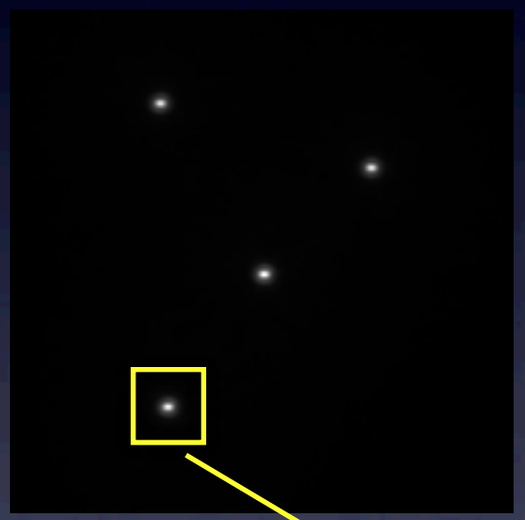
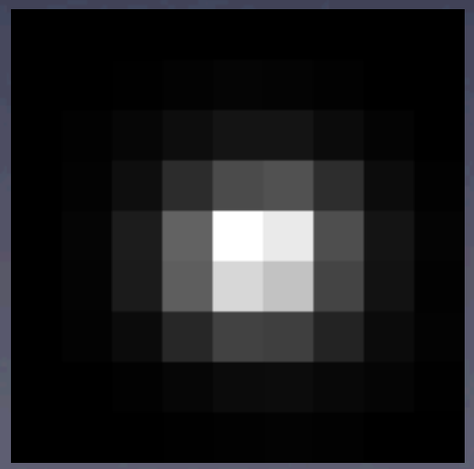
Image Analysis

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

What is a digital Image?

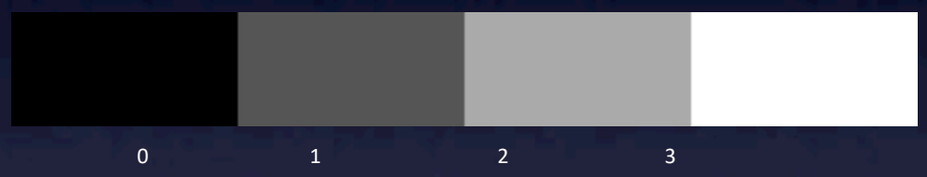
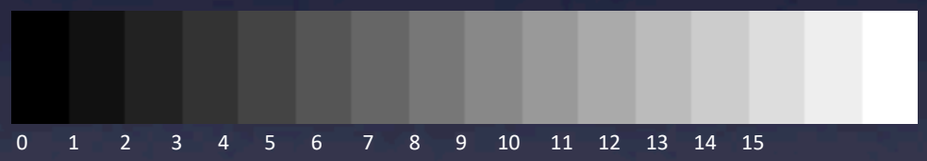
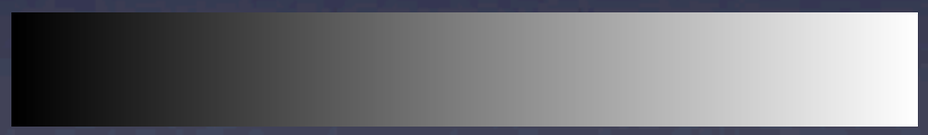
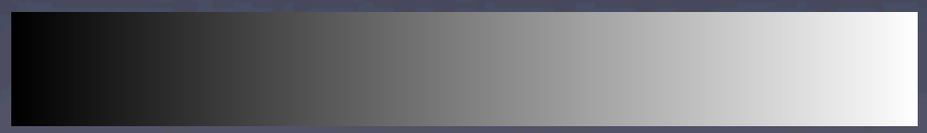
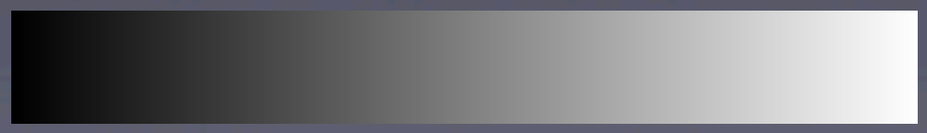
Many measurements of photon flux





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

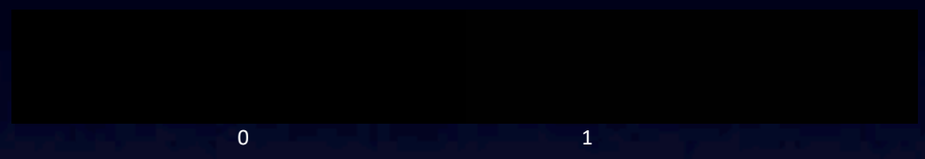
Bit depth and dynamic range

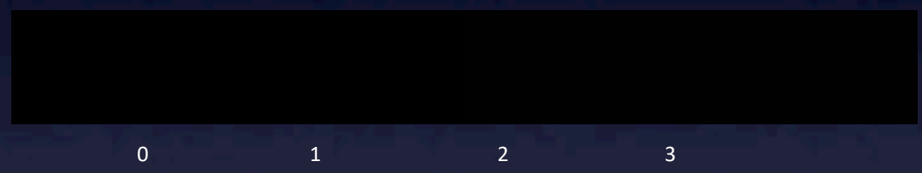
Nr. bits	range	
1	2	 Binary Image
2	4	 Grayscale Images
4	16	
8	256	 1-byte
12	4096	 2-bytes
16	65536	

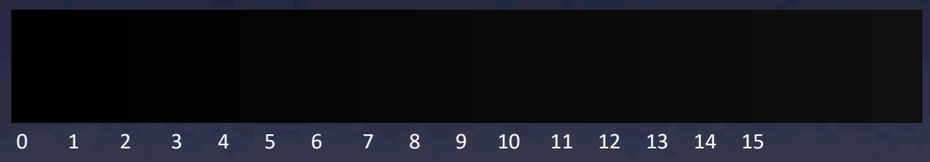
Bit-depth and resolution

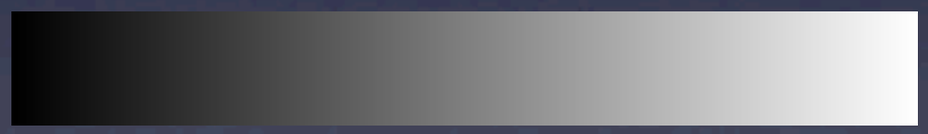
Bit depth and dynamic range

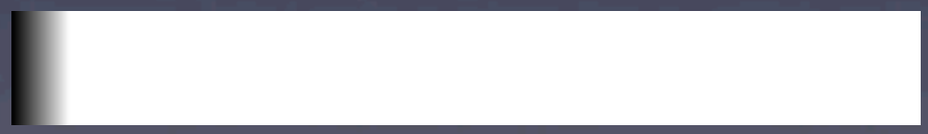
Nr. bits	range
1	2
2	4
4	16
8	256
12	4096
16	65536

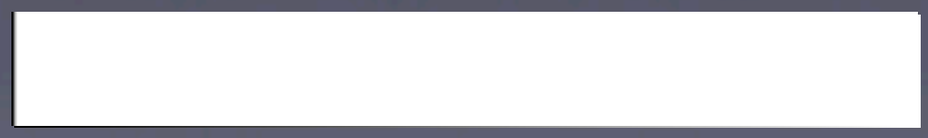








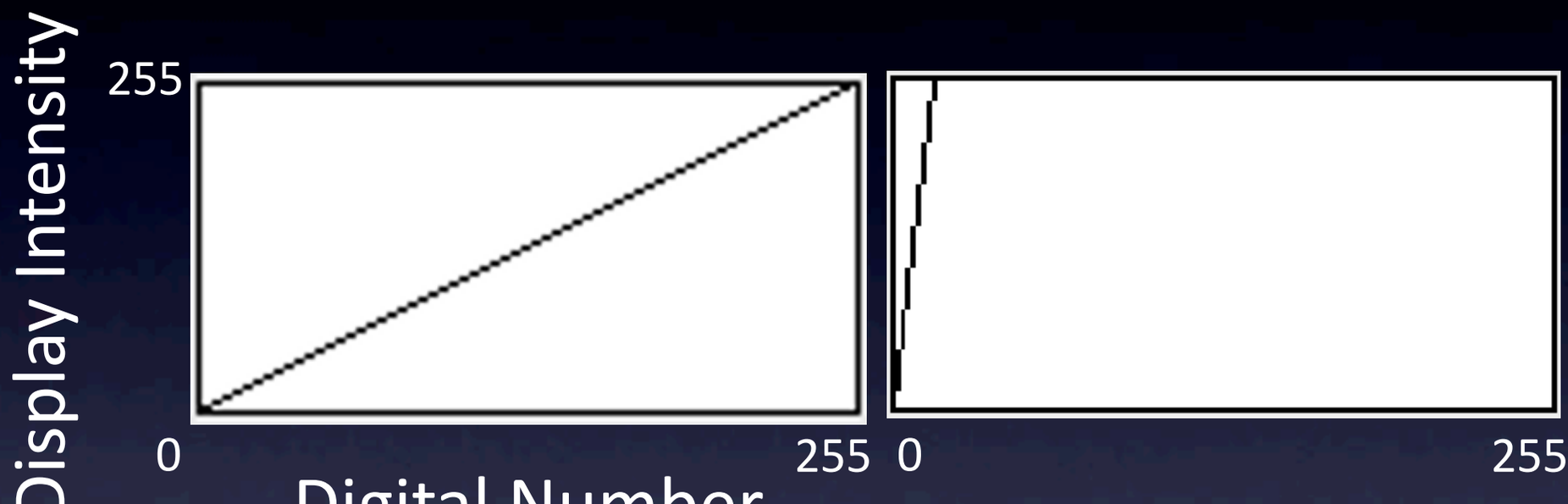




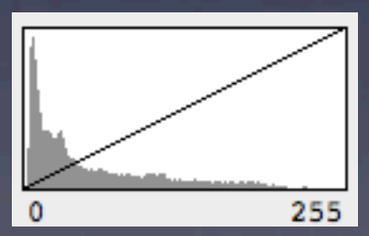
Binary Image

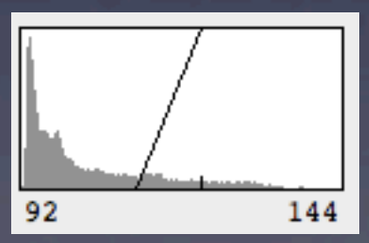
Grayscale Images

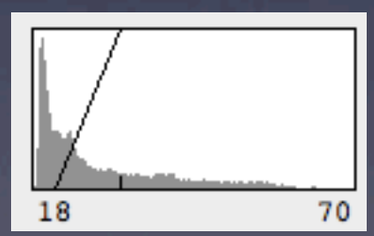
Mapping values onto display

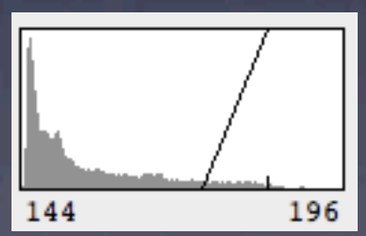


Mapping values onto display: Brightness/contrast





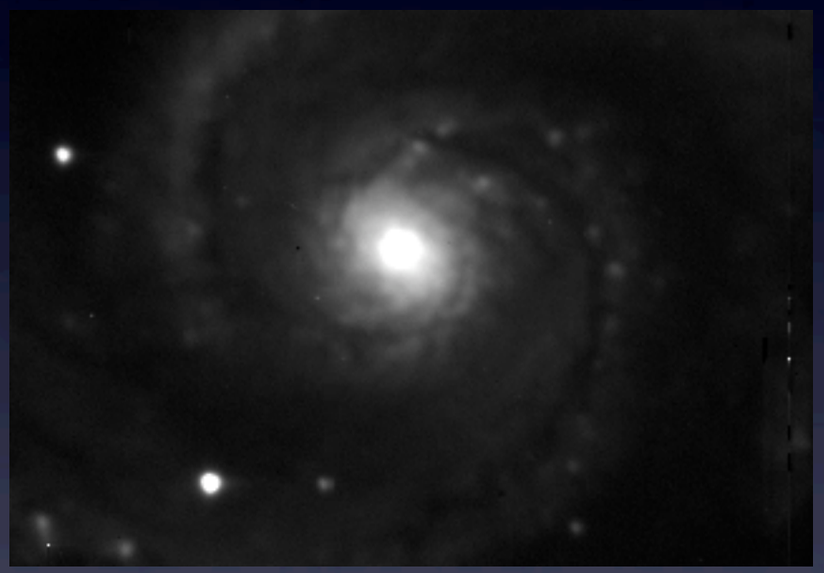


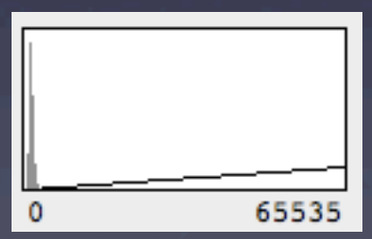


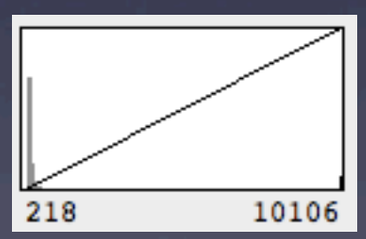
Slope = contrast

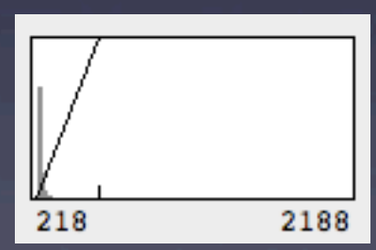
Brightness

Brightness/contrast









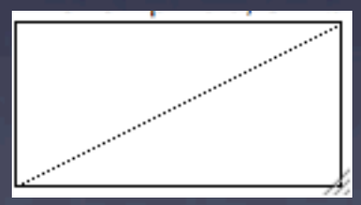
Full Range

Auto-Scale

High B/C

Be aware of software Auto-scaling for you!

Gamma adjustment



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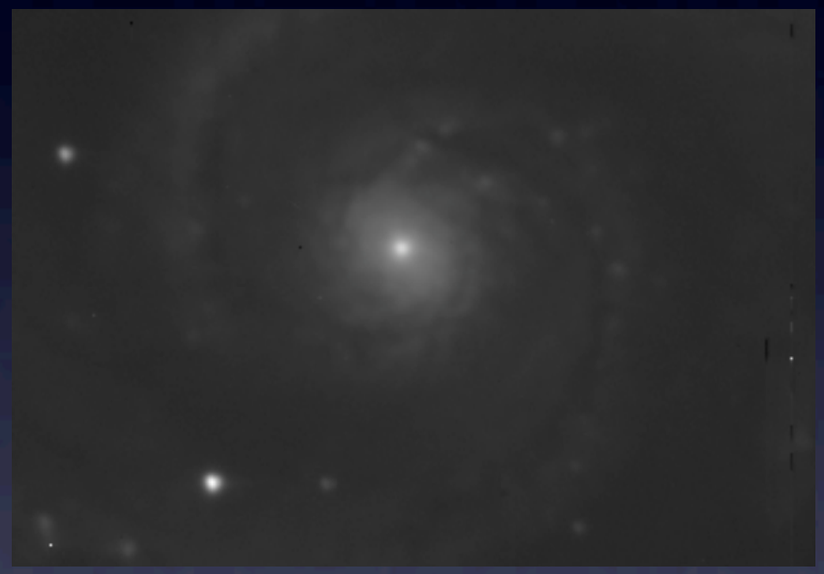
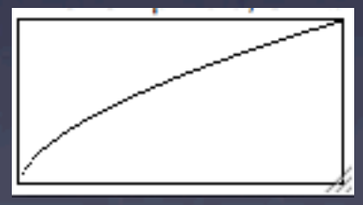
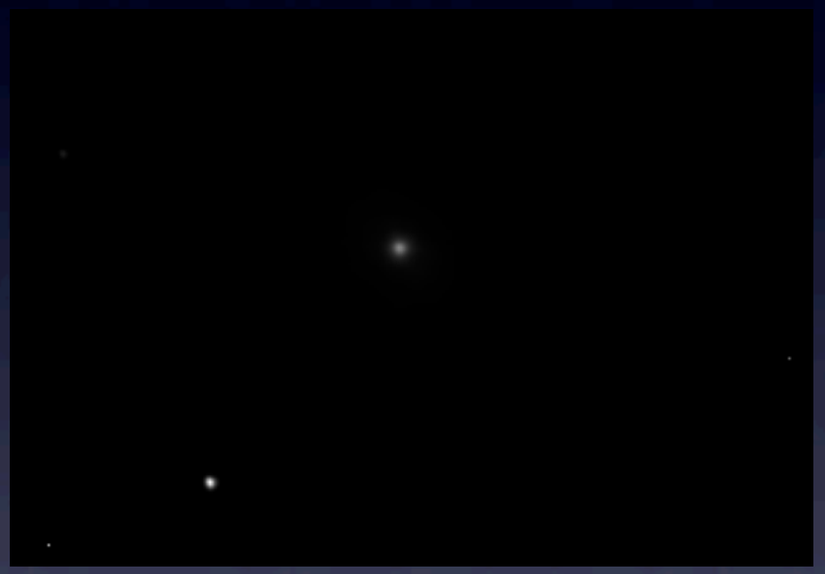
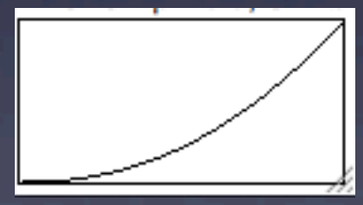




0.6





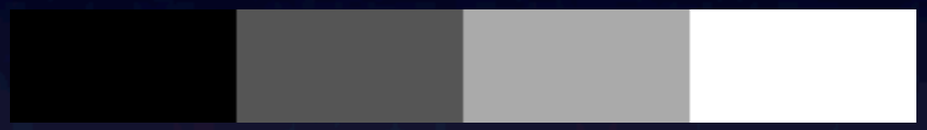
2.2

What are acceptable image manipulations?

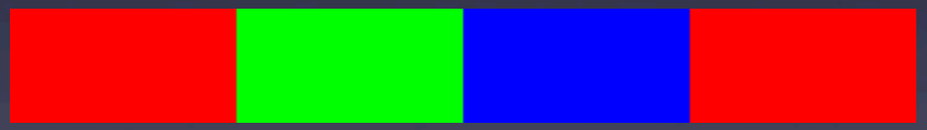
- JCB has the best guidelines
  - http://jcb.rupress.org/content/172/1/9.full
  - http://jcb.rupress.org/content/166/1/11.full
- Brightness and contrast adjustments ok, so long as done over whole image and don't obscure or eliminate background
- Nonlinear adjustments (like gamma) must be disclosed
-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

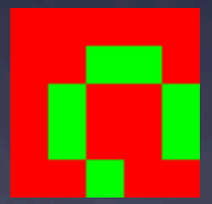
Lookup Tables (LUTs)

0 1 2 3

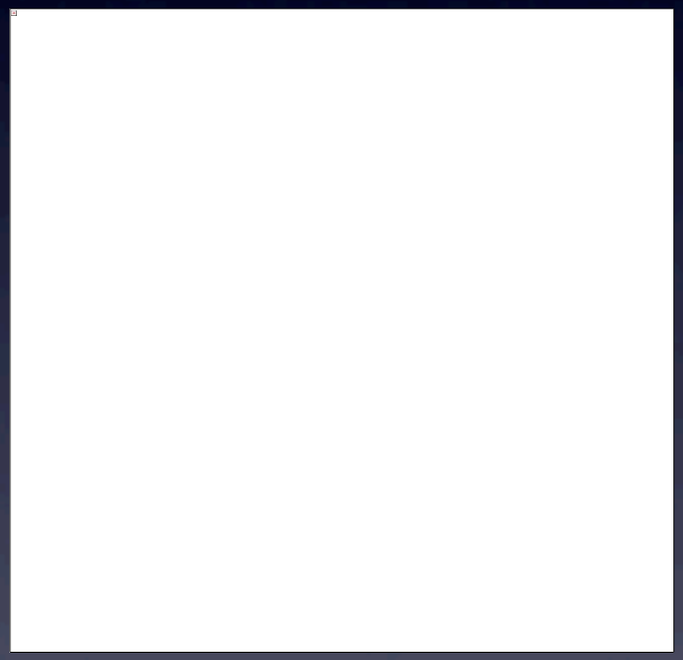


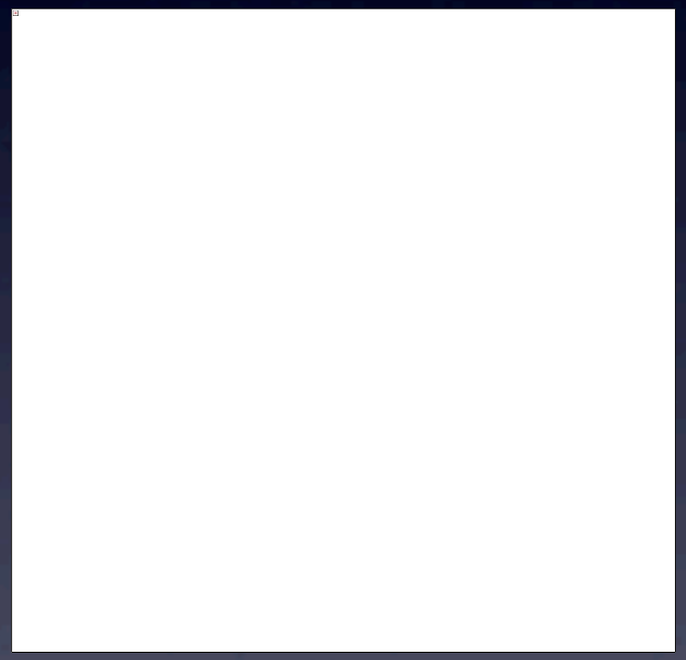
0	0	0	0	0
0	0	1	1	0
0	1	3	3	1
0	1	3	3	1
0	0	1	0	0

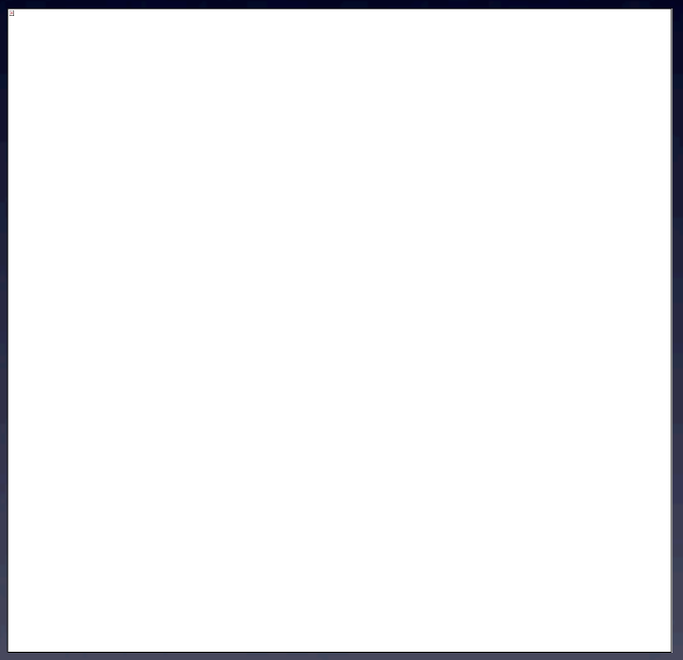


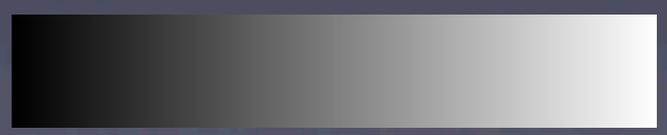


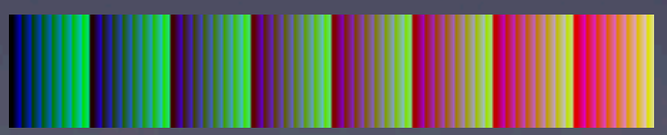
Lookup Tables (LUTs)



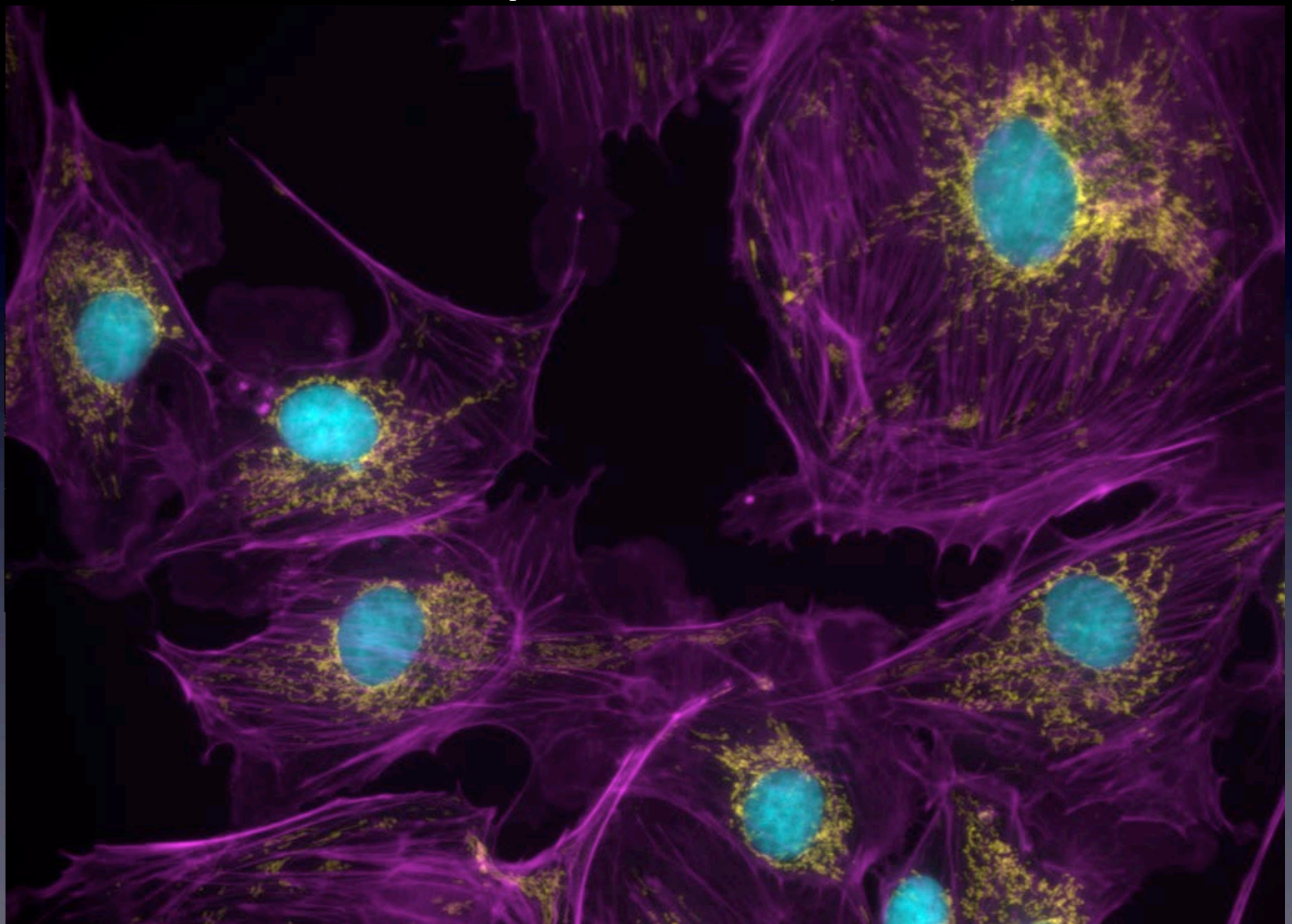




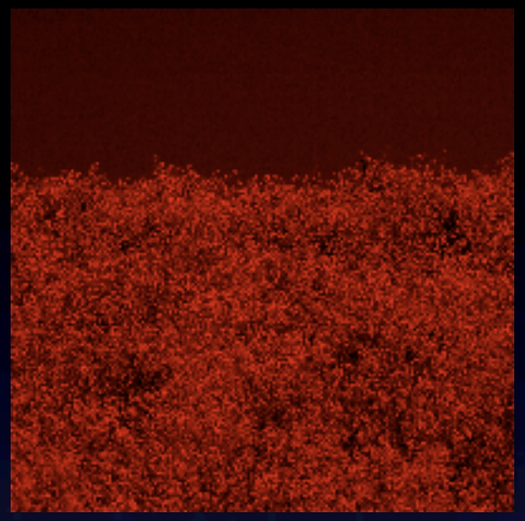


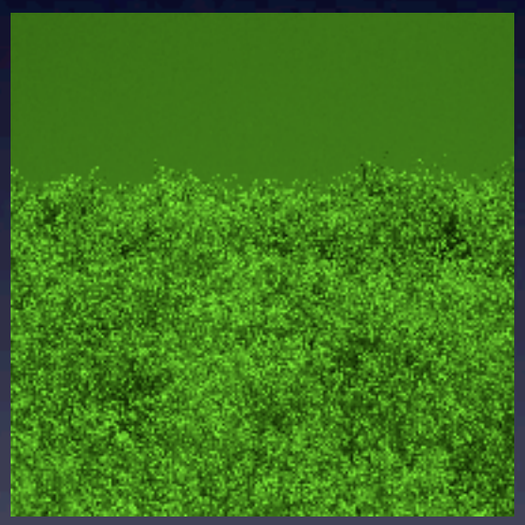


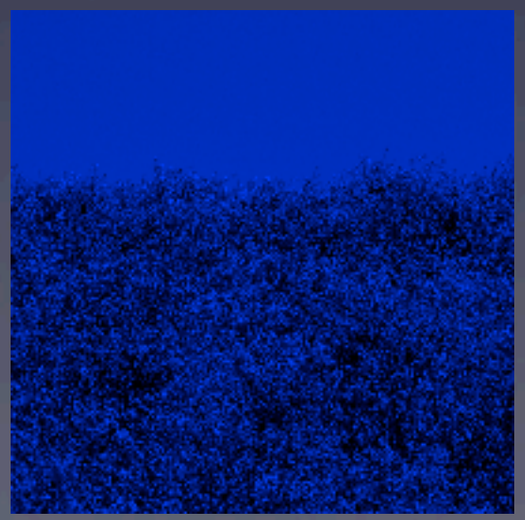
Lookup Tables (LUTs)



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

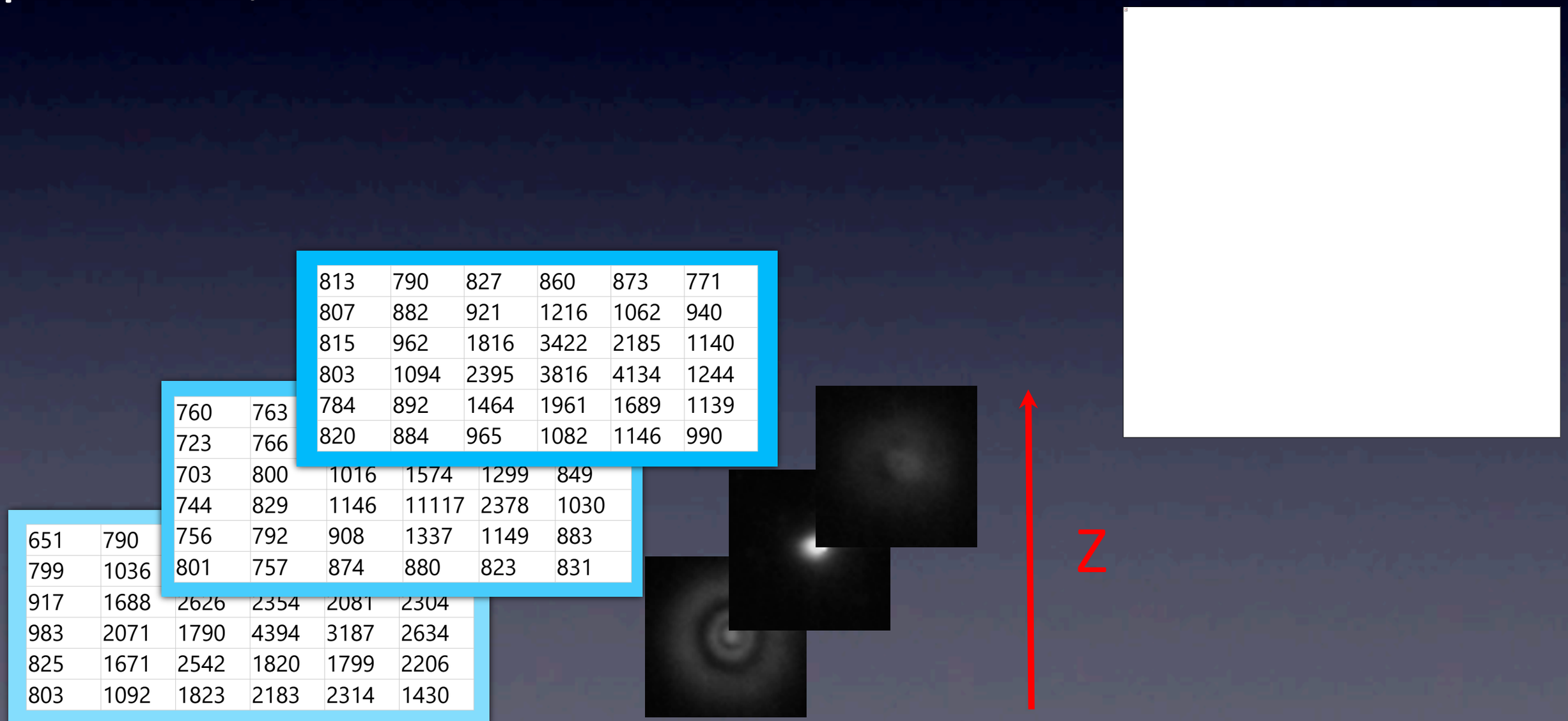
209	89	255	0
0	0	0	90
39	255	255	0
90	0	0	185

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:

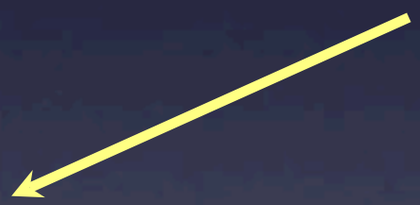
1392x1040x2 = 2.8MB

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

2.8 * 3 * 15 * 200 = 25.2GB

Compression

Losless versus Lossy



None (raw)
Run-length encoding
Dictionary approaches, etc..

Discards data not essential for visual appearance

http://dvd-hq.info/data_compression.php

File Formats

There are many!

Desired:

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

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 losless 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

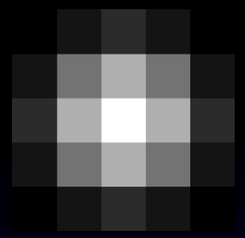
- Matlab
- IDL
- ImageJ (free, many plugins) http://rsb.info.nih.gov/ij/
- Imaris (3D visualization)
- Priism (Agard/Sedat labs) http://msg.ucsf.edu/IVE/
- CellProfiler http://cellprofiler.org

Linear Filters

Neighborhood convolution

Kernel

1	1	1
1	1	1
1	1	1



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

Simple Smoothing

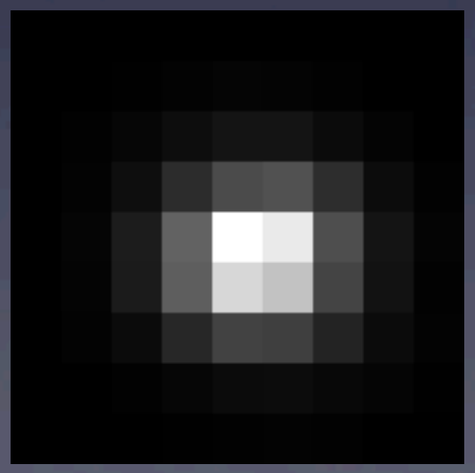
0	1	113	1	255	1	0	0	0	0
0	113	1	1	255	1	0	0	0	0
0	113	1	1	1	255	0	0	0	0
0	113	0	0	0	255	0	0	0	0
113	0	0	0	0	255	0	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	0	255	0	0
0	113	0	0	0	0	0	255	0	0

Gaussian Smoothing

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

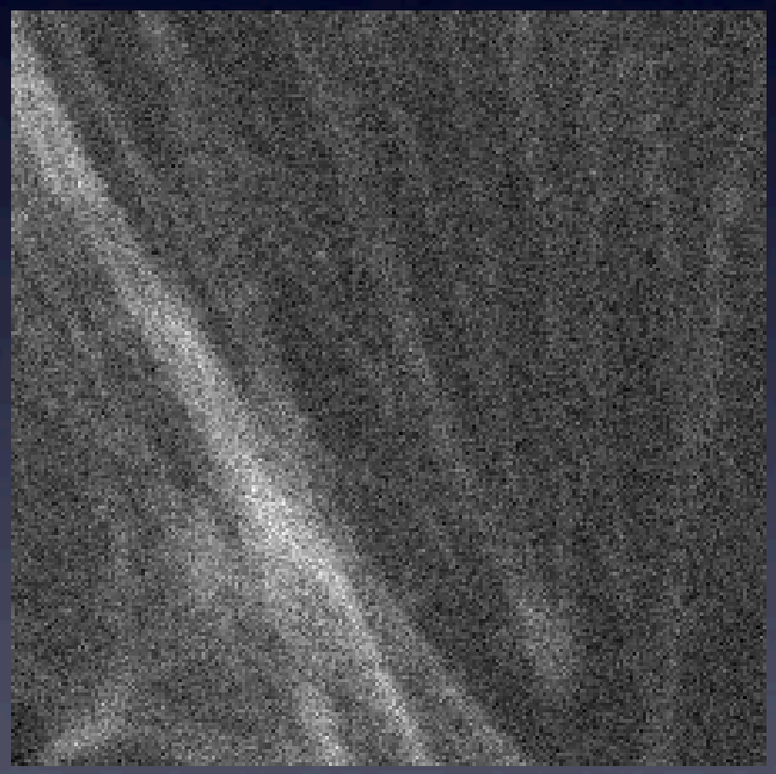


Measured PSF

Gaussian Filter

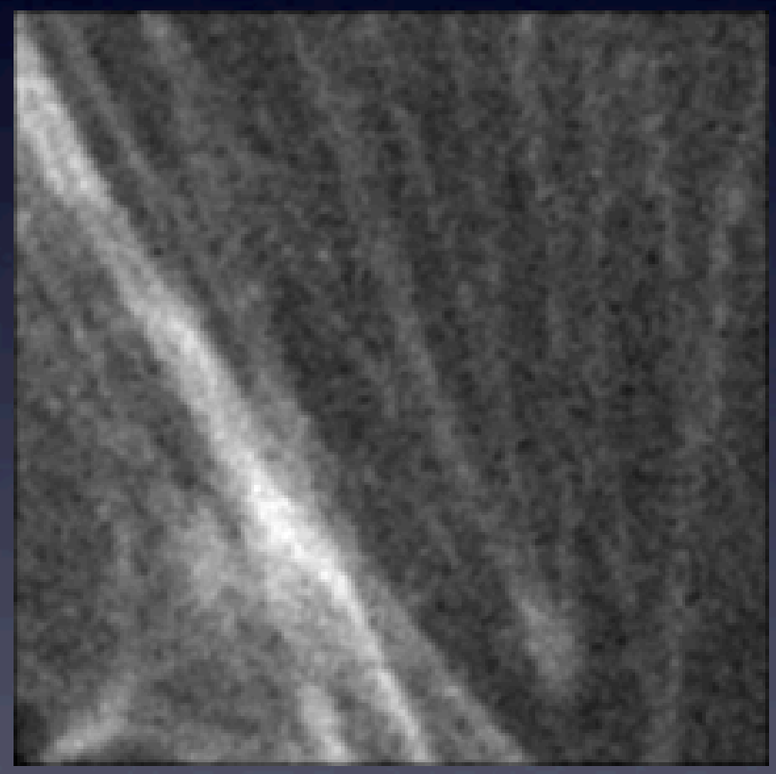
Why smooth?

Averages redundancy and suppresses noise



10 photons/pixel average

5 e- read noise



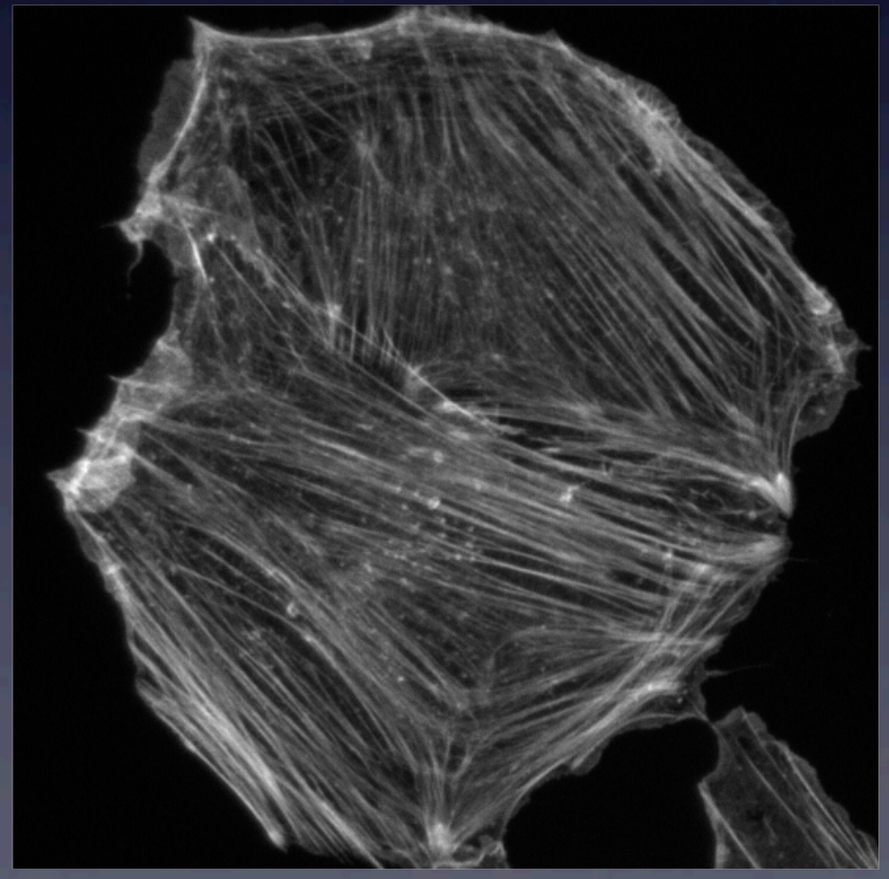
Gaussian smoothing filter,
σ = 1 pixel

Other Filters

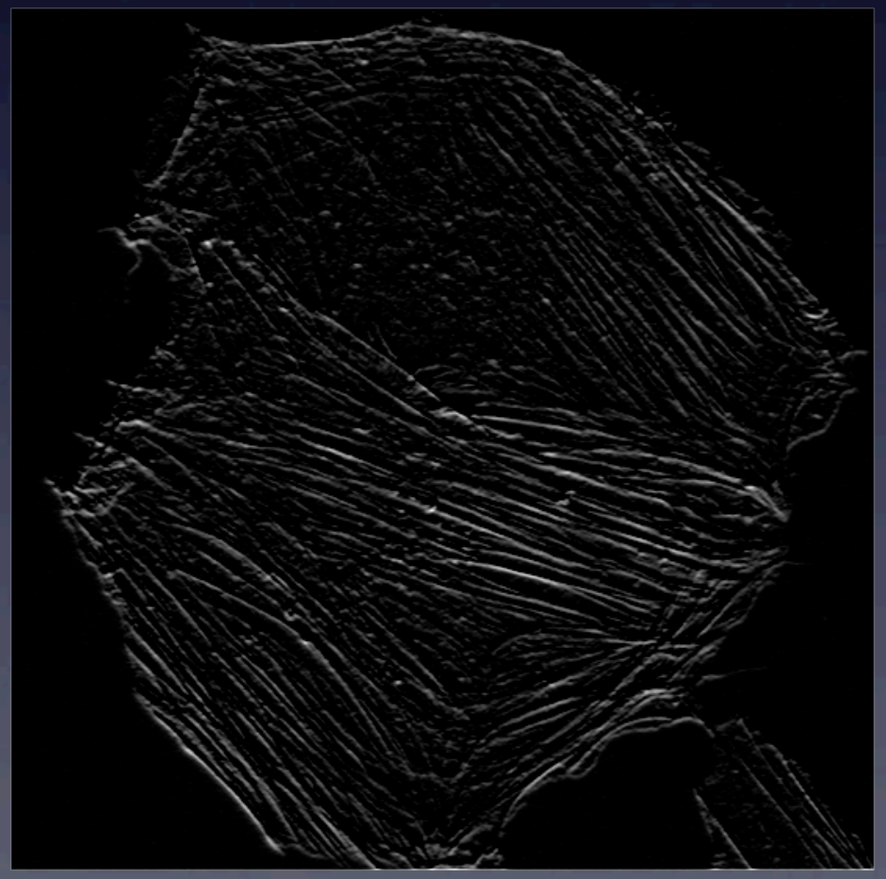
Edge Detection

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

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



Original



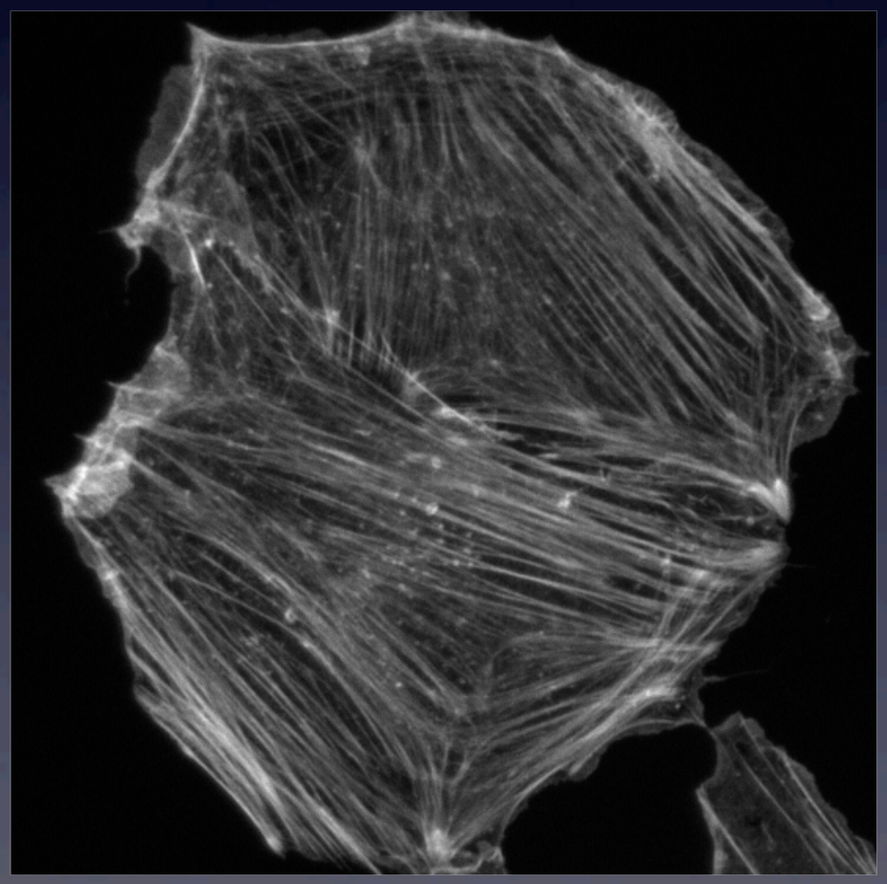
Horizontal edge detection

Other Filters

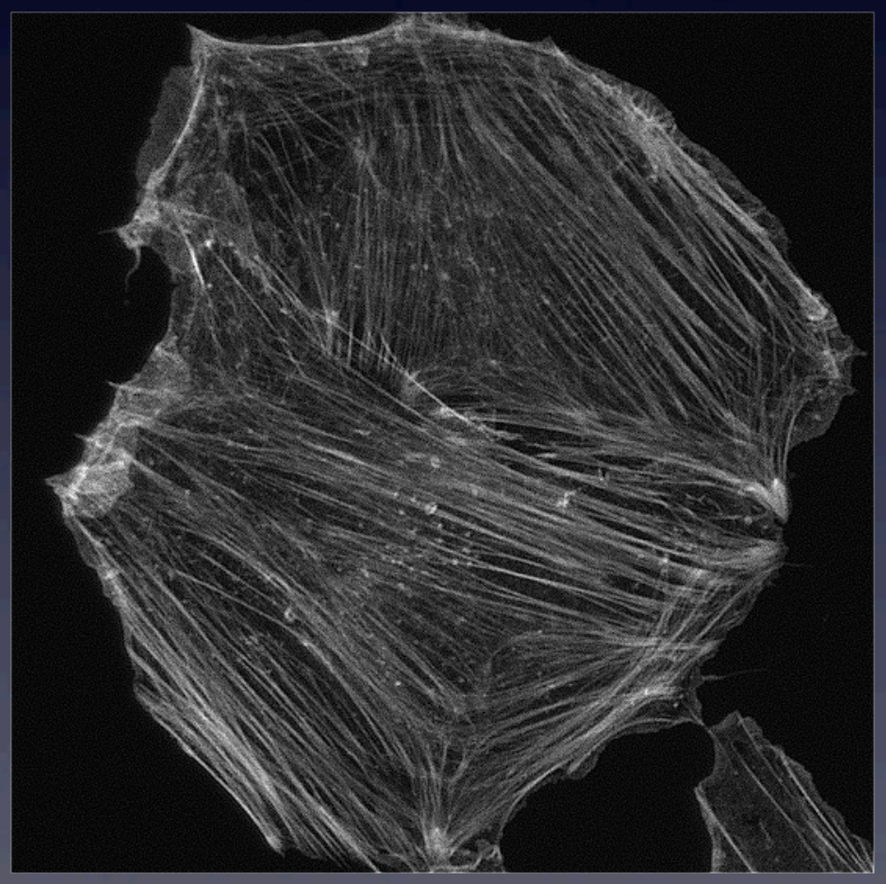
Unsharp Masking

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

(Laplacian)



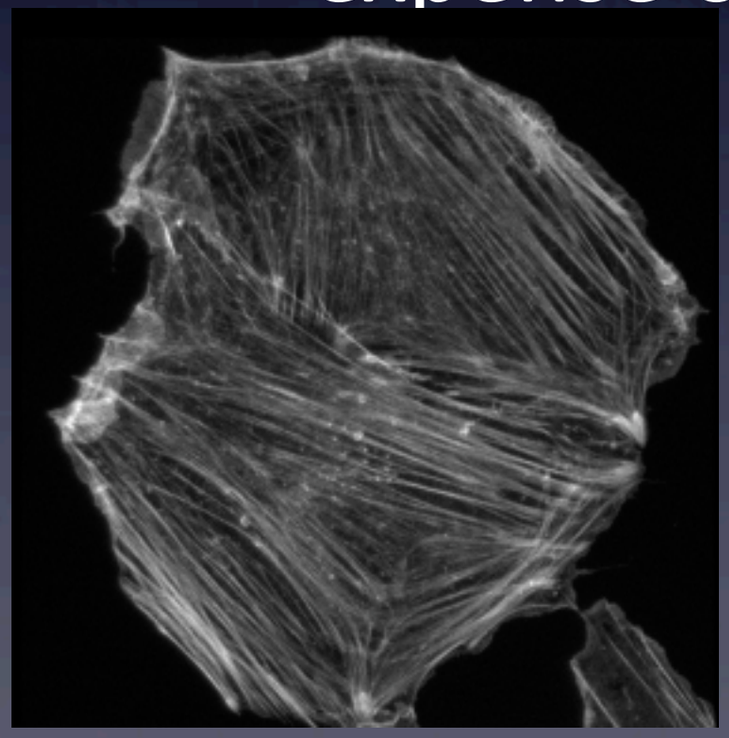
Original



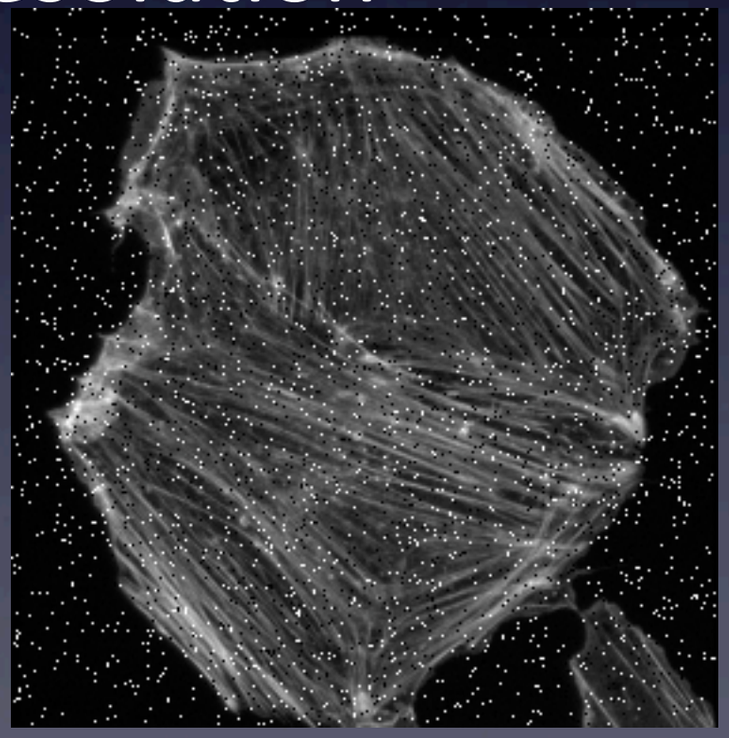
Unsharp masked

Non-linear Filters

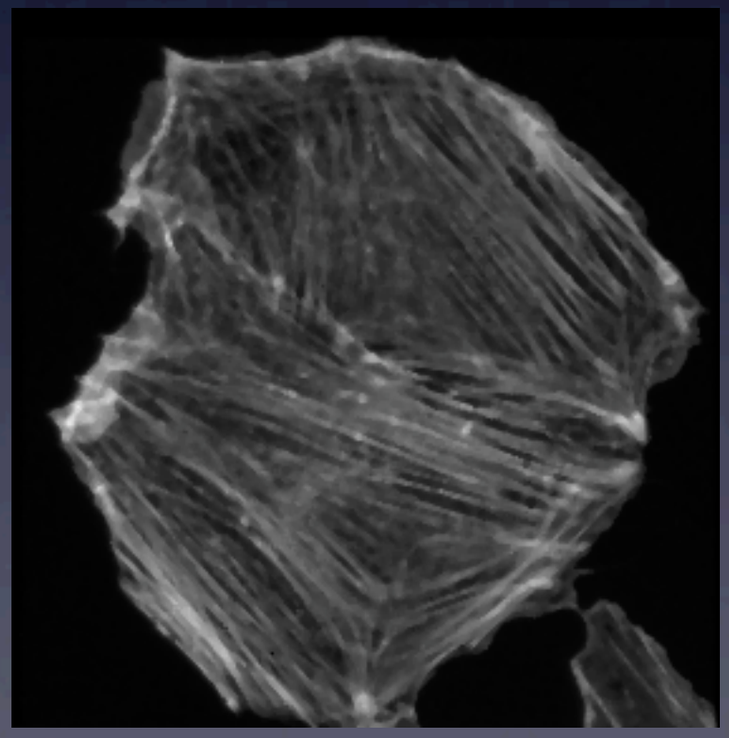
- Replace central pixel with min, max, median
- Median filter is a good noise filter, at the expense of resolution



Original



Artificial Noise

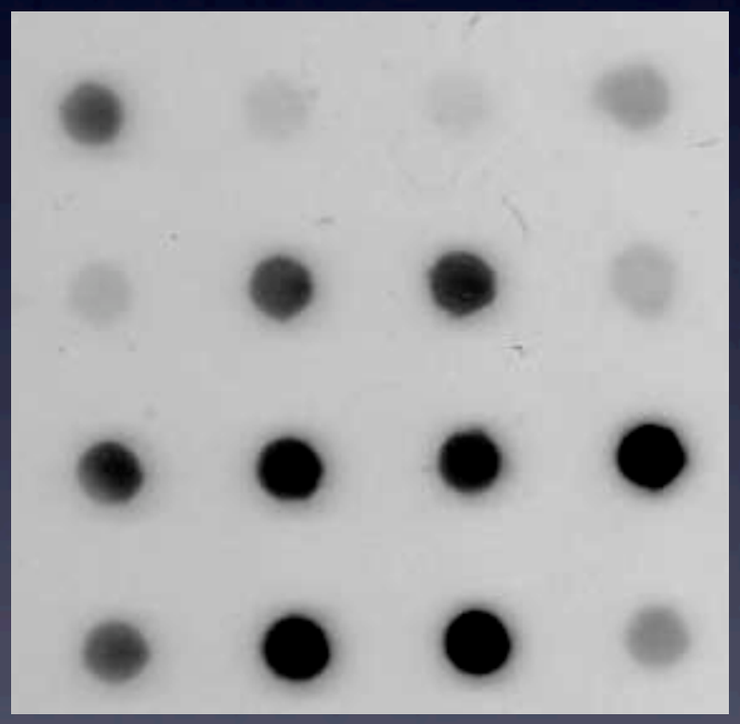


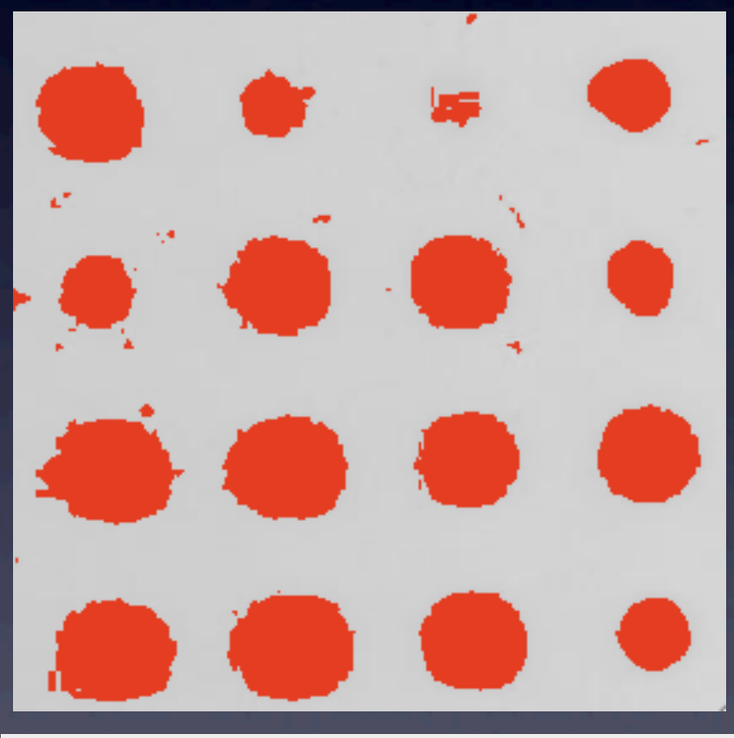
Median Filter

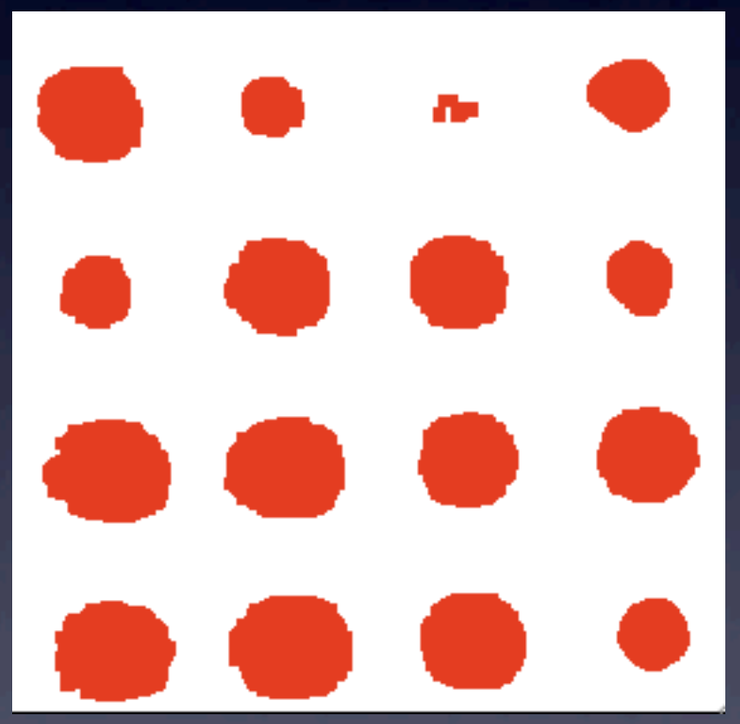
Segmentation

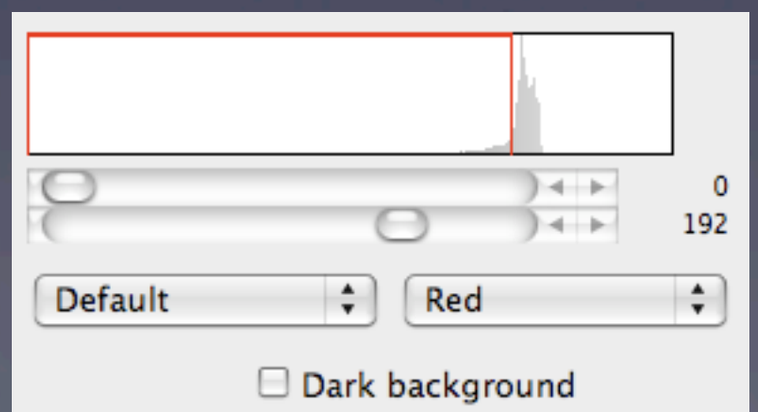
How to define the object that
you want to measure?

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







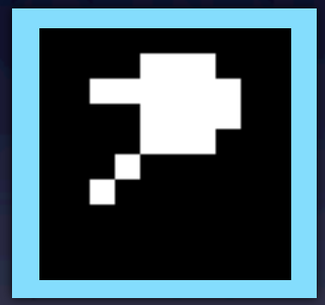


2x erosion
2x dilation

Binary Operations: Erosion/Dilation

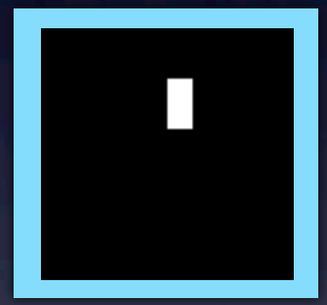
Structuring Element:

1	1	1
1	1	1
1	1	1



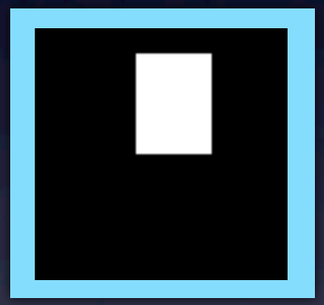
0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	0	0	0
0	0	1	1	1	1	1	1	0	0
0	0	0	0	1	1	1	1	0	0
0	0	0	0	1	1	1	0	0	0
0	0	0	1	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Erosion



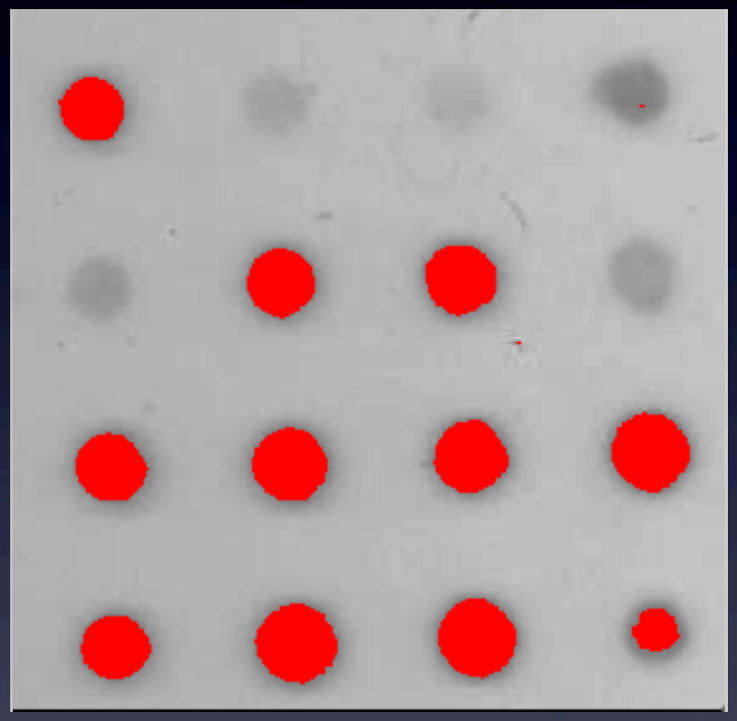
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

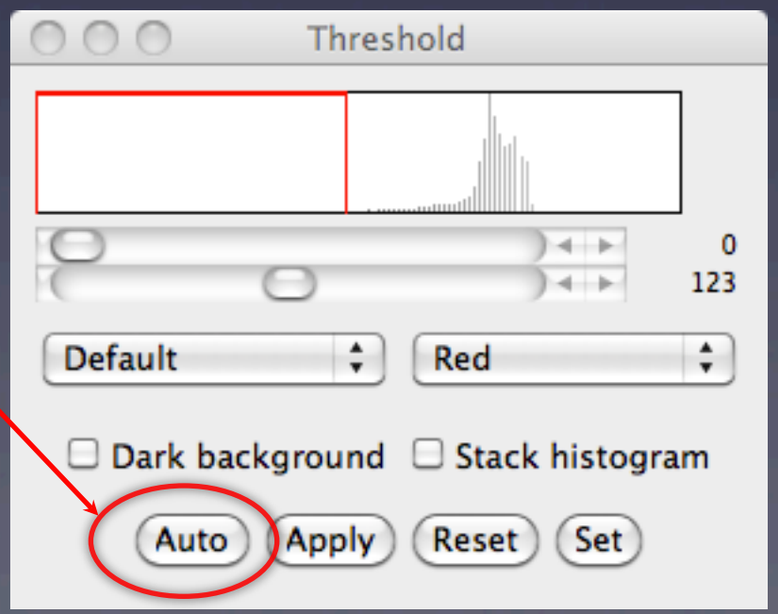
Dilation

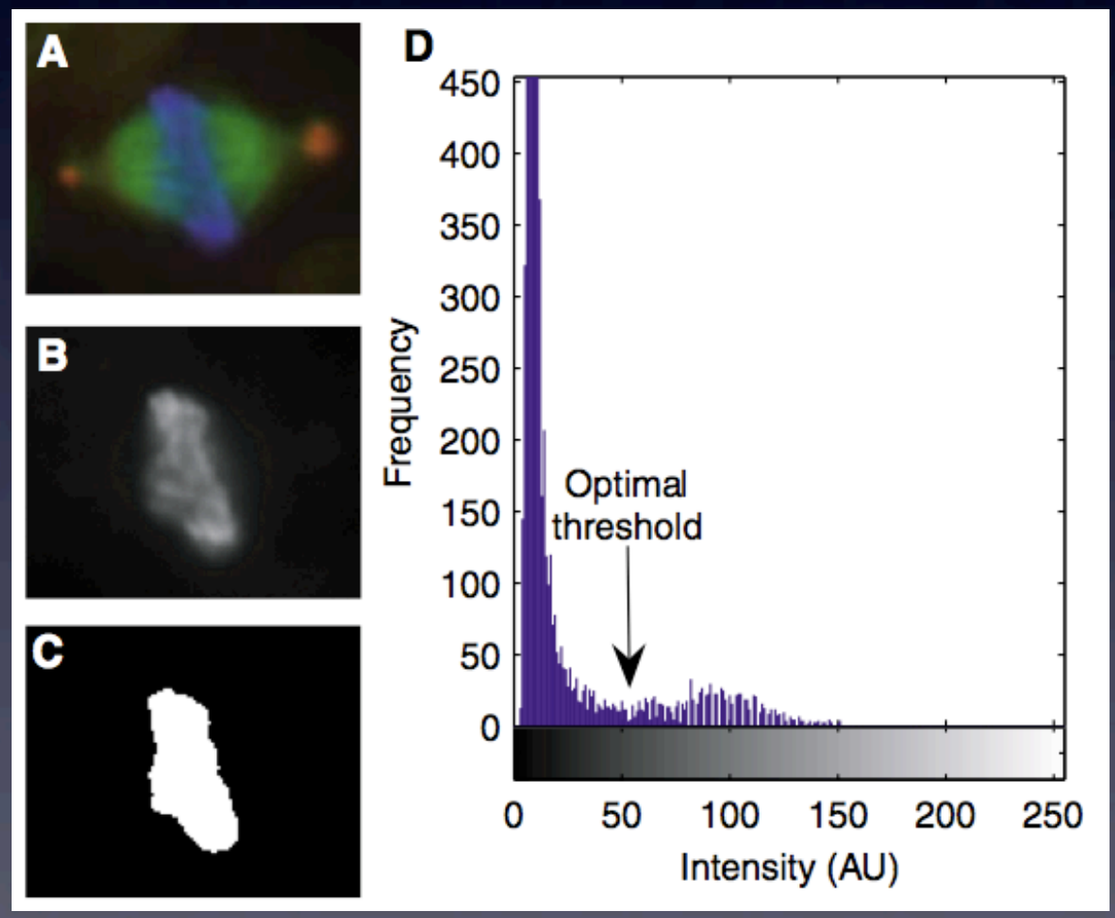


0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	1	1	0	0	0
0	0	0	0	1	1	1	0	0	0
0	0	0	0	1	1	1	0	0	0
0	0	0	0	1	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Thresholding, where to set the cutoff?

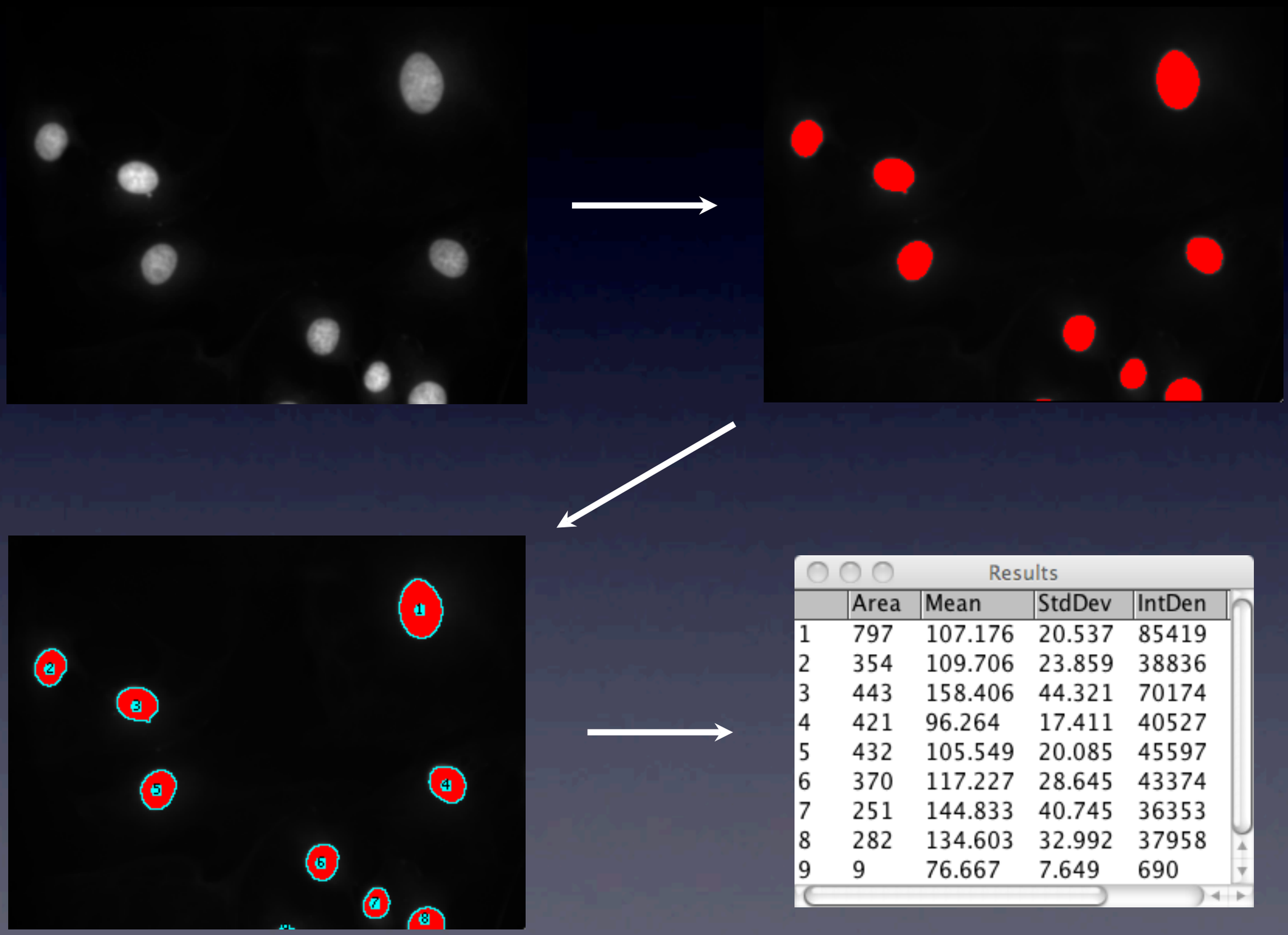






Automatic segmentation using Otsu's method

Measure objects



Results

	Area	Mean	StdDev	IntDen
1	797	107.176	20.537	85419
2	354	109.706	23.859	38836
3	443	158.406	44.321	70174
4	421	96.264	17.411	40527
5	432	105.549	20.085	45597
6	370	117.227	28.645	43374
7	251	144.833	40.745	36353
8	282	134.603	32.992	37958
9	9	76.667	7.649	690

Acknowledgements/References

Kurt Thorn

John C. Russ, The Image Processing Handbook

Gonzalez, Woods and Eddins, Digital Image Processing using Matlab

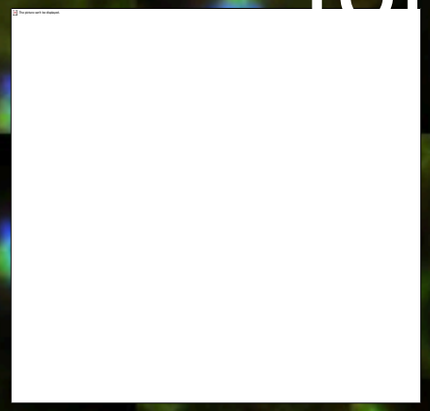
Burger and Burge, Digital Image Processing, An Algorithmic Introduction using Java (ImageJ)

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



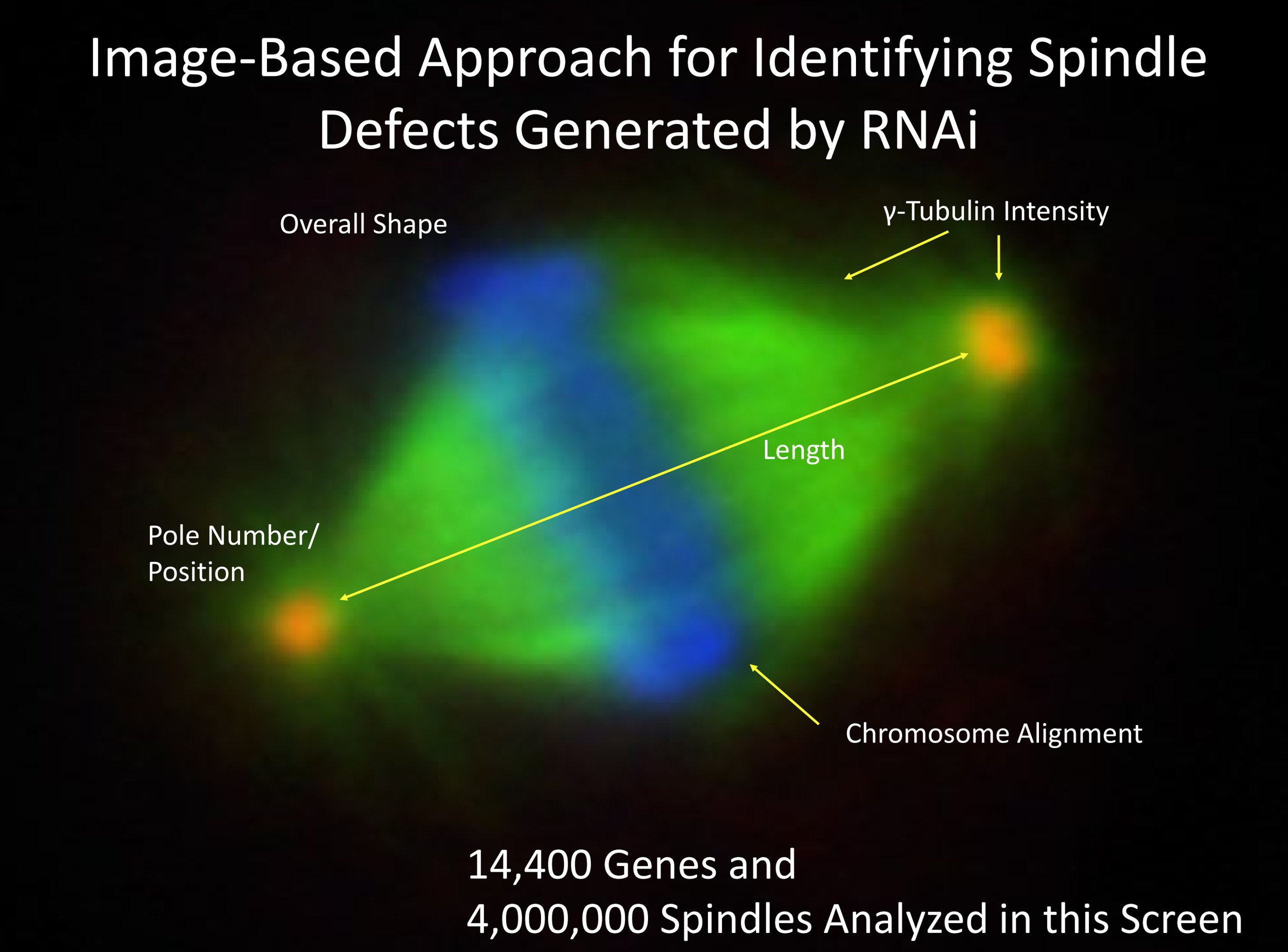
Ron Vale

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

Roy Wollman,
Jon Scholey (UC Davis)

Goshima et al. Science, 417 316 (2007)
Wollman and Stuurman. J. Cell Sc. 120 3715 (2007)

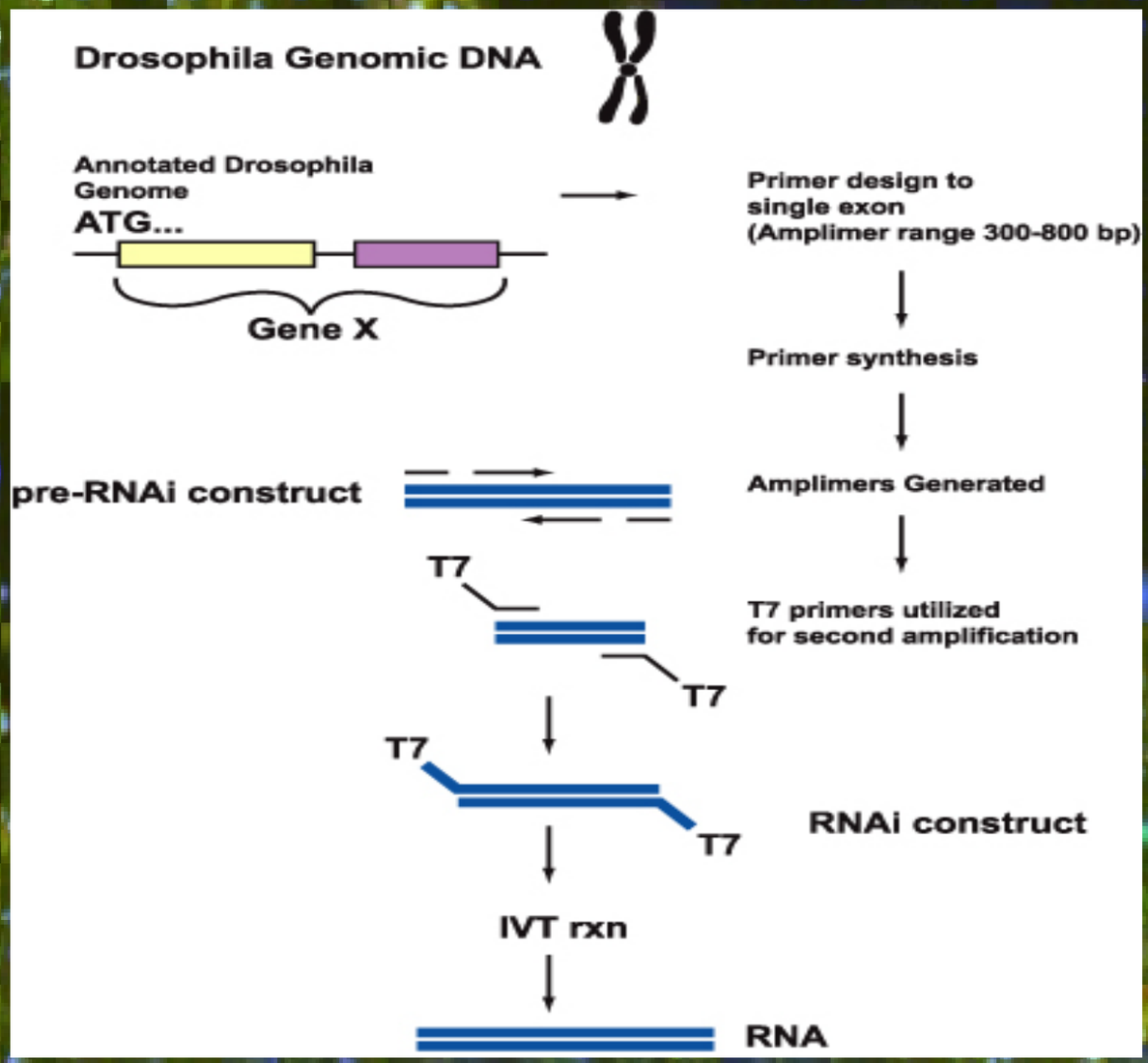
Image-Based Approach for Identifying Spindle Defects Generated by RNAi



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

High-throughput RNAi Screen

1. Full Fly Genome dsRNA Library:



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

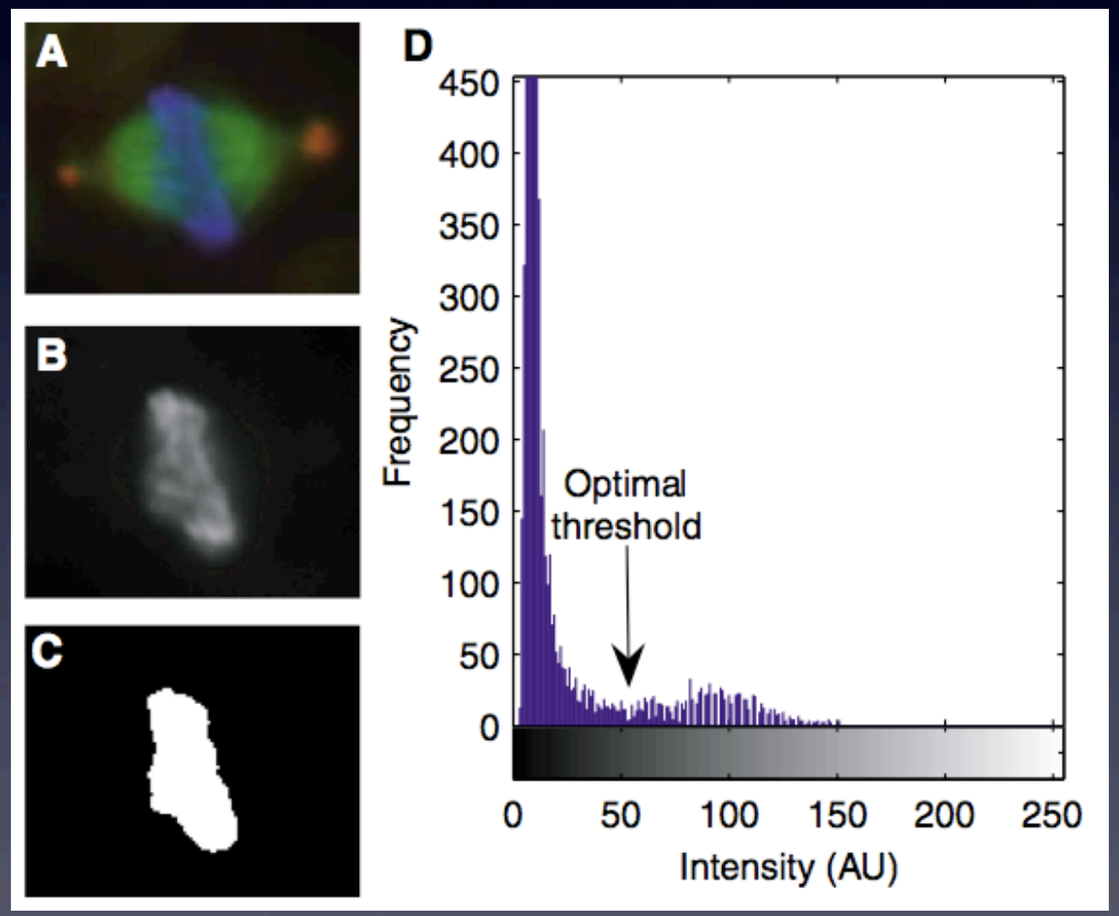
High-throughput RNAi Screen

3. High-throughput Microscopy Images

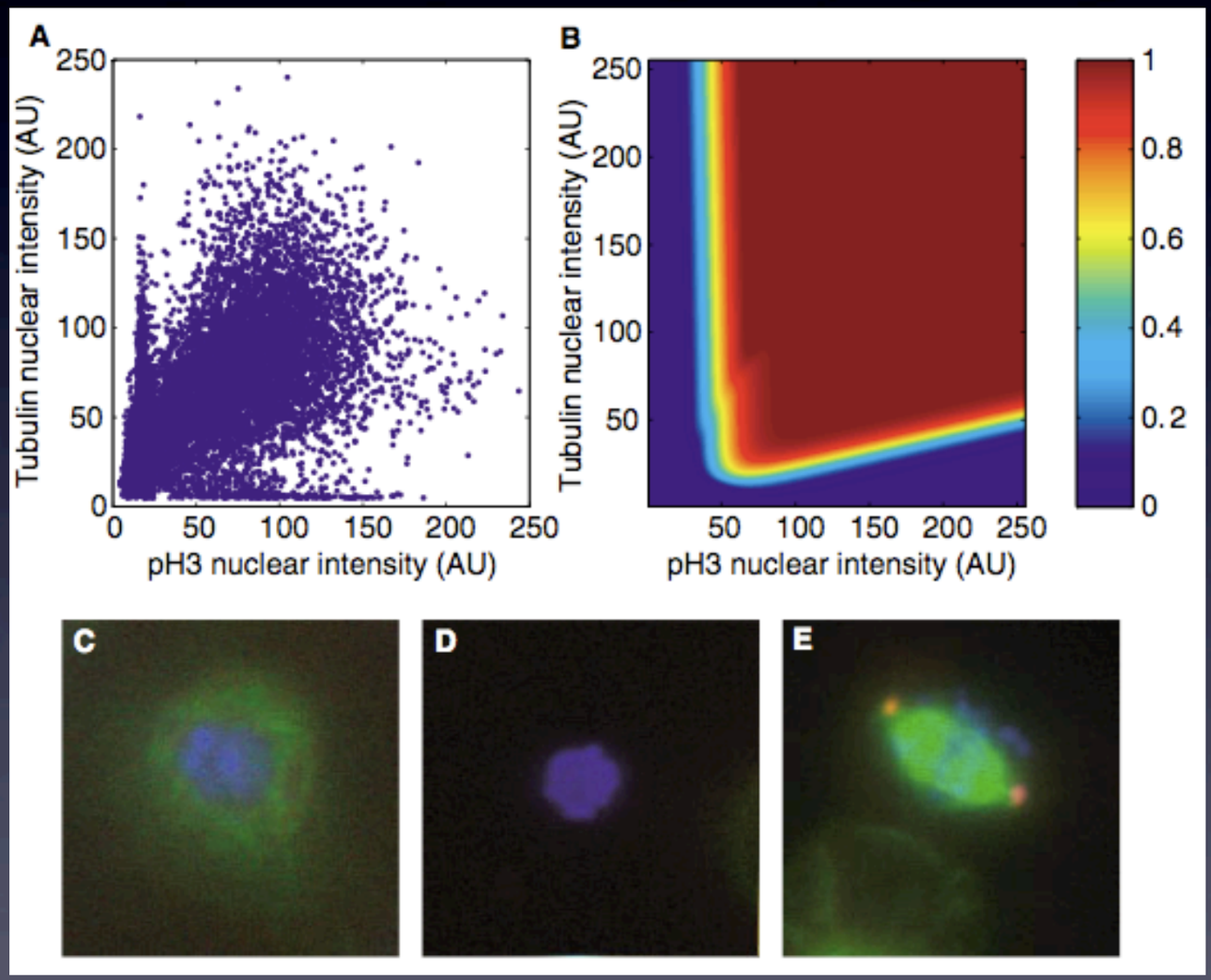
30-70 sites
8-bit BMP!
~25GB/plate
4TB total

High-throughput RNAi Screen

4. Automatic detection of metaphase cells



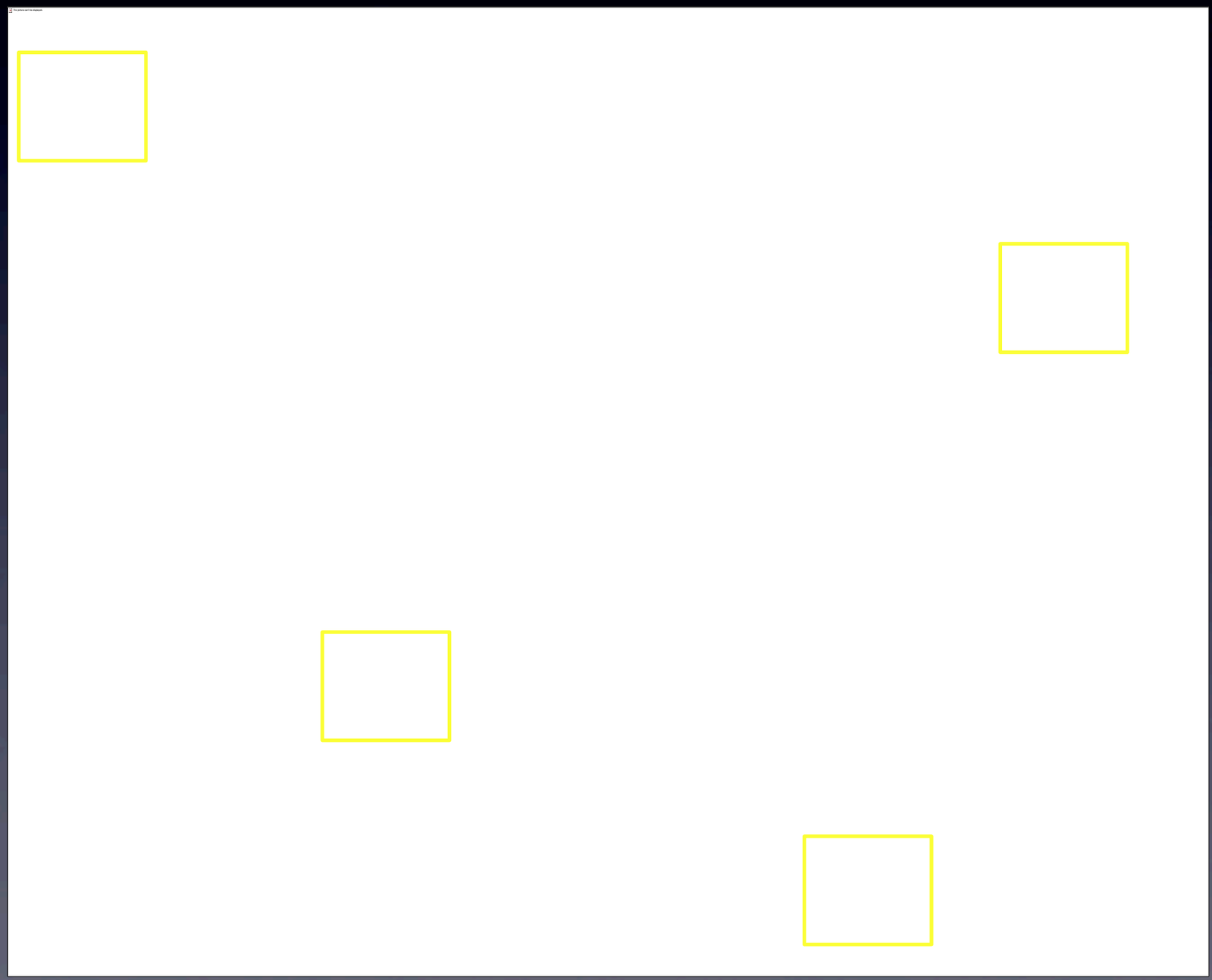
Automatic segmentation using Otsu's method



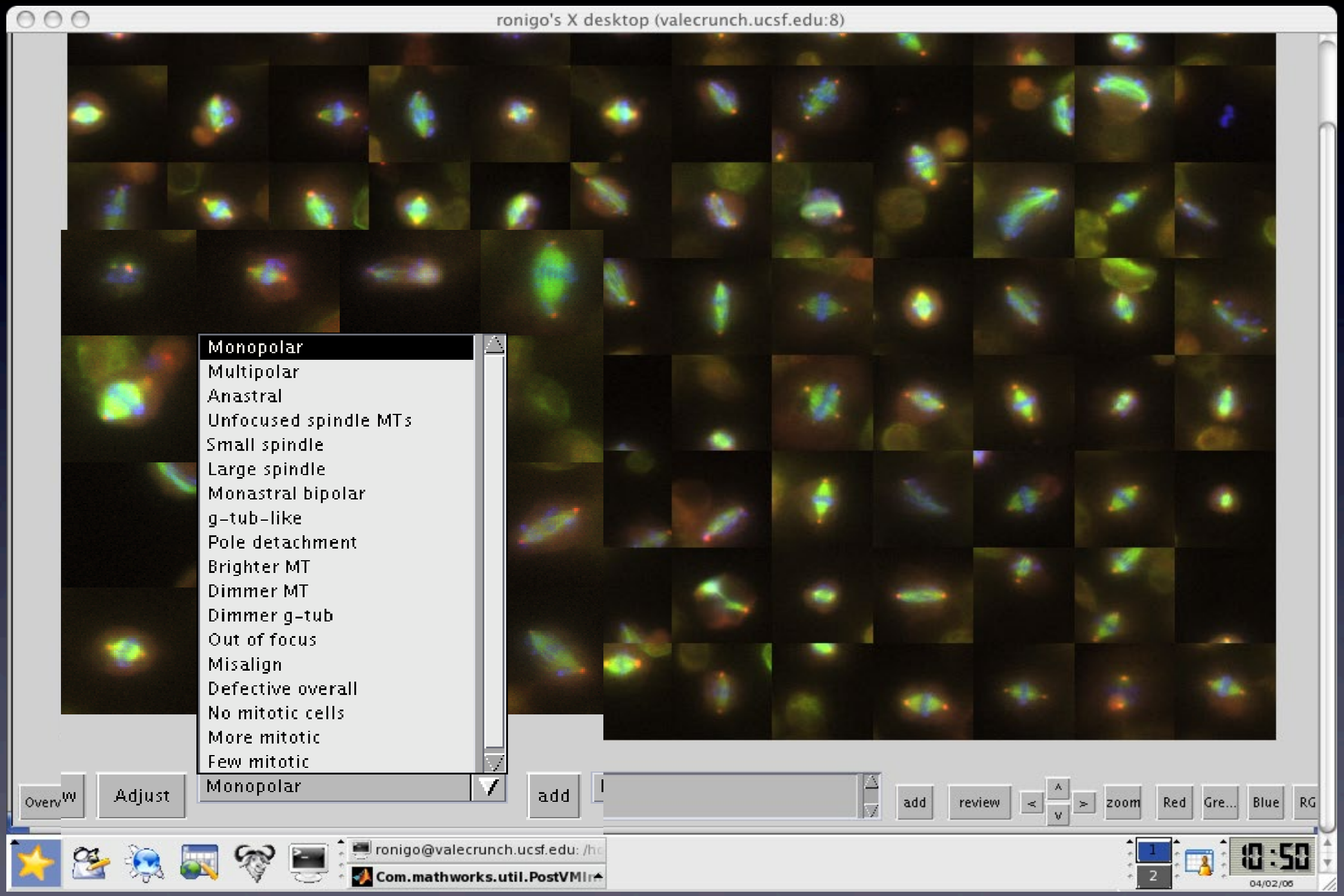
Classification using a trained neural network

High-throughput RNAi Screen

4. Automatic detection of metaphase cells



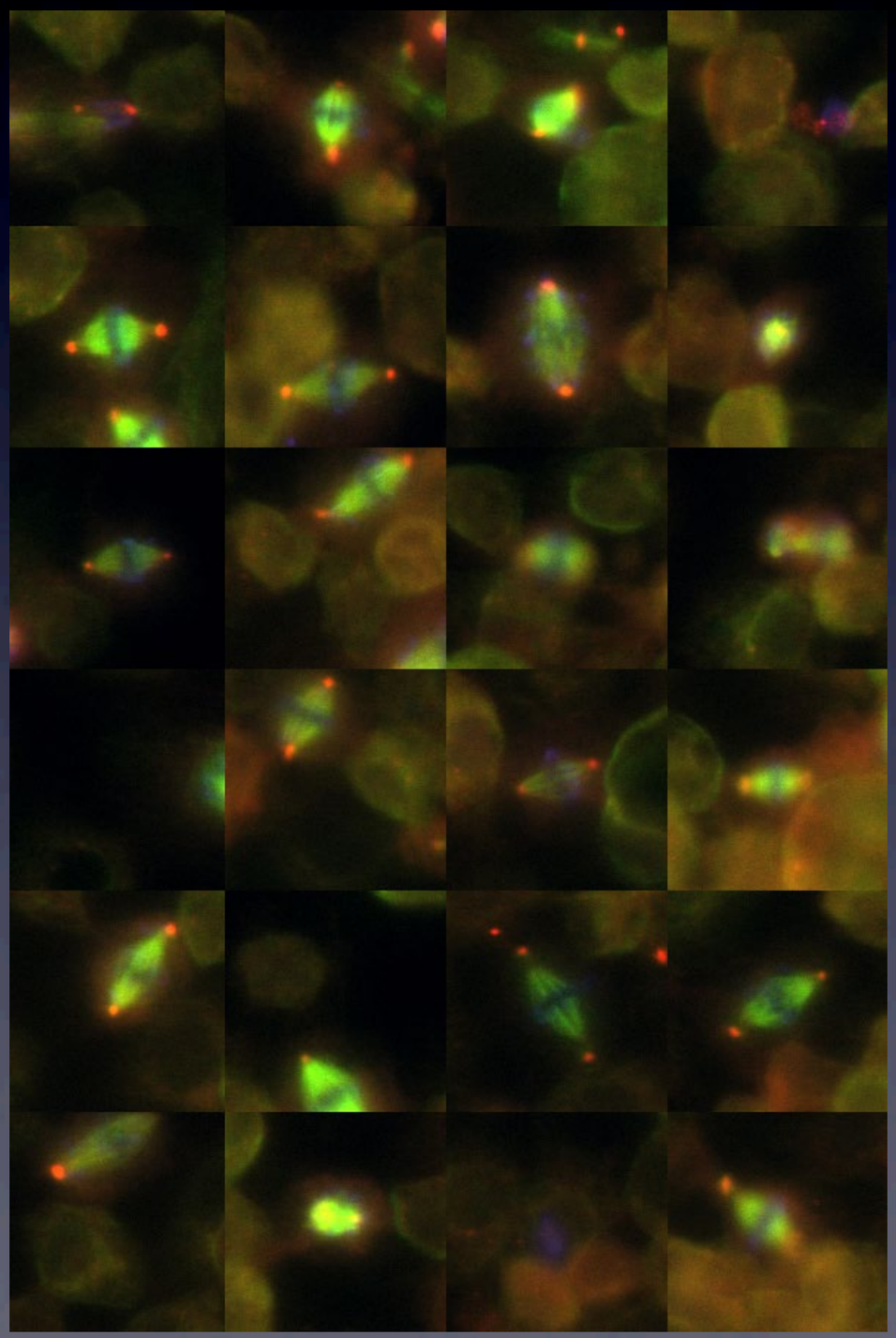
Analysis 1:
Galleries of Spindles (~200 per gene)
Scored Visually



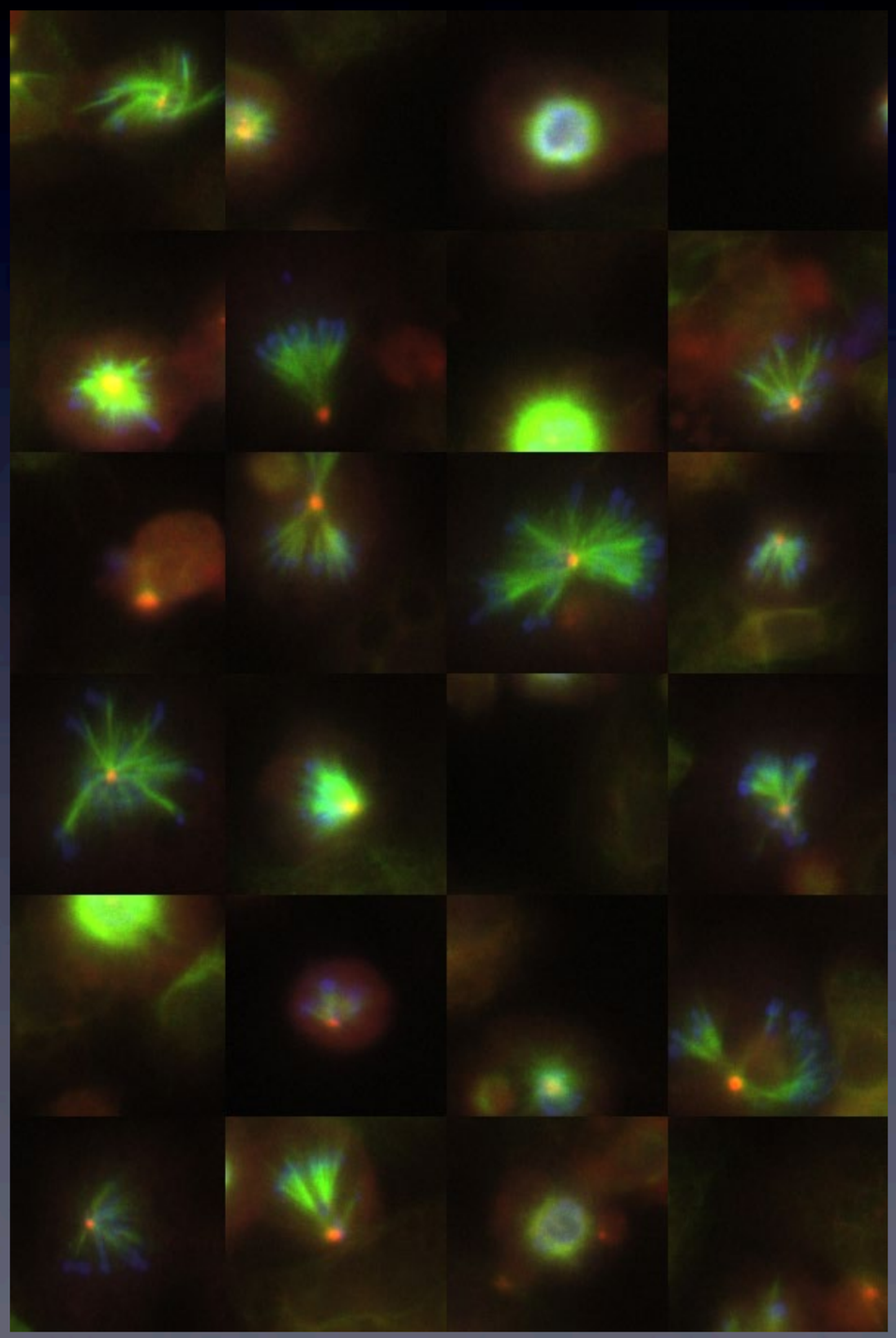
Side-by-side comparison of spindles made visual phenotype screening possible!

Example

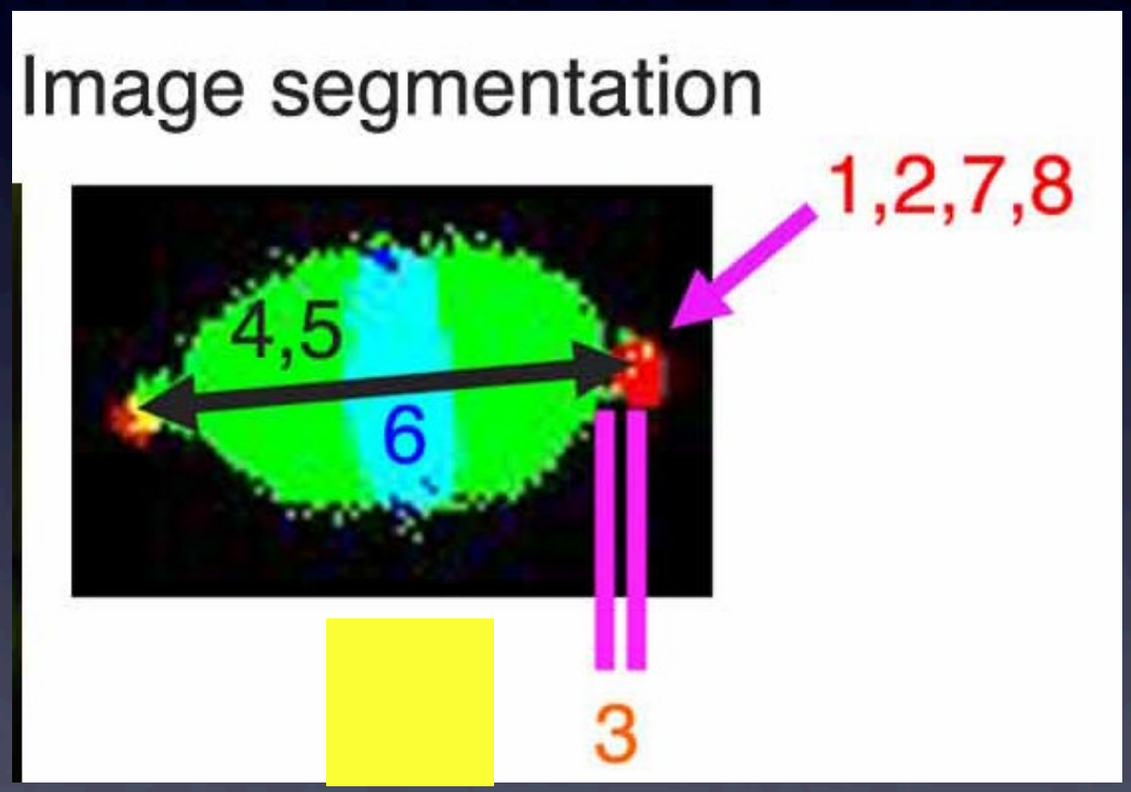
Control



Monopolar spindle (Kinesin-5/Klp61F)

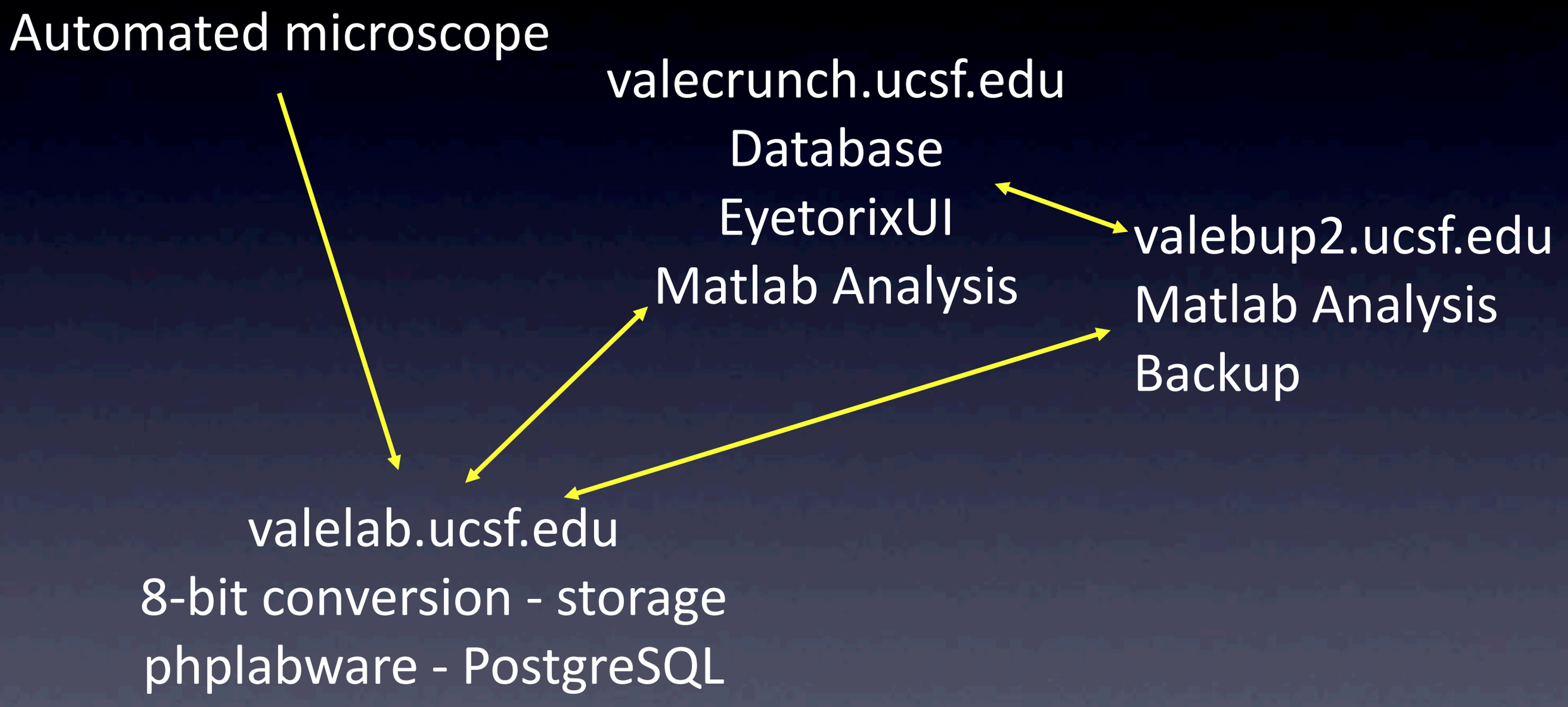


Analysis 2:
Image Segmentation and Computational Analyses



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

Workflow


Automated microscope
valecrunch.ucsf.edu
Database
EyetorixUI
Matlab Analysis
valebup2.ucsf.edu
Matlab Analysis
Backup
valelab.ucsf.edu
8-bit conversion - storage
phplabware - PostgreSQL

Vale Lab Screen Data Base

(http://rnai.ucsf.edu)

PhpLabware: MitoSpindleScreen

https://valelab.ucsf.edu/phplabware/general.php?tablename=mitospindle&md=&viewid=65&reportlinks=&reportoutput=1&num_p_r=++++96&searchj=&title=&sym=&cg=&plate=129&row=&columnplate=&rnaiprobe=&repeats=&ce

August 1, 2006, 7:59 pm — PhpLabWare version 0.6

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

Add Record | Now Viewing Table: **MitoSpindleScreen** (to edit mode) | View: Gohta | Edit views | Import Data

Report: ---Reports--- Send to: screen file Edit reports | 95 Records found. Showing 1 through 95. | 96 Records per page

all none reset: Misalign, --Clear, --Likely, --Weak

all none reset: multipolar, gtub area, monopolar, short spindles

Search | Show All

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								119.71	9.43%								
								96.02	9.79%								
								140.21	8.47%								
								144.57	8.23%								
yell								73.62	7.21%								
								72.67	4.88%								
								88.19	10.88%								
								98.67	7.55%								
lethal								91.90	8.89%								
								97.05	8.59%								
								101.95	9.90%								
								99.76	8.88%								
								71.93	7.55%								
								87.88	8.51%								
								74.88	9.70%								
	CG					probe info		76.07	11.86%								
	Ravus	15669	129	C	1	probe info		77.60	7.46%								
Suppressor of variegation 3-7	Su(var)3-7	8599	129	C	2	probe info		81.07	7.40%								
	CG15888	15888	129	C	3	probe info		82.71	6.30%	No	No				1 2		
	CG15887	15887	129	C	4	probe info		85.48	8.04%	No	No				1 2 3		

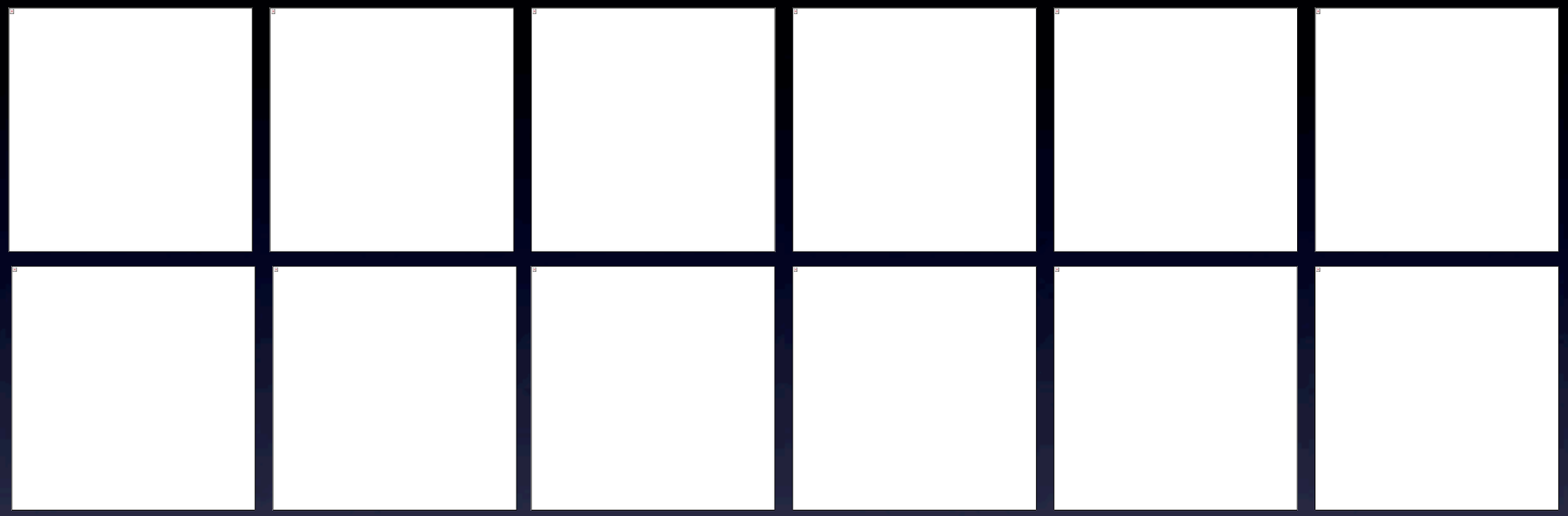
Link to dsRNA information

Link to phenotype gallery

Done — valelab.ucsf.edu

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)

The picture can't be displayed.

Lookup Tables (LUTs)