GNU Octave for Microscope Image Processing

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Micron

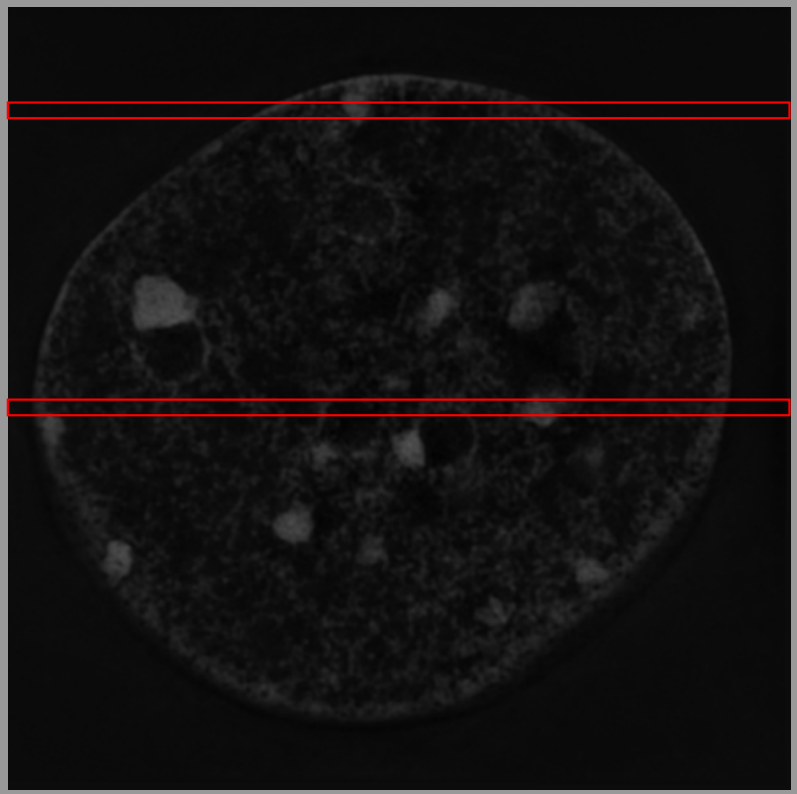
Develop and apply advanced microscopy methods for cell and developmental biology at the University of Oxford.

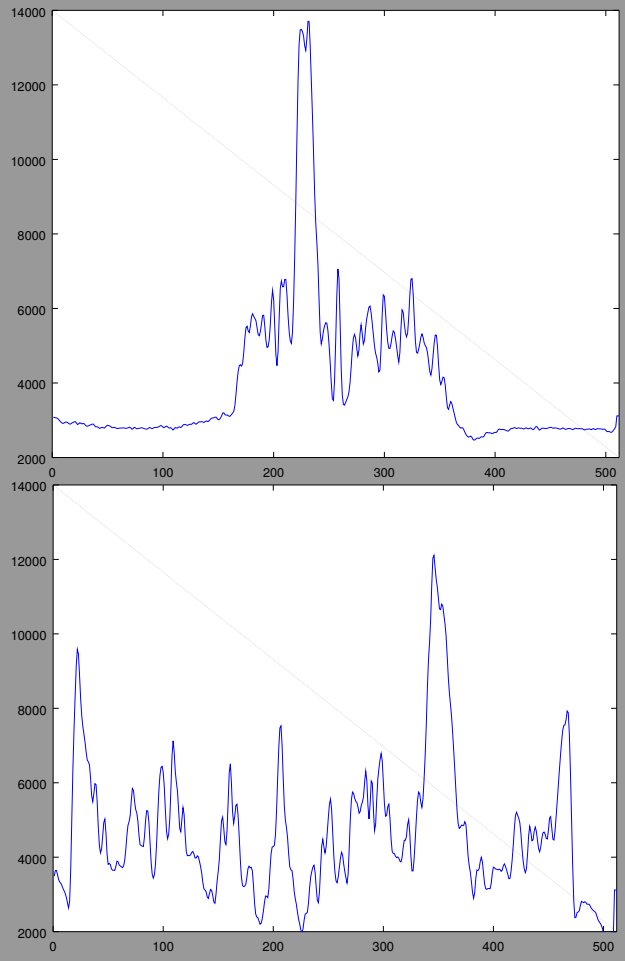
development Building new microscopes

facility Support researchers

Micron

Typical microscope images





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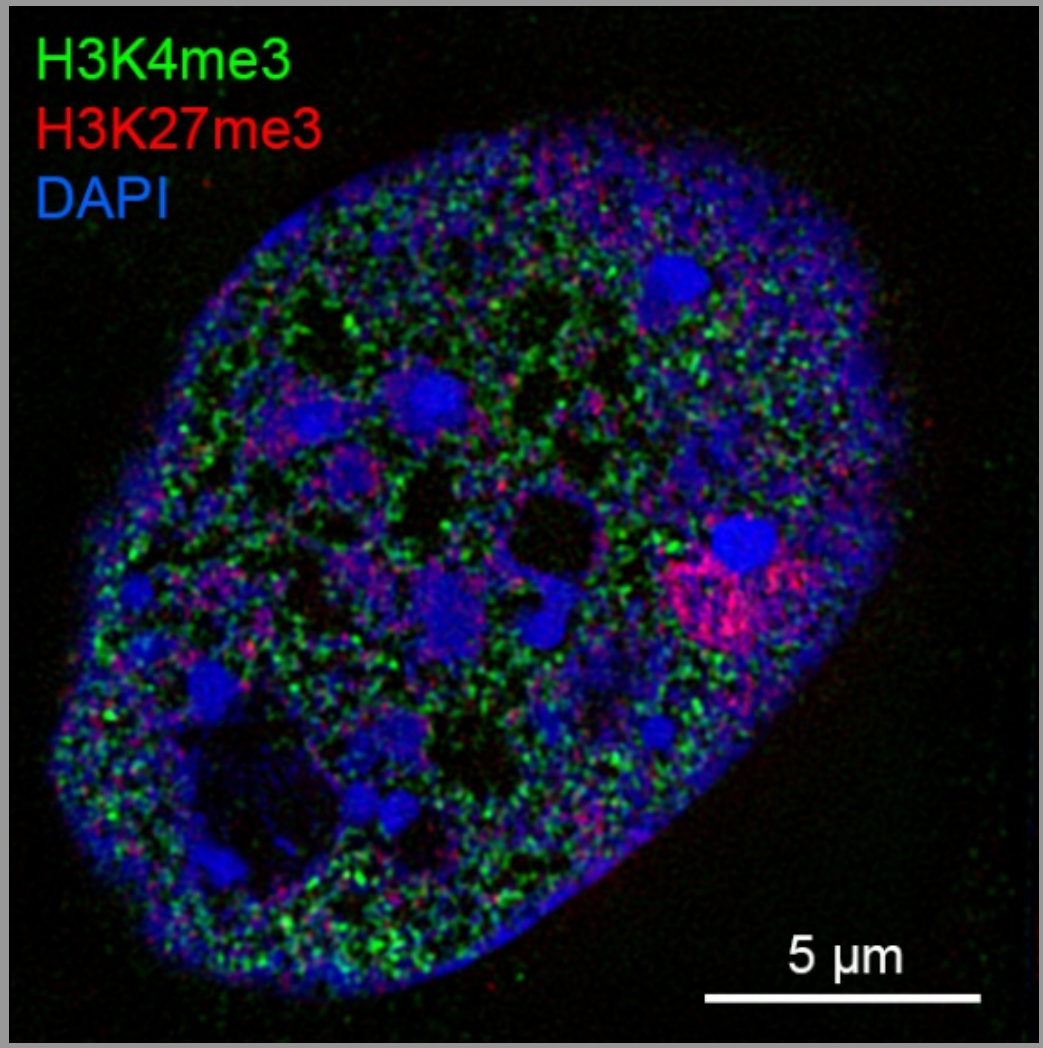

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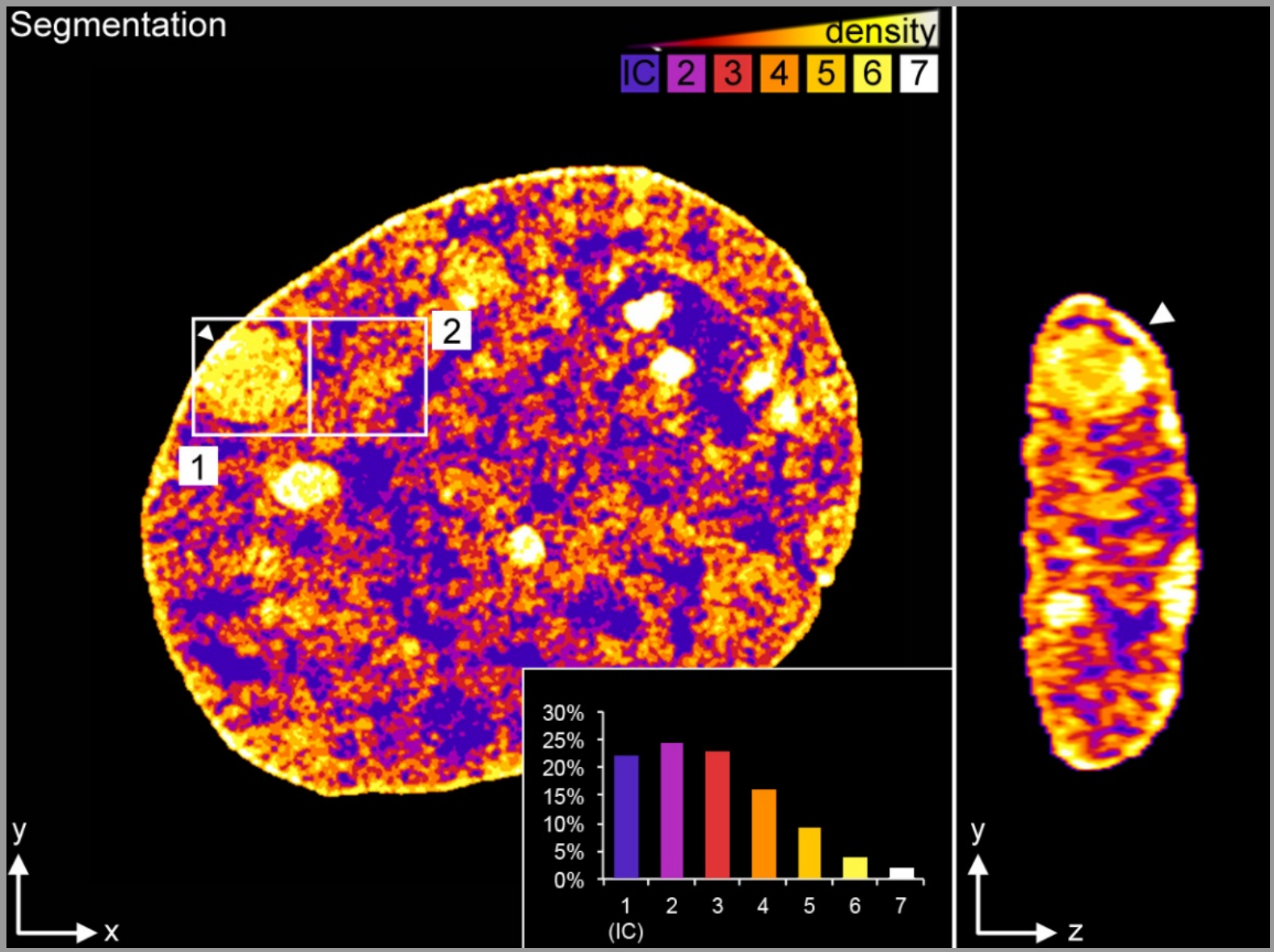
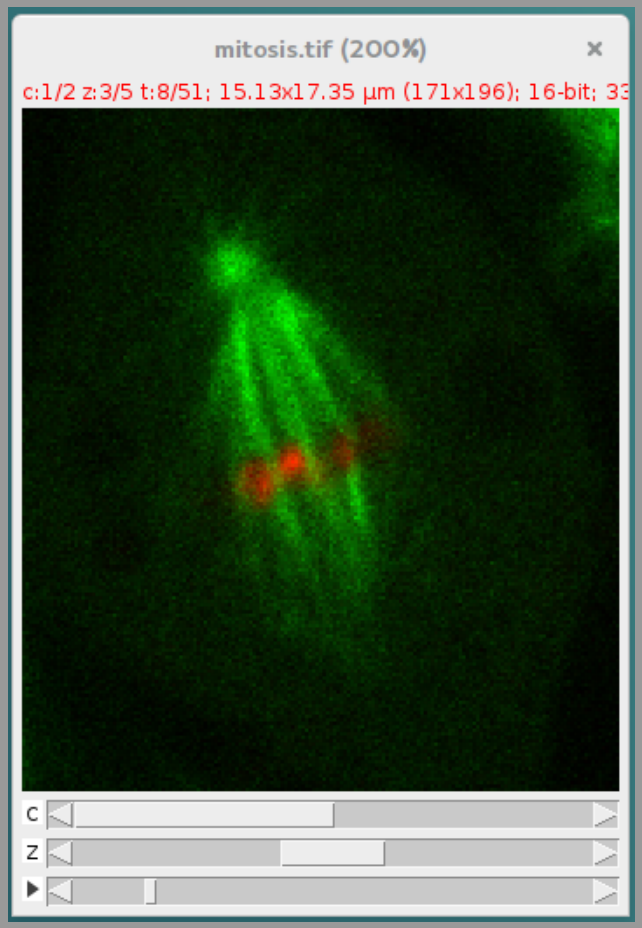


Typical microscope images

- 1024 * 1024 pixels
- 2 colours
- 21 Z stack
- 16 bit
- total of 44MB

- 512 * 512 pixels
- 1 colour
- 1 focal plane
- 2500 time frames
- 8 bit
- total of 655MB

Images as ND Arrays



- x and y
- time
- z (volume)
- wavelength
- phase
- angle
- lifetime

Think “data”, not “picture”

Absolutely barbaric

- `binary_dilation`
- `grey_dilation`
- `conv2`
- `convn`
- `bwlabel2`
- `bwlabeln`

Image functions

- When colour matters
  - imread
  - imwrite
  - rgb2hsv
  - lab2xyz
- Image related algorithms
  - imdilate
  - watershed
  - bwlabeln

Typical problems

Concentration
: Protein expression, number of complexes in a cellular compartment.

Co-localization
: Do two signals overlap and correlate?

Dynamics
: How fast does it move?

Why Octave

- Array programming language
- Logical indexing
- REPL
- Community
- Image package
- Octave Forge
- Free software

Analysis steps

1. Open image file
2. Identify regions of interest
3. Perform measurements

GraphicsMagick

Not really suitable for science

- No real image values
- No reliable bitdepth
- No reliable image type

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Surprisingly good enough.

GraphicsMagick

- TIFF is de facto standard in biological imaging
- TIFF is a multi-page

```
img4d = imread (fpath, "Frames", "all");
imwrite (img4d, fpath);
```

Bio-Formats

- Java library
- Reading of microscope images
- Focus on metadata
- Free software under GPL
- Matlab toolbox

`http://www.openmicroscopy.org/site/products/bio-formats`

Outcome

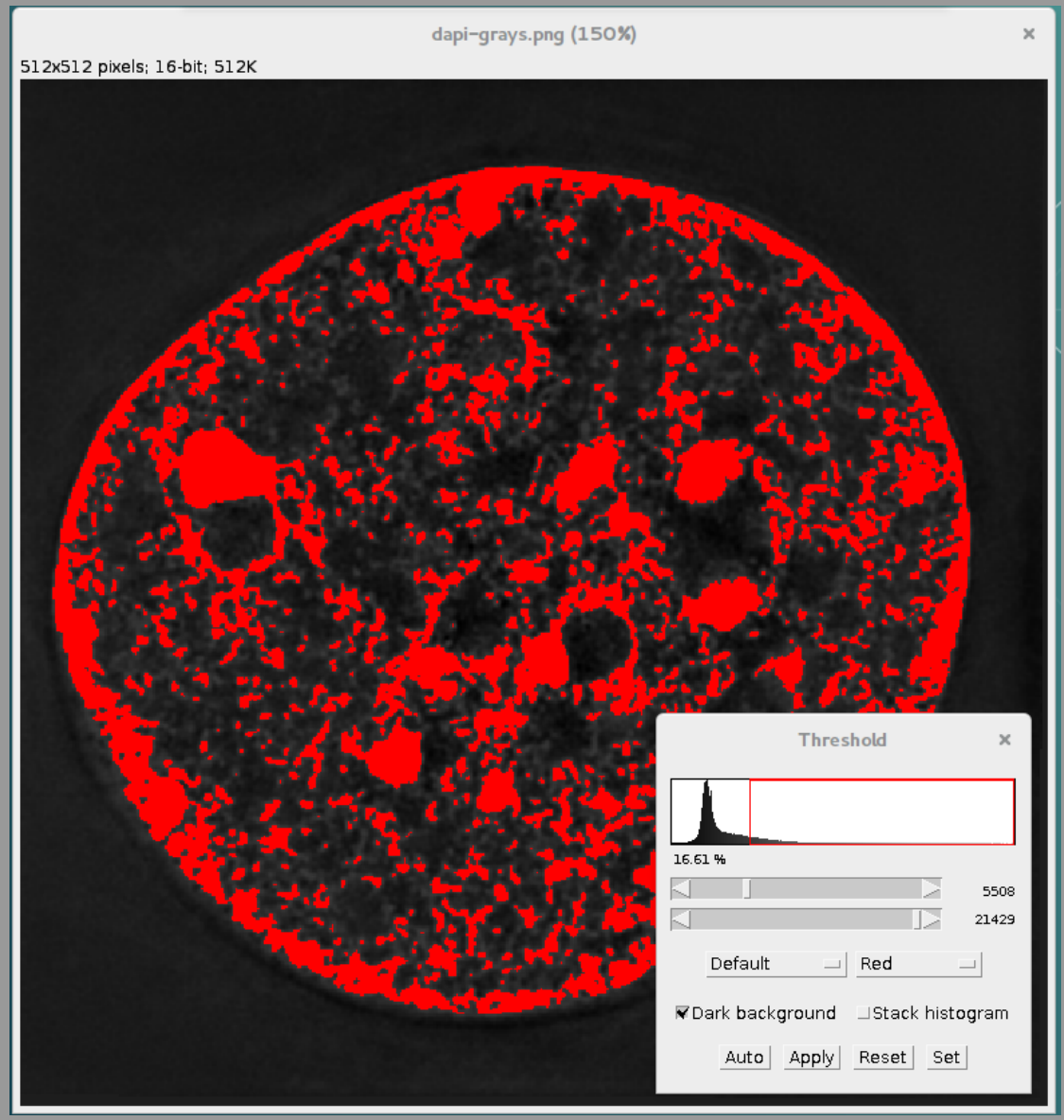
Changes in Octave:

- conversion between Octave and Java data types
- conversion of Java arrays
- fieldnames and isa
- get and set static class properties

Changes in Bio-Formats:

- use of `javaObject` and `javaMethod`
- 2 cases of `isoctave`
- release of an Octave package in their build system

Manual threshold



Automatic threshold

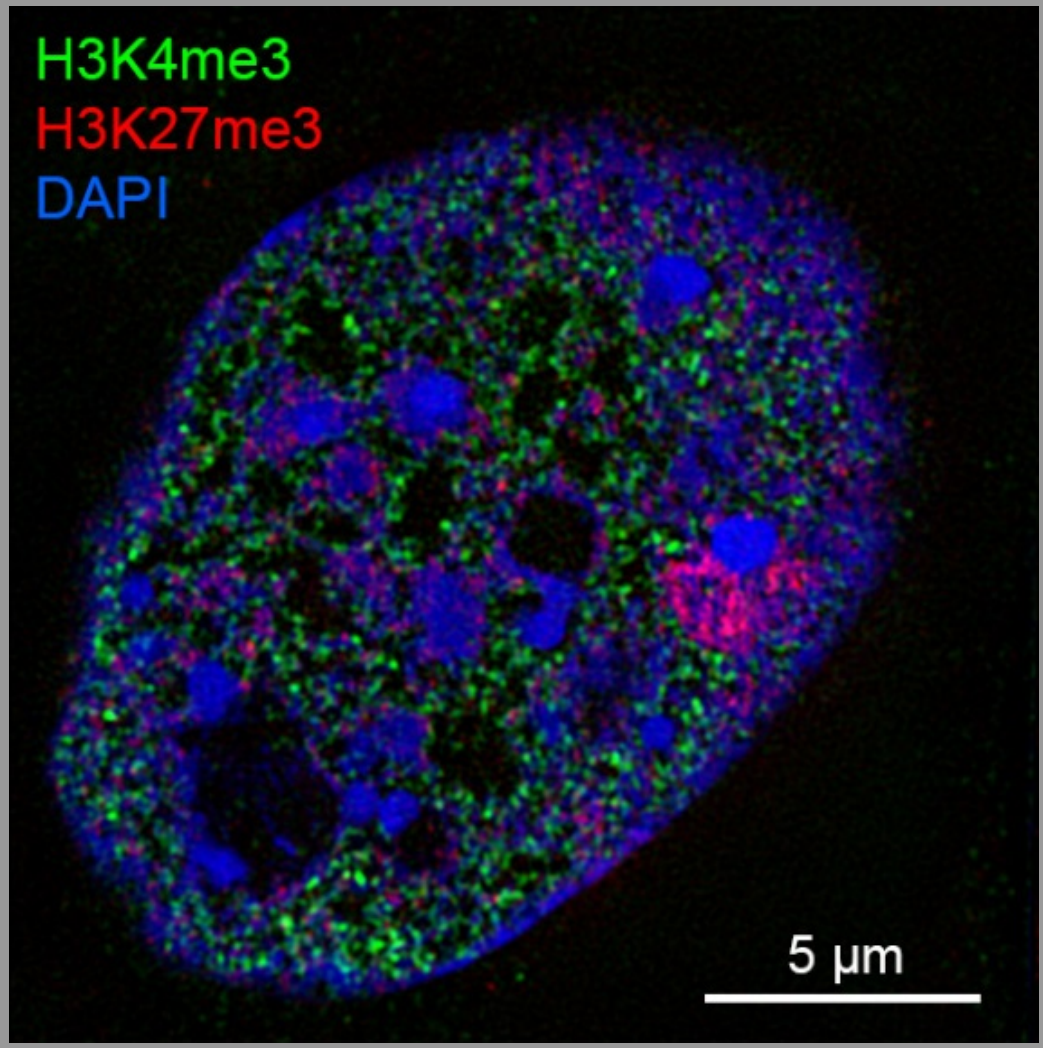
HistThresh by Antti Niemistö:

- MaxEntropy
- MaxLikelihood
- MinError
- Otsu
- concavity
- intermeans
- intermodes
- moments

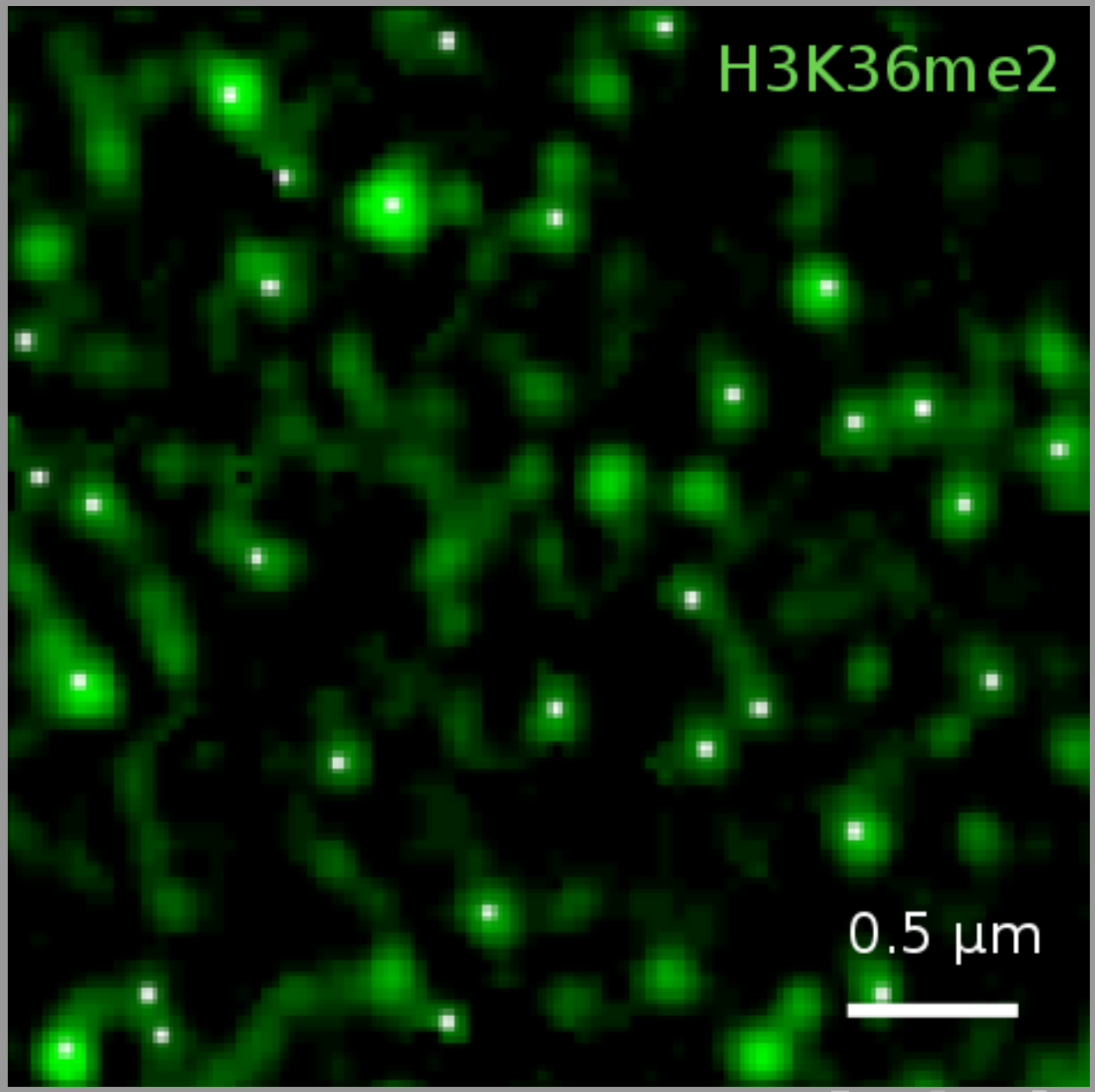
Automatic threshold

```
thresh = graythresh (img, "moments");
bw = im2bw (img, thresh);
bw = im2bw (img, "moments");
thresh = graythresh (hist, ...);
```

The problem



The solution

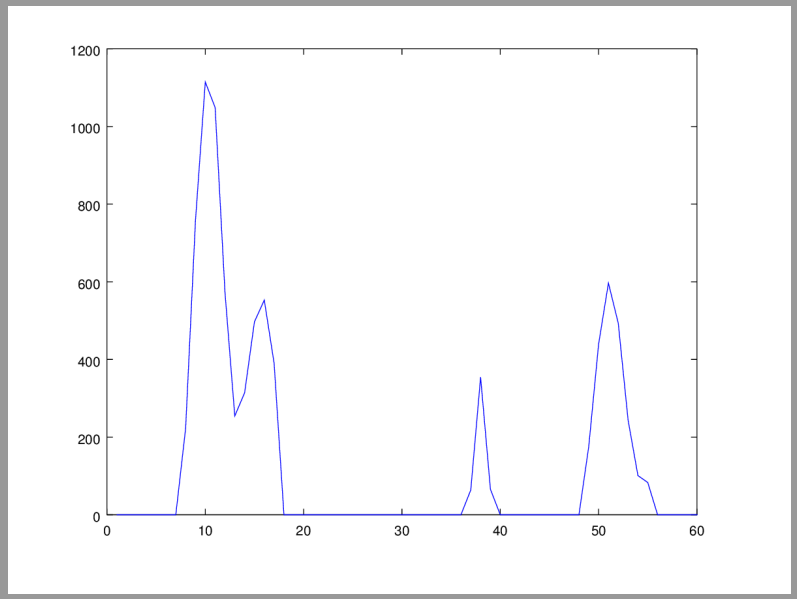


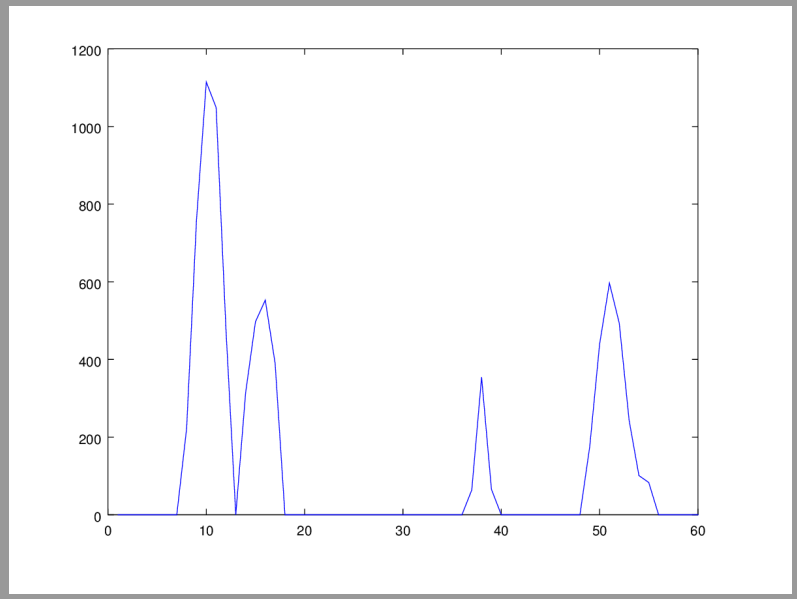
Nuclei ROI

1. `convn` + `fspecial` for blur
2. `graythresh` for threshold
3. `imfill` for filling holes

```
im2bw (foci, graythresh (foci(nuclei_mask)));
```

Watershed





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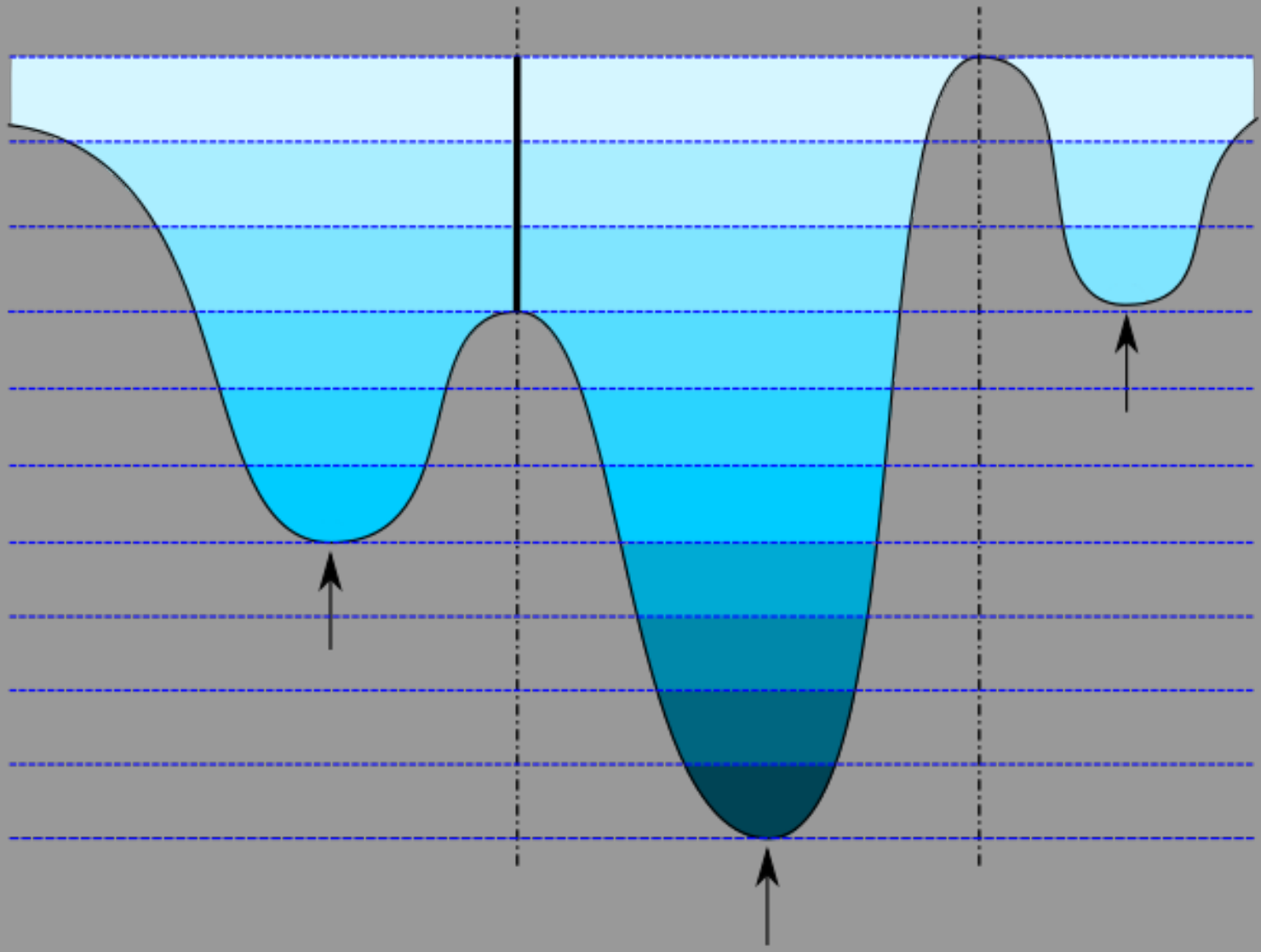

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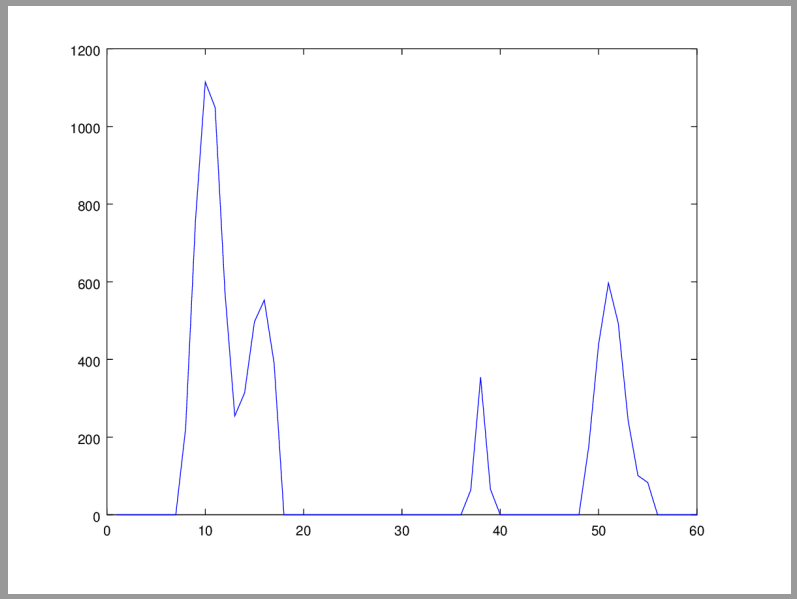
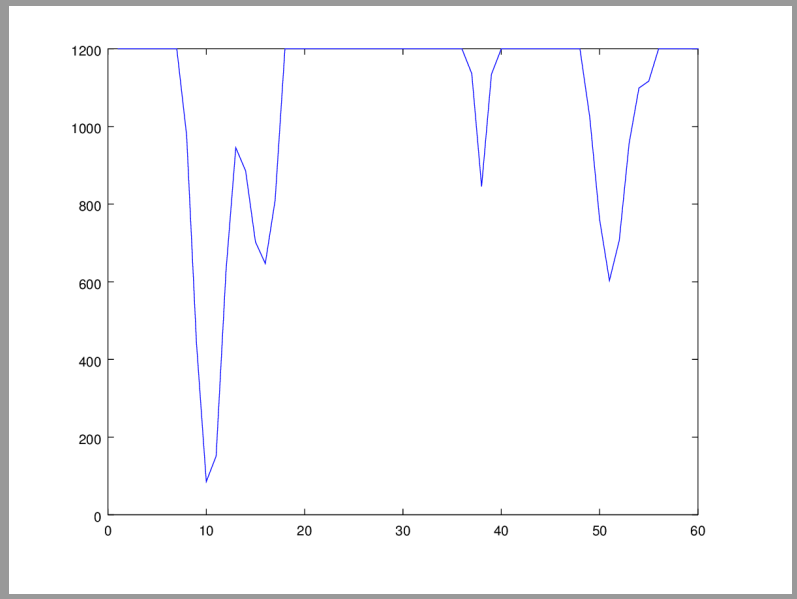


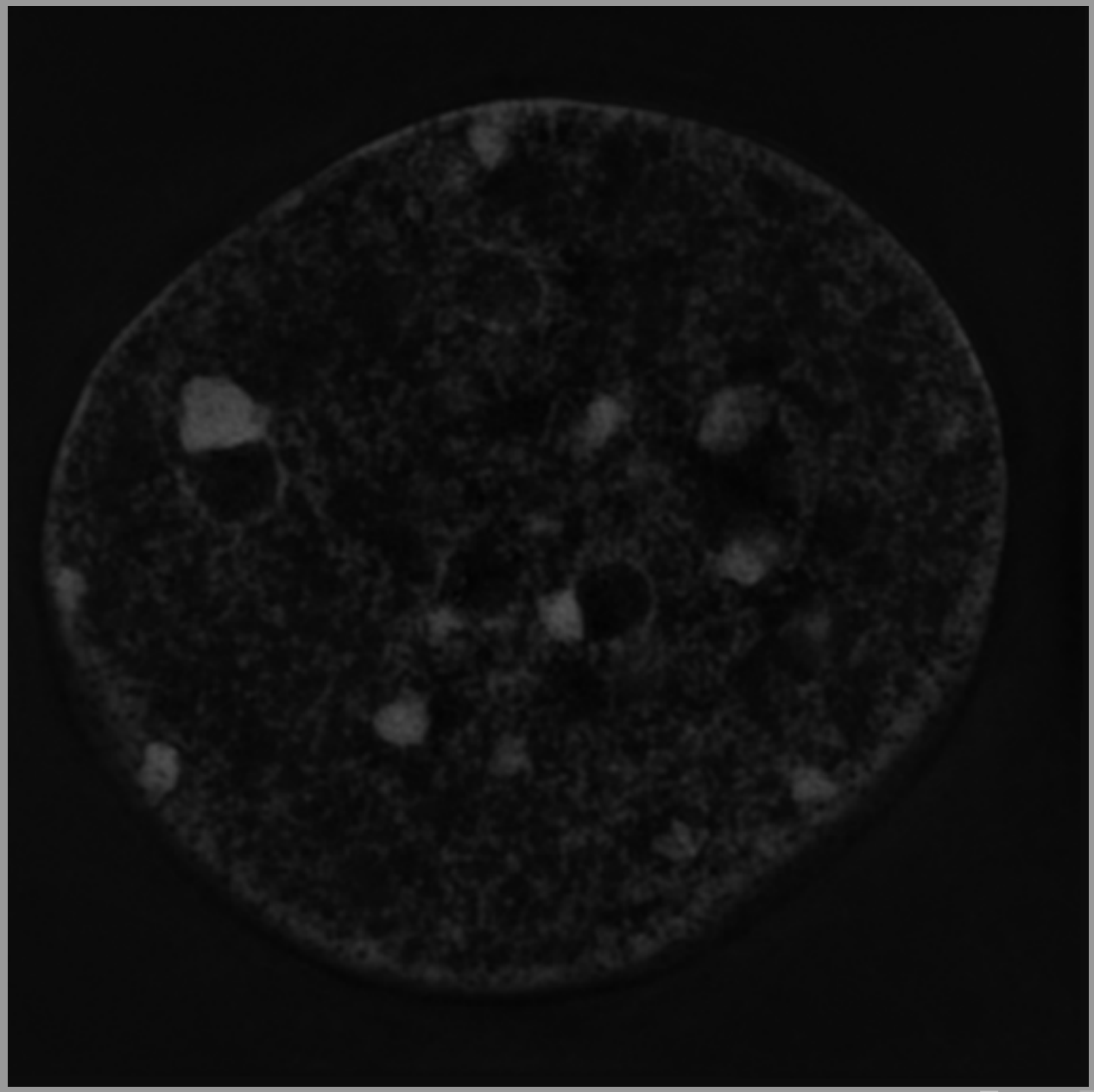


regionprops

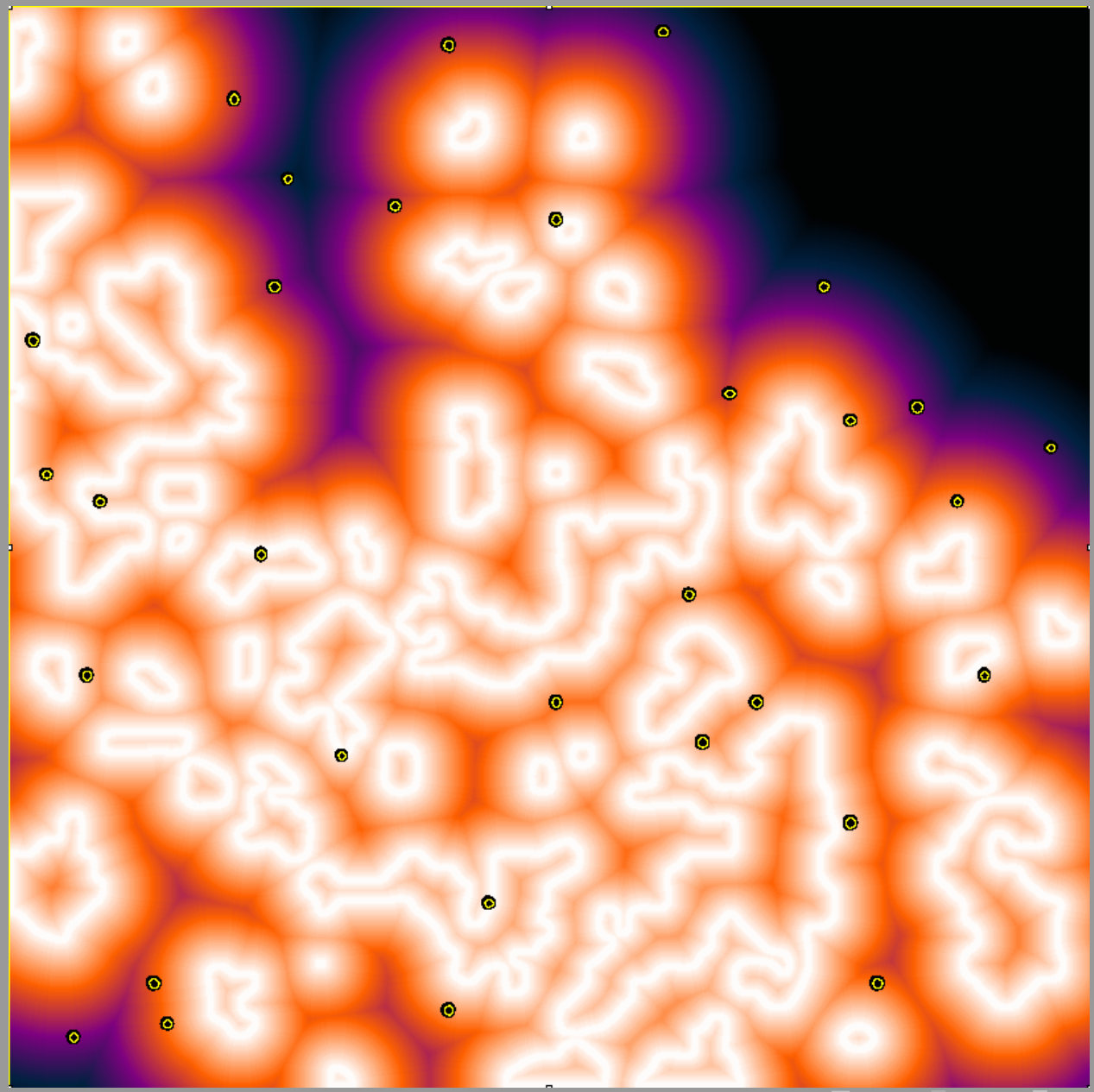
```
regionprops (bw, img, props);
```

- Eccentricity
- Image
- Perimeter
- Area
- WeightedCentroid

DAPI



Distance to IC



Outcomes

- `regionprops`
- `bwconncomp`
- `imregionalmin`
- `imreconstruct`

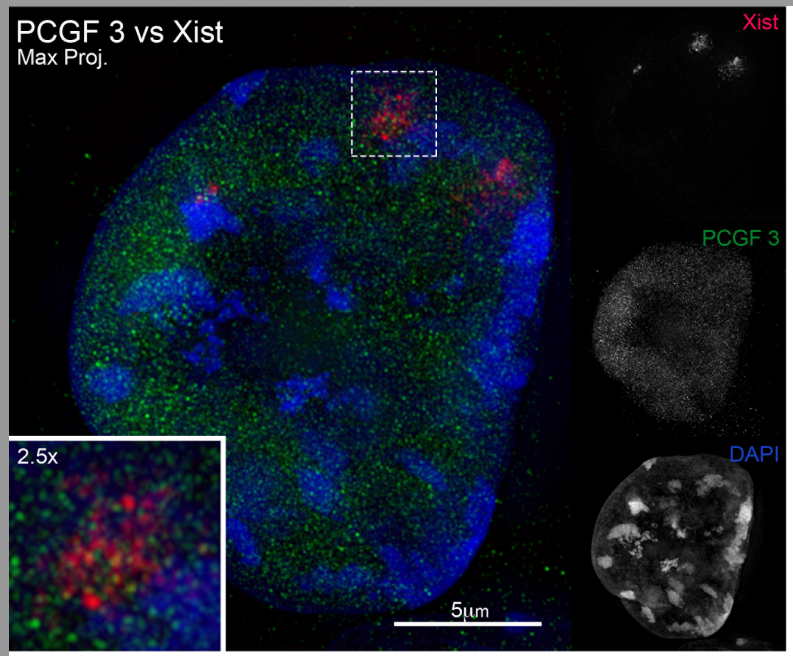
Outcomes

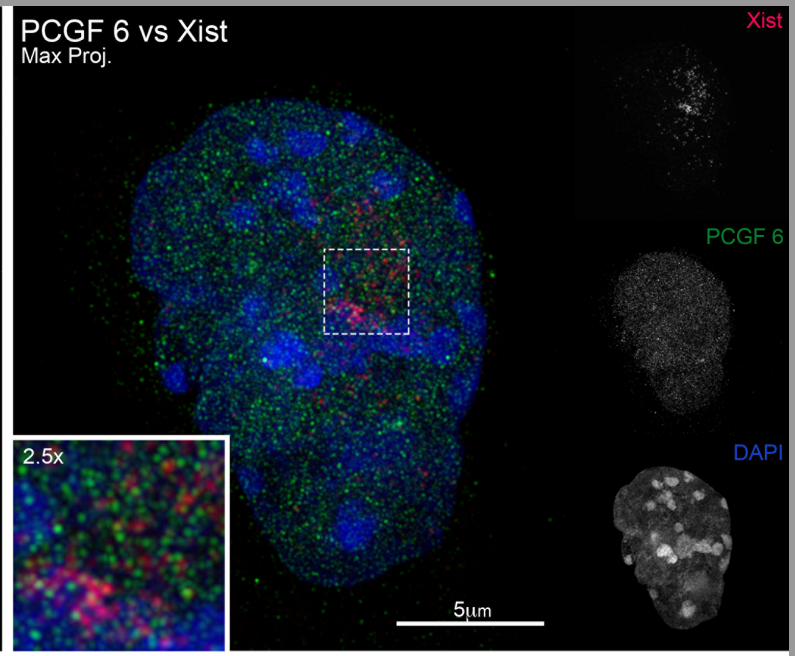
- tif log file
- CSV with center coordinates and nearest neighbour.

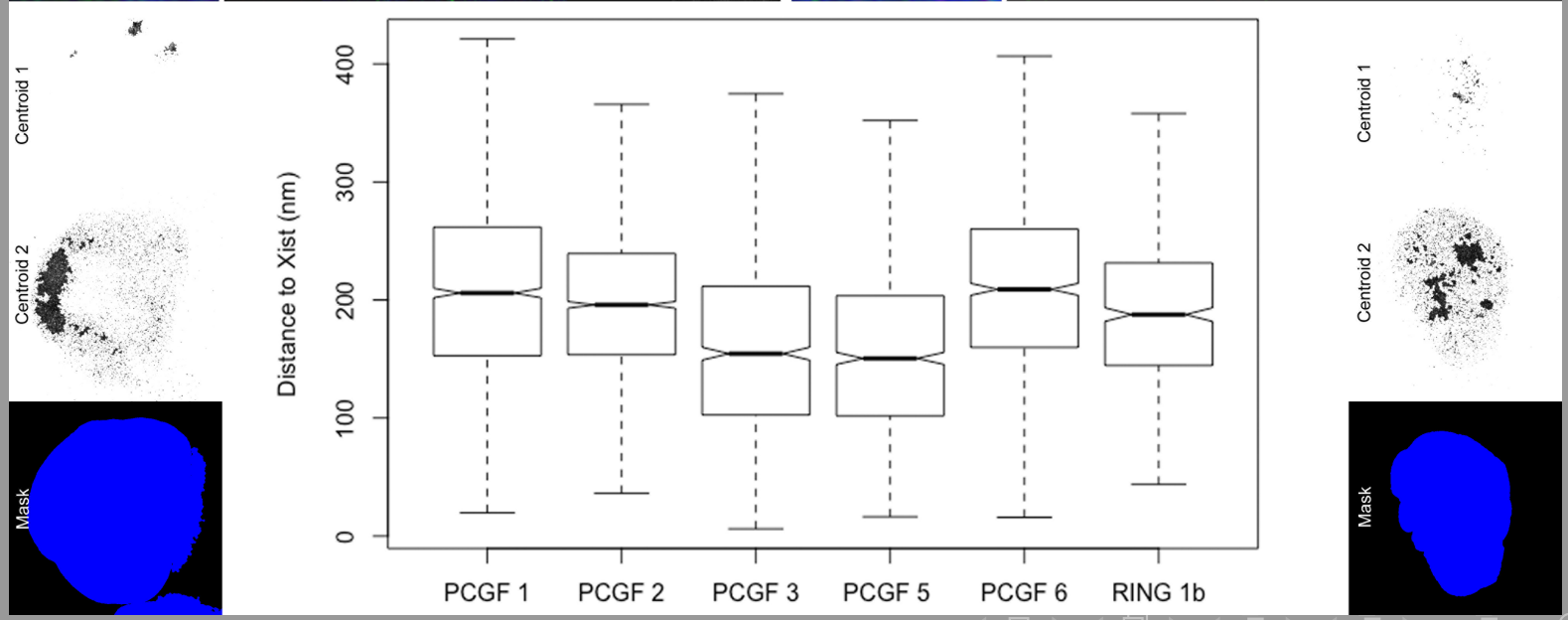
x	y	z	NN dist	NN idx	Voxels
5642.53	9789.85	484.85	1012.14	31	23
6135.53	10192.63	441.54	518.02	30	14
...					

- boxplot of distances

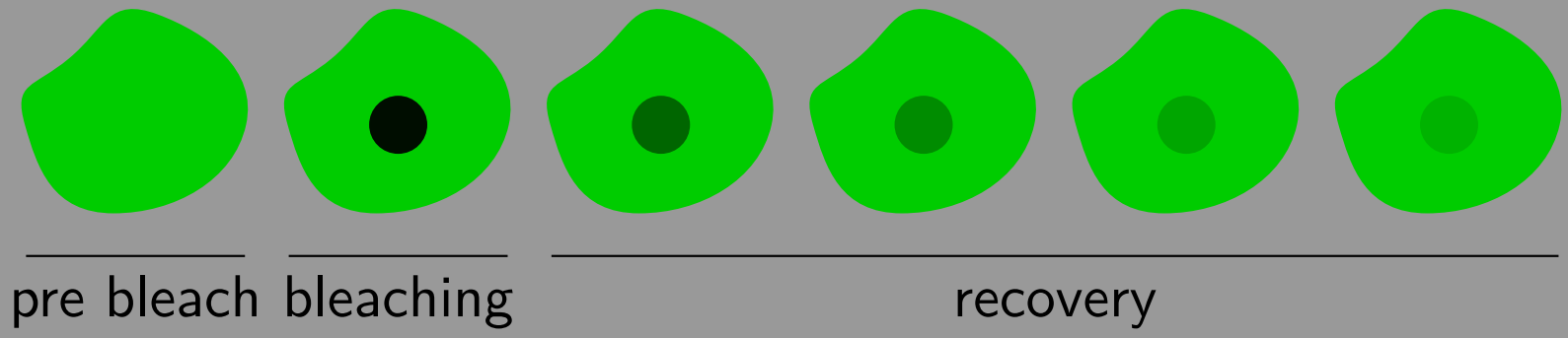
Outcomes

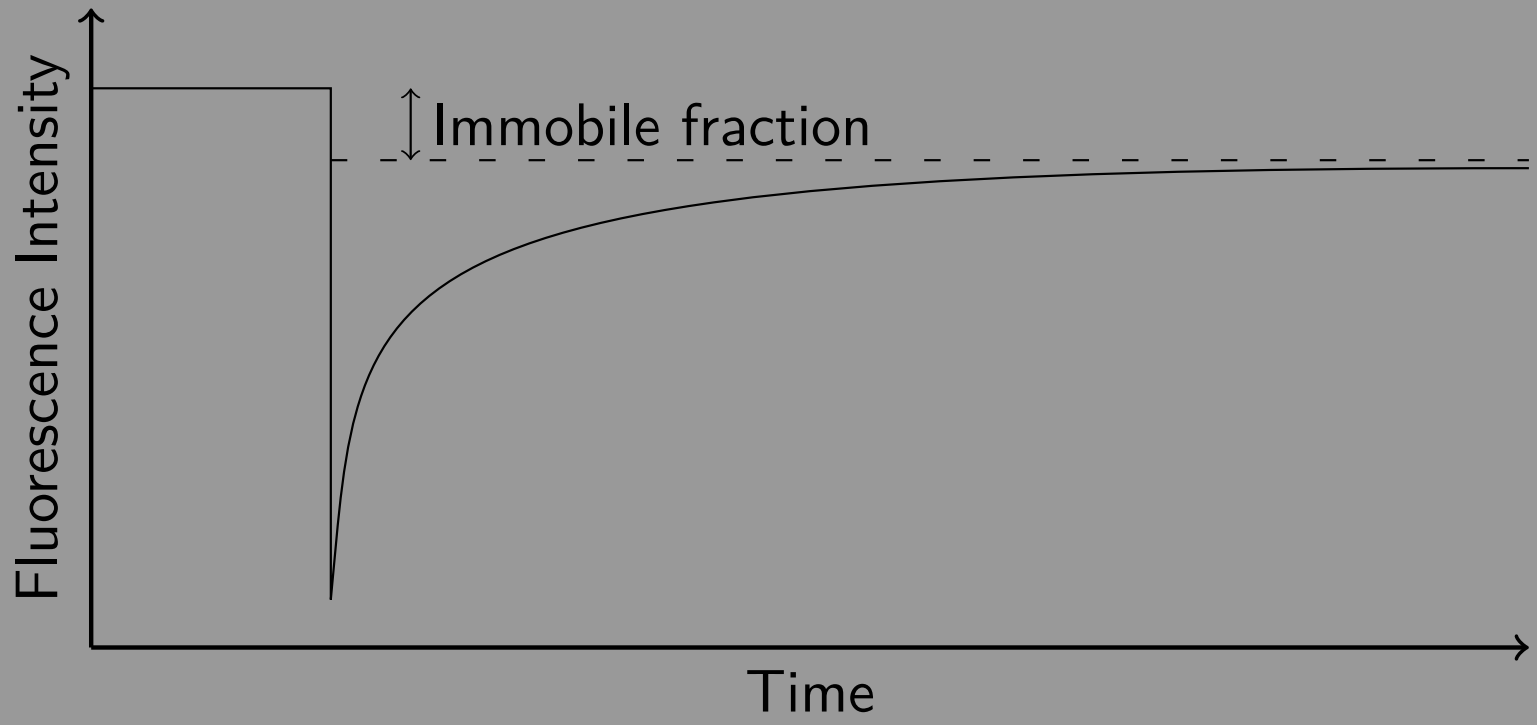




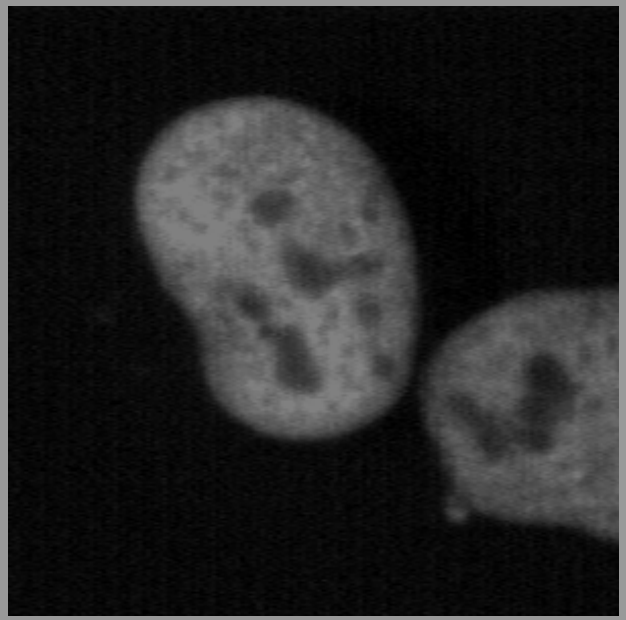


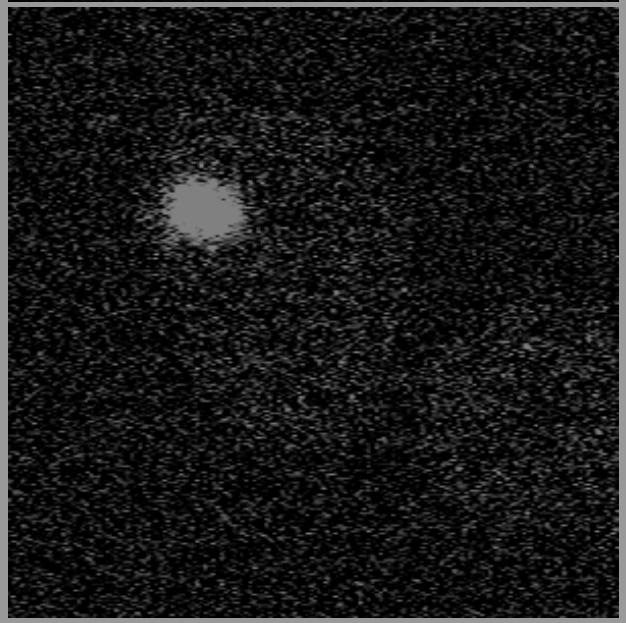
Fluorescence Recovery After Photobleaching (FRAP)

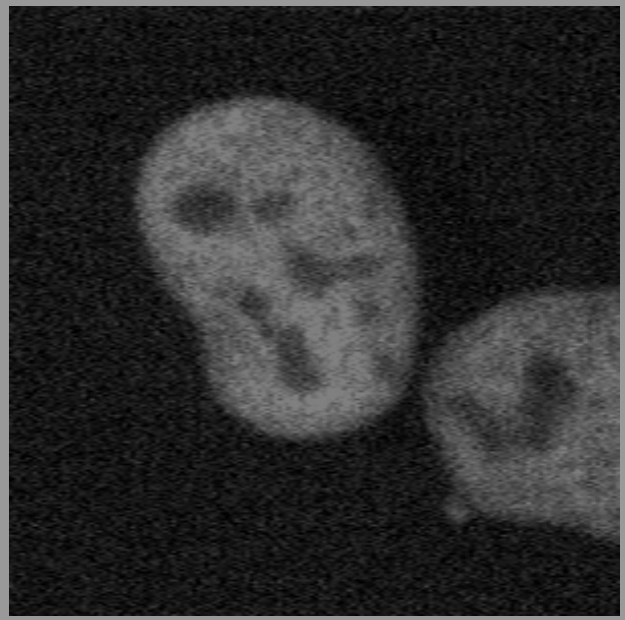


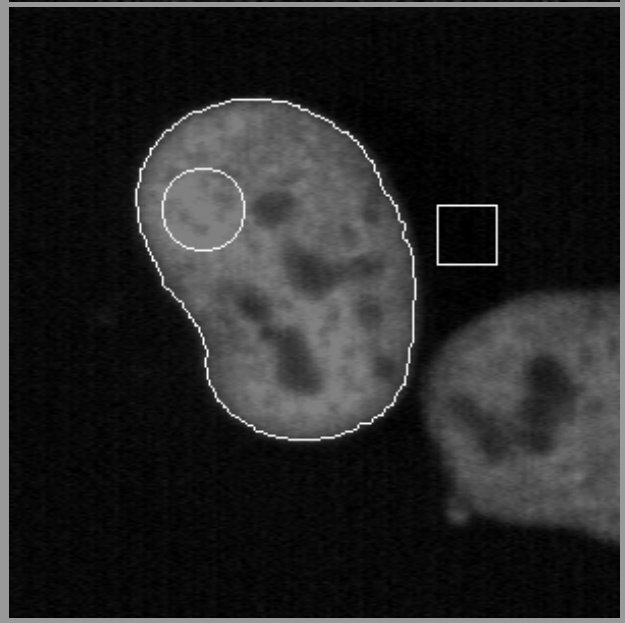


frapinator









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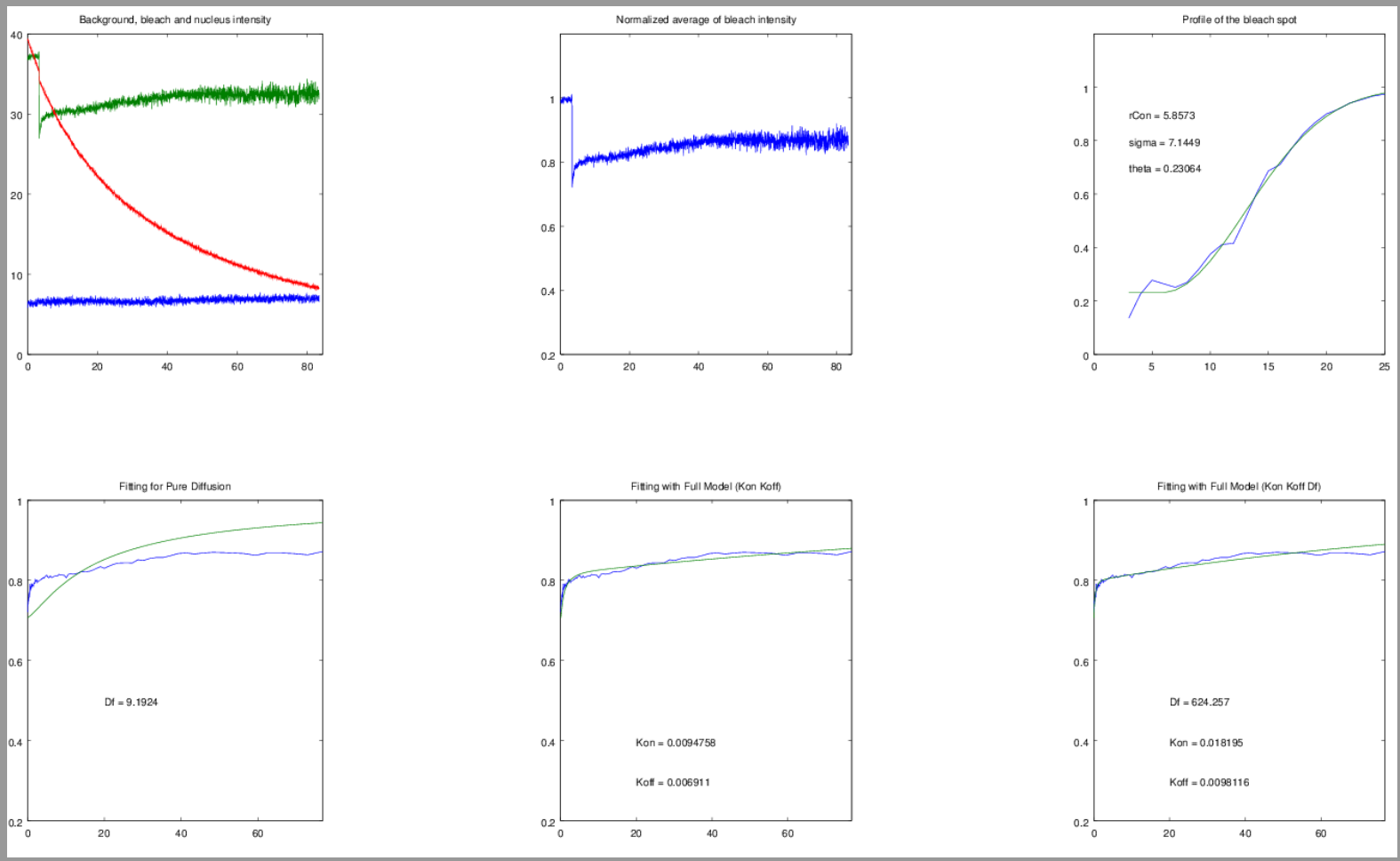

Don't port from Matlab

- More people win
- No need to backport upstream changes
- If you wait long enough, you don't even need to do anything

Future work

- more java for OMERO
  - Java multi-dimensional arrays
  - Java import statement and dot notation

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Acknowledgements

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