HORIBA Raman Microscope Operating and Training Manual

University of Arizona Nanofabrication Facility

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Quick Operating Steps

Use this section only after hands-on training. Detailed explanations, screenshots, definitions, and LabSpec 6 guidance are provided later in this manual.

Step 1 — Confirm authorization and sample suitability

• Confirm your training/session and that the sample and holder are approved.
• Do not continue with an unknown, leaking, shedding, or unapproved sample.

Step 2 — Mount the sample

• Secure the sample flat and stable in the approved holder.
• Check objective clearance and stage travel before focusing.

Step 3 — Start the system

- Turn on the Marzhauser XY-stage controller if it is not already operating.
- Turn on the Olympus brightfield lamp and set the brightness above zero.
- Open LabSpec 6 and wait for the instrument controls to load.
- Confirm Exit mirror → CCD (steady state) before acquisition. See the LabSpec 6 Software Guide for the exact controls..

Step 4 — Find and focus the sample

- Start with the 10× objective and live camera image.
- Use XY to center the target and slowly adjust Z/focus until the surface is sharp.
- Switch to 50× and refocus carefully for the first Raman measurement.

Warning: Do not make a large Z movement after switching to 50×; the objective is closer to the sample.

Step 5 — Configure the Raman optical path

- Pull the trinocular selector fully out to the camera position..
- Select the documented 785 nm Raman routing positions.
- Do not open optical covers or manually adjust exposed mirrors or laser alignment.

Step 6 — Enable the 785 nm laser

- Keep the laser-head aperture Closed during startup..
- Turn the 785 nm emission key from Standby to On..
- Open the laser-head aperture only after the sample and optical path are ready..

Step 7 — Confirm acquisition settings

- Use only facility-approved settings for the sample type.
- Confirm 785 nm, 50×, grating, slits, spectral range, Exit mirror → CCD (steady state), and save location..
- Auto Exposure may adjust acquisition time; it does not establish safe laser power.
- If approved power, exposure/Auto Exposure, or accumulation settings are unknown, stop before exposure.

Step 8 — Collect and monitor

- Collect the first short Raman measurement to verify usable signal and check for saturation before the intended measurement.
- Watch for movement, heating, visible damage, strong fluorescence, or signal drift.

- If the sample changes unexpectedly, stop acquisition and close the laser-head aperture immediately.

Step 9 — Save the data

- Keep the original data.
- Save the LabSpec file and required metadata.
- Export a text copy when needed for external analysis.

Step 10 — Leave the instrument safe

- Close the laser-head aperture.
- Return the 785 nm emission key to Standby.
- Follow the detailed Shutdown Procedure in this manual.
- Remove the sample and clean only by the approved method.

Before You Begin

Purpose, Scope, and Sample Compatibility

This manual is for trained users performing routine Raman and photoluminescence measurements.

Sample compatibility

Use clean, dry, stable, non-shedding samples that can be secured with safe objective clearance. Common examples include wafers, chips, thin films, crystals, ceramics, glasses, polymers, coatings, and other stable solids.

Ask facility staff before using powders, flakes, fibers, nanoparticles, liquids, gels, pastes, reactive or hazardous materials, unusual holders, or external equipment. Do not load leaking, wet, unidentified, loose, shedding, or unapproved samples.

Instrument OverviewW

The microscope focuses on the sample, the selected laser excites it, the iHR320 separates the collected light, and the Synapse/Symphony II detector records the spectrum. LabSpec 6 controls the measurement and data.

Physical Setup and System Components

The system consists of an Olympus microscope, motorized Marzhauser XY stage, camera, optical-routing controls, laser sources, iHR320 spectrometer, Synapse/Symphony II detector, and LabSpec 6 computer.

For routine Raman use:

- 10× is used to find and initially focus the sample; 50× is the starting Raman objective.
- 785 nm is used for Raman. The 532 and 633 nm lasers are reserved for photoluminescence.
- The camera is used for viewing and focusing; pull the trinocular selector fully out to the camera position before enabling a laser..
- LabSpec 6 controls acquisition and records the data.

Warning: Do not force the motorized stage, open optical/electronic housings, move aligned mirrors, disconnect cables, or adjust unlabeled controls.

Safety and Required Training

Operate the system only after required facility training and authorization, with an active facility session and an approved sample.

Keep optical/electronic housings closed and never bypass an interlock. A triggered interlock means the safety system interrupted laser operation because a required safe condition was not met. Recovery steps are under Laser and Acquisition Settings.

Obtain facility approval before using hazardous, reactive, volatile, wet, loose, shedding, biological, or otherwise unusual samples. Follow the approved containment, PPE, cleanup, and waste procedure.

Startup Procedure

1. Inspect the instrument and clear the stage area. Confirm each laser-head aperture is Closed and each emission key is in Standby.
2. Turn on the Marzhauser XY-stage controller and Olympus brightfield lamp if needed, then open LabSpec 6.
3. In Acquisition → Instrument setup, confirm Exit mirror → CCD (steady state). In Info → Parameters → Acq, confirm Detector reports Synapse/Symphony II.
4. Open the live camera view. If the hardware does not respond normally, stop and contact facility staff.

Sample Preparation and Loading

1. With the laser aperture Closed and key in Standby, place the 10× objective in position and create enough clearance to load the sample safely.
2. Secure the approved sample in the approved holder with the measurement surface facing upward.
3. Check from the side that the sample, holder, and objective cannot collide. Move the stage slowly and confirm the expected travel area is clear.

Microscope Setup and Focusing

1. Set the system for brightfield viewing and open the live camera image.
2. With the 10× objective, use XY to center the target and slowly adjust Z until the surface is sharp.
3. For Raman, switch to 50× and refocus carefully. Confirm the physical objective and the LabSpec objective setting match.

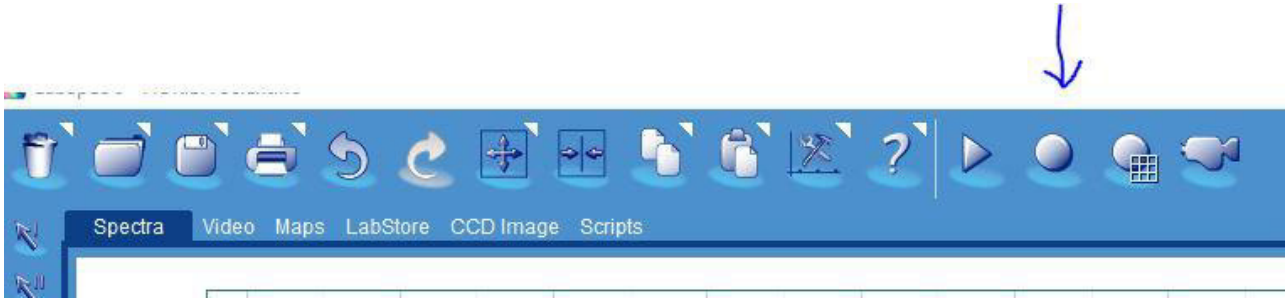
Warning: Do not use large or rapid Z movements after switching to 50×, and never allow the objective to contact the sample.

Laser and Acquisition Settings

785 nm Raman setup

1. Confirm the sample is focused and pull the trinocular selector fully out to the camera position..
2. Set the Raman path: laser selector → 785 nm; filter wheel → 1 (Spectroscopy/Signal); lower filter → II (785 nm); rear selector → I.
3. Keep the laser-head aperture Closed and turn the 785 nm emission key from Standby to On..
4. Set the upper method control to Laser Viewing (II), open the laser-head aperture, and verify the spot on the live camera. Do not use Z to shrink the spot..
5. Close the laser-head aperture when viewing is complete. For acquisition, stop live video if required and set the upper method control to Spectroscopy (III)..
6. Confirm the physical 50× objective and x50 in LabSpec, 785 nm, grating, slits, spectral range, CCD (steady state) exit mirror, and Synapse/Symphony II detector readout.

7. Click Spectrum acquisition (Ctrl+A) to collect the first spectrum. Check the first result before continuing with additional measurements.



Triggered interlock

If the interlock trips, close the laser-head aperture, pull the trinocular selector fully out to the camera position, and turn the 785 nm emission key to Standby. Re-enable only after the safe configuration is restored. If it trips again, stop and contact facility staff. Never bypass the interlock.

To stop the beam temporarily, close the laser-head aperture. To disable emission, turn the 785 nm emission key to Standby..

LabSpec 6 Software Guide

Use the screenshots as visual references. Values shown are examples; use approved settings for the actual measurement.

Main Page

Use the main window to view the spectrum and active configuration.

LabSpec 6 Software Guide

Use the screenshots as visual references. Values shown are examples; use approved settings for the actual measurement.

Main Page

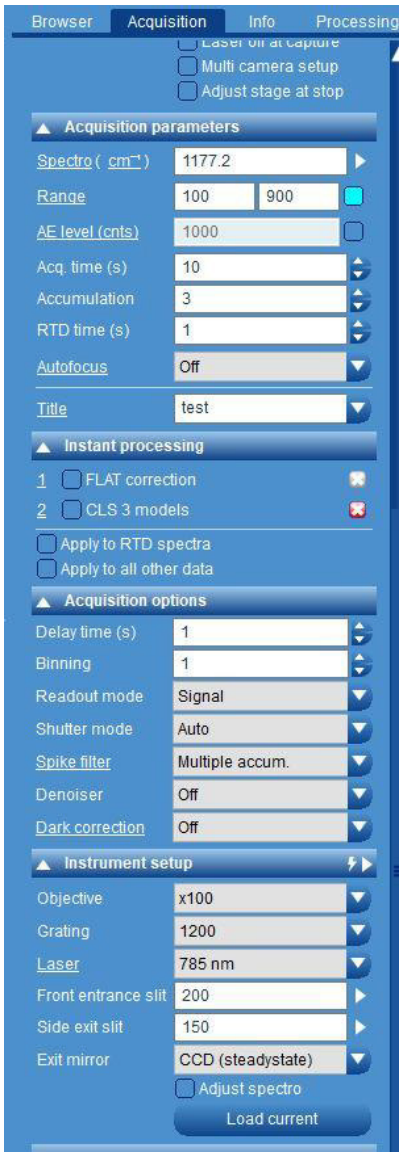
Use the main window to view the spectrum and active configuration.

Browser

Use Browser to select and manage spectra already open in the current LabSpec session.

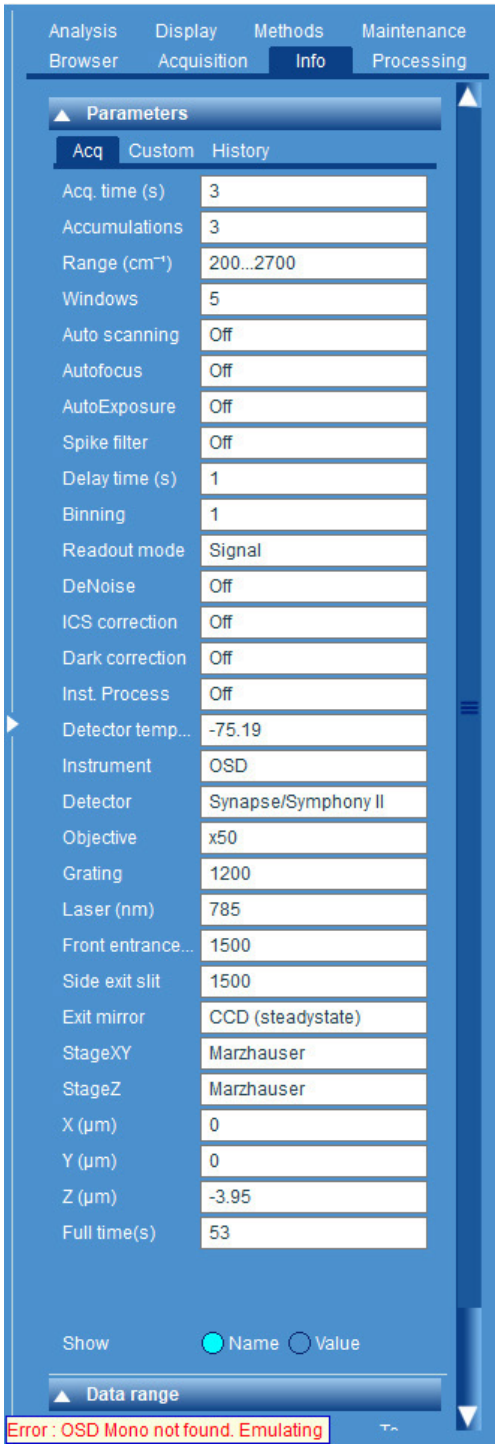
Acquisition

Use Acquisition for measurement parameters and instrument configuration. Confirm approved exposure and accumulations, then check Objective, Grating, Laser, slits, and Exit mirror. Exit mirror must be CCD (steady state).
The Acquisition parameters panel contains Range (spectral range), Acq. time (exposure/integration time), and Accumulation. The Instrument setup panel contains Objective, Grating, Laser, slits, and Exit mirror. Use the Video control for the live camera view.



Info

Use Info → Parameters → Acq to verify settings recorded with the data. Detector should report Synapse/Symphony II. This is a readout, not a selector.


Analysis
Display
Methods
Maintenance
Browser
Acquisition
Info
Processing
Parameters
Acq
Custom
History
Acq. time (s) 3
Accumulations 3
Range (cm⁻¹) 200...2700
Windows 5
Auto scanning Off
Autofocus Off
AutoExposure Off
Spike filter Off
Delay time (s) 1
Binning 1
Readout mode Signal
DeNoise Off
ICS correction Off
Dark correction Off
Inst. Process Off
Detector temp... -75.19
Instrument OSD
Detector Synapse/Symphony II
Objective x50
Grating 1200
Laser (nm) 785
Front entrance... 1500
Side exit slit 1500
Exit mirror CCD (steadystate)
StageXY Marzhauser
StageZ Marzhauser
X (µm) 0
Y (µm) 0
Z (µm) -3.95
Full time(s) 53
Show
Name
Value
Data range
Error : OSD Mono not found. Emulating

Processing

Use Processing for corrections, filtering, baseline, calibration, and data-range tools. Keep the original spectrum.

Analysis

Use Analysis for peak finding, fitting, and map characterization after checking the raw spectrum.

Display

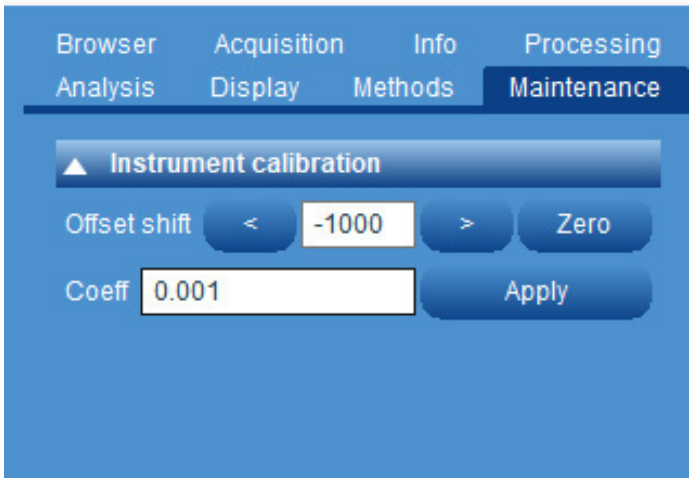
Use Display for axis units, scale, labels, legend, and plot appearance. Display changes do not alter the raw measurement.

Methods

Use only an approved saved method for the intended measurement.

Maintenance

Use calibration controls only according to the facility calibration procedure. Do not change calibration values to make a spectrum appear correct.



Calibration and Performance Check

Before collecting data, confirm the 785 nm Raman path, 50× objective, grating, and Exit mirror → CCD (steady state). Check the LabSpec calibration status for the current configuration..

Collecting a Raman Spectrum

Use the facility-approved power, exposure, and accumulation settings. Collect a short first measurement and check for usable signal, saturation, movement, heating, discoloration, fluorescence, or signal drift.

If the sample changes unexpectedly, stop acquisition and close the laser-head aperture immediately. Increase settings only within the approved method.

Raman Mapping

Use mapping only after a stable point spectrum has been collected. Define the approved line or area, confirm stage clearance across the full map, select the spacing and acquisition settings, then start the map.

Monitor the first points for sample movement, damage, saturation, or loss of focus. Stop the map if the sample or signal changes unexpectedly.

Saving and Exporting Data

Save the original LabSpec data with the sample name and required measurement information. Keep the unprocessed data.

Export a text copy when needed for external analysis. Record any processing applied to a spectrum used for reporting.

Shutdown Procedure

1. Stop acquisition and close the laser-head aperture.
2. Return the 785 nm emission key to Standby.
3. Return the system to the facility-approved safe state and remove the sample only after adequate objective clearance is established.
4. Clean the holder/stage only by the approved method, save all data, close the facility session, and report any abnormal condition.

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Understanding Raman Spectroscopy

Raman spectroscopy measures small energy shifts in laser light caused by molecular or crystal vibrations. The resulting peak pattern can help compare materials, phases, bonding, stress, disorder, or contamination.

Peak position, intensity, width, background, substrate, focus, and acquisition settings can all affect the spectrum. Use the interpretation section and appropriate reference spectra when identifying materials.

Photoluminescence Measurements

Photoluminescence (PL) measures light emitted after optical excitation and can provide information about electronic transitions, band-edge emission, defects, and recombination.

On this system, 532 nm and 633 nm are reserved for PL. Use only the facility-verified PL optical path and acquisition settings; do not adapt the 785 nm Raman route by assumption.

Independent PL procedure

1. Focus and center the sample using the microscope and live camera.
2. elect the facility-verified PL laser and optical path. Use 532 nm or 633 nm only for PL; do not use the 785 nm Raman route by assumption.
3. LabSpec 6, confirm the objective, grating, slits, spectral range, exposure time, accumulations, and detector path for the PL method.
4. Stop live video when required, then start Spectrum acquisition (Ctrl+A).
5. heck the first spectrum for usable signal, saturation, movement, heating, or other sample changes before continuing.
6. Save the original LabSpec data and required measurement information. Warning: Do not infer PL routing or laser-power settings from the Raman procedure. If the PL path or power control is not already established by training, stop and confirm it with facility staff.

Interpreting Common Materials

Interpret the overall spectrum rather than a single peak. Consider peak position, relative intensity, width, background, substrate, and acquisition conditions. Compare unknowns with appropriate reference spectra.

Silicon

Crystalline silicon commonly shows a strong feature near 520 cm^{-1}. On thin films, this may come from the silicon substrate.

Glass and amorphous materials

These often show broader features. A glass substrate can contribute strongly when the sample is thin or weakly scattering.

Polymers

Use the overall pattern and an appropriate reference; different polymers can share individual peaks.

Carbon-based materials

Carbon materials commonly show D- and G-region features. Use an established reference or analysis method for detailed interpretation.

Thin films and layered samples

Signals can come from both the film and underlying substrate or layers.

Strong or rising background

A broad rising background can indicate fluorescence and may obscure Raman peaks.

Weak or nearly flat spectrum

Recheck focus, sample position, optical path, and substrate/background before changing acquisition settings.

Saturated or clipped peaks

Do not interpret flattened or detector-limited peaks quantitatively. Adjust acquisition using the approved method.

Unexpected peaks

Consider contamination, substrate, holder, or another layer. A bare-substrate spectrum can help identify the source.

Before reporting an identification

Confirm that multiple features agree. If the result remains ambiguous, report it as inconclusive rather than assigning a material from one peak.

Troubleshooting

No signal or very weak signal — Recheck focus, sample position, Raman path, objective, laser selection, Exit mirror → CCD (steady state), and acquisition settings..

Strong background or fluorescence — Confirm the signal is from the intended sample area and compare with the substrate/background when useful.

Saturated or clipped signal — Stop and reduce the acquisition using the approved method.

Interlock trips — Close the laser-head aperture, pull the trinocular selector fully out to the camera position, and turn the 785 nm emission key to Standby. Re-enable only after the safe configuration is restored. If it trips again, contact facility staff..

Hardware, calibration, or configuration remains unclear — Stop and contact facility staff. Do not move cables, mirrors, or internal adjustments.

Quick Reference Checklist

Before acquisition: sample secure; objective clearance confirmed; trinocular selector fully out to camera position; correct 785 nm Raman path; 50× objective; Exit mirror → CCD (steady state); Synapse/Symphony II readout; save location set..

After acquisition: laser-head aperture Closed; 785 nm emission key in Standby; data saved; sample removed safely; holder/stage left clean; abnormal conditions reported..

Facility Contacts and Revision History

For unresolved instrument, safety, calibration, sample, or access questions, contact University of Arizona Nanofabrication Facility staff.

Record manual revisions here when facility-confirmed settings, procedures, screenshots, or safety requirements change.

Definitions

Acquisition — The process of collecting and recording a Raman or photoluminescence spectrum.

Accumulation — One of multiple repeated acquisitions that are combined to improve the measured signal.

Auto Exposure — A LabSpec 6 function that automatically adjusts acquisition time. It does not determine a safe laser power for the sample.

CCD (steady state) — The detector output used for routine steady-state Raman and photoluminescence measurements on this system.

Confocal — An optical arrangement that limits light collected from outside the selected focal region, improving spatial selectivity.

Detector — The component that converts collected light into an electronic signal that can be displayed as a spectrum. This system reports the steady-state detector as Synapse/Symphony II in LabSpec 6.

Exit mirror — The LabSpec 6 Instrument setup control that routes the spectrometer output toward the selected detector. For the documented steady-state workflow, select CCD (steady state).

Filter set — The optical filter configuration used with a particular laser wavelength to reject unwanted laser light while allowing the measurement signal to reach the spectrometer.

Grating — A spectrometer component that separates collected light by wavelength.

iHR320 — The HORIBA imaging spectrometer that separates the collected light before it reaches the detector.

LabSpec 6 — The HORIBA software used to control the instrument, configure acquisitions, display and process spectra, and save measurement data.

Laser-head aperture — The physical Open/Closed control at the laser head that allows or blocks the laser beam from entering the optical path.

Objective — A microscope lens used to view and focus on the sample and collect the Raman or photoluminescence signal. This procedure starts with 10× for finding and focusing the sample and uses 50× for the first Raman measurement.

Photoluminescence (PL) — Light emitted by a material after optical excitation. On this system, 532 nm and 633 nm are reserved for PL measurements.

Raman shift — The difference in energy between the incident laser light and the Raman-scattered light, normally displayed in cm^{-1}.

Raman spectrum — A plot of measured Raman signal versus Raman shift. The pattern of peaks can provide information about molecular or lattice vibrations and material composition.

Spectral range — The Raman-shift or wavelength interval recorded during an acquisition.

Substrate — The underlying material that supports a film, coating, particle, or other sample. A substrate can contribute its own features to the measured spectrum.

Synapse/Symphony II — The detector name reported in LabSpec 6 under Info → Parameters → Acq for the documented steady-state detector configuration.

Trinocular selector — The microscope viewing selector that directs the image toward the camera or eyepieces and is associated with the laser interlock on this system.

XY stage — The motorized sample stage used to move the sample horizontally in the X and Y directions.

Z/focus control — The control used to change the vertical focus position so the sample surface appears sharp

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