**PREPARE TILES FOR STITCHING – 11/01/2021**

**Input:** Raw tiles saved by the CX7 high-content microscope (one folder per well, inside the raw folder).

**Output**: Processed tiles, stored in a folder called tiles inside the output well folder. If the user chose to threshold the tiles, the thresholds are stored in the thresholds folder inside tiles.

**Code:** The Fiji macro 1\_prepareTiles.ijm inside the folder  
CellContactNetwork > Fiji macros > HeLa processing.

This macro…

* Loops over the wells specified by the user. For each well, it…
* Combines the channels of each tile into one tile with multiple channels.
* Performs operations on specified channels if the user asked for it (possibilities are: enhance contract, equalise histogram, subtract background.)
* Sets an (automatic) threshold on the channels that the user asked for, using the thresholding method specified by the user. Thresholds per tile are combined into a multi-channel image and stored in thresholds.
* Renames the tiles (and the thresholds) so their names form a column-grid (instead of a spiral grid) so they can be used for stitching later on.

**How to run it:**

* Make a root folder (= output directory). Example:

M:/tnw/bn/dm/Shared/Lukas/BEP/Experiments/WKS024/M20

* Open the macro in Fiji and run.
* Fill in the parameters:

**Raw folder**: the directory where the well folders that contain the raw images are stored. Example: N:/tnw/bn/dm/Shared/Kasper/Experiments/WKS024/MFTP\_01234

**Root folder**: the output directory where the well folders that contain the processed images are stored. Example:  
M:/tnw/bn/dm/Shared/Lukas/BEP/Experiments/WKS024/M20

**Wells**: the wells of this experiment you want to prepare the tiles of, separated by commas.

**Magnification**: Magnification at which the images were made. The only possible values are 10, 20.

**Width/height of fused image**: Number of tiles that constitute the fused image, on one axis only. For example, if the fused image consists of 8x8=64 tiles, then fill in 8 here.

**Number of channels**: number of colour channels.

**Spiral clockwise**: The CX7 high content seems to vary in which direction it spirals (I haven’t yet found out why). You can check this by opening the first three tiles and see in what direction it spirals. Default is anti-clockwise, tick the box to make it clockwise.

**Thresholds and operations**: Specify on which channels you want to do these operations, or fill in “-” to do no operation at all. Channel numbers correspond to the numbers in the image names of the raw image.

**Sigma for Gaussian blur**: A Gaussian blur is applied before applying a threshold. Set the sigma for the Gaussian blur here.

**Rolling ball radius**: is only necessary if you want to subtract background. To try different rolling ball radii, use the Fiji operation Process > Subtract Background on an example tile.

**Downscale:** Tick the box if you want to reduce the pixel size of the image (in x and y) by 2.

**Display**: Tick the box if you want to see the images (is slower).

Graphical user interface, text, application

Description automatically generated