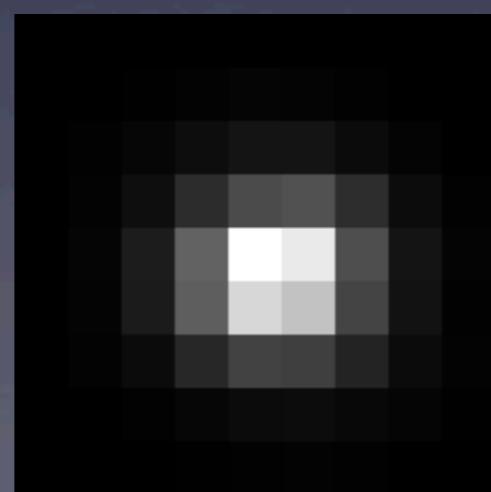


Image Analysis

Nico Stuurman and Kurt Thorn
UCSF Microscopy Course
3/30/2012

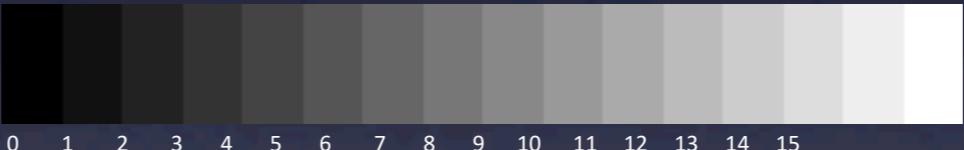
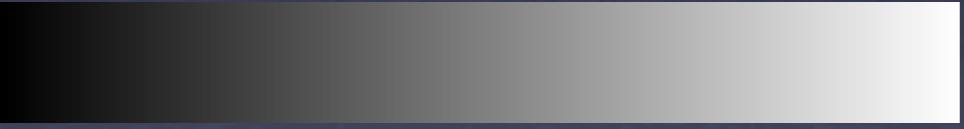
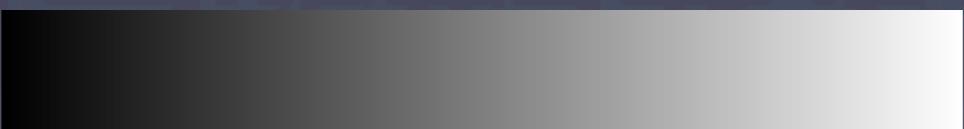
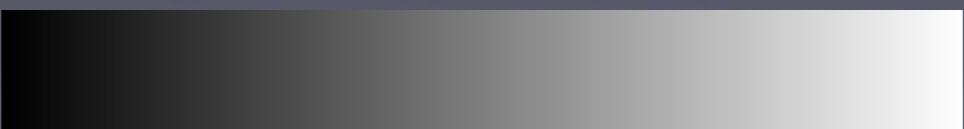
What is a digital Image?

Many measurements of photon flux



0	0	0	0	0	0	0	0	0	0
0	0	1	3	5	4	2	0	0	0
0	2	6	13	20	20	11	4	0	0
0	3	14	44	75	81	45	12	2	2
0	5	28	98	255	234	78	20	4	4
0	4	27	94	215	194	68	18	2	2
0	3	11	39	66	63	35	11	3	3
0	0	2	6	11	12	8	5	1	1
0	0	0	1	2	3	2	0	0	0

Bit depth and dynamic range

Nr. bits	range		
1	2		Binary Image
2	4		Grayscale
4	16		Images
8	256		1-byte
12	4096		2-bytes
16	65536		

Bit-depth and resolution



8-bit



4-bit



6-bit



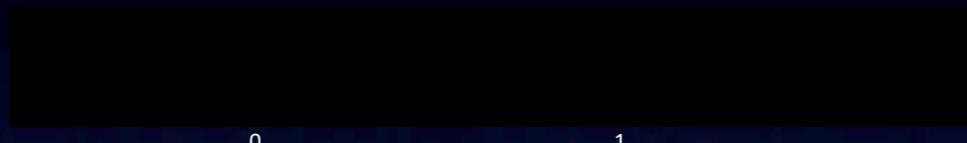
2-bit

Bit depth and dynamic range

Nr. bits range

1

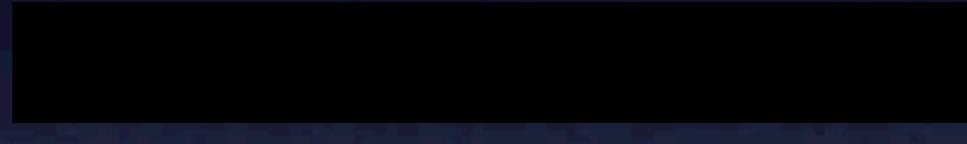
2



Binary Image

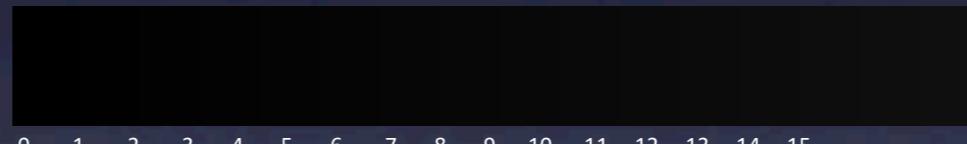
2

4



4

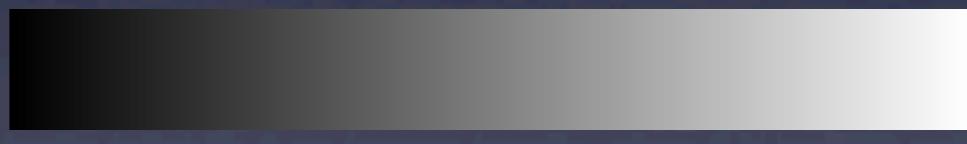
16



Grayscale
Images

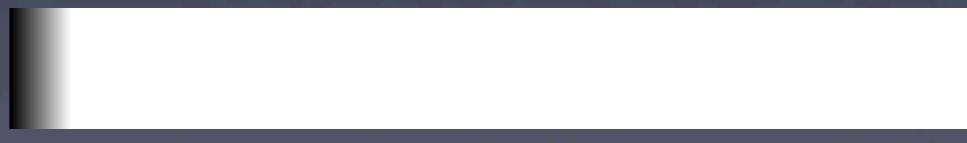
8

256



12

4096

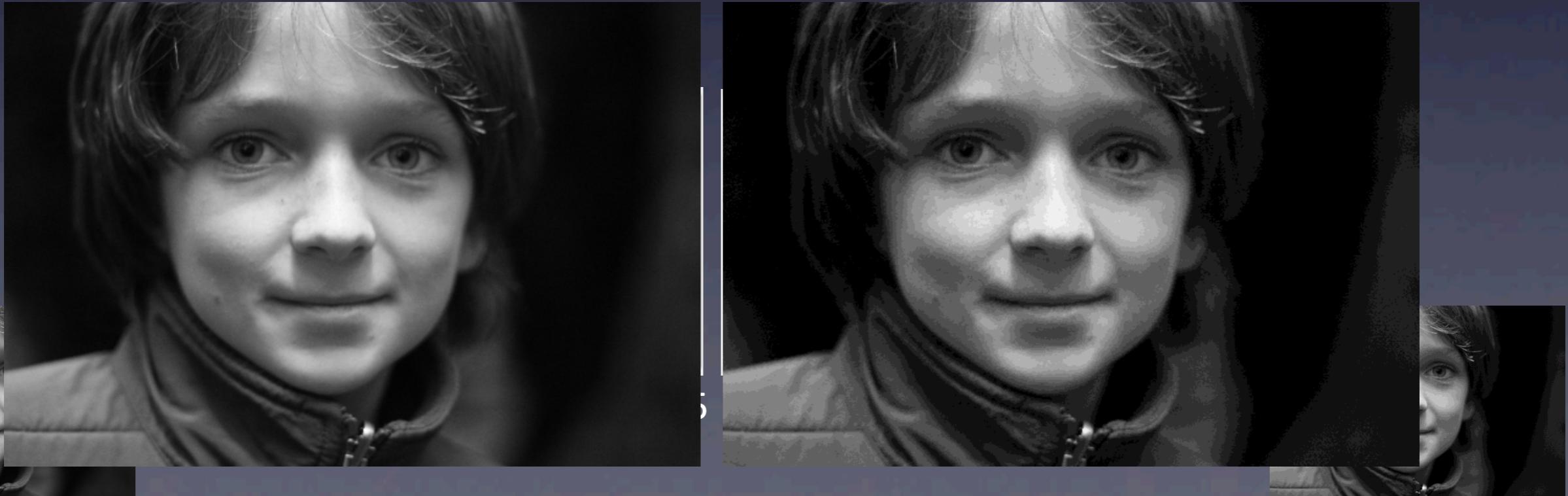
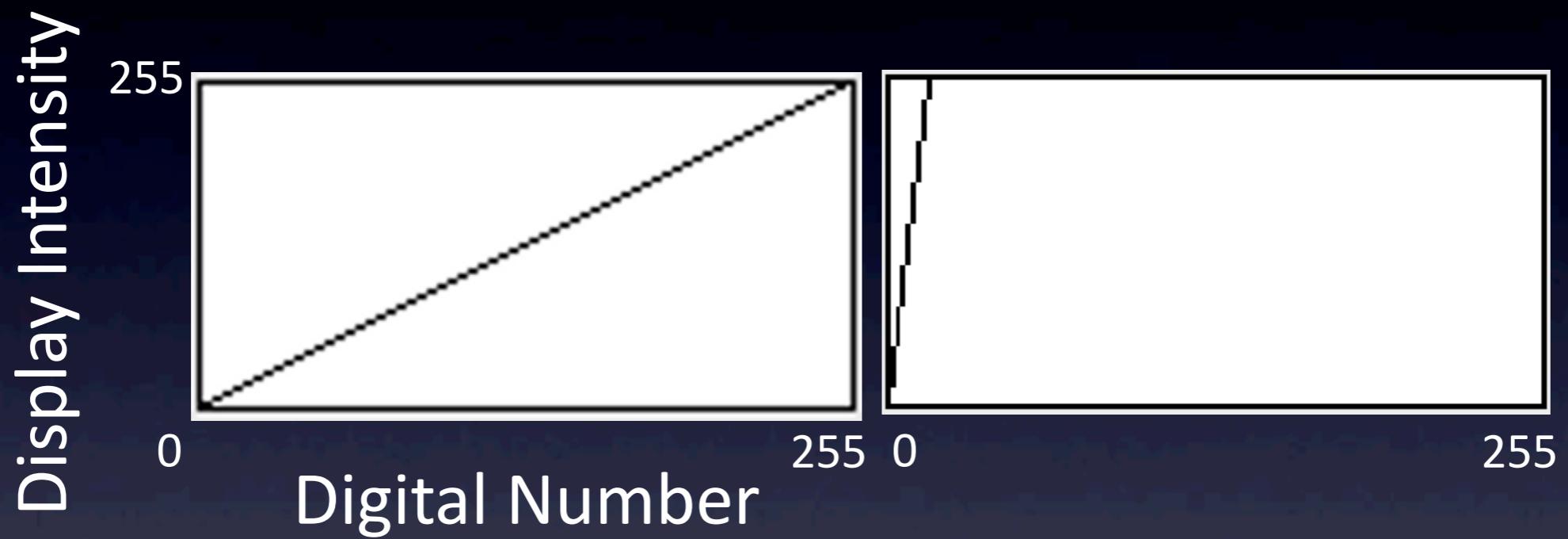


16

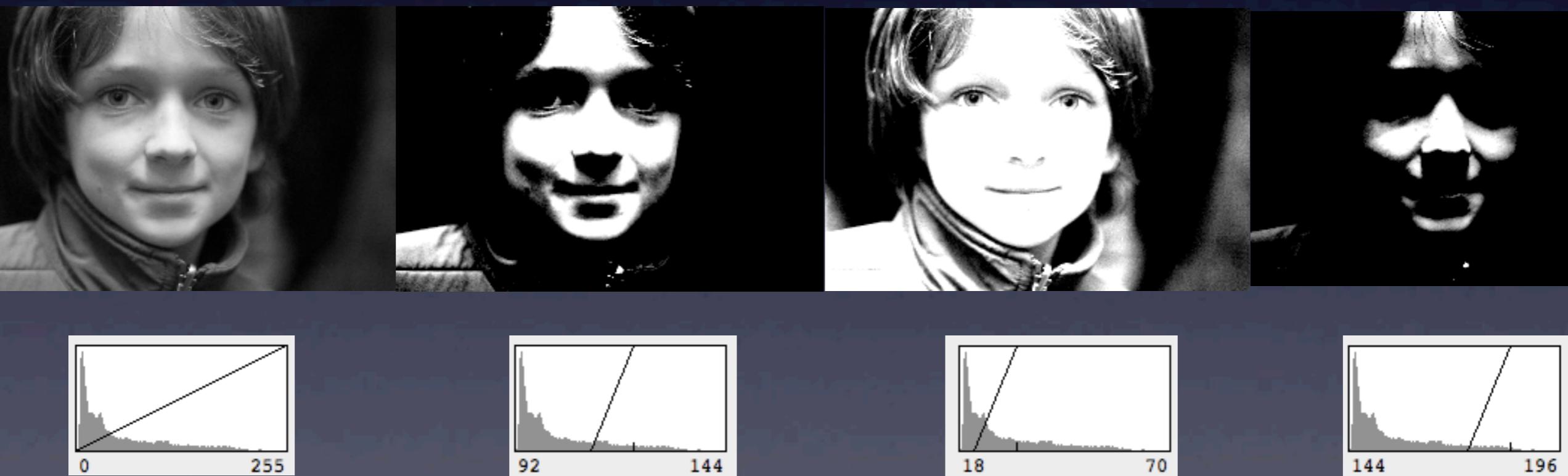
65536



Mapping values onto display



Mapping values onto display: Brightness/contrast



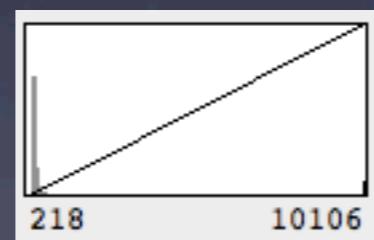
Slope = contrast

Brightness

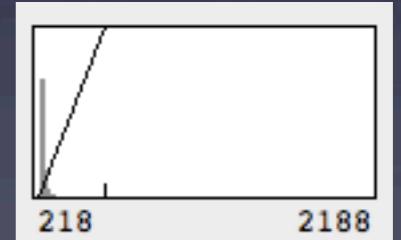
Brightness/contrast



Full Range



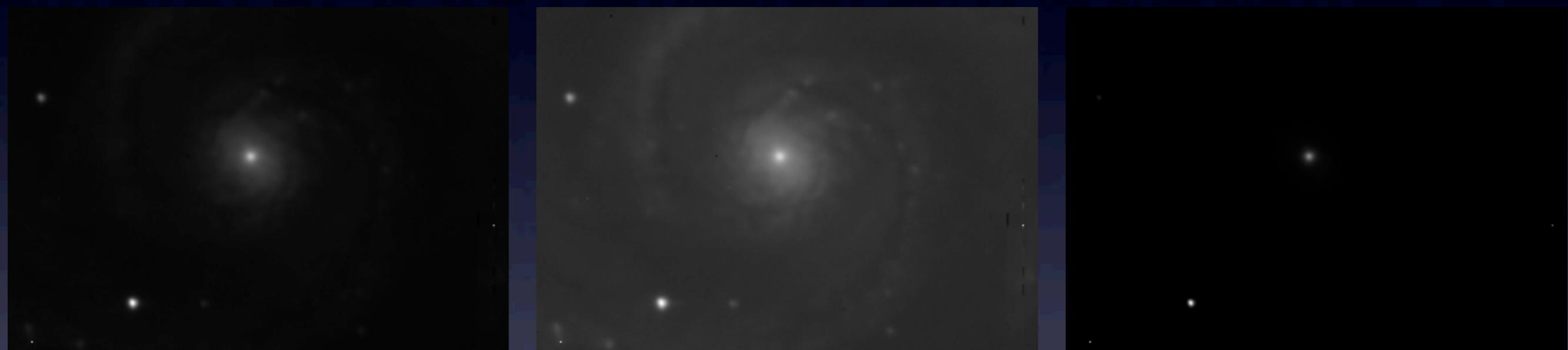
Auto-Scale



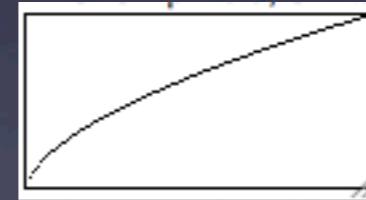
High B/C

Be aware of software Auto-scaling for you!

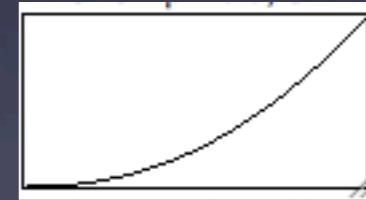
Gamma adjustment



1



0.6



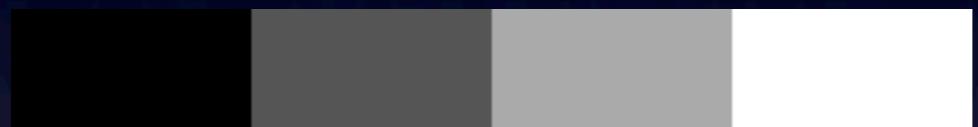
2.2

What are acceptable image manipulations?

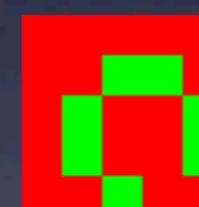
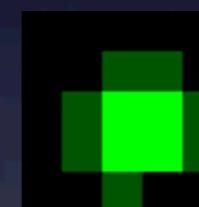
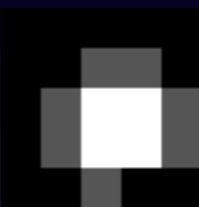
- JCB has the best guidelines
 - <http://jcb.rupress.org/content/172/1/9.full>
 - <http://jcb.rupress.org/content/166/1/11.full>
- Brightness and contrast adjustments ok, so long as done over whole image and don't obscure or eliminate background
- Nonlinear adjustments (like gamma) must be disclosed
- Controls should be treated the same as experimental

Lookup Tables (LUTs)

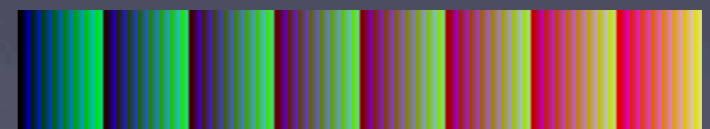
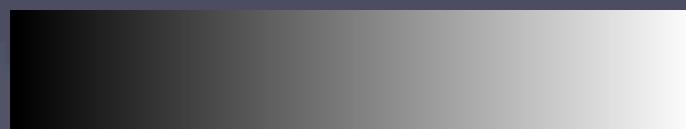
0 1 2 3



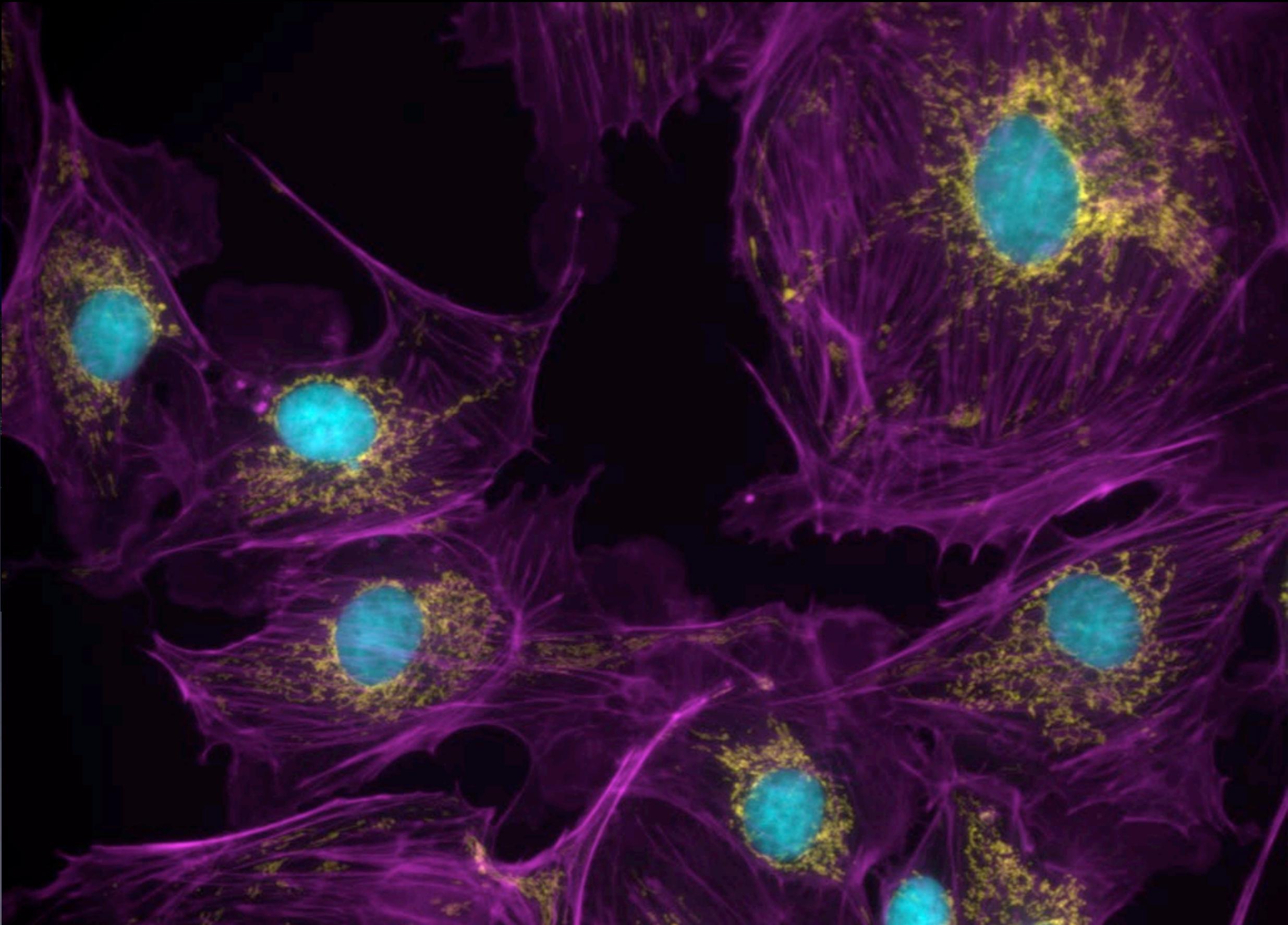
0	0	0	0	0
0	0	1	1	0
0	1	3	3	1
0	1	3	3	1
0	0	1	0	0



Lookup Tables (LUTs)



Lookup Tables (LUTs)



Color Images



Either:

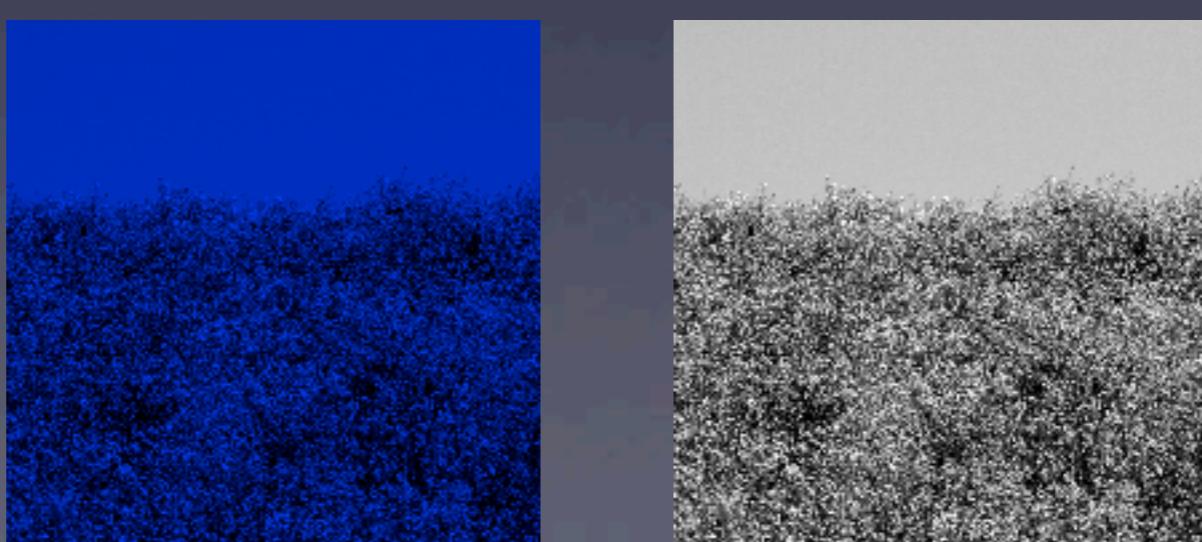
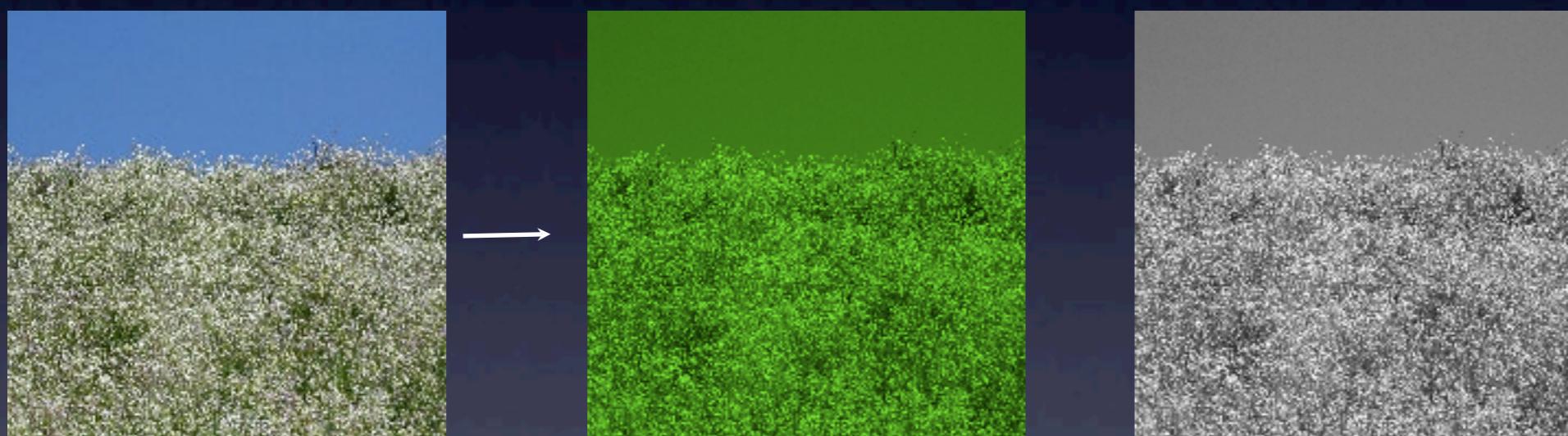
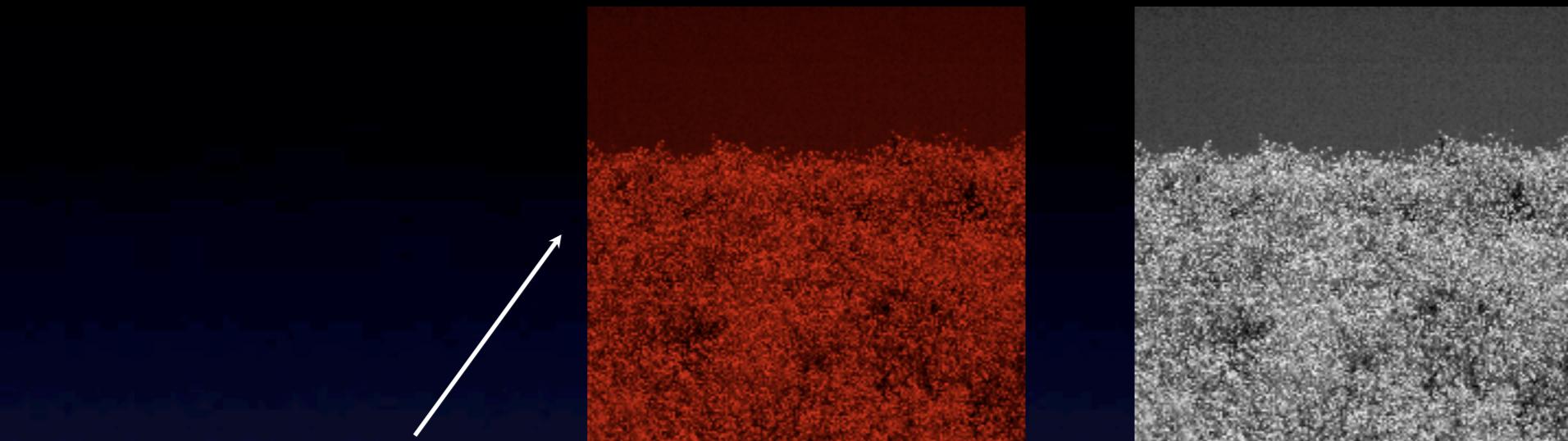
255 209 139	0 89 93	93 255 231	255 0 0
0 0 0	255 0 0	134 0 185	93 90 0
0 39 185	0 255 255	214 255 0	137 0 255
93 90 0	255 0 0	0 0 0	249 185 255

Or:

255	0	93	255
0	255	134	93
0	0	214	137
93	255	0	249

209	89	255	0
0	0	0	90
39	255	255	0
90	0	0	185

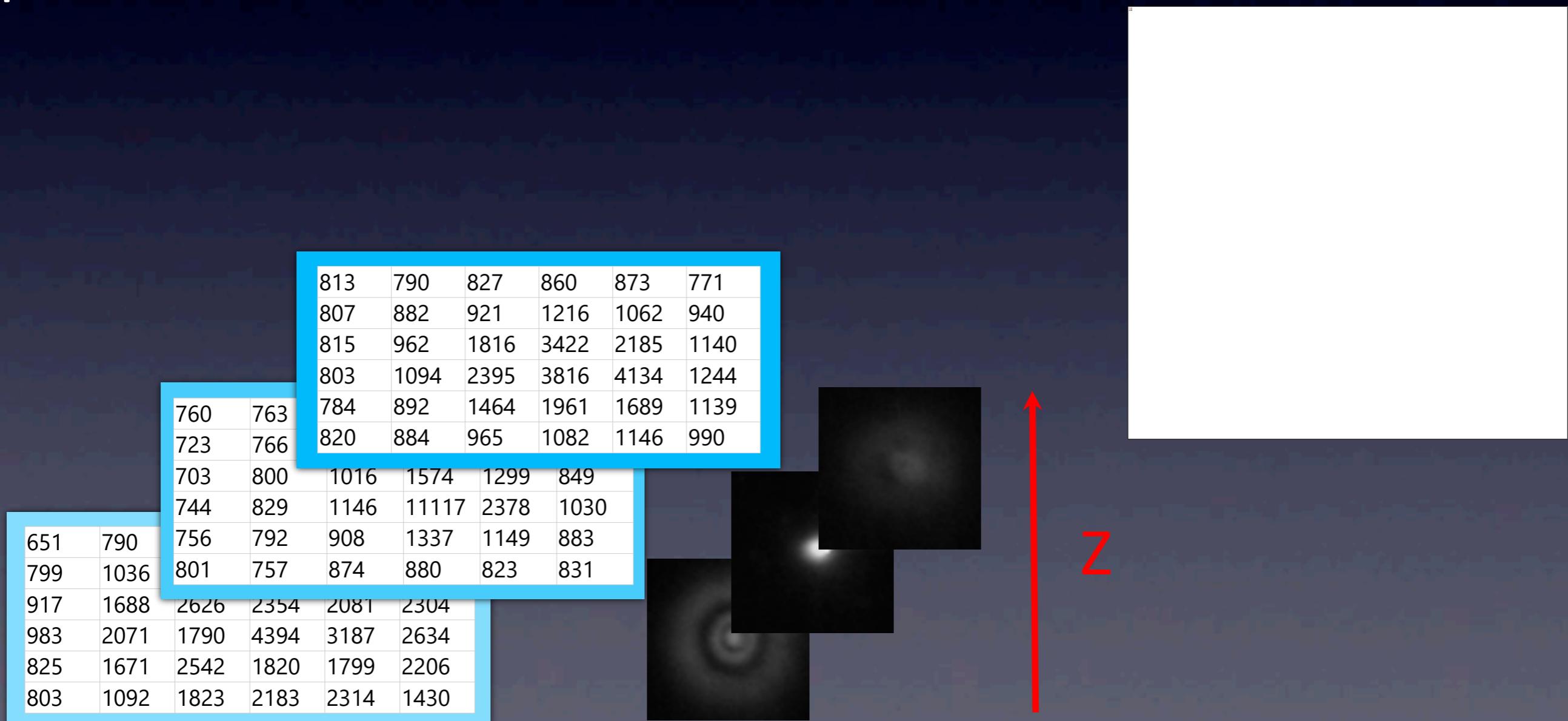
139	93	231	0
0	0	185	0
185	255	0	255
0	0	0	255



Stacks:

Sequences of images

Can represent time series(movies), z-positions, or other variables



File Formats

Data sets can be big:

$$1392 \times 1040 \times 2 = 2.8\text{MB}$$

3-channels, 15 image z-stack, 200 time points:

$$2.8 * 3 * 15 * 200 = 25.2\text{GB}$$

Compression

Original data can be restored

Loose original data!

Losless versus Lossy

None (raw)

Run-length encoding

Dictionary approaches, etc..

Discards data not
essential for visual
appearance

File Formats

Desired:

- Widely used
- No compression (or lossless)
- Works with 16-bit

There are many!

OME-TIFF

The swiss pocket knife for microscopy image data format:
Bioformats: <http://www.loci.wisc.edu/software/bio-formats>

Often good:

- Tiff: Container format, supports 16-bit and no compression, stacks

Often useful:

- ics/ids, JPEG2000, nd2, zvi, lsm: Less widely used/proprietary

Sometimes useful:

- JPEG (bad!), GIF, Png, BMP (although no or lossless compression, 8-bit only)

Software Tools

Acquisition + Analysis

- NIS Elements
- AxioVision
- MetaMorph
- Zen
- Slidebook
- many more...

Micro-Manager

<http://micro-manager.org>

Presentation

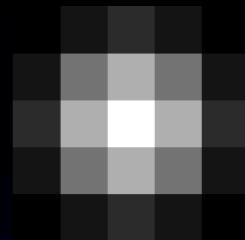
- Photoshop
- Gimp

Analysis

- Matlab
- IDL
- ImageJ (free, many plugins) <http://rsb.info.nih.gov/ij/>
- Imaris (3D visualization)
- Priism (Agard/Sedat labs)
<http://msg.ucsf.edu/IVE/>
- CellProfiler <http://cellprofiler.org>

Linear Filters

Neighborhood convolution



Kernel

1	1	1
1	1	1
1	1	1

Simple Smoothing



0	1	2	1	0
1	6	10	6	1
2	10	16	10	2
1	6	10	6	1
0	1	2	1	0

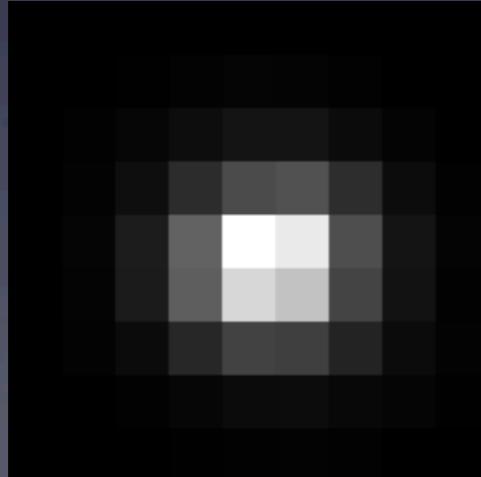
Gaussian Smoothing

0	1	113	1	255	0	0	0	0	0
0	113	1	1	255	0	0	0	0	0
0	113	1	1	1	255	0	0	0	0
0	113	0	0	0	255	0	0	0	0
113	0	0	0	0	255	0	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0

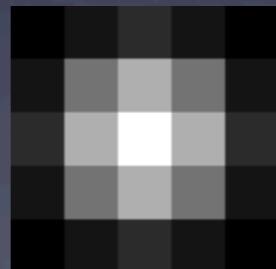
12	37	37	110	85	85	0	0	0	0
25	37	37	69	85	85	28	0	0	0
37	37	37	28	85	85	56	0	0	0
50	37	25	0	85	85	85	0	0	0
50	37	25	0	56	85	85	28	0	0
50	37	25	0	28	85	85	56	0	0
37	37	37	0	0	85	85	85	0	0
37	37	37	0	0	56	85	85	28	0
37	37	37	0	0	28	85	85	56	0
37	37	37	0	0	0	85	85	85	0

Why smooth?

- If your image is sampled appropriately (at Nyquist) the point spread function will be spread out over multiple pixels
- Properly exploiting this redundancy requires deconvolution
- But smoothing helps
- Also reduces single pixel noise artifacts that can't be real



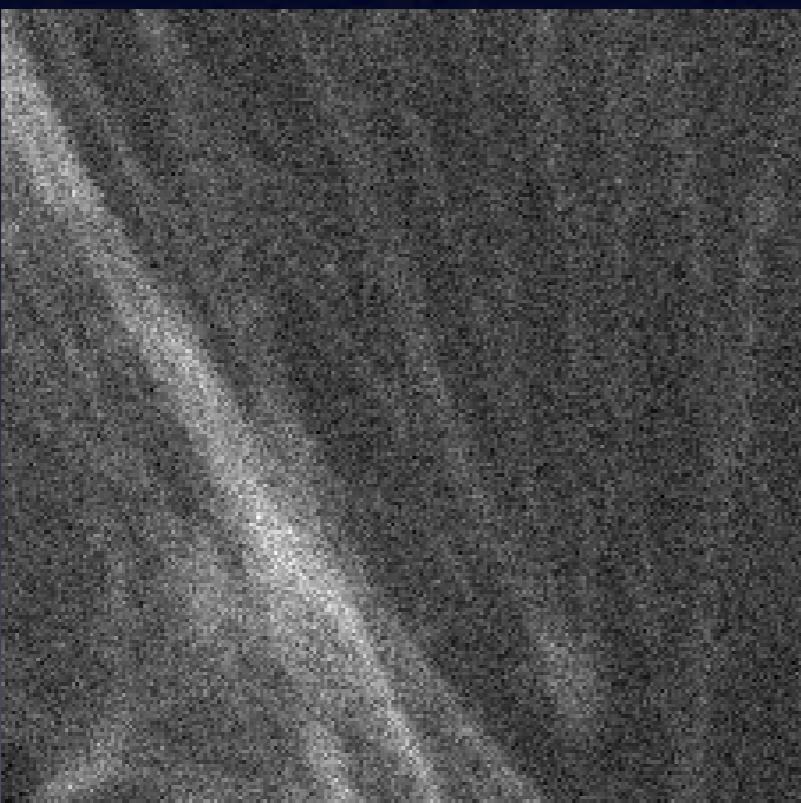
Measured PSF



Gaussian Filter

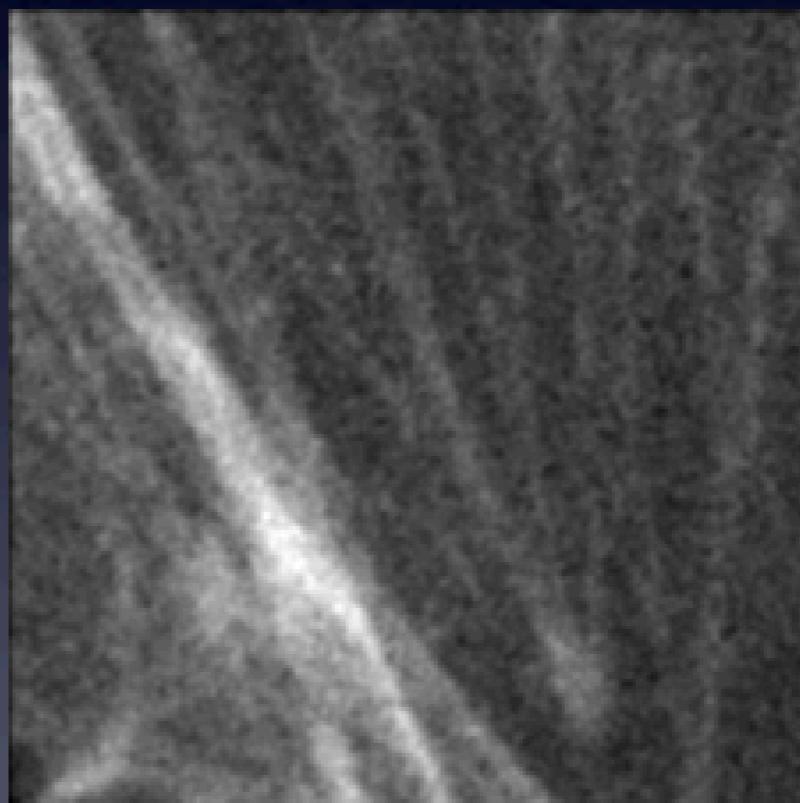
Why smooth?

Averages redundancy and suppresses noise



10 photons/pixel average

5 e- read noise



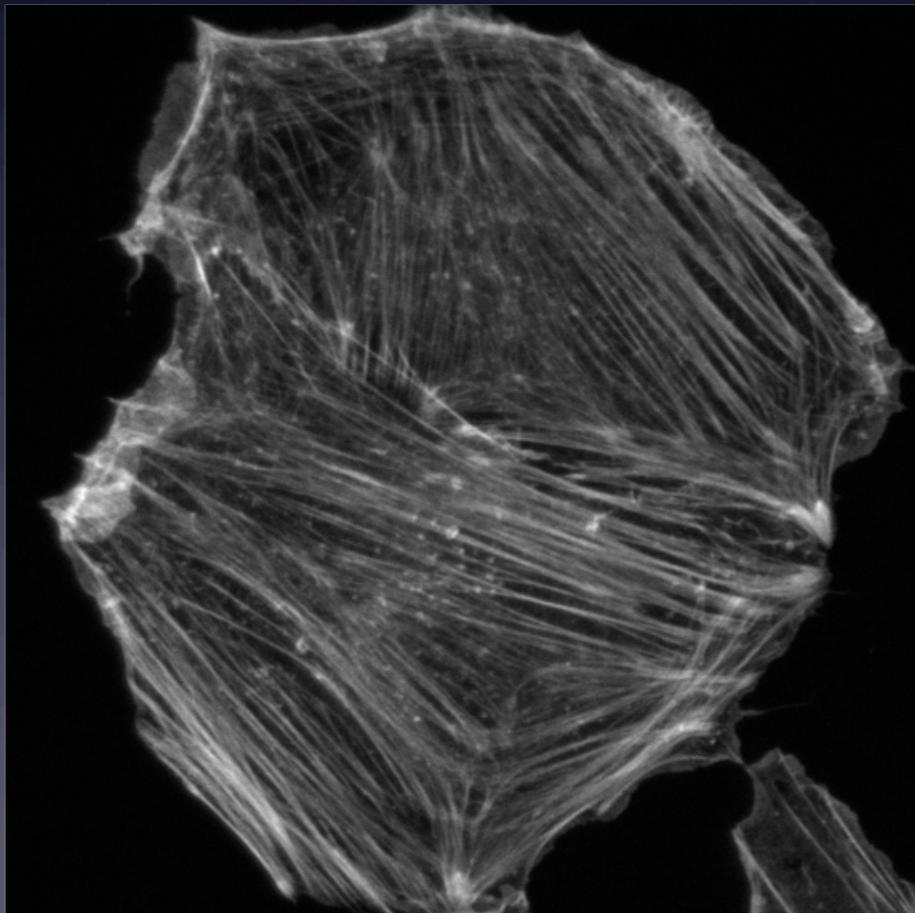
Gaussian smoothing filter,
 $\sigma = 1$ pixel

Other Filters

Edge Detection

1	1	1
0	0	0
-1	-1	-1

1	2	1
0	0	0
-1	-2	-1



Original



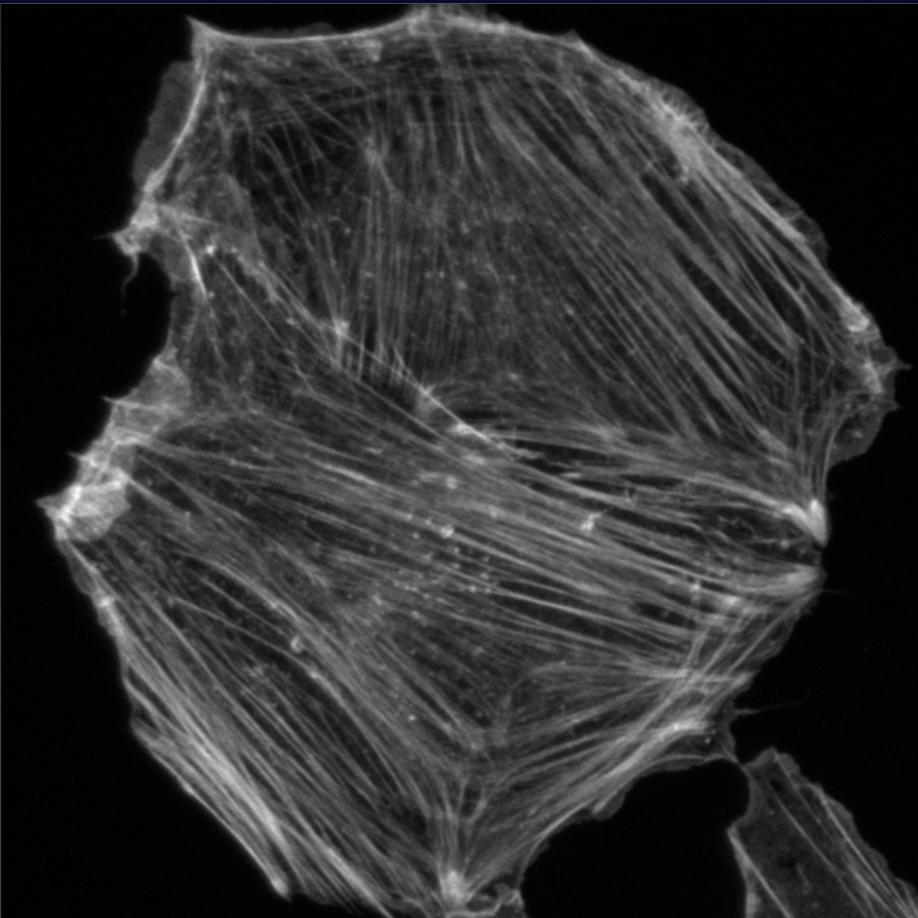
Horizontal edge detection

Other Filters

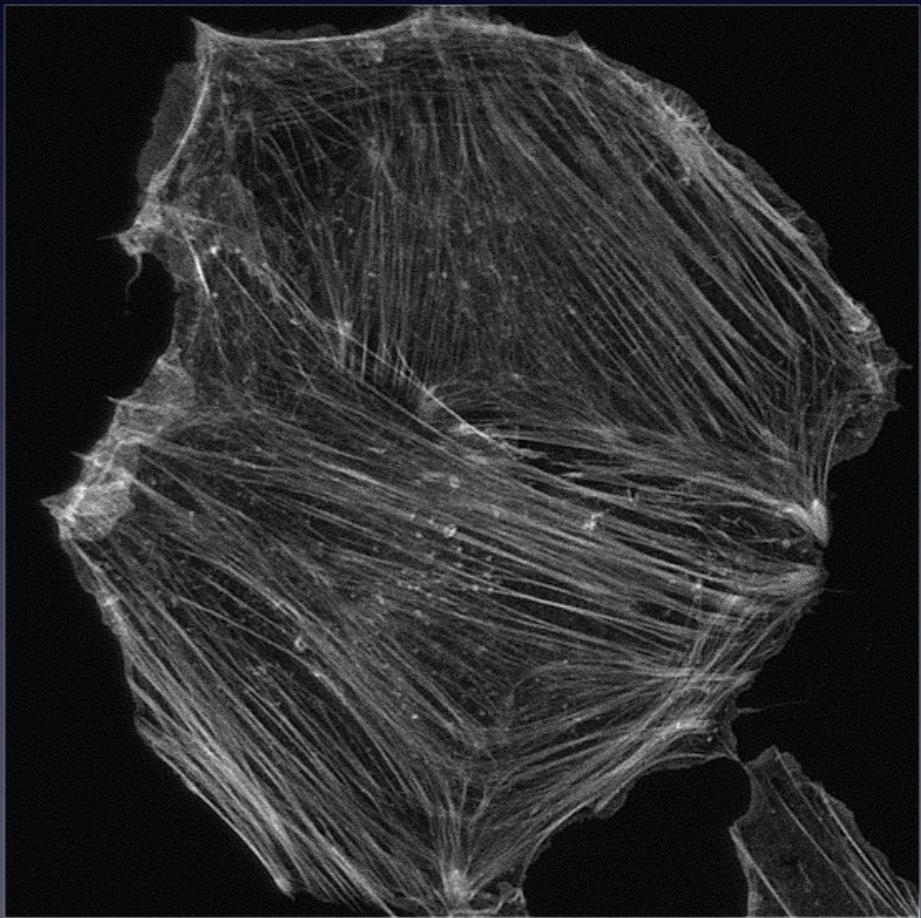
Unsharp Masking

-1	-4	-1
-4	26	-4
-1	-4	-1

(Laplacian)



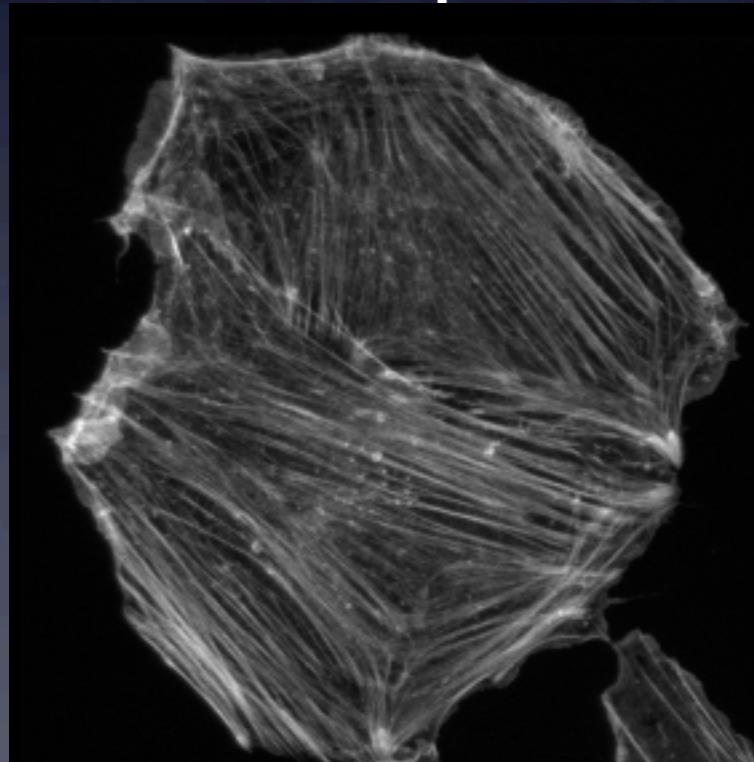
Original



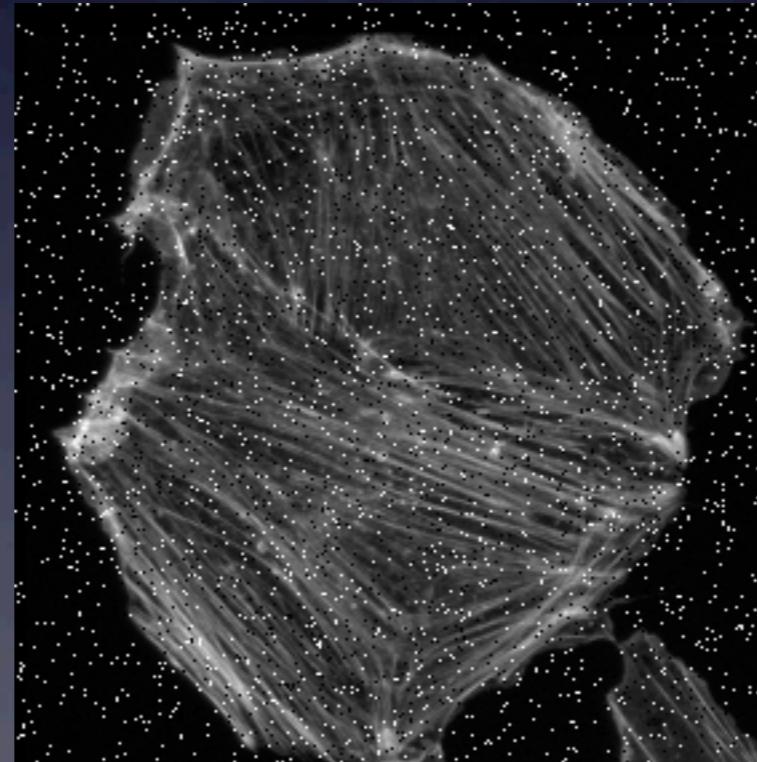
Unsharp masked

Non-linear Filters

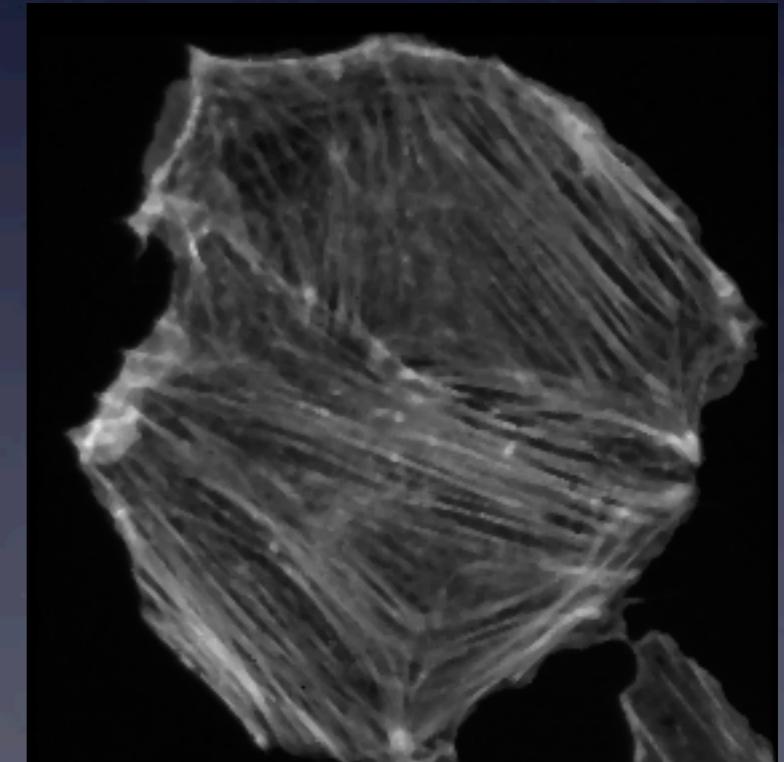
- Replace central pixel with min, max, median
- Median filter is a good noise filter, at the expense of resolution



Original



Artificial Noise

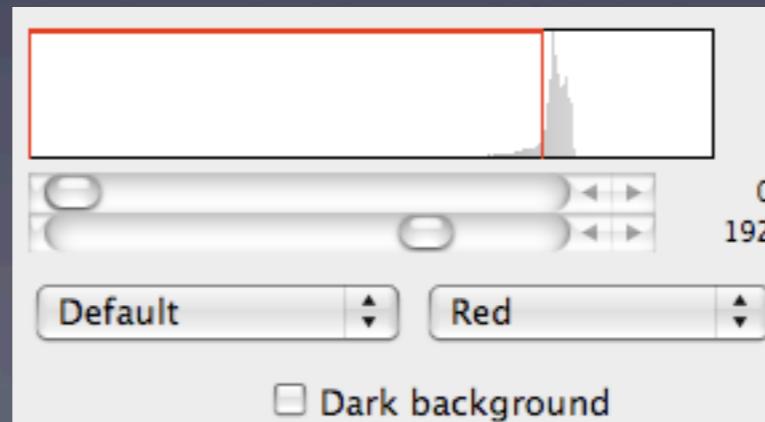
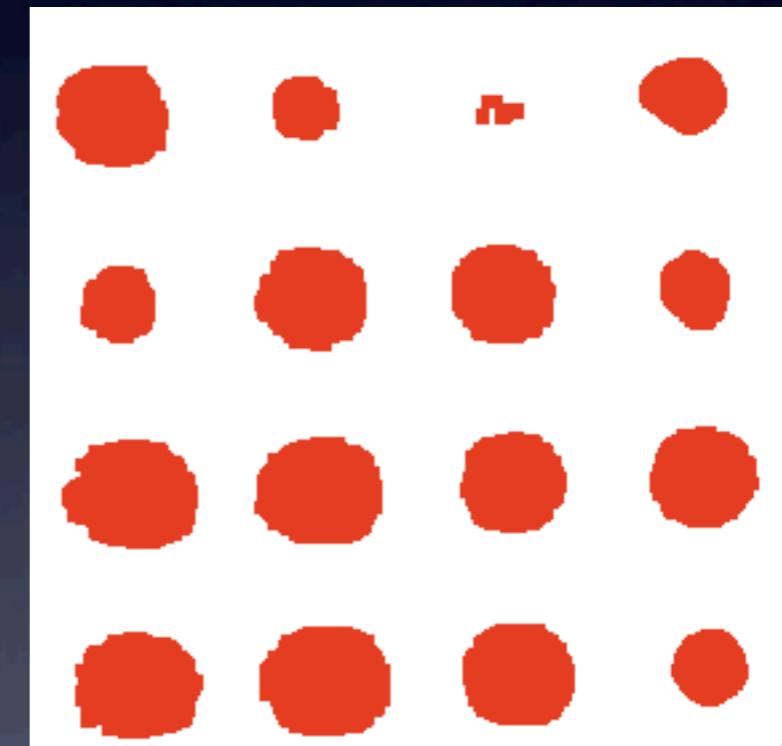
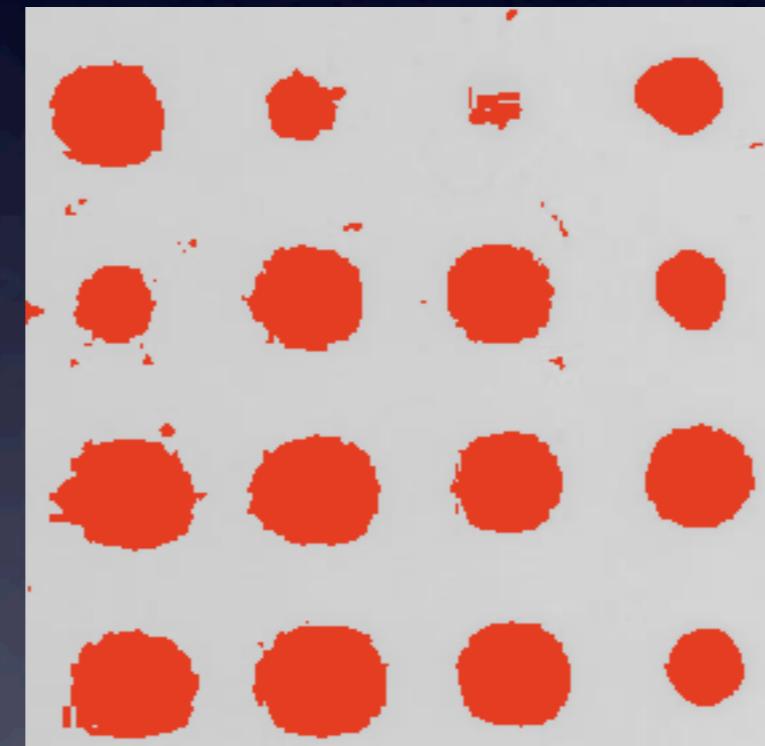
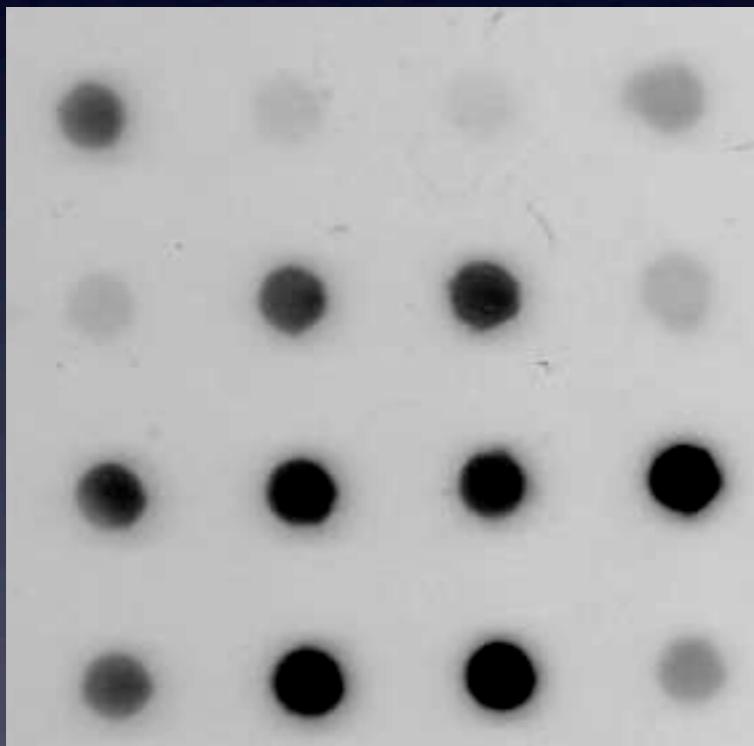


Median Filter

Segmentation

How to define the object that you want to measure?

Technique: make a binary image (mask) where your object=1 and background=0

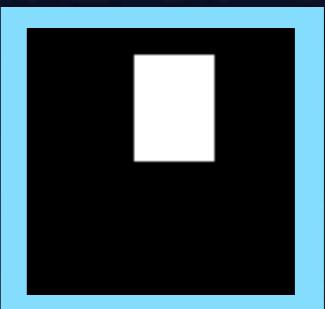
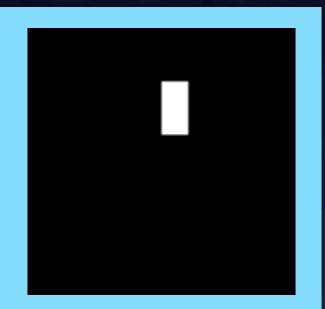
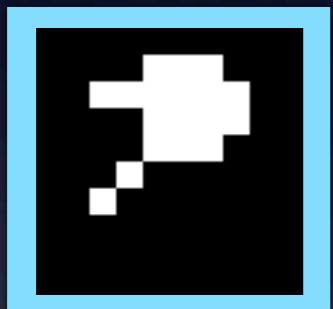


2x erosion
2x dilation

Binary Operations: Erosion/Dilation

Structuring Element:

1	1	1
1	1	1
1	1	1



0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	0	0	0
0	0	1	1	1	1	1	1	0	0
0	0	0	0	1	1	1	1	1	0
0	0	0	0	1	1	1	1	0	0
0	0	0	0	1	1	1	0	0	0
0	0	0	1	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0



0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

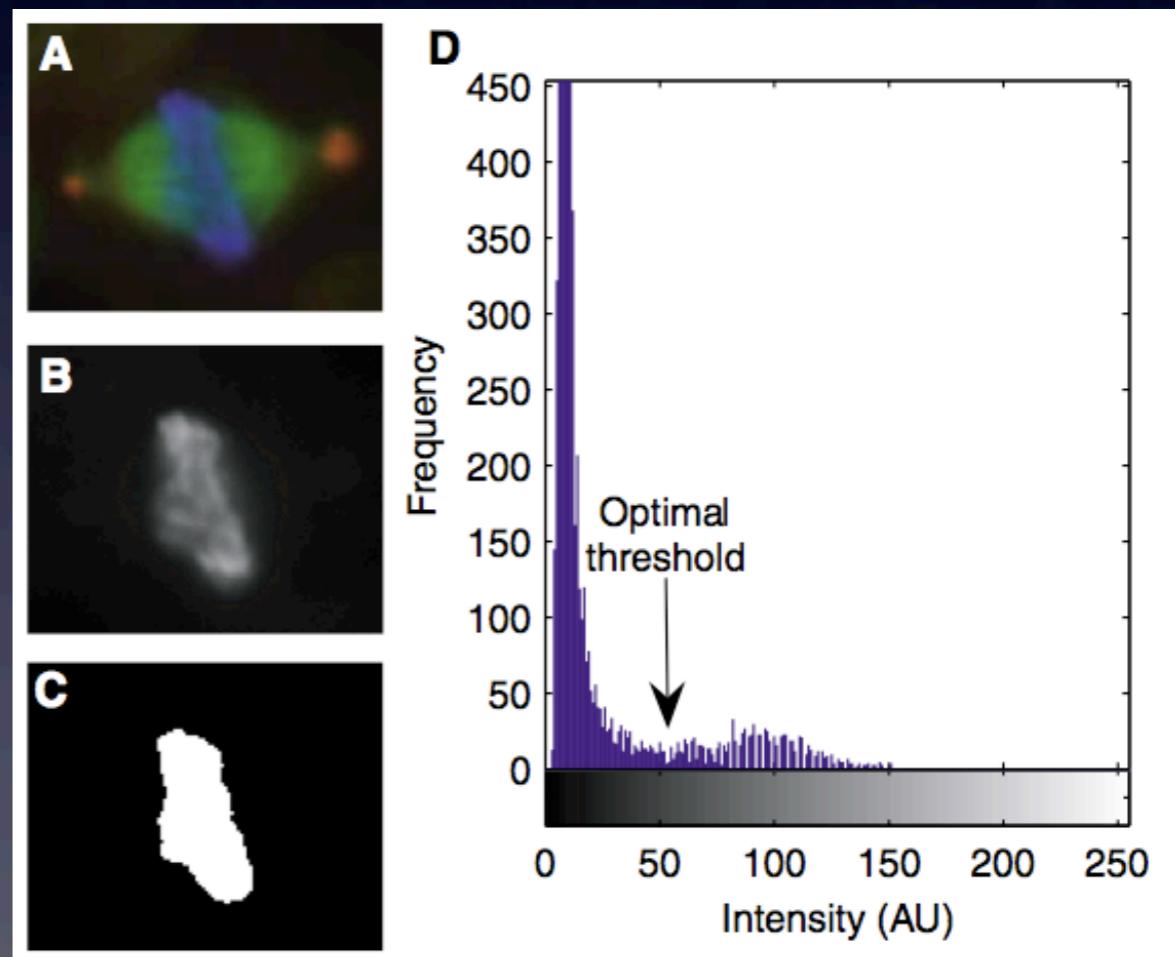
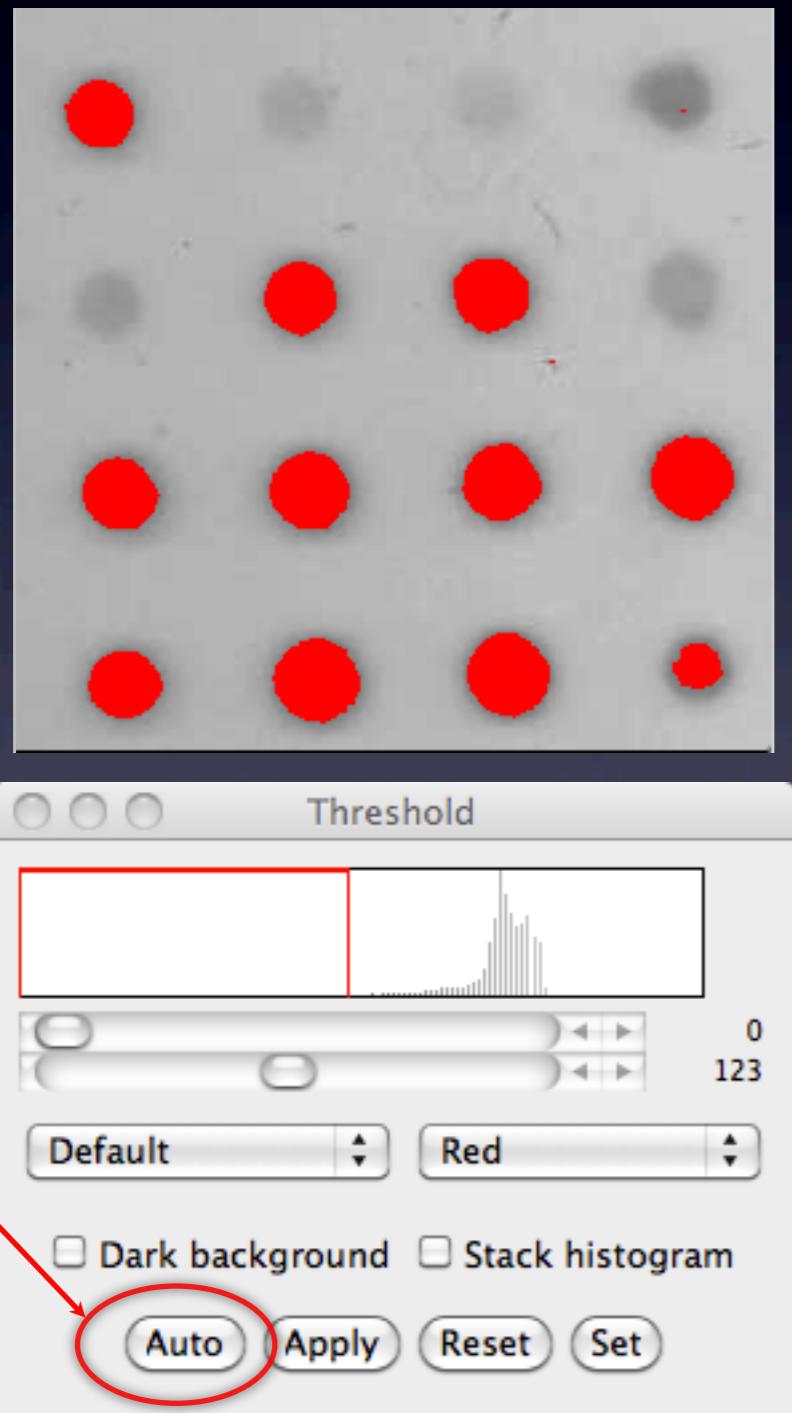


0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	1	1	0	0
0	0	0	0	0	1	1	1	0	0
0	0	0	0	0	1	1	1	0	0
0	0	0	0	0	1	1	1	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Erosion

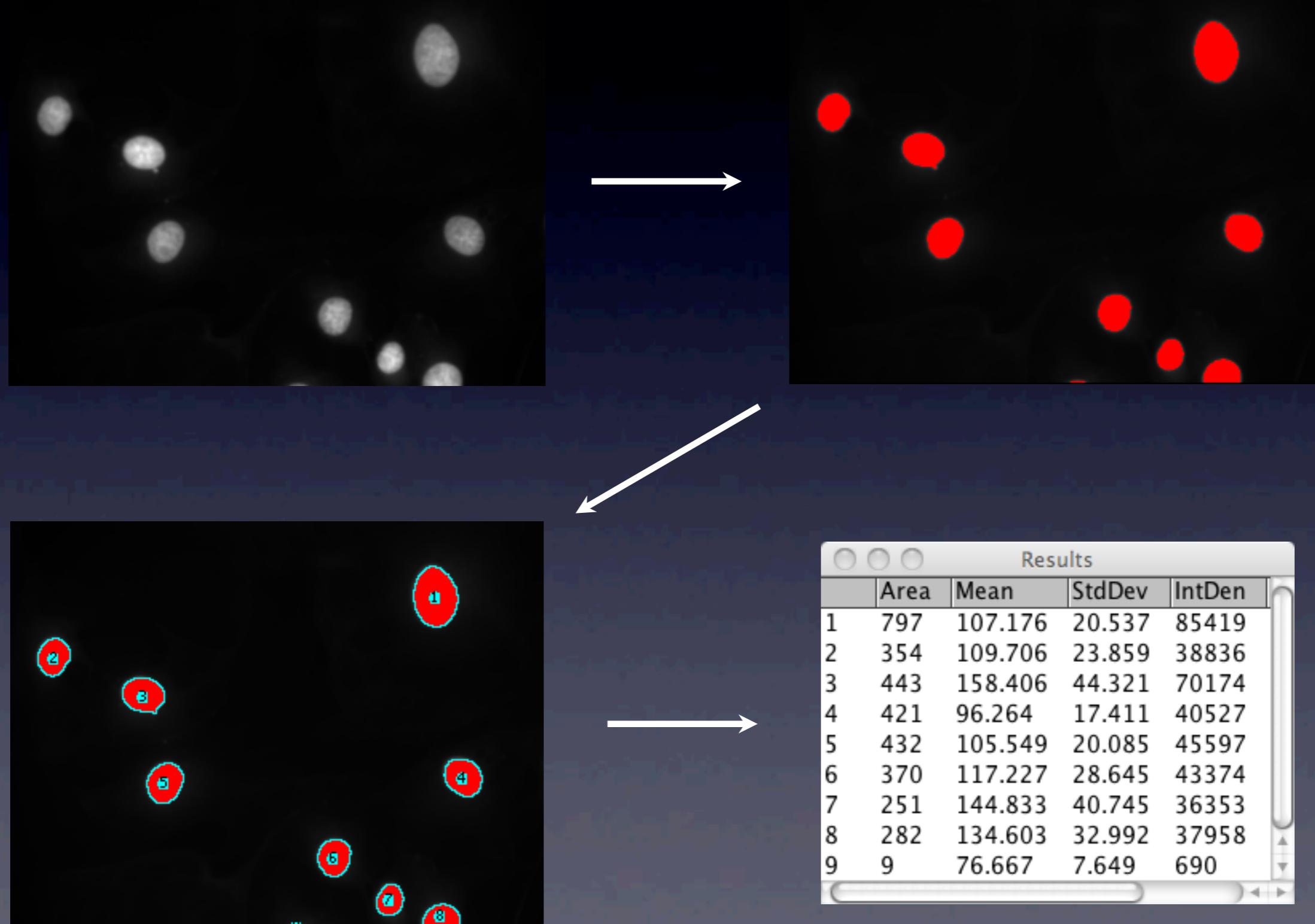
Dilation

Thresholding, where to set the cutoff?



Automatic segmentation using Otsu's method

Measure objects



Acknowledgements/Reference

S

Kurt Thorn

John C. Russ, The Image Processing Handbook

Gonzalez, Woods and Eddins, Digital Image Processing
using Matlab

Burger and Burge, Digital Image Processing, An
Algorithmic Introduction using Java (ImageJ)

High-throughput Imaging

Example: Whole genome RNAi screen in Drosophila S2 cells
for genes involved in mitotic spindle assembly



What are the molecules and molecular interactions that
build the metaphase spindle?

Whole Genome RNAi Screen in Drosophila S2 Cells for Mitotic Spindle Assembly

Ron Vale



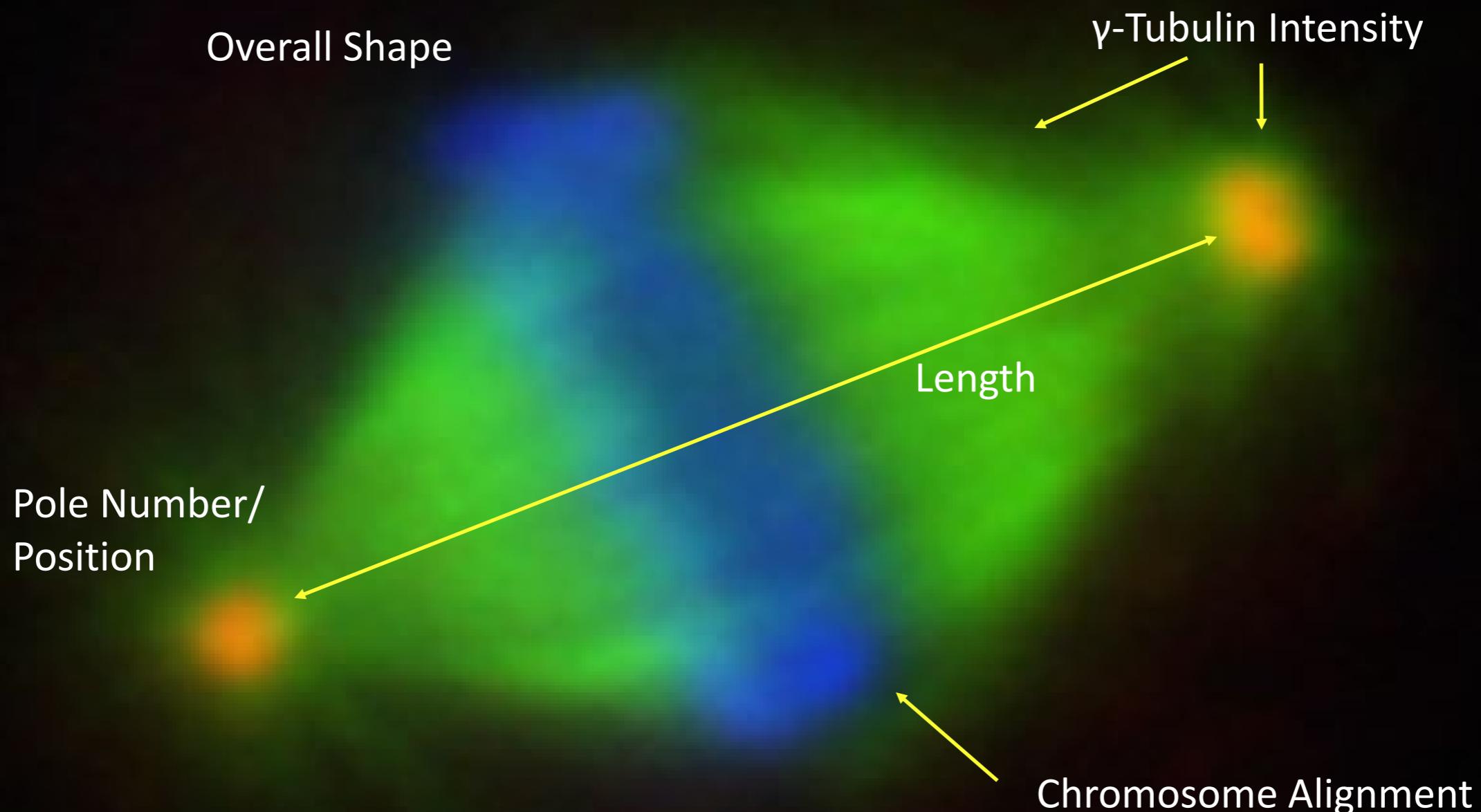
Gohta Goshima, Nico Stuurman, Nan Zhang, Sarah Goodwin (UCSF)

Roy Wollman,
Jon Scholey (UC Davis)



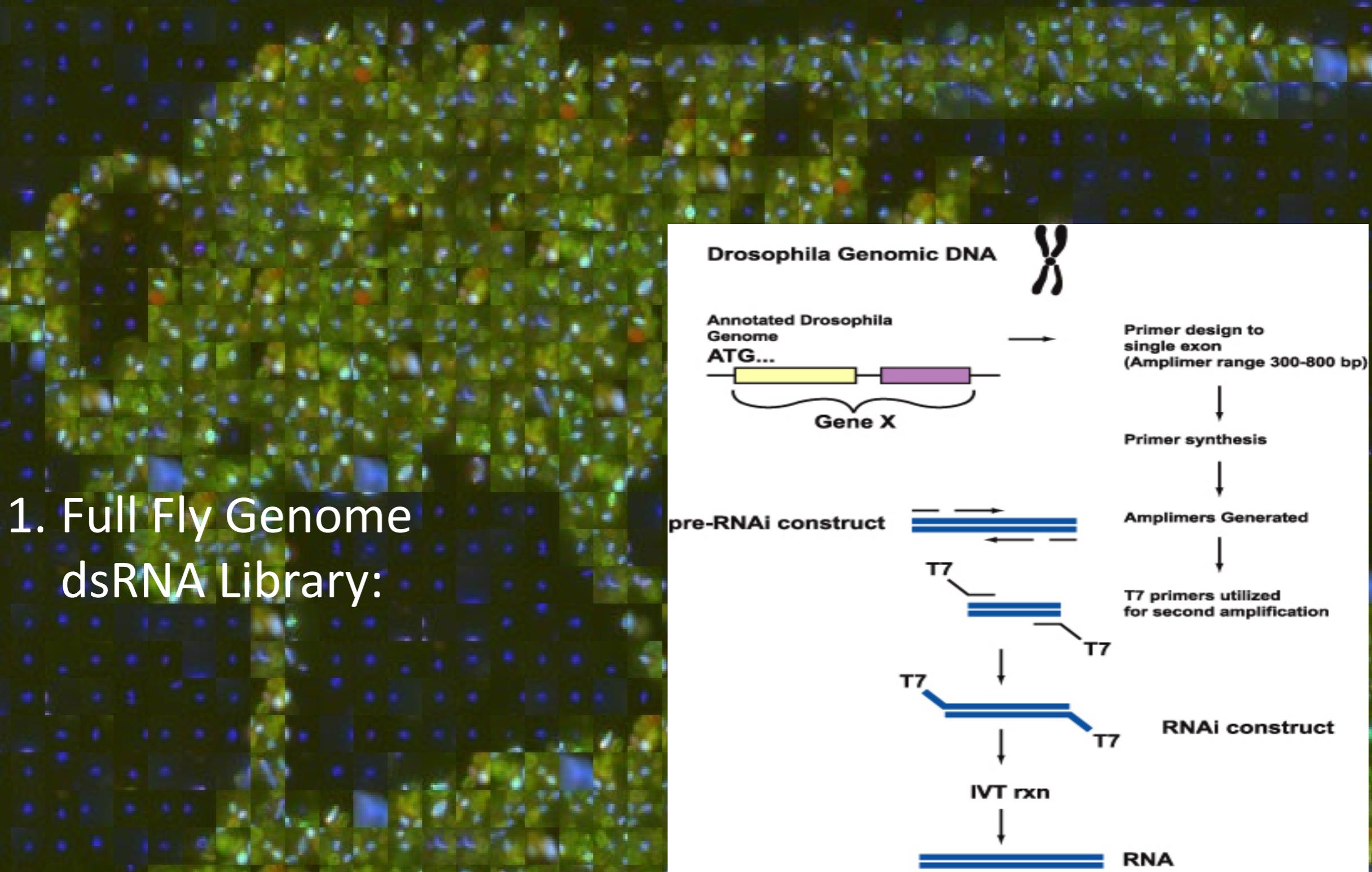
Goshima et al. Science, 316 (2007)
Wollman and Stuurman. J. Cell Sc. 120 3715 (2007)

Image-Based Approach for Identifying Spindle Defects Generated by RNAi



14,400 Genes and
4,000,000 Spindles Analyzed in this Screen

High-throughput RNAi Screen



High-throughput RNAi Screen

2. Treat S2 Cells with dsRNA for 4 days

96-well, plastic dish x 146
(each well has dsRNA for one gene)



+ APC dsRNA to
induce metaphase arrest

High-throughput RNAi Screen

3. High-throughput Microscopy

96-well, glass-bottom dish
for 40X, 0.95 NA imaging

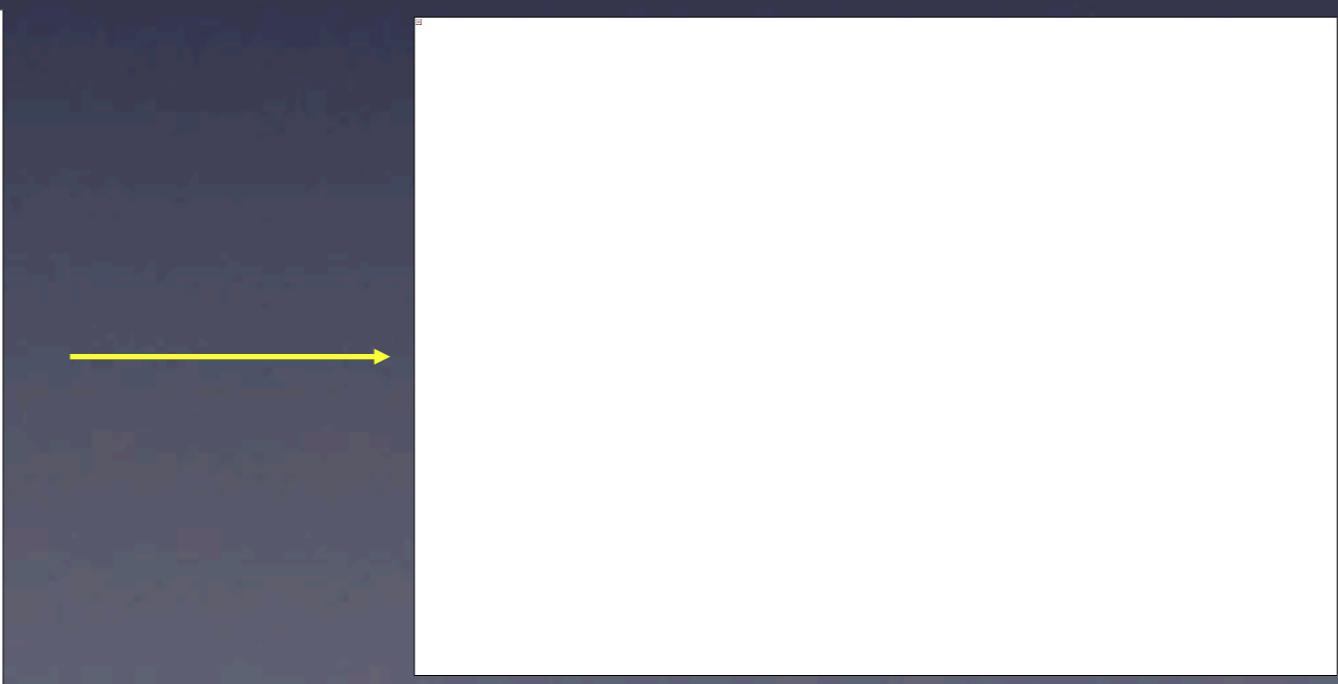


Fix & Stain
and Image



Autofocus

- Image-based
- Reflection-based



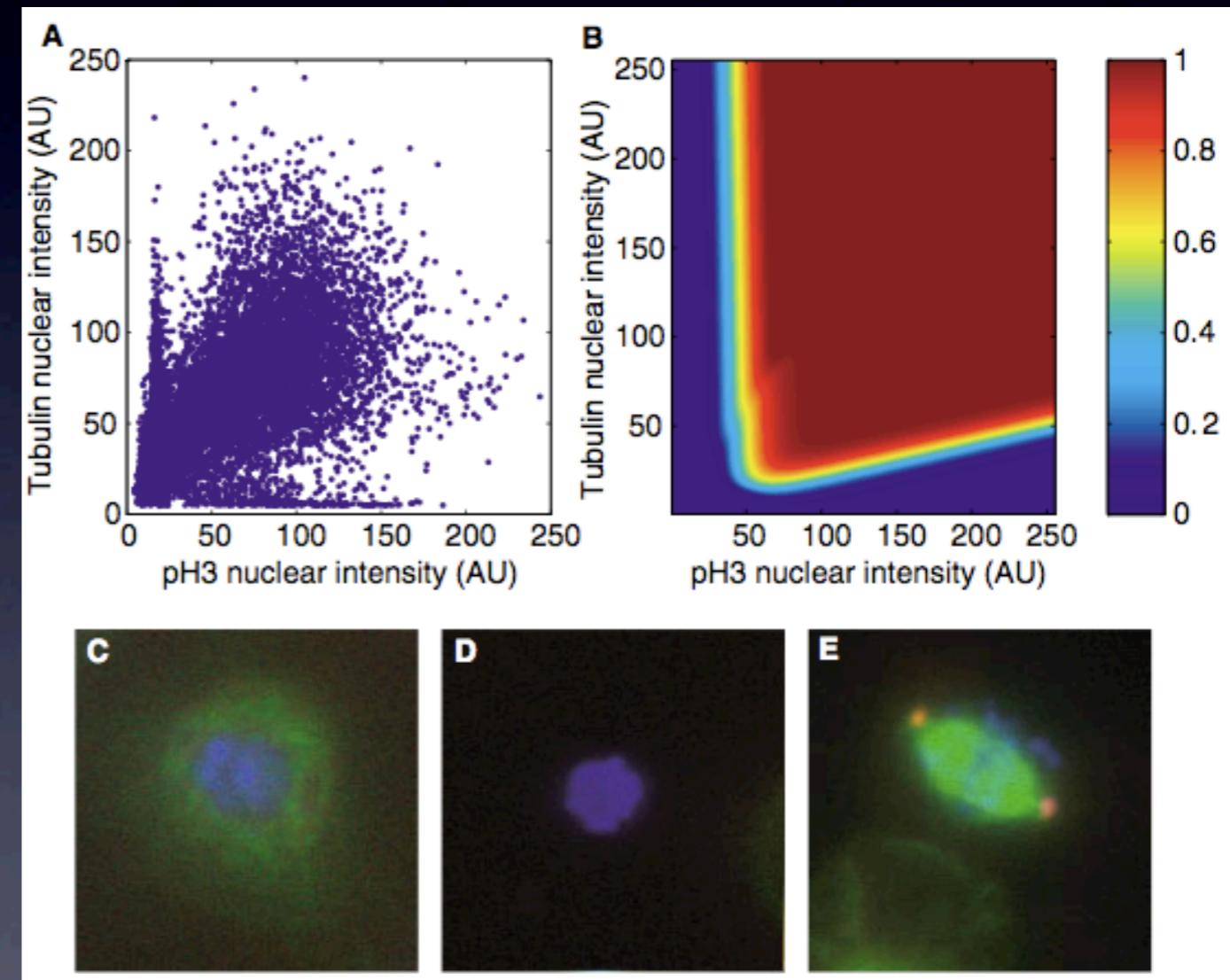
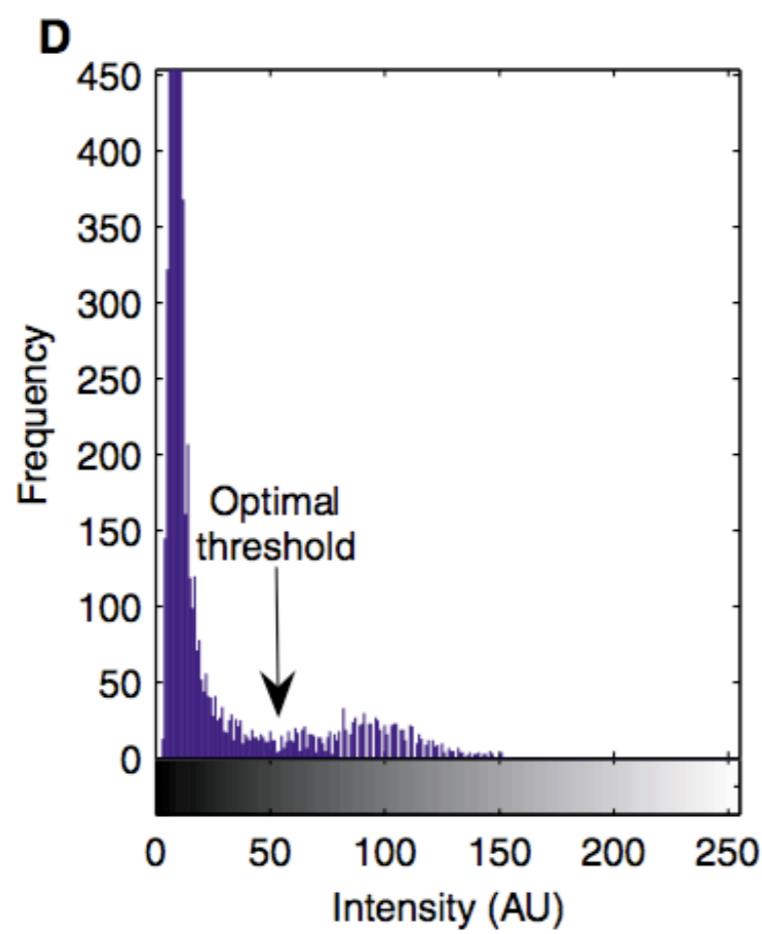
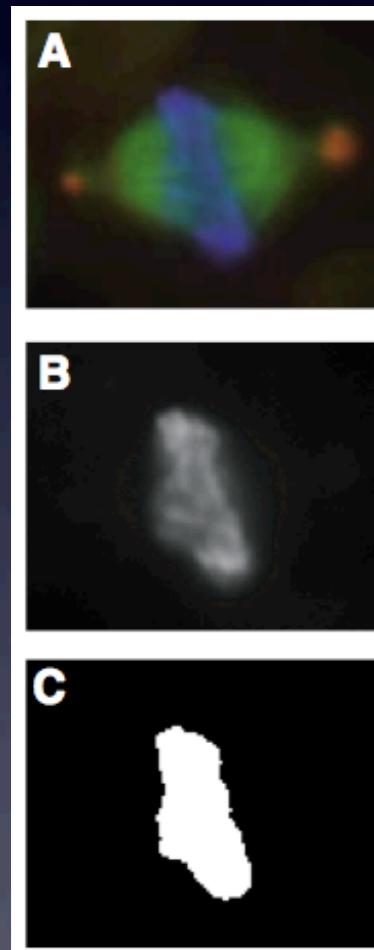
High-throughput RNAi Screen

3. High-throughput Microscopy Images

30-70 sites
8-bit BMP!
~25GB/plate
4TB total

High-throughput RNAi Screen

4. Automatic detection of metaphase cells

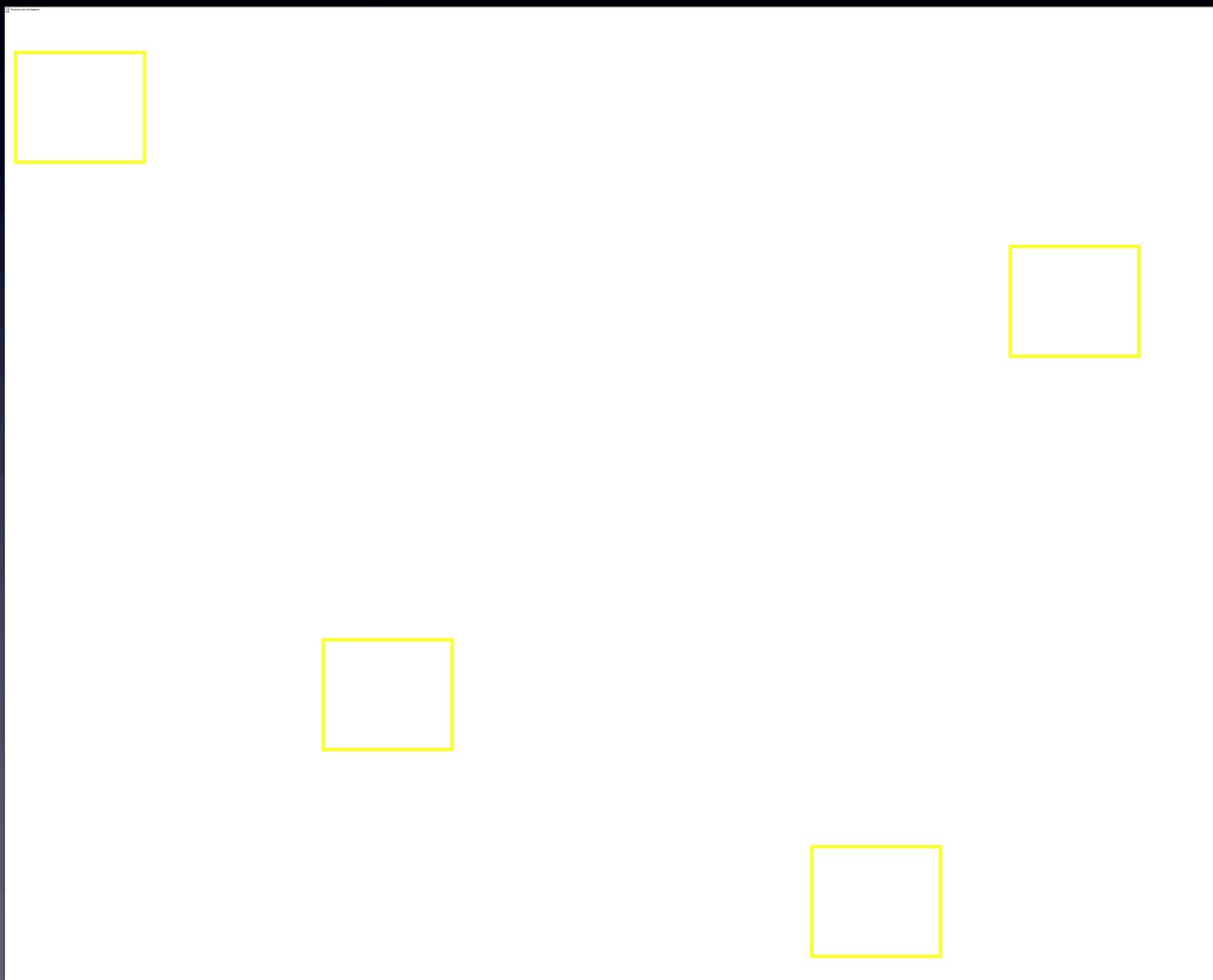


Automatic segmentation using Otsu's method

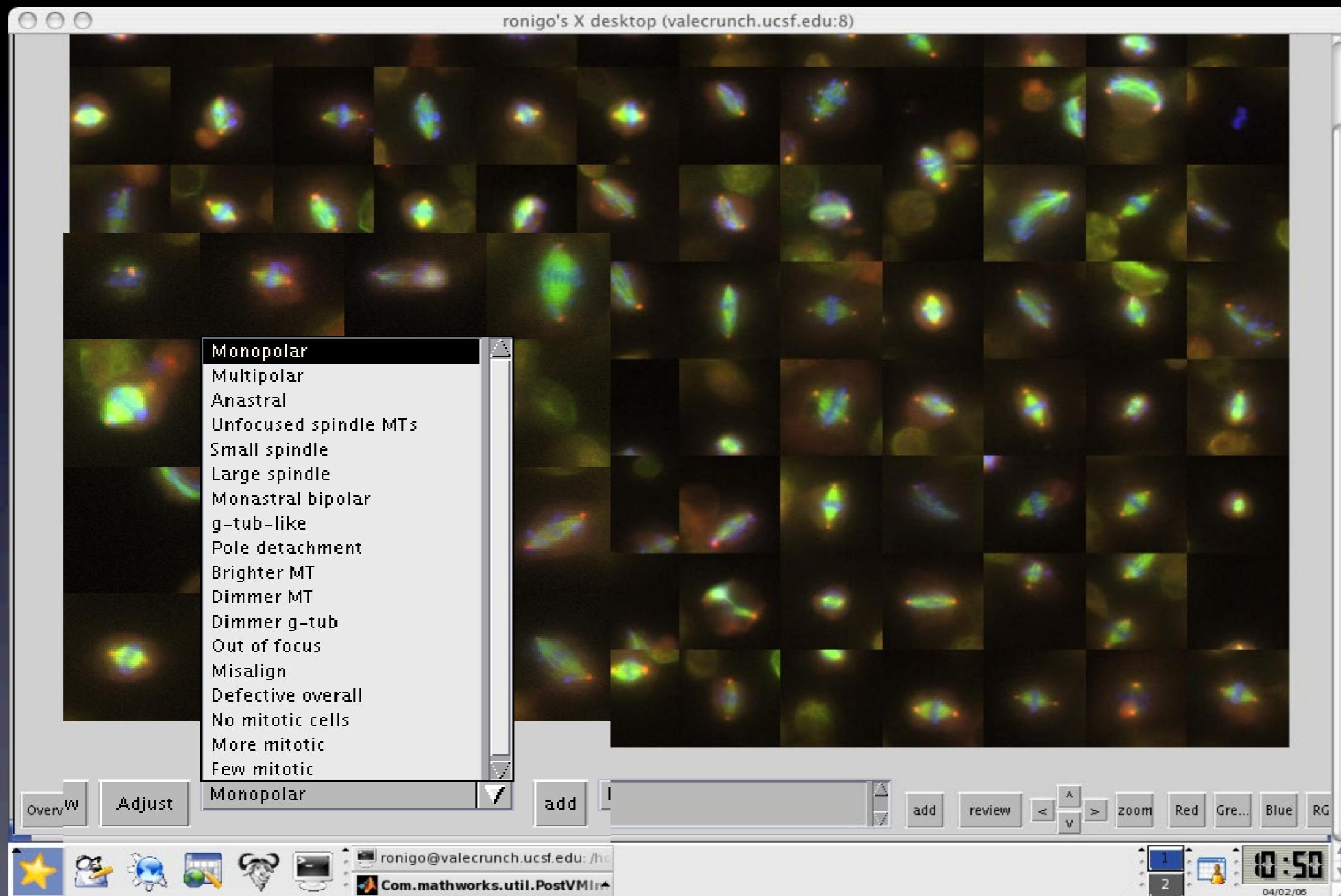
Classification using a trained neural network

High-throughput RNAi Screen

4. Automatic detection of metaphase cells



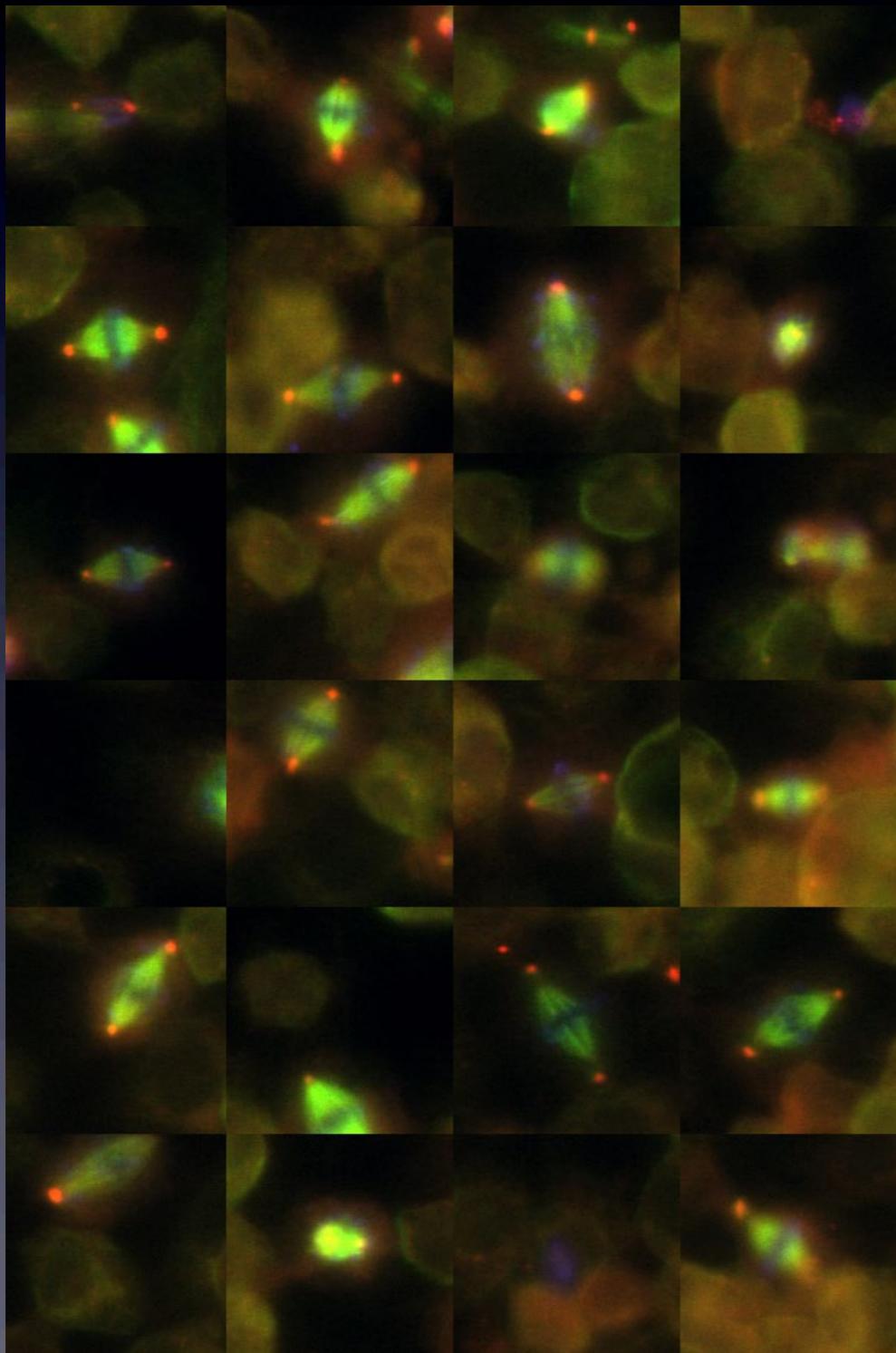
Analysis 1: Galleries of Spindles (~200 per gene) Scored Visually



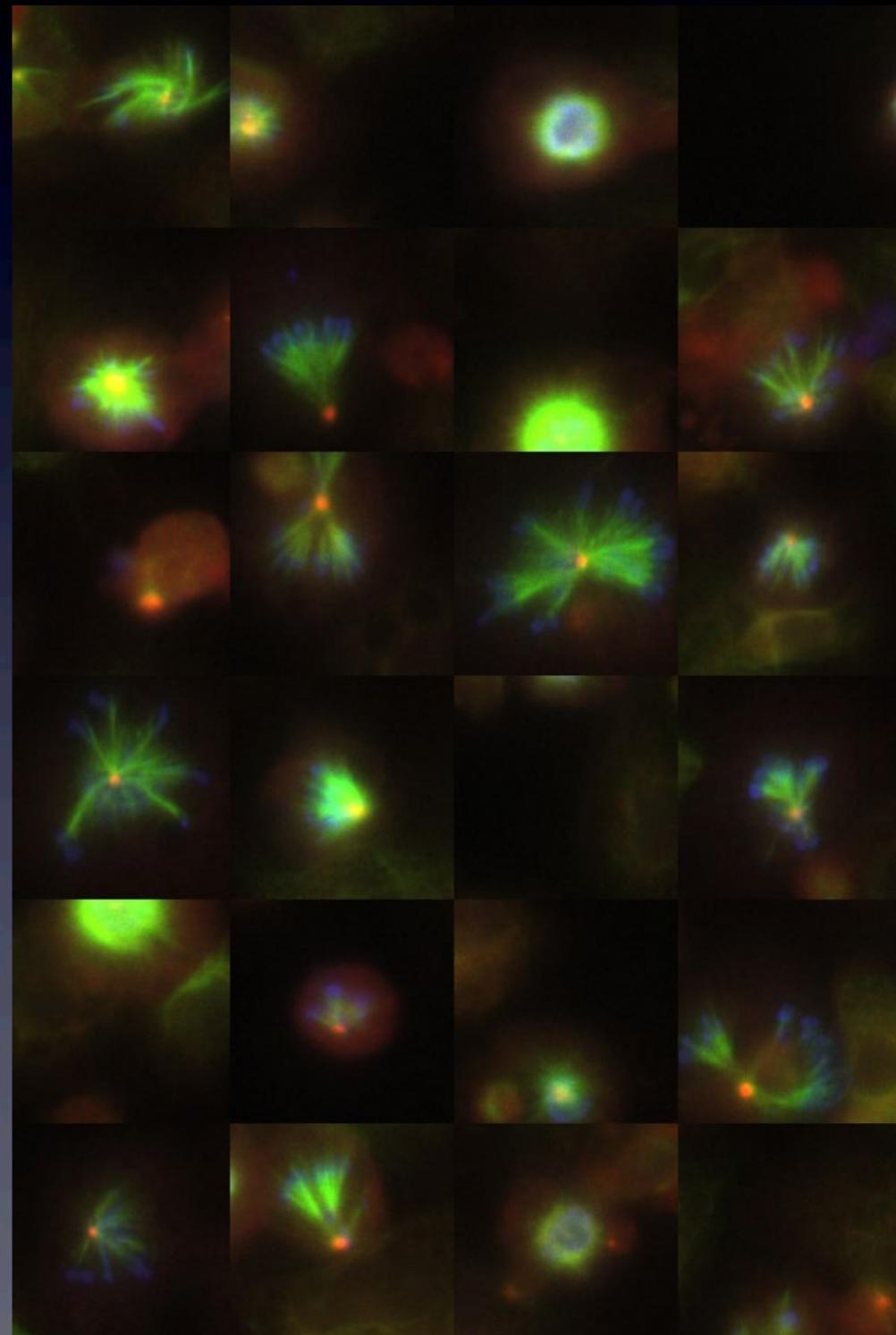
Side-by-side comparison of spindles made visual phenotype screening possible!

Example

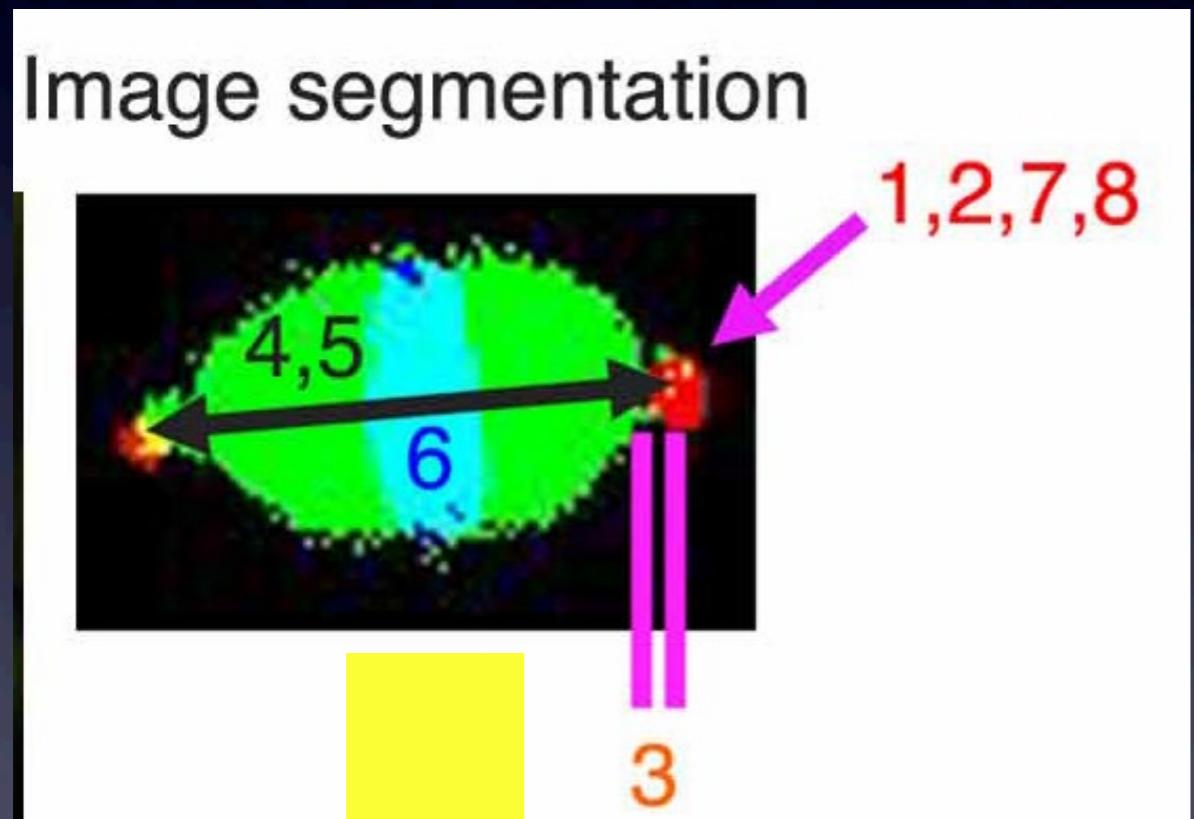
Control



Monopolar spindle (Kinesin-5/Klp61F)



Analysis 2: Image Segmentation and Computational Analyses



MatLab Code by Roy Wollman (UC Davis)
(publically available)

Workflow

Automated microscope

valecrunch.ucsf.edu

Database

EyectorixUI

Matlab Analysis

valelab.ucsf.edu

8-bit conversion - storage

phplabware - PostgreSQL



Vale Lab Screen Data Base

(<http://rnai.ucsf.edu>)

August 1, 2006, 7:59 pm

PhpLabWare version 0.6

--Links-- --Databases-- --System--

Add Record Now Viewing Table: MitoSpindleScreen (to edit mode)

Report: ---Reports--- Send to: screen file Edit reports

View: Gohtha Edit views

95 Records found. Showing 1 through 95.

Import Data 96 Records per page

Search Show All

all none reset Misalign multipolar
--Clear gtub area
--Likely monopolar
--Weak short spindles
Long spindles

name	symbol	CG	plate	row	col	RNAI probe	repeats	cells/image	mitotic index	hit	positive control	manual hits	computer hits	remarks	galleries	GFP localization	Action
	CG31347	31347	129	A	1	probe info		95.45	5.81%	No	No				1 2		
	CG14391	14391	129	A	2	probe info		94.43	8.50%	No	No				1 2 3		
	CG14394	14394	129	A	3	probe info		97.69	10.97%	No	No				1 2 3 4		
	beat-Vc	14390	129	A	4	probe info		91.29	5.27%	No	No				1 2		
	beat-Va	10134	129	A	5	probe info		68.64	6.00%	No	No				1 2		
	beat-Vb	31298	129	A	6	probe info		108.02	7.14%	No	No				1 2 3		
Spc25? - GG	CG7242	7242	129	A	7	probe info	Repeat	112.86	9.14%	Yes	No	Long spindle - Clear Long spindle Misalign Misalign - Clear	long spindles high circ2num	Large spindle - Clear Misalign - Clear	1 2 3		
	CG14384	14384	129	A	8	probe info		96.90	5.95%	No	No				1 2		
polyA-binding protein interprot								119.71	9.43%								
yellow	CG7242	40494	7242														
lethal(1)																	
Suppressor of variegation 3-7	Su(var)3-7	8599	129	C	2	probe info		81.07	7.40%	No	No				1 2		
	CG15888	15888	129	C	3	probe info		82.71	6.30%	No	No				1 2 2		
	CG15997	15997	129	C	4	probe info		95.40	9.04%	No	No						

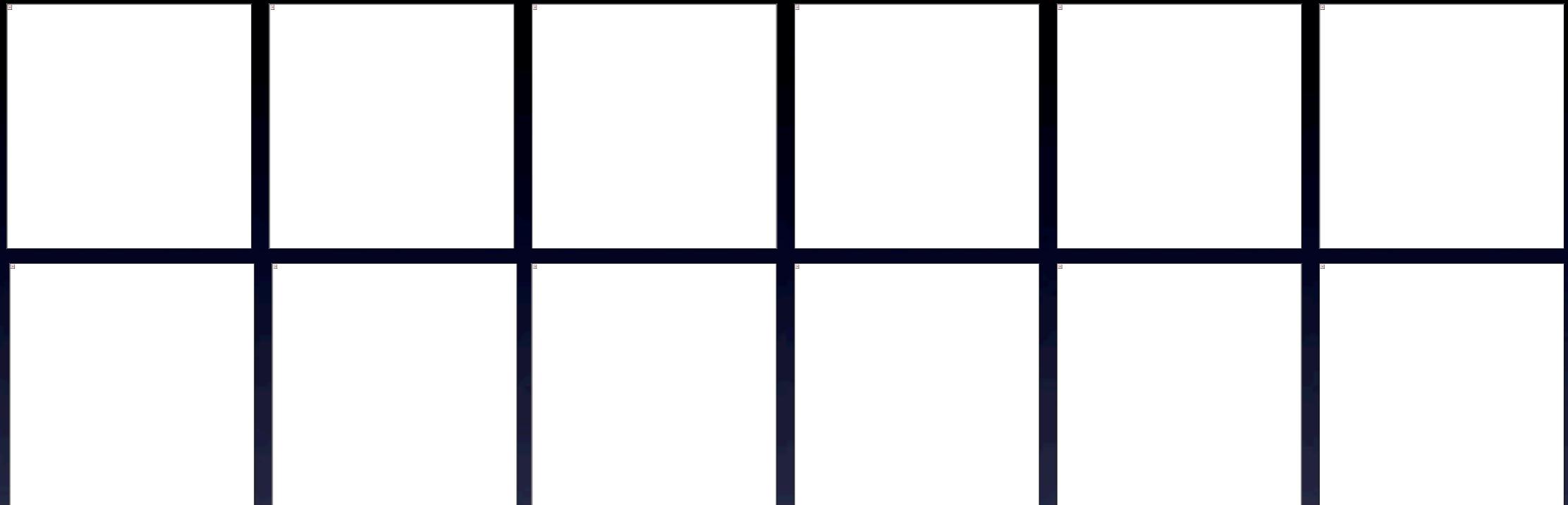
Link to phenotype gallery

Link to dsRNA information

Done

Results: RNAi of ~200 genes produced metaphase spindle defects

Phenotypes



45 of 49 known mitotic genes in S2 cells identified

~70 Novel or Unexpected Genes

Follow-ups: Ssp4-Patronin (Sarah)

Augmin complex (Gohta and Sabine)



Lookup Tables (LUTs)

