encontro CIÊNCIA '16

Multimodal Microscopy Image Processing

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Outline

- Motivation
- The problem
- Image processing methods
  - Image segmentation
  - Bacteria tracking
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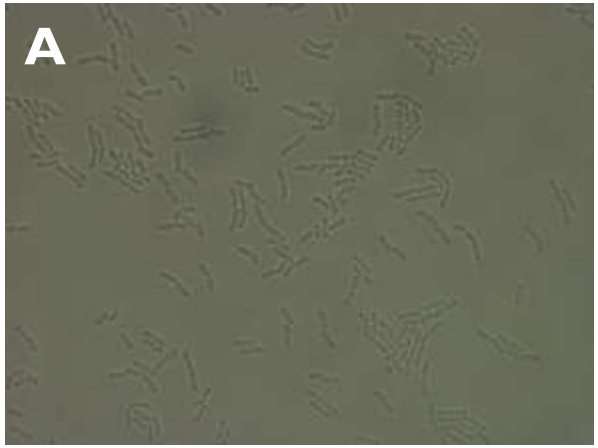
  - Spot detection
  - Nucleoid segmentation
- Conclusions and Next work

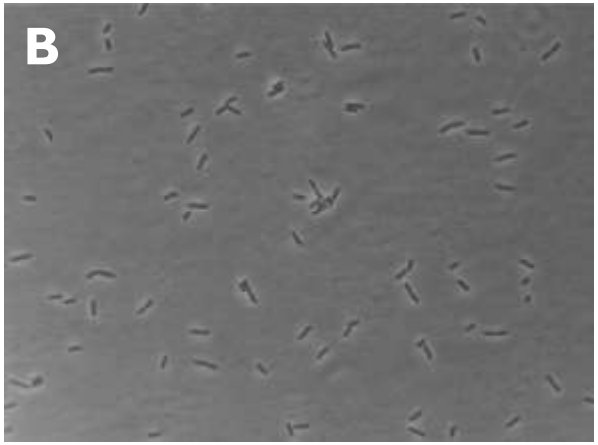
Motivation

- The need of fast and reliable methods of single cell evaluation for statistically significant studies
- Extraction of individual cells information from bacterial populations with large number of elements
- Avoid (as much as possible) manual analysis which is fastidious, time consuming and subject to observer variances

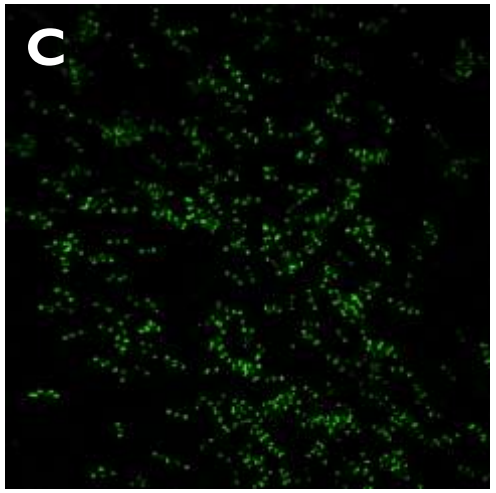
The problem



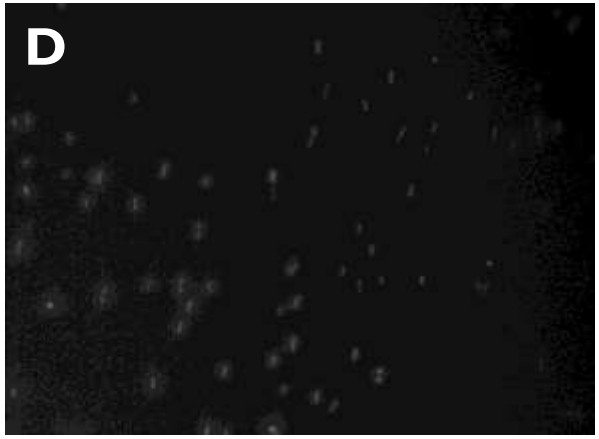
Diferential Interference Contrast Microscopy image



Phase contrast / Bright field image



Confocal Fluorescence Microscopy image



Epifluorescence image of DAPI stained nucleoids

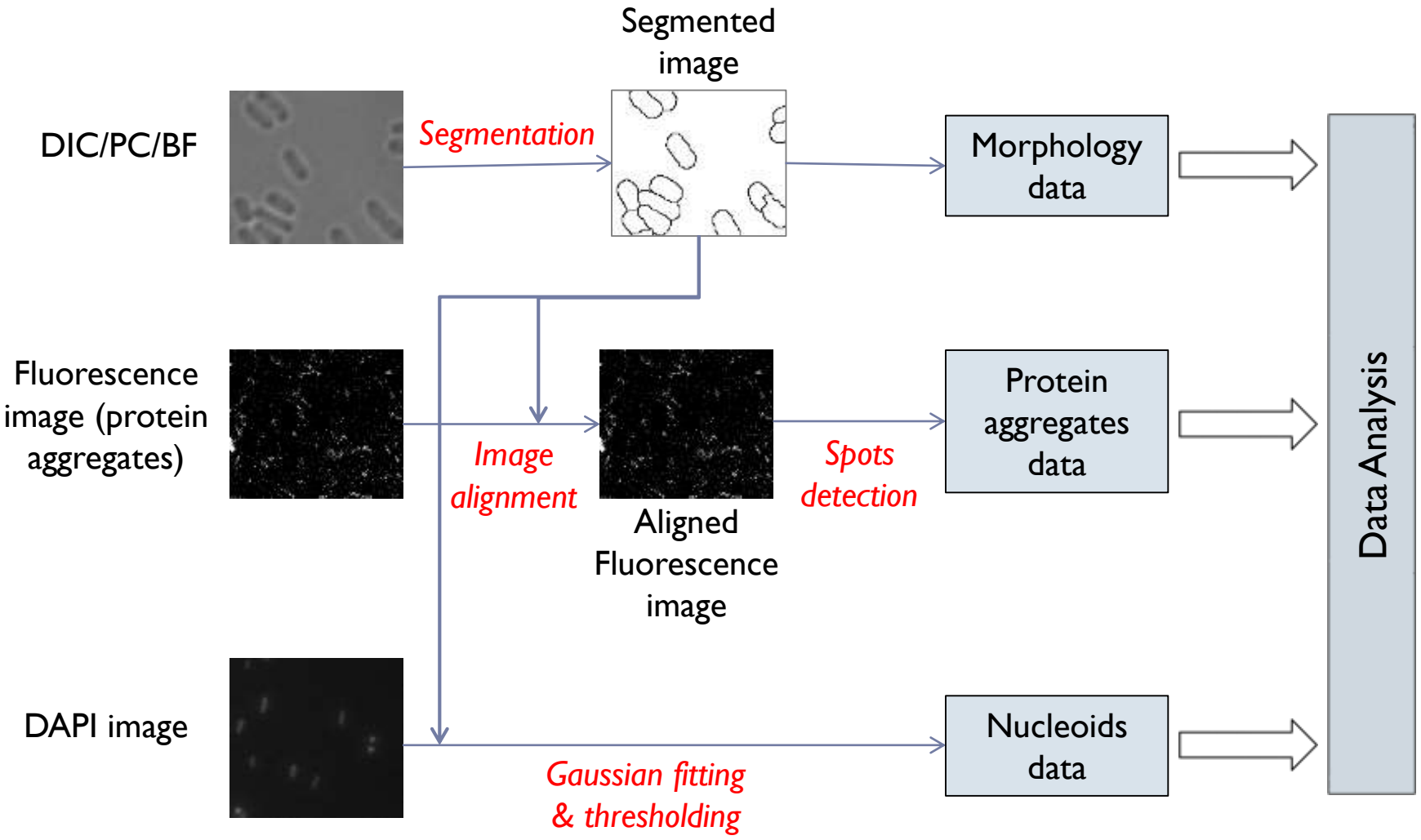
a) From A or B - extract morphology

b) From C – extract protein aggregates intensity, position and movement

c) From D – extract nucleoid dimensions and position

d) Analyse results and reach conclusions

Typical image processing pipeline



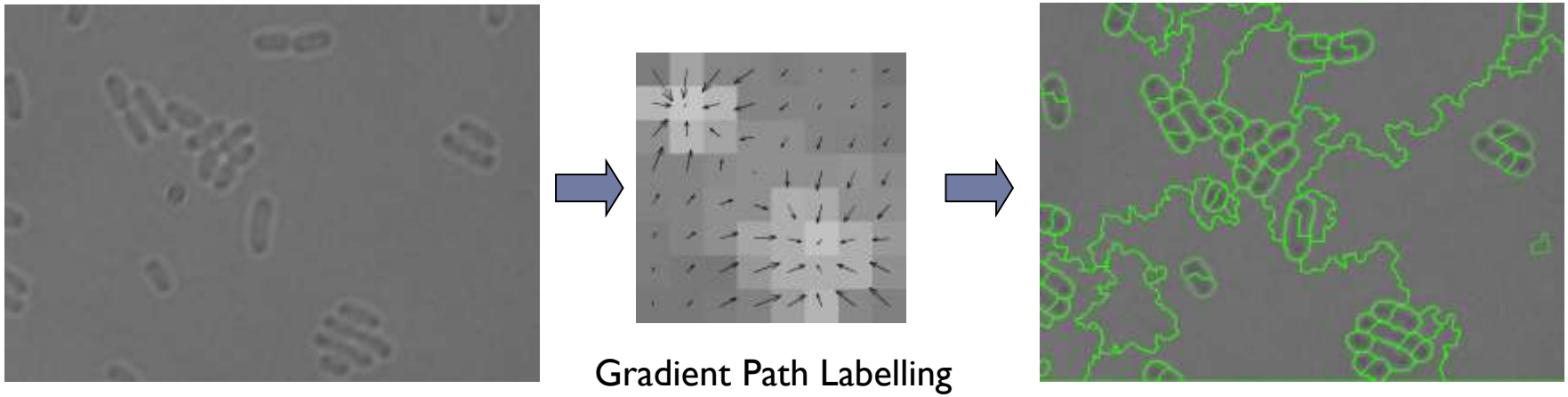
Bacteria segmentation

- Differential Interference Contrast
  - GPL performance is very good
  - Tuning to specific images characteristics is still important

- Phase contrast / Bright field
  - Modest GPL performance
  - Important manual segmentation/correction

The Gradient Path Labeling algorithm (GPL)

- An algorithm for maximums detection that was developed based on the labelling of ascending gradient paths



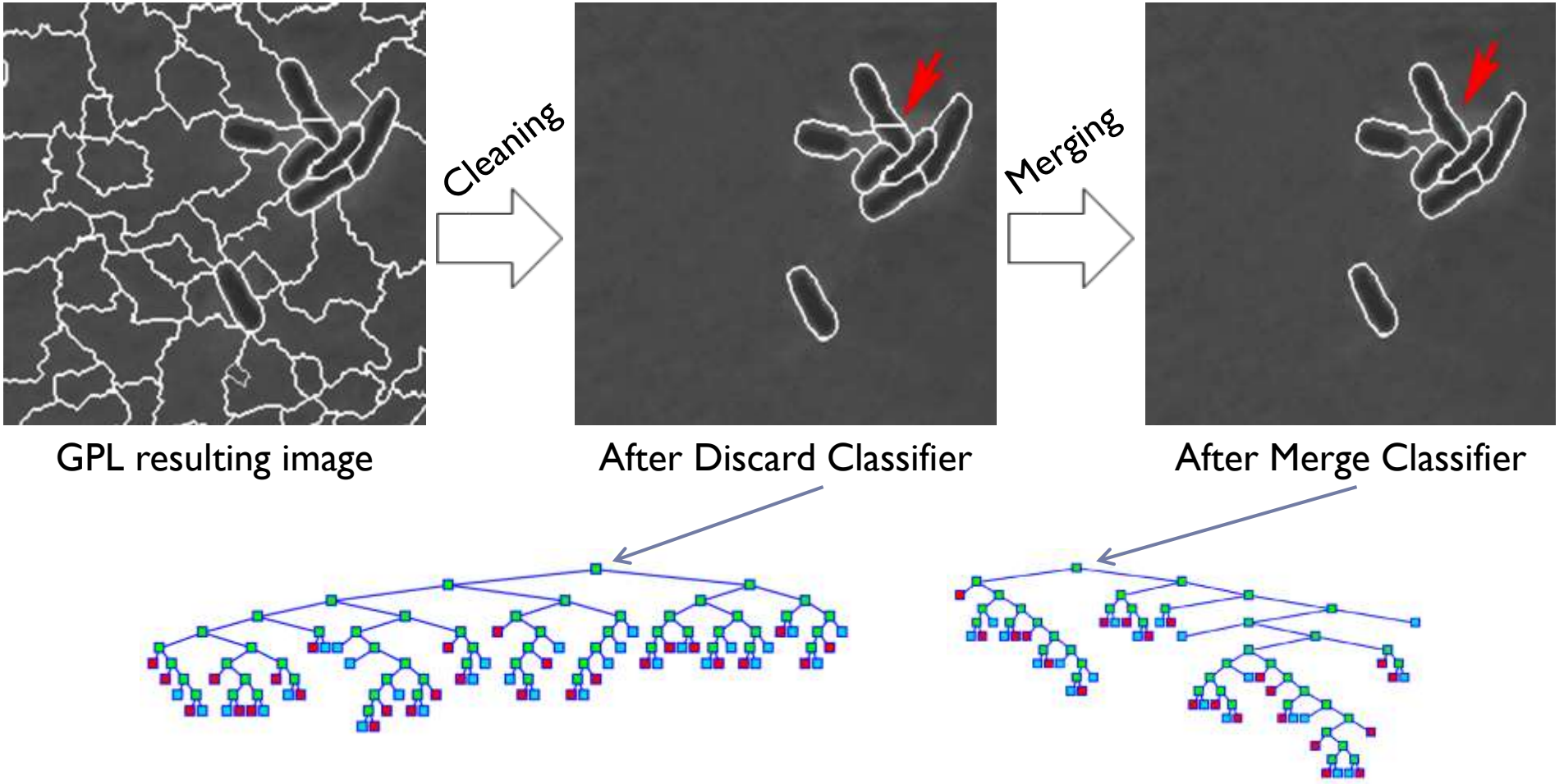
DIC image

Gradient Path Labelling

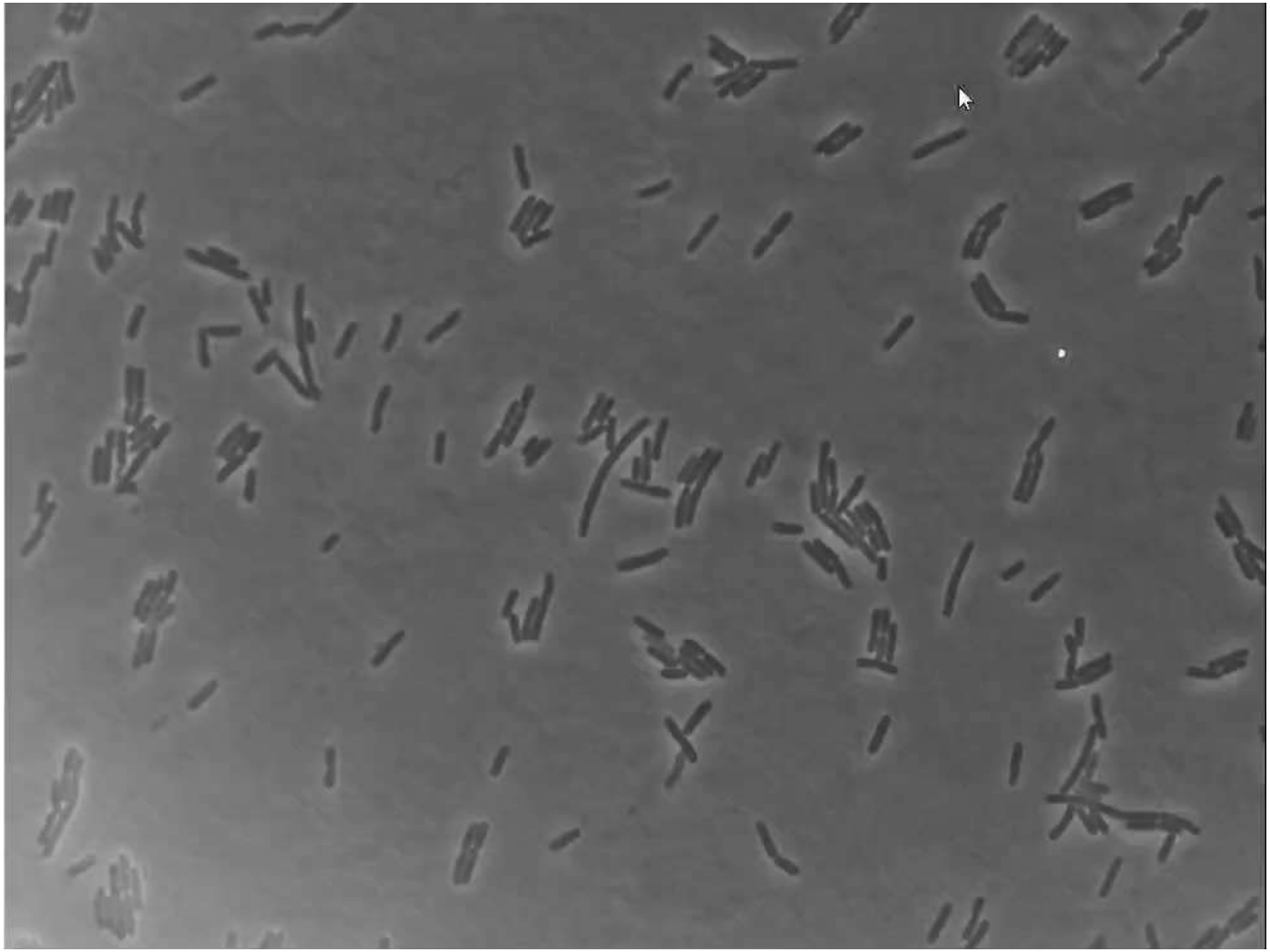
Segmented image

GPL segmentation cleaning and merging

(Phase Contrast images)



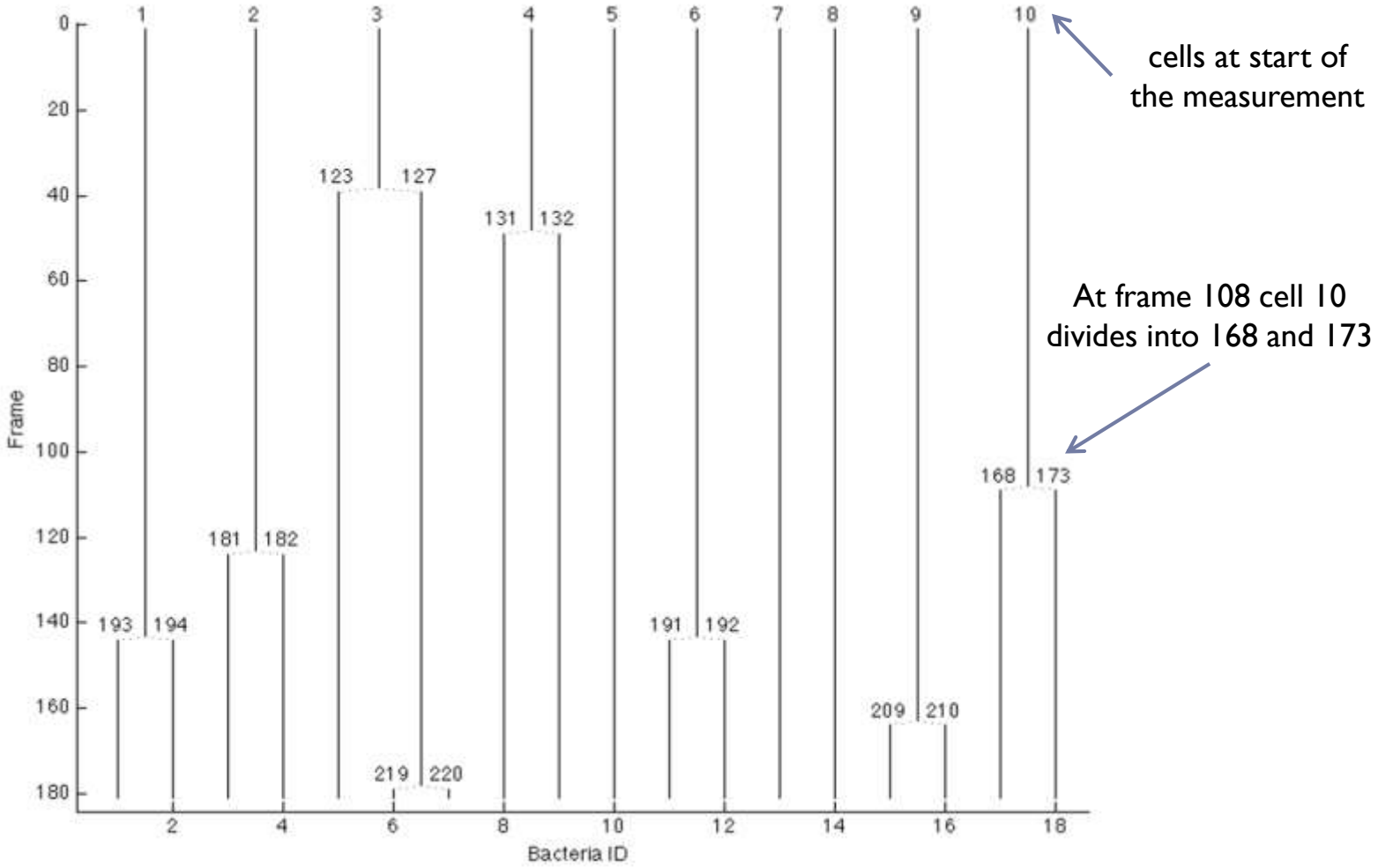
Computer aided manual segmentation



Morphology data extraction and cell tracking

- After segmentation, bacteria morphology is calculated
- Major axis and minor axis length and bacteria center are calculated by PCA
- Cell lineage is calculated from the segmented images
- The parent of a cell is the cell in the previous frame that most overlaps with it
- When two cells share the same parent it is assumed that they descend from it

Example of cell lineage plot



Cell tracking success

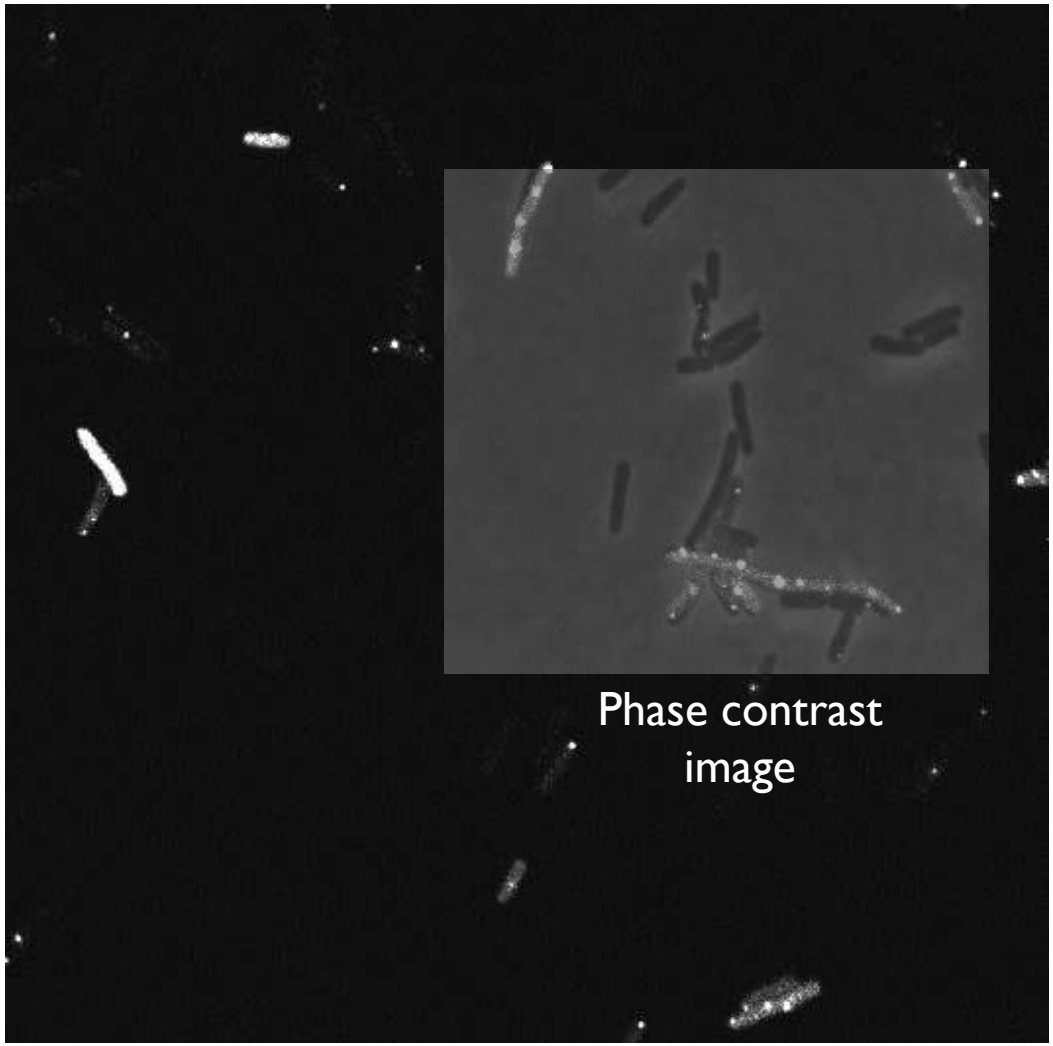
	22 °C	37 °C	43 °C	Total
Cell Tracking Error Percentage (Number of bacteria)	0% (162)	0% (115)	0.660% (909)	0.506% (1186)
Division Error Percentage (Number of divisions)	0% (58)	0% (52)	0.331% (302)	0.243% (411)

Cell tracking success depends on cell aggregates density

Multimodal image alignment

- After segmentation DIC/PC/BF images are aligned and scaled to fit fluorescence images
- Multi-modal images have usually different field of view and resolution
- Correlation based fitting produces good results in many cases
- Automatic alignment is sometimes more efficient or required

DIC/PC/BF vs Fluorescence images alignment

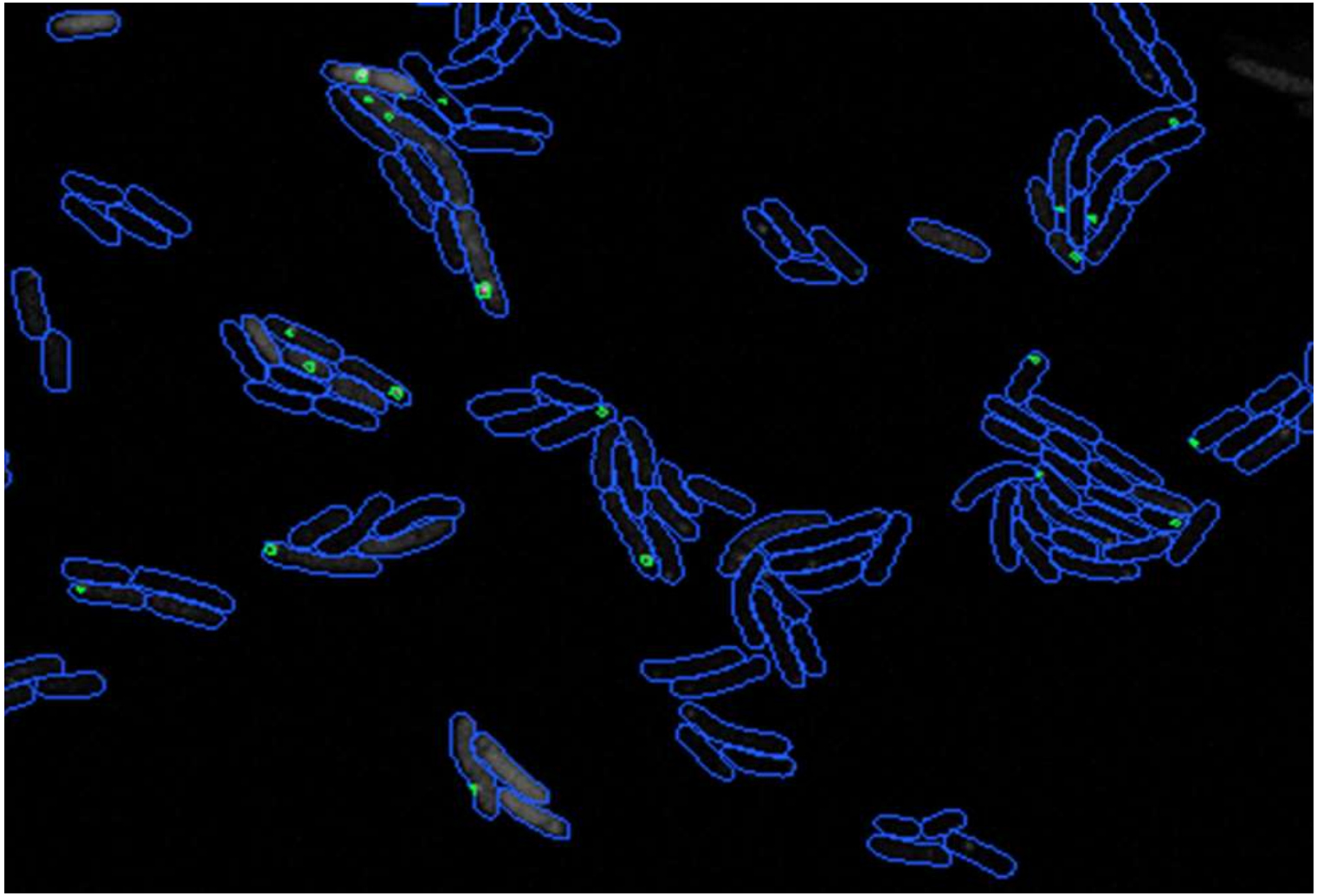


Multimodal image alignment

Spot detection

- For spot detection cell background intensity distribution is calculated
- Spots are thresholded assuming a Gaussian intensity distribution
- Threshold is pre-defined

Spots detection example



Spots detection success

	22 °C	37 °C	43 °C	Total
Number of spots	426	196	180	802
Number of false positives (FP)	16	11	5	32
Number of false negatives (FN)	0	1	1	2
Sensitivity	1,000	0,995	0,994	0,997
Precision	0,963	0,944	0,972	0,960
F1 score	0,981	0,969	0,983	0,978

Nucleoid Segmentation

- Nucleoid detection is done based on the GPL segmentation inside each cell (after segmentation)
- A modified three-dimensional modified Gaussian to fit each nucleoid
  - One nucleoid: $F(x,y) = G_1(x,y) + z_0$
  - Two nucleoids: $F(x,y) = G_1(x,y) + G_2(x,y) + z_0$

with

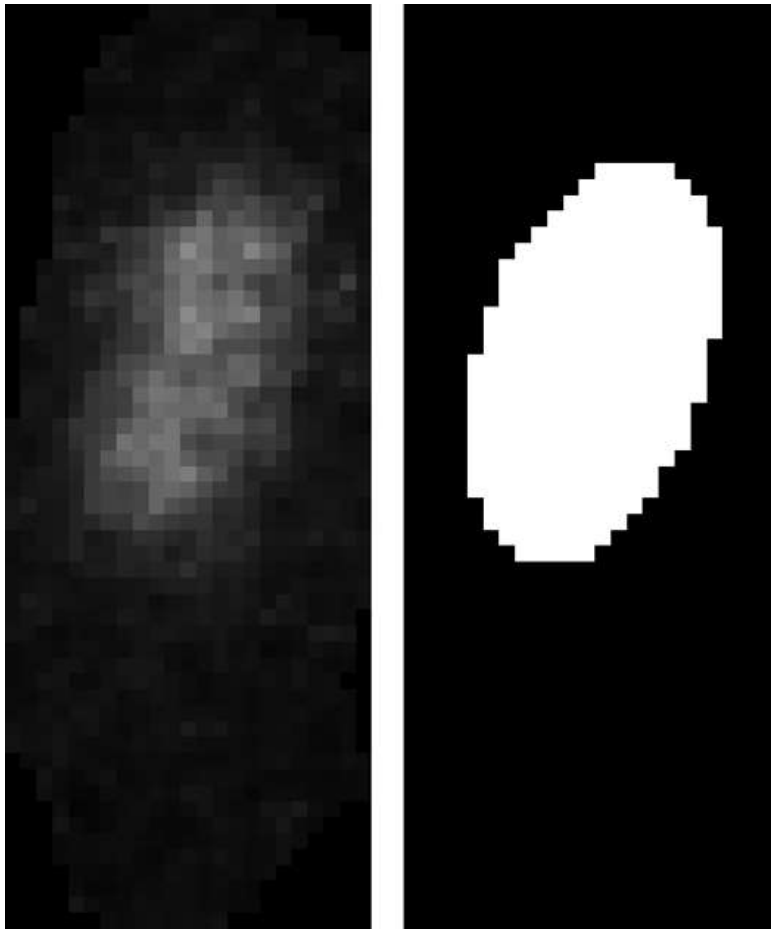
$$G_i(x,y) = A_i \cdot exp(-(a_i(x - x_{0i})^2 + 2b_i(x - x_{0i})(y - y_{0i}) + c(y - y_{0i})^2)^{(2/d)}$$

where

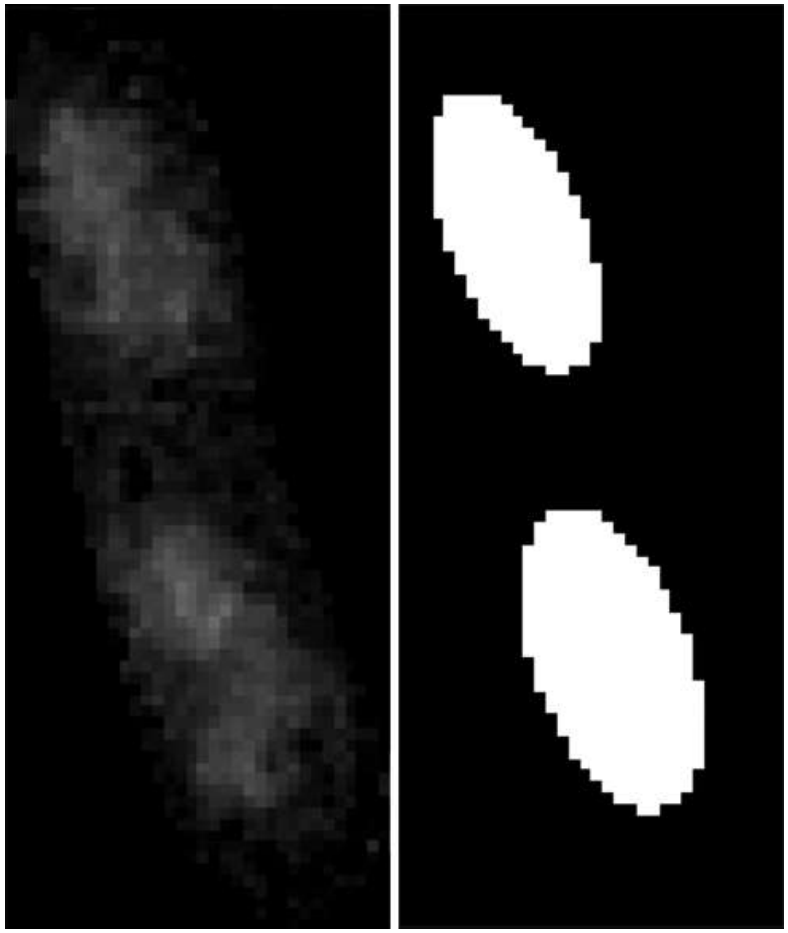
$$a_i = cos^2\theta_i / 2\sigma_{xi}^2 + sin^2\theta_i / 2\sigma_{yi}^2,$$
$$b_i = -sin\ 2\theta_i / 4\sigma_{xi}^2 + sin\ 2\theta_i / 4\sigma_{yi}^2,$$
$$c_i = sin^2\theta_i / 2\sigma_{xi}^2 + cos^2\theta_i / 2\sigma_{yi}^2,$$

Nucleoid Segmentation Results

One Nucleoid Detection:



Two Nucleoids Detection:



Example of nucleoid analysis results

Temperature	Numb. of Cells	Relative nucleoid **major axis** length	Relative nucleoid **minor axis** length
10 ℃	206	0.63 (0.12)	0.63 (0.09)
24 ℃	509	0.56 (0.11)	0.69 (0.09)
37 ℃	367	0.53 (0.11)	0.63 (0.09)
43 ℃	231	0.47 (0.12)	0.70 (0.12)

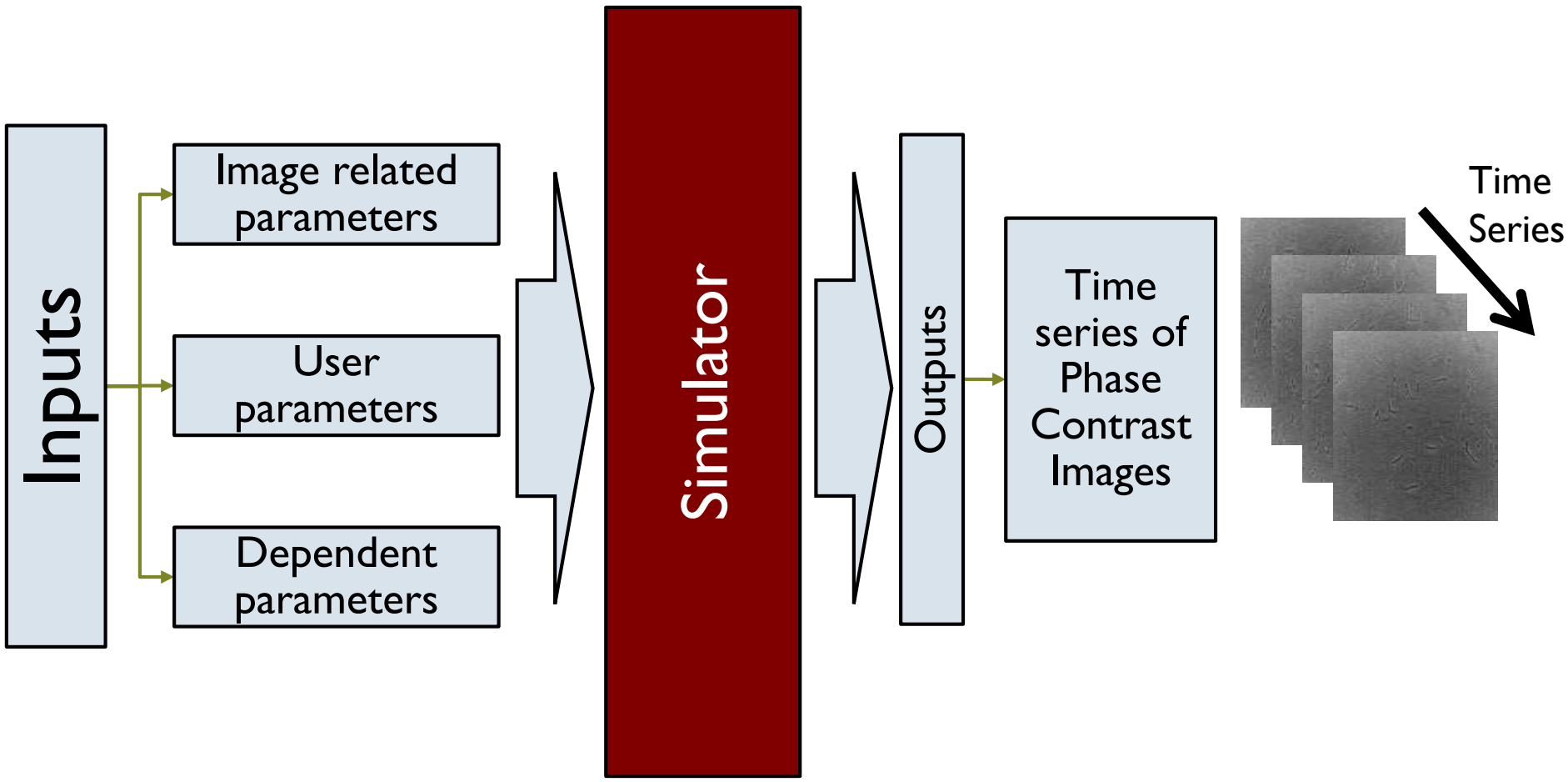
We can conclude that when temperature changes nucleoids keep their minor axis relative size while they reduce their major axis relative size.

Conclusions and future work

- General conclusion
  - The proposed methods shown to be able to extract useful information from individual cells with acceptable error

- However
  - Automatic bacteria segmentation should be improved – is Deep Learning the solution?
  - Improved cell tracking
  - Spots intensity evaluation
  - Software validation tools

miSimBa - *Microscopy Image Simulator of Bacterial Cells*



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