Lecture 15:

Applied Image Analysis

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Open Positions

- Group Leader position
- Assistant Microscopy Manager
- Image Analysis Specialist
- Image Data - System Administrator
- Correlative Light-EM Specialist

Contacts:

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1. Image restoration & filtering

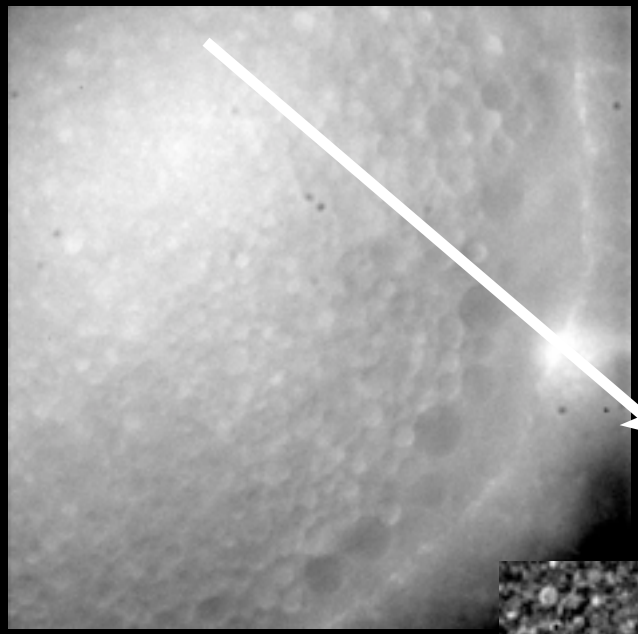
2. Feature extraction (segmentation)

3. Analysis and quantification

(4. Automation)

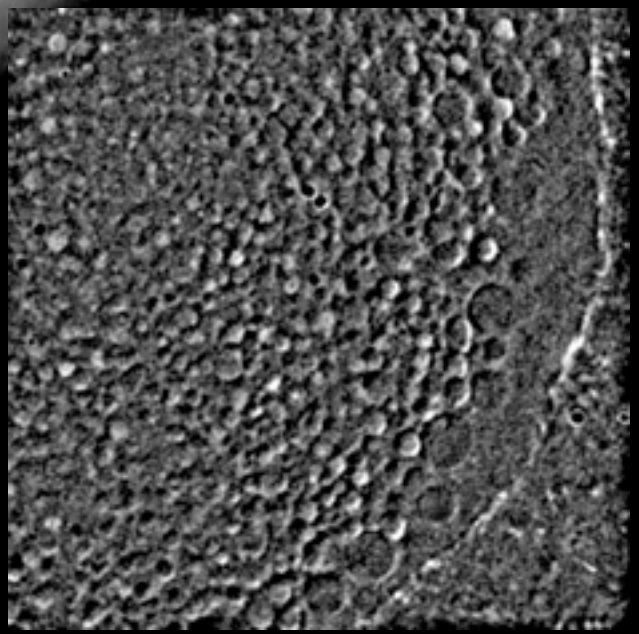
Improving and quantifying image data

raw data

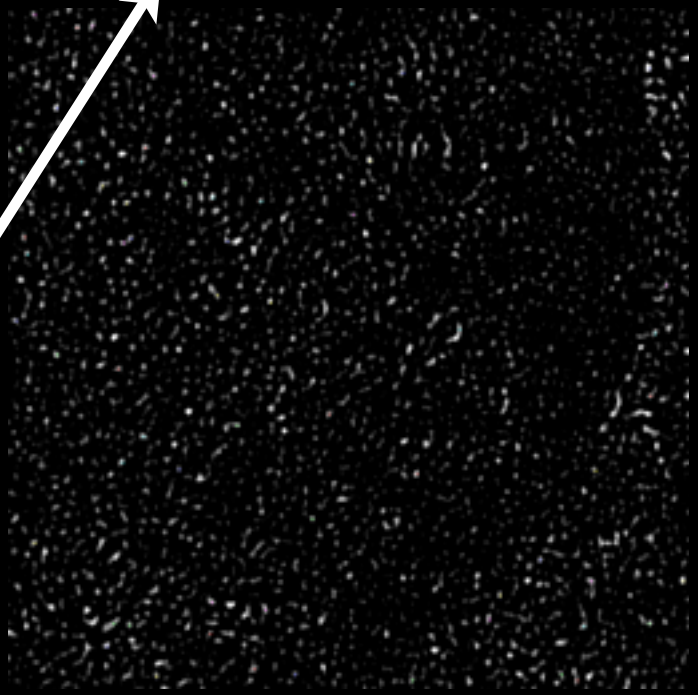


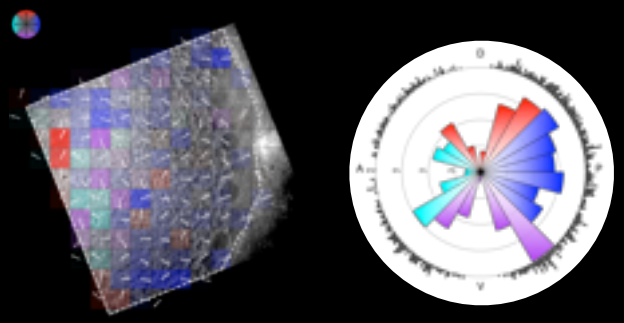
(Richard Parton)

processing/
filtering



analysis
(tracking)



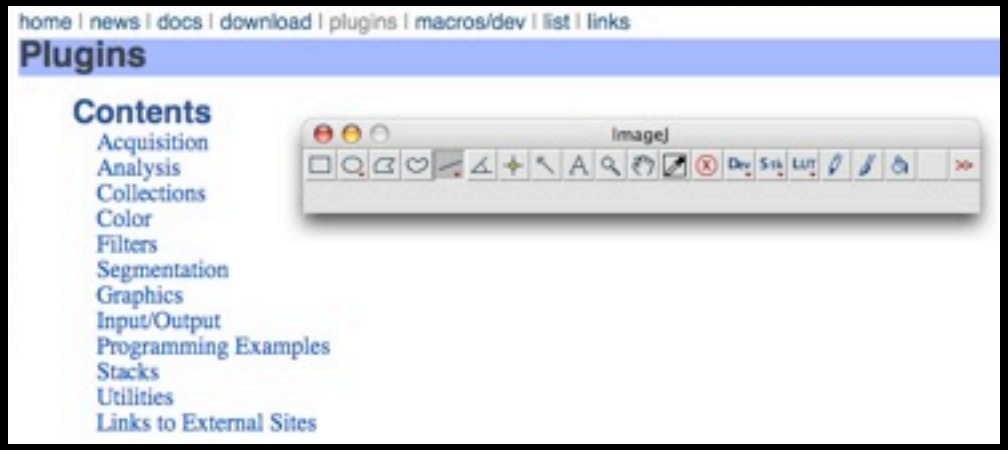


(Russell Hamilton)

Tools for image analysis

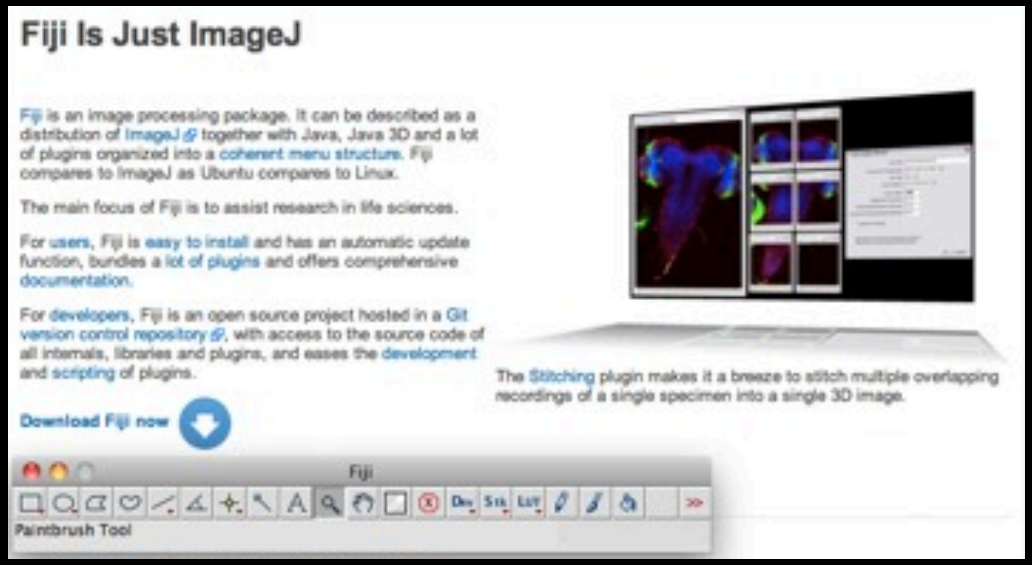
ImageJ

http://rsbweb.nih.gov/ij/



Fiji Is Just ImageJ

http://pacific.mpi-cbg.de/wiki/index.php/Fiji



Tools for image analysis

MATLAB

http://www.mathworks.co.uk/

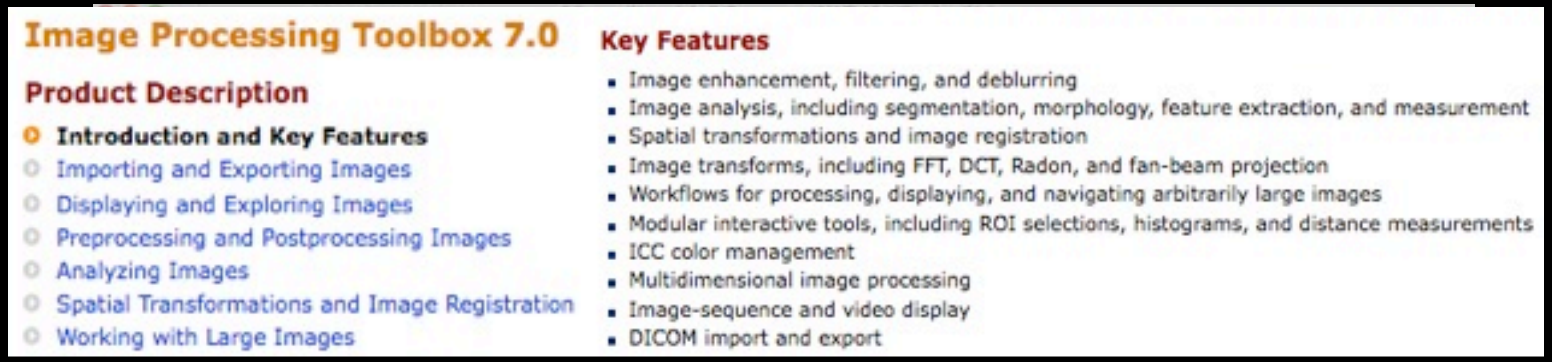


Image Processing Toolbox 7.0

Product Description

- **Introduction and Key Features**
- Importing and Exporting Images
- Displaying and Exploring Images
- Preprocessing and Postprocessing Images
- Analyzing Images
- Spatial Transformations and Image Registration
- Working with Large Images

Key Features

- Image enhancement, filtering, and deblurring
- Image analysis, including segmentation, morphology, feature extraction, and measurement
- Spatial transformations and image registration
- Image transforms, including FFT, DCT, Radon, and fan-beam projection
- Workflows for processing, displaying, and navigating arbitrarily large images
- Modular interactive tools, including ROI selections, histograms, and distance measurements
- ICC color management
- Multidimensional image processing
- Image-sequence and video display
- DICOM import and export

- prototyping, task automation

- plotting functions

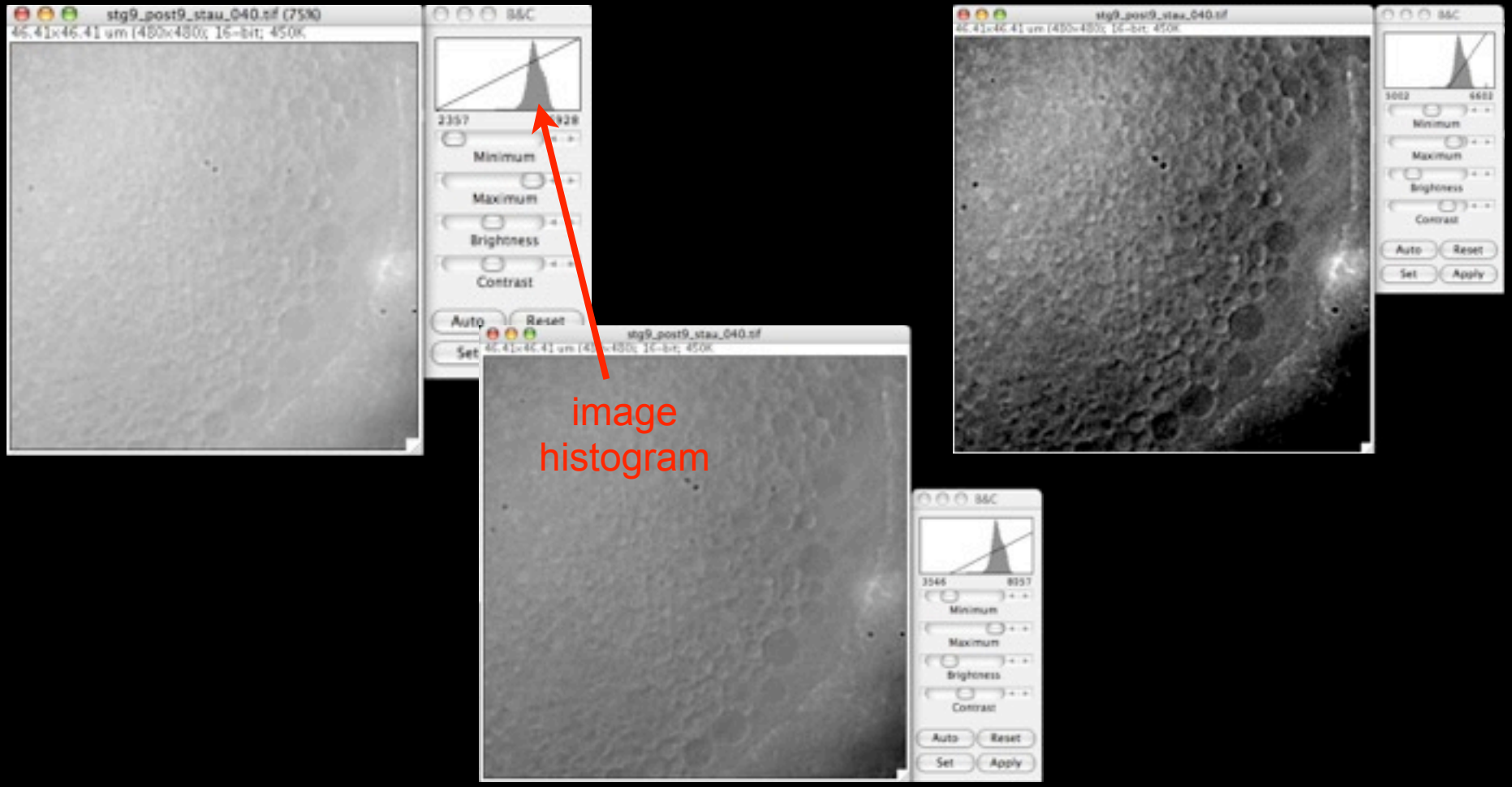
1. Image restoration / filtering

1. Image restoration / filtering

Intensity Transforms

- same operation applied to each pixel

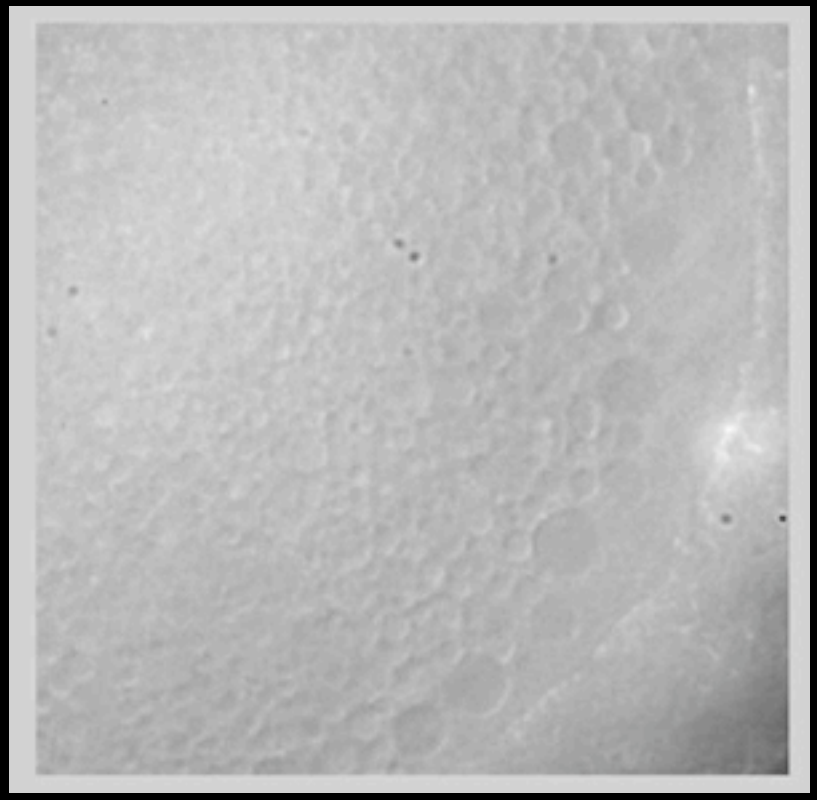
  => changes to image brightness and contrast

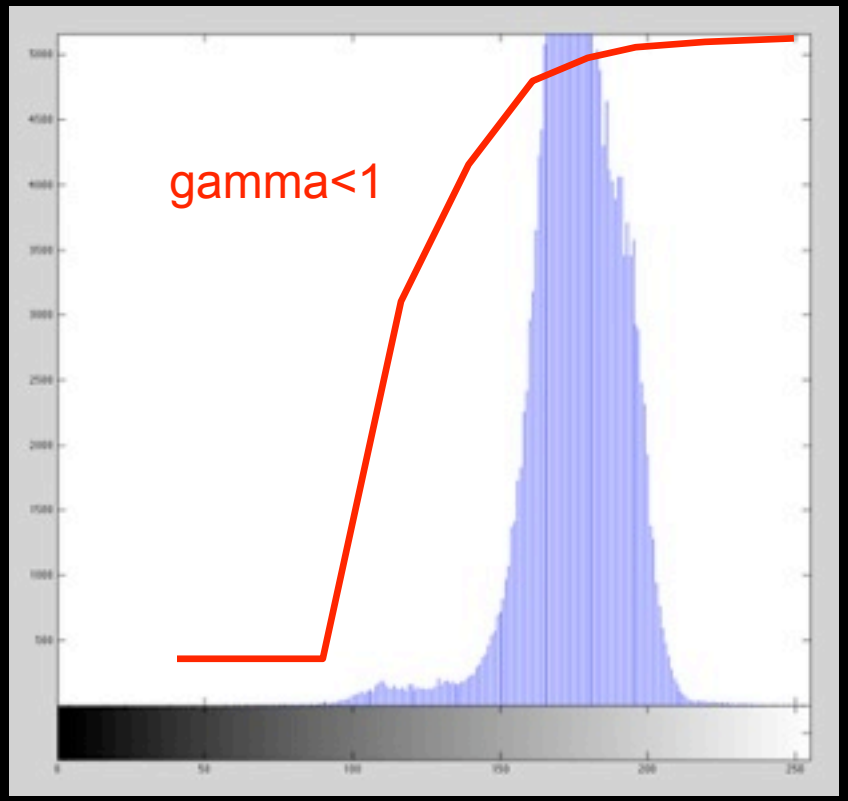


1. Image restoration / filtering

Intensity Transforms

- contrast stretching: gamma, histogram equalization





MATLAB - imshow to display image, imhist to display its histogram

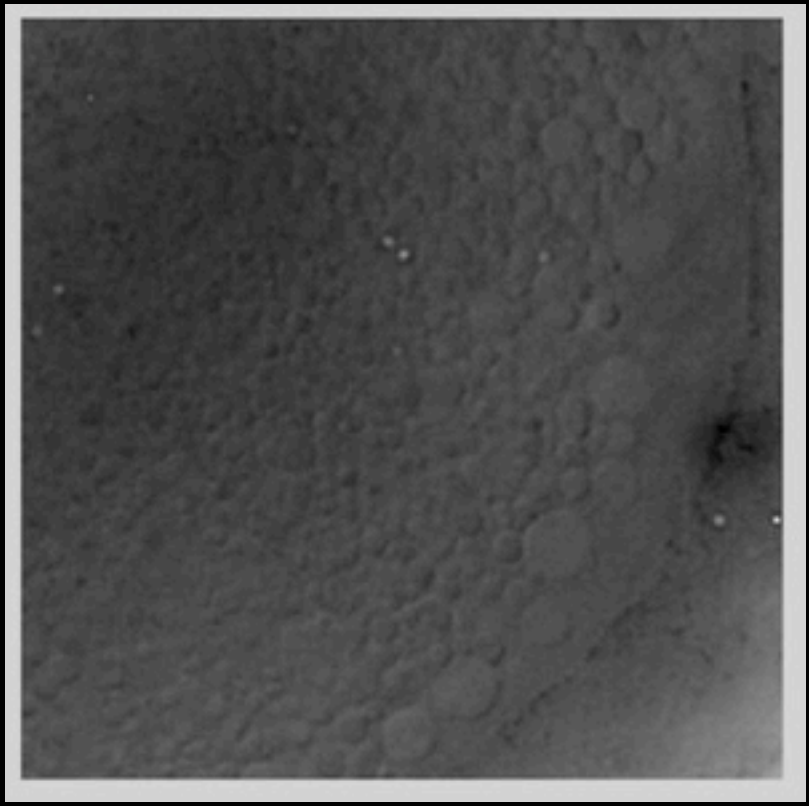
imadjust(f, [low_in high_in], [low_out high_out], gamma)

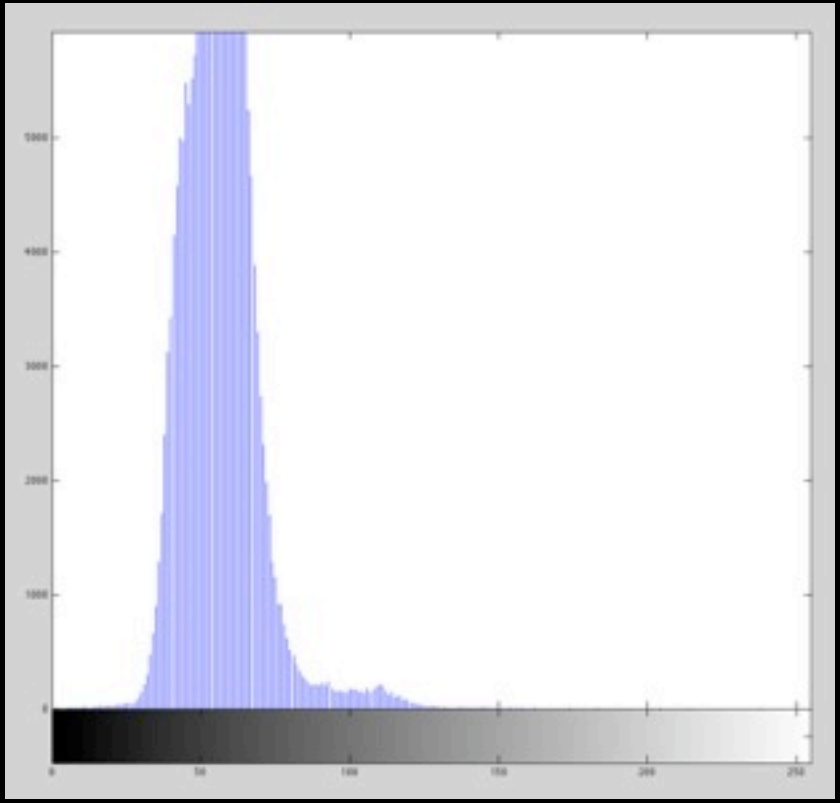
histeq to do histogram equalization

1. Image restoration / filtering

Intensity Transforms

- contrast stretching: gamma, histogram equalization





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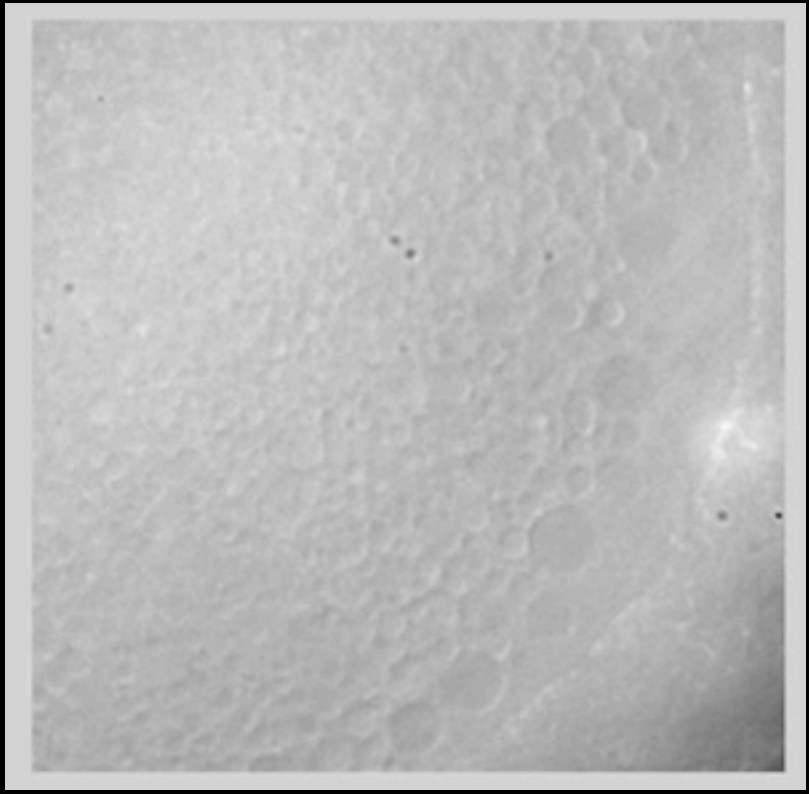

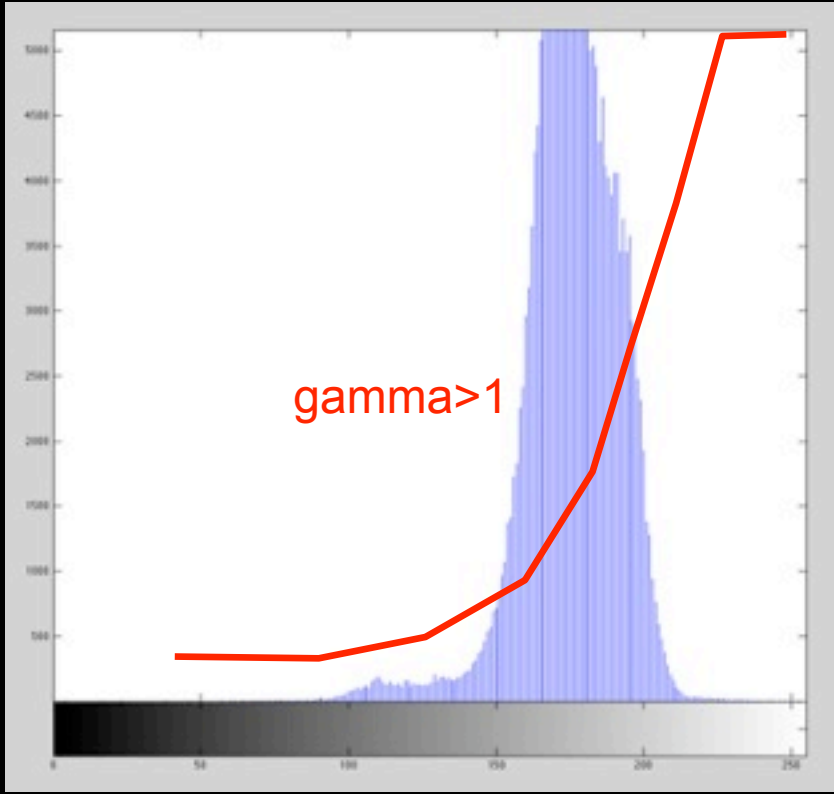

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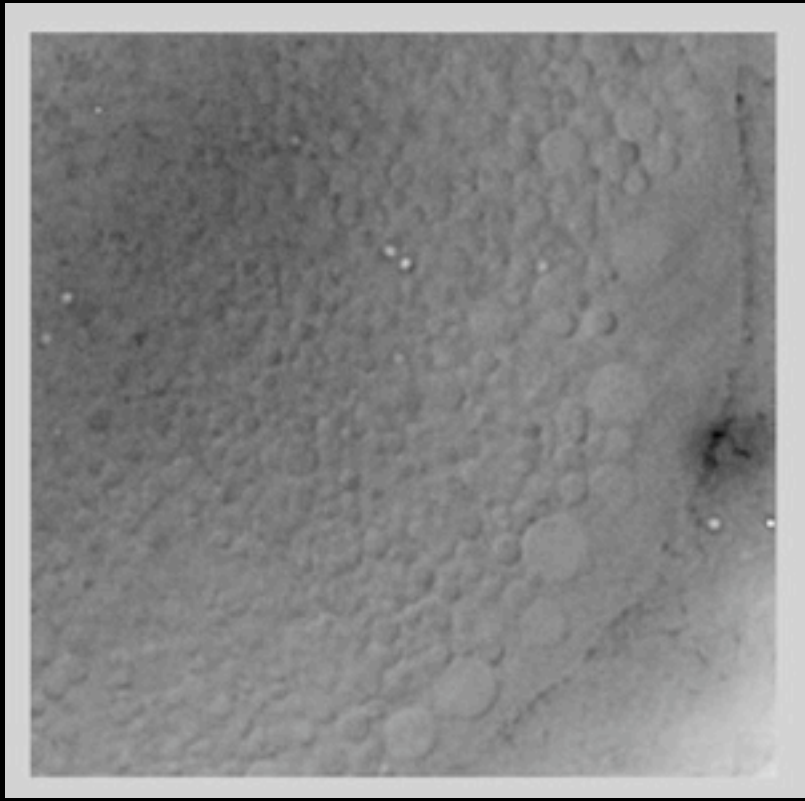

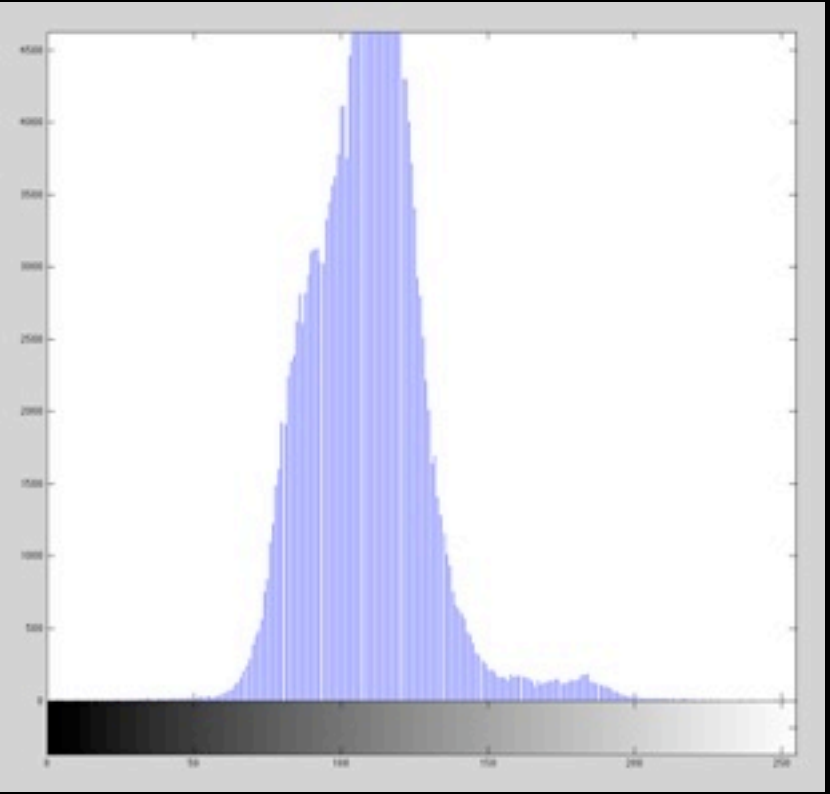

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1. Image restoration / filtering

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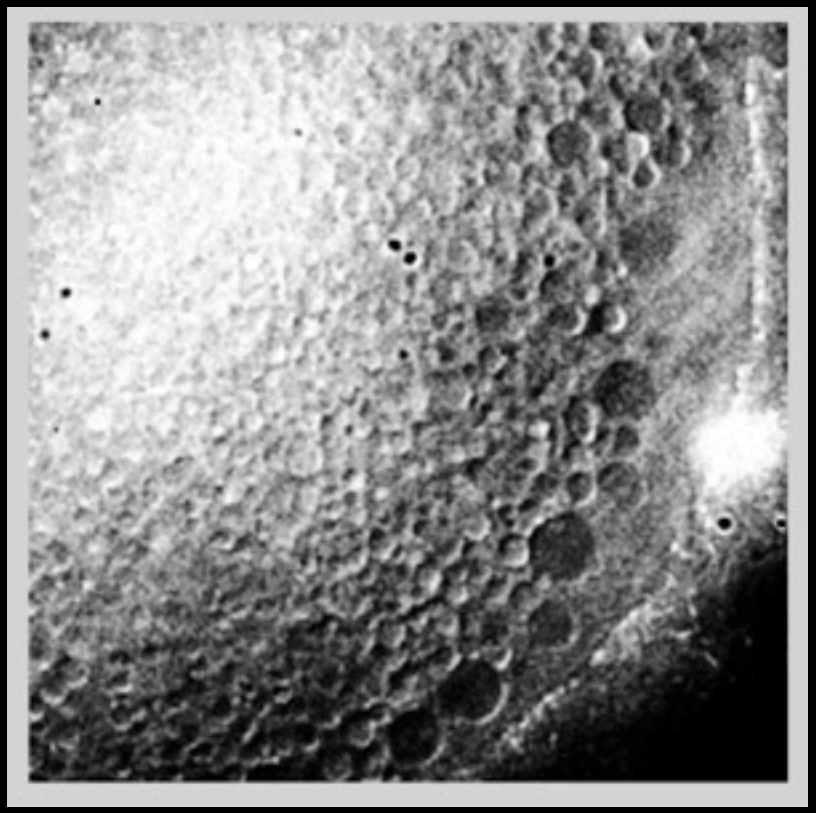
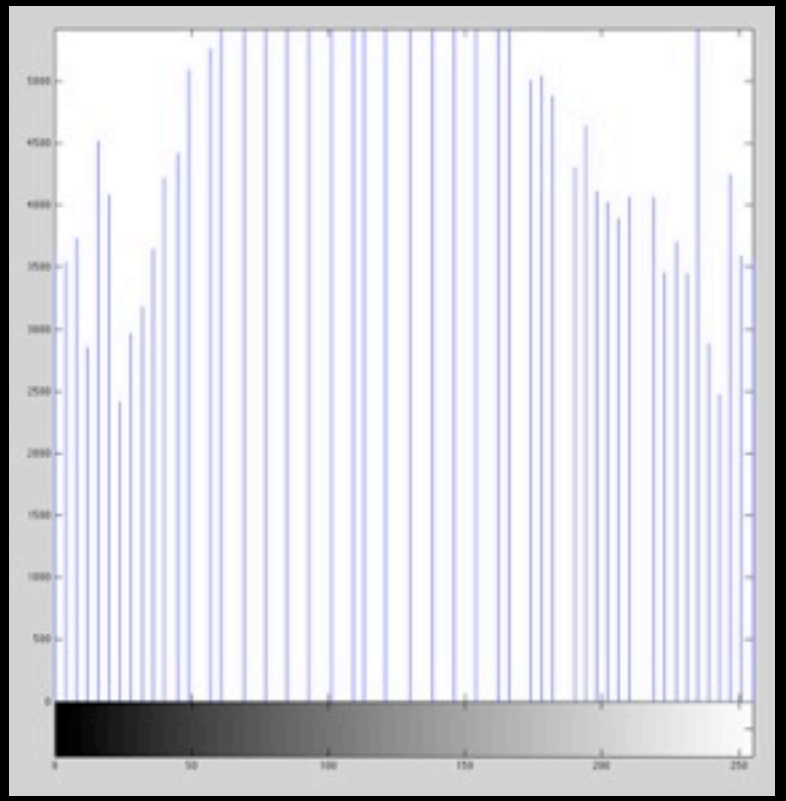




MATLAB - imshow to display image, imhist to display its histogram

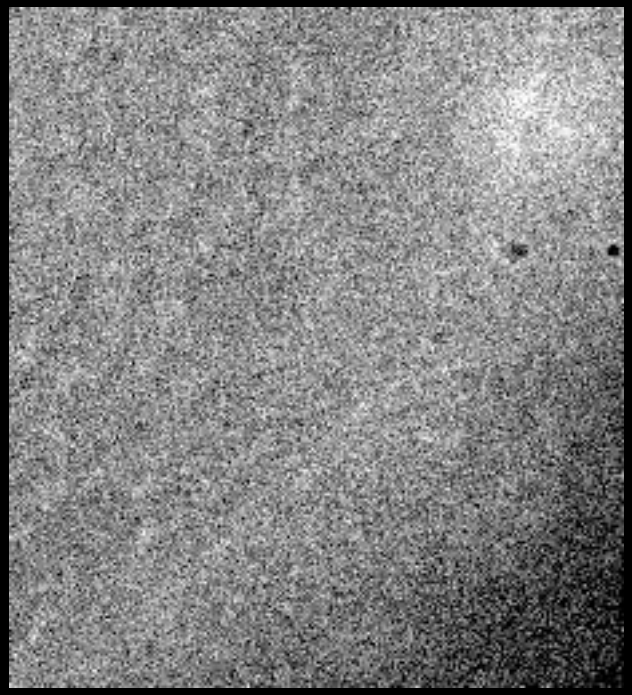
imadjust(f, [low_in high_in], [low_out high_out], gamma)

histeq to do histogram equalization

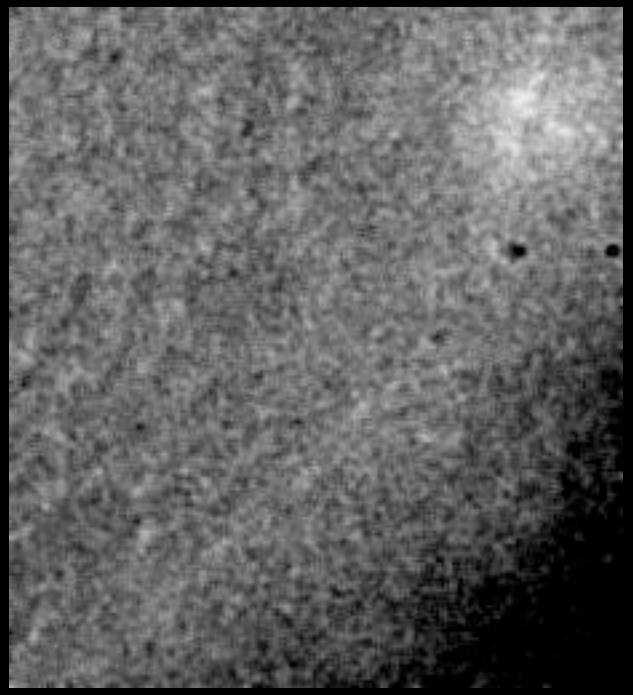
1. Image restoration / filtering

Spatial Filtering

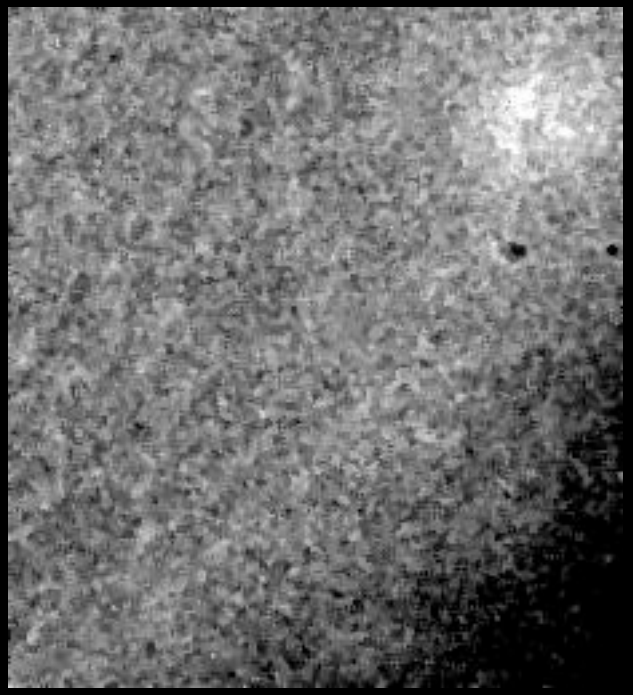
- operation applied to a pixel and its neighbours
- useful for removing noise / enhancing features of a defined size
- linear: e.g. mean filter, convolution with a kernel
  or non-linear: e.g. median filter



noisy image



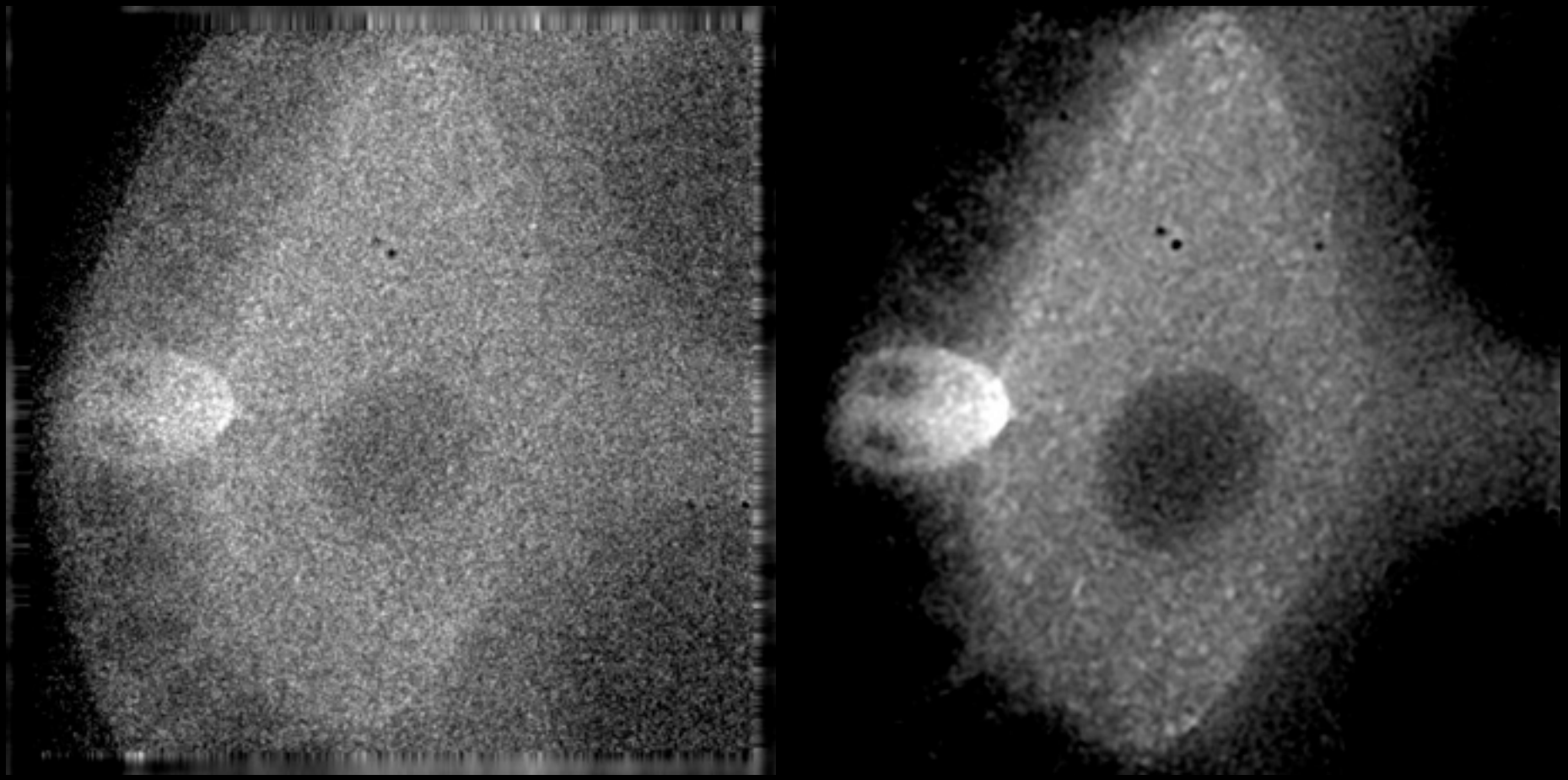
3x3 mean filter



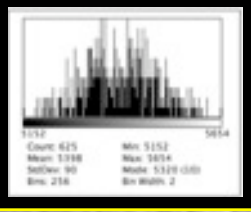
3x3 median filter

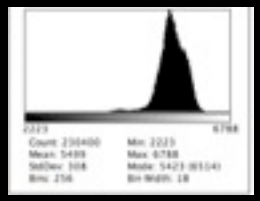
1. Image restoration / filtering

Spatial Filtering: denoising



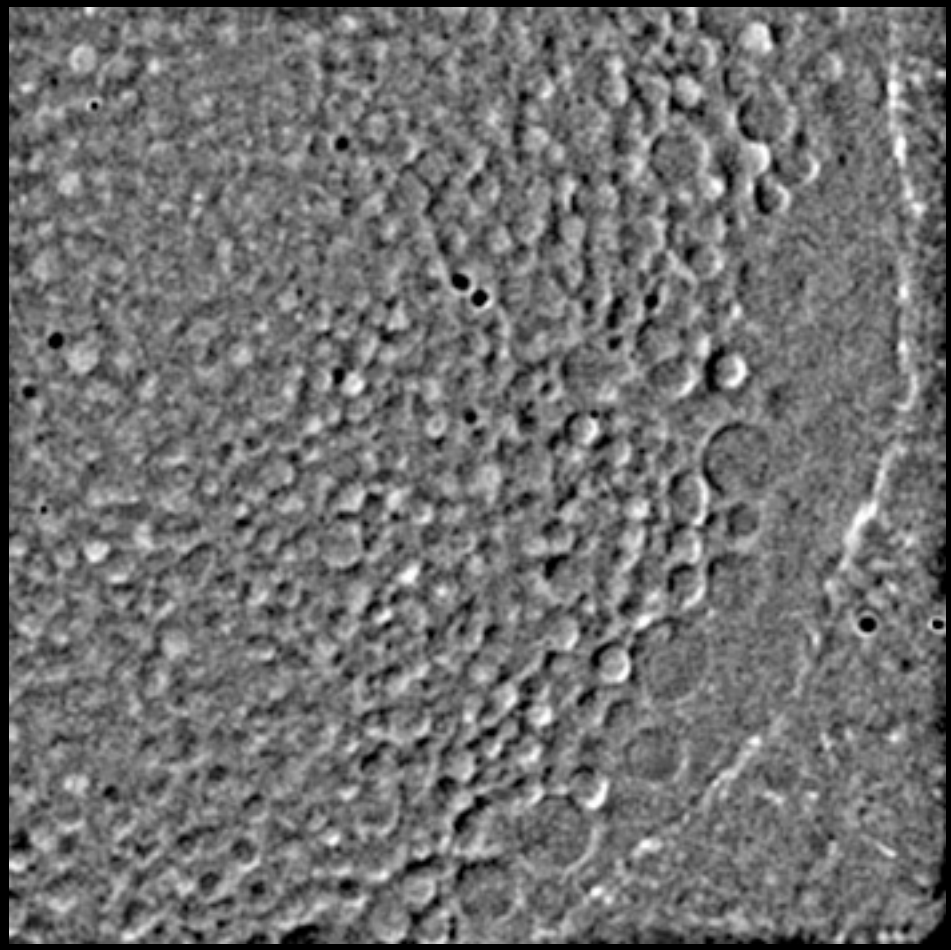
1. Image restoration / filtering





example of a custom intensity transform: scale according to local median

raw data showing uneven illumination



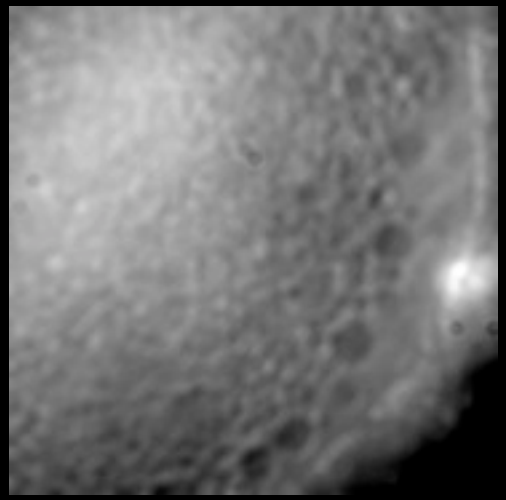
‘normalized’ image

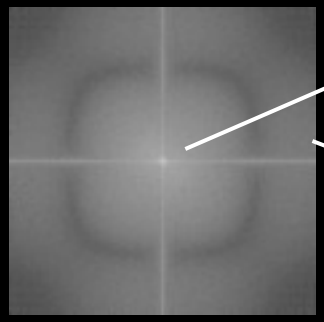
1. Image restoration / filtering

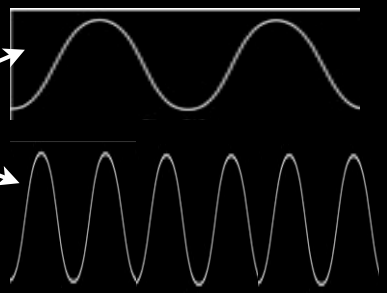
Frequency domain processing

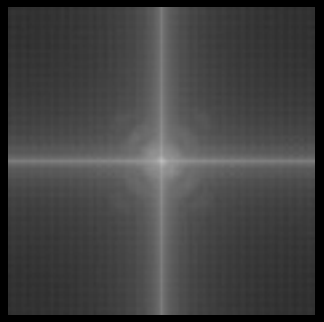
- Fourier spectrum shows frequencies present:
  large features = low frequency
  small features = high frequency (noise included!)

15x15
mean
filter





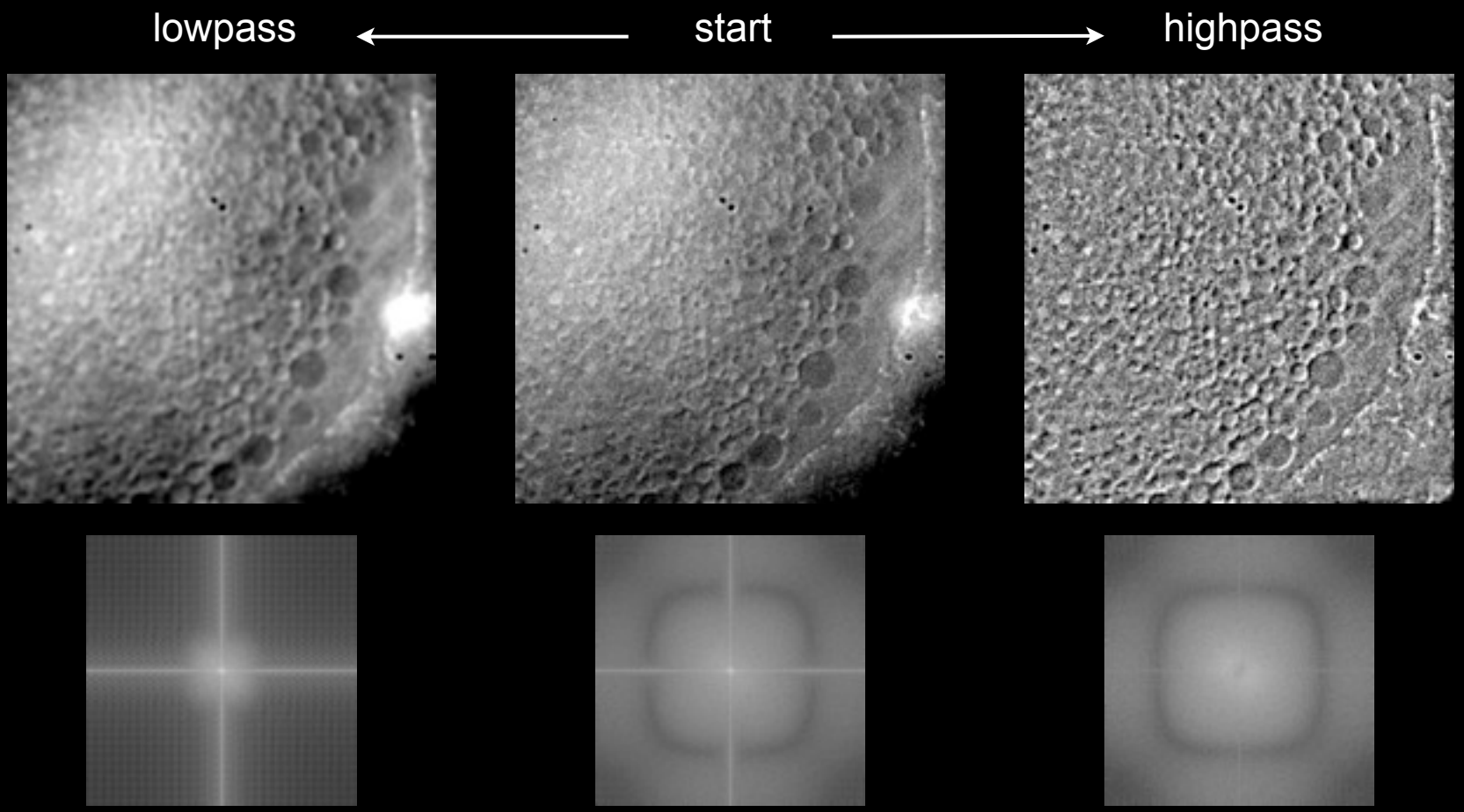




1. Image restoration / filtering

Frequency domain processing

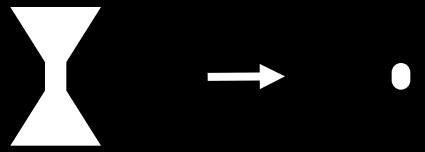
- filtering applied in Fourier space
- e.g. lowpass and highpass frequency domain filters

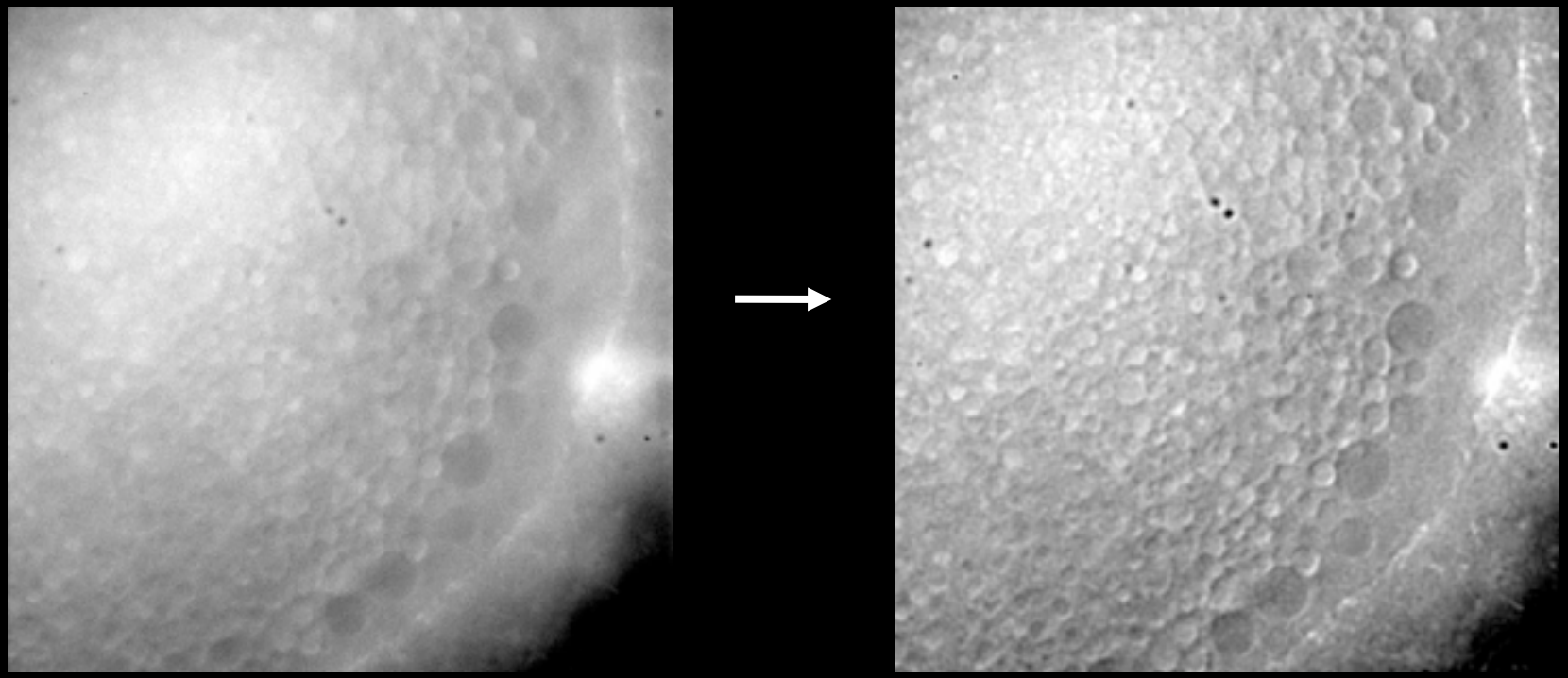


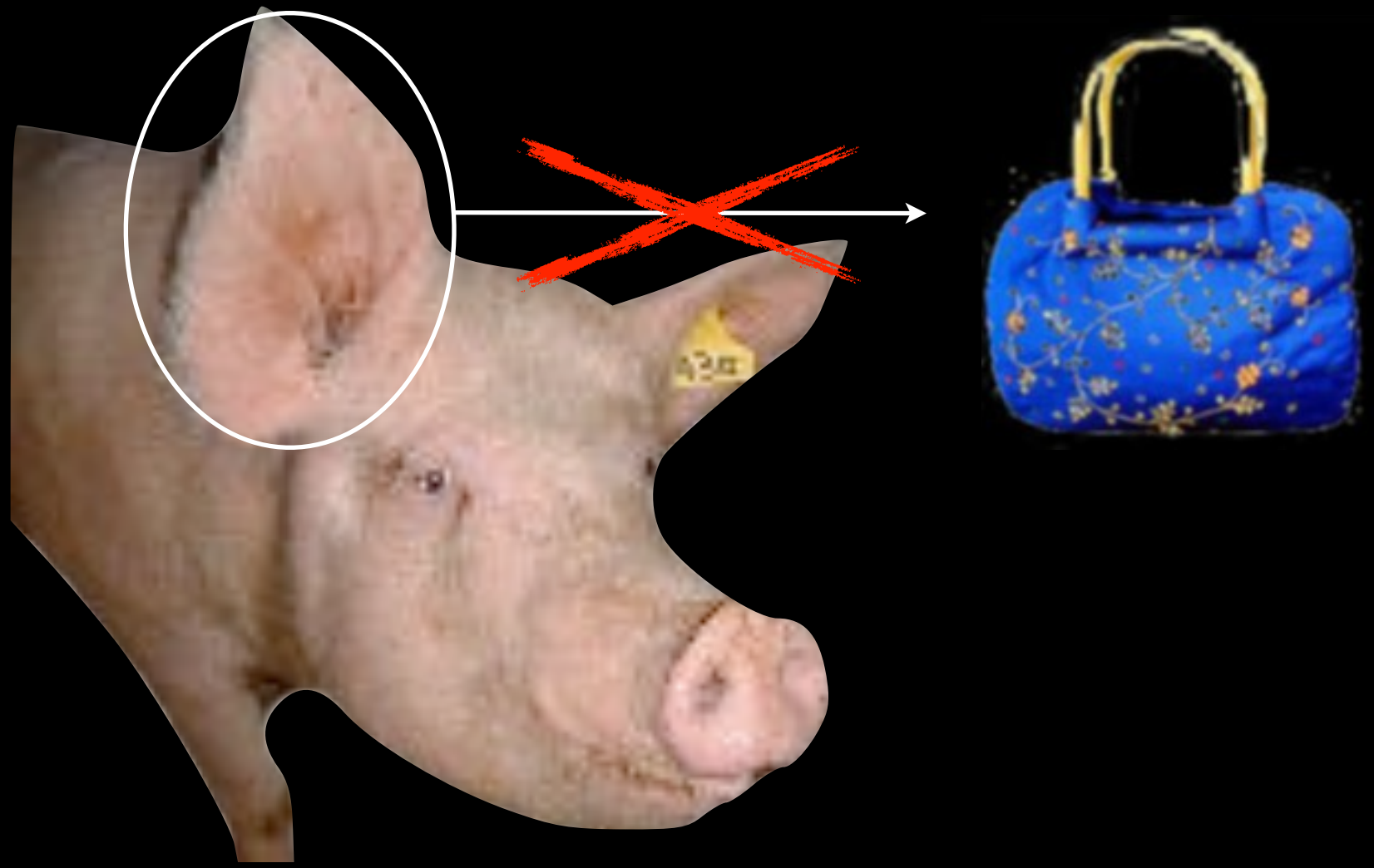
1. Image restoration / filtering

Deconvolution

- light from a point source spreads out (PSF) +noise







can I improve my data acquisition?

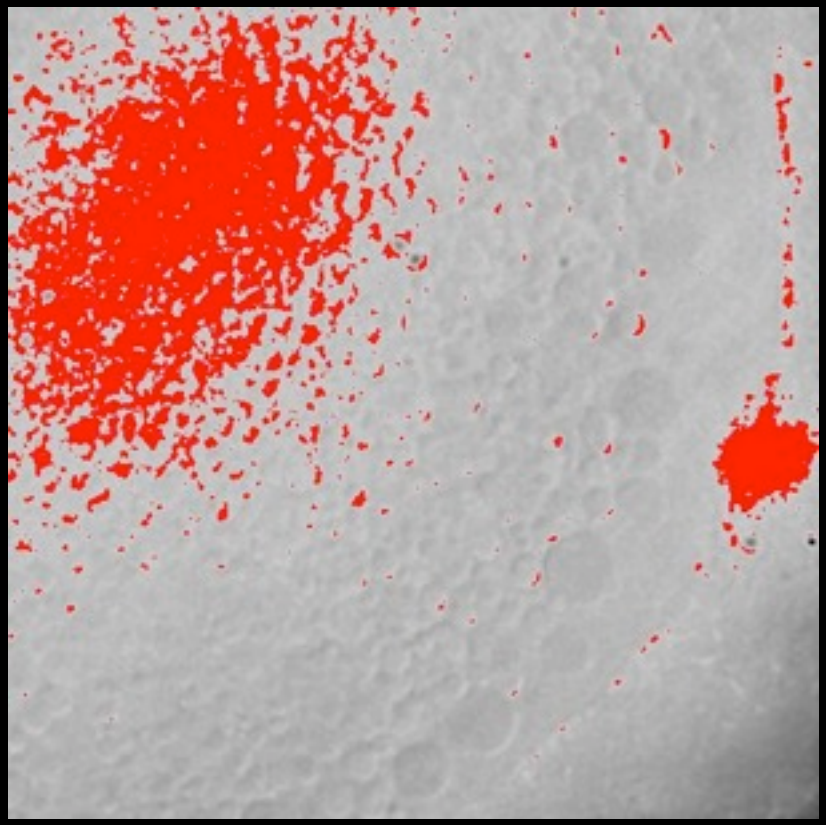
2. Feature extraction

2. Feature extraction

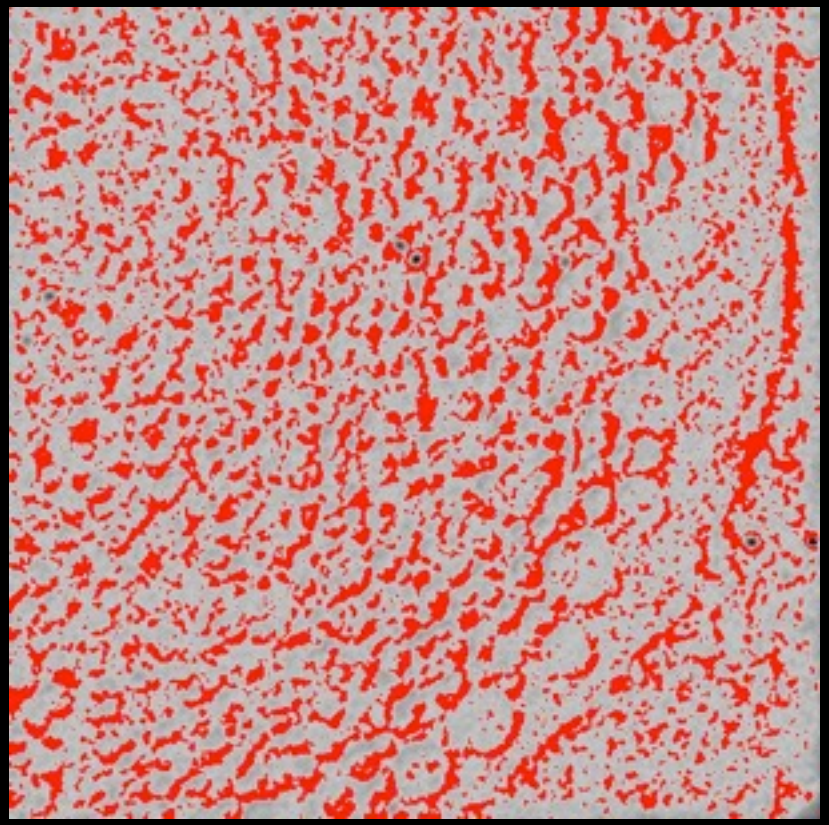
Image segmentation: thresholding

- a global threshold only works if the image is very ‘even’

using raw data



using ‘normalized’ data



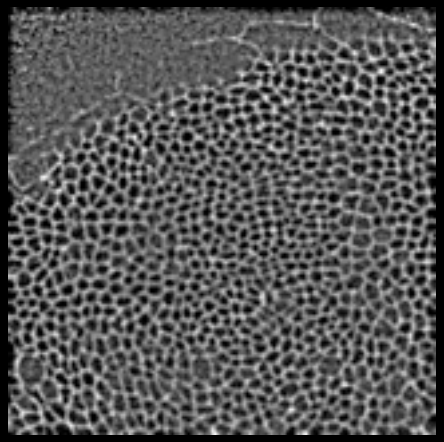
=> ‘adaptive thresholding’, or prior normalization of the image

2. Feature extraction

Morphological image processing

- dilation and erosion of binary images

ImageJ: Process->Binary menu ; MATLAB: bwmorph function

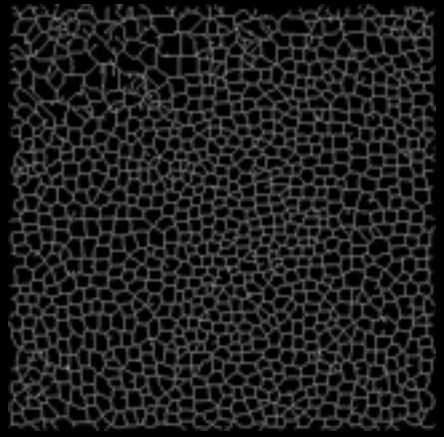


(Yanlan Mao, CRUK)

binarized

dilated

skeleton

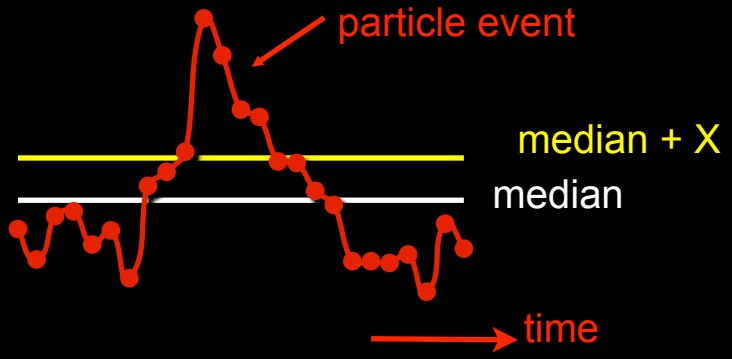


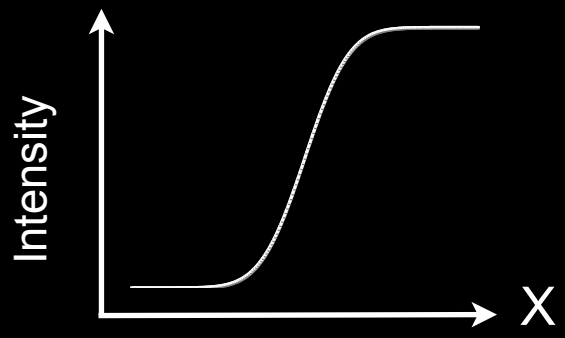
2. Feature extraction

Image segmentation: identifying 'foreground' features

- easy to implement custom filters in MATLAB, like this

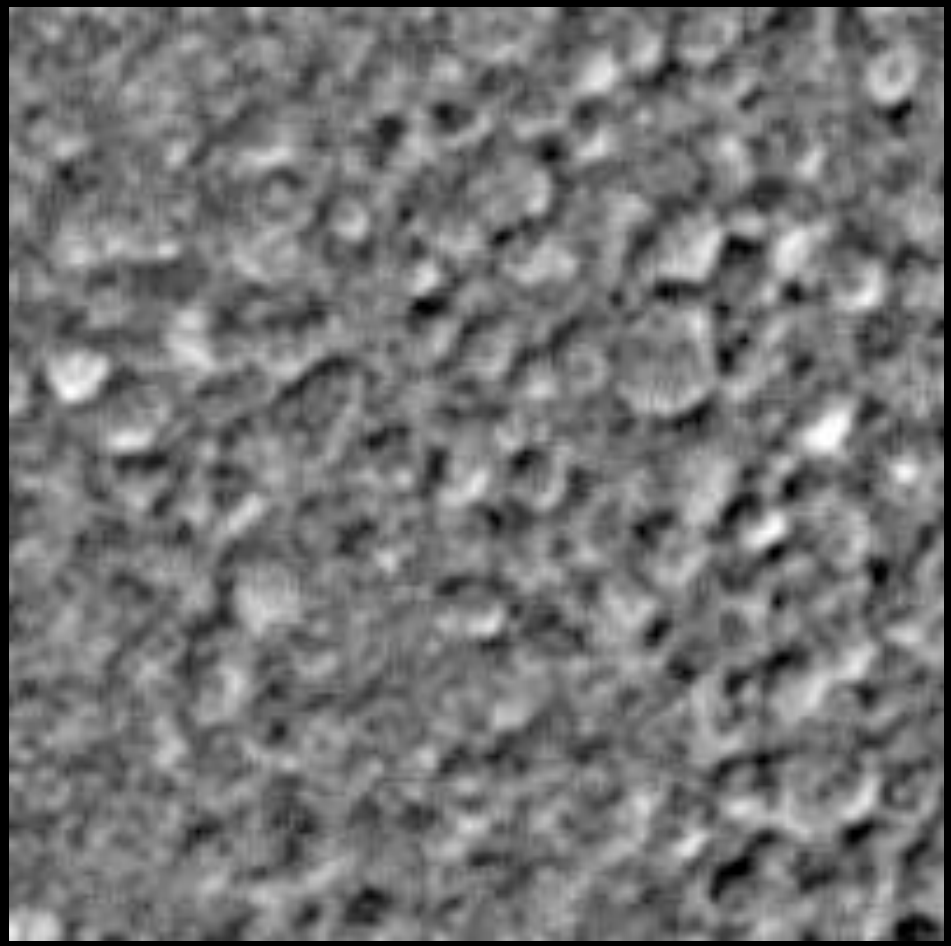
temporal median filter to identify moving foreground



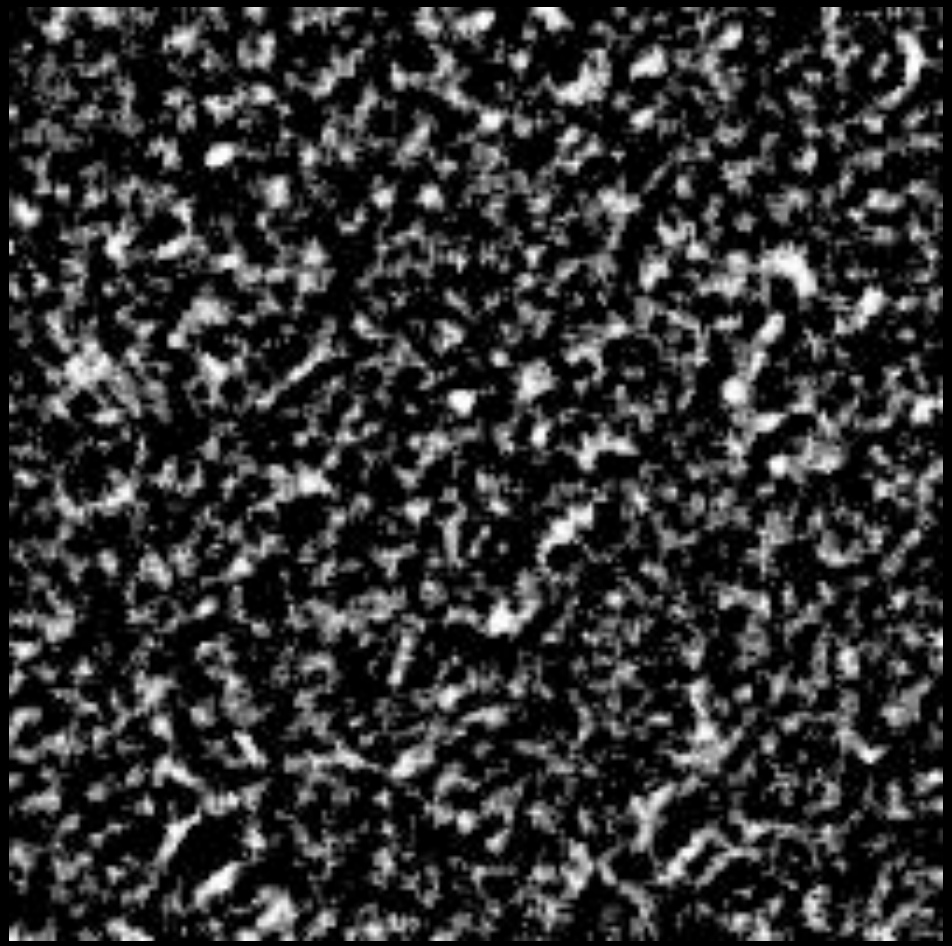


2. Feature extraction

Image segmentation: identifying ‘foreground’ features



200x200 area, normalized



200x200 area, non-background (‘foreground’)

2. Feature extraction

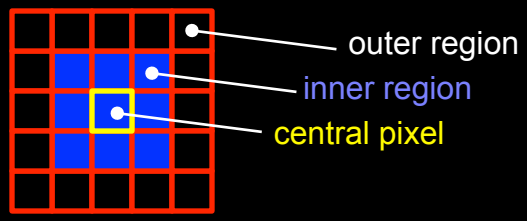
Object recognition

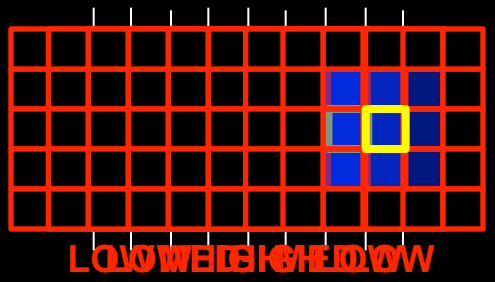
- many tools for point, line & edge detection in MATLAB
- generally work by either:
  - applying a mask to find maxima

    or
  - calculating intensity gradient (steep gradient = edge)

e.g. detection of Haar-like features to find particles

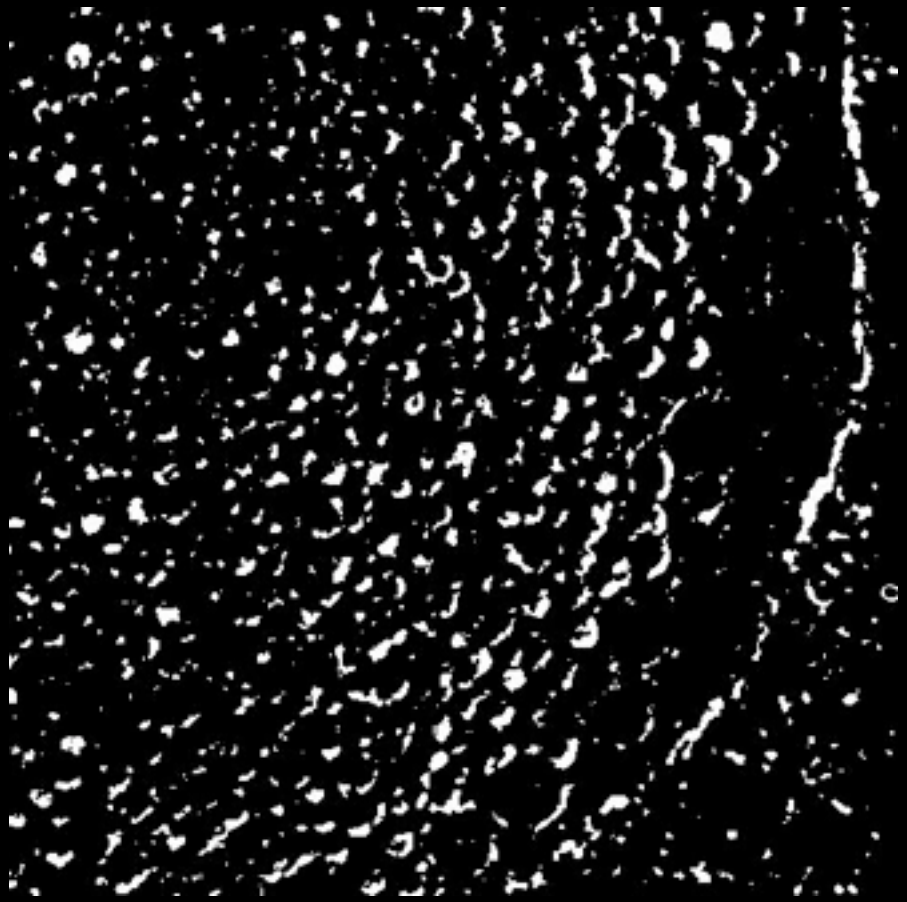
square Haar-like feature





2. Feature extraction

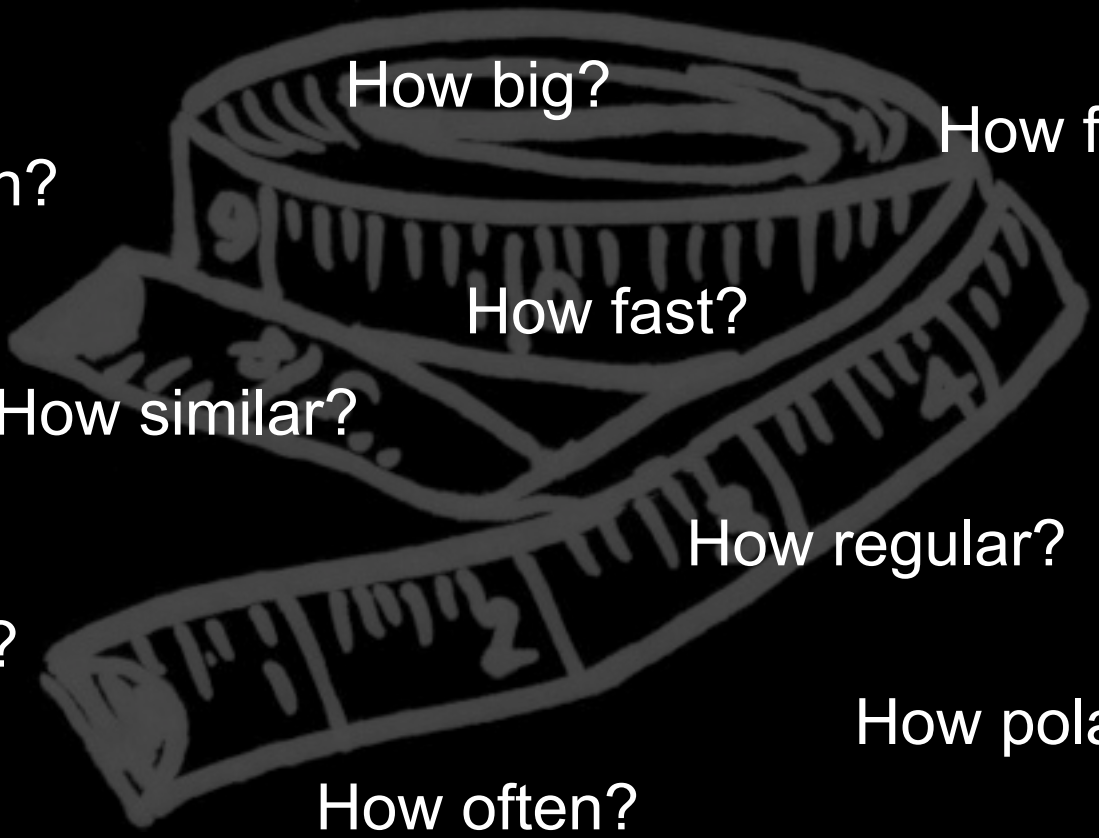
segmentation of raw data versus ‘feature image’



‘normalized’ data

detected features

3. Analysis and quantification



How big?

How far apart?

How much?

How fast?

How similar?

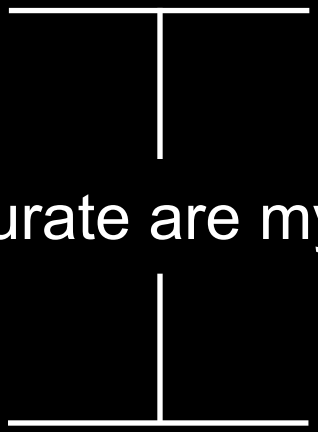
How regular?

How many?

How polarized?

How often?

3. Analysis and quantification



How accurate are my results?

- repeat measurements & calculate SEM

- test statistical significance of result: p-value < 0.05

- are two sets of results different? Student's *t*-test

3. Analysis & quantification

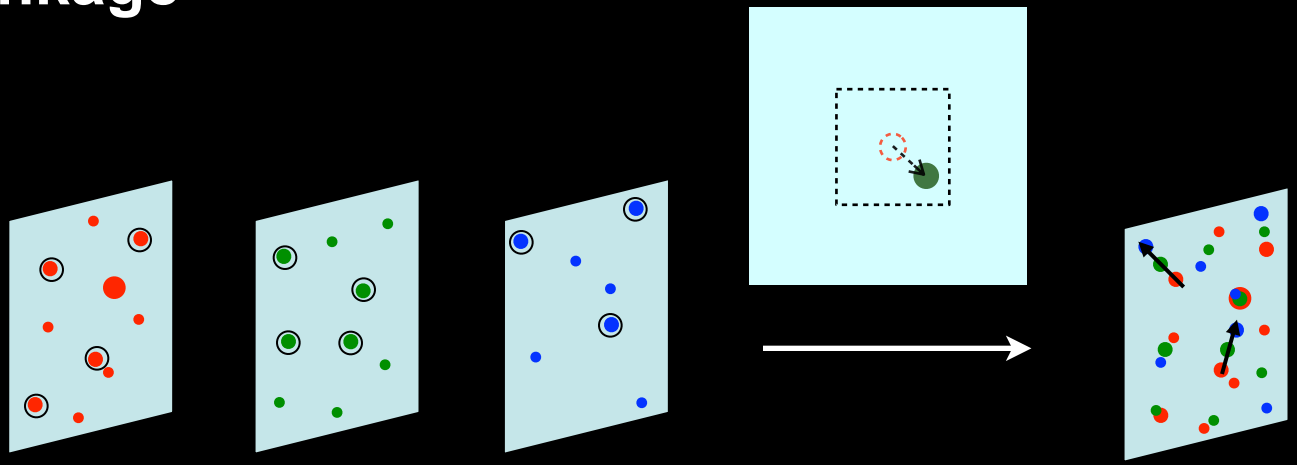
Problem 1: Particle Tracking

- simple particle tracking scheme:-

1. pre-filtering

2. segmentation

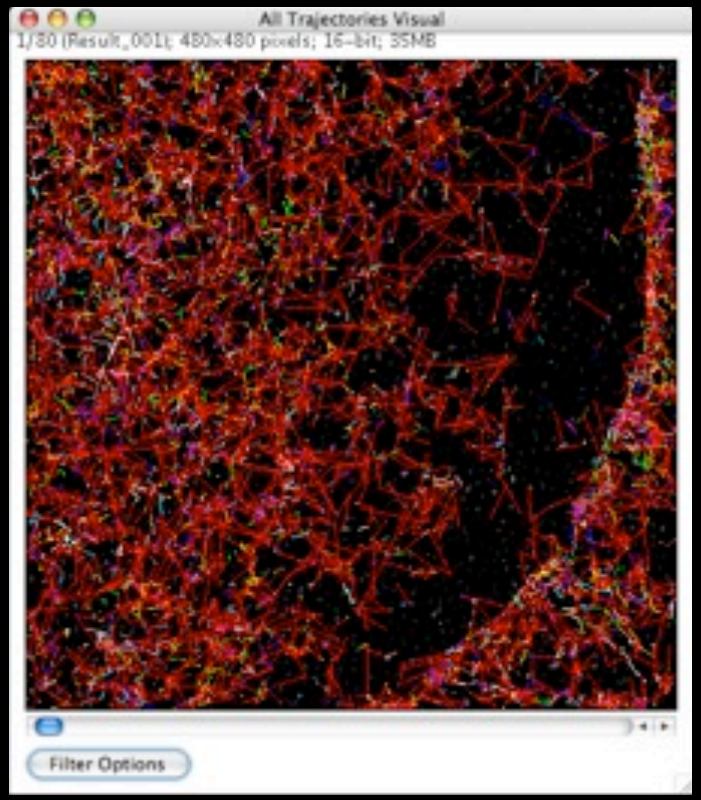
3. linkage

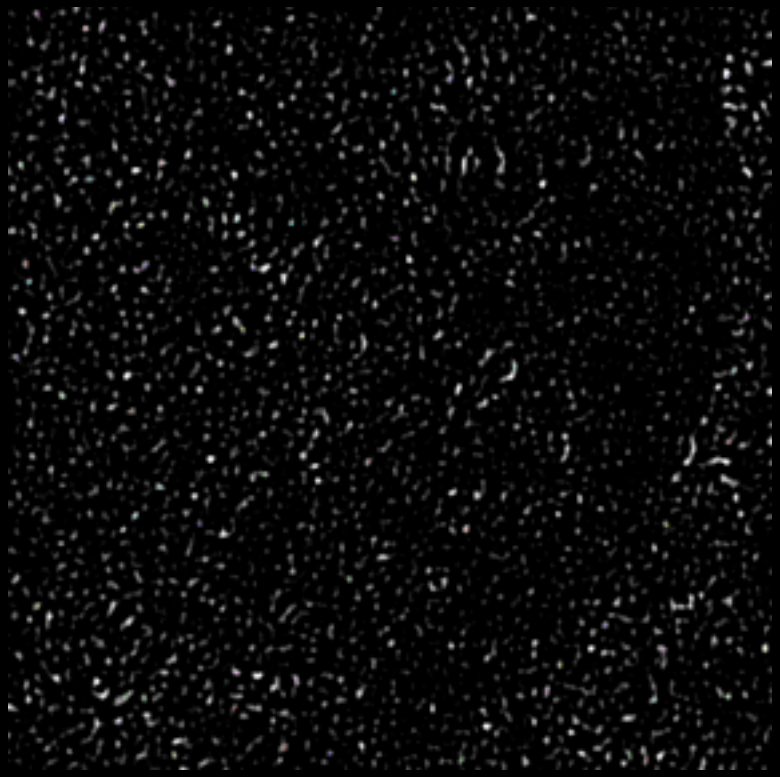


3. Analysis & quantification

Problem 1: Particle Tracking

- Single Particle Tracker from the MOSAIC group, available as ImageJ plugin and MATLAB code



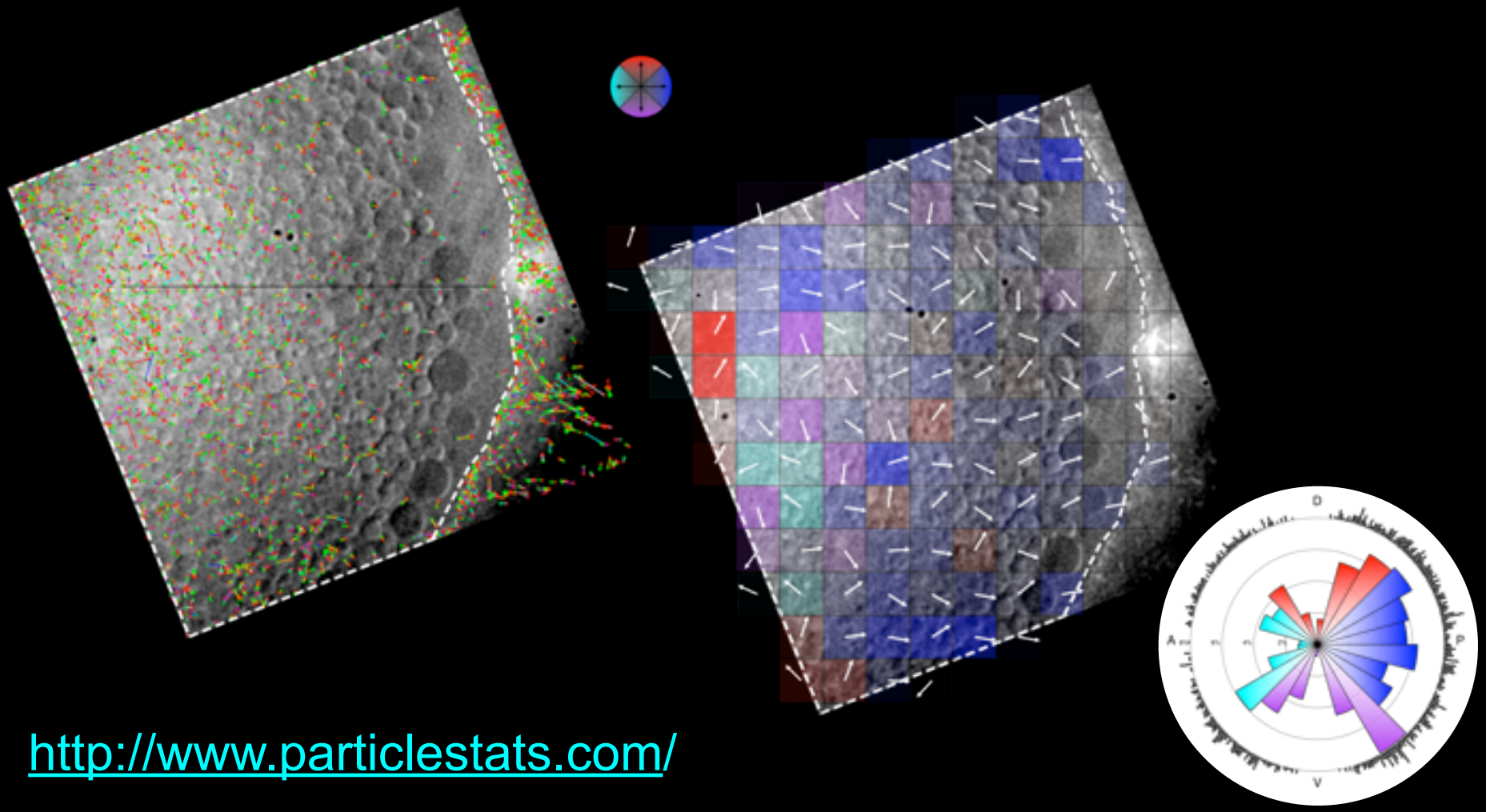


I. F. Sbalzarini and P. Koumoutsakos. Feature Point Tracking and Trajectory Analysis for Video Imaging in Cell Biology, *Journal of Structural Biology* 151(2):182-195, 2005.

3. Analysis & quantification

Analysis of directionality

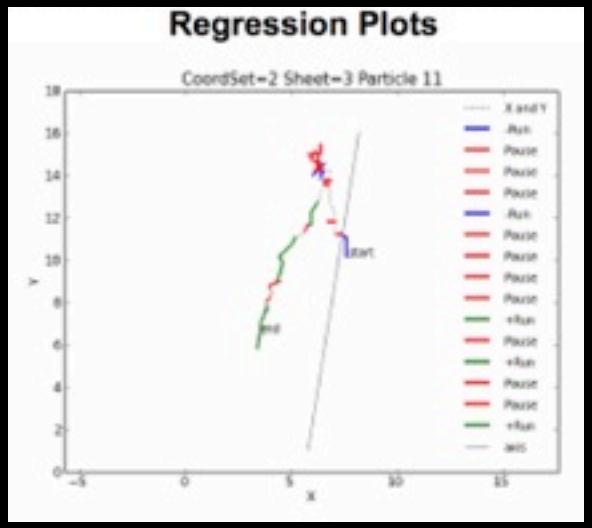
- using ParticleStats (Russell Hamilton)

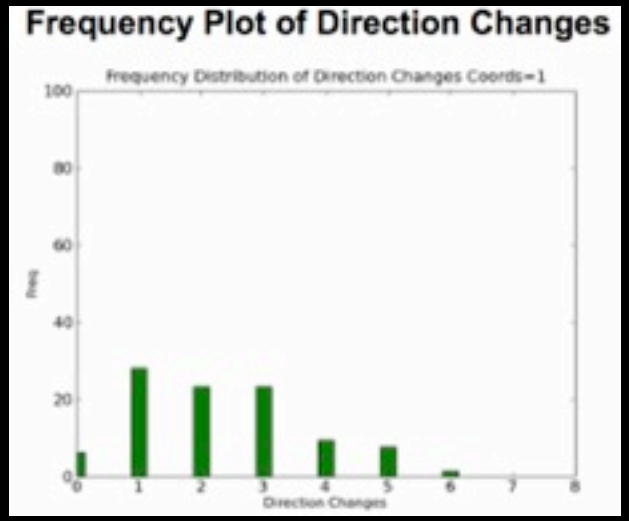


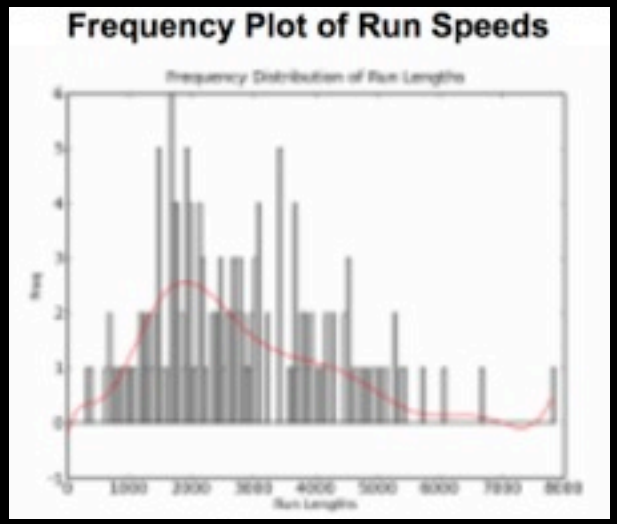
http://www.particlestats.com/

3. Analysis & quantification

Analysis of motility statistics







http://www.particlestats.com/

3. Analysis & quantification

Problem 2: Colocalization

where to start -

- Colocalization Tutorial for Volocity
http://www.perkinelmer.co.uk/pages/020/cellularimaging/training/theorycolocalizationanalysis.xhtml

- Manders et al. (1993). Journal of Microscopy 169:375-382
"Measurement of co-localisation of objects in dual-colour confocal images."

- http://www.macbiophotonics.ca/PDF/MBF_colocalisation.pdf

- check for bleed-through

- check that the two channels are properly registered

3. Analysis & quantification

Problem 2: Colocalization

Pearson's correlation coefficient, Rr:

$$R_r = \frac{\sum (R_i - \overline{R}) \times (G_i - \overline{G})}{\sqrt{\sum (R_i - \overline{R})^2 \times \sum (G_i - \overline{G})^2}}$$

For pixel i in the images, R and G are intensities of the red and green channel respectively.

1 means high colocalization,

0 and -1 are difficult to interpret

3. Analysis & quantification

Problem 2: Colocalization

Manders' overlap coefficient, R:

$$R = \frac{\sum_i (R_i \times G_i)}{\sqrt{\sum_i (R_i)^2 \times \sum_i (G_i)^2}}$$

between 0 and 1; 1 means high colocalization, 0 low

only reliable where 'red' and 'green' channels contain approximately equal number of pixels

3. Analysis & quantification

Problem 2: Colocalization

Manders' colocalization coefficients, M_{red} & M_{green}:

$$M_{red} = \frac{\sum_i R_{i,coloc}}{\sum_i R_i} \qquad M_{green} = \frac{\sum_i G_{i,coloc}}{\sum_i G_i}$$

$R_{i,coloc} = Ri$ if $Gi > 0$; $G_{i,coloc} = Gi$ if $Ri > 0$.
i.e. M_{red} is the sum of the intensities of red pixels that have a green component divided by the total sum of red intensities.

how much each channel overlaps with the other

between 0 and 1; 1 means all, 0 none

3. Analysis & quantification

Problem 2: Colocalization

Intensity Correlation Quotient:

$$PDM = (R_i - \overline{R}) \times (G_i - \overline{G})$$

PDM = Product of the Difference from the Mean for each channel.
For pixel *i* in the image, *R* and *G* are the respective intensities in the red and green channel.

$$ICQ = \left(\frac{N_{+ve}}{N_{total}} \right) - 0.5$$

N_{+ve} = number of positive values for PDM.
N_{total} = total number pixels that do not have a value of zero in each channel.

ICQ ~ 0, unrelated staining

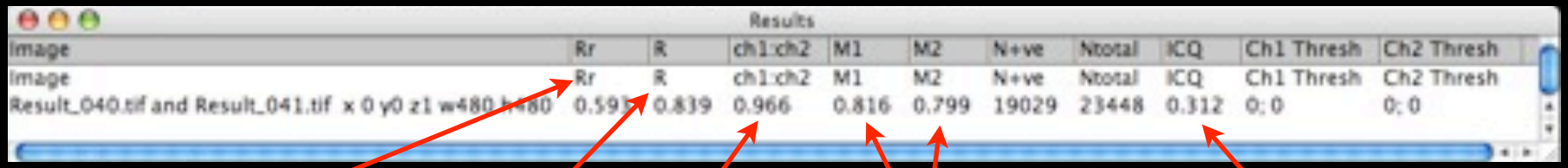
0 < ICQ < 0.5, dependent staining

-0.5 < ICQ < 0, segregated staining

3. Analysis & quantification

Colocalization

using ‘Intensity Correlation Analysis’ plugin:-



Rr = Pearson’s correlation coefficient

R = Mander’s overlap cofficient

ratio of ch1:ch2 pixel intensity

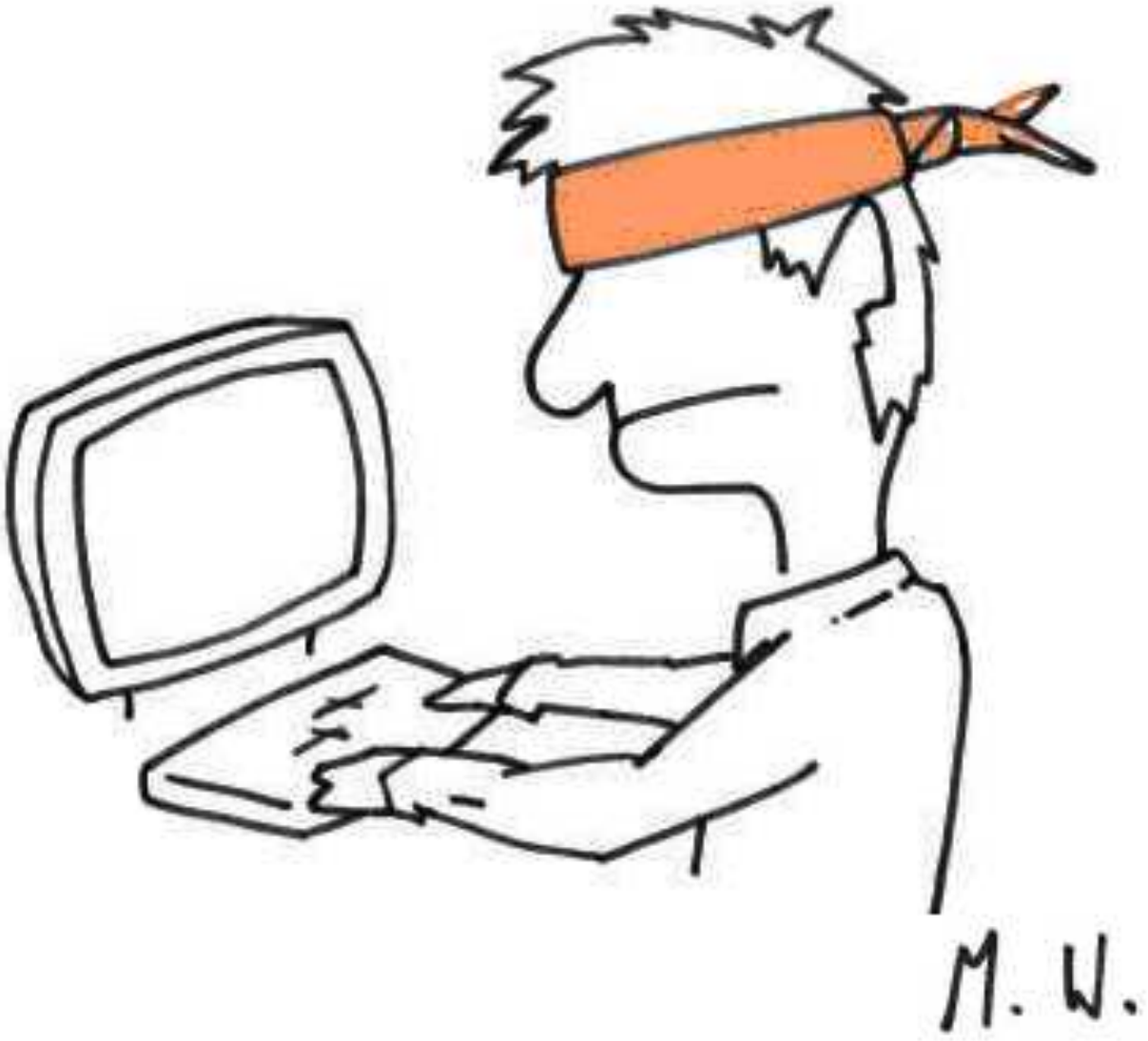
Mander’s colocalization cofficients (ch1&2)

Intensity Correlation Quotient

select ROI & threshold to exclude background

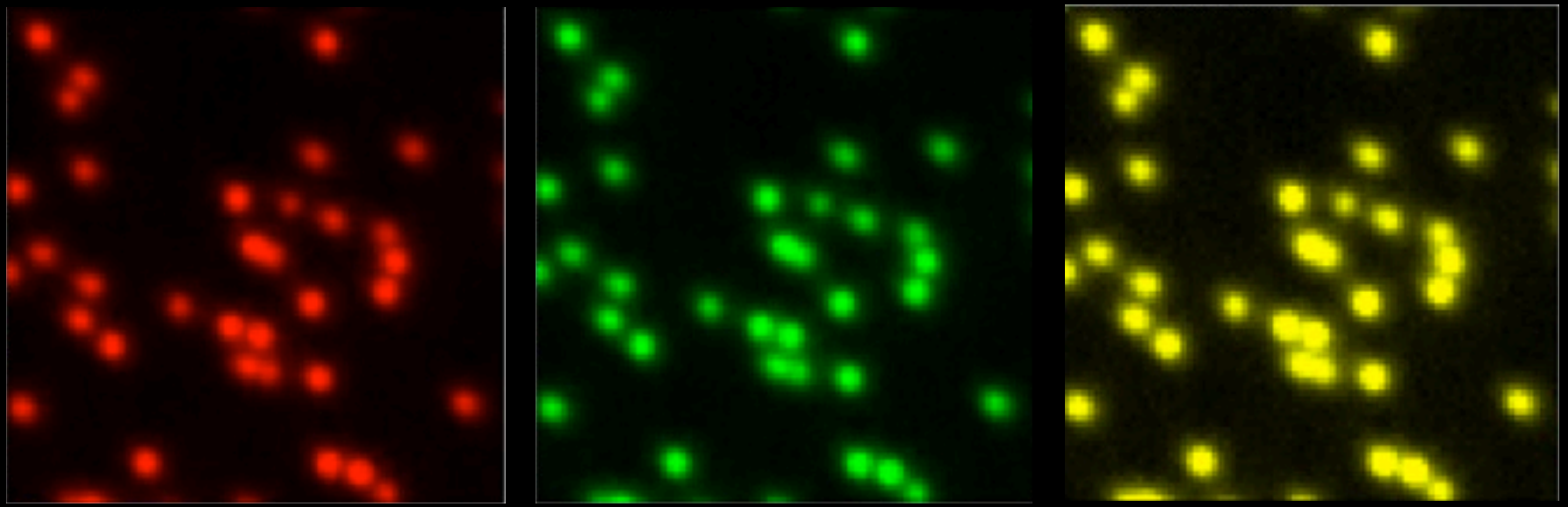
http://www.uhnres.utoronto.ca/facilities/wcif/imagej/colour_analysis.htm

Blind experimental analysis



3. Analysis & quantification

Colocalization



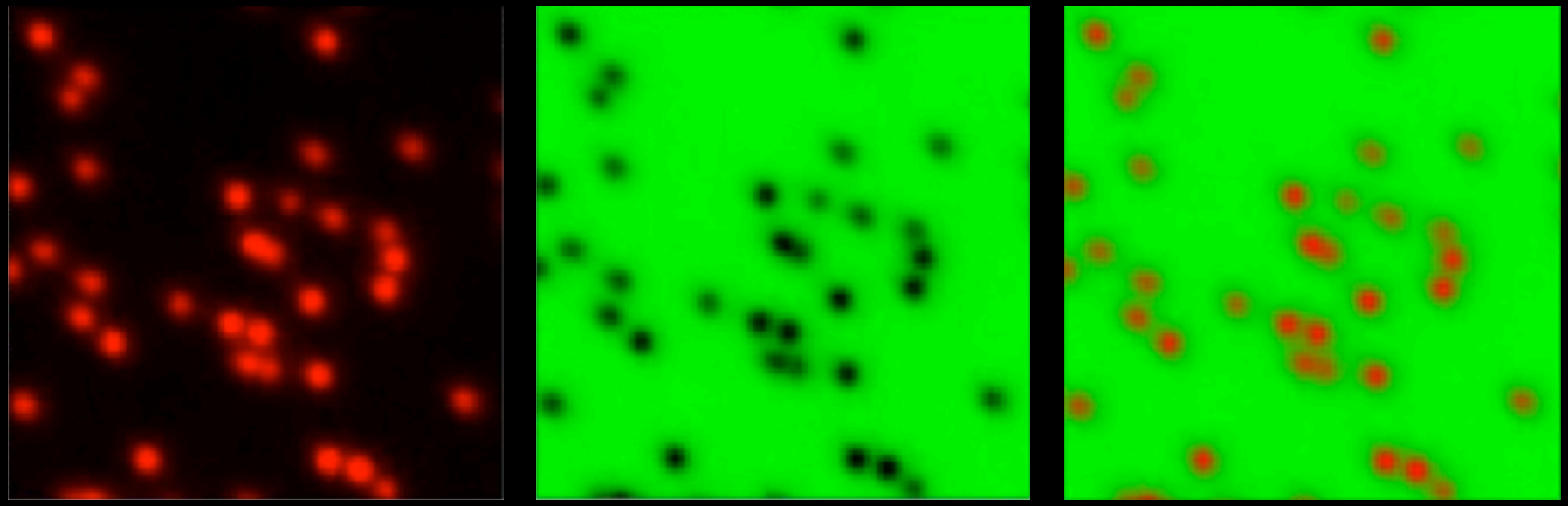
$R_r = 1$; $R = 1$; $M_{red} = 1.0$; $M_{green} = 1.0$; ICQ = 0.5

green dependent on red

http://www.uhnres.utoronto.ca/facilities/wcif/imagej/colour_analysis.htm

3. Analysis & quantification

Colocalization



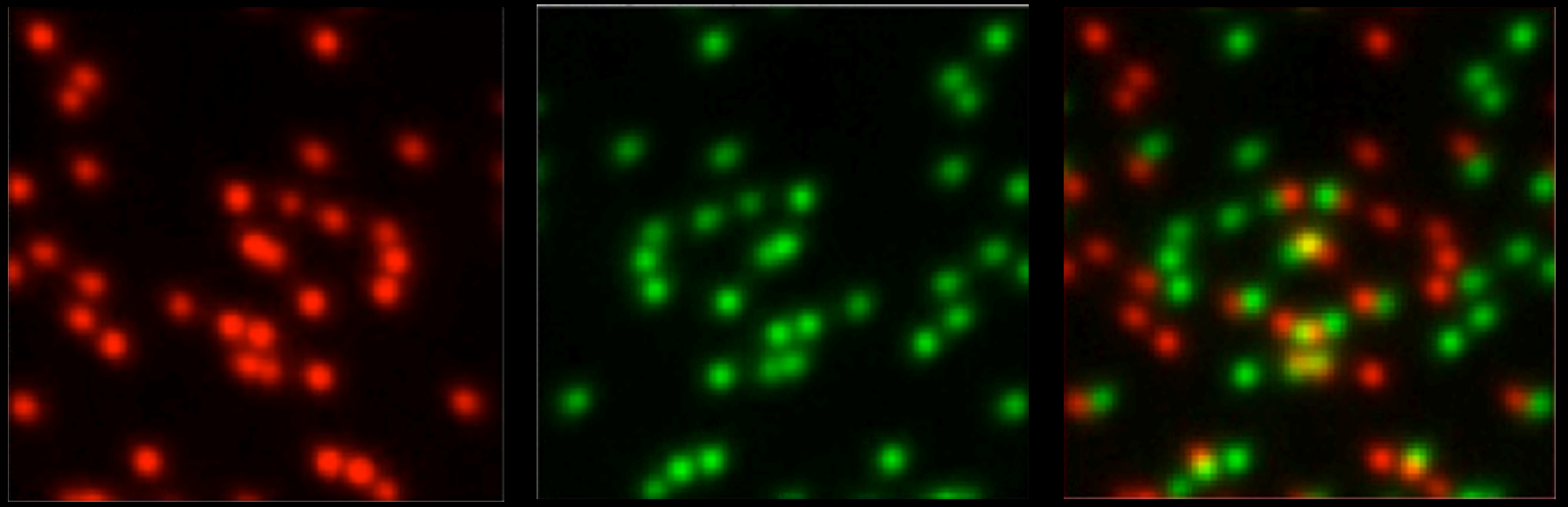
Rr = -1 ; R = -0.62 ; ICQ = -0.5

green and red segregate

http://www.uhnres.utoronto.ca/facilities/wcif/imagej/colour_analysis.htm

3. Analysis & quantification

Colocalization



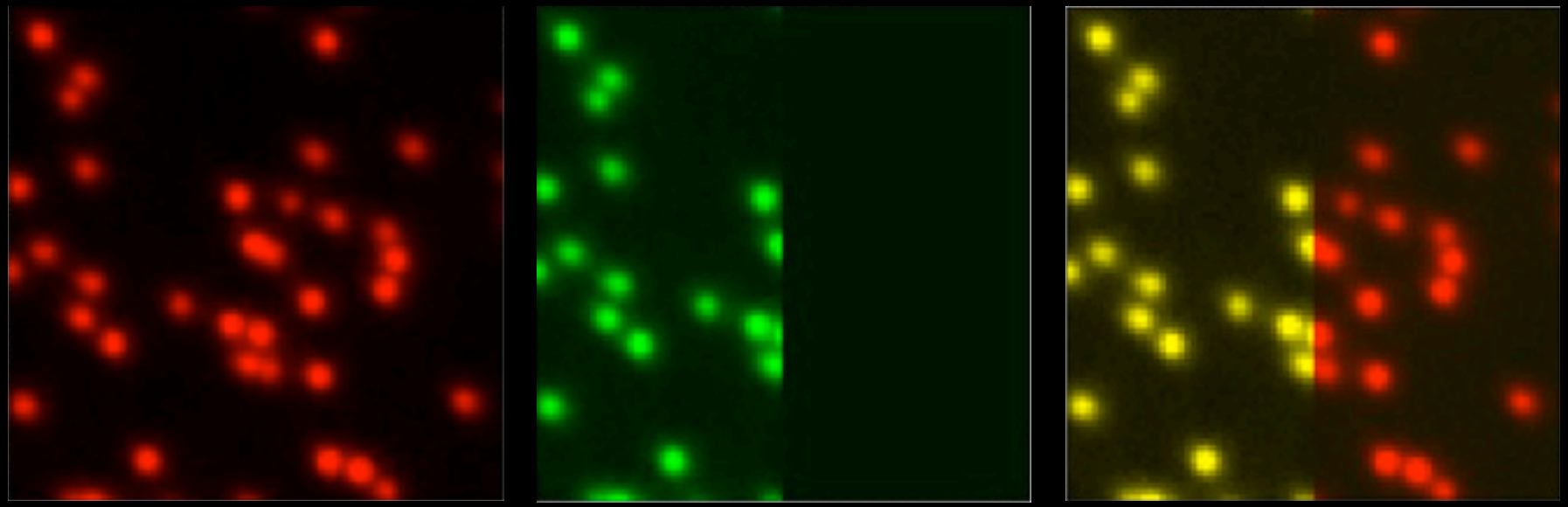
Rr = 0.38 ; Mred = 0.15; Mgreen = 0.15 ; ICQ = 0.18

green and red are unrelated

http://www.uhnres.utoronto.ca/facilities/wcif/imagej/colour_analysis.htm

3. Analysis & quantification

Colocalization



Rr = 0.62 ; Mred = 0.5; Mgreen = 1.0 ; ICQ = 0.30

half of red coincident with green, all of green with red

http://www.uhnres.utoronto.ca/facilities/wcif/imagej/colour_analysis.htm

Resources

ImageJ

http://rsbweb.nih.gov/ij/download.html

ImageJ plugins

http://rsbweb.nih.gov/ij/plugins/index.html

Fiji Is Just ImageJ

http://pacific.mpi-cbg.de/wiki/index.php/Fiji

MATLAB

http://www.mathworks.co.uk/

Digital Image Processing using MATLAB

R. C. Gonzalez et al., Prentice Hall, ISBN 0-13-008519-7

Octave (open source clone of MATLAB)

http://www.gnu.org/software/octave/

Image Processing: Principles and Applications

T. Acharya & A. K. Ray, Wiley, ISBN 0-471-71998-6

ParticleStats (Russell Hamilton)

http://www.particlestats.com/

WCIF ImageJ plugins

http://www.uhnresearch.ca/facilities/wcif/fdownload.html