

ENDSTATIONS WORKFLOW

Starting up the end station upstream or downstream

Hardware


- Make sure the beamline is open,
- When using Hyperion Microscope, before starting:
 - Fill the single channel detector with liquid N₂ (refill every 7h at max for the upstream endstation and 11h for the downstream endstation), **OR**
 - If you want to work with the Focal Plane Array (FPA) detector then fill both detectors with liquid N₂ (FPA to refill every 5h at max).

IMPORTANT! Do not switch on the FPA detector if the FPA is not cooled with liquid nitrogen. FPA refill in time is essential for detector safety when powered on! Top up FPA first as switching on the uncooled detector will cause permanent damage!

- Check that the N₂ gas valve on the wall is open (fully turned anti-clockwise) without touching the regulator, this is needed to operate the air bearing in the interferometer and the gas purging. The pressure on the regulator should be between 1-2bar.
- Check that the vacuum pump plug-switch at the back of the optical table is on (red light on).
- Turn on the microscope by inserting the plug at the back where the label says microscope on/off. Turn on the camera monitor (the switch is at the right side panel of the monitor).

N.B. Sometimes the detector holding time is low, and this will be indicated by a sign next to the detector indicating the current recommended refill time. If you notice the detector is warming up before this time, please advise the local contact, and fill up more regularly.

Software

- Turn on the endstation PC and Log in as **B22 User**:
On downstream: b22-dwnstrm\b22user, Password: b22userb22user123
On upstream: b22-upstrm\b22user, Password: b22userb22user1234
- Open Opus 7.2; enter the password OPUS (in capital letters), press “enter” twice.
- Calibrate the stage , tick activate joystick then click calibrate stage.

N.B. before calibrating the stage ensure the condenser is lowered and there are no obstructions under the objectives which could crash into the objectives as the stage moves to home positions.

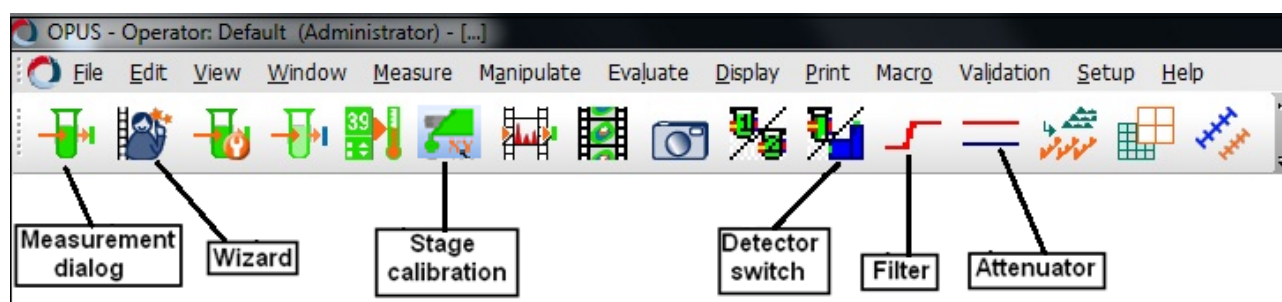
- Choose the detector you need to work with (single channel or FPA) by clicking “DET switch



N.B. Before selecting "FPA" ensure the FPA is cooled with the MCT, and then switched on.


Figure 1 shows the important icons in OPUS toolbar to set up an experiment.

Figure 1



Setting up the experiment

- Go to the *Measurement* dialog (also called advanced data collection) to set up the experiment

by clicking on the icon .

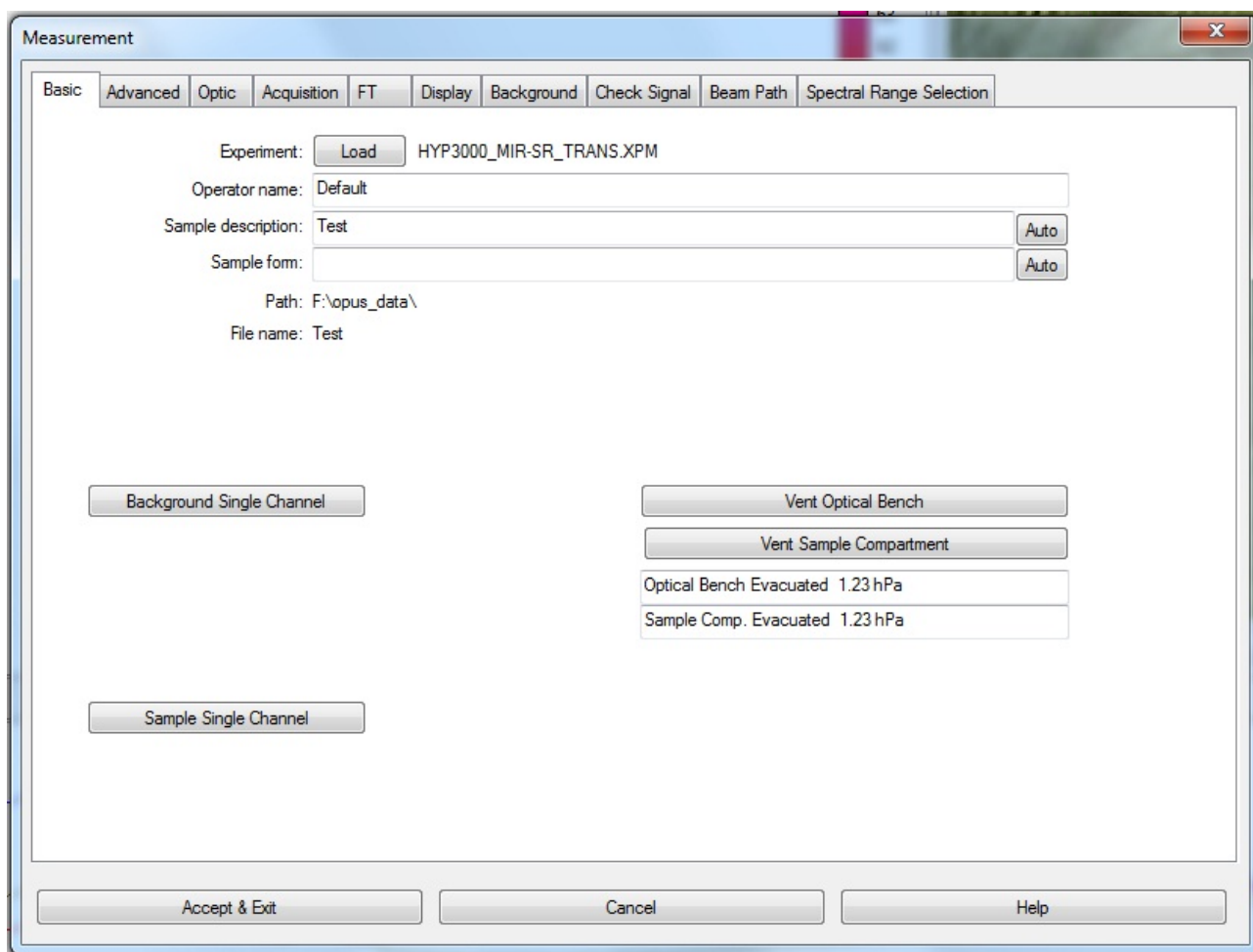
Basic Tab (Figure 2)

- Load the required experiment file
N.B. such a file will be set by B22 staff at the beginning of beamtime
- Specify:
 - * Operator (optional),
 - * Sample description (file name)
 - * Sample form (optional, extra sample information not included in file name)
- Check the pressure: Evacuate Optical Bench to get down the pressure to ~2 hPa. When pressed, the button changes to Vent Optical Bench. This may take ~10 minutes from a fully vented FTIR bench, e.g. after changing beamsplitters.

If you need to change the settings of the measurement parameters, go to the “*Advanced*” tab, in this case save the experiment file after changes have been made.

- When ok with all the settings, click “accept and exit”.

Figure 2



Advanced Tab (Figure 3)

- Specify the data file name of the spectra to be saved (as long as <snm> is the File name in the “advanced” tab, the sample description in “Basic” will be used as the filename).
For multiple single spectra, the data file extension will be incremented .0, .1, .2....., indicating the current number of measurements.
- The destination folder where the file is saved is under Path, click on the button showing “...” and check it is: F:\opus data. This is a shortcut to your experiment folder already set up by your local contact. If you want to create a new sub-folder, you can type \subfolder name after the existing path.

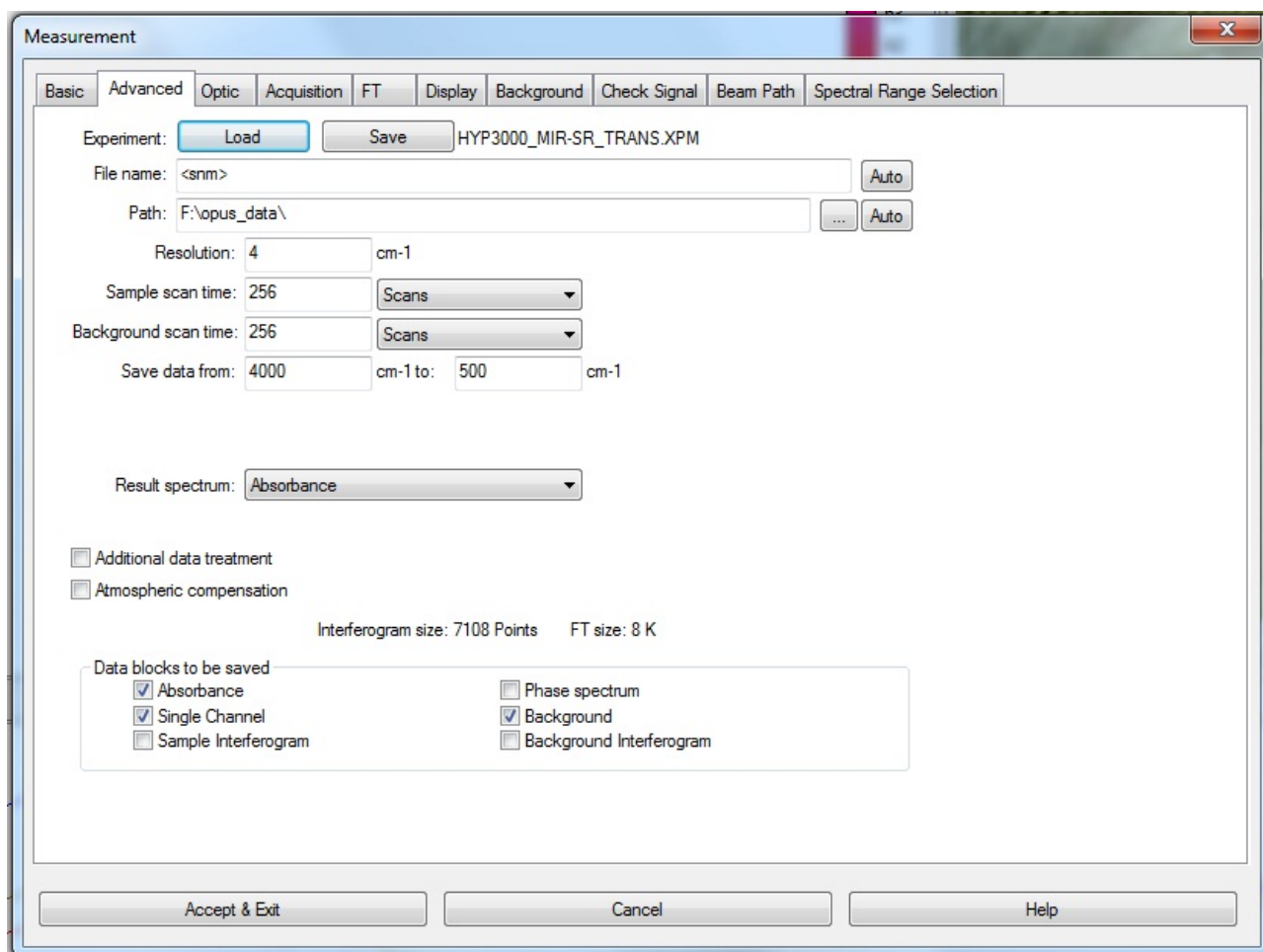
Generally the text boxes will turn yellow if a parameter is changed, and red if a value is not appropriate (for example the spectral range selected is outside that of the beamsplitter). Move the cursor over a red box to read the relevant warning/error message.

N.B. If the box is highlighted in red, it is a major problem. Contact your local contact.

- Define the resolution, the spectral range, and the number of scans for both the sample and the background.

- Choose the option for the result spectrum i.e. absorbance, transmittance, etc...
- If compensation for the CO₂ and water band is required, tick the “Atmospheric compensation” box.

Figure 3



Optic Tab (Figure 4)

In this section, you just need to choose the source setting:

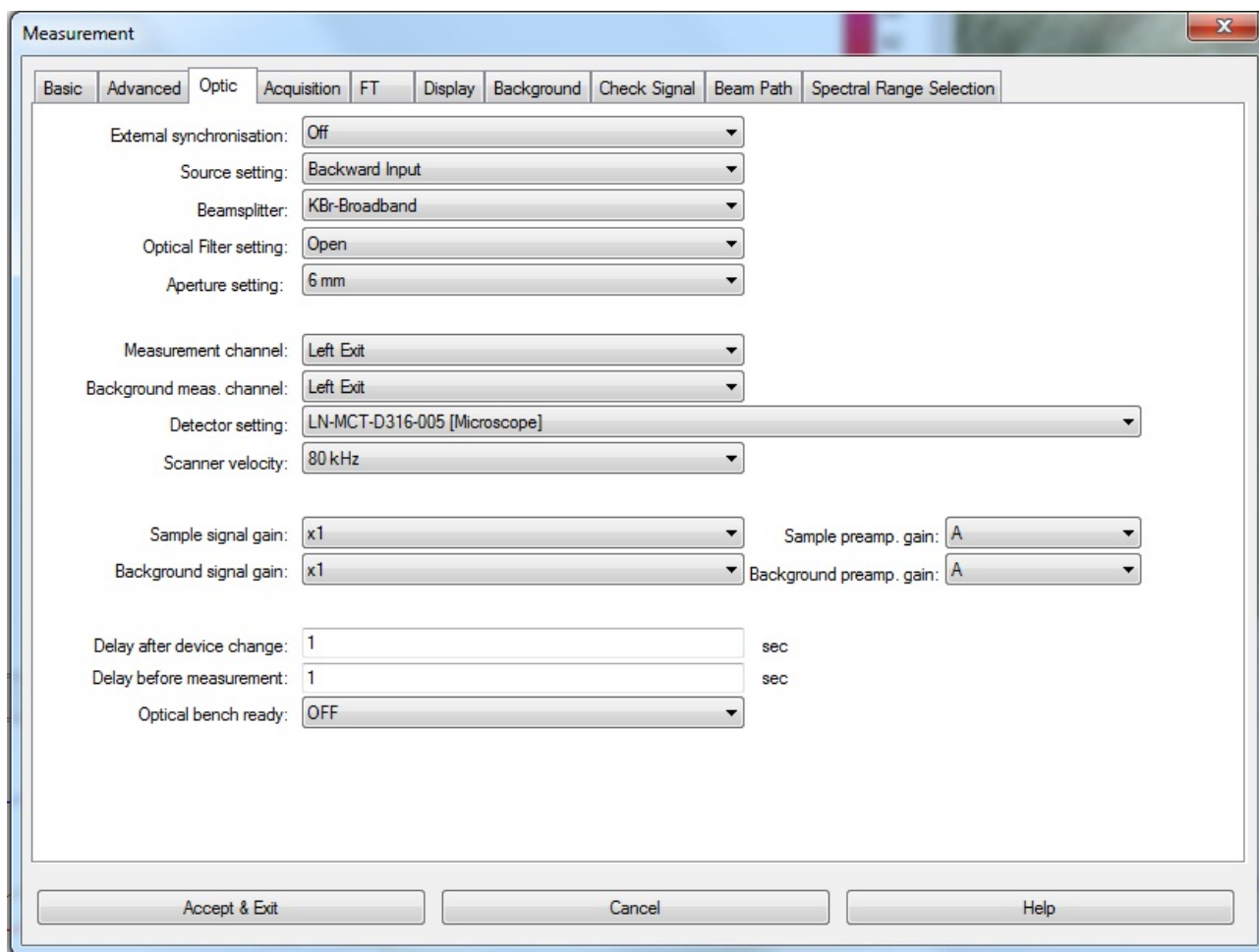
- MIR: internal Globar source.
- NIR: internal halogen lamp source.
- Backward Input: Synchrotron source.
- Right Input: Hg lamp (this far-infrared lamp is external to the FTIR. To use it, the lamp power supply and cooling unit – located under the optical table - must be plugged in and connected to the lamp housing on the metal optics box on the right hand side of the FTIR).

Important! After selecting the source, or when changing it from one option to the other, you need to click “check signal” for the mirrors to move into position.

Normally the other “optic” parameters will be fixed for mid-IR micro-spectroscopy.

The screenshot below shows the standard set up for synchrotron source measurements

Figure 4



If you download the proper experiment (XPM file) in the basic or advanced tab, you don't need to change the other parameters (acquisition, FT, display, background, and beam path). These will be loaded automatically. Just go to check signal so the instrument can switch to the proper source.

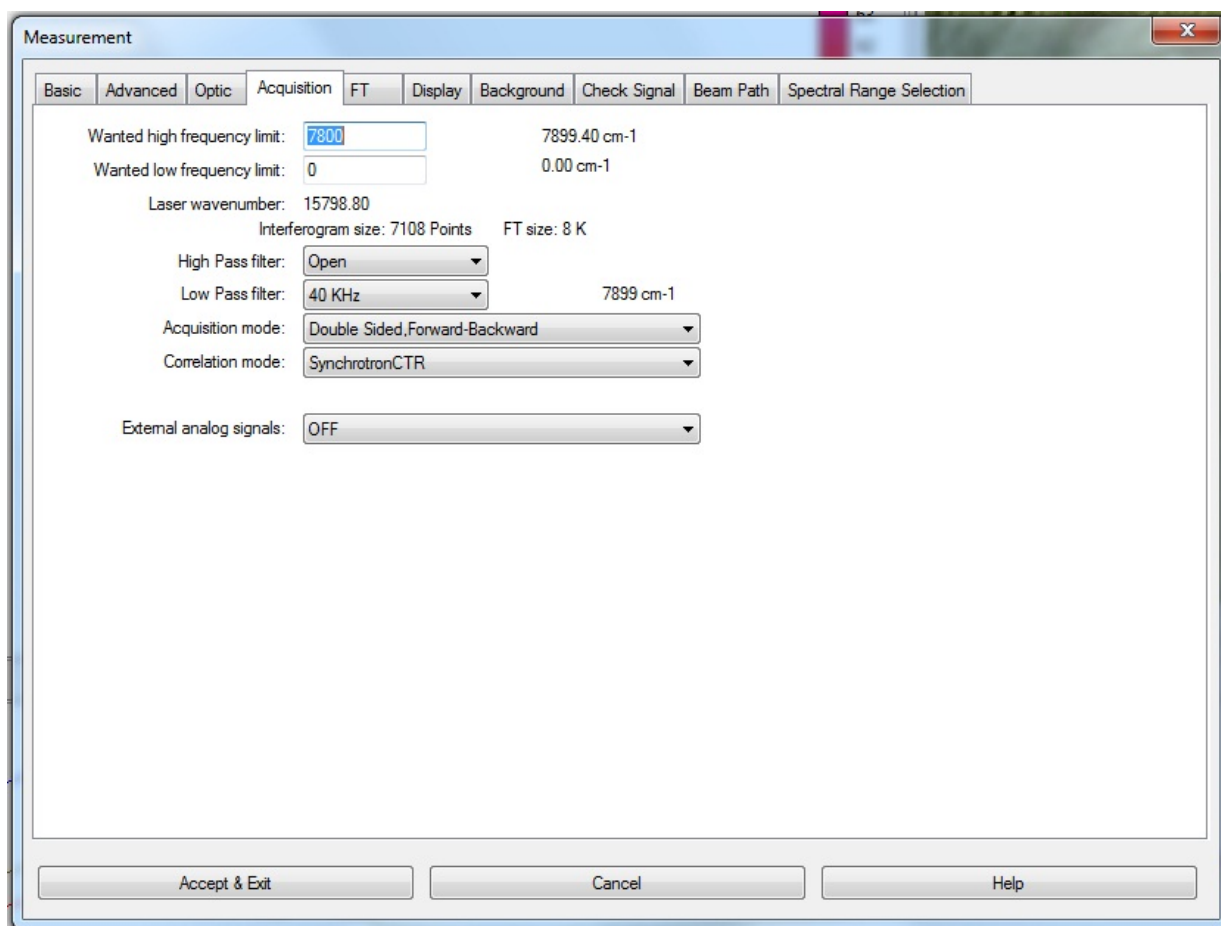
N.B. Don't forget to accept and exit when setting up or changing the measurement parameters.

Acquisition Tab (Figure 5)

Pay attention that the correlation mode is on "synchrotron CTR" when using the synchrotron source. This setting automatically rejects scans during the synchrotron electron beam top-up which happens for a few seconds every 10 minutes. The full number of requested scans will be acquired. The correlation mode should be set to "OFF" when using the global source to avoid unnecessary scan rejections.

The other parameters should remain unchanged for Mid-IR work.

Figure 5



FT Tab (Figure 6)

This should not need to be changed for a particular experiment. Figure 6 is an example for a standard mid-IR measurement . In case of doubt reload the correct experimental file in “*Advanced*”. Note that far-IR THz and other experiments may have different parameters and for example the phase resolution should be different for different spectral resolution. (The Bruker software should suggest that phase resolution 8 x the spectral resolution is the maximum, so at 1cm⁻¹ resolution phase resolution should be 8 or lower).

Display Tab (Figure 7)

You can change the display limits of your spectra (normally in mid IR use 4000-400 cm⁻¹). The example below shows the display from 10000 to 500 cm⁻¹ which is better when checking the signal to have a full view of the spectrum and assessing the stability when using the SR source.

If you want to display the spectrum during acquisition, tick “Display during measurement” (not recommended for long maps).

N.B. do not tick “Display single scan before measurement”.

Figure 6

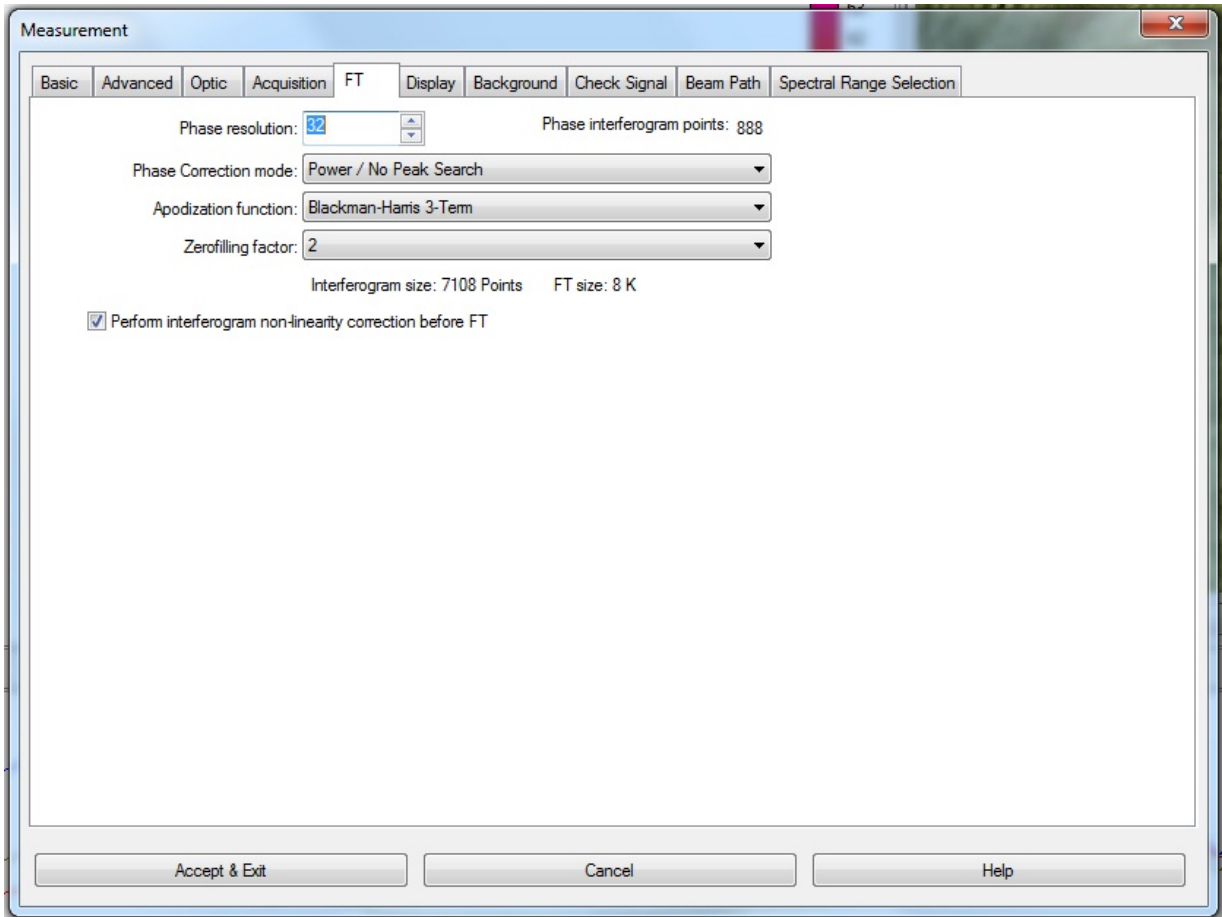
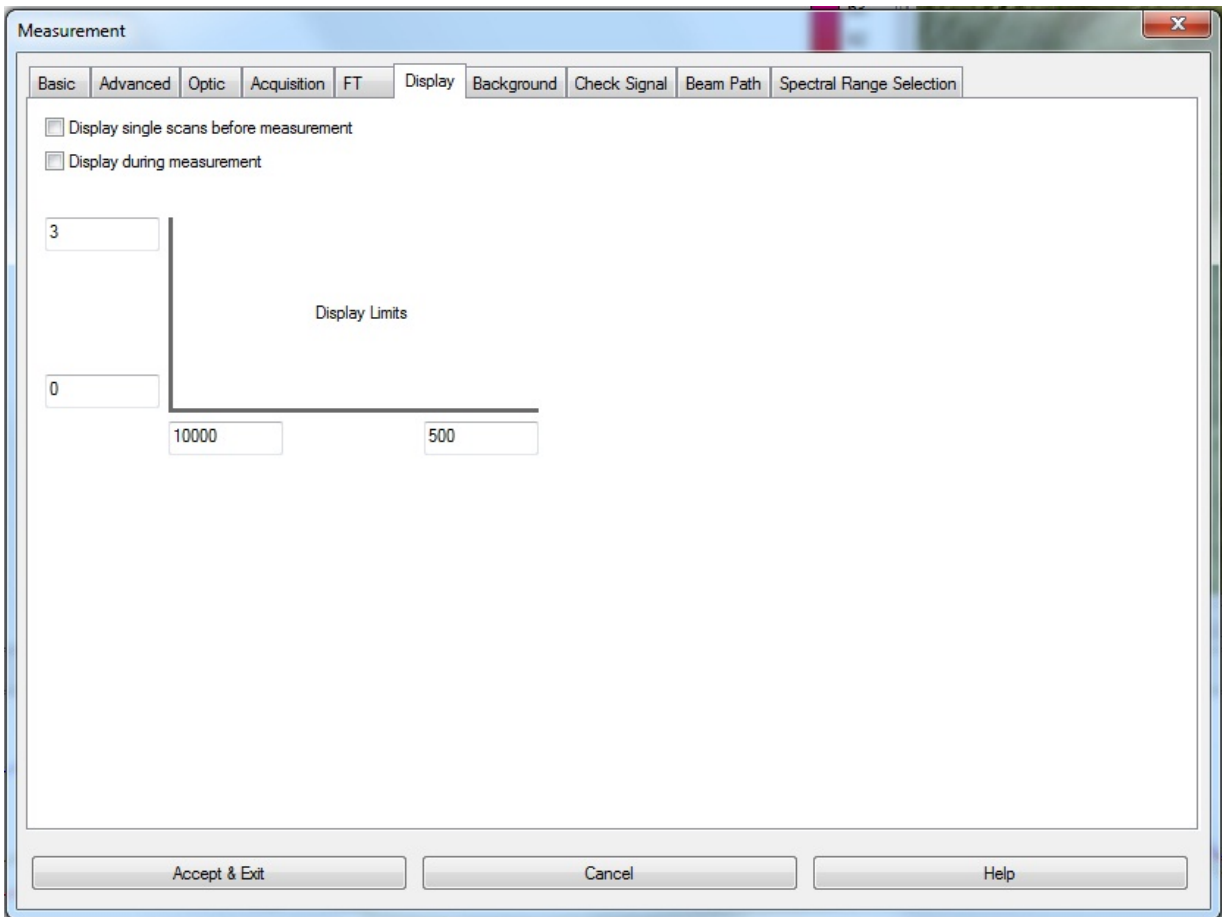


Figure 7



Working with the Microscope Single channel detector


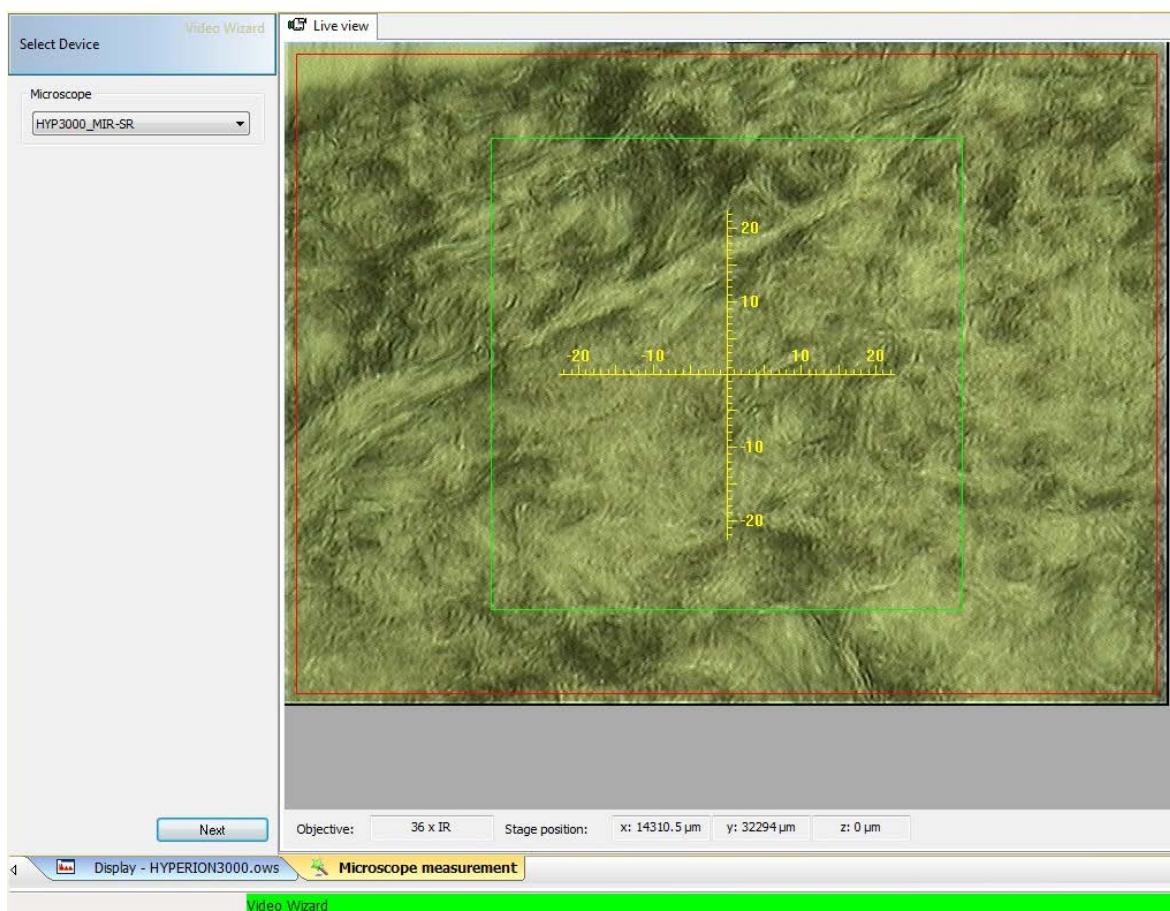
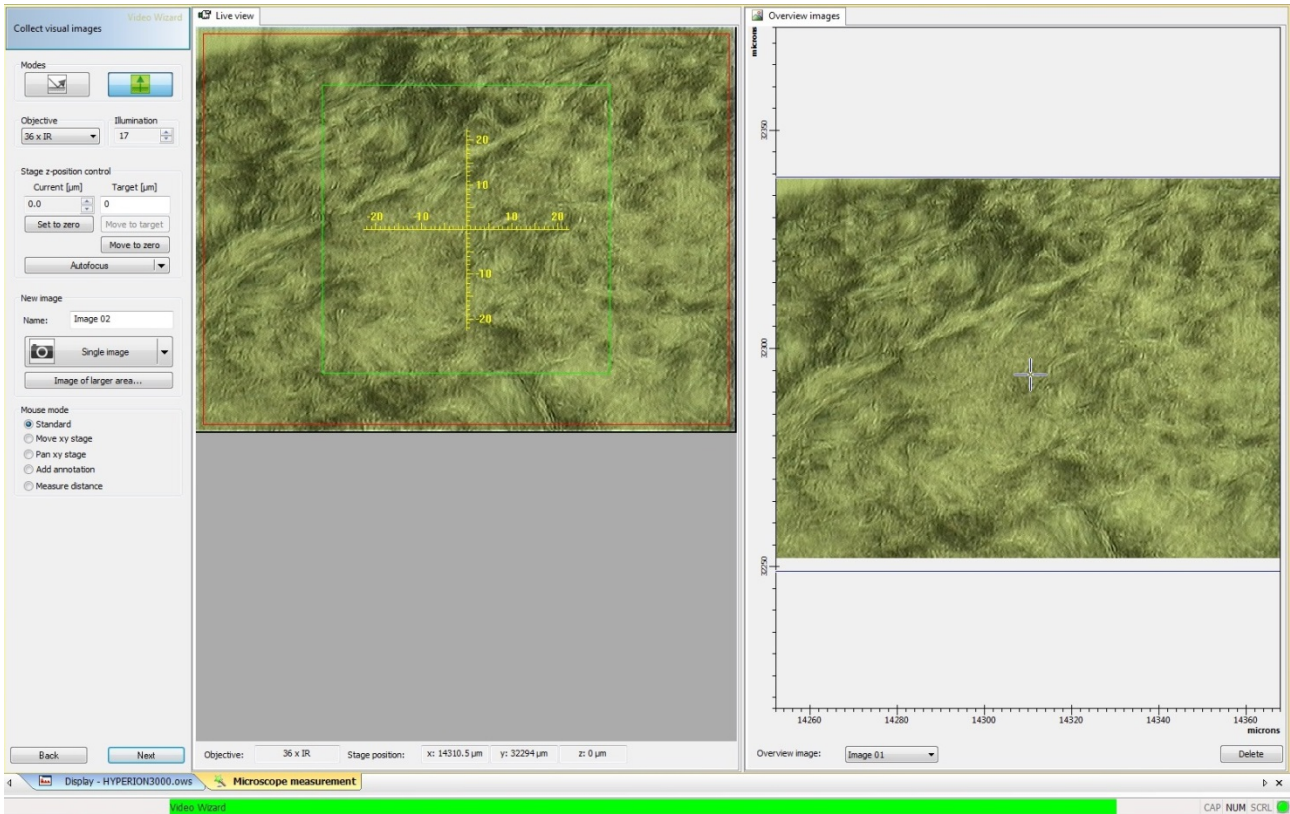
- Make sure the desired experimental file is selected (in measurement dialog).
- Click once on *the video wizard* icon  in the toolbar. The microscope measurement mode in figure 8 below should show the proper configuration after choosing the experiment file in the measurement dialog. For example for a synchrotron transmission measurement, this should read HYP3000_MIR_SR when the experimental file HYP3000_MIR_SR_TRANS.XPM is set in the measurement dialog.
- Do not touch anything (especially the joystick) when the wizard is opening, wait till it's completely opened (Risk of OPUS crashing). Normally, the slits will be closed and reopened automatically before the software is ready to use again.
- Once the correct configuration is shown click next. If not, close the wizard and go to the measurement dialog to load the proper experiment file as described before.
- When you click next the reset XY stage dialog will appear: do not click on reset if you have already done the stage calibration before to start.

Figure 8

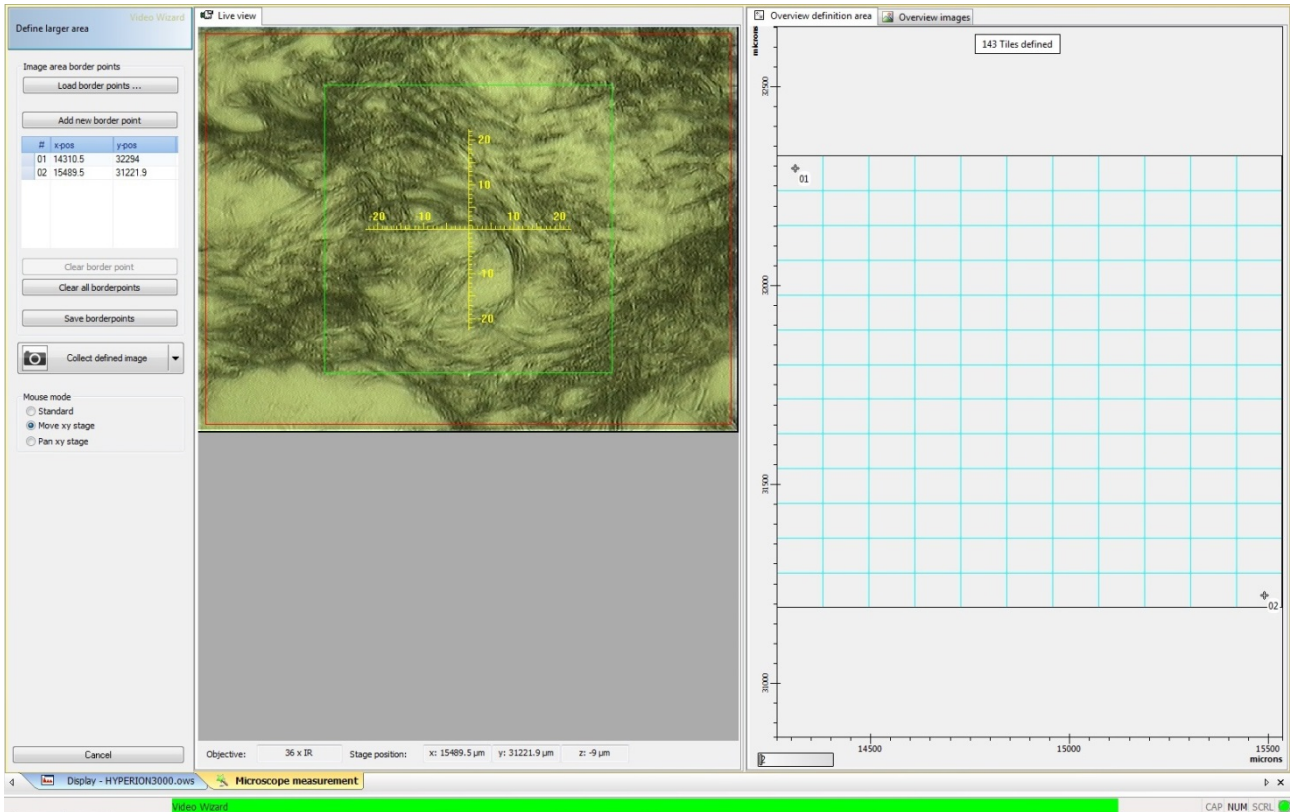


The next step will allow you to take a visible image of your sample. Make sure you choose the correct magnification objective on the left, and the mode (transmission or reflection) you will be using. By default the wizard when opened will set the microscope optics to reflection mode.

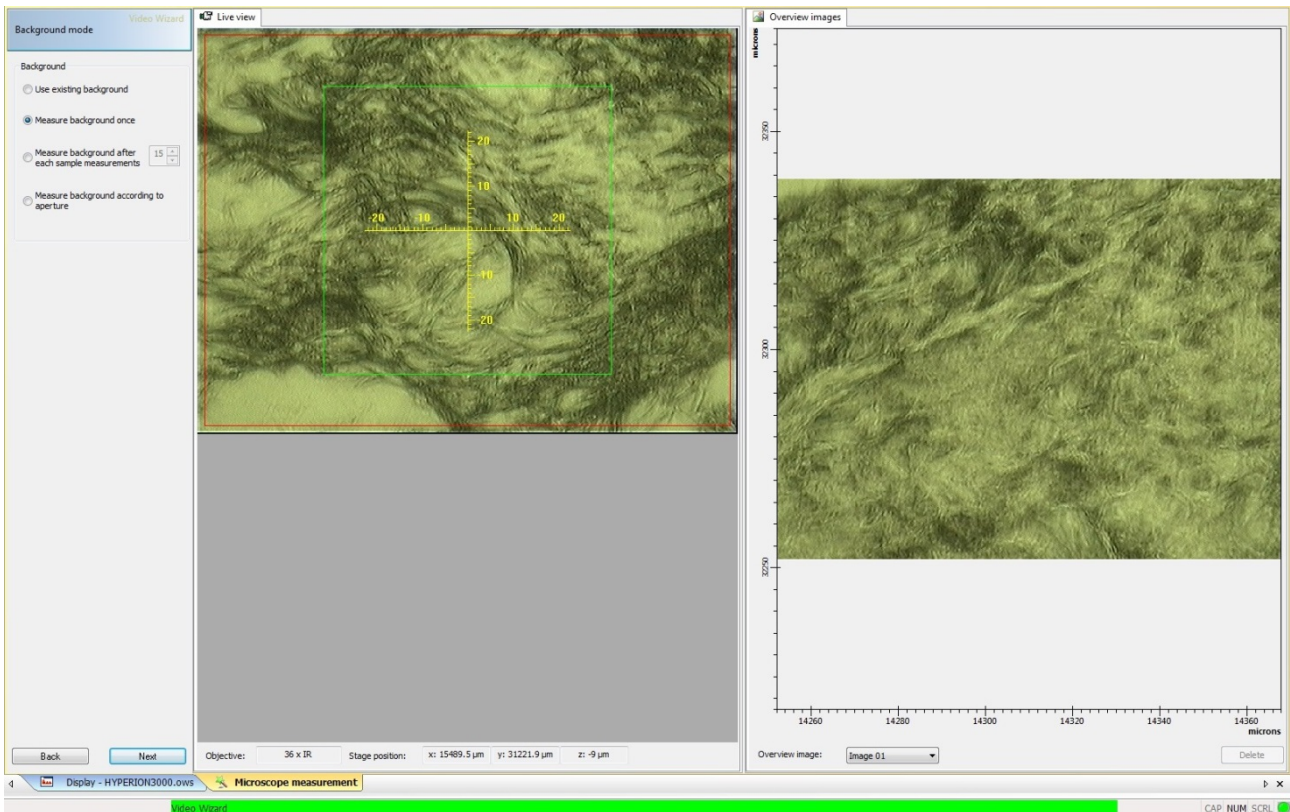
There are two ways of collecting visual images: a single shot by clicking on single image,



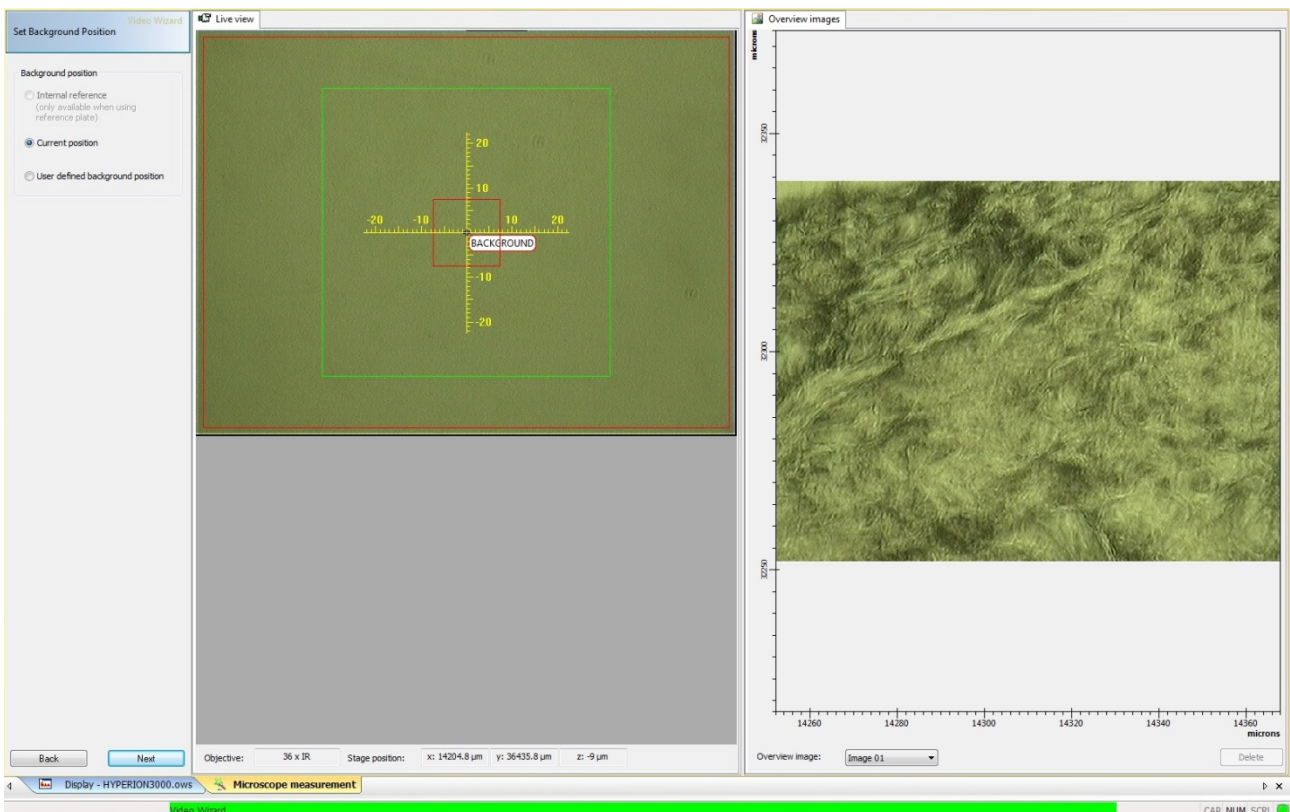
or image of larger area where you need to define few points for a bigger area of your sample and then collect the defined image. As a minimum for a larger area you need to define two diagonally opposite corners of the area you want to image.



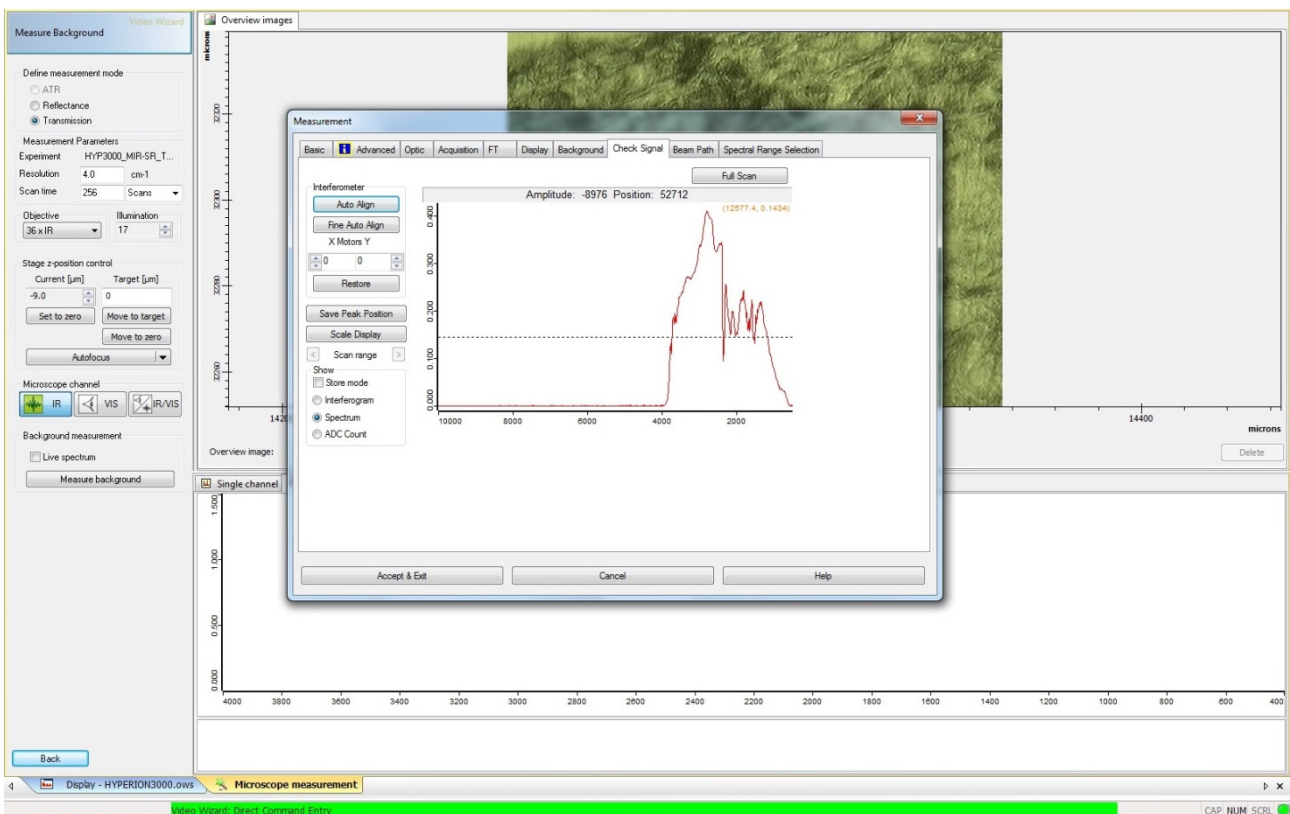
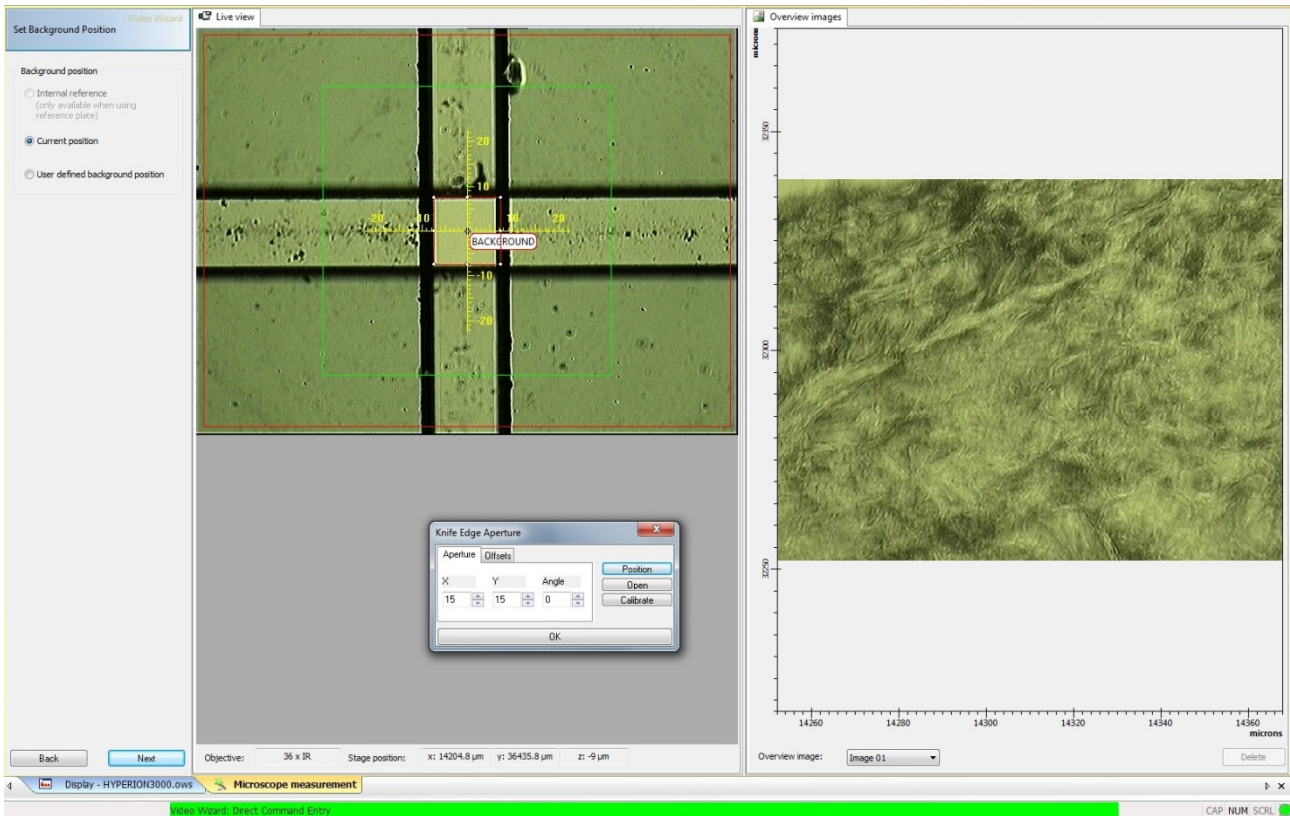
When you click next you will have the background mode dialog where you can see four options, choose the one you need and then go to a background position by moving the joystick.



Once you've moved to the background you can set the position by clicking "current position". With OPUS 7.2 version, the background position from the previous measurement is not saved, so for each measurement you need to set a new background or you can click use existing background. Then click next.




Before measuring the background, remember to choose the aperture size if you are working with synchrotron source. There is a red box on the live view image which defines the aperture, left click on it then right click to edit the aperture size, enter the required numbers then click position and wait till you see the actual microscope slits closing to the correct size. **Note** - remember the exact numbers you entered for aperture size and angle, as you will need these later to define the sample measurements. Click next to collect the background after checking the signal.



Click “IR” on the left. You should hear the motors moving in the microscope. Then click on the measurement tube in the OPUS toolbar to check the signal.

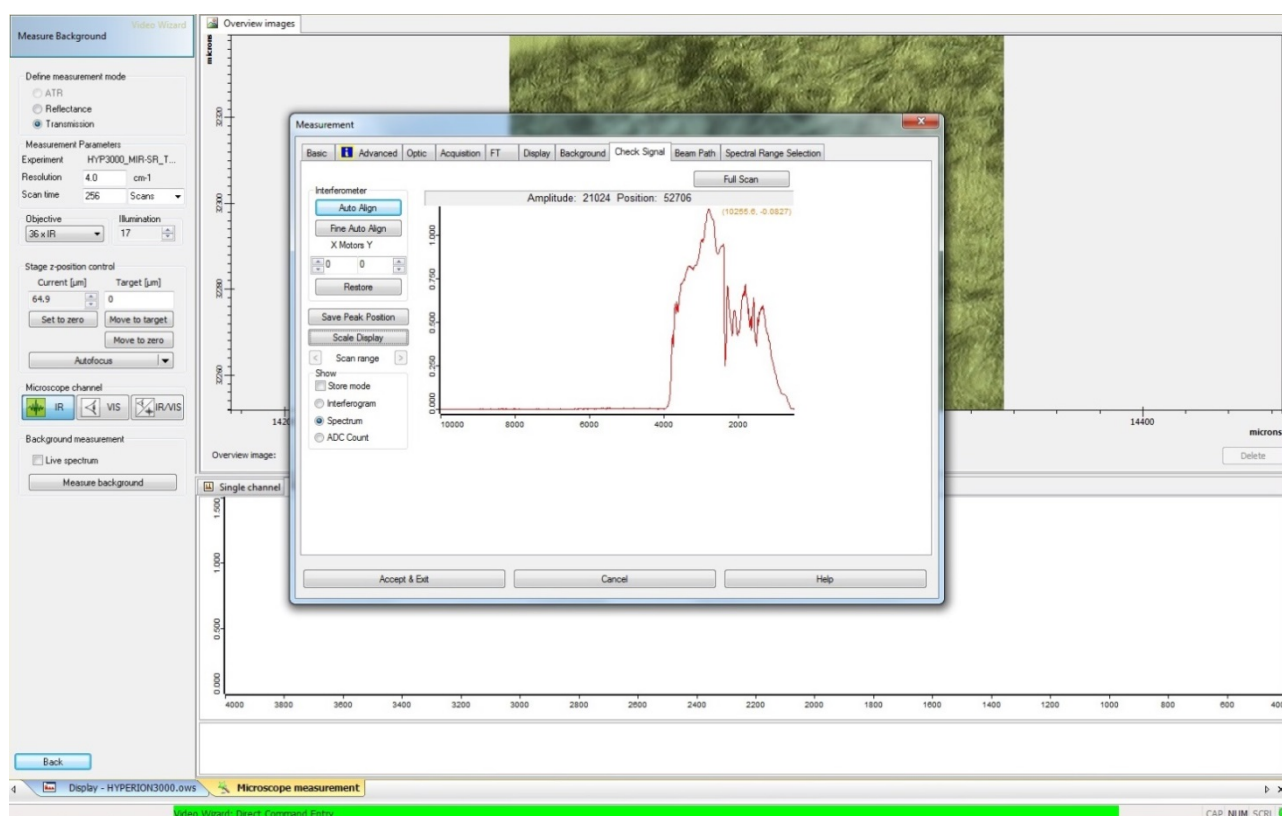
Check Signal for single channel

Every time you change the experimental conditions (new sample holder or new substrate, new slit size or change of source) check the actual IR signal by clicking on the tube  and “check signal” for intensity and stability on the background position.

The intensity of the single beam spectrum (proportional to the power of the radiation reaching the detector) is given by the Amplitude when “Interferogram” or “Spectrum” is shown.

Whenever checking the signal, ensure the aperture slits are in position and set to the correct size.

- Tick “Spectrum” to display single beam spectrum so the amplitude and stability of the signal in the whole wavenumber range can be assessed. Click “Scale Display” to display the whole spectrum in y axis range.



When using the synchrotron source and need to optimize the signal:

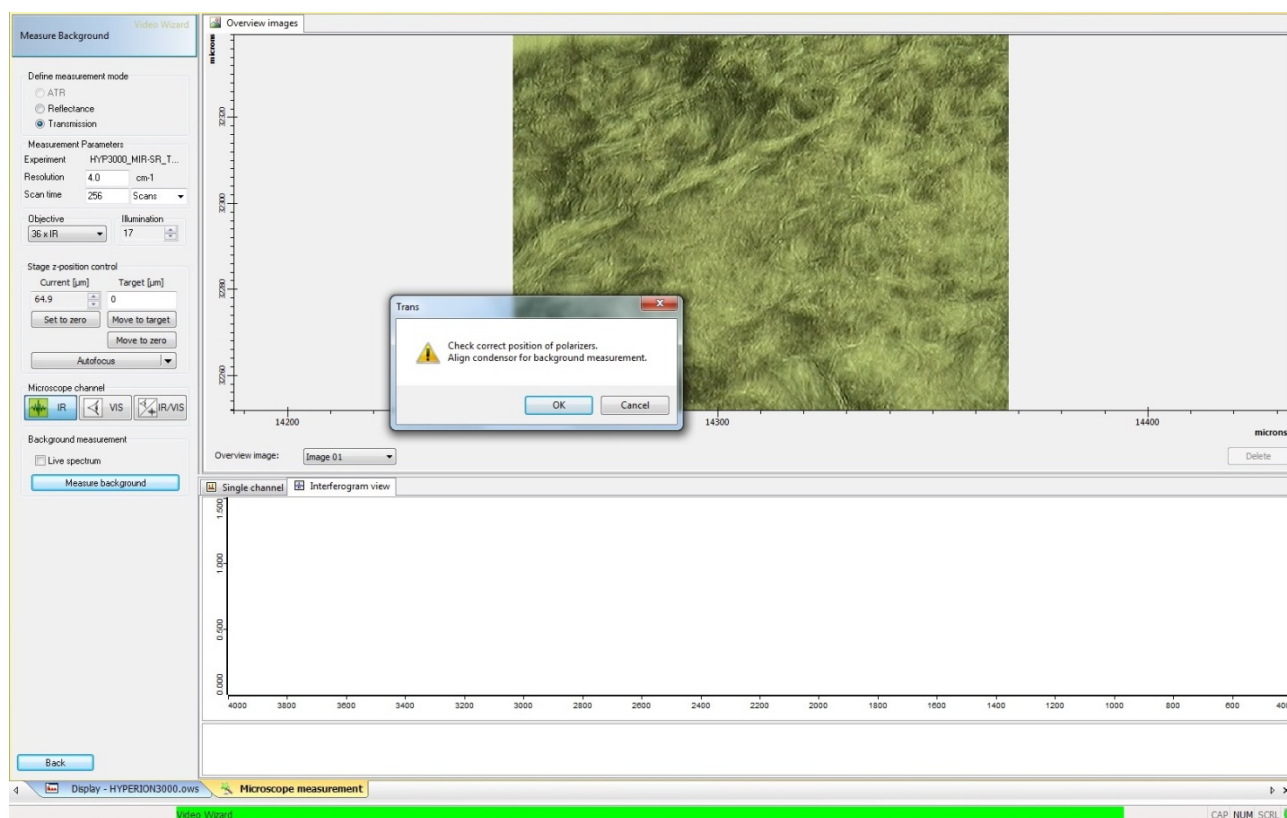
- Aim for the best possible number of counts in the whole mid-IR region and for stable spectrum in the whole range image display. 32,000 counts is the absolute maximum, above which the signal is saturated and this will cause artefacts in the spectra. The maximum recommended amplitude in any condition is 31,000 counts.
- Reduce any fluctuations in the intensity of the spectrum, in particular, the baseline of the spectrum in the near-IR region above 4000 cm^{-1} should be flat at the zero level, and stable.

- This is done by adjustments of the beamline secondary optics mirrors (see “SR beam optimization” section in the manual).

N.B. when changing source or beamsplitter (in the “optic” window) click “check signal” then show “Interferogram”, then scale display, and “save peak position” to calibrate the FTIR accurately.

When working in transmission mode, the condenser should be checked and aligned. When on a background position of the sample in the visible mode, close the metal condenser slits, and adjust the illuminated square to be centered on the glass slits by slightly moving the X and Y screws of the condenser. The default alignment of the Z micrometer should be done to bring the metal slits into sharp focus. Open the metal slits and return to IR mode then check signal. Depending on the substrate material and thickness, you may need to defocus the condenser by turning the Z micrometer to have the best signal configuration (e.g. a thick dispersive substrate like 2 mm ZnSe will not have the same focus for visible and IR light).

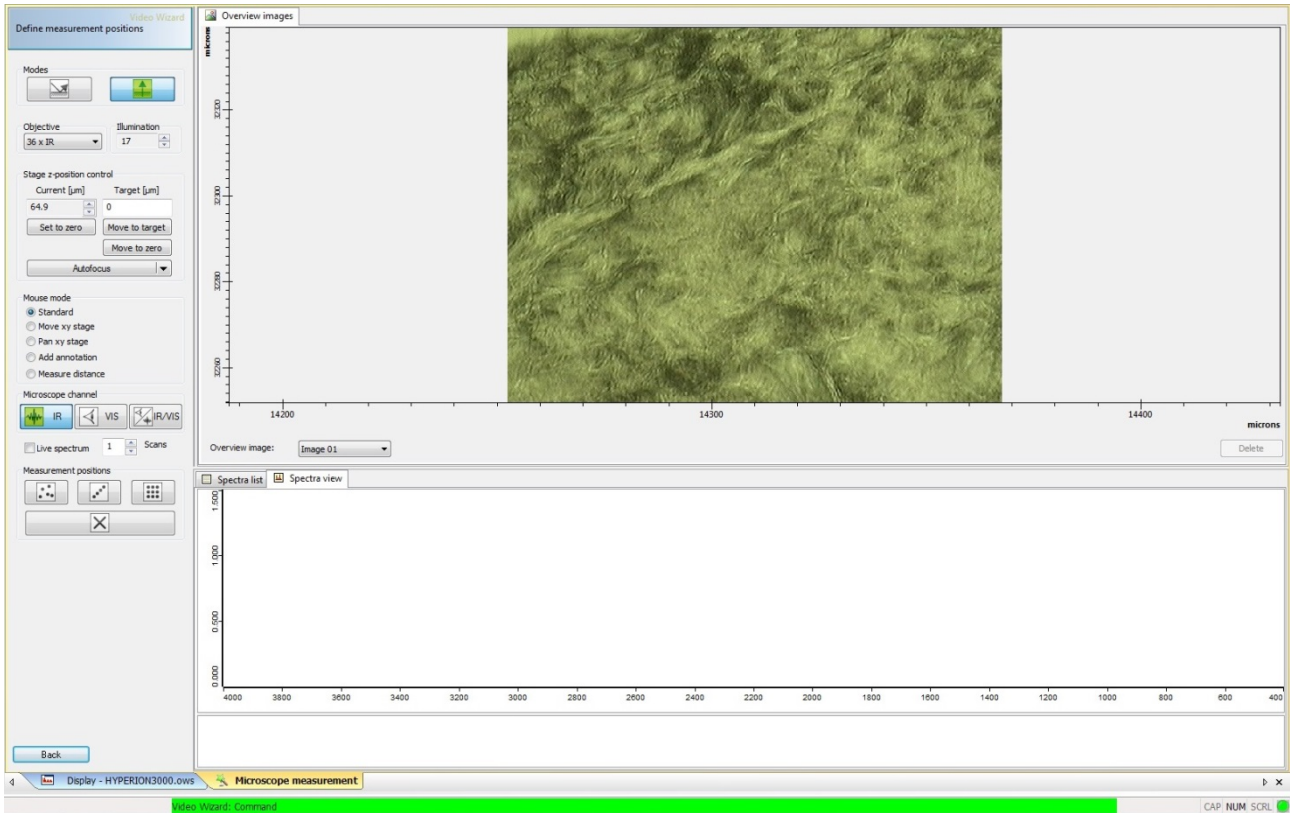
Once the signal is stable, you can measure the background. Make sure you have the correct parameters of the resolution and the scan time. For the message shown below “check correct position of polarizers, align condenser for background measurement” click Ok. This prompt is important if the sample and background conditions are very different.



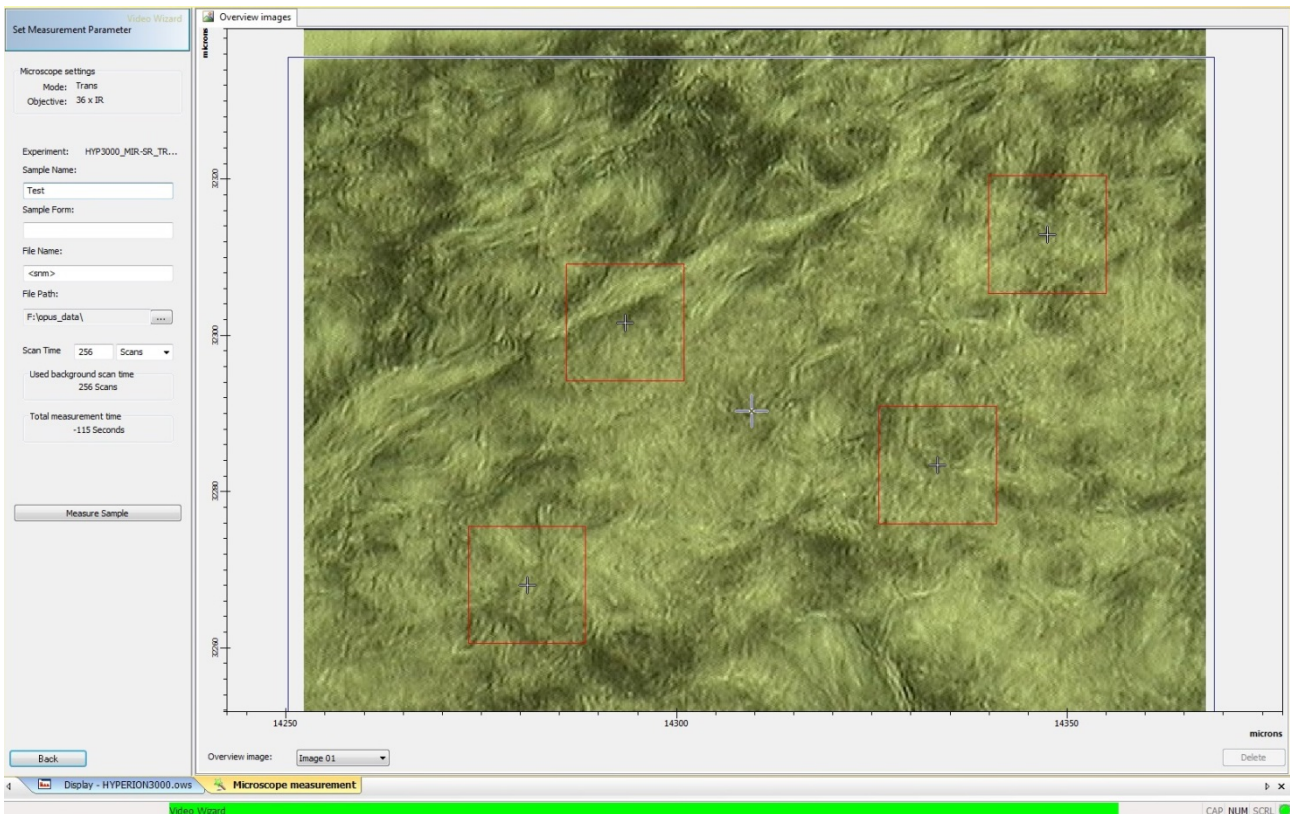
When the background is done, choose the IR measurement/map positions to acquire. There are three options: random point, line or 2D map. For the map (grid) you need to choose the X and Y numbers taking care of the step size which depends on the aperture used. **IMPORTANT!** Estimate the time required for the map (the time given by OPUS is not accurate), and ensure that the detector will not warm up during the measurement. None of the spectral data is saved until the very

end of the map, and if the detector warms up, a “detector not ready” error can cause OPUS to crash with the loss of all data!!”

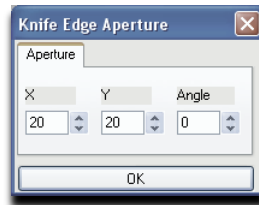
“Autofocus” is not currently in use on the system and should not be clicked.



Next, set measurement parameters, change the sample name and check the aperture size. The visible light image will be saved from when you entered the file name before launching the measurement.



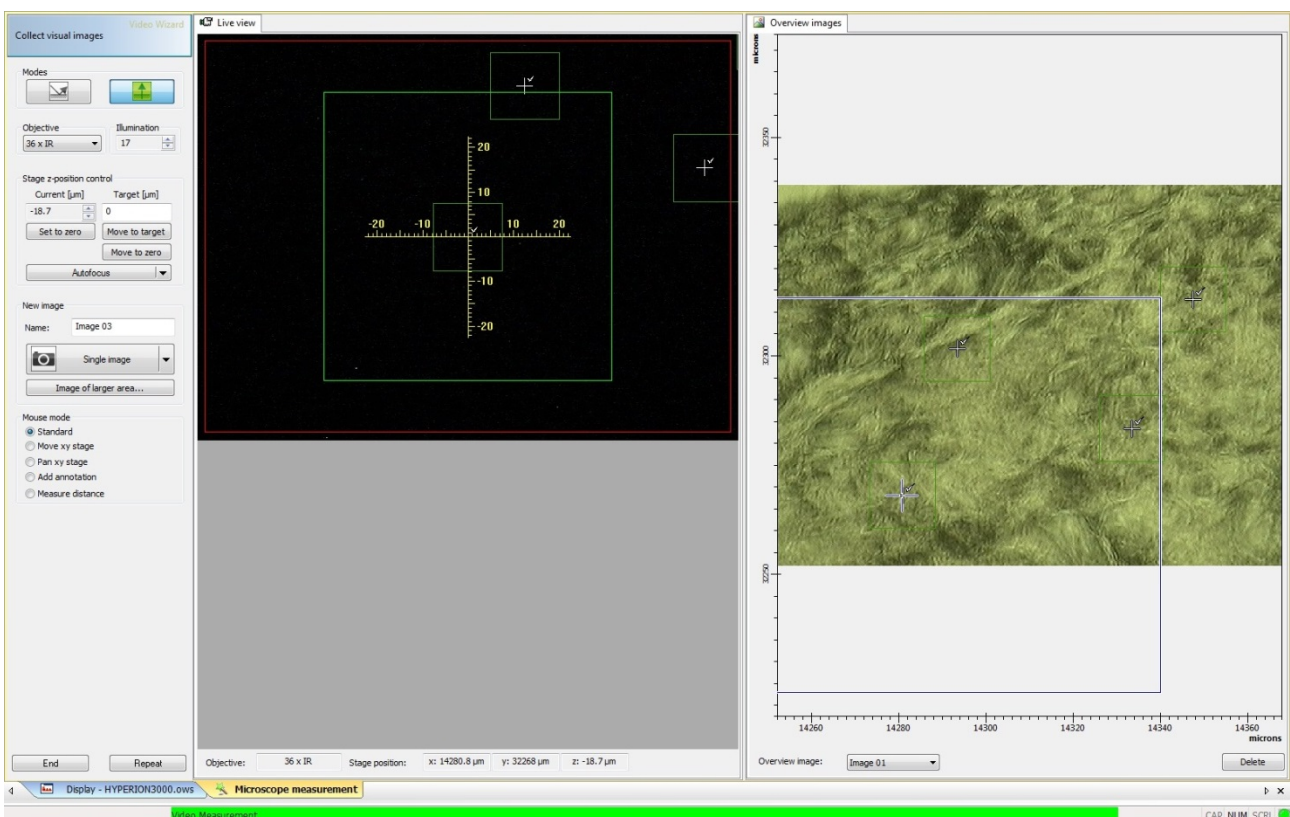
IMPORTANT: If you are doing a **grid or line** and you need to set up a step size between the points, before measuring the sample, left click on one of the defined red squares and then right click to check the aperture size. A little box (figured below: “knife edge aperture”) should appear where you can define the aperture size. Per default this will be the step size you set up for your grid so you need to change it to the correct slit size you used for the background.



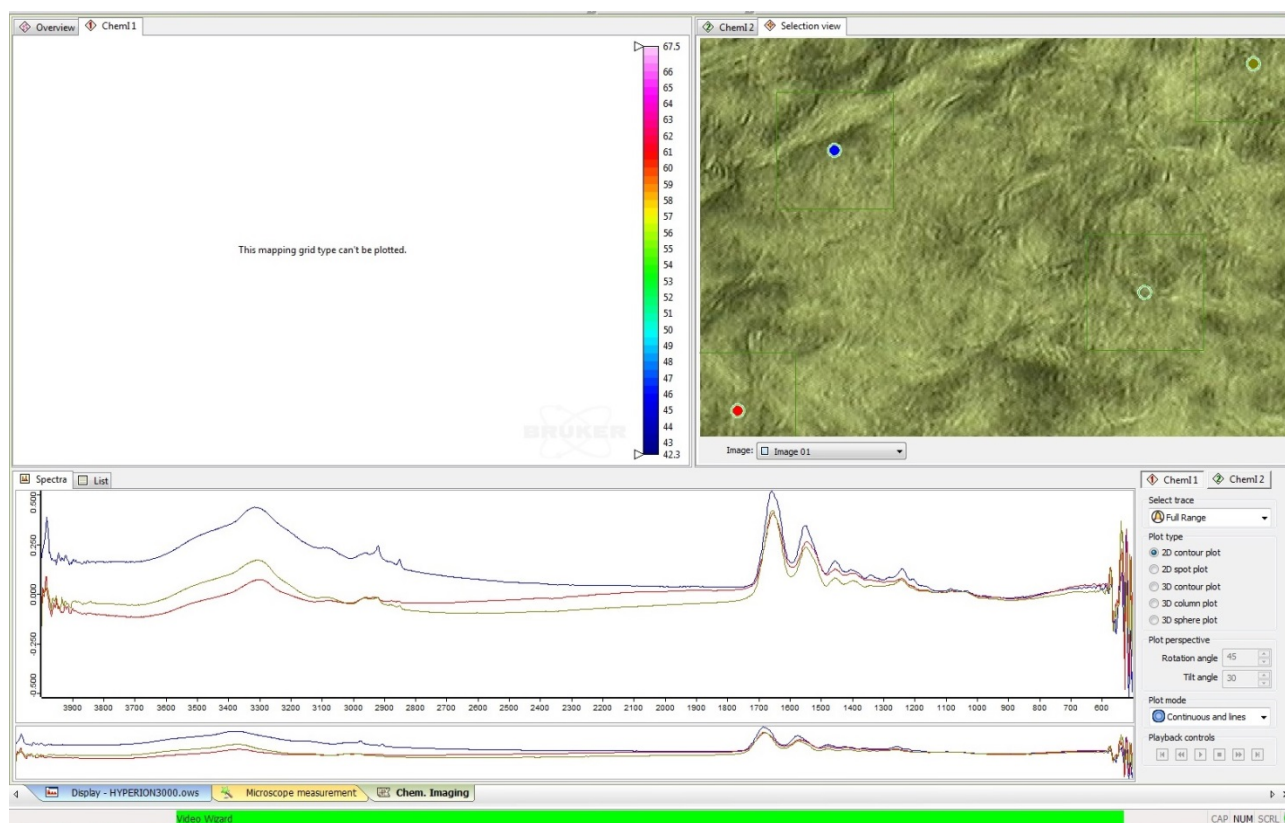
In case you change it right click again on the same red square and click “set to all” to set the new size to all apertures. Note for a diagonal line map, the default rotation of the aperture is along the line, and this should be changed to zero to be compatible with the background measurement. If this is not done, severe instability of the spectrum can occur.

After changing the sample name and checking the file path and the number of scans, click “measure sample”.

Once the image is acquired, click on “end” or “repeat” to finish. The data is only saved when one of these options is selected. Click end if you want to change the sample, and repeat if you want to take another measurement on the same sample. In this case the wizard will save the points you have chosen in the beginning to collect the large visible image but will not save the background position. Though, you can still use existing background option if you don't want to collect a new background for your next measurement (e.g. you are still using the same sample but want to measure a different area not far from the previous one).



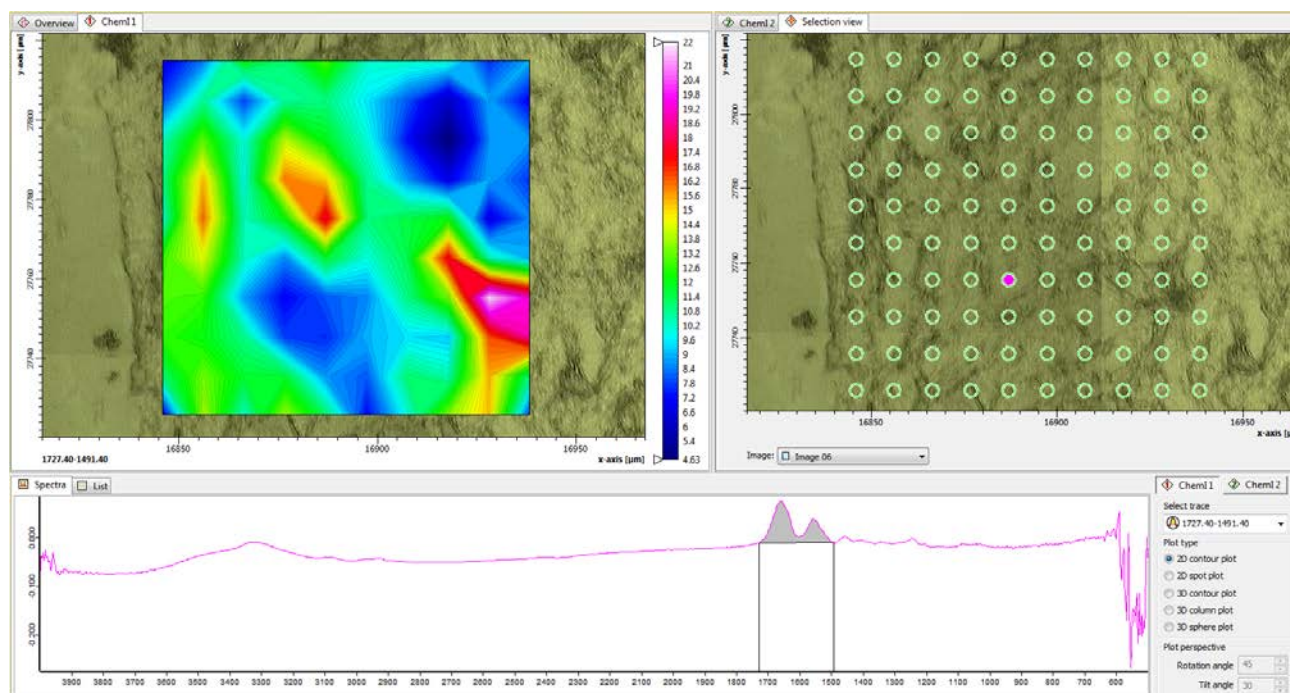
The data is saved in a Bruker proprietary 3D format, but single spectra can be extracted later as single OPUS spectra or e.g. CSV files if required.



Alongside the data collection wizard "Microscope Measurement" window/tab, a new "chemical imaging" window will then be opened. In the top right corner of the chemical imaging window is the data selection view. A circle is shown for each measurement position, and clicking on this shows the appropriate spectrum in the "Spectra" window at the bottom. You can do a quick band integration by holding down the left mouse button on the left side of the band to be integrated and dragging the mouse across to the far end of the band as shown in figure 9." This defines the integration wavenumber range, and several bands can be selected before performing the integration. Once all bands are defined, position the mouse just above the spectrum (not under the curve) and right click: this brings up a new dialog and you can click on "integrate". The top left panel "Chem1" will then show the false colour map of the band integration for line or 2D maps.

Here is an example for a map collected with SR showing the integration of the protein area on the spectrum and the false color map in the "chem1" window.

Figure 9



If you only want to select few spectra and extract those, you can select them from the “List” tab (in the spectrum window) and then extract.



It is strictly not recommended to analyze the data or overwrite the acquired data on the endstation PCs. This is just for giving you an idea on how you can adjust the measurement of your samples later on, in case you need to change few parameters for the next measurement. When you try an integration and you close the file in the “chem window”, the software will ask you if you want to save the changes done to your file, please say no otherwise you overwrite the raw data.

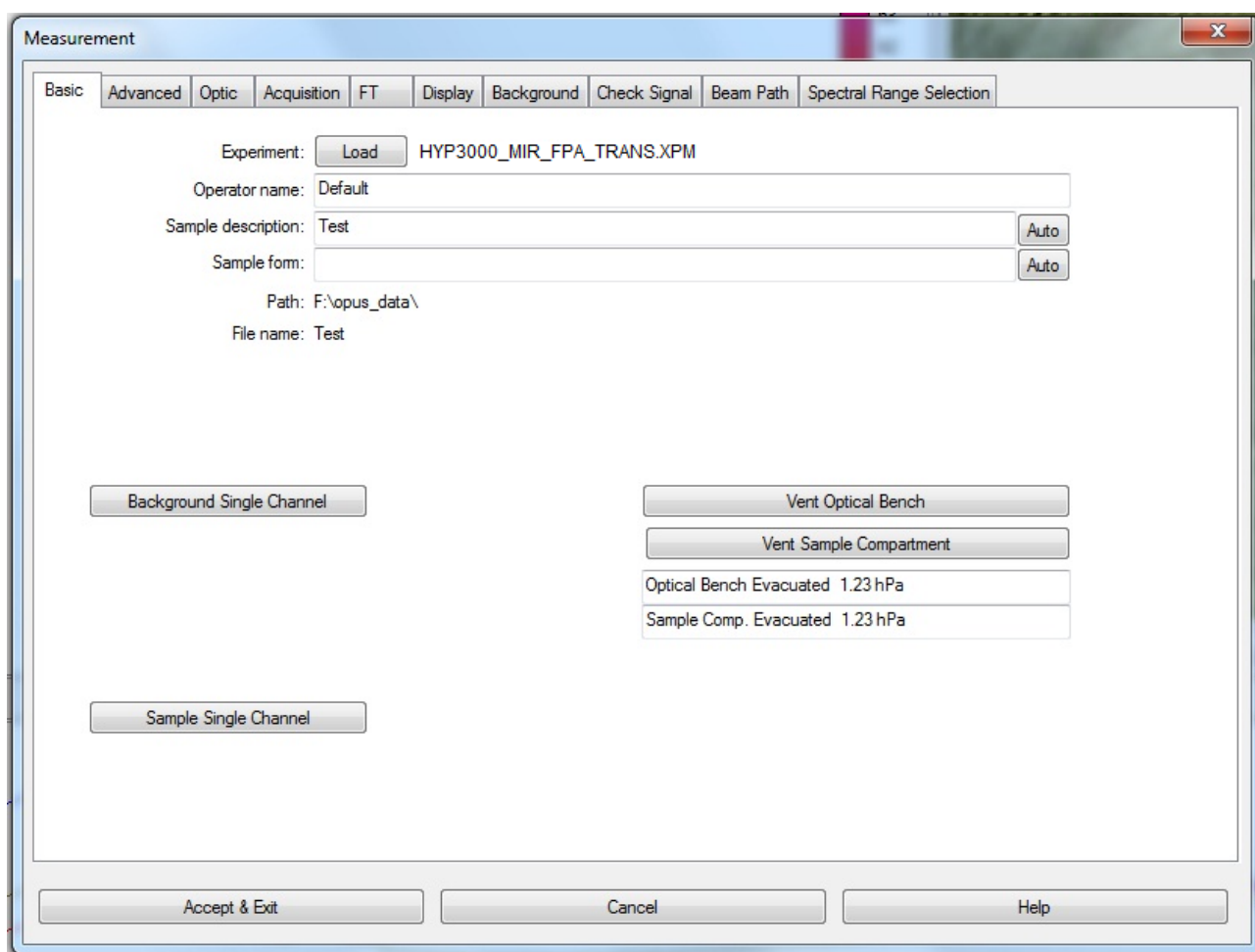
If you collect many maps, it’s always better to close them once acquired (i.e. after clicking end or repeat to save them) so the software won’t struggle as if there are many files open in the display window.


Working with the Focal Plane Array detector

IMPORTANT! First, make sure the wizard is closed and the detector is cold i.e. Do not switch on this detector unless cooled/filled in with liquid nitrogen.

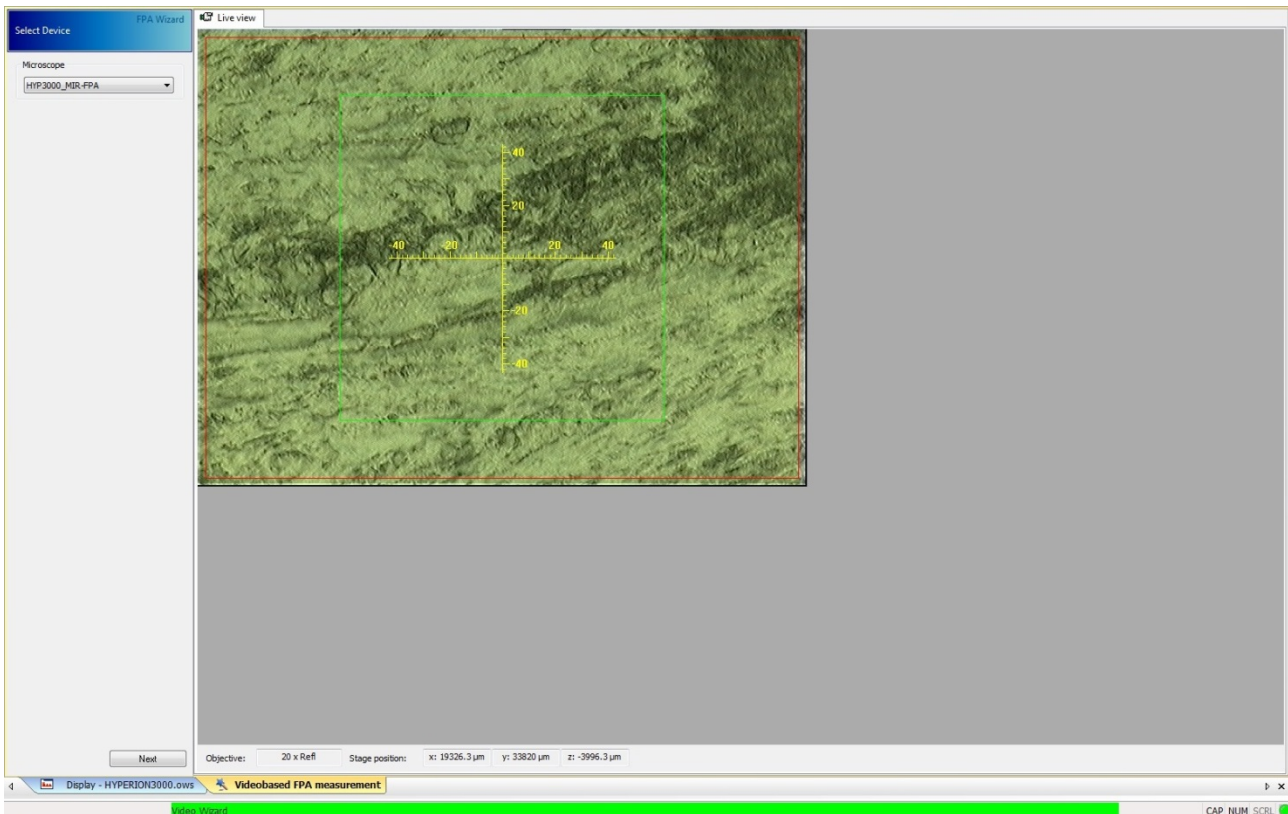
To turn on the focal plane array (FPA) detector, flick the metal switch (back of the microscope in the bottom of the detector) to the “Ein” position, it will give a green LED when on.

- If you were previously using the single element detector, it is necessary to switch to the FPA detector by clicking “DET switch”  in OPUS toolbar menu. Then open the measurement dialog by clicking on the tube .



- From the basic in the measurement dialog, load a file with an FPA experiment (.xpm) (with global (MIR) or synchrotron (SR), transmission, reflection) and all the parameters should be set up). You just need to change the resolution, number of scans in the “**Basic**” and “**Advanced**” tabs.
- Accept and exit and then you can open the video wizard .

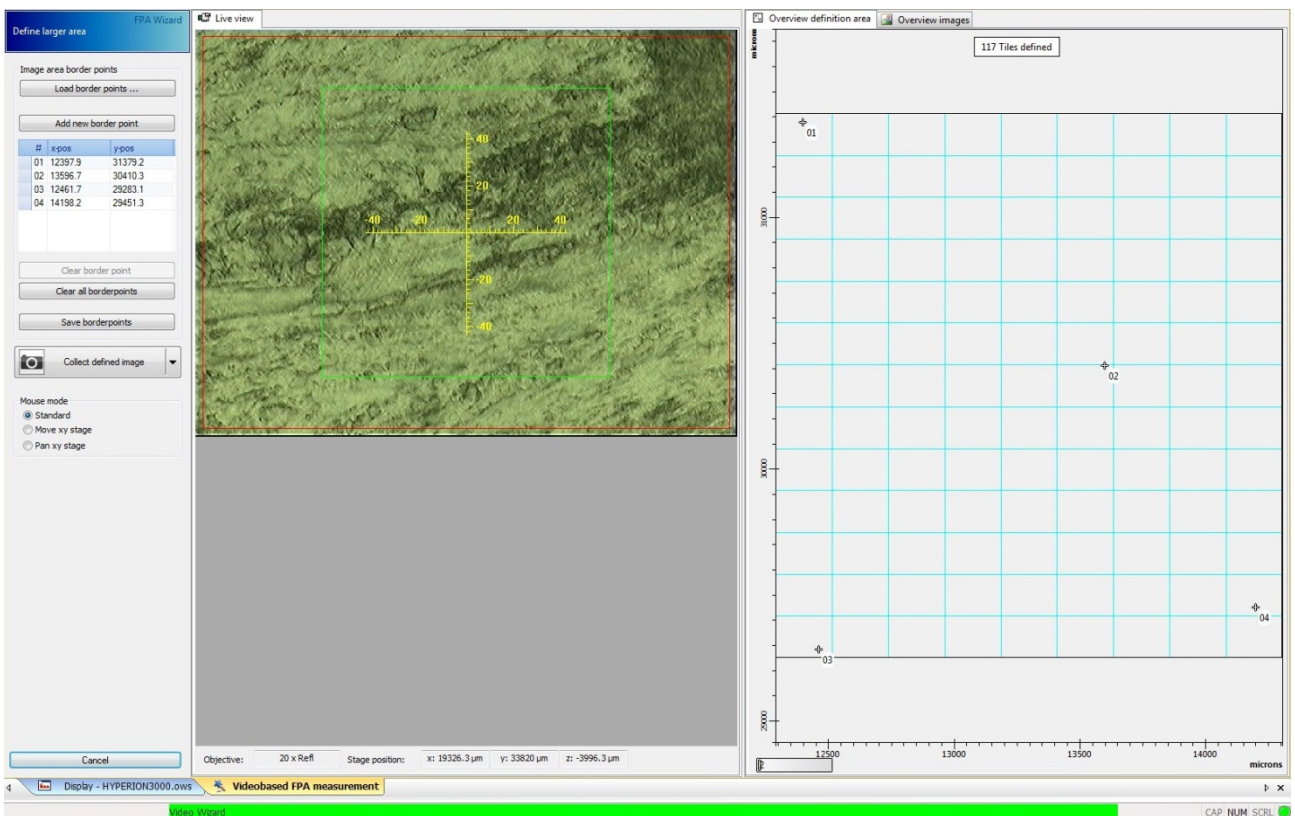
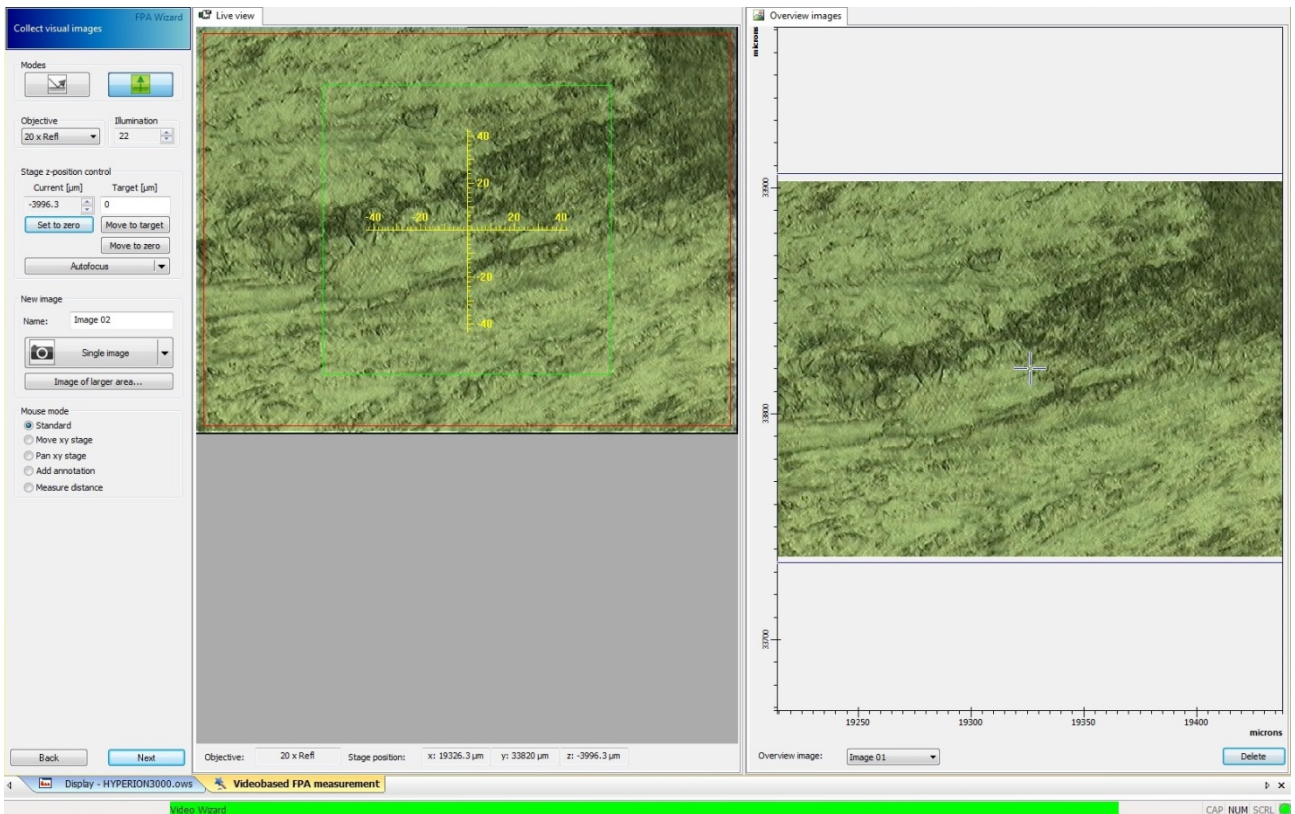
The wizard should show the proper device: FPA (MIR or SR). If not close the wizard, switch again to the FPA detector in the DET switch, and again load the proper experiment file in the measurement dialog. If yes click next, the reset XY stage dialog will appear, do not click on reset, you just click next as you have already done the stage calibration before to start.



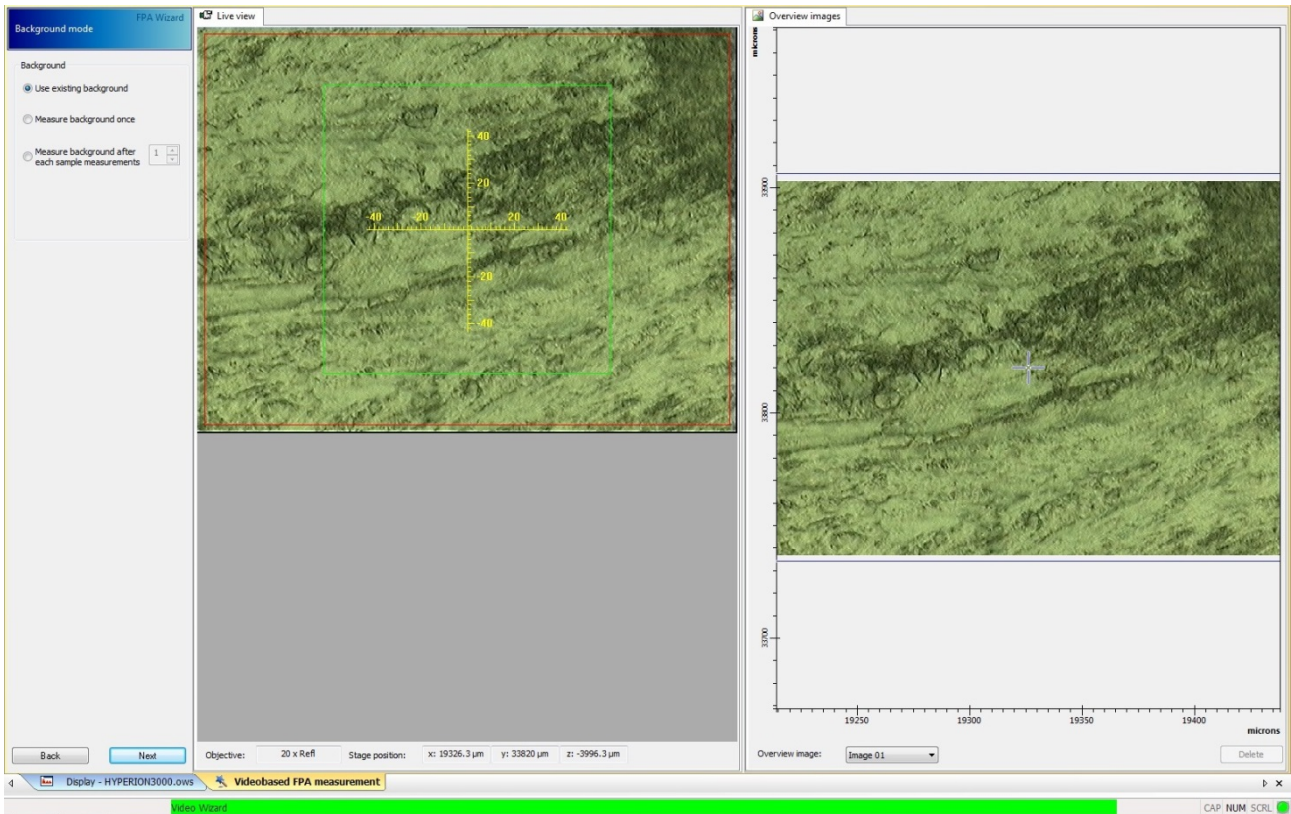
The next step will show the visible image of your sample. Make sure you choose the correct magnification objective on the left, and the mode (transmission or reflection). By default the wizard when opened will set the microscope optics to reflection mode. There are two ways of collecting visual images: a single shot by clicking on “single image”, or “image of larger area” where you need to define a number of points to scan a larger area and then collect the defined image. As a minimum for a larger area you need to define two diagonally opposite corners of the area you want to image.

N.B. For collecting a large image, you need to “Clear all border points” from the previous image, then chose your first point and click “Add new border point”, and repeat the same for next points till you make the grid you want, then click on “Collect defined image”.

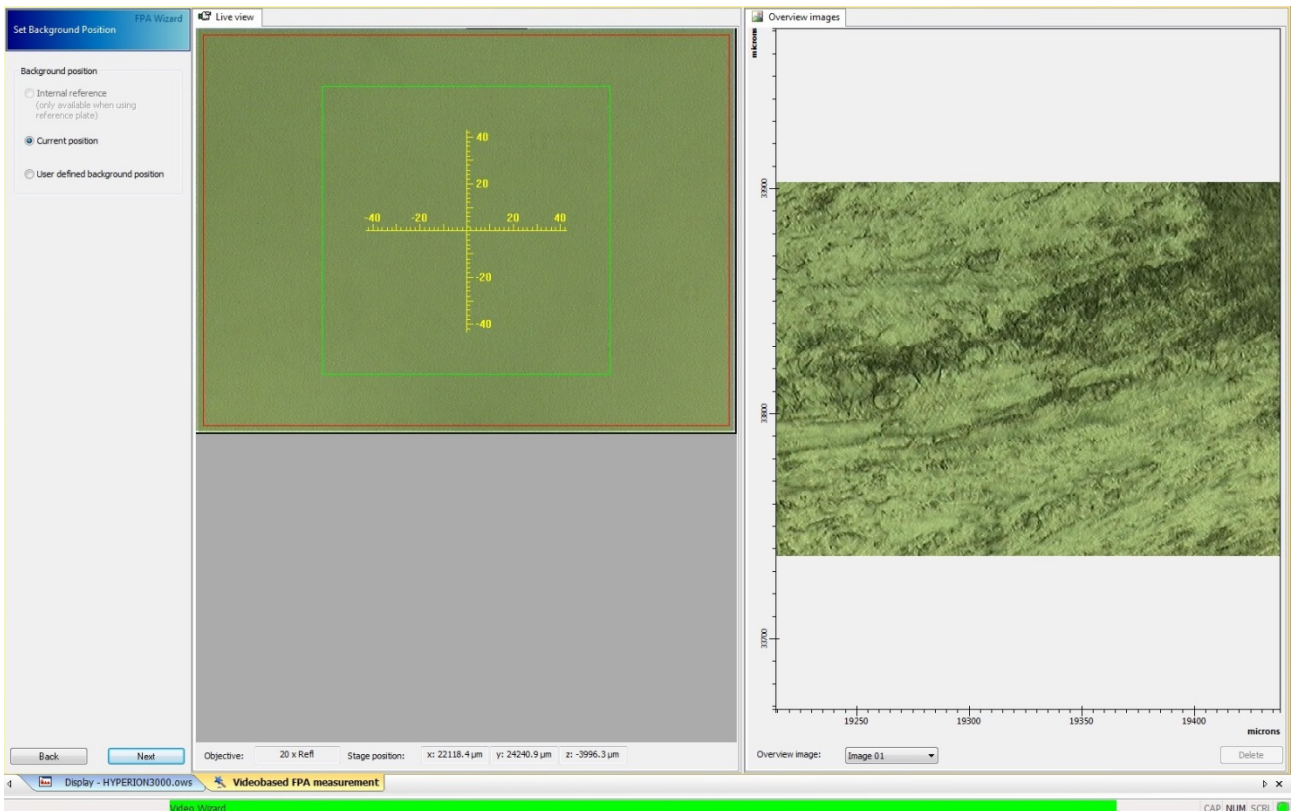
If you take more than one image, they will all be saved with your file unless you delete it from the previous screen before you start the measurement.



When you click next you will have the background mode dialog where you can see three options, choose the one you need and then go to a background position by moving the joystick.



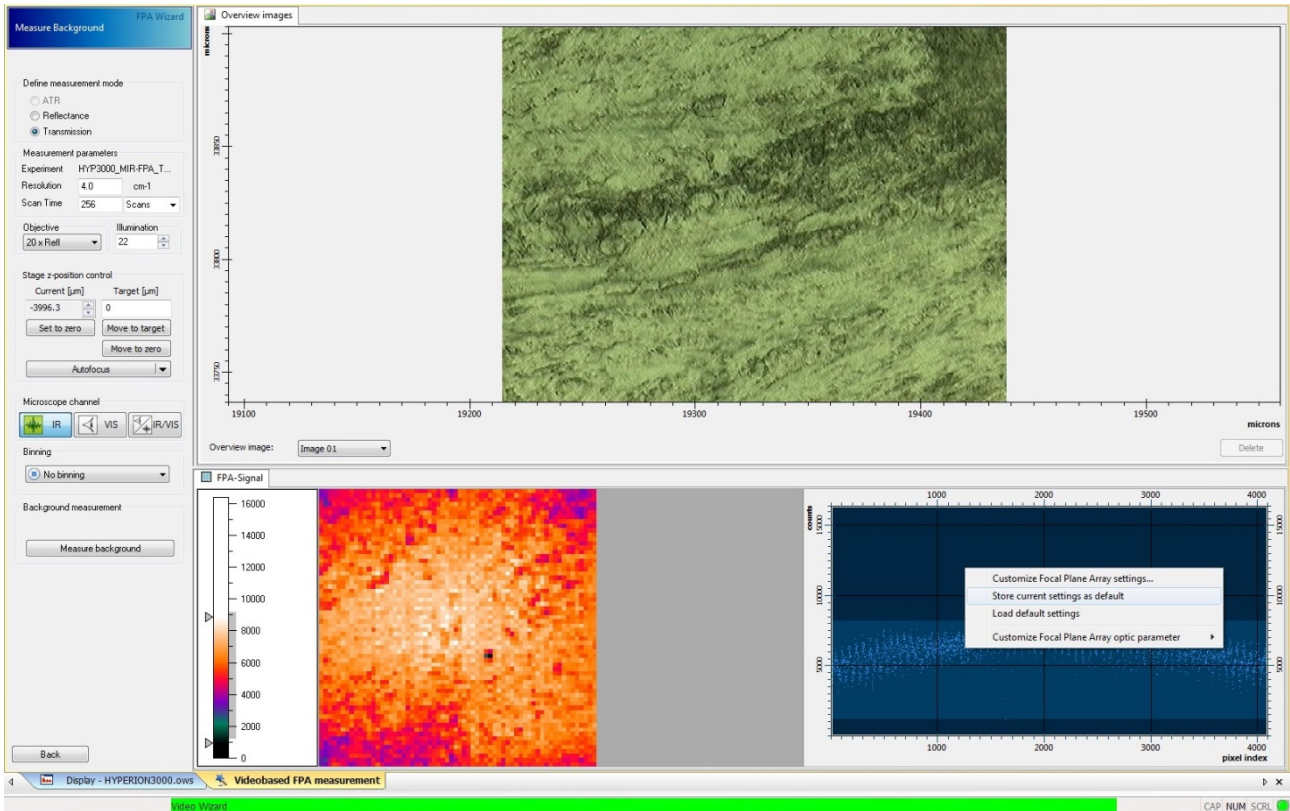
Once you've moved to the background you can set the position by clicking current position. If you don't want to collect a new background by choosing use existing background on the previous screen, this step won't be shown and the next step where you choose the measurement area will be shown directly.



Go next and click IR to check the signal.

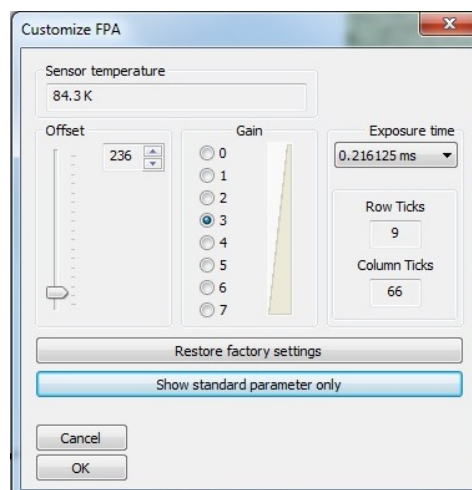
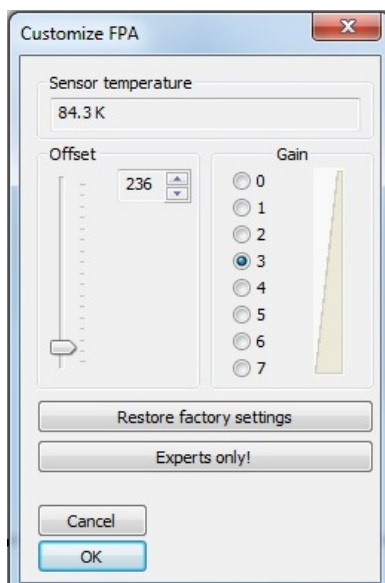
Check Signal for FPA

The “*Check Signal*” page of the FPA wizard differs significantly from the corresponding page for the single element detector. In the FPA signal box, there are two graphs as shown below.

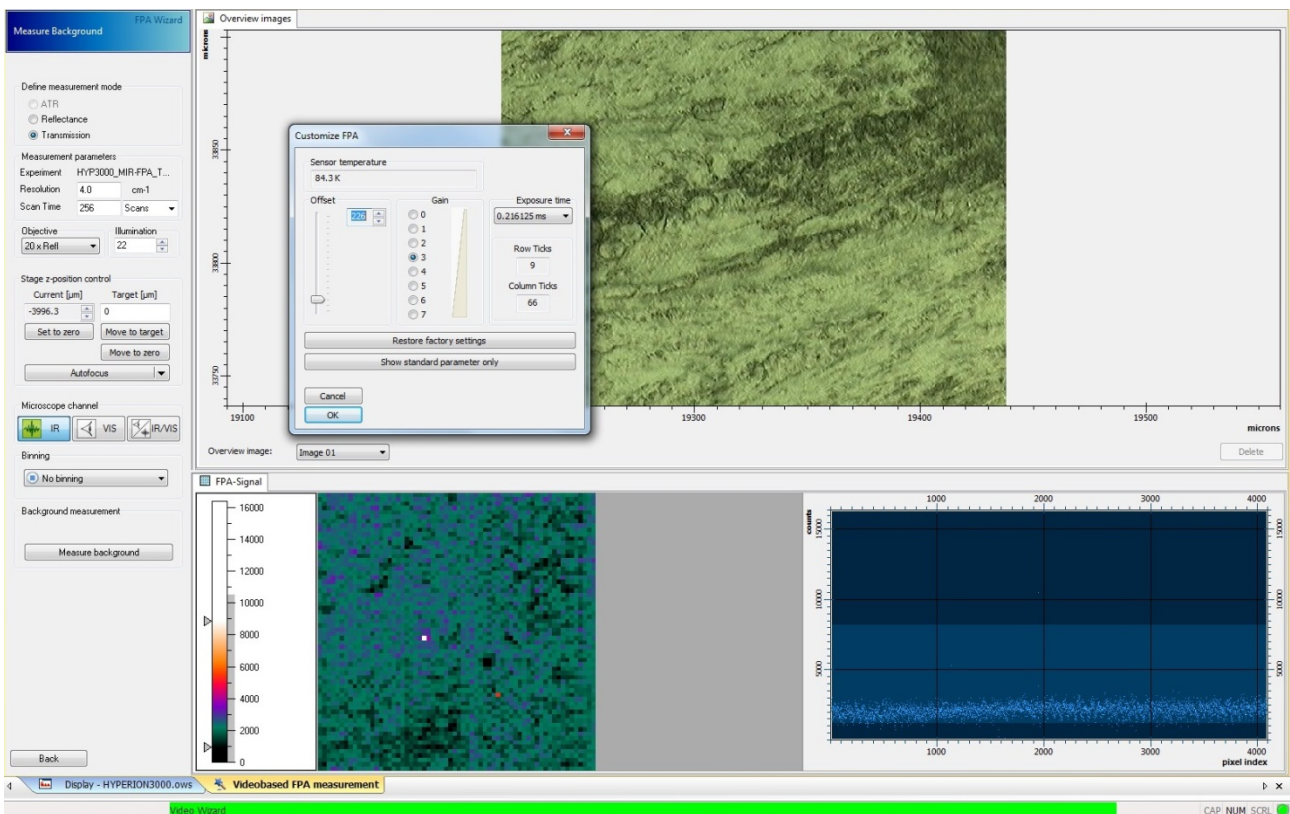
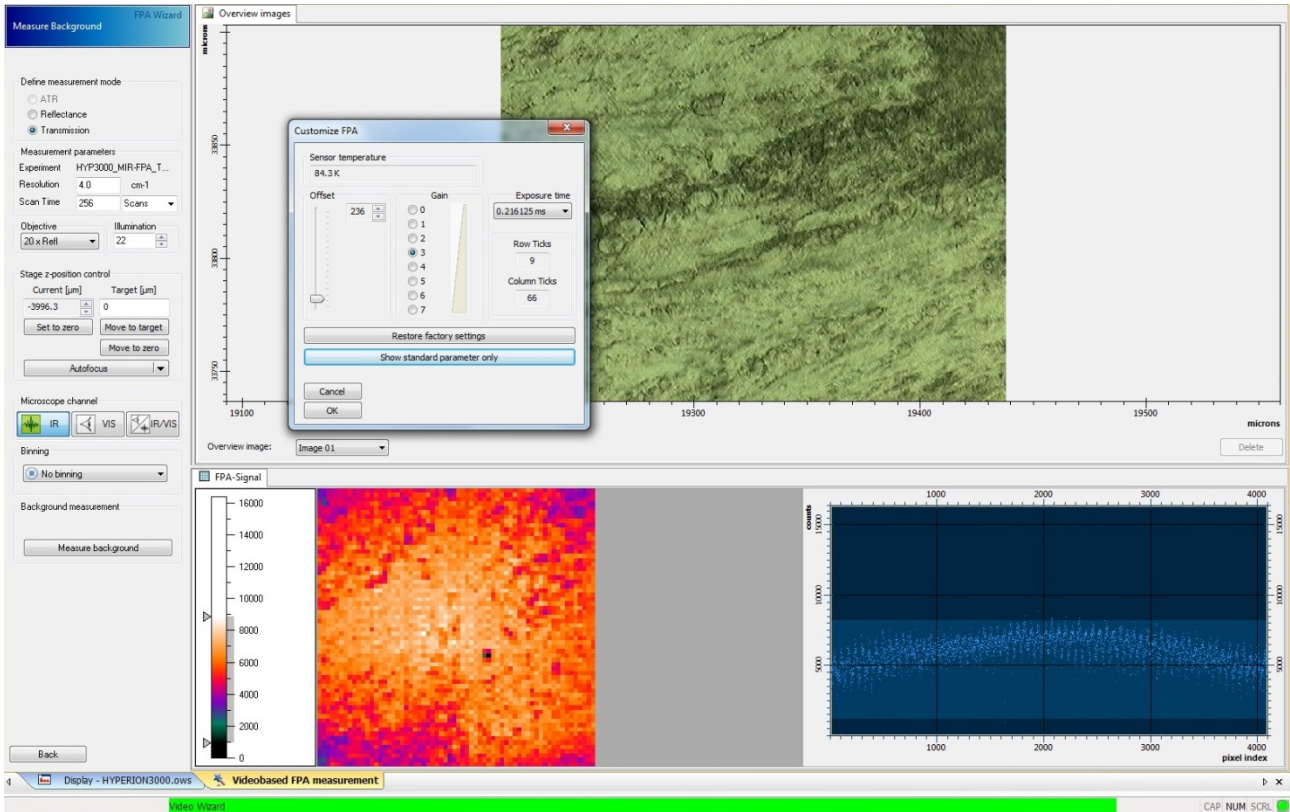


The graphs correspond to the live FPA IR image on the left and the pixel signal vs index on the right. The live image helps to adjust the condenser when working in transmission mode to fill the whole area and centering the spot. Right click on the pixel index box to have the drop box shown (as in the image above), choose “customize focal plane array settings”.

A new dialog box will appear (left box below), click on “experts only” to have the right box below. You can now change the exposure (integration) time, the gain and the offset to optimize the signal.



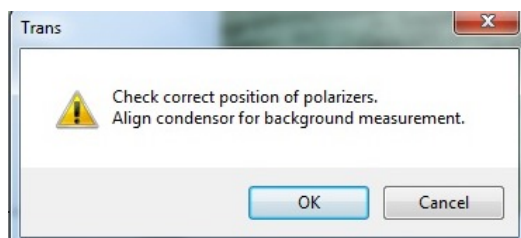
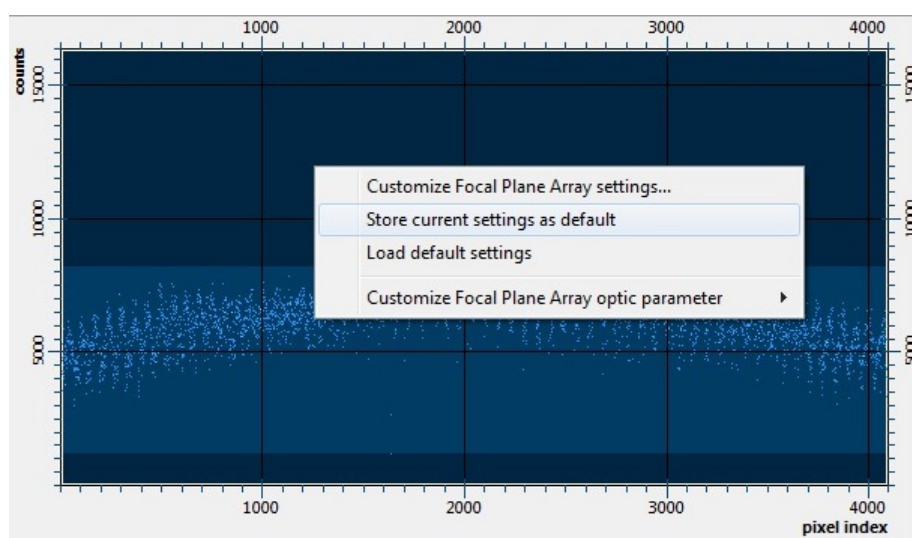
If you are working with transmission mode, close the condenser slits to see if the signal goes down. When optimized, the pixels signal with closed slits should be similar to the image below. The pixel values should always be in the lighter blue box in the pixel index graph (on the bottom of the box when the condenser slits are closed and between the middle and the top of the box when the slits are opened). This gives the best configuration which corresponds to the best signal.



In the 'oscilloscope display', the intensity values of all pixels (i.e. detector elements of the FPA detector) are displayed versus the pixel number. This kind of display allows you to recognize immediately pixels with too high signal intensity. You can optimize the signal by changing the parameters "Offset" and "Gain". The optimum is to have the absolute maximum and minimum pixel values at the top and bottom of the light shaded area of the pixel index vs counts plot. For correct operation of the FPA no pixels should fall outside this range, so the majority of the pixels will be well within these limits".

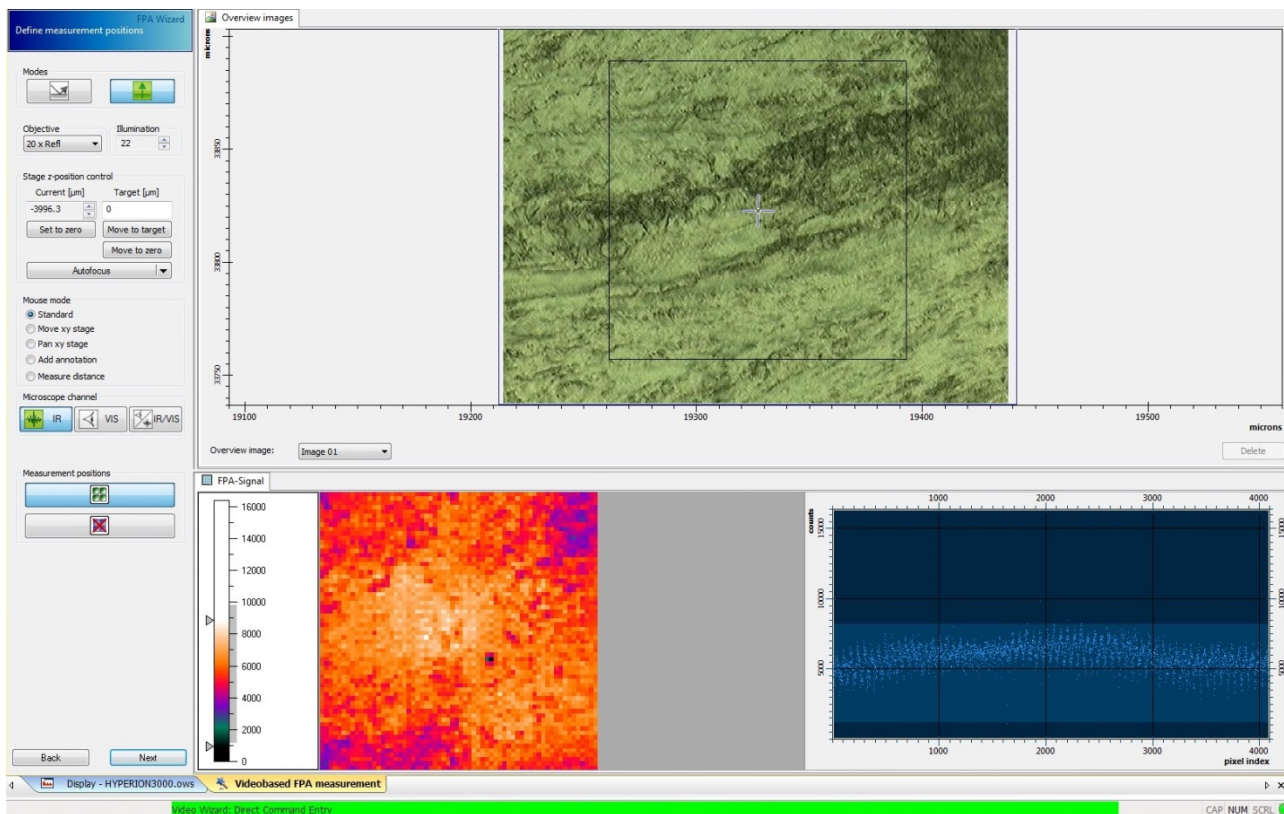
N.B. the optimum is to have the max signal around the mid position (~8000 counts) of the FPA set up display and the minimum (i.e. in visible mode) at about 2000 counts. To produce these values, adjust the "offset" and "gain" parameters. The reason for these settings is that during the FTIR scan, each pixel will jump between zero signal and double the time averaged (displayed) signal at the centreburst of the interferogram. For the 36X objective, this range may not be achievable, as the signal is weaker and it will drop just slightly between visible and IR.

Once you are happy with the signal, set the parameters to be the default ones by right clicking on the pixel index graph and selecting "Store current settings as default", otherwise the values will revert to the default values for each measurement. Then measure the background. Make sure you have the correct parameters of the resolution and the number of scans. For the message shown "check correct position of polarizers, align condenser for background measurement" click Ok.

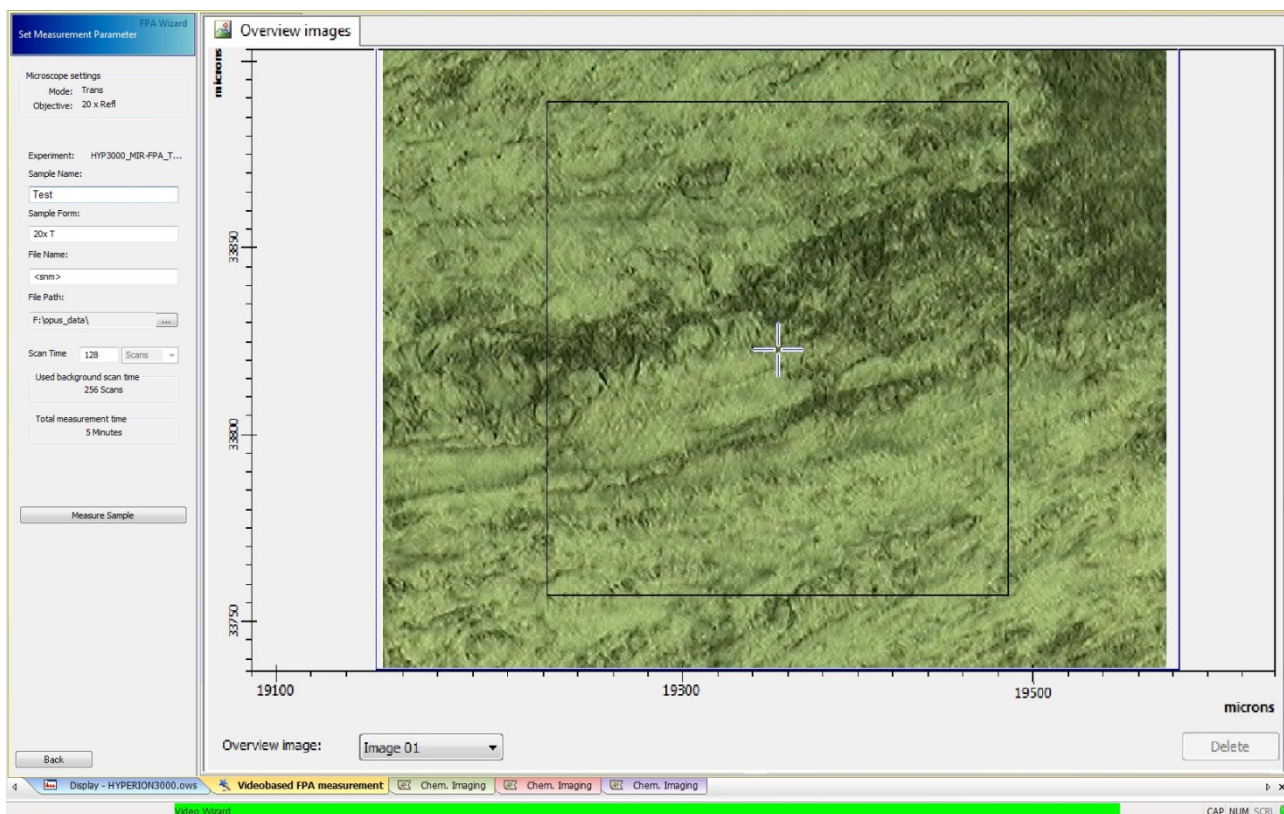


When the background is done, choose the sample area, by clicking the top button under "measurement positions". Double click on the location in the visible image to perform a single FPA shot at that position. If you are mapping a larger area, increase the numbers in X and Y directions.

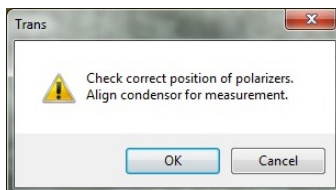
IMPORTANT! Estimate the time taken to acquire the map and ensure that the detector will not warm up during the map. This can cause serious damage to the detector, and all the map data will be lost. In practice this limits the mapping time to around 5 hours if the FPA dewar was just filled.



You now need to set measurement parameters, change the sample name and then click measure sample. Here the image is saved in the file path shown (check if it is correct, e.g. "F:\opus data" to save into your user folder).



For the message shown “check correct position of polarizers, align condenser for background measurement” click Ok.



Once the IR image is acquired, click on “end” or “repeat” to finish. Click end if you want to change the sample, and repeat if you want to take another measurement on the same sample. In this case the wizard will save the points you have chosen in the beginning to collect the large visible image but the background position is not saved.



Alongside the data collection wizard "Microscope Measurement" window/tab, a new "chemical imaging" window will be opened. In the top right corner of the chemical imaging window is the data selection view. A circle is shown for each measurement position, and clicking on this shows the appropriate spectrum in the "Spectra" window at the bottom. You can do a quick band integration by holding down the left mouse button on the left side of the band to be integrated and dragging the mouse across to the far end of the band." This defines the integration wavenumber range, and several bands can be selected before performing the integration. Once all bands are defined, position the mouse just above the spectrum (not under the curve) and right click, this brings up a new dialog and you can click on "integrate". The top left panel "Chem1" will then show the false colour map of the band integration.

Please remember to not overwrite the raw data if you try an integration so do not save the changes when closing the “chem window” and close the files as soon as they are finished (i.e. after clicking “end or repeat”) so the software won’t struggle with many opened files.

Check the FPA is turned off (switched to "Aus" position and the green led light off at the back of the microscope under the FPA detector) at the end of the measurements !!!

Hints for IR Microscope usage

XY Button/Calibrate

After a computer reboot, clicking on this button calibrates the motorized stage, i.e. the stage moves to its predefined home position. Tick the *Activate Joystick* checkbox to be able to move the stage position after that. **This should be done when the wizard is closed.**

Aperture in the microscope

Always re-check the aperture size on the background and on the defined sample points before starting the measurement. If you have set multiple measurement positions e.g. a line map, you click on the displayed aperture itself (red box) and set up the size you want as it may not take it automatically when you set it up for the background (the default is the same as the step size).

For further help, press the help button in the toolbar for more information about OPUS software.

Synchronization and data transfer/archiving

At the end of the measurement and in order to collect all the data acquired, you should perform a synchronization to move the data to the B22-storage server. It is best to organize your files into a useful structure before synchronization. Click the “synchronize data” icon on the desktop.

Test the synchronization first by saving your .XPM file into “F:\opus data” and check this file is moved to the B22-storage server on one of the analysis computers (ws002 or ws004).

Troubleshooting: Note: this section is for B22 staff use only; for users please speak to your local contact. If “SUDO” error occurs on synchronization the sudo service needs to be restarted. To do this, firstly log on as B22 admin (Log Off – Change User) then go to Start - Control panel - Administrative Tools - Services, find the sudo helper service and right-click to select start.

User Work Flow: The Endstation computers are for data collection not analysis. If users wish to analyze their data they should perform a synchronization of the data and work on the other beamline computers or in the B22 data room (1.42, zone 3). If a user wishes to save any modifications they make to the raw data, this should be saved in the processing folder, not on the hard drive of the desktop computer. This will make the data accessible from their home institution and will not fill up the archive with processed data.

Warning! Processing of synchronized data on the Endstation computers will cause the original data to be overwritten by the processed one on the next synchronization.

At the end of your beamtime

After finishing all measurements, it is very important to leave the instrument in a safe condition:

Close Opus, turn off the microscope but leave the PC on. If you have been using the FPA, check it is turned off.

Please consider the environment and turn off the beamline cabin lights.