OpenRAMAN Spectrum Analyzer V1.0.0 Manual

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1/ INSTALLING SPECTRUM ANALYZER

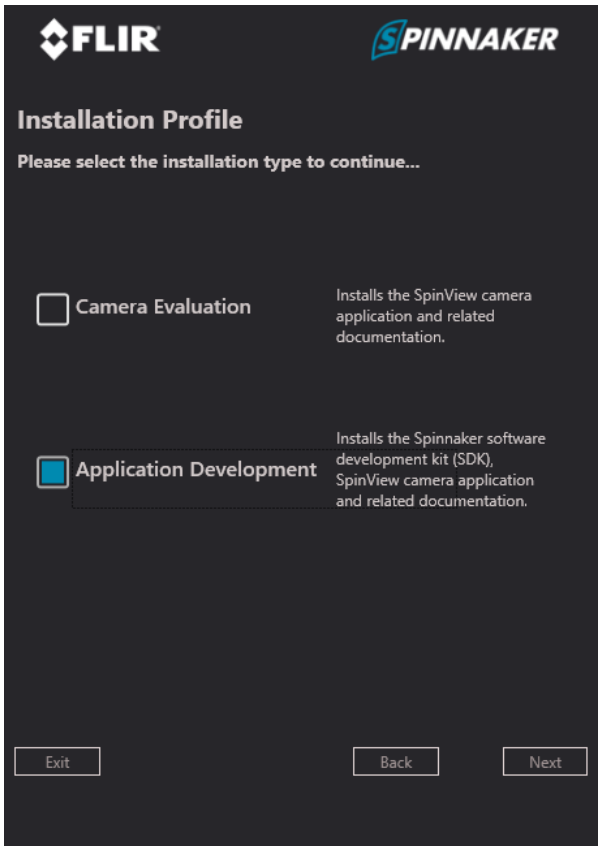
To install the Spectrum Analyzer suite, extract the .zip archive anywhere you want on your computer. If you are unsure where to extract the files, we recommend extracting them at C:/SpectrumAnalyzer location.

To run Spectrum Analyzer, go to the folder where you extracted the files and open the “bin” or “bin64” folder depending on if you are running a 32bits or 64bits version of Windows. If you are not sure if your computer is 32 or 64 bits, visit https://support.microsoft.com/en-us/help/15056/windows-32-64-bit-faq. Run SpectrumAnalyzer.exe to launch the software. We recommend that you place a shortcut to the application on your desktop for quicker launch.

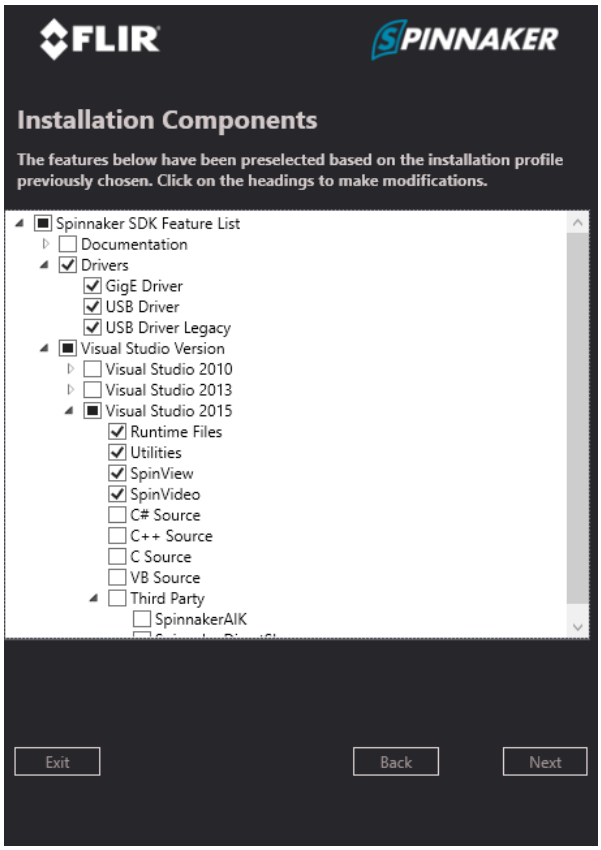
Important: To enable connection to the spectrometer, you will have to install the PointGrey Spinnaker SDK. Go to https://www.flir.com/products/spinnaker-sdk/ and download the Spinnaker SDK for Windows. We recommend using the version 2.0.0.147 as we have had issues when using a different SDK

version than the one the application was compiled with. You may also recompile the project pointgrey.dll project if you wish to use a different SDK version.

When installing the Spinnaker SDK, select “Application Development”:



Then select the following modules:

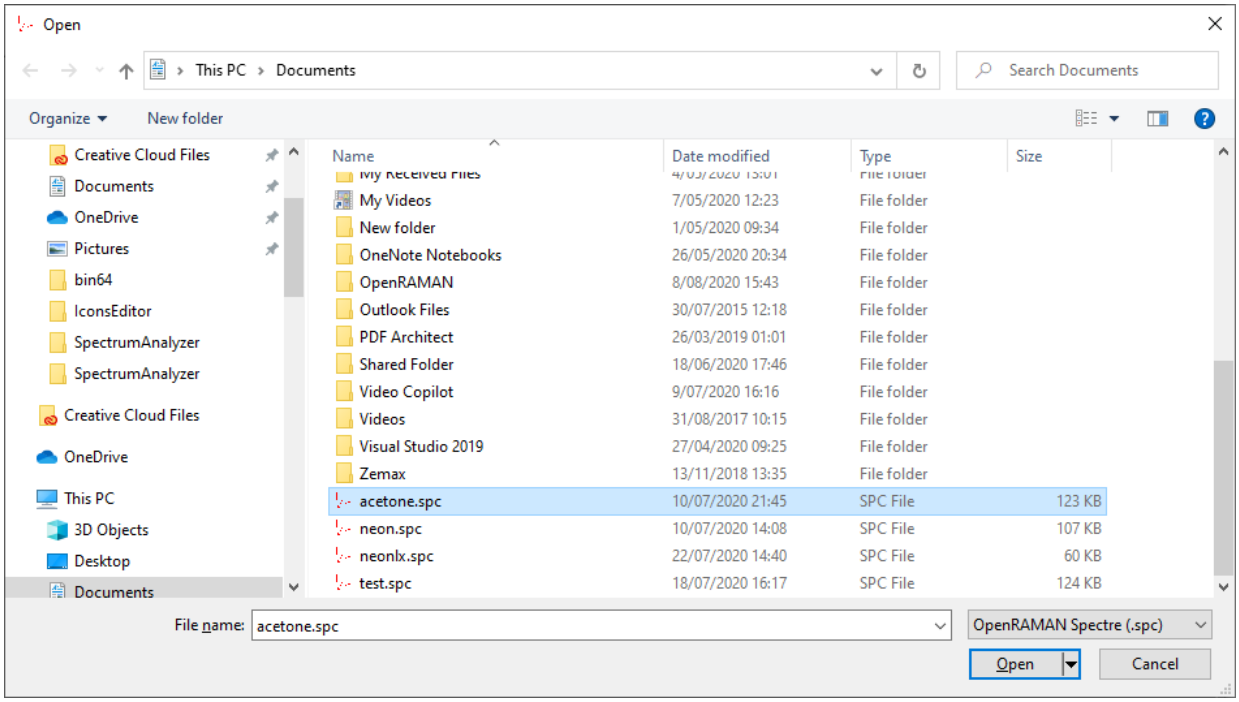


When using GiGe camera, run the configuration tool to set up your Ethernet adapter to work with cameras and connect to the camera using the SpinView program to set up a correct IP address. This should be done only once when you setup your system to work with the spectrometer.

2/ Opening and Saving Files in Spectrum Analyzer

To open a file in Spectrum Analyzer, first start the software (spectrumanalyzer.exe) and click on the "Open" button icon in the toolbar:

This will open a popup window that will ask you to select the file you wish to open:



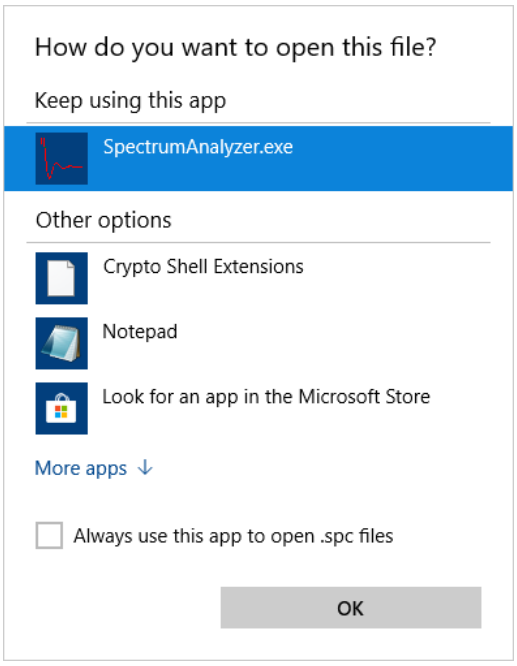
Spectrum Analyzer supports two types of files : (1) Spectre files (.spc), and, (2) CSV files:

- Spectre files are the own file format of Spectrum Analyzer and enables you to save all information concerning your spectrum such as camera and plot settings, blank data etc. Spectre files can only

be read by the Spectrum Analyzer application although it is not a proprietary file format since we have published its source code such that developers can integrate them in their own applications if they wish to.

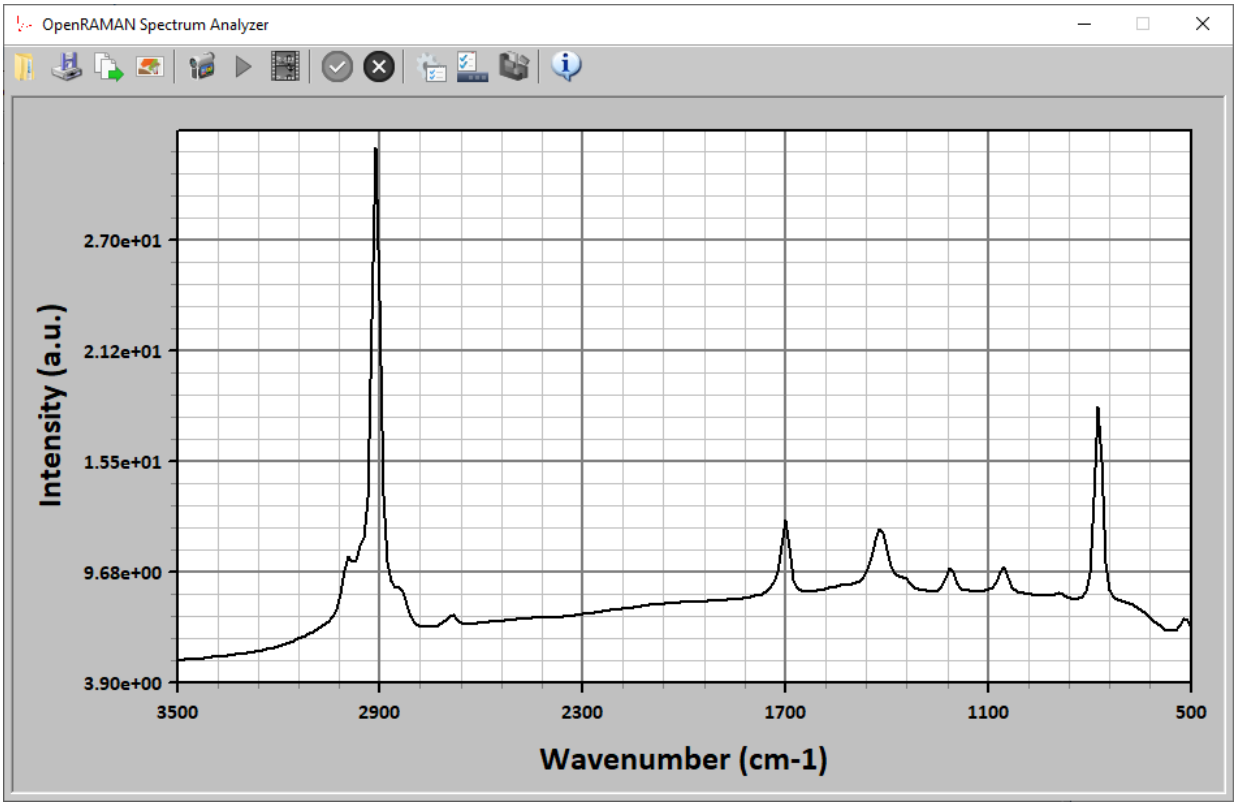
- CSV files are text files where data are separated by a comma symbol. Most commercial softwares (e.g. Microsoft Excel, Matlab…) support CSV files import which makes it an ideal format to exchange spectra with other people or software like Matlab for post-processing of the data. CSV files exported by Spectrum Analyzer only contains the plot data and does not contain program settings nor separate blank data.

You may also import files into Spectrum Analyzer by dragging the file from Windows to the application or by selecting it as the default application to open .spc or .csv files:



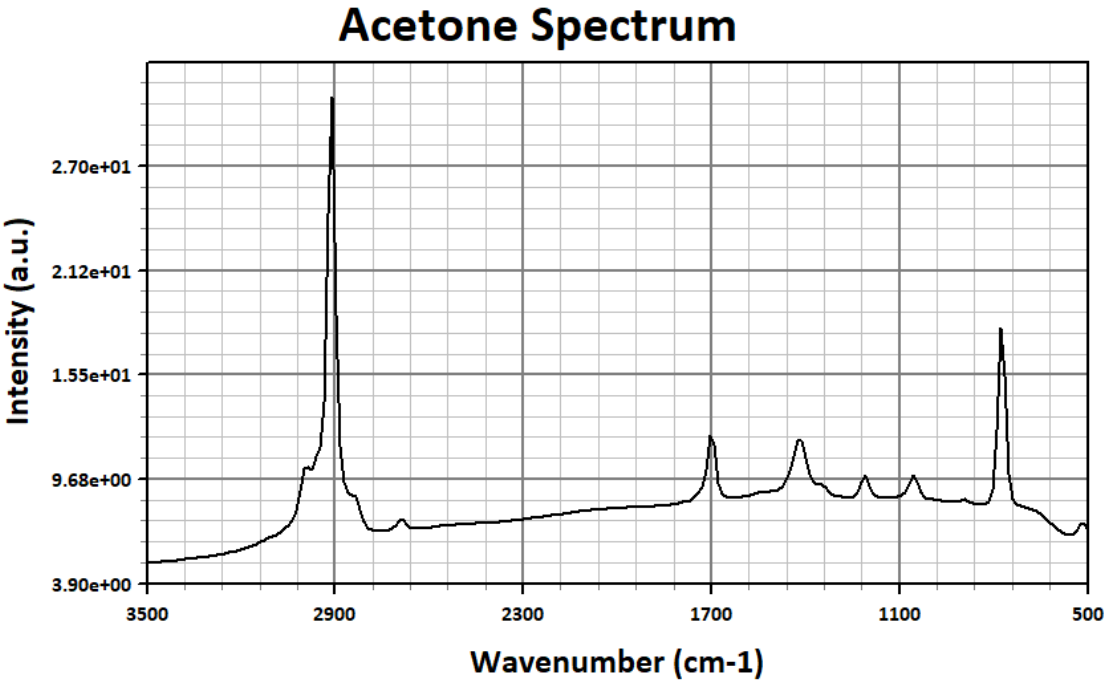
Once the file has been loaded the application toolbar will enable some of the actions:

- Saving the data (either in .spc or in .csv). Data imported as CSV cannot be exported as Spectre file because they lack the contextual information when initially importing the CSV.
- Copying data to the clipboard. After copying data to the clipboard you can paste it in other softwares like Microsoft Excel.
- Saving as image (see below).
- Configuring the plot data. This is discussed in section 4/.
- Calibrating the spectrometer. This option is discussed in section 6/.



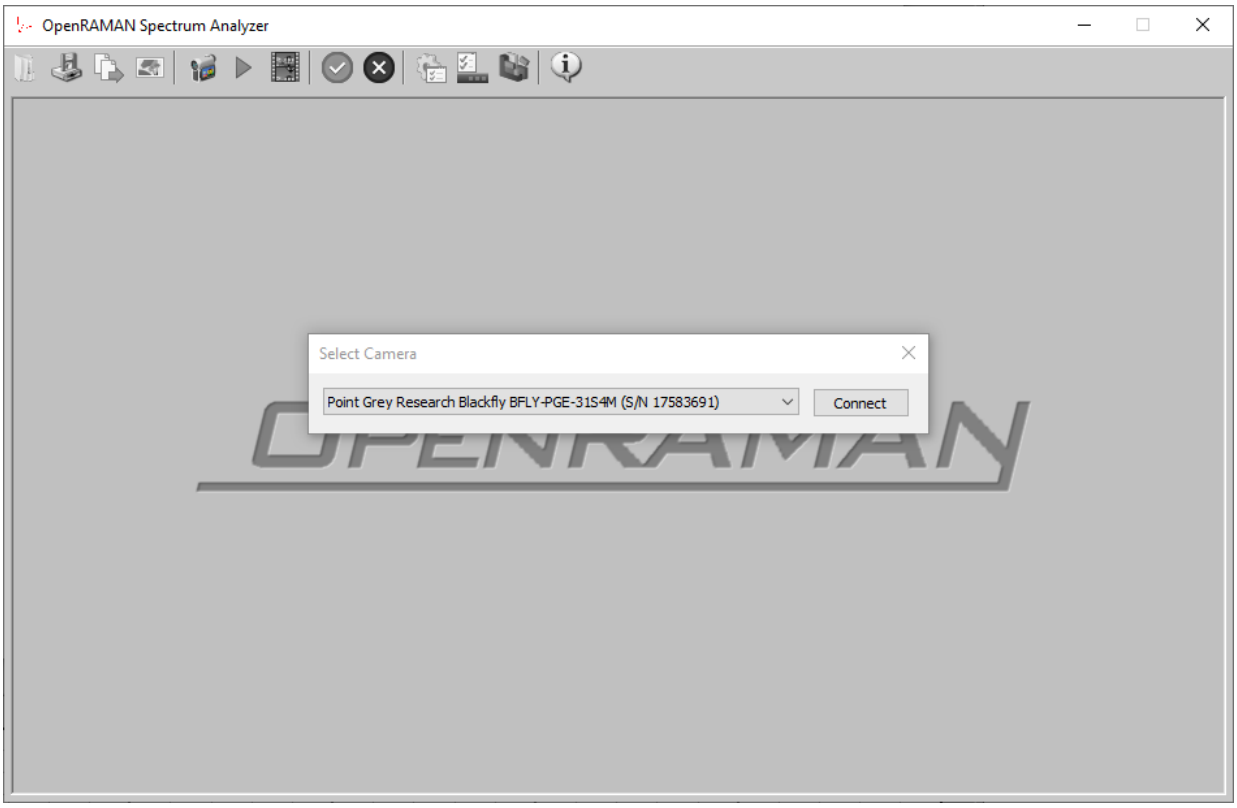
When using the image export functionality, the software will ask you which title you would like to put on your spectrum. You can either enter a title or leave the field empty. The program will then export a Bitmap (.bmp) of your spectrum with the title you selected. This option is handful when you need to

generate images for reports or to exchange data with other people without having to disclose the data by sending a CSV or having to generate a plot in Excel.



3/ CONNECTING TO THE SPECTROMETER AND ACQUIRING IMAGES

To connect to a spectrometer, click on the connect button. A popup will ask you to select the camera you will want to connect to. If you receive the error message that no camera has been found, check the IP settings of the camera if it is of GiGe type (see troubleshooting #8.1/).



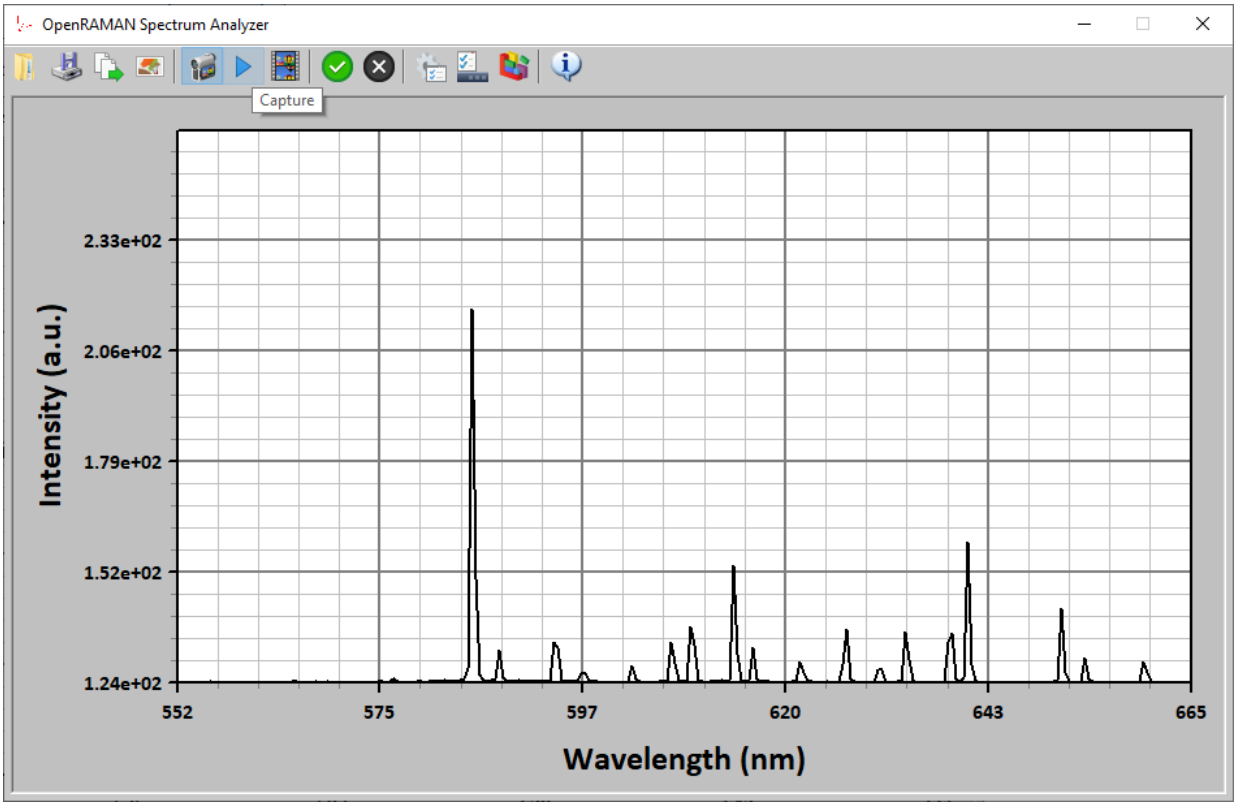
Spectrum Analyzer is compatible with both the Starter and Performance Editions but also support any spectrometer for which a .dll library has been included to connect to. By default, the application will load almost any recent PtGrey monochrome cameras. So far, the program has been validated with the following camera models:

Manufacturer	Model	Compatibility
Point Grey Research	BFLY-PGE-31S4M-C	Fully Compatible
Point Grey Research	BFLY-PGE-23S6M-C	Fully Compatible
Point Grey Research	BFLY-PGE-12A2M-CS	Fully Compatible
Point Grey Research	BFLY-PGE-05S2M-CS	Partially Compatible, cannot save calibration data

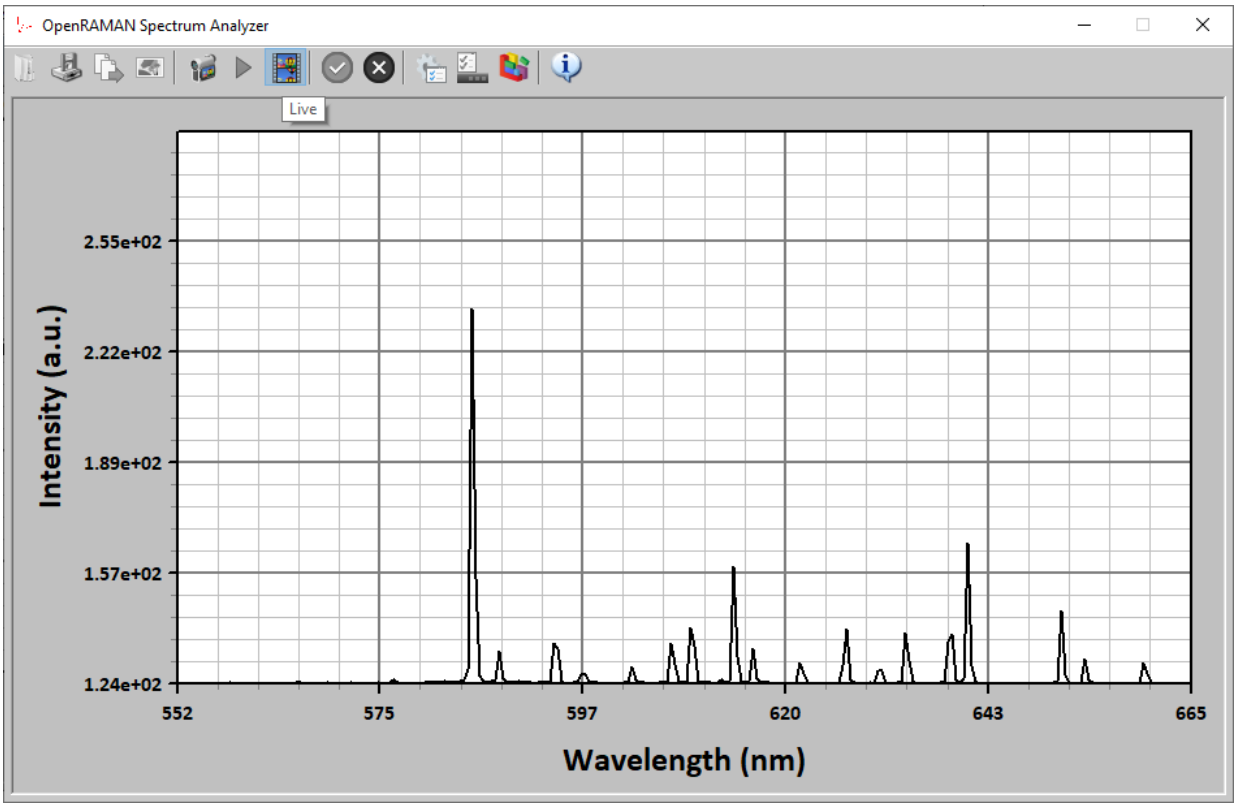
The first time you connect to the spectrometer you will get a notification that no calibration could be imported from the camera. In the absence of calibration, some options of the program are disabled and the axis will be restricted to Pixel type. You may still acquire and save spectra. When spectra are saved as Spectre files, you may later import a calibration to these files to re-enable the missing settings. Spectrometer calibration is discussed in more detail in section 6/. When connecting the camera for the first time, you may also get a warning message that the ROI is larger than the recommended value of

128 px and that it may slow down processing. Select the “yes” option. Consult section 7.1/ for tuning ROI.

To capture a spectrum, click on the single capture button:



You may also acquire images in live by clicking on the live capture button:



Live capture will usually be necessary when aligning the spectrometer. Some of the functionalities are disabled in the live capture mode.

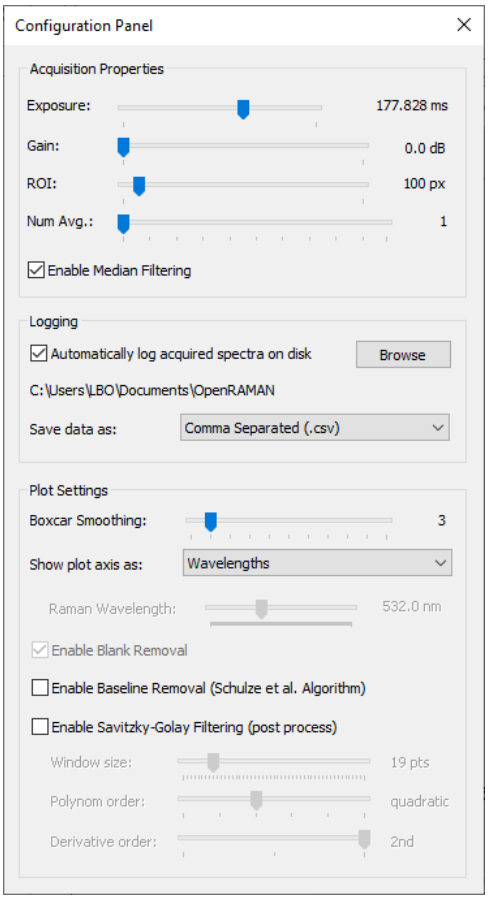
When exiting the live capture mode, the last spectra will be kept and you will be granted the same settings access as when using the single capture action.

It is also possible to capture a single image by pressing the F5 key on your keyboard.

4/ CONFIGURING SPECTRUM ANALYZER

Program settings can be accessed in the toolbar as soon as the application is connected to a spectrometer or that a file has been loaded:

When clicking the toolbar button, the configuration panel will open:



Program settings are divided into different groups.

4.1/ Acquisition Properties

Acquisition properties let you configure the camera settings such as exposure, gain, region of interest, number of average and filtering.

Exposure will control the camera exposure time. It is recommended to select the highest exposure possible without saturating the camera sensor. Saturation is discussed in details in section 7.1/.

Gain will control the camera gain. When saturation has been set to its maximum value (either limited by the camera or by the experiment), increase the gain without saturating the camera. Saturation is discussed in details in section 7.1/. Gain will increase noise but low ADC values will yield even more noise. A good strategy is therefore to first set exposure and only then increase gain such that the saturation of the camera goes to roughly 80%.

ROI will control the region of interest of the camera sensor. It is recommended to adapt the ROI value to image entirely the slit but no more than that to avoid accumulating noise in the spectra. Typical values are within 16-100 pixels for the Starter and Performance Editions. It is not recommended to use values larger than 128 pixels as it may strongly slow down the acquisition when median filtering is enabled.

Num Avg. controls the number of images taken for each spectrum. Increasing this number will decrease noise in faint spectra but will take a longer time to acquire the data.

Enable Median Filtering will remove hot and cold pixels of the sensor using a 3x3 median filtering kernel on the image data. It is recommended to keep this settings on.

4.2/ LOGGING

The logging panel will enable you to automatically save spectra to your disk every time the single capture button is pressed. The data can be saved as either CSV or Spectre files.

Automatic logging needs to be enabled to run the timed acquisition program. Timed acquisition is described in section 7.2/.

4.3/ PLOT SETTINGS

Plot settings will let you configure the plot data.

Boxcar smoothing will apply a boxcar kernel to the data. Boxcar smoothing will reduce noise at the expense of resolution. We recommend using a value of 3 for the Performance Edition and a value of 5 to 7 for the Starter Edition as a good trade-off between SNR improvement and resolution losses.

Show plot axis as will let you change the x axis. You may choose between pixels, wavelengths and Raman shift. In Raman shift mode, you will also be able to select the wavelength of your laser source to correct the offset of your spectra. The wavelength and Raman shift axis types are only available once a calibration has been performed or imported.

Enable Blank Removal will let you select if you wish to disable blank subtraction. We recommend to leave this settings on unless you really need to. The option will be grayed-out when no blank data has been acquired.

Enable Baseline Removal will perform a baseline removal on your spectrum using the algorithm described in Schulze *et al.* paper. Baseline removal should be applied on a need-to basis and we discourage you to leave this setting on all the time. Typical applications of baseline removal are strong fluorescence background on faint signals. Baseline removal can introduce artefacts in your spectra or even remove large weak bands like –OH groups which is why we recommend to avoid using it when peaks are readily visible even if fluorescence signal is present in the data.

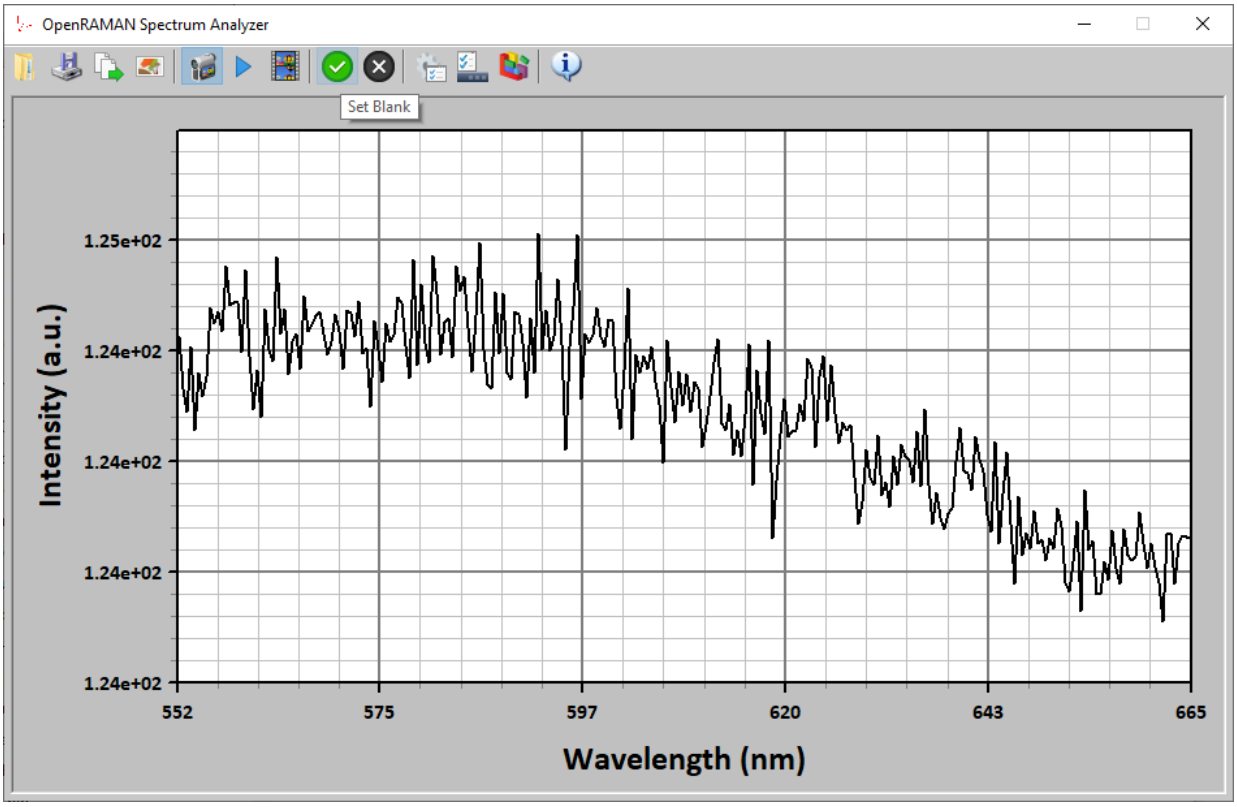
Enable Savitzky-Golay Filtering will perform Savitzky-Golay filtering on your data. Savitzky-Golay filtering is a popular technique in spectroscopy to either smooth data plots (“smooth” option in derivative order settings) or to compute derivatives of the signal. Application of second order derivate includes discrimination of two nearby peaks that have Gaussian shapes. As the results strongly depends on the window and polynom order settings, we recommend you to play a bit with these settings depending on your specific data.

5/ SETTING AND CLEARING BLANK DATA

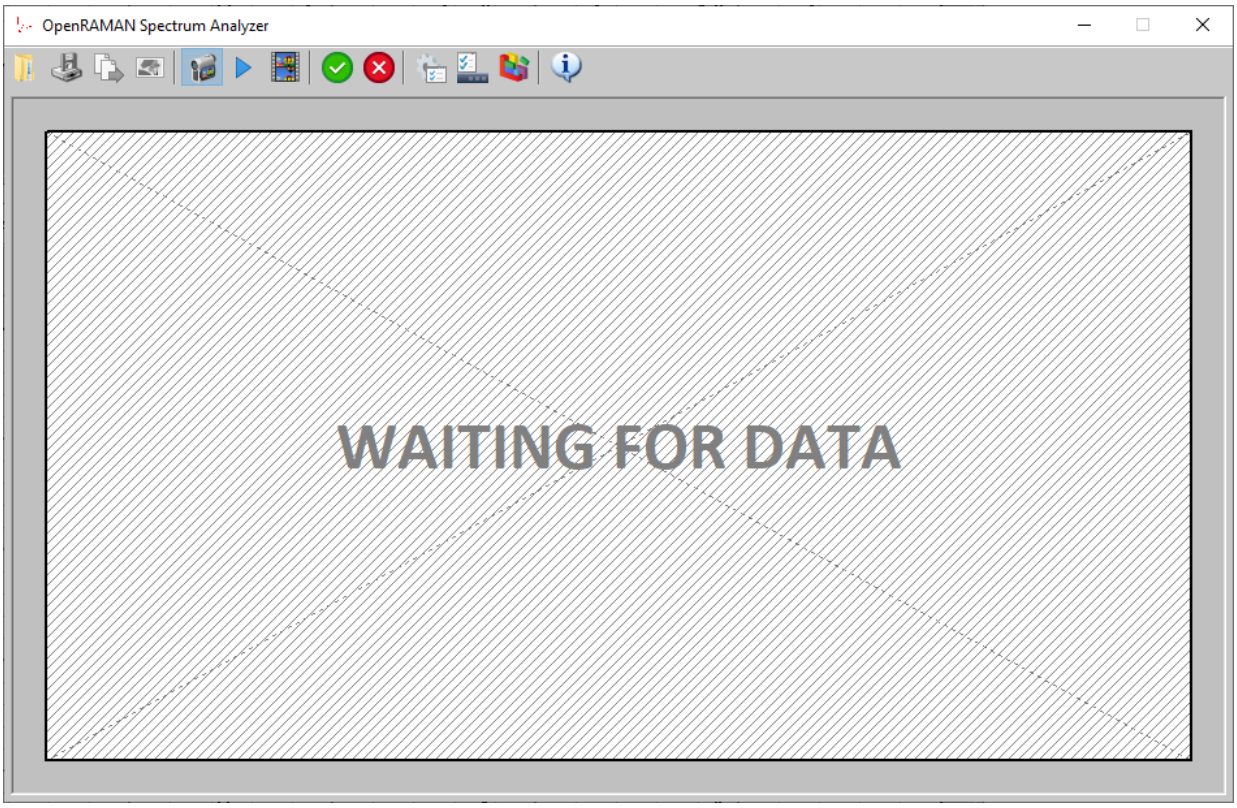
When long exposures or high gain will be used, you may want to remove a blank from your spectra. Expert spectroscopists will recommend you to always remove a blank spectra taken in the same conditions (exposure time, gain, averaging…).

Once you have identified the exposure and gain settings that you need to use, disable your laser source and take an acquisition. The resulting noise spectrum will be your blank measurement. Some users may refer to this as a “dark image” but it refers to the same concept.

Spectrum Analyzer allows you to remove this spectrum from all the spectra that you will later acquire by clicking the set blank button:

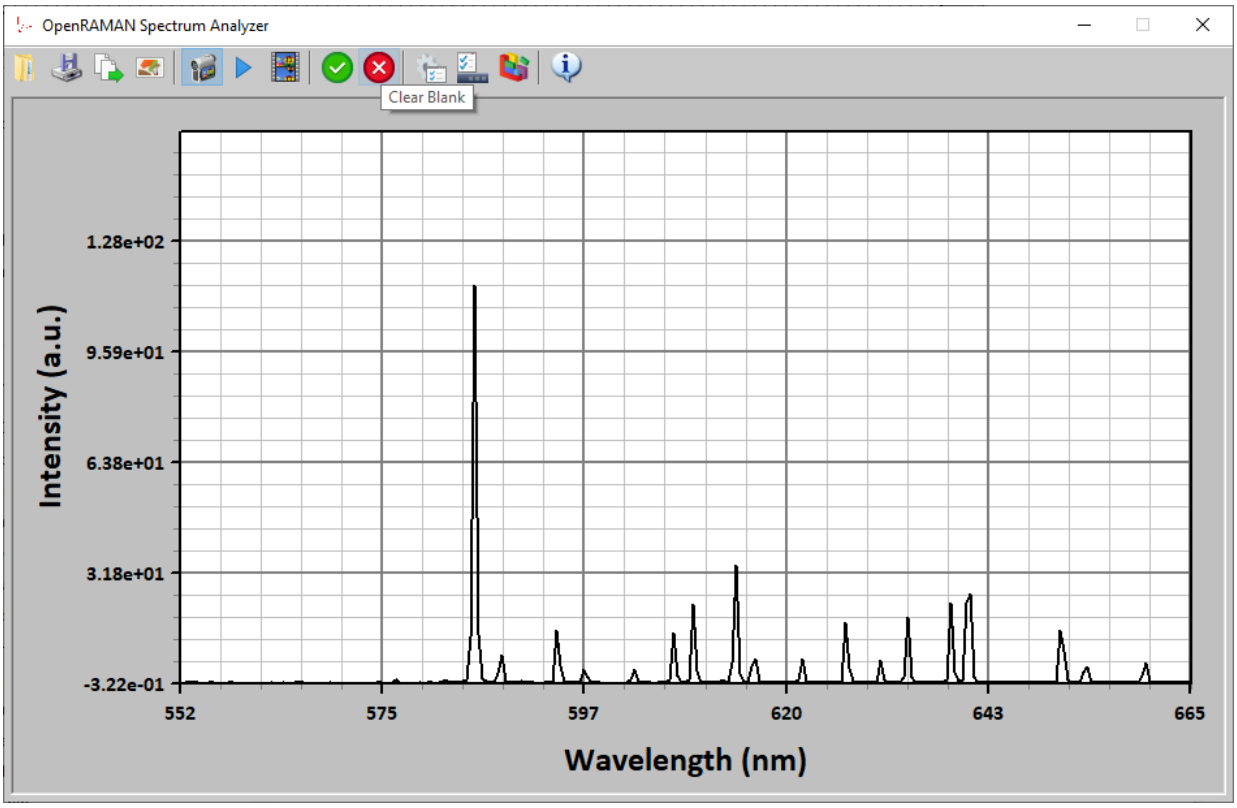


Once you clicked the button, the program will tell you that it is waiting for data:



You may then acquire your spectra using either the single or multiple acquisition buttons.

You may also discard the blank data if it is not applicable anymore (different exposure/gain settings etc.) by clicking on the clear blank button:



Blank data will be saved in the Spectre file next to the data so that you can eventually disable it later. Blank data will not be saved separately to CSV files and CSV or clipboard data will always have the blank subtraction enabled.

6/ CALIBRATING YOUR SPECTROMETER USING SPECTRUM ANALYZER

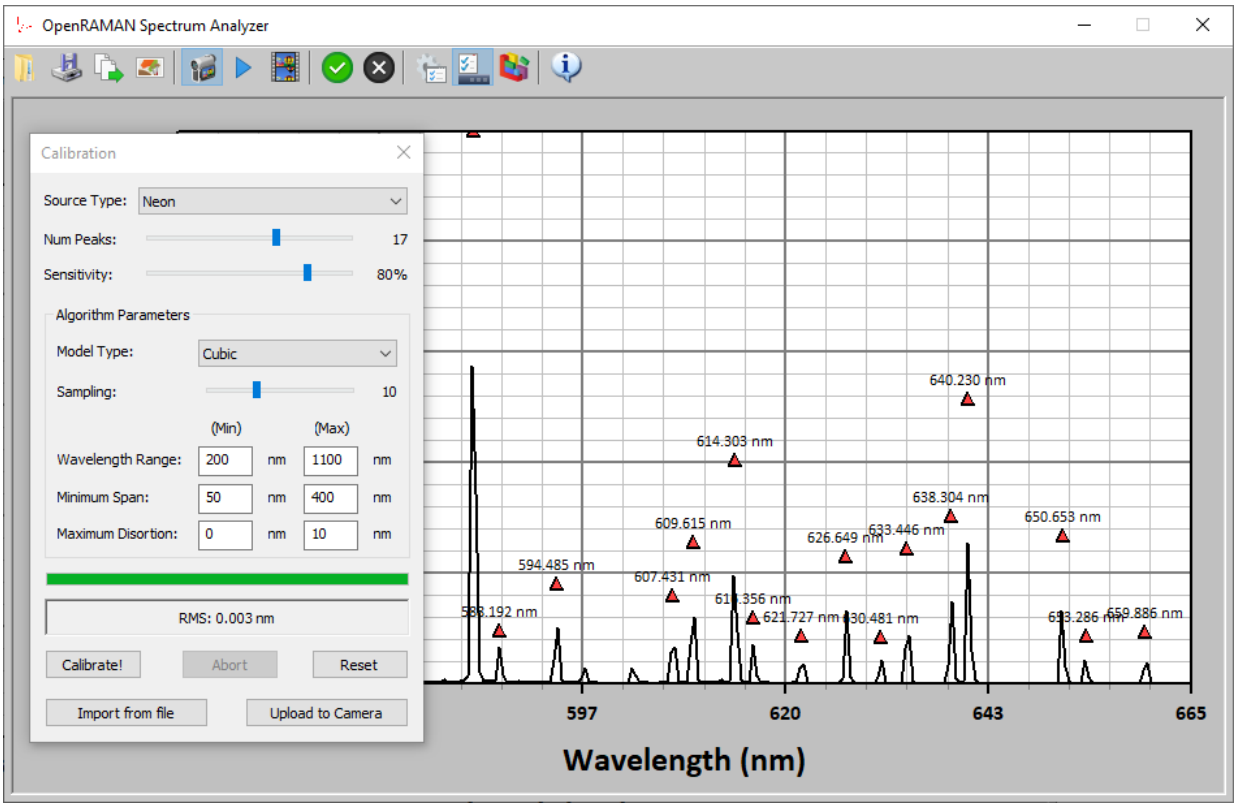
To convert pixel axis to wavelengths or Raman shifts, the spectrometer needs to be calibrated. It is recommended to perform regular checks on the calibration, especially for sensitive measurements.

Calibration is done using a calibration lamp. Currently, Spectrum Analyzer supports two type of calibration lamp: Mercury Argon and Neon. For the Starter and Performance Editions, we recommend using a Neon calibration lamp.

To calibrate your spectrometer, first take a clean spectrum of your calibration lamp using both blank measurement and the baseline removal of the software. You should also allow the lamp to warm-up according to its manufacturer recommendations.

Once you have acquired a clean spectrum, open the calibration window by clicking the button in the toolbar. Adjust the number of peaks and sensitivity such that the software correctly identifies your peaks. Note that the software is robust to missing peaks but does not tolerate erroneous peak.

Next, set the algorithm parameters and click the “Calibrate!” button. The quality of the fit will be shown in terms of a RMS figure. A RMS much below the resolution of the spectrometer indicate a good fit quality. When you are confident in the quality of the fit, you can upload the calibration data to the camera.



The algorithm that was developed to calibrate the system is relatively robust but may need some tweaking. You can either choose from a Linear model or from a Cubic model. Cubic model offers more flexibility but is slower to fit. If the algorithm does not fit properly, you can try increasing the sampling parameter. Increased sampling will run slower.

The algorithm works by a global search method that will reject solutions that are outside boundaries. You can define boundaries for the following parameters:

- Wavelength range of the spectrometer. By default, the algorithm will cover all the visible range of silicium detectors plus some margin. If the algorithm fails to converge, you can narrow these fields towards the theoretical value of your spectrometer design.
- Minimum Span is how much wavelength your camera sensor will cover. By default, the software will cover a relatively large span.
- Maximum distortion will limit the deviations to the linear model. This mode is available only in cubic model type. It is recommended to leave the minimum value at zero.

When tweaking these values, always use some safety margins. For example, if you spectrometer has a theoretical range of 550-670 nm, use values such as 520-700 nm or even more.

Calibration data will be saved with the file when saving in the Spectre file format. It is then possible to import the calibration from a Spectre file from the calibration window. When trying to import data from a file that contains a calibration performed with a different camera, the software will warn you that the calibration was not performed on the same system and that the result may therefore be incorrect.

7/ ADVANCED OPERATIONS

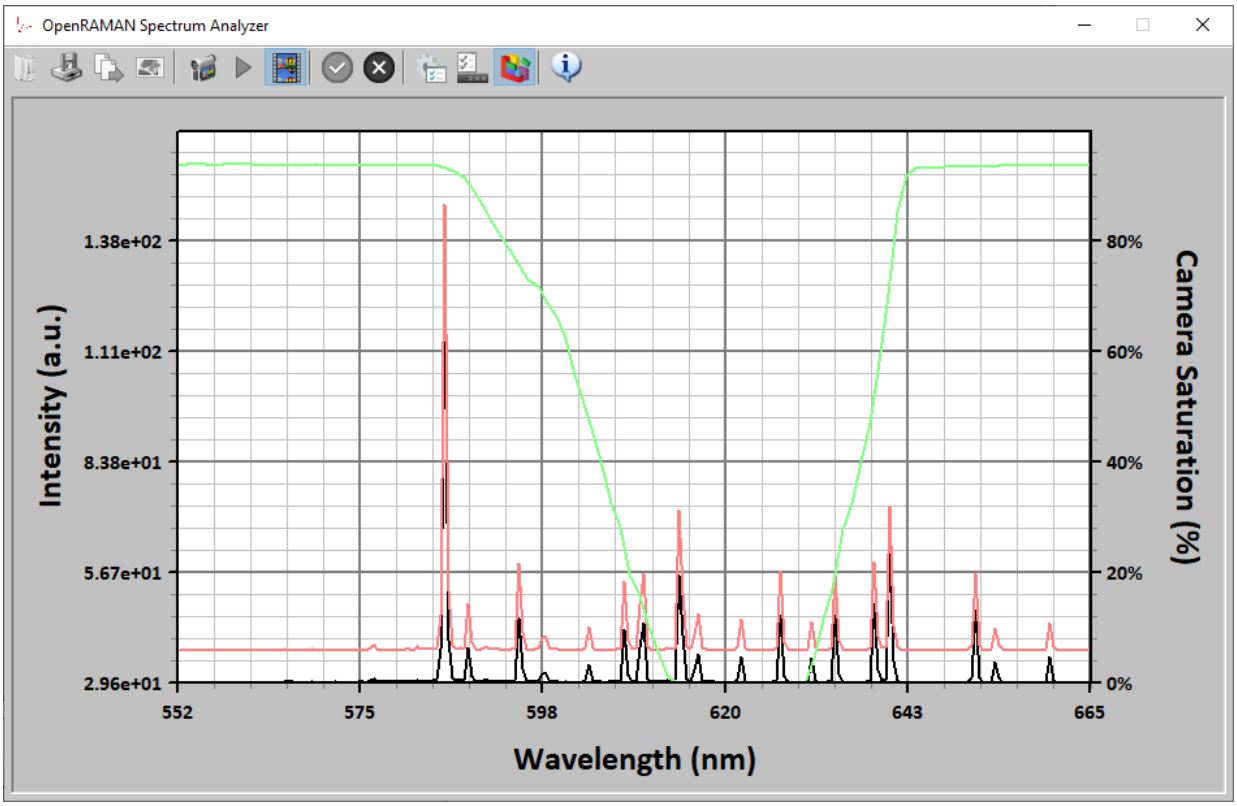
7.1/ VISUALIZING CAMERA SATURATION & CENTERING

The “show saturation” option in the toolbar allows you to visualize the saturation and the centering of the camera sensor. The option is available for both single acquisition and live mode but is best used in live mode to tweak the exposure and gain settings.

When acquiring spectra, it is important that the sensor does not saturate, *i.e.* that the electron well composing each photosite is never overflowing. In practice, you will want to keep the signal at about 80% of the ADU limit.

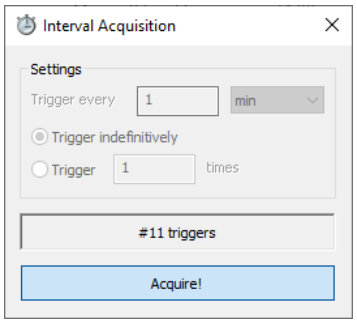
Also, the image of the slit only occupies a small fraction of the height of the sensor. The software then integrates each row of the sensor to yield a single value. It is recommended to crop the region of interest of the sensor to the actual image of the slit because non-illuminated area only bring noise to the plot. On the other hand, cropping too much will loose information and will also increase the noise value.

In the software, the saturation data is shown in red and the slit image data in green:



7.2/ TIME-LAPSE ACQUISITIONS

The Spectrum Analyzer software comes with a companion program called Interval.exe that allows you to acquire spectra at regular interval defined in either seconds, minutes or hours scale:



To perform time-lapse acquisitions, start the Spectrum Analyzer program and connect to the spectrometer. Adjust the exposure and gain for your experiment and enable the automatic logging of spectra. Eventually take a blank measurement if you wish to.

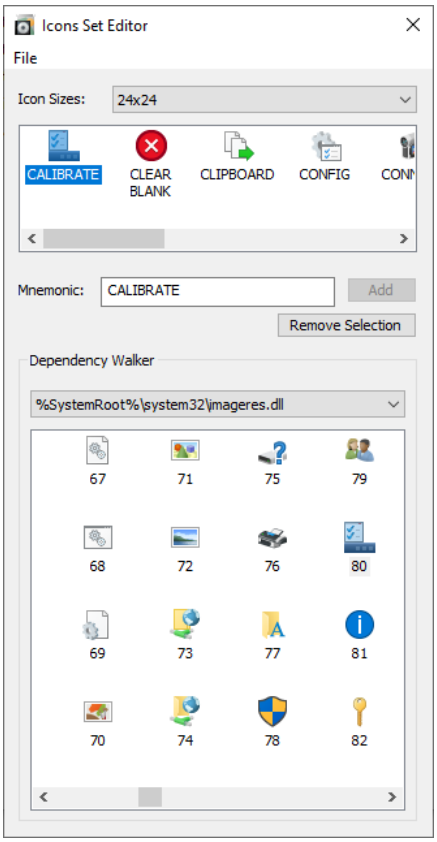
Then, parameter the Interval.exe program for the delay and number of repetitions that you would like. Press the “Acquire!” button to start acquisition and press again to stop the acquisition.

The Interval Acquisition program works by sending “F5” strokes to the Spectrum Analyzer program which will trigger an acquisition. Because the automatic logging is enabled in Spectrum Analyzer, all the spectra will be recorded at your specified location with a time stamp.

You can configure Spectrum Analyzer to save in either Spectre or CSV format.

7.3/ CHANGING PROGRAM ICONS

The Spectrum Analyzer software comes with a companion program called IconsEditor to allow you to change the icons of the toolbar. The Icons Set Editor program will help you modify a descriptor file but you will need to recompile the Spectrum Analyzer software or use a resource editor program to actually update the icons.



To change the toolbar icons, open the toolbar.iis file in the Spectrum Analyzer source code folder using the Icons Set Editor program. The program will list all the icons by their mnemonics and let you replace the current icon by selecting them from a list in the dependency walker window. Once you have modified all the icons, save the file by overwriting the toolbar.iis file and recompile the Spectrum Analyzer program.

Note that the program does not actually copy the icon, which would infrige the copyright of their original author. Instead, the program dynamically load the icons from their initial resource and refer to these using an official and legal mechanism implemented in Windows. This is similar to changing the icon of a file in Windows by right-clicking > Properties > Change Icon.

8/ TROUBLESHOOTING

8.1/ UNABLE TO CONNECT TO THE CAMERA/SPECTROMETER

If the application is unable to detect the camera check the following:

- When using a GiGe camera, check that the IP of the camera matches your current subnet using the camera manufacturer software.
- Some GiGe camera can be powered via Ethernet (POE) but only if the Ethernet card supports it. Check that your card support POE or that the camera has an external power supply.
- Check that the manufacturer camera software was installed properly and that all the necessary dll are installed.

8.2/ NO IMAGE CAN BE ACQUIRED

If the application connects correctly to the camera but fails to acquire image, check that you have installed the SDK version recommended in section 1/ as we already had issues when running the software with different SDK versions.

You may also check in SpinView that the following settings were set correctly to the camera and that SpinView can acquire image correctly:

Setting	Value
AnalogControl > Gain Auto	Off
AnalogControl > Gamma Enable	unchecked
AnalogControl > Sharpness Enable	unchecked
AnalogControl > Blacklevel Enable	unchecked
AnalogControl > Hue Enable	unchecked
AnalogControl > Saturation Enable	unchecked
AcquisitionControl > Acquisition Mode	Continuous
AcquisitionControl > Exposure Auto	Off
AcquisitionControl > Exposure Mode	Timed
AcquisitionControl > Framerate Enable(d)	unchecked
AcquisitionControl > HDR Enable	unchecked
AcquisitionControl > Framerate Mode	Off
ImageFormatControl > Pixel Format	Mono16
ImageFormatControl > Reverse X	checked
ImageFormatControl > Color Processing	unchecked
AcquisitionControl > Trigger Mode	Off