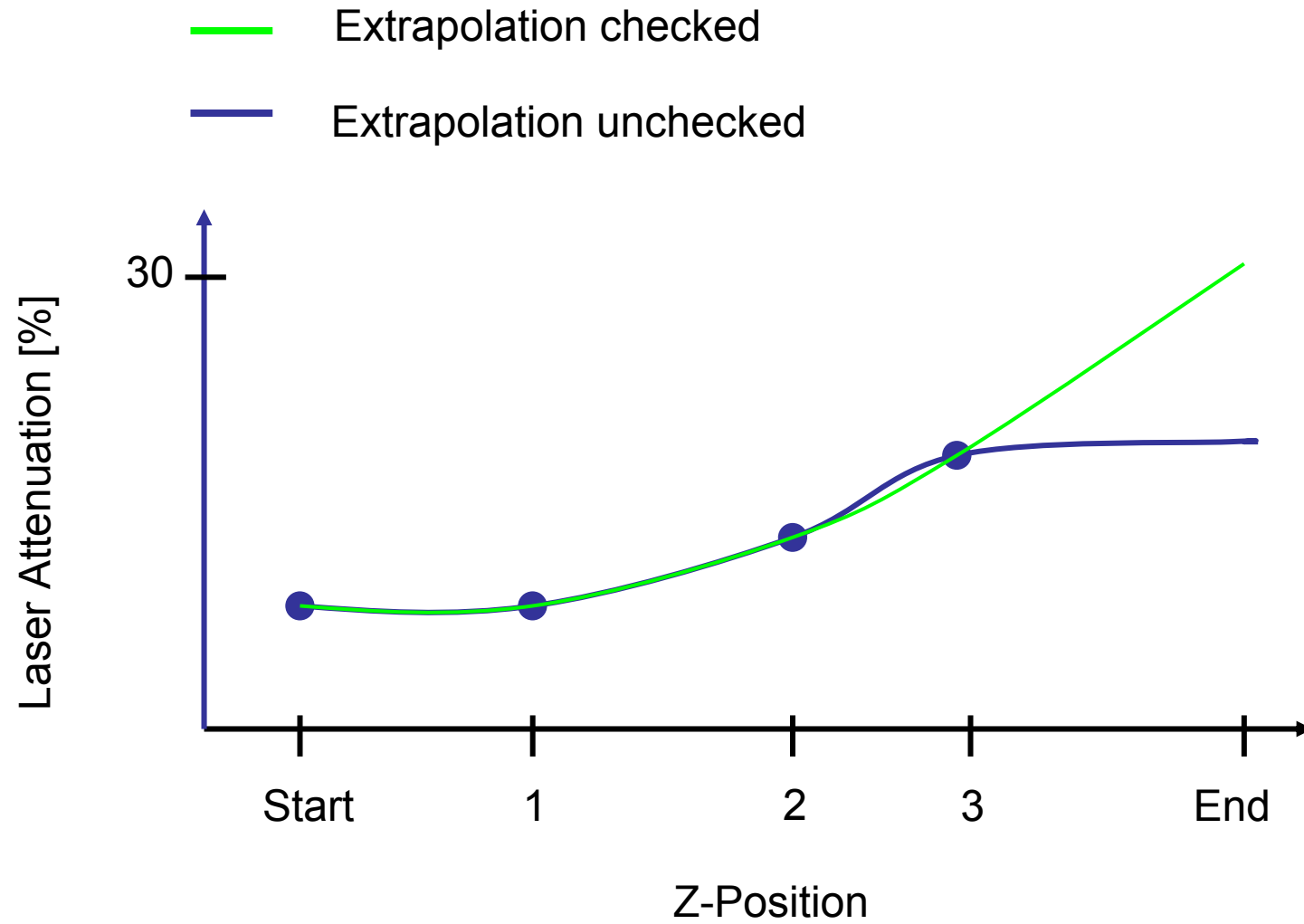


Auto Z-Brightness Correction

Auto Z Brightness Correction (Fig. 81) allows setting of different values for the scan parameters **Detector Gain**, **Ampl. Offset**, **Ampl. Gain** and **Laser Attenuation** of up to 10 freely selectable slices within a Z-Stack. During the scan procedure, the values of these parameters are automatically linearly interpolated between the neighboring positions within the Z-Stack. This function can be activated and deactivated with the check box **Use Correction**. **Add** takes the above mentioned parameters and correlates them with the current Focus Position which is then added as a **Position** in the list (for example the first slice of a stack). Adding a new position with a defined set of channel parameters cannot be done while scanning. The positions don't need to be listed in a specific order, the program will sort the values automatically in the background. **Move to** moves the actual focus to the highlighted **Position** in the list without scanning. Starting a **Continuous** scan then allows setting or correcting the parameters and storing them again hitting **Add**. Highlighted positions can be removed (**Remove**) or the whole list can be removed (**Remove all**). The interpolation between the **Positions** in the list can be extrapolated (check the box next to **Extrapolate**) to the actual first and last slice of a Z-Stack if those are not part of the range of the **Positions** in the list.

When **Enable Test** is active, scanning at single positions of the stack or while focusing through the stack is done using the settings of the **Position** list or the interpolated values calculated for the respective focus position. This allows controlling the settings before starting the actual acquisition of the stack.

Example for laser attenuation over z



How to use the Z Correction with LSM on Axio Examiner (upright, fixed stage microscope)

1. Focus onto the surface of the sample; this position is the **LAST** slice of the Z-stack as the acquisition of the whole stack is always performed with the objective being moved against gravity (so from inside the sample up to the surface).



2. Set this surface position to “0” in the **Focus** tool
3. Set this position as the last position of the Z- stack clicking “**Set Last**” in the **Z-Stack** tool

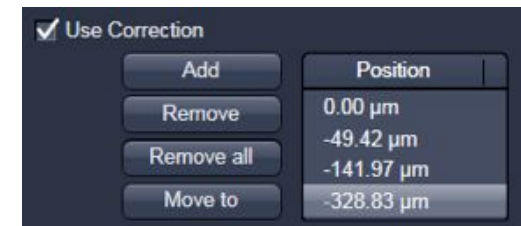


How to use the Z Correction with LSM on Axio Examiner (upright, fixed stage microscope)

4. Focus through the sample and adjust the parameters for the image acquisition using laser power, detector gain, offset or digital gain. Store the adjusted parameters for each position using the Correction function on the **Z-Stack** tool.

Note: Between the values of neighboring positions a spline interpolation will be performed; if the calculated values between two positions differ too much from a linear interpolation, add one additional position in between; Extrapolate will extend the interpolation to the borders of the stack if the correction positions are not defined for these.

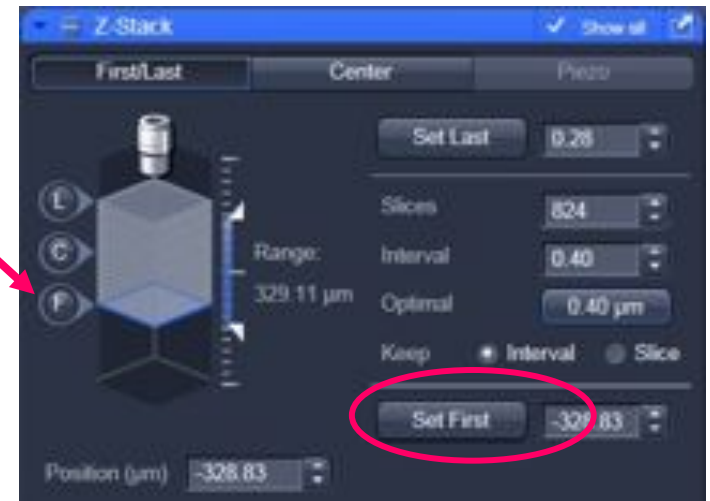
Enable test will adjust the parameters also when focusing manually and will show the updated values when the stack is actually acquired.



How to use the Z Correction with LSM on Axio Examiner (upright, fixed stage microscope)

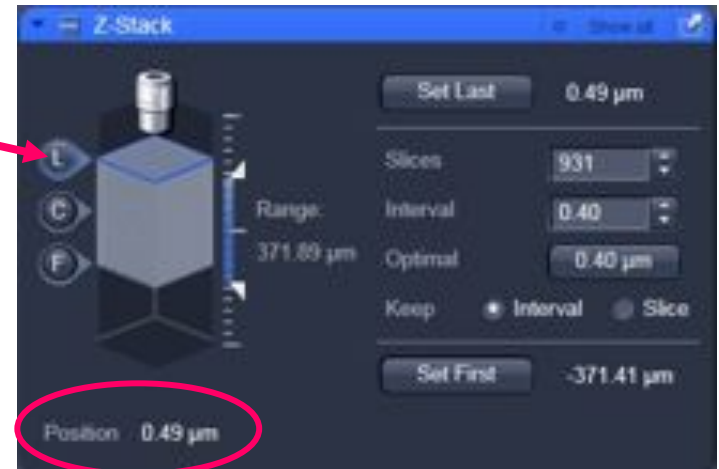
5. At the position which should be the end of the stack, mark this position with “**Set First**” in the **Z-stack** tool

Note: When adjusting the Optimal distance, the slice positions are slightly adjusted to match the number of slices and the distance in between. Check the manual on a more detailed description for these settings.



How to use the Z Correction with LSM on Axio Examiner (upright, fixed stage microscope)

6. Before the acquisition of the Z stack, **move back to the surface of the sample or the last slice of the Z-stack!** When at Z position “0” (or slightly adjusted; and which is the surface of the sample or the last slice of the stack) start the acquisition of the Z-stack.
When the stack is completed, save the image.



How to use the Z Correction with LSM on Axio Examiner (upright, fixed stage microscope)

7. For the next sample, focus to the surface of the sample and set the position to “0”. Stay at this position when reusing the image parameters of the stored image.

Note: The position of the stack will always be acquired in relation to the current position as otherwise severe damage to the sample could occur as the system does not know the absolute Z position as this changes when resetting a position.

The correction parameters are linked to absolute positions, so when reusing the parameters, **the relative position of the new sample has to be identical to the absolute position of the sample before**. To make that easy always mark the last slice on the surface as position “0” and perform the acquisition and the reuse from this position.

