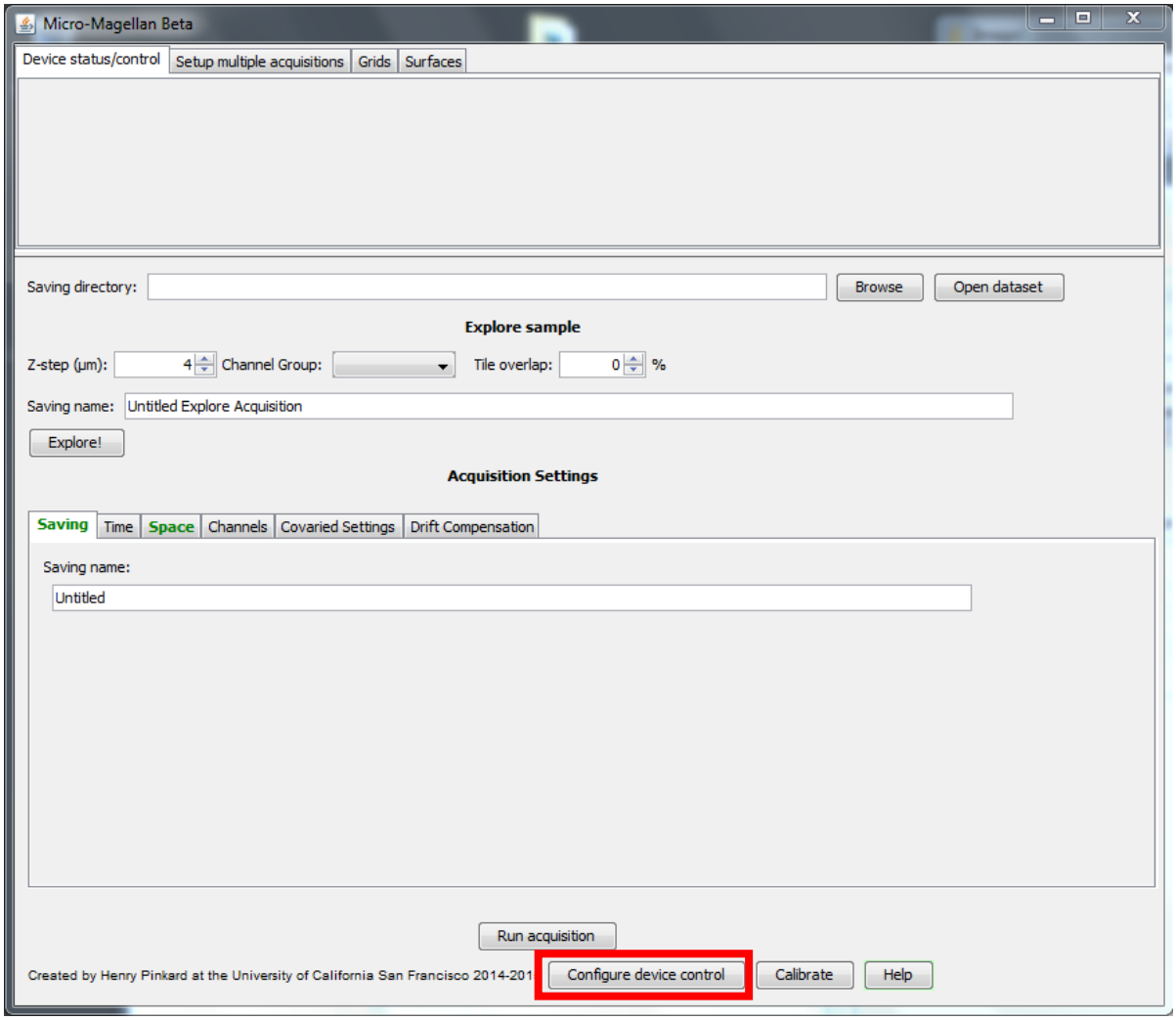
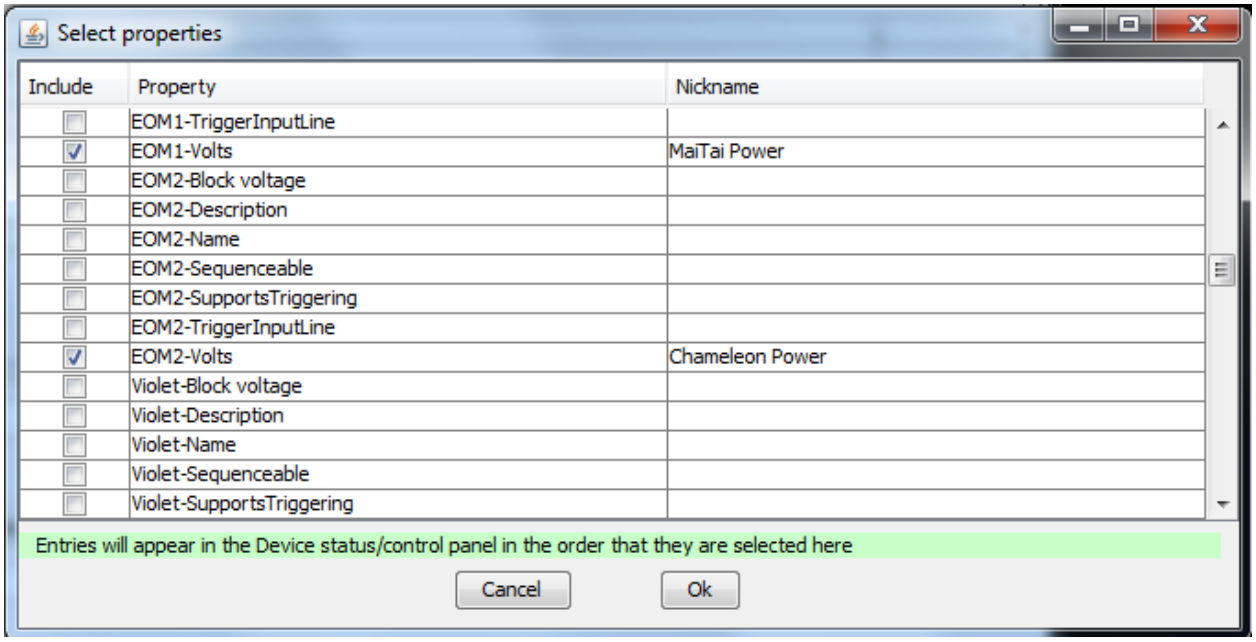
Setting up Micro-Magellan for Device control

From the plug-in menu, select Micro-Magellan and the unpopulated window opens

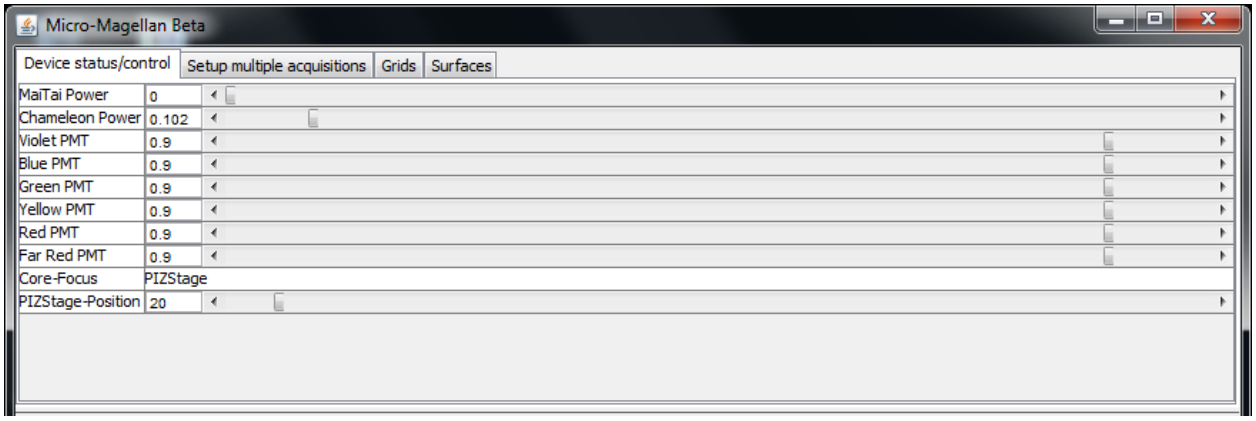


Select “Configure device control”

The window below will open, select devices to appear in the control window and the “nickname” that will appear

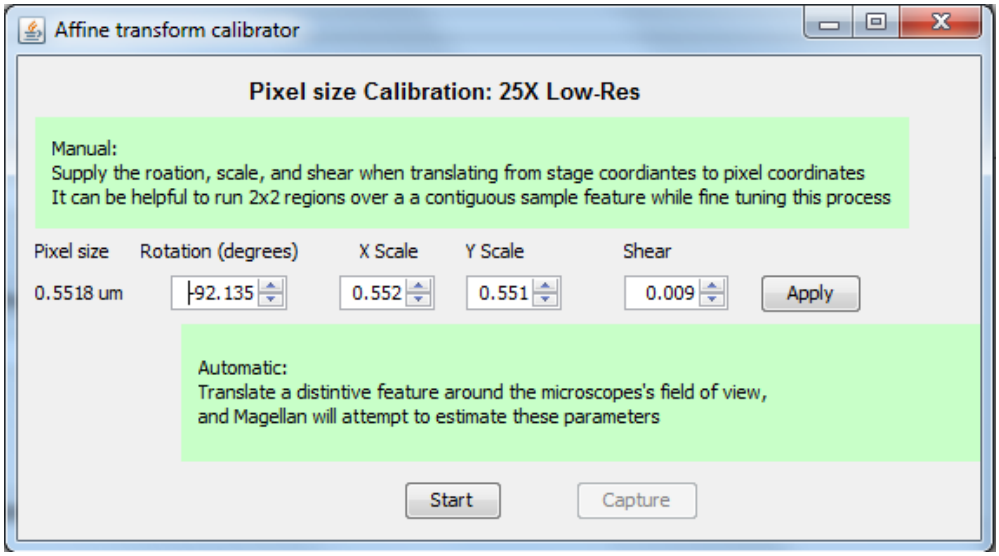


Press “Ok” and the selected devices will appear in the control window



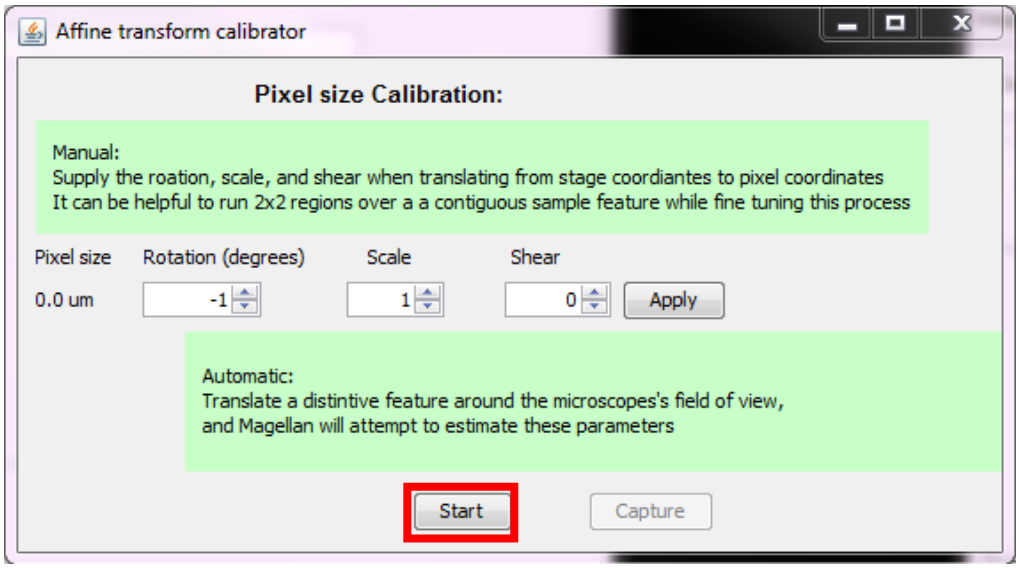
Setting up for Explore & Acquisition

In order for Micro-Magellan to acquire tiled images and assemble them, it must have an accurate pixel size calibration for the objective in use. If you have already calibrated your pixel size using the “Pixel size calibrator” plug-in from Micromanager, this should automatically propagate to Micro-Magellan. Before using Explore mode, verify that the calibration is correct by pressing “Calibrate”.



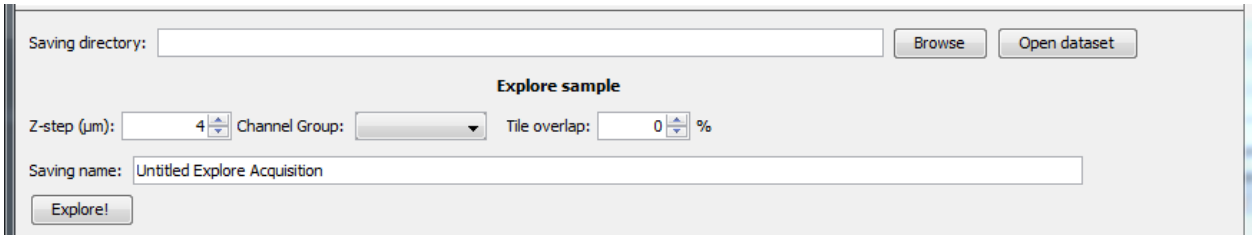
If the fields are populated as above, Micro-Magellan has the necessary information. These parameters can be adjusted manually to fine tune stage movement for improved stitching.

If the fields are not populated (as below), you can run click Start and follow the wizard instructions to generate an affine transform.

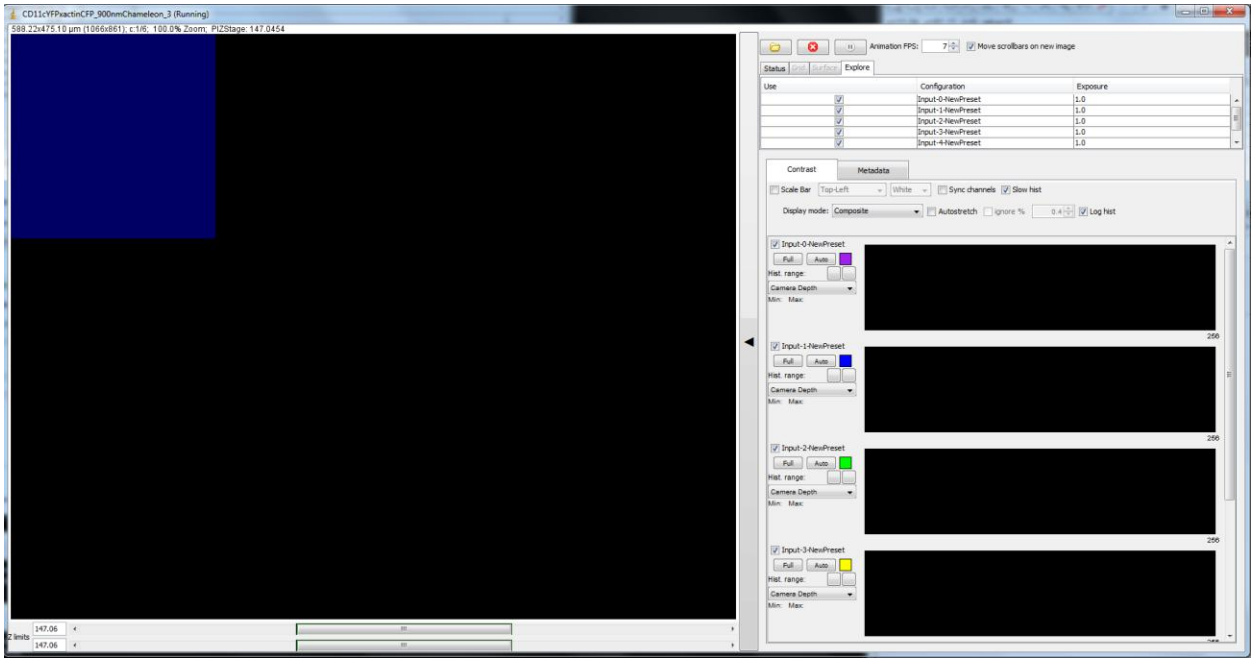


Using Explore Mode

Before exploring your sample you will need to enter your desired Z-step size and tile overlap. The channel group drop down is analogous to the channel group drop down within MicroManager's Multi-Dimensional Acquisition plug-in and will be pre-populated with the configurations groups you have created. Select the settings group that contains your channel configurations.



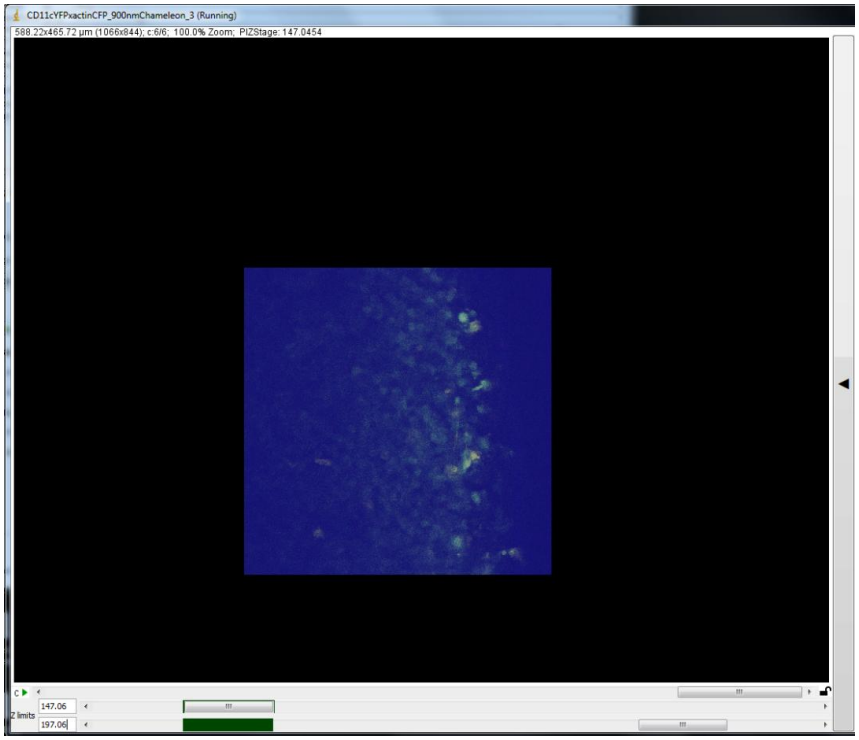
Pressing "Explore!" will open a new window:



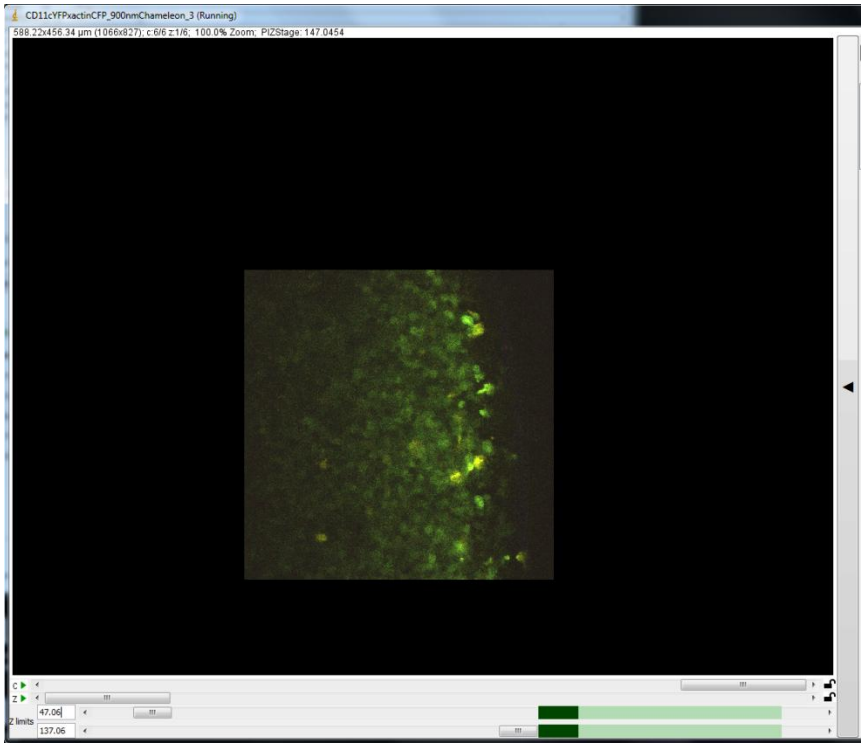
On the left side is your image area; a field of view will highlight blue as you hover over it. On the right, are look-up tables (LUTs) for each channel and several other acquisition set-up parameters.

To acquire an image in that area click to select (area will highlight green during active selection), once your selection is highlighted magenta, click again to confirm. You can click and drag to select multiple fields of view to be imaged.

The “Z limits” scroll bars below the image window control what Z-locations will be imaged, and will adjust automatically to accommodate the selected Z-step size.



Once you have begun to image, the Z-plane currently being displayed will appear dark green in the Z-limits scroll bars. Other acquired Z-planes will appear light green. In the example below, we have already collected a Z-stack (light green) and are extending our acquisition by acquiring additional Z-planes above what we have already.

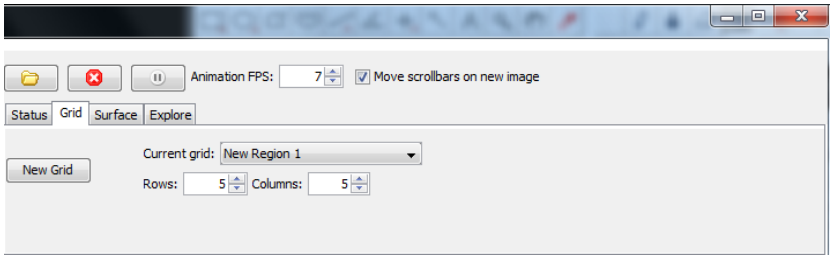


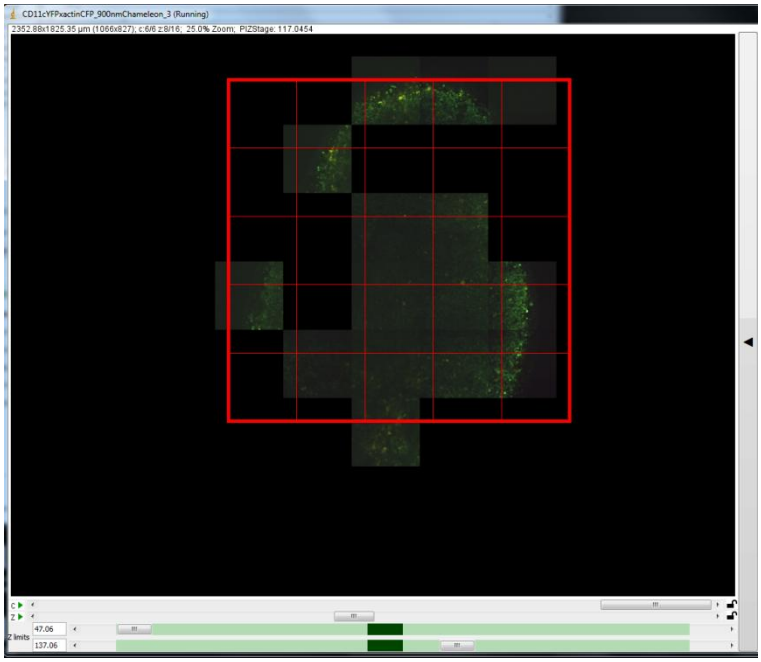
You can review acquired images by scrolling with the “Z” bar, and right-clicking and dragging to navigate in the XY plane. You can also zoom in and out by using the mouse scroll wheel with the image window selected.

Once you have explored your sample and would like to select areas to image over time, with more optimized parameters, better Z-resolution, etc. you can use either the “Grids” or “Surfaces” tab above the LUTs to do so.

Grids

In the Grids tab, you can define an N x N grid, which will appear over your explored image. With the Grids tab active, you can drag this grid over the area you would like to image. You may generate multiple grids of different sizes by pressing “New Grid”, and then move them to different areas.



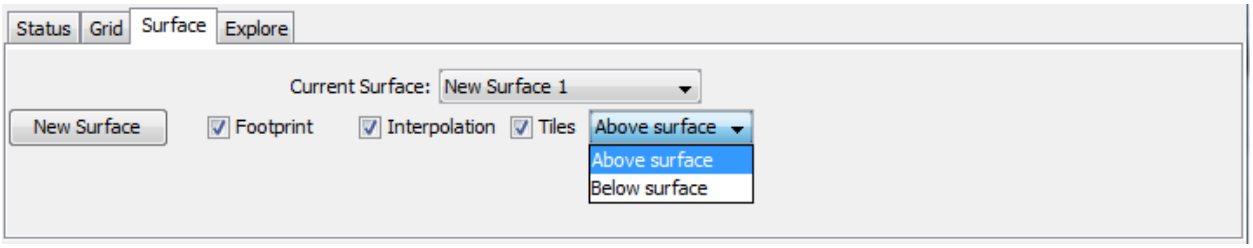


All of the grids generated in explore mode will populate the “grids” tab at the top of the Micro-Magellan main window, next to the Device status/control tab. If you are creating multiple grids, it may be helpful to toggle back to the Micro-Magellan main window, and in the select and rename the grids with their identifying features (ie: airway, duct, tumor).

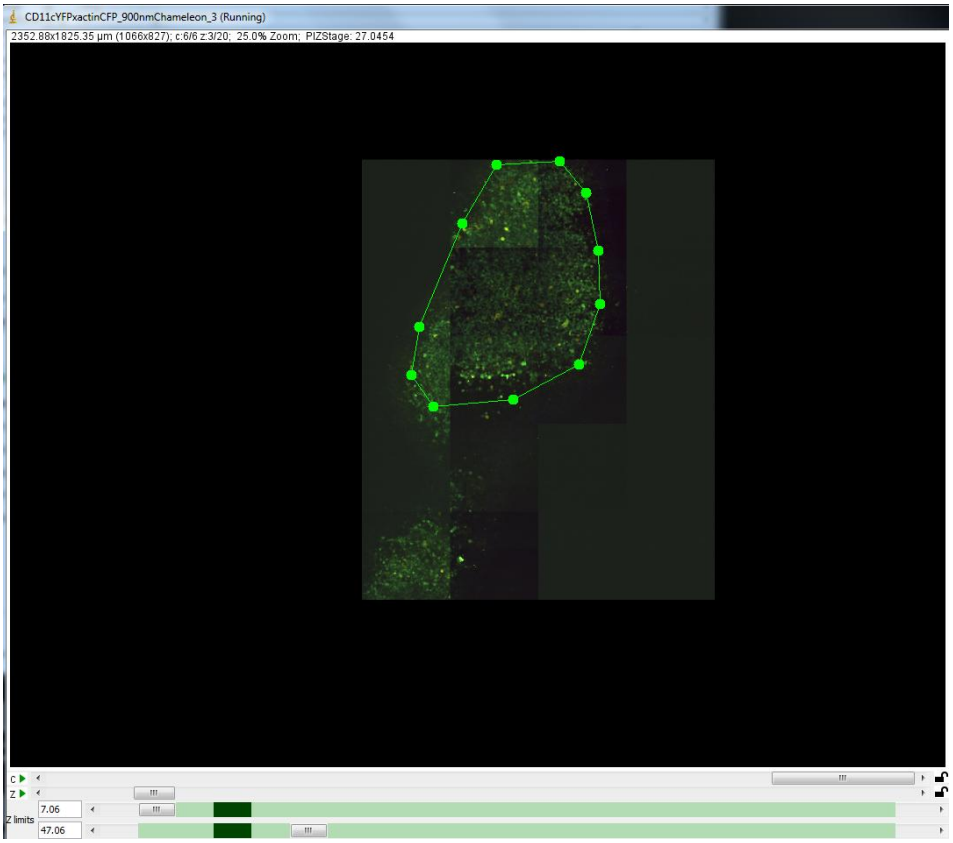
Surfaces

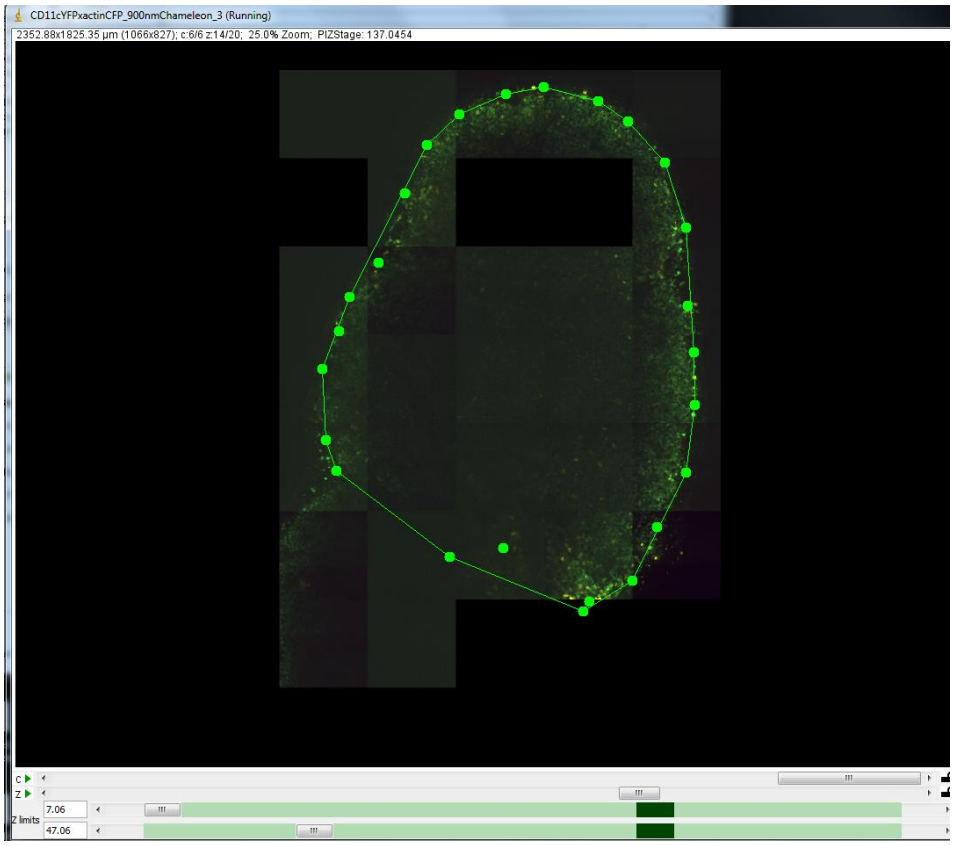
The surfaces tab allows you to define the morphology of the sample by defining the edges at multiple Z-planes; Micro-Magellan then interpolates between the outlines to generate a 3D representation of the sample which can then be used to adjust which fields are acquired to avoid collecting empty space, or vary hardware settings in a sample dependent manner.

To begin drawing your surface, first select (surface above/below)



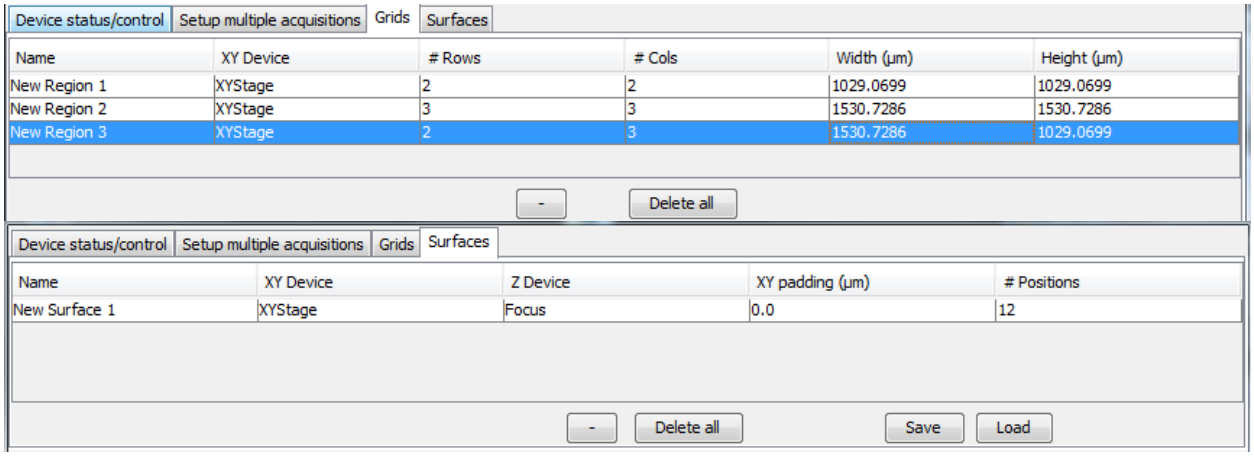
Here we have selected Surface Below, so we begin at the top of the sample and add points around the perimeter, then move the Z-slider down and adjust the outline.





Once you are satisfied with grids or surfaces generated, you can return to the main Micro-Magellan window to set up acquisition.

All of the grids and surfaces generated in explore mode will populate the “grids” and “surfaces” tabs at the top of the Micro-Magellan window, next to the Device status/control tab

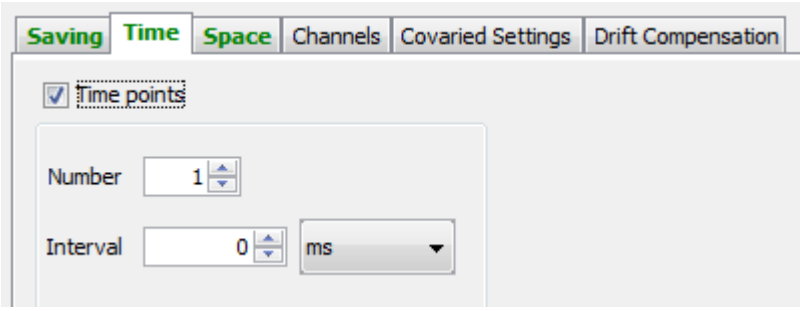


Acquiring Images

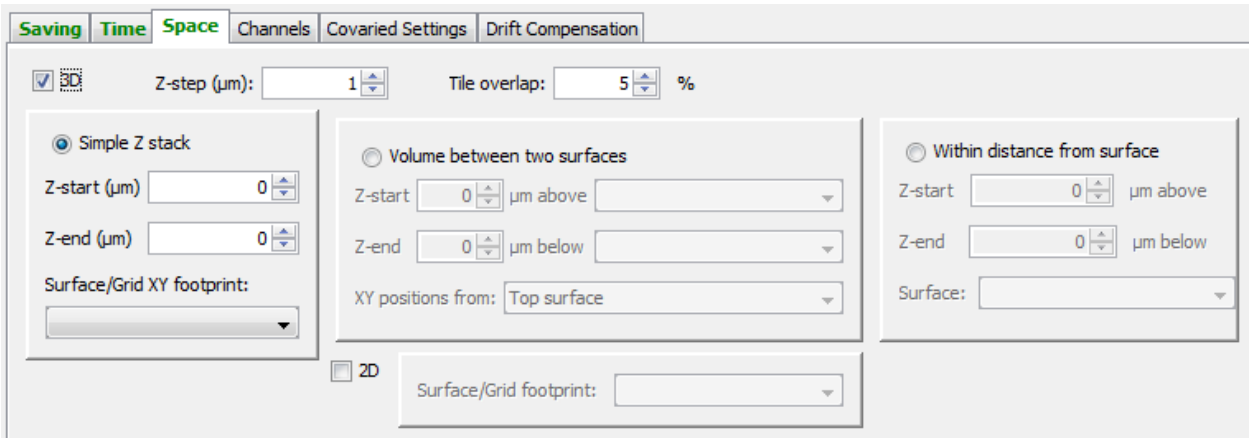
There are a series of tabs in the lower half of the Micro-Magellan window to help manage acquisition set up.

The “Save” tab allows you to define the name of the acquisition, and uses the same directory as the explore mode. To set up a simple, single area acquisition, enter a name for the file.

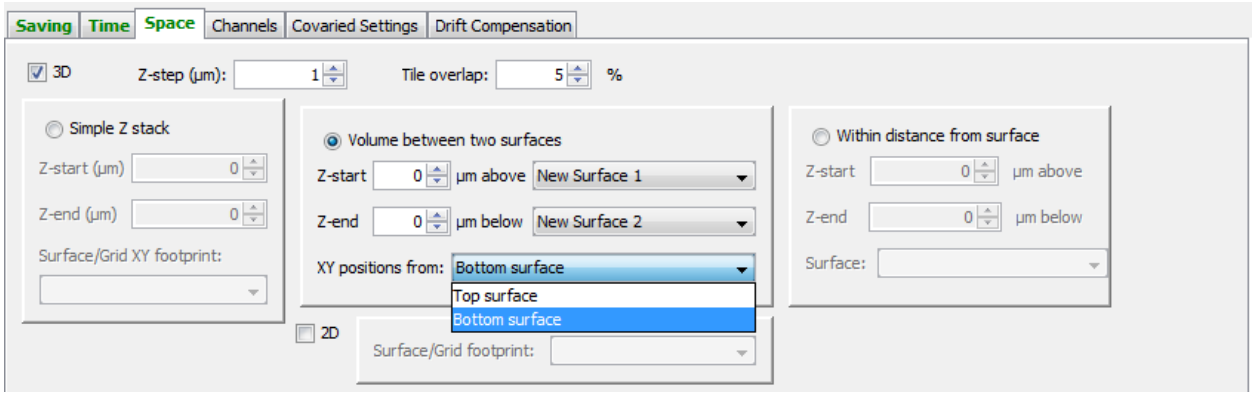
In the “Time” tab, you can enter time interval between image acquisition and the total number of time points to acquire.



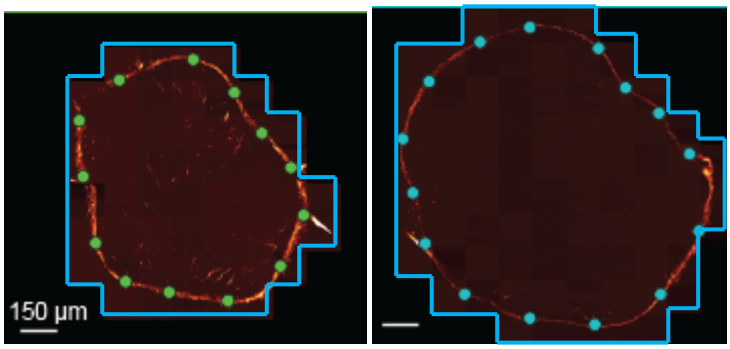
In the “Space” tab there are several options for defining the volume you want to image. In a simple Z-stack you enter start and end Z positions, and either the grid or surface to define XY location and positions.



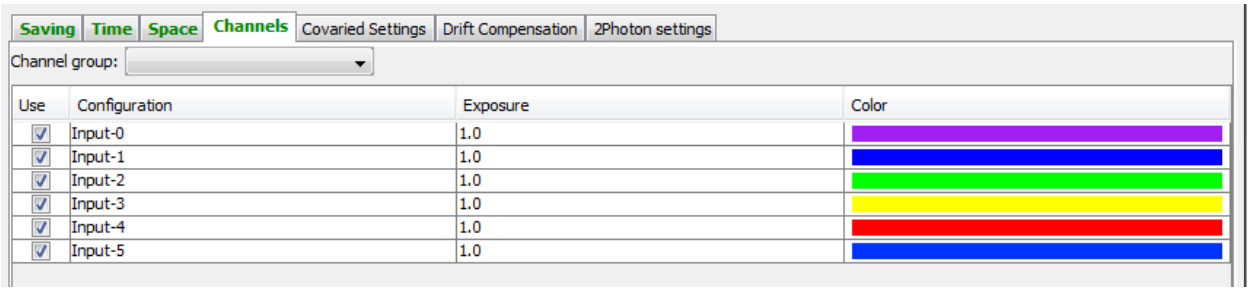
If you have created surfaces, you can also elect to image the area between two defined surfaces with a buffer on either side.



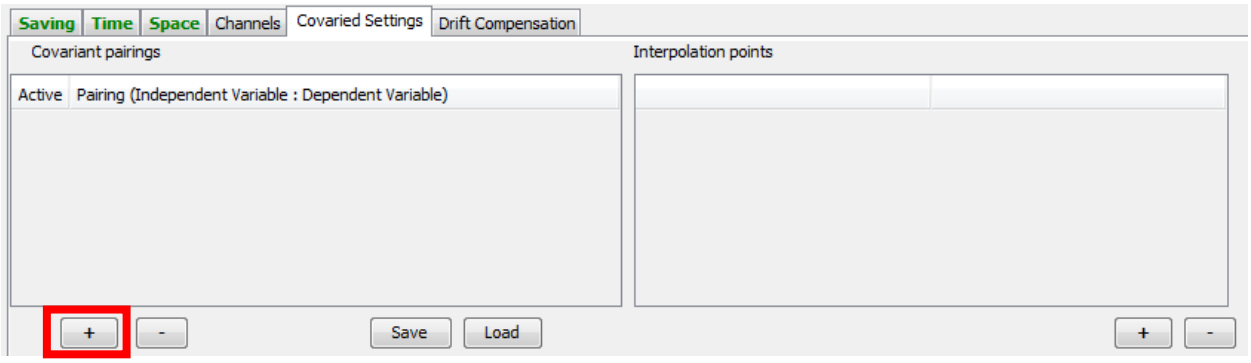
Imaging within a distance from a defined surface allows you to collect non-cuboidal volumes by using the edges of your surface to choose the XY positions that are collected at each Z-plane (example of XY footprints below). By selecting a Z-distance below a surface to image, you can also avoid collecting "blank" data in deep areas of the sample where there is no information due to scattering.



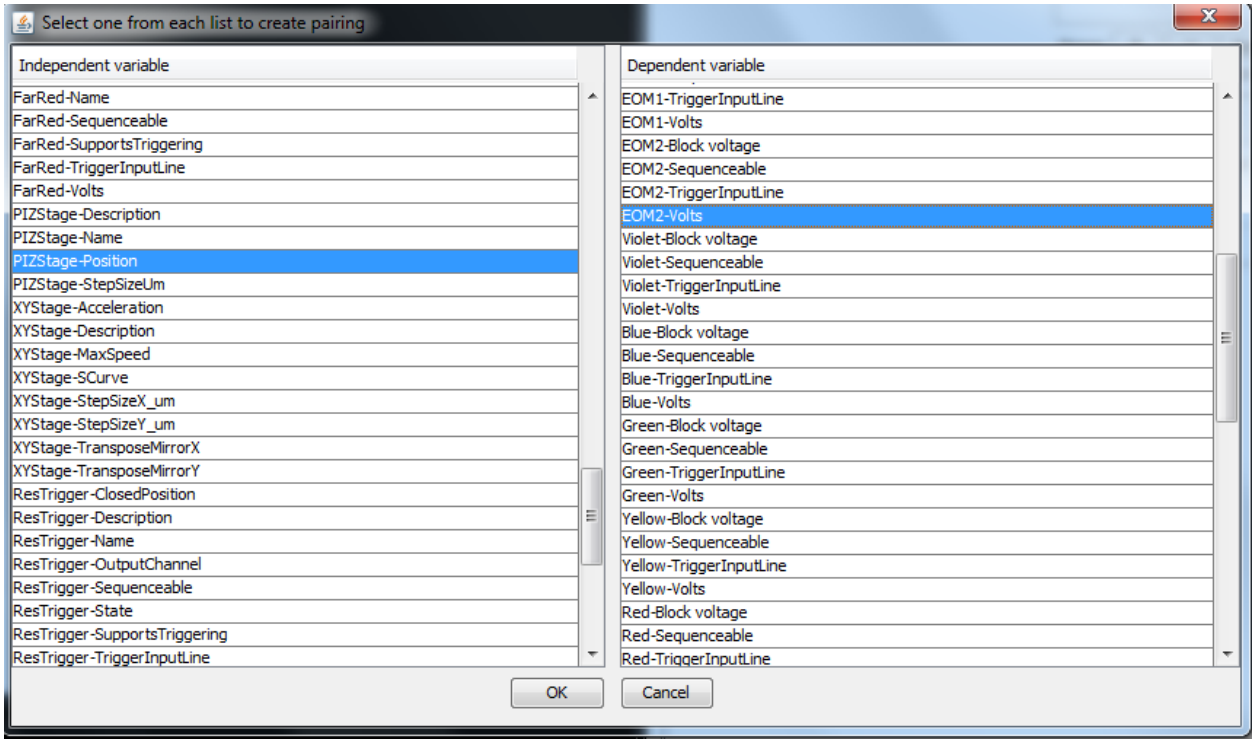
The "Channels" tab allows you select which of the available channels to collect, and the exposure time for each.



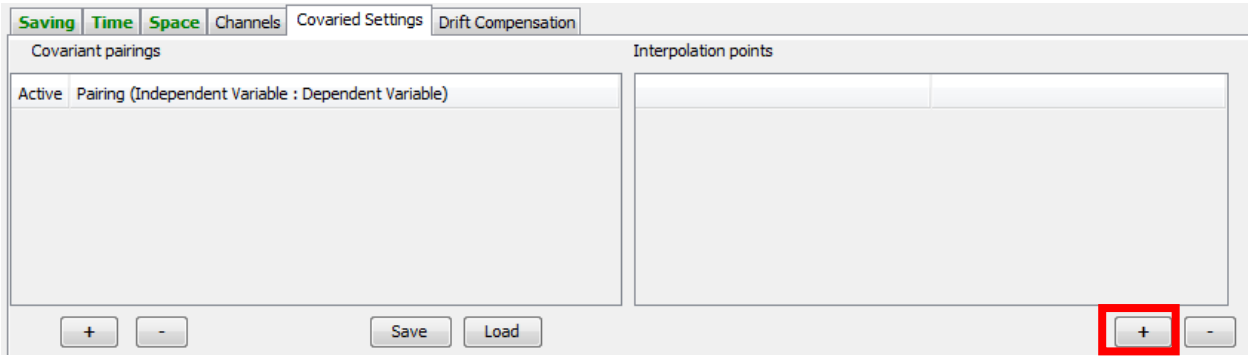
The "Covaried Settings" tab allows you to vary one hardware setting depending on the state/value of another hardware device/setting.



The "+" button on the right brings up the window below, allowing you to select one hardware setting as the independent variable, and a second as the dependent variable.

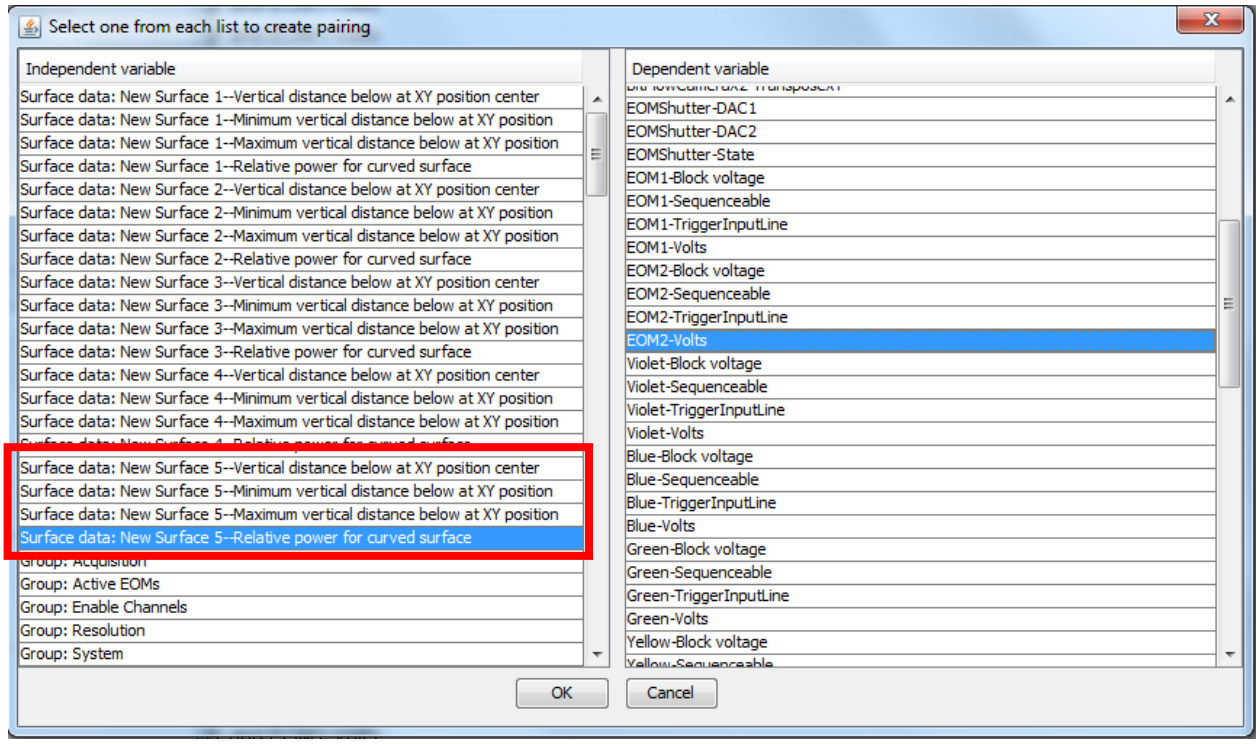


In this example, we have selected the Z stage position as the independent variable, and our EOM (laser power) will vary depending on its position allowing us it increase laser power as we get deeper into our sample.

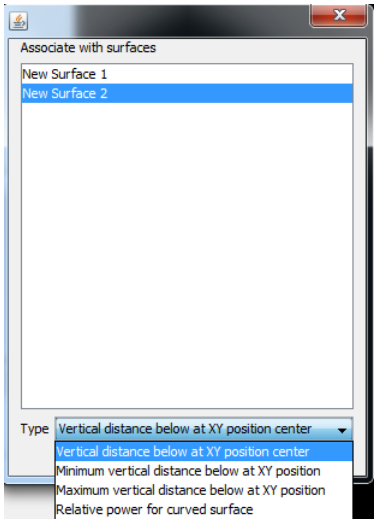


Once you have selected the two variables, clicking the “+” button on the right under Interpolation points allows you to enter settings for them. Micro-Magellan interpolates and applies values between the points entered.

If you have defined surfaces, you can also choose to vary hardware settings based on surface data.

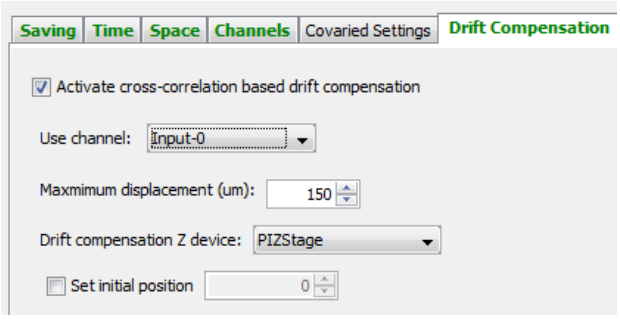


For a given tissue type and morphology, these parameters may be optimized and saved for use across samples. To load a saved set of interpolation points and settings, click “load” and select the saved parameter file. You will be prompted to select which surface you would like to assign the settings to, and which surface parameter you would like to use.



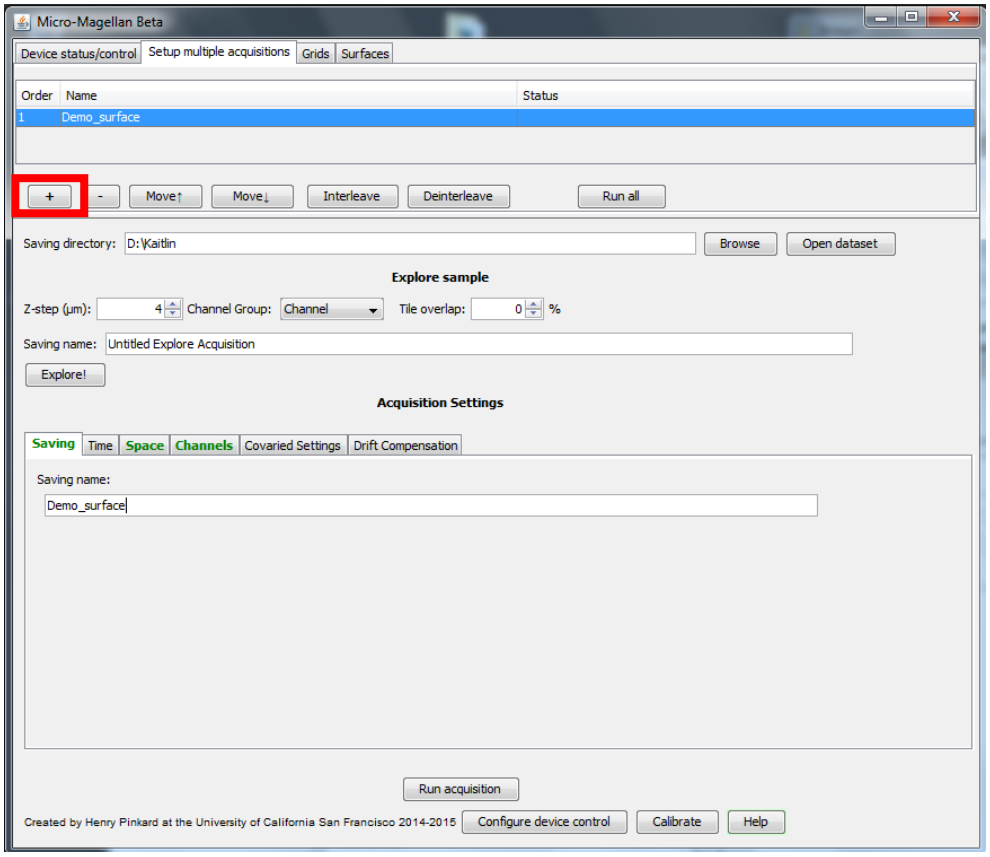
Drift Compensation

Drift compensation allows you to use a single channel that represents the static features of your sample, and use it to correct for Z drift.



Setting up multiple acquisitions

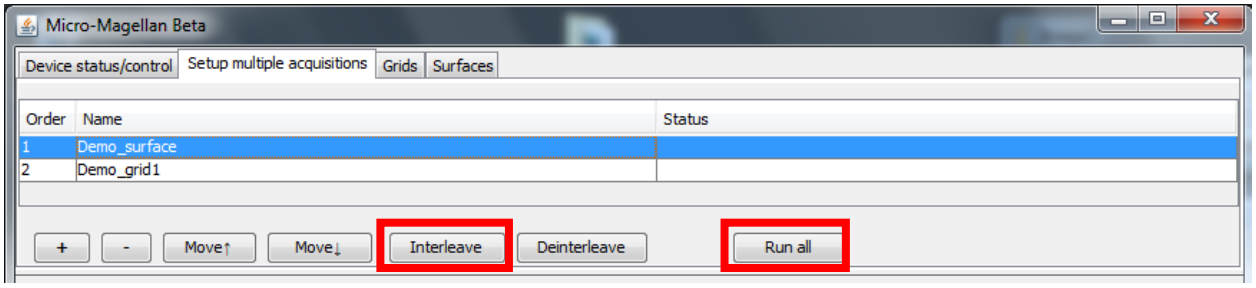
To acquire more than a single grid or surface at a time, select the "Setup multiple acquisitions" tab. Click "+", this will add a line with the same name as is in the "Saving" tab below. Enter the parameters for this acquisition in the other acquisition settings tabs.



Clicking "+" again will add another line, it will appear with the same name. Highlight the second (new) acquisition and change its name in the "Saving" tab below, and change necessary parameters in the other acquisition settings tabs.

"Run all" will sequentially acquire all acquisitions listed.

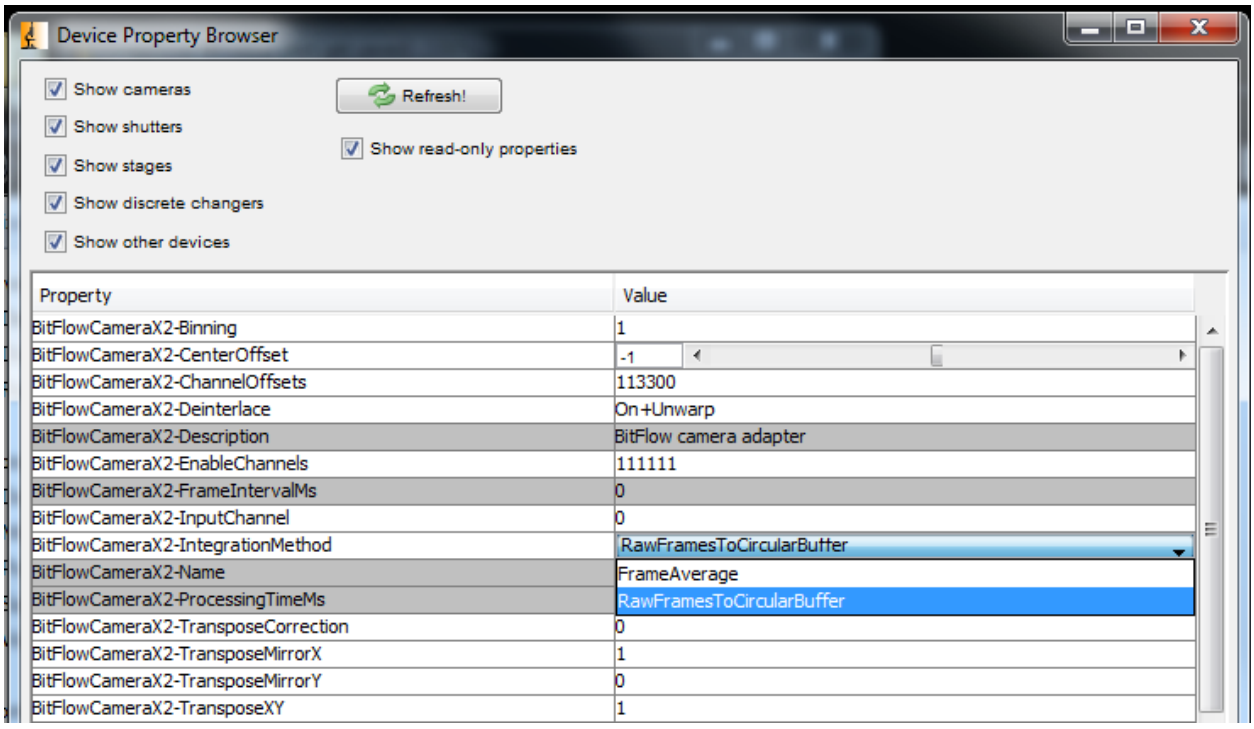
If you would like to collect time data in different areas concurrently (rather than completing a time lapse in one area, and then beginning the second time lapse in another area), select “interleave” and then “Run all”



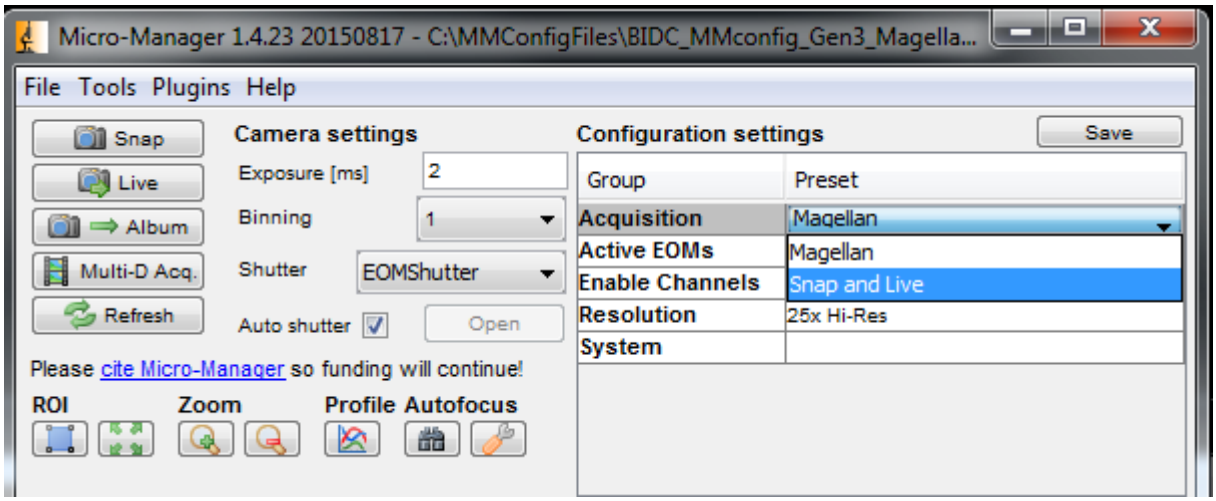
Line Scanning Systems

If you are using Micro-Magellan on a line scanning system, this plugin replaces the previous “100X TwoPhoton” plugin.

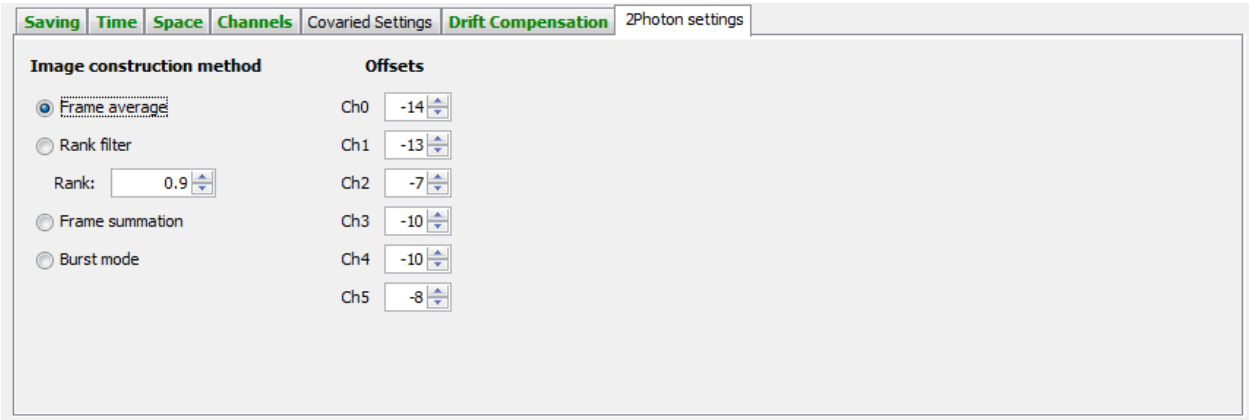
Because Micro-Magellan uses its own acquisition engine within MicroManager, when switching between using the Snap/Live buttons and Micro-Magellan, you must switch the BitFlowCamera-IntegrationMethod device parameter. For Snap and Live modes, select “FrameAverage”; for Micro-Magellan select RawFramesToCircularBuffer.



We simplify this for users by creating an “Acquisition” configuration with presets.



On startup, Micro-Magellan looks for the presence of a Bitflow board in the configuration file. If you it finds one (or multiple), it also loads a 2Photon Settings tab in the Micro-Magellan main window.



This tab gives you several options for image construction/processing. All options here pull the number of frames used from the “Exposure [ms]” field in the main MicroManager window.
The offsets fields allow you do adjust the interleave offset for each channel individually, although maximum difference between any two channels is 9 pixels.

Outputs

Micro-Magellan will create a directory with the name entered in the “saving” tab. Within this directory are subfolders with downsampled versions of the acquired images, these are used to create a “zoomable” image. Within each folder is a single TIF image for each XY position acquired, which stores all the channels, Z-planes, and time points for that XY location. These TIF files can be automatically stitched and saved as either an Imaris (.ims) file, or output to a FIJI window using the Imaricumpiler plugin for FIJI found here:
http://biomicroscopy.ucsf.edu/mediawiki/index.php?title=Analysis_Software_Repository