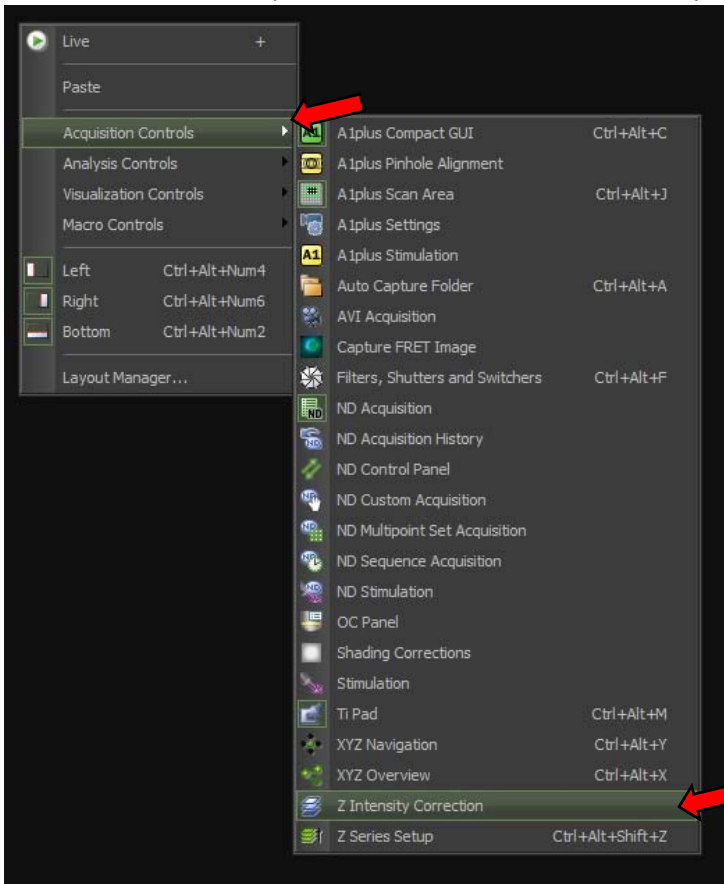


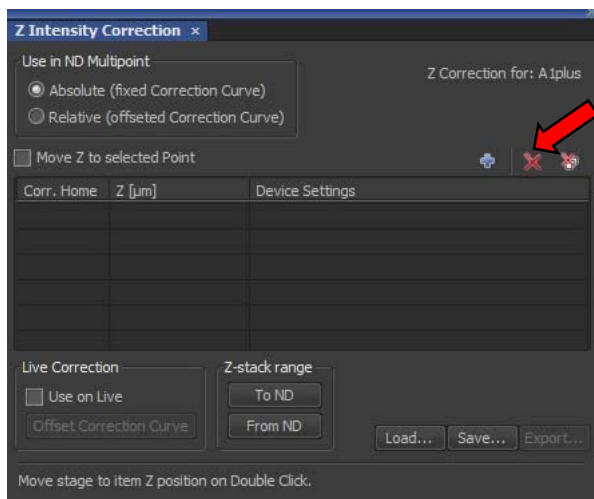
UConn Advanced Light Microscopy Facility Technical Note: Linear Z Compensation on the Nikon A1R

Chris O'Connell, Facility Scientist

- Set up Optical Configuration for the fluorescent probes in the sample
- Open the linear z compensation dialogue by right clicking on the background of NIS Elements to bring up the menu. Under “Acquisition Controls” select “Z Intensity Correction.”



- This will open up a new window

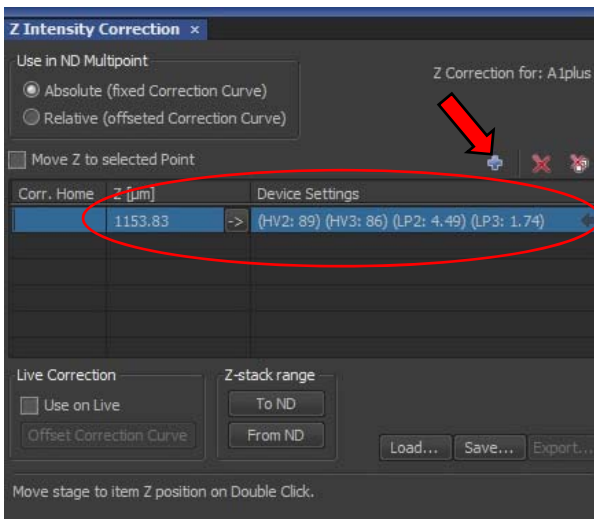


Select any old settings and click here to remove them.

- If there are any previous settings in the table, select them and click the red “X” to delete.
- Go to the bottom (near the coverslip) position of your z stack. Adjust the laser power and/or detector gain to appropriate levels. In this example there are two channels, 488 (green) nm and 558 nm (red).



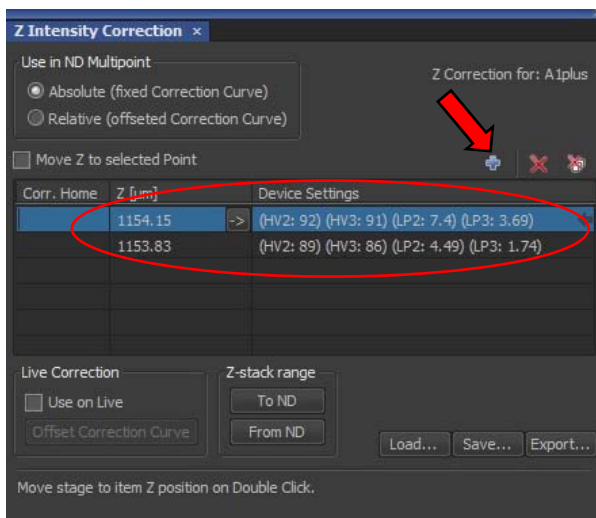
- Stop any live scanning and click the blue “+” button in the Z Intensity Correction window to add the settings for this bottom z position. The z position, laser power, and gain for all active channels at this z are now displayed in the z compensation window.



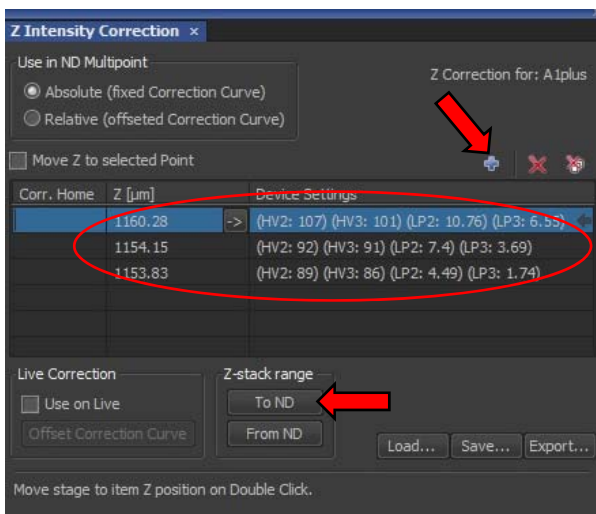
- As you focus up into the specimen the signal will likely decrease. Move deeper to an intermediate point in your z range. Adjust laser power and/or gain to higher settings to compensate for the decreased signal.

Note: You can just include the top and bottom positions, but one or more intermediate points may improve the result of intensity compensation.

- Stop scanning and click “Add” to include the settings for the current channel’s z position. You will now see the information for both the first and second z positions.

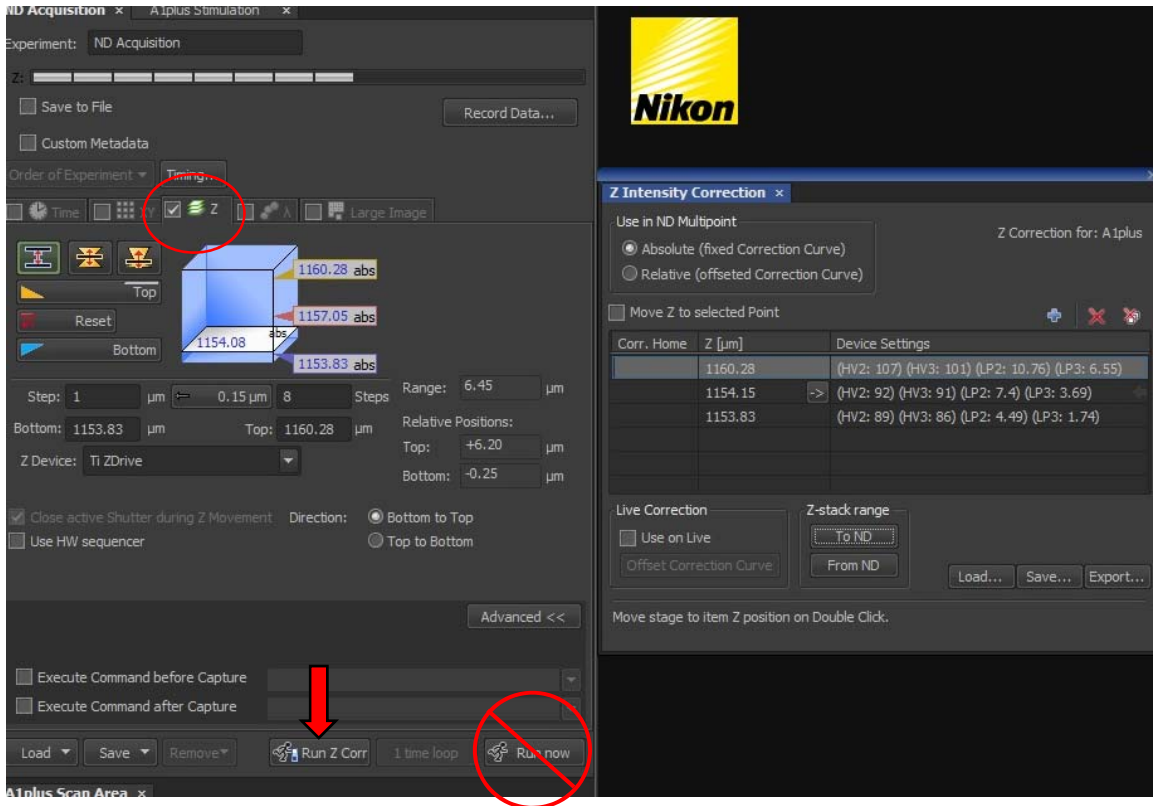


- If your specimen is very thick, you can include more than one intermediate z position.
- Move to the top of the z range you want to collect. Increase laser power and/or gain of the channels to compensate for further decreased signal.
- Stop scanning and click “Add” to include the settings for all channels at this final z position. You will now see the information for all z positions.

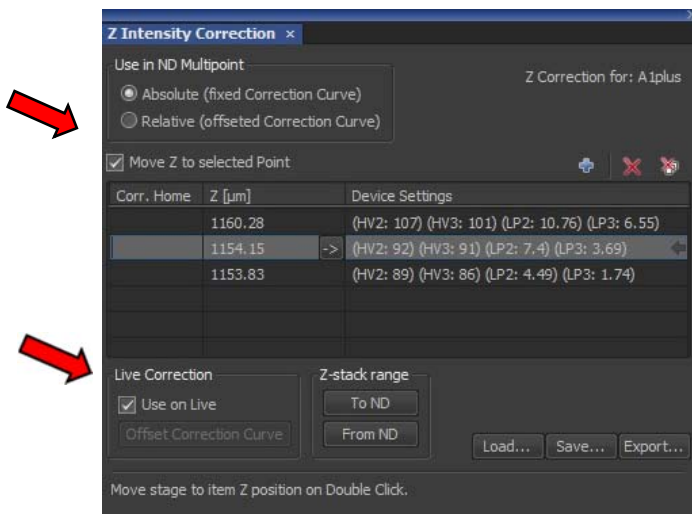


- Click “To ND” to send the z range information over to the ND Acquisition window. Make sure you have the “Z” tab checked before you run the experiment and set the step size to your desired interval.

- Click “Run Z Corr” in the ND Acquisition window to run the z stack and apply the corrections.
NOTE: Do not click “Run now” which is the usual method of starting. If you do this, the corrections will not be applied. You must use the “Run Z Corr” button.



Notes:



- Check “Move to selected Point” to move to any previously recorded z position when you select it
- Check “Use on Live” to apply the settings you specified at each z position when you reselect a z position that is recorded.
- Intermediate points can be added or removed by selecting the position and using the appropriate buttons in the z compensation dialogue.
- Avoid ramping up the power and gain too high. This will help minimize bleaching and image noise. It helps if your staining is bright so you can begin at low settings and have room to increase without going to extremes.