Bio-LIF

Resonon

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ONE

BIO-LIF OVERVIEW

Bio-LIF lets you scan biological samples. For example, you can scan cultures of microbes or any other tangible material sample you want to analyze. After scanning your sample culture, Bio-LIF produces a full-spectrum fluorescence image you can analyze.

Bio-LIF uses Resonon's **SpectrononPro** software to control a hyperspectral imaging system and to analyze the data you collect from your samples.

CHAPTER

TWO

UNPACK THE EQUIPMENT

Bio-LIF includes the following items:

- Bio-LIF scanner
- Computer with SpectrononPro software installed
- Two power cords including:
  - One scanner power cord
  - One computer power cord
- USB cable
- Two petri dish carriages
  - One carriage to hold a circular petri dish (16.5 cm by 11.4 cm)
  - One carriage to hold a rectangular petri dish (16.5 cm by 10.2 cm)

CHAPTER

THREE

LIFTING AND MOVING BIO-LIF

The scanner weighs approximately 65 lb (29.48 kg). A minimum of two people are required to lift and move the scanner.

Before you begin, you will need:

- A minimum of two people to lift the scanner out of the shipping box and move it to a workstation.
- A level tool to ensure the scanner is level on your workstation.

For safety, follow proper lifting techniques.

- Stand as close to the load as possible.
- Bend your knees and keep your upper body upright so your legs do the lifting rather than your back.
- Look straight ahead and keep your back straight and shoulders back so you have a slight arch in your lower back.
- Get a good grip on the load and use your feet to change direction, taking small steps as you go.
- Keep the load close to your body with your elbows at your sides.
- Lower the load in reverse by lowering your legs and keeping the load close to your body.

Workstation requirements

Bio-LIF dimensions measure 18.5w by 27h by 11d inches (46.9 by 68.6 by 27.9 cm) and weigh approximately 65 lb (29.48 kg). A suitable workstation for the scanner includes the following:

- A level workstation that supports 65 lb (29.48 kg).
- Workstation that is at least 18.5w by 27h by 18d inches. (These dimensions allow for 7 inches behind the scanner.)
- Minimum of 7 inches of space behind the scanner for air flow.
- Power outlet (120 or 240VAC).

CHAPTER

FOUR

SET UP BIO-LIF

Before you begin Bio-LIF must be level on your workstation. You can adjust the threaded leveling feet underneath the scanner and then use a level tool to ensure the scanner is level.

To set up the equipment 1. Connect the Bio-LIF power cord from the scanner to a wall outlet (120 or 240VAC). 2. Connect the computer power cord to a wall outlet. 3. Connect the USB hub located on the back of the scanner to the USB port on the computer. 4. Press the Bio-LIF Power button. The button lights up, indicating the scanner has power. 5. Adjust the leveling feet underneath the scanner until the scanner is level on your workstation. 6. To test level placement of the Bio-LIF, open the scanner door by pushing the handle on the right side of the scanner. If the scanner is level, the door will spring open and stay open. If not, keep adjusting the leveling feet and use a level tool until Bio-LIF is level.

Note: When the scanner door is open, the laser is disabled.

CHAPTER

FIVE

GET STARTED

This user manual is a quick start guide that describes how to use Bio-LIF to scan biological samples that you can analyze with Resonon's SpectrononPro software. To learn how to use additional options that are available in SpectrononPro, see User Manual for Spectronon.

5.1 Load your sample

Bio-LIF includes two carriage shapes, one that holds a rectangular petri dish and one that holds a circular petri dish.

To load your sample

1. Push the handle on the right side of the scanner to open the door. The door opens with the viewing light, inside the scanner, illuminated. The laser is disabled.

2. Place your sample within the indentation on the circular or rectangular carriage.

    a. Slide the carriage stabilizing lever to make room for your sample while you place it on the carriage.

    b. Release the lever to secure your sample on the carriage.

3. Place the carriage on the stage inside the scanner by aligning the magnets on the bottom of the carriage with the magnets on the stage.

Note: Each magnet is a different shape to ensure correct placement of the carriage.

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4. Close the scanner door.

5.2 Scan your sample

When you scan your sample, Bio-LIF creates a hyperspectral image as a cube and SpectrononPro captures the reference data you can analyze.

After you load your sample onto the carriage and place the carriage on the stage inside the Bio-LIF, you are ready to scan.

Calibrating the scanner SpectrononPro automatically captures a response cube to remove laser nonuniformity, and a dark cube to remove image background noise. Keep in mind that the default integration time for the imager may not be optimal for your specific medium. For the best data quality, you can refine the scanning process by adjusting the integration time values and then analyzing your results until you get a scan that works well for your medium. You can also manually calibrate the scanner. See Manually calibrate the scanner.

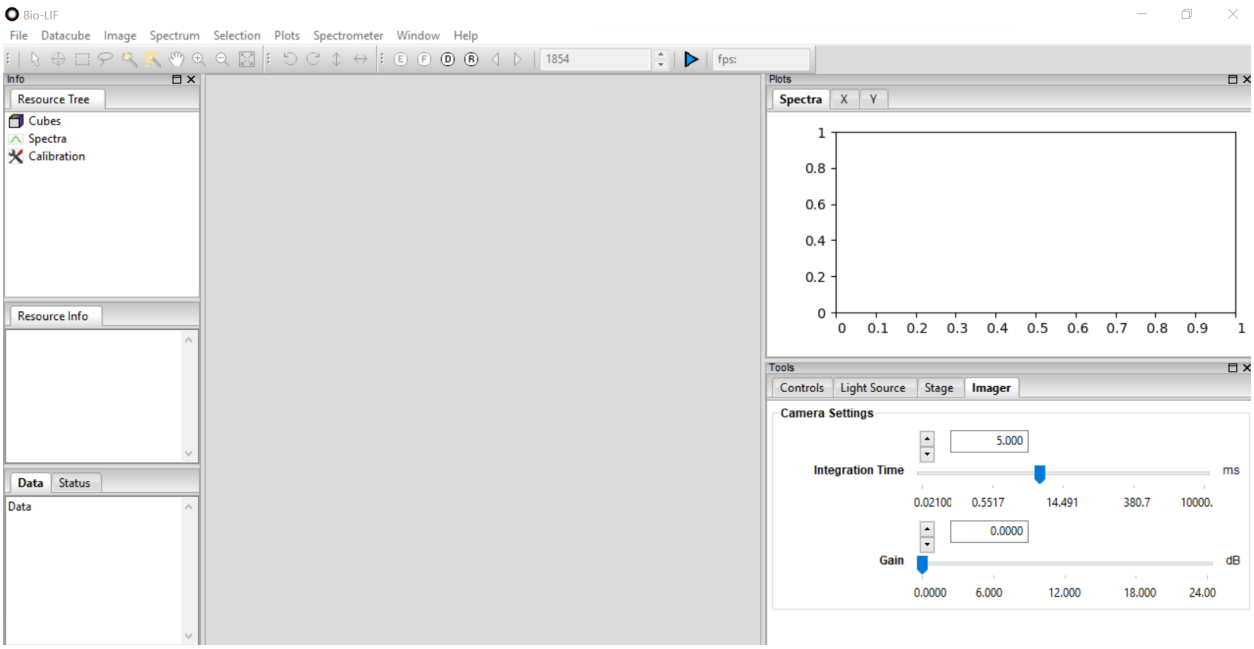
To scan your sample

1. Press the Bio-LIF Power button located above the scanner door. The button lights up, indicating the scanner has power. The laser is disabled when the door is open.

Hint: Let Bio-LIF warm up for five minutes. You can open the door while the scanner is warming up.

2. Start the computer and double-click the SpectrononPro icon on the desktop to open the scanning software. SpectronPro opens with the following options enabled.

- D button (Dark Current Correction) and R button (Response Correction Cube) on the ribbon.
- Arrow button on the ribbon. (This button is used to start a scan.)
- Controls, Light Source, Stage, and Imager tabs on the Tools section of the workspace.



Note: The scanner door must be closed for these options to be available.

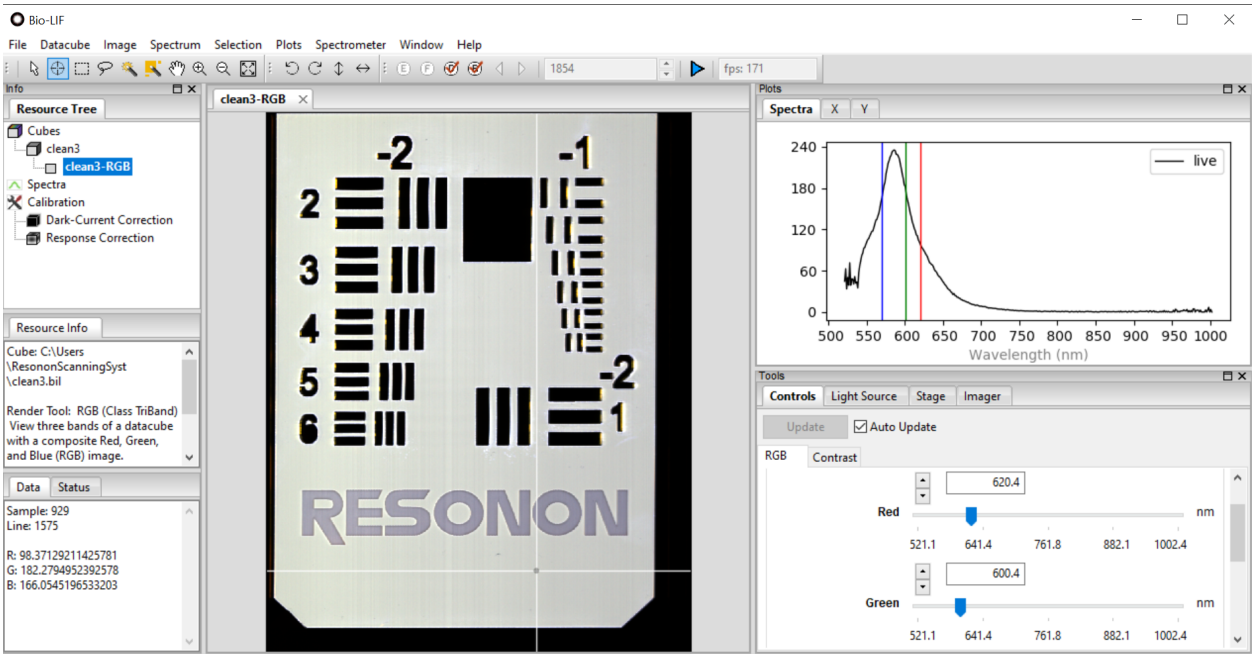
3. Push the handle on the right side of the scanner to open the door. The door opens with the viewing light, inside the scanner, illuminated. The laser is disabled.

4. Load your sample onto a carriage and place it on the stage inside Bio-LIF. See Load your sample.

5. Close the door. After you close the door, the laser is enabled.

6. Click the **Arrow** button (triangle icon) on the ribbon to start scanning. The arrow changes to a square during the scan. After Bio-LIF is done scanning, your image displays on the content pane and you get the following results.

- Current Scan-RGB is added to the Resource Tree tab under Cubes.
- Dark Current Correction and Response Correction are added under Calibration.
- Resource information, for cubes and calibration corrections, populates on the Resource Info tab. Resource information includes data from the header file, including the number of lines, cube size, integration time, and the system serial number.
- For a selected pixel, sample data displays on the Spectra tab in the Plots section.



7. To view resource information, select an item on the Resource Tree. The information displays on the Resource Info tab. For more details about resource information, see User Manual for Spectronon.

8. To interrupt the scan, click the Stop button (square icon) that replaced the arrow during the scan.

Note: The system automatically stops scanning after the petri dish on the carriage has been scanned.

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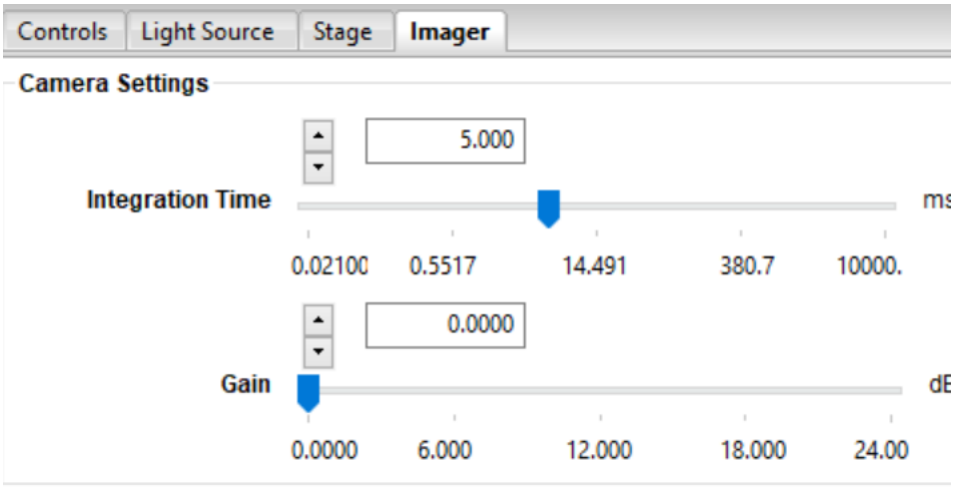
9. To adjust integration time of your scan, select the Imager tab in the Tools section.

    a. Enter a new value in the Integration Time field.



    b. Press Enter.

Hint: You can also use the Up and Down arrows or move the slider to adjust the integration time. (These options do

not require pressing Enter.)

10. To manually calibrate the scanner by experimenting with different integration times that may work better for your sample medium, see Manually calibrate the scanner.

11. To save the cube of your current image, select Cubes > Current Scan on the Resource Tree tab.

    a. Right-click Current Scan and select Save Cube As. The Enter Filename for Saving window opens.

    b. Enter a name in the File Name field.

    c. Click Save.

Note: If you do not save your currently scanned image, it will be overwritten by the next scan you perform.

After a calibration scan is collected, it displays in the Resource Tree. Unless you replace the data, SpectrononPro uses the reference data and dark cube from that specific scan for any subsequent scans you perform during the current session. If the system detects an issue with your data, SpectrononPro asks you to replace the data.

See User Manual for Spectronon to learn how to use additional options that are available in SpectrononPro.

5.3 Manually calibrate the scanner

Because Bio-LIF lets you scan various types of sample mediums, the SpectrononPro default settings for the imager may need to be adjusted. When you manually calibrate the scanner, you can also fine-tune your image by experimenting with different integration times until you get the best data quality of your specific medium.

Best practice When you change the integration time value of your image, the system also asks you to retake a dark current correction so that the image settings for your new integration time are correct.

Response correction data does not change when you rescan a sample.

To manually calibrate the scanner

1. **Click the D button on the ribbon to repeat the scan for dark current correction.** The Dark Correction: Replace Existing window opens.

    a. Click Ok to repeat the scan using a new dark cube.

    b. Click Cancel to keep your current dark cube scan.

Note: If you skip this step, the Warning: Invalid Corrections window opens to recommend that you retake the dark current correction so that the image settings for your new integration time are correct.

Response correction does not change when you rescan.

2. **To replace the response correction, click the R button on the ribbon.** The Response Correction: Replace Existing window opens.

    a. Click Ok to repeat the scan using a new response correction.

    b. Click Cancel to keep the existing response correction.

3. To save your new scan, right-click Current Scan and select Save Cube As. The Enter Filename for Saving window opens.

    a. Enter a name in the File Name field.

b. Click Save.

Note: If you close your new scan without saving, it will be overwritten by the next scan.

After you finish rescanning your image, compare your results to an example of a scan that has produced quality data. See *Examples of scanned samples*.

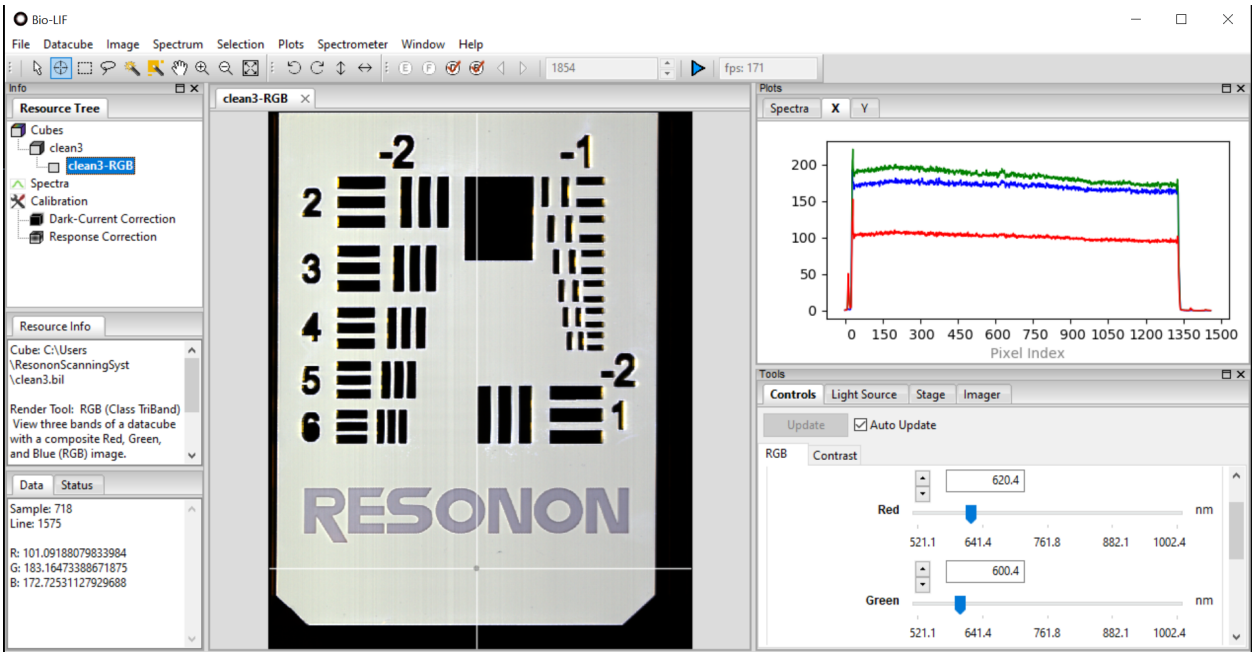
CHAPTER

SIX

EXAMPLES OF SCANNED SAMPLES

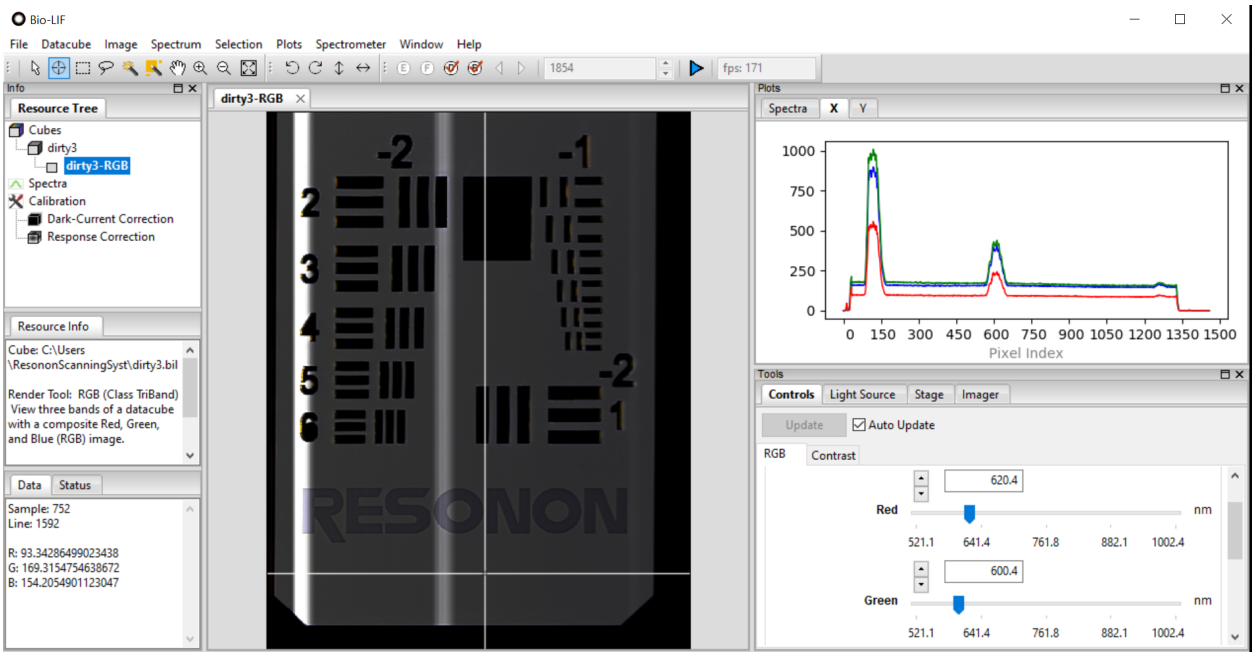
This section describes what to look for in a quality scan with good data and the steps you can take to improve the quality of your data. After you get the results of your scan, compare your image and the collected data to the images included here.

When a sample has uniform fluorescence, we expect the cross section on the X and Y tabs to be flat across a single row or column of pixels at every spectral channel. The following image shows variation on the order that we expect from a well-calibrated scan.

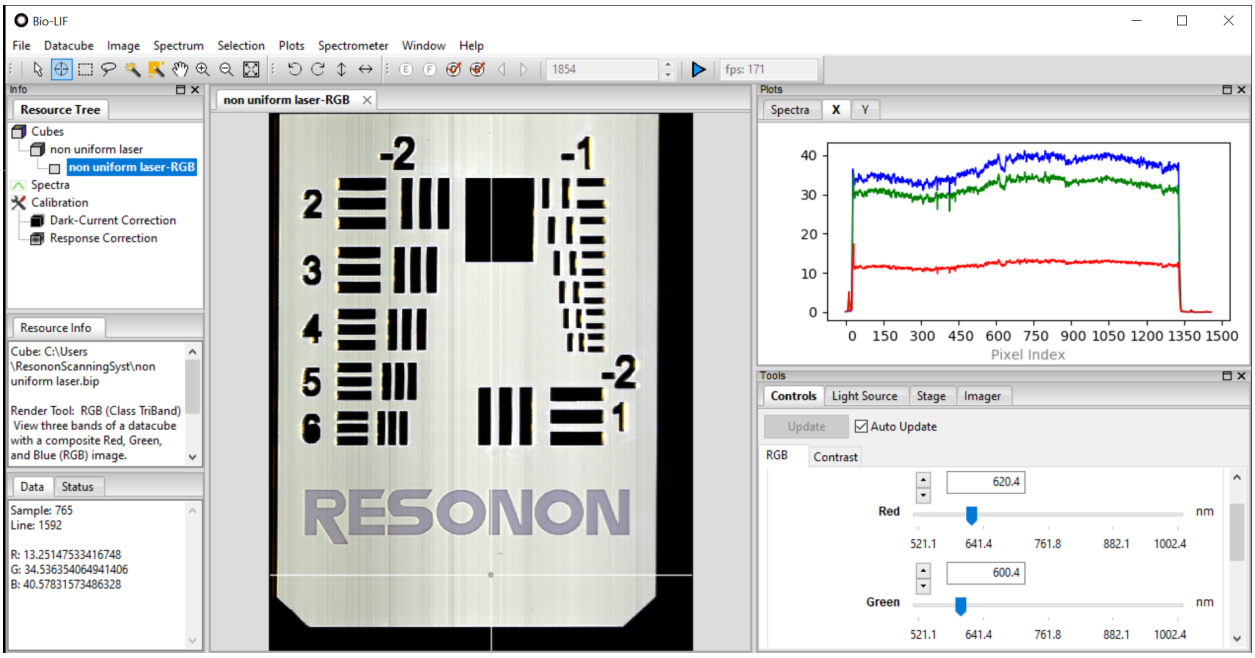


The following two images are examples of poor quality scans.

The image below shows a scan that was calibrated from a dirty reference tile. Notice the striped pattern that displays on the image, indicating a dirty reference tile. This leads to spikes or dips in the cross section of a sample along the scanning dimension.



The image below shows a scan with laser drift, meaning the plot of the cross section appears with lines that are not flat. Notice how the intensity varies across the cross section, but compared to the dirty tile, it has a smooth profile. Typically, recalibrating the scan can remove this type of variation. If recalibration does not work, try to clean or realign your calibration material.



CHAPTER

SEVEN

USE CASE SCENARIOS

Specific customer interactions with Bio-LIF, including best practices for how to handle commonly experienced scenarios are described in this section.

7.1 Troubleshooting

If SpectrononPro opens without the Laser tab or in 'Benchtop' mode. Check that the required petri dish flags necessary are enabled in the Properties >Target path to run SpectrononPro.

7.2 Best Practices

There are a number of methods or techniques that are generally found to be superior to those achieved by other means or that have become a standard mode of operation. Best practices as they apply to using Bio-LIF with SpectrononPro are included here.

CHAPTER

EIGHT

GLOSSARY

auto expose Automatically sets the longest integration time without saturation for a given camera framerate.

auto framerate Based on the integration time, the fastest camera framerate.

auto scan Sample scan that automatically returns a dark current correction and a response correction of the image.

auto scale Sample scan that automatically scales the image by the ratio of the integration times for the response correction and sample scan.

auto speed Stage speed that is automatically set for square pixels based on the default imager and camera settings. This ensures a correct aspect ratio without adjusting integration times.

carriage Component that holds the petri dish for scanning.

dark cube Cube that represents all background signals present in the detector, including read noise and dark current.

datacube Multidimensional array of values. For example, images taken by hyperspectral imagers are stored as datacubes. Similar to RGB images, datacubes have two spatial dimensions and one spectral dimension, except that the spectral dimension can also have hundreds of colors. Datacubes can also include metadata that store additional information. For example, metadata can include the size of each dimension and the spectral wavelengths.

imager Hyperspectral imaging system that collects fluorescent light from a sample medium.

laser Illumination source that uses optics to create a line that produces a uniformly fluorescent image of a sample medium.

response cube Calibration used to correct nonuniform illumination.

session Continuous time frame when taking a series of scans that use the same dark current and response corrections.

stage Component inside the Bio-LIF that holds the petri dish carriage and moves under the camera while the scanner captures an image.

CHAPTER

NINE

RELATED LINKS

User Manual for Spectronon

Resonon Product Documentation