PHOTOTHERMAL
SPECTROSCOPY CORP

mIRage®

System Manual

Part #00-0031-11
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Chapter 1

1. Cautions, Warnings, and Notices

1.1. Photothermal Spectroscopy Corp (PSC) Office

Photothermal Spectroscopy Corp
325 Chapala St.
Santa Barbara, CA 93101
Email: support@photothermal.com
Tel: (805) 845-6568

For information on our latest products and more, see our web site at **www.photothermal.com.**

Support

For assistance with applications or instrument service and repairs, please email us at support@photothermal.com or call the PSC Help Desk at: (805) 845-6568.

For product information, to order accessories and/or consumables, contact your sales associate or info@photothermal.com.

Feedback

Please send any comments, suggestions, feature requests, bug reports or any other feedback to support@photothermal.com.

1.2. Safety

This section outlines safety considerations when operating the mIRage.

1.2.1. High voltage 

Hazardous voltage exists inside various enclosures of the instrument. Do not make any attempt to remove the enclosures of the mIRage instrument or its other system components. Contact the Photothermal service group for troubleshooting and repair of any electrical components. AC power must be turned off and disconnected before replacement or repair of any system component. The user should never touch any wire or electrical contact within the mIRage enclosure with any part of the body or any electrically conductive tools.

1.2.2. Pinch Point/Crush Hazard 

There are potential pinch point/ crush hazards on the mIRage system between microscope objectives and the sample/sample XY stage and the XY stage and the microscope frame as shown in the figure below. Points marked A indicate possible pinch points between microscope objectives and the sample stage. Points marked B indicate possible pinch points in the objective slider mechanism. Points marked C indicate possible pinch points between the XY stage and microscope frame. Keep hands and fingers away from these areas when the objective(s) and/or XY stage are in motion. Both the microscope objectives and XY stage can move automatically.

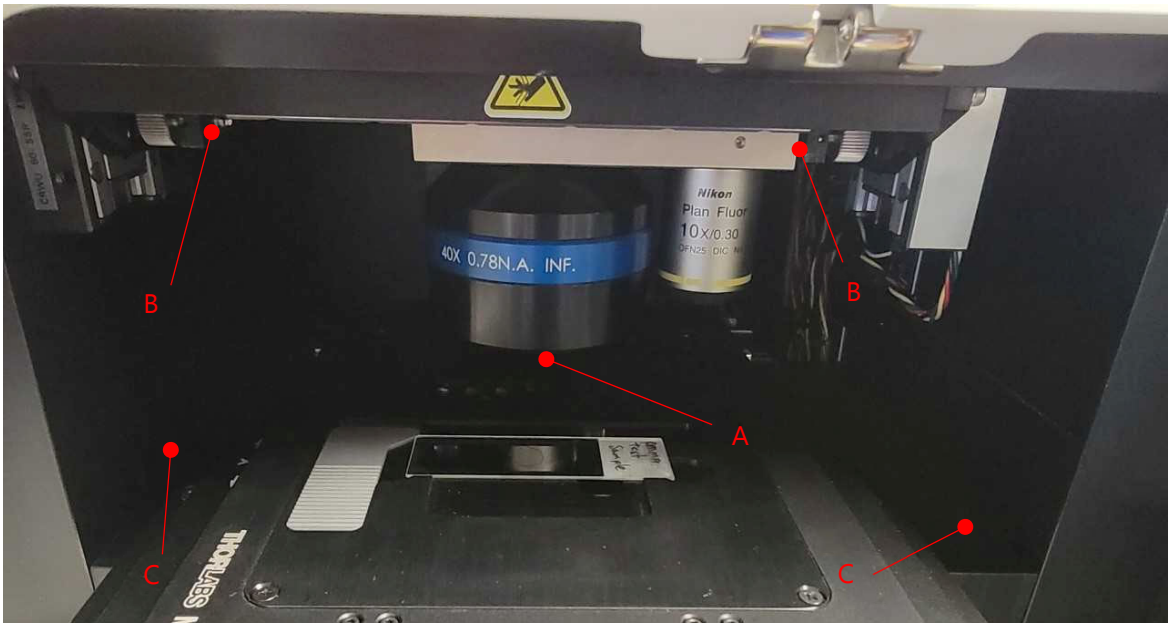

Figure 1-1. Locations of potential pinch points/crush hazards.

1.2.3. Laser Safety


The mIRage system contains one or more internal lasers (Class 3B at 532nm) and works with one or more external IR lasers. The external lasers are typically tunable within a range of 2.5 to 12 microns, with laser class between Class 1 and Class 4, depending on the type of laser.

During normal operation, the user is protected from exposure to these lasers by system covers, door interlocks, and physical blockage of the beam such that no exposure to unsafe laser radiation is permitted. The laser class of the combined mIRage system is Class 1.


Caution: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous laser energy exposure.


Higher laser power could be accessible in the interior of the product if covers are removed or the interlock is defeated, one or more of the following laser radiation beams could be exposed:
QCL IR laser: Class 4 laser, average power <100 mW at 3-12µm in 1000 ns pulses
OPO IR laser: Class 4 laser, average power <250 mW at 2.5-3.7 µm in 10 nsec pulses
532 nm probe laser: Class 3R laser, power <200mW CW (certain models <400 mW)


Do not open the housing or covers on any part of this product. There are no user serviceable parts inside the system. If a laser malfunction is suspected, immediately contact PSC for repair or replacement.


Once a month, visually inspect the IR laser system housing to verify that no panels or covers are loose or distorted

In accordance with laser safety requirements, the following laser precautions are affixed to the system:

Laser hazard labels are located on the mIRage system at each removal housing or cover, with the exact number and type of labels depending on the IR laser(s) selected.

1.2.4. Laser Safety Interlock

The mIRage utilizes an interlock system to ensure safe laser conditions at all times during normal use. The interlock system uses two miniature-snap action lever switches, mounted on each side of the front door, wired in series to a laser shutter mechanism. The laser shutter mechanism is in the laser path along the top of the mIRage, beneath the top cover. When both switches are closed a motor is energized that lifts a weighted beam block, allowing the beam to pass. The interlock system is designed and tested to be "Fail Safe" in that no single point of failure can lead to an unsafe condition.

NEVER attempt to defeat the interlock system. NEVER modify or adjust any component of the interlock system. NEVER intentionally or unintentionally manually press both switches at the same time. The interlock does not need to be defeated to perform normal maintenance tasks. Contact Photothermal Spectroscopy Corp immediately if there is any reason to believe that the interlock system is not functioning appropriately.

1.3. Notices

The material contained in this manual, and in the online help for the software used to support this instrument, is believed adequate for the intended use of the instrument. If the instrument or procedures are used for purposes other than those specified herein, confirmation of their suitability must be obtained from Photothermal Spectroscopy Corp (PSC). Otherwise, PSC does not guarantee any results and assumes no obligation or liability. PSC also reserves the right to revise this document and to make changes without notice. PSC may have patents, patent applications, trademarks, copyrights, or other intellectual property covering subject matter in this document. Except as expressly provided in the written license agreement from PSC, the furnishing of this document does not give you any license to these patents, trademarks, copyrights, or other intellectual property. PSC operating software, the associated manuals and online help, are proprietary and copyrighted. Purchasers are granted a license to use this software program on the module and controller with which they were purchased. These programs may not be duplicated by the purchaser without the prior written consent of PSC. Each licensed program shall remain the exclusive property of PSC, and no rights or licenses are granted to the purchaser other than as specified above.

1.3.1. Patents

The mIRage system and PTIR Studio software are covered by many U.S. and international patents. For a list, see https://www.photothermal.com/home/patents/.

1.3.2. Trademarks

mIRage® and featurefindIR™ are trademarks of Photothermal Spectroscopy Corp, 325 Chapala St., Santa Barbara, CA 93101.

1.3.3. Copyright

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The Company shall not be liable for any special, incidental, indirect, or consequential damage of any kind or other damages whatsoever (including, but not limited to damages for loss of business profits, business interruptions, or other pecuniary loss) arising out of or in connection with the use or non-use of the Software, even if the Company has been notified of the possibility of such damages.

Governing Law and General Provisions

This Agreement will be governed by the laws of the State of California, U.S.A., excluding the application of its conflicts of law rules. This Agreement will not be governed by the United Nations Convention on Contracts for the International Sale of Goods, the application of which is expressly excluded. If any part of this Agreement is found void and unenforceable, it will not affect the validity of the balance of the Agreement, which shall remain valid and enforceable according to its terms. You agree that the Program will not be shipped, transferred, or exported into any country or used in any manner prohibited by the United States Export Administration Act or any other export laws, restrictions, or regulations. This Agreement shall automatically terminate upon failure by you to comply with its terms. All copies of the Software under your control shall be destroyed upon termination. This Agreement may only be modified in writing signed by an authorized officer of Photothermal Spectroscopy Corp.

Chapter 2

2. mIRage System Overview

This section introduces the mIRage key capabilities and system components.

2.1. Introduction

The m**IR**age® IR Microscope is an innovative new system, providing submicron IR spectroscopy and imaging for a wide variety of applications. Using Optical Photothermal IR (O-PTIR) spectroscopy, a proprietary technology developed by Photothermal Spectroscopy Corp, Mirage solves two of the biggest problems in IR spectroscopy:

- Achieving submicron IR spectroscopy and chemical imaging without the need for contact-based ATR accessories
- Measurement of thick samples in non-contact reflection mode, providing transmission quality IR spectra that correlate to industry standard FTIR databases

m**IR**age provides IR spectroscopy and chemical imaging, independent of IR wavelength. Using O-PTIR, highly sensitive IR measurements are enabled, providing transmission quality absorption without surface contact. This eliminates the need for thin samples and improves turnaround times.

O-PTIR operates via high speed, pulsed, tunable IR laser light focusing onto the sample surface (below). When the IR laser wavelength is tuned to an absorption band in the sample, IR absorbing regions of the sample rapidly heat up. A visible laser beam, focused to a much smaller spot than the IR beam, measures the photothermal response of the IR absorbing regions with submicron spatial resolution.

For more information see www.photothermal.com

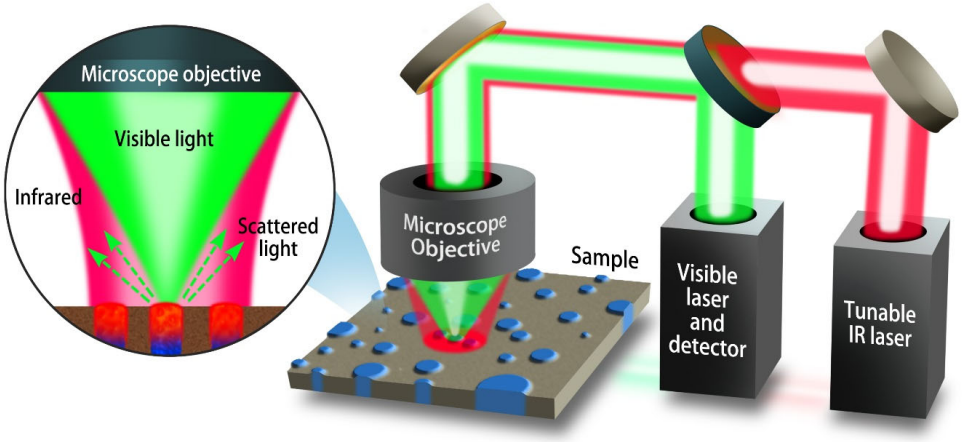


Figure 2-1. The O-PTIR technique of mIRage.

O-PTIR enables multiple IR techniques, including Transmission FTIR quality Hyperspectral reflection mode absorption spectra (below, left), Reflection mode high speed single spectra (below, middle) and submicron resolution IR microscopy (below, left).

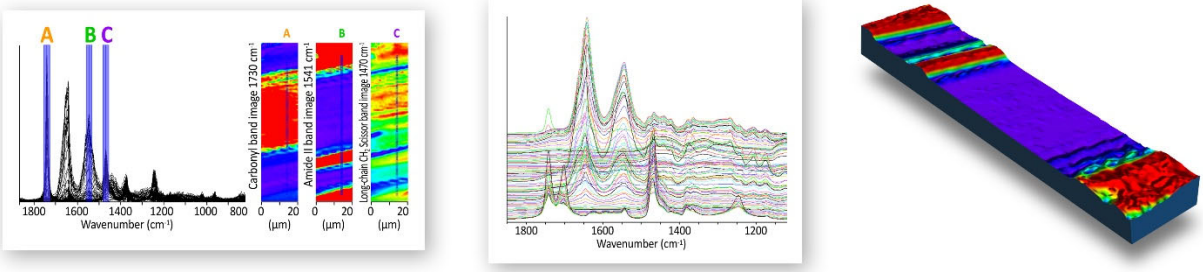


Figure 2-2. Examples of mIRage data sets.

2.2. System Components

This section outlines system components. Refer to this section for power up/power down instructions.

2.2.1. The mIRage Microscope.

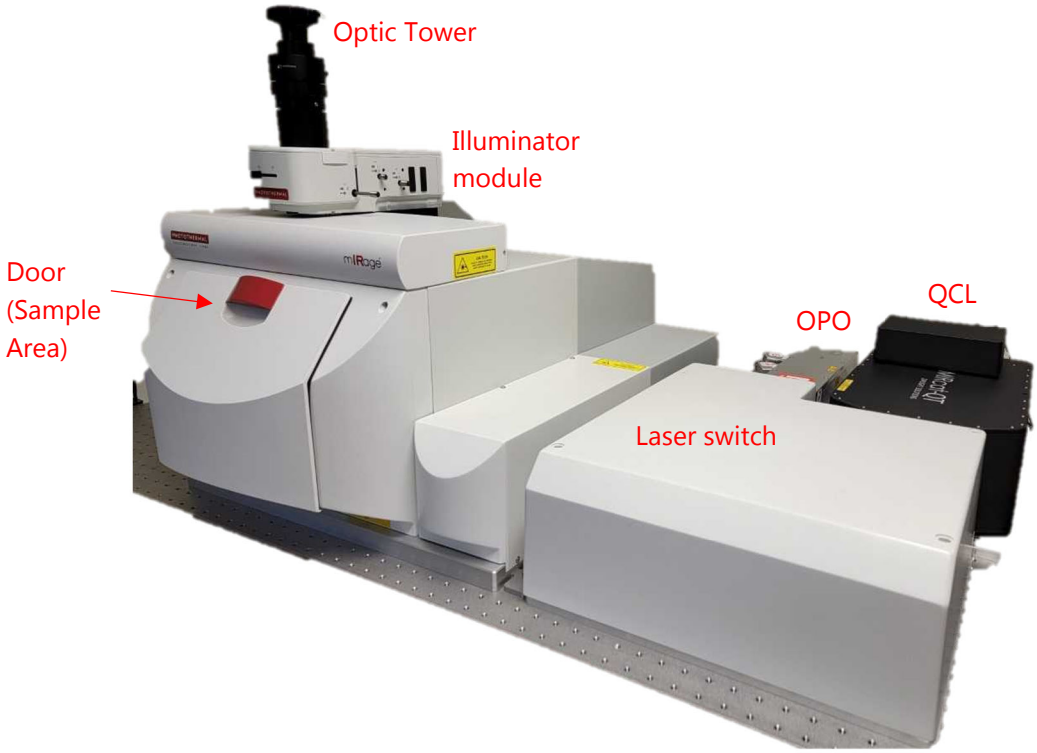


Figure 2-3. mIRage microscope

2.2.1. Computer and monitors

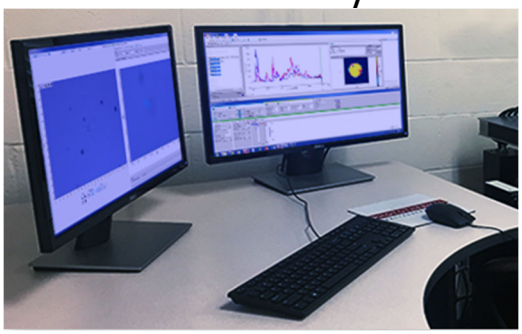


Figure 2-4. mIRage LS computer monitors

2.2.2. mIRage Controller and Power Supply

- The mIRage Controller has the DSP, FPGA, memory, communication interfaces, A/Ds and D/As. It is responsible for sending and reading the voltages/signals to and from the microscope as well as communicating with the computer.
- The power supply supplies DC voltages to the Controller and microscope.

*Figure 2-5. The **mIRage** controller and power supply.*

2.2.3. Chiller

Figure 2-6. Chiller for QCL and OPO IR laser sources.

2.2.4. QCL Laser

The power switch for the QCL laser is on the back as shown below. Press once to power up the QCL. Press and hold to power down the QCL.

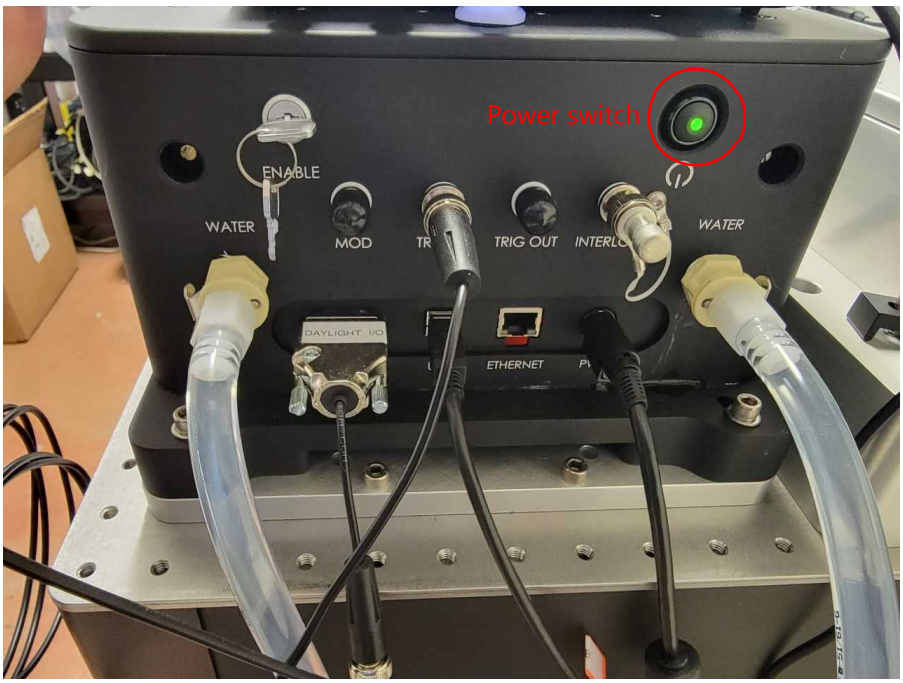


Figure 2-7. QCL IR laser power switch location

If your system has an OPO laser, see Section 6.5 for instructions on how to power up this IR laser.

2.2.5. Zurich Instruments Lock-in Amplifier

Figure 2-8. Zurich Lock-in Amplifier

2.2.6. Olympus Power Supply

- This supplies power to camera's illumination.
- A knob on the front controls the intensity of the illumination.

Figure 2-9. The Olympus power supply

2.2.7. Thorlabs XY stage controller

Depending on your system configuration, your mIRage system will have one of the two Thorlabs XY stage controllers shown below. **Note:** If your system has a BBD302 controller, do not adjust any settings or use controls on this controller.

Figure 2-10. Thorlabs XY stage controllers.

2.2.8. OPO Controllers

If the mIRage system is supplied with an OPO IR laser, it will have the two OPO controller boxes as shown in the figure below. One is labeled TC-4 and the other is labeled DD-40. Power up using this sequence:

1) Turn on TC-4 with key switch on its front panel front panel.
2) Turn on the DD-40 by turning the key.
3) Wait for the LAN lights on the front panel of the TC-4 to illuminate.

Figure 2-11 OPO IR laser controller boxes

2.3. Monthly system power cycle

Note: It is recommended to power cycle the complete system roughly once per month to clear any latent errors. To power cycle the system, use the system shut down process in Section 9 followed by the power up sequence described in Section 3.1.

2.4. Maintenance

There are no user serviceable parts inside the system and the only maintenance required is to perform visual inspections, keep the system clean and to maintain the fluid level in the laser chiller. All maintenance must be performed with the entire system turned off and the power cord to the main power strip unplugged. See Energy Isolation: Lockout / Tagout procedure below in Sec. 2.4.6

2.4.1. Chiller maintenance

Details on filling the chiller and the fluid required are in the included manual for the chiller. Please consult this manual before operating or filling the chiller. Always turn off the IR laser before stopping or servicing the chiller. Always turn off and remove the power cord from the chiller before checking the fluid level or adding fluid.

2.4.2. Enclosure inspection

All laser enclosure panels on the mIRage system should be visually checked for any gaps, at least once per year. Do not remove any source or cover components during this visual inspection. Notify PSC if any gaps are observed.

2.4.3. Cleaning

The mIRage system should be kept clean of dust. If cleaning is required, care needs to be taken. The system should not be connected to the main power supply during cleaning. Cleaning should first be attempted with a vacuum with soft brush on the intake hose, or other air sources to remove any debris. If this does not work, the unit can be wiped down on the exterior using a cleaning cloth and isopropyl alcohol. Take care when doing this to prevent any liquid from going inside the enclosures of the system.

2.4.4. Annual service visit/preventative maintenance

PSC offers an optional annual service visit for the purpose of optimizing the performance of the system and IR source and to return the system to original factory performance. Please contact PSC for additional information.

2.4.5. Fuse replacement

The user can replace the external fuses at the power entry module if required with the system. There are no internal user replaceable fuses in the system. To replace the fuses, the power cord attaching the system to the main supply must be disconnected first. The cover on the power entry module on the back of the Power Supply box can then be opened by inserting a small flat bladed screwdriver in the slots on the top of the power entry module. This cover will angle open from the top and remain connected to the power entry module. The fuse holder can then be removed by using the small flat bladed screwdriver to pry out the fuse holder. Once the fuses are replaced, return the fuse holder to the correct orientation so that the correct voltage is displayed for the voltage of the supply in your installation. Push the cover of the fuse holder back in place and reconnect the power cord.

Caution: For continued protection against the risk of fire, replace only with the same type and rating of fuses. All the fuses are 250V type “T” slow blow fuses. The current rating of the fuses should be 6.3A for both 100 and 115VAC supplies and 3.15A for 230VAC supply.

2.4.6. Energy Isolation: Lockout / Tagout

Before performing any of the above-mentioned maintenance, power must be disconnected from the system. First turn all system power switches to the OFF position, then switch off the power and all power strips and finally unplug the power strip power cords from the wall outlet. Tag the power cord and/or the wall outlet as appropriate so that it is clear a Tagout condition is present.

2.5. Installation

Before shipment, the mIRage system is tested, aligned, and specified parameters set for each system. The mIRage system requires careful alignment during the installation process and so PSC requires that the mIRage system be only installed or moved by PSC service personnel.

2.5.1. Inspecting the System

When you receive your mIRage system, look over the shipping container carefully for any signs of damage. We recommend that you do not open the shipping containers before the PSC representative arrives for installation. If there is a requirement to open the shipping containers, please check the parts received against the enclosed packing list.

- If the shipping containers are damaged, notify the carrier and contact the PSC office immediately.
- If the shipping containers are intact but parts are missing, contact the PSC office. The address of the PSC office can be found at the front of this manual.

The mIRage system and the IR source/sources are carefully packed and shipped in custom foam boxes. Please retain these shipping containers for shipping the unit for any required service.

2.5.2. Choosing a Location

The weight and dimensions of the mIRage system are included in the mIRage Facilities Requirements document (00-0035). Please contact PSC for the most current version of this document and review this document to make sure the location for the system meets the complete requirements in this document. A table for the system electronics, computer and monitors is not supplied with the system and must be supplied by the user.

The mIRage electronics should be placed on a stable surface and the cooling vents on the side of the electronics boxes should not be blocked by any obstructions. Also, the user needs to have easy access to the power switch and power cord on the back of the unit to allow the unit to be disconnected should that be required.

The location of the mIRage system is also of great importance for the performance of the system. The primary signals in the O-PTIR measurements are high resolution measurements over a relatively long time. Strong vibration, air currents or large temperature changes can disturb the measurement. So, the system should be placed in a location with appropriate consideration. A ground strap is supplied with the system. This should be attached to the optical table top and then to a suitable earth ground.

2.5.3. Power Requirements

The mIRage system is set in the factory to 100V, 115V or 230VAC (+/-10%), depending on the country and can be run from 50-60 Hz frequency. The unit requires minimal power (current less than 5A for 100 or 115VAC and less than 2.5A for 230VAC) and can typically be connected to a standard wall outlet. To minimize damage to the unit, if installed in a location that has intermittent power, we recommend a surge suppressor or in the extreme case a power conditioner. An uninterruptible power supply can also be useful to keep the system operational in cases of a power outage. If using a UPS, make sure to choose one that has sufficient current capacity for the LS system and enough runtime to arrange to save your data and safely shut down the system.

2.6. System purging

The mIRage system is supplied with purge gas ports that can optionally be supplied with purge gas to remove water vapor/CO_2 from the instrument enclosure. Purging is not always required but may be helpful in preventing the effects of strong water vapor absorption lines from causing spectral distortions. See the mIRage facilities requirements manual for instructions on how to connect purge gas to the instrument.

If your instrument is connected to a purge gas supply, contact the individual responsible for supervising instrument use to learn how to turn on the purge gas supply if necessary.

Warning! Purge gas must only be used in compliance with applicable safety standards and oxygen monitoring if required. The use of purge gases in enclosed spaces without sufficient ventilation or oxygen monitoring could lead to unconsciousness and/or death if excess purge gas displaces oxygen in the room.

Chapter 3

3. Preparing for a measurement

This section overviews common steps that will be used for all measurement modes. Specific measurement modes are detailed in following chapters.

NOTE: This manual describes software operation as of version 4.5.1. Certain features may be unavailable in earlier software versions. If you are running an earlier version of software contact PSC to see if a software upgrade is appropriate.

3.1. System preparation

To prepare the system for use, follow the steps below.

1. Power up system components. Use Section 0 to identify components to turn on and their power switch locations. **Power up the components in this order:**
   - Power up the computer and monitors first.
   - Power up all other system components
   - (This power up sequence is intended to avoid conflicts when Microsoft Windows assigns COM ports.)
2. Double-click the PTIR Studio icon 

to open the software.
3. Click Initialize 

on the top toolbar.
   This verifies communication between all the components and readies the hardware and software for use. The bottom status bar changes from "Not Initialized" to "Idle" when initialization is complete. (Initializing may take a couple of minutes.)

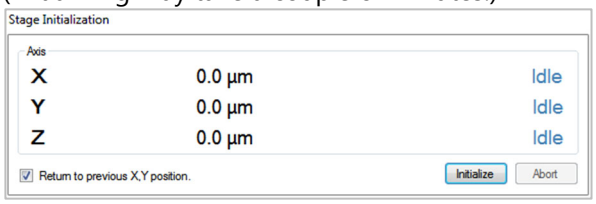


Figure 3-1. System initialization.

   - When prompted to initialize the Stage:
     - ♦ Click Initialize.
     - ♦ Select OK when the status changes for all the axes to "Initialized and ready".

3.2. PTIR Studio Software Overview

The interface has two windows: the Document window (usually on the left) and the Live window (usually on the right). The two windows are positioned independently on each of the two monitors. The Document window (Figure 3-2) is where acquired spectra and images will be displayed for analysis. The Live window (Figure 3-3) is used for live microscope video, sample navigation, focusing, real-time controls, settings, and system monitoring. The Live window uses a tabbed user interface that dynamically changes depending on the selected task. The three tabs are Navigate, Spectra, and Image as shown in Figure 3-4.

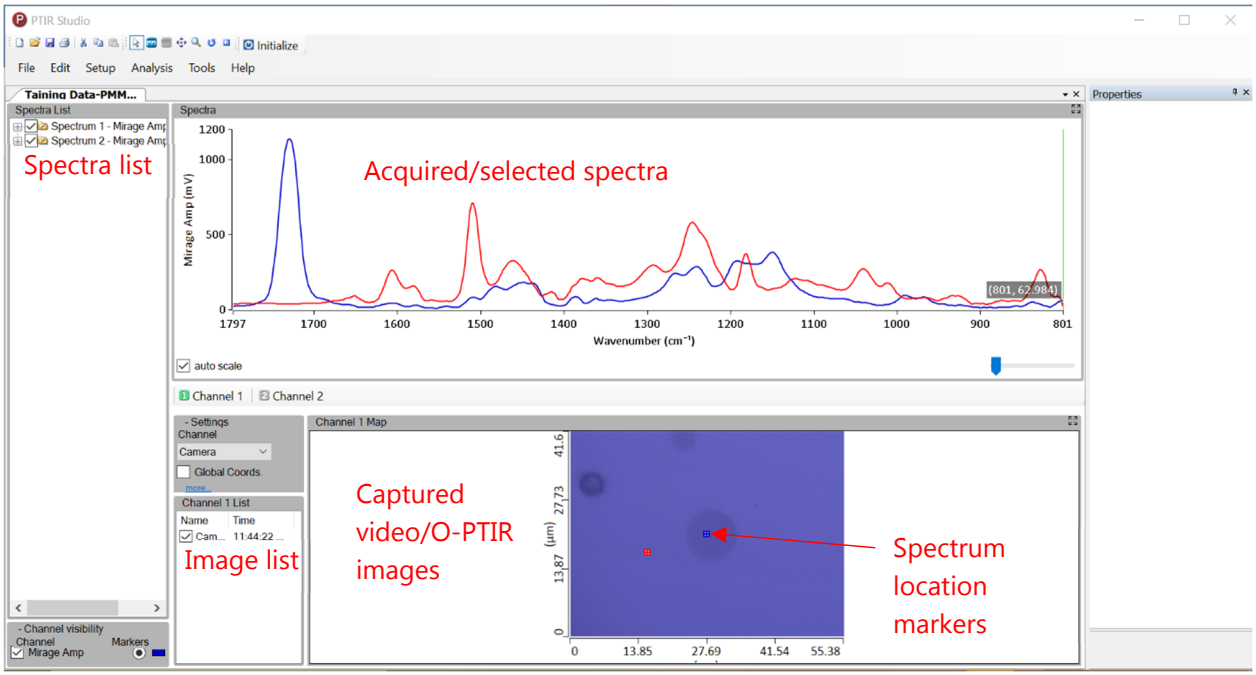


Figure 3-2. The Document window (Left monitor)

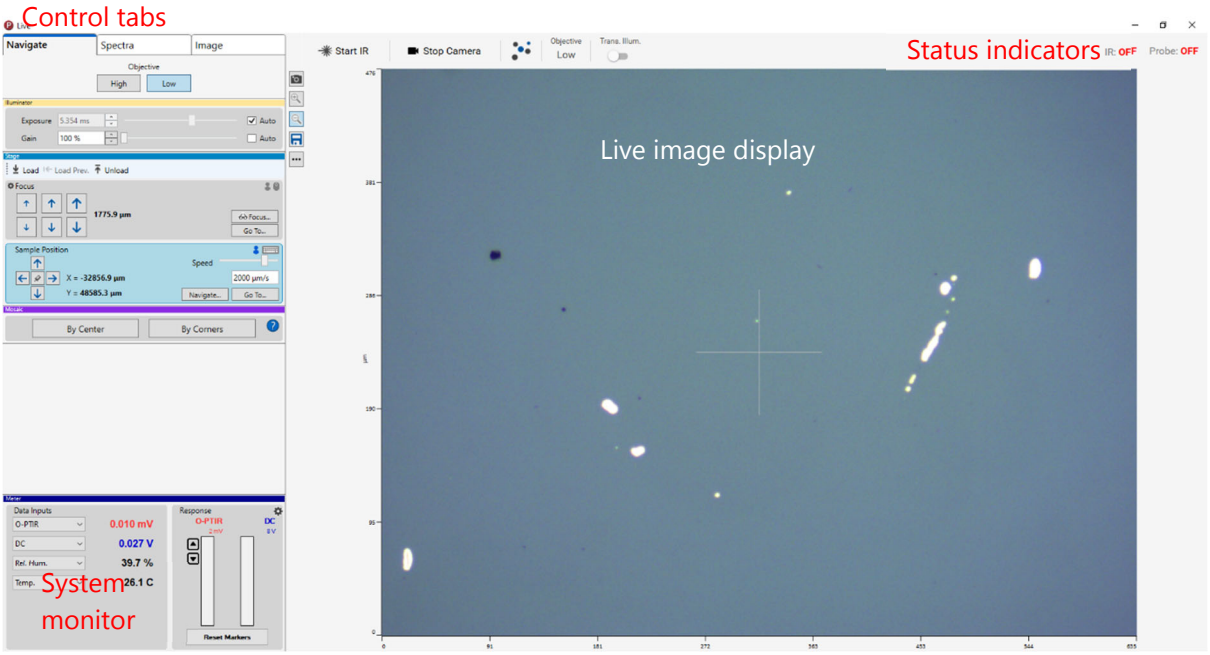


Figure 3-3. The Live window (right monitor).

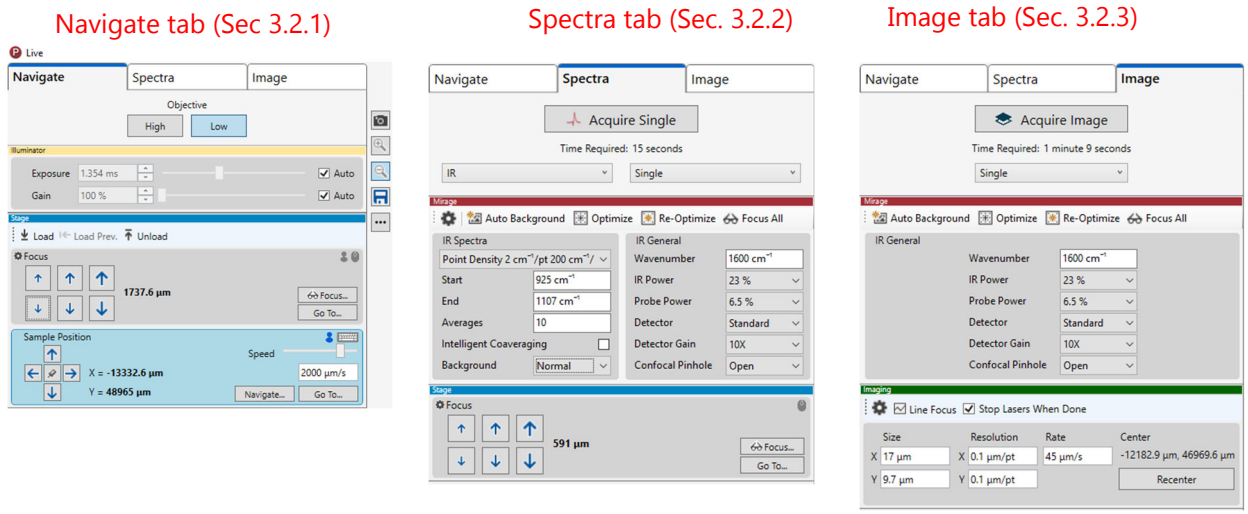
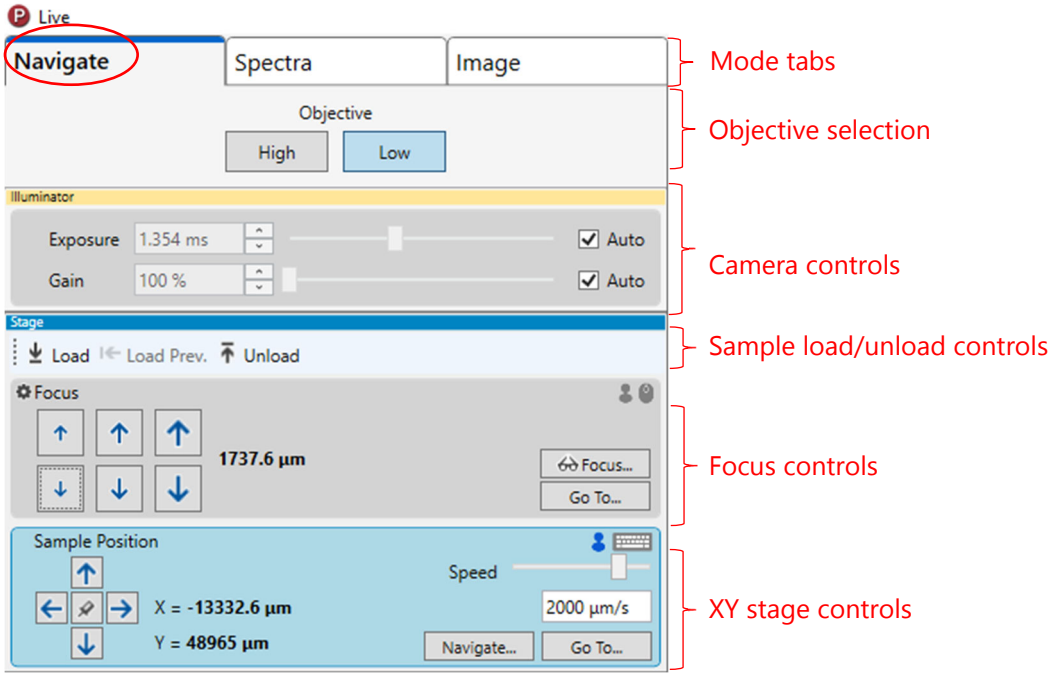


Figure 3-4. Live window with tabbed user interface.

3.2.1. Live window Navigate tab

Below is a summary of the Navigate functions available on the Live window. Click on the Navigate tab to bring these controls into view. These controls are for selecting an objective, controlling illumination parameters, loading/unloading samples, focusing on the sample, and moving around the sample on the XY stage. Each of these controls will be described in more detail below.



3.2.2. Live window Spectra tab

The Spectra tab contains all the controls and settings for acquiring O-PTIR spectra (and Raman spectra if supplied). Spectra acquisition is detailed in Sec. 4.8.

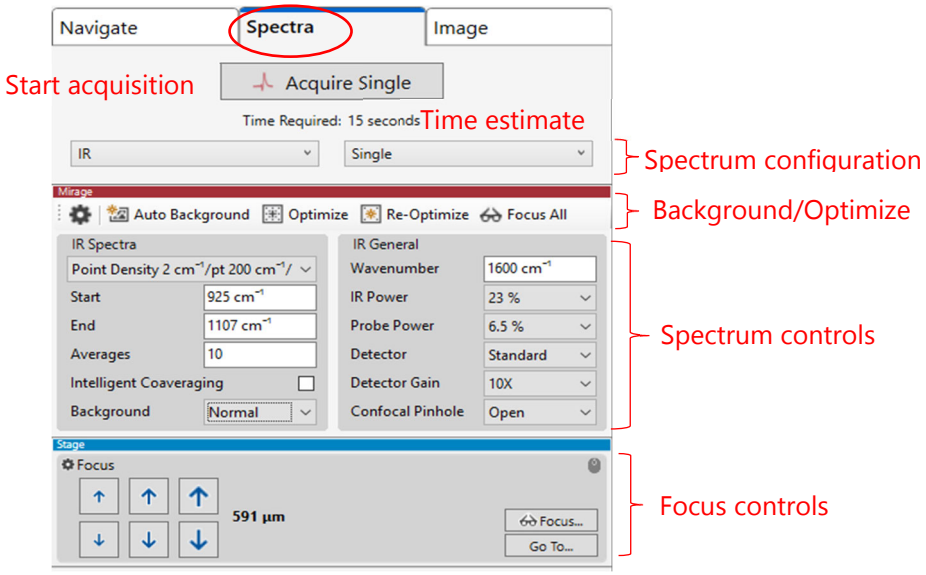


Figure 3-5. Spectra tab on the Live window.

3.2.3. Live window Image tab

The Image tab contains all the controls and settings for acquiring O-PTIR images. Image acquisition is detailed in Sec. 4.9.

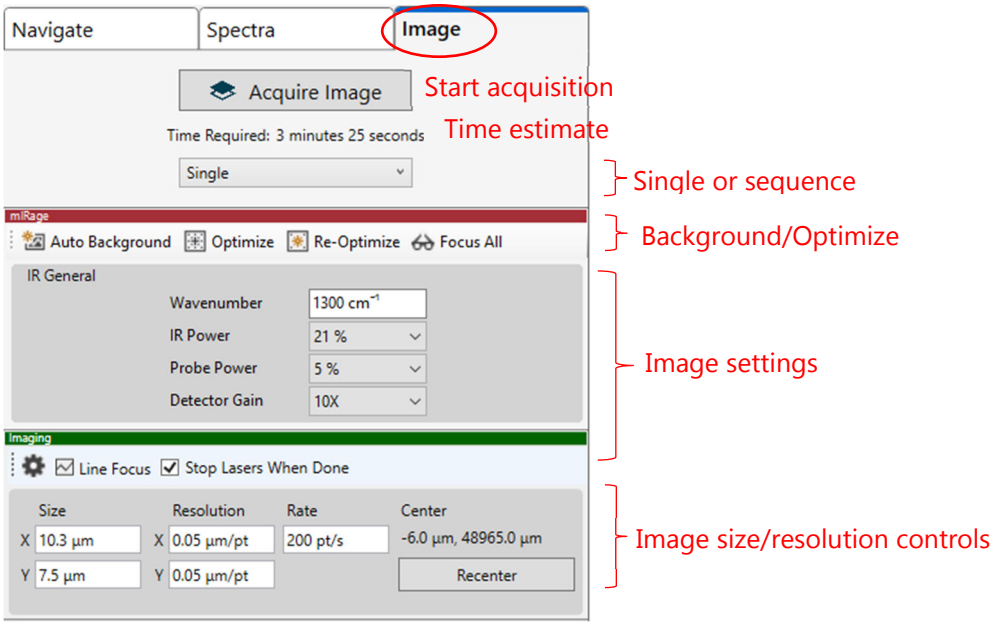


Figure 3-6. Image tab on the Live window.

3.3. Sample preparation

The most successful MIRage O-PTIR samples:

- Are greater than 200 nm thick.
- Have features of interest wider than 500 nm.
- Have organic materials of interest or other materials with strong IR absorption bands (or Raman if enabled)
- Have surface roughness/flatness less than 500 nm. Samples with larger surface corrugation can be measured with O-PTIR but may require refocusing due to the small depth of focus of the probe beam.
- Are rigidly mounted and/or adhered to a solid substrate. For example, samples like fibers, thin films, powders, etc. should ideally be adhered/attached in a way that they will not move or vibrate during a measurement. Contact PSC applications support for specific suggestions on sample mounting.
- Biological cells should be well adhered to a glass slide, cover slip or calcium fluoride flat.

Samples should be mounted on a glass slide or supplied sample holder so they can be easily secured onto the XY stage.

3.4. Sample loading

If a sample is already loaded, use the Unload button on the Stage panel to unload the sample. Clicking on Unload will retract the microscope objective turret and move the XY stage to the front of travel for easy access for sample loading/unloading.

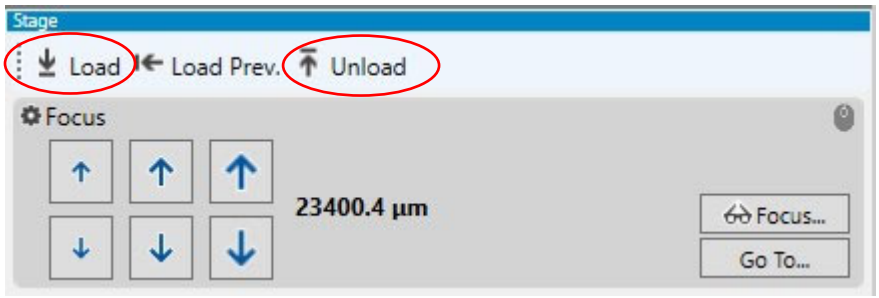


Figure 3-7. Sample loading and unloading

Place a sample or sample holder onto the XY stage and secure with the spring clip. When the sample is firmly secured on the XY stage, click on the Load or Load Prev. button. The Load button will recenter the XY stage under the objective turret but will not lower the objective turret. The Load Prev. button will move to the last XY sample position and then lower the objective to the last Top Focus position. Only use the Load Prev. button when you are re-installing the same sample or a sample identical thickness as the previous sample.

Warning: Using the Load Prev. button on a sample that is taller than the last loaded sample can cause the objective to crash into the sample, risking damage to both the sample and objective. Only use the Load Prev. button when loading a sample that is the same thickness as the previous sample (e.g., last sample and previous sample are both 1 mm thick glass slides).

3.5. Sample navigation

Sample navigation is controlled by the Stage panel and if desired by the Stage Navigation pop-up control. To open the Stage Navigation pop-up, click on the Navigate… button on the Stage panel as shown in the figure below.

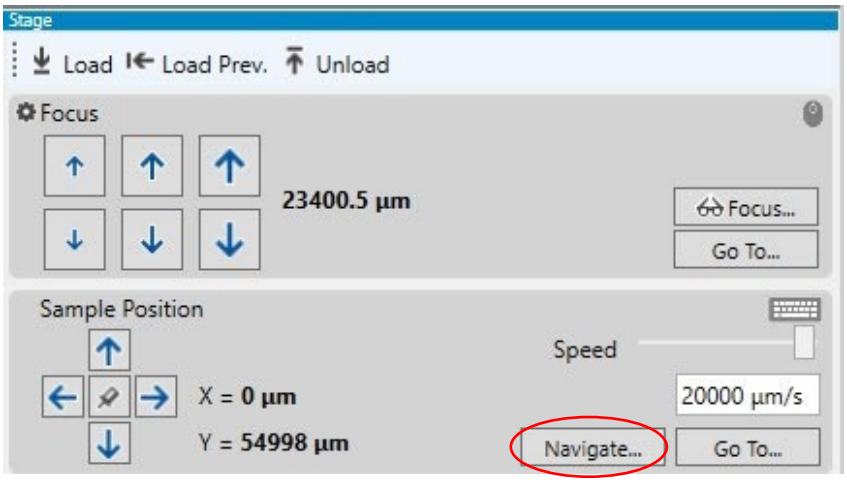


Figure 3-8. Stage panel with controls for XY stage and objective focus controls and Navigate button.

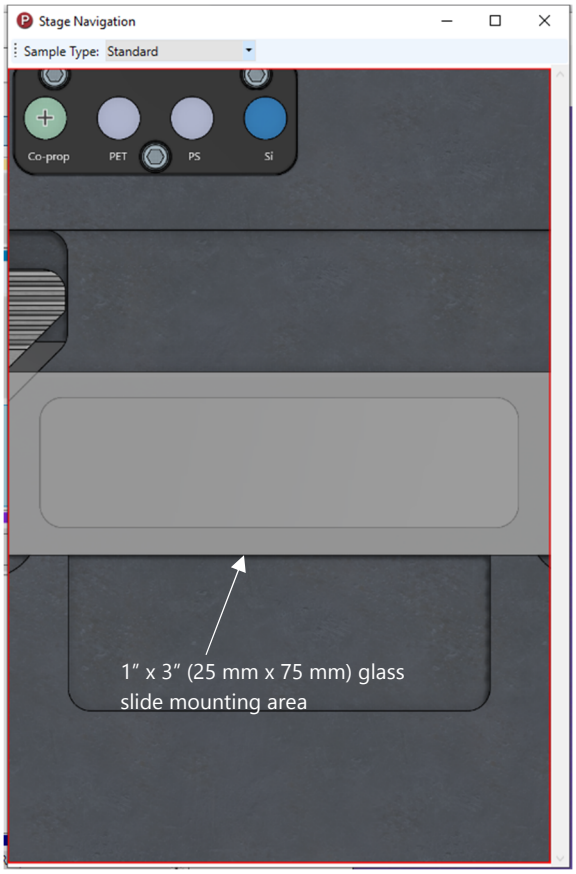


Figure 3-9. Stage Navigation pop-up control with sample holder and reference sample locations noted. PET= Polyethylene Terephthalate, PS=Polystyrene, Si=silicon.

The Stage Navigation pop-up is a graphical view of the sample stage. Clicking on any location in the Stage Navigation image will move the XY stage to position the selected location under the mIRage microscope objective. Figure 3-9 shows the mounting locations of the 1" x 3" (25 mm x 75 mm) sample holder as well as background and reference samples.

3.6. Start Camera

If the camera video view is not already started, click Start Camera to enable live video mode for focusing and sample XY stage motion. (The camera normally starts automatically unless disabled.) Unless trying to visualize the probe beam spot, make sure the Start IR/Start Raman buttons are not clicked as the Start IR/Start Raman buttons block the camera video.

3.7. Changing objectives

Objectives can be changed using the Objective selection buttons. Up to four objectives can be selected depending on the configuration of your system. Generally, start with the 10X (or optionally 4X) low mag objective for initial sample navigation and then switch to an appropriate high mag objective for O-PTIR and Raman measurements, depending on your measurement mode. The Zoom in/Zoom out icons will increase/decrease the magnification to the display screen via digital zoom. These buttons do not change the microscope objective.

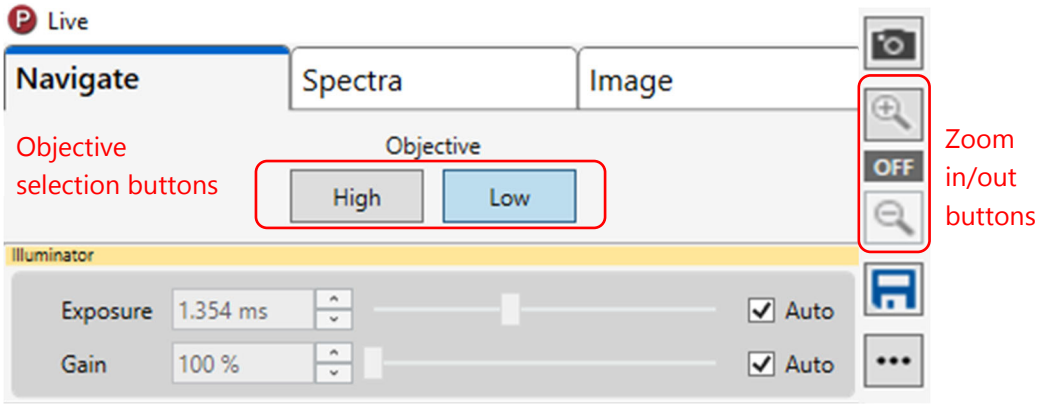


Figure 3-10 Objective selection and digital zoom controls.

3.1. Zoom controls

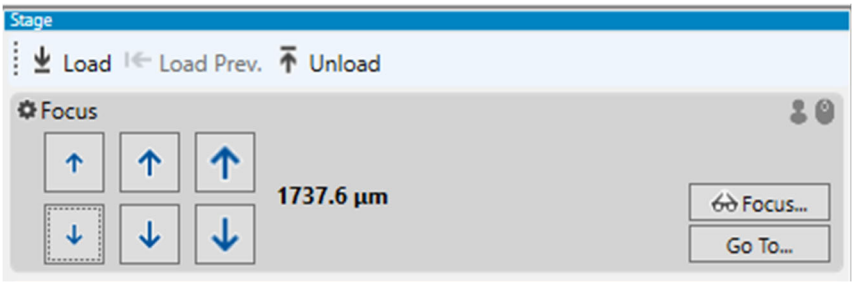
Depending on the camera model, the mIRage may have two or more digital zoom settings so set the optical field of view displayed in the video window. To zoom in or out, click on the appropriate icon shown in the figure above. The current zoom setting (Off, Low, Med, and Max) is displayed between the zoom in/out icons. It can be useful to use higher zoom settings for mosaics and/or for featurefindIR particle images to create images with more even illumination across the field.

3.2. Focusing on a sample

3.2.1. Focusing with the microscope camera

To focus on a sample, use the arrow keys on the Focus control panel to bring the sample into focus. The three sets of arrows move the objective at fast, medium, and slow focus rates. Generally, use the fast focus buttons to get close to focus and then the medium and/or slow for fine focusing.

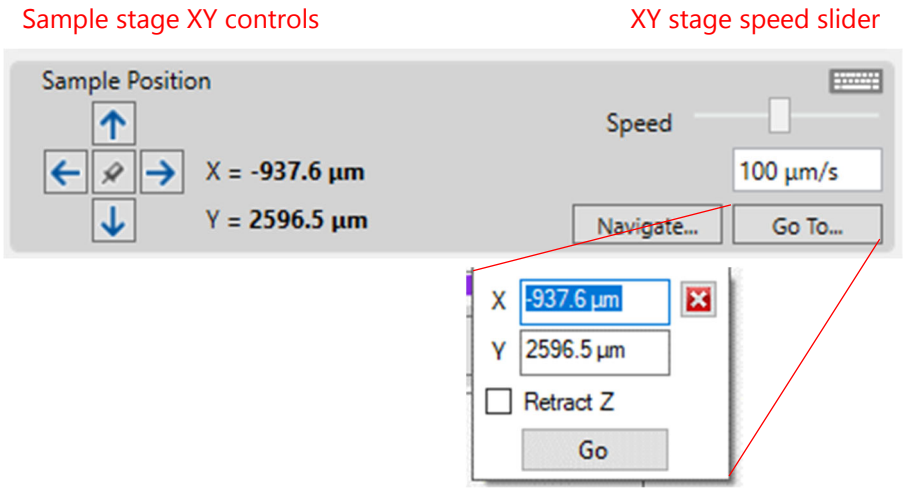
The Go To… button can be used to drive the focus to a desired height. This must only be used when returning to focus on a sample with a known focus height. The Focus… button is only used for optimizing the O-PTIR signal level and is not used during sample navigation/visual focus.

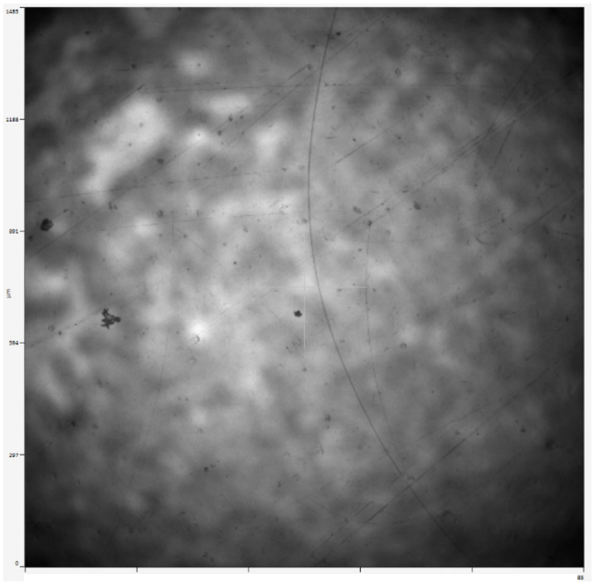


Caution: Extreme care must be used when focusing on tall samples to prevent the objective from crashing into the sample causing damage to the sample and/or objective. Familiarize yourself with the working distances of your objectives and make sure not to lower the objectives below the working distance.

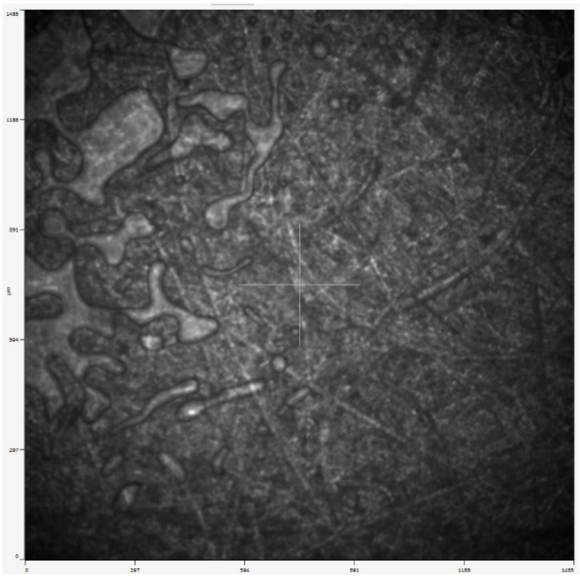
Make sure to focus on the top surface of the sample

When focusing on a thin sample, ensure that you are focused on the **top surface** of the sample. Become familiar with the typical z focus value for the samples you use. To focus on the top surface, start well above the expected focus position and move the focus down using the medium or slow speed until a surface comes into focus. For samples with few surface features, it is useful to look for scratches or dust to indicate where the surface is. Once you have found a surface in focus, click on the live video image to attempt to center a feature in the video view. If the feature moves to center, you have found the surface of the sample. If the feature does not move, you are focused on an intermediate image plane of another optic in the microscope system. Continue moving the focus down until you find the top surface. If you overshoot, you may find a second surface which is the bottom surface of the sample. Depending on your sample mounting, you may see different features on the bottom surface, for example adhesive or other features of the underlying substrate. Learn to recognize the expected features of the top surface such that you don't accidentally focus on the bottom surface. The focus range is generally limited to prevent objective crashing for standard 1 mm thick glass slides, but when using thicker samples use extreme caution when focusing to avoid crashing the objective into the sample, especially when using high NA/shorter working distance objectives.





Polystyrene reference sample **top** surface



Polystyrene reference sample **bottom** surface

Figure 3-11. Video images of top and bottom of polystyrene (PS) reference sample.

3.2.2. Focus by minimizing probe spot size

It is also possible to rapidly optimize the O-PTIR spatial resolution on some samples by minimizing the apparent spot size of the probe beam at the sample surface. To visualize the probe beam at the sample, take the following steps:

1) Select the appropriate objective for the O-PTIR mode you are using.
2) Click on Start IR to direct the probe beam to the sample.
3) Click on Start Camera to also turn on the camera

The camera is now looking at the sample through the probe beam mirror. The sample will not be visible as it is generally too dim, but the brighter probe beam should be visible on many samples.

4) Optionally set illumination intensity to zero for better viewing of the IR laser spot.
5) Click on the Zoom in icon
6) Adjust the fine focus buttons to get the sharpest image of the probe laser spot

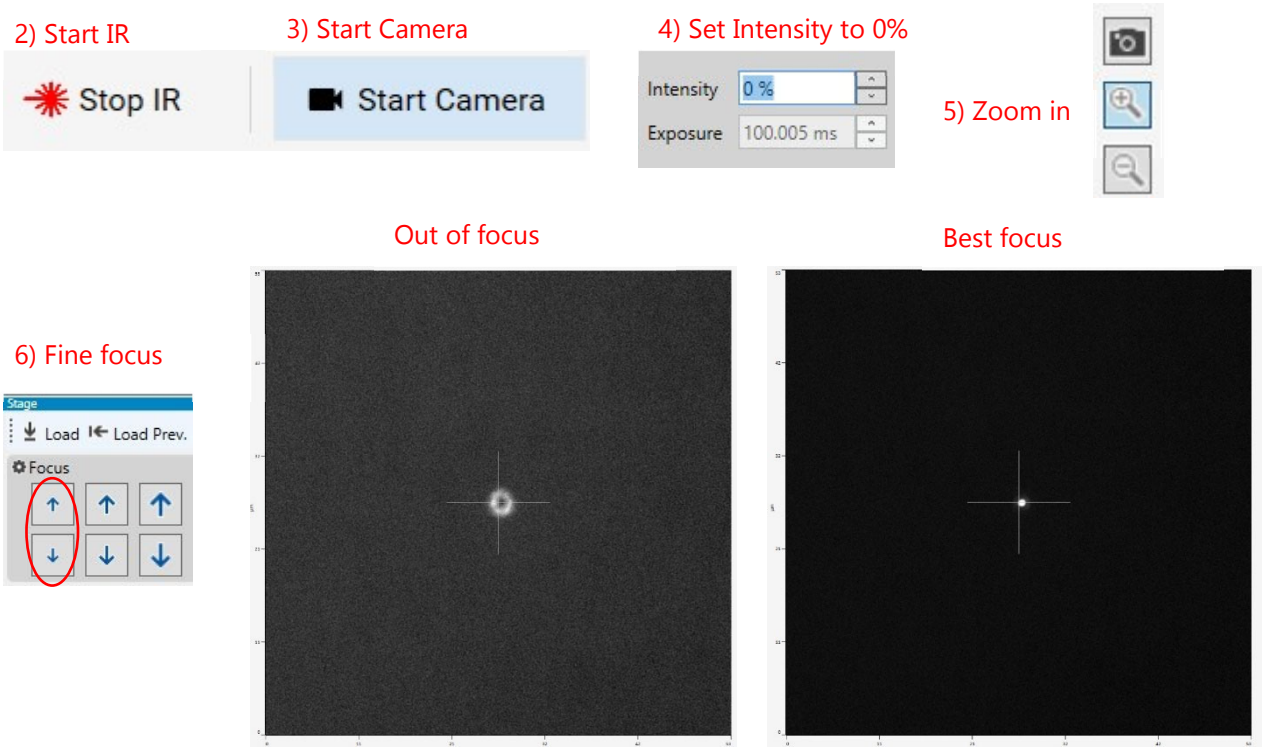


Figure 3-12. Adjusting focus using minimum probe beam size

3.3. Camera exposure/gain controls

The camera exposure controls are shown below. There are two Auto check boxes, the first for camera auto exposure and the second for camera auto gain. It is generally recommended to use the auto exposure only whenever possible. If the sample is very dark or the auto-exposure leads to excessively long exposure times, it can be useful to turn up the gain or turn on auto gain control.

Figure 3-13. Camera exposure/gain controls

3.4. Capturing camera images

Microscope images can be saved using the icons to the left of the video display in the Live window while the Navigate tab is selected. To capture an image to the current document, click on the camera icon . To save an image outside the document to a file on your computer or network, click on the disk icon .

Figure 3-14. Capturing images to the document and saving images to disk.

3.5. Capturing mosaics

In addition to individual optical images a Mosaic of the sample surface can be collected using the Mosaic function shown below. This function will automatically move the stage in an array and capture optical images to generate an image over a larger area than allowed by the field of view of the objective.

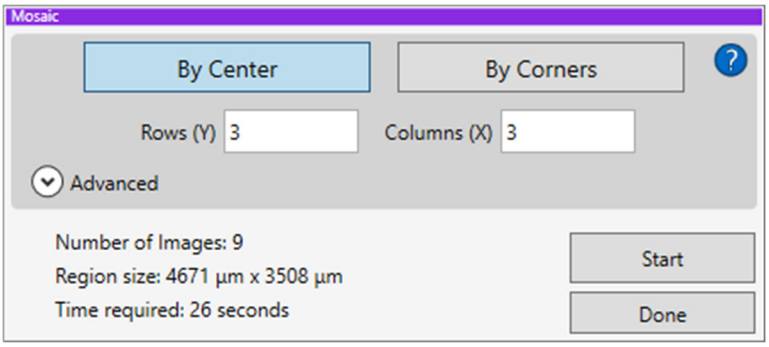


Figure 3-15. Capturing optical image mosaics

3.5.1. Mosaic advanced options

Clicking on the down arrow next to the word Advanced will open up additional advanced options, including the ability to automatically perform flatfield corrections.

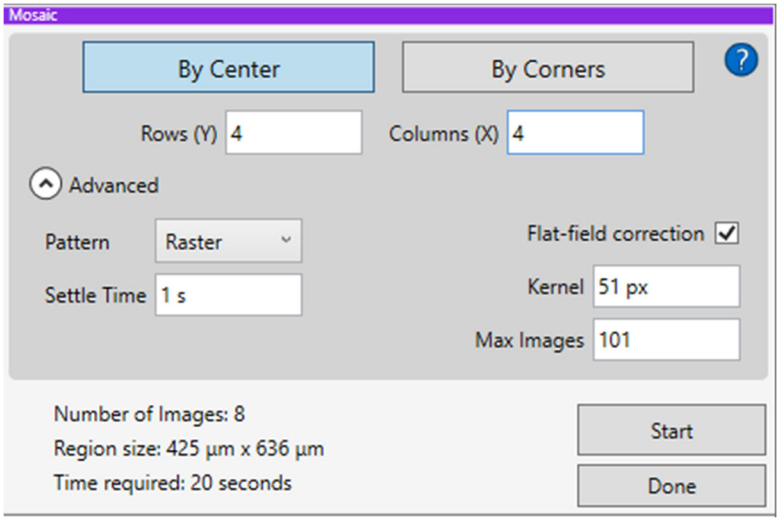


Figure 3-16. Mosaic advanced options

Flatfield correction for mosaics

Some objectives can show some darkening towards the edge of the field, especially at low zoom and/or with large field of view cameras. PSC has implemented a flat field correction algorithm that can be used to post process optical images to produce a more even field. The flat field correction can also be applied automatically in mosaic collection by clicking on the Flat-field correction checkbox under the Advanced option. Use of higher zoom levels for mosaics can also improve the brightness uniformity.

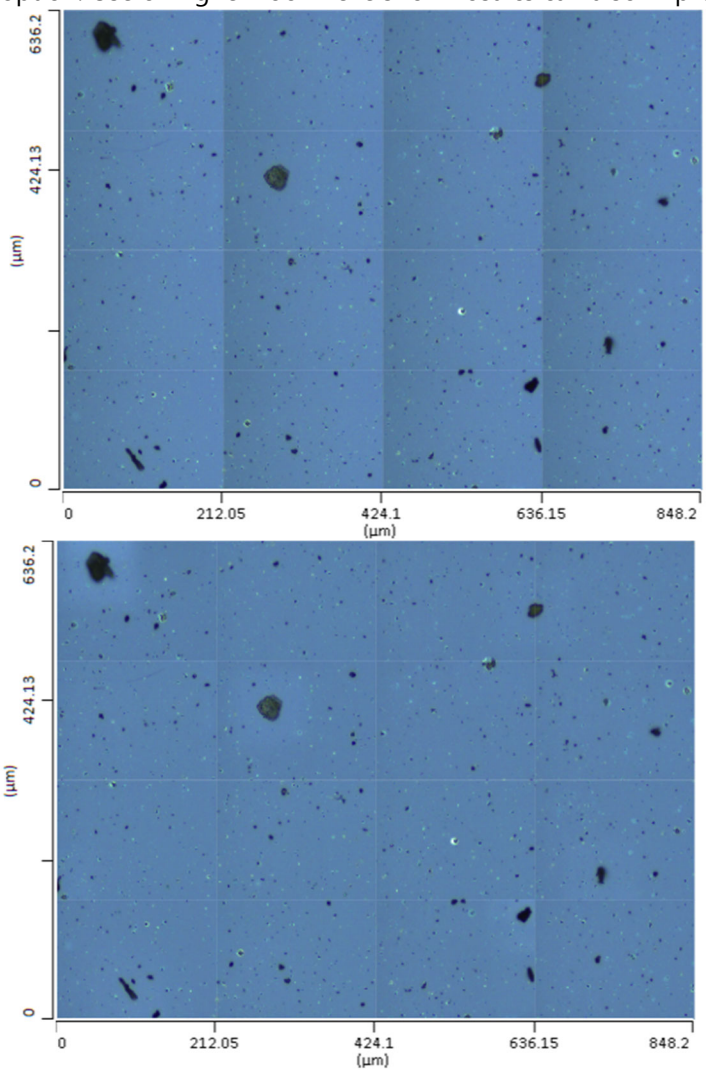


Figure 3-17. Optical image mosaics without (top) and with (bottom) flatfield correction

Mosaic Settle Time

The settle time field in the mosaic advanced options is a wait time after the stage move and before the image acquisition. Using a larger settle time will cause the mosaic acquisition time to increase, but will give more time for the automatic exposure control to stabilize. If AEC is turned off, this settle time can be made smaller.

3.6. Setting waypoints

Sample stage positions can be saved using the waypoints function within the PTIR Studio software.

- Click on the pin icon  located in the center of the sample position arrows to open the waypoints popup window.
- Waypoints can be added by clicking the + icon and deleted using the − icon.
- To move to a particular waypoint, click on the waypoint and then click the move to waypoint button.
- Waypoints can be named by clicking on the waypoint number and entering a desired text label.
- Waypoints can be saved to a user specified file and reloaded using the save/open icons. 

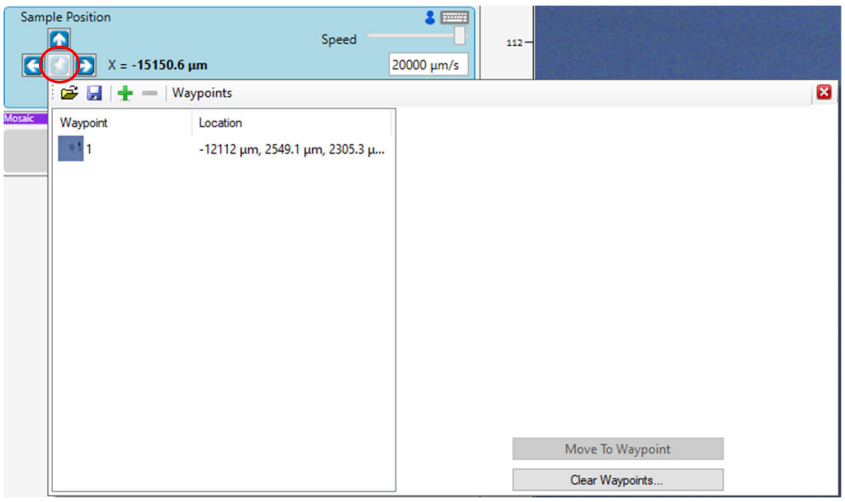


Figure 3-18. Waypoint tool

Chapter 4

4.O-PTIR measurements

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4.1. O-PTIR introduction

The mIRage instrument operates O-PTIR in a co-propagating mode where the IR pump beam and the visible probe beam are focused onto the top of the sample with a reflective (Cassegrain) objective.

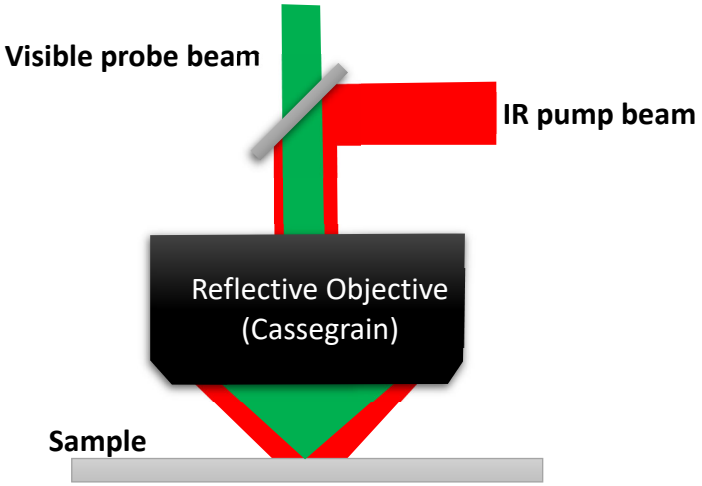


Figure 4-1. O-PTIR co-propagating mode

The Optimize steps described later involve arranging for optimal overlap of the IR pump beam and visible probe beam at the sample.

4.2. O-PTIR measurement checklist

Following is an abbreviated list of the steps to use for O-PTIR measurements. This section is intended as a reminder for experienced users or as an outline for newer users. Detailed descriptions of each step follow later in this chapter as indicated in the Section Reference column.

Table 4-1 Initial setup checklist

Step	**Section Reference**
Purge system if desired	2.6
Select low mag objective	4.3
Load a sample	3.4
Navigate to a region of interest	3.5
Collect an Auto Background	4.5
Open a blank document	4.7.1
Select 40X (high mag) Cassegrain objective	4.7.2
Capture a video image of sample	3.4
Select detector	4.7.4
Set initial measurement parameters	4.7.5
Tune IR laser to an absorbing wavelength	4.7.6
Start IR	4.7.7
Check signal saturation/adjust gain	4.7.8
Optional: O-PTIR autofocus to maximize signal OR Focus to achieve minimum probe spot size	4.7.9 OR 3.2.2
Check signal stability	4.7.10
Adjust IR/probe power and gain	4.7.11
Acquire test spectrum	4.7.12
Ensure no sample damage	4.7.13

Table 4-2 Spectra measurement checklist

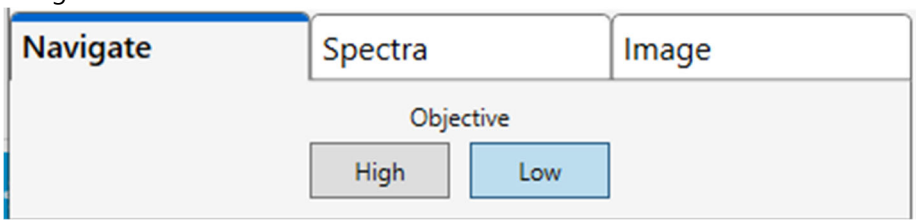
Step	**Section Reference**
Select image for spectra map reference	4.8.1
Set spectral range and number of averages	4.8.2
Select spectral measurement mode (Single or Array)	4.8.3 or 4.8.8
Select spectra measurement point(s)	4.8.3
Click Acquire to acquire spectra to document	4.8.3
Save data after acquisition	4.8.4
Optional Steps	
Enabling automated spectral array collection	4.8.8
Point arrays	4.8.9
Line arrays	4.8.10
Grid arrays	4.8.11
Hyperspectral arrays	4.8.12

Table 4-3. O-PTIR imaging checklist

Step	**Section Reference**
Select O-PTIR image scan area	4.11.1
Select Single image mode	4.11.2
Minimize probe beam size OR Optimize focus using Line Focus tool	3.2.2 OR 4.11.5
Adjust image settings as desired	4.11.3
Click Acquire to acquire image to document	4.11.3
Save data after acquisition	4.8.4
Optional steps	
Image Sequence	4.11.3
Interleaved images	4.11.4

4.3. Objective selection

The mIRage system is supplied with two objectives, a low magnification objective (typically 10X) and a 40X reflective objective (Cassegrain). Either objective may be used for sample navigation. The 40X Cassegrain is always used for O-PTIR measurements. Typically start with the low mag objective for initial sample navigation.



4.4. Load a sample and navigate to a sample location

Load a sample, navigate to a desired location, and focus on the sample as described in Sections 3.4-3.2 and optionally capture one or more optical images to the document.

4.5. Acquiring Auto Background

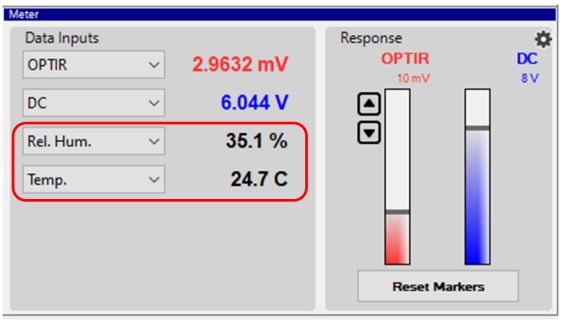
The Auto Background is a tool for automatically optimizing the performance of the mIRage instrument and acquiring a measurement of the optical power curve (the "background") for the selected IR laser source. The background is used to normalize O-PTIR spectra for variations in the IR power as a function of IR wavelength. The background will change over time based on temperature and humidity and therefore it is recommended to acquire a new background before each measurement on a new sample or at least once every few hours if continuously measuring the same sample.

4.5.1. Optionally purge your O-PTIR system

Before acquiring an auto background, it may be desirable to purge the system to reduce the effects of IR absorption by water vapor. See section 2.6 and the mIRage facilities manual regarding system purging.

4.5.2. Humidity and temperature indicator

The humidity and temperature of the mIRage system are monitored whenever PTIR Studio software is running. The current relative humidity and temperature are displayed on the Meter panel of the Live window and are stored with each acquire spectrum and image. A purged system should generally be able to achieve <5% internal humidity. If the system is not purged, get in the habit of checking the humidity and make sure to acquire a new IR power background if the humidity changes by more than 2%.



4.5.3. Starting an Auto Background

To start a background, on the document window, Click on Auto Background from the mIRage panel as shown below.

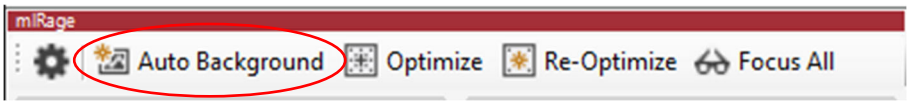


Figure 4-2. Starting an auto background.

In Step 1, the system will move to the location of a reference sample on the XY stage. Make sure nothing is blocking access to the background sample (e.g., no tall sample on the stage that would cause an objective to crash into the sample when it moves to the background sample position) and then click Next.

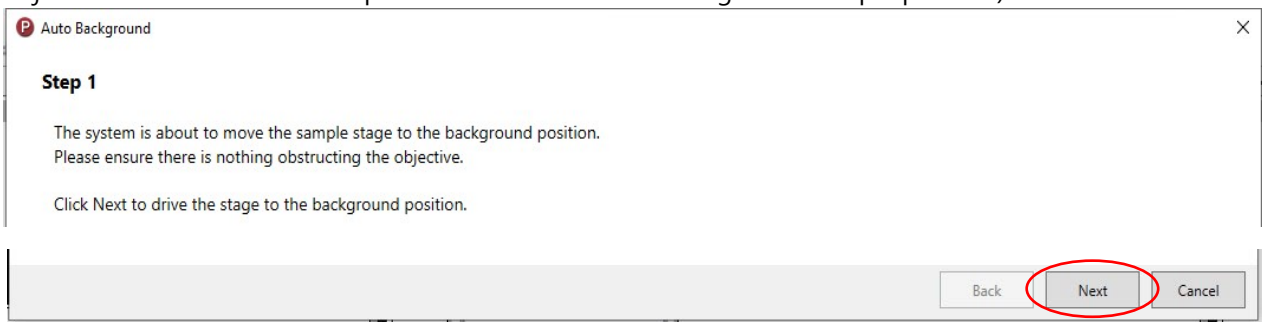


Figure 4-3. Moving to the background sample.

In Step 2, adjust the objective focus using the up/down arrow keys in the dialog box to bring the background sample into sharp focus. Usually there will be some small defects or small particles on the background sample that can be used to judge best focus. The focus range is limited such that it will stop after moving a maximum of 50 µm in either direction. If you don't find the background sample focus by the time the focus axis stops moving, click on the other arrow to reverse direction until you find the sample focus. Once the surface focus is found, click Next.

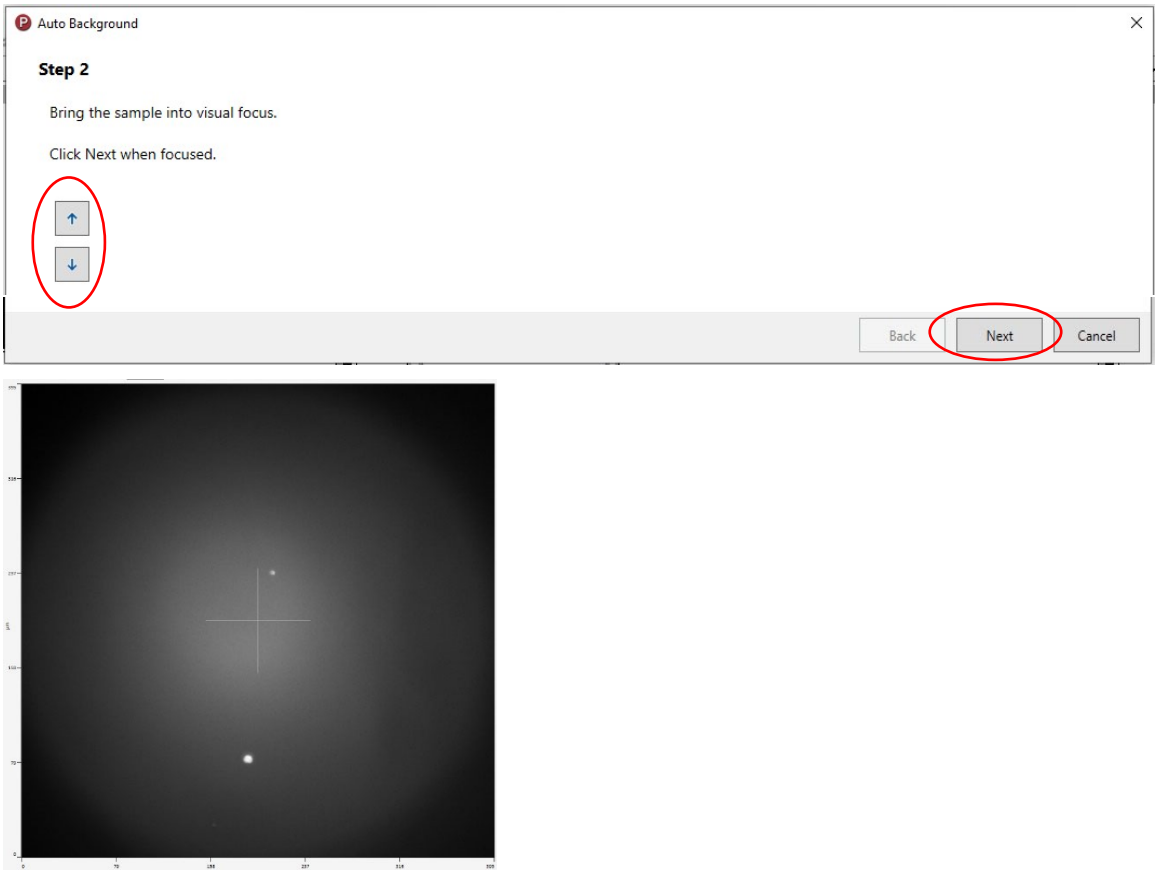


Figure 4-4. Step 2, focusing on the background sample.

In step 3, the Auto Background tool will automatically proceed through a series of stages to optimally align the system and acquire a background. The three stages are: 1) Optimize; 2) Focus; and 3) Background. In the Optimize stage the system will automatically align the IR and probe beam for optimal overlap. In the Focus stage, the system will adjust the top objective focus to maximize the O-PTIR signal. In the Background stage, the system will measure the optical power of the IR laser as a function of wavenumber. To monitor these stages, you can click on the small show/hide arrows next to each stage label, as circled in the figure below. The figure shows what successful Optimize, Focus, and Background stages should generally look like. If the Optimize and/or Focus stages do not look similar to those shown below, contact Photothermal for support.

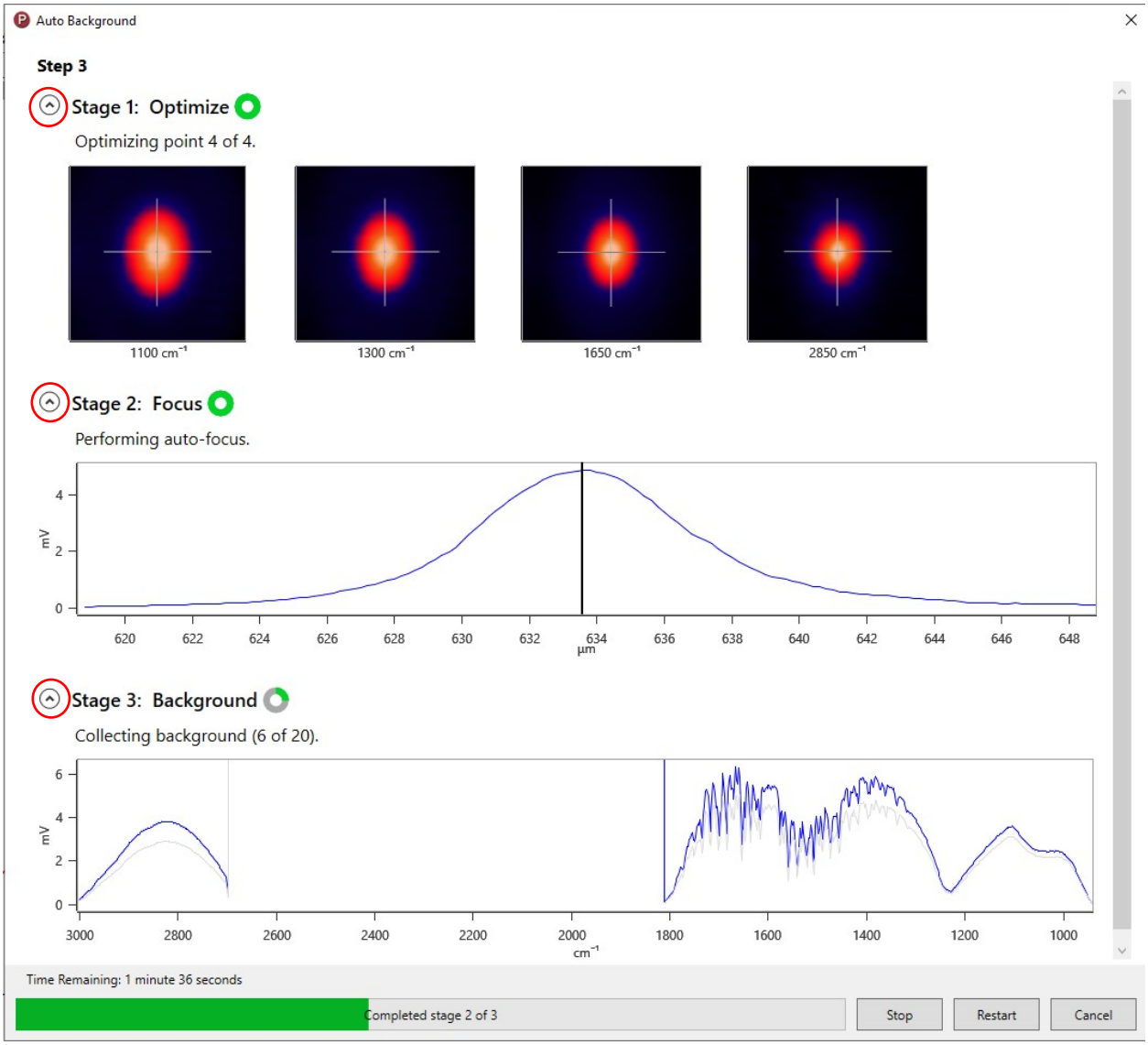


Figure 4-5. Auto background in progress.

Once the auto background is completed, inspect the background curve. The shape should generally resemble the factory reference shown in light gray, though it is completely normal to see some variation, e.g., somewhat higher or lower power than the factory reference. The ripples in the region from 1300-1800 are associated with IR absorption by water vapor so the depth of these ripples depends on the humidity of the laboratory and mIRage system. To reduce the depth of the water absorption lines, purge your system with dry nitrogen or clean dry air as described in the purging section 2.6.

If you are satisfied with the background results, click Save & Apply. You can optionally specify a filename for the background and save it to a location of your choice. Otherwise, the system will automatically save the background to a default location using a date/timestamp for the file name. Once you click Save and Apply, the XY stage will move back to the last sample location.

4.6. Re-optimize periodically

The Auto Background described above will optimize the overlap of the IR and probe beams. This overlap can change over time, especially in environments where the temperature changes significantly e.g., due to air conditioning or other laboratory temperature changes. It is recommended to periodically run a full Auto Background (for example every few hours), but for a quicker re-optimization use the Re-Optimize button on the mIRage panel on the Spectra tab. The Re-Optimize function will move the XY stage to the background sample position, automatically adjust the overlap between the IR and probe beams, but **not** acquire a new background. It is recommended to use Re-Optimize before measuring any new sample or every hour or so when measuring the same sample over an extended period.

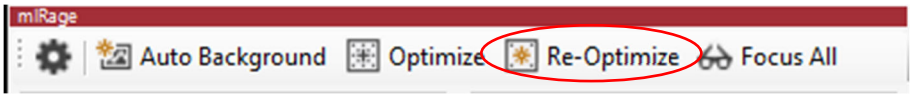


Figure 4-6. Re-Optimize: automated re-optimization of IR/probe beam overlap at the reference sample

Note that the Optimize button on this toolbar is a manual optimization process that is generally not needed because of the automatic optimize steps in the Auto Background and Re-Optimize functions.

4.7. Getting ready for a measurement

4.7.1. Opening a document

To open a new document, click on File/New/New IR Document or click on the Create new document icon on the Document window as shown below.

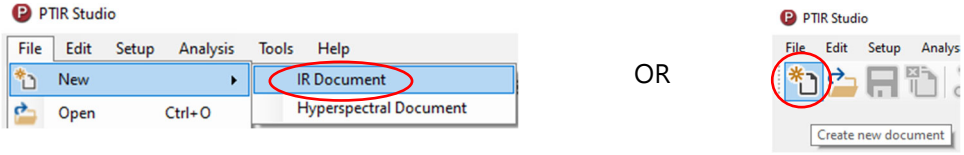
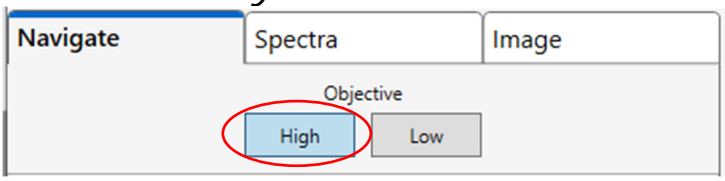


Figure 4-7. Opening a new document.

4.7.2. Objective selection



It is best to select the high mag Cassegrain objective C40X before starting O-PTIR measurements. Once you have located an area of interest with the low mag objective, switch to the 40X Cassegrain (High Mag) objective and focus on the sample before starting O-PTIR measurements.

4.7.3. Optimizing O-PTIR measurement settings

The basic approach for optimizing settings is shown in the Figure 4-8 below.

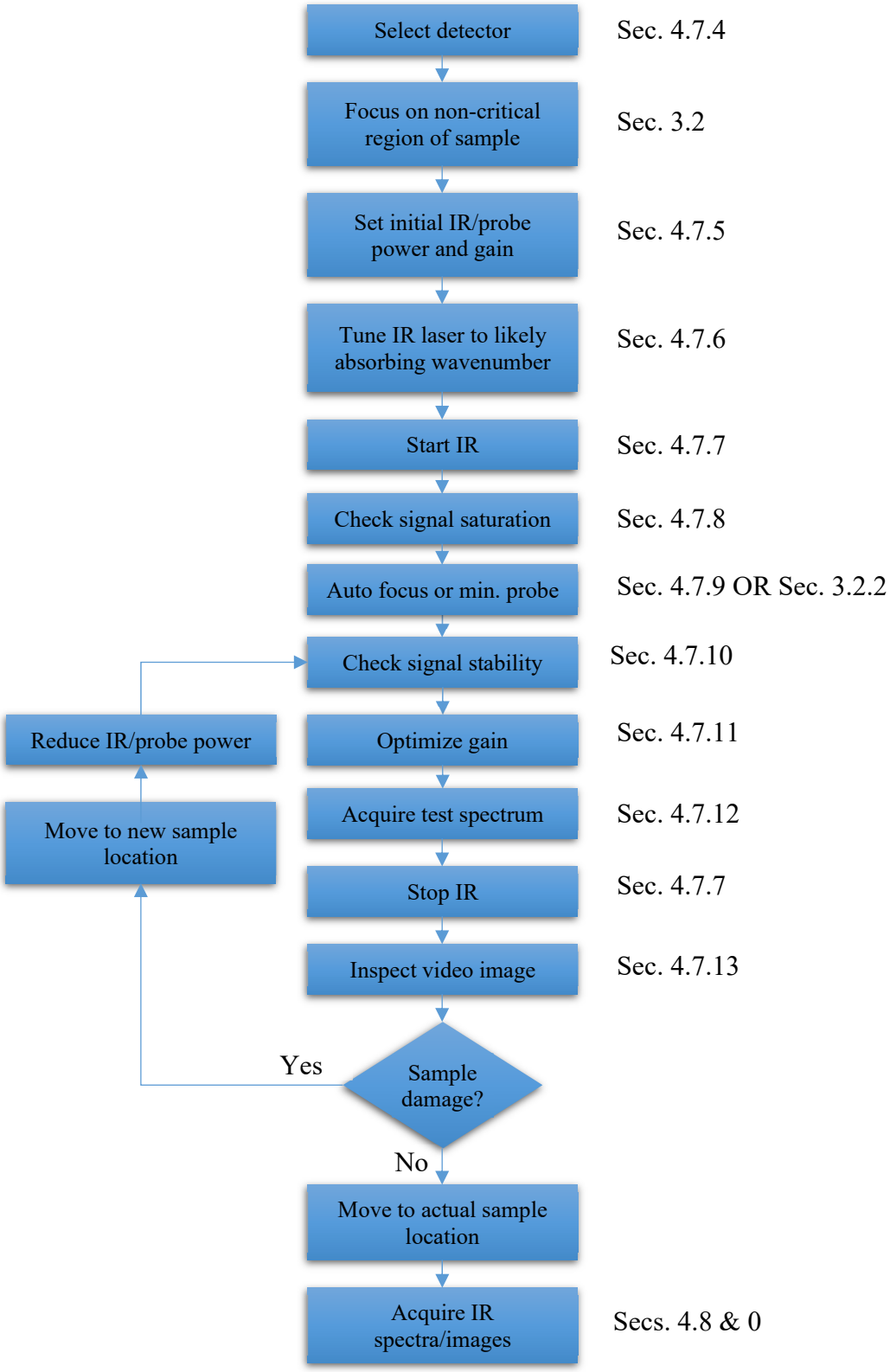


Figure 4-8. Flow chart for optimizing measurement settings

4.7.4. Detector selection

Select an appropriate detector for your measurement as shown in the figure below.

Detector	Use for	Notes
Standard	Most samples in air that are not easily photodamaged	
APD	Colored, dark, and/or delicate samples that may be damaged by higher intensity probe light	Max probe power is limited with APD to avoid detector damage
Transmission	Samples in liquid or other transparent samples with very low surface reflectivity	

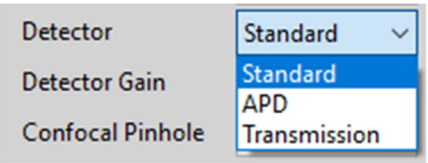


Figure 4-9. Detector selection

4.7.5. Setting initial parameters

Before acquiring IR spectra or images, it is important to select optimal measurement settings that optimize the signal-to-noise ratio for O-PTIR measurements and IR and probe beam power levels that do not damage the sample. Good starting values are shown in the figure below. Note that the IR and probe power level choices on your system may be slightly different, so choose the values closest to the ones shown below. If enabled, set the IR polarization to 0 degrees. IR and probe power can be adjusted to optimize signal to noise/minimize sample damage as described in the steps shown in Figure 4-8 and sections 4.7.8-4.7.13.

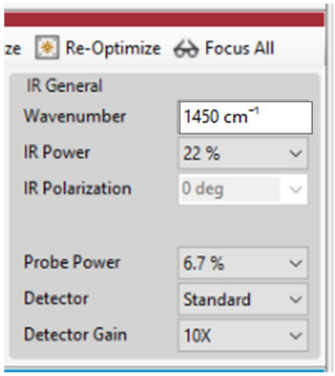


Figure 4-10. Good starting values for IR images and spectra.

4.7.6. Setting initial wavenumber

On the IR General panel, set the Wavenumber to a value corresponding to a likely strong absorption band in your sample. If you don't know strong absorption bands of your sample in advance, 1450 cm^{-1} is a good starting point which corresponds to a methyl group C-H bending excitation. If you know your sample has a strong carbonyl band, you may wish to start at 1725 cm^{-1}. **Note:** If your sample is very delicate or irreplaceable, you may wish to avoid strong absorption bands when performing the initial measurement optimization to avoid accidental sample damage during optimization.

4.7.7. Start/Stop IR

To turn on the IR and probe beams to start an O-PTIR measurement, click on Start IR. When the IR beam is on, an indicator is shown on the real time screen. IR: ON Probe: ON To turn off the IR beam, click Stop IR.

4.7.8. Check for signal saturation/adjust gain

On the Meter screen under the Spectra tab, check the DC signal level. For the standard detector, the DC signal level must be below 8V. It is best to leave some "headroom" for variations in sample reflectivity, so usually it's best if the DC is roughly between 4-6 V. Adjust the Gain and/or probe power to ensure the DC signal is not saturated.

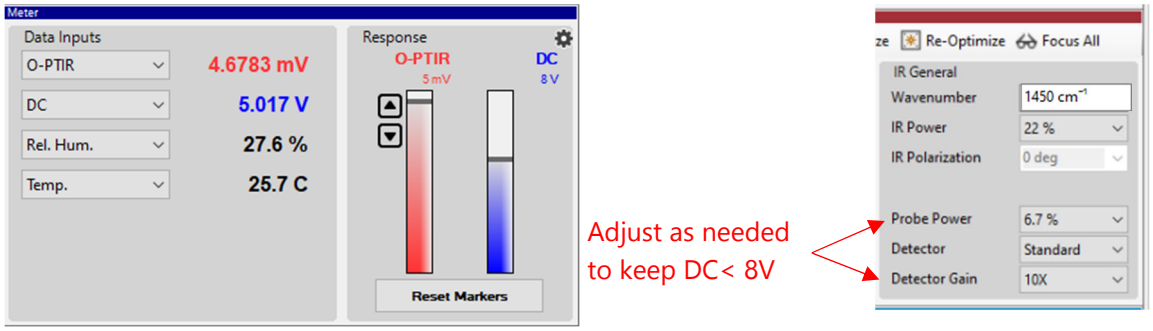


Figure 4-11. Check for saturation of DC signal and adjust probe power and detector gain as needed.

4.7.9. Autofocus to maximize O-PTIR signal

To maximize the O-PTIR signal at the current sample location, click on the Focus… button. The Focus… button will execute a sweep of the Cassegrain objective focus while monitoring the strength of the O-PTIR signal. If a sufficient peak is found, the system will automatically move to the focus position with the strongest signal. The Focus All button works best with samples with relatively smooth surfaces. Samples with lots of scattering features may require manual focusing using the focus controls on the Stage Panel. The O-PTIR autofocus can also be accessed through the Focus… button on the Stage panel which provides access to advanced controls.

Note: If the autofocus does not provide a good clear peak, leave the focus set to where the video image is sharpest.

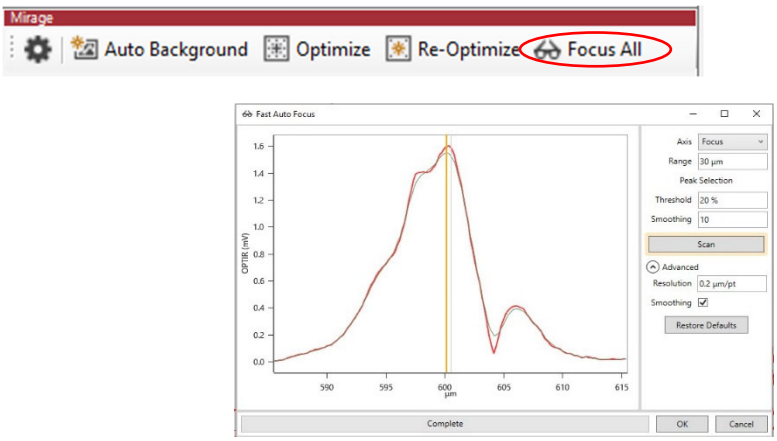


Figure 4-12. Optimizing the O-PTIR signal with autofocus.

4.7.10. Check signal stability

Also check that the O-PTIR and DC signals are stable in the MIRage response strip chart after turning on the IR beam with the Start IR button. This strip chart is available on the Spectra tab. Ignore the initial transient where the IR and probe beam are being turned on but review the response after a few seconds. If the DC and/or O-PTIR signal fluctuates strongly, this indicates the IR and/or probe power is too high. In this case, stop the IR beam, reduce the IR and/or probe power and try again. After the initial transient, the DC and probe power should appear stable as in the second chart in the figure below.

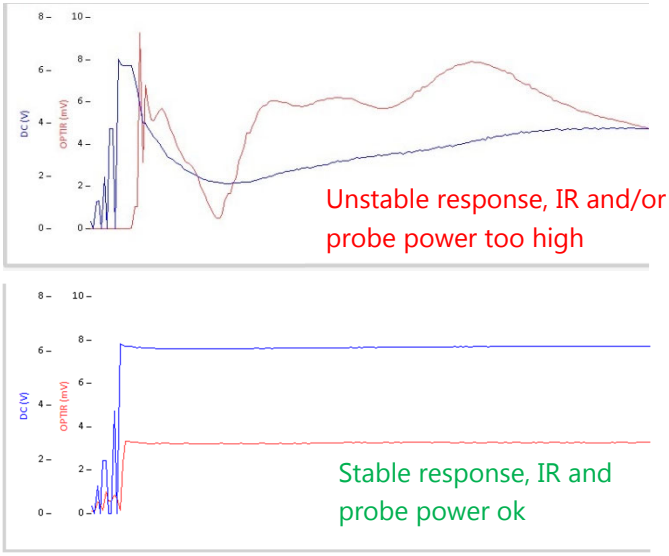


Figure 4-13. Confirming O-PTIR signal stability

If the signal is stable, but low, consider increasing the IR and/or probe power to maximize the signal level while maintaining a stable signal.

4.7.11. Turn up gain if needed

Once an appropriate probe power is selected, increase the detector gain if necessary to select the highest gain that does not saturate the detector. Ideally the DC signal will be between 4-6 V, but somewhat higher or lower is ok. Adjust the gain as described in Sec. 4.7.8.

4.7.12. Acquire test IR spectrum

To prepare to acquire spectra, select the Spectra tab and choose a Start/End wavenumber for the spectra and a number of Averages. The estimated time to acquire a spectrum is shown under the Spectra tab. When satisfied with the settings, click on Acquire Single to acquire a spectrum.

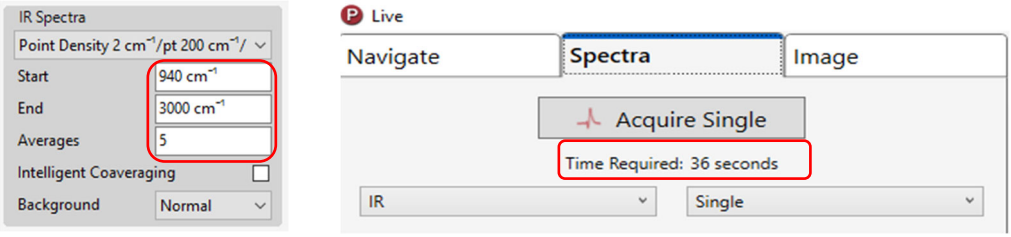


Figure 4-14. Acquiring a spectrum

4.7.13. Check for sample damage

Turn off the IR beam by clicking on the Stop IR button. Inspect the video image of the sample to ensure there is no photodamage. If you observe photodamage, turn down the IR and/or probe power and repeat steps 4.7.7-4.7.8. Note that damage from excess IR power tends to make larger damage spots (several microns across) due to the larger wavelength, whereas excess probe power usually results in smaller damage spots.

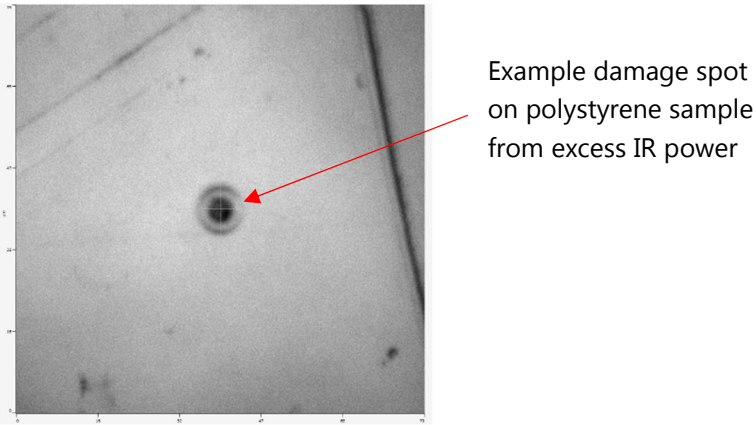


Figure 4-15 Inspecting sample for photodamage. This step is best done with the Zoom In button selected.

If there is no evidence of sample damage and the DC and O-PTIR signals are stable, you are ready to begin acquiring spectra and/or IR images of your sample. See section 4.8 for IR spectra and 4.11 for IR imaging.

4.8. Acquire IR spectra

This section details the steps for acquiring O-PTIR spectra with the mIRage. The same basic steps apply for acquiring Raman spectra. Details of Raman operation are outlined in Chapter 5.

4.8.1. Selecting an image for locating spectra

Once you have captured a video image to the document, the software will generally use the last captured image as a reference to let you select locations for IR spectra or images. If you want to manually choose a specific video image to use as your location reference, select the Camera channel and right click on the desired image, and the click on Send to Array… as shown below.

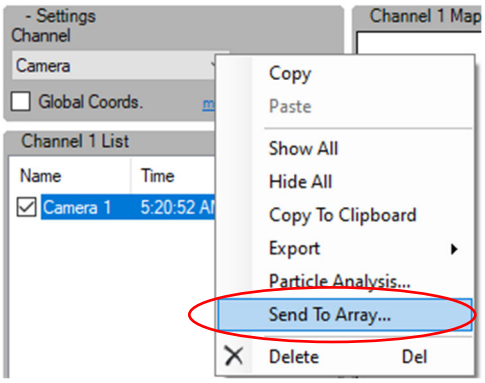


Figure 4-16. Sending a camera image to the Spectra tab for selecting measurement points

4.8.2. Spectral range and averaging

Set the spectral range (Start and End) parameters and the number of averages, as shown below. Note that increasing the Averages setting will increase the signal to noise ratio of the collected spectra by co-averaging the selected number of spectra at each location. The SNR will generally increase with the square root of the number of averages. For example, to increase the SNR by 3X, set the Averages to 9.

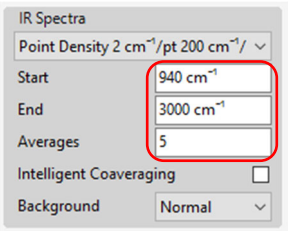


Figure 4-17. Setting the spectra range and number of co-averages for spectral acquisition

Intelligent Co-averaging

Intelligent Co-averaging is a special acquisition mode that uses statistical denoising techniques to separate signal from noise during the acquisition process. Specifically, this technique looks for features that are common between successive spectra while rejecting uncorrelated noise. See Section 7.1 in the Advanced Operations chapter for more details.

4.8.3. Single Point Spectra

To acquire a single spectrum manually, perform the following steps:

1) Select Single
2) Select the Navigate (bullseye) icon from the toolbar
3) Position the bullseye to select a measurement point
4) Adjust the spectral range and number of averages as desired.
5) Click the Acquire Single button to acquire a spectrum

The spectrum will be captured to the document and associated with the measured location.

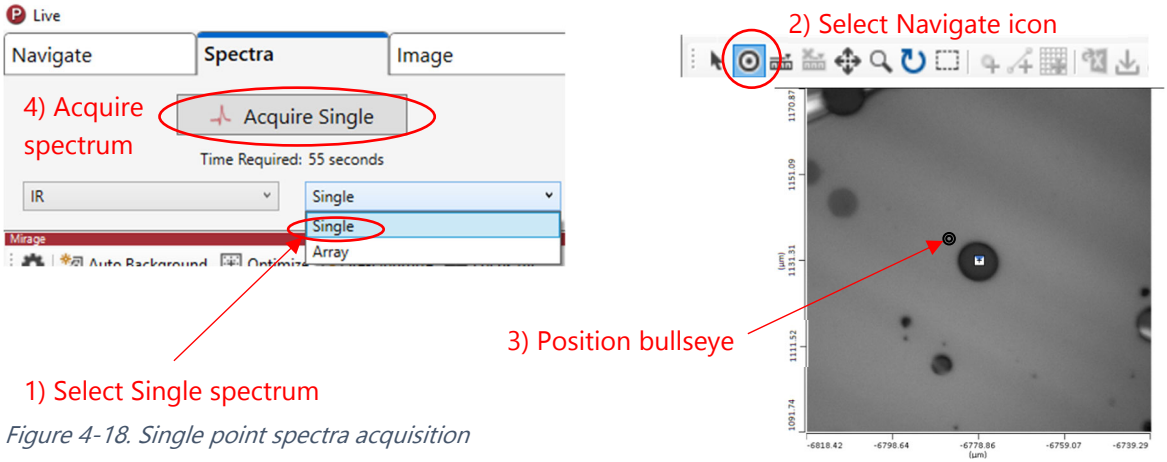


Figure 4-18. Single point spectra acquisition

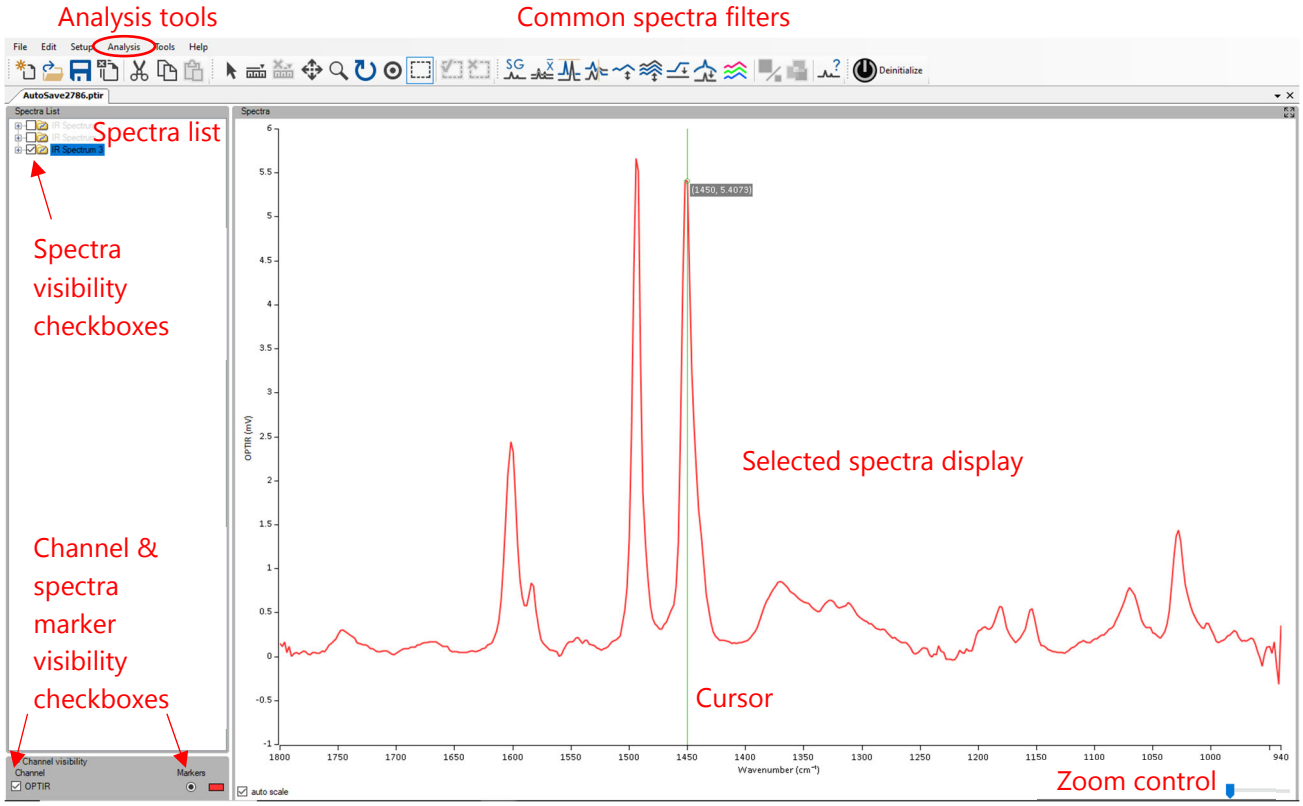
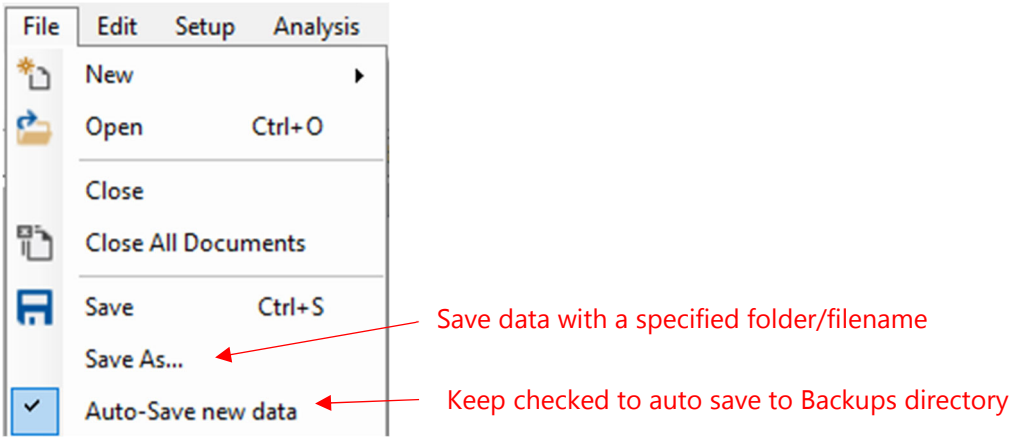


Figure 4-19. Spectrum captured into the Document window.

4.8.4. Saving data

By default, data is automatically saved to the C:\ProgramData\Photothermal\PTIR Studio\Backup using a filename AutosaveNNNN.ptir, where NNNN is an automatically generated sequential number. If you have more than 10,000 unsaved data files, older Autosave files will be overwritten.



To save acquired data to a filename and location of your choice, click on File/Save As..., choose an appropriate save location, and filename. **PSC strongly recommends saving data to a location that is regularly backed up by your IT department.**

4.8.5. Spectra Configuration Recipes

PTIR Studio supports two different recipes for acquiring Spectra, one intended to maximize the SNR of acquired and another intended to minimize spectrum acquisition time. The key differences between the two recipes are the sweep speed of the QCL laser and the resulting integration time constant used by the lock-in amplifier (calculated automatically). The High SNR recipe is generally recommended when trying to maximize SNR and spectral resolution. The High Speed recipe is recommended when performing large array measurements where it is desirable to reduce the measurement time. Some systems may be enabled with other recipes, for example with coarser spectral resolution which can reduce the sensitivity to humidity in unpurged systems and reduce impact of water vapor IR absorption lines. Contact PSC applications support if you need help choosing the best recipe or creating additional recipes.

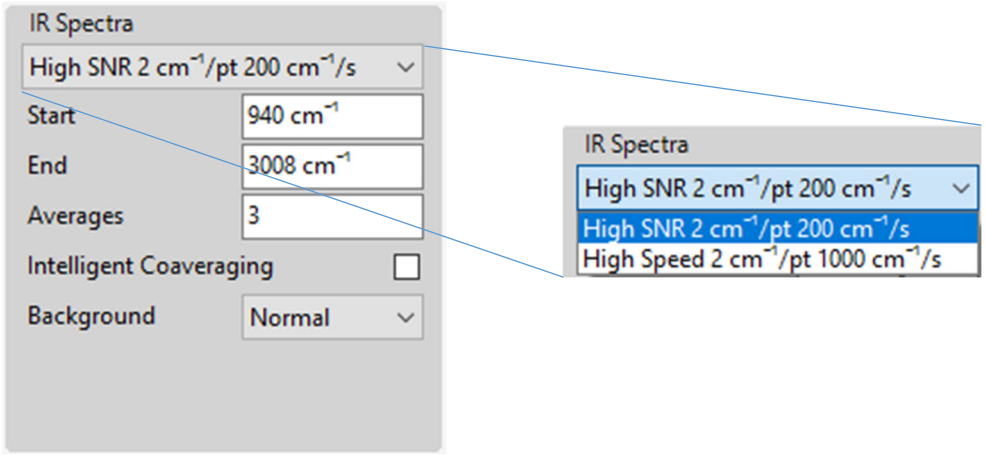


Figure 4-20. Selecting spectra configuration recipes

IMPORTANT: If you change the spectra configuration recipe, you must take a new background using the newly selected configuration recipe. If you attempt to take a spectrum using a configuration recipe that does not match the current background, the software will issue a warning.

4.8.6. Basic spectral analysis

Once a spectrum is acquired, it appears in the Document window (left monitor). The spectra can then be filtered, analyzed, and displayed as desired. Detailed documentation of the spectral analysis tools is described separately in the mIRage Software Manual, but Figure 4-19 and Figure 4-21 show a brief overview of available functionality.

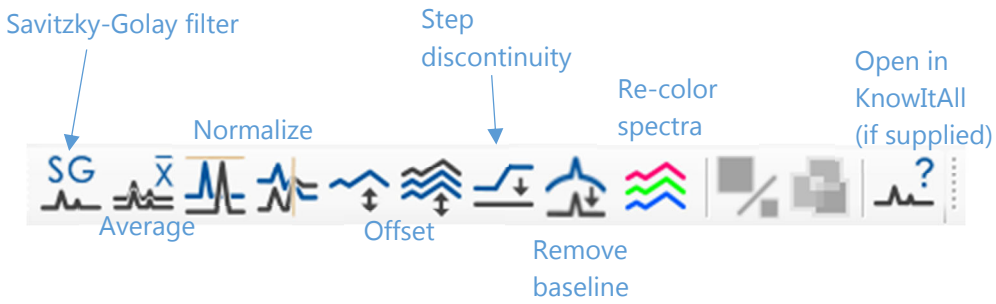
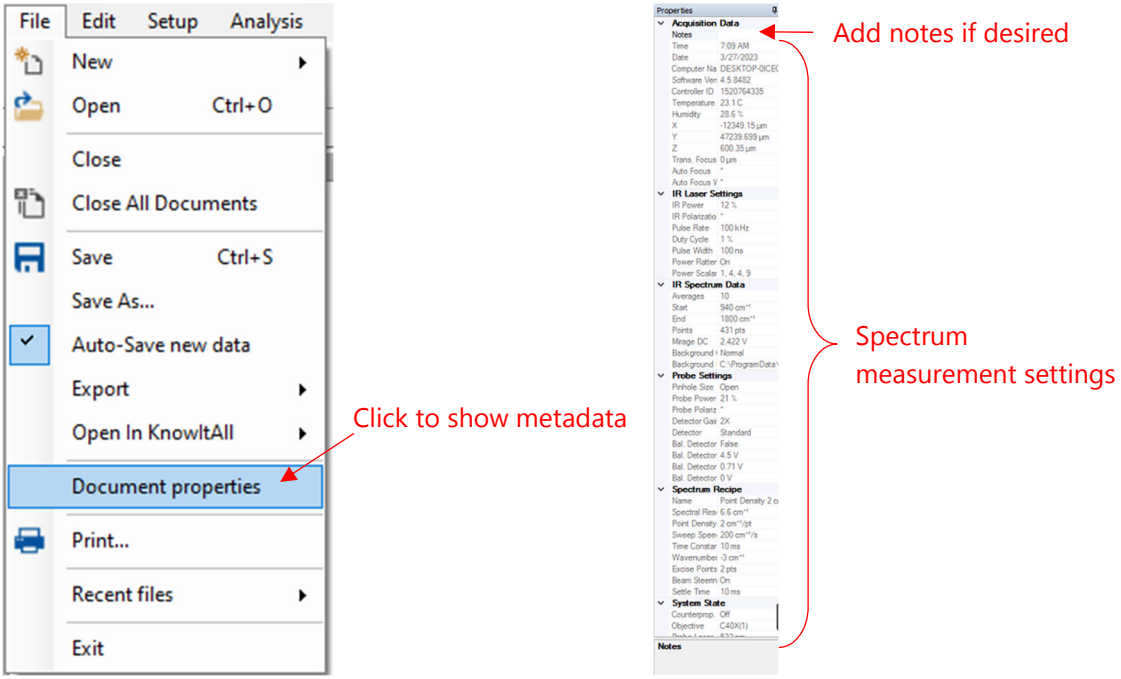


Figure 4-21. Commonly used spectral filter and display tools available on the Document window toolbar.

4.8.7. Spectral metadata

Measurement settings for each spectrum are automatically saved with each acquisition. To view the spectral metadata, select a spectrum and then go to File/Document properties. A Properties box will open to the right of the displayed spectrum containing all settings used. There is also a Notes field where additional sample/measurement details can be entered manually.



4.8.8. Automated spectral array collection

There are several ways for the user to collect a series of automated spectra. Point selection, line arrays and full hyperspectral images can be obtained. To enable spectral arrays, change the system from single spectra mode to array mode in the Spectra Tab.

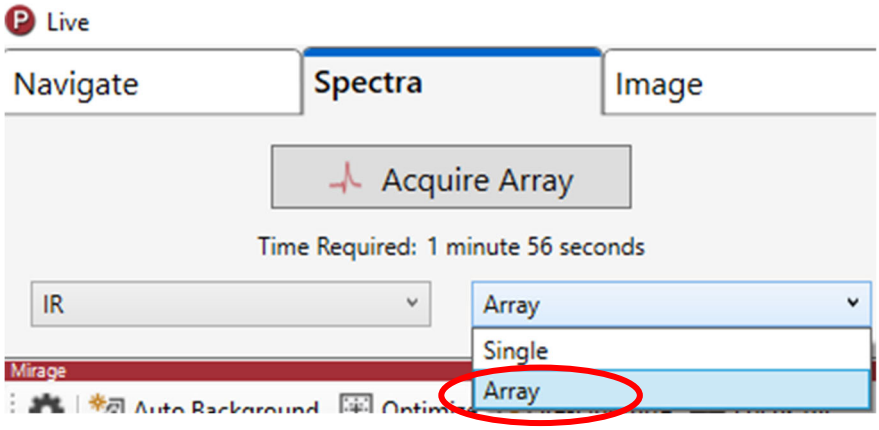


Figure 4-22. Enabling spectral array collection.

The optical view shown in this tab will be the last image captured while in the navigate tab or an image manually selected using the Send to Array… right click.

Spectra can be selected in one of three ways: (1) Point arrays, (2) Line arrays, (3) Grid arrays as shown in the figure below.

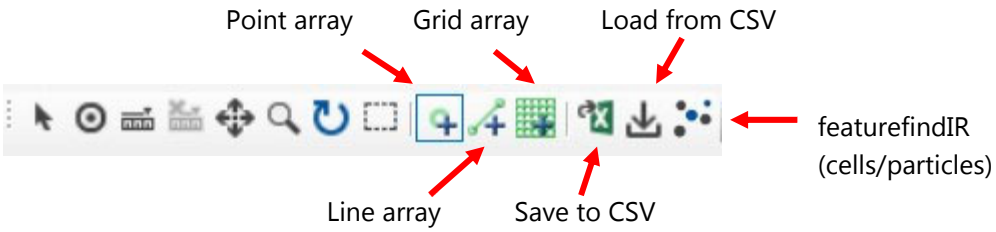


Figure 4-23 Spectrum array selection tools.

The next several figures show examples of each of these array modes.

4.8.9. Point arrays

A point array is an array of spectra at arbitrary manually selected points. To create a point array, select the point array icon and then click on desired locations in the optical image. The XY coordinates of each point selected appear in the left panel.

Note, there are two check boxes on the bottom left.

- The first is for an autofocus before each point in the array, this is useful for rough samples.
- The second option will turn off the IR laser when the array is complete

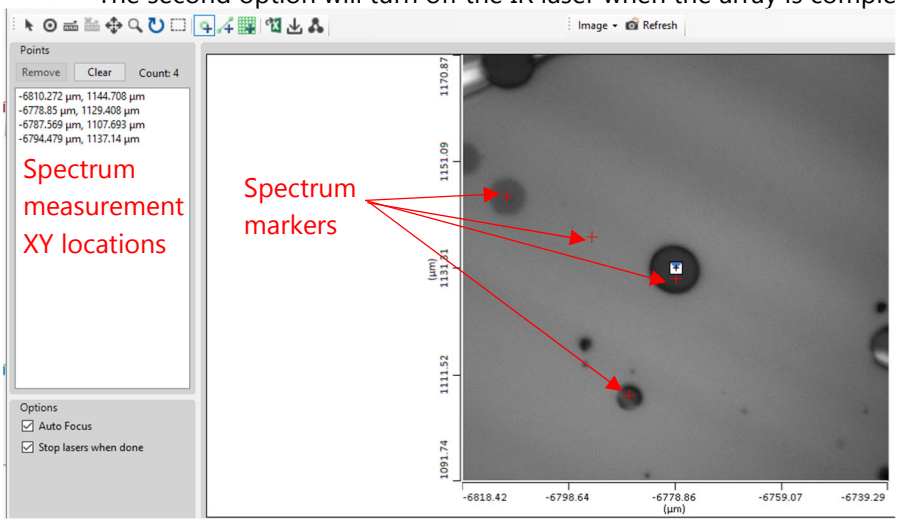


Figure 4-24. Point spectral array.

Click on Acquire Array to begin data collection.

4.8.10. Line arrays

To configure a line array, select the line array icon and draw a line across the region to measure. The left-hand panel now shows the parameters of the line drawn, which may be adjusted further by the user if desired. For example, the spectrum spacing, length of the line, and/or number of points can be adjusted.

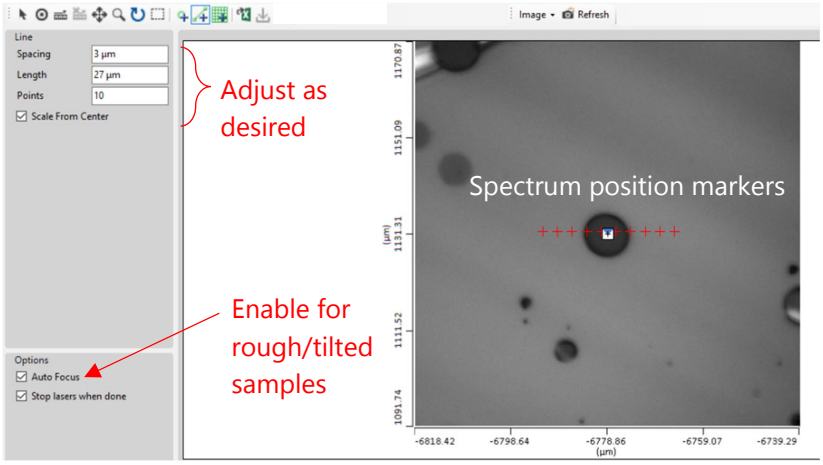


Figure 4-25 Settings for a line spectral array

Click on Acquire Array to begin data collection.

4.8.11. Grid Arrays

For a grid array, select the grid array tool, click anywhere on the optical image and drag a box over the area of interest. The panel on the left side shows the grid parameters which can be modified by the user.

Note the check boxes on the bottom left.

- For a simple grid array, leave the hyperspectral check box unchecked. Hyperspectral arrays are described in the next section.
- The use of autofocus is generally recommended for the collection of larger grid arrays, especially on rough or tilted samples.
- If using autofocus, if possible, pick a wavenumber that is a common absorption band to all regions within the array. For organic/polymeric materials, 1450 cm^{-1} is a good choice. For biological materials, 1660 or 1550 cm^{-1} (Amide I/II bands) usually produce the strongest signals. Set the wavenumber for the autofocus in the IR General/Wavenumber control on the Spectra tab.

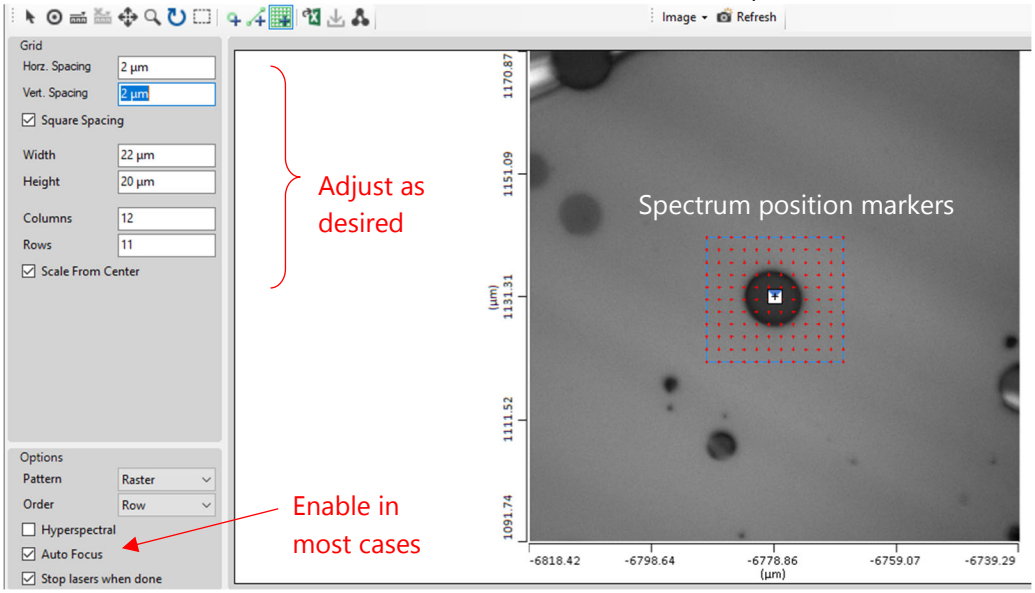


Figure 4-26. Grid array spectra settings

Click on Acquire Array to begin data collection.

4.8.12. Hyperspectral Arrays

Hyperspectral arrays are a special form of grid arrays that create a different document type that is better configured for acquiring/displaying large numbers of spectra. The setup is the same as for grid arrays, except that the Hyperspectral checkbox must be clicked.

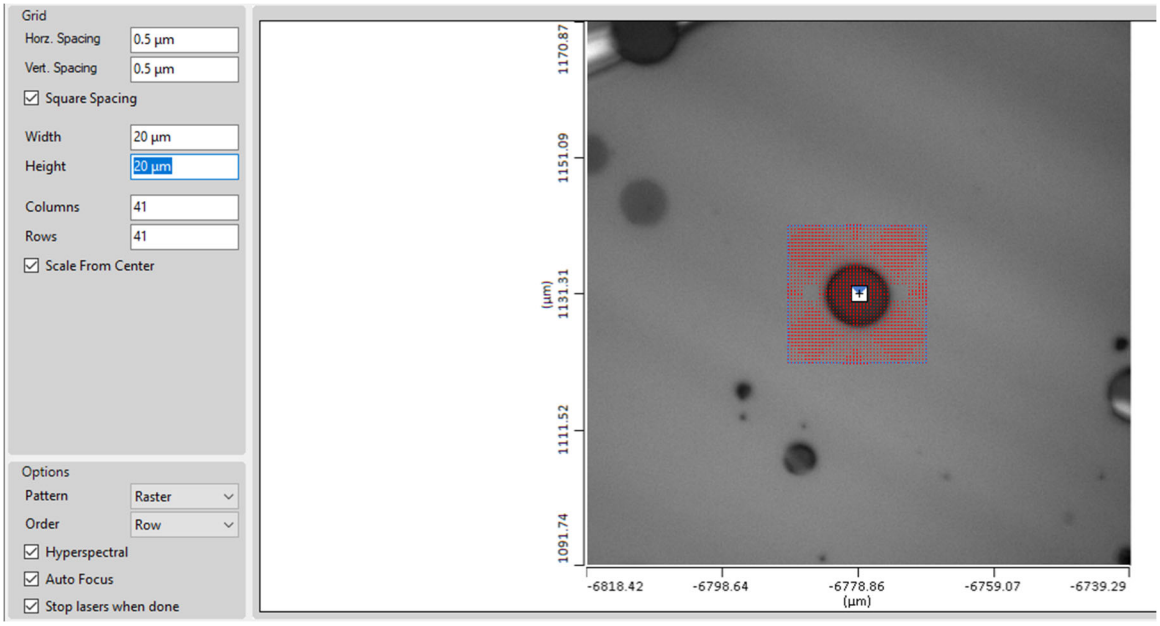


Figure 4-27. Hyperspectral array collection

Pay special attention to the time estimate for hyperspectral arrays as it is possible to configure an array that would take much longer than desired to complete. If your initial configuration will take longer than desired, reduce the number of points and/or increase the spacing between points on the Grid Array settings and/or reduce the number of averages in the Spectra tab.

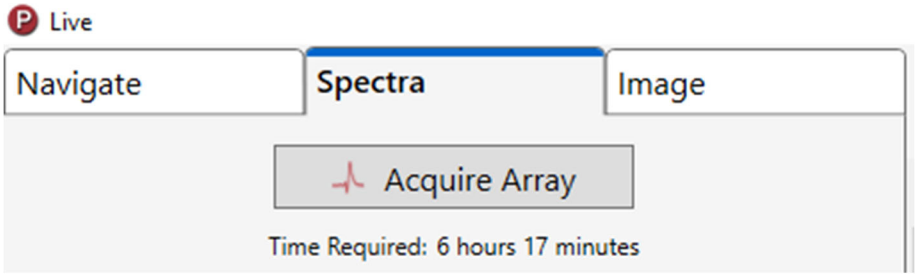
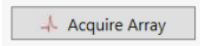


Figure 4-28 Check the time required for a hyperspectral array before starting and reconfigure settings if desired.

Click on 

to begin data collection.

Hyperspectral data analysis

See the mIRage Software Manual Chapter 4 for details on hyperspectral data analysis available within PTIR Studio. Third party software packages can be useful for analyzing large datasets from hyperspectral arrays. Two packages to consider are Quasar (https://quasar.codes/) and Cytospec (https://www.cytospec.com/).

4.9. Automated IR Spectra with featurefindIR

featurefindIR™ is a tool that can be used to **automatically identify the positions and sizes of cells/particles** in an optical microscope image and then save those positions for automated spectral acquisition. To use featurefindIR, use the following steps:

1) Select the Navigate tab, focus on a sample, and navigate to a region of interest.
2) Adjust the camera illumination/exposure settings as necessary to get a clear optical image with low noise. (Longer exposure times may be helpful as long as the image doesn't saturate.)
3) Click on the featurefindIR icon at the top of the Live Window.
4) If the optical image has uneven illumination, click on Flatfield Correction.
5) Adjust the threshold cursors to highlight particles as desired. Move the left cursor to select particles that are darker than the background (histogram peak) and the right cursor for particles that are brighter than the background.
6) Adjust the threshold cursors until particles are highlighted in gold, while the background is not.
7) Adjust the Min and Max particle size settings as desired.
8) Click Calculate to calculate the locations and sizes of identified particles.
9) Click on Export to CSV… if you want to save the particle table to disk.
10) Click on Accept to use the identified particle locations to Array tool on the Spectra tab. This imports the particle positions to the Spectra tab for automated acquisition.
11) Review the spectrum array table on the Spectra tab to deselect any locations you want to omit and if desired, manually add any additional points.
12) Review spectra acquisition parameters and the estimated time required.
13) Click Acquire to start automated acquisition of spectra at each point in the array table.

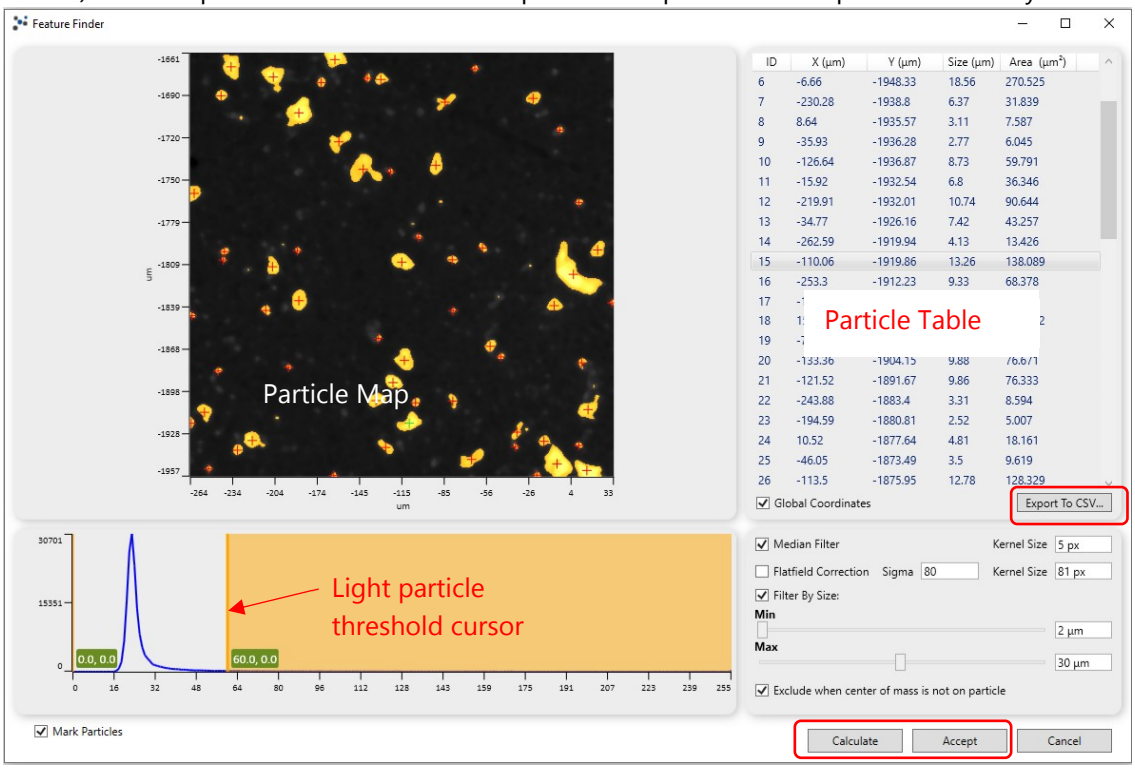


Figure 4-29. featurefindIR interface

4.9.1. Selecting dark or light particles

featurefindIR has two cursors, one for selecting particles that are darker than the background and one for selecting particles that are lighter than the background. Either or both can be used to select particles of interest.

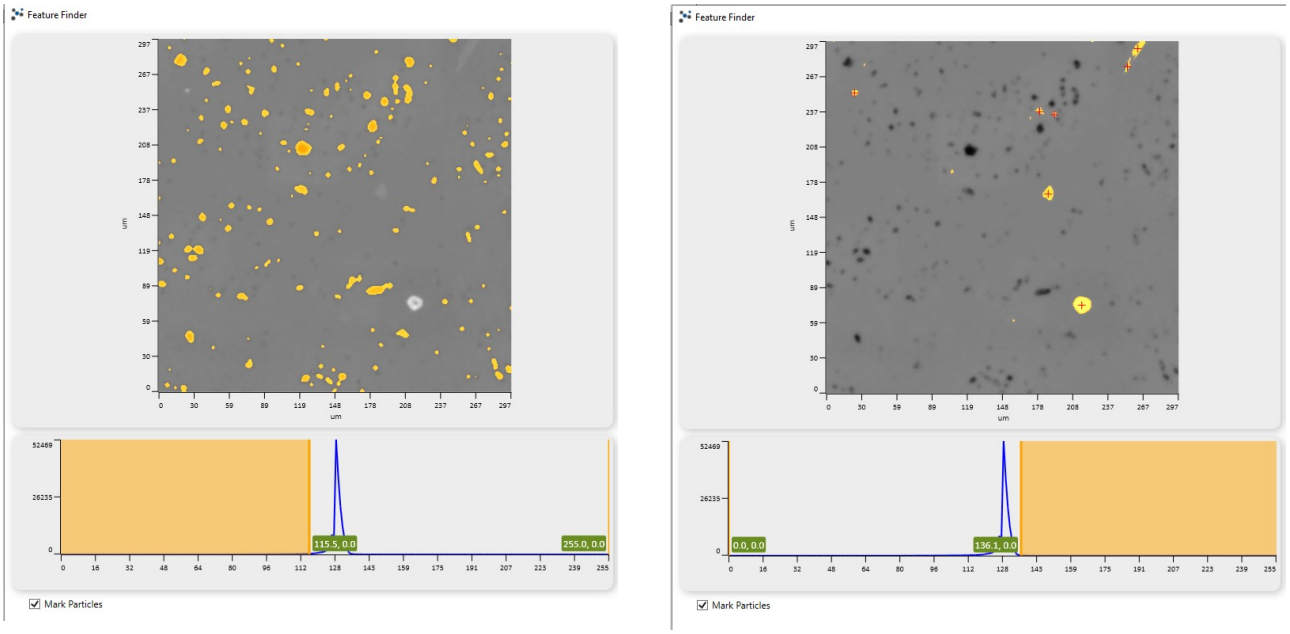


Figure 4-30. featurefindIR, cursor positions for dark or light particle

4.9.1. Adding/deleting measurement points

To delete a particle from the spectral array, click on the particle crosshair in the featurefindIR image window. The selected particle will highlight in the particle table at right. Right click and select Delete to remove the particle from the spectral array.

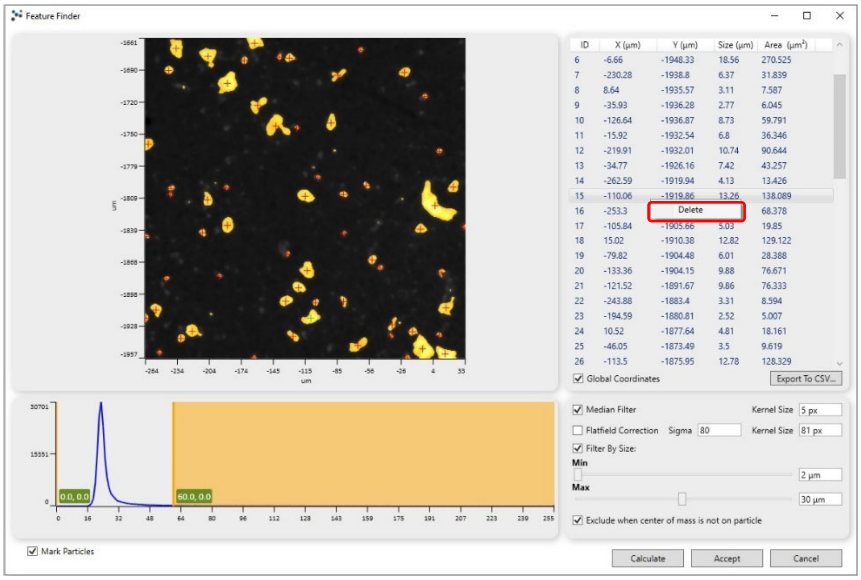


Figure 4-31 Deleting particles from a featurefindIR array.

To add a spectrum measurement point, right click on any point in the featurefindIR image and select Add Point Here as shown below.

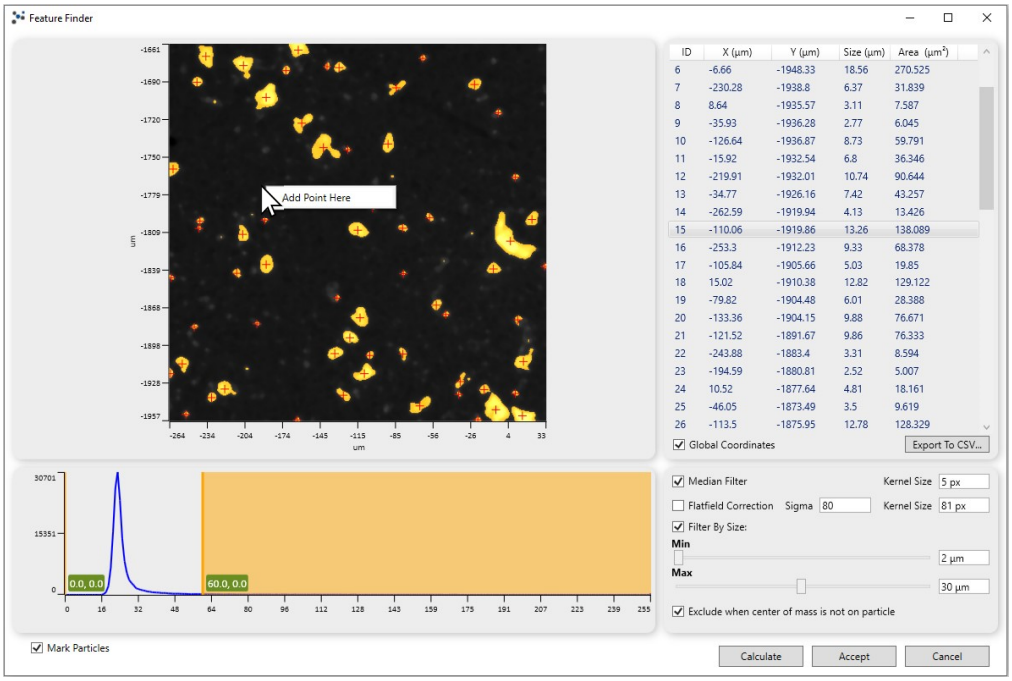


Figure 4-32. Adding a point to featurefindIR spectra array

4.9.2. Spectral array map

Once the Accept button is selected, the coordinates for each particle is automatically loaded into the spectra array interface as shown below.

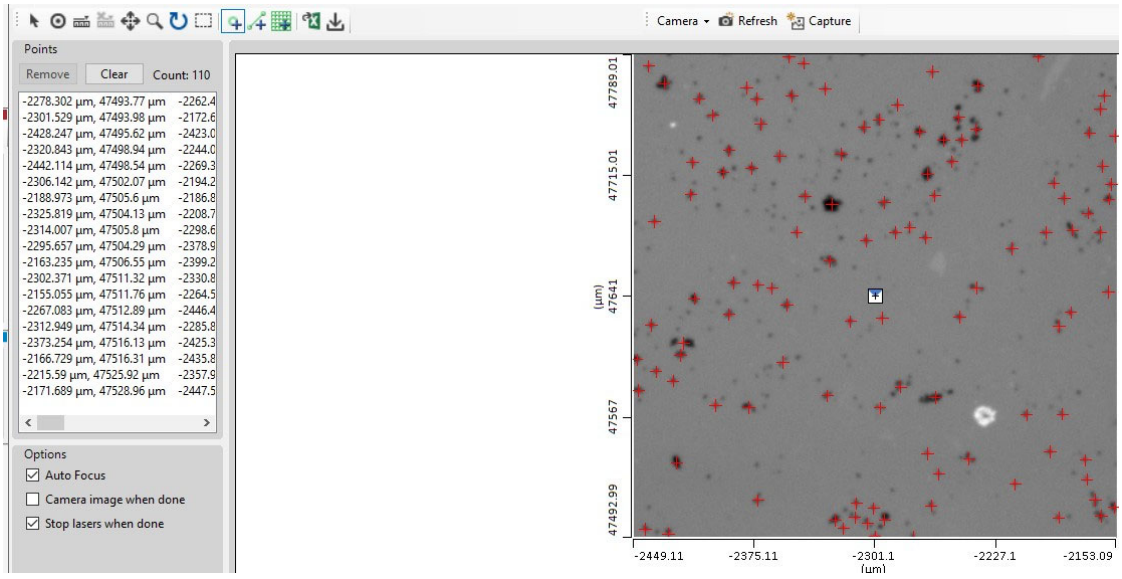


Figure 4-33. featurefindIR particle map loaded as a spectral array on the Spectra tab.

4.9.3. Calibrating objective offsets

If the featurefindIR image was acquired using a different objective than the measurement objective, it is necessary to calibrate the offsets between the two objectives. This will occur, for example, if you select a 10X objective for the featurefindIR image, yet the system will measure with the 40X Cassegrain objective in co-propagating mode. In this case, the system will step through a process to measure and update the offsets between the objectives to ensure optimal registration between the featurefindIR particle map and the measurement points. Here are the steps for objective offset calibration:

1) After clicking Acquire Array, the system will prompt that objective offset calibration will be performed as shown below. Click on Calibrate to continue.

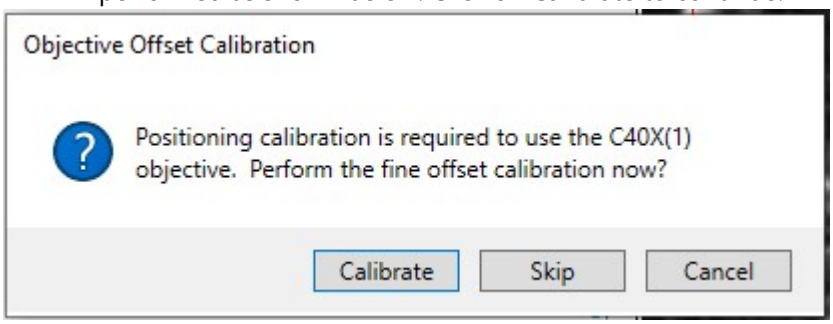


Figure 4-34. Objective Offset Calibration Prompt

2) The system will move to a saved point on a reference sample with a particle or other small feature roughly centered in the field of view of the first objective, e.g. the 10X objective. Use the arrow keys to focus and carefully center the feature on the crosshairs indicated as shown in the figure below. Once centered, click Next.
3) The system will then move to the measurement objective, typically a higher power objective, for example the 40X Cassegrain objective.
4) Repeat the process of focusing and centering the feature in the high mag view.
5) Click on Accept to complete the calibration.
6) The spectral array will now begin using the just calibrated offsets between the two objectives.

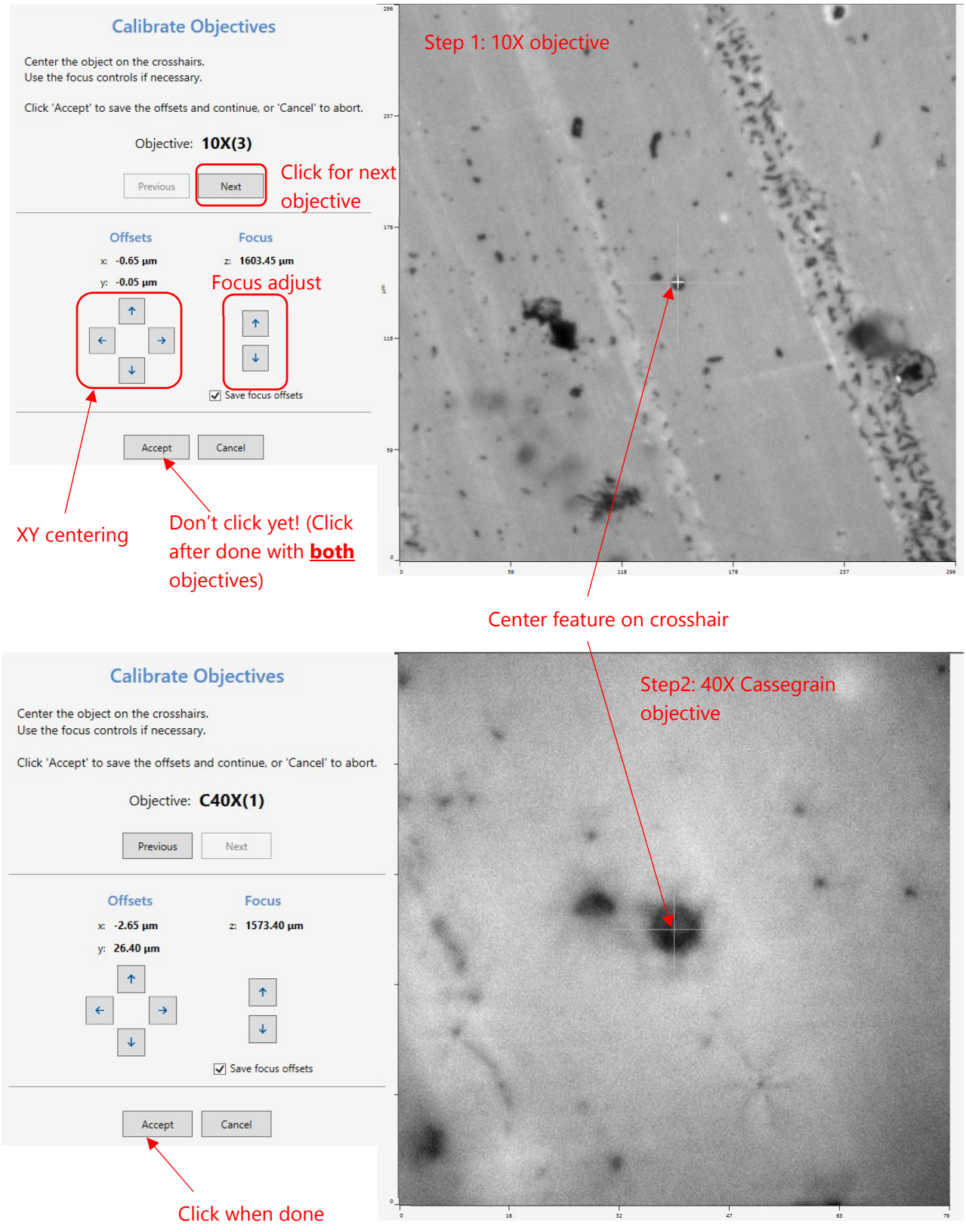


Figure 4-35. Calibrating offsets between objectives before spectral array

At completion of the array the particle array map will be captured to the document along with spectra from each point in the array table.

4.9.4. Particle Table

After completion of the spectral array the featurefindIR particle summary can also be accessed in the spectral document. To view the summary, click on View Particle Info at the bottom of the spectra list as shown below.

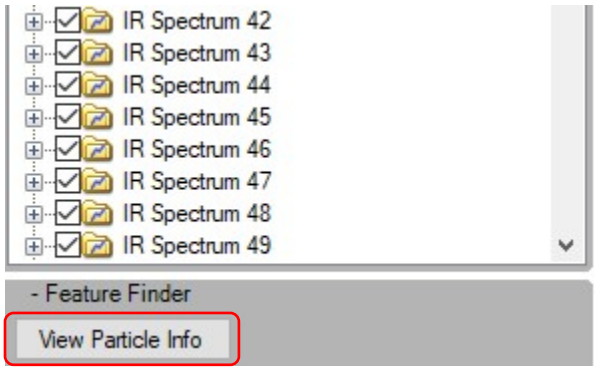


Figure 4-36. View Particle Info

Clicking on the View Particle Info button will open the Particle Info summary as shown below. This table lists the positions and sizes of each particle where an automated spectrum was acquired. This table can be exported by clicking on the Export to CSV... button.

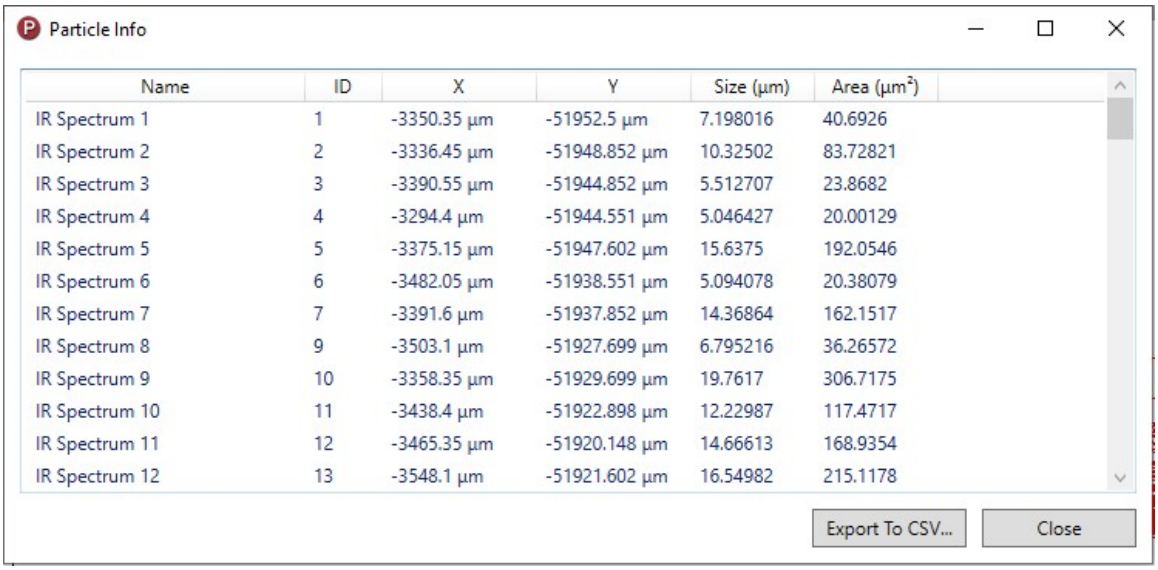


Figure 4-37. featurefindIR Particle Info Summary

4.9.5. Advanced featurefindIR Options

Global vs Image coordinates

The particle table can either display Global Coordinates (i.e. XY stage locations) or Image Coordinates relative to the bottom left of the image. Global Coordinates are generally recommended as it makes it easier to navigate back to a specific particle location for later review.

Excluded When Center of Mass Not on Particle

featurefindIR calculates a spectrum measurement location based on the center of mass of the particle. For some complex particle shapes or aggregates of particles, the calculated center of mass may be off the particle. When this checkbox is selected, featurefindIR will not include such locations in the spectral array.

Note: featurefindIR capabilities are being expanded. Contact PSC if you see additional capabilities that are not documented here.

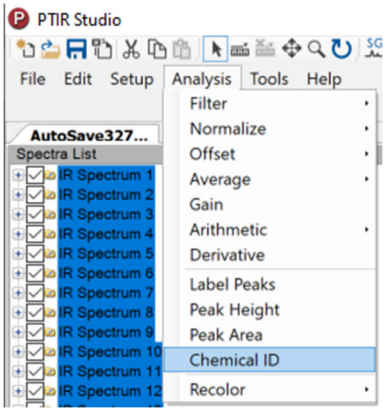
4.10. Chemical ID

4.10.1. Chemical ID introduction

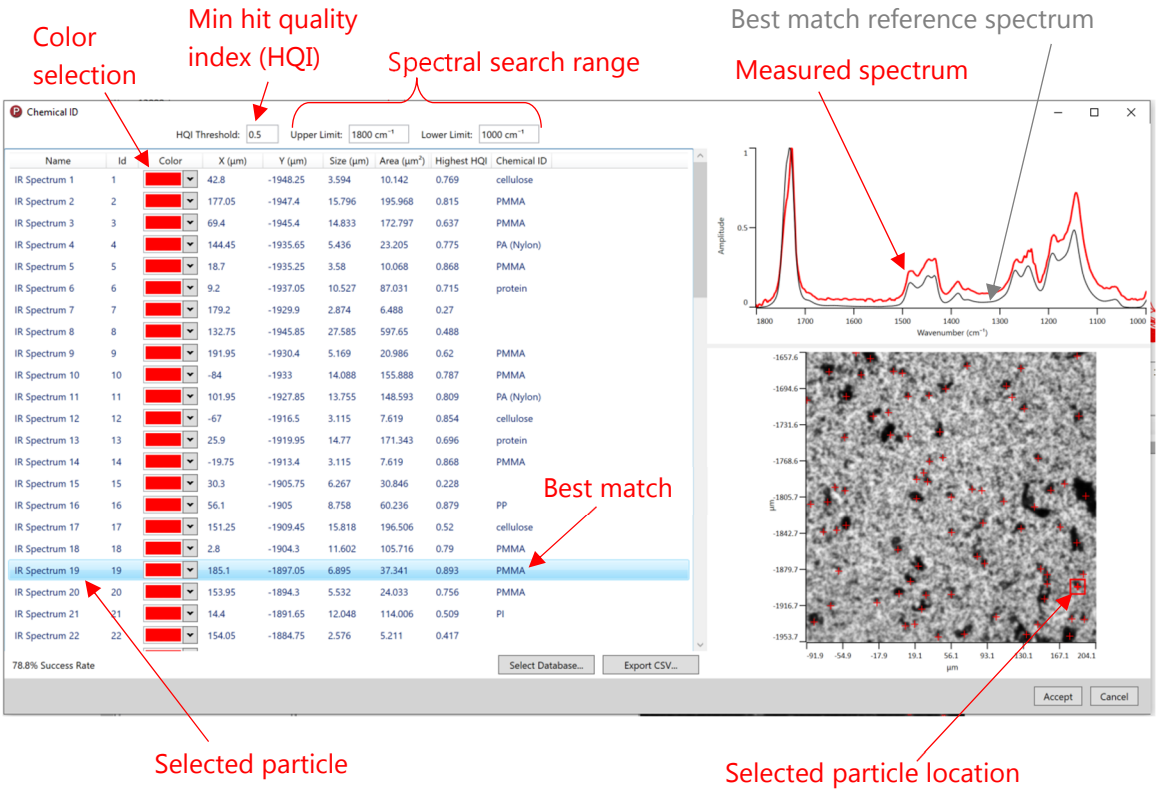
Chemical ID is an optional software capability available from PSC via separate license that provides the ability to search acquired spectra against one or more databases of reference spectra to identify potential spectral matches. The current Chemical ID search capabilities is specifically targeted at microplastic particle identification and includes high quality reference spectra of 18 of the most common polymers as well as spectra from some common non-plastic interferents like cellulose, protein, bone, seashell, and sand. Contact your sales associate if you are interested in adding this capability to your system.

4.10.2. Using Chemical ID

To use Chemical ID, select one or more spectra and then click Analysis/Chemical ID as shown below.



After execution, a Chemical ID summary table will appear as shown below. Adjust the spectral search range and the minimum hit quality index (HQI Threshold) as desired. The results will automatically update with any changes to these parameters.



4.10.3. Recoloring spectra by material type

To recolor any spectrum/particle marker in the Chemical ID summary table, click on the Color drop-down menu for a specific spectrum as shown below and then click on any desired color. To apply the same color to all spectra/spectra markers of the same chemical type, right click on the recolored spectrum and select "Apply Color to Chemical ID". Once complete adjusting colors as desired, click on the Accept button. Clicking on Accept will recolor the markers and spectra in the document file to match your choices. Note it will also update a system configuration file to use the same colors for the same chemical IDs in the future. E.g. if you select blue for PMMA, the Chemical ID tool will assign all PMMA spectra/markers to be colored blue the next time you run Chemical ID.

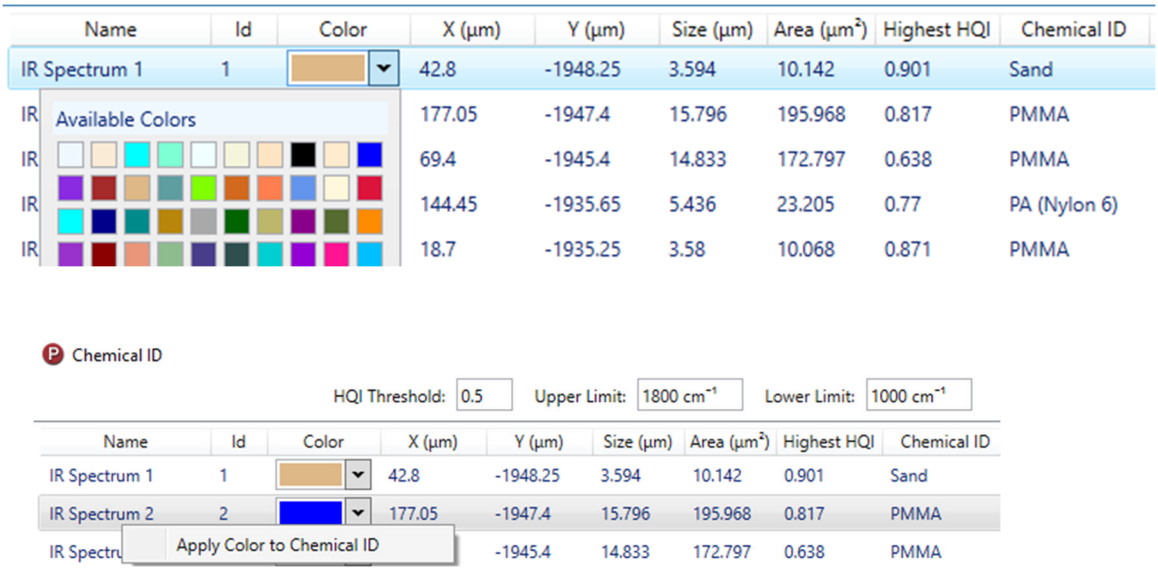


Figure 4-38. Recoloring a single spectrum (top) and right click to apply the same color to all spectra with the same chemical ID (bottom)

Name	Id	Color	X (µm)	Y (µm)	Size (µm)	Area (µm²)	Highest HQI	Chemical ID
IR Spectrum 1	1		42.8	-1948.25	3.594	10.142	0.901	Sand
IR Spectrum 2	2		177.05	-1947.4	15.796	195.968	0.817	PMMA
IR Spectrum 3	3		69.4	-1945.4	14.833	172.797	0.638	PMMA
IR Spectrum 4	4		144.45	-1935.65	5.436	23.205	0.77	PA (Nylon 6)
IR Spectrum 5	5		18.7	-1935.25	3.58	10.068	0.871	PMMA
IR Spectrum 6	6		9.2	-1937.05	10.527	87.031	0.902	PA (Nylon 6)
IR Spectrum 7	7		179.2	-1929.9	2.874	6.488	0.309	
IR Spectrum 8	8		132.75	-1945.85	27.585	597.65	0.483	
IR Spectrum 9	9		191.95	-1930.4	5.169	20.986	0.622	PMMA
IR Spectrum 10	10		-84	-1933	14.088	155.888	0.781	PMMA
IR Spectrum 11	11		101.95	-1927.85	13.755	148.593	0.841	PA (Nylon 6)
IR Spectrum 12	12		-67	-1916.5	3.115	7.619	0.937	Cellulose
IR Spectrum 13	13		25.9	-1919.95	14.77	171.343	0.72	PA (Nylon 6)
IR Spectrum 14	14		-19.75	-1913.4	3.115	7.619	0.876	PMMA
IR Spectrum 15	15		30.3	-1905.75	6.267	30.846	0.337	
IR Spectrum 16	16		56.1	-1905	8.758	60.236	0.858	PP
IR Spectrum 17	17		151.25	-1909.45	15.818	196.506	0.704	Sand
IR Spectrum 18	18		2.8	-1904.3	11.602	105.716	0.79	PMMA
IR Spectrum 19	19		185.1	-1897.05	6.895	37.341	0.897	PMMA
IR Spectrum 20	20		153.95	-1894.3	5.532	24.033	0.76	PMMA
IR Spectrum 21	21		14.4	-1891.65	12.048	114.006	0.599	PI

Figure 4-39 Chemical ID summary after recoloring spectra by Chemical ID

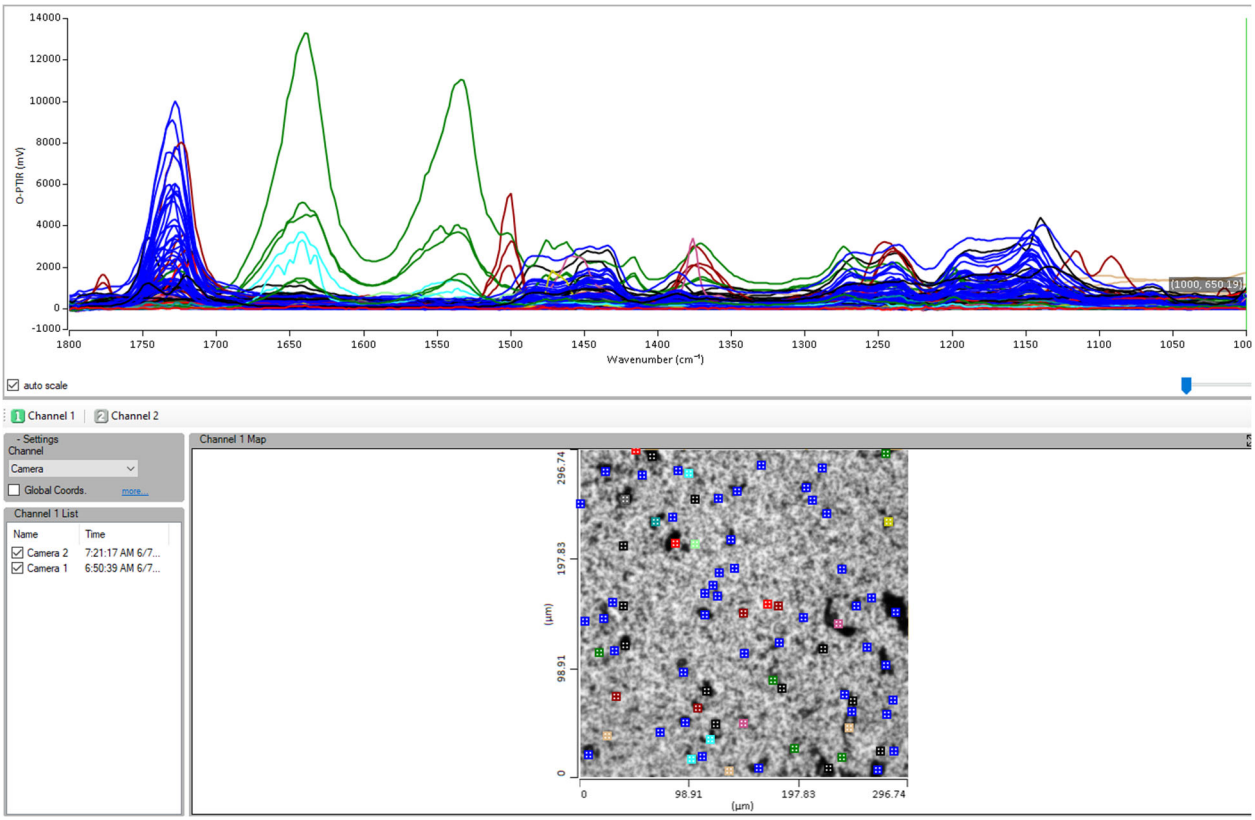


Figure 4-40 Spectra and markers in the document recolored to match selection in Chemical ID tool

4.10.4. Adding spectra to the Chemical ID database

User acquired spectra can be added to the Chemical ID database using the steps described below. Note that the PTIR Studio installer installs the default spectral database in this location:

C:\ProgramData\Photothermal\PTIR Studio\database\reference_spectra.xml

To add user acquired spectra to the Chemical ID database, follow these steps:

1) Open a PTIR Studio file with a spectrum you would like to add to the Chemical ID database.
2) Click to highlight the spectrum you would like to add to the database and then right click and select Add to Database as shown below.
3) Add any metadata you would like to add about the material measured for the reference spectrum.
4) When done, click Add to Database.
5) The added spectrum will be available for the next and all future uses of the Chemical ID tool.

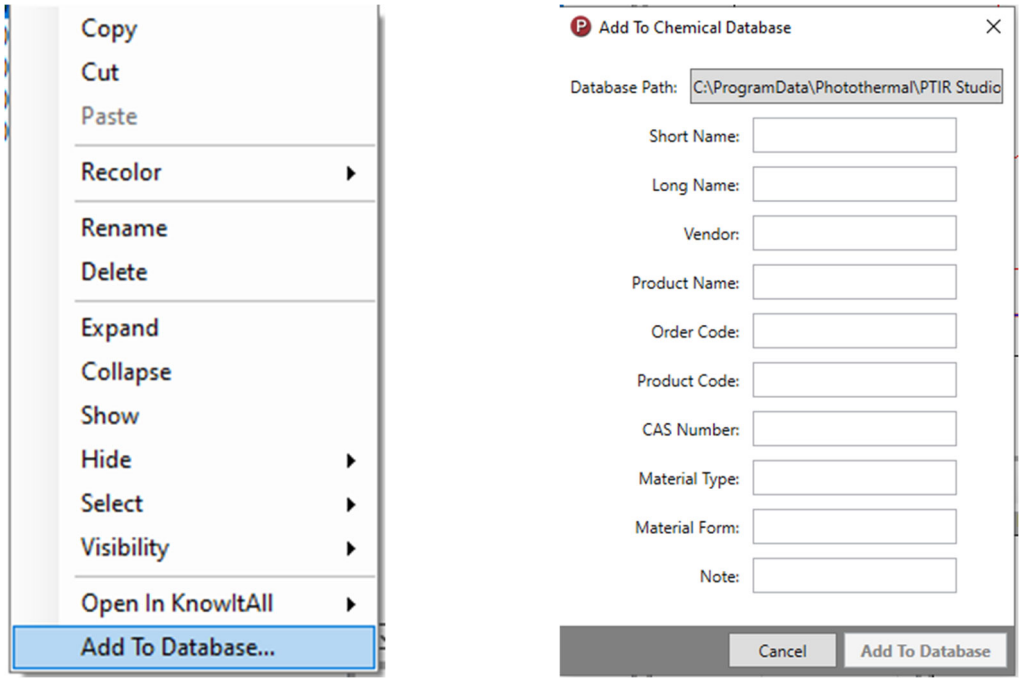
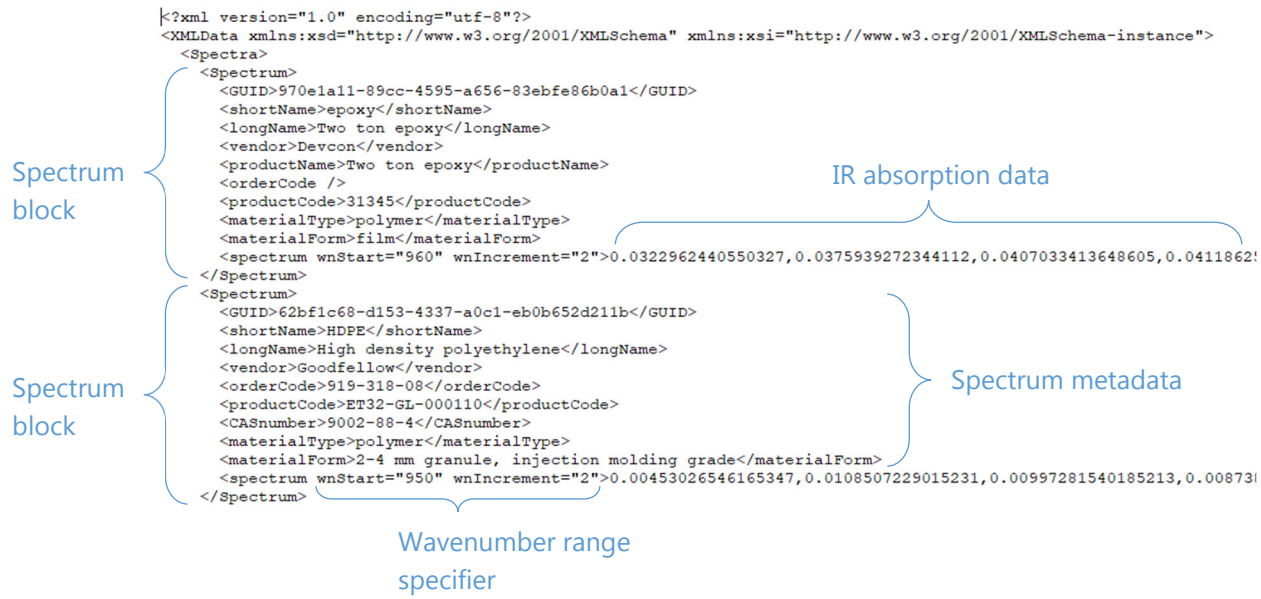


Figure 4-41. Adding user acquired spectra to the Chemical ID spectral database

4.10.5. Inspecting spectral metadata

If you would like to inspect the spectral metadata for any material in the database, open the reference database in a text editor.

Important note: Use extreme caution when editing the reference database file as formatting errors can cause the database to become inoperable.




```
<?xml version="1.0" encoding="utf-8"?>
<XMLData xmlns:xsd="http://www.w3.org/2001/XMLSchema" xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance">
  <Spectra>
    <Spectrum>
      <GUID>970e1a11-89cc-4595-a656-83ebfe86b0a1</GUID>
      <shortName>epoxy</shortName>
      <longName>Two ton epoxy</longName>
      <vendor>Devcon</vendor>
      <productName>Two ton epoxy</productName>
      <orderCode />
      <productCode>31345</productCode>
      <materialType>polymer</materialType>
      <materialForm>film</materialForm>
      <spectrum wnStart="960" wnIncrement="2">0.0322962440550327,0.0375939272344112,0.0407033413648605,0.0411862
    </Spectrum>
    <Spectrum>
      <GUID>62bf1c68-d153-4337-a0c1-eb0b652d211b</GUID>
      <shortName>HDPE</shortName>
      <longName>High density polyethylene</longName>
      <vendor>Goodfellow</vendor>
      <orderCode>919-318-08</orderCode>
      <productCode>ET32-GL-000110</productCode>
      <CASnumber>9002-88-4</CASnumber>
      <materialType>polymer</materialType>
      <materialForm>2-4 mm granule, injection molding grade</materialForm>
      <spectrum wnStart="950" wnIncrement="2">0.00453026546165347,0.0108507229015231,0.00997281540185213,0.00873
    </Spectrum>
```

Figure 4-42. Chemical ID database format

4.11. Acquiring O-PTIR images

To acquire O-PTIR images, first capture a video image to a document and then in the document window, select a region of interest for imaging using the Select Scan Area tool as shown below.

4.11.1. Selecting a scan area

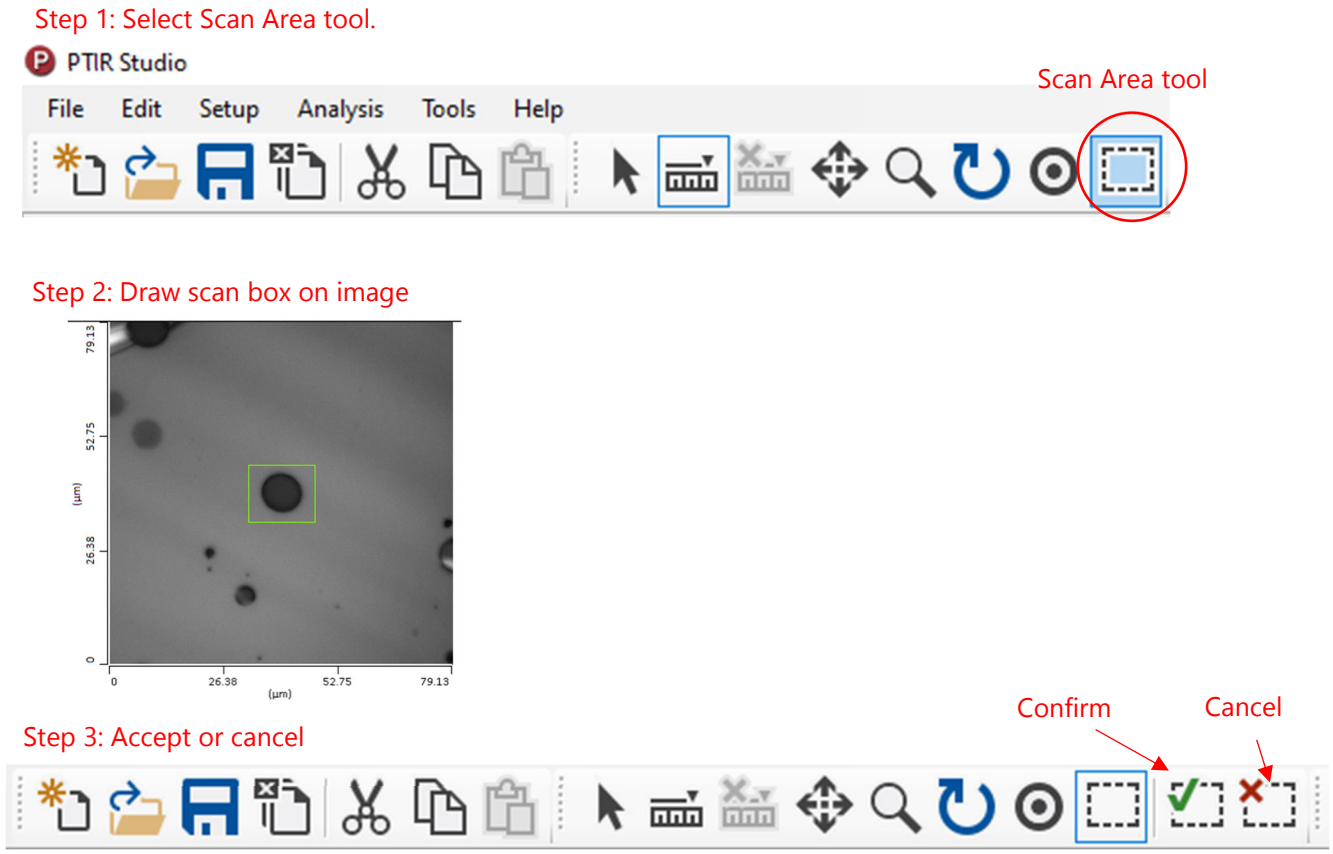
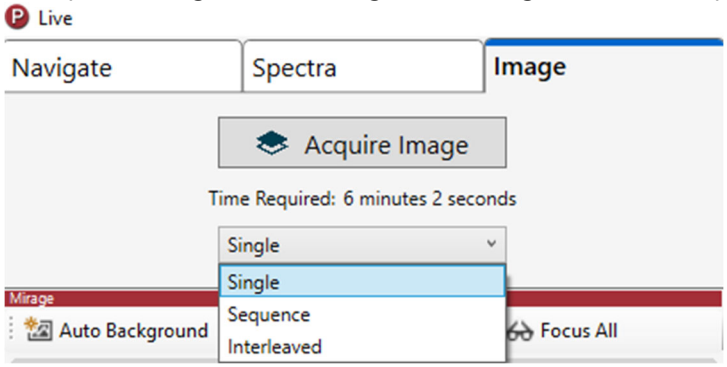


Figure 4-43. Selecting a scan area for an O-PTIR image.

4.11.2. Capturing single O-PTIR images

To capture a single O-PTIR image, select Single from the drop-down menu on the Image tab.



4.11.3. Adjust image settings

Next, review the settings for the image on the Live window, under the Image tab as shown below.

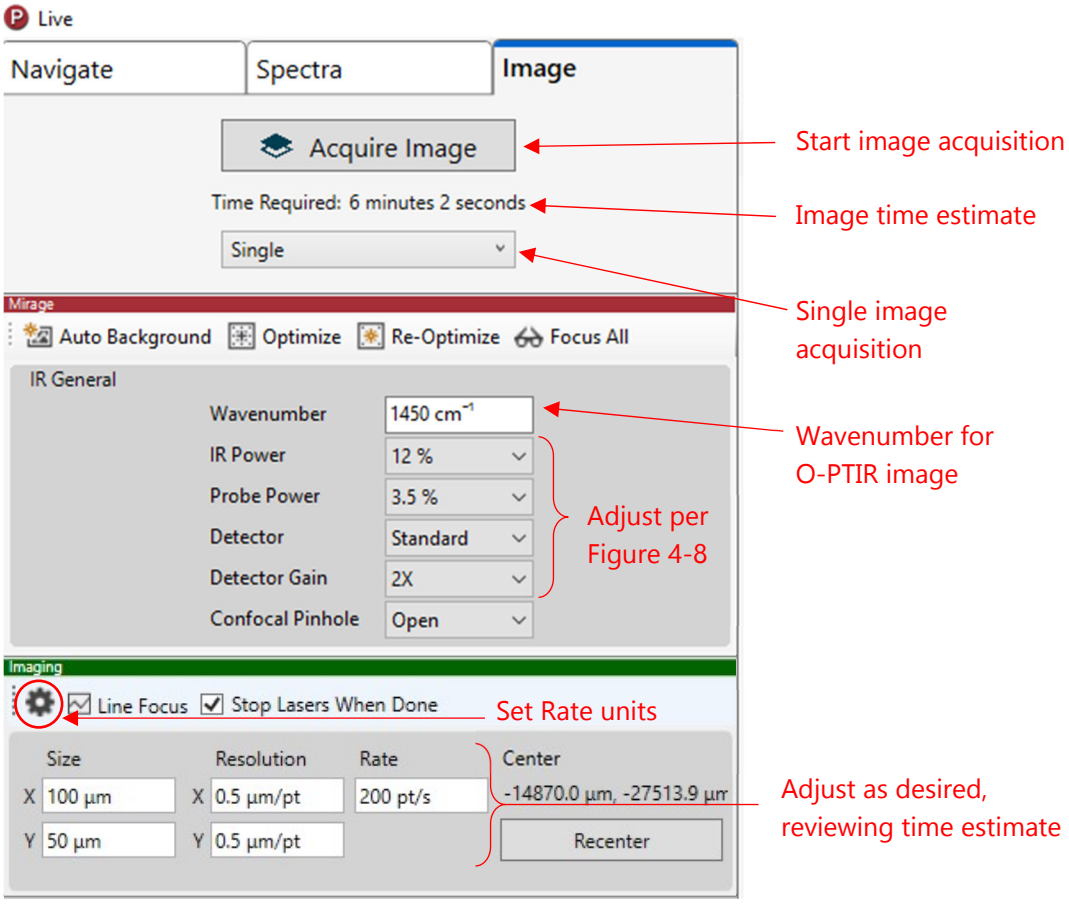


Figure 4-44. O-PTIR image settings

Resolution adjustment

The resolution sets the pixel size (or pixel resolution) for the image. The spatial resolution of the O-PTIR technique is around 300-700 nm depending on objective and probe laser wavelength. For high resolution measurements, it is recommended to use a pixel resolution at least 2X smaller than your desired spatial resolution. Decreasing the pixel resolution will increase the total image acquisition time. For larger area images/survey scans, pixel resolution can be made larger to reduce image acquisition time. Guidelines for pixel resolution are shown in the table below. Higher or lower Resolution settings may be used, but at the expense of coarser image resolution or longer imaging times.

Table 4-4. Recommended pixel resolution settings for different image sizes

Image size	Recommended default resolution	Recommended resolution range
10 µm	0.05 µm	0.05-0.1µm
100 µm	0.5 µm	0.2-1 µm
1000 µm	5 µm	2-10 µm

Image scan rate adjustment

The Rate setting in the Imaging panel controls how quickly the sample is scanned. A good default value is 200 points/sec, and the usable range is normally between 100-1000 points/sec. In general, lower rates will provide better signal-to-noise ratio (SNR), but at the expense of longer image acquisition times. In general, the SNR goes like $\frac{1}{\sqrt{imaging\ rate}}$. So, to increase the SNR by 2X, it is necessary to decrease the imaging rate by 4X. Refer to the Time Required estimate under the Acquire button when adjusting the imaging rate to see if the acquisition time is acceptable. For lower quality survey scans, it can be useful to increase the imaging rate. Higher image rates will decrease the image acquisition time, but only up to a point. Due to scan overhead times, increasing the image rate above a certain threshold **will not** significantly decrease image acquisition time. Image rates>1000 pts/sec generally provide diminishing improvements in image acquisition time.

To avoid needing to change the Rate between different scan sizes, it is recommended to set the imaging rate in **points/sec**. To access the units for the Rate setting, click on the gear icon in the Imaging panel.

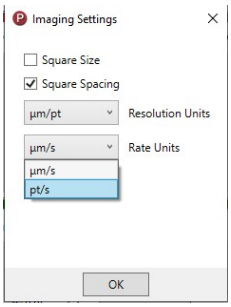
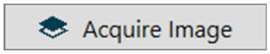


Figure 4-45. Setting Rate Units

Selecting desired image channels

Next, ensure that desired channels are selected for the images. The standard channels are O-PTIR and DC. The O-PTIR image will show the relative IR absorption at the selected wavenumber and the DC image shows the brightness at each image pixel. The DC image is similar to a brightfield image when using the Standard detector and similar to a transmitted light image when the Transmission detector is used. It is generally recommended to keep the Auto Range and Use Min/Max check boxes selected. The Palette can be changed if desired for different color tables for display. The Palette and display scales can also be changed in the document after image acquisition.

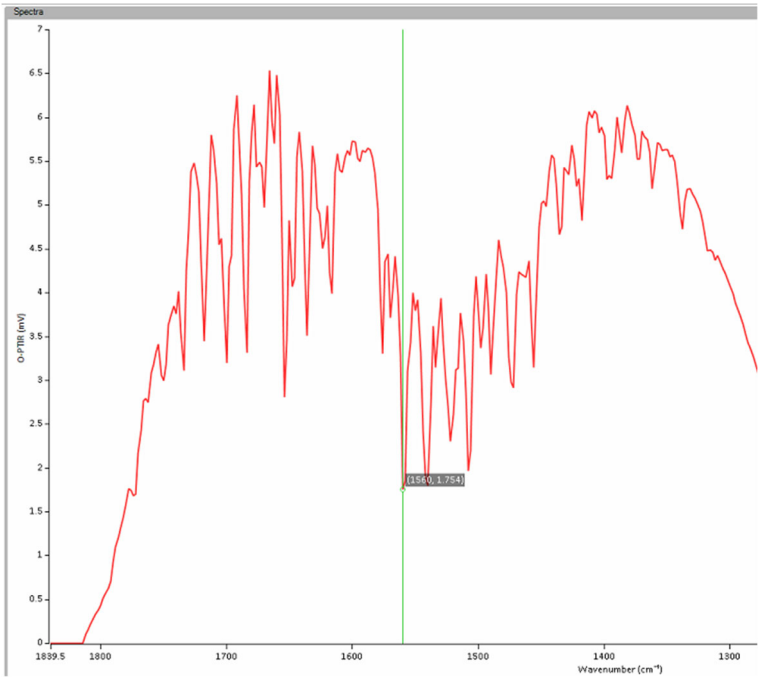
Figure 4-46. Channel selection for O-PTIR imaging.

Once satisfied click the 

button.

4.11.4. Avoiding water vapor absorption lines

Atmospheric water vapor has strong IR absorption bands that can significantly reduce the IR power at certain wavelengths in unpurged systems. If your system is un-purged, avoid acquiring O-PTIR images at the strong IR absorption bands. A portion of an O-PTIR background is shown below at left that shows a series of sharp downward spikes between 1300-1800 cm^{-1}. These spikes are absorption bands associated with atmospheric water vapor. Especially at high relative humidity, these absorption bands can dramatically reduce the IR power delivered to the sample. For this reason, it can be useful to purge the mIRage with clean dry air and/or avoid acquiring O-PTIR images at the strong water absorption bands. The table in the figure below lists some strong water vapor bands and the percentage of maximum IR power measured at 38% relative humidity. Typically, it is sufficient to shift the IR wavenumber by just a few cm^{-1} to avoid a strong absorption band.



Wavenumber	% of max power
1457	29%
1473	49%
1489	55%
1507	31%
1521	37%
1541	28%
1559	24%
1653	41%
1685	51%
1701	50%
1717	51%
1735	44%

Figure 4-47 O-PTIR background showing water absorption bands and table of strong absorption lines

To determine which water absorption bands may be problematic on a specific system or on a specific day, it can be helpful to examine your IR power background for low power points associated with water vapor absorption. The easiest way to do this is to open a document and then select Tools/IR Background Calibration/Add to Document as shown below.

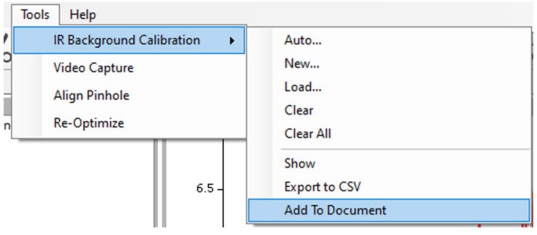


Figure 4-48. Adding IR background to document

You can then examine the background in the document window to identify strong water vapor absorption peaks to avoid.

The background file can also be exported to a CSV file if desired using Tools/Background/Export to CSV.

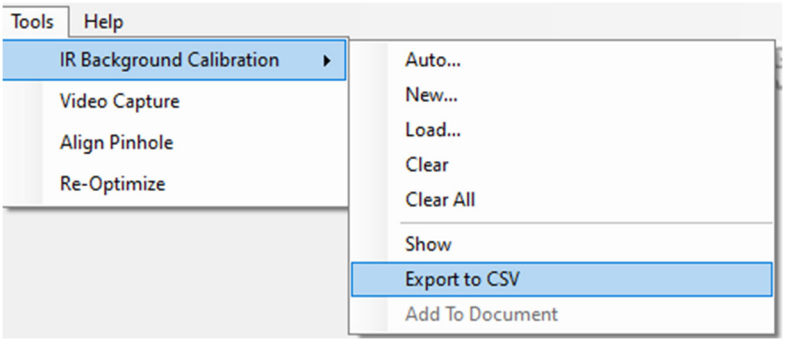


Figure 4-49. Exporting background file to CSV

4.11.5. Line Focus tool

One method of optimizing spatial resolution has already been described in in Section 3.2.2, involving minimizing the size of the probe beam spot in the camera view. Another approach is using the Line Focus tool which allows the user to optimize the focus position while watching profile traces of the Image channels while the scan stage scans over the same line continuously. The Line Focus function can be accessed by clicking on the Line Focus button in the upper left of the Imaging panel, shown below.

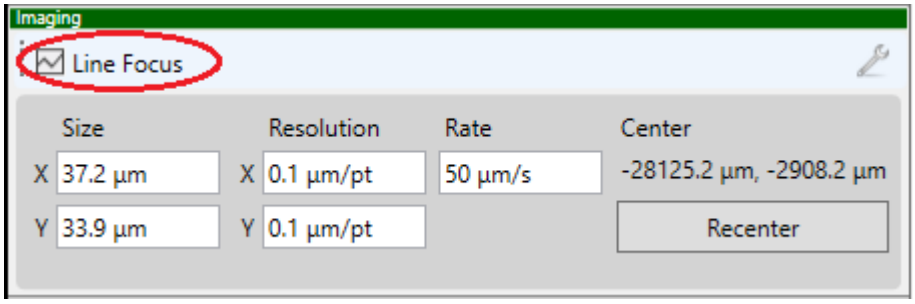


Figure 4-50. Line Focus tool

Clicking on this button will stop the vertical image scan at the current position and bring up a menu, shown below which displays scope traces of the Image channels, typically the O-PTIR (MIRage Amp) and DC channels. Prior scan lines will be displayed while the color gradually fades to white. The user can then click on the Focus Position up or down arrows to move the objective up or down to obtain the sharpest contrast in the image. Typically, the focus increment is set to 0.5 µm. In addition, the user can select a different location in the image by clicking on the image with the bullseye tool and the horizontal light blue line indicating the location where the Line Focus function is collecting data will move to this new position. Once the focus position has been adjusted to achieve the steepest slope between features the X

can be clicked on which will exit the Line Focus menu and restart the image at the beginning to collect a complete image at the new focus position.

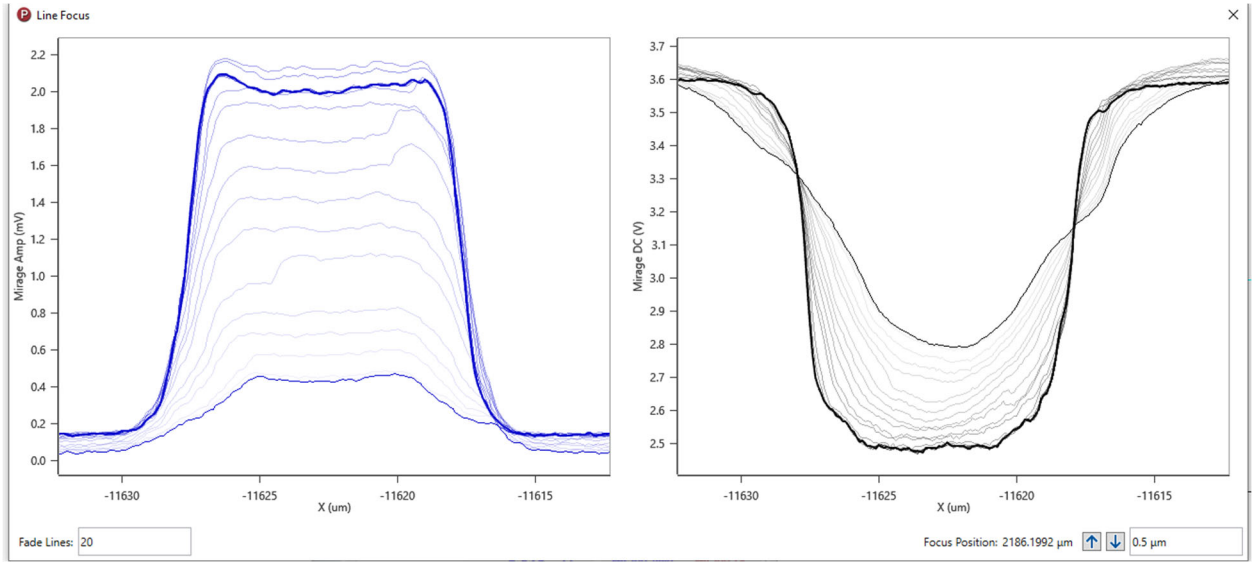


Figure 4-51 Line Focus tool image profiles during focus adjustment

4.11.6. Changing image center (Recenter button)

To change the center of an O-PTIR image after already acquiring one, select the bullseye icon from the toolbar on the Live window and click on the desired center location on the O-PTIR image on the Image tab. The sample will move the selected location under the IR/probe lasers. To choose this point as the center of a new image, click on the Recenter button on the Imaging panel as shown in the figure below.

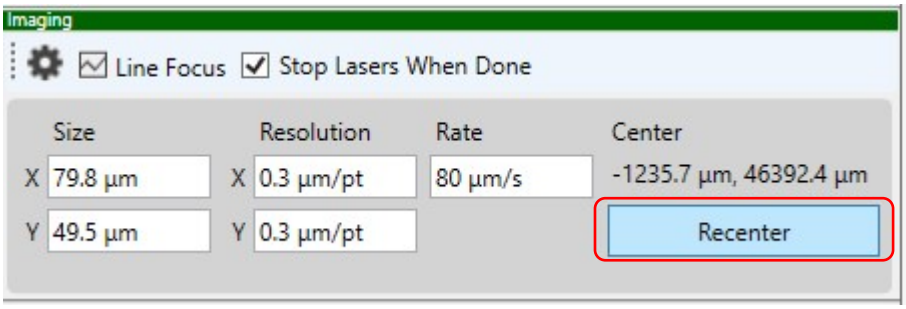
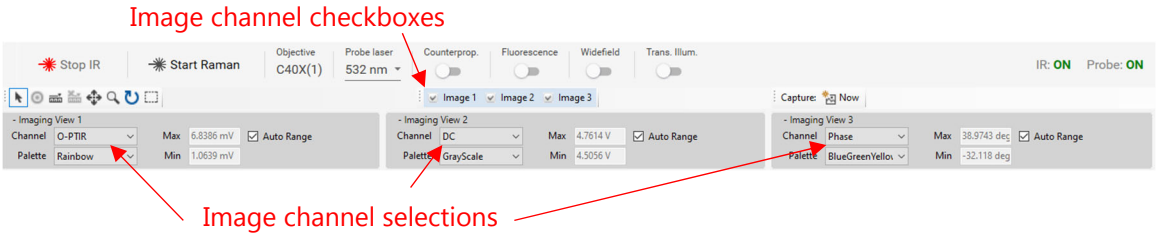


Figure 4-52 Image Recenter

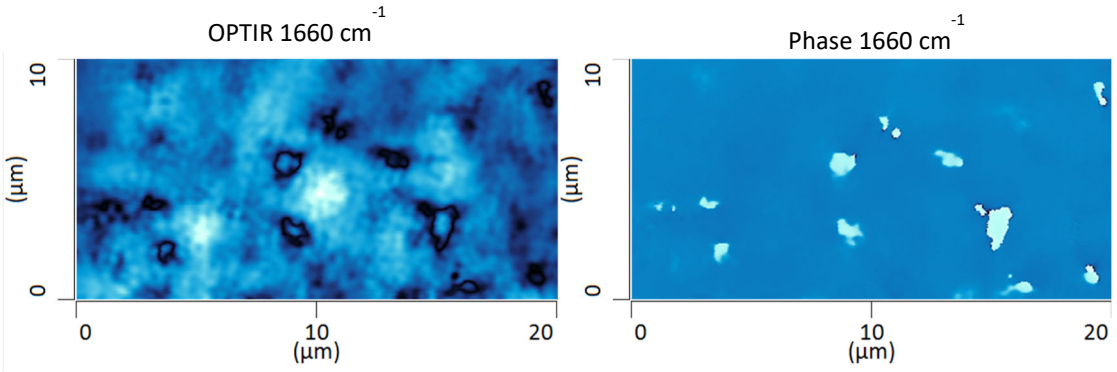
4.11.1. Changing the number of acquired images

The current software version permits capturing up to three image channels simultaneously which permits simultaneous imaging of IR absorption (O-PTIR channel), brightness image (DC channel) and Phase. To enable to disable any of the images, click on the check boxes next to the image number.



4.11.2. The Phase Signal

The Phase channel plots the phase delay of the O-PTIR signal from the lock-in amplifier. Changes in phase indicate a difference in the delay between the IR pulse and the photothermal response of the sample. The phase signal is primarily indicative of the thermal time constant of the material under study. In some cases the phase signal can provide additional/complementary contrast to help identify regions that are different from their surroundings and may warrant additional investigation. The figure below shows an example of using the phase channel to identify different material components in a biological cell. In this case the lipids in the cell showed significantly different phase than the surrounding protein.



4.11.3. Image sequences

PTIR Studio also offers the ability to automatically collect a series of different O-PTIR images at different wavenumbers and optionally with different settings. To enable sequence mode, select Sequence in the drop-down menu on the Image tab as shown below.

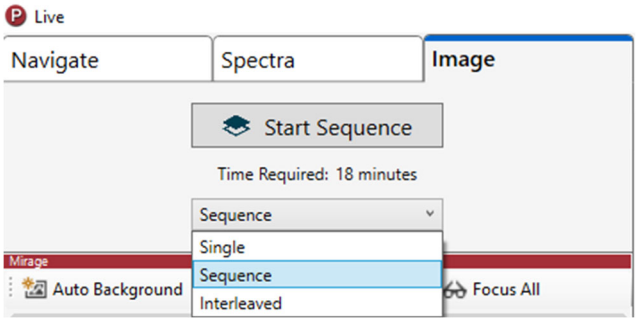


Figure 4-53. Selecting Sequence mode

Selecting Sequence mode will display an Image Sequence control panel shown below.

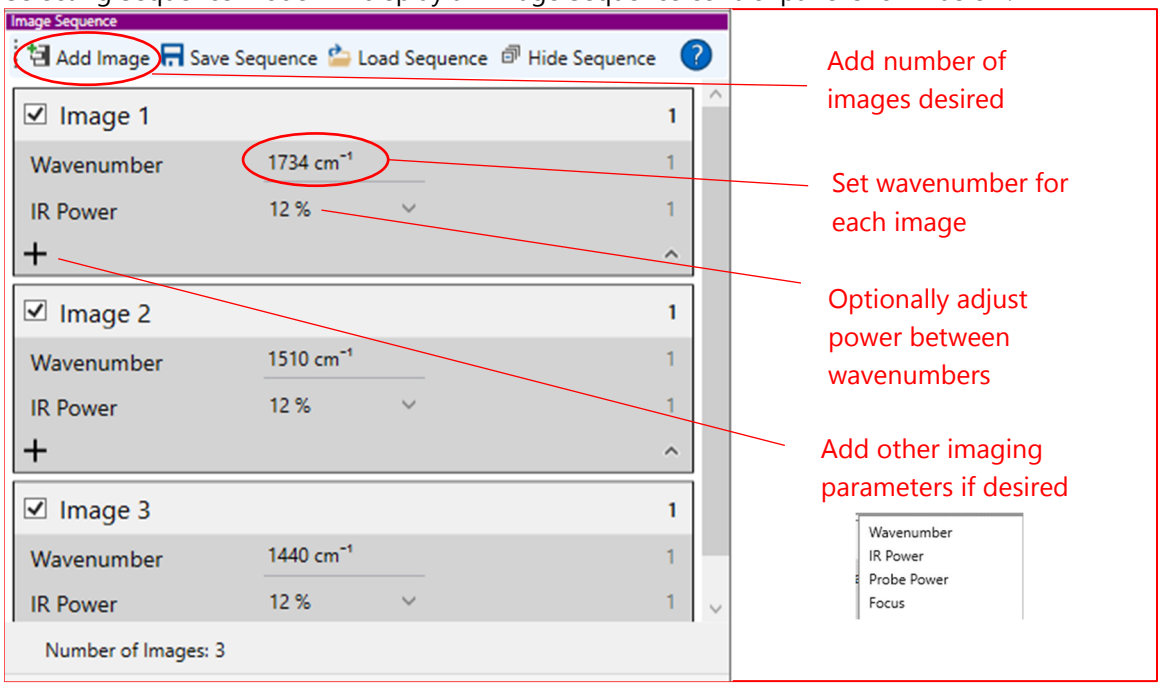


Figure 4-54. Settings for image sequence mode

Review the sequence time estimate and adjust imaging parameters if desired. Click 

to acquire the image sequence.

4.11.4. Interleaved images

Interleaved mode automatically acquires two O-PTIR images at two different IR wavenumbers interleaving scan lines, one at the first wavenumber, one at the second wavenumber until both images are collected. Interleaved mode is very useful for ratiometric imaging, i.e., calculating the ratio of two O-PTIR images at different IR absorption bands. The use of interleaved scanning ensures that the two measurements are

performed very close in time to reduce or eliminate potential effects from thermal drift. Interleave mode works in the following way:

1) The first line of the image is scanned at Wavenumber 1 (ν1)
2) The IR laser is changed to Wavenumber 2 (ν2) during the retrace scan
3) The first line of the image is rescanned at Wavenumber 2
4) Steps 1-3 are repeated for all lines in the image

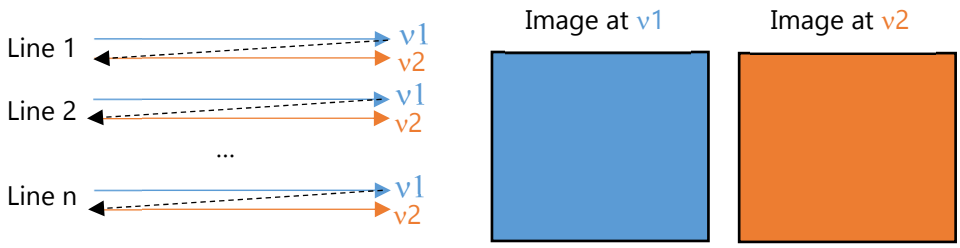


Figure 4-55. Illustration of interleaved mode that simultaneously acquires two images at two different wavenumbers.

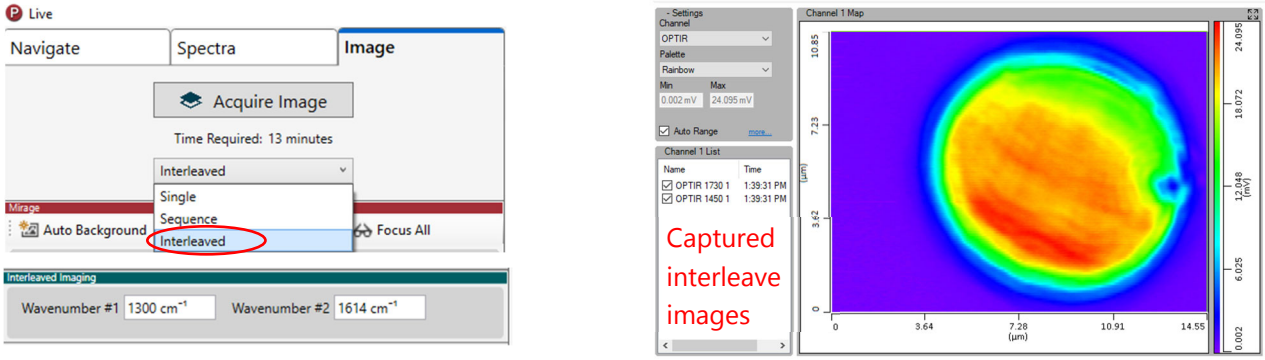


Figure 4-56. (Left) Enabling Interleaved mode. (Right) Captured interleave images in document window.

4.11.5. Calculating a ratio image

Why should I calculate a ratio image?

The contrast in O-PTIR images can depend on several sample properties (e.g., reflectivity, thickness, and thermal/mechanical properties), thus a single wavelength O-PTIR image does *not* necessarily accurately portray variations in IR absorption alone. For this reason, it is often useful to calculate image ratios that compare the O-PTIR signals at two different wavenumbers.

Consider an O-PTIR image with a signal intensity $S(x, y, \nu)$, where (x, y) are the locations on the sample, and ν is the IR wavenumber used to collect the O-PTIR image. This signal can be broken down into two separate functions:

Eq. 1: $S(x, y, \nu) = A(x, y, \nu)B(x, y)$

(1) $A(x, y, \nu)$ which represents the IR absorption as a function of (x, y) position and wavenumber ν

(2) $B(x,y)$ which represents the other sources of variation in the O-PTIR signal, for example reflectivity, sample thickness, and thermal/mechanical properties.

Calculating the ratio of the signal *S* at two different wavenumbers *ν1 and ν2 gives:*

Eq. 2: $$\frac{S(x,y,\nu 1)}{S(x,y,\nu 2)} = \frac{A(x,y,\nu 1)B(x,y)}{A(x,y,\nu 2)B(x,y)} = \frac{A(x,y,\nu 1)}{A(x,y,\nu 2)}$$

In this case the reflectivity, sample thickness, and thermal/mechanical properties are constant at each point in the sample, thus the $B(x,y)$ term in the numerator and denominator are the same and cancel out in the ratio. The ratio image thus reveals the variation in IR absorption while eliminating effects from other sample properties.

Calculating ratio images

To calculate a ratio image, do the following:
1) Capture two images at different wavenumbers using Interleaved Mode (Section 4.11.4)
2) In the Document window image list, select the image you want to use as the numerator.
3) Select Analysis/Ratio or click on the ratio icon  .
4) Click and drag an image at a different wavenumber to the denominator selection "Ratio Image B" as shown in the figure below and then click Continue.

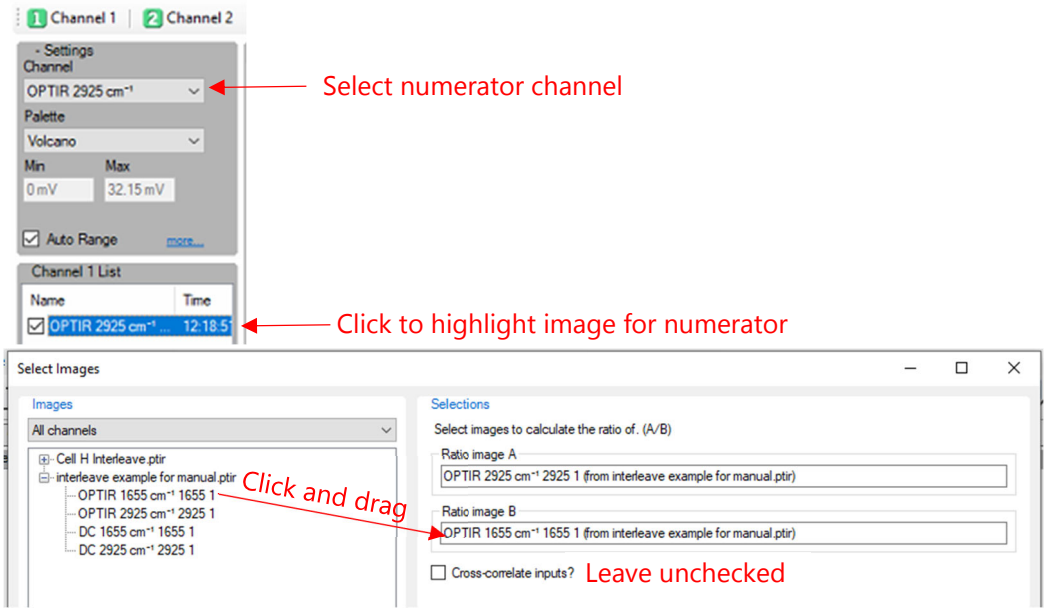
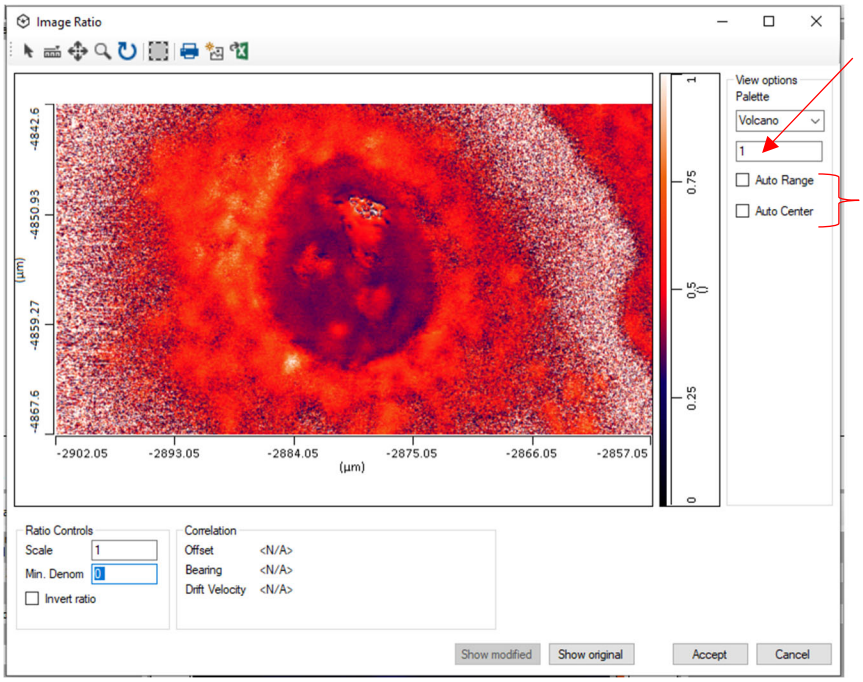


Figure 4-57. Setting up an image ratio calculation.

5) An image ratio display will open, an example shown below.



6) If desired, adjust the Min. Denom to exclude areas from the ratio calculation where the denominator has minimal O-PTIR signal. This will avoid excessive noise in a ratio image due to dividing by a number close to zero. The figure below illustrates ratio images calculated with different minimum denominator settings.

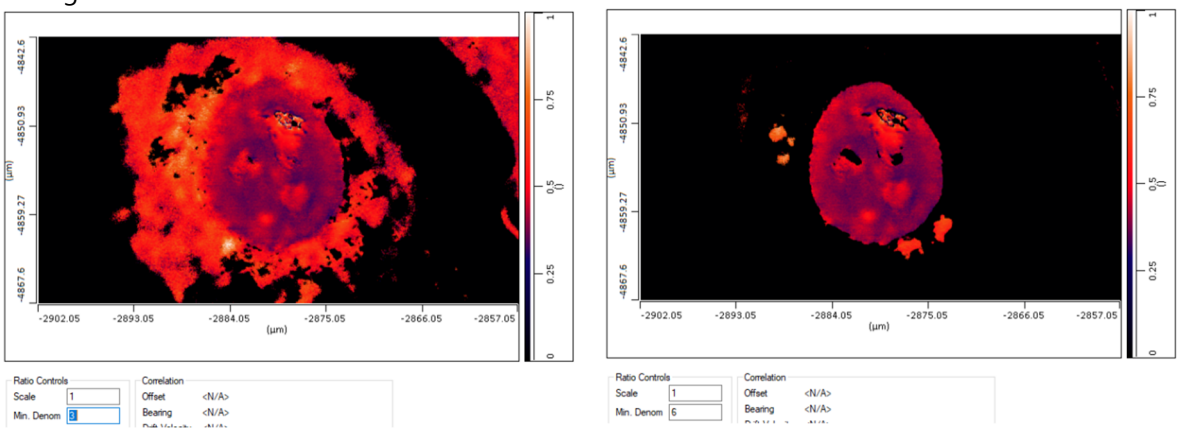
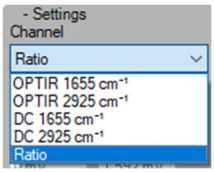


Figure 4-58. Adjusting the minimum denominator setting in an image ratio calculation

7) Click Accept to accept your selection and save the ratio image to the document. The image will be saved in the document under the Ratio channel.



4.11.6. Using O-PTIR images as reference maps for spectra

Either optical microscope images or O-PTIR images can be used as the reference to choose locations for O-PTIR (and/or Raman) spectra. To use an O-PTIR image, capture an O-PTIR image to the document. If not already selected, click on Image in the Image type drop-down menu as shown below. To use a Camera image, select Camera instead.

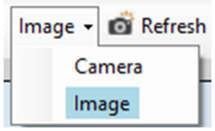
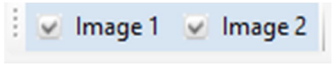


Figure 4-59. Toggling between Camera and Image choices for reference map

4.11.7. Image display settings

The Live window image display can be configured to display either one or two images using the radio buttons shown below.



The image channel displayed for the left or right image window can be selected in the Channel dropdown menu. The min/max range on the live display can also be adjusted, but Auto Range is recommended.

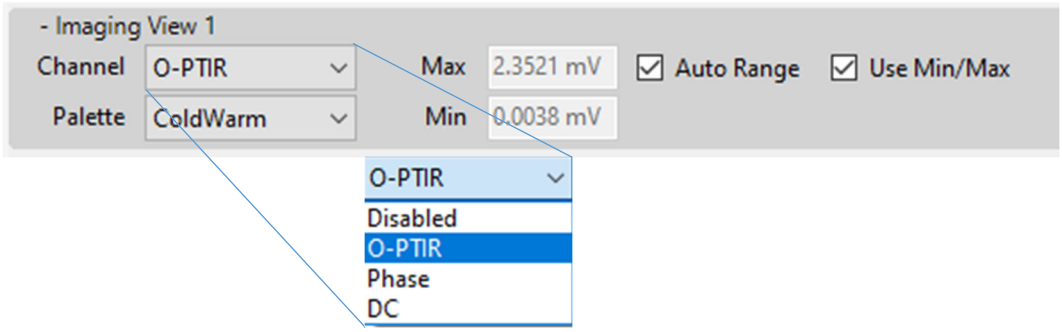


Figure 4-60. Selecting an image channel for display on the Live window

The color palette for an image display on the Live window can be selected from the Palette drop down menu on the image view. This palette selection can be changed on acquired image data in the Document window.

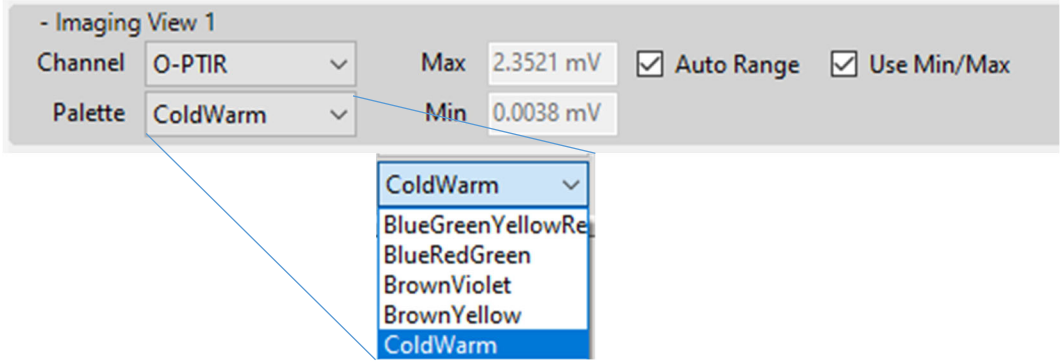


Figure 4-61. Changing the image display color palette.

4.11.8. Image toolbar icons

The Live window has the following toolbars for use with the currently active image. Similar tools are on the Document window for use with acquired images.

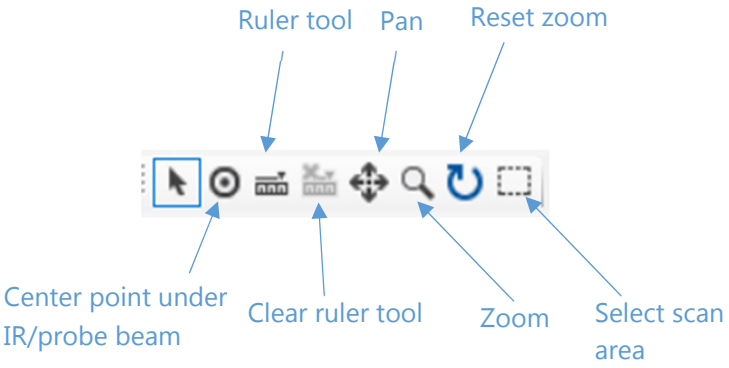


Figure 4-62. Live window image toolbar icons

4.11.9. Capture Now

O-PTIR images are automatically captured to the document on completion. In some cases, it may be desirable to capture an image to the document before it has completed (for example if you wish to abort the current scan but save the already acquired image data). To capture an incomplete document at any time, click on the Capture Now button. Note that Capture Now may have incorrect image metadata due to the incomplete image acquisition.

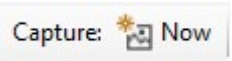
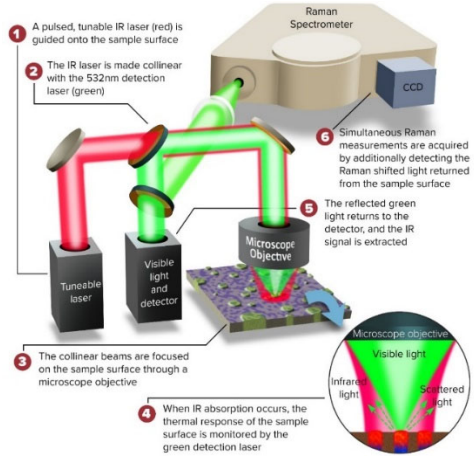


Figure 4-63 Capture Now button

Chapter 5

5. Raman

This section details simultaneous acquisition of Raman and O-PTIR spectra.
Certain mIRage systems may also come coupled to a Raman spectrometer. Due to the fact a visible probe laser is used during O-PTIR measurements, Raman scattering is always occurring on the sample surface. By filtering off only the Raman shifted light and sending it to a spectrometer this enables the collection of simultaneous O-PTIR and Raman spectra. A schematic diagram of this principle is shown below.



5.1. Enabling Raman

To enable the simultaneous mIRage and Raman measurements select the IR+Raman option on the drop-down menu on the Spectra tab of the Live window as shown below. For samples that are only Raman active, it is possible to select Raman only without enabling O-PTIR measurements.

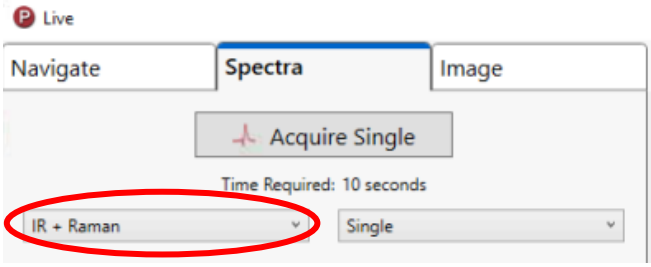


Figure 5-1. Enabling Raman measurements

This opens an additional panel that appears in the spectra tab that contains the Raman controls.

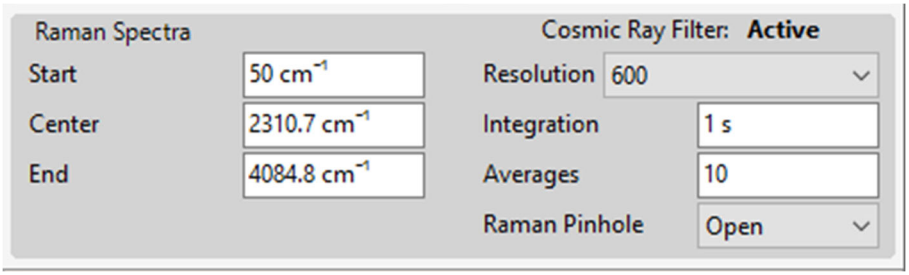
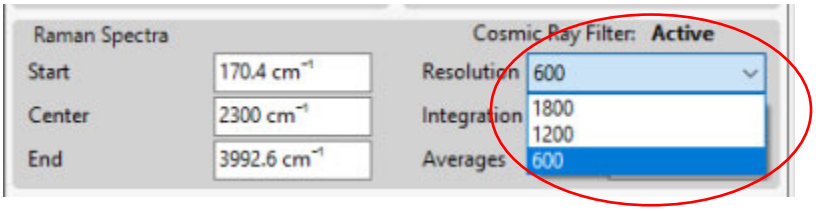


Figure 5-2. Raman spectra settings

5.2. Raman grating selection

The first choice when collecting a Raman spectrum is the choice of grating. The grating is changed by clicking on the resolution tab and accessing the grating drop down list. Available gratings may depend on your system configuration.



The choice of grating affects both the resolution of the data and the range of Raman shift covered by the measurement. The grating, resolution and range for each grating are shown in the table below.

Grating	Range	Resolution
600 lines/mm	3870 cm^{-1}	0.12 nm/pixel
1200 lines/mm	1722 cm^{-1}	0.06 nm/pixel

5.3. Setting Raman spectral range

After choosing a grating, we now select a "Center" value. This will set the start and end points of the spectral collection window. This number is usually selected to have a start value of ~ 200 cm^{-1}. The specific start value can be chosen depending on the Raman edge filter cutoff (the filter used to block the Raman excitation beam from reaching the spectrometer).

5.4. Setting Raman integration time

The integration time is how long the collected Raman beam is exposed to the CCD camera. The CCD detector will saturate at a value of > 65,000 counts, so ensure the integration time is set low enough to ensure this doesn't happen.

The integration time should be set based on the strength of signal from the sample. In general, use an integration time of 0.1-0.2 seconds for focusing/pinhole alignment where you need real-time feedback. For actual sample measurements, an integration time of 1 second is a good default value to start with. Adjust as needed for desired SNR, while making sure the Raman spectrometer sensor is not saturated (see Section 5.7). When collecting simultaneously IR and Raman spectra it can be useful to match the integration time for IR and Raman spectra so that neither measurement is waiting on the other to complete.

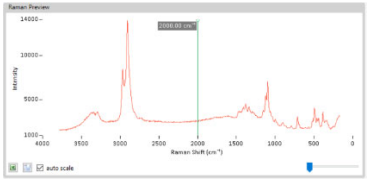
The final setting is the number of averages collected when acquiring a spectrum. In general, the SNR will increase like the square root of the number of averages. So, 4 averages will provide 2X better SNR. Using 2 or more averages enables the use of the Cosmic Ray Filter, described in Section 5.10.2.

5.5. Fluorescence bleaching delay

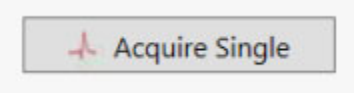
The "Fluor. Bleach Delay" parameter will optionally turn on the probe laser and dwell at a location for a specified time to attempt to photobleach sample fluorescence. Note that this delay will be applied to every point in a spectral array so it can have a major impact on measurement throughput. If you have substantial sample fluorescence, try an experiment first to see how much time is required to bleach the fluorescence to an acceptable level and determine if your measurement permits the required amount of fluorescence bleaching delay. As an alternative on multi-laser systems, much less sample fluorescence is typically observed at 785 nm excitation wavelength.

5.6. Starting Raman measurements

Once the desired Raman parameters have been set, the Raman signal from the surface may be displayed in real time by clicking on the "Start Raman" button at the top of the panel.



The real time Raman spectrum will now be displayed in the Raman preview window in the bottom right of the Live window. The Raman parameters can then be adjusted to optimize the spectral quality. Once the desired parameters for both IR and Raman have been entered, simply click on Acquire single to collect simultaneous spectra.



5.7. Avoiding Raman saturation

The Raman signal is integrated in a camera-based sensor that has a 16-bit limit, i.e., 65535 counts. Ensure that your max signal in the Raman Preview window is not over this limit. Note that a fluorescence

background can boost the total signal up near saturation for long integration times, so check this with the baseline compensation off. If your signal is saturated, reduce the integration time and if desired increase the number of averages.

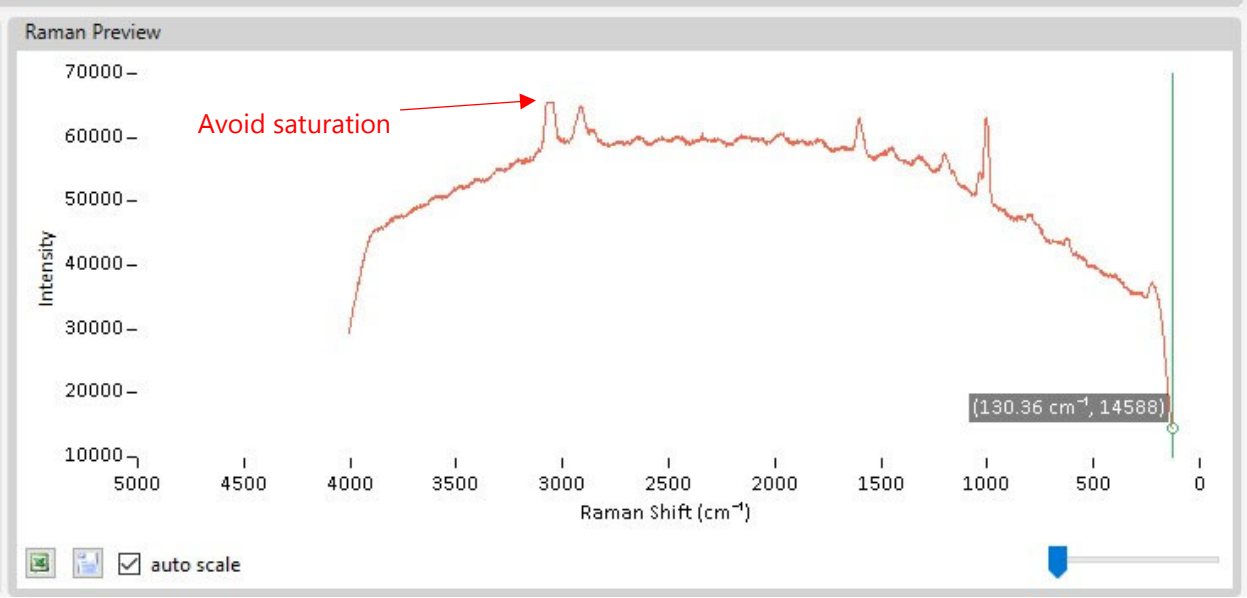


Figure 5-3. Raman spectrum illustrating signal saturation

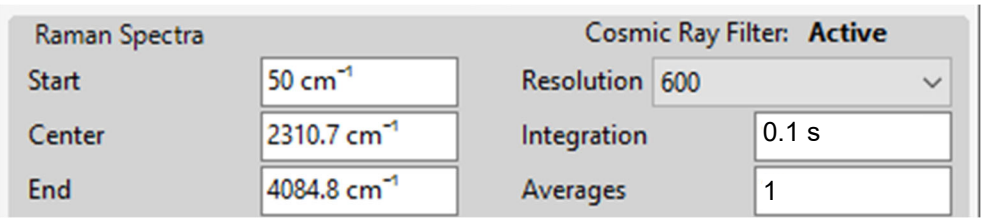
5.8. Raman focus adjustment

To adjust the focus for Raman measurements, either use the minimum probe beam adjustment as described in Section 3.2.2 or alternately adjust the focus while monitoring the Raman intensity in the Raman preview window. Note that in some cases the Raman signal level will increase when the IR beam is defocused, so for best spatial resolution, use the minimum probe beam size adjustment.

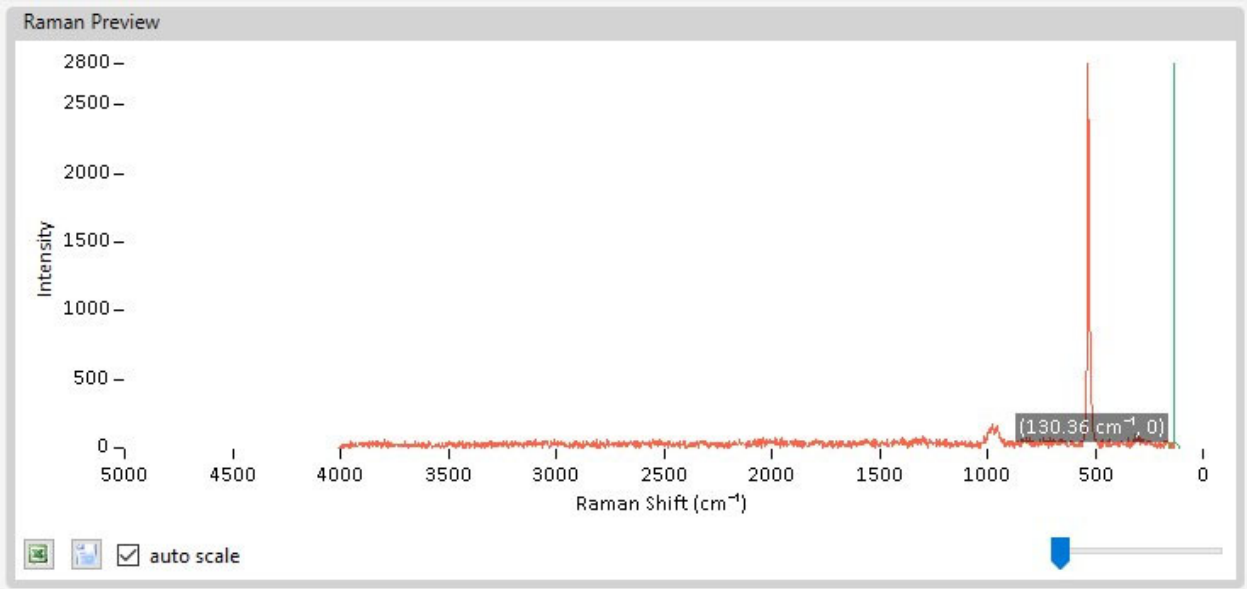
5.9. Aligning the Raman pinhole

To align the Raman pinhole, do the following steps.

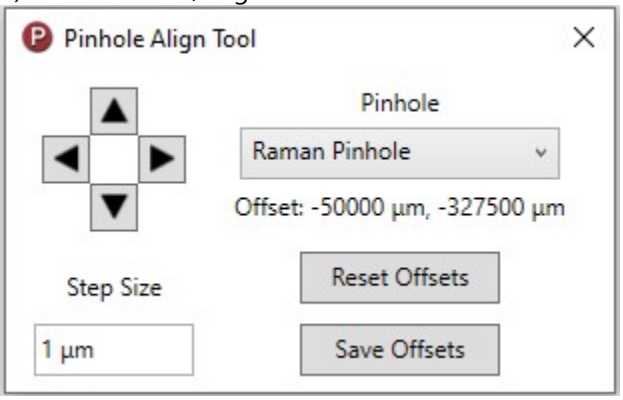
1) Navigate to the silicon reference sample (see Sec. 3.5 and Figure 3-9)
2) Adjust the Raman settings as shown below



3) Click Start Raman on the Spectra tab and observe the Raman spectrum preview and specifically the silicon Raman peak around 520 cm^{-1}.



4) On the Raman settings window, reduce the pinhole size to the smallest size where there is still a signal.
5) Click on Tools/Align Pinhole on the Document window menu bar.



6) Select the Raman Pinhole from the drop-down menu.
7) Adjust the step size to be around 10% of the current pinhole size. E.g., for a 200 µm pinhole, set the step size to 20 µm. (Note: Some mIRage systems will have pinhole alignment units listed in "pts" which is the number of motor steps to move the pinhole. For systems that use "pts" units, start with a step size of 50-500 pts, and adjust as necessary. Contact PSC support if you would like your system recalibrated to use microns instead of motor steps.)
8) Click on the up/down/left/right arrows while monitoring the amplitude of the silicon Raman peak. Adjust until the Raman peak amplitude is maximized. It is sometimes easier to turn off the auto scale on the Raman spectrum preview to see the peak size grow and shrink. Alternately, leave the auto scale on and observe the maximum intensity of the vertical graph scale.
9) Change the step size to around 1% of the current pinhole size.
10) Repeat step 8 until the signal is maximized.
11) Once the peak is successfully maximized, click on Save Offsets.
12) If the Raman signal is lost, click on Reset Offsets to return to the original starting position.
13) If you started with a larger pinhole for step 4 than you want to use for your measurement, reduce the pinhole size to the desired diameter and repeat the above steps for the smaller pinhole.

5.10. Advanced Raman settings

Advanced Raman settings are available by clicking on the settings button (gear icon) on the mIRage panel on the Live window and then selecting the Raman tab.

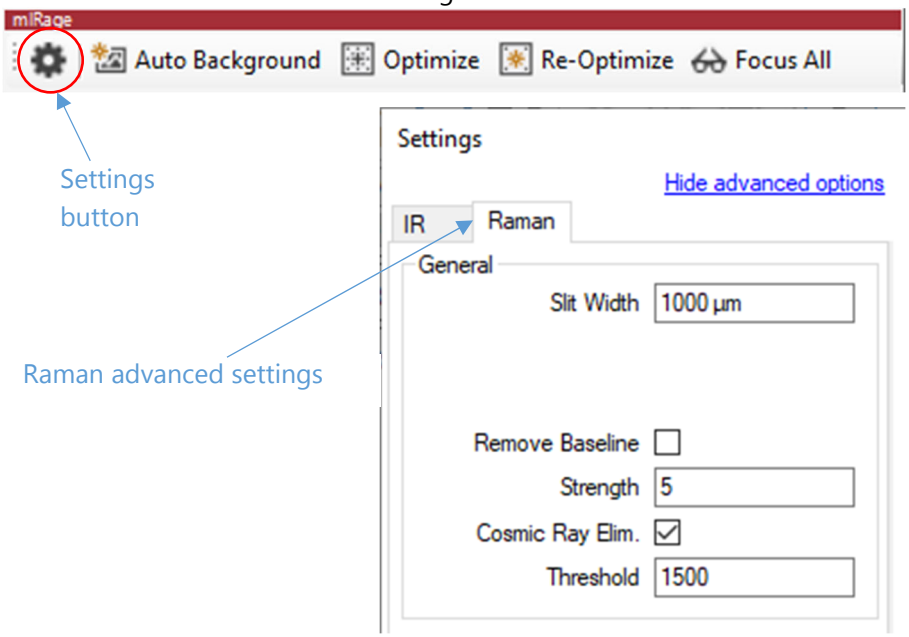


Figure 5-4. Raman advanced settings

5.10.1. Remove Baseline

Remove baseline can be used to remove at least partially suppress a fluorescence background. Use Remove Baseline with caution as it can alter Raman peak shapes if used too aggressively or on data without a smoothly varying baseline. Note that there is also an offline baseline removal tool in the Analysis/Filter menu on the Document window. If you wish to preserve your raw Raman data, leave the Remove Baseline unchecked, and post-process the spectra using the Remove Baseline capability in the Analysis/Filter menu. This function is described in more detail in the mIRage Software Manual.

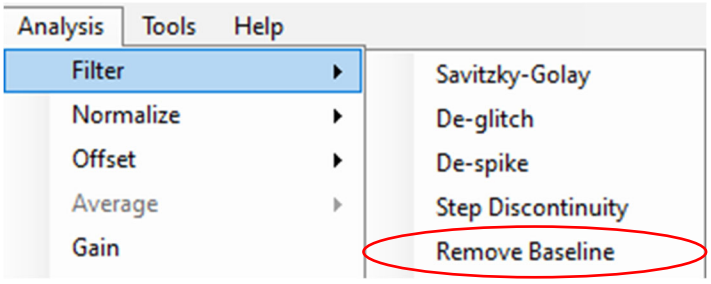


Figure 5-5. Offline Remove Baseline filter tool available on the Document window

5.10.2. Cosmic Ray Filter

Cosmic rays are high-energy particles originating from outer space. When a cosmic ray hits the Raman detector during a measurement, it will create a sharp spike in the data, as shown below.

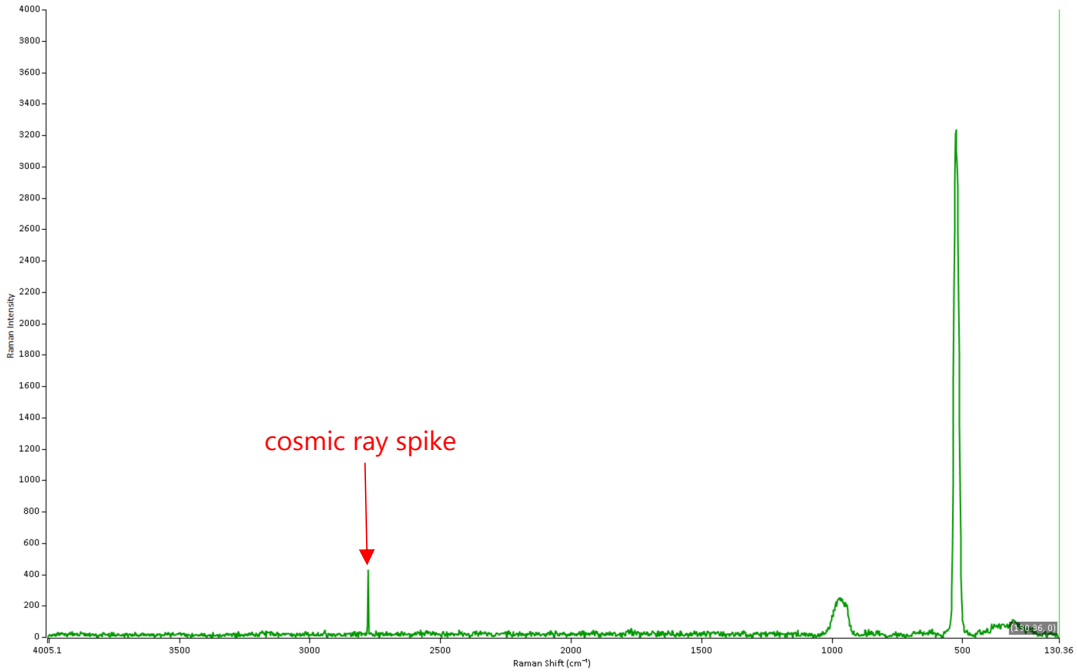


Figure 5-6. Example cosmic ray spike

PTIR Studio offers different options to remove cosmic ray spikes, either in real-time at the time of data acquisition or in post-processing.

Real-time cosmic ray suppression

To enable real-time cosmic ray suppression, click on the Cosmic Ray Elim. checkbox. Note that the cosmic ray filter is only active when the number of averages is set to 2 or more. The cosmic ray filter compares two or more spectra to each other and rejects portions of the data that exceed a threshold value from the median. Thus, a cosmic ray spike on one Raman spectrum will generally not occur at the same detector pixel on another spectrum and thus will be detected and eliminated from the data. The Threshold value sets the number of counts above which a cosmic ray spike is detected and eliminated.

Cautions about using the Cosmic Ray Filter

Because the cosmic ray filter is used to reject portions of the spectra where one spectrum is very different from another, this can cause issues when Raman spectra are unstable. For example, samples with high fluorescence and a high bleaching rate can lead to successive Raman spectra that are substantially different from each other, thus causing the cosmic ray filter to reject real data and cause spectral artifacts.

For samples with no auto-fluorescence, a reasonable threshold for the cosmic ray filter is 500. For samples with high fluorescence, it can be better to use higher cosmic ray thresholds, e.g., around 1500.

Offline Cosmic Ray Filter

Because of the potential for the real time cosmic ray filter to generate artifacts in the presence of autofluorescence, it can be desirable to apply a cosmic ray filter in a post-processing step. There are three options available. Both the De-spike and De-glitch filters on the Analysis/Filter menu on the Document window can be used to post process single Raman spectra to remove cosmic ray spikes. There is also a cosmic ray removal tool under the Analysis/Average menu, although this must be applied to at least two Raman spectra because it uses the same approach as the real time cosmic ray filter.

Chapter 8

6. Options and Accessories

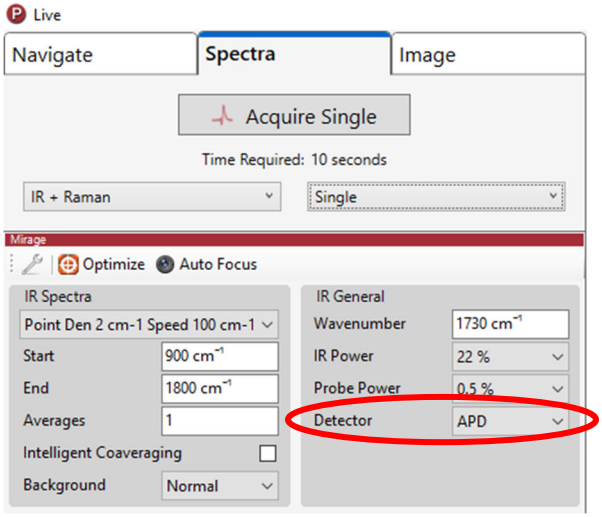
This section documents the use of certain optional features and accessories. Most optional features and configured at the time of purchase, but some options are available as an upgrade. Contact your sales associate if you are interested in acquiring optional features and/or accessories.

6.1. Additional detectors

Some mIRage systems may be equipped with a variety of detectors. There are a maximum of three detectors in the system, Standard, APD, and Transmission depending on purchased configuration.

6.1.1. APD Detector

The APD detector is typically used when a sample has a strong interaction with the probe laser, causing sample damage. This detector can operate with very low light conditions, allowing for spectral collection on these challenging sample types. The spectral collection and IR imaging procedure for the APD detector is essentially the same as for the Standard detector, with some mirror differences.



1. To select the APD, choose it from the detector drop down menu on the Spectra panel.
2. When the APD detector is selected, the software limits the levels of probe power that can be used. available. This is a safety feature to protect the detector, as it can be damaged with higher probe laser powers. For 532 nm, the max power for APD operation is 1% of the total laser power and for 785 nm the APD limit is < 5% of the total power.
3. Optimize the signal and collect data in the same manner as when using the standard detector.
4. It is good practice to switch back to the standard detector when done using the APD.

6.1.2. Transmission Detector

While both the APD and Standard detectors operate in reflection mode, there is also an option to add a transmission detector below the sample stage. The transmission detector is especially useful on samples with little reflectivity, for example samples in liquid.

1. When using the transmission detector, if possible, it is best to initially optimize the O-PTIR signal with the standard detector prior to switching to transmission. Start by acquiring an auto background with the standard detector to ensure good alignment between the IR and probe beam.
2. To select the transmission detector, select it from the detector drop down menu on the Spectra tab.
3. Using the transmission detector will normally require lower detector gain than the standard detector because most samples will transmit >90% of the incident probe light.
4. After ensuring the detector signal is not saturated, use the Focus All button to optimize the signal level.
5. It is good practice to switch back to the standard detector when done using the transmission detector.

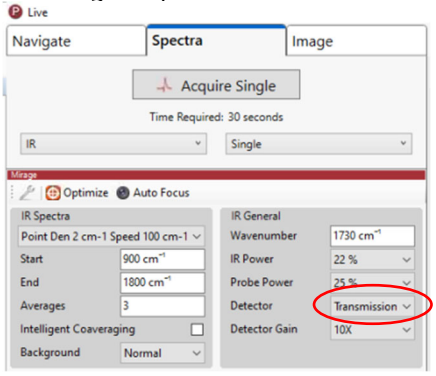


Figure 6-1. Selecting the transmission detector

6.2. Transmission illumination

Transmission illumination is an optional feature of the mIRage that can be configured at the time of purchase. Transmission illumination uses the condenser/objective underneath the sample to deliver white light illumination for the optical microscope video view. Transmission illumination is useful for samples with low reflectivity that do not show good contrast in epi-illumination, for example samples in liquid.

Note: Since the Transmission Illumination module uses the same reflective condenser as is used for focusing the IR beam for counter-propagating mode, these two modes cannot be used simultaneously. When counter-propagating mode is enabled and the Start IR button is pressed, transmission illumination is inactive. Also note that the optimal Trans. Focus position for transmission illumination and counter propagating mode may be different, so make sure to set the Trans. Focus position as appropriate.

6.2.1. Enabling transmission illumination

To enable Transmission Illumination on systems with this capability, turn on the Trans. Illum. slider at the top right of the Live window as shown in the figure below.

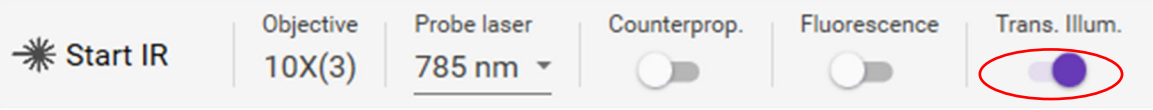


Figure 6-2. Enabling transmission illumination

6.2.2. Trans illumination focus adjustment

Once enabled, use the Trans. Focus controls on the Stage panel to adjust the focus position for best contrast.

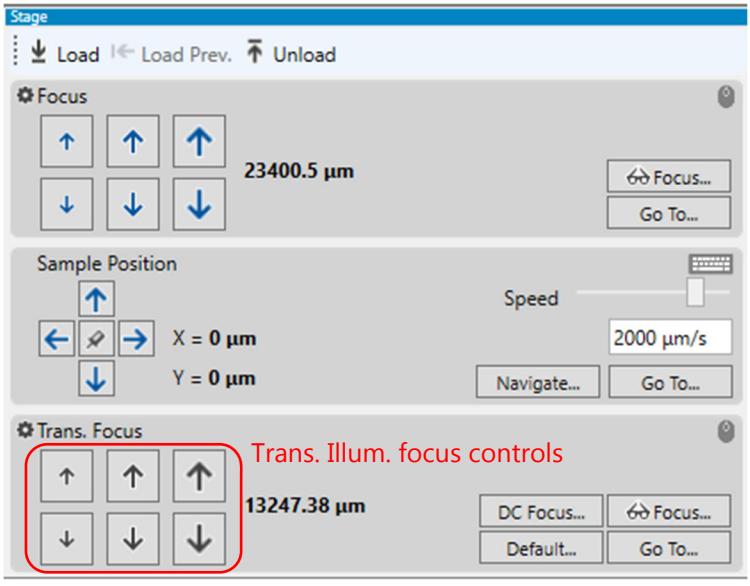


Figure 6-3. Adjusting focus for Transmission Illumination

6.3. Infrared polarization control

This section outlines the use of the optional feature of infrared polarization control.

6.3.1. Polarization control background

Polarization is an optional feature of the mIRage that can be configured at the time of purchase. The polarization control system consists of a wire grid polarizer placed in the IR beam path that can be rotated to different angles under computer control. Acquiring spectra and/or O-PTIR images as a function of polarization can give information about molecular orientation. Note that rotating the polarizer away from 0° will result in reduced IR power. For that reason, it is recommended to maintain the polarization angle between ±45°.

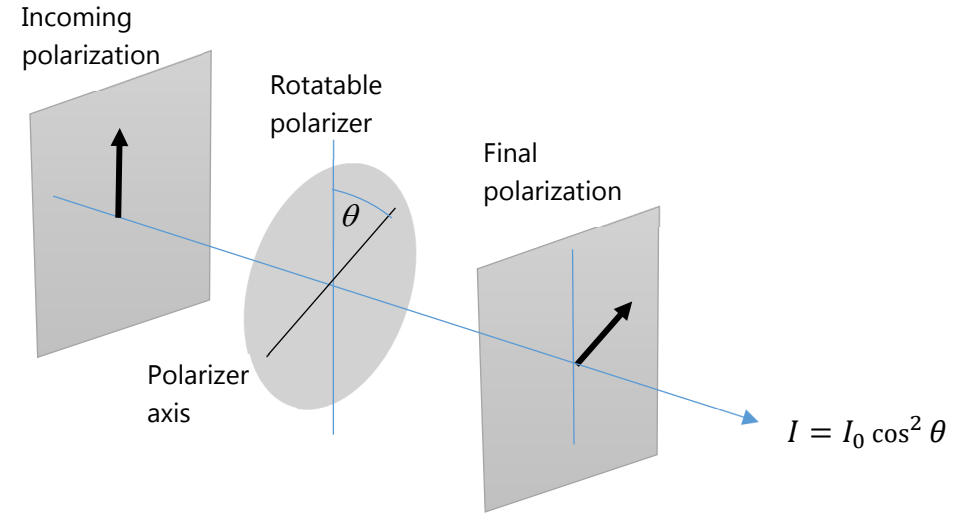


Figure 6-4. Concept of the IR polarization control

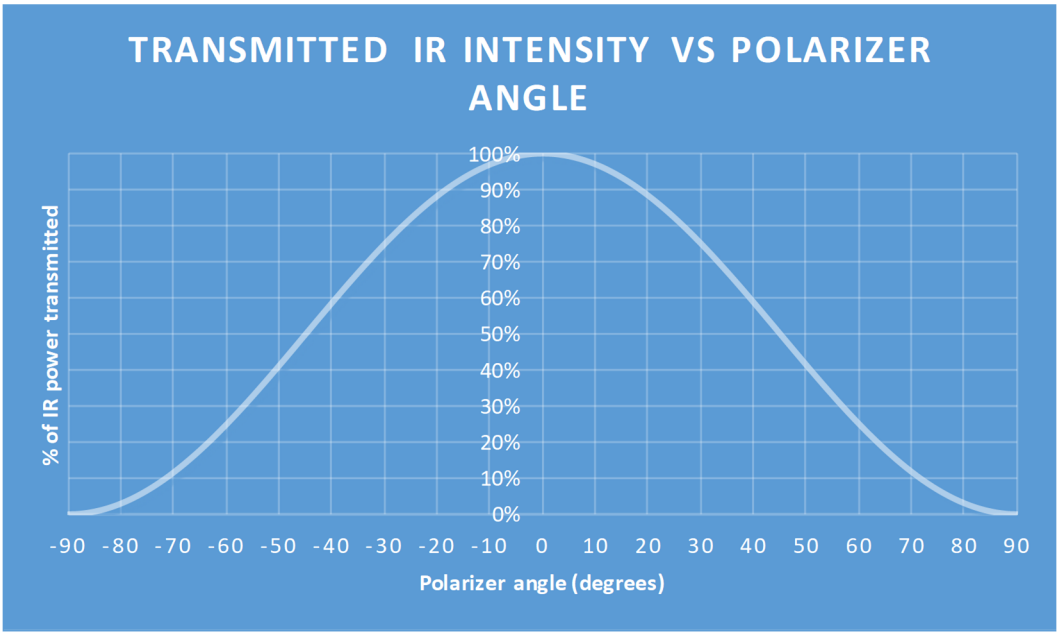
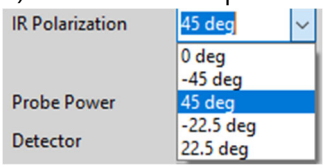


Figure 6-5. IR power versus polarization angle.

6.3.2. Using Polarization Control

1) Enable polarization control using the Polarization slider on the top of Live window. 

2) Select a desired polarization from the IR Polarization drop-down menu.



3) Collect an Auto Background using the procedure described in Section 0.

4) Save the Auto Background with a name that includes the polarization angle.

6.4. Raman polarization control

Raman polarization is a special optional feature of the mIRage that can be configured at the time of purchase. If your system is configured with Raman polarization control, the polarization of the probe beam angle can be adjusted as well on the IR General Panel on the Spectra tab of the Live window.

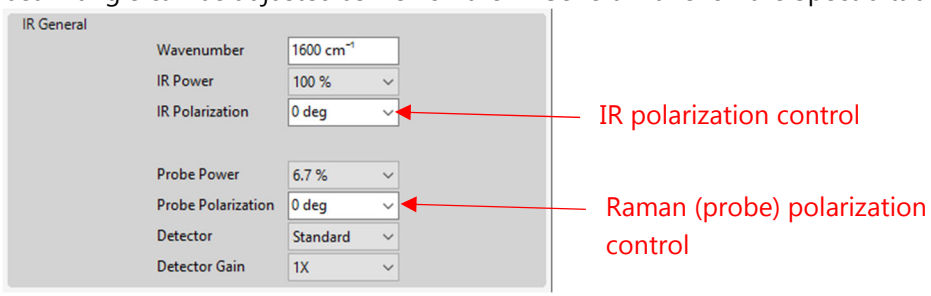


Figure 6-6. IR and probe beam polarization control

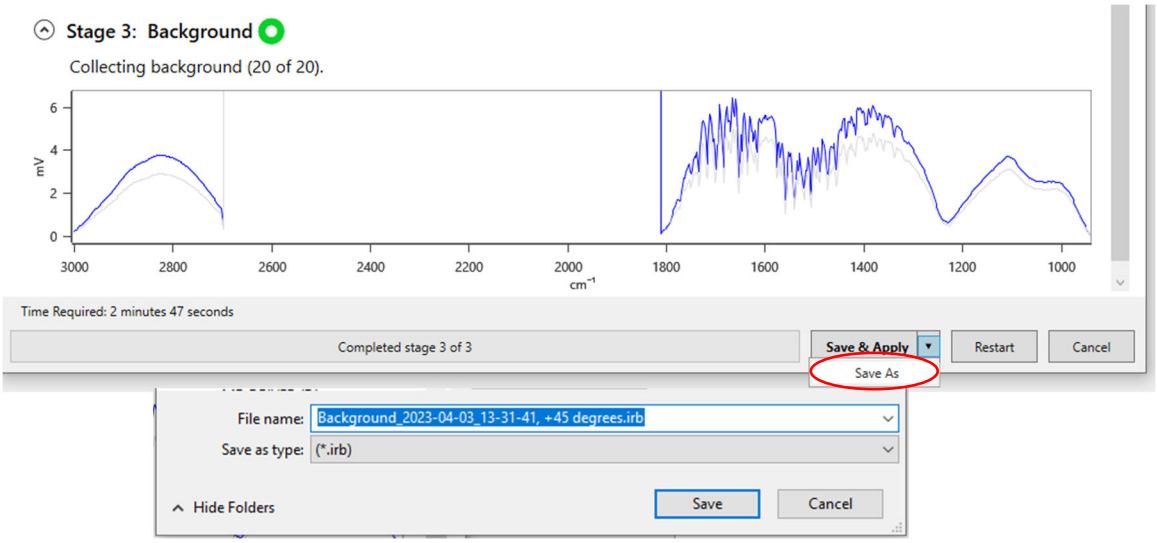


Figure 6-7. Saving auto-background with polarization angle.

5) Click on the Optimize button on the Spectra tab and save the current Optimize points to a file including the current polarizer angle as shown in the figure below.

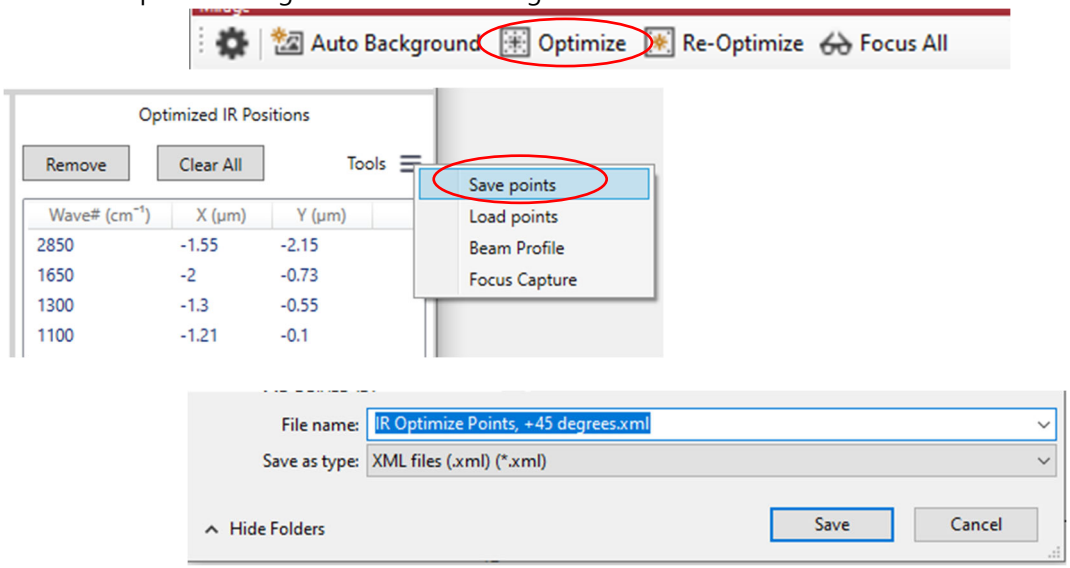


Figure 6-8. Saving optimize points to a file containing the polarization angle in the name.

6) Repeat steps 2-5 for all desired polarizations. Recommended polarizations are -45°, 0°, and +45°. If more polarizations are required, it is recommended not to go outside the range ±45°.

7) Select the polarization angle desired for the first measurement.

8) Load the previously saved background for the current polarization.

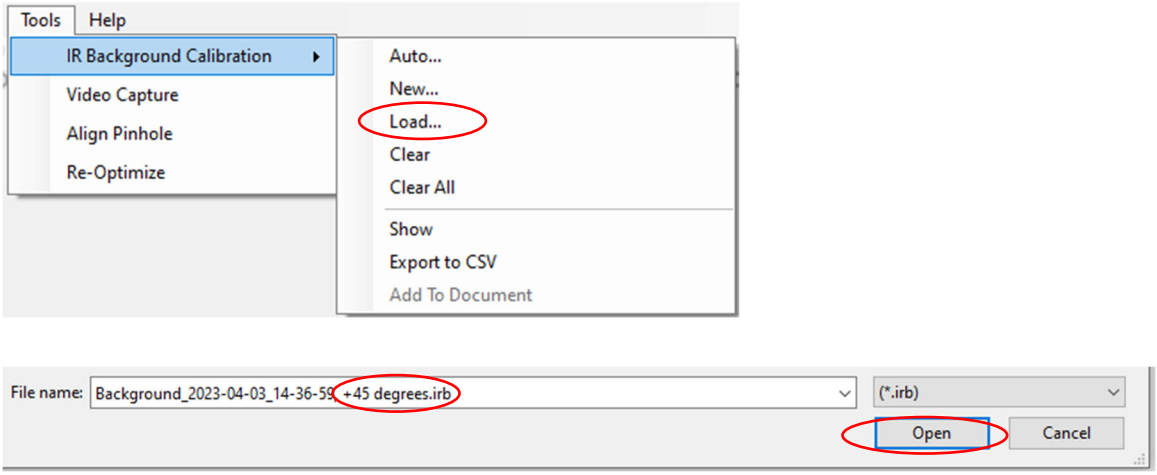


Figure 6-9. Loading a previously saved Auto Background.

9) Load the previously saved Optimize points for the selected polarization.

10) Acquire spectra and/or images of sample at the selected polarization.

11) Repeat steps 7-10 for any other desired polarization angles.

6.5. OPO Laser

Some mIRage systems can optionally be configured with an additional optical parametric oscillator (OPO) laser. To use this laser, do the following:

1) Deinitialize the mIRage system.
2) On the Document window select Setup/Hardware Configuration.
3) Select OPO as the Active config as shown below.
4) Initialize the software.

After reinitialization, the software will display the available tuning range and tuning speeds for the OPO. Note that the OPO tunes much slower than the QCL, so acquiring IR spectra will take longer.

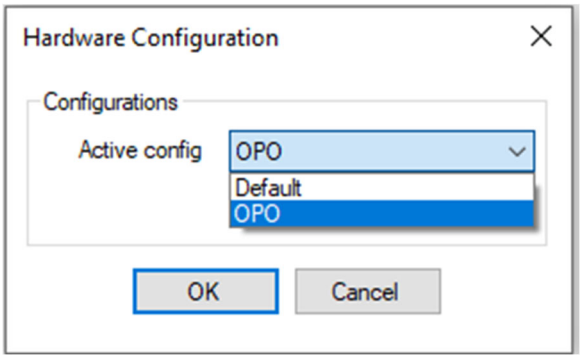


Figure 6-10. Selecting OPO IR laser (if supplied)

To switch back to the QCL, repeat the above process, but select the Default or QCL option for the Active config.

Chapter 9

7.Advanced operation

This section documents certain advanced features. These features provide advanced users additional flexibility/capability for certain measurements.

7.1. Intelligent Co-averaging

Intelligent Co-averaging is a patented[1] technique for significantly improving the signal-to-noise ratio (SNR) of co-averaged spectra. The Intelligent Co-averaging approach analyzes a series of co-acquired spectra to separate features that correspond to either signal or noise and then suppresses the noise. Specifically, this approach analyzes a series of acquired spectra to find spectral features that are common between the co-acquired spectra (the signal) and rejects features that are different (the noise). Example results of Intelligent Co-averaging are shown below in Figure 7-1. Intelligent co-averaging can reach SNR values that are far higher than can be achieved with additional co-averaging.

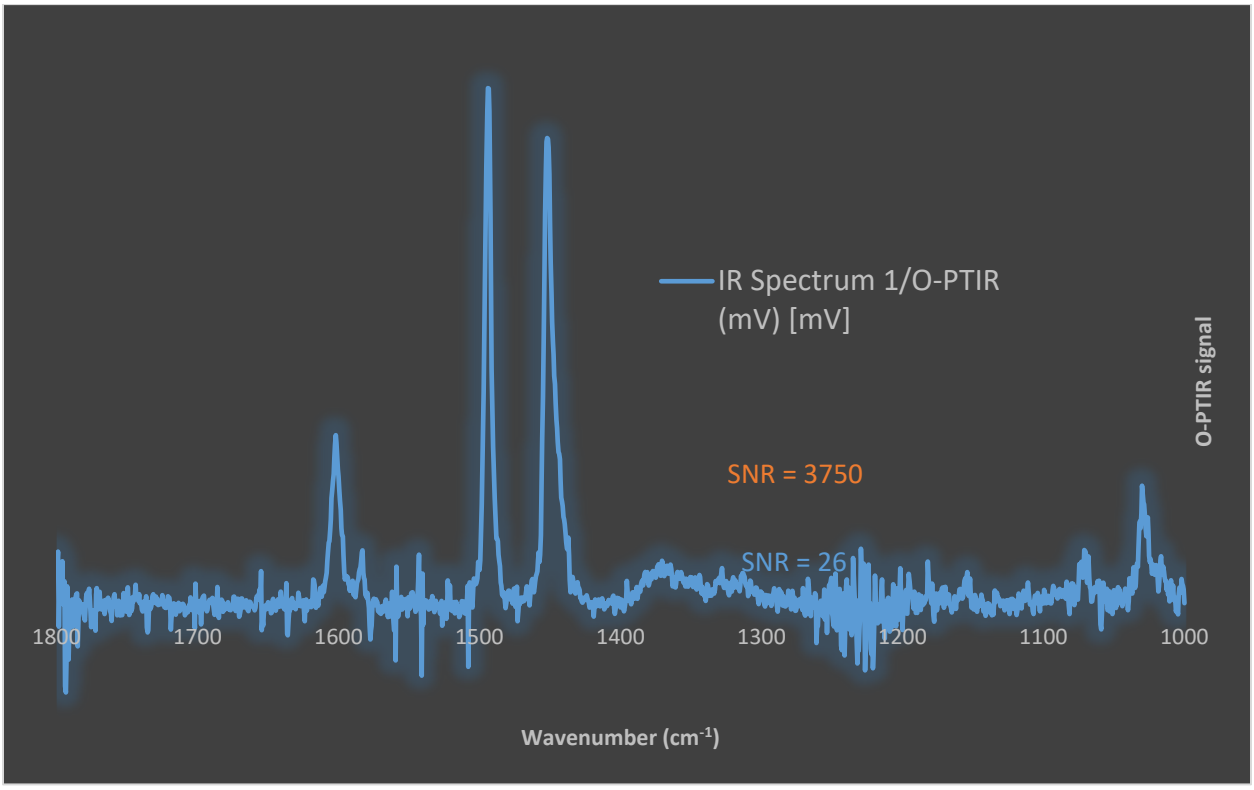


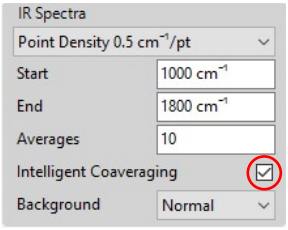
Figure 7-1. Intelligent Co-averaging

[1] U.S. Patent No. US10809184B1

7.1.1. How Intelligent Co-Averaging Works

Each spectrum is deconvoluted into a series of wavelets and corresponding wavelet amplitudes. A signal to noise ratio (SNR) is calculated for each of the wavelet amplitudes. A high SNR indicates that a specific wavelet/wavelet amplitude is part of the signal whereas a low SNR indicates the wavelet comprises noise. The wavelet amplitudes are sorted by SNR into a so called "scree plot" of decreasing SNR. A cursor is used to set the dividing line between signal and noise. Then the spectrum is recalculated using only the wavelets and wavelet amplitudes to the left of the cursor. This approach can provide dramatically better results than simple averaging because it maintains the features that are common between spectra (the signal) and rejects those that are different (the noise).

7.1.2. Using Intelligent Co-averaging



To use Intelligent Co-averaging, do the following:

1) On the Spectra tab, click the Intelligent Co-averaging check box on the IR Spectra panel on the mIRage window.
2) Select the number of co-averages desired. Usually, 5-10 is a good number.
3) Click on Acquire.
4) When acquisition is complete, the Intelligent Co-Averaging window pops up.
5) Slide the scree plot cursor left and right while monitoring the resulting spectral quality and residual. Generally, the best results will occur when the cursor is positioned at a knee in the scree plot. Be careful not to position the cursor too far to the left or wavelet artifact spikes will appear in the filtered data.
6) Click on the Both Averages button when done to write the traditional co-averaged and Intelligent Co-averaged data to the document.

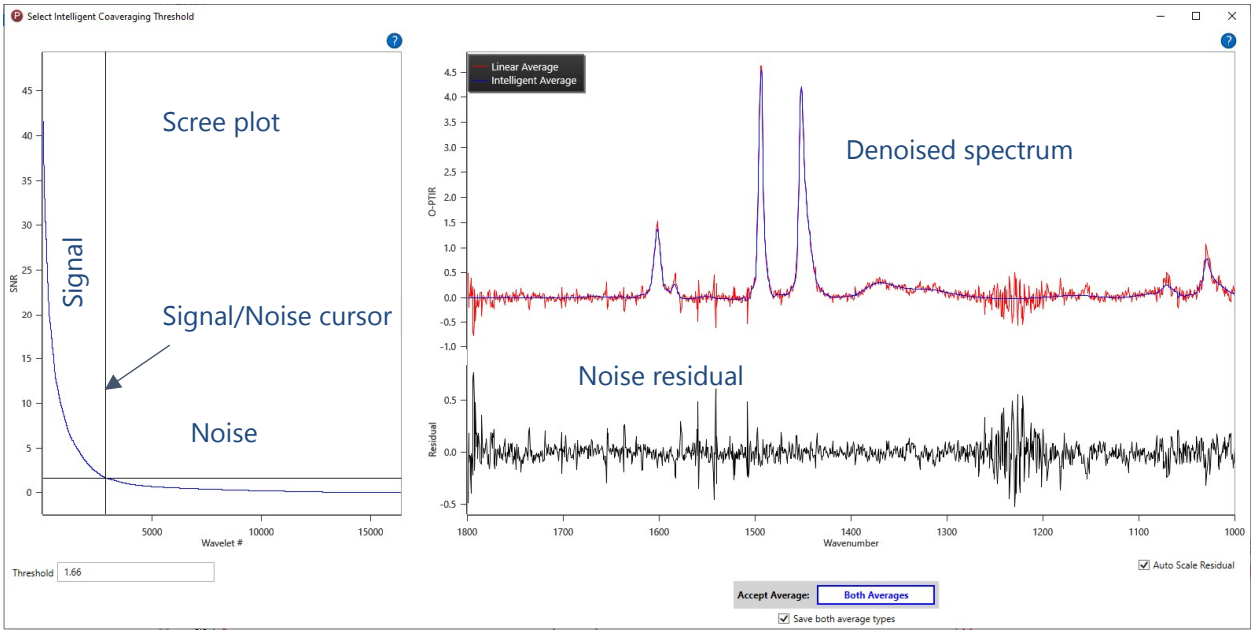


Figure 7-2. Intelligent Co-averaging

7.2. Data channel editor

The Data Channel Editor can be used to change the display settings of O-PTIR data channels for spectroscopy and can be used to add custom data channels for O-PTIR imaging and spectroscopy.

The Data channel editor is preconfigured with three standard data channels, O-PTIR, Phase, and DC. The O-PTIR signal is the amplitude of modulation of the probe beam intensity in response to IR absorption by the sample. The Phase signal is a measurement of the relative time delay between the onset of an IR pulse and the photothermal response in the O-PTIR signal. The Phase signal can be useful for visualizing differences in thermal relaxation times between different regions of a sample. The DC signal is a measurement of the average intensity of probe light scattered from the sample and is similar to a bright-field image of the sample. (The DC signal will represent an epi/reflection image when using the standard detector and a transmitted light image when using the transmission detector.)

These three standard channels should not be modified (except if you wish to change the display color).

Please contact PSC support if you are interested in adding other custom data channels.

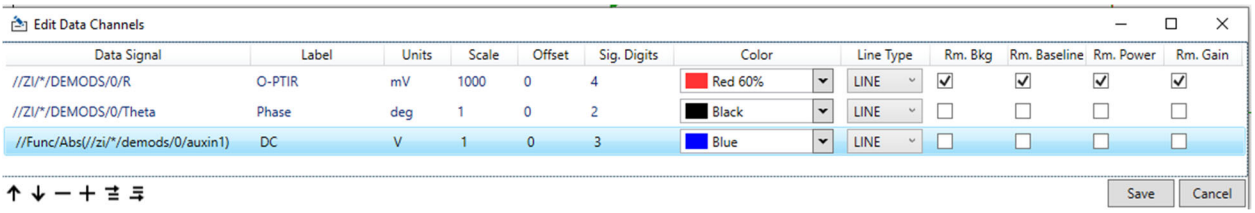


Figure 7-3. Data channel editor

7.3. Manual Background

Generally, PSC recommends using the Auto Background feature described in Section 0. If there is a compelling reason to perform a manual background, it can be accessed using Tools/Background/New…

The manual background will use the currently selected detector, which may not be the detector configured for the Auto Background. Adjust the background settings as desired, e.g., the Start/End of the IR sweep and the number of averages and then click Acquire. Click Save… to save the background and Done to exit.

Figure 7-4. Starting a manual background.

Figure 7-5. Running a manual background

7.4. Manual Optimize

The Optimize function is used to adjust the overlap between the IR and probe beams. In general PSC recommends using the Auto Background (Sec. 0) and/or the Re-Optimize (4.6) functions as both include automated Optimize routines on the appropriate reference sample. In specialized cases it may be desirable to perform an optimize directly on a sample of interest, e.g., when using a water immersion objective or on a sample of non-standard thickness. In this case, the Optimize function can be accessed manually using the Optimize button on the mIRage panel of the Live window.

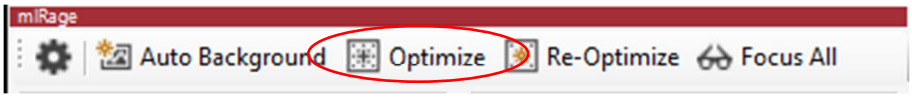


Figure 7-6. Manual optimize button

The Optimize button opens a pop-up window with controls for optimizing the IR/probe beam overlap. The table in the upper right of the IR Optimize window shows the current Optimized IR Positions, a table of XY offset positions for each stage of the IR laser. To update optimize positions for any QCL stage:

1) Click on one of the wavenumbers in the position table to select that wavenumber.
2) Click the Scan button. The system will raster the IR beam position while measuring the O-PTIR signal to produce an image of the signal vs. XY offset (essentially an image of the IR spot).
3) Adjust the position of the IR spot image until it is centered on the cursor
4) Click Update.
5) Repeat steps 1-5 for other QCL stages as desired. Note only one wavenumber can be used for each QCL stage. The QCL stage tuning ranges are shown at the top of the window.

6) Click OK to exit.

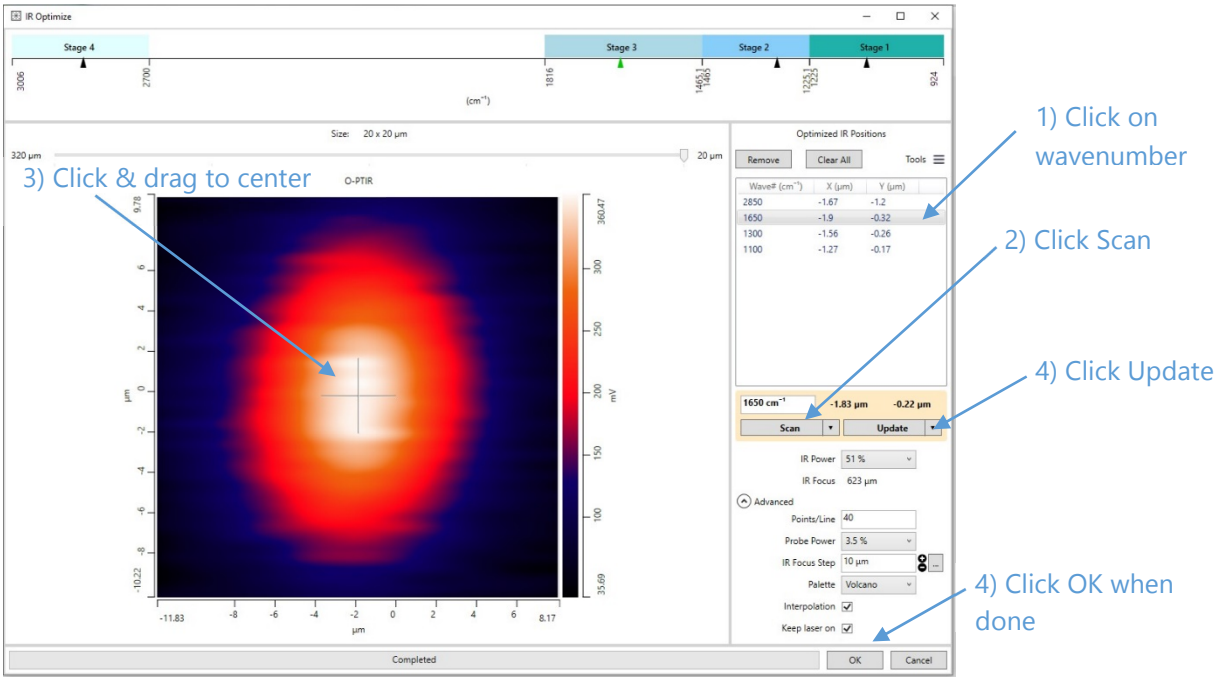
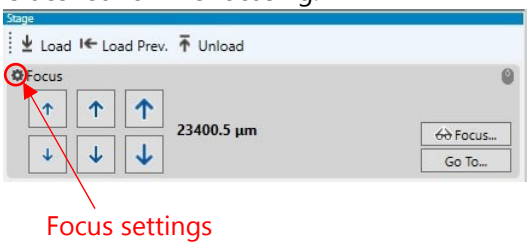
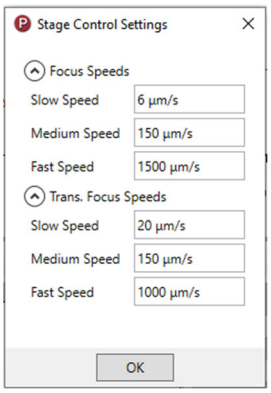


Figure 7-7. Manual Optimize

7.5. Adjusting focus speeds

Focus speeds can be adjusted by clicking on the settings (gear icon) on the Stage panel. The default focus speeds are shown below. It is generally recommended to keep these default values unless a slower speed is desired for fine focusing.





7.6. Changing the backup directory

Unsaved data is automatically saved to the backup directory. The default location for the backup directory is C:\Program Data\Photothermal\PTIR Studio\Backup.

It is recommended to choose a backup directory location that is automatically backed up by your IT department to prevent any data loss in the case of a computer hard drive failure. To change the backup directory, select Setup/Change Backups Directory… and then navigate to a desired backup location and click OK to select. Unsaved data will be placed in the backup directory using a naming convention of AutosaveNNNN.ptir, where NNNN is a four-digit sequential number as described in Section 4.8.4.

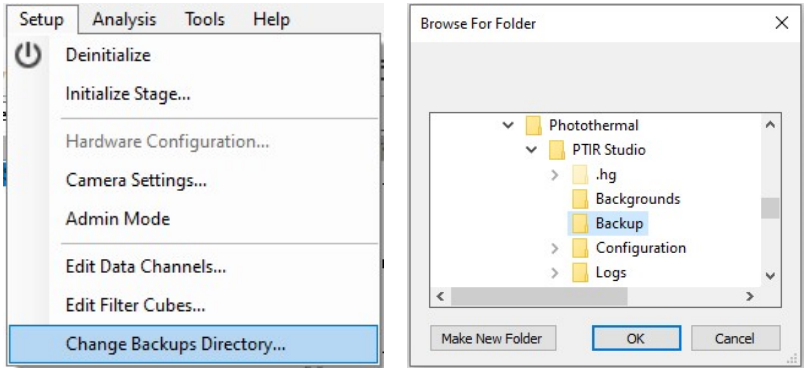


Figure 7-8. Changing the backup directory

Chapter 10

8. Troubleshooting

This section details outlines some potential sources of trouble and potential remedies. For additional assistance contact the PSC support hotline. Email: support@photothermal.com Tel: 1-(805) 845-6568.

8.1. Troubleshooting table

Problem	Potential Cause	Potential solutions	Section references
No O-PTIR signal	DC signal saturated	Turn down probe power and/or gain	4.7.8
	Sample holder blocking IR beam (counter-prop mode)	Move away from sample holder lip	3.5
	Check pinhole	Open or align pinhole	**Error! Reference source not found.**
Weak signal	Sample not at best focus	Focus All	4.7.9
	Poor IR/probe beam overlap	Run Re-optimize	4.6
	IR and/or probe power too low	Increase IR and/or probe power	4.7.10
Unstable DC or O-PTIR signal	IR or probe power too high	Turn down IR and/or probe power	4.7.10
Noisy spectra	IR/probe power too low	Increase IR and/or probe power	4.7.10
	Weak sample absorption	Increase number of spectra averages	4.8.2
Poor spectral quality	IR power background change	Take new auto-background	0
Poor spatial resolution/O-PTIR image blurry		Use Line Focus Tool to optimize	4.11.5
Streaks in O-PTIR image	Image scan speed too high	Reduce image speed	4.11.3

8.2. System Errors

In the case of a system error, try the following steps in order until the issue is resolved

1) Quit and restart the PTIR Studio.
2) Reboot the computer and then restart PTIR Studio
3) Power cycle all the system components, reboot the computer, and then restart PTIR Studio

If the steps below do not resolve your issue, please contact Photothermal Support or your local representative for further assistance. Please capture a screen shot of any error message(s) you see and share this information along with any details of steps taken to produce the error.

Email: support@photothermal.com
Tel: 1-(805) 845-6568

9. System shutdown procedure

The mIRage system is designed to run continuously day and night. If the system is not being used overnight, it is fine to leave it powered on. For times when the system is not in use, but remains powered, we recommend leaving the system in the De-Initialized state.

1. Select “Stop IR” to stop any measurements
2. Select the Deinitialize button

For when the system will not be used for extended periods the system should be powered down. Also, if desired, the system may be powered down over weekends for improved energy efficiency. To power down the system, use the following procedure:

1. Select “Stop IR” to stop any measurements
2. Select the Deinitialize button.
3. Close PTIR Studio.
4. Turn off the power to the mIRage power supply, using the switch on the front of the power supply.
5. Turn off the QCL laser by pressing and holding down the power button. A tool may be required to access the power button inside the mIRage enclosure.
6. Turn off the IR laser chiller.
7. Turn off the Thorlabs stage controller.
8. Turn off the Zurich lock-in amplifier.
9. Turn off the Olympus illuminator power supply.
10. Turn off the computer using the normal computer shutdown procedure.
11. Turn off the computer monitors.
12. Turn off the main power strip to the system, using the switch on the power strip.

Refer to Chapter 2 to locate each of these system components and their power buttons.

10. List of Figures

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