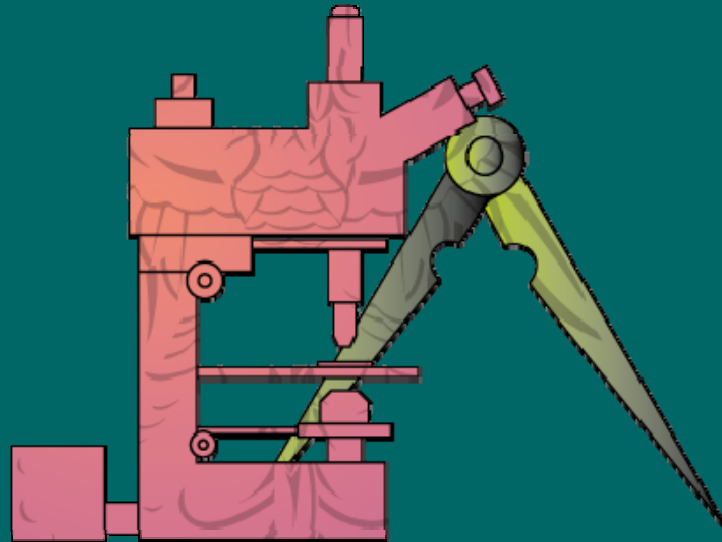


IPF Course 2008



basic course on image processing techniques



Schedule – Course

Day 1

- intensity operation / measurements
- introduction to ImageJ

Day 2

- filtering images in the spatial, frequency and time domains

Day 3

- Segmentation - finding and measuring objects in images



Schedule – Day 1

first half

Theoretical Session A

- Bit Depth
- RGB Color Space
- Lookup Tables
- Line Profile

Practical Session A

Break

second half

Theoretical Session B

- Histogram
- Scatterplot
- Scaling

Practical Session B



Before you start writing...

Presentations soon available at:

<http://tu-dresden.de/med/ifn>



Image Processing?!

255	255	255	255	255	255	255	255	255	255
255	255	255	255	50	50	50	50	255	255
255	255	255	50	50	50	50	50	255	255
255	255	255	50	50	50	50	50	255	255
255	255	255	72	50	50	50	50	255	255
255	255	255	255	50	50	50	255	255	255
255	50	50	50	50	50	50	50	50	255
255	255	255	255	255	50	255	255	255	255
255	255	255	255	50	255	255	255	255	255
255	255	255	255	50	50	50	50	51	168
255	255	255	255	50	255	255	255	255	255
255	255	255	50	255	255	255	255	255	255
255	255	255	50	255	255	255	255	255	255
255	255	50	255	255	255	255	255	255	255



Bit Depth

Measured intensity
by
detector



digitization

Corresponding level
in
image

Dwell depth: 10 electrons

5 electrons counted



Bit depth: 10 levels

Level 5 selected *

* RAW data

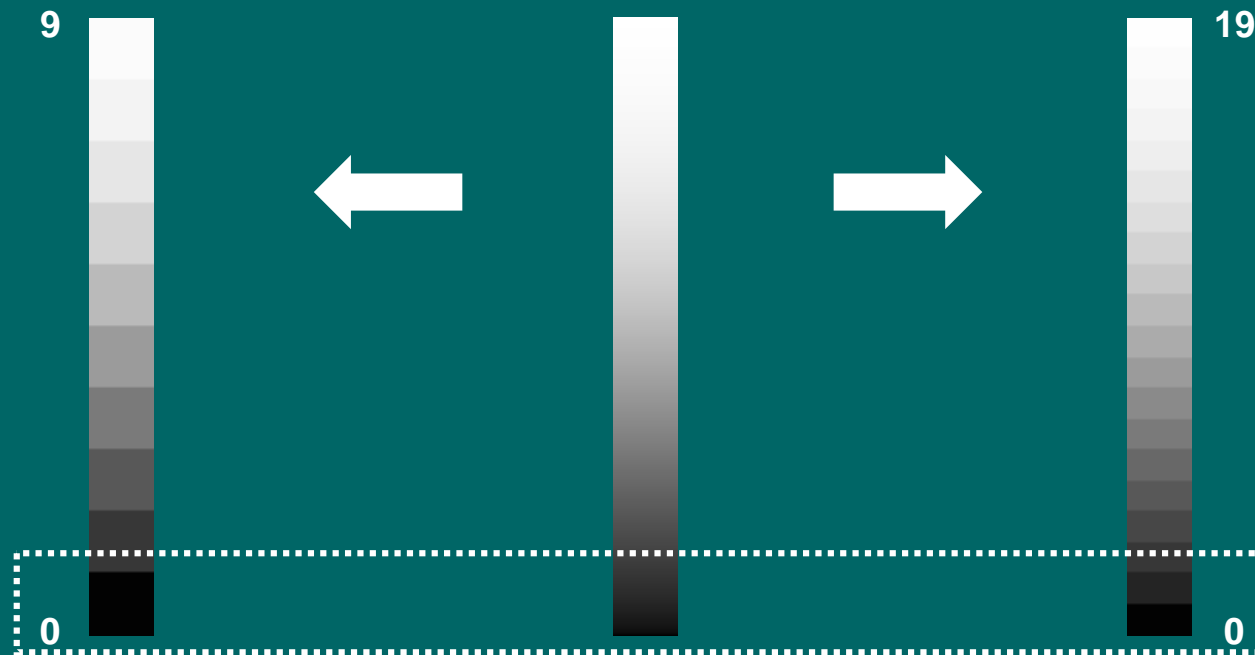


Bit Depth

„digital“ int.
Bit depth: 10

„real“
intensities

„digital“ int.
Bit depth: 20



Bit Depth

1 bit	2^1	2
8 bit	2^8	256
<hr/>		
12 bit	2^{12}	4096
14 bit	2^{14}	16384
16 bit	2^{16}	65536

...

← segmentation

~ limit of human eye, displays...

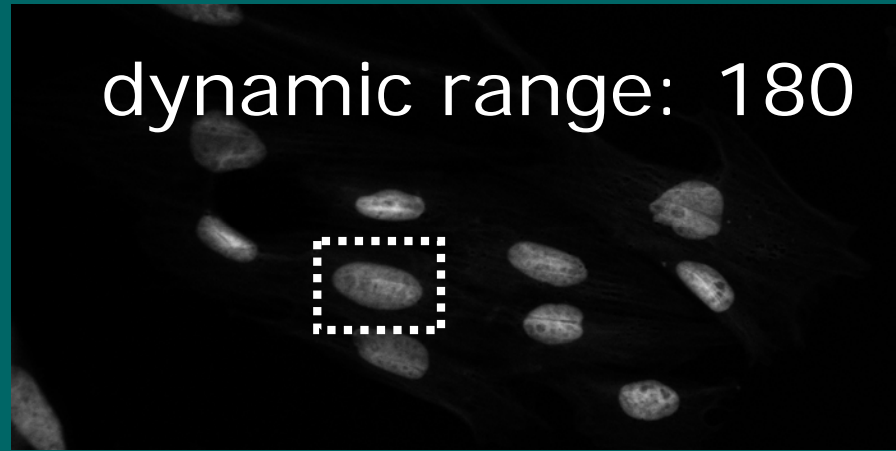
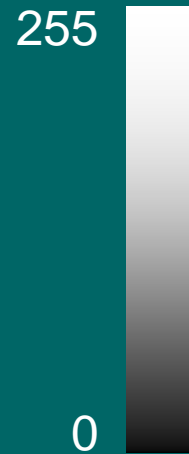
↓ intensity-related measurements



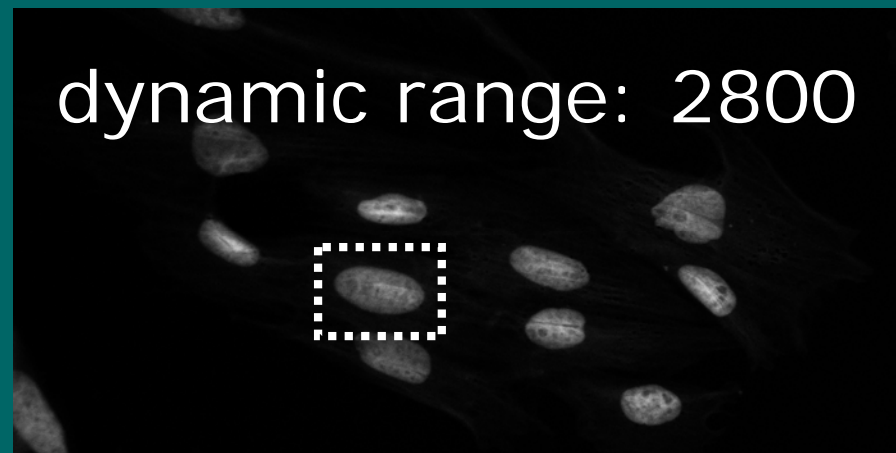
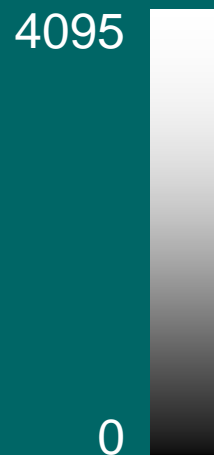
Bit Depth

for intensity-related measurements

8 bit



12 bit



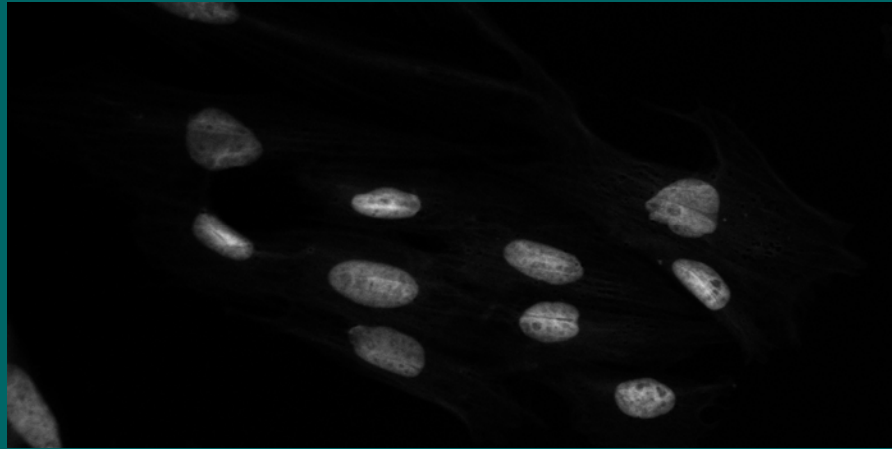
Bit Depth

for segmentation

8 bit

255

0



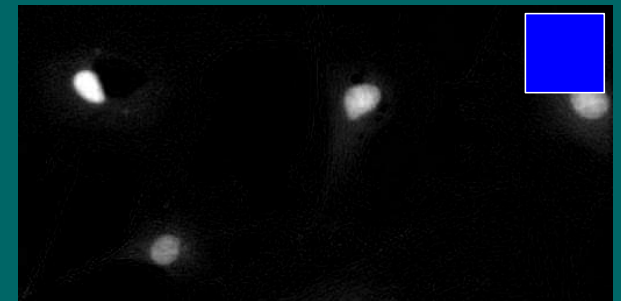
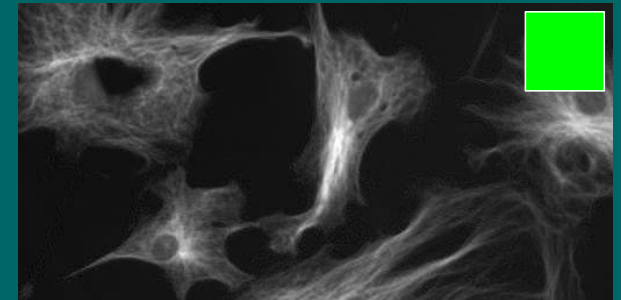
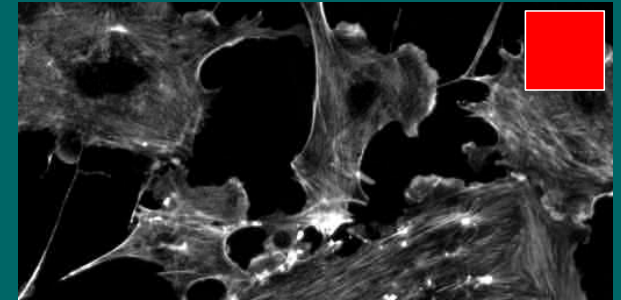
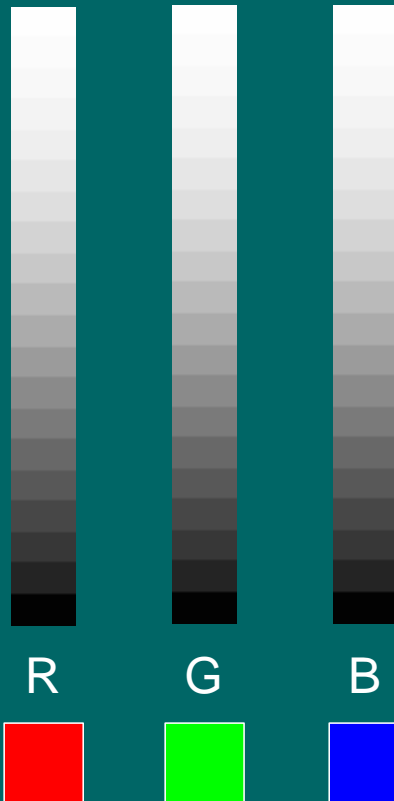
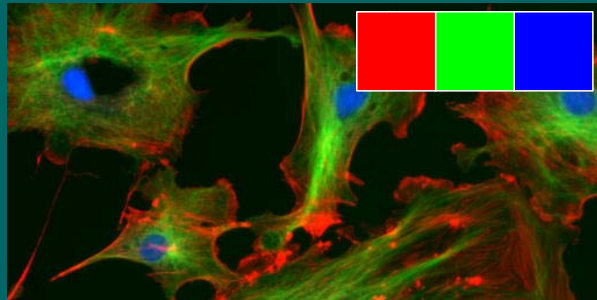
1 bit

1

0

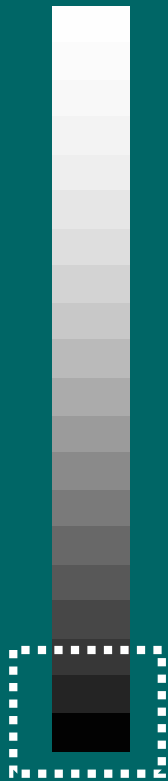


RGB Color Space

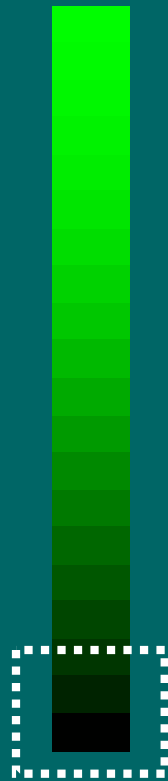


Lookup Tables

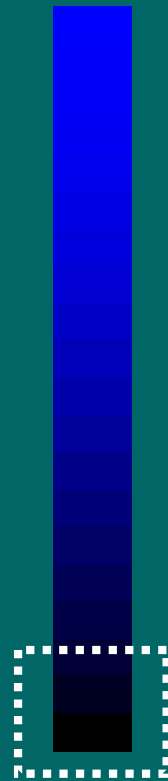
for better contrast



„gray“



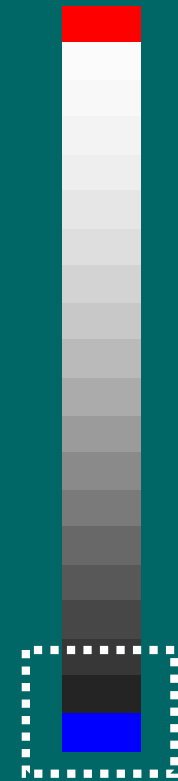
„green“



„blue“

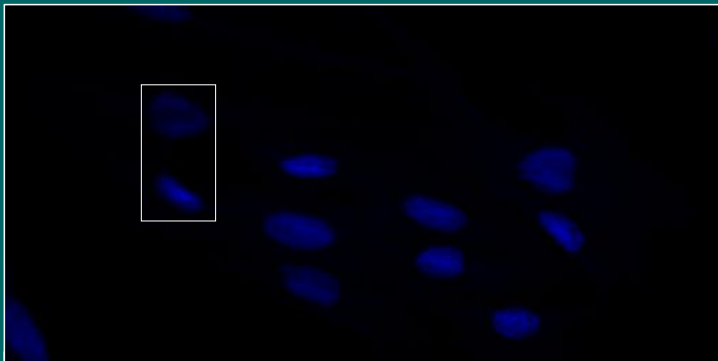


„fire“

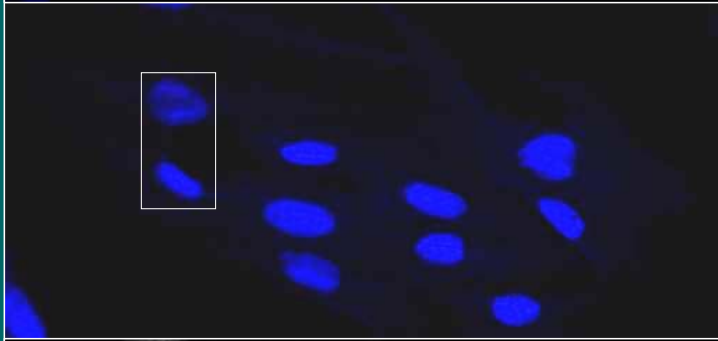


„HiLo“

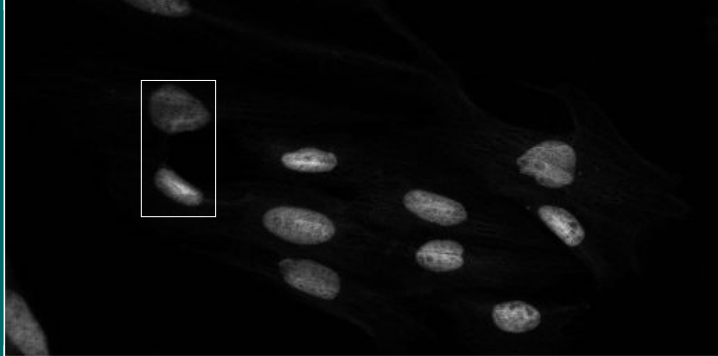




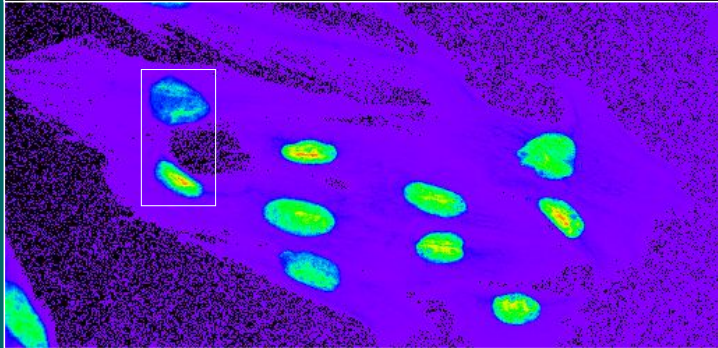
„original“ blue
linear



brightness + contrast
data changed/lost!



grayscale
linear

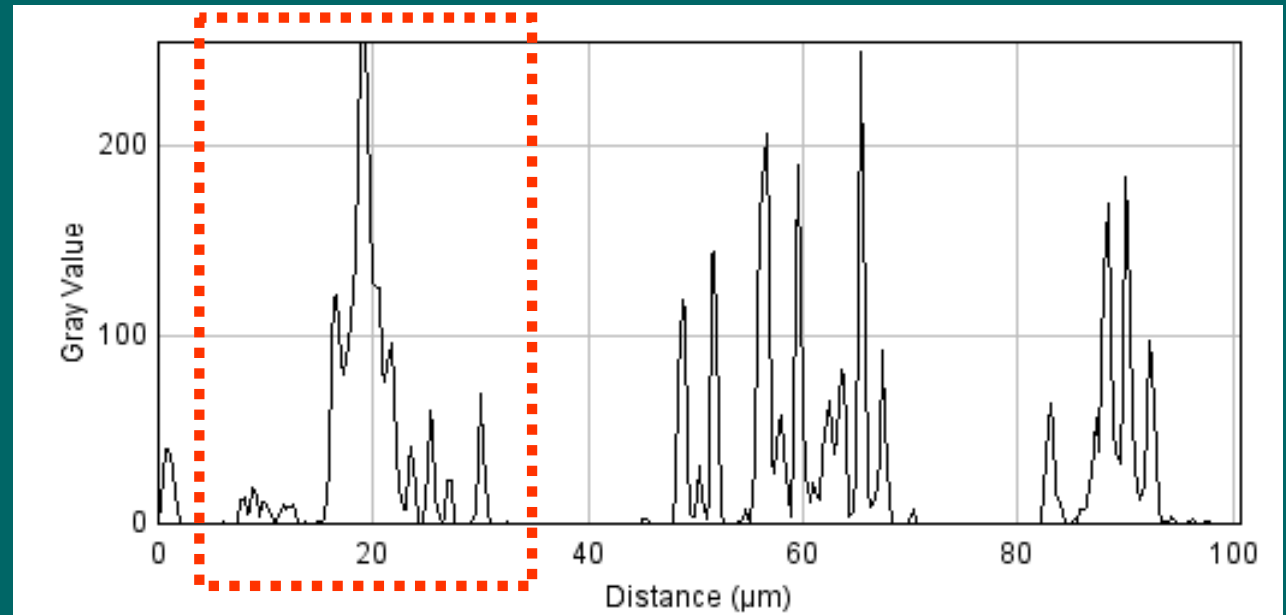
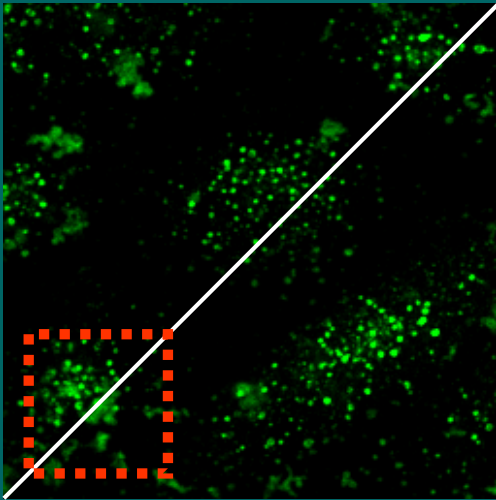


lookup table
not linear



Line Profile

alt.: „kymograph“

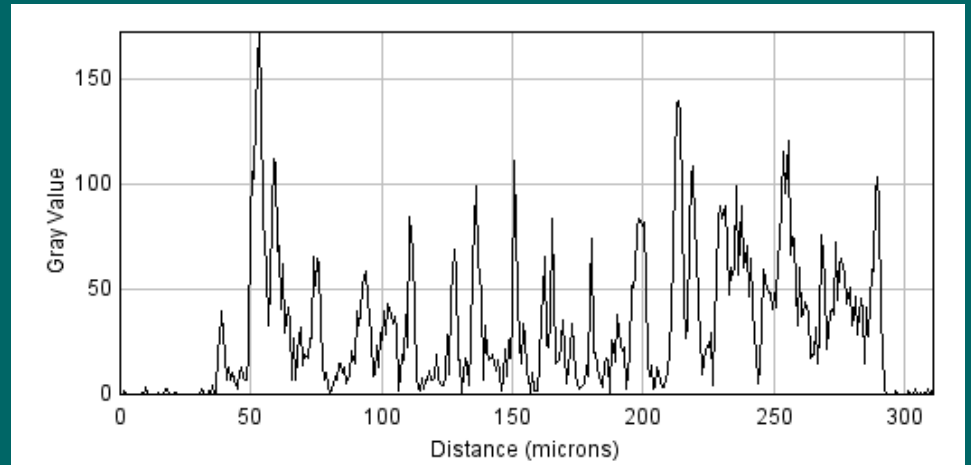
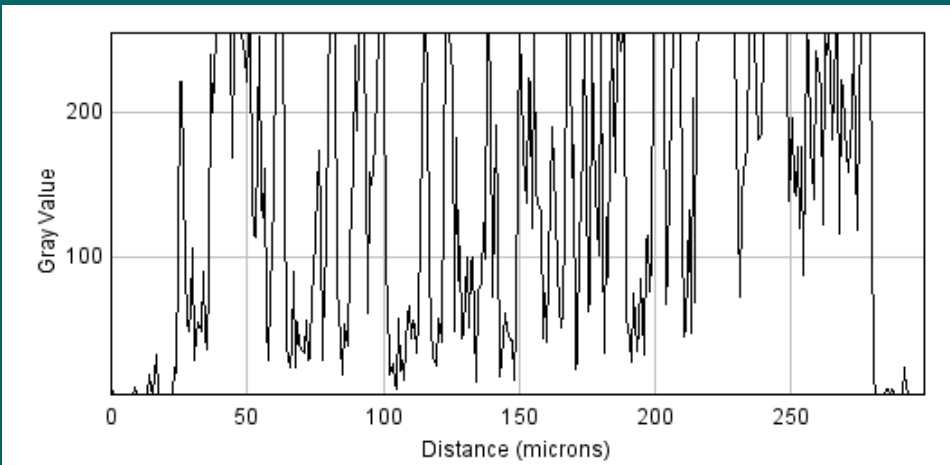
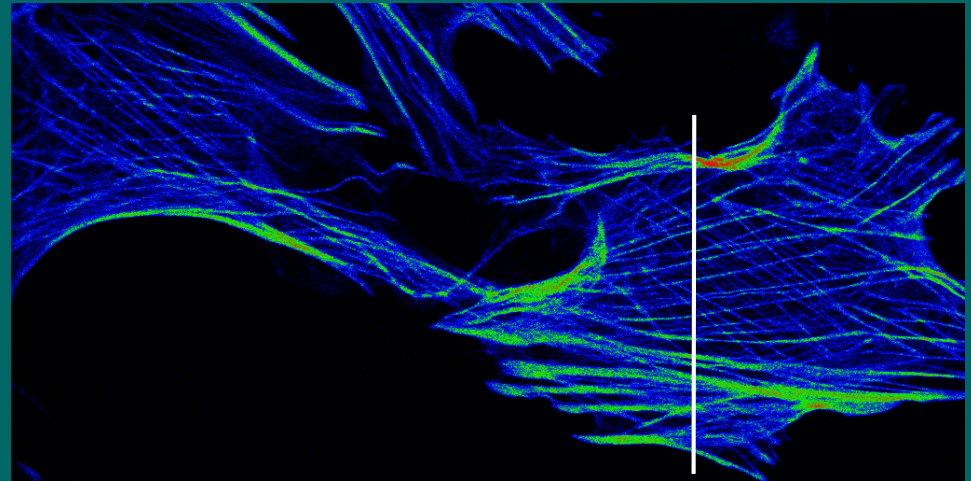
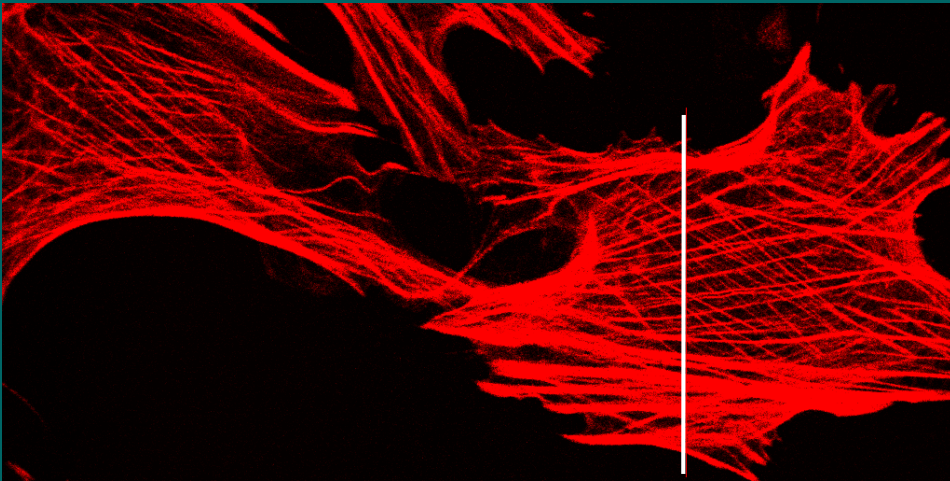


intensity measurements



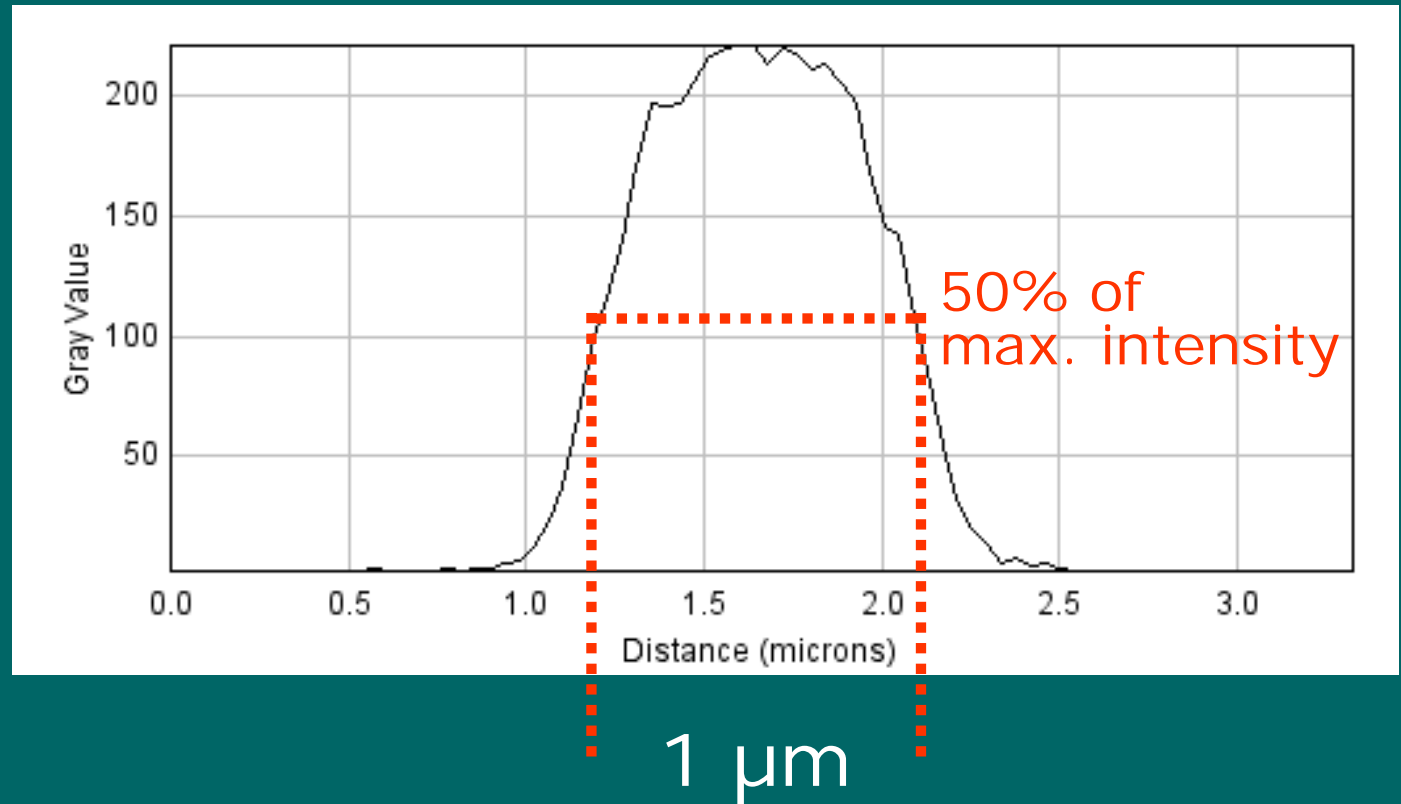
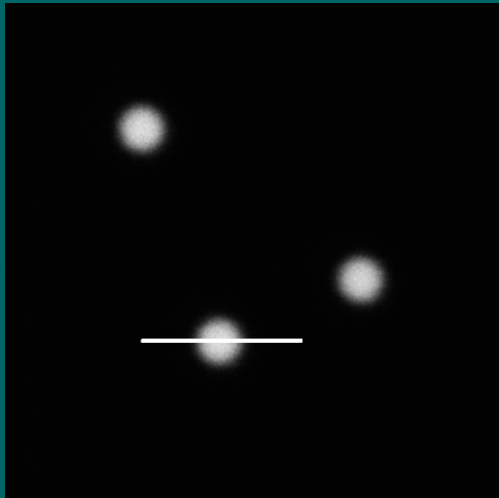
Line Profile

for quality evaluation



Line Profile

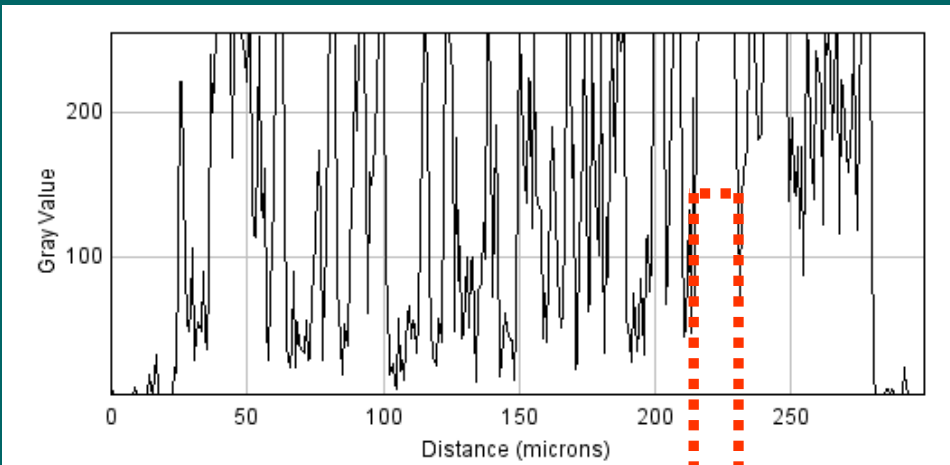
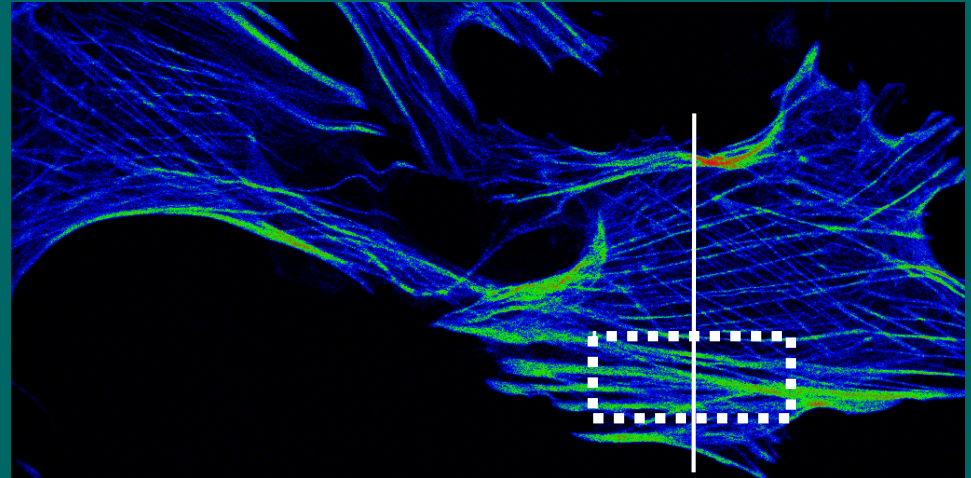
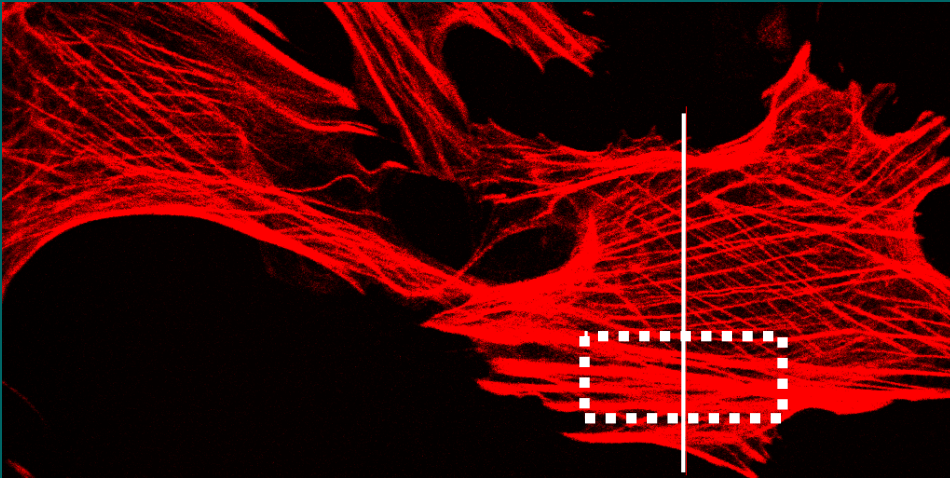
for size & distance measurements



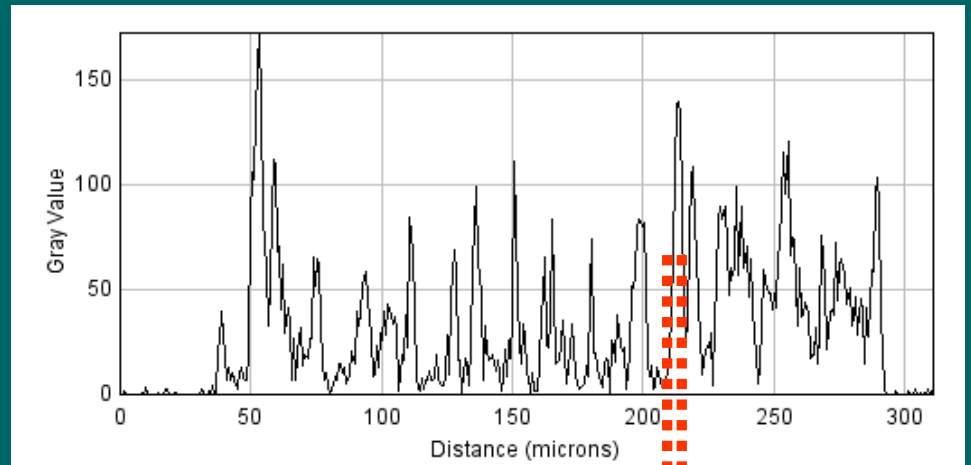
FWHM („Full Width at Half Maximum“)



Line Profile



correct ?



correct !



Practical Session A

- Bit Depth
- RGB Color Space
- Lookup Tables
- Line Profile

fluocells6.tif



ImageJ

toolbar

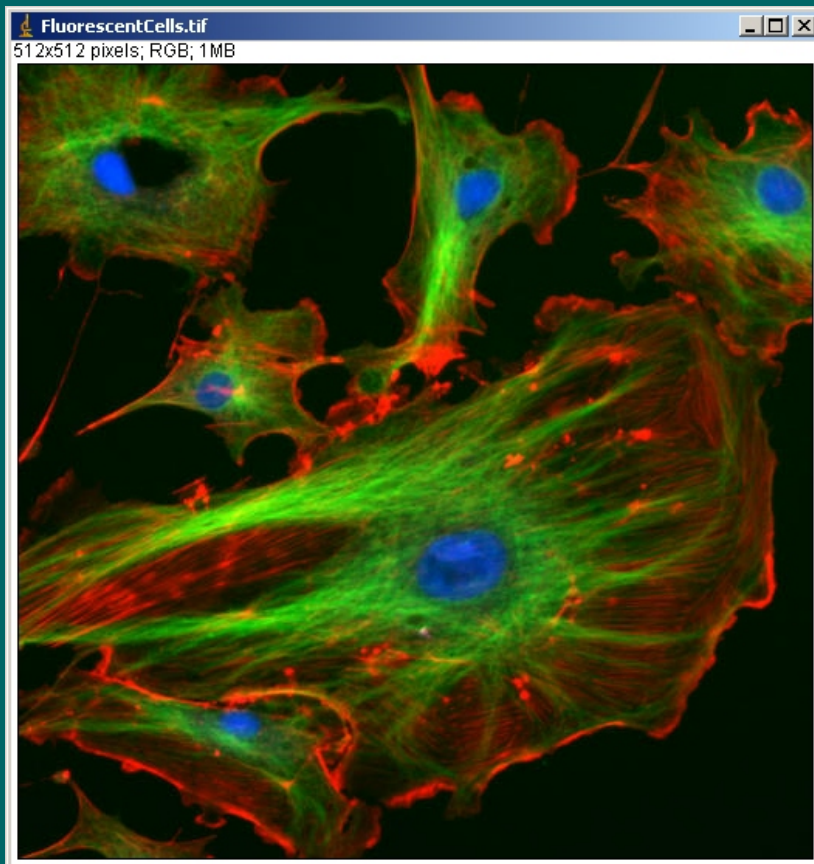
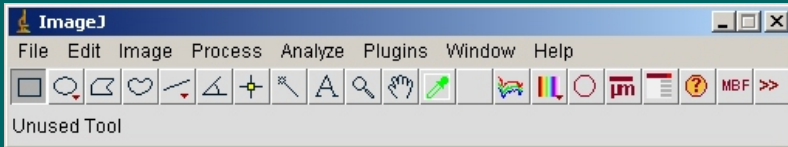
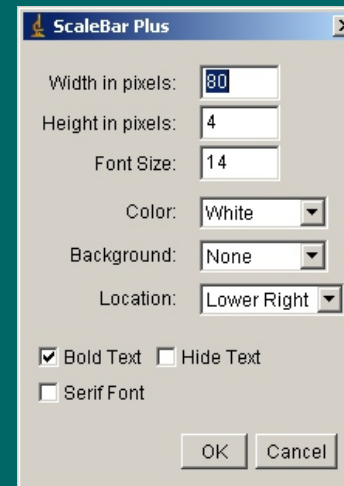


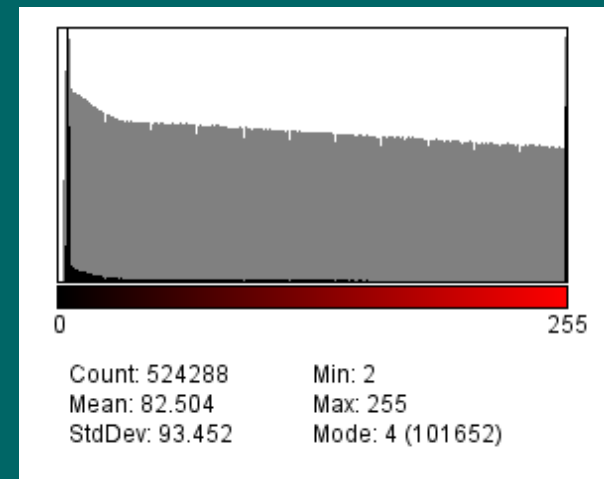
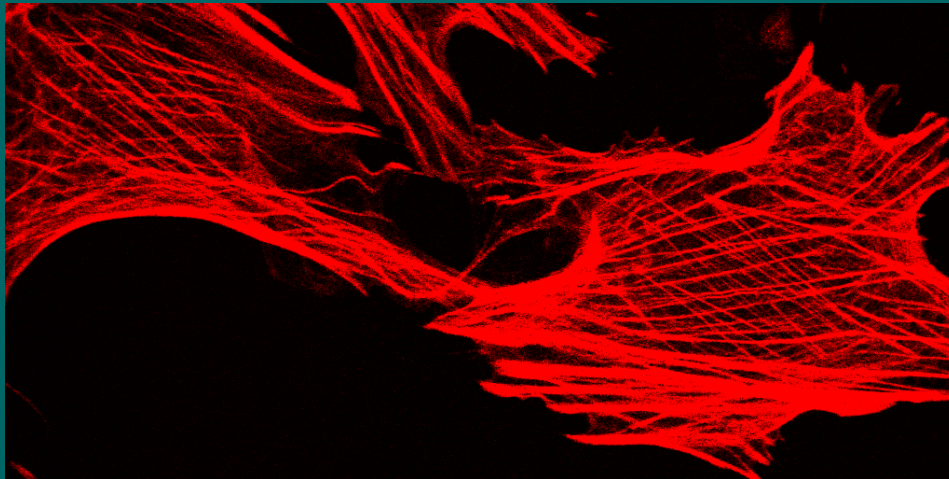
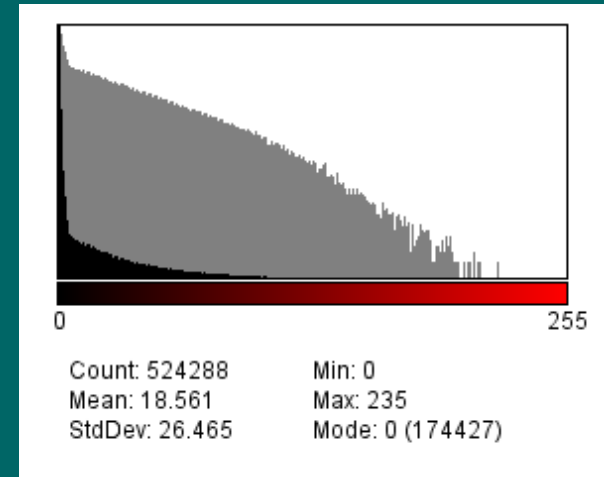
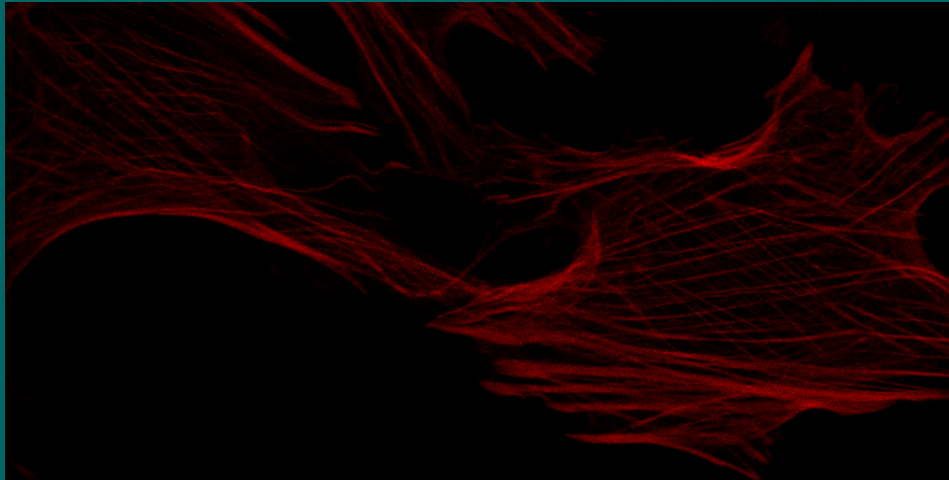
Image window



Settings window

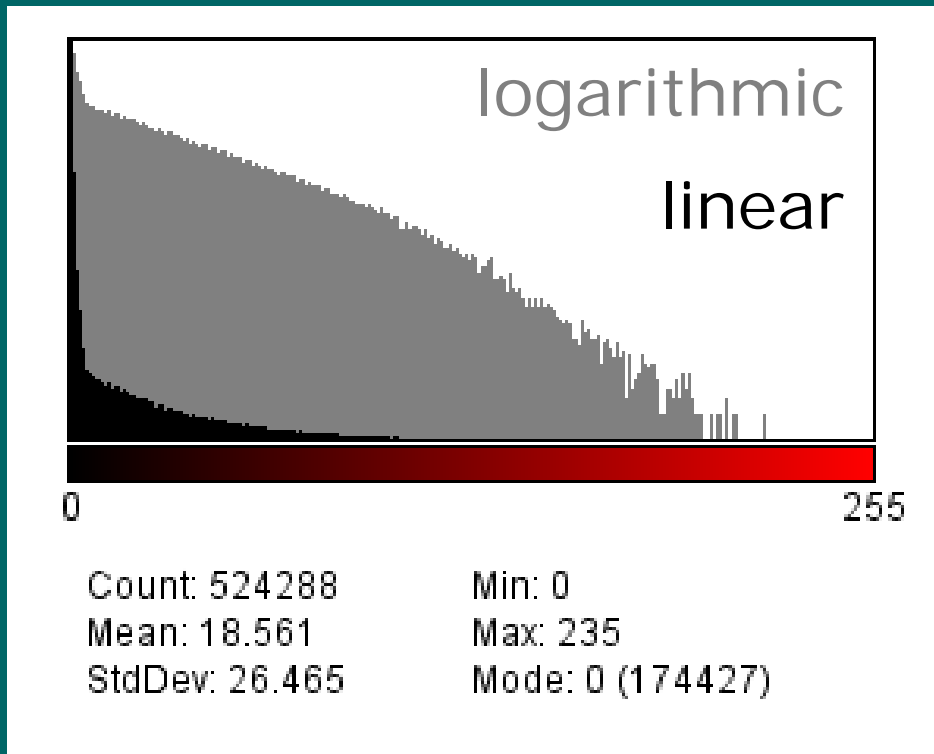


Histogram

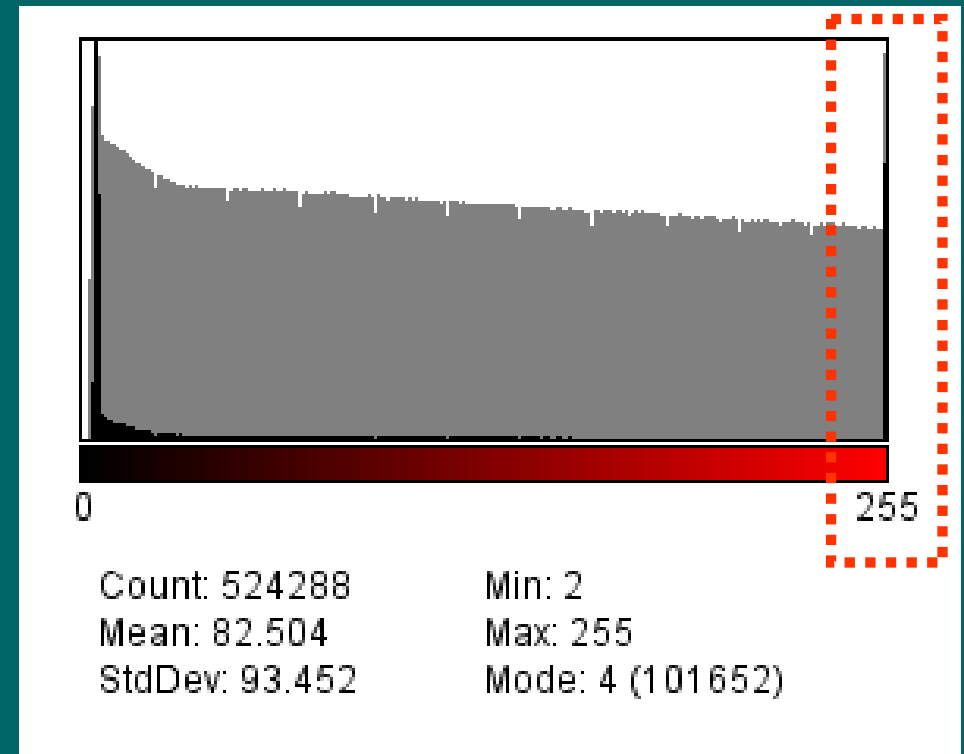


Histogram

fluorescence microscopy



OK

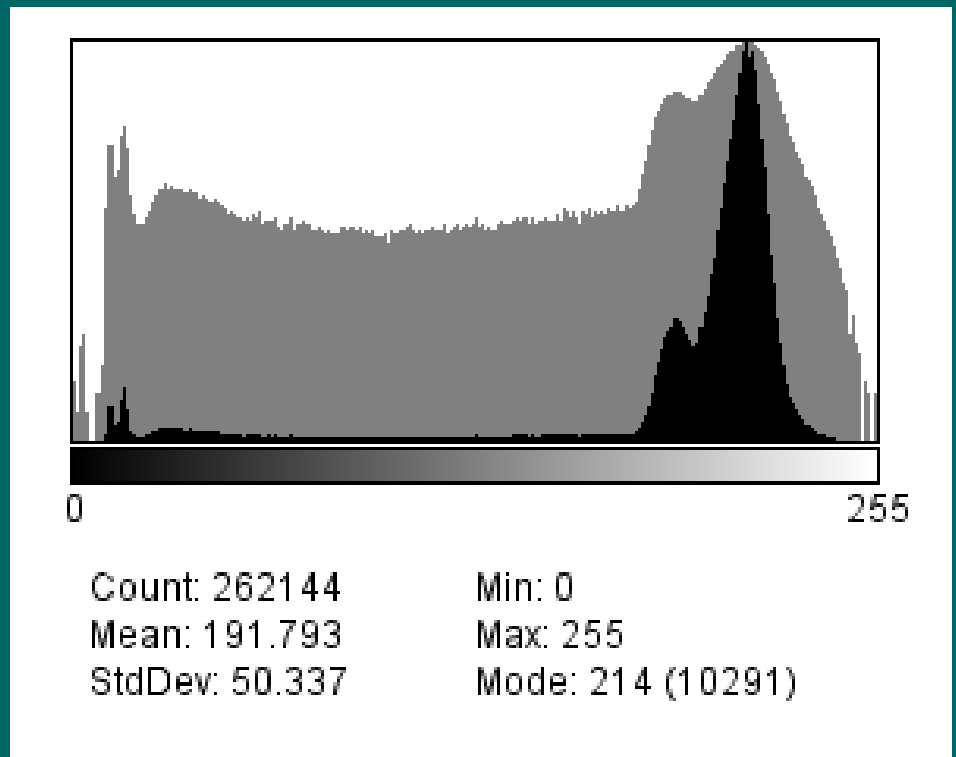
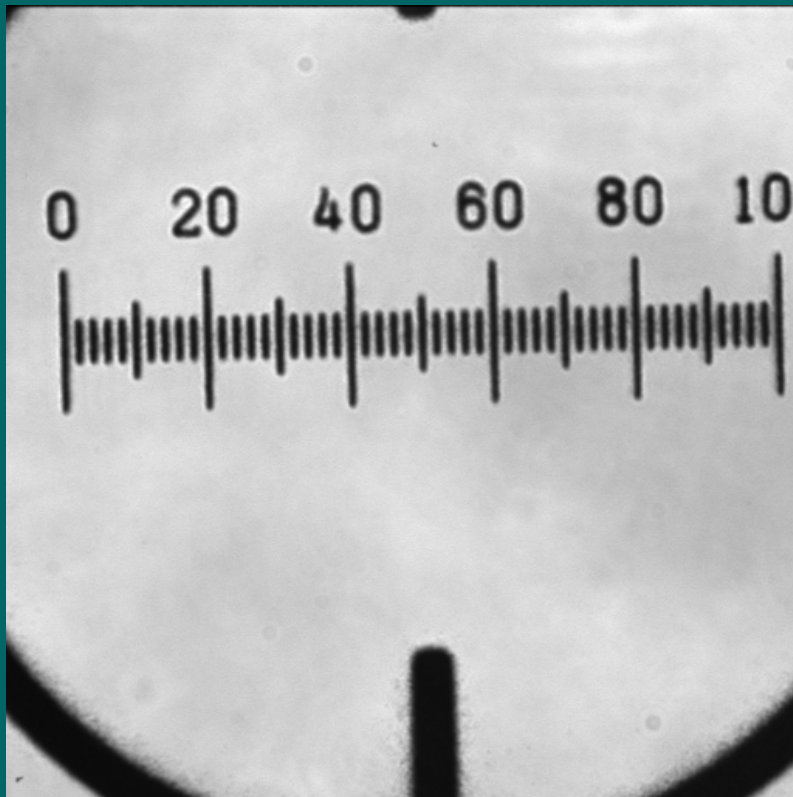


not OK, data clipped

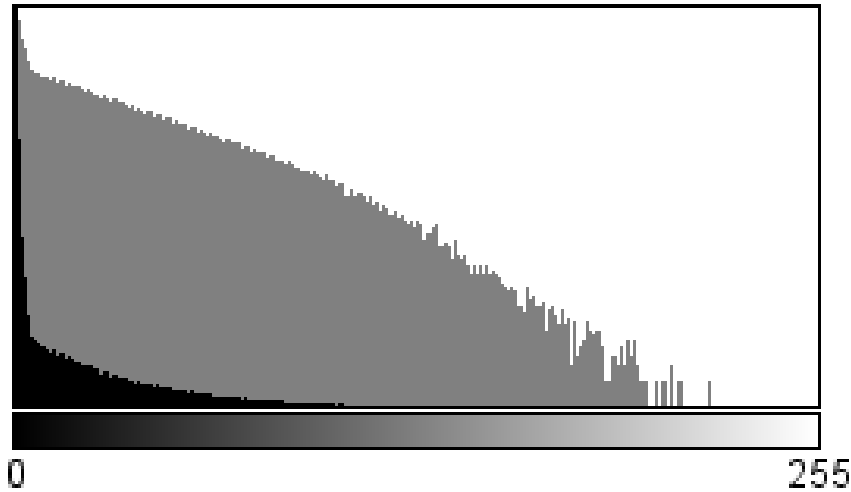


Histogram

brightfield microscopy

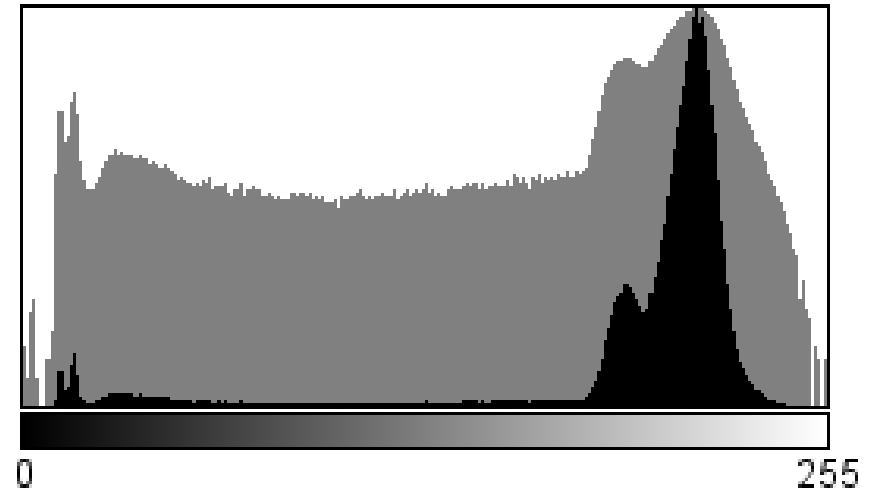


Histogram



Count: 524288
Mean: 18.561
StdDev: 26.465
Min: 0
Max: 235
Mode: 0 (174427)

fluorescence

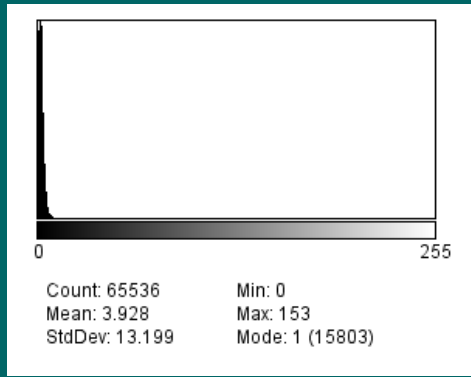
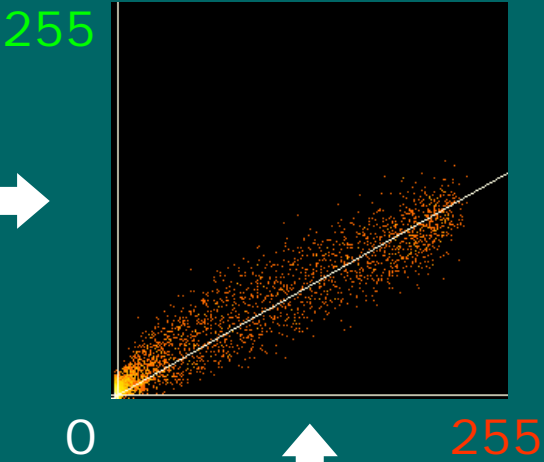
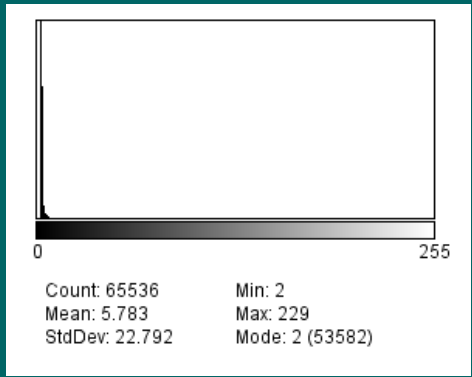
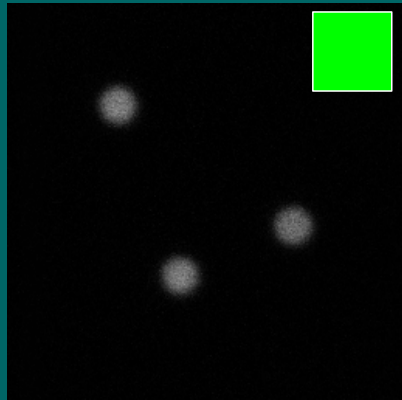
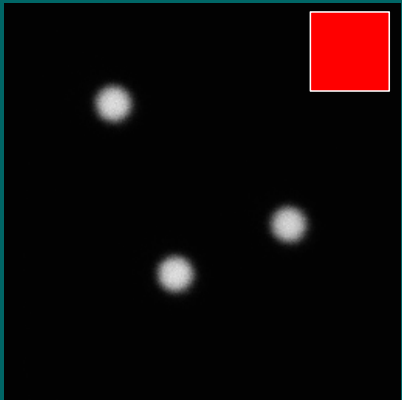


Count: 262144
Mean: 191.793
StdDev: 50.337
Min: 0
Max: 255
Mode: 214 (10291)

brightfield



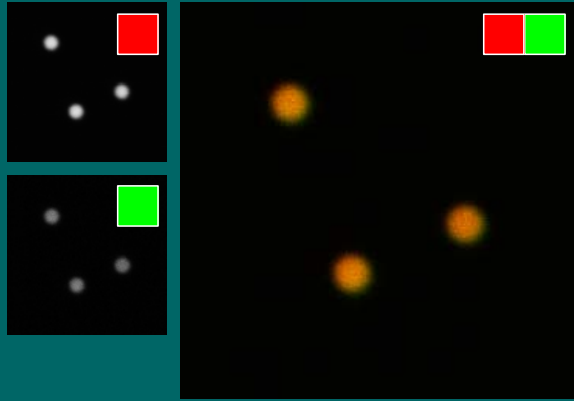
Histogram > Scatterplot



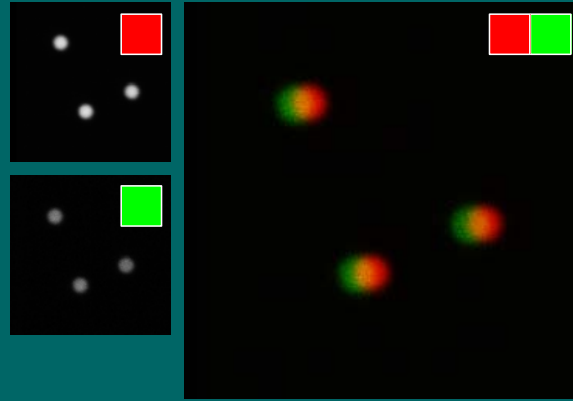
pixel localization



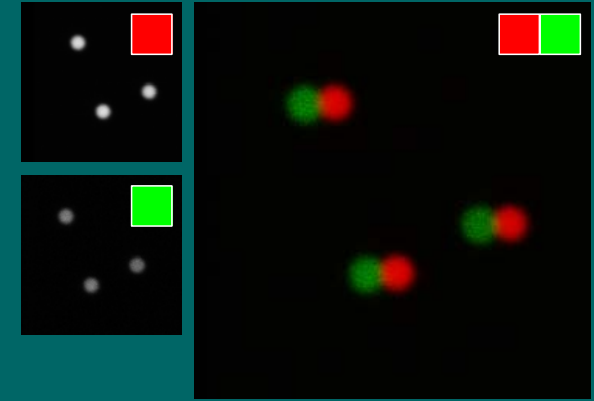
Scatterplot



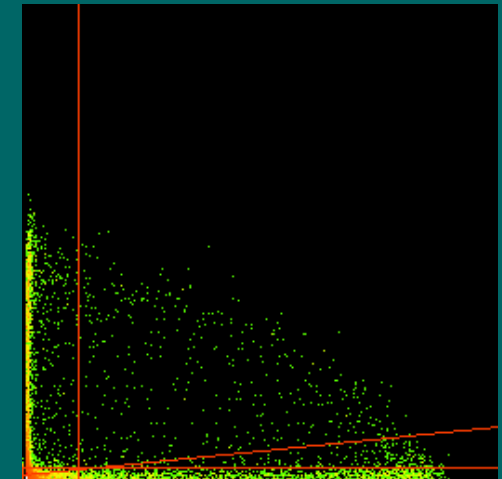
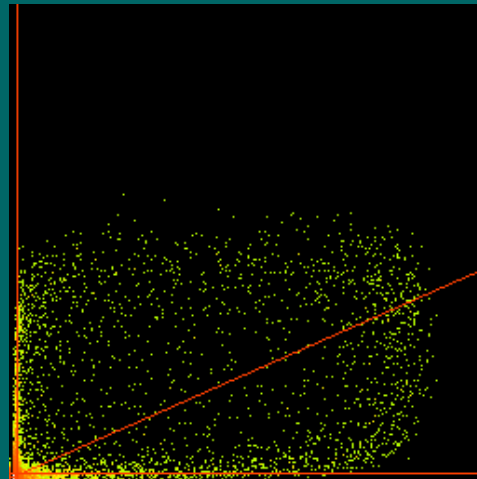
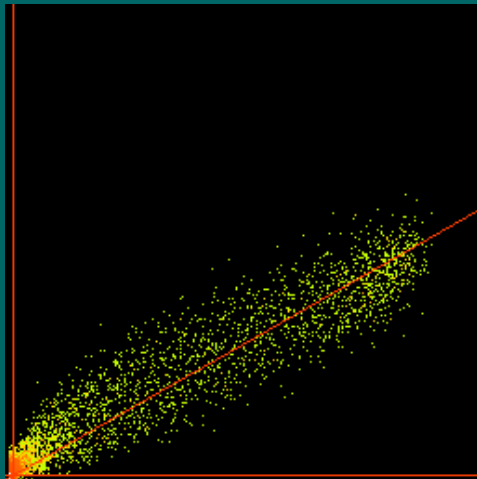
original R+G

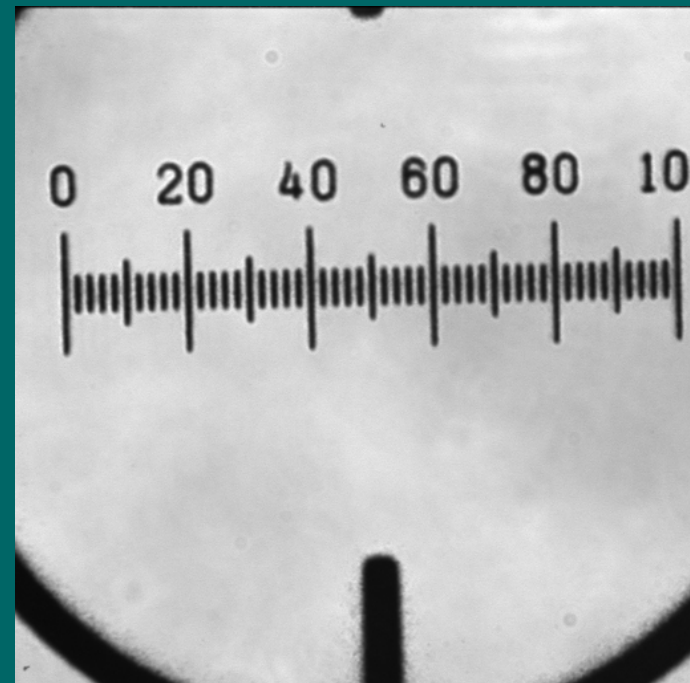
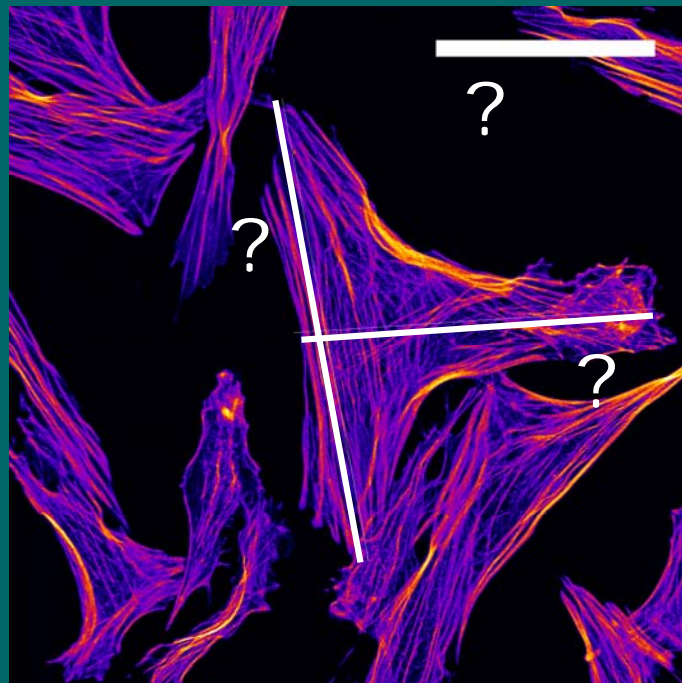
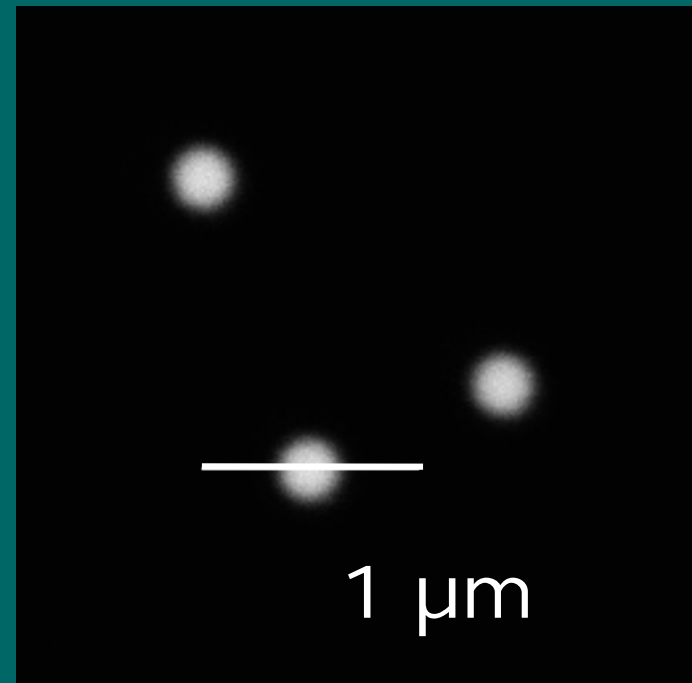


R shifted +10 pix



R shifted +20 pix





↓
?

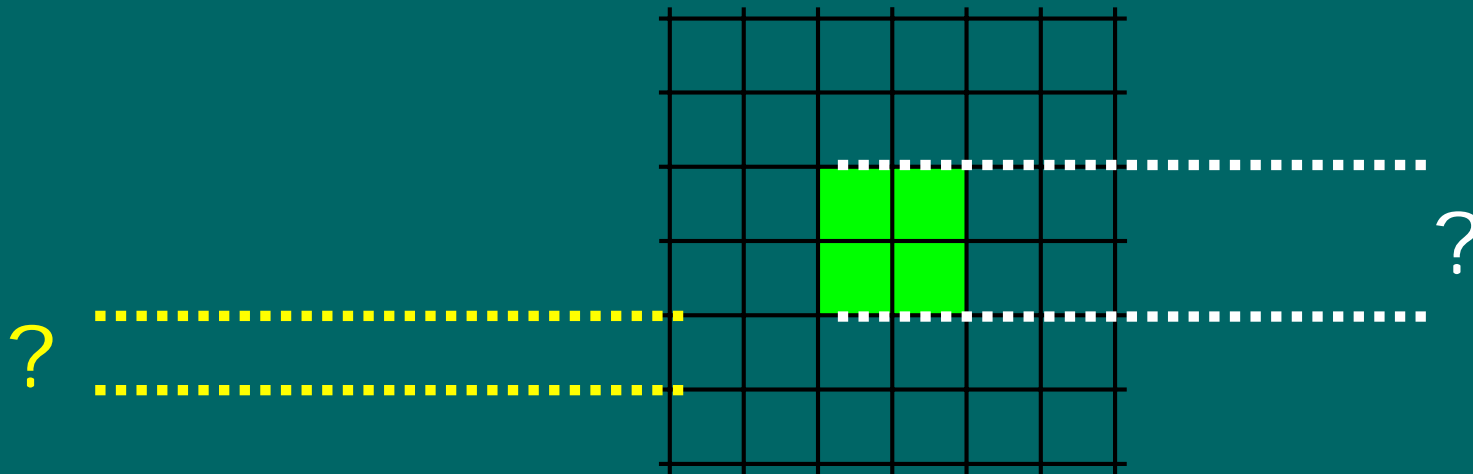
↓
?

↓
?



Pixelsize & Scaling

- How big is a structure that is represented in my image?
=
• How big is one pixel?



Pixelsize & Scaling

- Pixelsize stored by acquisition system in „metadata“
- might be changed / lost during processing
- dataset for image processing:
 - image data
 - metadata



Practical Session B

- Histogram
- Scaling

fluocells6.tif

ruler.tif

