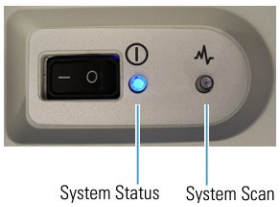
OPT5: Operating Procedure for RaptIR+ Microscope
Thermo Fisher Nicolet iS50 FT-IR Spectrometer

1. **Ensure you have an active OPT5 reservation.**

2. **Add liquid nitrogen to the detector** on top of the microscope and wait ~20 minutes for the detector to stabilize before taking any data.
    a. This step may not be necessary if another user has used the microscope earlier in the same day. Once the software is on, you can click on the green check mark in the top right to check the detector temperature.



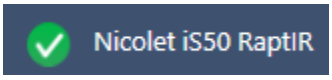
3. **Verify that both the system and the microscope are on.** There should be a blue power light on the back left of the iS50 and a blue power indicator on the RaptIR+.

4. **Check the desiccant on the main module.** The round indicator on the desiccant compartment cap should be blue. If the indicator has turned pink or white, notify SMIF staff and do not use the instrument.

5. **Turn on the Paradigm OMNIC computer.** The black box on top of the two instrument computers has a red light that indicates which computer is active. If the iS50 computer (1) is active, ensure that the OMNIC software is closed and press the gray "SELECT" button to switch to the computer for the RaptIR+ (2), which runs Paradigm OMNIC software.

6. **Open the OMNIC Paradigm software.**

7. **Set the instrument configuration to the RaptIR+ microscope.** This is shown in the top right next to the instrument status indicator. If the instrument is set up for transmission or ATR, you can configure it for the RaptIR+ microscope by (a) touching the RaptIR+ touchpoint or (b) navigating to the Configure menu, then setting the Sample Location to "Right Microscope."



8. **Verify or modify the experiment settings.** There are default settings recommended for all three microscope modes that you can choose from the drop-down menu.
    a. Checking **"Autofocus Before Capture"** will automatically focus the 4x objective on your sample before capturing the initial mosaic image.
    b. **Final Format** - determines the y-axis units that will be used to plot your data. This can be changed after data collection as well.
    c. **Sample Scans** - changes the number of scans taken at each point/step.
    d. **Mosaic Capture** – Current Location
    e. **Camera Profile** – Auto
    f. **Resolution** – 8cm^{-1} Wavenumbers
    g. **Profile** – Peak

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h. ***Analyze Using* –** This determines if you will be doing transmission, reflection, or ATR data collection and CANNOT be changed later.
i. Aperture – Can be changed later, determines the size of the area sampled at one point
j. Step Size – Can be changed later, determines how far the aperture moves before taking another data point

9. **Click on "More"** and verify or modify the rest of the experiment settings.
    a. **Background Scans / Frequency –** it is recommended to take the same number of background scans as sample scans
    b. **Advanced**
        i. **Range Limits –** This is where you can edit the range of wavenumbers you'd like to sample with your spectra
        ii. **Atmospheric Suppression –** It is recommended to turn this on
        iii. Most settings are either determined by the instrument specifications (beamsplitter, detector, source) or pre-set to the recommended values

10. Hit 'eject stage' after verifying there's nothing underneath/near the stage it will run into.

11. Depending on if you will be collecting transmission, reflection, or ATR data, proceed to the relevant section below.

Reflection Mode

12. **Load your sample.** Remove the sample holder from the stage. Pull back the metal slider to put your slide in place, then release to secure the slide in place. Replace the sample holder, ensuring the red dots are lined up.

13. **Ensure the 4x (smaller) objective is active.** If the 15x objective is active, click 'acquisition' and then under microscope, hit the 'change objective' button.

14. **Click "Live Display" to get a live camera view.** Use the top right knob on the joystick panel to turn up the reflection light intensity. Use joystick to position and focus the sample. Rotate the joystick head to adjust the z-position (stage height/focus).

15. **Click 'Start Session'.** The stage will automatically move and capture a mosaic image of your sample, centered at the current location.

16. **Turn on the aperture illumination.** You can do this by clicking on camera view and then the aperture settings tab. Click the box next to 'aperture illumination' and increase the value until the aperture size is visible. This will aid you in fine-tuning the aperture width, height, and rotation to best fit your sample. The aperture size directly correlates to microns.

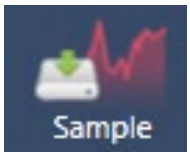
17. **If needed, change the step size.** The step size is only relevant if you are collecting line/area data, as it determines how far the aperture moves between data points. It is recommended that your step size is 5 units smaller than your smallest aperture setting (if your aperture is 20 units wide and 30 units tall, the recommended step size would be 15 units).

18. **Measure a background spectrum.** From the floating toolbar, select the background tool. This will pull up a live view of a single-scan spectrum of the background where the crosshairs are. Click the mosaic at the point where you want to measure the background. You can click around until you are happy with the preview. Once you are happy with the background, hit "Accept Background". Then click "Measure Background."

19. **Specify the areas, lines, and/or points you want to analyze.** Using the floating tool bar, hover over "Background" and select "Point", "Line", or "Area". You can add multiple areas and points to a single analysis.

20. If you want to preview the spectrum in a particular location, hit live spectrum at the top of the window, use the floating toolbar to select the stage tool, and click on the mosaic where you want to see the spectrum

21. If you want to delete a selection region/point, click "Region Queue" in the floating toolbar, right click on the region, and remove it from the queue.



22. The estimated measure time will appear above the 'sample' button at the top of the window. Change the parameters or scan regions/points to change the sampling time, if needed. **Start measurement by hitting "Sample".**

Transmission Mode

23. **Load your sample.** Remove the sample holder from the stage. Pull back the metal slider to put your slide in place, then release to secure the slide in place. Replace the sample holder, ensuring the red dots are lined up.

24. **Ensure the 4x (smaller) objective is active.** If the 15x objective is active, click 'acquisition' and then under microscope, hit the 'change objective' button.

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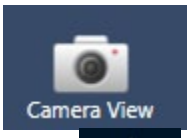
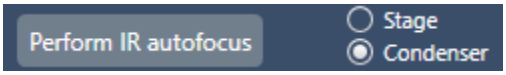
25. **Click "Live Display" to get a live camera view and navigate to your sample.** Use the top left knob on the joystick panel to turn up both the transmitted light and reflected light intensities. This will aid in initially locating your sample.
Use the main joystick to position and focus the sample. Rotate the joystick head to adjust the z-position (stage height/focus).

26. **Click 'Start Session'.** The stage will automatically move and capture a mosaic image of your sample, centered at the current location.



27. **Align the condenser.** Click on "Camera View" and choose the IR Autofocus tab. Use the knob to turn the reflected light all the way down. Instead of selecting 'stage', select 'condenser' and then click "Perform IR autofocus". The condenser will move up and down to find the optimized condenser height. Once this is complete, adjust the knob to optimize the amount of transmitted light so that you can clearly see your sample



28. **Turn on the aperture illumination.** You can do this by clicking on camera view and then the aperture settings tab. Click the box next to 'aperture illumination' and increase the value until the aperture size is visible. This will aid you in fine-tuning the aperture width, height, and rotation to best fit your sample. The aperture size directly correlates to microns.

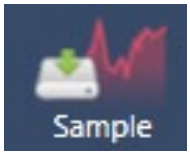
29. **If needed, change the step size.** The step size is only relevant if you are collecting line/area data, as it determines how far the aperture moves between data points.
It is recommended that your step size is 5 units smaller than your smallest aperture setting (if your aperture is 20 units wide and 30 units tall, the recommended step size would be 15 units).

30. **Measure a background spectrum.** From the floating toolbar, select the background tool. This will pull up a live view of a single-scan spectrum of the background where the crosshairs are. Click the mosaic at the point where you want to measure the background. You can click around until you are happy with the preview. Once you are happy with the background, hit "Accept Background". Then click "Measure Background."

31. **Specify the areas, lines, and/or points you want to analyze.** Using the floating tool bar, hover over "Background" and select "Point", "Line", or "Area". You can add multiple areas and points to a single analysis.

32. If you want to preview the spectrum in a particular location, hit live spectrum at the top of the window, use the floating toolbar to select the stage tool, and click on the mosaic where you want to see the spectrum

33. If you want to delete a selection region/point, click "Region Queue" in the floating toolbar, right click on the region, and remove it from the queue.



34. The estimated measure time will appear above the ‘sample’ button at the top of the window. Change the parameters or scan regions/points to change the sampling time, if needed. **Start measurement by hitting “Sample”.**

ATR Mode

ATTENTION: small hard particles will leave pits in the surface of the crystal, do NOT use ATR for hard samples like glass beads, cement, or sand.

35. **Load your sample.** Remove the transmittance/reflectance sample holder from the stage. Take the ATR sample holder out of the case. Pull back the metal slider to put your slide in place, then release to secure the slide in place. Place the ATR sample holder with your sample on the stage, ensuring the red dots are lined up.

36. **Ensure the 4x (smaller) objective is active.** If the 15x objective is active, click ‘acquisition’ and then under microscope, hit the ‘change objective’ button.

37. **Click “Live Display” to get a live camera view.** Use the top right knob on the joystick panel to turn up the reflection light intensity. Use joystick to position and focus the sample. Rotate the joystick head to adjust the z-position (stage height/focus).

38. **Click ‘Start Session’.** The stage will automatically move and capture a mosaic image of your sample, centered at the current location.

39. **Turn on the aperture illumination.** You can do this by clicking on camera view and then the aperture settings tab. Click the box next to ‘aperture illumination’ and increase the value until the aperture size is visible. This will aid you in fine-tuning the aperture width, height, and rotation to best fit your sample. The recommended aperture size for ATR is 100 x 100 units. Because Germanium has a refractive index of 4, this will probe a 25 μm^2 area.

40. **If needed, change the step size.** The step size is only relevant if you are collecting line/area data, as it determines how far the aperture moves between data points. It is recommended that your step size is 5 units smaller than your smallest aperture setting (if your aperture is 20 units wide and 30 units tall, the recommended step size would be 15 units).

41. Ensure that the 15x objective (the larger, bulkier objective) is now in use. Change the objective if needed.

42. **Lower the stage.** Click on the Focus Settings tab in Camera view. Take note of the value for Stage Z (height). *You may want to copy the value to the clipboard or write it down.* Replace the stage height value with "1" and hit enter.

43. **Install the ATR crystal.** Remove the ATR crystal from the carrying case and remove the plastic cover from the ATR without rotating it. Rotating the cap may alter the z-axis alignment of the crystal. Inspect the crystal to ensure it is clean. If you need to clean the crystal, use a cotton pad to wipe off the surface. When you are ready to mount the ATR onto the objective, hold the ATR with both hands and keep it horizontal while slowly and gently pushing it into place.

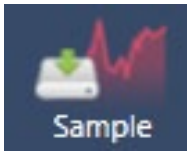
44. **Raise the** stage by replacing the Stage Z (height) value with the value you noted earlier. Note: You will no longer be able to see the sample or rotate the turret to the 4x objective to see the sample with the ATR module in place. From this point, you will rely solely on the initial mosaic captured for navigating around your sample.

45. **In the acquisition tab, under microscope, click the ATR Contact icon.** 

From this tab, you can fine-tune the amount of pressure you would like to put on your sample during collection and the release distance, or how far the ATR will move away from the stage between data points. It is recommended to use a target pressure of 30, but this may vary depending on your sample. A 2,000 micron release distance is also recommended. Longer release distances increase measurement times for batch measurement but ensure that the sample is not dragged, and the ATR crystal is fulling detaching from the sample.

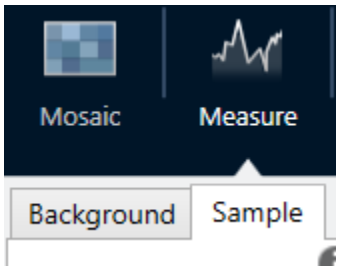
46. **Specify the areas, lines, and/or points you want to analyze.** Using the floating tool bar, hover over "Background" and select "Point", "Line", or "Area". You can add multiple areas and points to a single analysis. You do not need to specify a location for the background, as it will be collected in air rather than on the slide.

47. If you want to delete a selection region/point, click "Region Queue" in the floating toolbar, right click on the region, and remove it from the queue.



48. The estimated measure time will appear above the 'sample' button at the top of the window. Change the parameters or scan regions/points to change the sampling time, if needed. **Start measurement by hitting "Sample".**

Finishing



49. **Process the data.** (If desired.)
    a. **Execute Report** - One way to save your data is to click "Execute Report" in the top left pull-down menu. You can save a pdf that includes the mosaic and your highlighted spectrum.
    b. **Open Spectrum in New Tab** - You can also navigate from the measure tab to the sample sub-tab and select a point/area/spectrum you want. Ensure that it is active/highlighted (it will be red), right click it and say "add selected spectrum to dashboard measurements". You can then go from the "analysis" tab to the "dashboard" tab, look at the most recently added measurement and right-click to rename it. Then right click the spectrum to open it in a new tab.
    c. **Library Search** – Once a spectrum is added to a new tab, you can navigate to that tab and then click 'search' to library search. The results will show up on the right-hand side. You can toggle the eye button to display the match as an overlay with the original spectrum.

    d. **Find Peaks** - Go to "Identify" and then "Find Peaks". You can then click and drag to edit the region you would like to label. You can use the slider at the bottom to adjust the sensitivity. Multiple regions can have different set sensitivities. Then hit save and your spectrum with the labeled peaks will be saved.
    e. **Save Spectrum** – Under the "File" menu, choose "Export Spectrum". SPA is the OMNIC / Paradigm OMNIC format. This saves your interferograms and it always recommended. TSV is compatible to move to excel. Choose your personal directory within the UserData folder on the desktop for the file location.

50. **When finished:**
    a. Go to the dashboard tab and hit **'Eject Stage'**. This will make it easier for you to unload your sample.
    b. **Unload your sample.** If you performed ATR, be sure to use both hands and to keep the plate fully horizontal while removing from the objective. If the plate falls and the crystal bounces against the stage, *there is a high probability the crystal will crack or shatter.* Gently wipe the crystal with a cotton pad (moistened with IPA, if necessary), cap the crystal, and put it in the case.
    c. **Re-install the transmission/reflectance sample holder** on the stage.
    d. **Put the silicon wafer back** over the stage. This prevents debris from falling into the condenser underneath the stage.
    e. **Exit the OMNIC Paradigm software.**
    f. **End your OPT5 reservation.**
    g. ***Leave the FT-IR system power and microscope power on.***