It is our recommendation to use journals instead of the normal controls via the MX interface.

The reason for this is that this will give us more control and there are some bugs and actions in our software that might make it less predictable.

For this you would use the **Devices > Focus > Auto focus tab > Configure Laser**

Configure Laser Autofocus Dialog Options

Action

Sets the type of autofocus used when the Autofocus command is called from the AutoFocus tab of the Focus dialog. The following options are available:

- Autofocus - Full Search - Performs a search across the full focus range, looking for either one or two peaks as specified in the Search Options field.
- Autofocus - Incremental - Performs a search across an incremental range, ranging around a previously found position.
- Preview Pass - Performs a preview pass across the full focus range and displays a graph of the results.

Search options

Find 2 surfaces

Attempts to find a second peak from the start position. For example, if you are using the MetaXpress Software to perform plate acquisition, this would search for both the bottom of plate and bottom of well focus values.

Find 1 surface

Attempts to find a single peak from the start position.

Perform iterative search

The search is broken up into increments based on the Thickness value, covering up to the Full Range value. The search ends when the desired peak is found within the last range searched. If not selected, the entire Fullrange is traversed and the desired peak is selected from within the entire search range.

Start position

Start from current z-position

Starts the search from the current z-motor position.

Start from (um)

lets you enter a specific z-motor position (in um) to use when starting the search.

Search parameters

Full range (um)

Specifies the full range to cover for a Full Search. If Perform iterative search is selected, this represents the maximum range to cover.

Incremental range (um)

Specifies the range to cover for an incremental search.

Thickness offset (um)

Specifies the distance to move after finding the first peak and before starting the search for the second peak. If Perform iterative search is selected, this also represents the range of each individual search.

Exposure 1st surface (us)

Specifies the exposure to use when searching for the first surface.

Exposure 2nd surface (us)

Specifies the exposure to use when searching for the second surface. This option is only enabled when Find 2 surfaces is enabled.

Coarse step (um)

Z-motor step size used during the initial stage of autofocusing. The default value varies for each objective.

Fine step (um)

Z-motor step size used during the second stage of autofocusing. The default value varies for each objective.

Laser intensity (%)

Sets the laser intensity. This option is not available for all LAF hardware platforms.

Post-focus offset (um)

Specifies the z-offset to apply after finding the target peak in a search. This is the offset from the laser focus z-position to the image focus z-position. The default value varies for each objective and wavelength

OK

Accepts the current values and closes the dialog.

Cancel

Closes the dialog without changing any settings.

Once you have determined the parameters you can change the journals and use it during Plate Acquisition Setup.
On the Acquisition tab, disable the built-in autofocus options (laser autofocus and image autofocus).
Enable the option to "Run Journals During Acquisition".
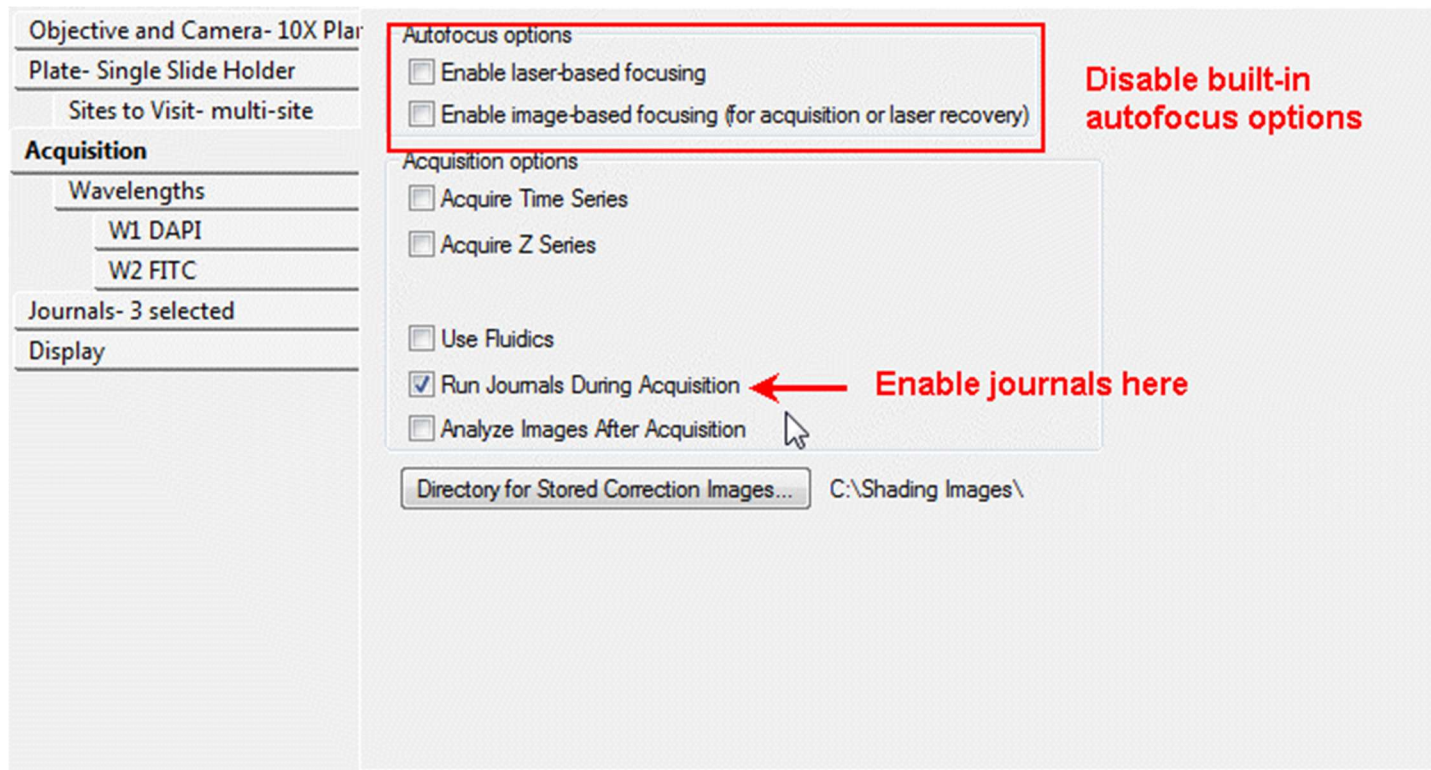
On the Journals tab, enable the two journals at the specified steps (Start of Plate and Before each Image).
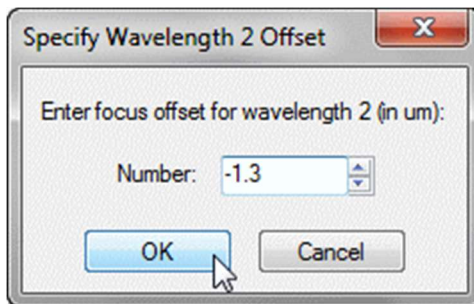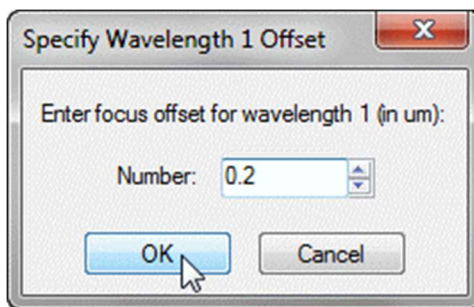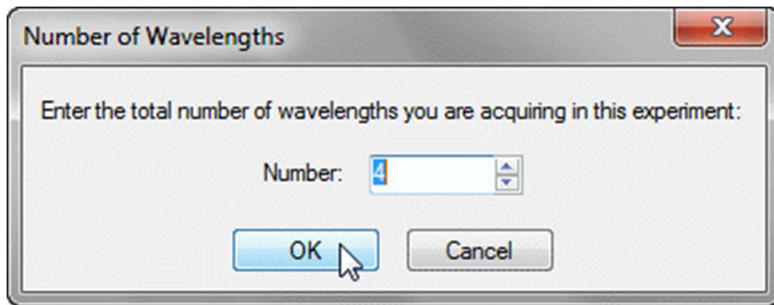Also enable the option to "Prevent asynchronous hardware moves".

The Start of Plate journal is for specifying the offsets for each wavelength.
As it is currently configured, this will bring up interactive prompts at the start of plate:

Number of Wavelengths

Enter the total number of wavelengths you are acquiring in this experiment:

Number: 4

[OK] [Cancel]

Specify Wavelength 1 Offset

Enter focus offset for wavelength 1 (in um):

Number: 0.2

[OK] [Cancel]

Specify Wavelength 2 Offset

Enter focus offset for wavelength 2 (in um):

Number: -1.3

[OK] [Cancel]

Etc.

Alternatively, you can specify the offsets directly in the journal. Set "InteractiveSetup" to "N" to skip the prompts.
You will need to go to the Journal Editor and select the specific journal to make changes (Control menu > Journal > Edit Journal or Journal menu > Edit Journal). Make sure to Save the modified journal.

Journal Editor

File Edit

Builtin Functions | Recorded Journals | Actions

Assign To Variable
Comment Field
Delete Variable
Exit Playback
For/Next
If/Then/Else
Pause Playback
Prompt User
Trace
While

Journal:

C:\Users\Paula.Gedraitis\Documents\Journals\Acquisition\LAFOffsets_StartOfPlate.JNL

Functions | Descriptions ← **1. Click on the Descriptions tab**

This journal prompts the user at the beginning of a plate to specify the offsets they want for channels 2 and higher once focus is found for channel 1 in their acquisition. Enable this journal when at Start of Plate, use in conjunction with the actual autofocus journal
Originally written by Vicki Racicot, significantly modified by Paula 2016-07-28

Non-interactive version - enter the offsets as variables here. Set InteractiveSetup to "N".
Set offset to 0 for wavelengths you are not using. Click on the Descriptions tab to view the actual settings.
W1Offset is the offset from the laser autofocus position

InteractiveSetup = "Y"
W1Offset = 0
W2Offset = 0
W3Offset = 0   **2. Click on specific setting to select**
W4Offset = 0
W5Offset = 0
W6Offset = 0
W7Offset = 0

Assign To Variable

Variable:

InteractiveSetup

Expression:

"Y" ← **3. Modify expression as appropriate**

☐ Disable   **4. Save modified journal**

Save   Run Journal   Exit

You will also want to edit the laser autofocus journal itself to customize the focus parameters.
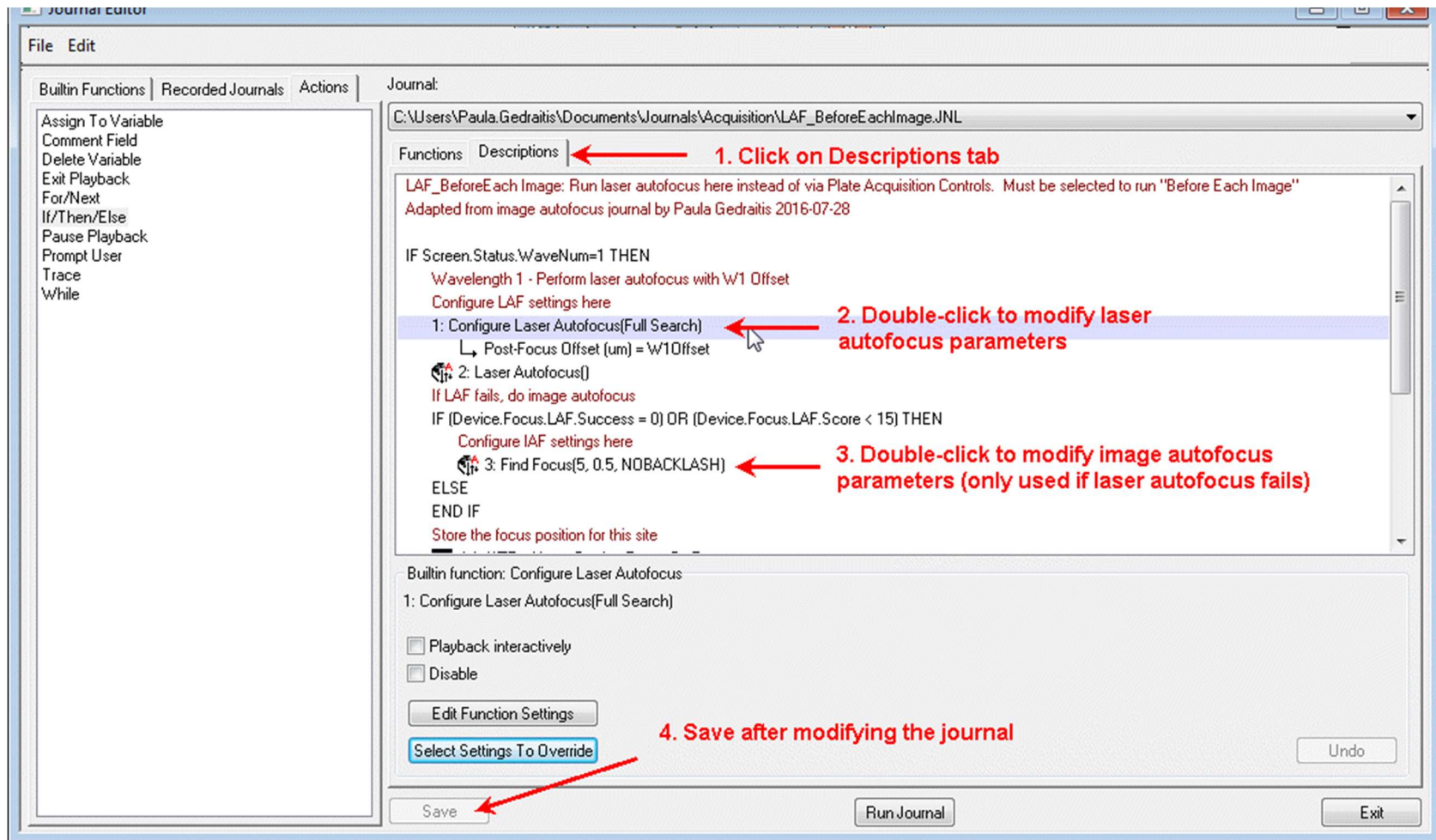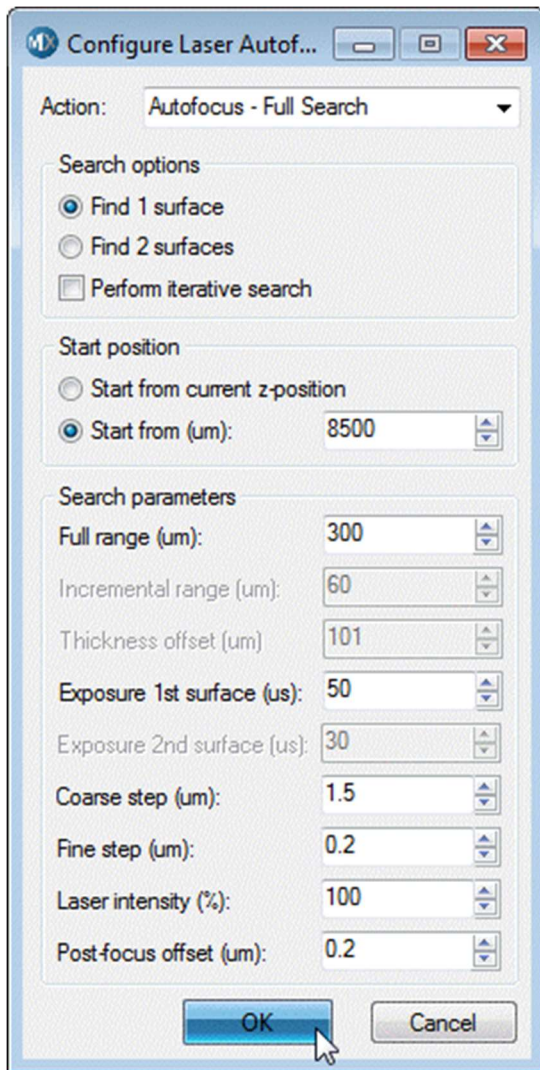
Step 1 is to configure the Laser Autofocus parameters.

Here are differences between this and the plate acquisition laser autofocus settings:
- Set to find 1 or 2 surfaces
- Do simple search or iterative search
- Start from specified Z position, current position, or you can override start position with a variable

- Set the full search range here to prevent crashing
- Minimum exposure time is 10 us. If your signal is bright (doubtful with your 100x objectives), you will have to turn down the laser intensity %
- Laser intensity is normally 100%. Only decrease this if signal is too bright even at 10 us.
- Only one exposure time is attempted in the journal, as opposed to plate acquisition.

Also, the journal is overwriting the 'Post-focus offset' with the W1Offset that is specified in the StartOfPlate journal. It would be simpler to specify all the offsets in one location.

Configure Laser Autof...

Action: Autofocus - Full Search ▼

Search options
- ⦿ Find 1 surface
- ◯ Find 2 surfaces
- ☐ Perform iterative search

Start position
- ◯ Start from current z-position
- ⦿ Start from (um): 8500

Search parameters

Full range (um):	300
Incremental range (um):	60
Thickness offset (um)	101
Exposure 1st surface (us):	50
Exposure 2nd surface (us):	30
Coarse step (um):	1.5
Fine step (um):	0.2
Laser intensity (%):	100
Post-focus offset (um):	0.2

OK   **Cancel**

Step 2 performs the actual laser autofocus.

Step 3 is to run an image-based autofocus, in the case that the laser autofocus fails (LAF success is 0 or LAF focus score is low).
You can configure the settings here.

Because the stage is voice coil driven, there is no backlash so do not enable the "Backlash compensation" option.
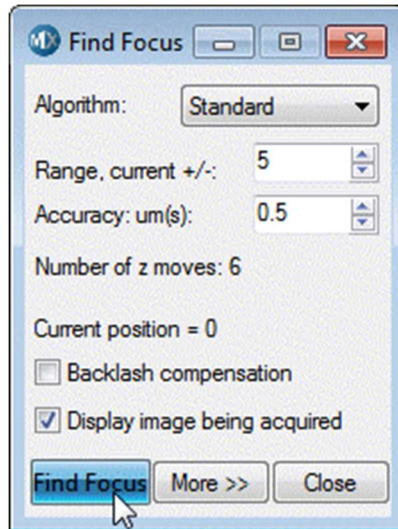
Again, save the journal after making modifications.

The journals can be further customized if needed (for example try LAF again with higher exposure time if it fails the first time).