**Get software**

Get most recent version from:

https:// bitbucket.org/microscopeguerrillas/schnitzcells\_tans.git

Also get the repository:

https://bitbucket.org/microscopeguerrillas/schnitzcells\_tans\_extensions.git

In both, take your updates from the branch "Martijn\_develop". (I.e. merge "Martijn\_develop" into your own branch.)

**Run analysis**

The mother machine data is currently analyzed using the following workflow:

**Step 1**

Update your schnitzcells config file (e.g. Schnitzcells\_Config\_2017\_01\_12\_pos1.xlsx)

In Matlab, run

>> Schnitzcells\_masterscript

The workflow in this GUI is from top to bottom, with the option to do a preliminary analysis (left) and a full analysis (right). (If you open Schnitzcells\_masterscript.m for editing, there are comments explaining what the GUI does at each button click – the parameter runsections is set by the GUI, followed by a call to the script to run a certain section.)

"Select and load config file"

Choose the file you just made/edited.

Then start with preliminary analysis (analysis on a selection of frames to get an impression of the data, usually take some fluor frames into this data set, and skip a load of frames, e.g. [1:30:300] for a dataset of 300 frames with fluor images at 1,15,30 etc.).

Press "Set the preliminary flag".

"Create p (parameter )struct".

"Crop".

Follow instructions, make sure that the cropped picture in the well looks like the one in figure 2 below.

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| **Figure 1.** Screenshot of GUI. | **Figure 2.** Note that at the bottom (where bacteria also exit the well) the crop is made right through the well. This is done intentionally. It can sometimes by convenient to only analyze cells that are deeper in the well (less frame-to-frame movement). |

Note here that if you have worked on this dataset before, the buttons marked in blue allow you to return to your dataset after Matlab has been closed down and is restarted. Also when you have e.g. reached buttons further down the workflow (for example "corrections and calculations"), always first press the blue buttons. After pressing the blue buttons from top to bottom, it is not nessecary to re-do other buttons of the work-flow (but you can if you like).

Now, when analyzing data from the mother machine, mother machine compatibility is not yet found in this GUI. (It is listed on the right under "extra options" for reference.) So activate this option manually. (Do this again any time you press the "create p (parameter) struct" button).

>> p.mothermachine=1

Or

>> p.mothermachine=2

Set to 1 if bacteria disappear at bottom, set ot 2 if bacteria disappear at the top. See also:

>> help PN\_segmoviephase\_3colors.m

This option will simply remove bacteria that touch the bottom or top of the image. This means you have to choose your crop window accordingly (see earlier at the text about cropping). See the example below. (This option is a "beta" option; i.e. there might be small glitches still.)

For analyzing data from the mother machine it is also convenient to activate the option "use full img". (This deactivates the attempt to identify a colony of cells, i.e. a mask with most contrast, a procedure that often leads to missing cells in mother machine data.)

Press "segmentation".

Once done, press "manually check segmentation".

Table 1 below shows a list of options that you can now use to make sure each frame is correct. The green circle in the left top of this picture indicates it has been saved (i.e. checked) before.

Press "quick analysis".

Press "placeholder tracking".

"Corrections and calculations"

"Preliminary plots"

The file yourfolder\posXcrop\data\posXcrop-Schnitz.mat now contains a file with the analysis on each cell. Because this is preliminary analysis, cell lineages are not tracked, and each line only contains information about an observation on a cell in a single frame. Some other data is exported to the directory yourfolder\outputSummary\, and also other folders in yourfolder\posXcrop contain some data.

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| **Figure 3.** Screenshot of manually checking segmentation. |  |

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| **Table 1.** A list of options that you can use whilst doing the manual check of the segmentation. Lines with a strikethrough are not used, lines with an asterisk I have not used before. |
| Instructions: press left mouse button (or key '4') on two consecutive cells to join them.  Press right mouse button (or key '5') to cut at that point.  Shift + left mouse button (or key '7') erases current cell.  When you're done, press <space> to go to the next frame.  press <escape> to redo this frame from the original autoseg file.  to save progress during work on a frame, press:  'w' to write a temporary partial correction to the file.  press 'x' to black out an area.  press 't' to mark terraced area.  press 'v' to define region of interest and restrict to this selection.  press 'c' to morphologically close each cell area (imclose)  ~~press 'r' to renumber the cell you are pointing to.~~  press 'o' to obliterate all but the cell you're pointing to.  press '.' to skip frame (without saving).  press ',' to go back a frame (without saving).  press 'a' to add a new cell where the mouse is pointing.  press 'q' to quit.    ~~press 'e' to expand image.~~  ~~press 'f' for "fine-tuning" (to avoid renumbering the image).~~  press 'g' to goto indexnum = ... .  ~~press 'R' to renumber all cells.~~  press 'i' to fill the cell you are pointing to.\*  press 'j' to fill all cells.\*  press 'h' to reseed a cell.\*  press 'd' to remove all 'cells' which look like dirt (large convex hull).  press 'u' to go back one step (possible once).  press 'm' to switch between phase contrast and segmentation image.  press 'l' to show perimeters of cell areas.  press 'b' to restrict segmentation to overlaps with previous segmentation image  (requires assistedCorrection mode, protential trouble after backstep ',')  press 'n' to toggle fullscreen mode (go to next frame after toggling it off).  's' to toggle no numbers, cell numbers, schnitz numbers. |

**Full analysis**

Even when you have just performed the preliminary analysis, it is convenient to start at the top. (Note that your framerange should now be the full frame range of your dataset. Set frameRangeFull in the Excel file accordingly.)

"Select and load config file"

"Set full flag"

"Create p (parameter) struct"

"Crop"

>>p.mothermachine=1

(Or >>p.mothermachine=2, see above.)

"Segmenation"

"Manually check segmentation".

"Quick analysis"

Now, since the tracking depends on correct segmentation, especially regarding cell divisions, you'll have to re-do the following part of the analysis a few times.

"(Re)track cells, correct manually"

Instead of the button above, you can use an alternative tracker. The "MW" is much faster for instance. Before using "MW", it is convenient activating

>> p.ignoreFailedChecksTracker=1

There is a bug somewhere in the script, which requires you to after having ran the "MW" tracker, you still have to press the button "(Re)track cells, correct manually". This will skip all the tracking, but will perform a few operations that are required after tracking.

There is another bug which causes the program to open an unresponsive window of "manually checking" Close this window.

Once you have tracked your frames, there will be issues. Some cells that have been tracked show suspicious behavior (like moving 200px from one frame to the next; likely to have been caused by incorrect tracking instead of something real). These issues will be listed in the command window, and in a notepad file that will open automatically (also to be found in yourfolder\posXcrop\..).

Press "manually check segmentation"

Try to resolve these issues.

Repeat the tracking with your favorite tracker (e.g. press "MW" and then "(Re)track cells, correct manually").

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Note that the "s" option is convenient here, because it will give you information about the tracking (schnitz numbers correspond to lineages, as do colors; this option is only available after tracking one or more times).

Note that cells with issues are highlighted by a checkboard white pattern to aid you.

Once your satisfied, you can close the segmentation and run the tracking a last time.

Press "make movie".

This will create a movie that allows you to double-check the lineage you have just made.

Press "Create backup segtrack".

Press "Corrections and calculations".

(Go for a cup of tea, this will take a while.)

Press "Create analysis plots".

Again, \the file yourfolder\posXcrop\data\posXcrop-Schnitz.mat now contains a file with the analysis on each cell. But now each line corresponds to a cell from birth to division. Type

>> schnitzcells

To get an impression of the information you can obtain.

Convenient fields to know are "frame\_nrs" which gives you the frames in which this cell lived, "time" which gives the time at which this cell lived, corresponds to frame\_nrs, "cellno" which gives you the number by which the cell is known in that frame (not that this is different from the schnitz number of the cell, which corresponds to the line number in the schnitzcells struct), and also:

length\_fitNew: currently used field that gives that cell's length at a point in time (corresponding to frame\_nrs and time).

muP5\_fitNew\_cycCor: currently used field with growth rate in doublings/hour.

dY5: currently used absolute change in Y fluorescent signal. (It has a corresponding time field schnitzcells.time\_atdY.)

Y6\_mean: currently used fluorescent signal concentration field (corresponding time field schnitzcells.time\_atdY).

The "Create analysis plots" outputs to the directory yourfolder\ (date)\_posXcrop\_(name)\ also a bunch of useful plots, like probability distribution functions and cross correlations of aforementioned parameters.

**More detailed information**

More detailed information can currently only be found in the comments of the scripts used here. Type

>> edit Schnitzcells\_masterscript

To take a look at the code that gets executed by the GUI. There is more information in the comments of that code. Also functions that get called by this code are usually commented and provide extra information and options.

Note that the "p" structure and the "ourSettings" together (and a bit redundant) hold a load of important information about your dataset when you are working on it.