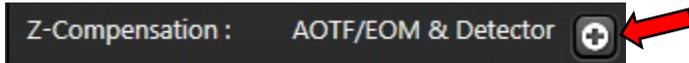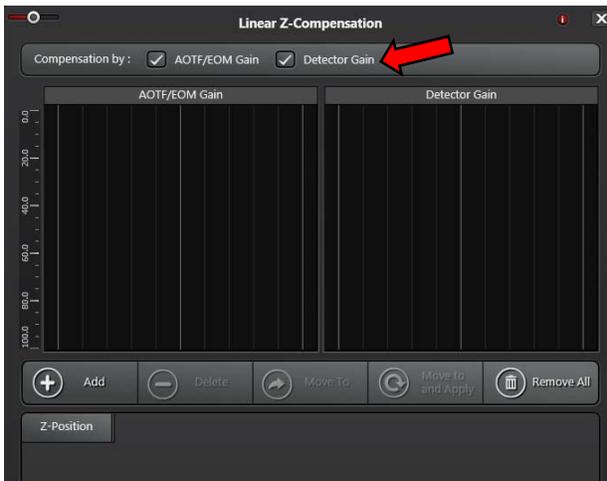# UConn Advanced Light Microscopy Facility Technical Note:
Linear Z Compensation on the Leica SP8

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- Set up imaging sequences to create separate channels
- Open the linear z compensation dialogue by clicking on the "+" button in the Z-Stack window


- This will open up a new dialogue

Check whether you want to ramp up laser power (AOTF Gain), detector gain, or both during acquisition. Note, if you collect sequences by line you can only compensate with laser power. If you collect by frame or by stack, both options are available.

- Go to the bottom (near the coverslip) position of your z stack. Click on your first sequence and adjust the laser power and/or gain. In this example there are two sequences (channels), 405 and 488 nm.
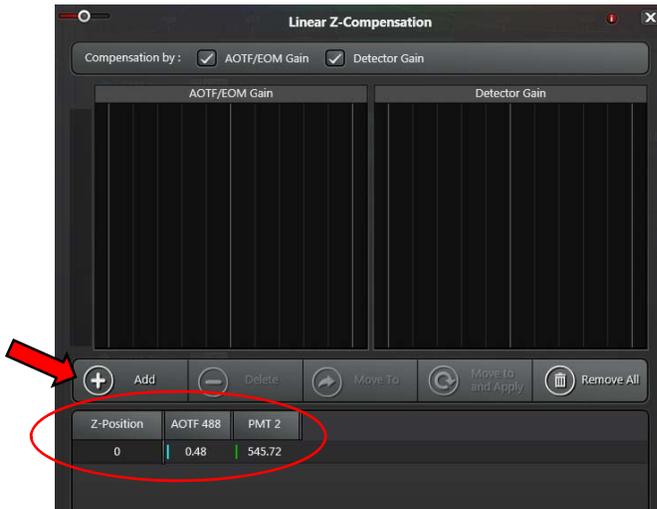

- Click "Begin" in the Z-Stack dialogue to set the bottom of the range. The laser power and gain for this channel at this z are now displayed in the z compensation window.
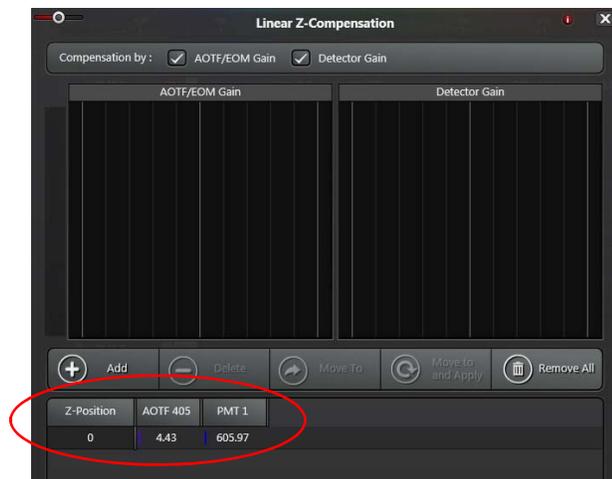
- Select the second sequence and set laser power and gain for channel 2.

- When this is done, click "Add" in the Z compensation window to add the information for channel 2 at this z position.


- Select and repeat this for each additional sequence (channel) at the bottom z position.

- As you move 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 instensity compensation.

- 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 for this channel.

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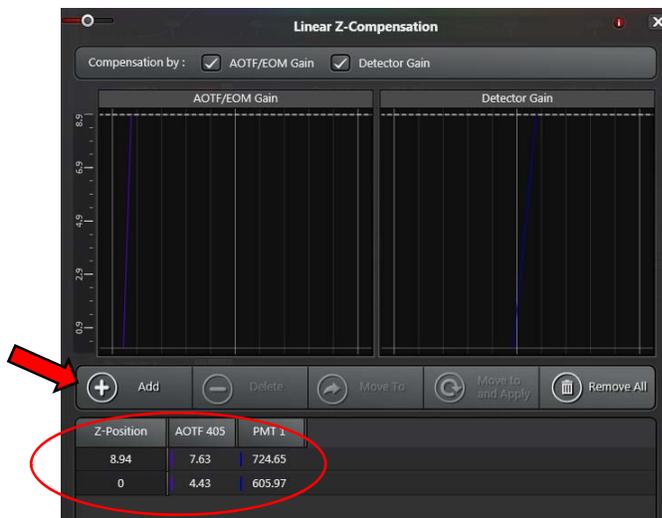
- Click on each of the other sequences. Adjust the laser power and gain and click "Add" while in each sequence to include the setting information for all channels at this intermediate z position.

- If your specimen is very thick, you can include more than one intermediate z positions.

- Move to the top of the z range you want to collect. Increase laser power and/or gain of the channel you are in to compensate for further decreased signal.

- Click "End" in the Z-Stack window to include the settings for the current sequence at this final z position. You will now see the information for all z positions for this channel.
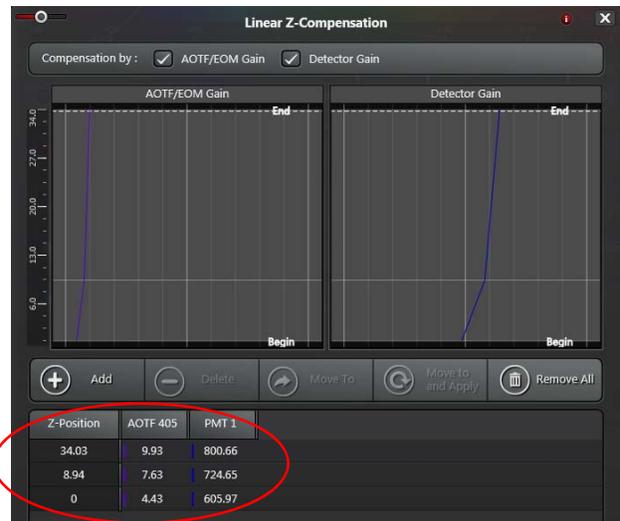
- Select the next sequence and adjust its laser power and gain.

- Click "Add" to include the settings for this z position. You will now see the information at all z positions for this channel.
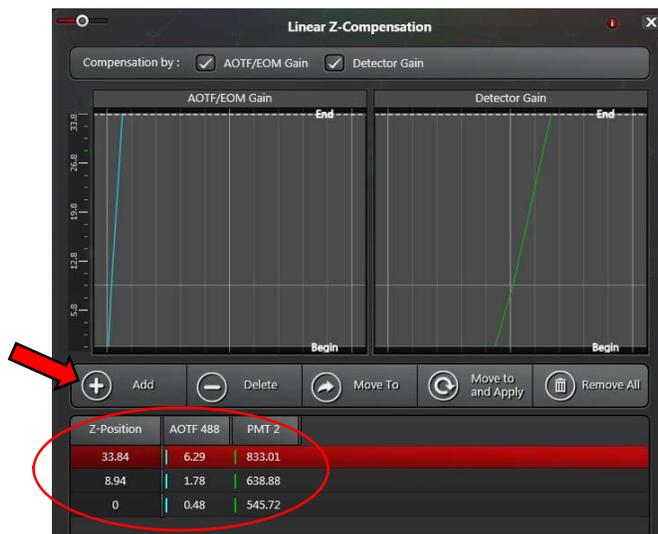
- Repeat for any additional sequences.

- Click "Start" to collect the z stack with the compensation.

Notes:

- Click "Move to and Apply" to move to any previously recorded z position and apply the settings you specified.

- Intermediate points can be added or removed by selecting the position and using the appropriate buttons in the z compensation dialogue.

- The plots show a graph of the linear compensation between points. Horizontal lines represent intermediate positions.

- 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.

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