

Technical note

Micro-Manager: Integrating solutions for micro-spectroscopy

Control of fully integrated systems for carrying out micro-spectroscopy is now available through Micro-Manager (μ -Manager). The necessary software plugins have been developed at Andor to allow for the synchronised control of a spectrograph and CCD detector along with the functionality of a full microscope system. This allows for the collection of spectral data over two and three dimensions resulting in the high information data-cubes that we associate with multispectral and hyperspectral analysis. Micro-Manager is a widely used and freely available open-source software application which controls the functions of a microscopy system^[1, 5, 6].

Micro-Manager has been developed primarily for microscopic imaging applications. This technical note outlines how it can enable an automated modular micro-spectroscopy solution all controlled centrally from one software application.

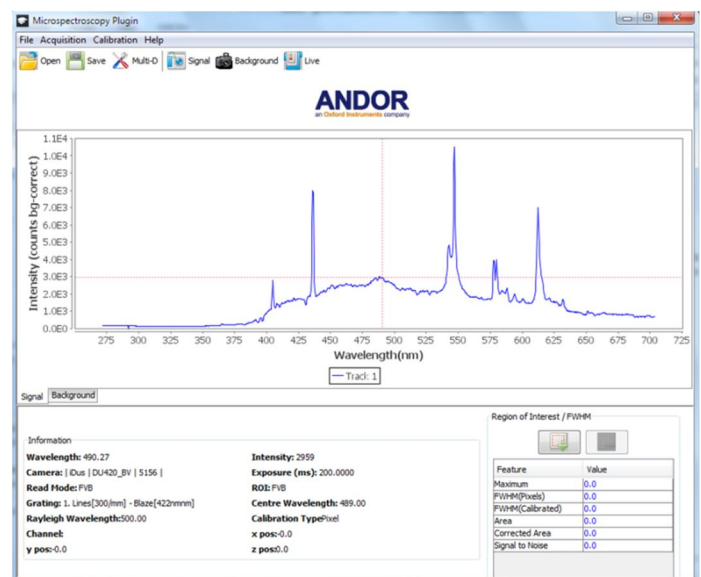


Figure 1: A typical system setup and above a view of the spectral acquisition window in Micro-Manager.

Getting started

The main steps in setting up a micro-spectroscopy system include:

1. Deciding on the type of spectroscopy measurement to be made and the requirements of those measurements for example if it is photoluminescence, Raman, or other type of modality and if it is to be a single scan or multidimensional scans of the sample.
2. Deciding upon and configuring the necessary hardware components within the system such as the microscope, XY sample stage, light sources, spectrograph and so on.
3. Determining the software control of the system – whether different components are controlled by separate software packages or the system is fully integrated through one software package.
4. Setting up the experiment through the software for the acquisition, handling and storage of the data for further analysis.

The configuration of a system for control through Micro-Manager is outlined in the ‘Quick Start Guide’ for Micro-Manager^[2] and should be referred to for the installation and set up of the software initially.

Details on possible configurations for micro-spectroscopy and how to go about selecting components for such a system are outlined in the ‘Micro-spectroscopy Specification sheet’^[3] and the technical note on ‘Micro-spectroscopy: Configurations for Modular Solutions’^[4]. These provide valuable information on getting started with setting up your own micro-spectroscopy system.

Micro-Manager addresses points 3 and 4 above – where it is outlined in this technical note how it can be used to give a fully integrated modular solution and allow for easy configuration to capture multidimensional spectral data.

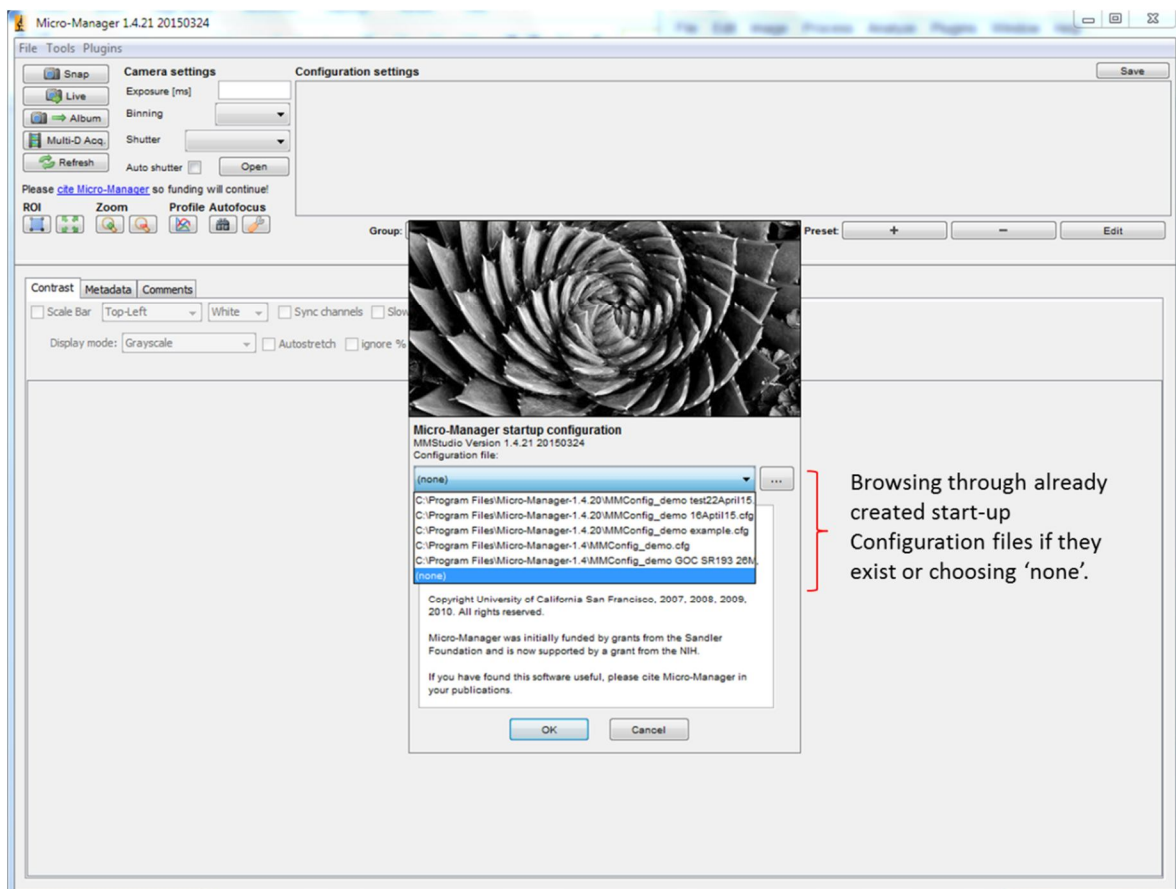


Figure 2: The main view screen of Micro-Manager displayed upon initiation of the software.

Configuring Micro-Manager for micro-spectroscopy

A number of steps are required to set up communication between the various hardware components in the system and the PC. This will involve the setting up of the system **'Configuration file'** to include all the components required and that are installed, and accessing the **'Device Property Browser'** menu where the correct parameter values can be set for the acquisitions to be taken such as spectral range, exposure time, number in series and so on. Finally the **'Andor plugin'** has to be selected and configured for the type of acquisition to be acquired such as single scan or kinetic series.

Configuration file: setting up the communications

When Micro-Manager software is first loaded up the main screen is displayed as shown in figure 2. There is an interactive menu presented up front which allows for previously created Configurations files to be loaded up or if the aim is to create a new configuration file then 'none' can be selected. This note outlines the setting up of a new configuration file so 'none' may be selected at this point, unless one wants to modify an already existing file.

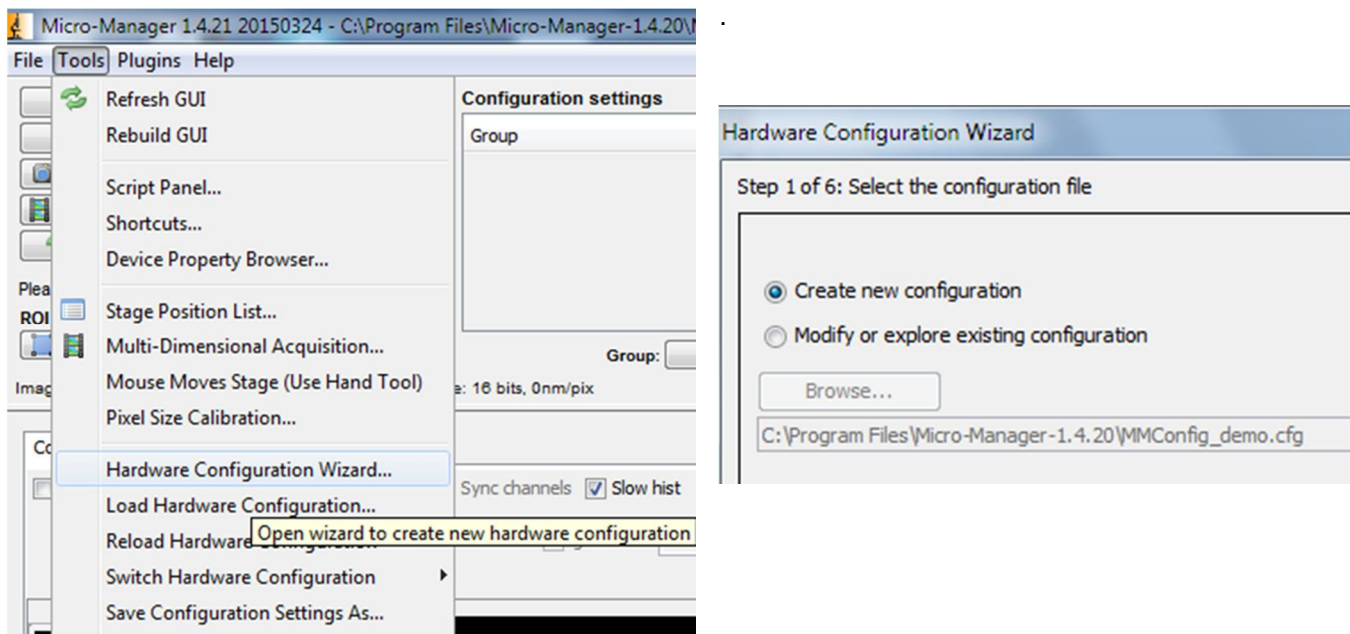


Figure 3: Selecting the Hardware Configuration Wizard, which when selected presents the options of either creating a new configuration file or modifying an existing one (see right).

To open the wizard tool for the Configuration file setup, go to **Tools** in the main menu bar, and then select **'Hardware Configuration Wizard'** in the drop down menu. The wizard gives a set of six steps for setting up the file (See figure 3). The first offers the option of either creating a new file or modifying an existing file.

The setup then moves to step 2 (by clicking on Next) where the various installed hardware devices can be added to the system. This is illustrated in figure 4 where the two Andor threads have been expanded. One corresponds to the installed Andor camera and the other to Shamrock spectrograph. To add a device it is highlighted in the drop down list on the bottom left and 'Add' is selected on the right. Figure 5 shows the steps to add the Shamrock to the configuration. The Shamrock is selected and 'Add' is activated which brings up the installed Shamrock that has been FOUND on the system. On clicking 'OK' the Shamrock is added to the list of installed devices.

Note in this process Micro-Manager searches for installed and active devices connected to the computer. What it finds it presents for selection. If the Shamrock is not connected or switched on it will NOT be possible to add it to the configuration. This applies to all devices. In the case of the Shamrock an error message would be displayed

indicating: **“Error in device (“Andor Shamrock”): Unable to communicate with the device.”**. If this occurs, check the connections and that the spectrograph is switched on.

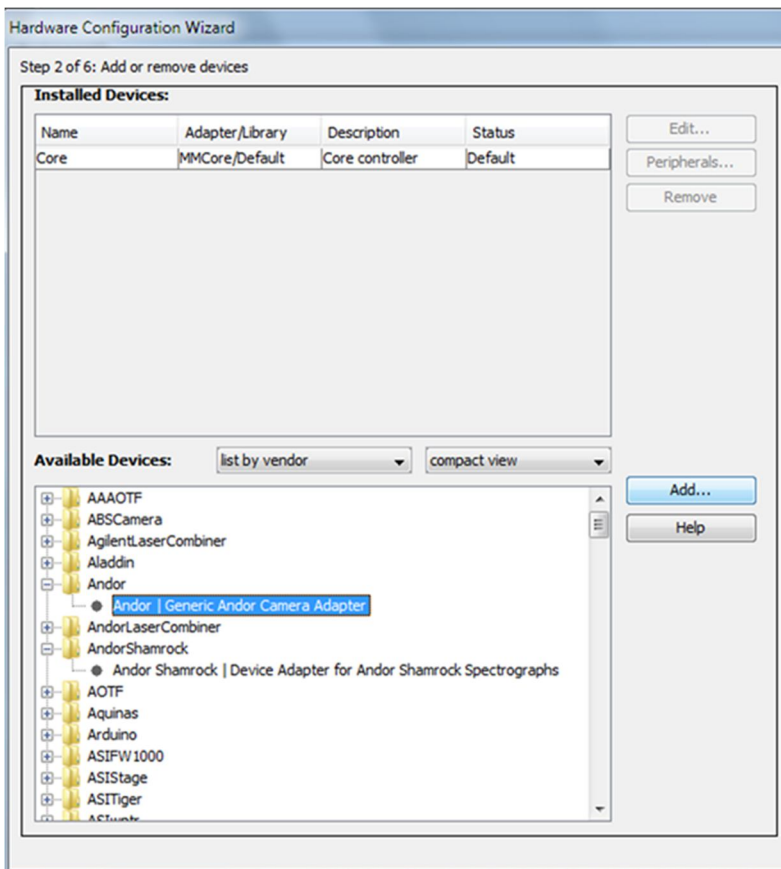


Figure 4: Adding devices to the configuration file.

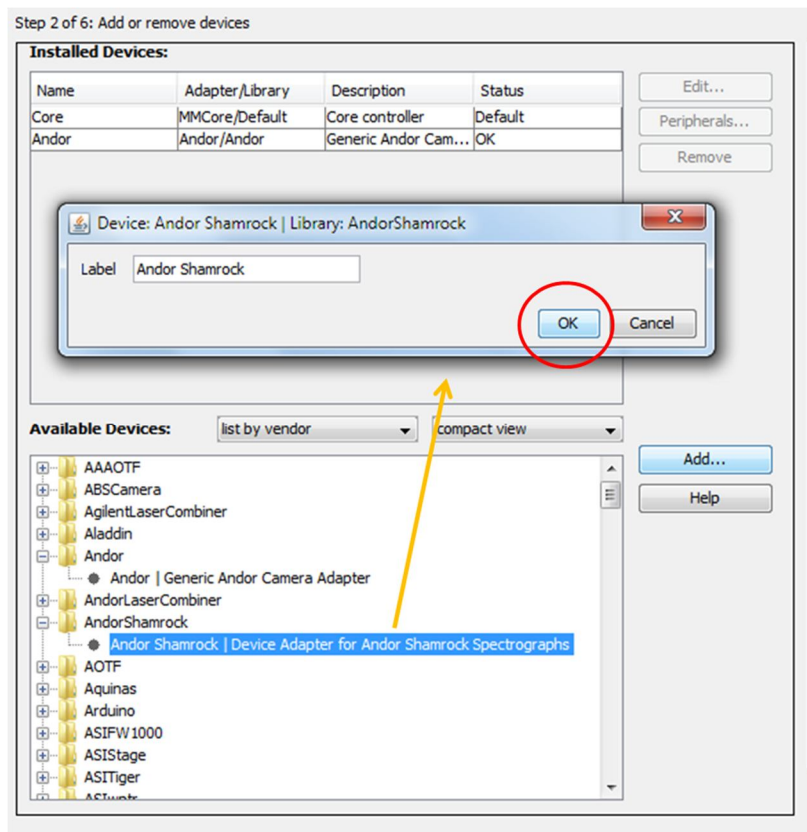


Figure 5: Adding an installed Shamrock to the Configuration file.

All the installed devices required to configure the system – such as microscope stage, objectives, filter wheels and so on - can be added in turn to complete the list of devices as illustrated in figure 6. Note if it is desired to remove a device from the system configuration then it is only a matter of highlighting the device in the installed list and clicking on 'Remove'.

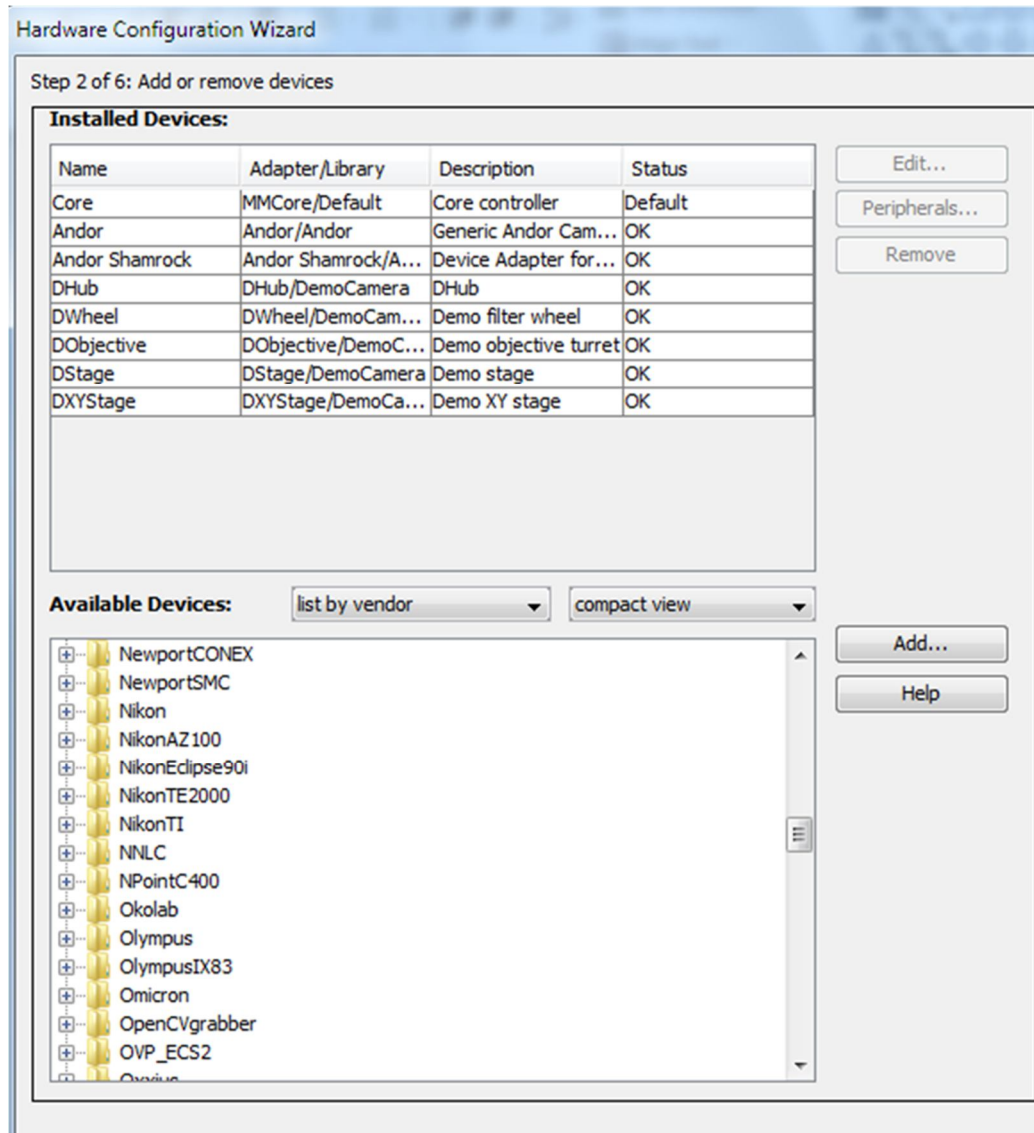


Figure 6: An example configuration showing a range of possible components including microscope parts and an 'XY' sample stage.

Steps 3 follows where default components to be used can be selected such as the camera, shutter and focus stage; this is relevant where there are more than one of each of these components installed for the system configuration.

Step 4 allows for the setting of delays for certain devices to aid synchronization of the various functions of the system. Some devices will execute a command when informed by Micro-Manager but do not return a signal to the control software to indicate the action has been completed. This can lead to problems if the required action has not completed before the next action has started. For example if a shutter has to be opened before an acquisition is taken a delay can be set for the shutter to ensure it is in correct state before acquisition occurs.

It is possible to assign labels to some components within the system to make usage more convenient and meaningful such as when there are multiple objectives or filters present; for example objective-A could be labelled 'Objective X20 NA-0.4'. It is possible to do this at Step 5 in the wizard setup.

Finally at step 6 the configuration file can be given a name and saved in a suitable folder such as the Micro-Manager installation folder in 'C:\Program Files'. Figure 7 shows views of how steps 3, 5 and 6 appear.

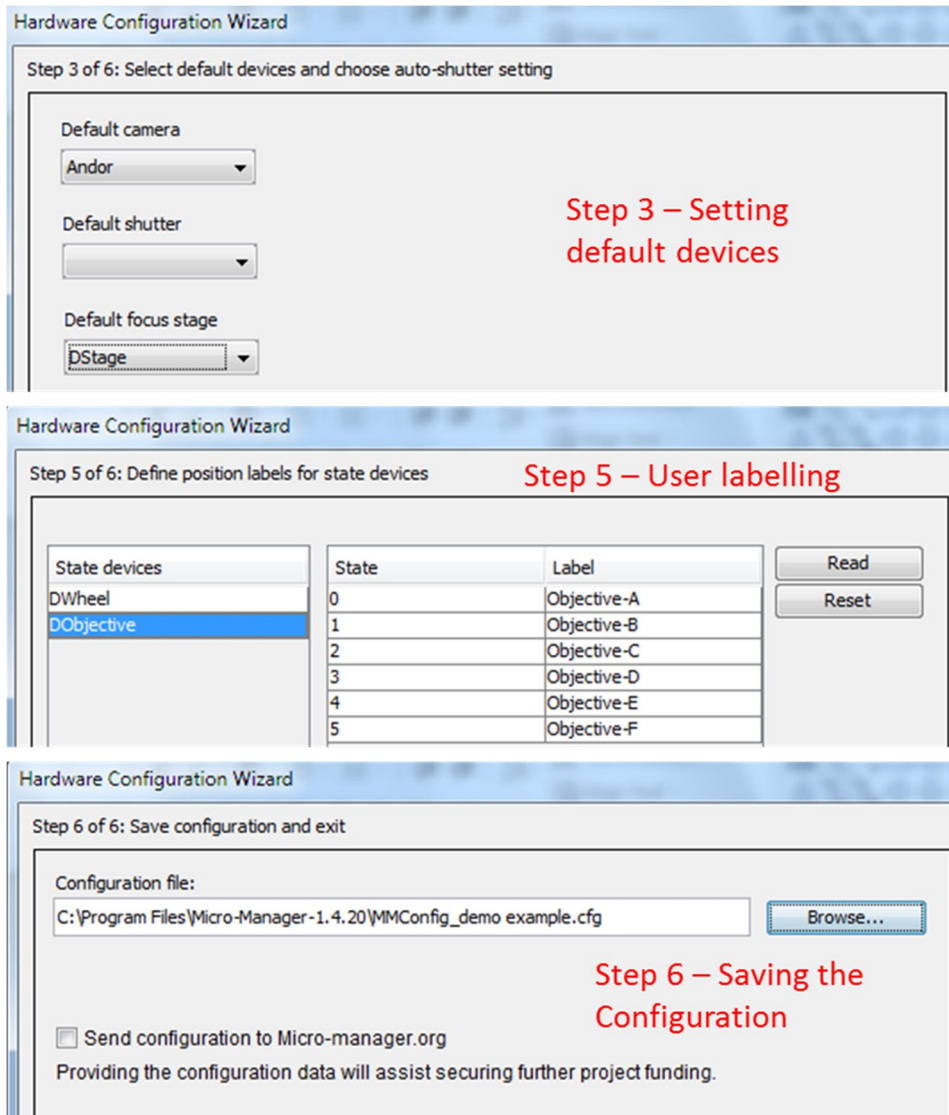


Figure 7: Illustrative views of the options presented at steps 3, 5 and 6 of the Configuration setup wizard.

Using the Device Property Browser to set parameters for the system

The values for the various parameters needed to configure a system for a series of acquisitions can be set through the **Device Property Browser**. This can be accessed from the **Tools** drop down menu as shown in figure 8. When the **Device Property Browser** is opened the various parameters for all installed devices are listed in a table with the parameter name on the left and its values on the right. This is illustrated in figure 9.

There are some parameters which are shown as greyed out bands. These either have fixed predetermined values which cannot be changed or are parameters that are determined by value settings for other parameters: in any case they remain inactive to be changed directly. All the active parameters can be changed and these will either have a number of predetermined values from which a selection can be made or have a range of values where a free entry may be made provided it lies within the acceptable or valid range.

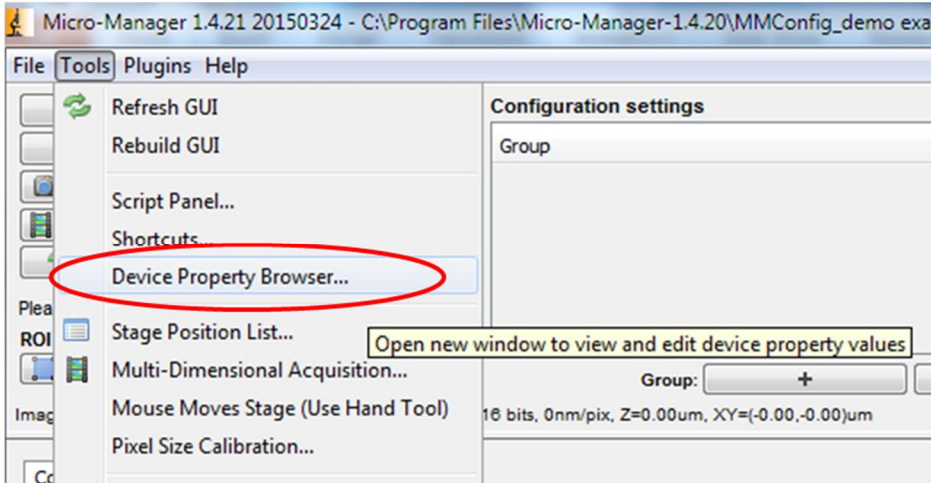
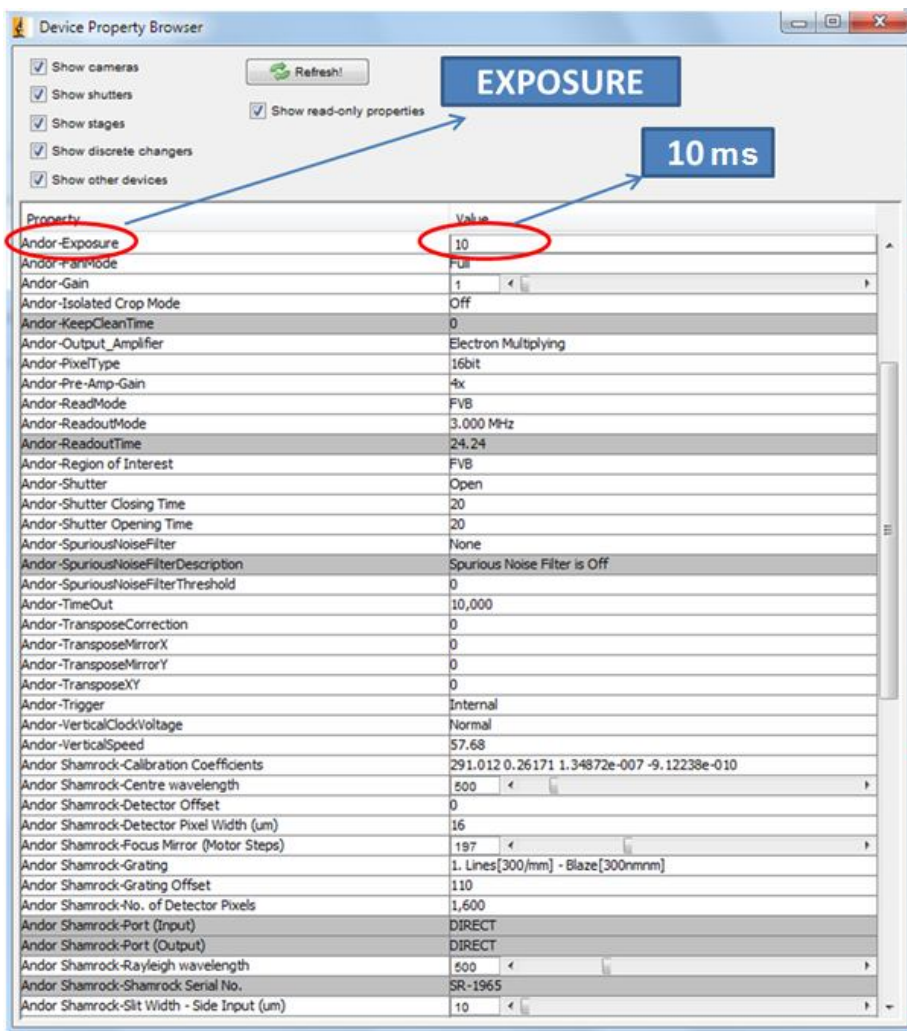


Figure 8: Accessing the Device **Property Browser** to set the various parameter values.



Andor camera acquisition settings

Andor spectrograph acquisition settings

Figure 9: The Device **Property Browser** where the current settings for acquisition parameters can be viewed and modified as needed. An Exposure setting of 10 ms is illustrated as an example entry in the table.

The Andor 'Microspectroscopy Plugin':

The '**Microspectroscopy Plugin**' utility is the new addition to Micro-Manager which allows for the capture of standard spectra as opposed to the usual 2D microscopy images. It is accessed – as shown in figure 10 – from the main **Plugins** drop down menu at the top, then selecting **Andor** and then clicking on '**Microspectroscopy Plugin**'. This brings up the main window as shown in figure 11.

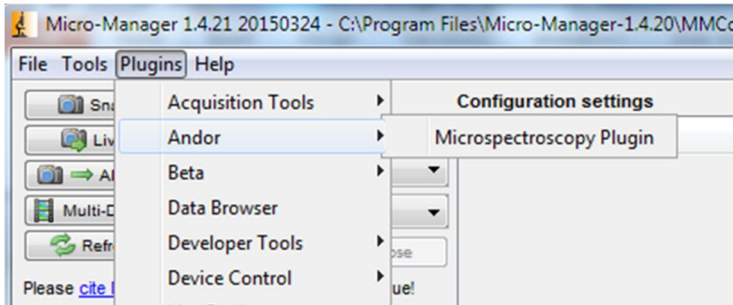


Figure 10: Accessing the Andor '**Microspectroscopy Plugin**' from the **Plugins** drop down menu.

At the top of the main window are the **Acquisition** controls for capturing the raw spectral data including background corrected data. A **Calibration** utility is available which allows for calibration of the spectral or x-axis in terms of wavelength (nm), energy (eV), Raman shift (cm^{-1}) or raw pixel values. Acquired spectra can be saved (and reloaded) as raw ASCII format files. The raw spectrum is displayed in the main display window where the horizontal and vertical axis can be scaled to ensure the optimum display of the data.

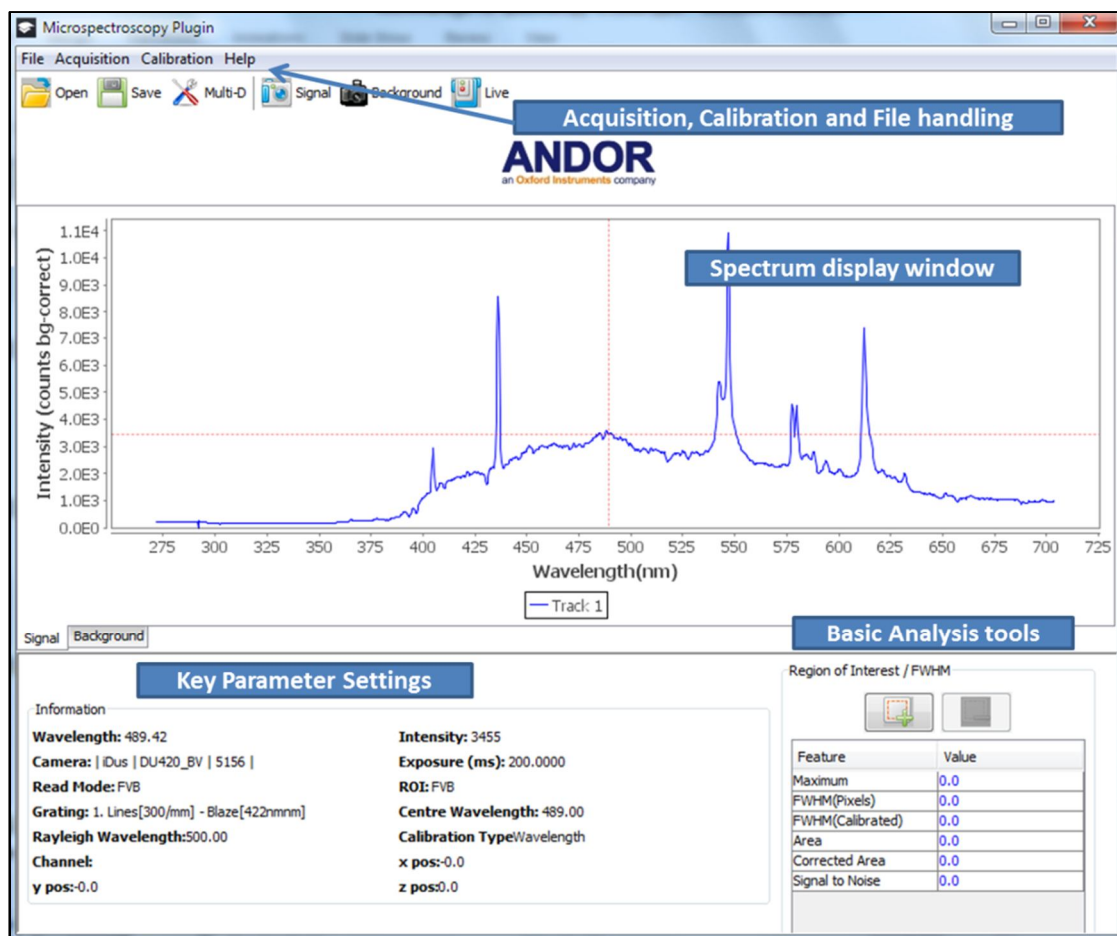


Figure 11: Main parts of the '**Microspectroscopy Plugin**' display.

The key parameters that have been used for the current acquisitions are summarised on the bottom left hand corner of the **Plugin** window; parameters include the grating used, the central wavelength setting for the spectrograph, and the camera exposure time.

Capturing and displaying a spectrum

When capturing a spectrum the normal read-out mode used for acquisition is Full Vertical Binning or FVB mode. This should be set in the **Device Property Browser**. If setting up for the first time there are a number of key parameters to be set before a satisfactory spectral acquisition can be taken. There is a means of setting the default values required each time when doing spectroscopy by defining a **Group** in the '**Configurations settings**' sub-window of the main Micro-Manager window. This will be discussed later in the technical note. The key default settings that will need set at the outset if they have not already been set are identified in Table 1.

Table 1: Some key default values to set for spectral acquisitions.

PARAMETER NAME	VALUE
Andor-ReadMode	FVB
Andor-Shamrock-Centre wavelength (nm)	A number >0 e.g. 500
Andor-Shamrock-Detector Pixel Width (µm)	Pixel width of sensor e.g. 16
Andor-Shamrock-No. of Detector Pixels	Width of sensor in pixels e.g. 1600

An illustrative spectrum is shown in figure 12 where the line spectrum is displayed when the x-axis is calibrated in wavelength in units of nanometers (nm). The spectrum is an example of a 'background corrected' spectrum and both the 'background' and 'corrected signal' have display tabs at the bottom of the data window.

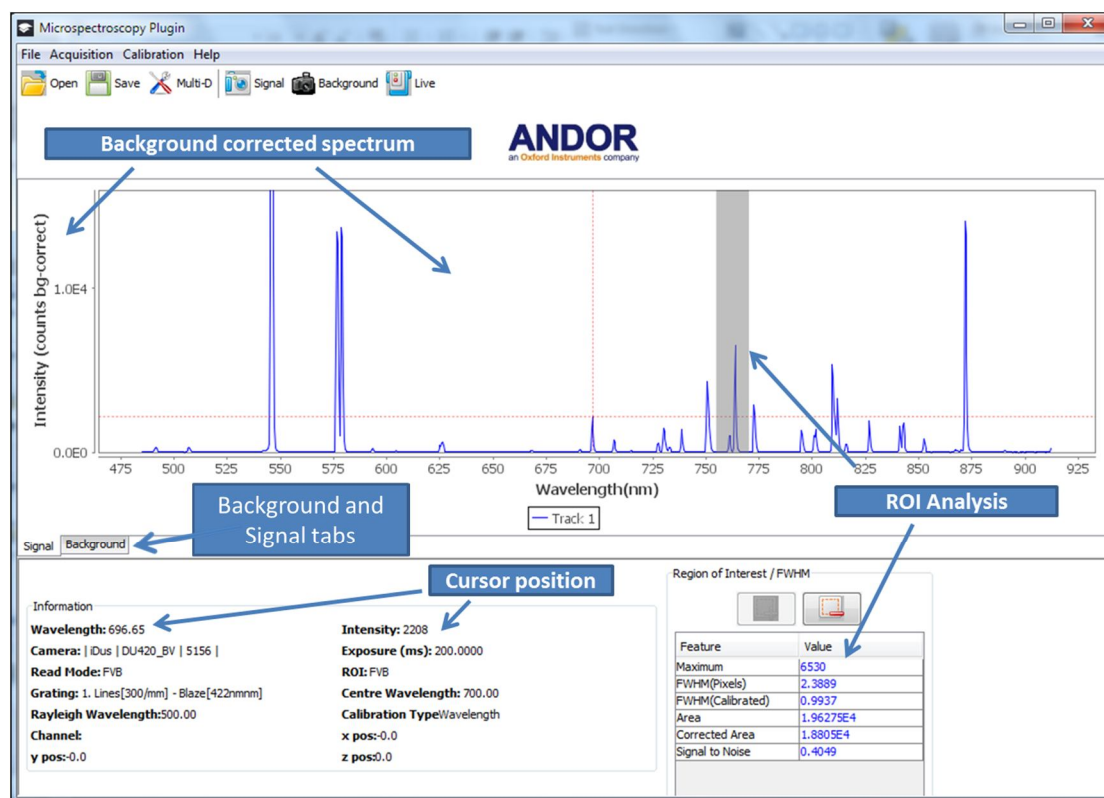


Figure 12: Display of a typical spectral acquisition in the plugin window.

The cursor can be moved along the spectral profile and its position and the intensity at that point is displayed in the information box at the bottom left. The **Region of Interest (ROI)** analysis tool is shown on the bottom right where a spectral line feature around 765 nm has been selected; its Full Width Half Maximum (FWHM) and integrated intensity are among the characteristics reported. A more detailed view of the ROI tool is shown in figure 13. The region of interest is defined by clicking on the button outlined in red which brings up a data entry window where the lower and upper wavelengths of the ROI can be entered. On clicking on 'OK' the ROI is highlighted on the spectrum and the extracted data is updated in the table as displayed on the lower right side. This tool can remain active when capturing spectra in real-time or continuous mode which makes it particularly useful when optimising the setup of the system.

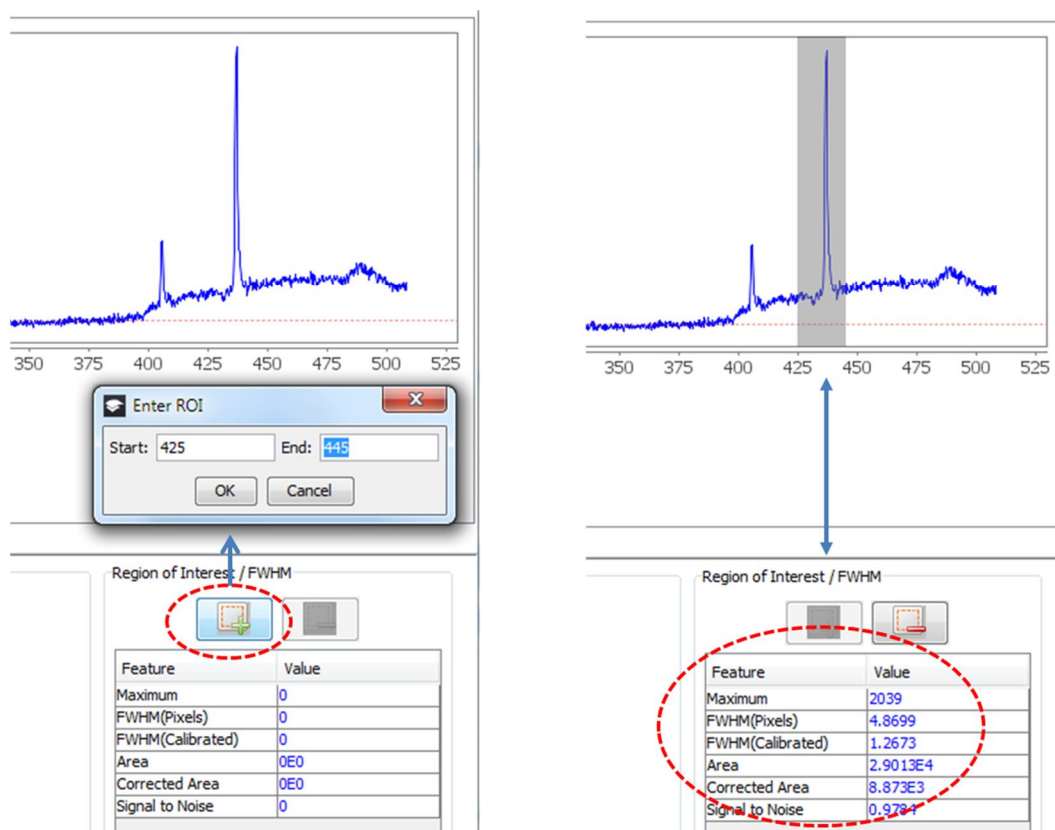


Figure 13: Accessing and using the ROI tool.

Multi-dimensional acquisitions - kinetic series of spectra

Series of spectra are needed when carrying out variation-with-time studies or building up 2D spectral images and chemical mapping. It is also possible to build up hypercubes of data with three dimensions in space and a spectrum at each voxel within the 3D space. The main utility within Micro-Manager is provided in the **'Multi-Dimensional Acquisition'** window which is accessed by selecting the **'Multi-D Acq'** tab on the upper left corner of the main display or from the short-cut **'Multi-D'** button at the top of the **plugins** window.

A view of the **'Multi-Dimensional Acquisition'** window is shown in figure 14. There are options to set up the spectral series acquisition as a function of a) time, b) spatial position in a 2D plane (XY), c) spatial position in depth (Z) to view slices, d) multiple channels, for example where multiple excitation sources or bandwidths are being used, or any combination of these. The activation box is just ticked for whichever functional dimension is needed. For example in figure 14 **'Time points'** has only been activated and the setup is configured to acquire 20 spectra in a kinetic series. When the **'Acquire'** button is pressed the acquired series is displayed in the **Micro-spectroscopy Plugin** window. By activating the **'Multiple positions (XY)'** and selecting **'Edit position list..'**, a table can be created as shown in figure 15 where the coordinates for different stage positions are defined as points where measurements may be made.

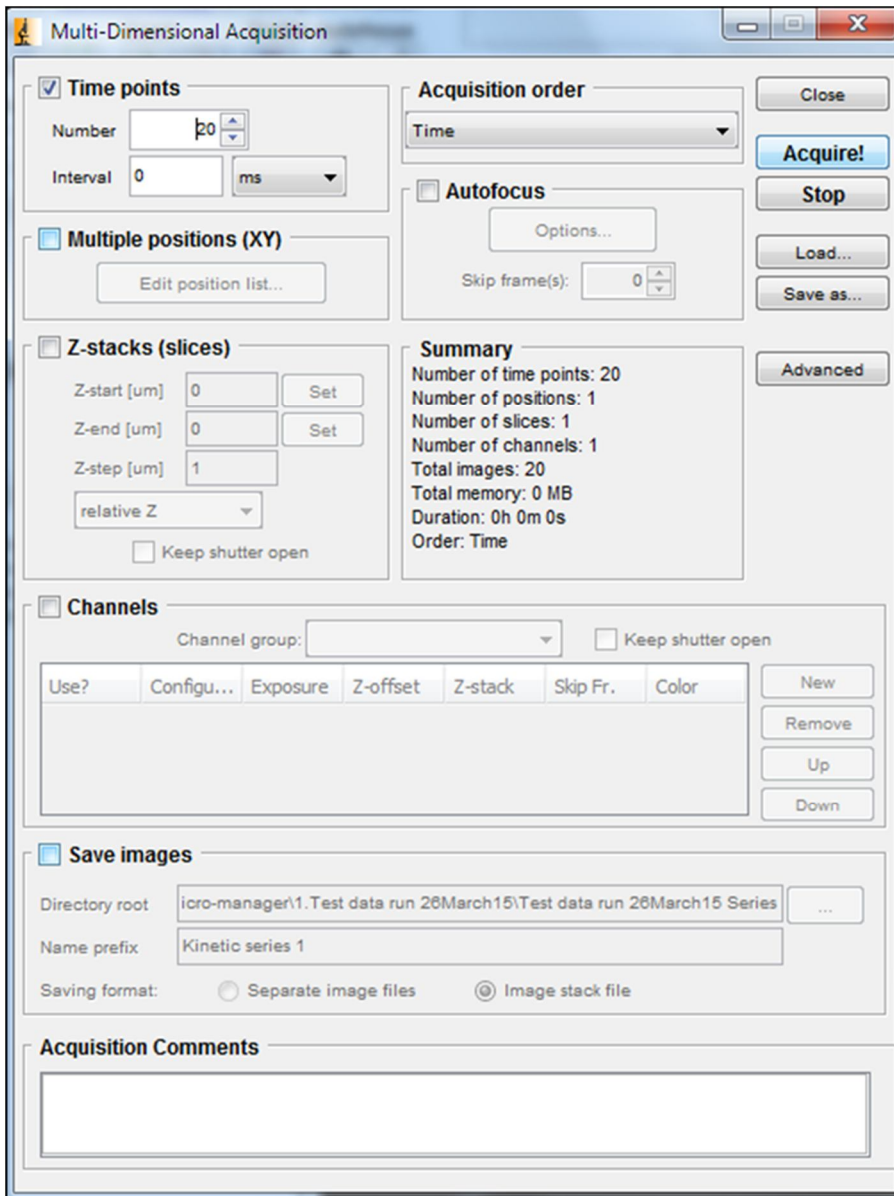
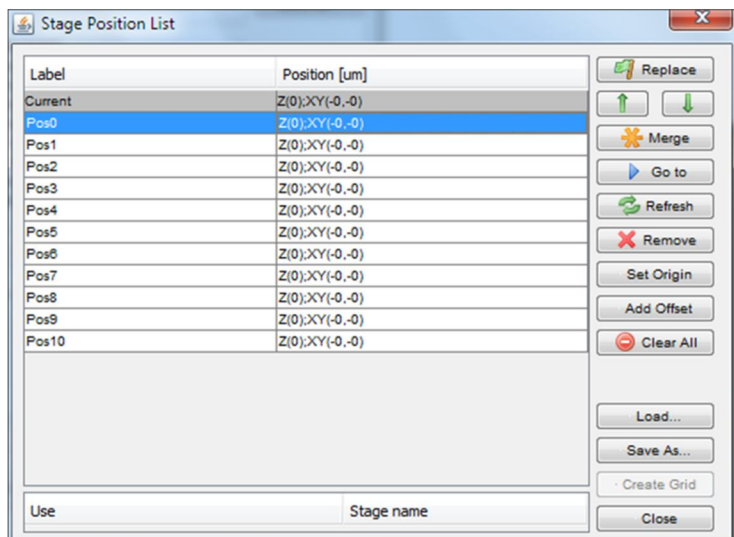


Figure 14: View of the 'Multi-Dimensional Acquisition' window. The system can be configured to take a spectral series as a function of a) time, b) position in 2D (XY), c) position in depth (Z), and d) for multiple channels where for example multiple excitation bandwidths or sources may be used.

Figure 15: Setting up points for different stage positions. Coordinates for several stages may be used.



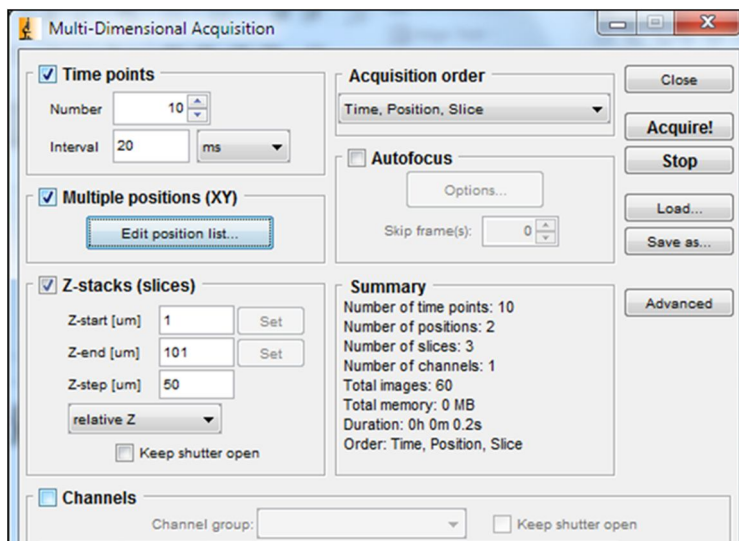
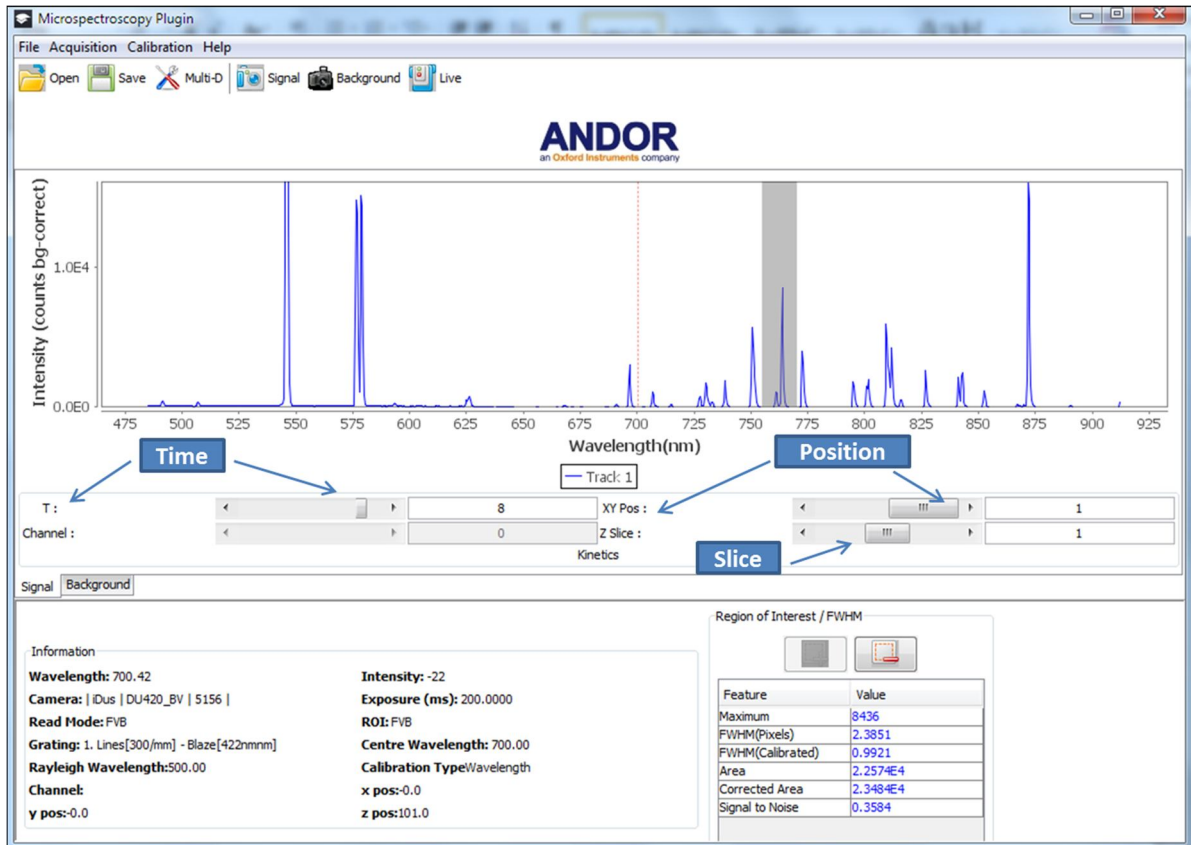


Figure 16: An example of a multi-dimensional acquisition series where variation in time, position (XY) and slice (Z) are used. The settings are shown to the left for a series of 60 spectra. On top is a view of the plugin acquisition window where the scroll bars are active for each parameter to allow stepping through each of the acquired spectra in the series.

An example of a multi-dimensional series is illustrated in figure 16. The series was set up to acquire 60 spectra in the series with variation steps in time, position and slice (Z) position. When a series is acquired scroll bars for each dimensional parameter appear below the spectral acquisition window. These can be used to step through, view and analyse each spectrum in the series.

Creating Configuration Settings

It is convenient to be able to set up the system with the required predefined parameter values for a given experiment without having to enter the values each time on starting up Micro-Manager. This is possible through the creation of 'Configuration settings' tables. This functionality is provided in the top right quadrant of the main window as shown in figure 17.

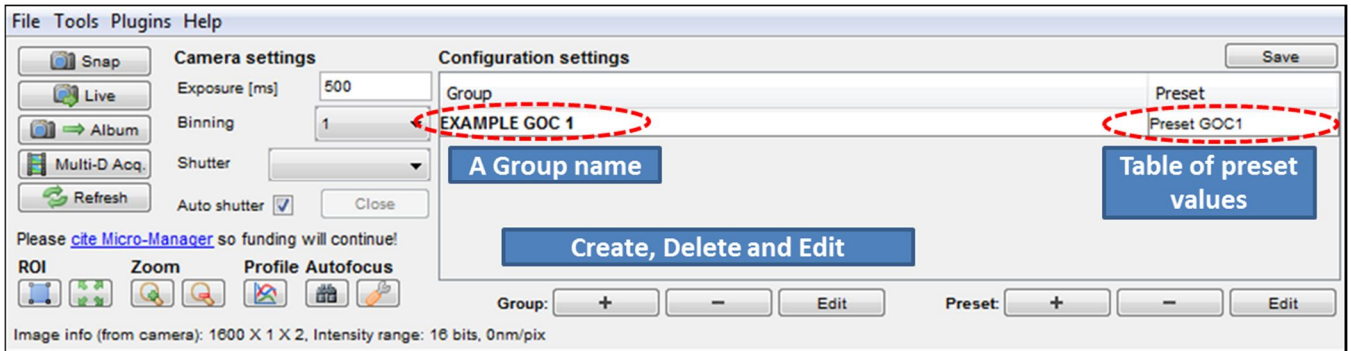


Figure 17: Setting up preset values in a **Configuration group**.

To create a **Configuration Group**, the '+' is selected which brings up a template with all the parameters and the values as they appear in the default uploaded Configuration as illustrated in figure 9 earlier. When a **Group** has been created and the table of **Preset** values have been set, it is possible to see the contents of the **Preset** table by simply hovering the mouse over the bar containing the **Group** name or **Preset** name as illustrated in figure 18.

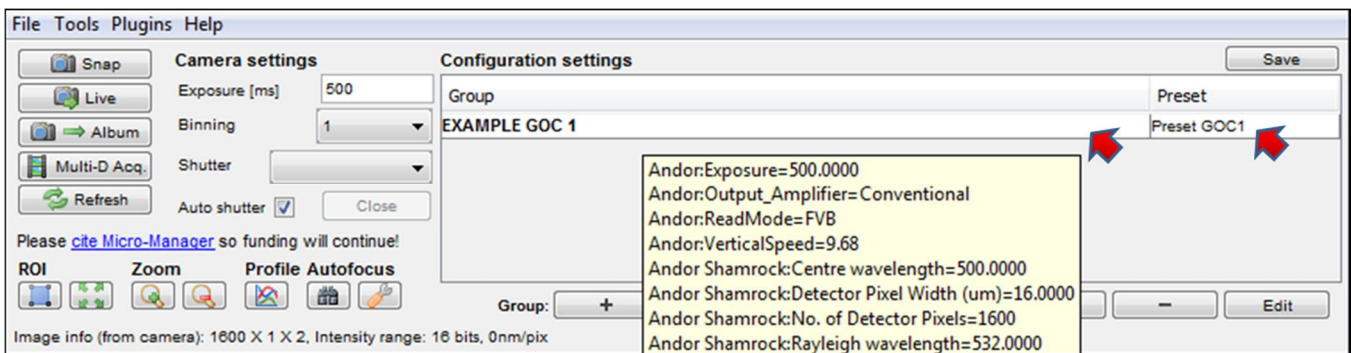


Figure 18: Using the mouse to see contents of the Preset table. Mouse is hovered as per red arrows.

When the '+' is selected to create a Group Configuration file, the '**Group Editor**' window is presented where first a name should be given to the group and then the relevant parameters for the '**Preset**' table are chosen. This is illustrated in figure 19. On clicking OK the Group file is created and the window for editing the **Preset** values of the chosen parameters is presented as illustrated in figure 20. Each '**Preset**' table of values should be given a name and the values set. This means that for a given '**Group**' configuration there can be multiple '**Preset**' tables depending on the different variations of measurement to be made; thus a given set of parameter values do not have to be set afresh each time but rather the particular '**Preset**' table or sub-file is chosen. '**Preset**' values can be saved within the main '**Configuration**' file by clicking on '**Save**'.

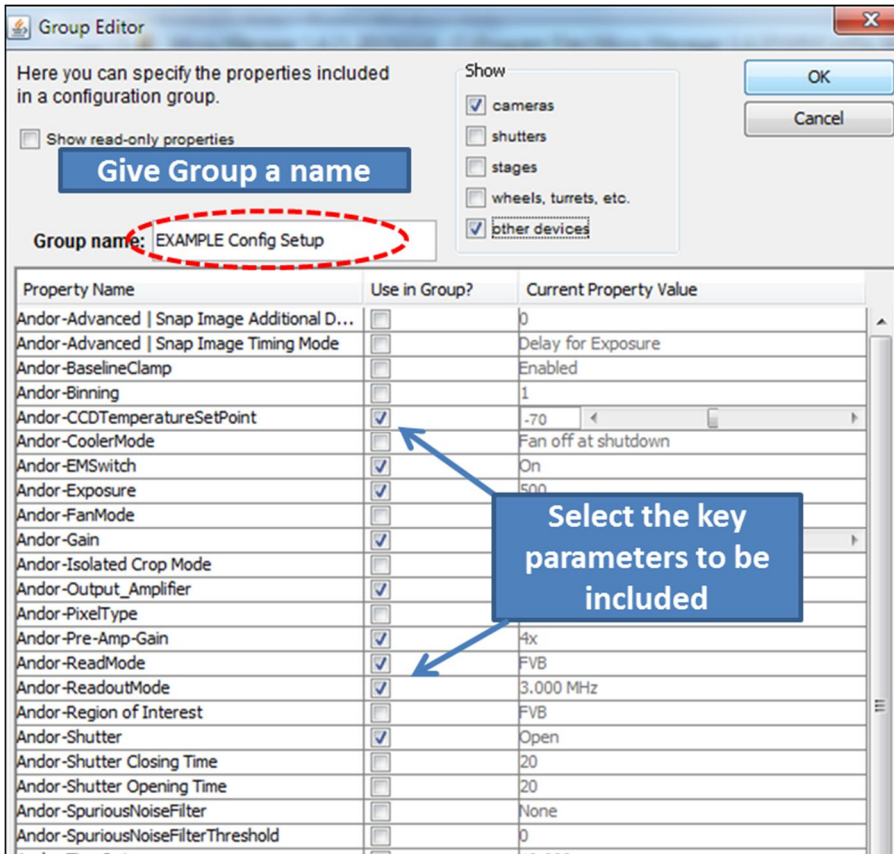


Figure 19: Using the **Group Editor** to set up a **Configuration Setup** table and **Preset** file which defines the parameter values for a particular experiment.

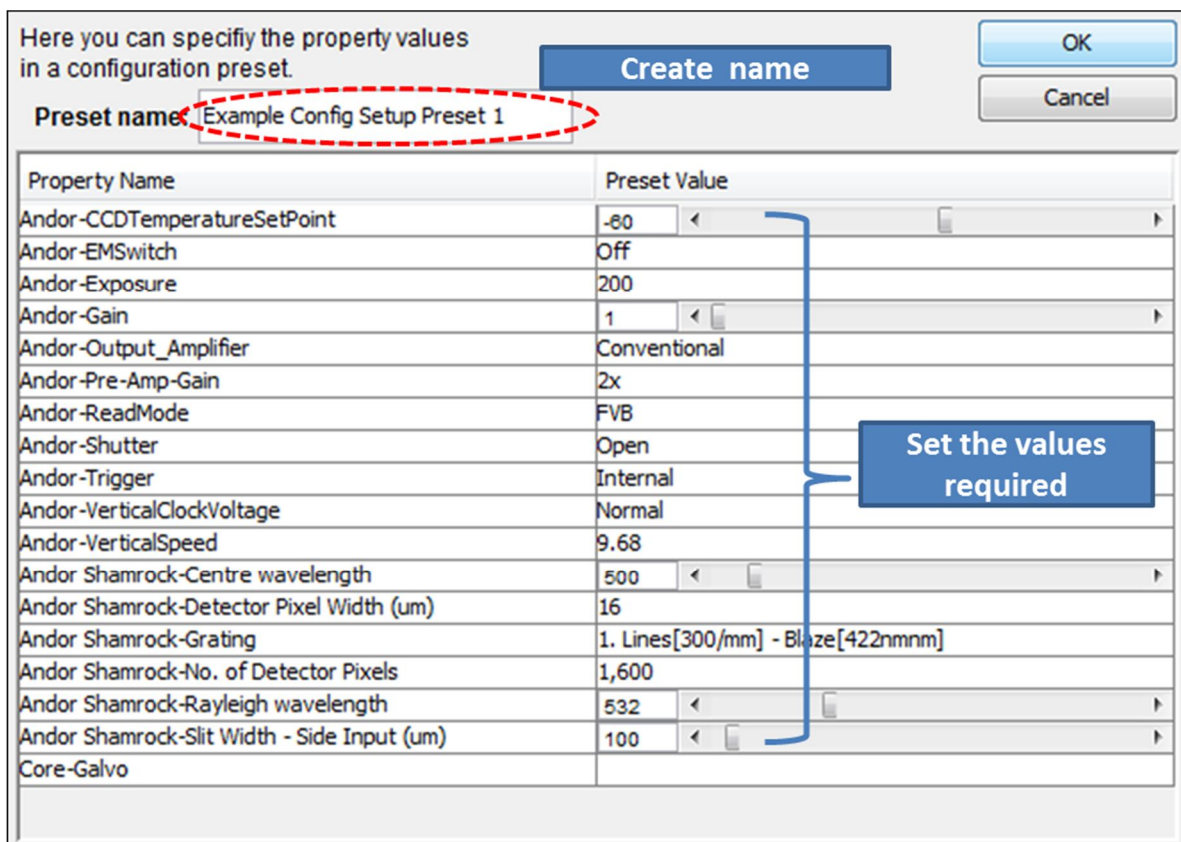


Figure 20: Modifying the values in the **Preset** file for key parameters to be used in an acquisition experiment.

Script files: Setting up scripts to automate experimental acquisitions

Experimental acquisitions can be automated through the use of small macro programs which control and synchronise the various components within the system. One such example is where it is desired to capture a series of spectra in a 2D map or scene. Micro-Manager has built-in scripting capabilities. It makes use of the interpretative language **Beanshell**, which has a syntax similar to **Java**. The scripting utility can be accessed through the 'Script Panel' which is available in the drop down 'Tools' menu at the top of the main window. A picture of the 'Script Panel' is shown in figure 21, where an example of a simple script file is shown. The example, when 'Run' is pressed, moves the xy stage to four positions in turn, capturing and saving a spectrum at each position.

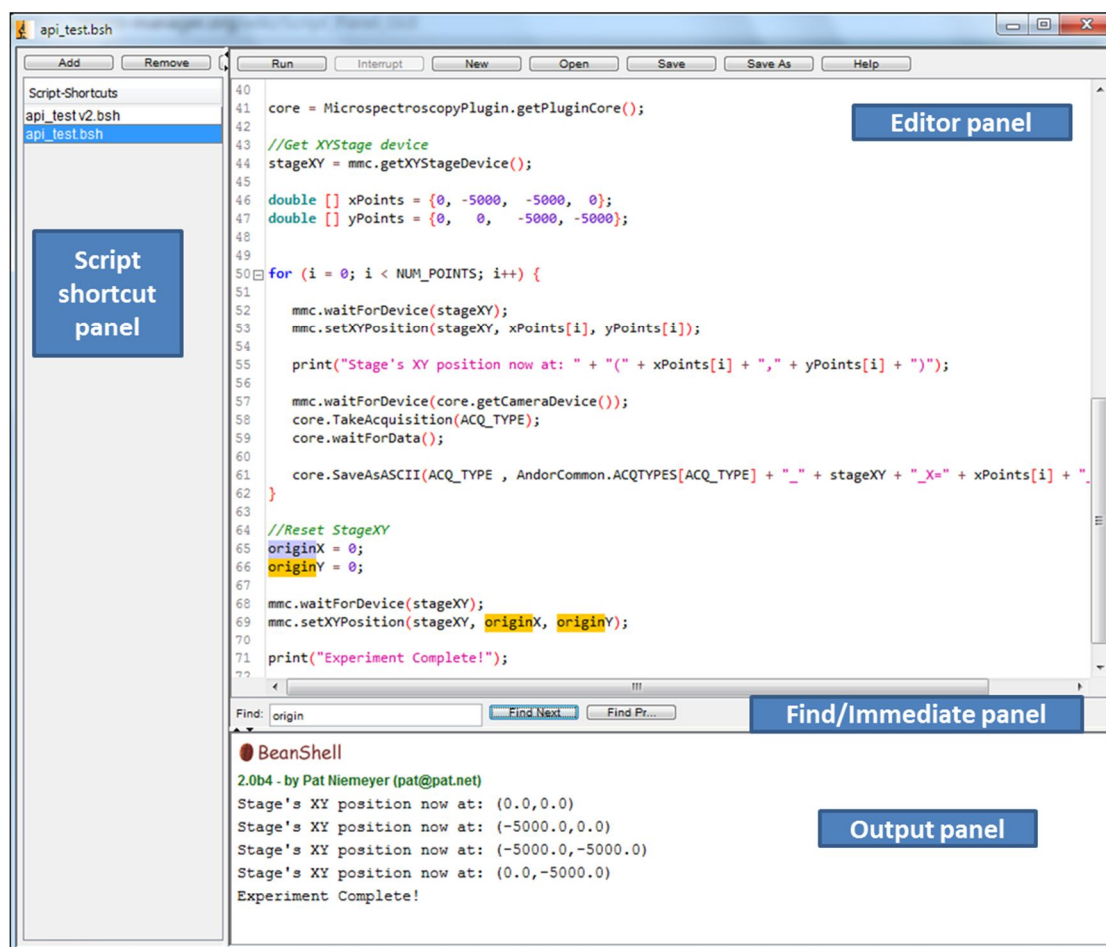


Figure 21: The 'Script Panel' with a simple example of a script file.

The Script panel consists of four areas or sub-panels -

- **Editor panel** – on top right allows for the creation and editing of script files or macros,
- **Script Shortcut panel** – on left side shows the names of script files that can be readily accessed without having to go to the folder where the file is stored to run each time,
- **Output panel** – at the bottom right shows output from the macro using for example the 'print' command or to relay error messages to the user,
- **Find panel or Immediate panel** – a tool sitting central on the right between the **Editor panel** and the **Output panel** – the tool functionality provided here will depend on the version of Micro-Manager used and its settings.

The script files facilitate the automation of multiple acquisitions in a typical experimental configuration, including the spooling of acquired data to the disk drive where large volumes of data are to be collected.

File handling: Saving, Loading and the format used

The '**Microspectroscopy plugin**' has been set up to deal with **ASCII** formatted files. This is one of the more basic formats for saving the raw data and which can be handled by most third party software applications where post process analysis may be carried out. In the case of a kinetic series the spectra may be either saved in one composite file or as individual files for each spectrum. Metadata is attached to end of the data files which summarises the acquisition conditions and other useful information. Figure 22 shows the window for saving the data files and the possible delimiters that may be selected.

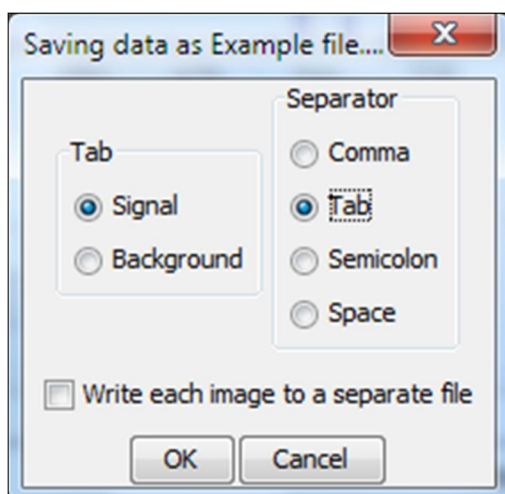


Figure 22: The data files are saved in ASCII format with a choice of delimiter that may be used. The Signal and background data have to be saved separately.

Conclusion:

The '**Microspectroscopy plugin**' offers a good workable solution to setting up an integrated micro-spectroscopy system. It is relative easy to implement and use. Micro-Manager is open-source and lends itself to people starting out to set up their own custom configurations with minimal expenditure outlay.

References:

- [1] <https://www.micro-manager.org> - Micro-Manager website
- [2] 'Quick start guide – Spectroscopy plug-in for Micro-Manager'
- [3] 'Modular Micro-spectroscopy Solutions' specification sheet
- [4] 'Micro-spectroscopy: Configurations for Modular Solutions' - technical note
- [5] Stuurman N, Edelstein AD, Amodaj N, Hoover KH, and Vale RD (2010), 'Computer Control of Microscopes using μ Manager', Curr Protoc Mol Biol. October 2010
- [6] Edelstein AD, Tsuchida MA, Amoda N, Pinkard H, Vale RD, and Stuurman N, (2014), 'Advanced methods of microscope control using μ Manager software', Journal of Biological Methods, Vol. 1(2), e10