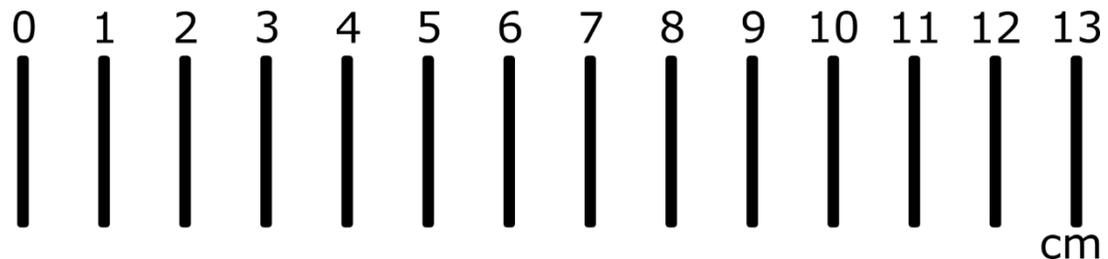


5. Set the calibration tile on the scanning stage directly below the camera.
6. In Spectronon, click on the (F) *Focusing* icon on the toolbar. Once activated, the button should be red, and a live view of the sensor will show up in the *Image* panel. Note that the vertical dimension represents by default the spatial extent of the scanner's imaging line. The horizontal dimension represents the spectral channels associated with each pixel.
7. The 6-fixture broadband halogen light source does not require alignment, but the line light (found on some benchtop systems) does require alignment. To align it:
 - a. Loosen the thumb screw behind the light arm, swivel the line light until you maximize the image brightness, and re-tighten the thumb screw to secure the light in its optimal orientation.
 - b. If you fail to observe a bright area on the focusing image, then the exposure time may be too low. If the image is extremely bright, the exposure time may be too long causing pixel saturation.
 - c. Click the (E) *Auto Exposure* icon on the toolbar.
 - d. Repeat steps a through c if necessary.

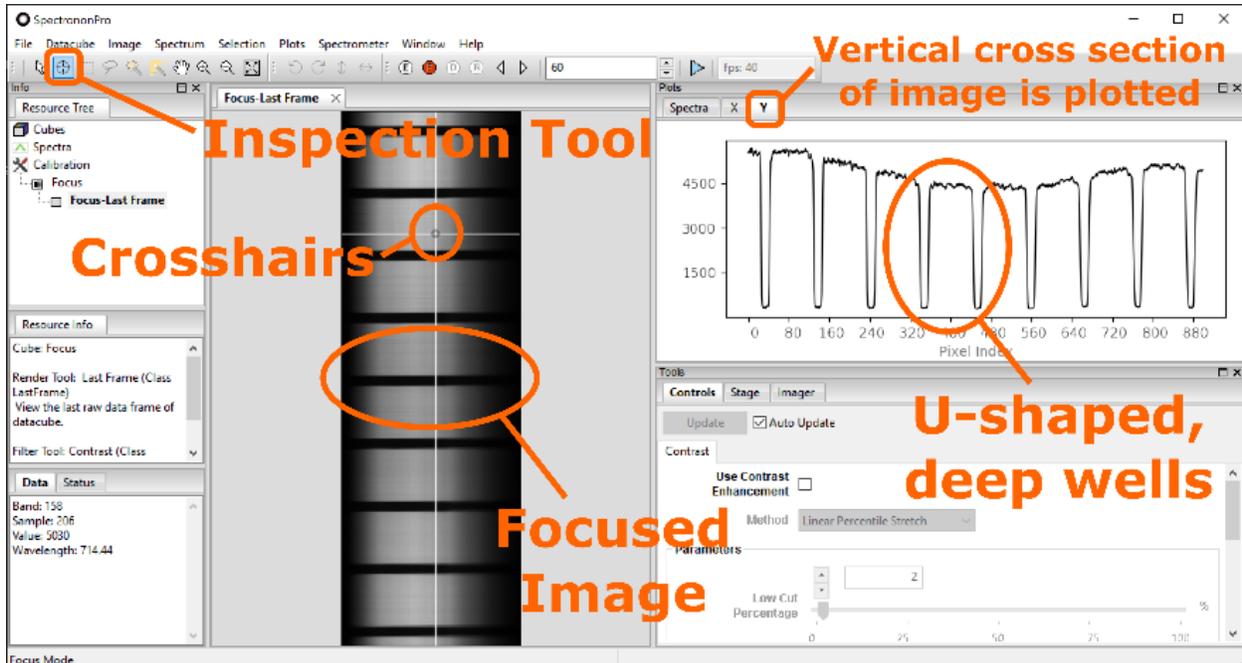
Note: The mechanical components are designed to ensure that the line light and the imager's field of view are always parallel to each other, ensuring that the brightness is uniform along the spatial direction. If not, check to ensure that the camera and line light brackets are properly seated in the tower.

Hint: To avoid shadows, it is best to have the light as close to the imager as possible (while ensuring that the imager and light do not interfere with each other in any way).

- Loosen the lens collar with a proper-sized Allen wrench. Place the *Focusing Sheet/Ruler* (below) on the stage with the lines pointing in the direction of the stage's travel and at the approximate height of the object to be scanned. Swivel the lens' focus ring until the target's lines come into sharp contrast on the image. Re-tighten the lens collar.



Hint: Click the *Inspection Tool* (crosshairs) on the toolbar, and then click somewhere on a white part of the image to display the *spectrum* associated with the current pixel in the *Plots* panel. You should see a broad peak, corresponding to the spectral makeup of light reflected at that point. Then click on the “Y” tab in the *Plots* panel to see a cross-section of the focusing image in the y (spatial) direction. Valleys in the spatial cross-section correspond to the dark lines on the *Focusing Target Sheet*. Focus is achieved when the valleys are deepest, and U-shaped as opposed to V-shaped.



- After focusing, you might need to auto-adjust the exposure again because focusing the imager can affect the amount of light reaching the sensor.

10. Cover the lens and click on the (D) *Dark Current Correction* icon in the toolbar. This will take a few seconds. This calibration corrects for background noise signal at each pixel.
11. Replace the calibration tile on the stage making sure it covers the entire field of view. Remove the lens cover and click on the (R) *Response Correction* icon. The tile serves as a “perfect” reflector to calibrate each pixel for wavelength-dependent sensor response and spatial lighting variability.
12. The dark current and response correction calibrations you just performed will automatically be applied to subsequent measurements. *It is still recommended to save the Dark Correction and Response Correction datacubes at this time for future reference or troubleshooting.
13. Next, we set the stage speed so that images are recorded with the correct (square) aspect ratio. We recommend using the *Auto Speed Mode* by following the steps below.
 - a. Place a ruler (or *Focusing Sheet/Ruler* above) on the scanning stage, parallel to the imager’s field of view, just to the right of it. Click the *Record Scan* button (blue triangle above the *Image* panel) and once the ruler had been imaged, click the blue triangle (now a red square) to stop the scan.
 - b. The scan width (swath) can be read on the image of the ruler.
 - c. Under the *Stage* tab of the *Tools* panel in the bottom right of your screen, check the *Auto Speed Mode* box and enter the value you just measured into the *Swath* field. Your scans should now have the correct aspect ratio.
- 14. If you make any changes** (moving the camera up/down the tower to change your field of view, adjusting the lighting intensity or orientation, or accidentally bumping the light out of alignment) **you should repeat this entire focusing routine so that your images are focused and properly calibrated according to the new lighting conditions.**

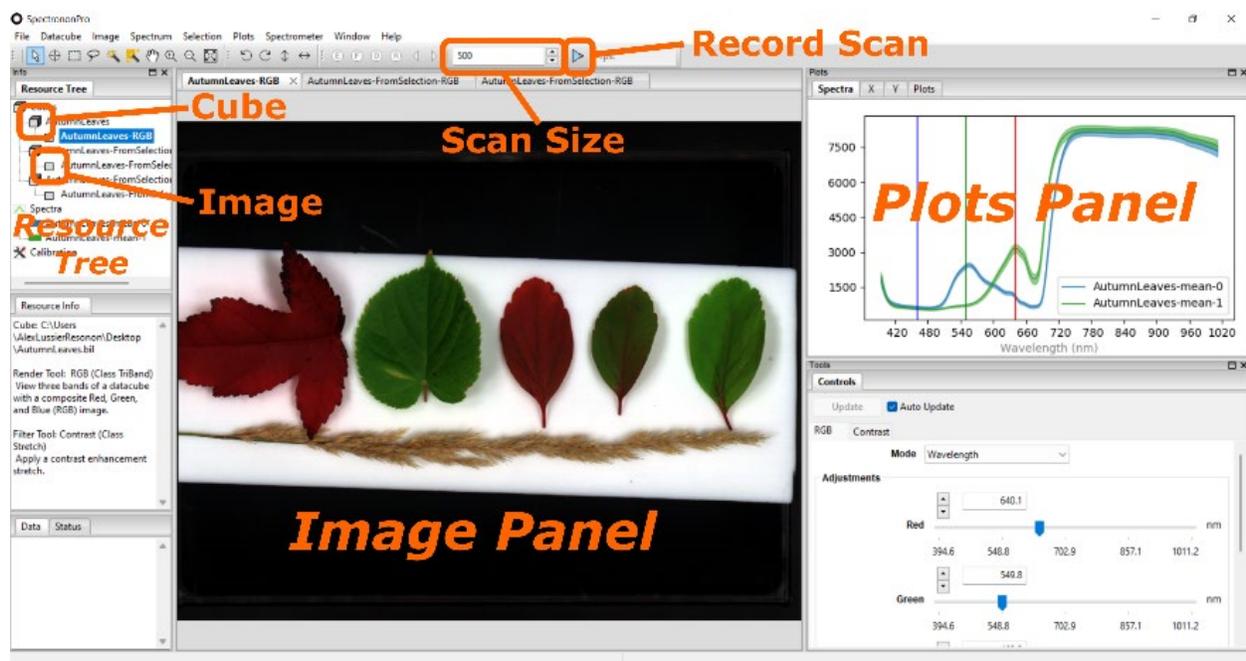
Data Acquisition

1. You are now ready to start scanning. Place your sample on the scanning stage to the right of the line light's footprint.

Hint: Samples with significant height variability can be challenging and require a careful choice of lens and f-stop setting to achieve a high depth of field. Defocusing the line light to maximize lighting uniformity at various sample depths can also help.

2. Set the *Scan size* (the number of lines you wish to scan) in the box above the *image* panel.

Hint: You can estimate the number of lines you need based on the imager's spatial resolution and the swath you measured when preparing to scan. If the aspect ratio was set correctly, the *cross-track* resolution and *along-track* resolutions should be the same. Optionally you can set the Scan Size to 0 and manually stop the data acquisition when the scan extent is satisfactory.



3. Click the blue triangle *Record* icon in the toolbar.

Note: Every time you press record, you will overwrite the previous scan. This allows you to reset the sample position or scan size until you have optimized the image. You can then save the current scan.

4. When you have recorded a suitable scan, go to the *Resource Tree* in the top left of your screen, right-click on the datacube that you have just acquired, click on *Save Cube As* in the drop menu, enter the name you wish, and click *Save*.

Hint: There is an important distinction between a datacube and an image. A datacube contains three-dimensional data (two spatial dimensions and a spectral dimension). It contains all the information acquired in a scan. An associated image is merely a 2D rendering of the data that utilizes a few discrete spectral channels. In the *Resource Tree*, datacubes are indicated with a *cube* icon while images are indicated with a *square* icon. Depending on which one you right-click, different options will be presented to you in the drop-down menu.

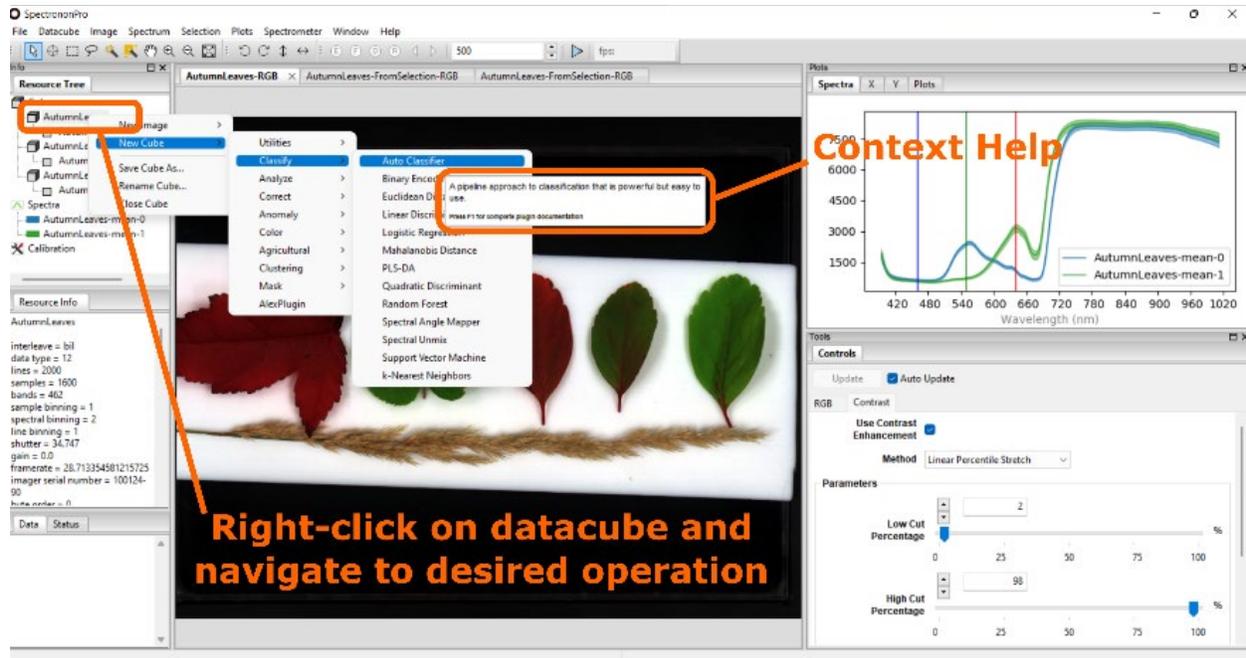
Data Analysis

Once you have acquired a datacube, Spectronon allows you to perform many different analyses of the data. Before you begin, make sure you understand the distinction between a datacube and an image (explained above).

Important Spectronon concept: Please understand that all analysis operations imply the creation of a *new* datacube that has been manipulated according to the chosen analysis. The new cube is listed in the resource tree as a branch below the original cube, but it is in fact a distinct and independent datacube. It can be saved, loaded, and manipulated independently of the original cube.

1. To perform an analysis, right-click a datacube in the *Resource Tree*, click *New Cube*, and then any of the available built-in tools in Spectronon (*Utilities, Classify, Analyze, Correct, etc.*)

Hint: Equivalently, you can left-click a datacube to highlight it, and then click *Datacube* in the main menu to select an analysis step of your choice.



Hint: Whenever a menu item for data manipulation is highlighted in Spectron, a short context help window opens. Clicking on F1 at this time will open an additional popup window with more detailed help and often some links for additional information.

2. Whenever a new cube is generated, an associated image is generated and presented under a new tab in the *Image* panel. The *Tools* panel at the bottom right of your screen allows you to manipulate that *image*. In the *Tools* panel you will find the following tabs:
 - a. The *RGB* tab allows you to modify the wavelengths used to render the image
 - b. The *Contrast* tab allows you to apply a contrast filter and adjust the parameters of that filter.
 - c. If the cube was generated via a *New Cube* operation (e.g. a *Normalize datacube* operation), you can click on the tab associated with that operation and change the parameters of the operation.

Note: the analysis tools available in Spectron are too numerous to describe here but the same general principles apply to all.

- i. Select a cube.
- ii. Create a *new* cube based on a specific algorithm of your choice.
- iii. A new *datacube* and associated *image* are generated.

Important Concept: Some operations, for example classifications, require you to have previously generated reference cubes or reference spectra. If so, when you select a *New Cube* operation, a popup window will ask you to select the cubes

or spectra you wish to use to perform that operation. For example, select a cube in the *Resource Tree*, click *Datacube* in the main menu, then navigate to *New Cube*, *Classify*, and then *Auto Classifier*. This classifier sorts each pixel as belonging to one of any number of classes, based on its spectral similarity to reference datacubes. A popup window opens asking you to specify how many classes you wish to use, and then to assign a cube for each of those classes. We will now learn how to generate those reference cubes.

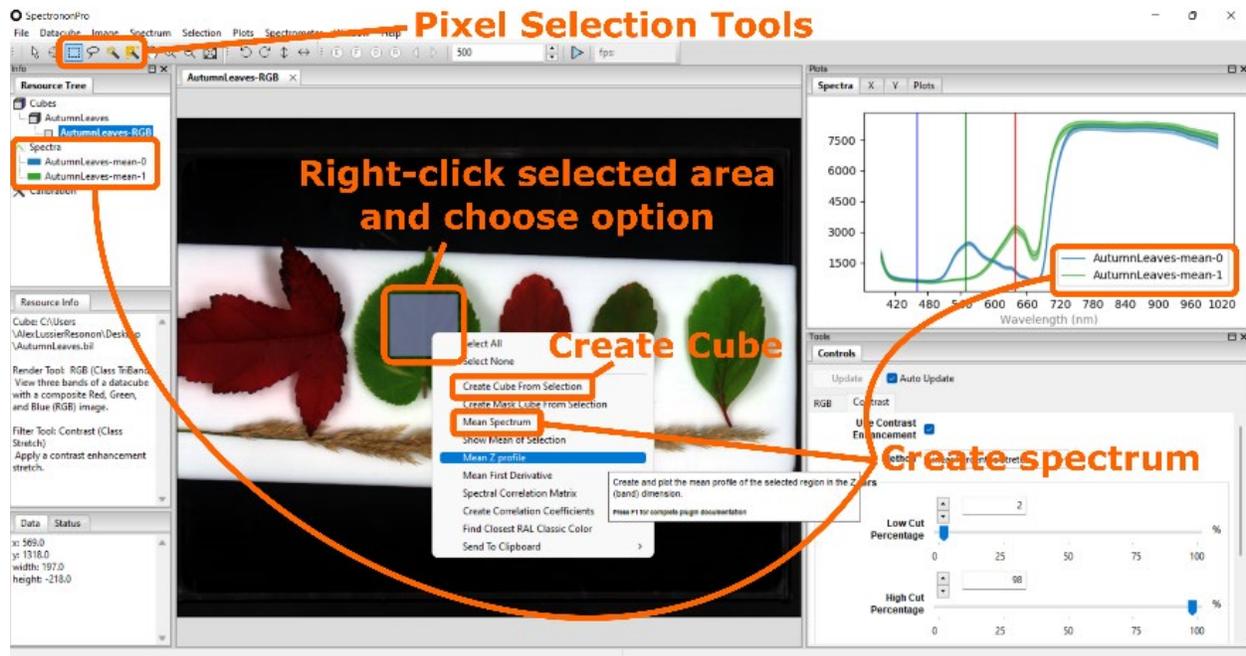
Creating a reference cube (or spectrum)

If you need to create one or more reference cubes for future analysis, it will unlikely be a cube generated from a full scan. Instead, you will create a sub-cube from a part of a scan that is known to accurately represent an element of interest. For example, if you are wanting to classify plant leaves as belonging to one of two types (A or B). You can acquire a scan that contains a leaf from each type and then follow the steps below:

1. While the image associated with your reference scan is open in the *Image* panel, click on one of the selection tools in the toolbar (for example the *select rectangle* selection tool).
2. Click and drag to delimit a region of the scan that is representative of type A leaves.
3. While hovering over the greyed-out area you selected, right-click and choose *Create Cube From Selection*. A new cube is created and appears in the *Resource Tree*. An image associated with this new cube shows up in the *Image* panel.

Note: If the Free Form selection tool is used, the new cube will have the spatial relationships between pixels altered in order to create a rectangular cube from the region.

Hint: The new cube has an automatically generated name with “-FromSelection” appended to the name of the cube it was generated from. You may want to rename it to something that is specifically meaningful to you, such as “Type A.”



- Go back to the original reference scan, select a region representative of type B, and generate a datacube from that region. Rename it "Type B." You now have reference datacubes for type A and type B plant leaves, which you can use to classify an unknown mix of type A and B leaves.

Note: Some classification operations may require reference spectra (instead of reference datacubes). To create a reference spectrum, select an area of the scan, hover over the greyed-out area, right-click, and choose *Mean Spectrum*. A spectrum will appear in the *Plots* panel. It will have a spectral line and an envelope that spans plus and minus one standard deviation from the average spectrum in the selected area.

Note about closing Spectron

Remember that no datacube or image is saved to disk unless you explicitly do so. When you exit Spectron, the program will let you know about any unsaved cube, image, or spectrum. If there is something in that list that you wish to save, click *Cancel*, save the files you need, and then close Spectron.