

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 **Channels** choose one channel to define the area.

In **Acquisition Mode** set pixel size 256x256, scanning speed 8, no averaging.



In **Tile Scan** set number of tiles.

Press **Start Experiment**

If you use Prescan overview image note the objective!

Uncheck **Tile Scan**.



Check **Z-Stack**.

In **Z-Stack** set Z stack parameters:

Interval, Set First, Set Last

Press **C** to move to the middle slice



In **Acquisition Mode** set scanning parameters:

Frame Size, Scan Speed, Scan Average

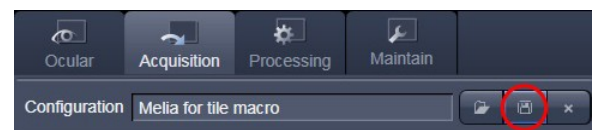


In **Channels** check all channels.



Save this configuration (channel, scanning, Z settings)
under your name.

Please overwrite each time.



In the **Macro** Menu choose **Multi Time Series**.



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.

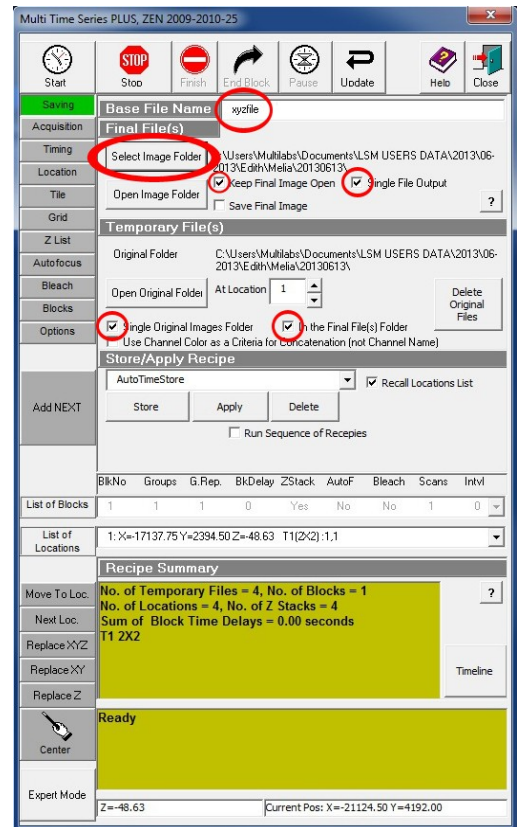
Check **Keep Final Image Open** and **Single File Output**.

Do not check **Save Final Image** (uses an inferior stitching algorithm).

In the **Temporary File(s)** segment below:

Check **Single Original Images Folder** and

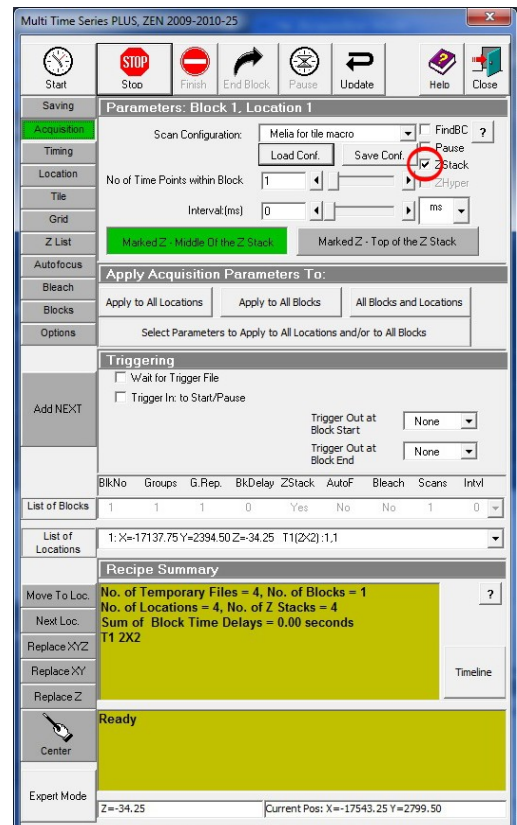
Check **In the Final File(s) Folder**.



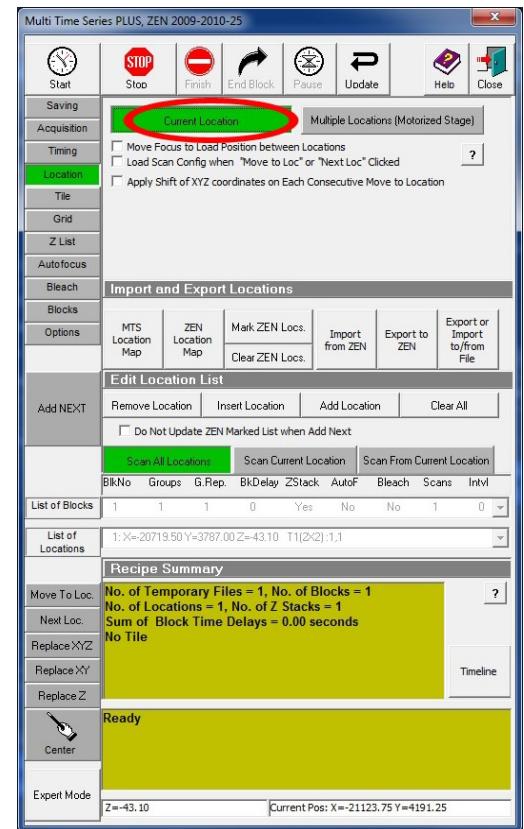
In the **Acquisition** Tab load your Scan Configuration.

Check **ZStack**

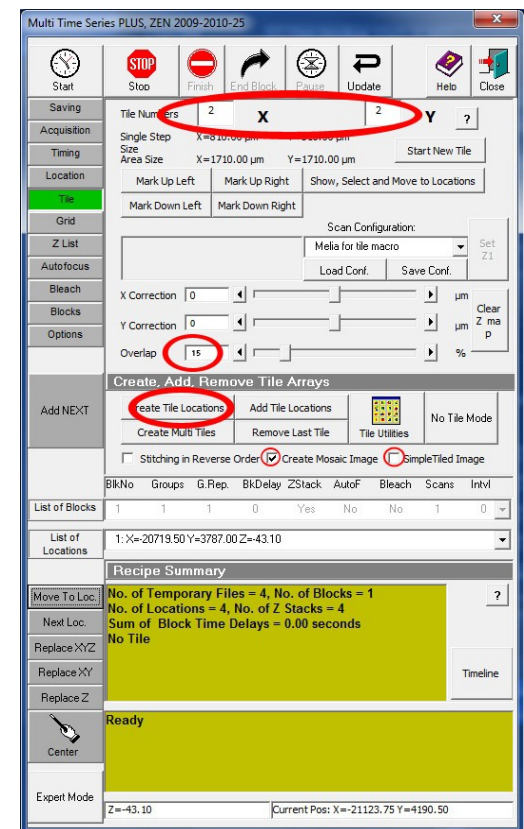
Press **Marked Z - Middle of Z Stack**



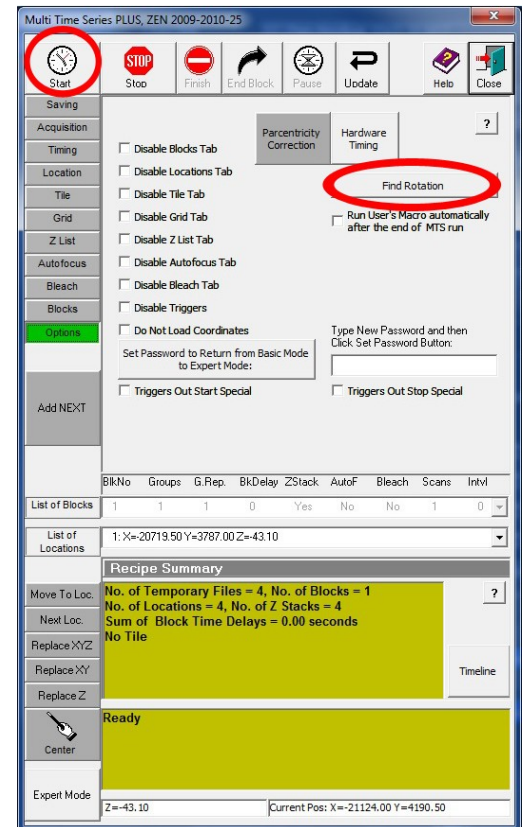
In the **Location** Tab press **Current Location**



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**.



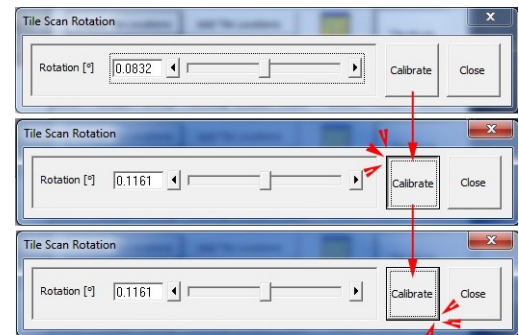
In the **Options** Tab press **Find Rotation**



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** → **Multi Time Series** → **Tile** Tab → **Tile Utilities** → **Create Tile**

