# Multi-time Tile Scans and Stitching

The Zeiss AIM software has the ability to make tile scans (put a number of images next to each other to create a large image). With the standard software you can only make 2D tile scans with a zoom of 1.

The Multi-time macro has extended capabilities.

- Every zoom setting can be used
- You can make 3D tile scans
- NEW you can stitch the tile scan with a correlation algorithm (Macro version 3.2t or later)

To setup the stitching algorithm for the first time proceed as follows:

1) Select the right lens
2) Put the convalaria slide on the microscope in the middle of the sample at the right focal plane
3) Make a nice image with a zoom of 1 and save your settings as a multi-track configuration under the name current
4) Make a database to store your results and make a folder to store your temporary results
5) Start the multi-time macro
6) Going from top to bottom of the multi-time macro
   a. Put all repetitions sliders to 1
   b. Deactivate autofocus
   c. Deactivate Bleach
   d. Select your Multi-track current configuration
   e. Put the number of scans to 1, if necessary activate ZStack
   f. Fill in a Base file name
   g. Select your Image Data Base
   h. Select your Tmp Image folder
7) Now press the Edit Location button
8) Select the Tile tab
   a. Fill in Tile Numbers 3 for X and 3 for Y
   b. Put Rotation Correction to 0
   c. Put the X Correction value to 0
   d. Put the Y Correction value to 0
   e. Put overlap to 0
   f. Press the Create Tile button
   g. Press Quit
9) Press the Start time button
10) The macro start to make the different images and stores the results in a Raw Image and a Tile Image
11) Check the Image to see if you have some overlap or your are missing bits
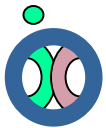
a. If you are missing bits go back to step 8 and fill in positive values for X and Y till you no longer miss bits (example 10x lens Microscope 1st floor X=35 Y=25)

　　　b. If you have a overlap go back to step 8 and fill in negative values for X and Y till you no longer have a overlap

12) Press edit location again and with the right X and Y Correction values put the Overlap value to 15%

13) Now you made the Raw data Image which will be used for the stitching

14) Open the Raw data Image

15) Press the Tile Time Series button in the multi-time macro

16) Normally the right tile numbers will be selected otherwise fill in the right Tile numbers

17) Put the Overlap at 15%

18) Measure in the Tile Scan how much the overlap of objects is (at the borders you should see structures twice) multiply this value by 2 and fill this in as Correlation Range (example 40 µm for a 10x lens). Don't forget to close this image because the Raw Image must be active for the following steps

19) Put the noise level at 20%

20) Press the Correlate button, a new Tile Image with a perfect stitching should be made. If the stitching doesn't look right go back to step 17 and change the values for overlap and Correlation range. Repeat until you find the perfect stitching parameters

21) Write down the X and Y correction and overlap percentage from the edit locations tile tab and write down the overlap and correlation range values the Tile Time series window (overlap and correlation range).

22) Put your own slide on the microscope and use the pre-recorded settings to make a perfect tile

If you change the zoom you should change the X, Y correction and Correlation range accordingly. Selecting more than 3x3 images should be no problem.
The correlation stitching can be done later on the offline work stations. It's important to use the right X, Y correction and 15% overlap when you make the images. Correlation stitching will not work when the overlap is less than 10%. The Noise level (%) is dependent on your sample.