The main focus of my PhD thesis is quantifying and interpreting bacterial multicellular behaviour in microscopy image data, primarily in the context of biofilms of *Bacillus subtilis*, but also in the firing of the Type VI Secretion System of *Serratia marcescens*. Storing and organising microscopy images on an OMERO server [1] allows convenient access to unprocessed intensity data from Matlab using the OMERO.matlab toolkit [2]. Within this environment I developed a set of tools designed to have generalised functionality for analysing intensity images and brought them together in a package called OMERO.mtools [3] with full source code available [4]. It is, however, sometimes necessary to perform bespoke analyses for very specific experiments. In this repository I present the code used in my thesis that fits this description.

Of the functions here, two have been published previously. ‘YuabExtentAnalysis.m’ (run from its GUI in Interfaces/YuabExtentLaunchpad.m) was used to determine the penetrance of the YuaB protein into floating biofilms of *B. subtilis* [5] and ‘fpBacteriaSeg3D.m’ is a multi-step procedure for segmenting fluorescent *S. marcescens* cells for automatic cell counting [6].

The remainder of the code here was used to quantify laser scanning confocal microscopy of *B. subtilis* colony biofilms in three distinct temporal windows of growth: during “Colonisation”, when individual cells develop into a coherent biomass; throughout “Expansion”, when a monolayer of chains of cells grow radially from the central mass; and “Maturation”, where radial expansion virtually stops and the biomass becomes thicker. Wild-type NCBI 3610 strain growth was compared to specific gene-deletion strains using combinations of non-fluorescent cells, constitutively fluorescent (GFP) cells and cells harbouring a GFP promoter reporter for the *eps* operon.

Biological processes probed at each of these stages include the colonisation rate, *eps* expression via GFP signal intensity, and physical measurements such as the motion of features relative to the leading edge of the biofilm.

To use the code a Matlab environment should be set up with the OMERO.matlab toolkit of a version matching the server where the images are hosted, and a session object should be declared as global. Table 1 below explains the period of biofilm formation each file was used to analyse as well as the arguments and returns of each function, which are defined in Table 2.

[1] <https://www.openmicroscopy.org/omero/>

[2] <https://www.openmicroscopy.org/omero/downloads/>

[3] <https://www.openmicroscopy.org/omero/features/analyze/>

[4] <https://github.com/mporter-gre/mtools>

[5] Hobley, L., Ostrowski, A., Rao, F. V, Bromley, K.M., Porter, M., Prescott, A.R., et al. (2013) BslA is a self-assembling bacterial hydrophobin that coats the Bacillus subtilis biofilm. Proc Natl Acad Sci U S A 110: 13600–5 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3746881&tool=pmcentrez&rendertype=abstract

[6] Gerc, A.J., Diepold, A., Trunk, K., Porter, M., Rickman, C., Armitage, J.P., et al. (2015) Visualization of the Serratia Type VI Secretion System Reveals Unprovoked Attacks and Dynamic Assembly. Cell Rep 12: 2131–42 http://www.cell.com/article/S221112471500950X/fulltext

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| --- | --- | --- | --- |
| **Colonisation** | **Data Type** | **Arguments** | **Output** |
| biofilmColonisationRate.m | Time-lapse | session, imageId, c | meanPatch, minMaxMeanPatch,  grads, fdhm |
| measureCellOrientationsInMiddle.m | Single plane | session, imageId | props |
| measureCellOrientationsInRing.m | Single plane | session, imageId | props |
| rotateImageAndImport.m | Single plane | session, imageId |  |
| roiIntensityOverTime.m | Time-lapse | session, imageId, c | meanPatch, minMaxMeanPatch,  FXMinMax, fdhm |
| **Expansion** |  |  |  |
| roiIntensityOverTime.m | Time-lapse | session, imageId, c | meanPatch, minMaxMeanPatch,  FXMinMax, fdhm |
| biofilmGrowthRate.m | Time-lapse | session, imageId,  c, tRange | grad, rsq, micronsPerMinute |
| distanceFromBiofilmFront.m | ROI data | session, imageId,  rightMost, pointCoords | distFromFront, stats, gof |
| distFromBiofilmFrontVsStepGrad.m | ROI data | distFromFront, steps | startDistVsGrad,  distFromFrontVsStep |
| trackPointROIDistances.m | ROI data | session, imageId | coords, distMat, steps |
| **Maturation** |  |  |  |
| biofilmVolumeSURF.m | Z-Stack | volumes | points |
| volumeUnderBiofilm.m | Z-Stack | session, dsId | volumes, volSum |
| XYZProjectionMakeAndImport.m | Z-Stack | session, imageId, c, flipDim |  |

|  |  |
| --- | --- |
| session | OMERO session object |
| imageId | OMERO image ID |
| c | Channel number |
| tRange | Time range e.g. [0-25] |
| rightMost | Vector of x-coordinates |
| pointCoords | Matrix of x-y coordinates, as columns |
| distFromFront | Vector of distances |
| steps | Vector of step distances |
| volumes | Volume map images |
| dsId | OMERO dataset ID |
| flipDim | Flip dimensions (Bool) |
| meanPatch | Mean intensity of an ROI |
| minMaxMeanPatch | Mean intensity of an ROI, normalised |
| grads | Vector of gradients |
| fdhm | Full duration half max |
| props | Matlab regionprops |
| FXMinMax | Normalised function of line |
| rsq | R-Squared of fit |
| micronsPerMinute | Speed of expansion |
| stats | Statistics of fit |
| gof | Goodness of fit |
| startDistVsGrad | Matrix of start distance from biofilm front and grandients, as columns |
| distFromFrontVsStep | Matrix of distance from biofilm front and grandients, as columns |
| points | Matlab SURFPoint objects |
| volSum | Sum of volume map image |