

Confocal Application Notes

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Z-stack with compensation of intensity loss in Z

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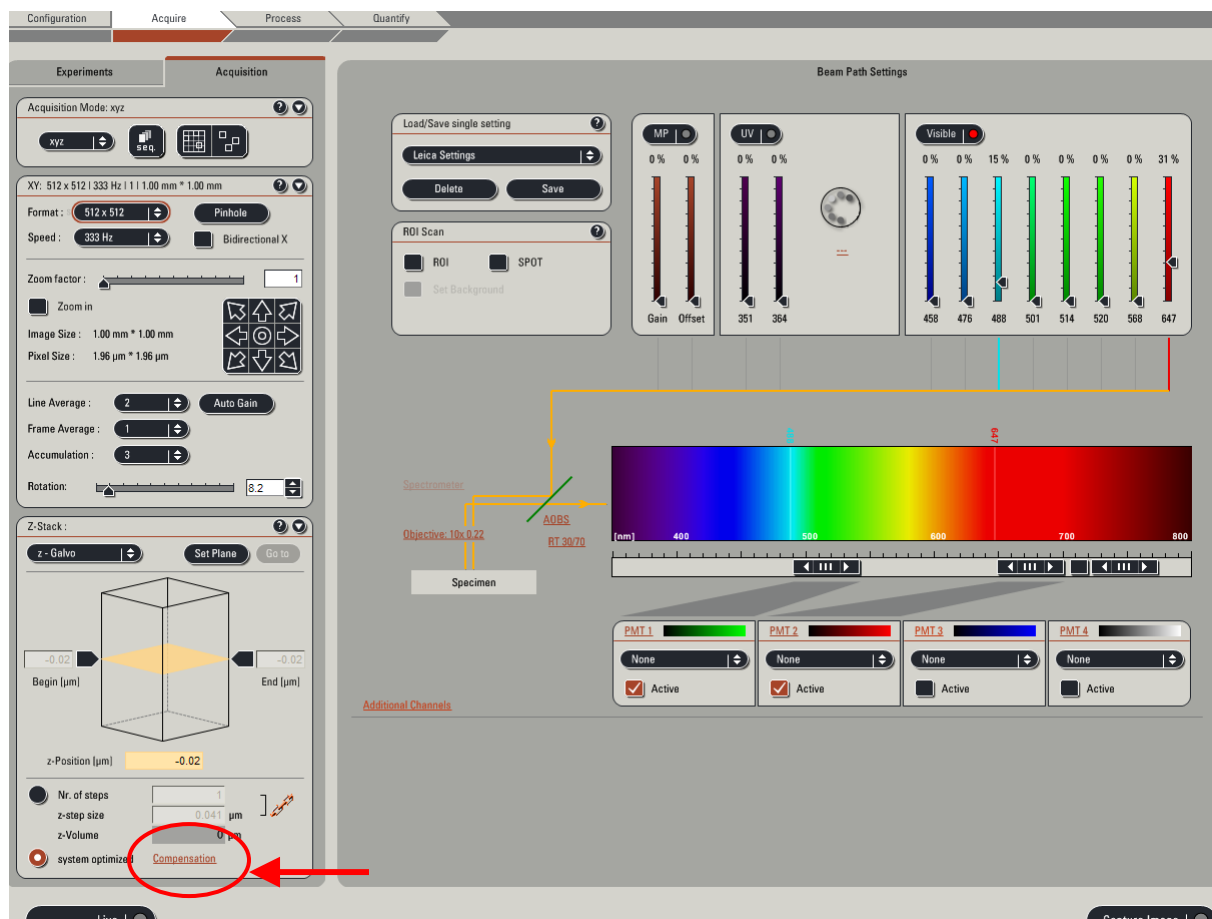
The compensation tool is used when your z-stack displays different intensities inside the stack. This tool will allow you to adjust the intensity as needed either by adjusting the laser power (**linear compensation by AOTF**), or the gain (**linear compensation by PMT**), or both (**linear compensation by AOTF and PMT**).

You will need to setup your system to compensate between different z-positions in your stack, allowing you to enhance (or decrease) linearly your power between these points.

In this example, we describe compensation of intensity loss by using the linear compensation by PMT (gain). You will just have to adjust the intensity by using the Smart gain knob on your control panel. If you need to use the compensation by AOTF, then the intensity can be adjusted using the laser slider in the Acquisition window.

The compensation tool is accessible from the acquisition screen (as shown below).

A. You should be in xyz mode (or xyzt) in order to have the Z-stack window appear. Activate the compensation tool in the Z-stack window.



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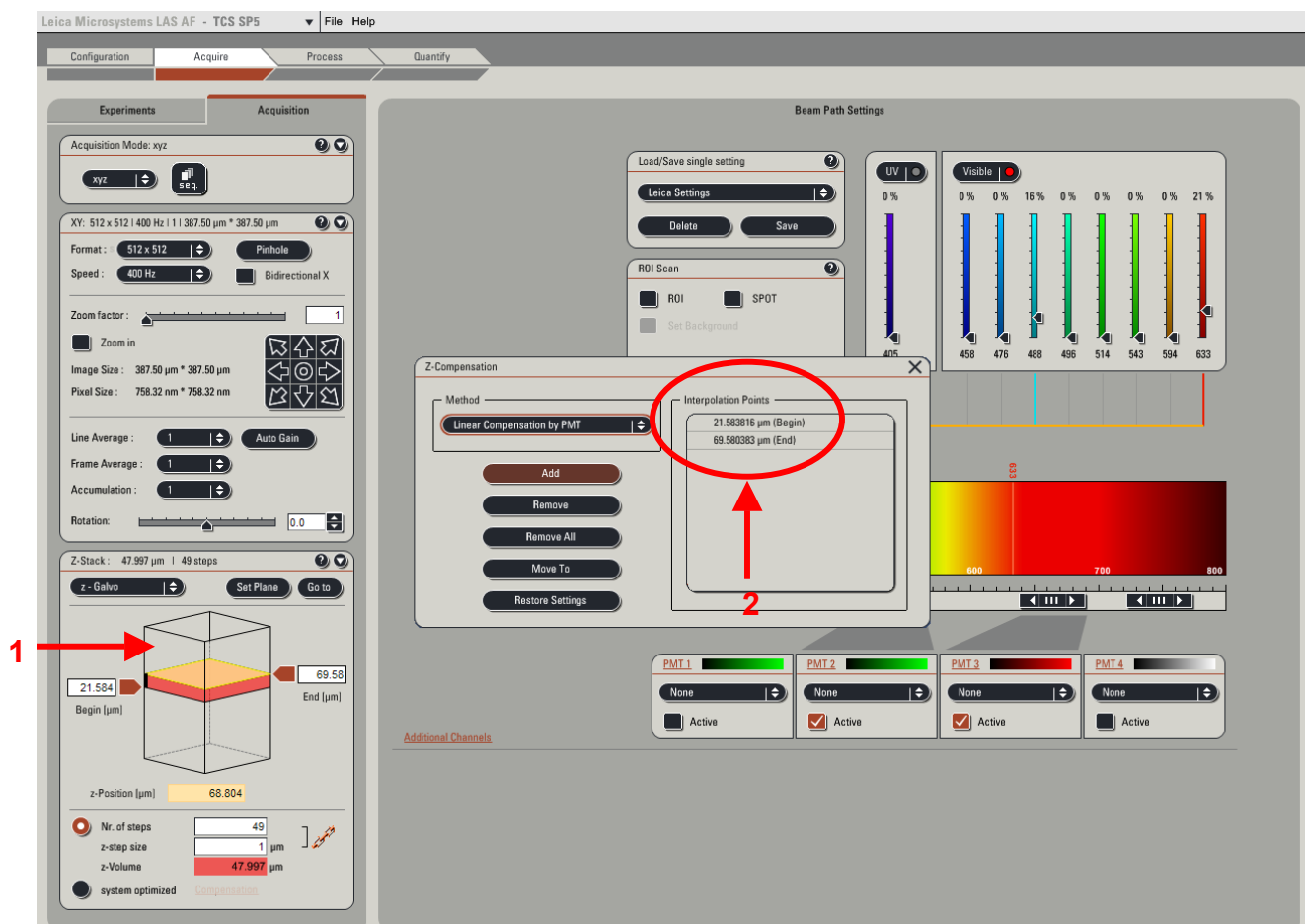
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- B.** Setup your Begin and End points for your Z-stack (1). The interpolation points noted (Begin) and (End) will appear in the Z-Compensation window (2).



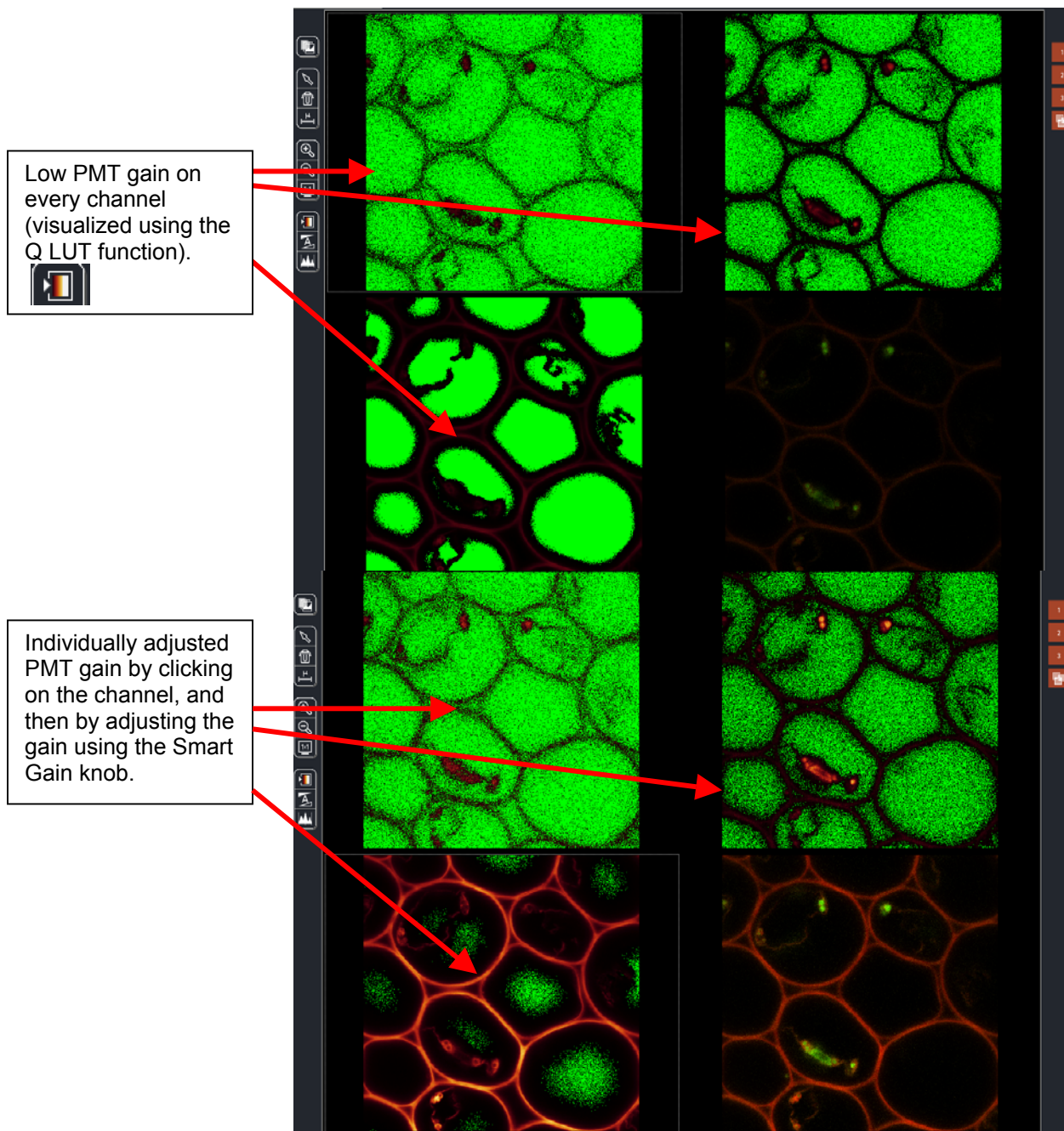
- C.** Click on **Live**  and move along your z-stack and identify where the intensity is decreasing.

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C. When the position is found, adjust your PMT gain.

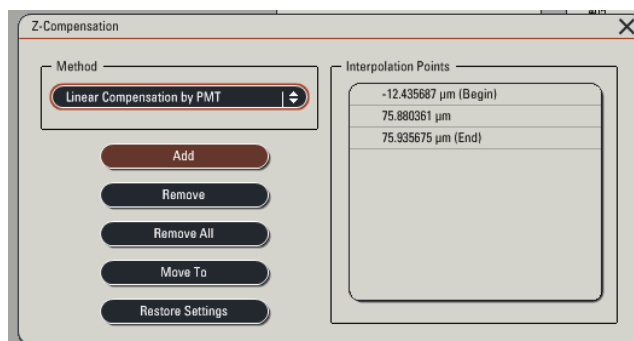


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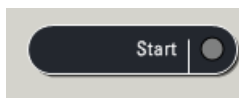
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- D. Click **Add** in the Compensation window. This position as well as the new gain intensity will be stored and used to extrapolate a linear compensation between the different points. You can add as many points as needed.



- E. Click on **Start** to begin your z-Stack with Compensation.



You can follow the linear compensation by looking at the Gain intensity number on the control panel at the top of your Smart gain knob. This should gradually enhance (or decrease depending on your settings) until it reaches its added point(s) between begin and end interpolation points.