## Imaging and Microscopy Center Biomedical Core Facility



## מרכז דימות ומיקרוסקופיה מרכז תשתיות ביורפואי

הפקולטה לרפואה ע"ש רות וברוך רפפורט הטכניון - מכון טכנולוגי לישראל

The Ruth & Bruce Rappaport Faculty of Medicine Technion-Israel Institute of Technology

2015-10-29 Auto Z Brightness Correction 20131121.docx



## To correct Z-stack brightness for thick specimens



Set optimal scanning parameters for each channel.

Set low resolution fast settings (256x256pixels, speed 8, average 1)

Set Z-stack limits with optimal interval (Z-Stack set first, set last).

Press Start Experiment

In Gallery view (left tabs), Gallery tab (bottom tabs) with Show Text checked, note the last satisfactorily bright slice (in this example 30.42)

*Move to first slice:* in <mark>Z-Stack</mark> <double-click> 🗊

In Z-stack, Auto Z Brightness Correction (bottom) check Use Correction

Add the current position+settings to correction list:

In Z-stack, Auto Z Brightness Correction (bottom) press Add



In Focus you cannot enter a value at Z-position to move the stage to a position, but you can enter a value at Step Size and then use the arrows next to Z-position to move the stage to the right Z. Do not press the arrows repetitively and fast, because the movement will not be precise.

In Focus change Step Size to the value of the last satisfactory slice.

Move to the last slice you wish to leave at original settings.

In Focus press once the Z-position up arrow

Add the current position+settings to the correction list:

In <mark>Z-stack</mark>, Auto Z Brightness Correction (bottom) press Add

Move to last slice In Z-Stack <double-click> Do not use the stage to the loading position!!!

Change laser/gain/offset settings at the last slice

Add the current position+settings to correction list: In Z-stack, Auto Z Brightness Correction (bottom) press Add

Uncheck any checked options in Auto Z Brightness Correction

Prescan Z-stack in low resolution, fast settings

Change to acquisition settings (e.g., 1024x1024 pixels, speed 7, average 2)

Press > Start Experiment





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