**Single bacteria identification**

1. The single bacteria pipeline uses as input 100%, non-compressed auramine subtracted (Cy3-FITC) images. Use **auramine\_aligned\_substracted image 100%.** Rename the image to base1\_c1\_ORG.tif and move it to folder called base1.
2. Tile the auramine image with **Sequencing\_v2\_Lite\_TilingBactSubtr.m** script.
3. Rename the tiled images (e.g with **Renamer)** so that all have a 3-digit number e-g Tile1->Tile001 so that they are placed in the correct order at cell profiler.
4. Use the Cell profiler 4.0.6 **210118 Filter Objects Test\_20 rules** script and add the **rules\_Mixed\_20.**txt file at the Filter objects modules.

(Rescale intensity input: 0.0-0.5, RI output: 0.0-1. Primary objects: Diameter 1-60. Threshold: Global/Otsu/3-classes/Foreground smoothing scale:1.3488, correction factor: 0.4, intensity, smoothing filter: 8, if too compartmentalized try RI input 0.008/0.014-0.5).

The output of the cell profiler is located within the tiled folder and the output files contain: i) all the tiles with identified objects (red), ii) all the tiles with classified objects (red and green), iii) 5 excel files: Bact, experiment, image and **FilterBacteria\_Single** and **FilterBacteria\_Cluster** that we use for further analysis.

1. Merge the FilterBacteria\_Single and FilterBacteria\_Cluster file (e.g. with Microsoft Powershell).
2. Use the **RelateBacteriaAndGene** matlab script with input i) decoded file from ISS (QT\_details), ii) bacteria segmentation file ->FilterBacteria file from Cell profiler (merged or not), iii) tilepos-> the tiled file after tiling of either ISS or BactTiled of Auramine images. The output is a ParentBacteria folder which contains a QT\_details\_ParentBact file and a ChildCounts file.