



**Quantification of dynamic recruitment of repair  
factors to DNA-Damage**

**and**

**Automated localization, tracking and classification of  
dividing cells in live cell movies**

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University of Konstanz



## **INCIDE**

### **Interdisciplinary Center for interactive Data Analysis, Modeling and Visual Exploration**

Bridge between:

- Computer Science
- Chemistry
- Biology
- Mathematics
- ...

Main topics:

- Analysis and visualization of large amounts of data
- High-throughput image processing



## INCIDE

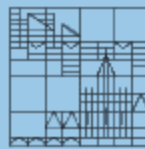
### Projects

- Molecule Tracking
- Neural Crest Cell Tracking
- Quantification of chromatin structure in neurons
- ...
- **Quantification of dynamic recruitment of repair factors to DNA-Damage**
- **Automated localization, tracking and classification of dividing cells in live cell movies**



# **Quantification of dynamic recruitment of repair factors to DNA-Damage**

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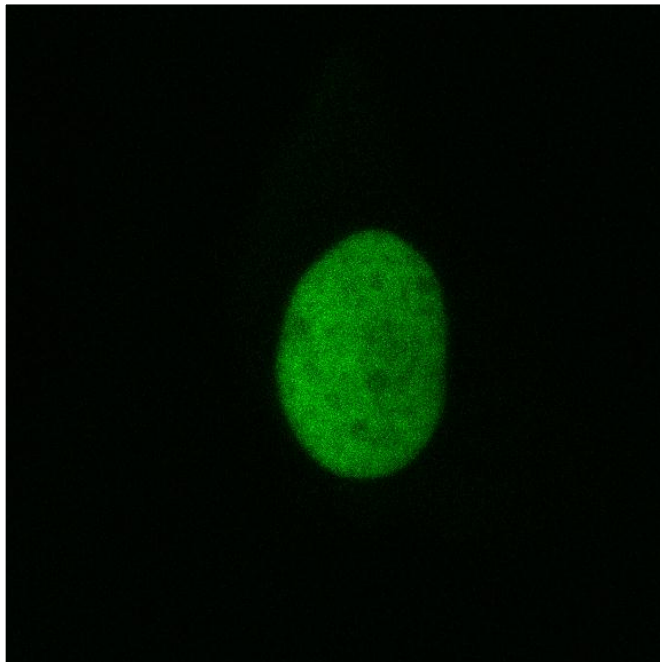


## Dynamic recruitment of repair factors to DNA-Damage

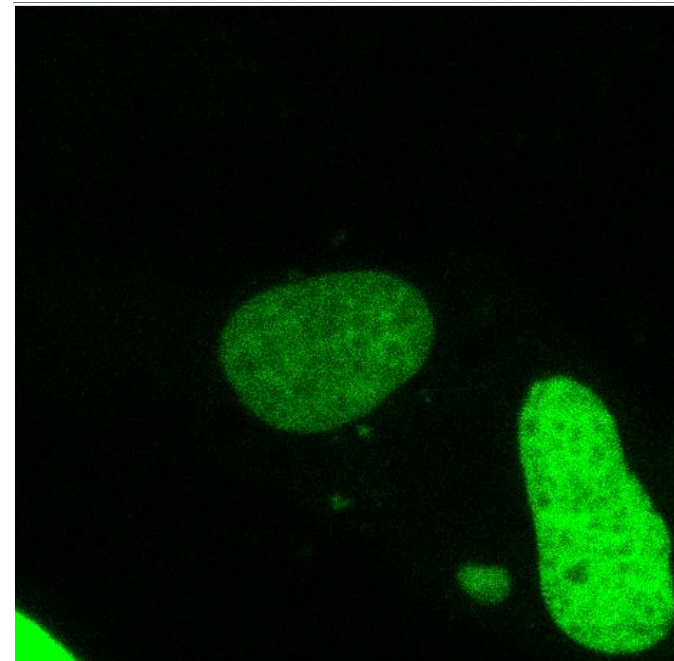
### Information

- Nucleus is shot and damaged by a laser
- Change of intensity over time in the damaged region quantifies the repair-protein

**A**



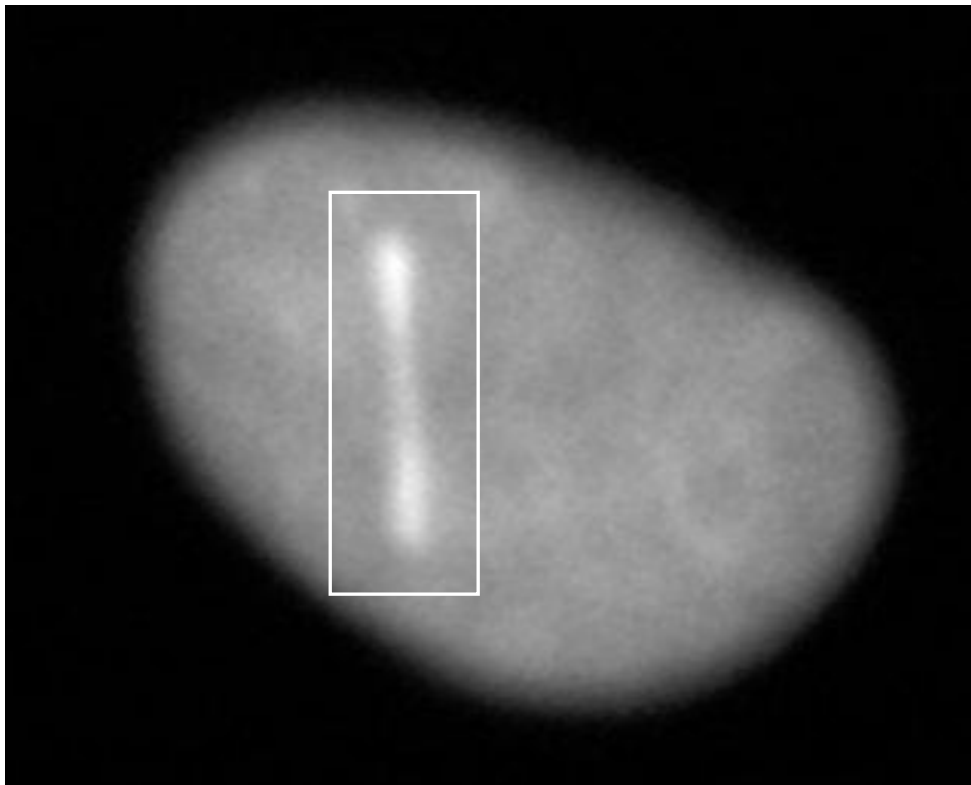
**B**





## Dynamic recruitment of repair factors to DNA-Damage

### Goal





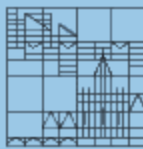
## Dynamic recruitment of repair factors to DNA-Damage

### Manual workflow - A lot of work to do...

- ImageJ helps
- Manual segmentation of the cell
- Manual selection of the damaged area
- Measurement and analysis with different tools (e.g. Matlab, Excel ...)

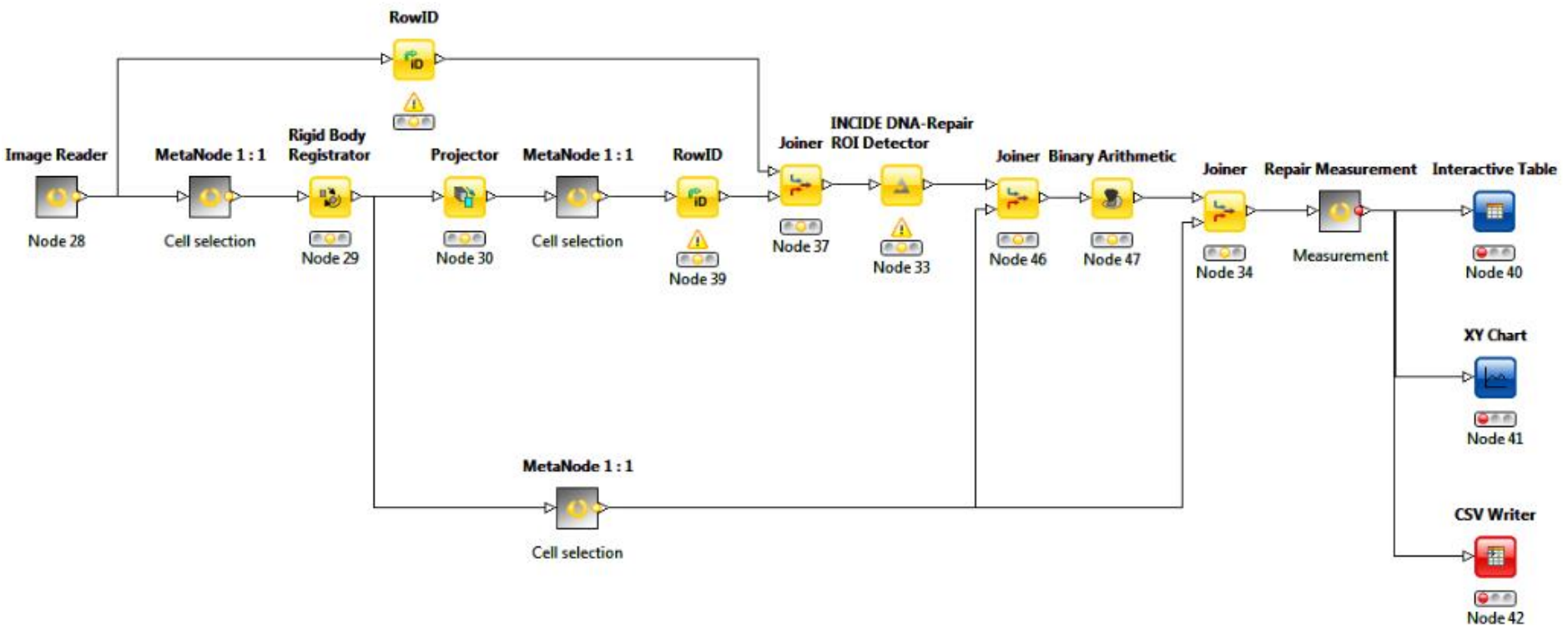
### Manual workflow - Problems

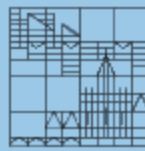
- Result is not reproducible
- Rater-depended
- But most important: **Time consuming!**



# Dynamic recruitment of repair factors to DNA-Damage

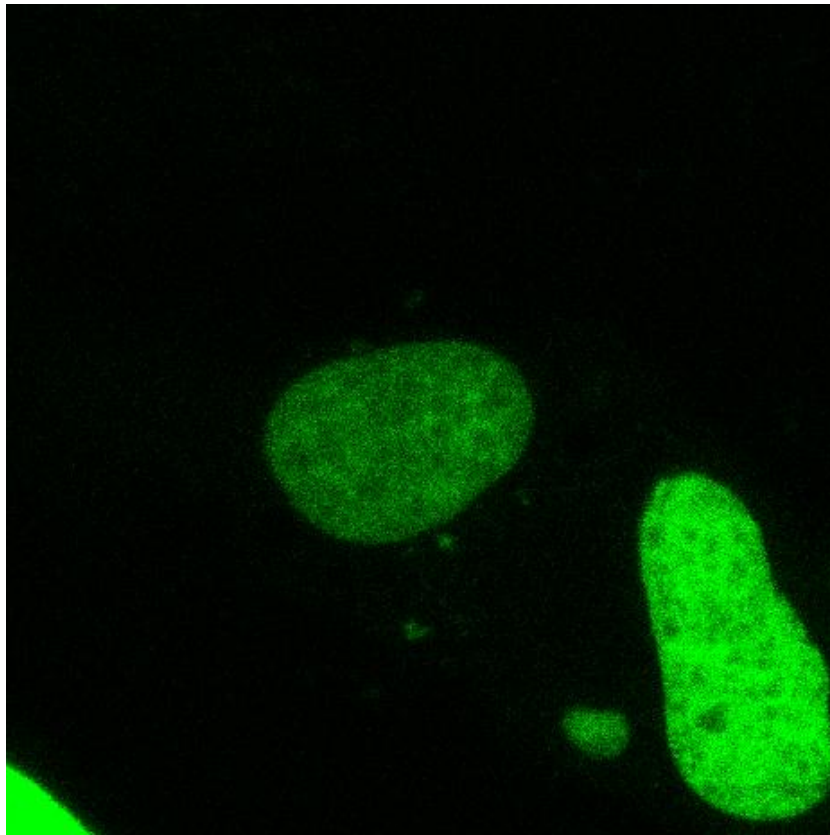
## KNIME Workflow - Overview





## Dynamic recruitment of repair factors to DNA-Damage

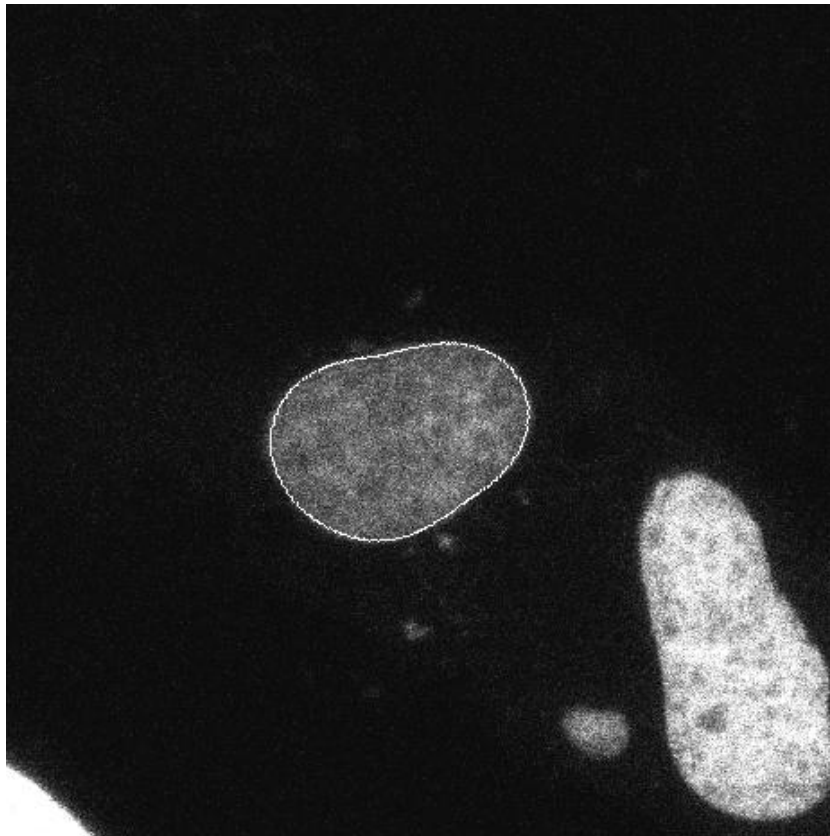
Algorithm: Segmentation of the cells

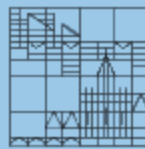




## Dynamic recruitment of repair factors to DNA-Damage

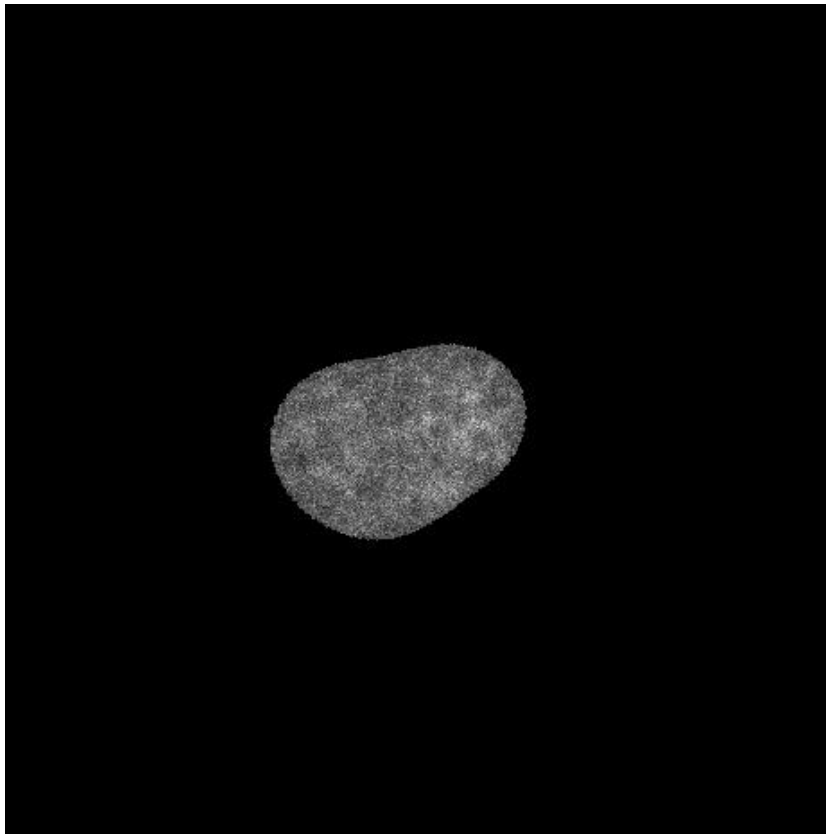
Algorithm: Segmentation of the cells

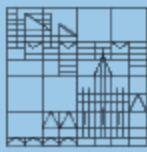




## Dynamic recruitment of repair factors to DNA-Damage

Algorithm: Cropping and Registration of the nucleus

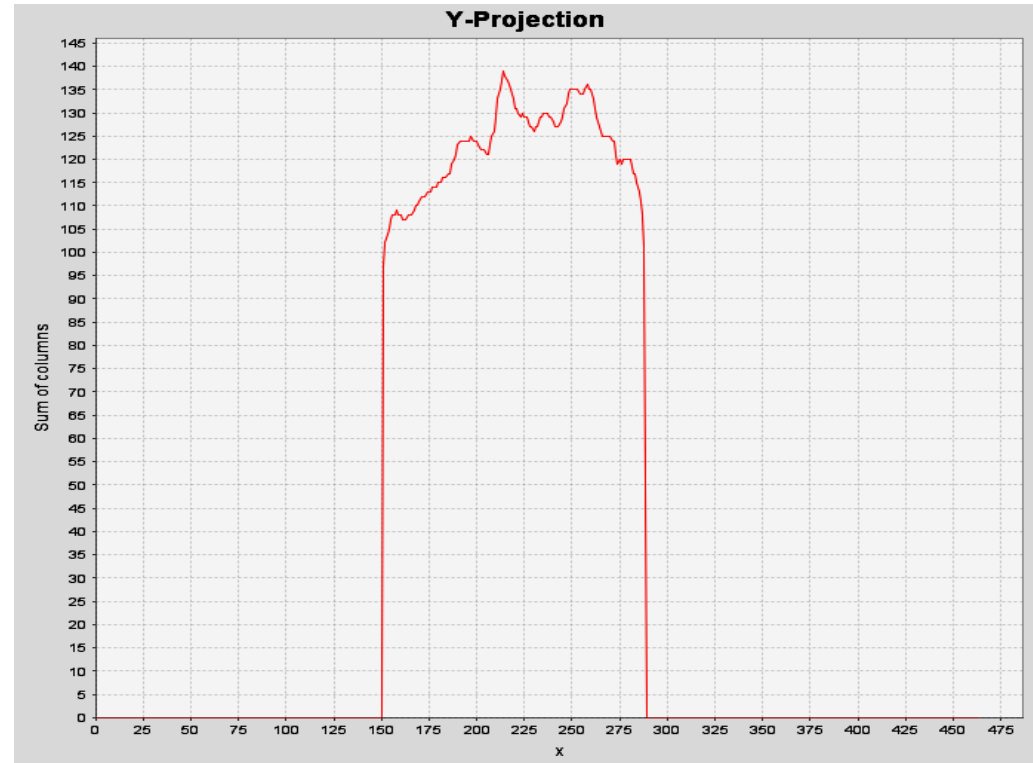
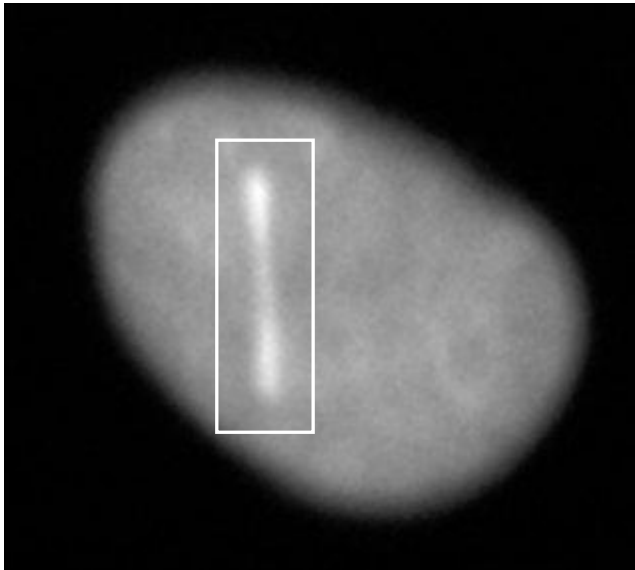




## Dynamic recruitment of repair factors to DNA-Damage

### Algorithm: Identifying the damaged part

- Score ( $\max_n$ ) = Intensity( $\max_n$ ) + Area gradient( $\max_n$ ) + CenterDist( $\max_n$ )





## Dynamic recruitment of repair factors to DNA-Damage

**Results** (101 Stacks with ~ 62 pictures per stack)

- Segmentation
  - ~ 99 % correct segmented
- Damage detection
  - ~ 99 % correct detected

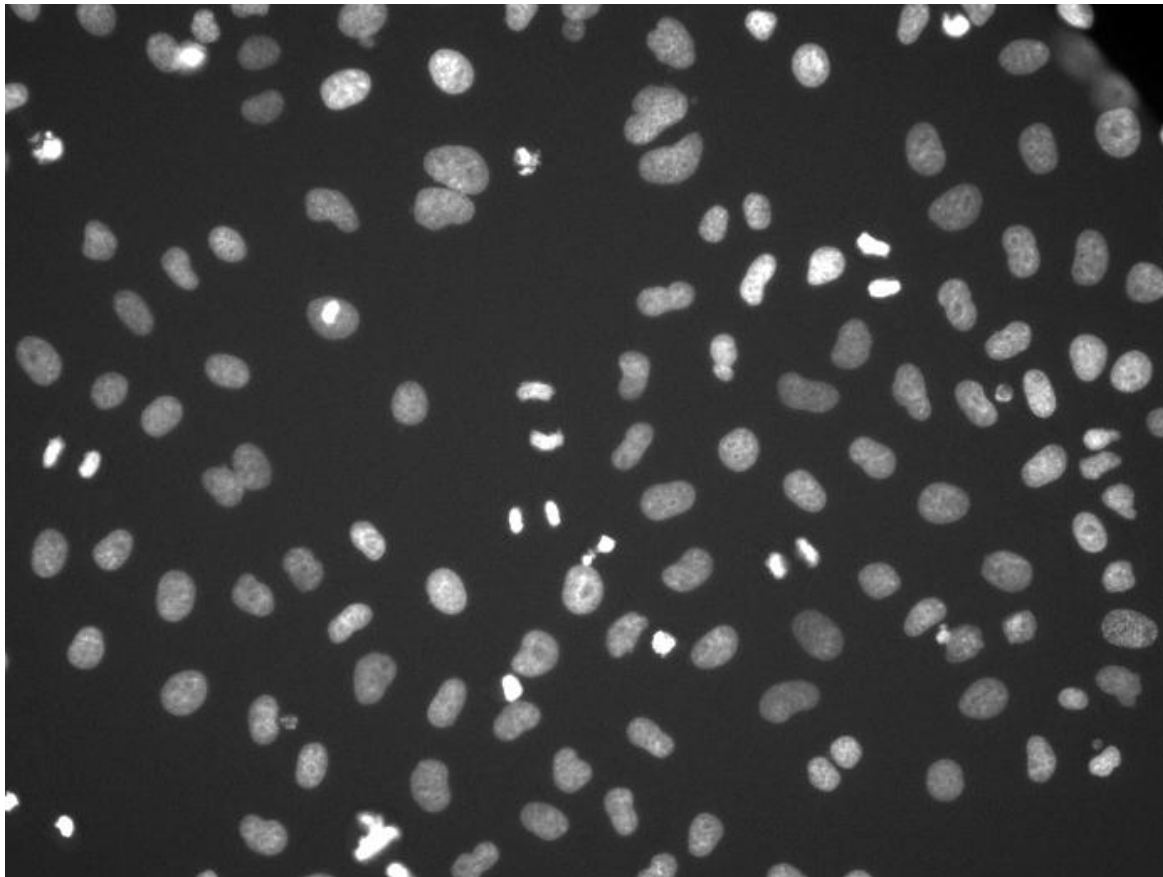


# **Automated localization, tracking and classification of dividing cells in live cell movies**



## Automated localization, tracking and classification of dividing cells

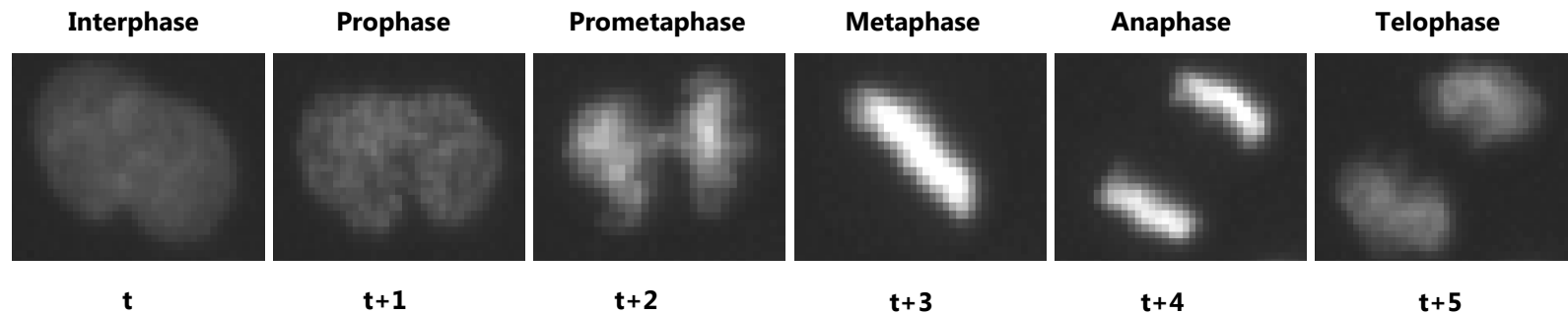
Movie





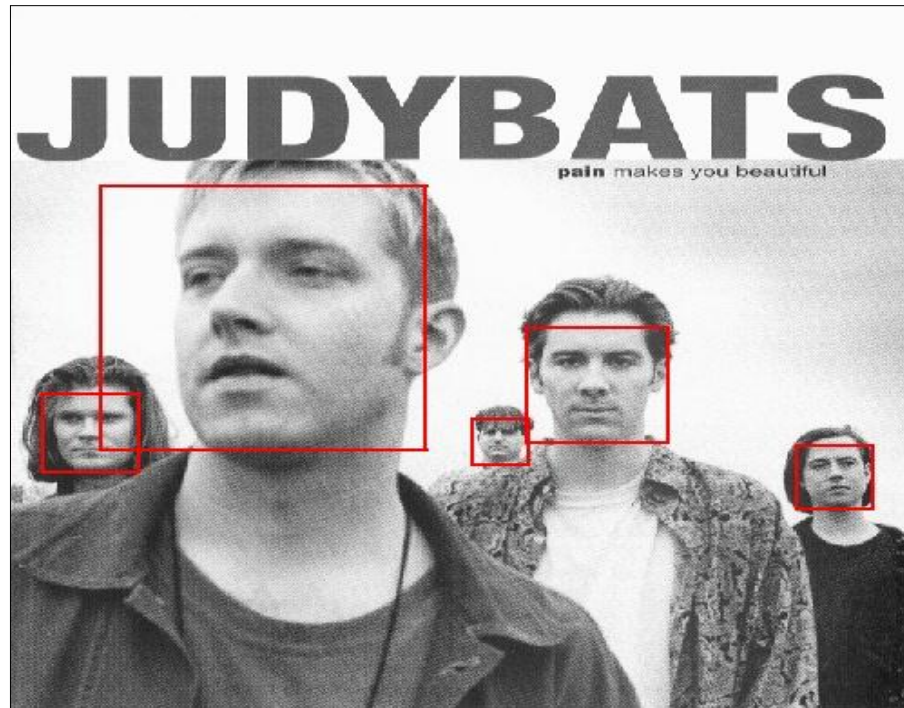
# Automated localization, tracking and classification of dividing cells

## Phases to classify





## Automated localization, tracking and classification of dividing cells



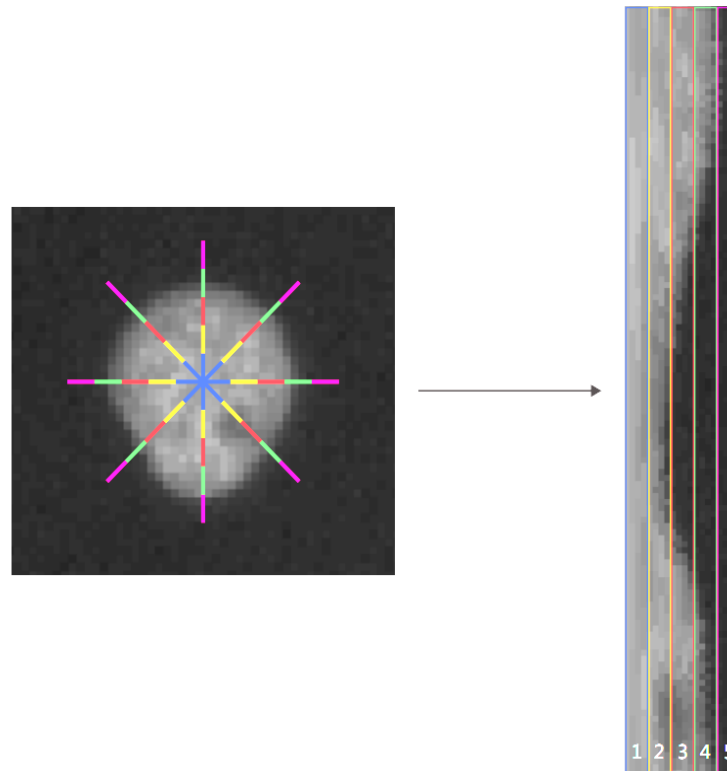
[Viola et al. 2002]

➔ **AdaBoost.SIP [Zhang 2009]: Localization, tracking and classification of cells**



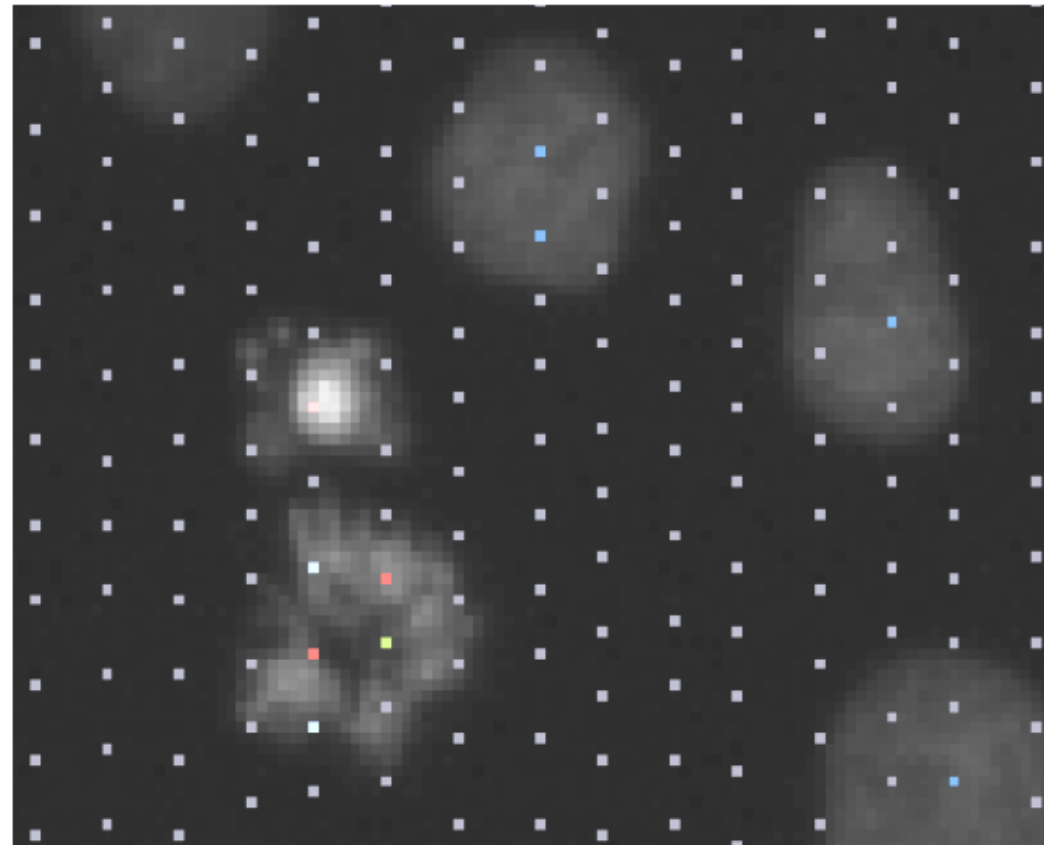
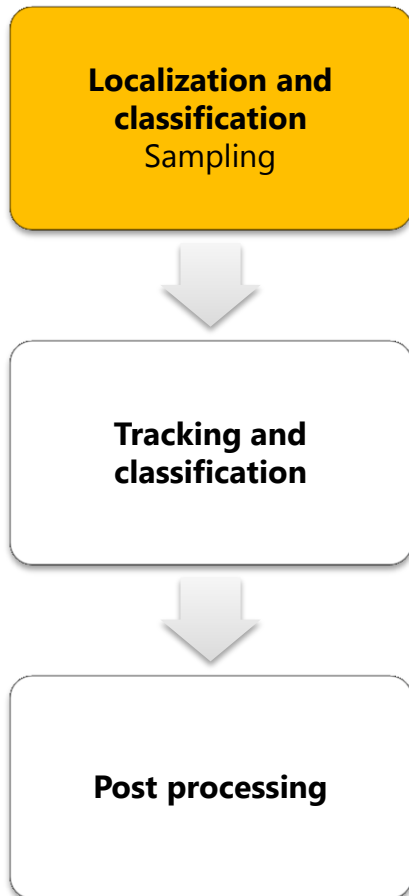
# Automated localization, tracking and classification of dividing cells

“Polar-Masks” for feature extraction





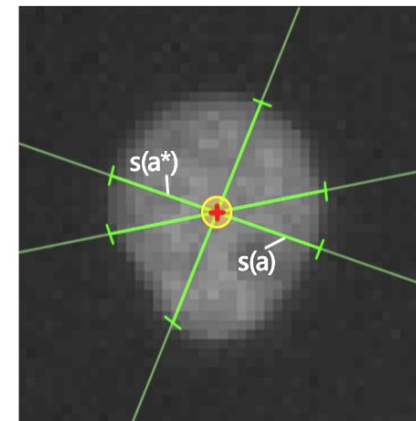
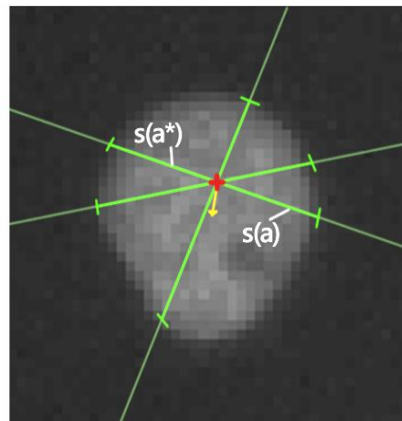
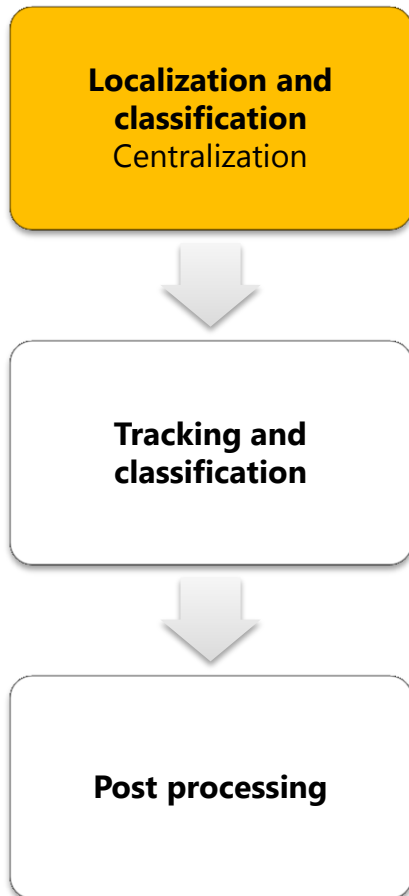
## Automated localization, tracking and classification of dividing cells



 Sampling the picture



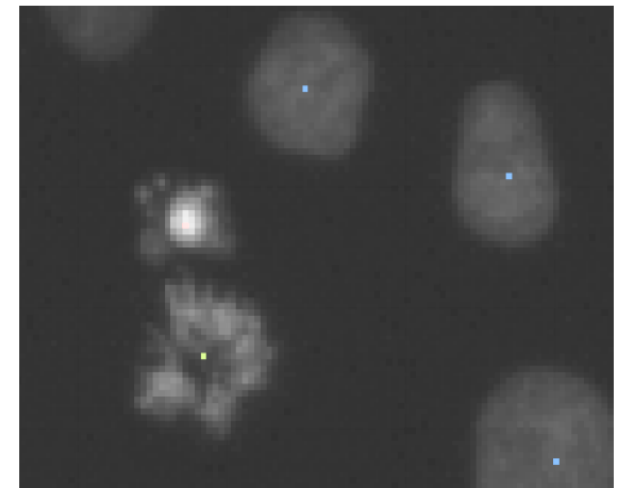
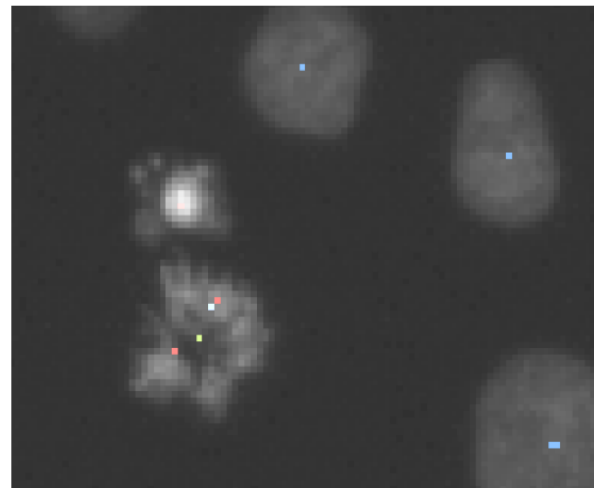
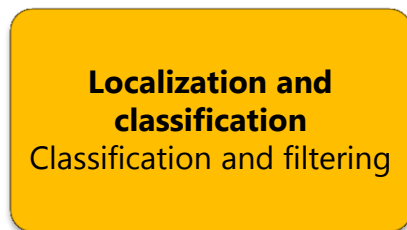
## Automated localization, tracking and classification of dividing cells



**➔ Adjust the sampling points towards the cell center**



## Automated localization, tracking and classification of dividing cells



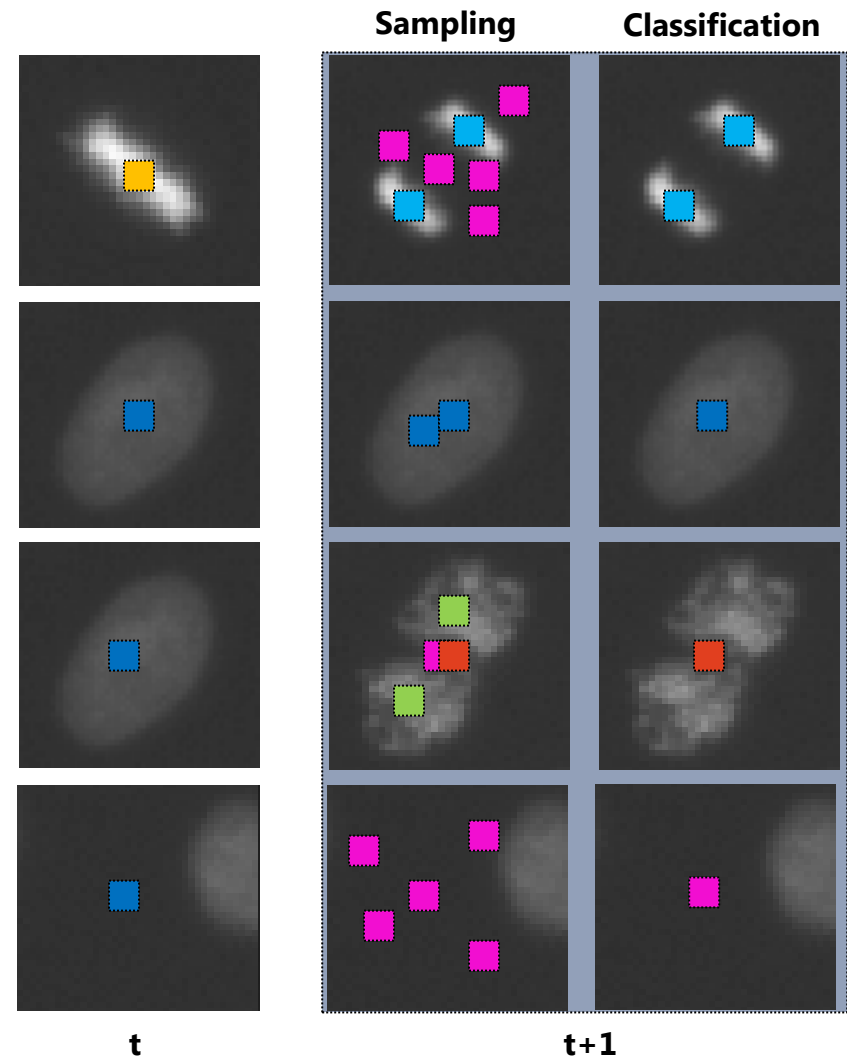
**Classify the sampling points and take the "best" ones**



# Automated localization, tracking and classification of dividing cells

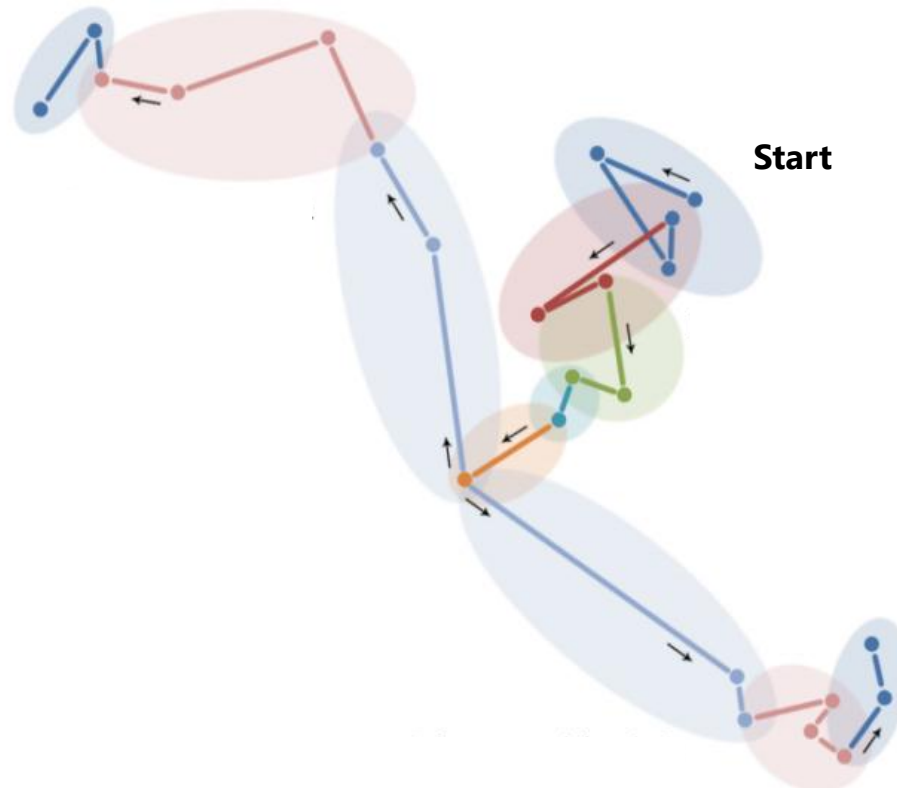


- Interphase
- Prophase
- Propmetaphase
- Normal Metaphase
- Abnormal Metaphase
- Anaphase
- Telophase
- Negativ



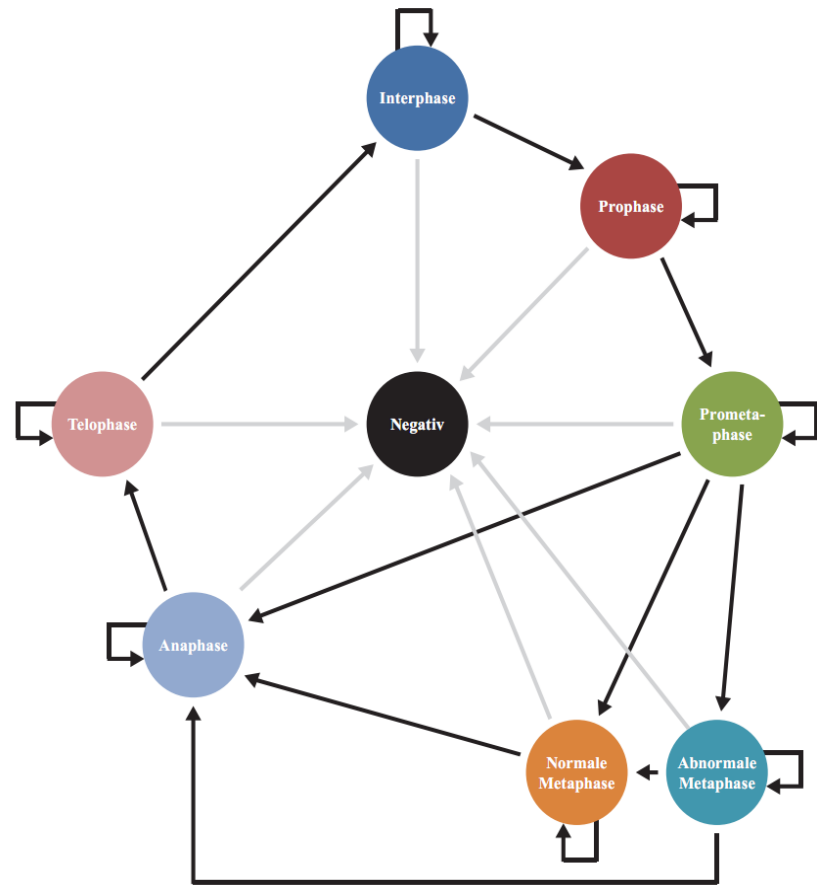
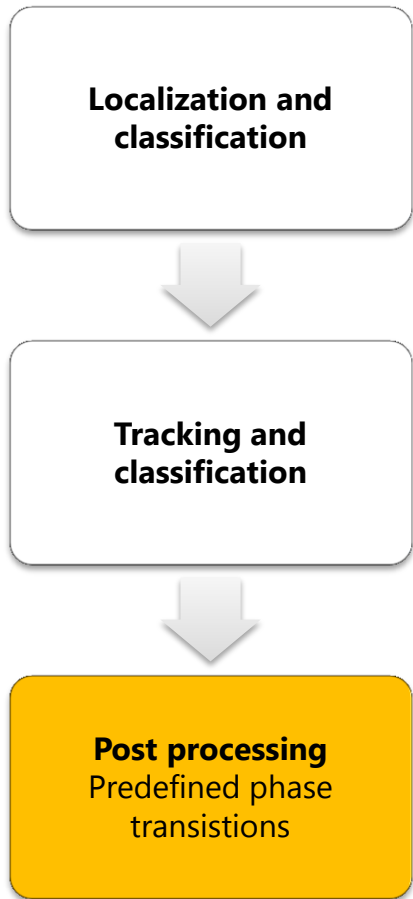


## Automated localization, tracking and classification of dividing cells





# Automated localization, tracking and classification of dividing cells

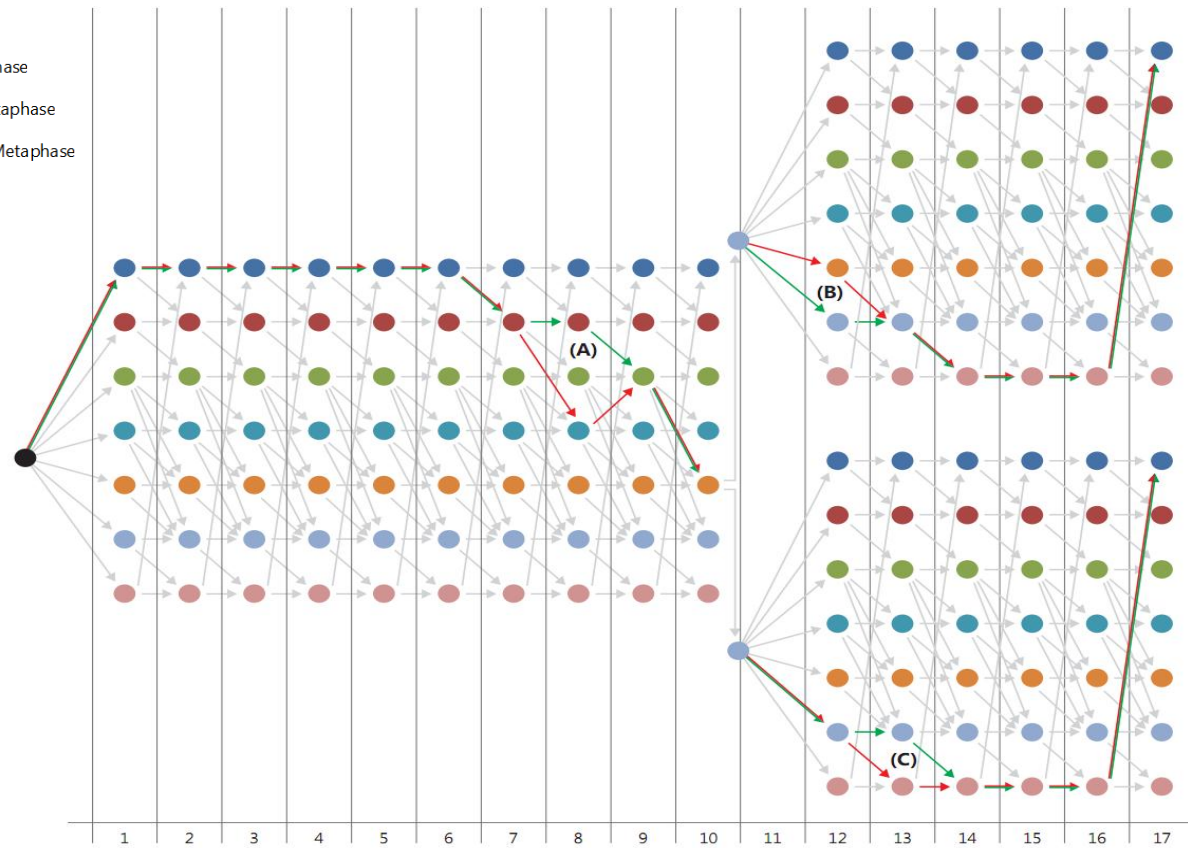




# Automated localization, tracking and classification of dividing cells



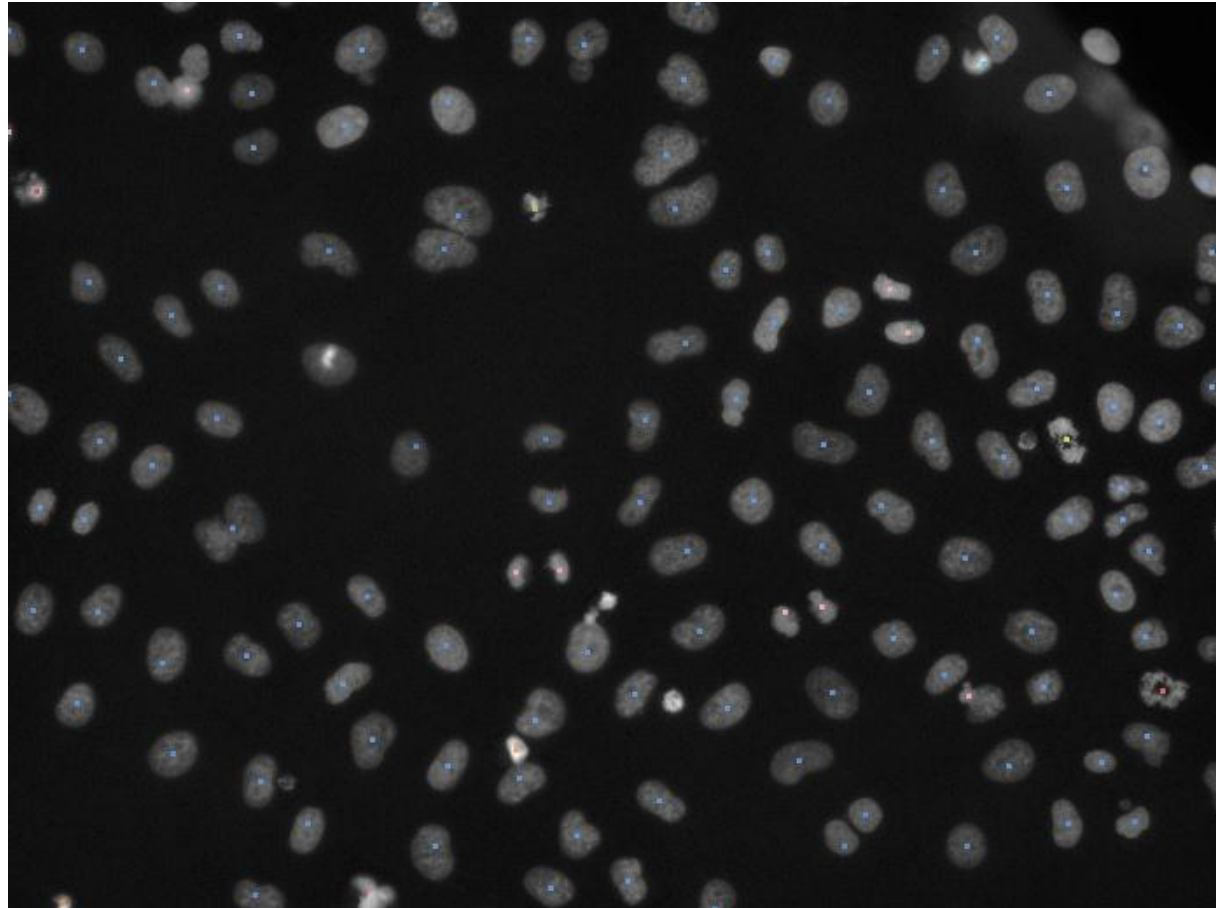
- Interphase
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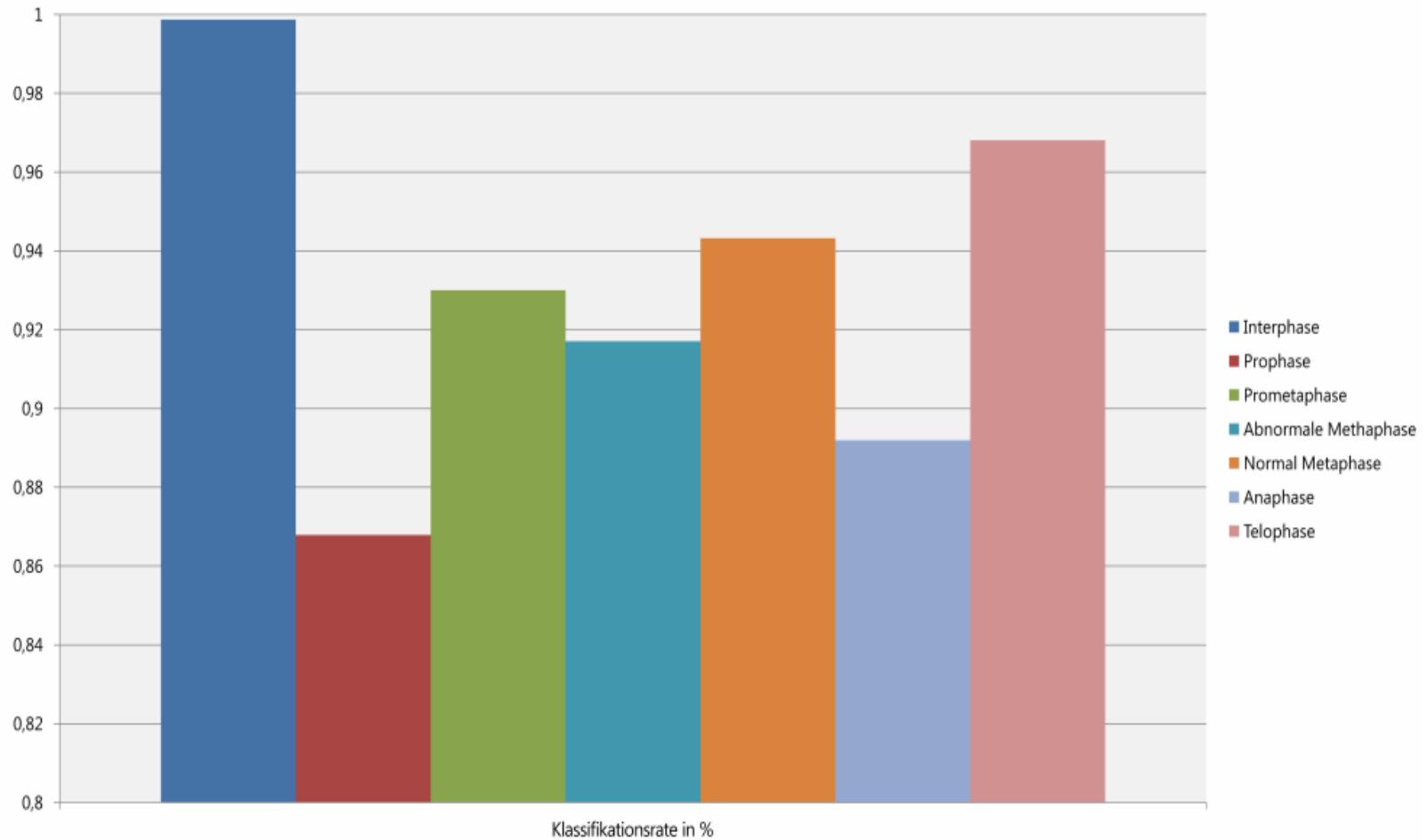
## Automated localization, tracking and classification of dividing cells

- Interphase
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- Negativ





## Automated localization, tracking and classification of dividing cells





## Automated localization, tracking and classification of dividing cells

### Advantages

- No precise segmentation needed
- Classification results improve the tracking
- Still many extensions and improvements possible (e.g. reducing number of parameters)

### Open questions

- How to handle dead cells?
- Crossing trajectories
- Completely overlapping cells

### Future work

- Extension of the post processing
- Improvement of the AdaBoost.SIP
- Learning trajectories



Thank you