

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

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Micron
Oxford wellcome trust UNIVERSITY OF OXFORD

Oxford Advanced Microscopy Unit
Imaging and Modulating Molecular Dynamics

Now recruiting

- Group leader position
- Assistant Microscopy Manager
- Image analysis specialist
- Image Data - System Administrator
- Correlative light - electron microscopy specialist

Micron Oxford is funded by a major grant from The Wellcome Trust to the Department of Biochemistry and the Sir William Dunn School of Pathology in association with the Wellcome Trust Centre for Human Genetics and the Oxford Centre for Integrative Systems Biology

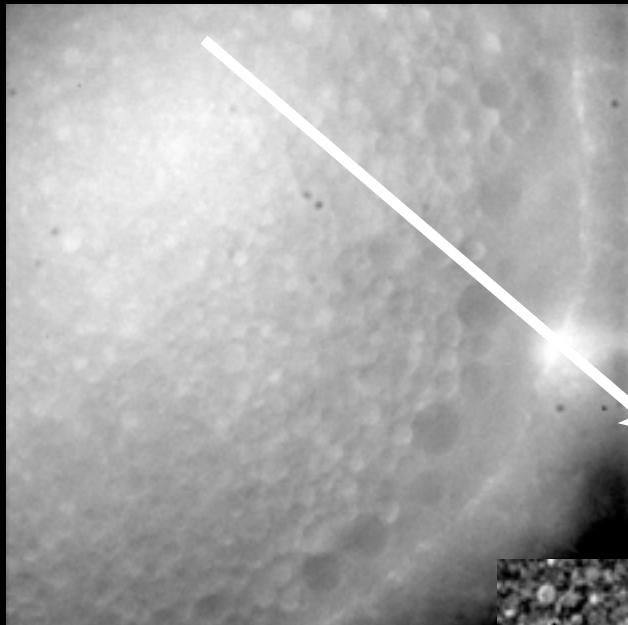
informal enquiries to: Kim Nasmyth, Ilan Davis, Liz Robertson, Jordan Raff, Neil Brockdorff or Nick Proudfoot (email sarah-jane.scard@bioch.ox.ac.uk)

Web site: <http://www.micronoxford.com>

1. Image restoration & filtering
 2. Feature extraction (segmentation)
 3. Analysis and quantification
- (4. Automation)

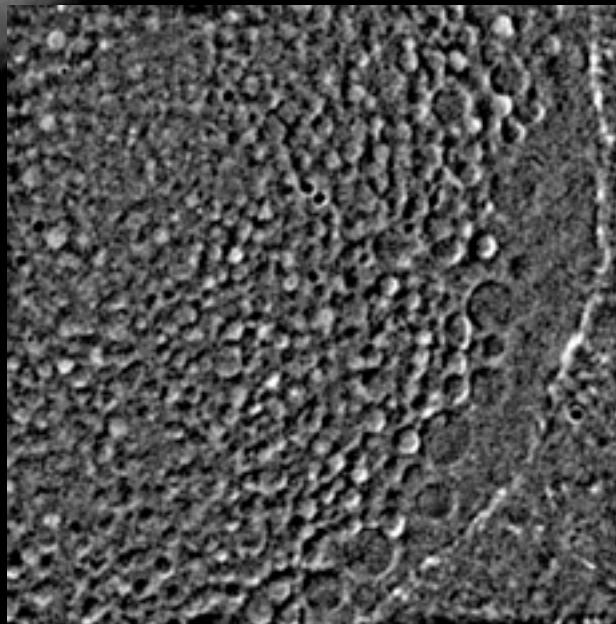
Improving and quantifying image data

raw data

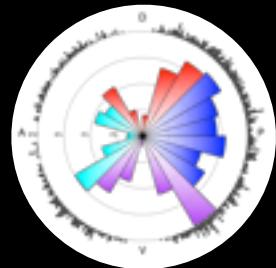
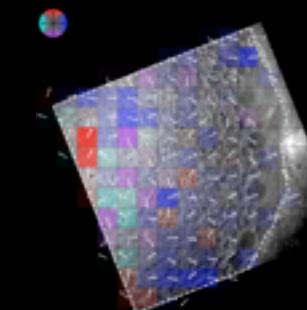


(Richard Parton)

processing/
filtering



analysis
(tracking)



(Russell Hamilton)

Tools for image analysis

ImageJ

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

The screenshot shows the 'Plugins' section of the ImageJ website. At the top, there is a navigation bar with links: home | news | docs | download | plugins | macros/dev | list | links. Below the navigation bar, the word 'Plugins' is displayed in a large, bold, blue font. To the left, a sidebar titled 'Contents' lists various categories: Acquisition, Analysis, Collections, Color, Filters, Segmentation, Graphics, Input/Output, Programming Examples, Stacks, Utilities, and Links to External Sites. To the right, there is a screenshot of the ImageJ application window. The window title is 'ImageJ'. The toolbar at the top contains various icons for file operations, selection, measurement, and analysis. The main workspace shows a grayscale image with some red and green overlays.

Fiji Is Just ImageJ

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

The screenshot shows the main page of the 'Fiji Is Just ImageJ' website. The title 'Fiji Is Just ImageJ' is displayed prominently. Below the title, there is a brief description: 'Fiji is an image processing package. It can be described as a distribution of ImageJ together with Java, Java 3D and a lot of plugins organized into a coherent menu structure. Fiji compares to ImageJ as Ubuntu compares to Linux.' There are three sections with text and images: 'The main focus of Fiji is to assist research in life sciences.', 'For users, Fiji is easy to install and has an automatic update function, bundles a lot of plugins and offers comprehensive documentation.', and 'For developers, Fiji is an open source project hosted in a Git version control repository, with access to the source code of all internals, libraries and plugins, and eases the development and scripting of plugins.' At the bottom, there is a 'Download Fiji now' button with a download icon, and a screenshot of the Fiji application window showing a multi-panel interface with a 3D rendering of a specimen.

Tools for image analysis

MATLAB

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

Image Processing Toolbox 7.0	
Product Description	
Introduction and Key Features	Key Features
○ Introduction and Key Features	<ul style="list-style-type: none">■ 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
○ Importing and Exporting Images	
○ Displaying and Exploring Images	
○ Preprocessing and Postprocessing Images	
○ Analyzing Images	
○ Spatial Transformations and Image Registration	
○ Working with Large Images	

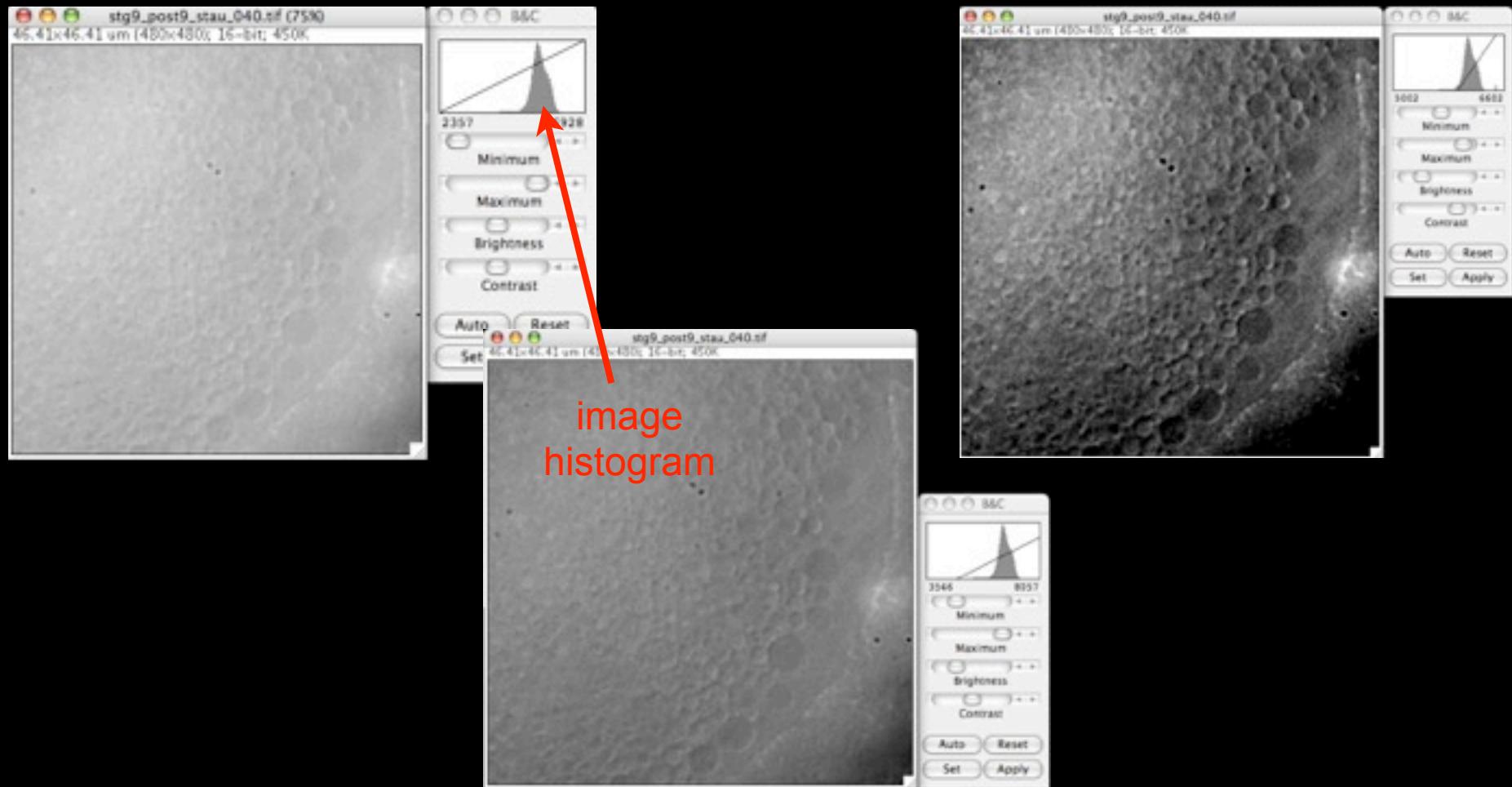
- prototyping, task automation
- plotting functions

1. Image restoration / filtering

1. Image restoration / filtering

Intensity Transforms

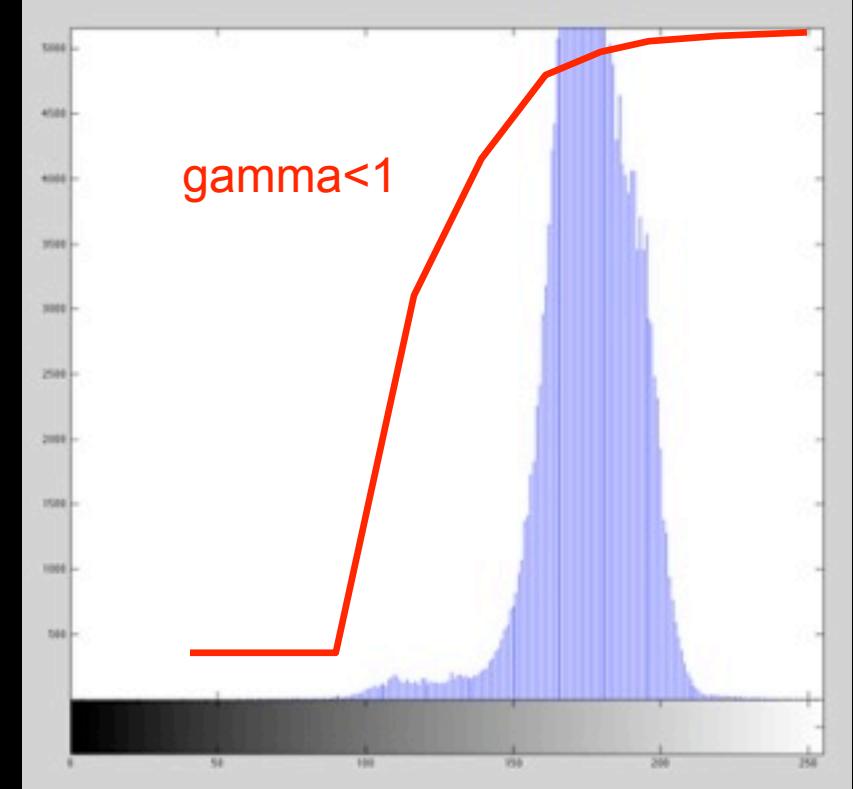
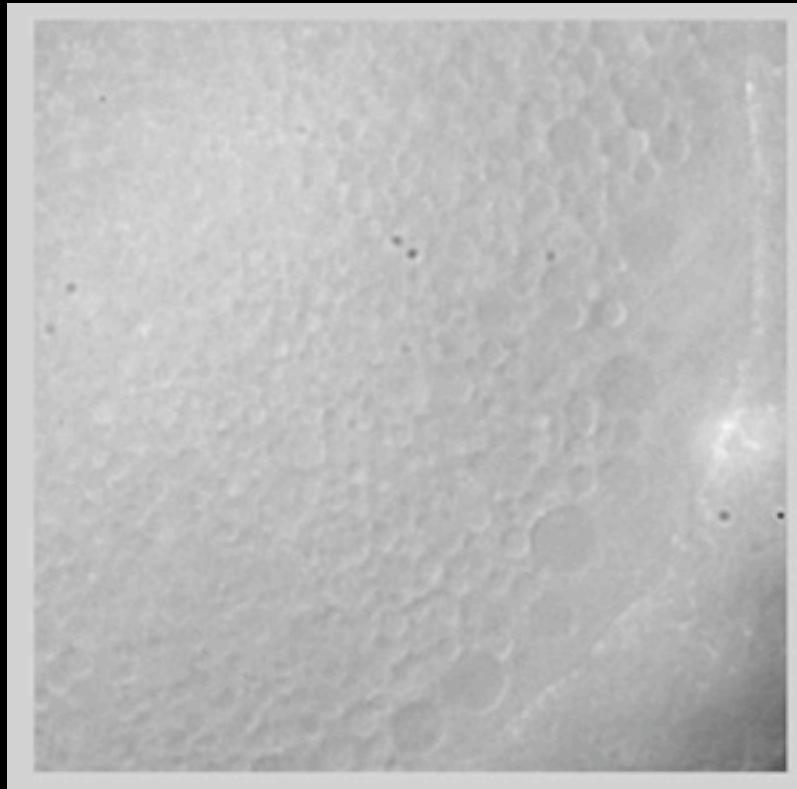
- same operation applied to each pixel
- => changes to image brightness and contrast



1. Image restoration / filtering

Intensity Transforms

- contrast stretching: gamma, histogram equalization



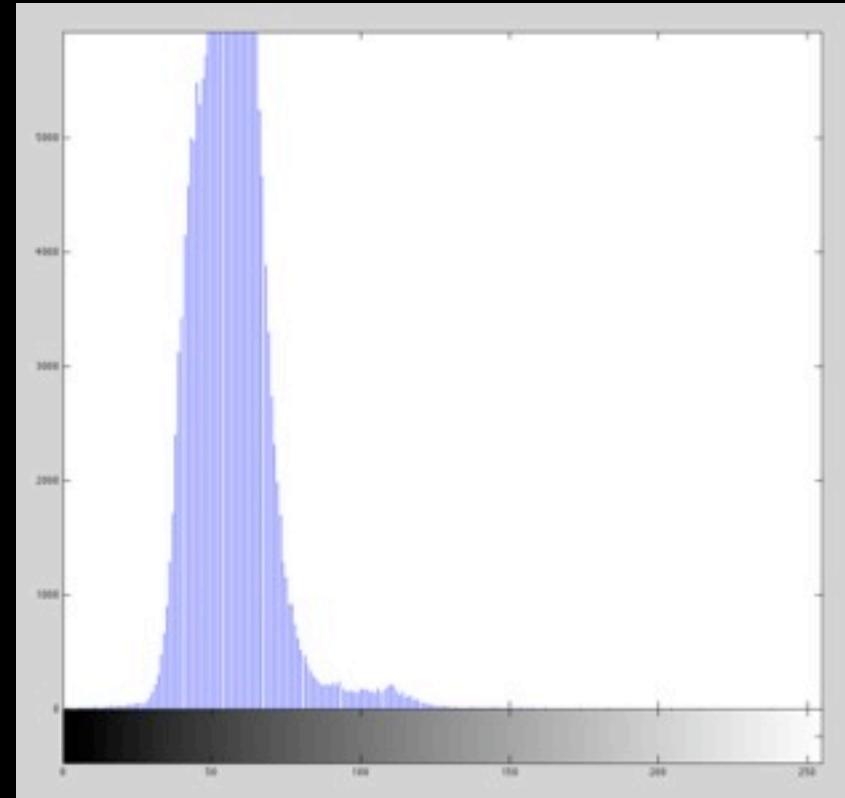
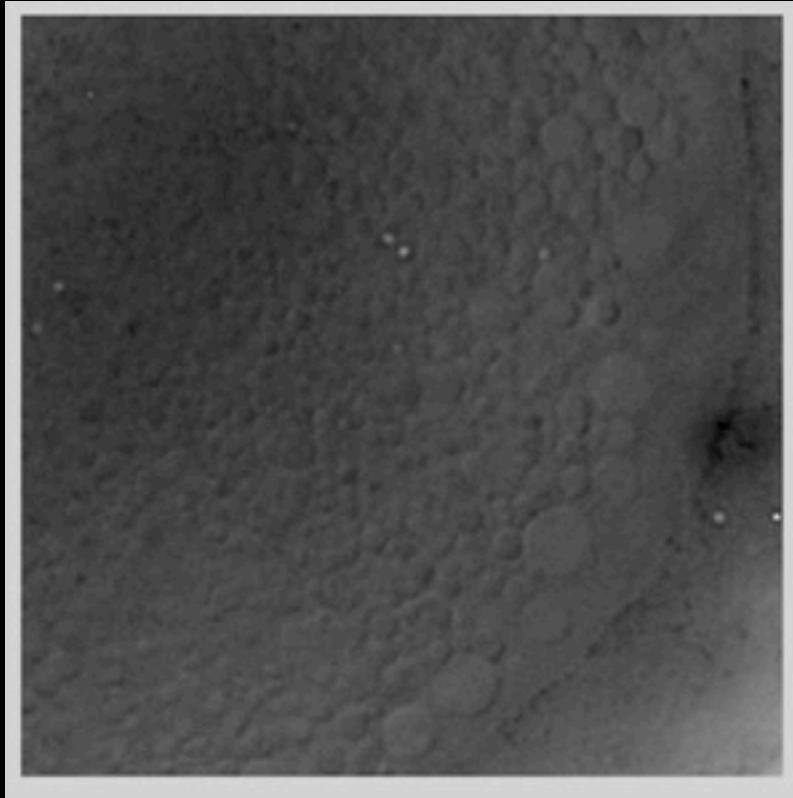
MATLAB - imshow to display image, imhist to display its histogram

```
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



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

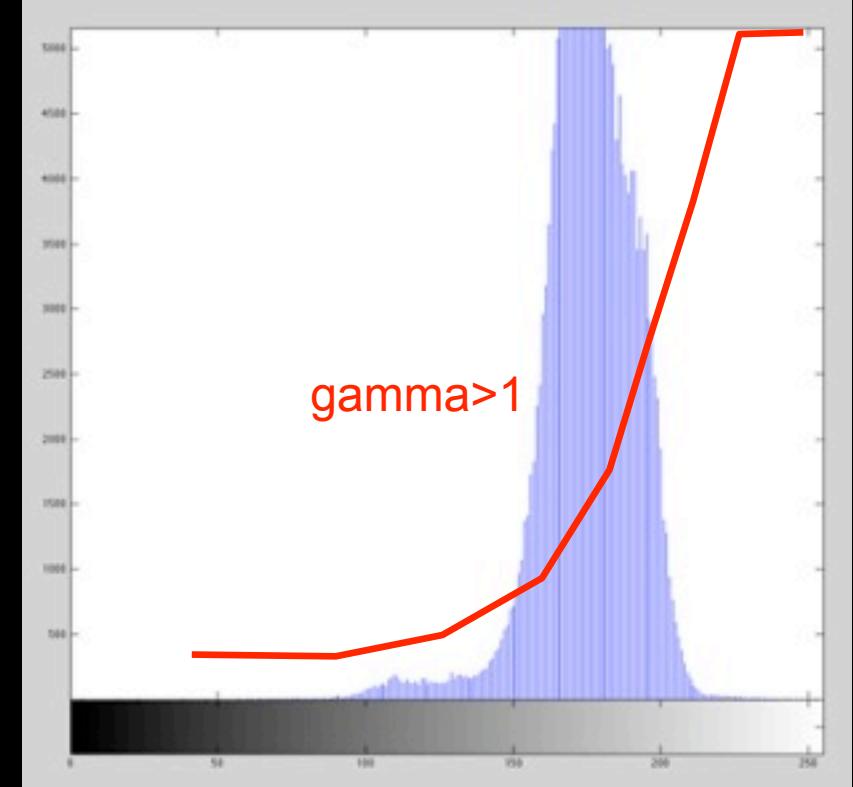
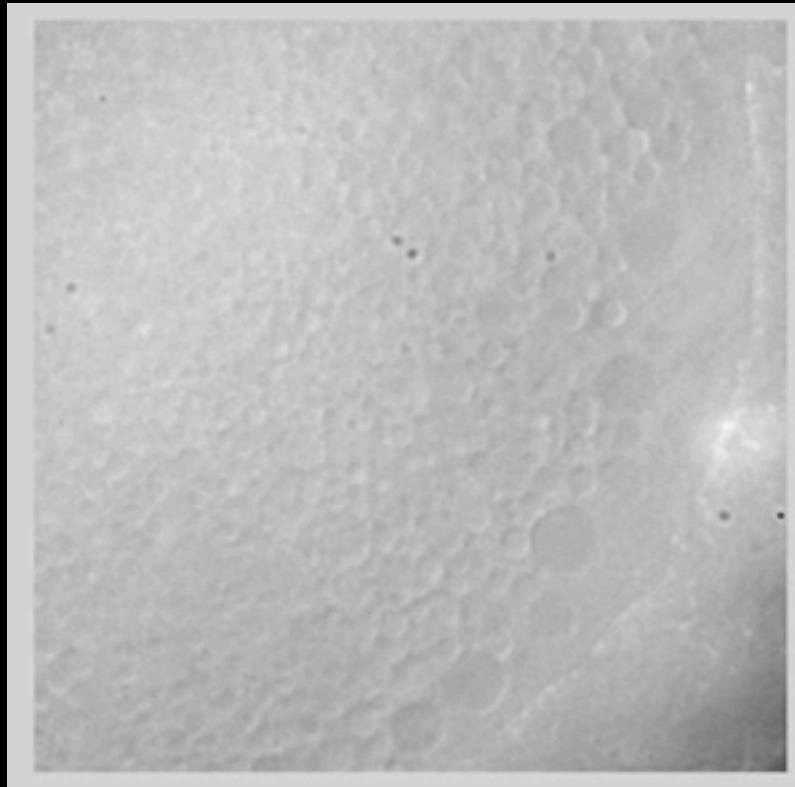
imadjust(f, [low_in high_in], [low_out high_out], gamma)

histeq to do histogram equalization

1. Image restoration / filtering

Intensity Transforms

- contrast stretching: gamma, histogram equalization



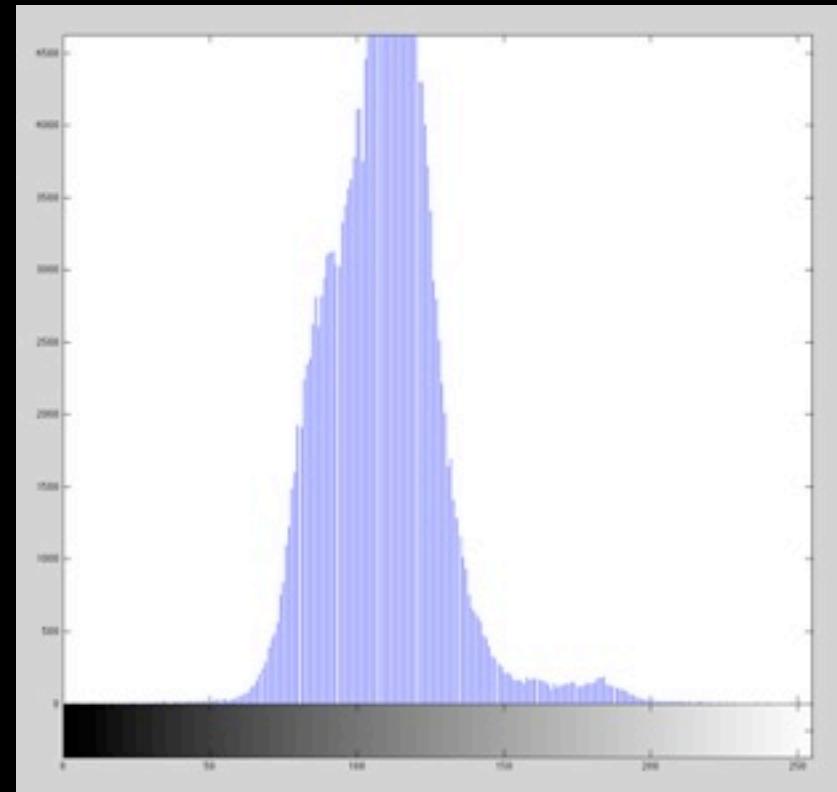
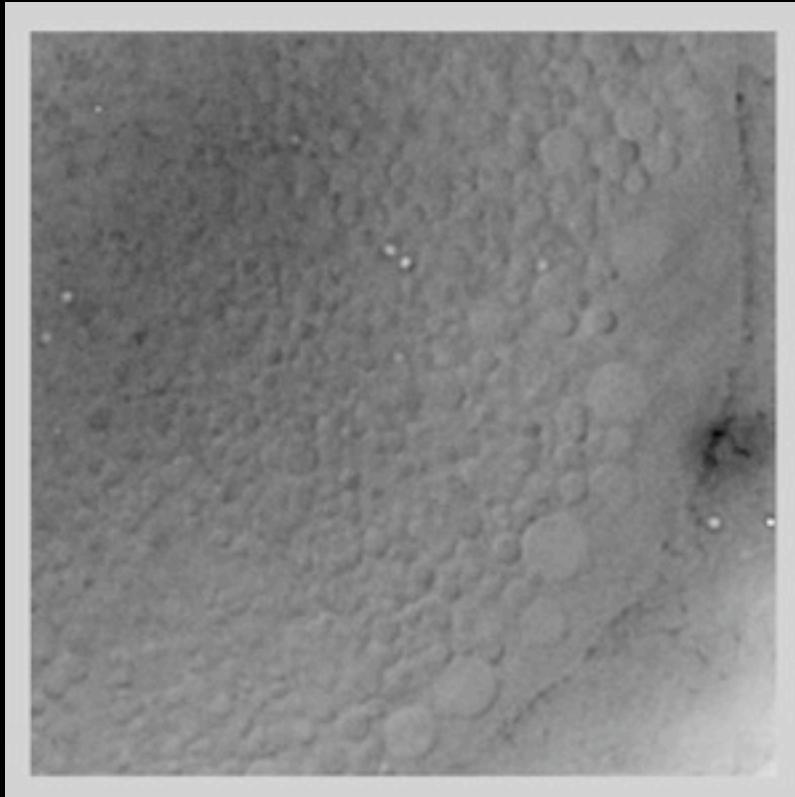
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imadjust(f, [low_in high_in], [low_out high_out], gamma)  
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1. Image restoration / filtering

Intensity Transforms

- contrast stretching: gamma, histogram equalization



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

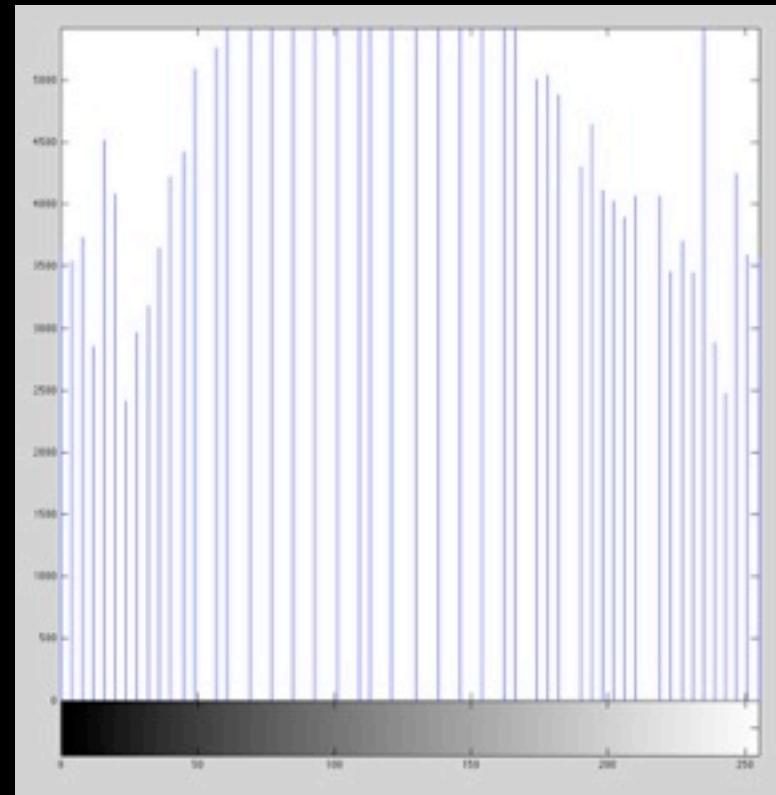
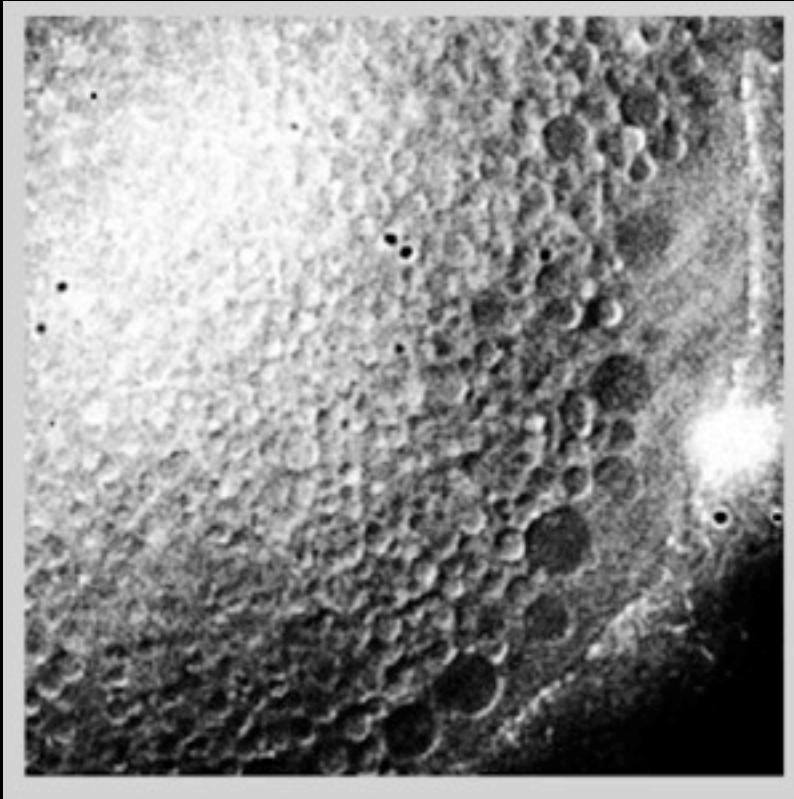
imadjust(f, [low_in high_in], [low_out high_out], gamma)

histeq to do histogram equalization

1. Image restoration / filtering

Intensity Transforms

- contrast stretching: gamma, histogram equalization



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

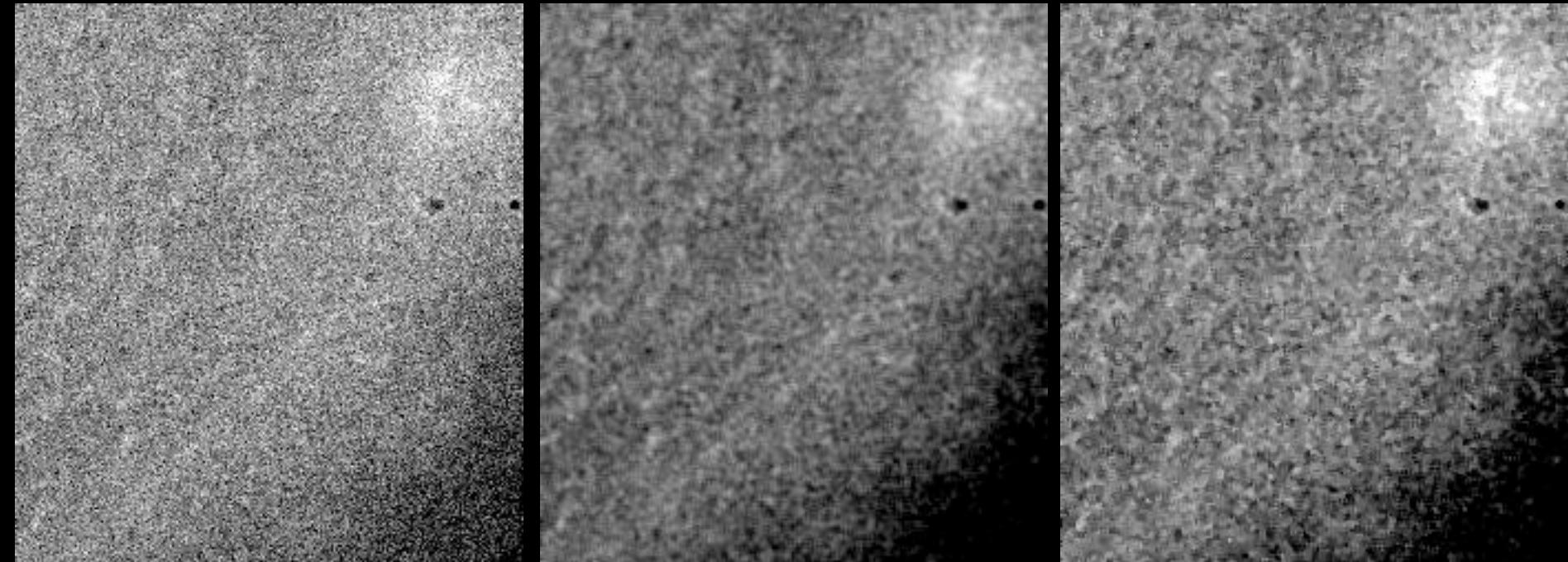
imadjust(f, [low_in high_in], [low_out high_out], gamma)

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



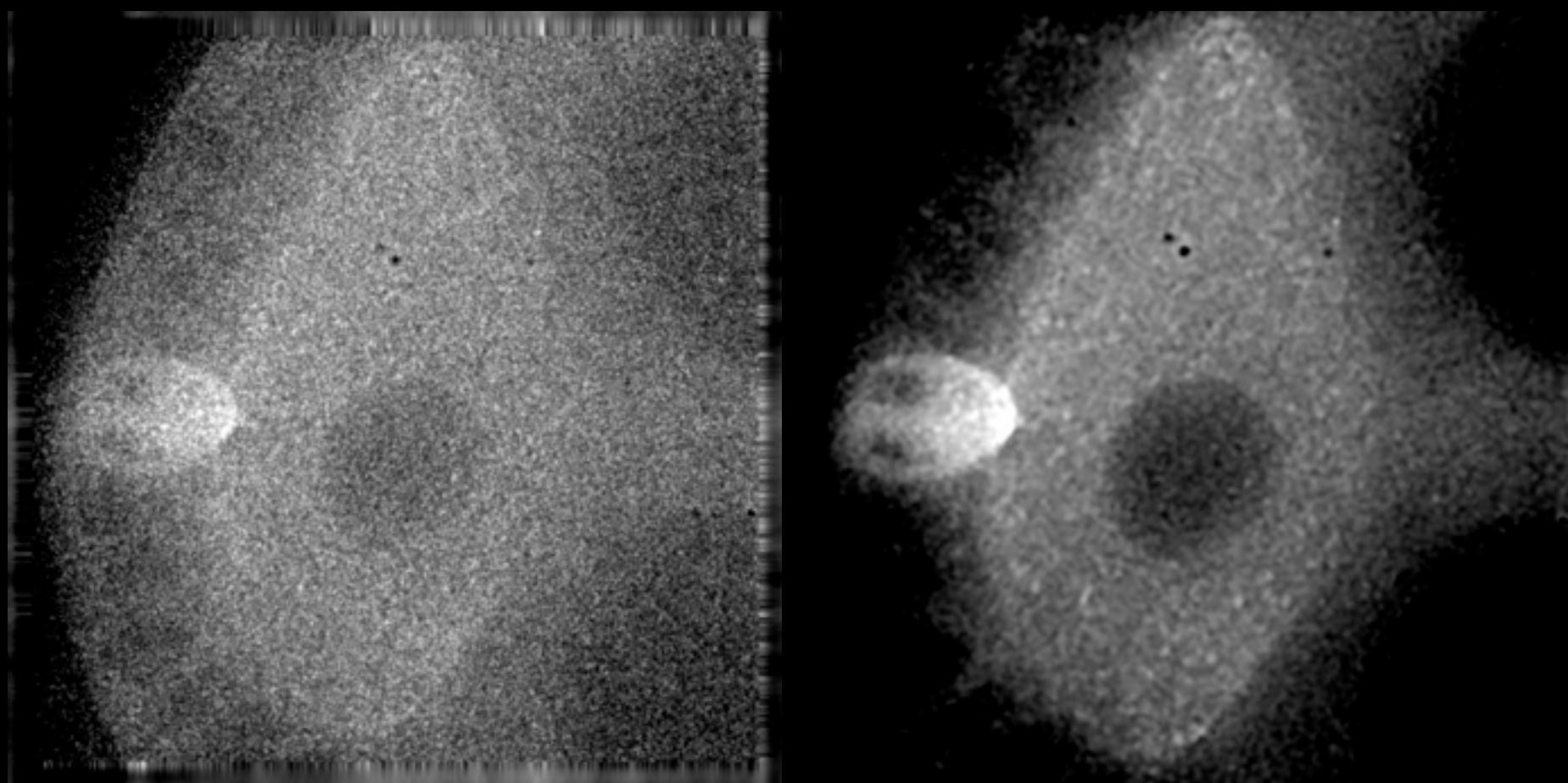
noisy image

3x3 mean filter

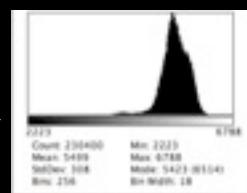
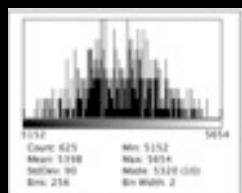
3x3 median filter

1. Image restoration / filtering

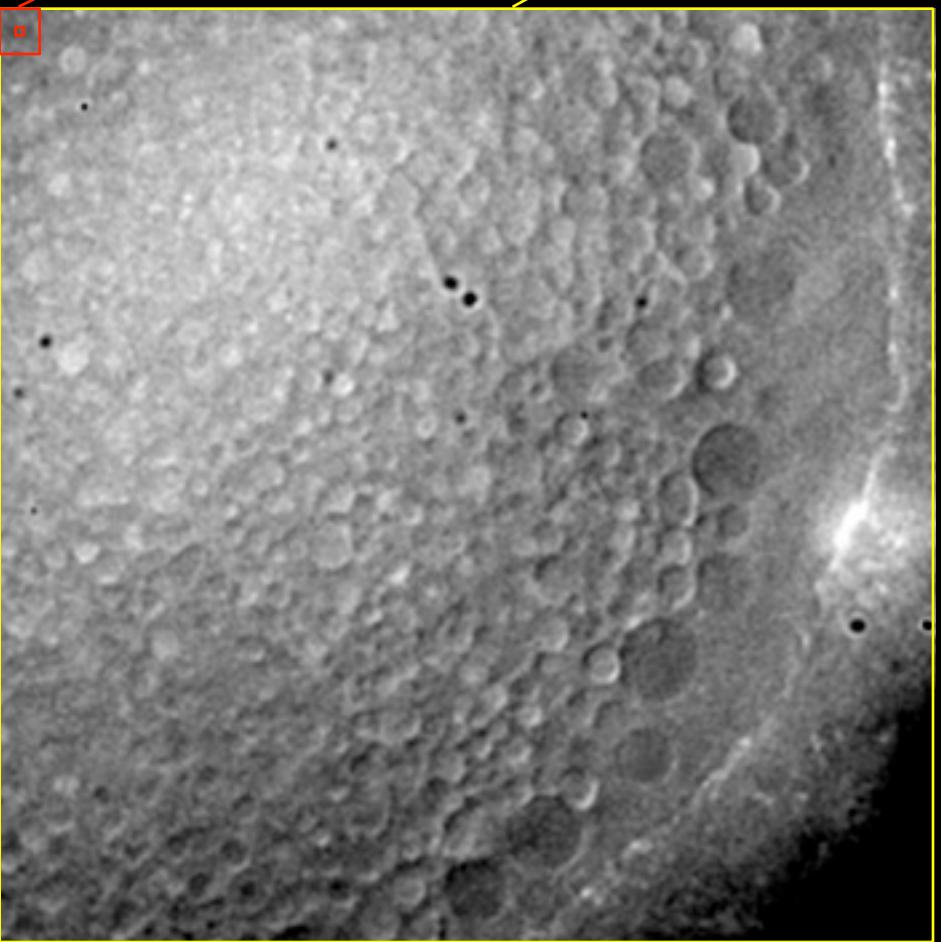
Spatial Filtering: denoising



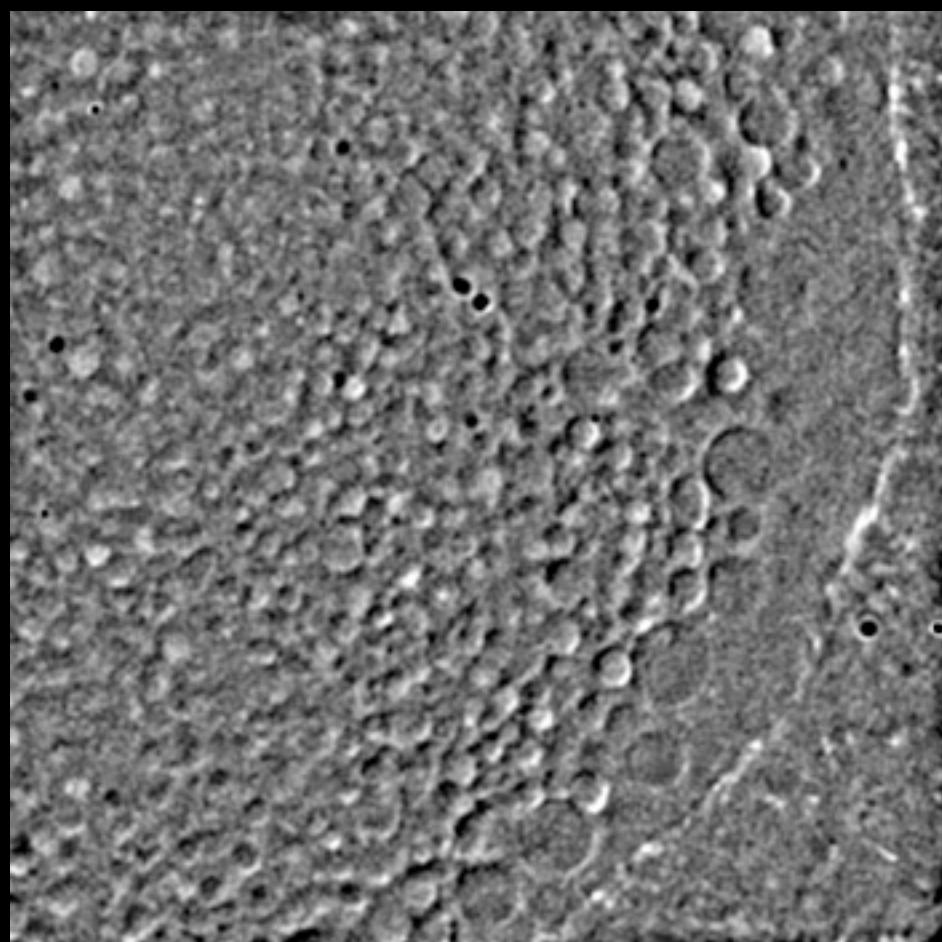
1. Image restoration / filtering



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



raw data showing uneven illumination

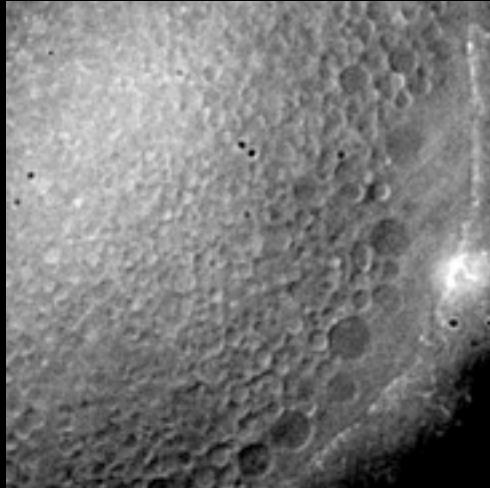


'normalized' image

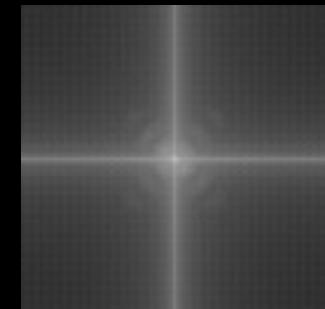
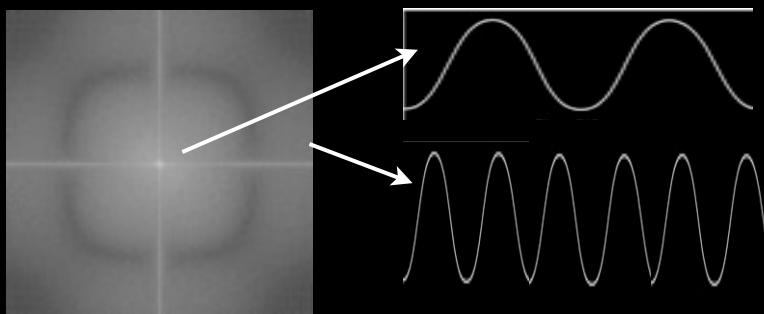
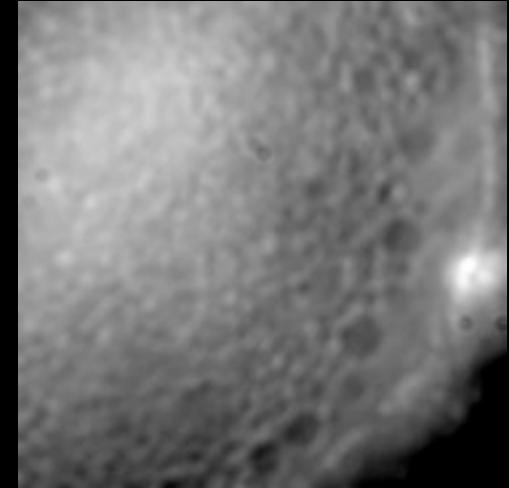
1. Image restoration / filtering

Frequency domain processing

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



15x15
mean
filter

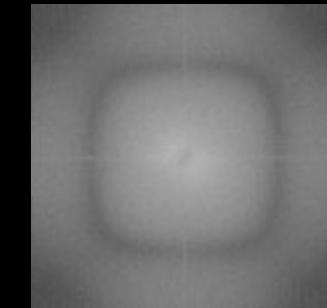
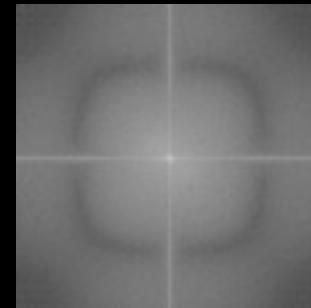
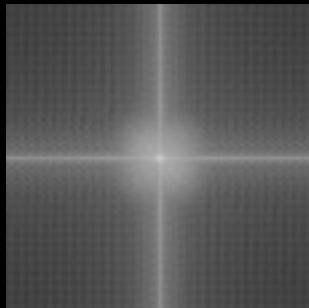
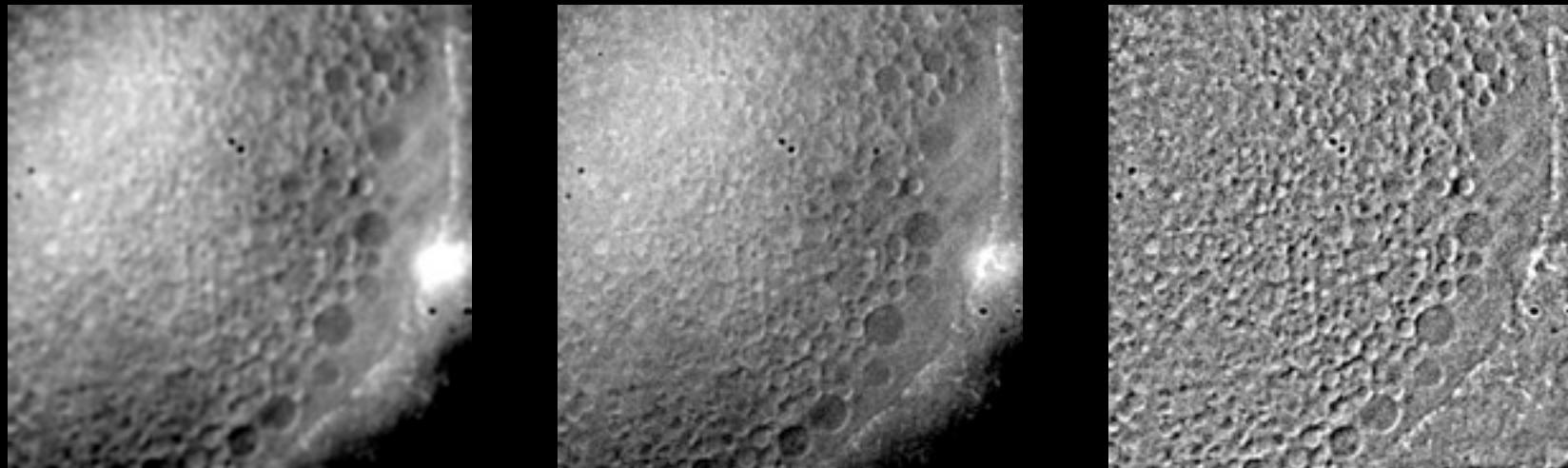


1. Image restoration / filtering

Frequency domain processing

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

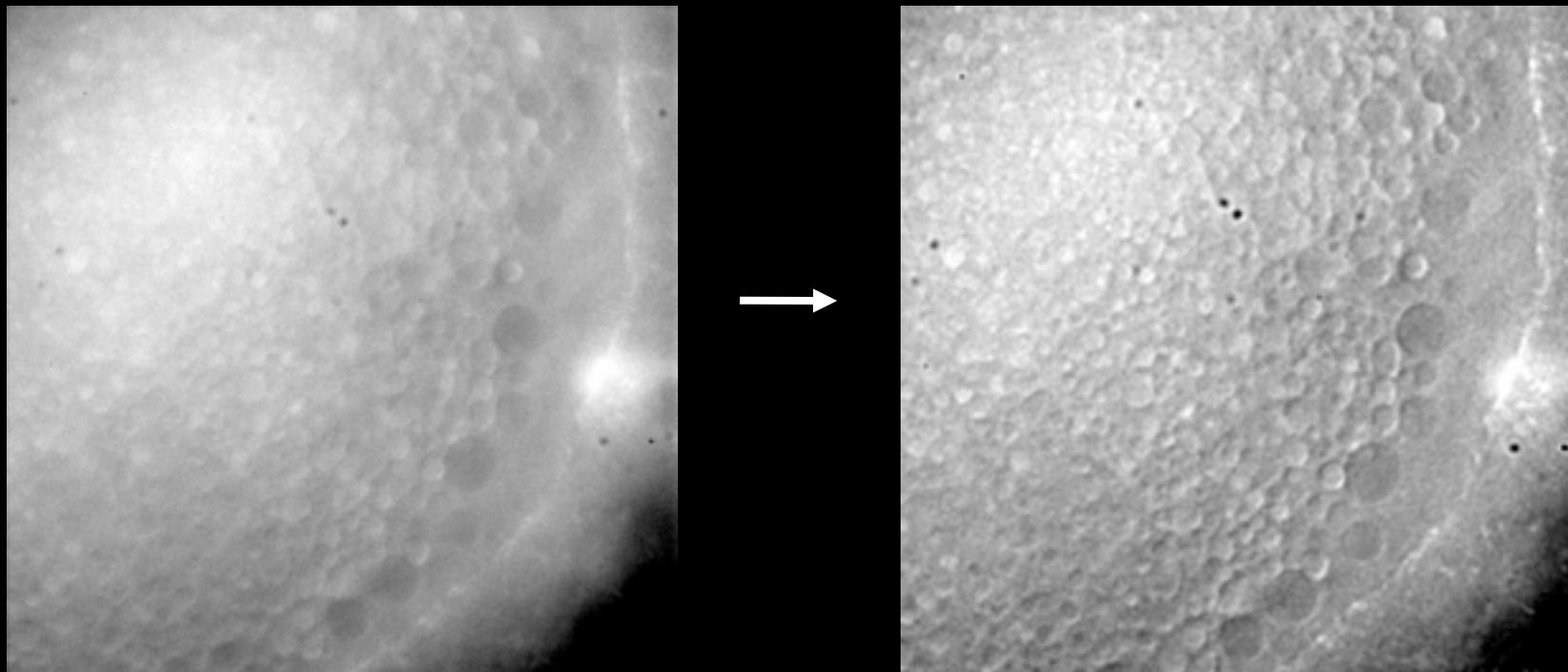
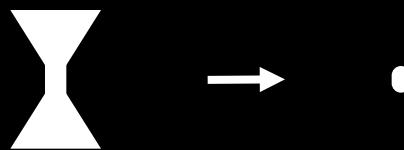
lowpass ← start → highpass

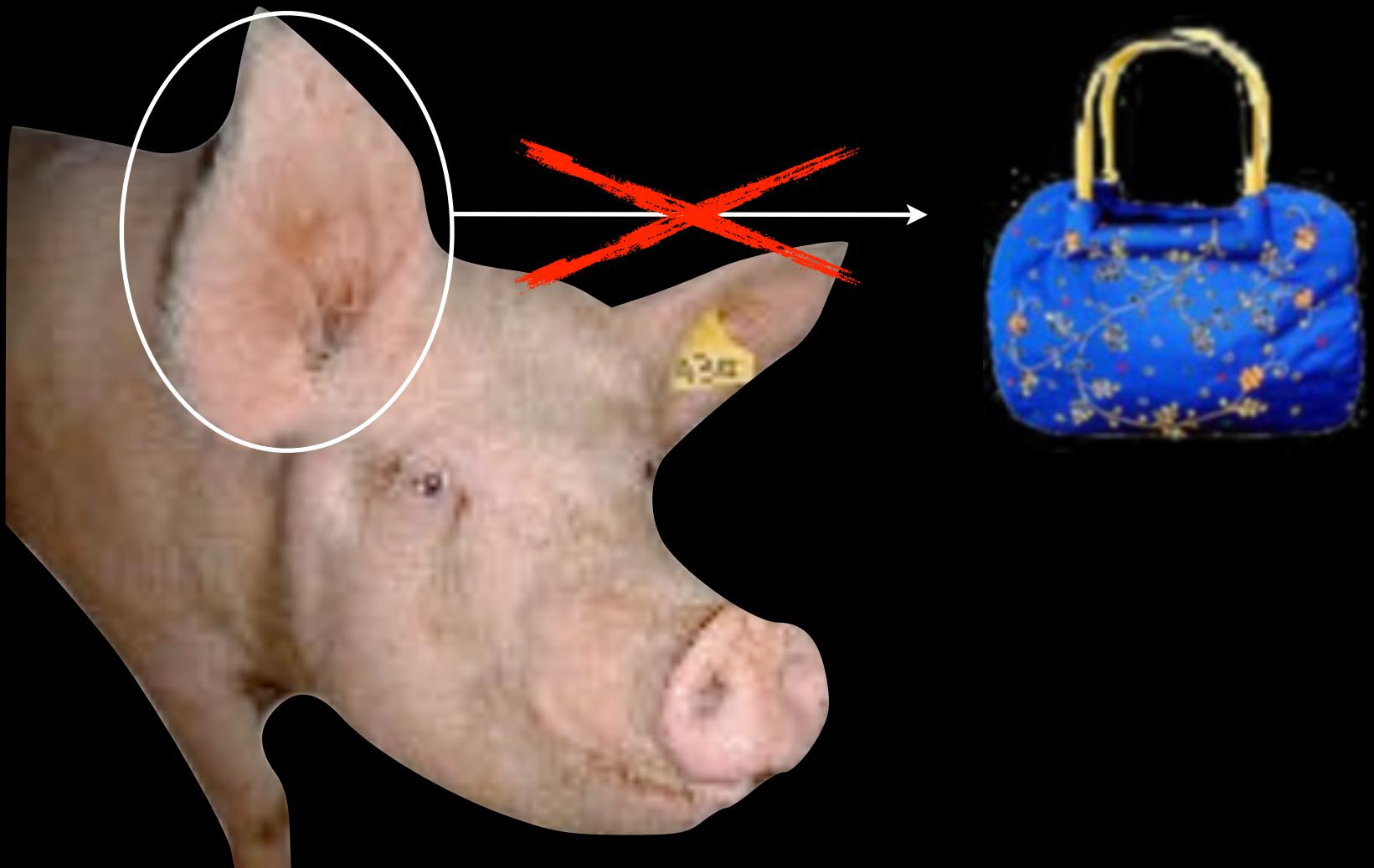


1. Image restoration / filtering

Deconvolution

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





can I improve my data acquisition?

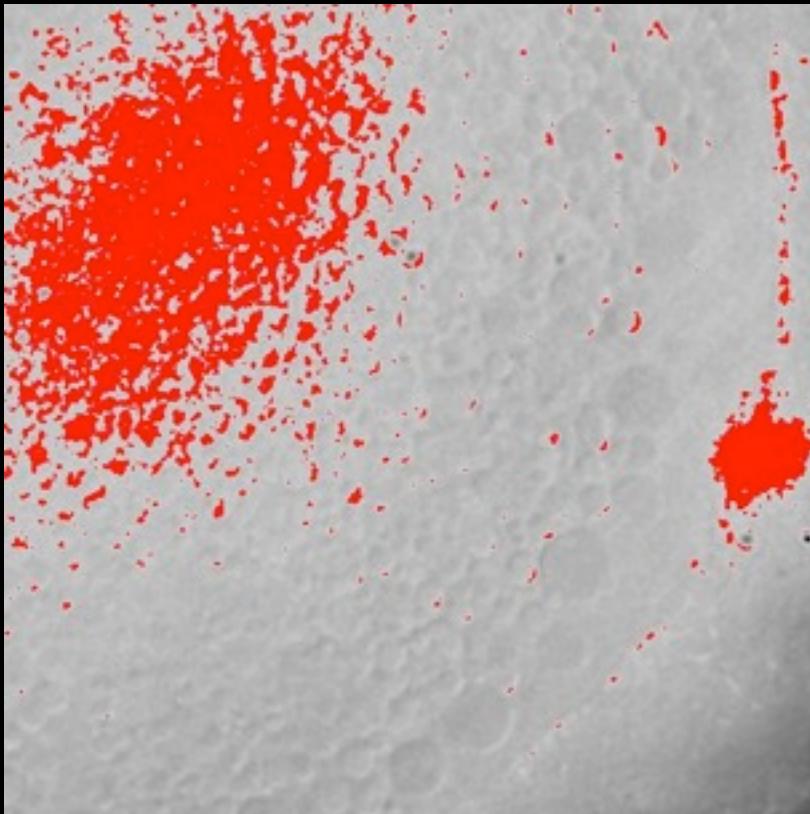
2. Feature extraction

2. Feature extraction

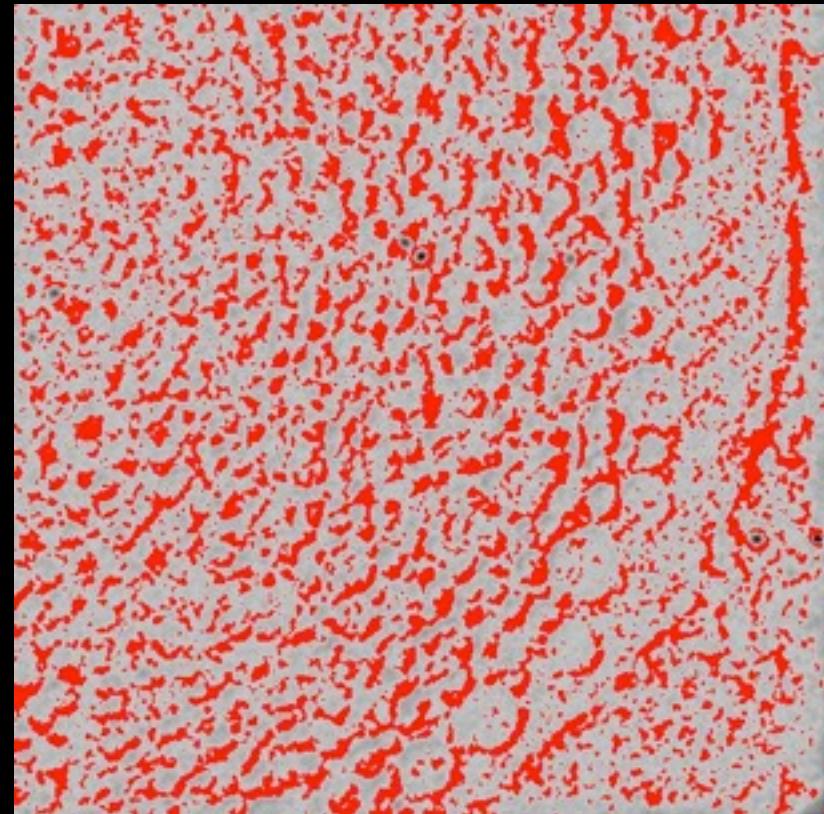
Image segmentation: thresholding

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

using raw data



using ‘normalized’ data



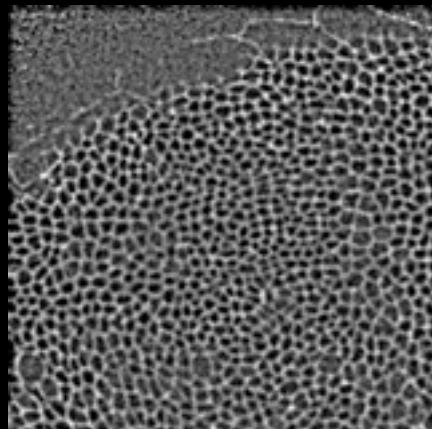
=> ‘adaptive thresholding’, or prior normalization of the image

2. Feature extraction

Morphological image processing

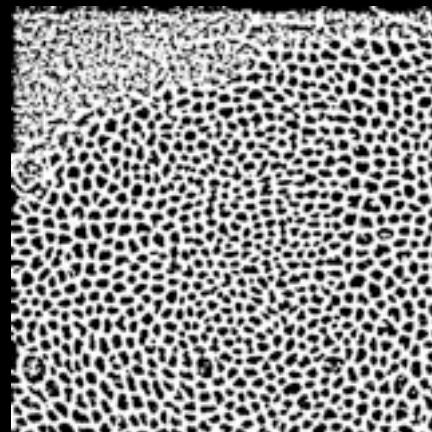
- dilation and erosion of binary images

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

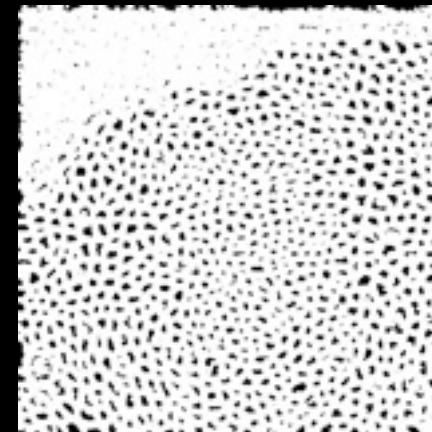


(Yanlan Mao, CRUK)

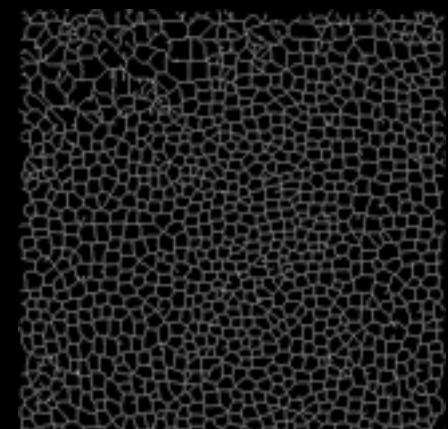
binarized



dilated



skeleton

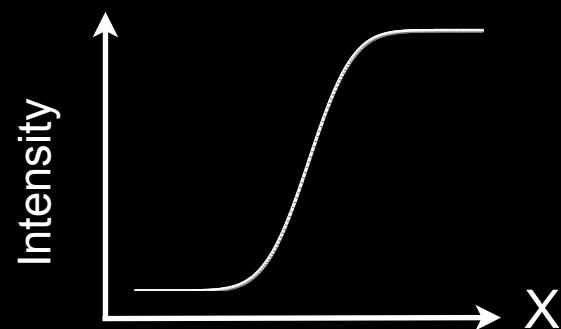
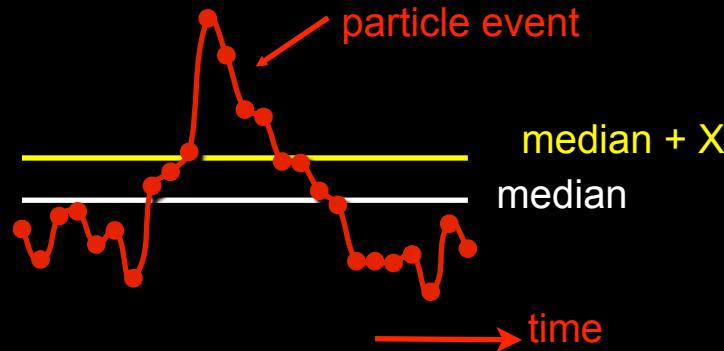


2. Feature extraction

Image segmentation: identifying ‘foreground’ features

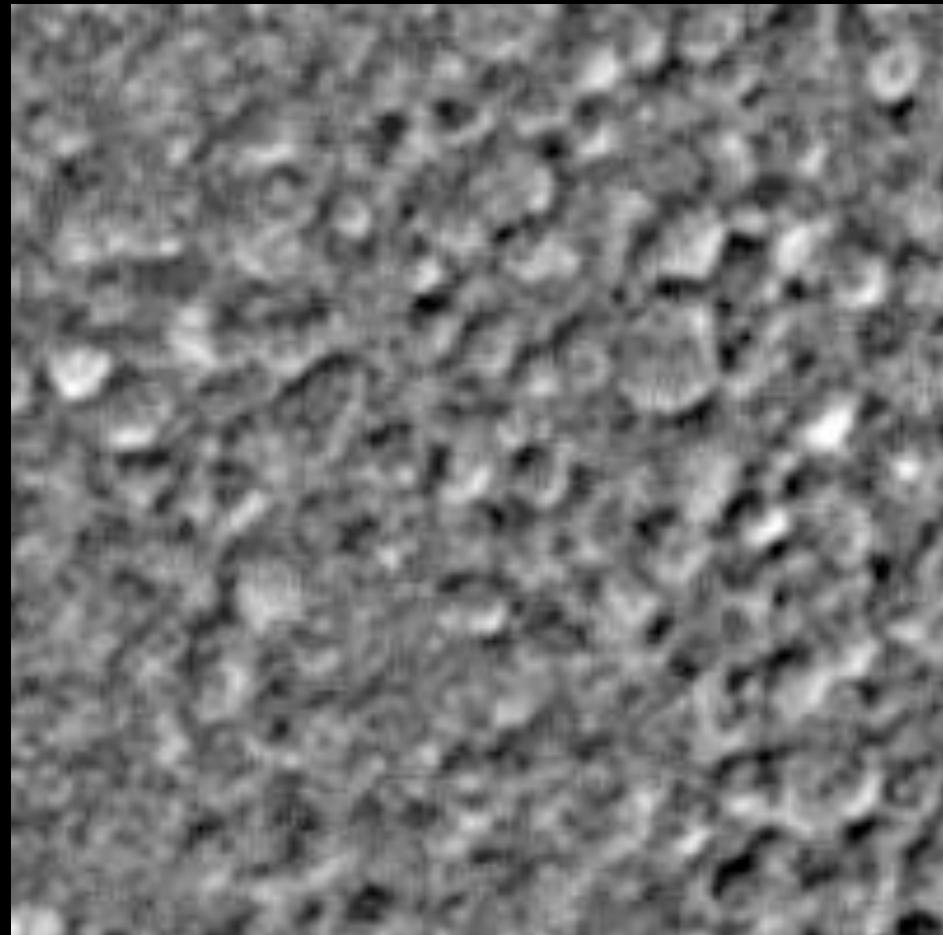
- easy to implement custom filters in MATLAB, like this

temporal median filter to identify moving foreground

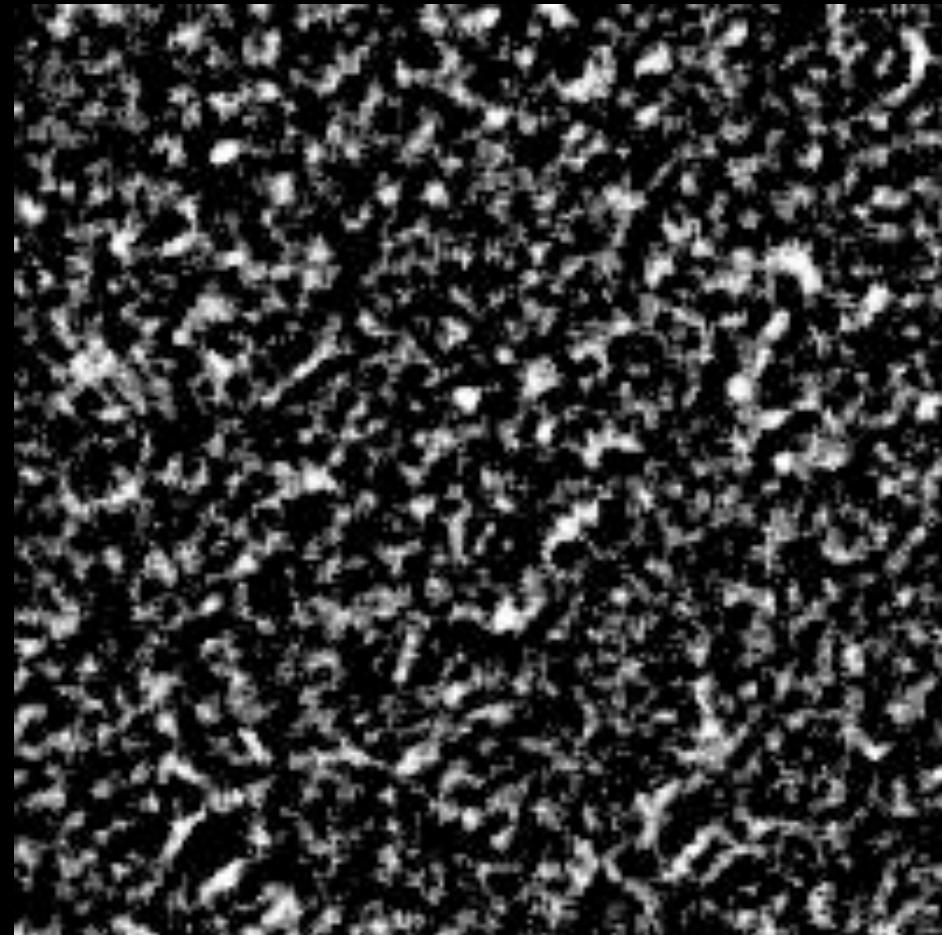


2. Feature extraction

Image segmentation: identifying ‘foreground’ features



200x200 area, normalized



200x200 area, non-background ('foreground')

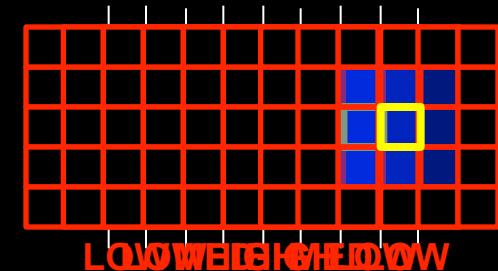
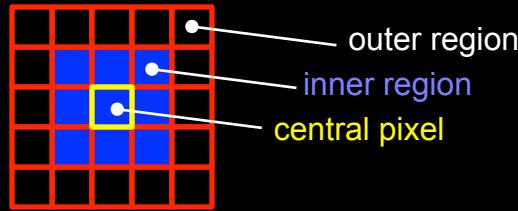
2. Feature extraction

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)

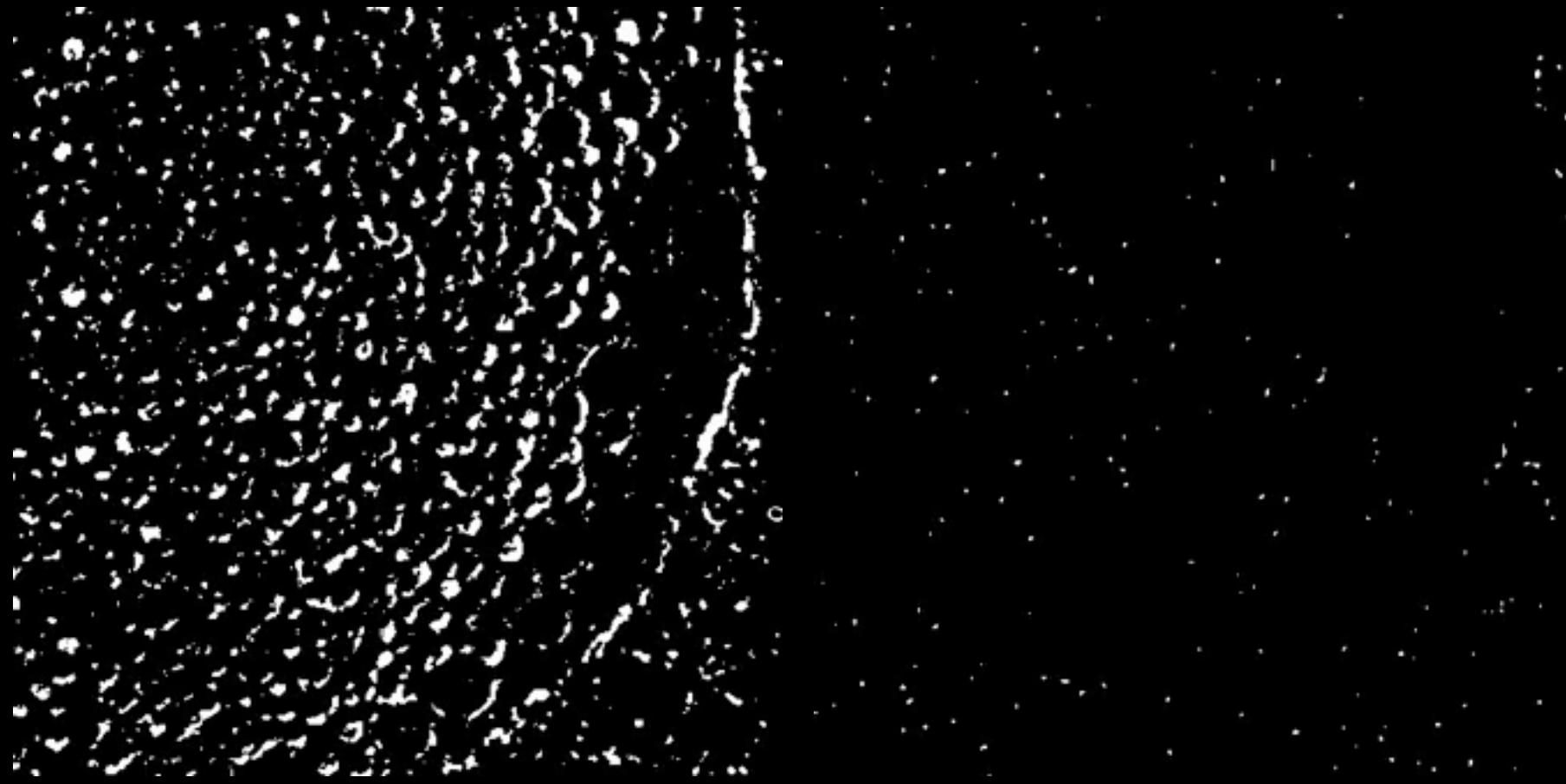
e.g. detection of Haar-like features to find particles

square Haar-like feature



2. Feature extraction

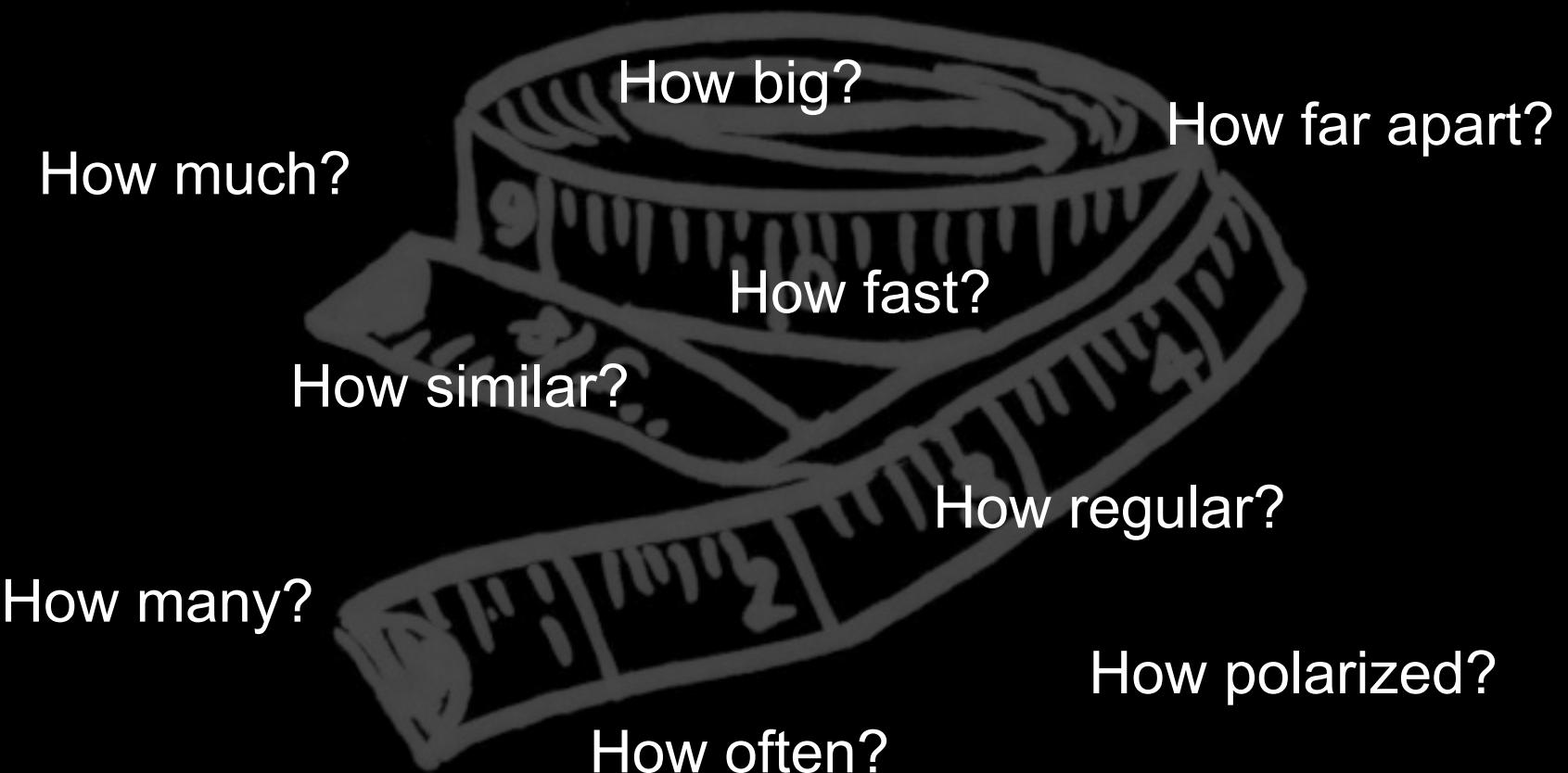
segmentation of raw data versus ‘feature image’



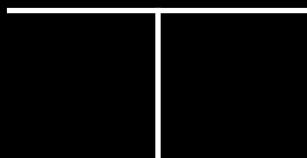
‘normalized’ data

detected features

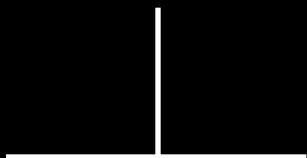
3. Analysis and quantification



3. Analysis and quantification



How accurate are my results?



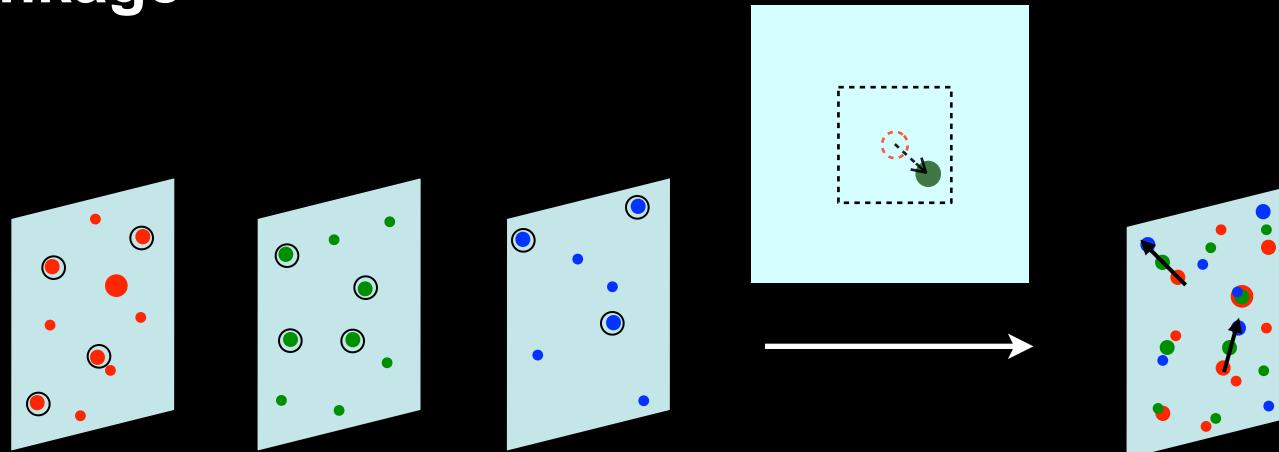
- repeat measurements & calculate SEM
- test statistical significance of result: p-value < 0.05
- are two sets of results different? Student's *t*-test

3. Analysis & quantification

Problem 1: Particle Tracking

- simple particle tracking scheme:-

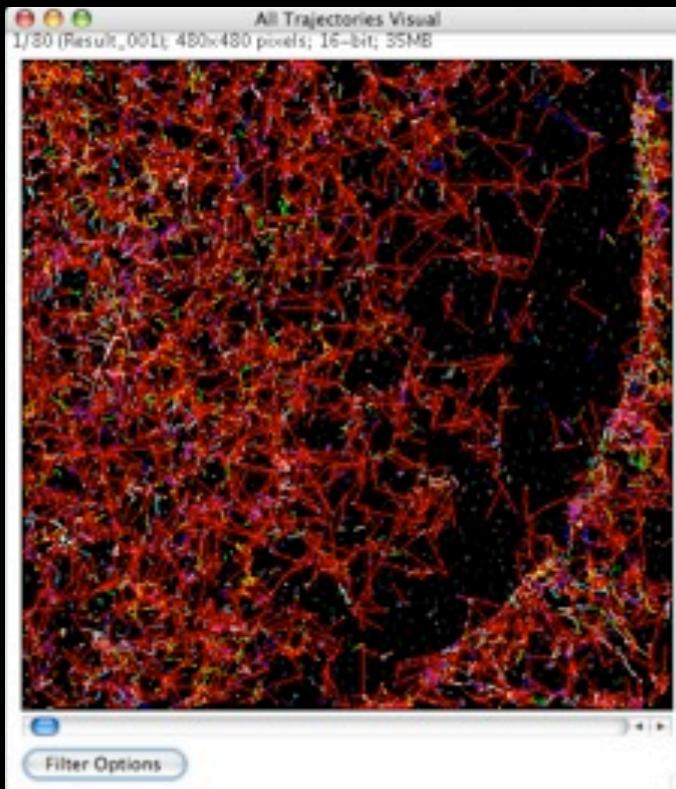
1. pre-filtering
2. segmentation
3. linkage



3. Analysis & quantification

Problem 1: Particle Tracking

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

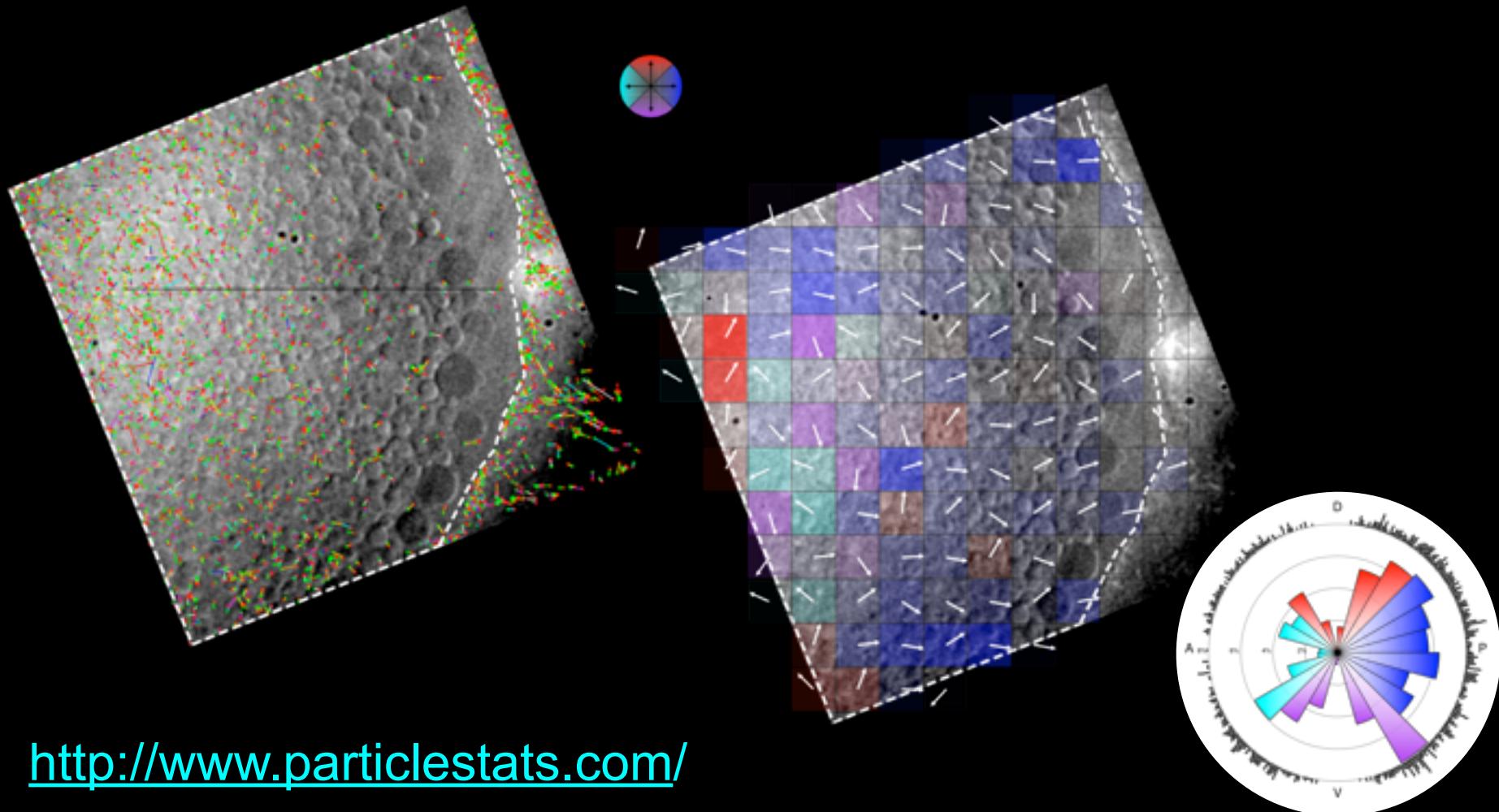


I. F. Sbalzarini and P. Koumoutsakos. Feature Point Tracking and Trajectory Analysis for Video Imaging in Cell Biology, *Journal of Structural Biology* 151(2):182-195, 2005.

3. Analysis & quantification

Analysis of directionality

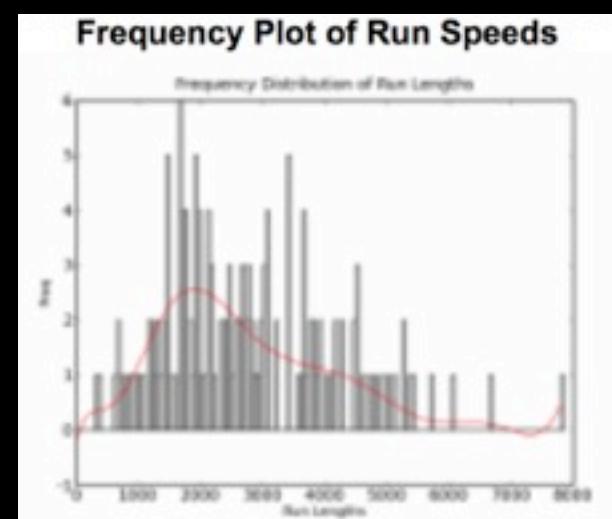
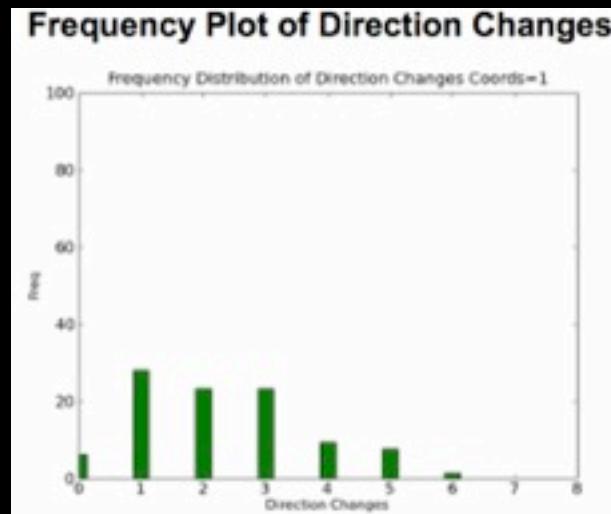
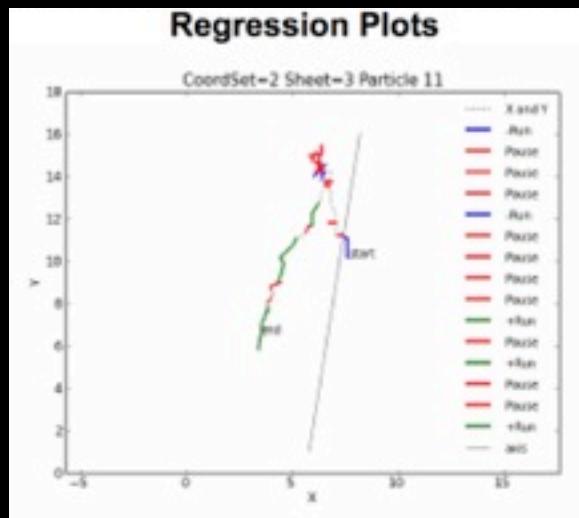
- using ParticleStats (Russell Hamilton)



<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:

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

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} :

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

$R_{i,coloc} = Ri$ 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:

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

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:-

Results												
Image	Rr	R	ch1:ch2	M1	M2	N+ve	Ntotal	ICQ	Ch1 Thresh	Ch2 Thresh		
Result_040.tif and Result_041.tif x 0 y0 z1 w480 h480	0.593	0.839	0.966	0.816	0.799	19029	23448	0.312	0:0	0:0		

Rr = Pearson's correlation coefficient

R = Mander's overlap coefficient

ratio of ch1:ch2 pixel intensity

Mander's colocalization coefficients (ch1&2)

Intensity Correlation Quotient

select ROI & threshold to exclude background

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

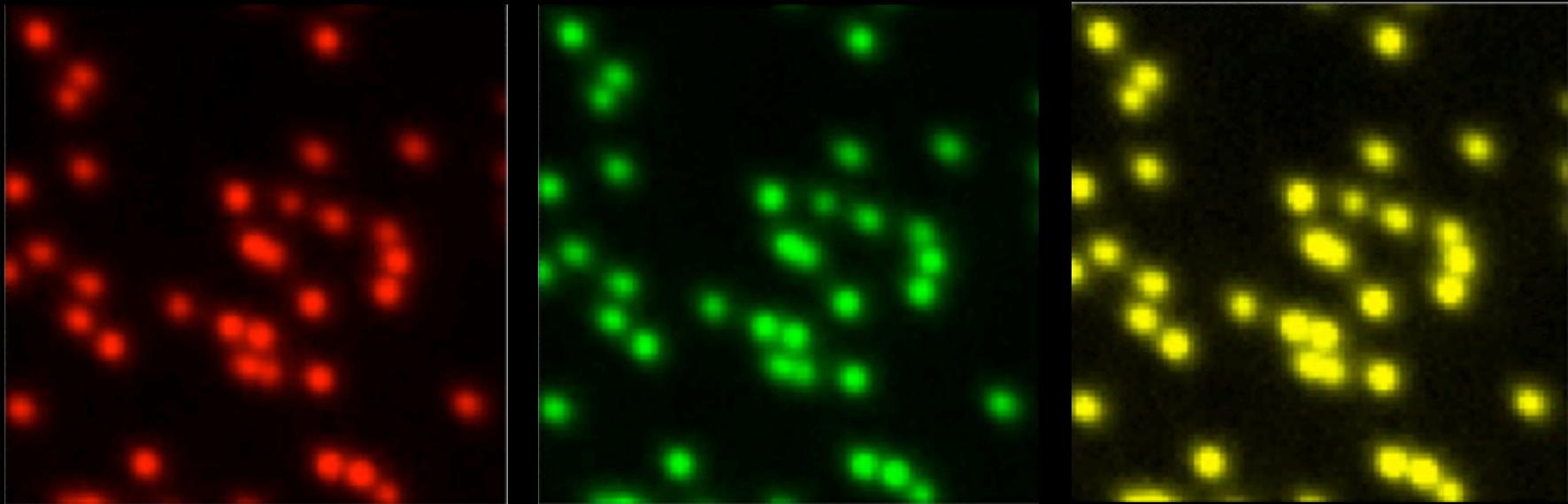
Blind experimental analysis



M. W.

3. Analysis & quantification

Colocalization



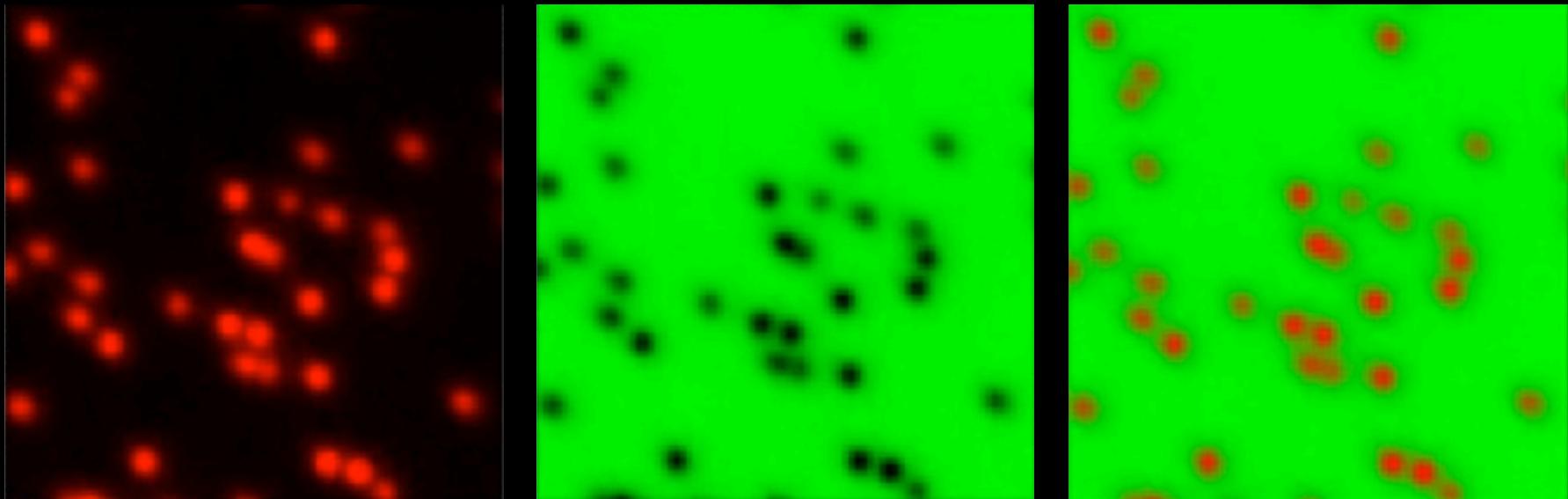
$$R_r = 1 ; R = 1 ; M_{red} = 1.0 ; M_{green} = 1.0 ; ICQ = 0.5$$

green dependent on red

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

3. Analysis & quantification

Colocalization



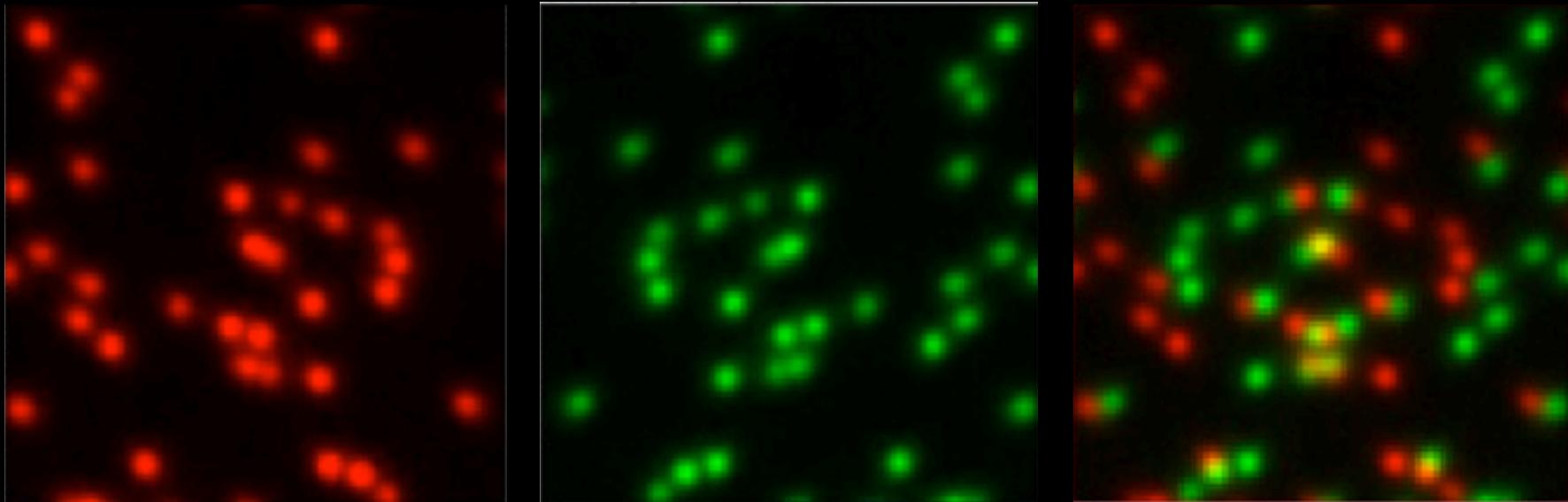
$$R_r = -1 ; R \cancel{= 0.62} ; ICQ = -0.5$$

green and red segregate

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

3. Analysis & quantification

Colocalization



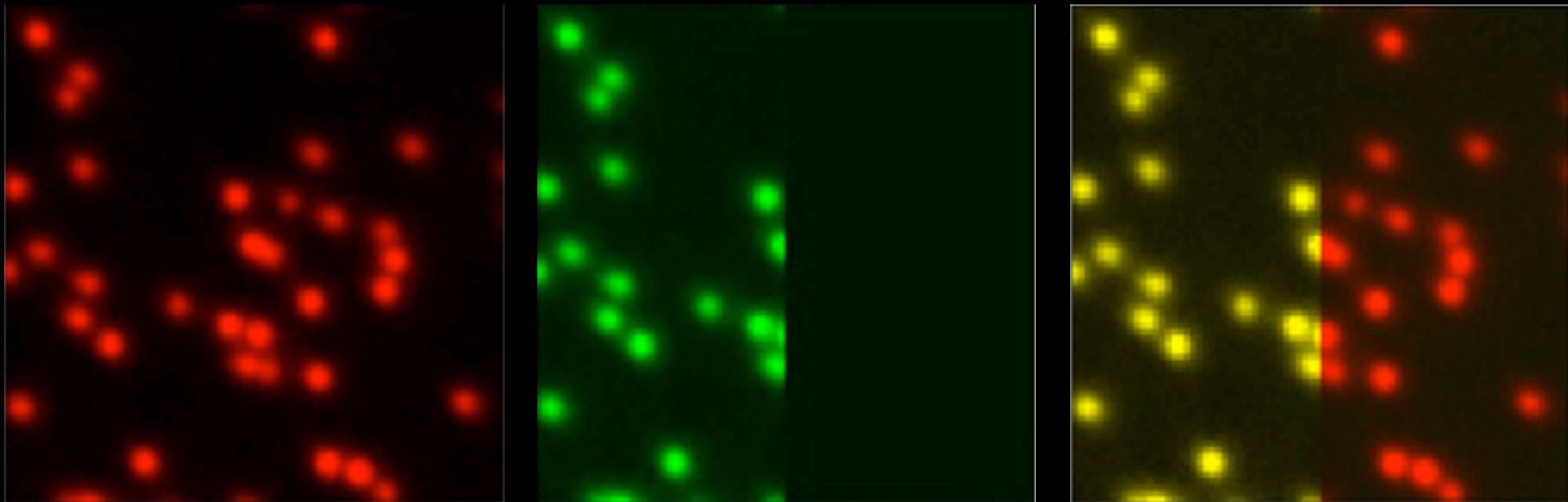
$R_r = 0.38$; $M_{red} = 0.15$; $M_{green} = 0.15$; $ICQ = 0.18$

green and red are unrelated

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

3. Analysis & quantification

Colocalization



$R_r = 0.62$; $M_{red} = 0.5$; $M_{green} = 1.0$; $ICQ = 0.30$

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>