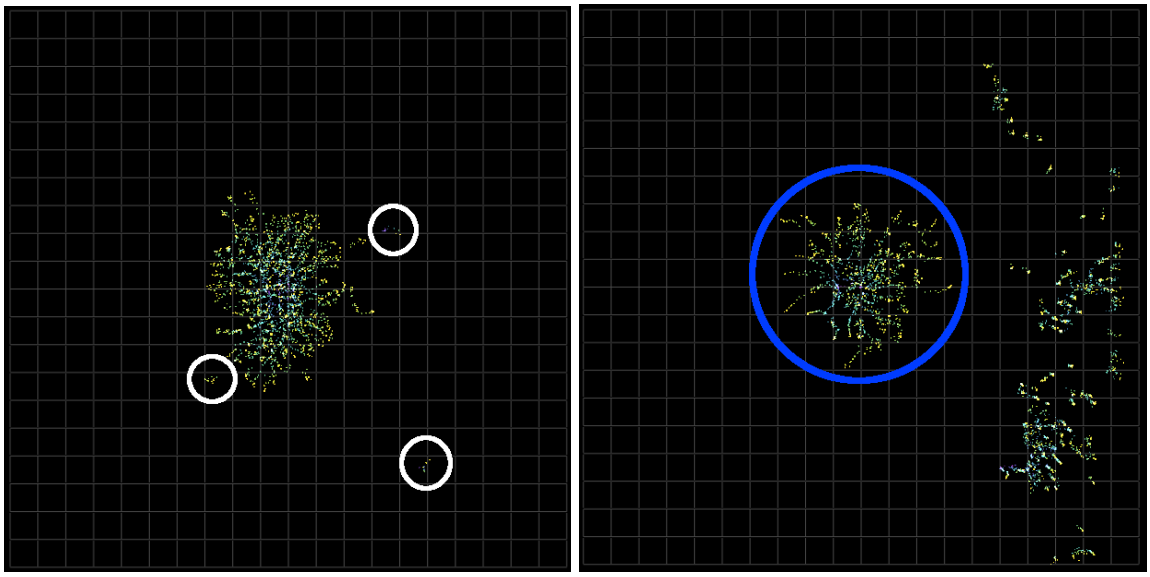

Implementation: The workflow was implemented in Python. The main function `seevis` runs the algorithm for one data set, or movie. To change the color mapping, the `-s` option is set to values from 1 to 3: NM (1), TM (2), and PM (3). We employ `PyQtGraph` to provide standard interactive capabilities including panning, rotation, zooming.

Input: The input data is one directory with multiple image files, i.e. the records of the growth of a single *S. meliloti* bacterium. It is accessed by `seevis` using the `-i` option. Provided a CSV has been precomputed, it is possible to parse the file using the `-f` option.

Output: Results of the first two steps are automatically exported. Preprocessing results are binary images saved in the local directory. Particle-related computations result in an exported CSV file containing all the particle coordinates and their respective trajectory. Once a color mapping is selected, SeeVis displays the particle trajectories and/or coordinates.

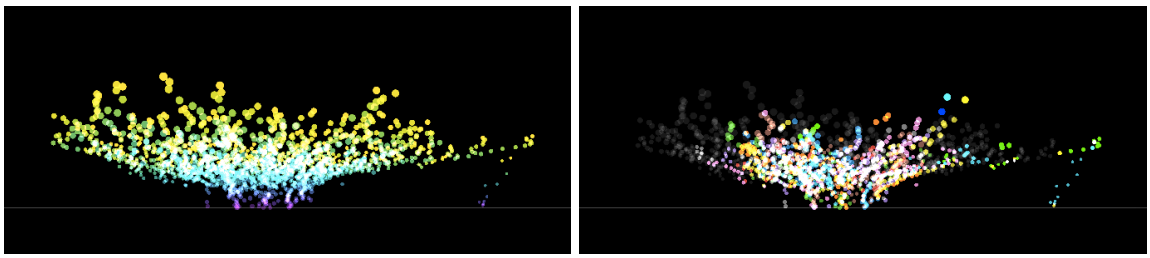
Remarks: The visualization displays a particle in the form of a spot that scales with the view (see `functions.py, line 111 and lines 393--396`). The spot size can be changed according to user preferences or image modalities. The spot size has been chosen such that the particle trajectory does not contain scattered spots and such that neighboring spots do not lead to visual occlusion.
The preprocessing parameters as well as the particle paradigm are detailed in Hattab G, Wiesmann V, Becker A, Munzner T, Nattkemper TW. A novel Methodology for characterizing cell subpopulations in automated Time-lapse Microscopy. Frontiers in bioengineering and biotechnology. 2018. doi:10.3389/fbioe.2018.00017.

Example experiment: In this example, microbiologists aim at understanding quorum sensing disruption for *S. meliloti*. The activity of a specific promoter (quorum sensing cell state) fused to the mVenus coding region is monitored. Disrupting the medium in which the bacteria grows permits to further analyze and understand adaptation to stress. High phosphate concentrations are introduced in the medium to disrupt cell communication by repressing quorum sensing signaling, in turn changing fluorescence signals and growth patterns.
Data: M. McIntosh and V. Bettenworth. Onset of quorum sensing and exopolysaccharide production in single cells within growing microcolonies. Philipps University of Marburg, 2017. doi:10.4119/unibi/2913120.



(a) A colony surrounded by artifacts **(b)** A colony at the edge of a microfluidics chamber

Figure S1: Time mapping demonstrated for two movies (44 frames) in this experiment. (a, b) The space-time cube is displayed with azimuth = 0 degrees and elevation = 90 degrees and a xy grid for two different movies: (a) The white circles highlight imaging artifacts at the interface (e.g. noise) or in the medium. Consequently, these may impact the detection and tracking of cells and/or features. (b) The blue circle shows the colony of interest. Although the imaging system was automatically able to detect and follow the growth of the mother cell, this colony did grow at the edges of the microfluidics chamber. The microfluidics system employs a pump to sustain the growing bacteria by replenishing the medium. Due to the bacterial cells' size, it is possible to see cells escaping the chambers (gradient and flow) circulating in the microfluidics medium flow. Since the colony is close the the chamber's edge it is possible that such a scenario may impede tracking results. SeeVis may be used to quickly verify if the system needs adjustment before recording another movie in this experiment.



(a) Time mapping **(b)** Progeny mapping

Figure S2: Zoomed view of the colony seen in Fig. S1 (a). (a, b) The space-time cube is displayed with azimuth = 90 degrees and elevation = 0 degrees. (a) In this view, one of the imaging artifacts is positioned on the right side of the growing colony. The space-time cube provides a spatiotemporal overview confirming that this artifact is not part of the colony. (b) The progeny mapping shows that this particular artifact was present throughout the whole movie. And that it might be beneficial to further invest time to investigate its source.