#### Imaging and Microscopy Center Biomedical Core Facility



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

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

2014-03-31 how to do tiling of Z-stack in LSM 510 Zen 20140331.docx

# Tiling of Z-stack in LSM 510 Zen MTS

Optimize all channel parameters (pinhole diameter, laser intensity, gain, offset, rotation, zoom). Consider zooming in (at least 1.5) to eliminate shading problems.

Navigate to the center of your field of interest and decide on number of tiles.

To prescan until field is satisfactory:

Check Tile Scan. In <u>Channels</u> choose one channel to define the area. In <u>Acquisition Mode</u> set pixel size 256x256, scanning speed 8, no averaging.	✓ Z-Stack 21 Slices Time Series Bleaching ✓ Tile Scan 5 x 5 Tiles Positions Regions St	1.28 GB art Experiment
In Tile Scan set number of tiles. Press ► Start Experiment If you use Prescan overview image note the objective! Uncheck Tile Scan.	🔽 <b>::</b> Tile Scan	✓ Show all
Check <mark>Z-Stack</mark> . In <mark>Z-Stack</mark> set Z stack parameters: Interval, Set First, Set Last Press © to move to the middle slice	💌 🗐 Z-Stack	Show all
In Acquisition Mode set scanning parameters: Frame Size, Scan Speed, Scan Average	🕨 🛥 Acquisition Mode	✓ Show all
In Channels check all channels.	Channels	✓ Show all
Save this configuration (channel, scanning, Z settings) under your name. Please overwrite each time.	Ocular Acquisition Processing Maintain   Configuration Melia for tile macro Melia Maintain	

#### In Multi Time Series PLUS

In the Saving Tab Enter a Base File Name for your images. Select Image Folder where your images will be saved. Check Keep Final Image Open and Single File Output. Do not check Save Final Image (uses an inferior stitching algorithm). In the Temporary File(s) segment below: Check Single Original Images Folder and Check In the Final File(s) Folder.

In the Acquisition Tab load your Scan Configuration. Check ZStack Press Marked Z - Middle of Z Stack



Start	Stoo Finish End Block Pause Update		
Saving	Parameters: Block 1, Location 1		
Acquisition	Scan Configuration: Melia for tile macro 🗸 FindBC ?		
Timing	Load Conf. Save Conf. Pause		
Location	No of Time Points within Block		
Tile			
Grid			
Z List	Marked Z · Middle Of the Z Stack Marked Z · Top of the Z Stack		
Autofocus	Apply Acquisition Parameters To:		
Bleach	Apply to All locations Apply to All Blocks All Blocks and Locations		
Blocks	Apply to All Locations Apply to All Dices All processing Locations		
Options	Select Parameters to Apply to All Locations and/or to All Blocks		
Add NEXT	Walf for Trigger File └ Walf for Trigger File └ Trigger In: to Start/Pause Trigger Out at Block Start Trigger Out at Block End None ▼		
	BlkNo Groups G.Rep. BkDelay ZStack AutoF Bleach Scans Intvl		
List of Blocks	1 1 1 0 Yes No No 1 0 🔽		
List of Locations	1: X=-17137.75 Y=2394.50 Z=-34.25 T1(2X2):1,1		
	Recipe Summary		
Move To Loc.	No. of Temporary Files = 4, No. of Blocks = 1		
Next Loc.	Sum of Block Time Delays = 0.00 seconds		
Replace XYZ	T1 2X2		
Replace XY	Timeline		
Replace Z			
Center	Ready		



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## In the Location Tab press Current Location

In the Tile Tab set the number of tiles you decided to collect. *In this order:* Set the Overlap value to 15%. Click Create Tile Locations Check Create Mosaic Image Uncheck Simple Tiled Image.

Multi Time Ser	es PLUS, ZEN 2009-2010-25
Start	Stop Finish End Block Pause Update Helo Close
Saving	
Acquisition	Current Location Multiple Locations (Motorized Stage)
Timing	Move Focus to Load Position between Locations ?
Location	Apply Shift of XYZ coordinates on Each Consecutive Move to Location
Tile	
Grid	
Z List	
Autofocus	
Bleach	Import and Export Locations
Blocks	Expert or
Options	MTS ZEN Mark ZEN Locs. Import Export to Import Location Location from ZEN ZEN to/from
	Map Map Clear ZEN Locs.
	Edit Location List
Add NEXT	Remove Location Insert Location Add Location Clear All
	C Do Not Update ZEN Marked List when Add Next
	Scan All Locations Scan Current Location Scan From Current Location
	BlkNo Groups G.Rep. BkDelay ZStack AutoF Bleach Scans Intvl
List of Blocks	1 1 1 0 Yes No No 1 0 🗸
List of	1: X=-20719.50 Y=3787.00 Z=-43.10 T1(2X2):1,1
Locations	Paoine Summan
	No. of Temperany Files = 1. No. of Pleake = 1
Move To Loc.	No. of Locations = 1, No. of Z Stacks = 1
Next Loc.	Sum of Block Time Delays = 0.00 seconds
Replace XYZ	
Replace XY	Timeline
Replace Z	
Center	Ready
Expert Mode	Z=-43.10 Current Pos: X=-21123.75 Y=4191.25
t.	

Multi Time Ser	ies PLUS, ZEN 2009-2010-25
Start Start	Stop Finish End Block Pause Update Heip Close
Saving	Tile Numers 2 V 2 V a
Acquisition	Single Step XES Upper
Timing	Size Area Size X=1710.00 µm Y=1710.00 µm Start New Tile
Location	Mark Up Left Mark Up Right Show, Select and Move to Locations
Tile	Mark Down Left Mark Down Right
Grid	Scan Configuration:
Z List	Melia for tile macro 👻 Set
Autofocus	Load Conf. Save Conf.
Bleach	X Correction 0
Blocks	Clear Clear
Options	Y Correction 10 y µm 2 min
	Overlap 15 1
	Create, Add, Remove Tile Arrays
Add NEXT	reate Tile Locations Add Tile Locations
	Create Multi Tiles Remove Last Tile Tile Utilities
1	
	Sottoning in Reverse Order Create Mosaic Image Simple filed Image
	BlkNo Groups G.Rep. BkDelay ZStack AutoF Bleach Scans Intvl
List of Blocks	1 1 1 0 Yes No No 1 0
List of	1: X=-20719.50 Y=3787.00 Z=-43.10
Locations	Pocino Summany
	No. of Tomporary Files = 4. No. of Blocks = 1
Move To Loc.j	No. of Locations = 4, No. of Z Stacks = 4
Next Loc.	Sum of Block Time Delays = 0.00 seconds
Replace XYZ	no me
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Replace Z	
	Ready
Center	
Contor	
Expert Mode	
Exheir worde	Z=-43.10 Current Pos: X=-21123.75 Y=4190.50
	4



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### In the Options Tab press Find Rotation



In Tile Scan Rotation press Calibrate. Wait a few seconds for it to perform the calibration (see button change below) Press Close

Skim through other tabs on the left to make sure there are no unwanted settings such as repetition, time, autofocus, bleach, block.

In Multi Time Series PLUS top row of buttons:

Press Update and press Start .

At the end of scanning (Ready) you will have two files: One with the original images, All\_[yourBaseName]\_Sum1.lsm which you can concatenate with any algorithm you choose and one, Tile\_Stitch\_All\_[yourBaseName]\_Sum1.lsm already concatenated with the Zen stitching algorithm. Save them!





To concatenate later: Macro  $\rightarrow$  Multi Time Series  $\rightarrow$  Tile Tab  $\rightarrow$  Tile Utilities $\rightarrow$ Create Tile



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