Digital Imaging

Data Handling

Basic Image Processing

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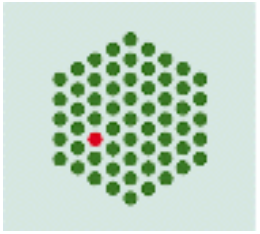


EMBL
European Molecular Biology Laboratory

Overview

Part I:

- File formats (data storage)
- Programs for
  image viewing / processing / representation

Part II:

- Basic Image Processing (using ImageJ)

File Formats – data storage

- Lossless image formats
- Lossy compression formats
- Custom formats (microscope companies)
- Sequence vs. single image per file
- 8bit, 12bit, 16bit, 32bit, RGB

- Storage:
  - Always have at least 1 copy of the data
  - Very suitable: fileservers (automatic backup)

Lossless Image Formats

- TIFF (with our without compression)
- BMP (windows uncompressed)
- GIF (graphics interchange format)
- PNG (portable network graphics)
- Raw data
- ‘text image’

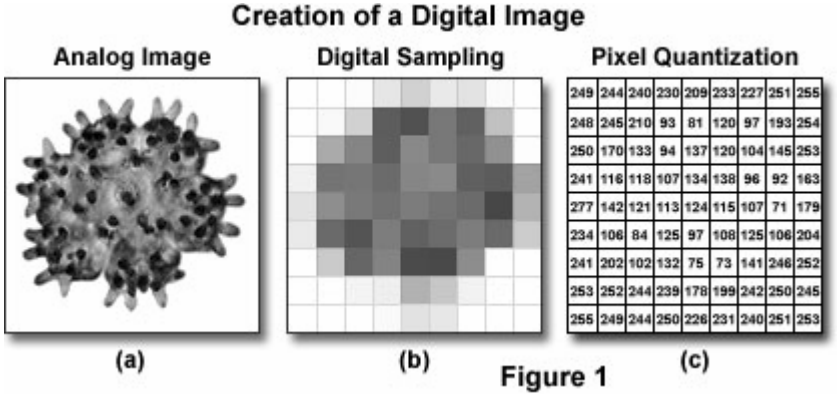


Figure 1

Microscopy Primer
http://micro.magnet.fsu.edu/primer

Image Format: TIFF

- Tag Image File Format
  - Image header with flexible set of ‘tags’ which can be used to store e.g. microscopic settings
- Flexible in color space and bit depth
  - Microscopy: grayscale 8bit, 16 bit (12bit data)
  - Color (e.g. Overlay): RGB (red green blue 8bit each)
  - Quantification: 32bit (floating point values)
- Always lossless: Uncompressed or compressed
- Multiple images possible in one file

Lossless image compression

- Redundant information can be compressed
- Identical information can be grouped
- Repeated patterns can be used to compress data size

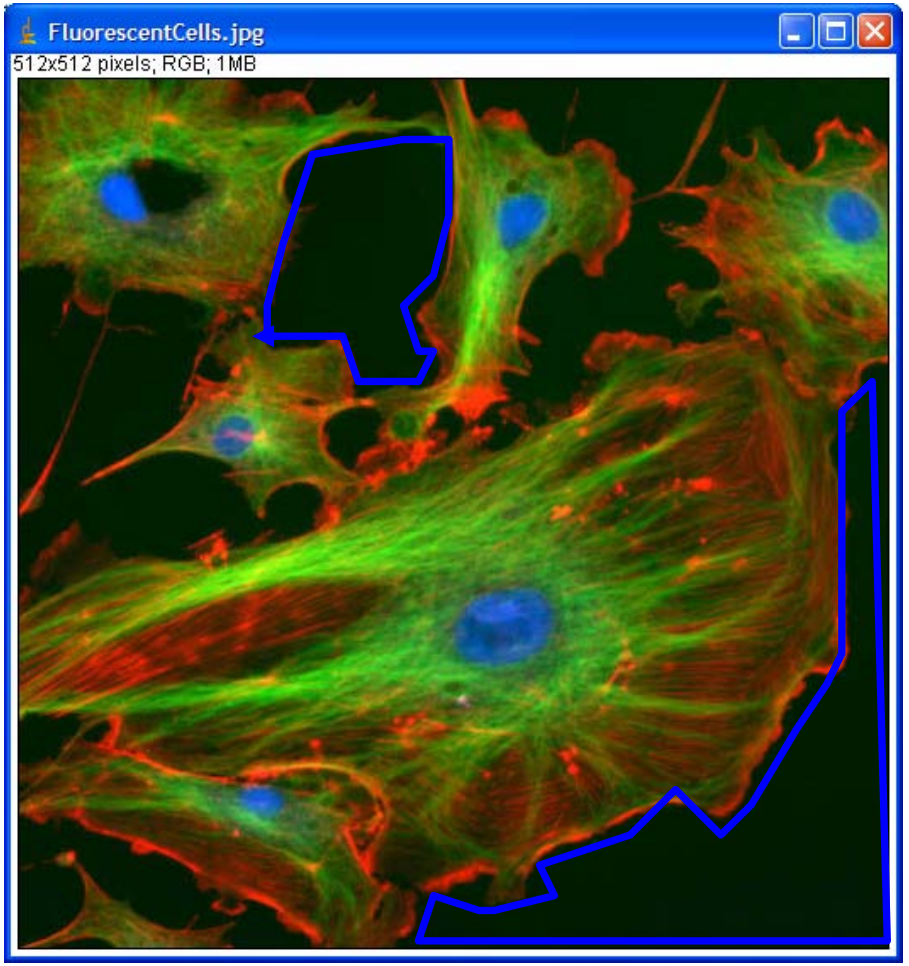
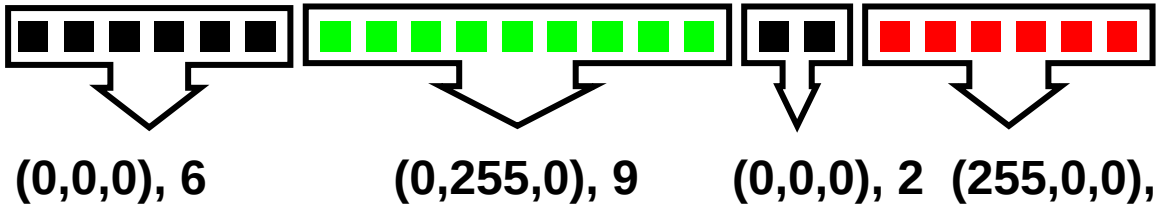


Image Compression: TIFF

- Run Length Coding (RLE): first number discribes the color, the second the number of following pixels having the same color.



- LZW (Lempel-Ziv-Welch): Find repetative patterns of values and give them a number which is points to an entry of a „dictionary“ (LUT).

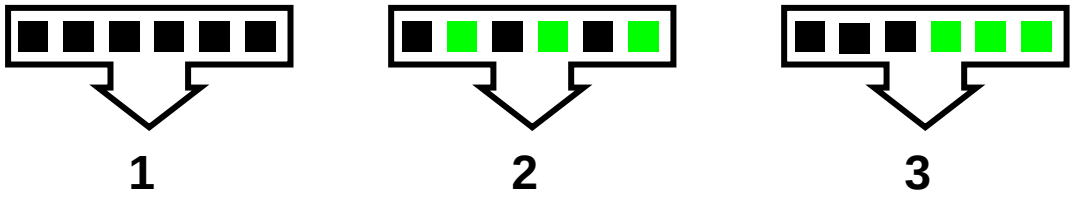
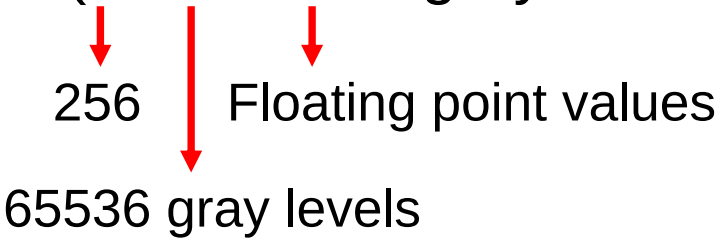


Image Compression: TIFF

Pros:

- Extra infos can be written in the ‚tags‘
  (e.g. microscope data, like objective lens, voxel size)
- Most programs can read it
- Lossless
- Flexible (8, 16, 32bit grayscale, 8:8:8bit RGB)



Cons:

- Big files
- Compressed files can´t be loaded by ImageJ

Lossy Image Formats

- The lossy compression algorithm takes advantage of the limitations of the human visual senses and discards information that would not be sensed by the eye. (like mp3 in audio).
- Compression level is usually flexible, but the more compressed the more information is lost and artifacts become visible by eye

Original Lena Image (12KB size)

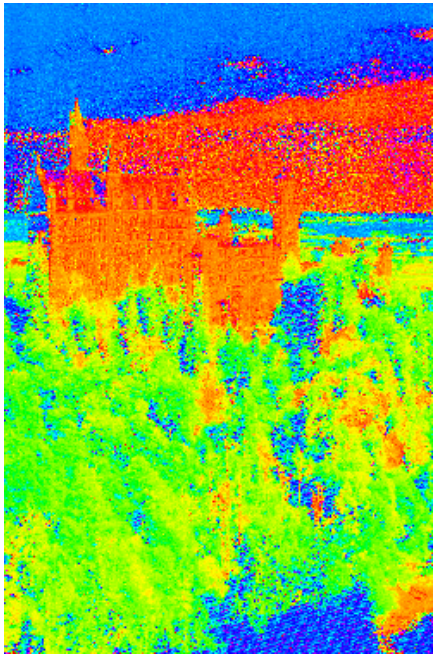
Lena Image, Compressed (85% less information, 1.8KB)

Lena Image, Highly Compressed (96% less information, 0.56KB)

From: www.wikipedia.org

Image Compression: JPG

- Split image into color and gray-scale information (color is less important than bounderies)
  → reduce high frequency color information.
- Group pixel into 8x8 blocks and transform through discrete cosine transform…



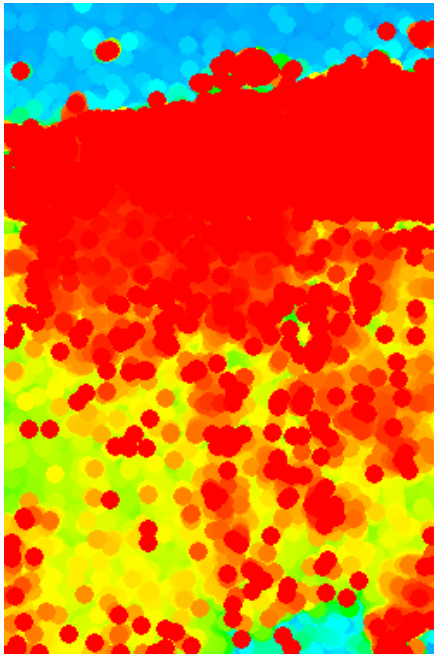
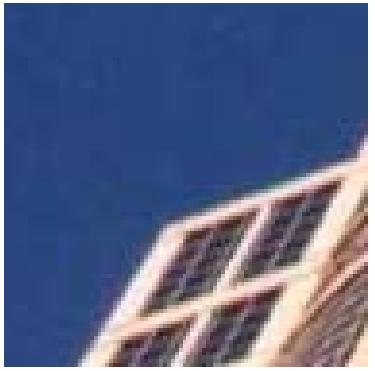



Image Compression: JPG

Pros:

- Small Files
- True Color
- Usable for most photos (real life) and presentations (powerpoint)



Cons:

- **Do not use for quantification !**
- „Unrelevant“ details get lost
- Every file-saving reduces the quality

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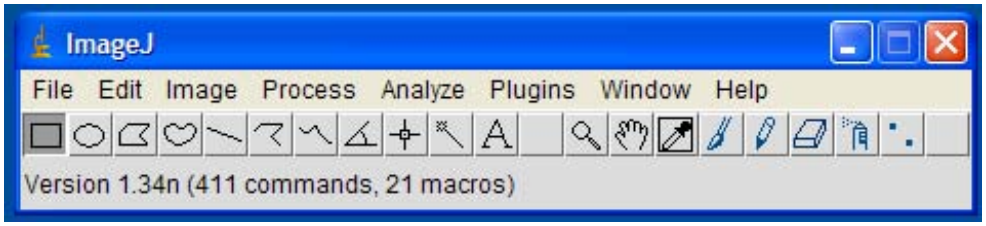
Image Viewers

- **ImageJ** (Java based, freeware, Win/MAC/Linux)
- **Irfanview** (www.irfanview.com/)
  - Freeware
  - Convert (e.g. tif → jpg)
  - Batch processing
- **ACDSee** (ACD Systems)
- Microscope companies
  - Zeiss Image Browser / Axiovision LE
  - Leica LCS Lite
  - Olympus Viewer

Image Representation

- **ImageJ**
- **Imaris** (Bitplane):
  - 2 floating licenses
  - installed on image processing workstations
- Photoshop, Paintshop, Illustrator, Corel Draw (, Powerpoint)
- **Volocity** (Improvision):
  - 1 License, installed on 1 image processing workstation
- **Amira** (Mercury computer systems)
- Custom software of microscopes

Image Processing



- **ImageJ** (http://rsb.info.nih.gov/ij/index.html)
  - installed on all image processing workstations
  - Installation: www.embl.de/almf/html/EMBL_ImageJ.htm OR www.macbiophotonics.ca/imagej/installing_imagej.htm
  - Manual: http://www.macbiophotonics.ca/imagej/
  - Additional plugins: http://rsb.info.nih.gov/ij/plugins/index.html
- **Metamorph** (Universal Imaging),
  - installed on one image processing workstations
- Custom software of microscopes

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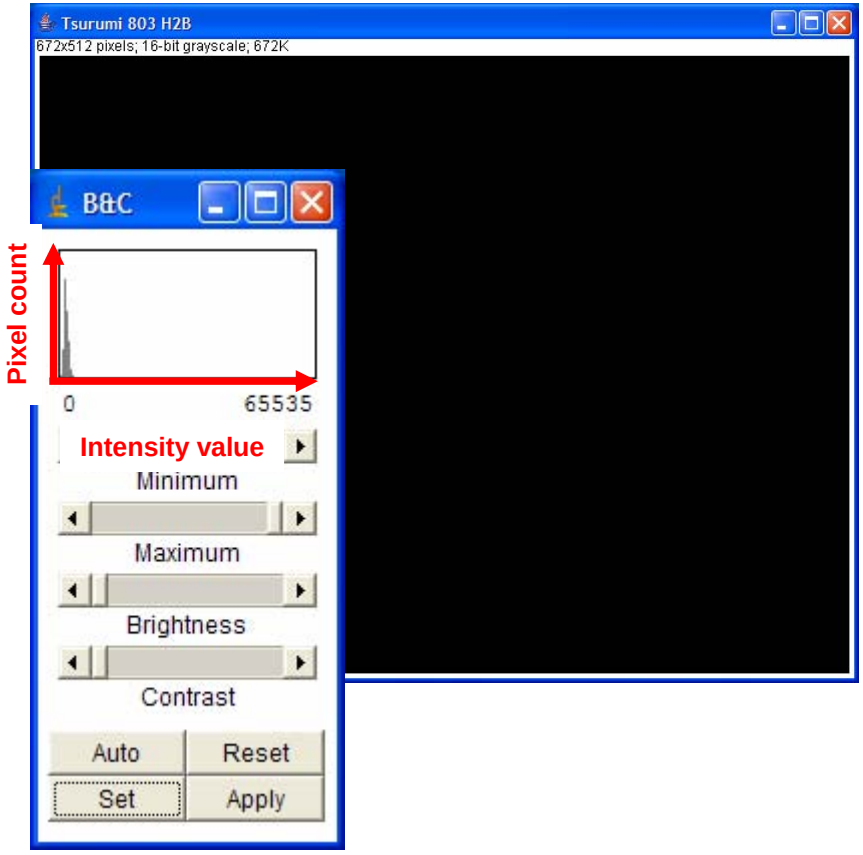
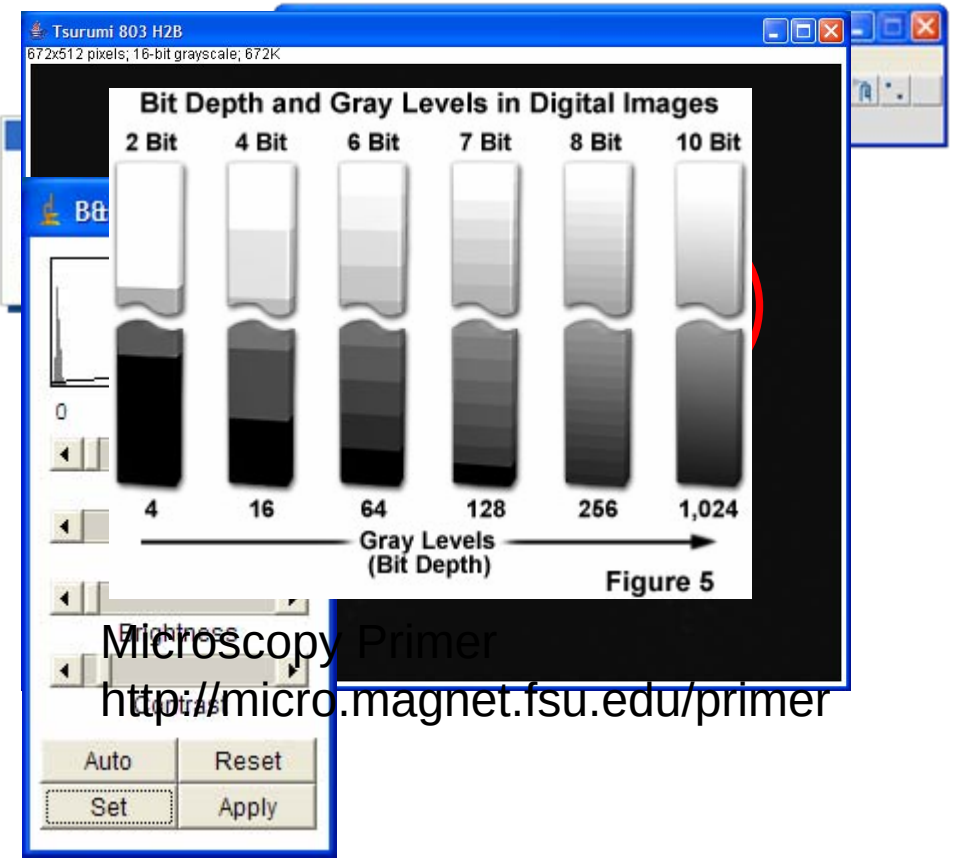
Image Processing Basics

- Visual Image Inspection
- Lookup tables (LUT) and LUT operations
- Histogram, brightness, contrast
- Filter
- Threshold
- Measurements
- Color functions

Visual Image Inspection

- Displaying images, histogram

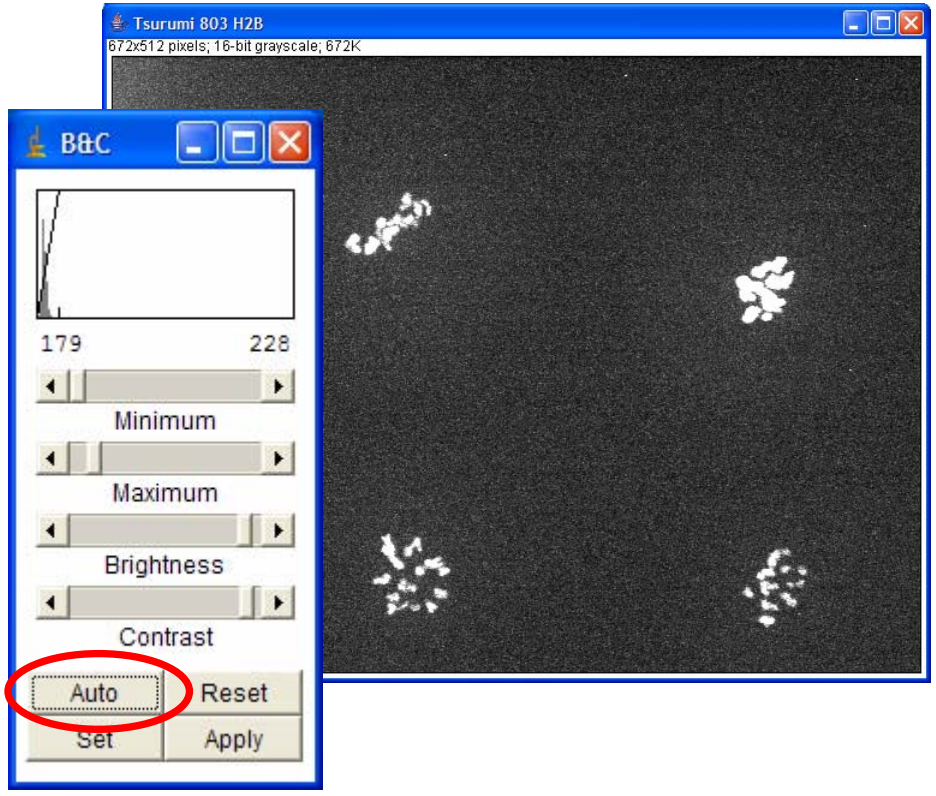




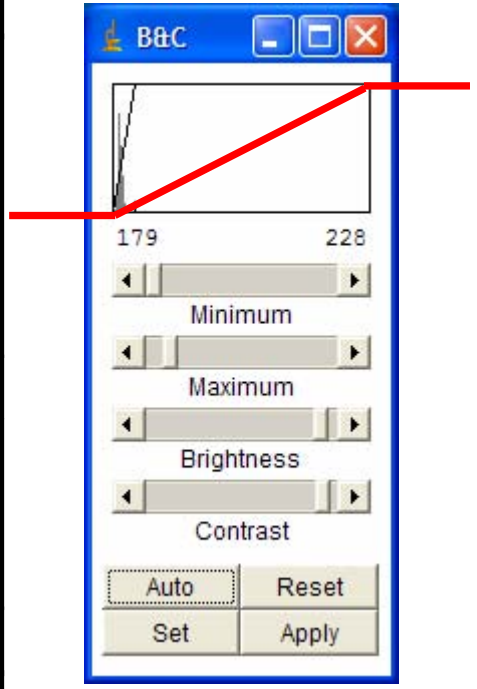
Microscopy Primer
http://micro.magnet.fsu.edu/primer

LUT operations

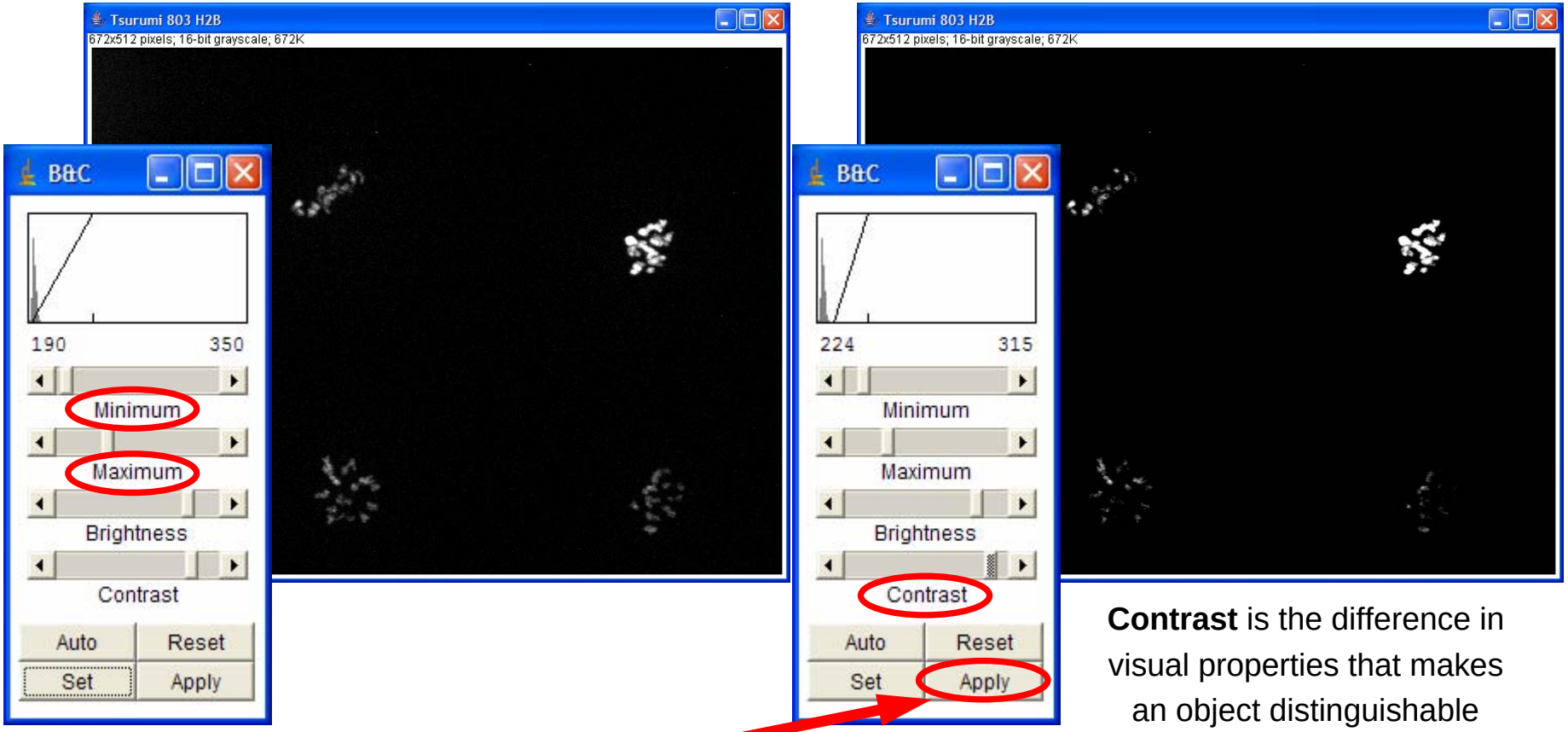
- Lookup table (LUT)
  - Displays can only show 256 gray values (8bit) per color
  - Data is unchanged, it´s only “mapped” differently



Data Intensity	Displayed Intensity
0	0
…	…
179	0
180	5
181	10
…	…
226	
227	
228	255
229	255
65535	255



Brightness, Contrast



Caution: Apply modifies the data!

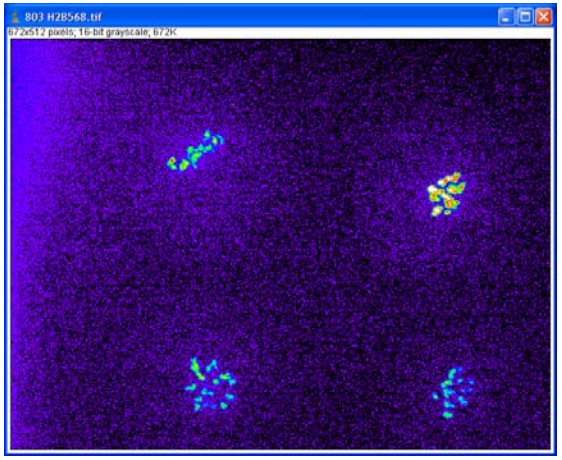
Contrast is the difference in visual properties that makes an object distinguishable from other objects and the background.

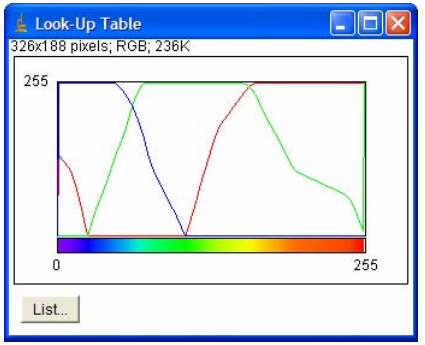
Color LUT

- The pixel contains a „pointer“ to an array, where the actual pixel values are stored

old LUT:
1: (0,102,255)
2: (51,102,255
3: (10,100,200)

new LUT:
1: (0,0,0)
2: (0,255,0)
3: (255,0,0)





“Rainbow” LUT

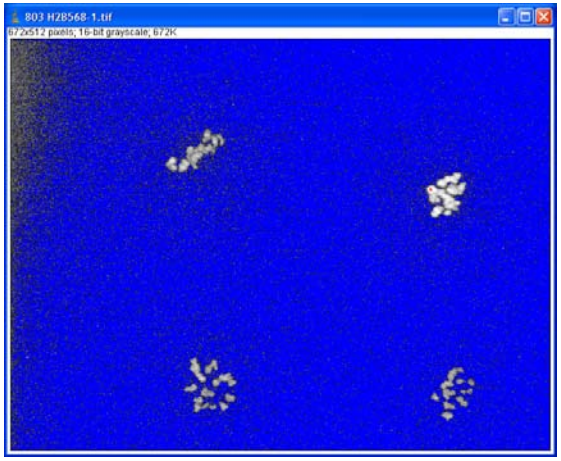
Non-linear Histogram Stretch

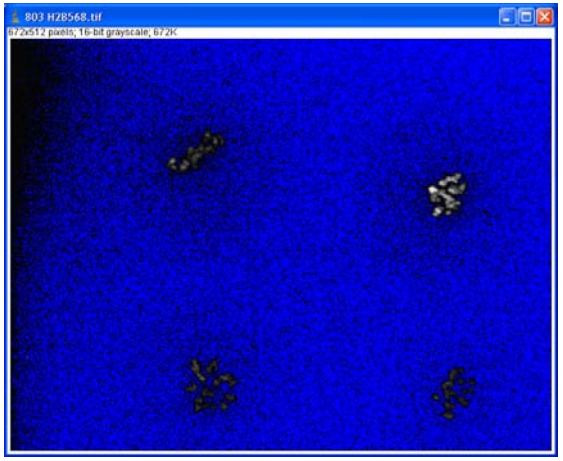
- Enhance contrast by (changing data):

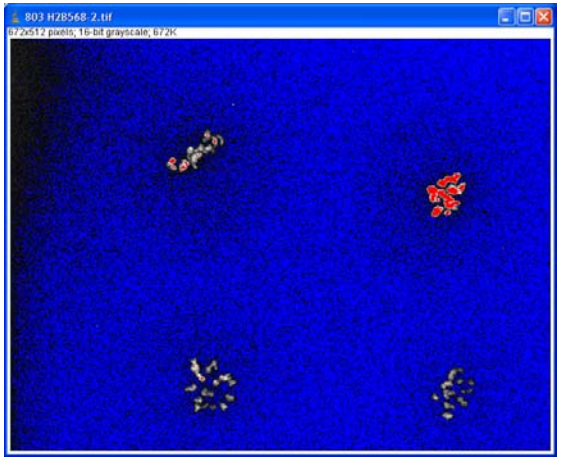
"**Equalization**" non-linear stretch based on square root of the intensity

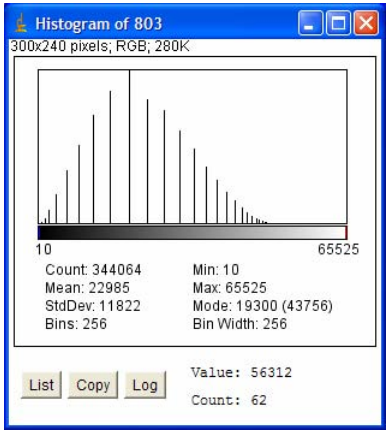
Raw data

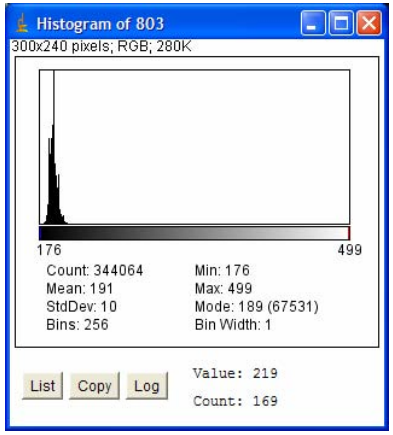
Linear stretch "**Normalization**"

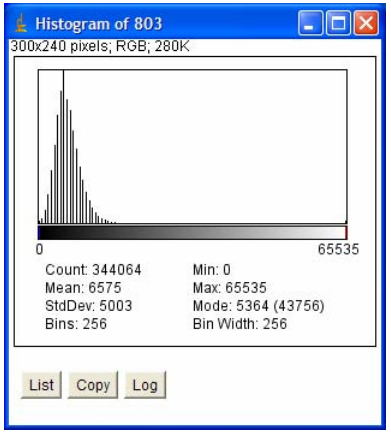




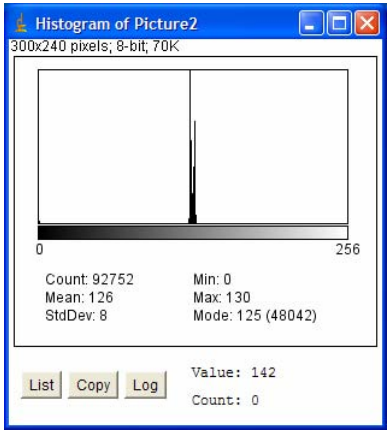


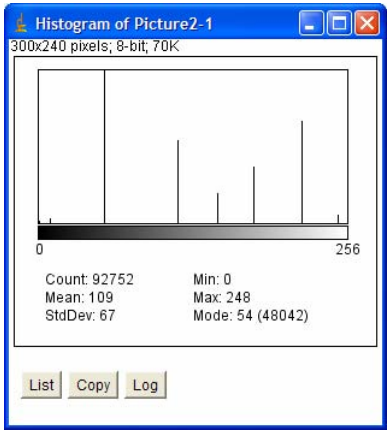






Equalization

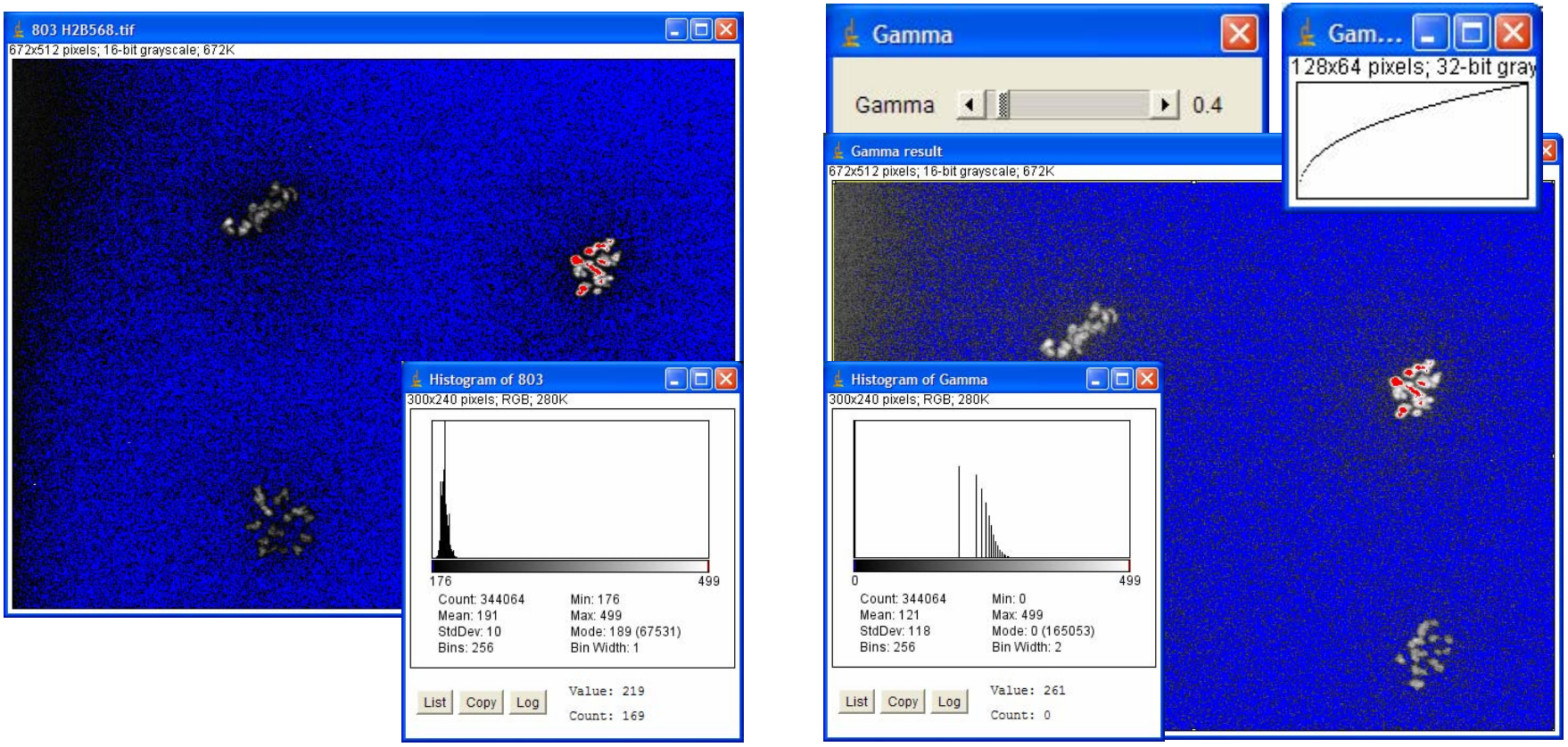




Gamma

- Gamma is a non-linear histogram adjustment
- 8 bit images:

  New intensity = 255 × *[(old intensity/255) gamma*]

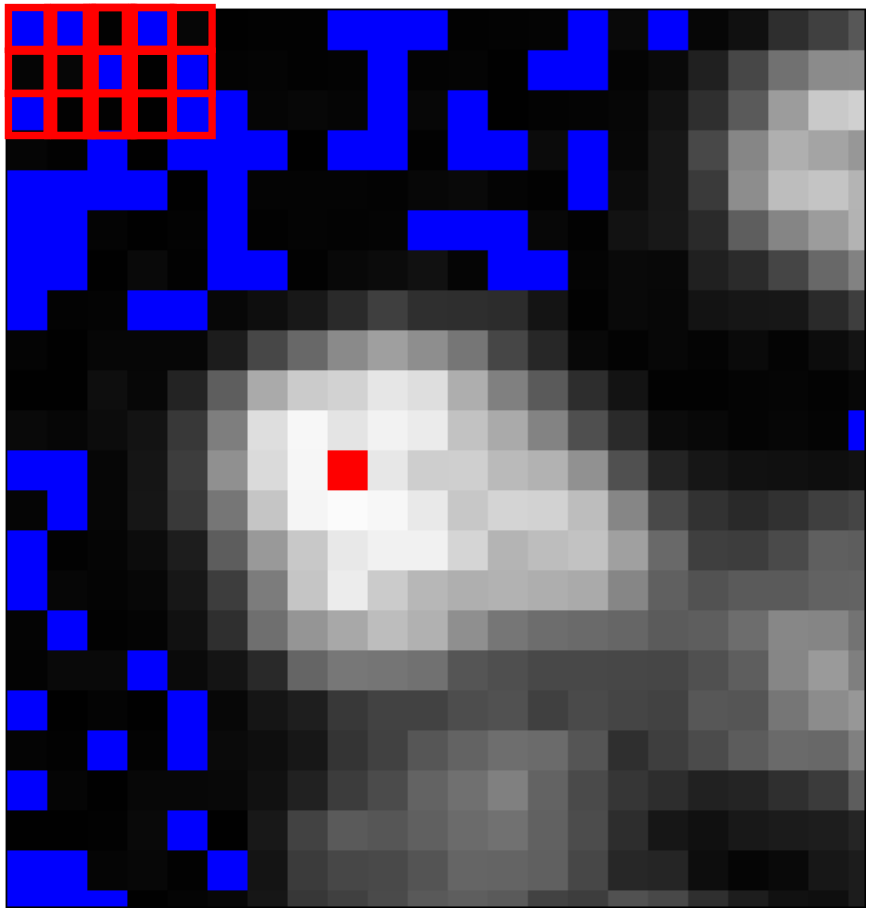


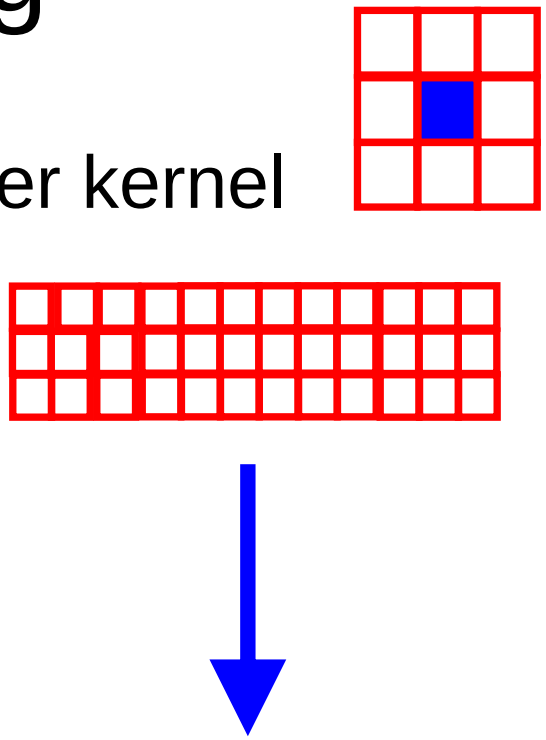
Filtering

- Image processing filters are mainly used to:
  - suppress the high frequencies in the image, *i.e.* smoothing the image, noise reduction
  - or suppress the low frequencies, *i.e.* enhancing or detecting edges in the image
- An image can be filtered either in the frequency or in the spatial domain.
  - Filtering in the frequency domain requires Fourier transform first and re-transformation after application of the filter.
  - Filtering in the spatial domain is done by convolving the image with the filterfunction.

Filtering

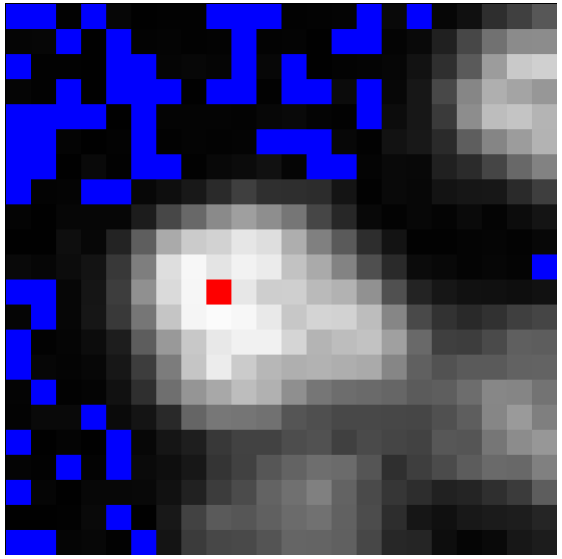
- Shifting and multiplying a filter kernel

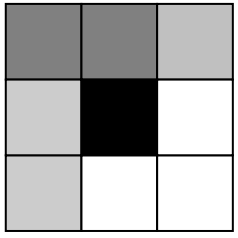




Filtered image

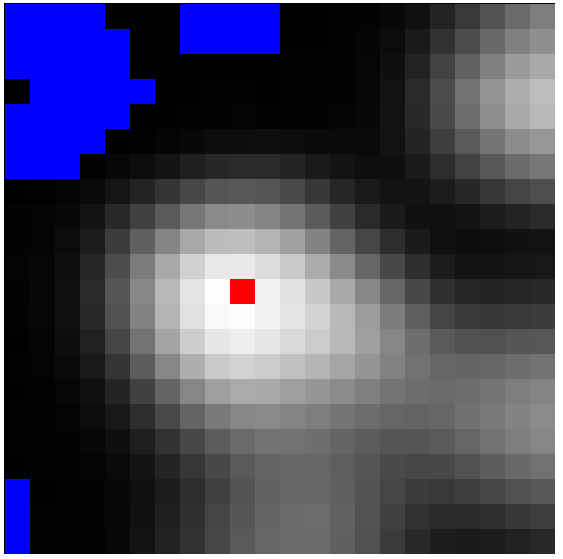
Noise Reduction: Mean

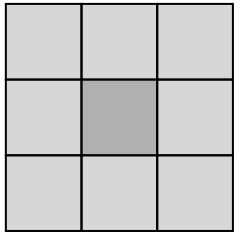




1/9	1/9	1/9
1/9	1/9	1/9
1/9	1/9	1/9

mean

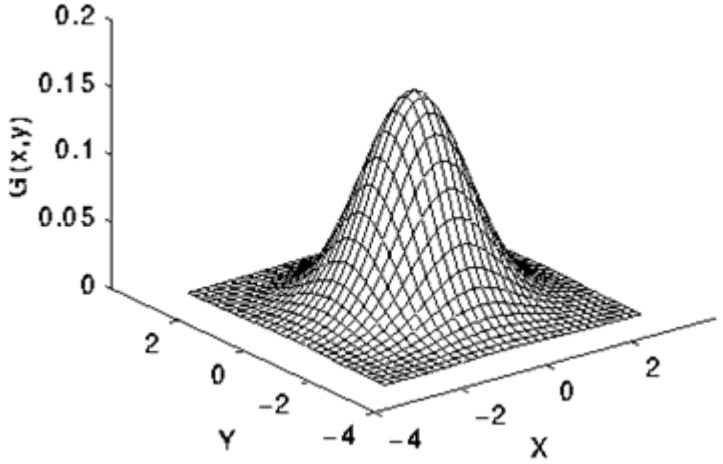




Mean 1pt

Noise Reduction: Gaussian

- Filtering with a gaussian bell-shaped kernel:



$\frac{1}{16}$

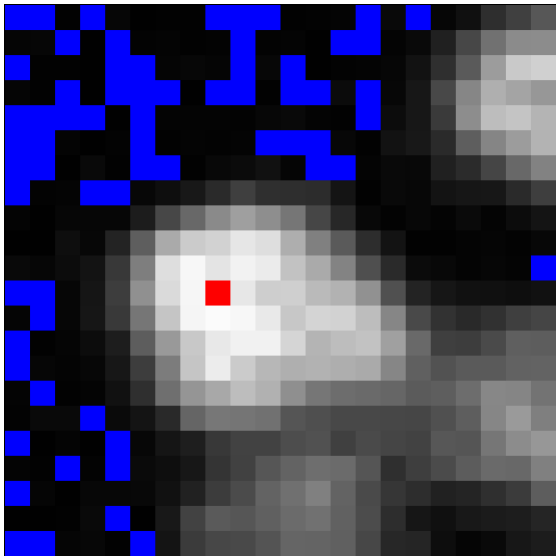
1	2	1
2	4	2
1	2	1

10	25	3
9	33	5

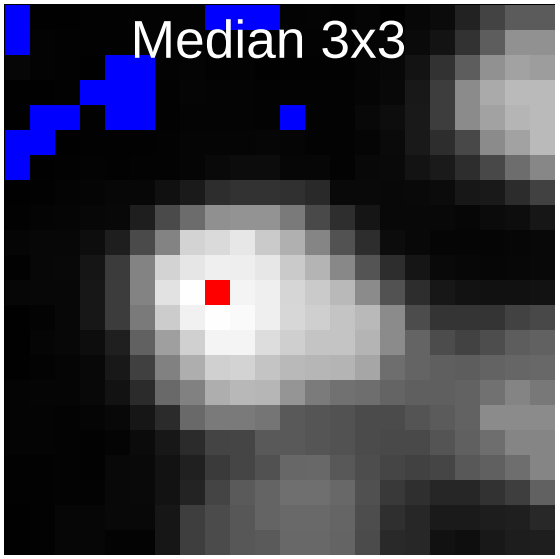
$\frac{1}{16}$

= 15

Noise Reduction: Median



median

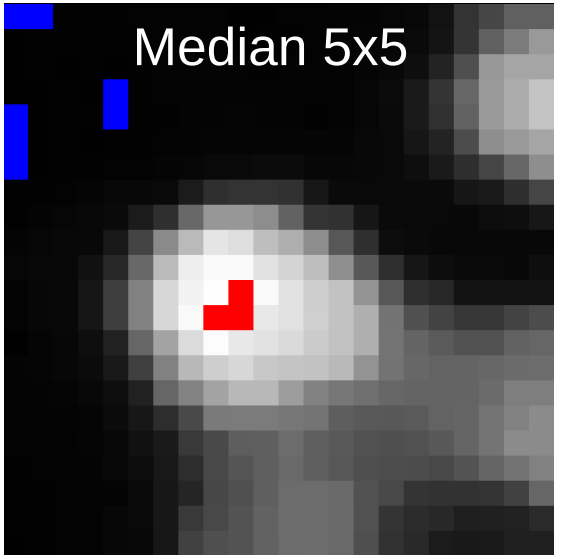


123	125	126	130	140
122	124	126	127	135
118	120	150	125	134
119	115	119	123	133
111	116	110	120	130

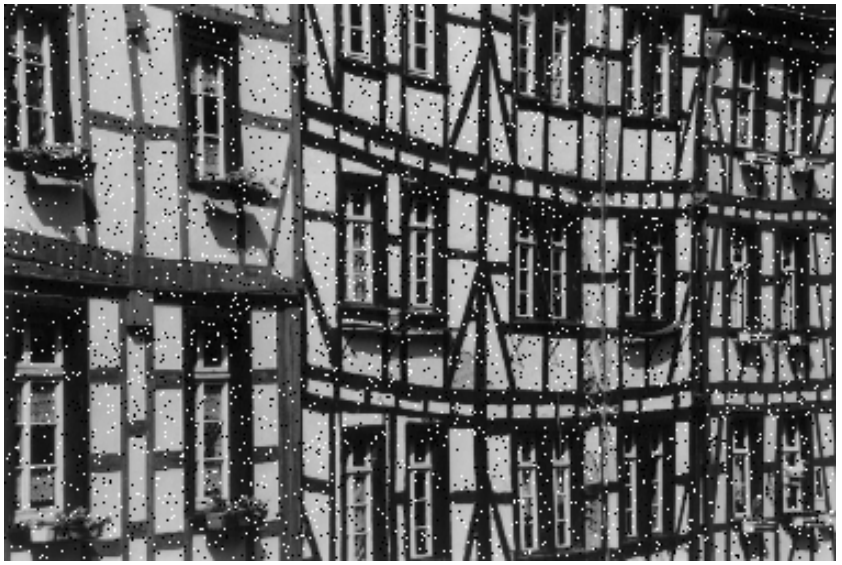
Neighbourhood values:

115, 119, 120, 123, 124,
125, 126, 127, 150

Median value: 124



Noise Reduction: Median, Mean



Median, 1pt

Mean, 1pt

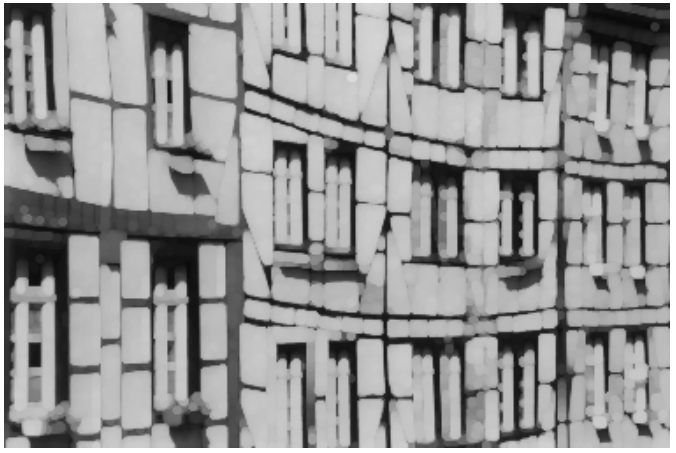
Median-, Mean-, Max-, Min-Filter

Median, 5pt

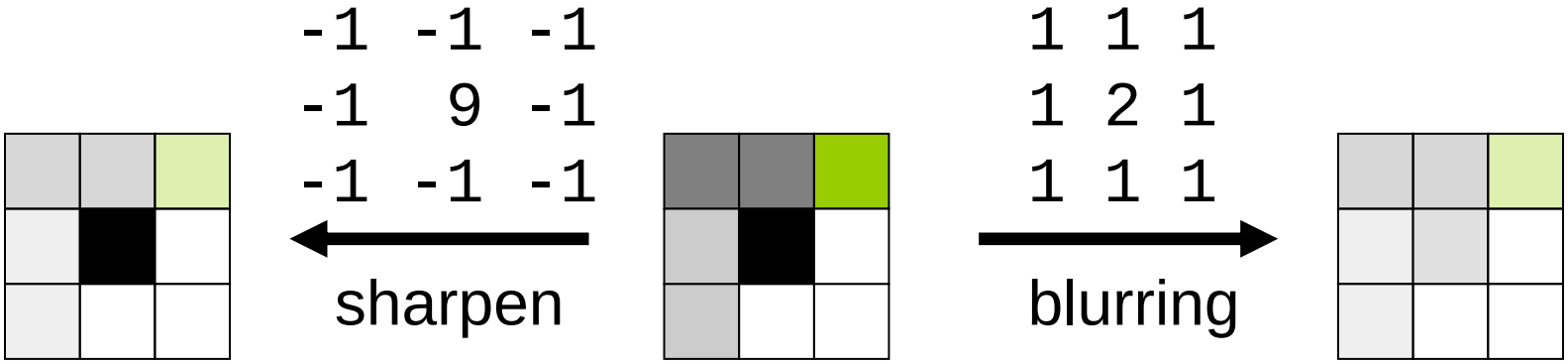
Mean, 5pt

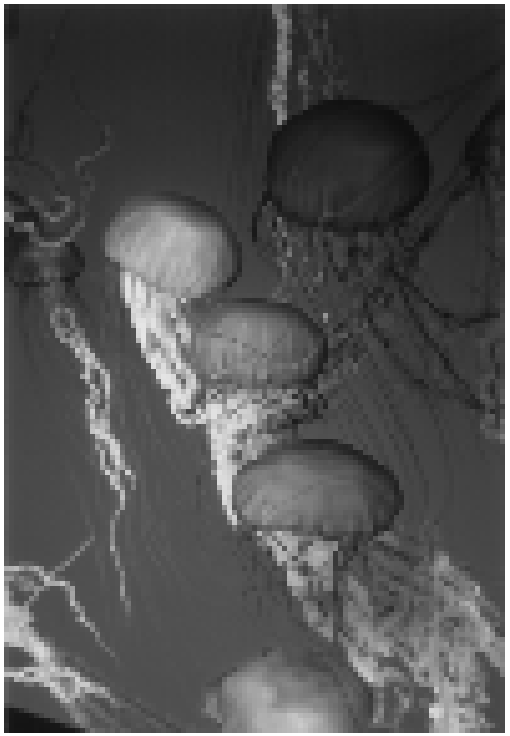
Min, 2pt

Max, 2pt

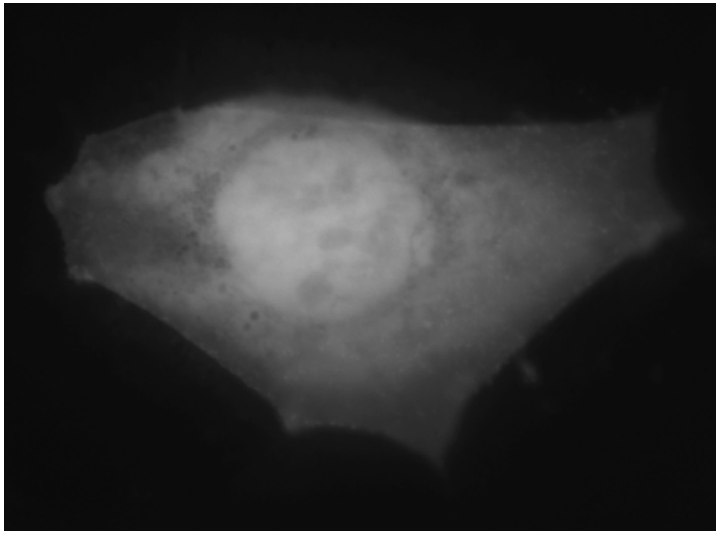


Sharpen / Blur



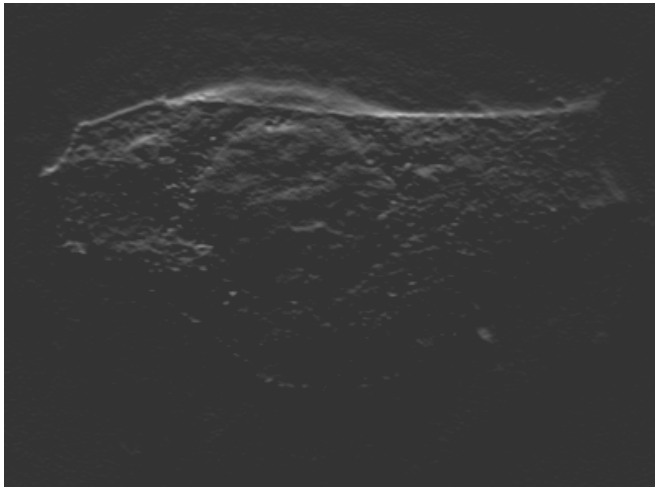


Example: Edge-Finding with derivatives



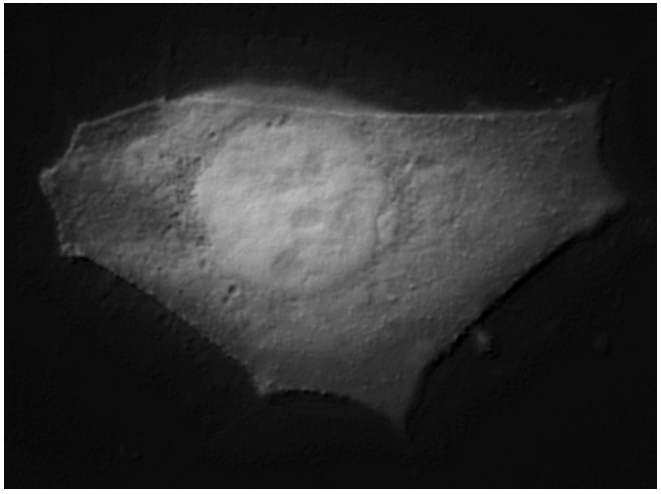
```
-1  -1  -1
 0   0   0
 1   1   1
```

→



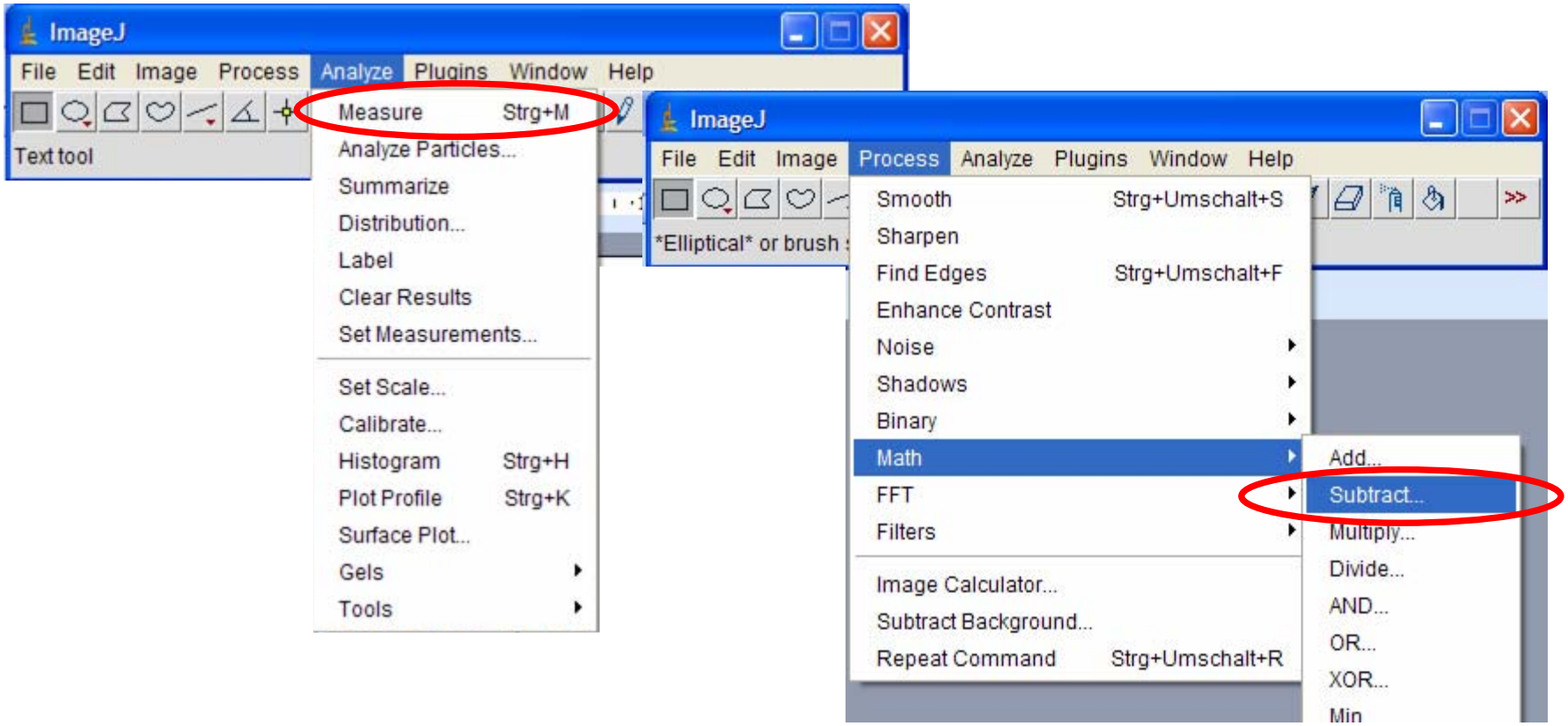
→

```
-1  -1  -1
 0   1   0
 1   1   1
```



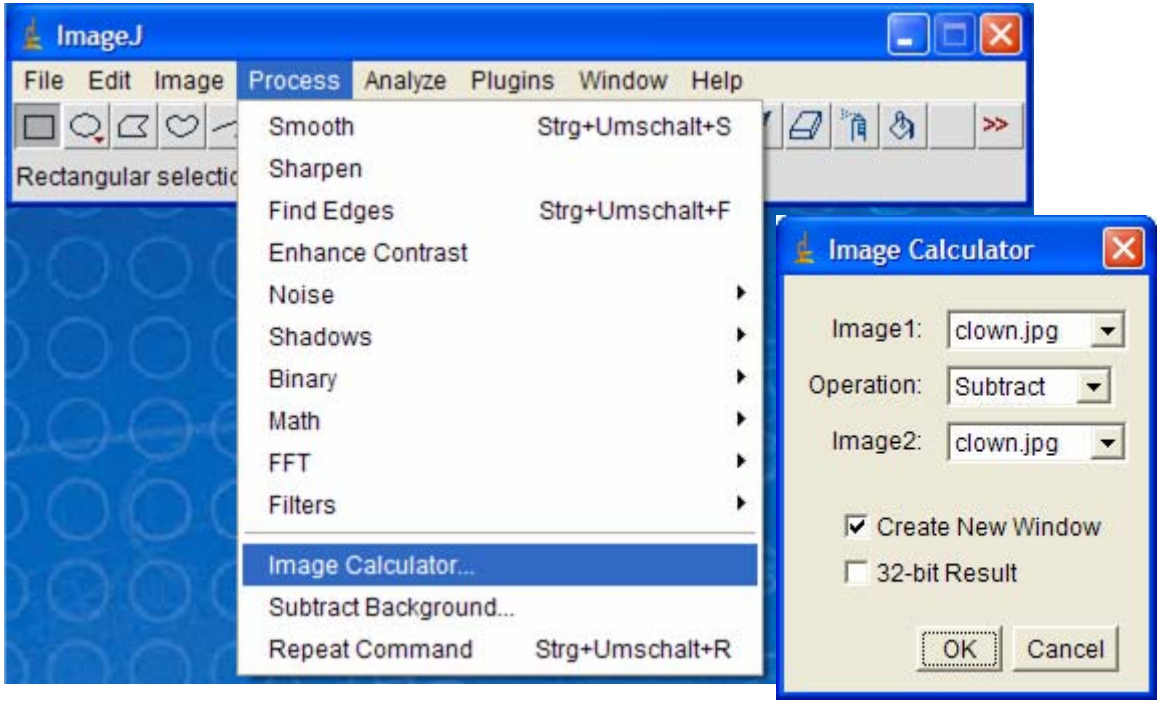
Background Subtraction

- Even background:
  - Measure the average intensity in the background and subtract this average from image



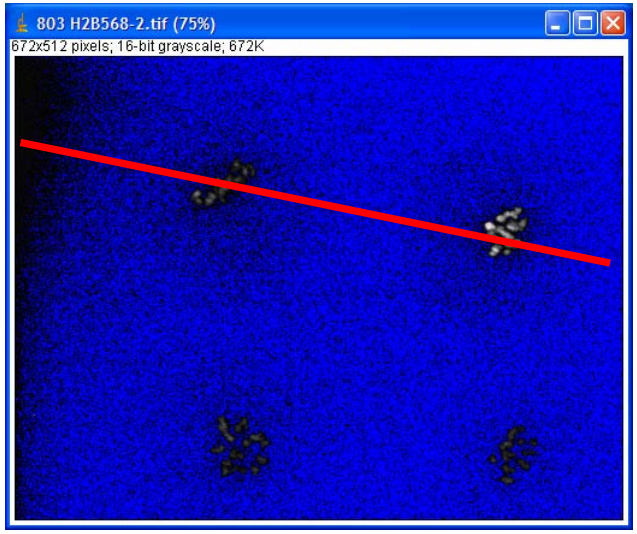
Background Subtraction

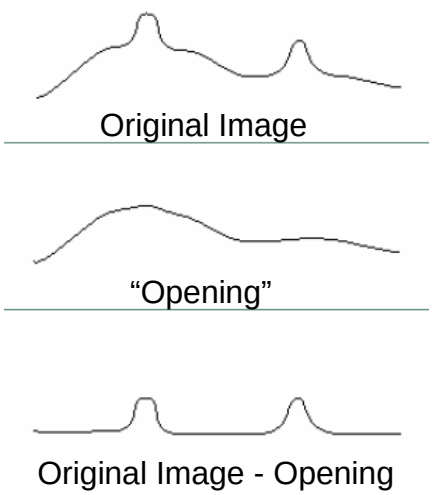
- Subtract “background image”
  (same exposure time without illumination)

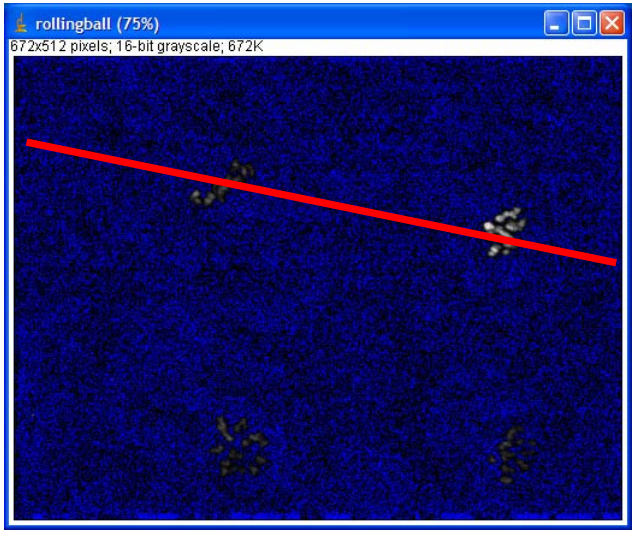


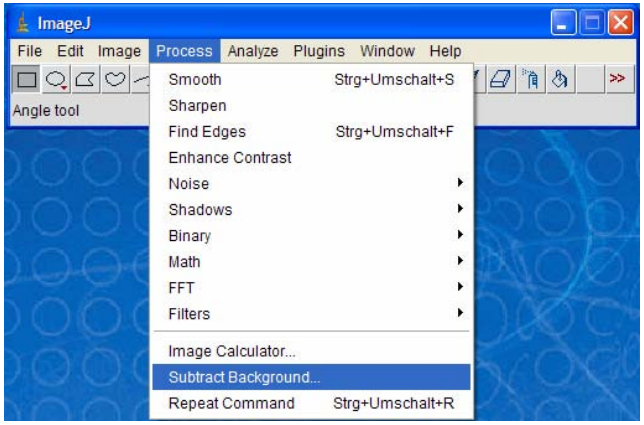
Background Subtraction

- Uneven background: Rolling ball filter
  - Use kernel larger than diameter of largest object

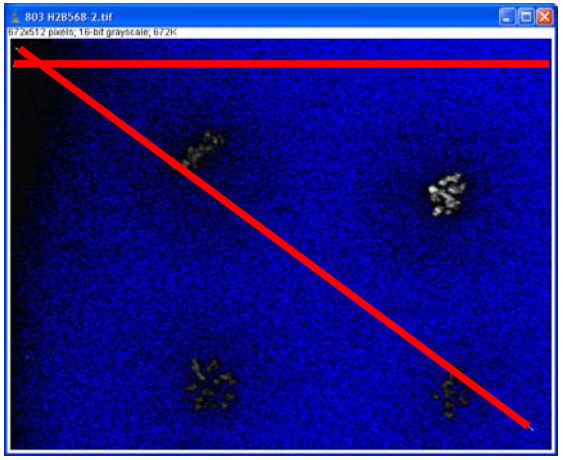




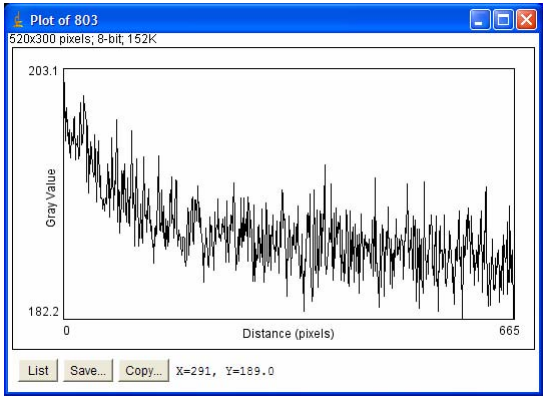


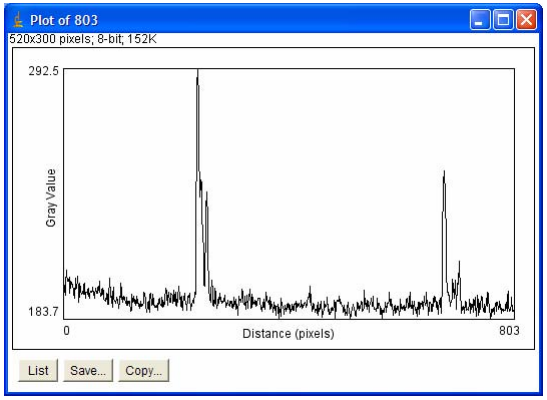


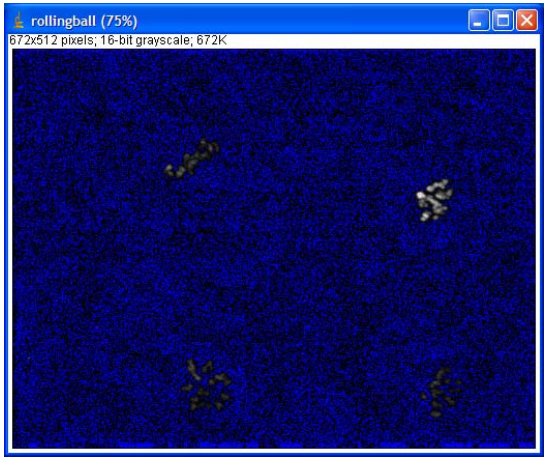
Line Profile



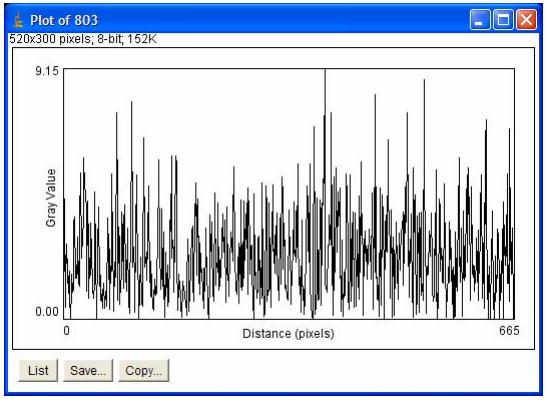
Without background subtraction

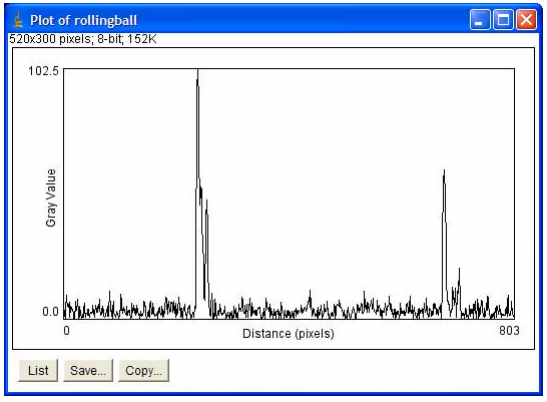






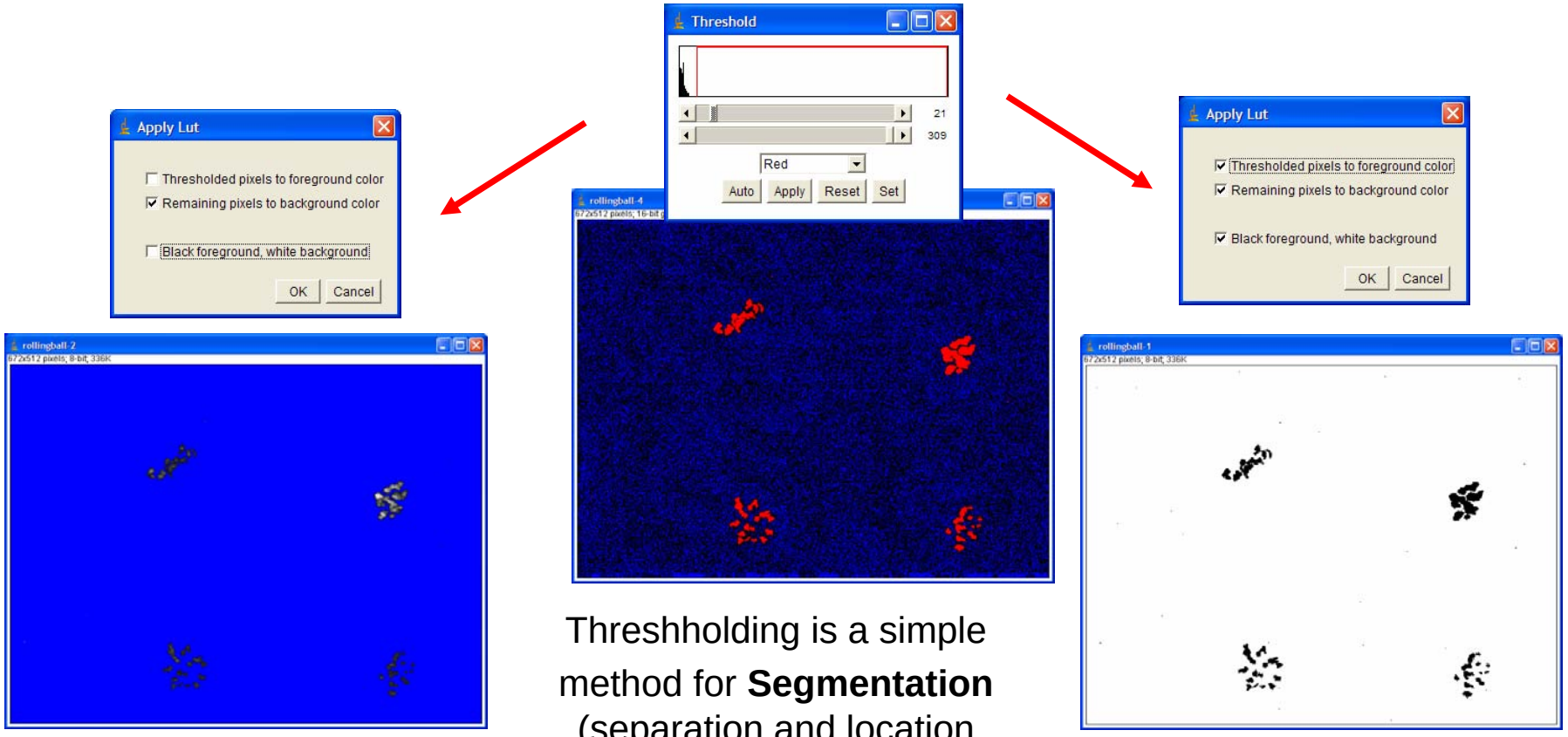
After rolling ball (50) background subtraction





Thresholding

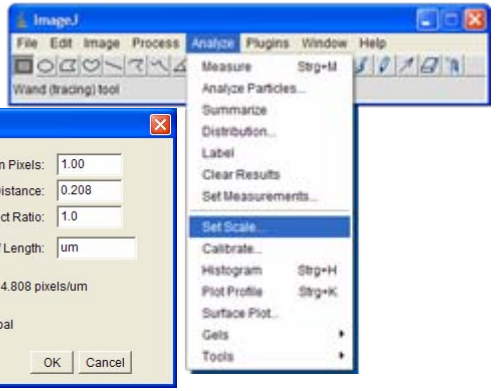
Thresholding is used to change pixel values above or below a certain intensity value (threshold):

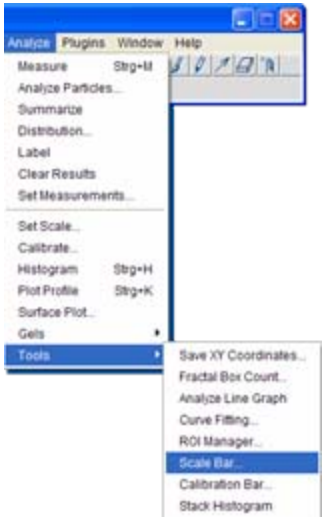


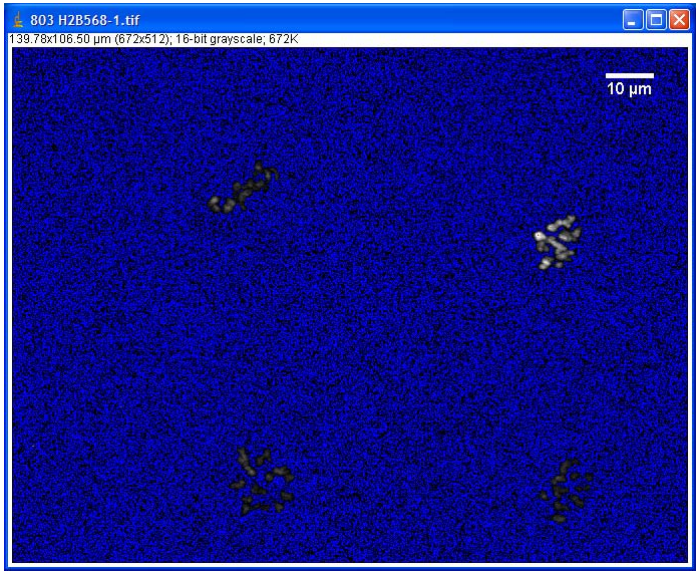
Threshholding is a simple method for **Segmentation** (separation and location of objects of interest)

Measuring Sizes

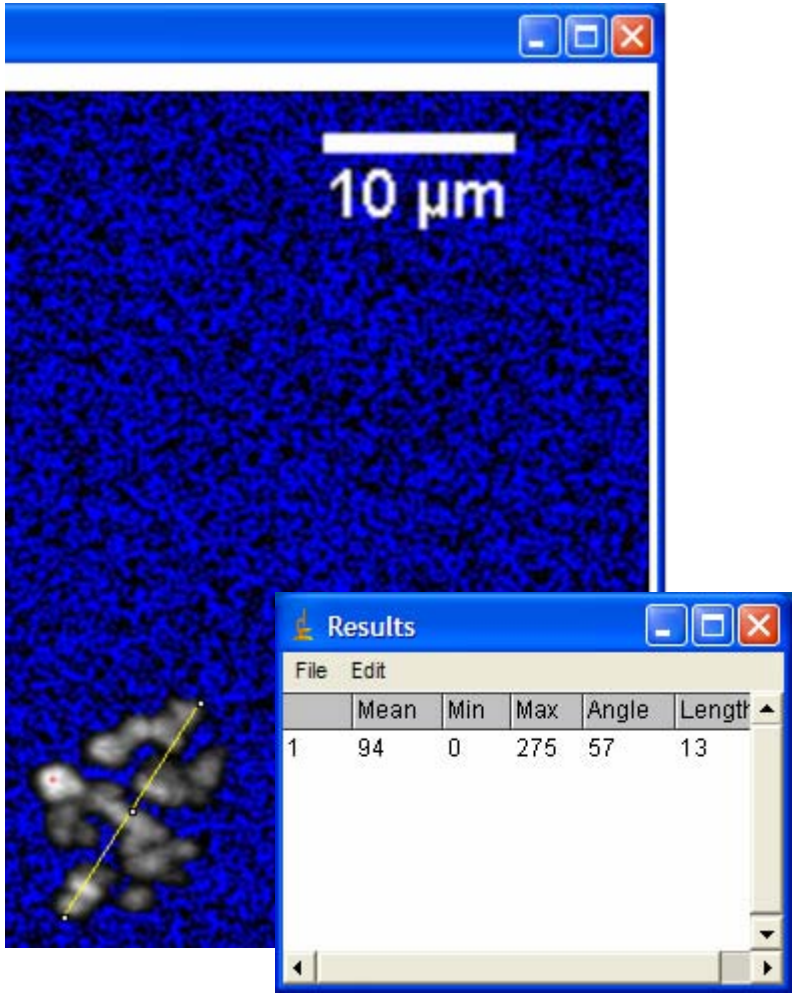
- Set Scale with pixel (voxel) size
- Include Scalebar

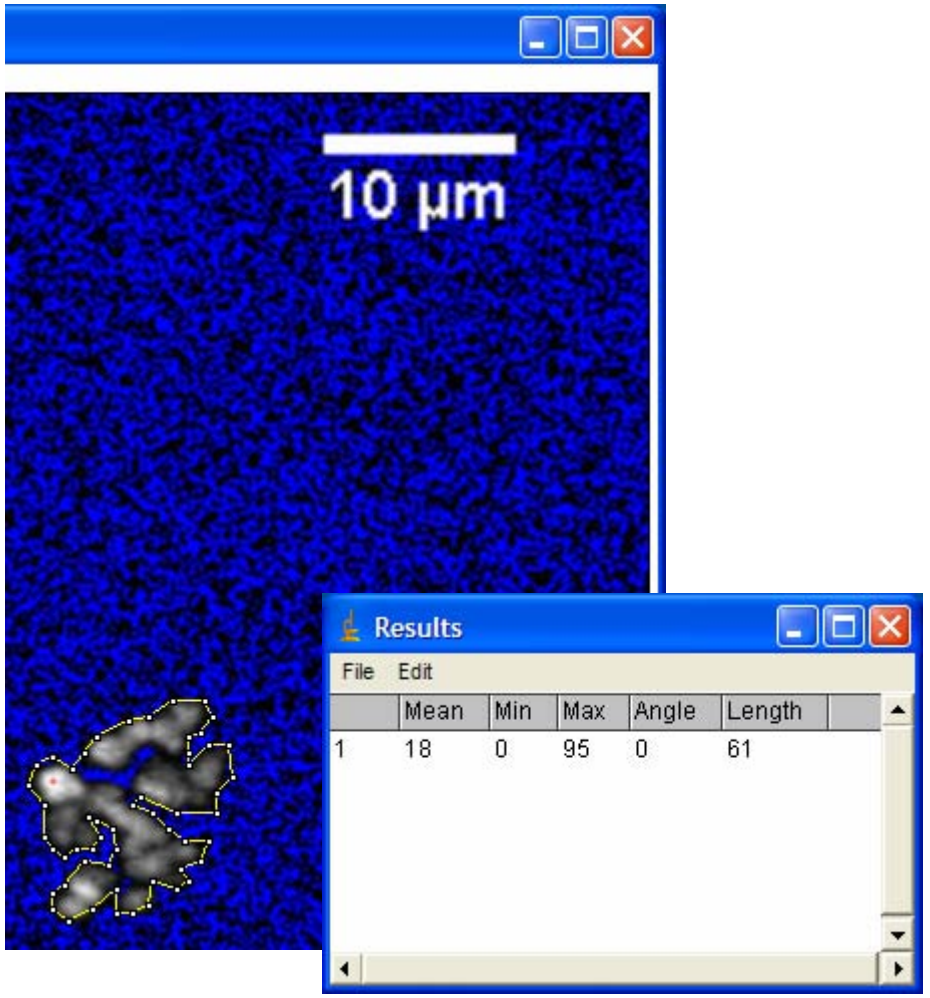




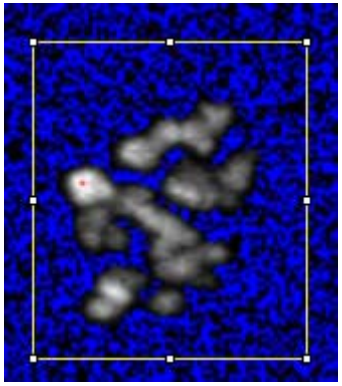


Measuring Length

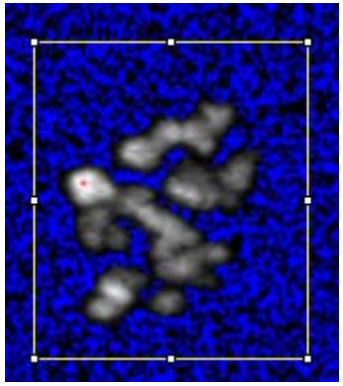




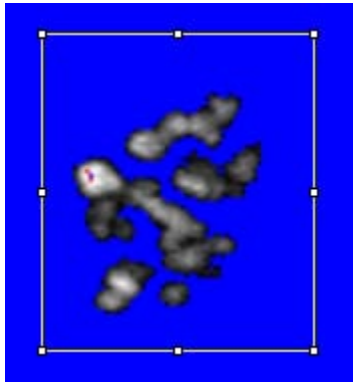
Area Measurement



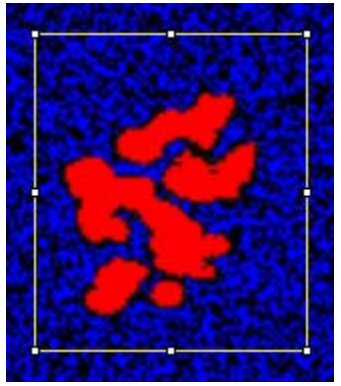
16bit image



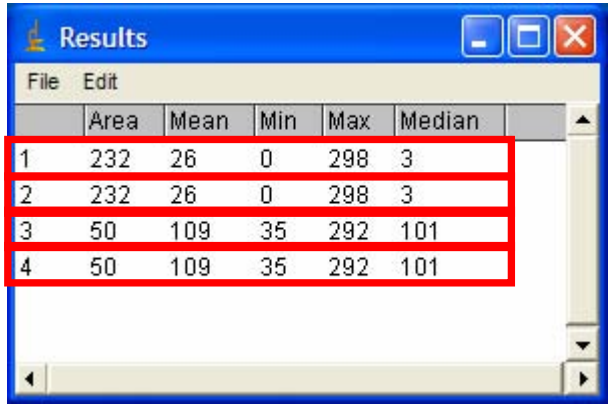
32bit image

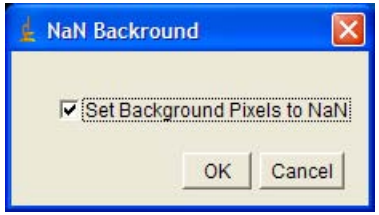


32bit image, background thresholded to “Not a Number”

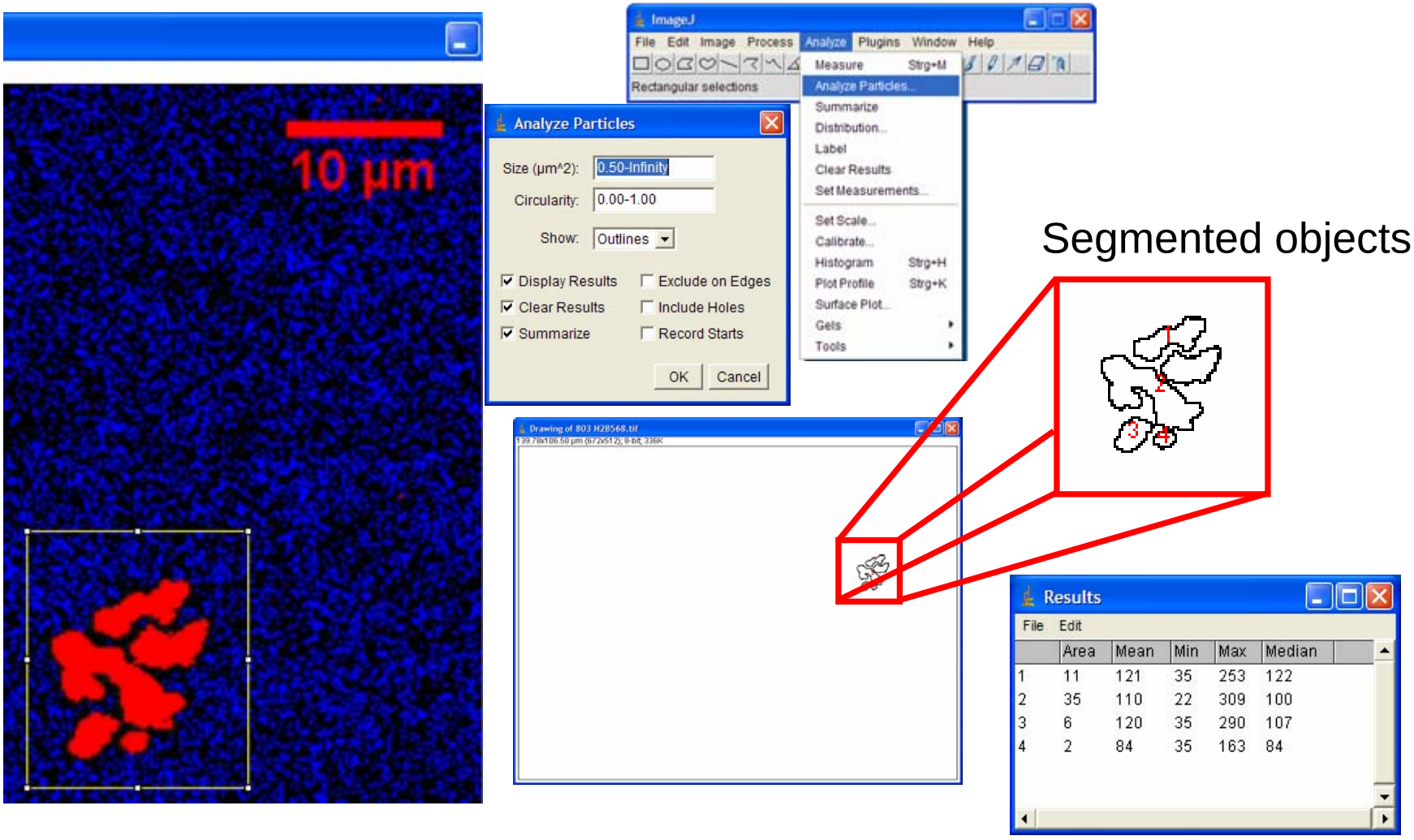


16bit image, same threshold as in 32bit image but not applied

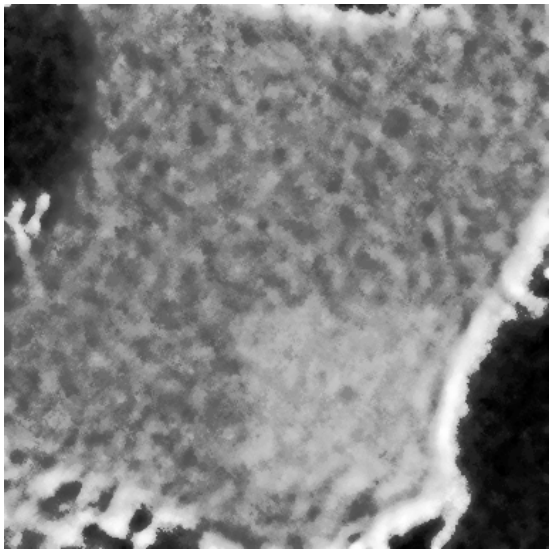


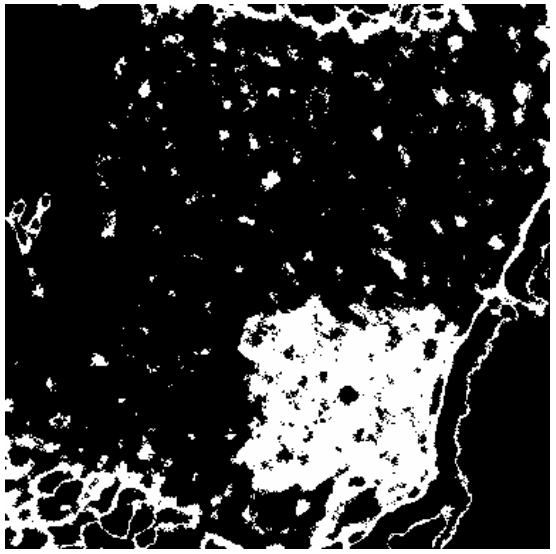


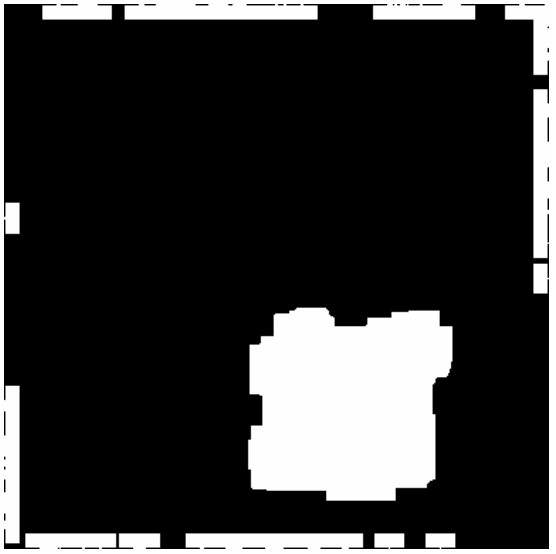
Analyze Particles

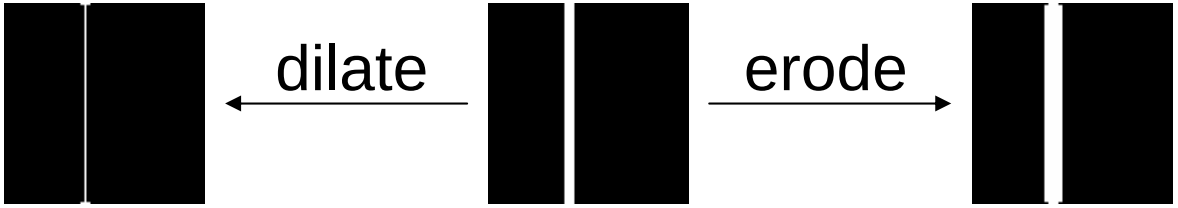


Threshold and Opening/Closing





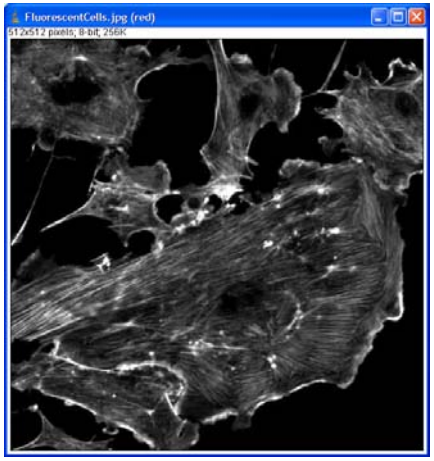


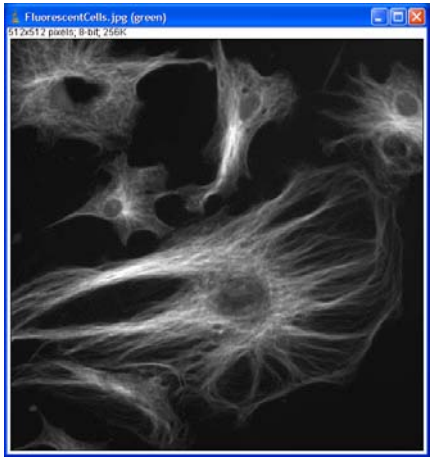


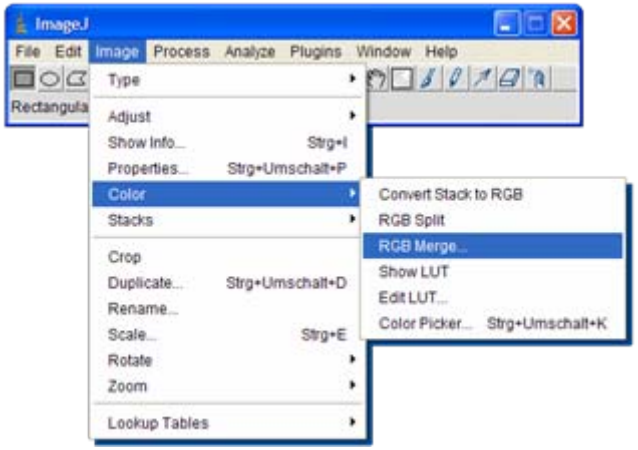
Closing: Dilate/Erode
Opening: Erode/Dilate

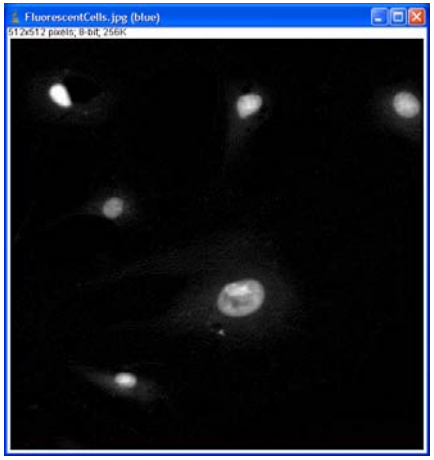
Color Functions

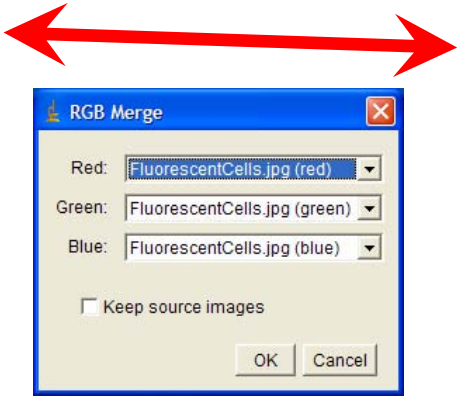
- RGB Merge /RGB Split

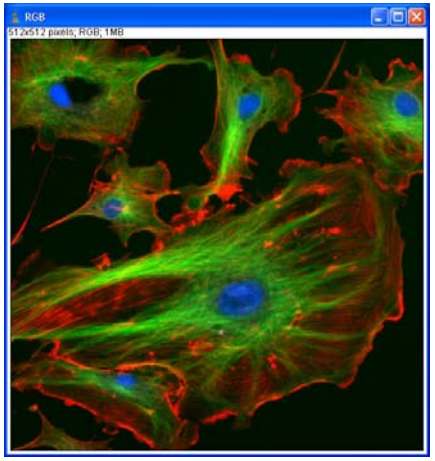












Acknowledgements

- ALMF: Rainer, Timo, Arne, Yury
- Andreas Schleifenbaum (some slides)
- Chizuko Tsurumi (images)

Further Information

- Microscopy Primer
  http://micro.magnet.fsu.edu/primer/index.html
- EAMNET Teaching Modules (Zeiss Basics!)
  http://www.embl.de/eamnet/html/teaching_modules.html
- Digital Filters:
  http://homepages.inf.ed.ac.uk/rbf/HIPR2/filtops.htm