Computational toolboxes for single cell imaging

Jian Wei Tay

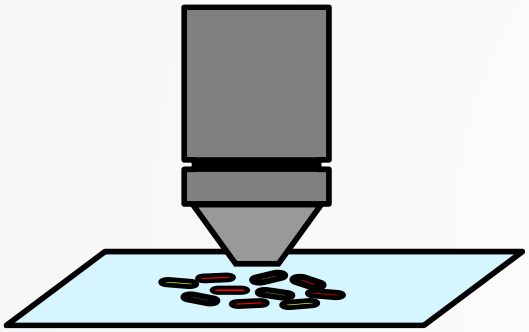
BioFrontiers Institute

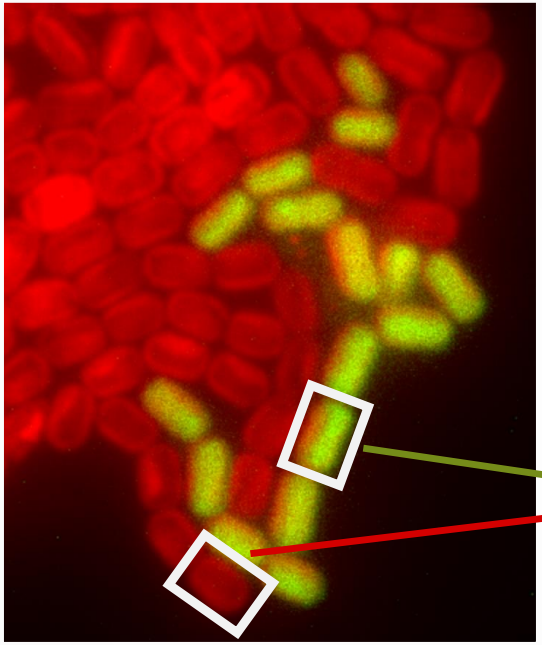
Advanced Light Microscopy Core

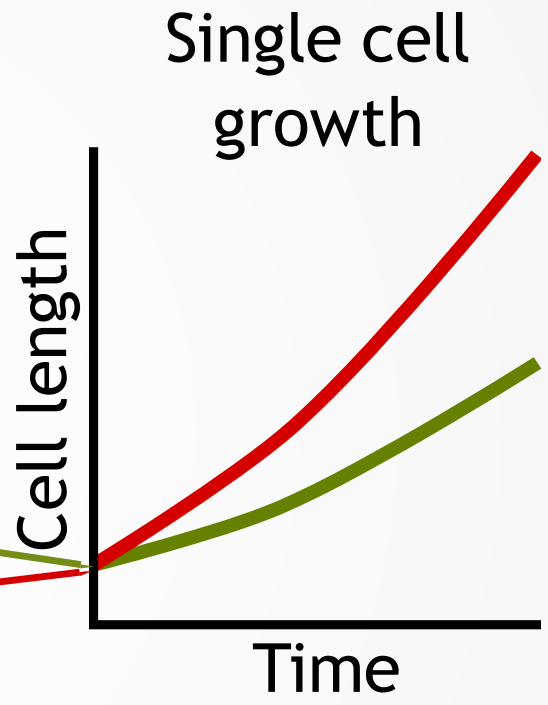
SCR Single Cell Single Molecule Supergroup

Feb 17 2020

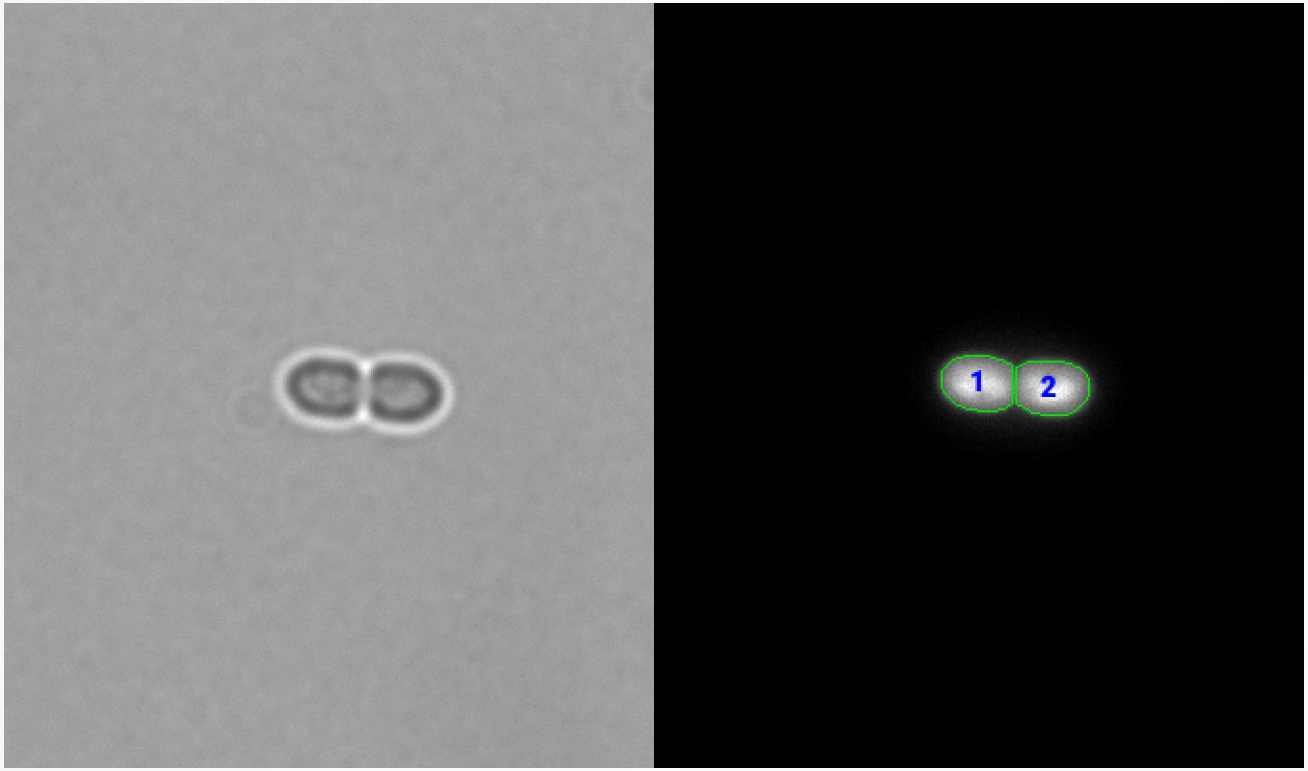
Microscopy enables individual cells to be observed







Computational tools are needed to analyze the images



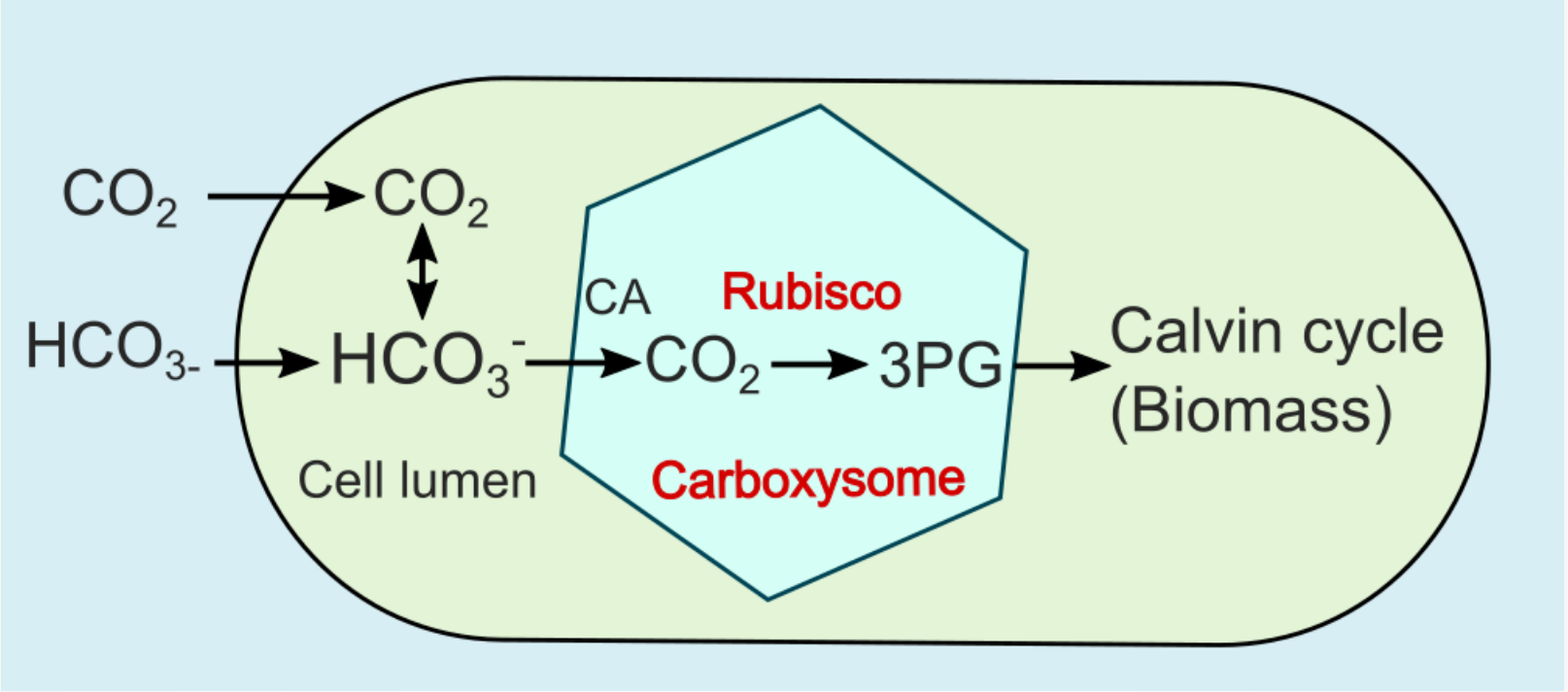
- Time-lapse images contain a lot of data (e.g. many cells, intensity, spatial organization, …)
- Helps avoid tedious analysis by hand as well as experimenter bias (i.e. by drawing expected cell outlines instead of from image data)

Motivation

- Introduce a few MATLAB toolboxes that I have been developing

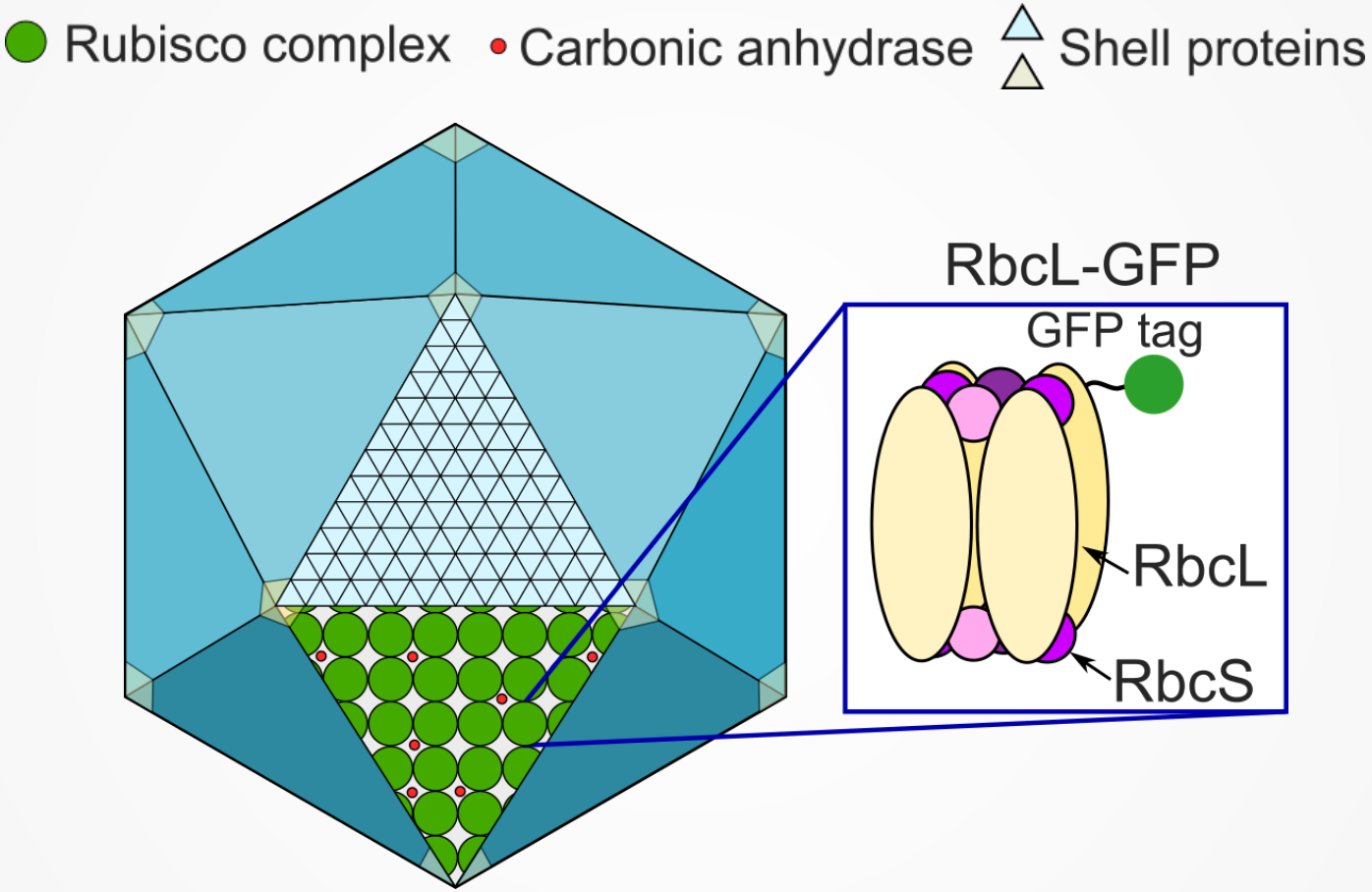
- Biological context:
  - Studying the carboxysome in cyanobacteria
  - Project with the Cameron lab

The carboxysome plays a central role in carbon fixation within cyanobacteria

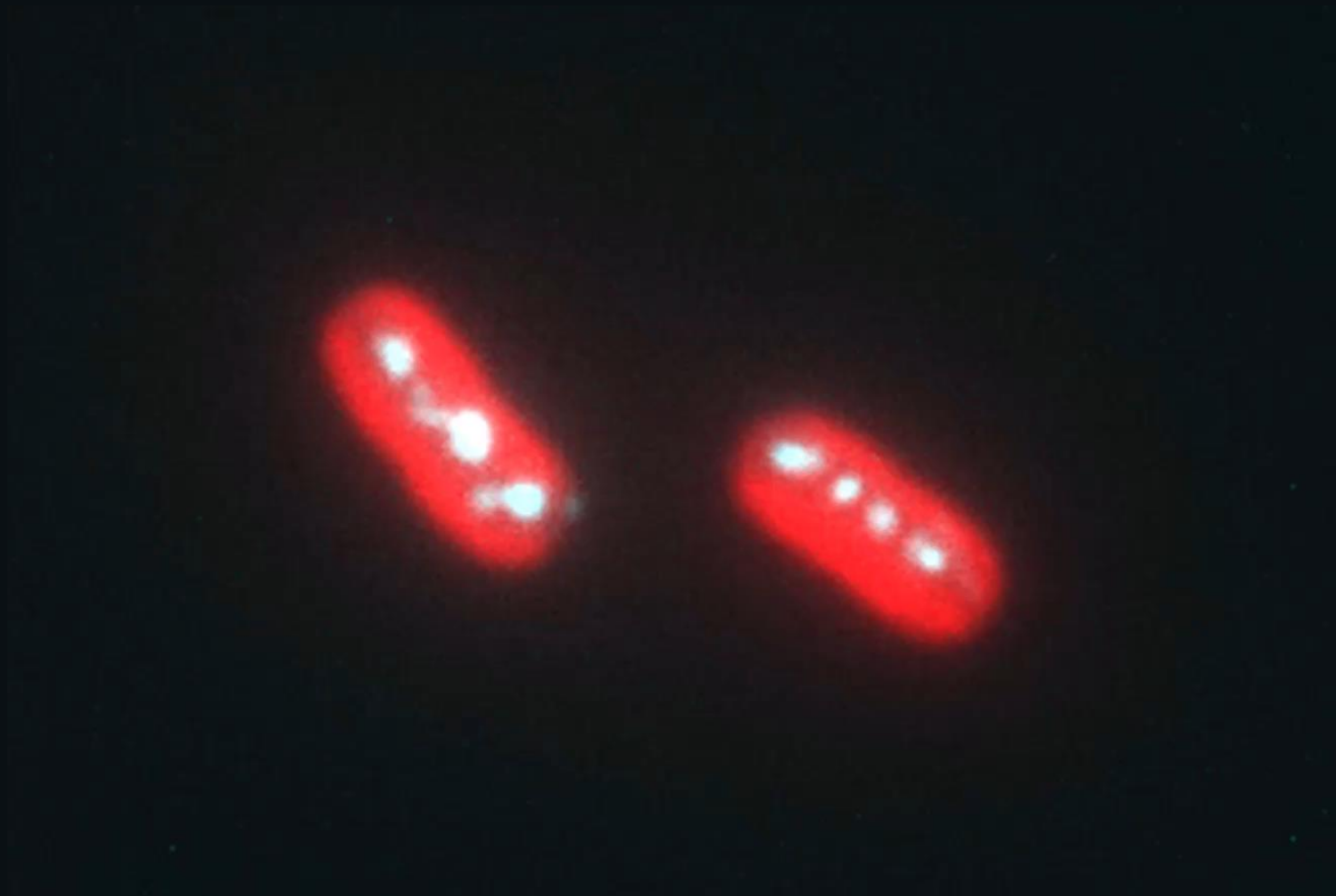


- Houses the enzyme Rubisco which catalyzes the primary reactions in carbon fixation
- Important for understanding photosynthesis and improving crop yields

Rubisco is tagged with GFP for visualization



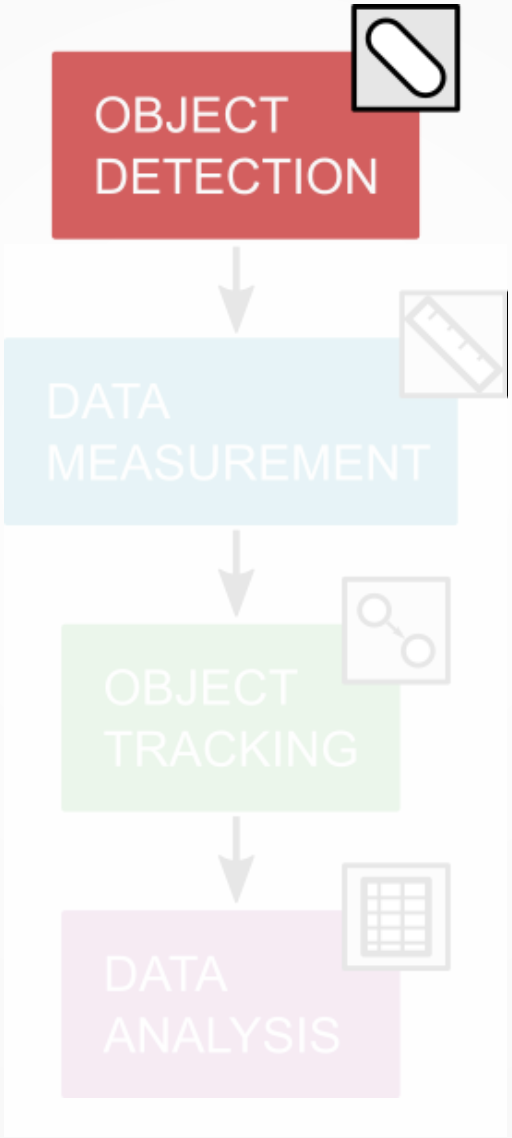
Carboxysome expression is stopped at start of movie



Chlorophyll

Carboxysomes

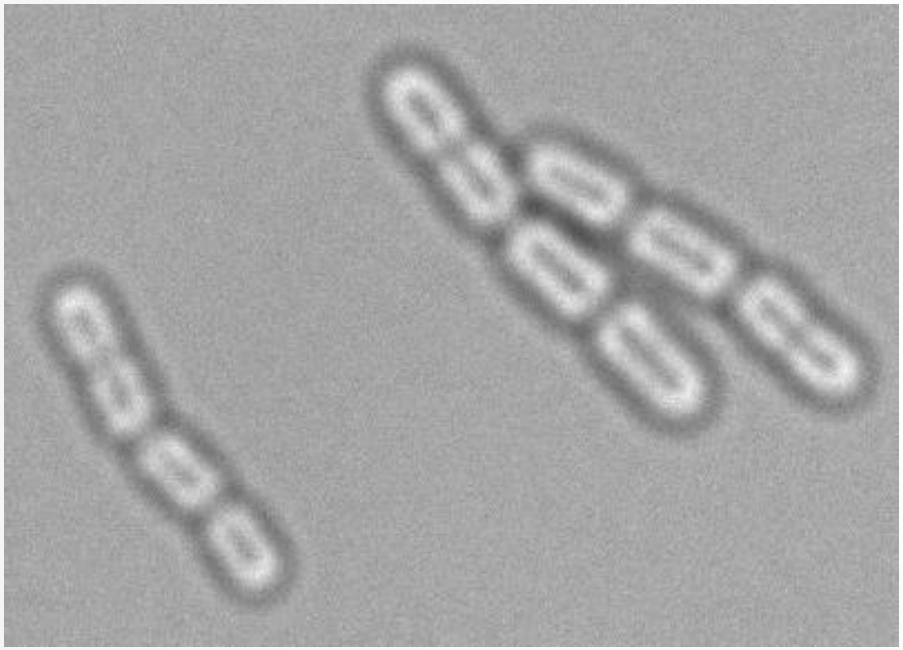
The image analysis pipeline



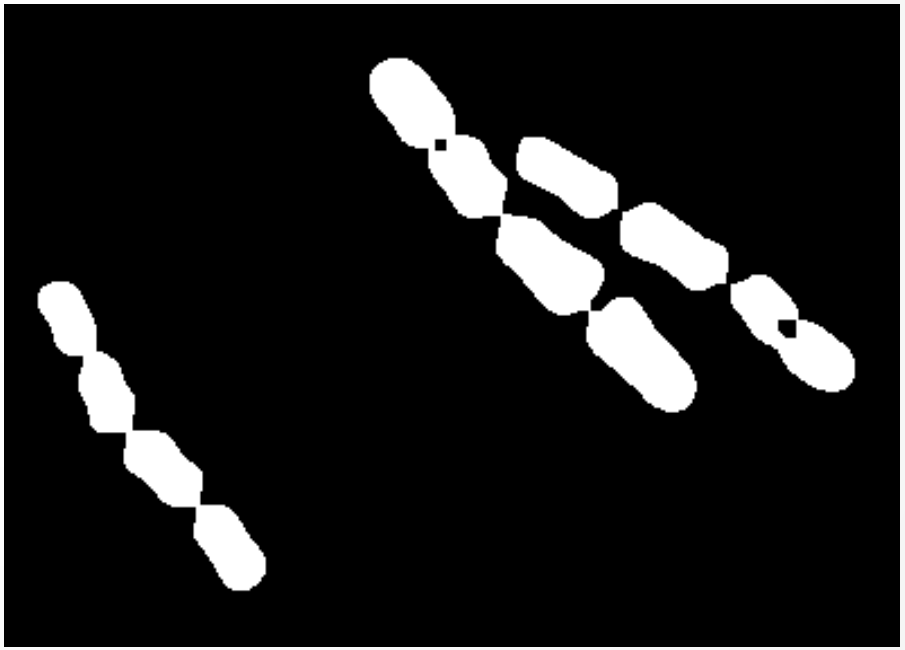
Detecting cells

- Identified cells using the brightfield image
- Generated mask by intensity thresholding

Brightfield image

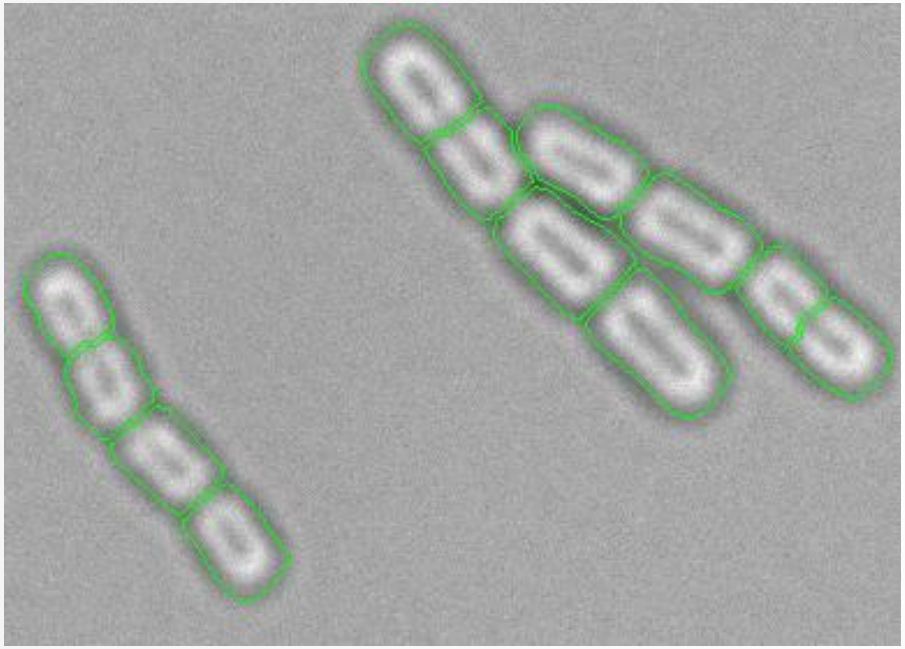


Binary mask

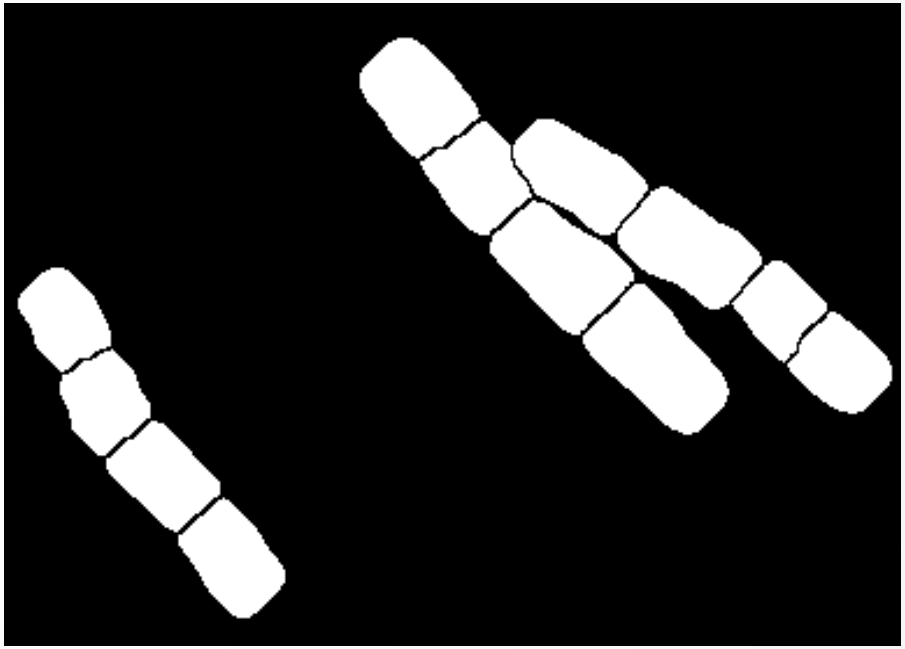


Cell clusters are separated by watershedding

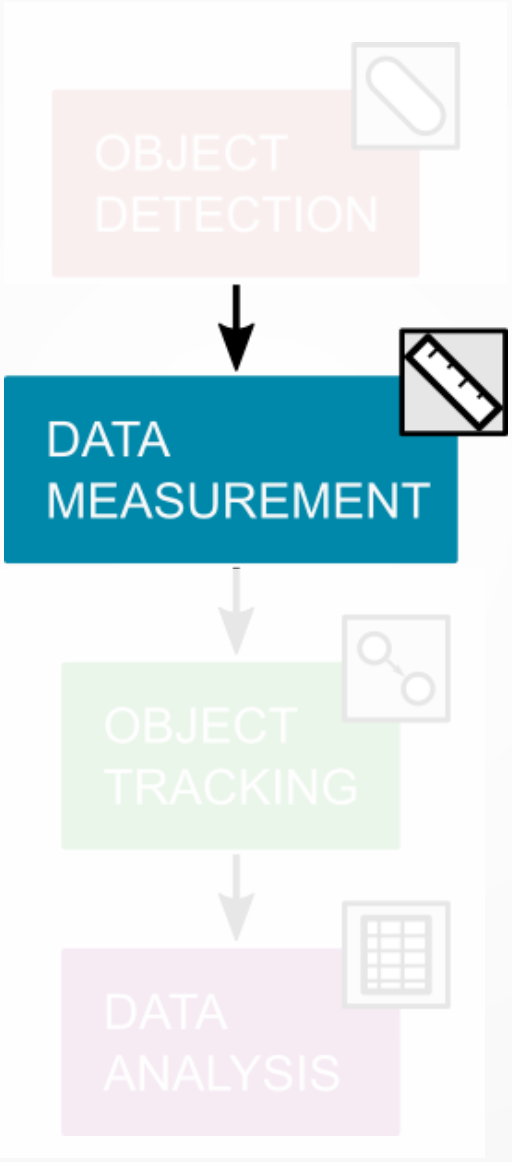
Brightfield image



Binary mask

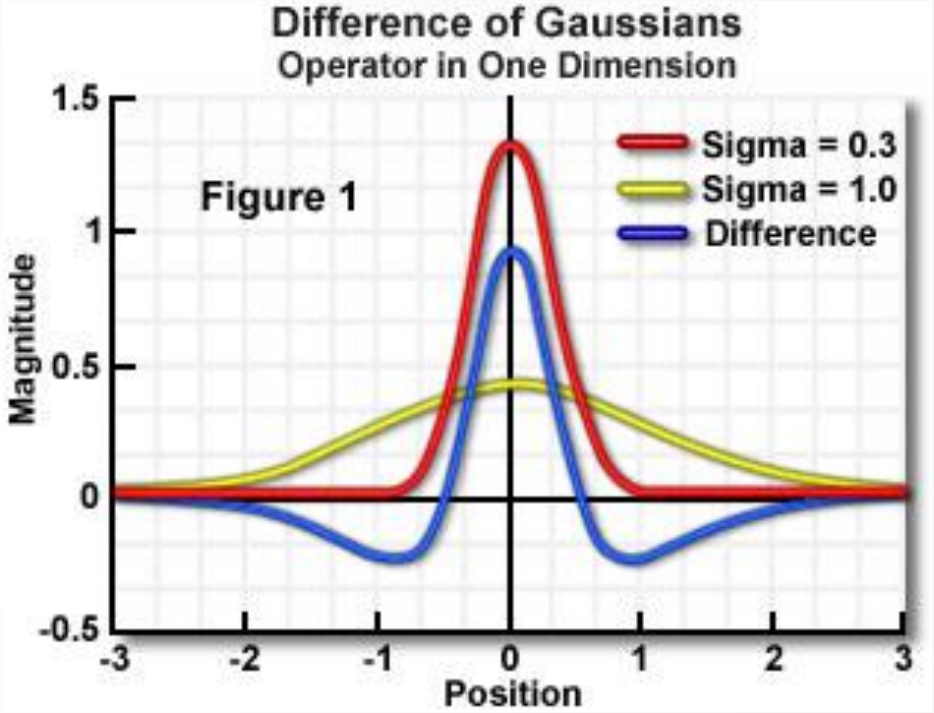


The image analysis pipeline



- Cell length
- Number and position of carboxysomes

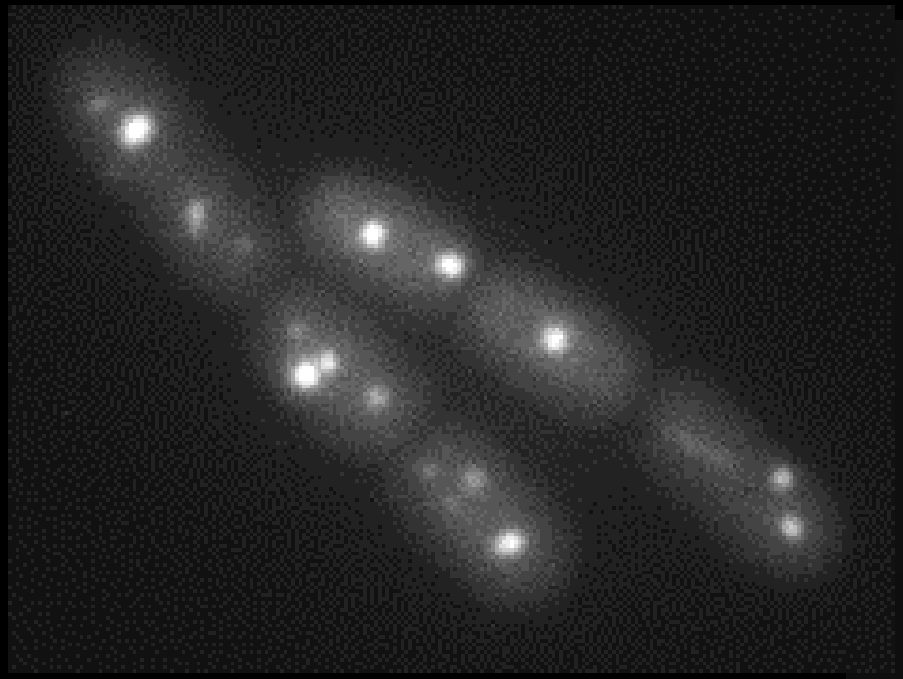
Carboxysomes (dots) are identified using the difference of Gaussians filter



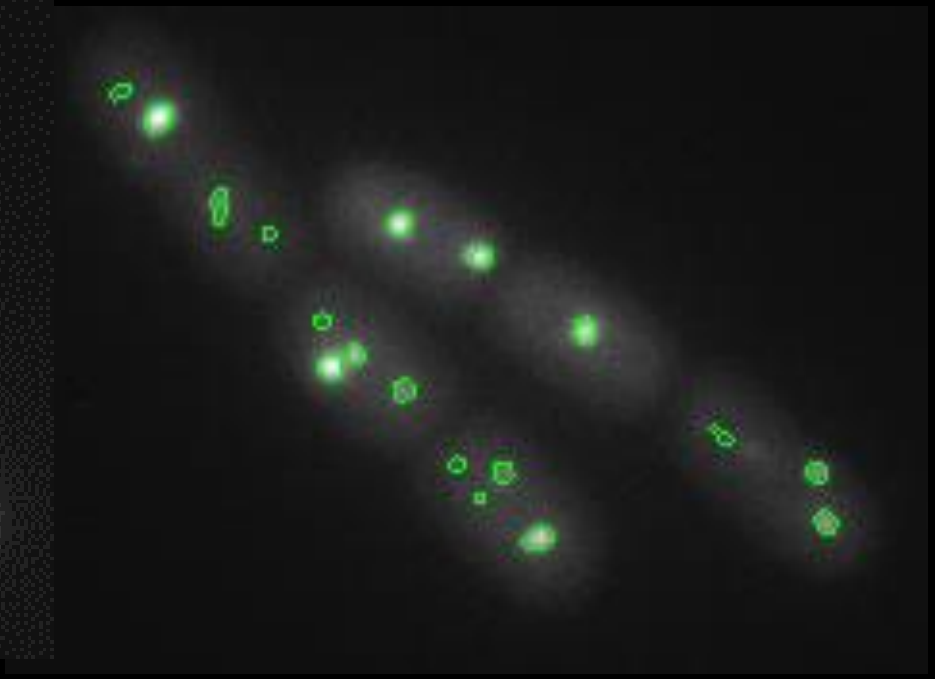
- Difference of Gaussians:
  - Blur image with two Gaussian filters with widths σ_1 and σ_2
  - Subtract the blurred images $I_{\sigma 2} - I_{\sigma 1}$
  - The filter keeps structures with size between σ_1 and σ_2

https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/diffgaussians/index.html

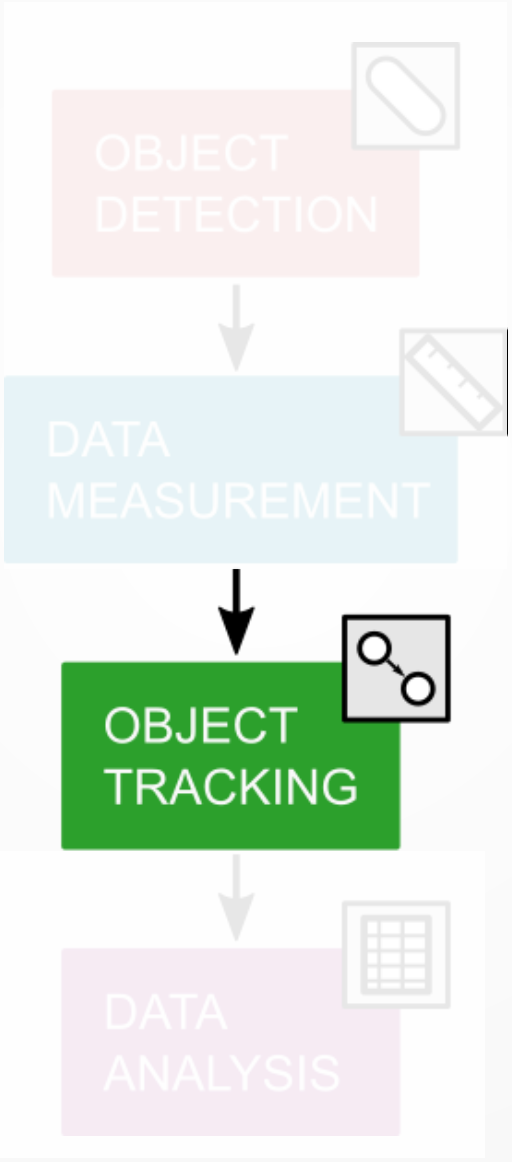
Carboxysome fluorescence



Mask overlay

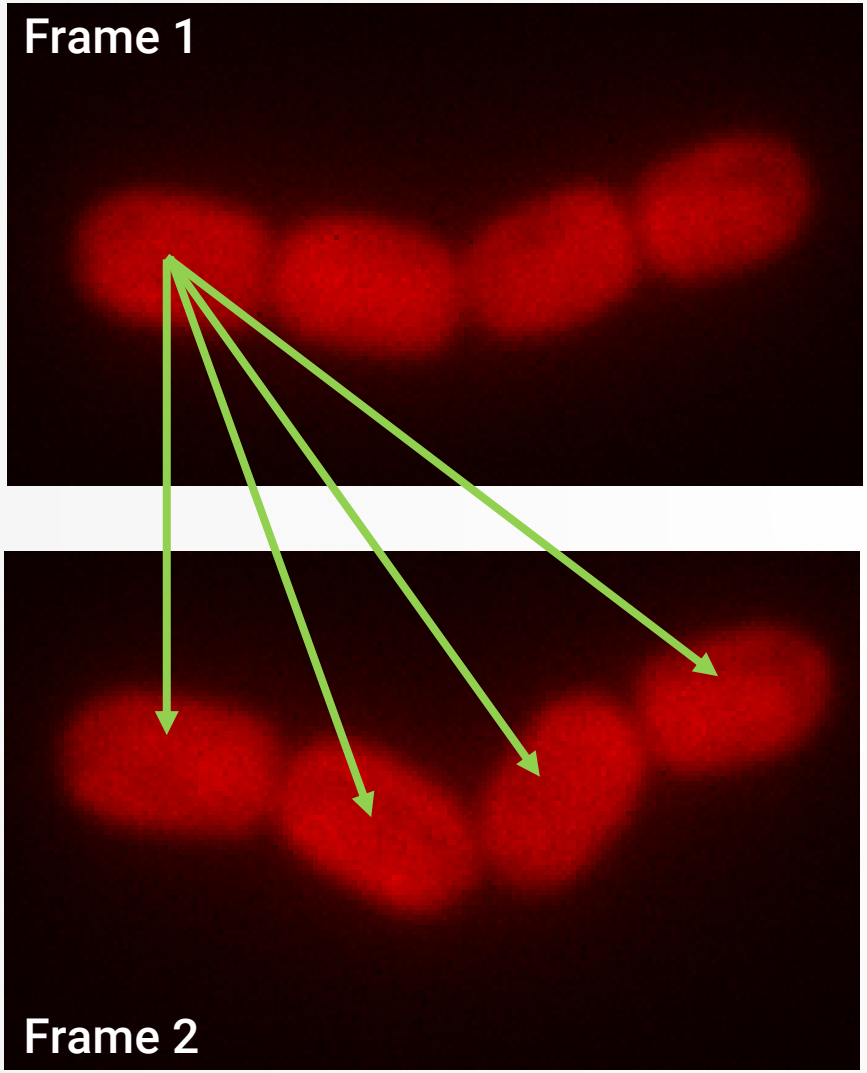


The image analysis pipeline



1. Follow individual cells over time
2. Identify cell division

The tracking problem

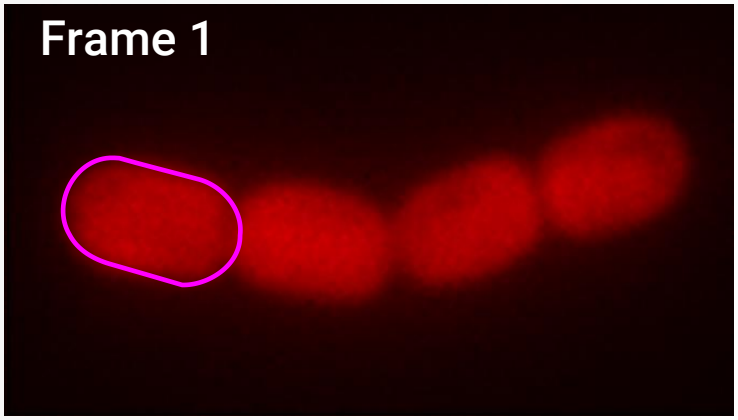


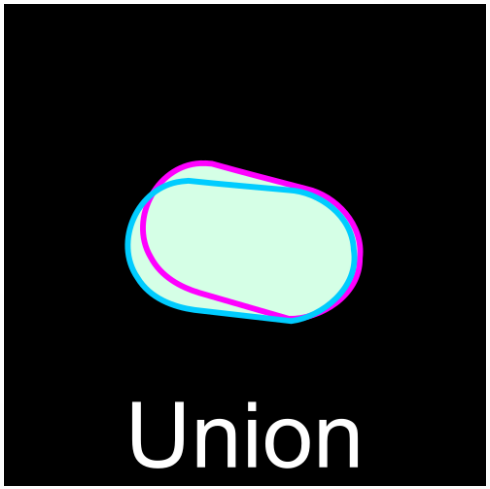
- Tracking using the linear assignment framework
- Each cell detected in frame 2 is given a score that it is the same cell in frame 1
- The lowest score = the same cell

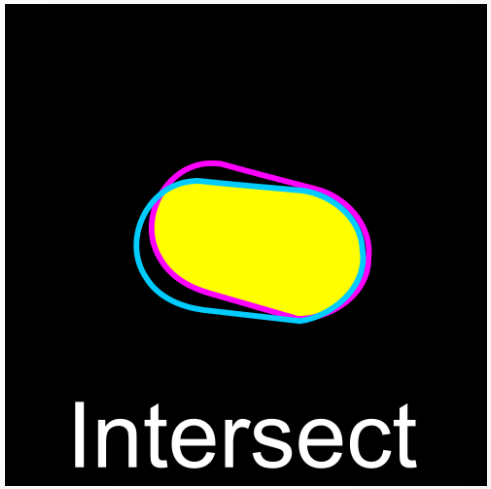
Jaqaman et al. Nature Methods 5, 695-702 (2008)

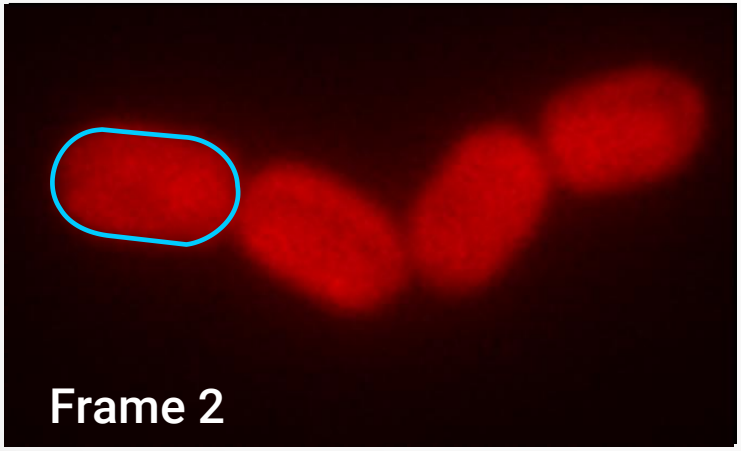
Linking score is ratio of overlapping pixels

$$\text{Overlap score} = \frac{|A \cup B|}{|A \cap B|} = \frac{\text{Number pixels union}}{\text{Number pixels intersect}}$$

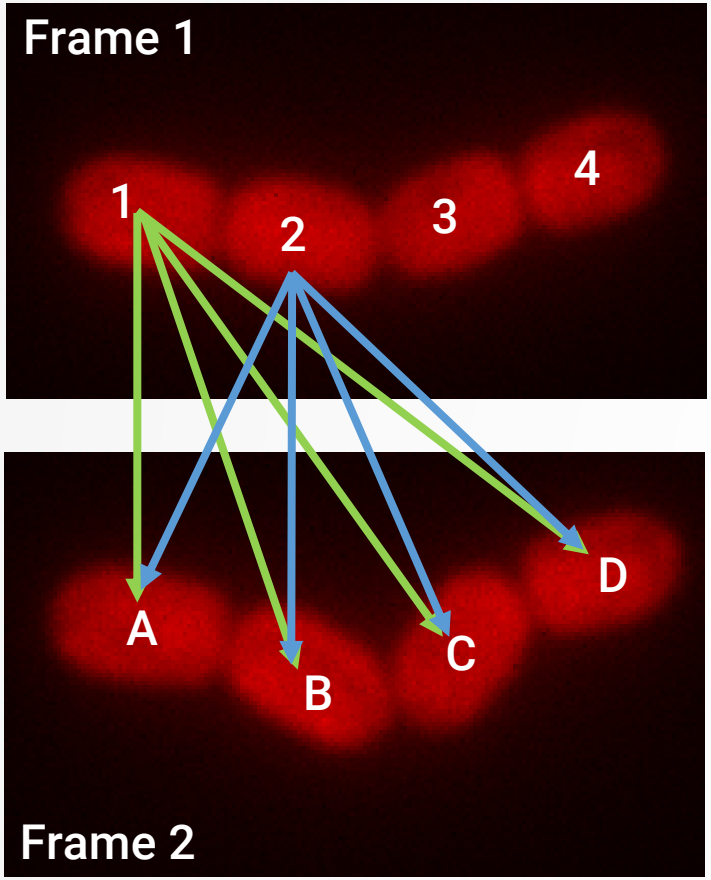








The linking cost matrix



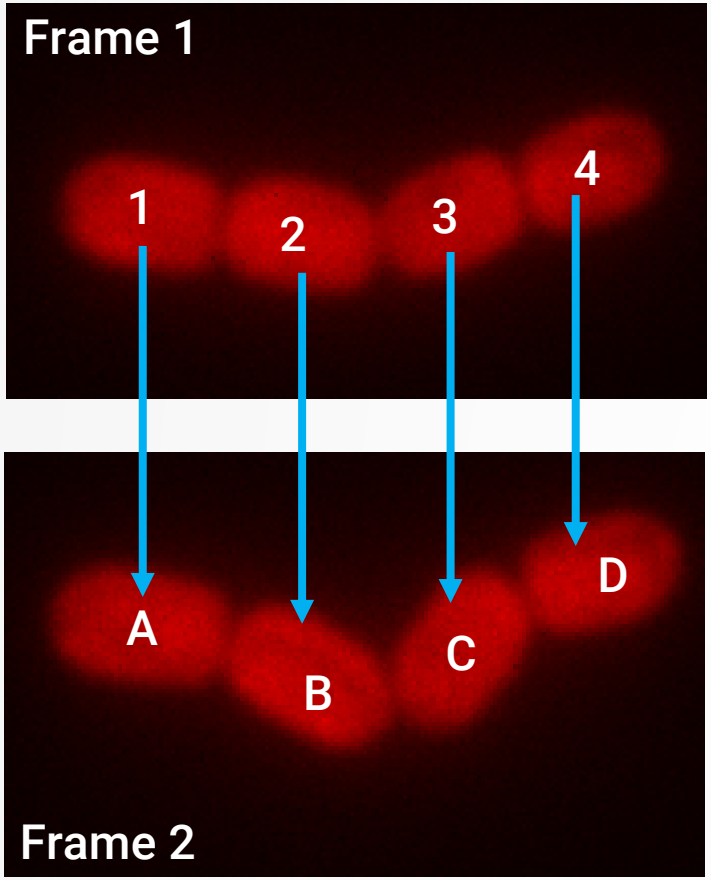
$$\text{Overlap score} = \frac{\text{Number pixels union}}{\text{Number pixels intersect}}$$

Frame 2

Frame 1	A	B	C	D
1				
2				
3				
4				

For each row, assign a column such that total score is minimized

The linking cost matrix



$$\text{Overlap score} = \frac{\text{Number pixels union}}{\text{Number pixels intersect}}$$

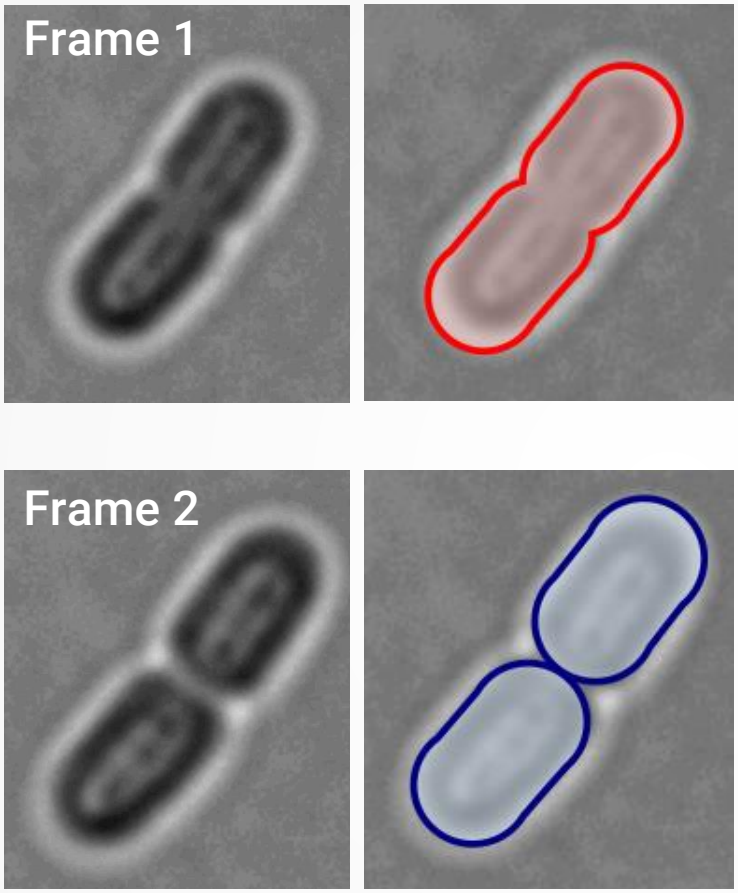
Frame 2

Frame 1	A	B	C	D
1	1.5	Inf	Inf	Inf
2	Inf	4	21	Inf
3	Inf	15	5	Inf
4	Inf	Inf	Inf	1.7

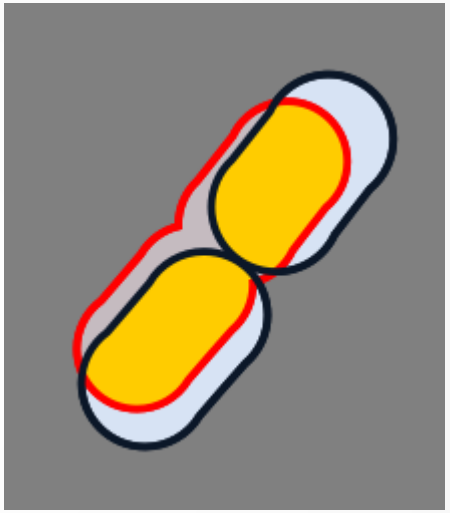
For each row, assign a column such that total score is minimized

Jonker, R, and Volgenant, A. Computing 38, 325-340 (1987)

Cell division identified by overlap score

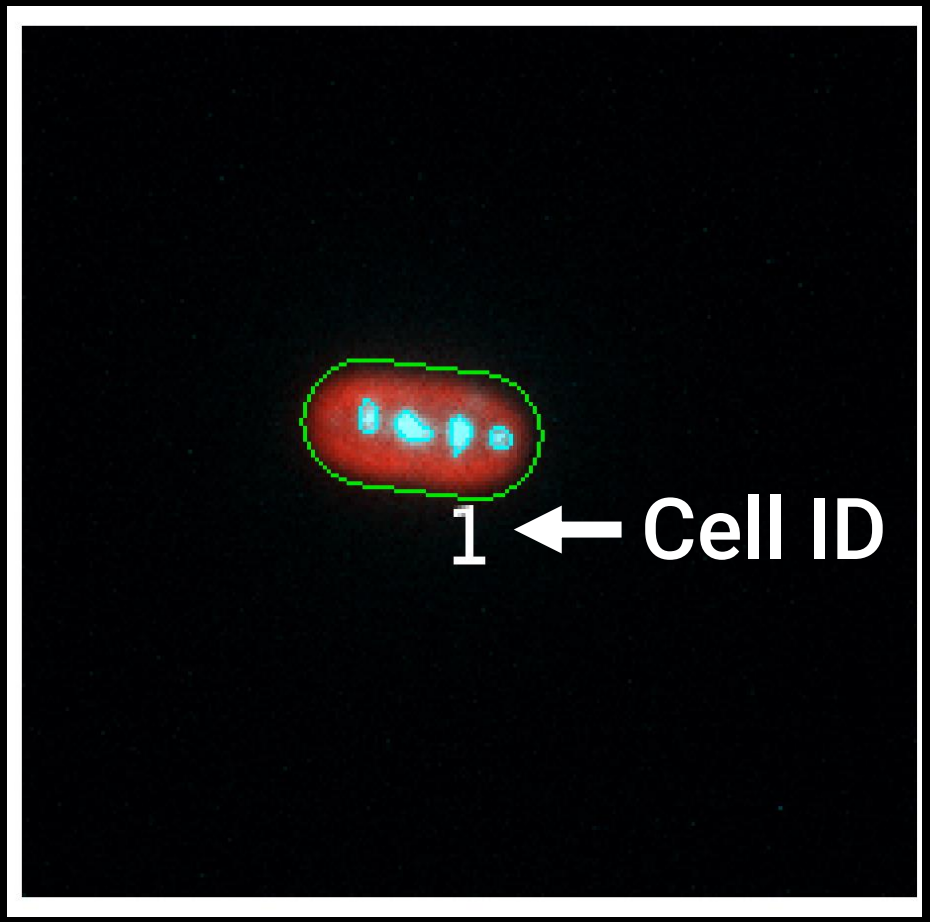


Merge of masks



- Division if two cells in frame 2 overlap the same cell in frame 1

Example of tracking output



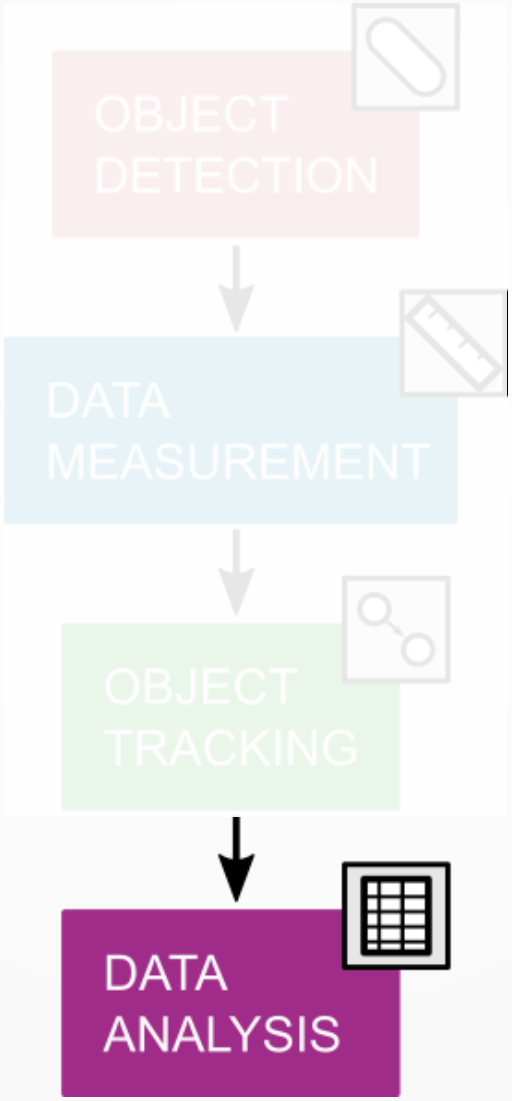
Chlorophyll

Carboxysomes

Output data structure

- ID – unique number assigned to each cell
- MotherID – ID of mother cell
- DaughterID – ID of daughter cell
- Number of carboxysomes
- Position of carboxysomes
- Cell length

The image analysis pipeline



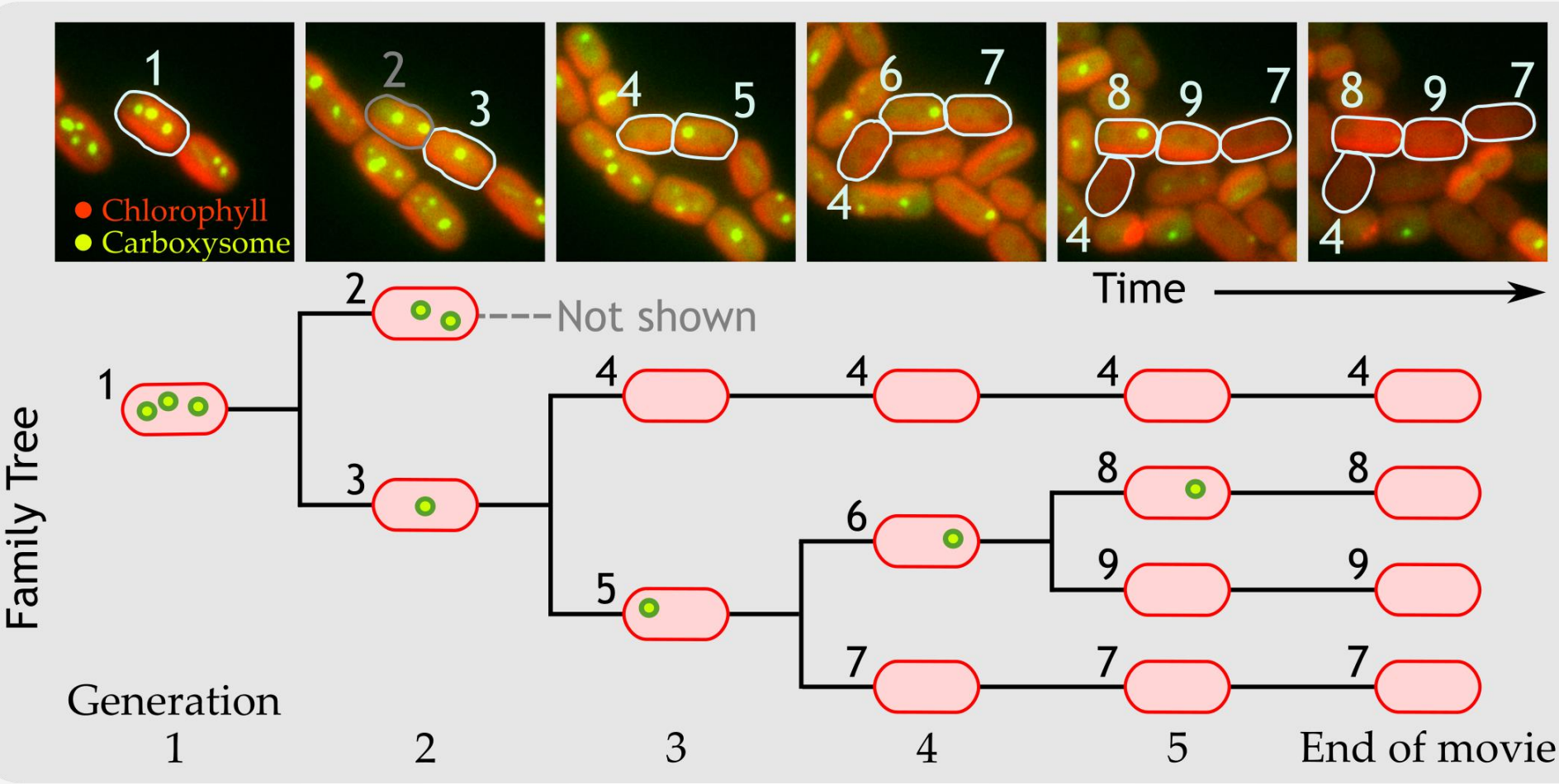
Output data structure

- ID – unique number assigned to each cell
- MotherID – ID of mother cell
- DaughterID – ID of daughter cell

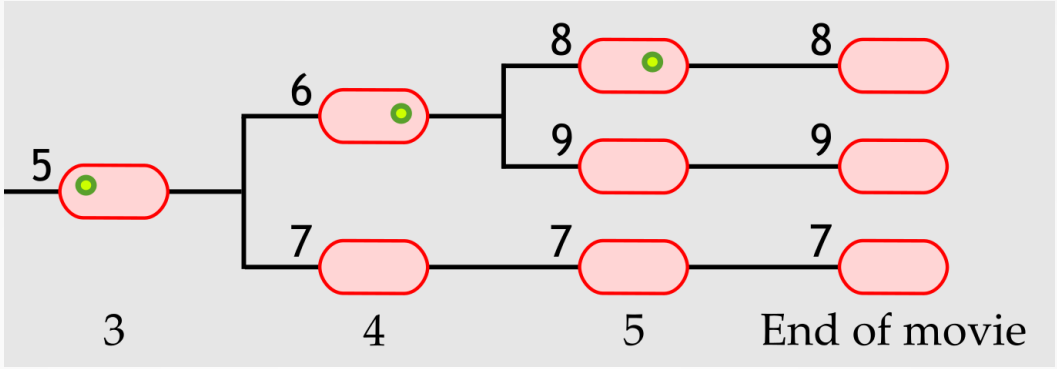
- Treat data as a binary tree (a common data structure)

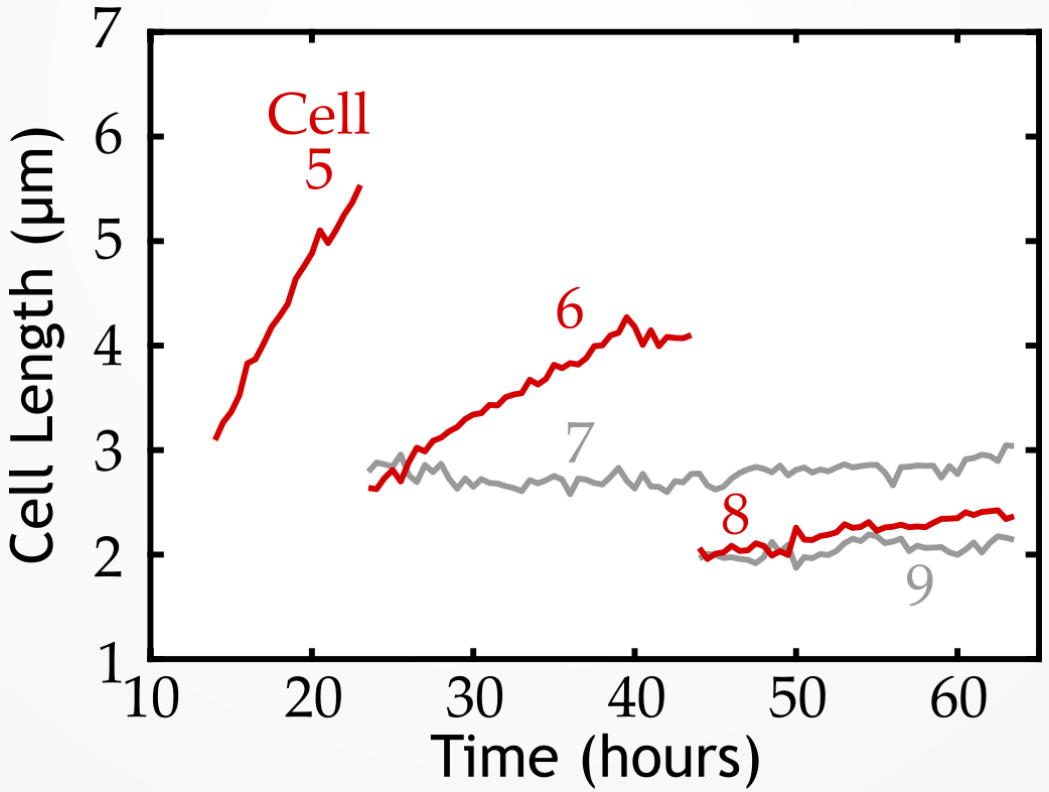
- We implemented existing traversal algorithms to analyze these trees

Tree traversal allow us to identify cells with one carboxysome



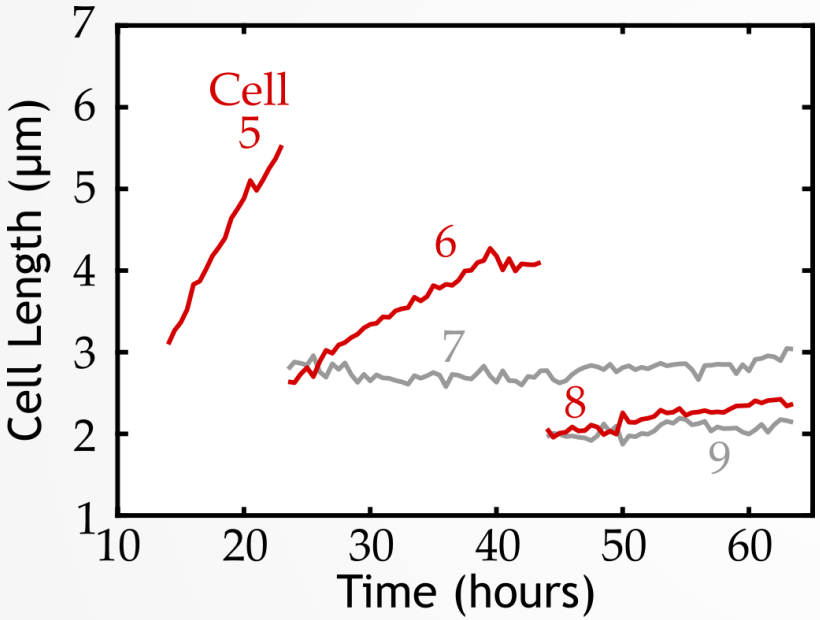
Cells without carboxysomes do not grow

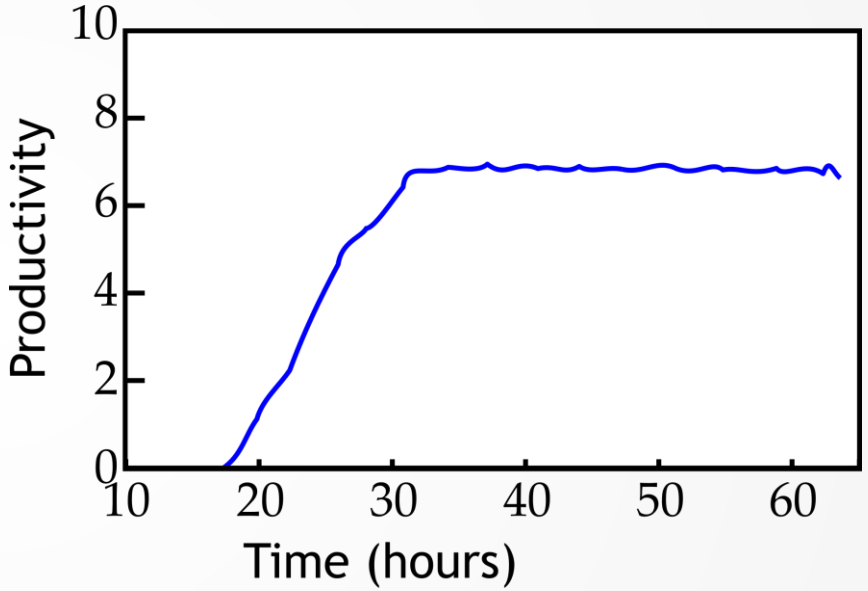




Quantification of carboxysome activity

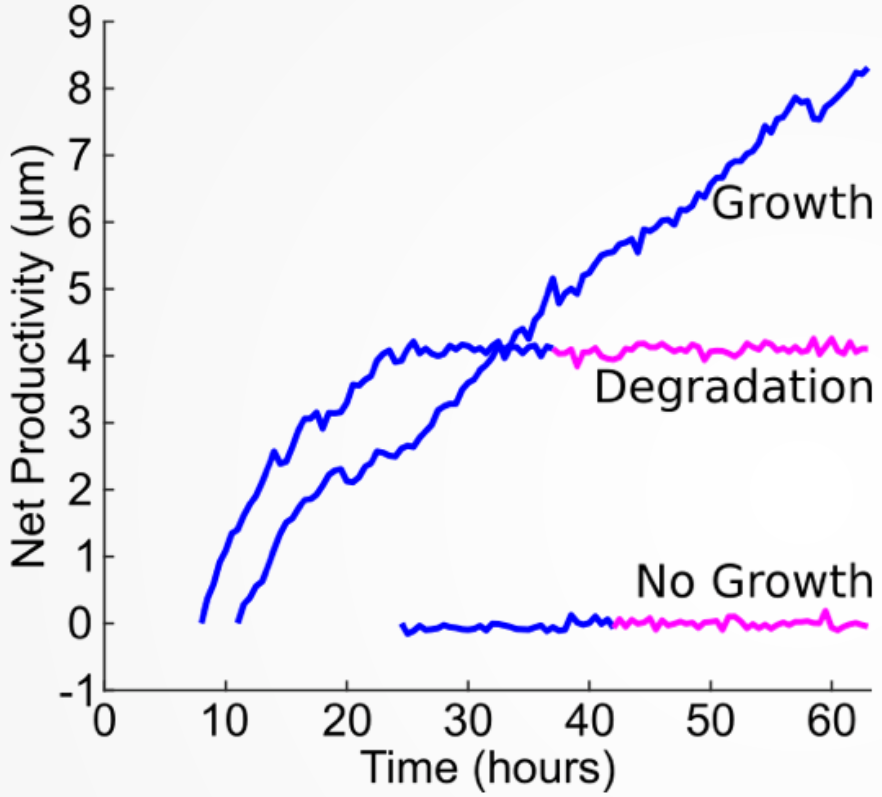
Productivity = $\sum_t Length(t)$





Productivity = biomass production of a single carboxysome

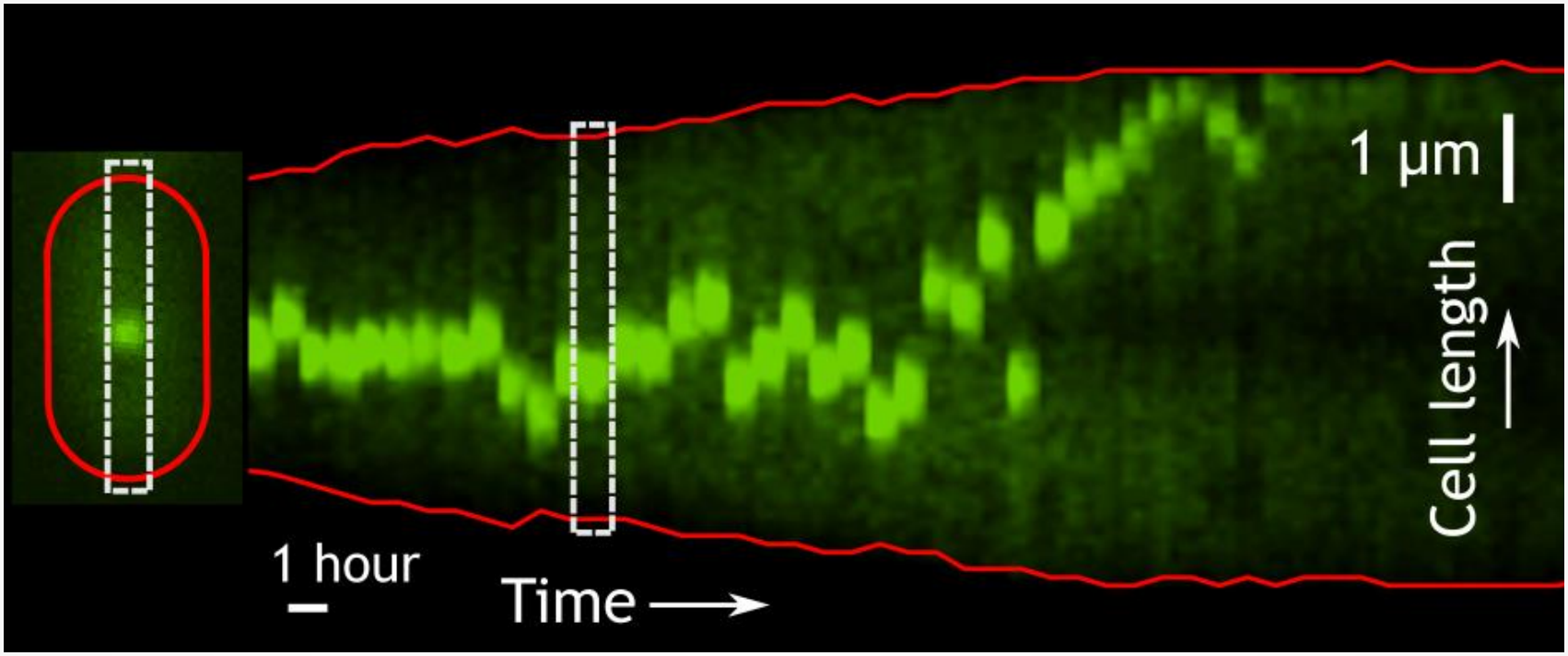
Clustering different net productivity patterns



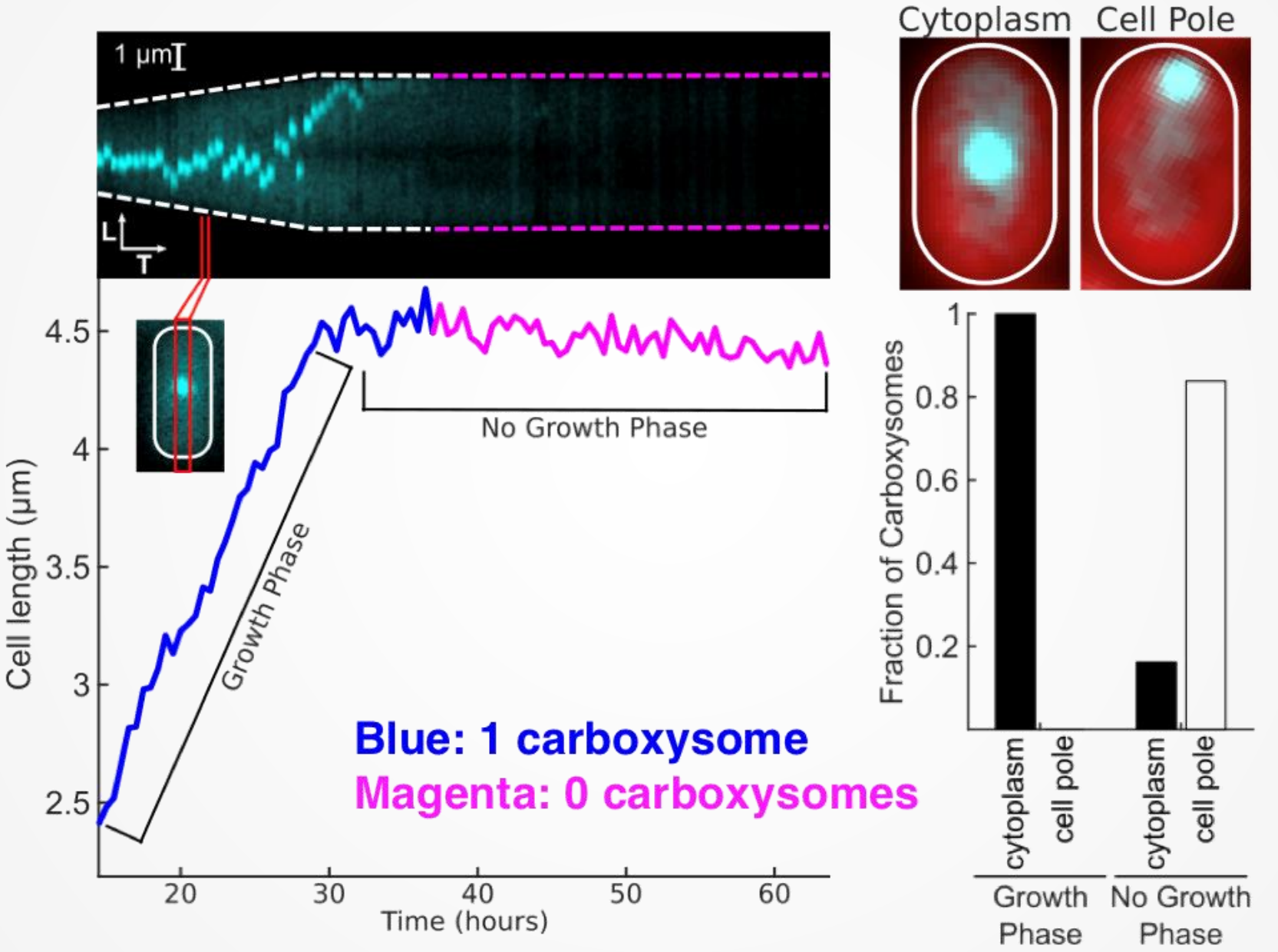
Blue: 1 carboxysome

Magenta: 0 carboxysomes

Kymograph shows carboxysome location



Inactive carboxysomes change localization and degrade



Summary

- Fluorescence microscopy enables individual cells and structures to be observed and quantified

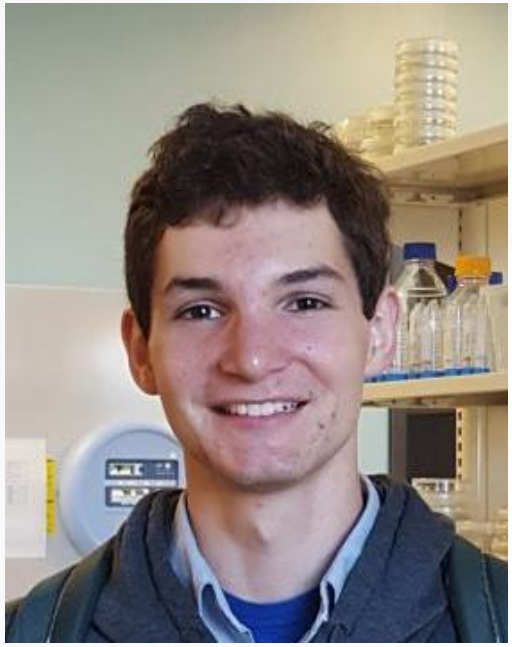
- Computational tools are necessary for analysis

Available toolboxes

https://biof-git.colorado.edu/biofrontiers-imaging

- **BioformatsToolbox** – Read ND2 (and other microscope) images
  - Uses the Bioformats Java library from openmicroscopy.org
  - Repackaged as a MATLAB class
  - https://biof-git.colorado.edu/biofrontiers-imaging/bioformats-image-toolbox
- **LAPLinker** – Linear Assignment Linking toolbox
  - Object tracking code
  - Lineage/Tree plots
  - https://biof-git.colorado.edu/biofrontiers-imaging/lap-cell-tracker
- **CyAn** – Cyanobacteria Analysis toolbox
  - Segmentation
  - Kymographs
  - Coming soon

Acknowledgements



Nick Hill

Jeff Cameron

- Early versions of the code were also tested by the Palmer and Kralj labs

Computational toolboxes for single cell imaging

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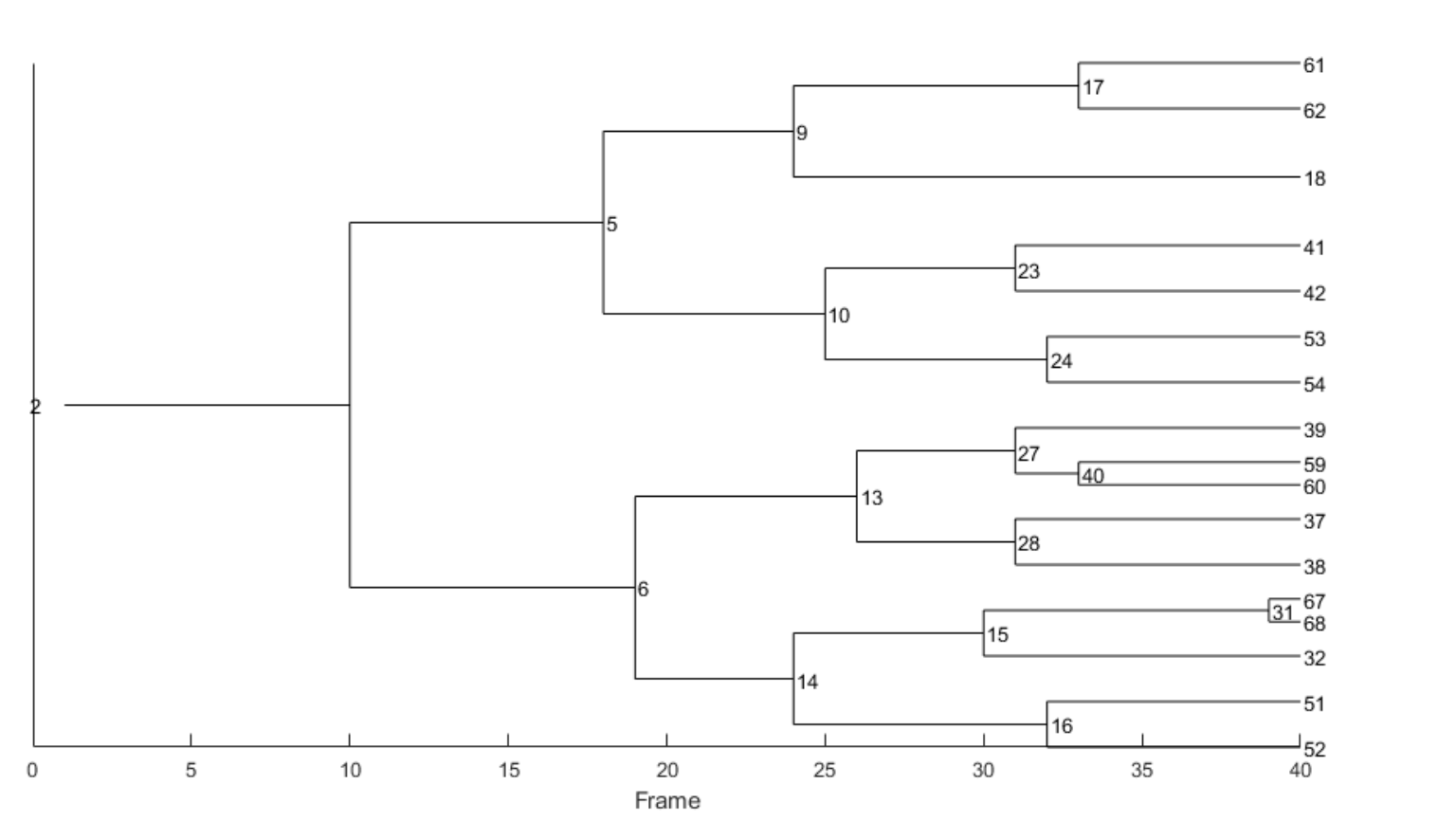
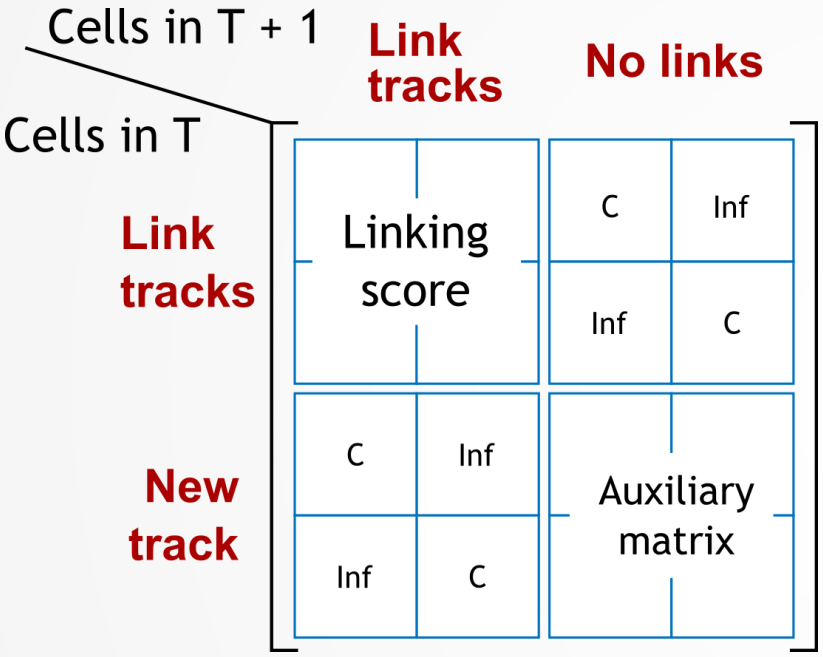
SCR Single Cell Single Molecule Supergroup

Feb 17 2020



- **Working on adding data overlays (e.g. number of spots) to the tree plot**

Tracking cells: The linear assignment approach



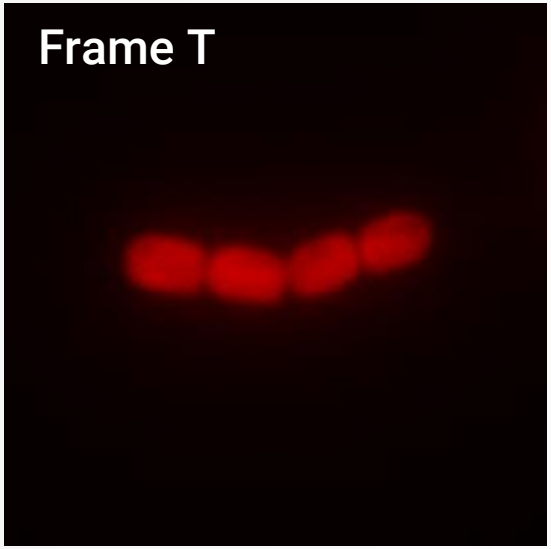
C = cost of alternative outcome

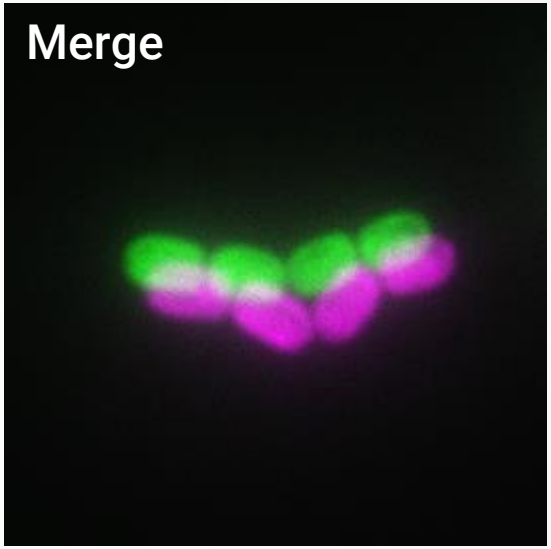
Alternative outcomes:

- No links – cell in frame T was not detected in frame T + 1 (drifted out of FoV or segmentation error)
- New track – cell in frame T + 1 was not previously found in frame T (division event or drifted into the FoV or segmentation error)

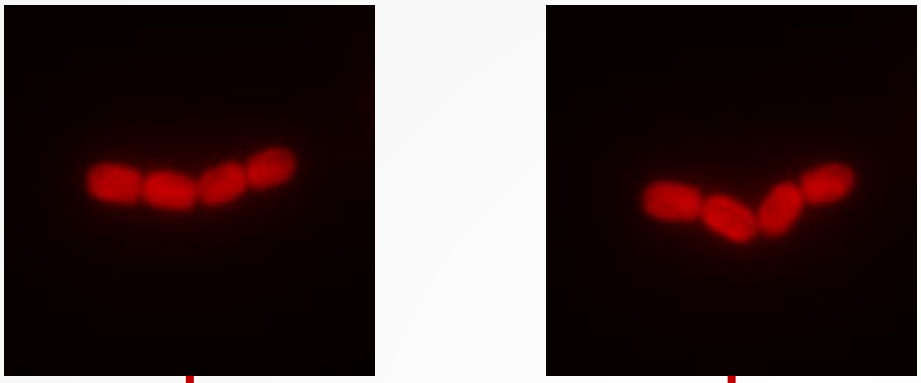
Jaqaman et al. Nature Methods 5, 695-702 (2008)

Example showing image drift between frames

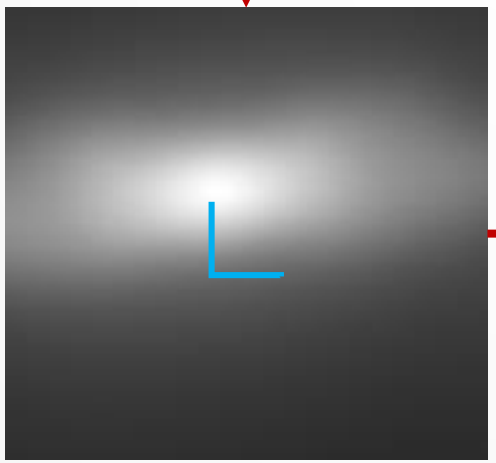




Drift correction

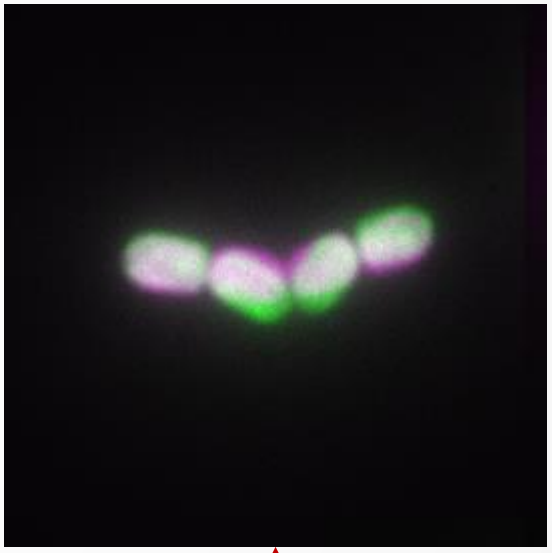


Compute product of 2D Fourier transforms
(cross-correlation)

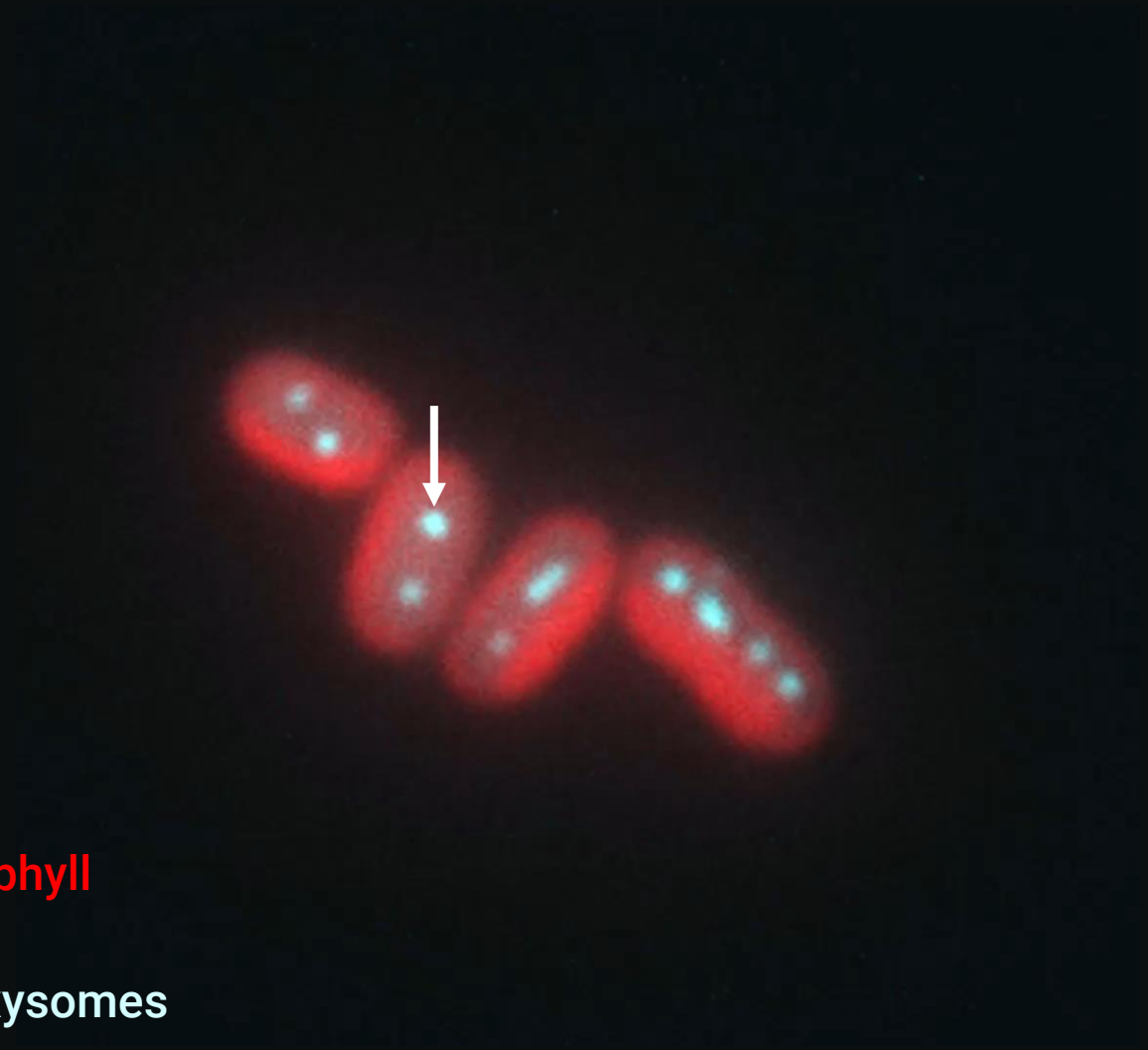


Distance from center to max
cross-correlation gives pixel shift

Drift corrected image



Why microscopy is important in SCSM research: An "ultraproductive" carboxysome?



Chlorophyll

Carboxysomes

Carboxysome activity were classified into three distinct phenotypes