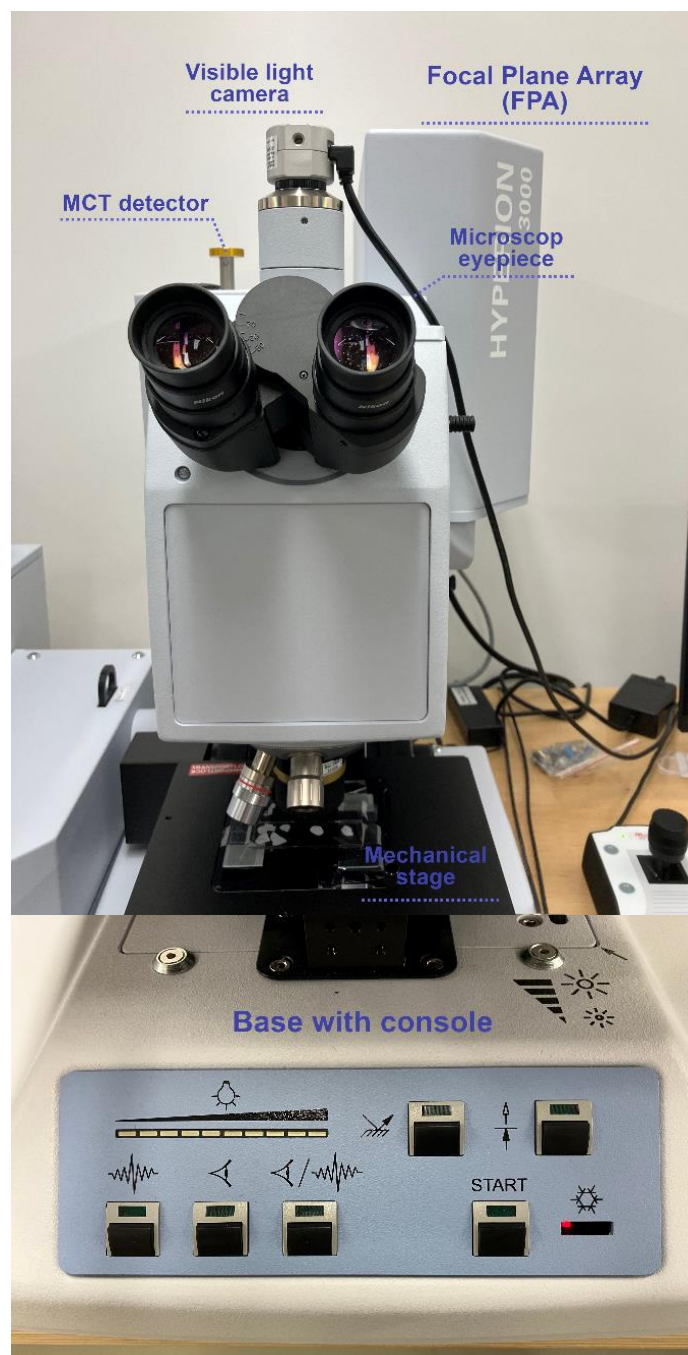


Bruker Hyperion 3000 Infrared Microscope with Vertex 80v Spectroscopy Microscopy Measurement Manual

The main elements of the microscope are two types of detector – MCT and Focal Plane Array (FPA), eyepiece, visible light camera, mechanical stage for sample, and microscope console (Fig. 1). The movement of the stage (left-right and up-down) is controlled by the joystick.



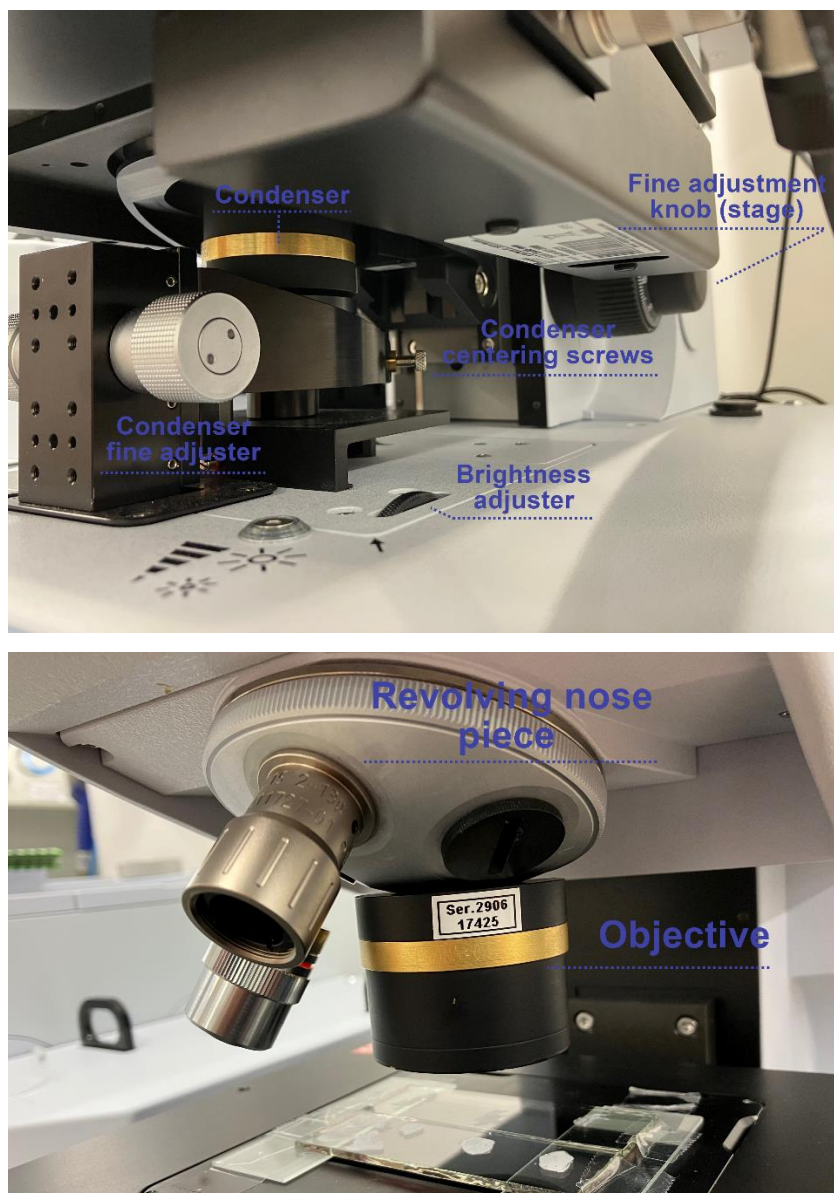


Fig. 1. FT-IR microscope elements

A compressed air source and a vacuum pump are connected to the spectrometer, which contains the standard radiation source, beamsplitter, interferometer, and mirrors system directing the beam to the measuring chamber or microscope (Fig. 2). The microscope is coupled with the spectrometer.

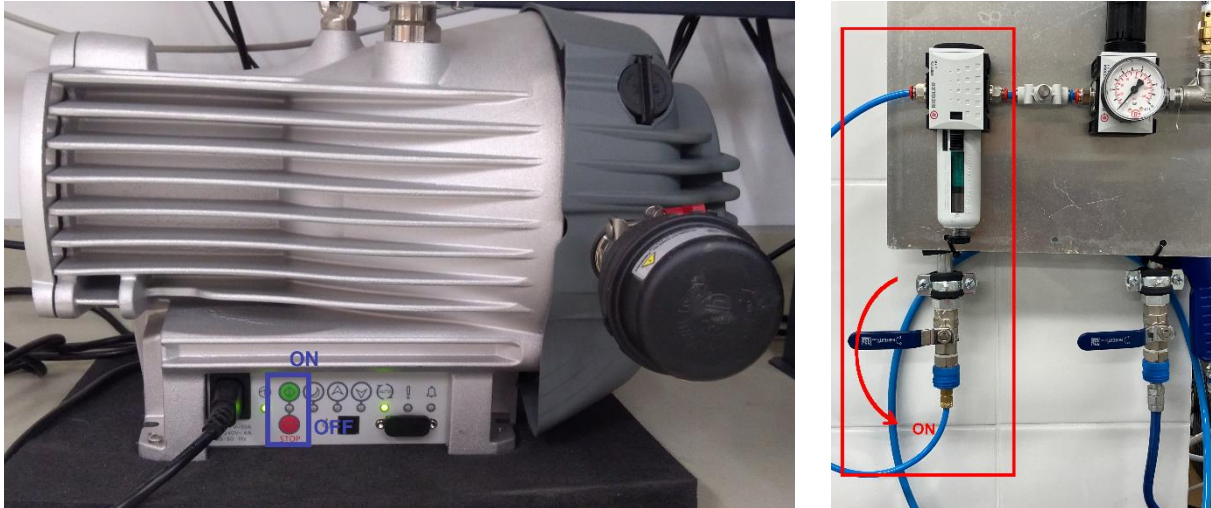


Fig. 2. Vacuum pump and valve of compressed air

Steps of turning on the microscope:

1. Check that the spectrometer is turned on. The LED system indicates the status of the device
2. Start compressed air flow (valve on the wall, turn on the vertical position)
3. Turn on the vacuum pump (green button) and start pumping down the system - open the Internet Explorer browser, select the "Direct Control Panel" item on the loaded page, and then "Evacuate instrument". The pressure and status of the device are continuously displayed. The air in the spectrometer has been pumped out correctly if the status "Evacuated" is displayed (Fig. 3)

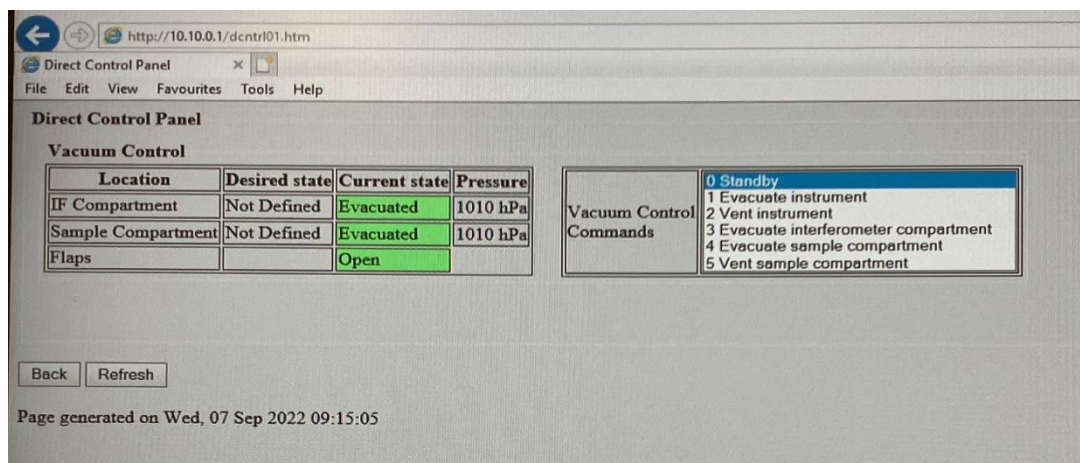


Fig. 3. "Direct Control Panel" of the vacuum pump

4. Turn on the microscope – the button on the rear part of the housing. The FPA detector has a separate switch (bottom part of the detector housing)

5. The detector used for the measurement (MCT, FPA, or both) should be cooled down with liquid nitrogen before starting the measurements*

* Please ask the CIRI beamline scientist for access to liquid nitrogen. Safety glasses, gloves, and protective clothing are required

I. Measurement in reflection or transflection mode with FPA detector

1. The measurement starts with choosing the interesting area of the sample. In the microscope software OPUS choose “Measure”, then “Video Guided Measurement” (Fig. 4),

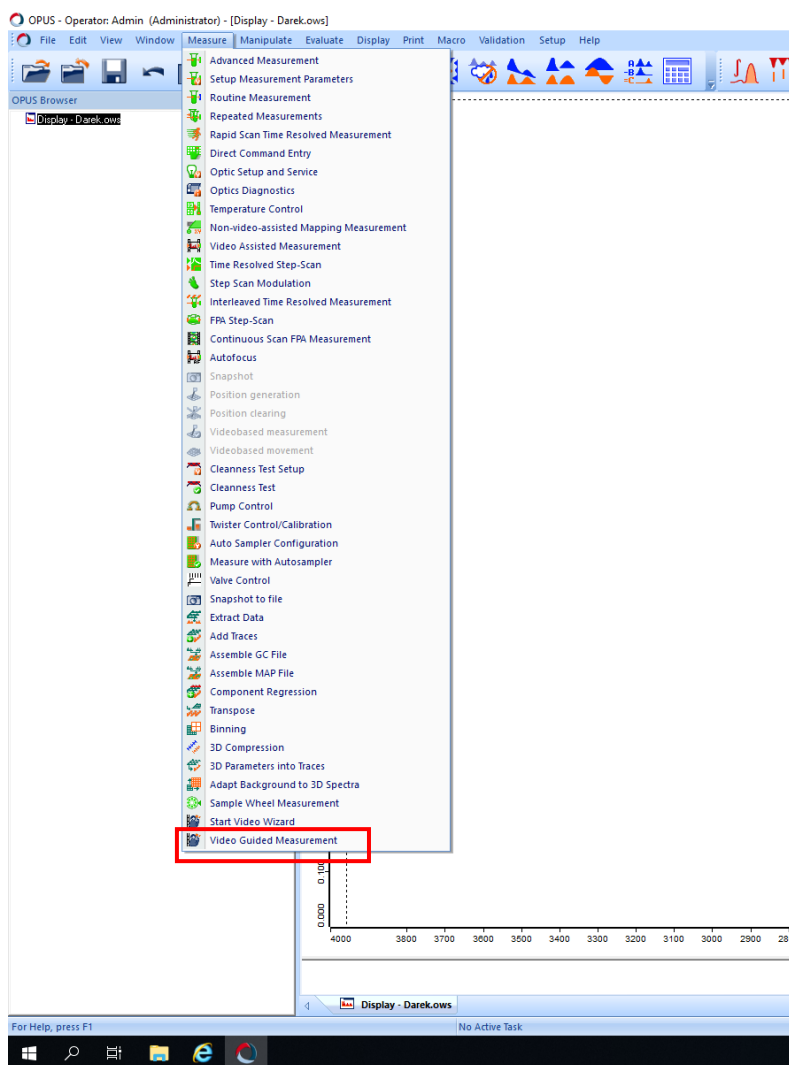


Fig. 4. The “Video Guided Measurement” option

and “Hyperion 3000-FPA”.

In the first panel, select the points that will be used to define the area for which a set of individual photos will be taken using a digital camera - "Collect defined image" (Fig. 5). Before taking a photo, find the focus on the sample surface by moving the table up and down with the joystick. Click “Next”.

Note 1: The microscope must be in reflection mode (See fig. 1). The reflection mode is shown as an arrow bouncing off a horizontal line. The diode should be lit. If this is not the case, use the button.

Note 2: The type of objective we use must match the objective selected in "Video Guided Measurement". Depending on the magnification of the objective, the image is collected with different steps. The program automatically combines individual "tiles" into a full image of the sample.

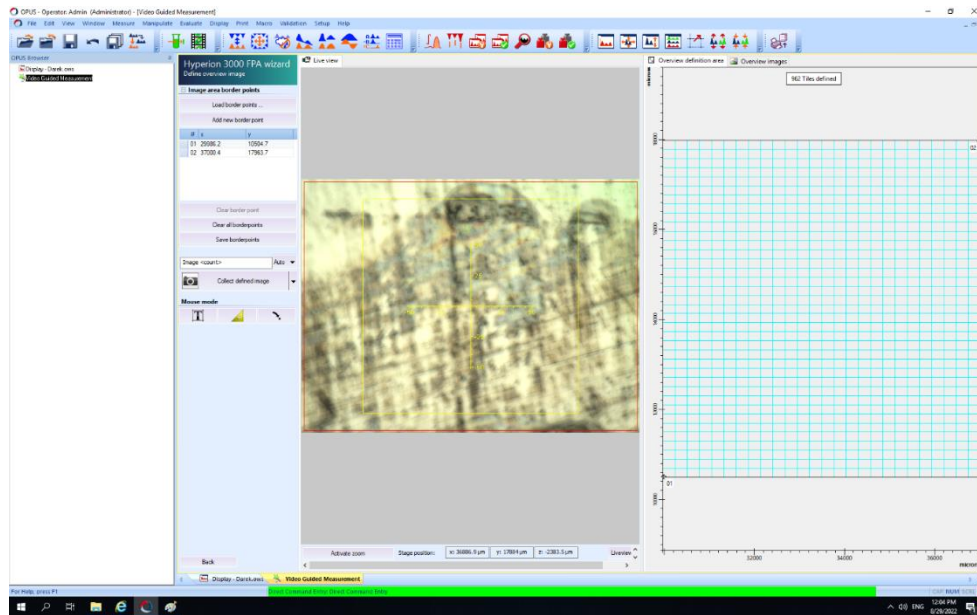


Fig. 5. Collecting defined image

2. The next step is choosing the place for background measurement and the parameters of the FPA detector. For transflection mode, the clean, reflective part of the sample's substrate is needed. For reflection reflective mirror (the microscope equipment). Because visible light and infrared light do have not the same paths, the correction of focus for IR is suggested. For correction use the “Live spectrum” option, which allows you to view the signal on the FPA live (Fig. 6). The lower left window shows the integration for each pixel of the entire spectrum. By moving the table up or down with the joystick, sharpen the edges of the sample

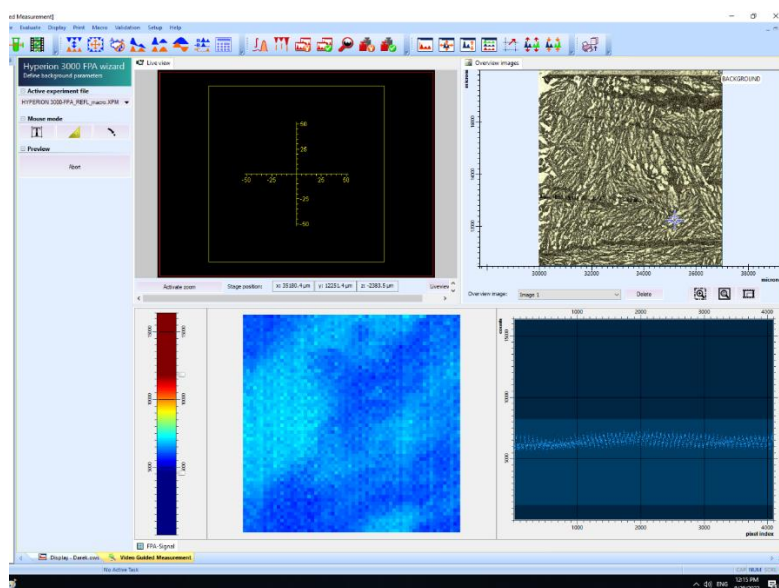


Fig. 6. The “Live spectrum” option

After adjusting the height of the table, return to the background-position. The lower right window shows the pixel count. Set the FPA parameters so that the "pixel line" is at the top of the allowed range (brighter, wide bar). After right-clicking in the lower right screen area, the option to select FPA parameters ("Customize FPA") appears. Set "Gain" to zero, select "Offset", and after entering "Expert only" the exposure time (Exp. Time) - Fig. 7.

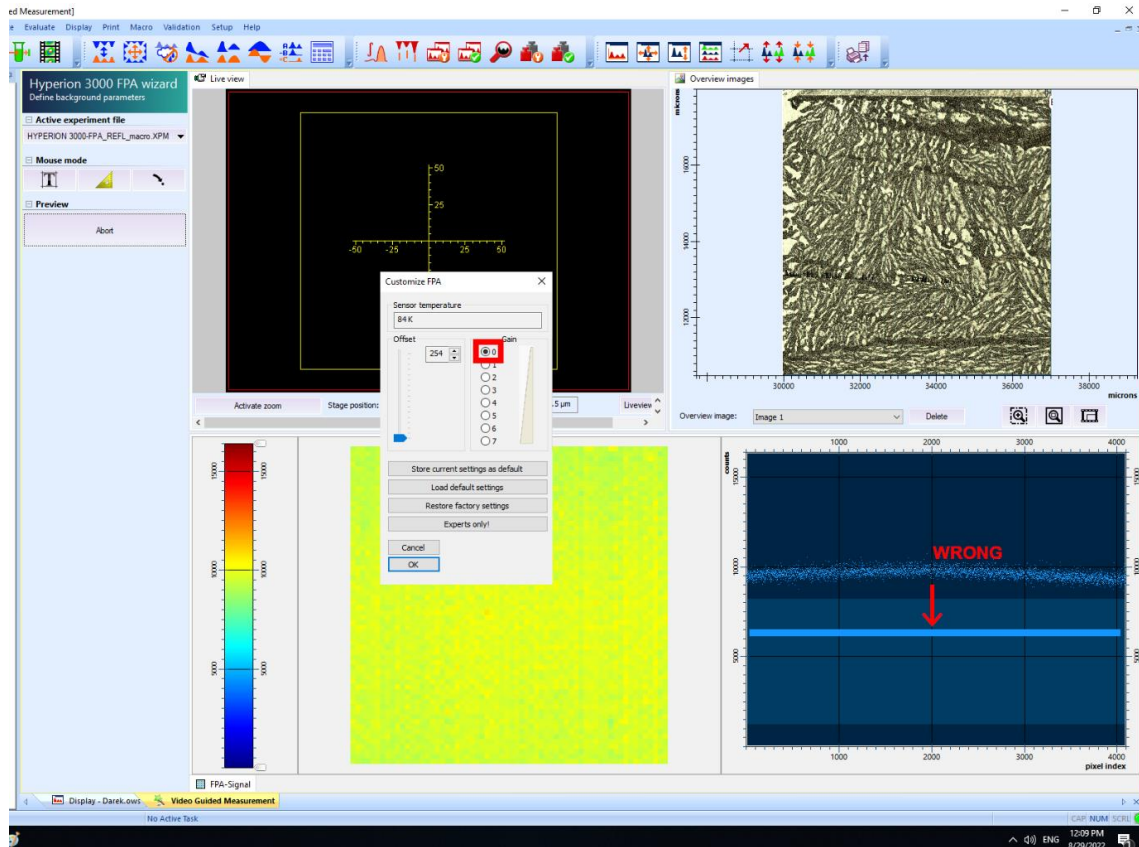


Fig. 7. The „Live spectrum” option – parameters of FPA

3. Selection of experiment parameters - spectral resolution, wavelength range, and the type of data (absorbance, interferogram, etc.) are selected and saved in the so-called "Experiment". In the upper left corner, there is information about the loaded experiment ("Active experiment file"). In the case of transfection or reflection measurements, select "HYPERION 3000-FPA_REFL.XPM" from the list of experiments (Fig. 8)

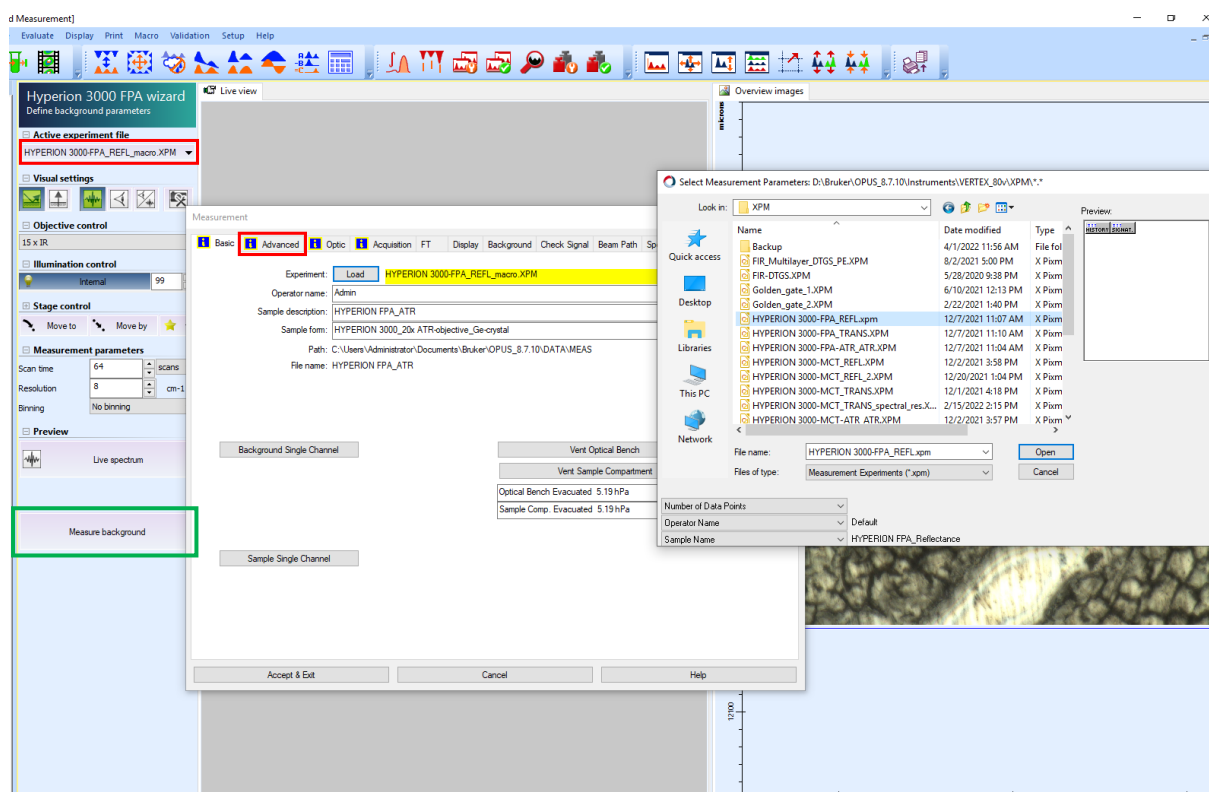


Fig. 8. Loading of chosen experiment

Parameters of measurements are chosen in “Advanced” (Fig. 9):

- Spectral resolution – standard 4 or 8 cm^{-1}
- Sample scan time
- Background scan time
- Save data from – the spectral range. For the MCT-based detectors, the maximum is 4000-650 cm^{-1} in MIR.

The changes have to be saved!

The actual measurement starts with the background: the “Measure Background” button in Figure 9. Check "Scan time" and "Resolution" and correct the values if they do not correspond to those previously selected in the experiment. In the next step, we define the area for measuring the sample. On the previously prepared optical photo of the sample, apply the mesh, as shown in Fig. 10, using the "Mapping Positions" option and select the number of tiles.

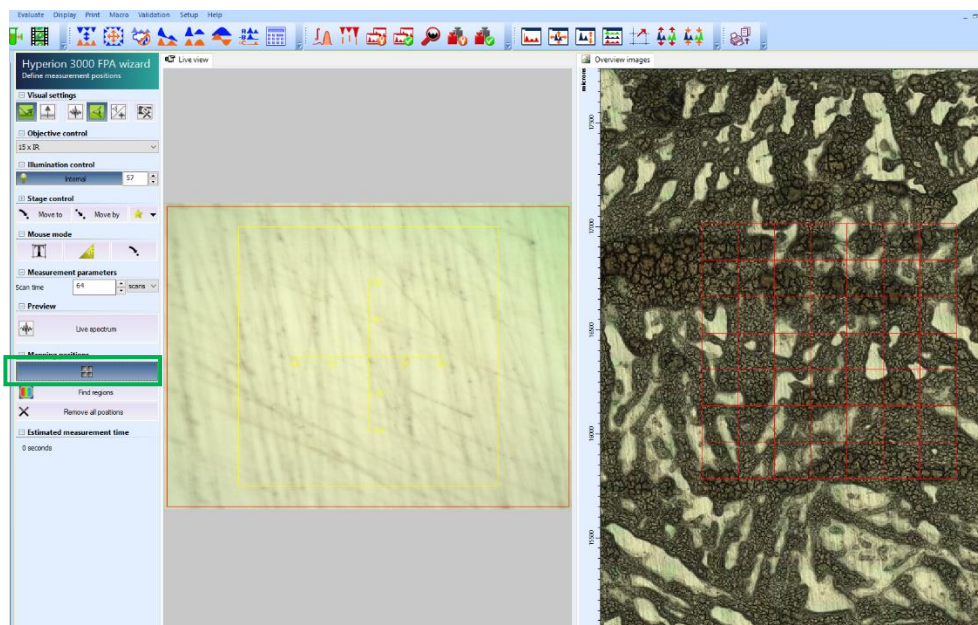


Fig. 10. Selecting of numbers of measured “tiles” – “Mapping positions”

In the next window, before starting the measurement, you must enter the path where the measurement results will be saved and the name of the sample.

II. Measurements in transmission mode with FPA detector

The measurement instruction in the transmission mode is an extension of the transfection /reflection mode instruction. Before starting the measurements, also read the previous part of the manual.

An additional step in the case of measurements in the transmission mode is the setting of the condenser from which the beam exits. After focusing (using the objective) the visible image by moving the stage (See: measurement instructions in transfective mode), take a photo of the sample and switch to the transmission mode (Fig. 1 - microscope console, button marked with two vertical arrows crossing the horizontal line).

Condenser setting for visible light:

1. Choose a place on the substrate (the substrate for measurements in transmission must be transparent to infrared radiation!) without any dirt
2. Close the condenser aperture (Fig. 11) by moving to the left. The image from the condenser with the circular aperture superimposed will appear on the screen and in the eyepiece. Using the condenser centering screws (Fig. 1), set the wheel so that its center coincides with the intersection of the two axes (Fig. 12)

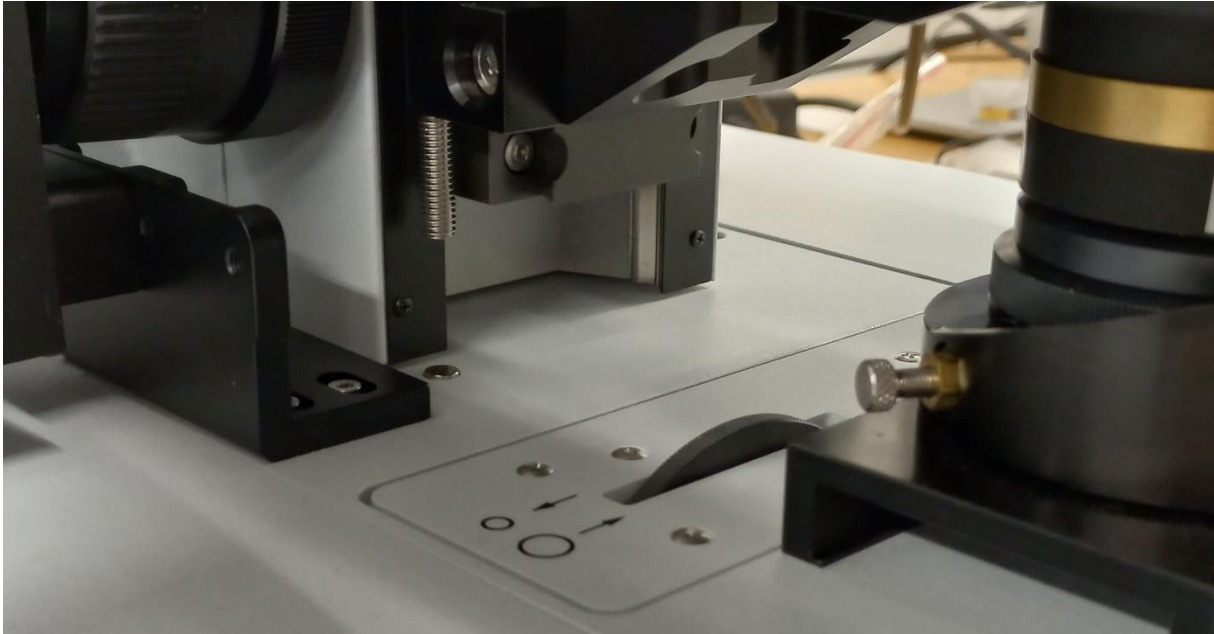


Fig. 11. Closing and opening of the condenser aperture

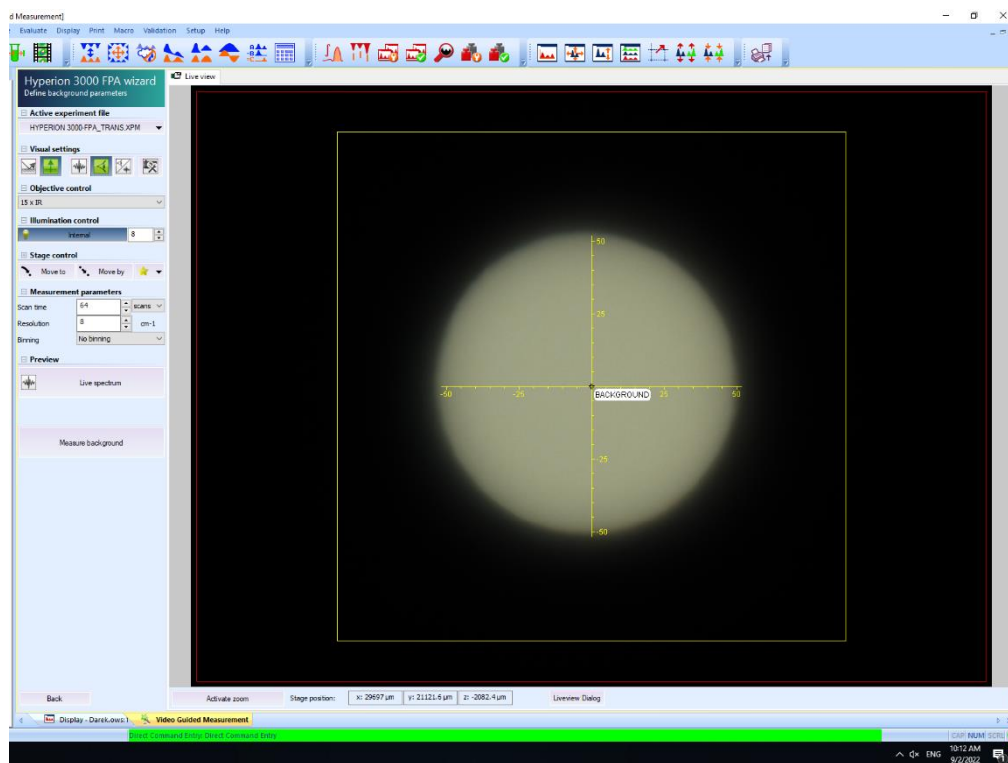


Fig. 12. The condenser after centering

3. The next step is focusing in transmission mode. Adjust the height of the condenser using the screw (Fig. 1) so that the circles are as small as possible and their edges as sharp as possible. Fig. 13 shows the image with well-focused, but not centered condenser. After completing the settings, you need to open the aperture.

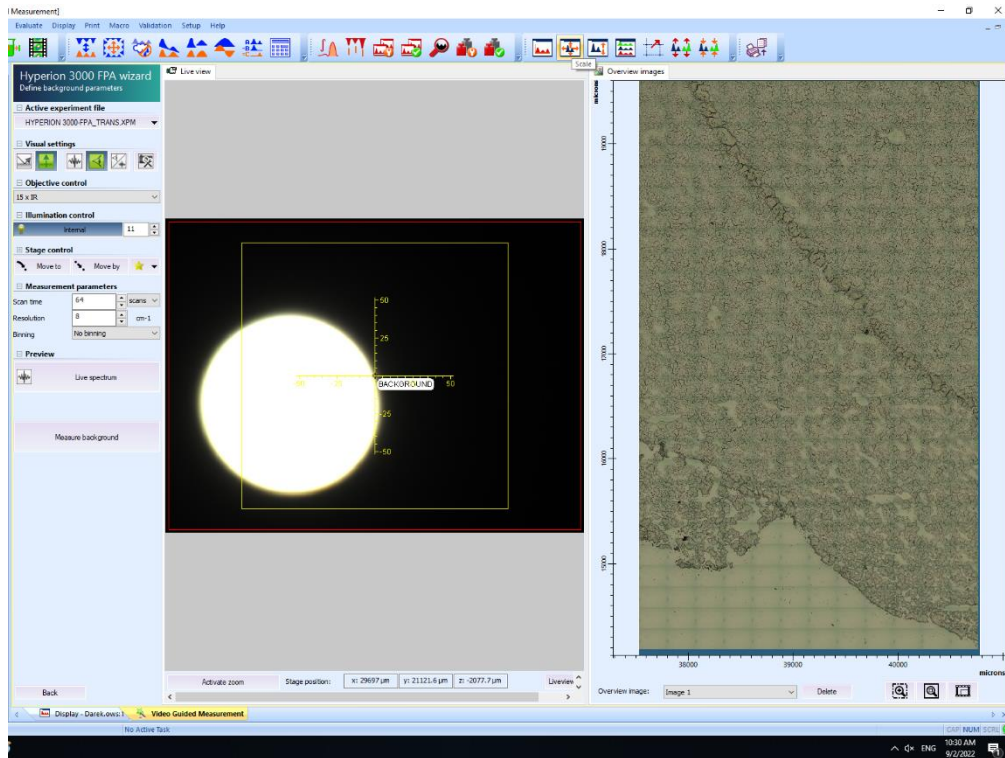


Fig. 13. Sharp image of the condenser with closed aperture but in the wrong, not centered position

The paths of visible light and infrared light are not the same, so the objective and condenser settings should be repeated for infrared light using the "Live spectrum" option. See point 2 for measurement in reflection/transflection mode.

1. Move using the joystick to the edge of the sample or a fragment of the substrate with a small "dust". The sharpness of the image should be increased by moving the stage with slow up-down movements. The sample should stand out sharply from the background (Fig. 14).

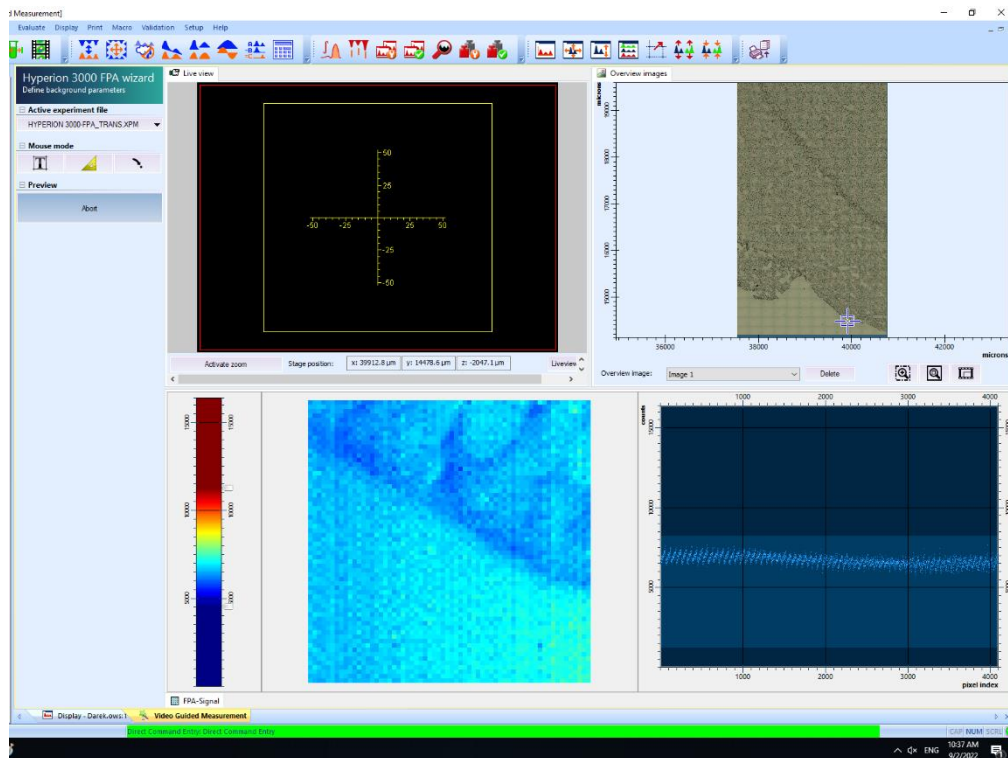


Fig. 14. The “Live spectrum” option

2. Come back to the background place and adjust the X, Y, and Z positions of the condenser. Close the aperture and use the big screw to adjust the height of the condenser and the small two to adjust the position in “Live spectrum”.
3. Selection of FPA detector parameters - after right-clicking on the lower, right image in the "Live spectrum" mode, select "Customize FPA". Set "Gain" to zero, select "Offset", and after entering "Expert only" the exposure time (Exp. Time). Pixels whose signal is collected from the background should form a straight line located in the upper part of the allowed area (Fig. 15).

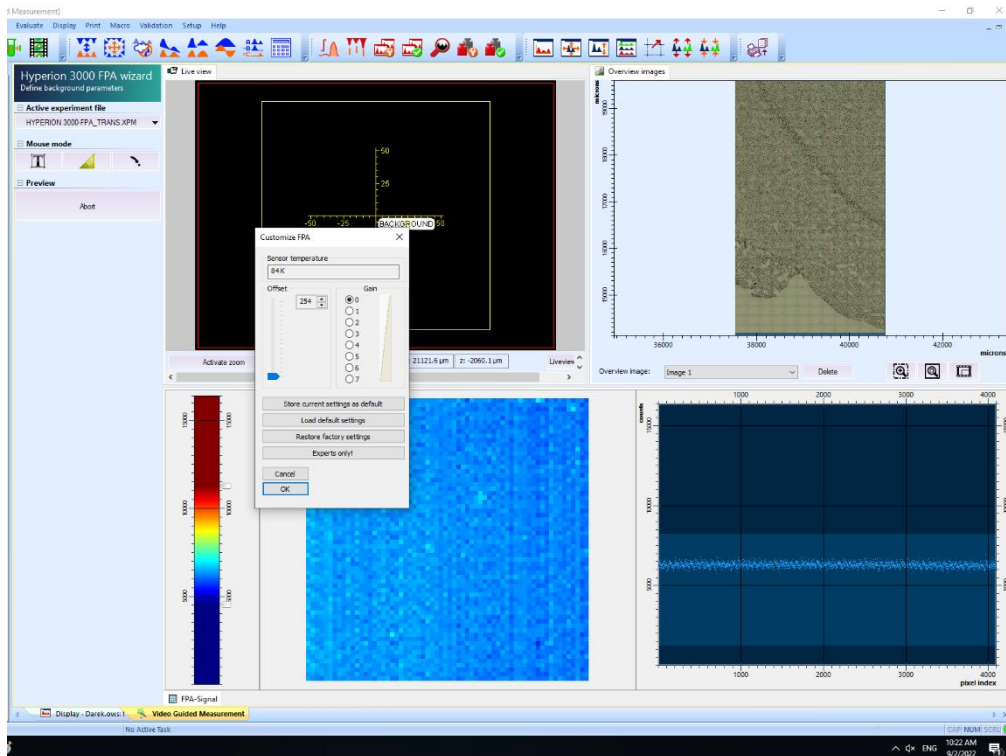


Fig. 15. The „Live spectrum” option – parameters of FPA

The measurement is proceed in transmission mode so the proper experiment has to be loaded in “Active experiment life” - „Hyperion 3000-FPA_TRANS.XPM”. The selection of parameters in the experiment is described in point 3 in the manual for measurements in the transfective/reflective mode.

The measurement starts from the background. Click “Measure Background” (Fig. 13). Check "Scan time" and "Resolution" and correct the values if they do not correspond to those previously selected in the experiment. Click “Next”. In the next step, we define the area for measuring the sample. On the previously prepared optical photo of the sample, apply the mesh, as shown in Fig. 16, using the "Mapping Positions" option and select the number of tiles.

In the last step, name the sample and add the path to the folder where the data should be saved.

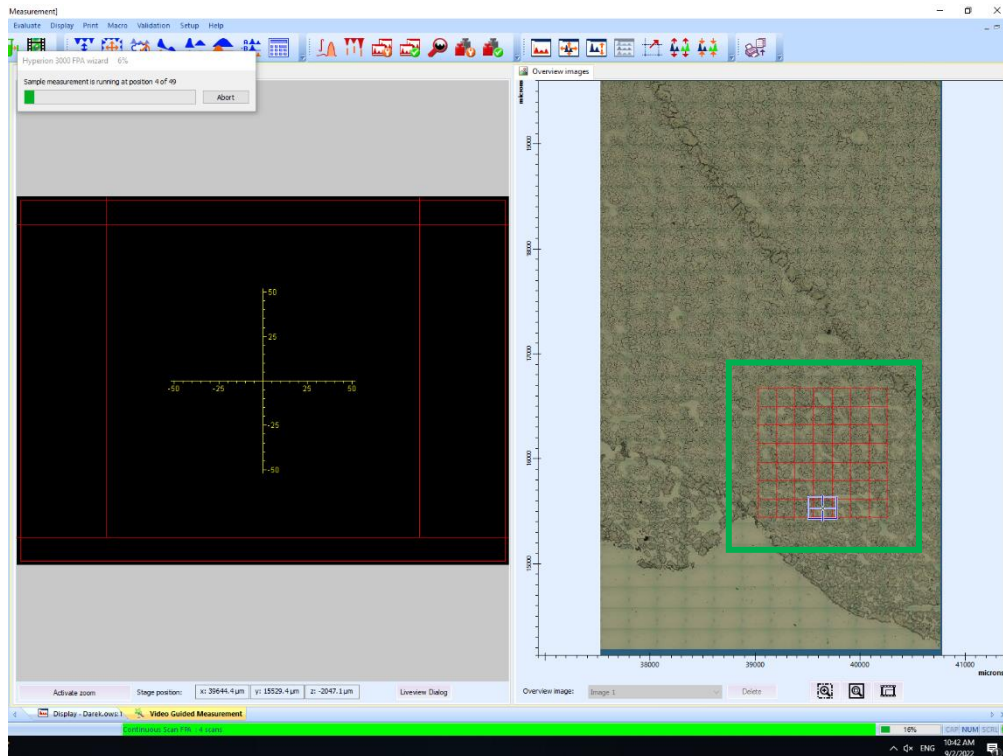


Fig. 16. Selecting of numbers of measured “tiles” – “Mapping positions”

1. Measurements with MCT detector

The steps of the measurements with the use of the MCT detector are analogous to the FPA measurements, except for the optimization of the signal detection parameters (Figs. 7 and 15). In the microscope software OPUS choose “Measure”, then “Video Guided Measurement” (Fig. 4), and “Hyperion 3000-MCT”.

An additional step is to choose the area from which the signal is collected using a mechanical aperture.

After selecting the place of interest and taking a picture with the camera, narrow down the measuring area using the apertures above the objective (Fig. 17). You can move the apertures (mode marked with two concentric squares) and rotate (mode marked with a circle).

Attention! For measurements in transmission mode, set the condenser before applying the aperture as described in section II "Measurements in transmission mode with an FPA detector".



Fig. 17. Mechanical apertures – Top: slide mode. Down: rotation mode

Figure 18 shows the area limited by a twisted aperture from which a signal is collected reaching the MCT detector (one spectrum).

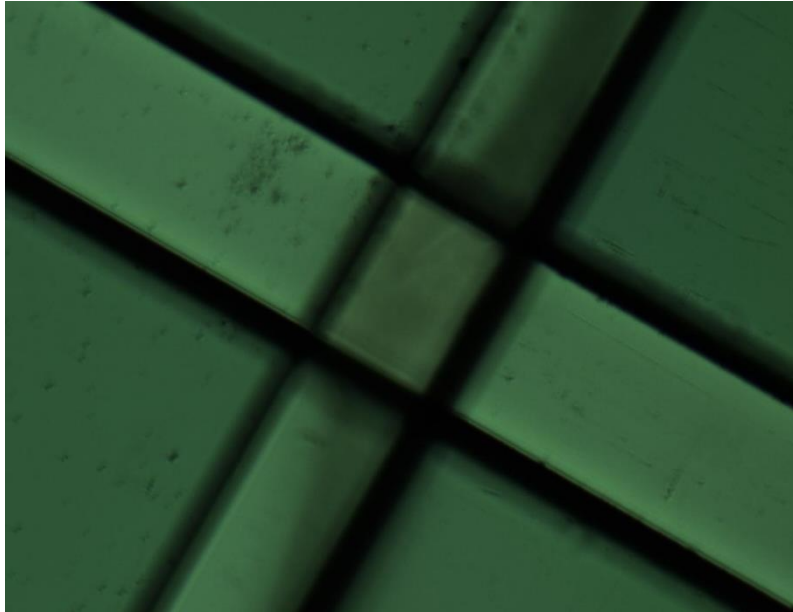


Fig. 18. Measured area limited by apertures

To measure the background, a single measurement is made with the aperture set previously. The appropriate experiment has to be chosen in the ("Active experiment file") (Fig. 8). It is "HYPERION 3000-MCT_REFL_2.XPM" for measurements in reflection and transflection mode, and "HYPERION 3000-MCT_TRANS.XPM" for transmission mode.

With the MCT detector, you can take single measurements or map a sample. Define the measurement area by marking individual measurement points, lines of points, or a mesh on the photo taken before (Fig. 19).

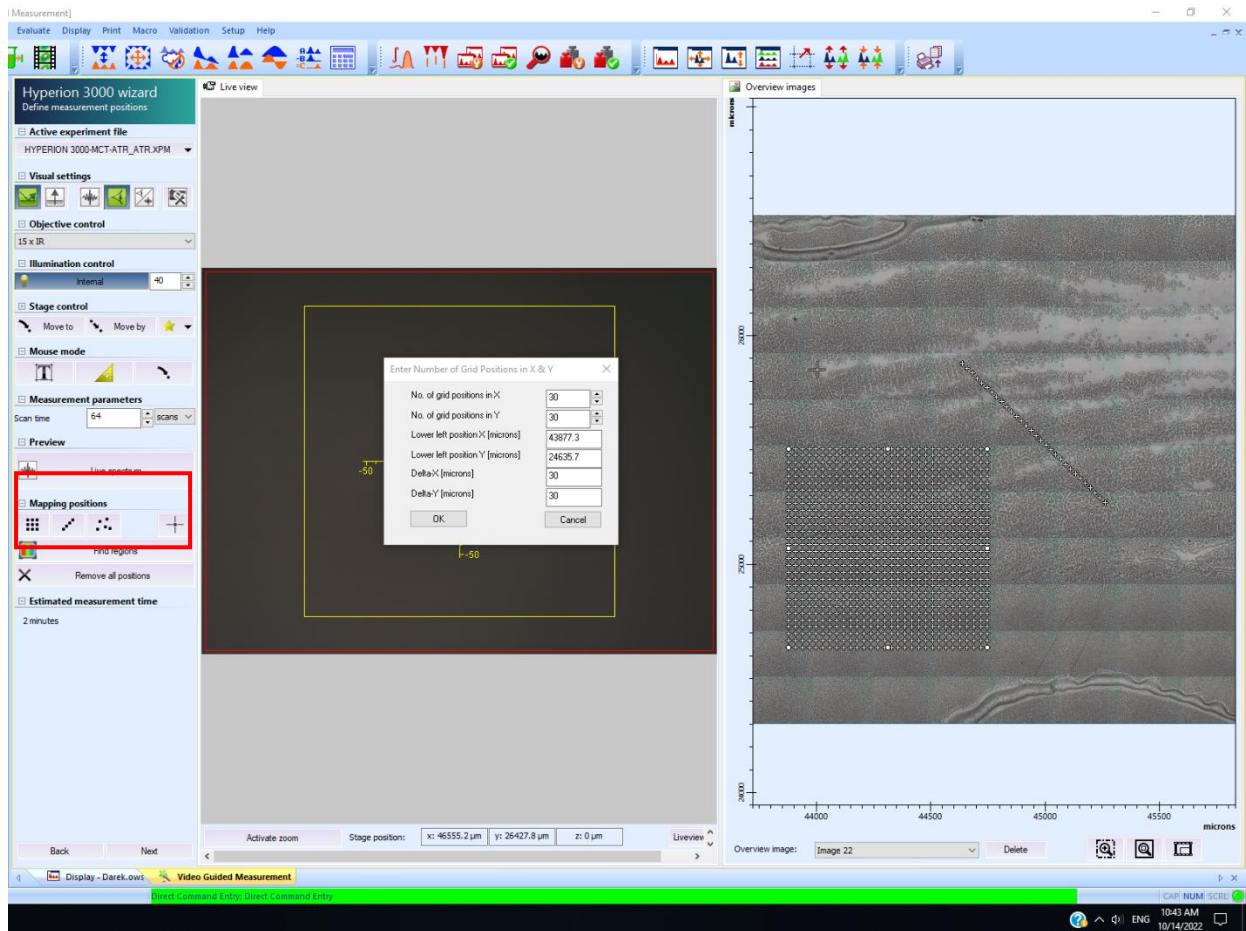


Fig. 19. Choosing mapping position

In the case of the straight line and mesh of points options, a window appears in which the number of points ("No. of grid positions") and the step ("Delta [microns]") are defined. Figure 19 shows the window for the mesh. If an entire area with no voids is to be mapped, the aperture size must match the step. If the aperture size is larger than the step, the measurement points will overlap.

Steps of turning off the microscope:

1. Close the OPUS software
2. Turn off the FPA detector and microscope
3. Choose the "Stand by" option in the "Direct Control Panel" on the loaded page in Internet Explorer (Fig. 3)
4. Turn off the vacuum pump (Fig. 2)
5. Stop the compressed air flow (Fig. 2)