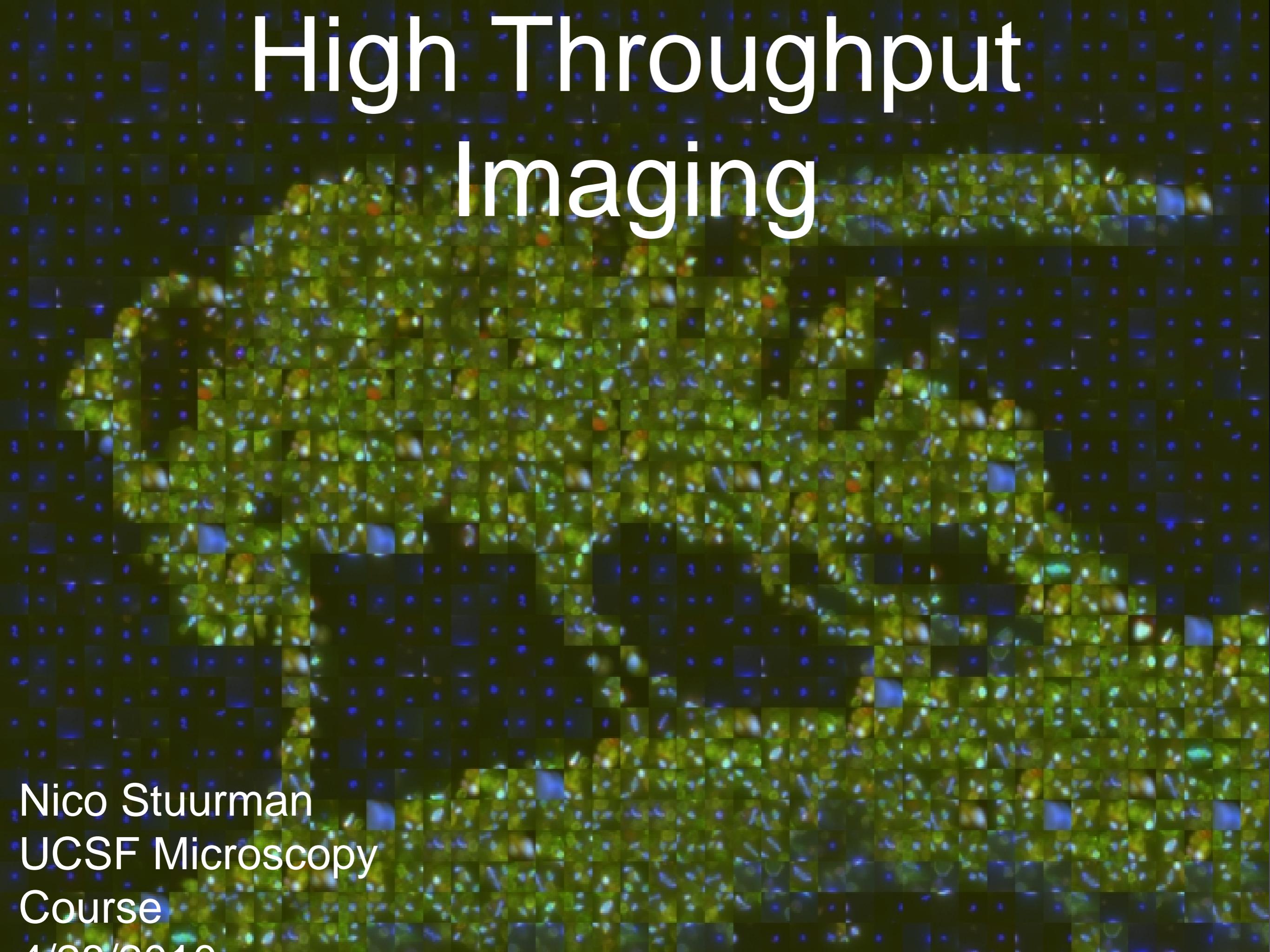


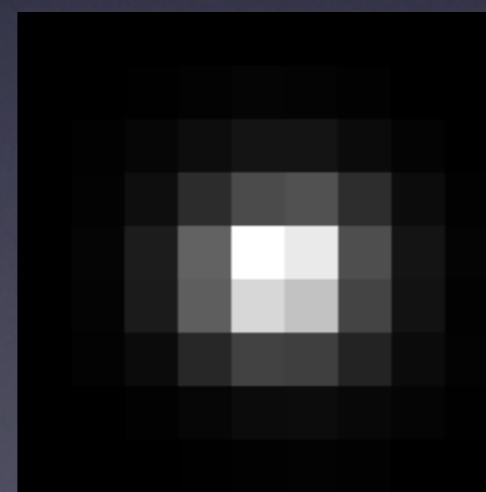
# High Throughput Imaging



Nico Stuurman  
UCSF Microscopy  
Course  
1/22/2012

# What is a digital Image?

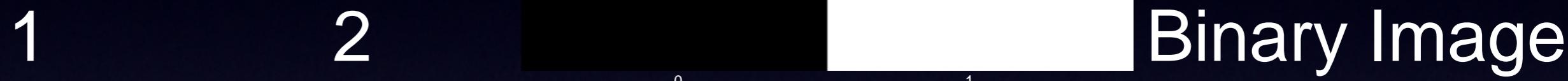
Many measurements of photon flux



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



# Bit-depth and resolution



8-bit



4-bit



6-bit



2-bit

# Bit depth and dynamic range

bit. bits

range

1

2

2

4

4

16

8

256

12

4096

16

65536

0 1

0 1 2 3

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

15

Binary Image

Grayscale  
Images

Display Inten

0

Digital Number

255 0

255

255

Histogram:

Nr. pixels with specific DN

0

255

255



play

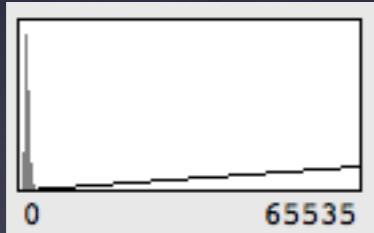
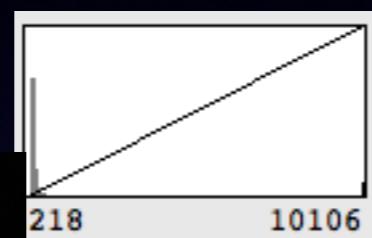
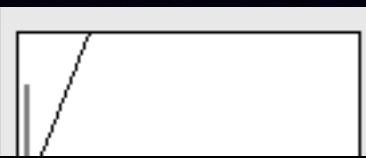
# Mapping values onto display: Brightness/contrast



Slope = contrast

Brightness

# contrast



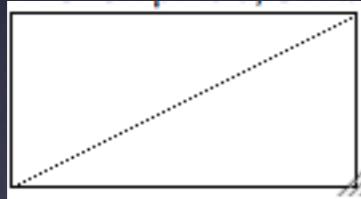
Full Range

Auto-Scale

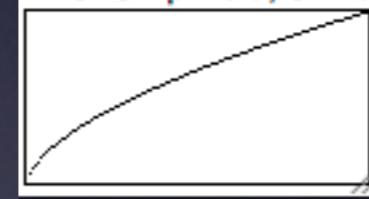
High B/C

Be aware of software Auto-scaling for you!

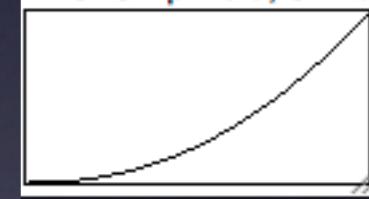
# Gamma adjustment



1



0.6



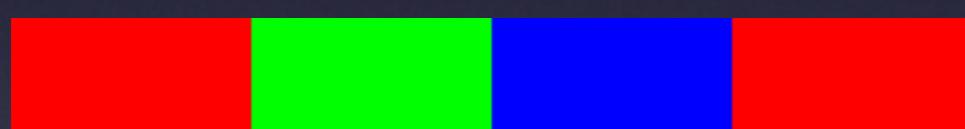
2.2

# What are acceptable image manipulations?

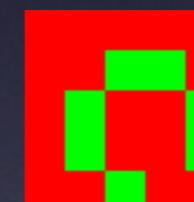
- JCB has the best guidelines
  - <http://jcb.rupress.org/content/172/1/9.full>
  - <http://jcb.rupress.org/content/166/1/11.full>
- Brightness and contrast adjustments ok, so long as done over whole image and don't obscure or eliminate background
- Nonlinear adjustments (like gamma) must be disclosed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

# Lookup Tables (LUTs)

0    1    2    3

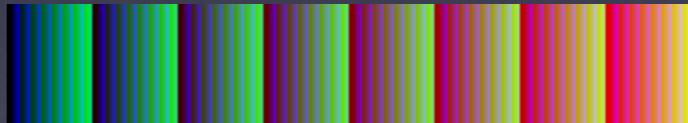
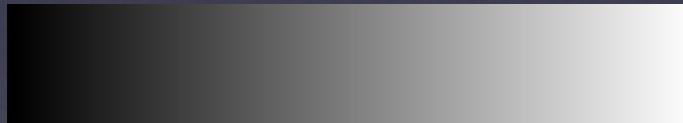


0	0	0	0	0
0	0	1	1	0
0	1	3	3	1
0	1	3	3	1
0	0	1	0	0



# Lookup Tables (LUTs)

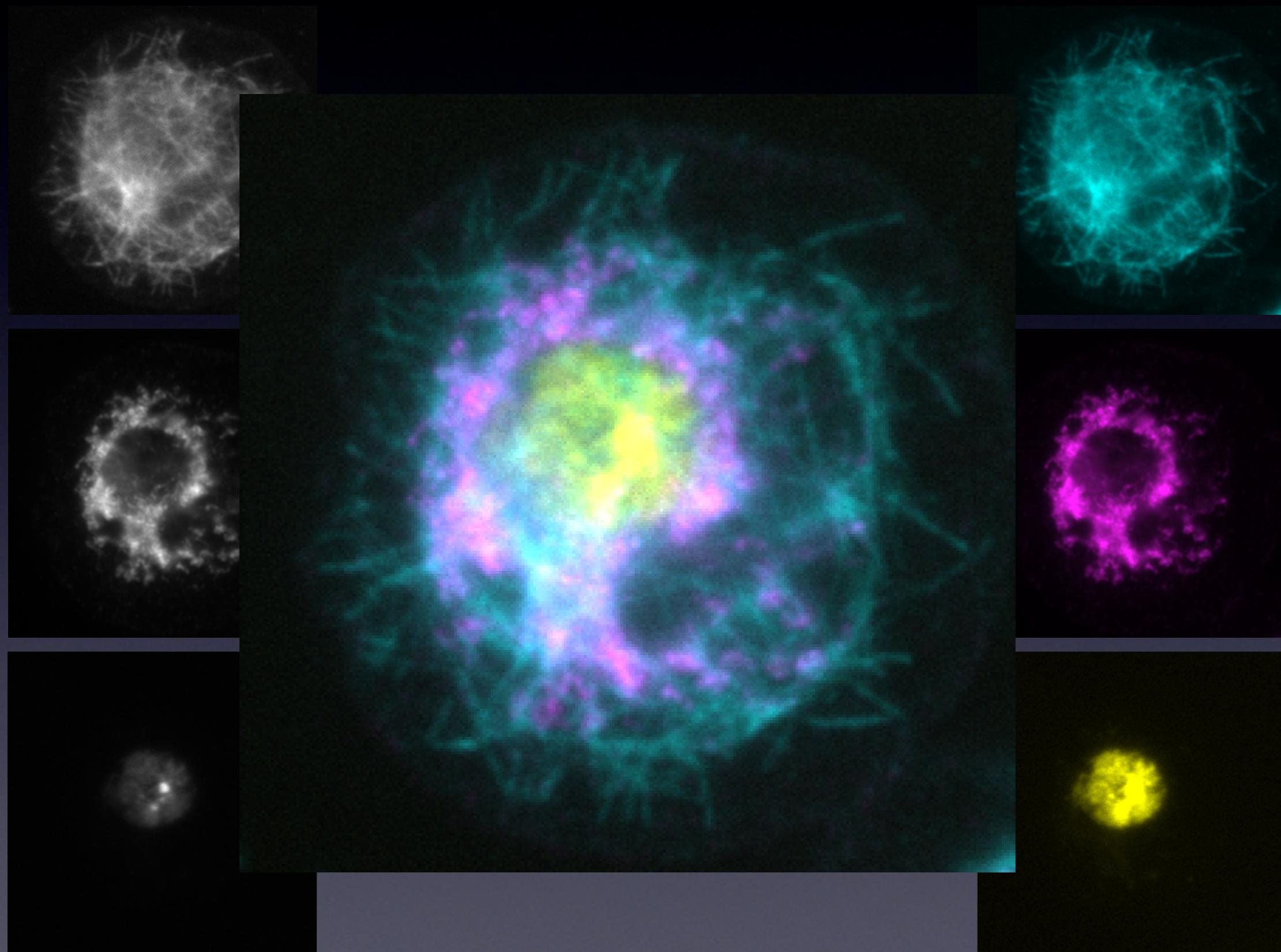
QuickTime™ and a  
MPEG-4 Video decompressor  
are needed to see this picture.



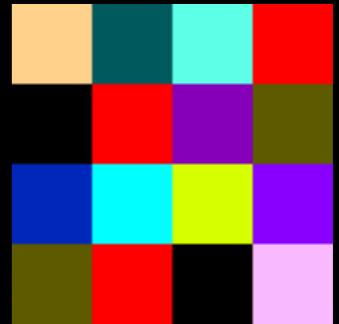
QuickTime™ and a  
MPEG-4 Video decompressor  
are needed to see this picture.

QuickTime™ and a  
MPEG-4 Video decompressor  
are needed to see this picture.

# Lookup Tables (LUTs)



# Color Images



Either:

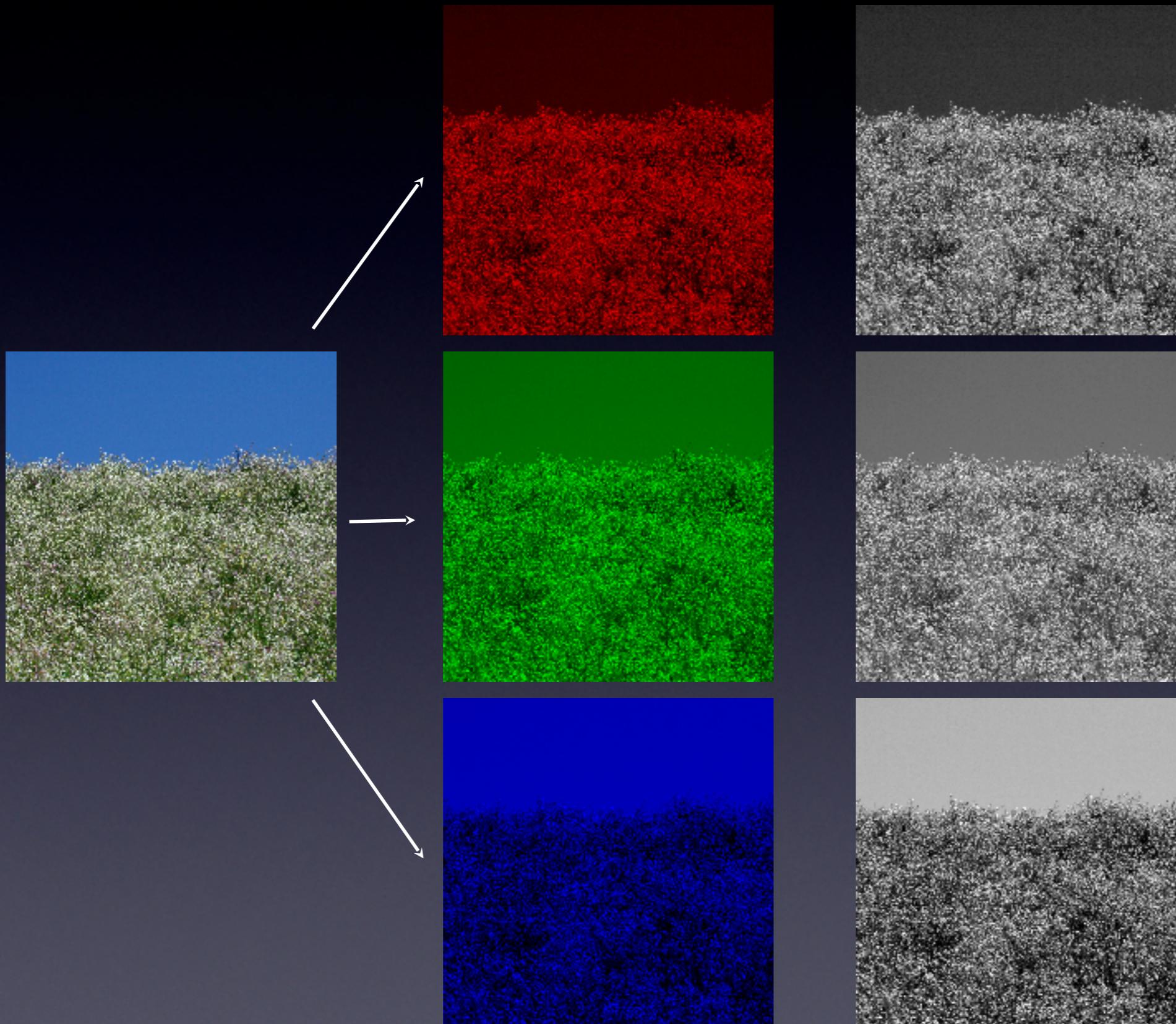
255	209	139	0	89	93	93	255	231	255	0	0
0	0	0	255	0	0	134	0	185	93	90	0
0	39	185	0	255	255	214	255	0	137	0	255
93	90	0	255	0	0	0	0	0	249	185	255

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  
Sequences of images

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



QuickTime™ and a decompressor are needed to see this picture.

# 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:

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

Original data can be restored



Compression

Loose original data!

## Lossless 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

## 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)

There are many!

OME-TIFF

The swiss pocket knife for microscopy image data format:

Bioformats: [http://www.loci.wisc.edu  
/software/bio-formats](http://www.loci.wisc.edu/software/bio-formats)

# 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/>
- Priism (Agard/Sedat labs) <http://msg.ucsf.edu/IVE/>
- CellProfiler <http://www.cellprofiler.org>

# Filters

## Neighborhood convolution

Kernel

1	1	1
1	1	1
1	1	1



Simple Smoothing



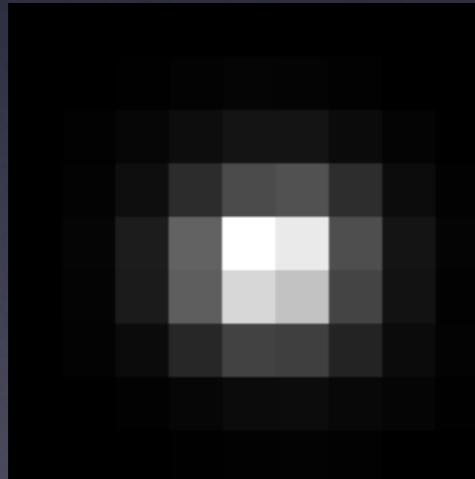
1	1	113	1	215	1	0	0	0	0
1	113	1	1	215	1	0	0	0	0
1	113	1	1	215	0	0	0	0	0
0	113	0	0	0	255	0	0	0	0
113	0	0	0	0	255	0	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	255	0	0	0
0	113	0	0	0	0	0	255	0	0
0	113	0	0	0	0	0	255	0	0

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



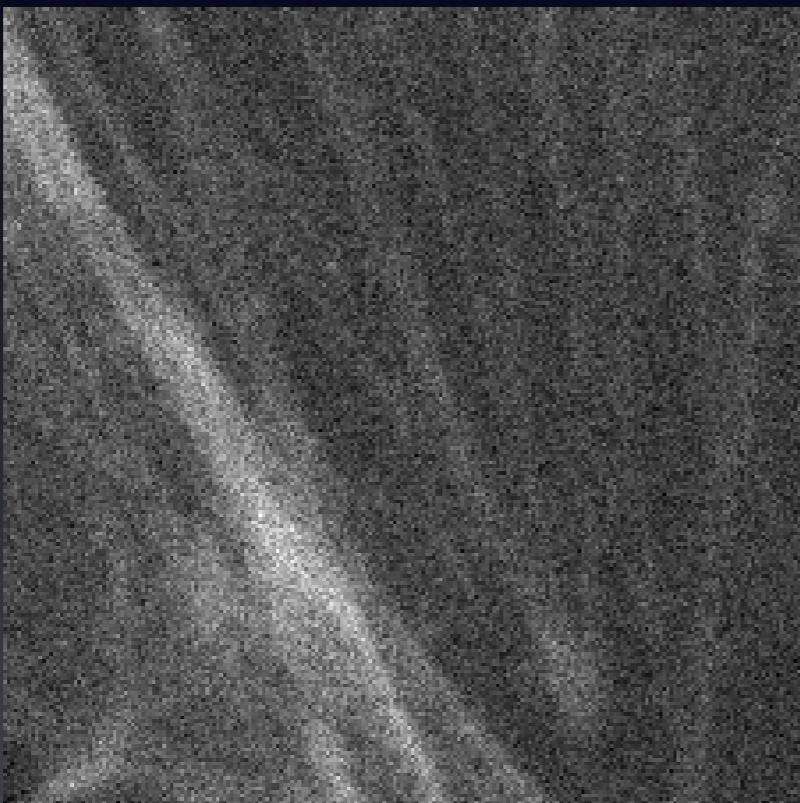
Measured PSF



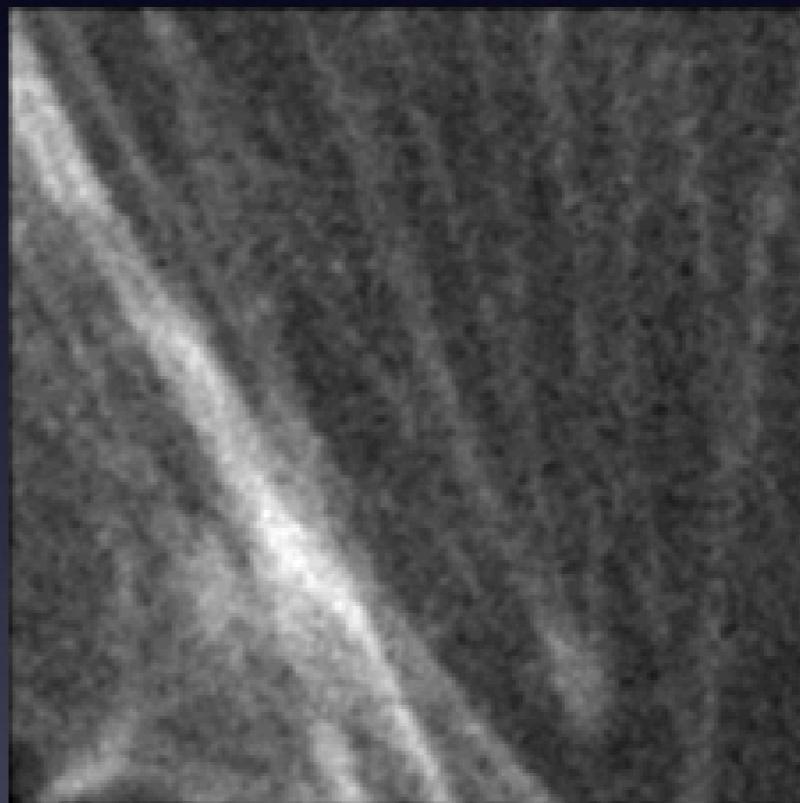
Gaussian Filter

# Why smooth?

- Averages redundancy and suppresses noise



10 photons/pixel average  
5 e- read noise



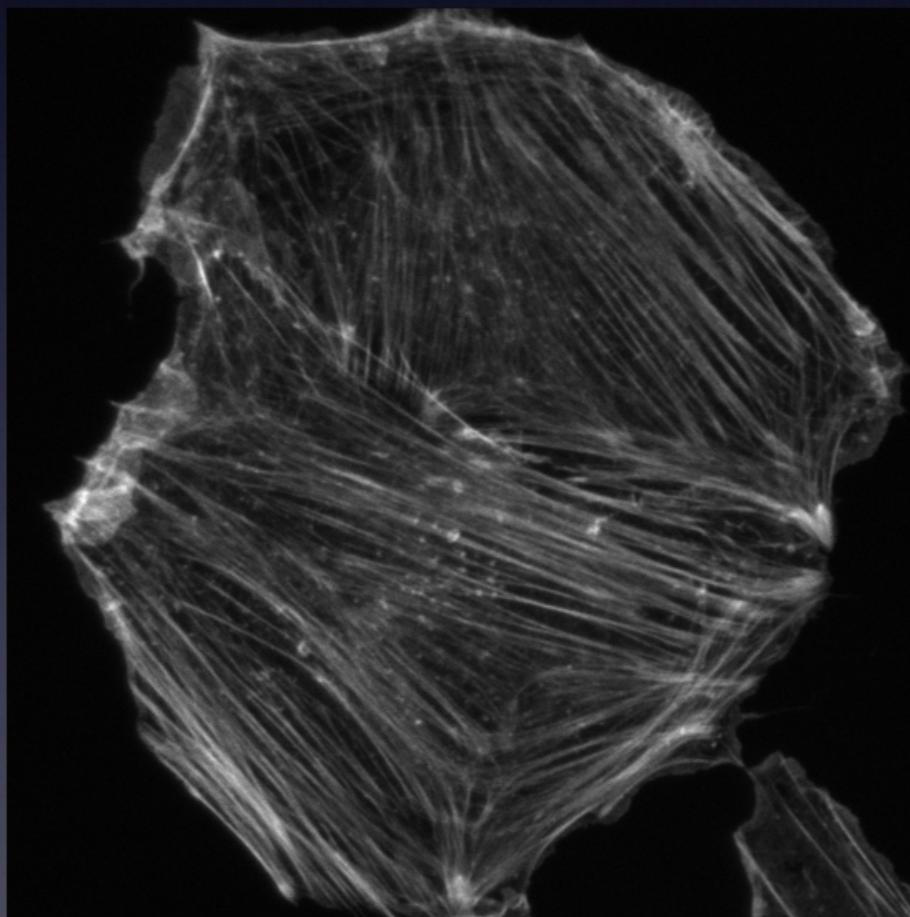
Gaussian smoothing  
filter,  $\sigma = 1$  pixel

# Other Filters

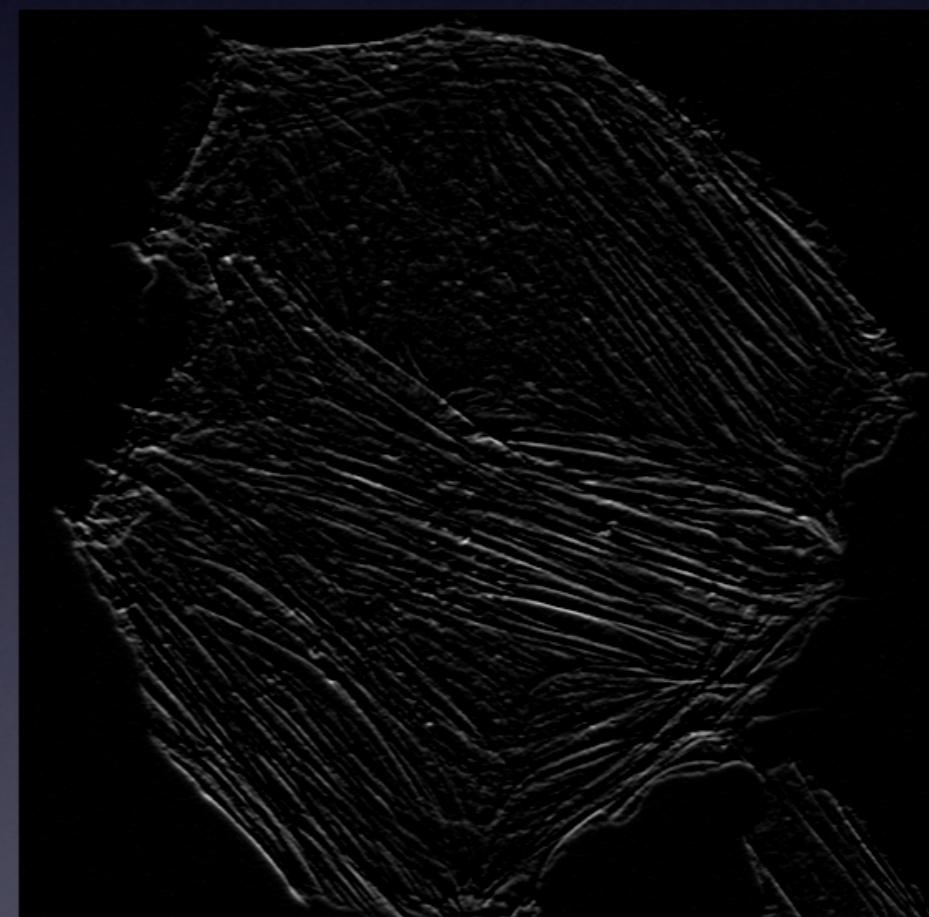
Edge Detection

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

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



Original

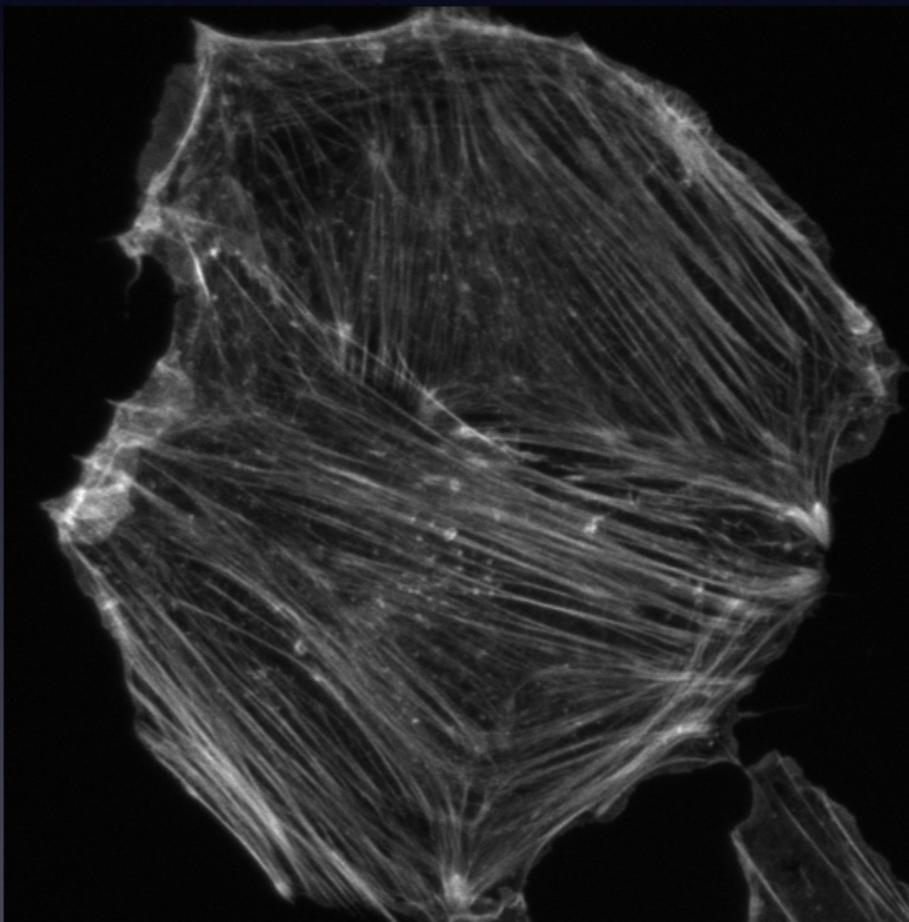


Horizontal edge  
detection

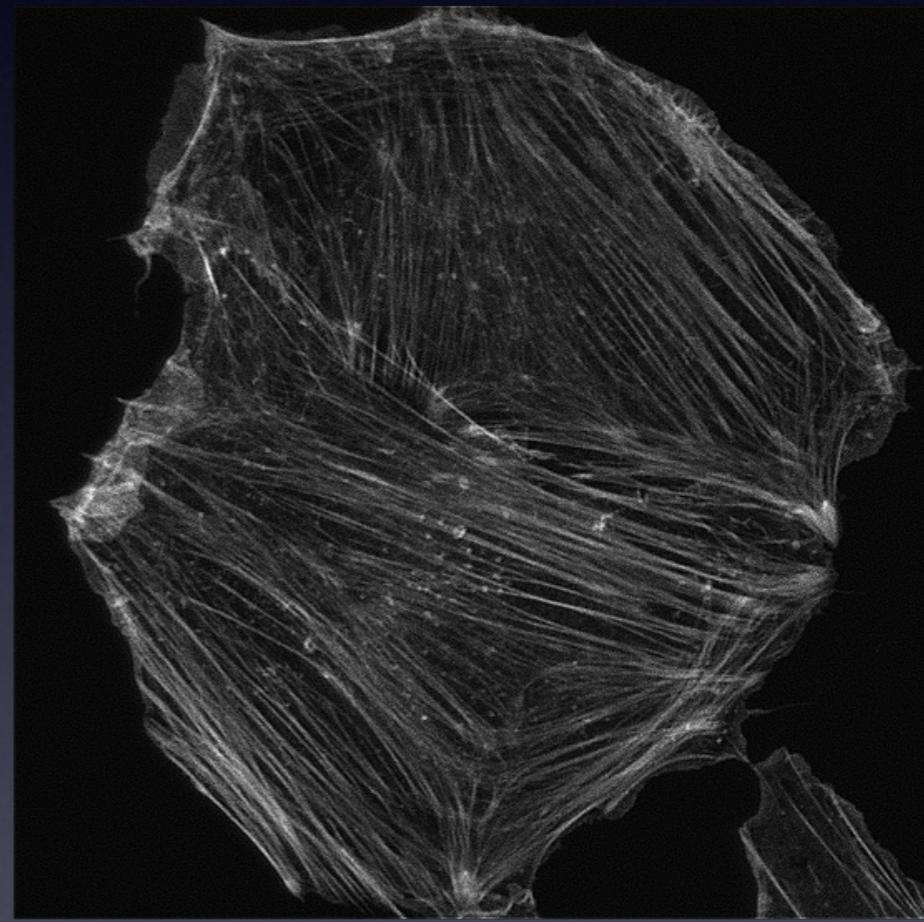
# Other Filters

## Unsharp Masking

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



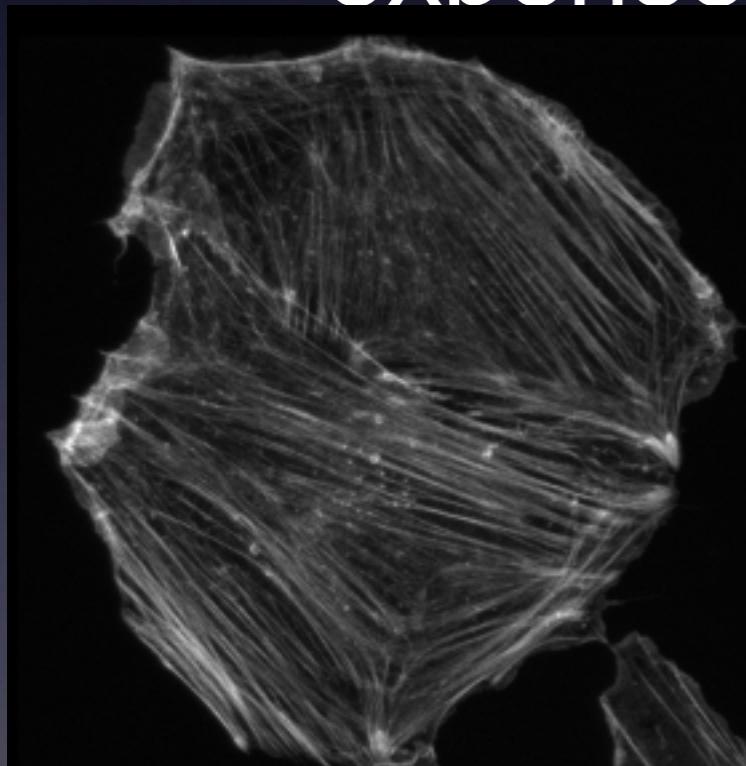
Original



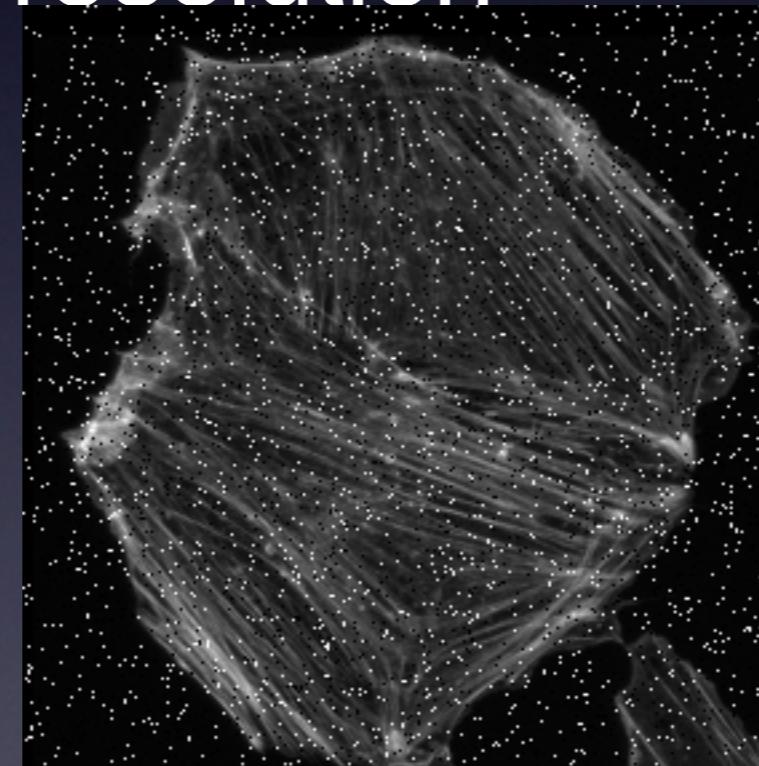
Unsharp masked

# Non-linear Filters

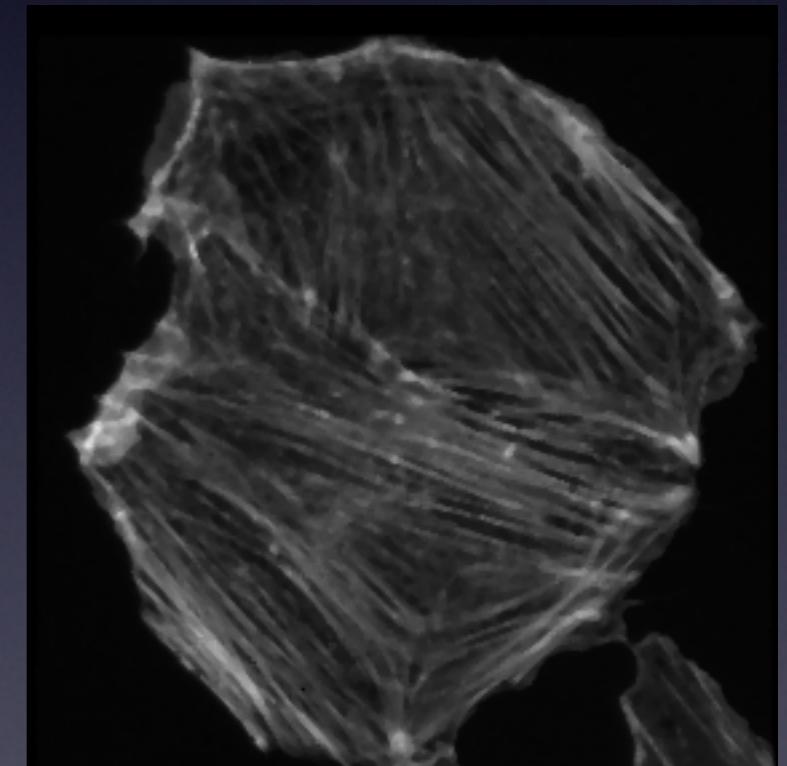
- Replace central pixel with min, max, median
- Median filter is a good noise filter, at the expense of resolution



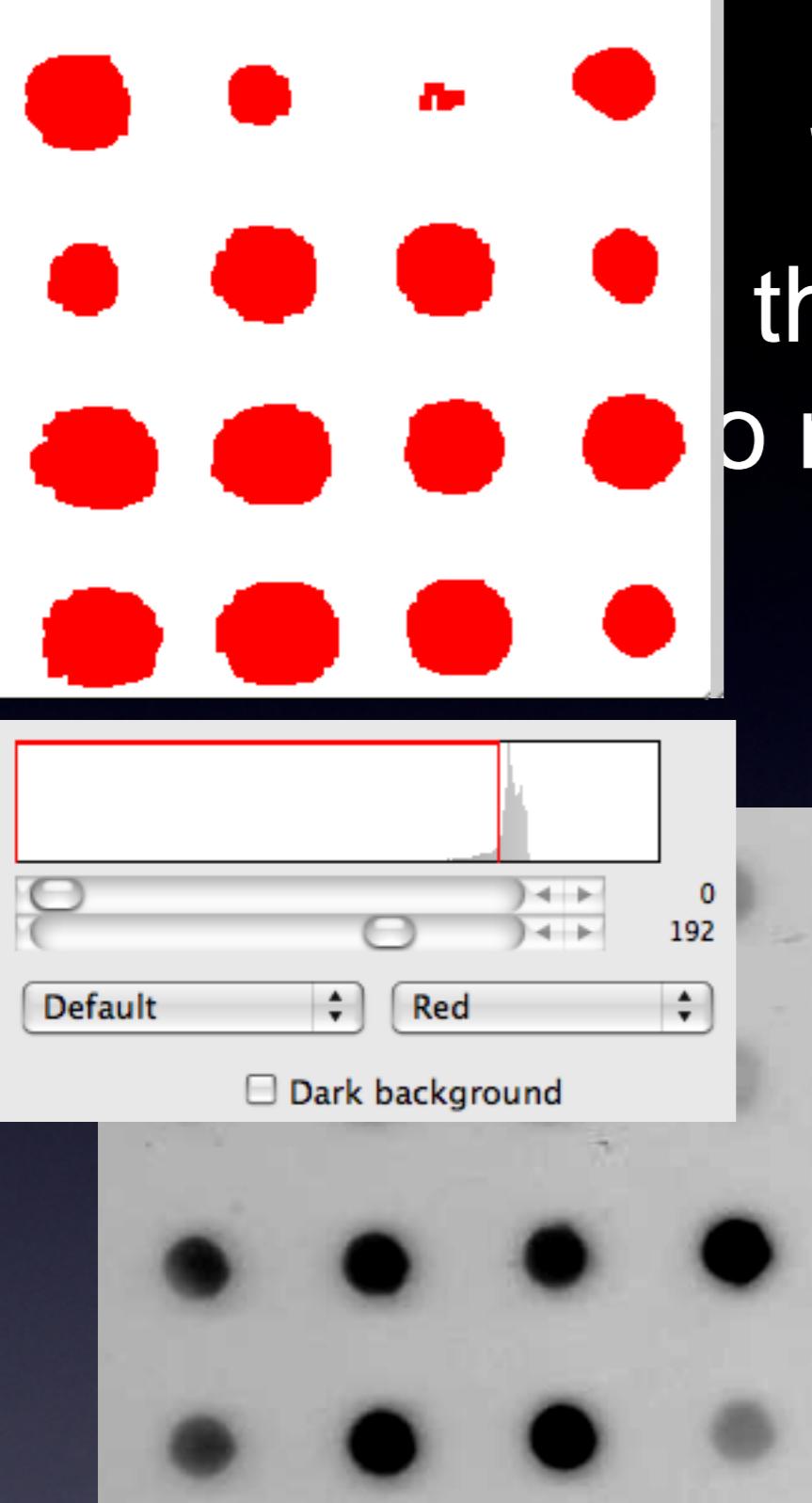
Original



Artificial Noise



Median Filter

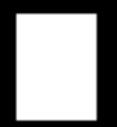


# Segmentation

the object that  
you want to measure?

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

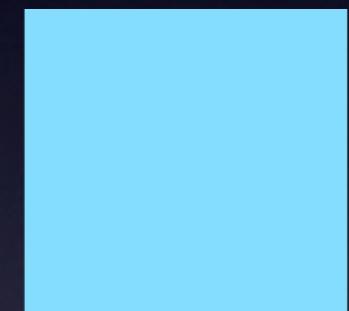
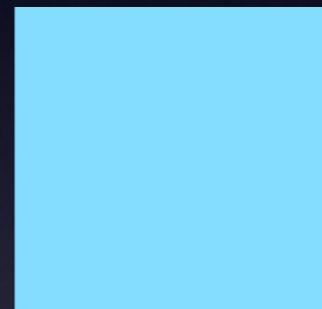
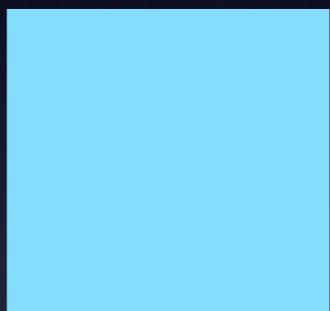
2x erosion  
2x dilation



# Binary Operations: Erosion/Dilation 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	0	1	1	1	0	0	0	0
0	0	1	1	1	1	1	0	0	0	0
0	0	0	0	1	1	1	1	0	0	0
0	0	0	0	1	1	1	0	0	0	0
0	0	0	0	1	1	1	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

Erosion

Dilation

# High-throughput Imaging

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

QuickTime™ and a  
MPEG-4 Video decompressor  
are needed to see this picture.

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

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

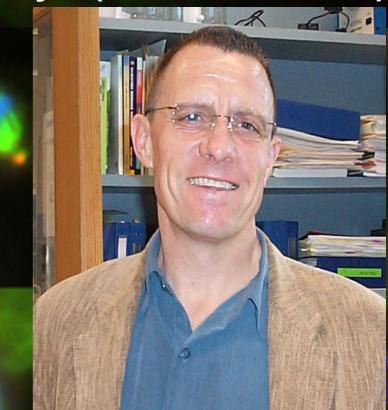


Ron Vale

Roy Wollman,  
Jon Scholey (UC Davis)

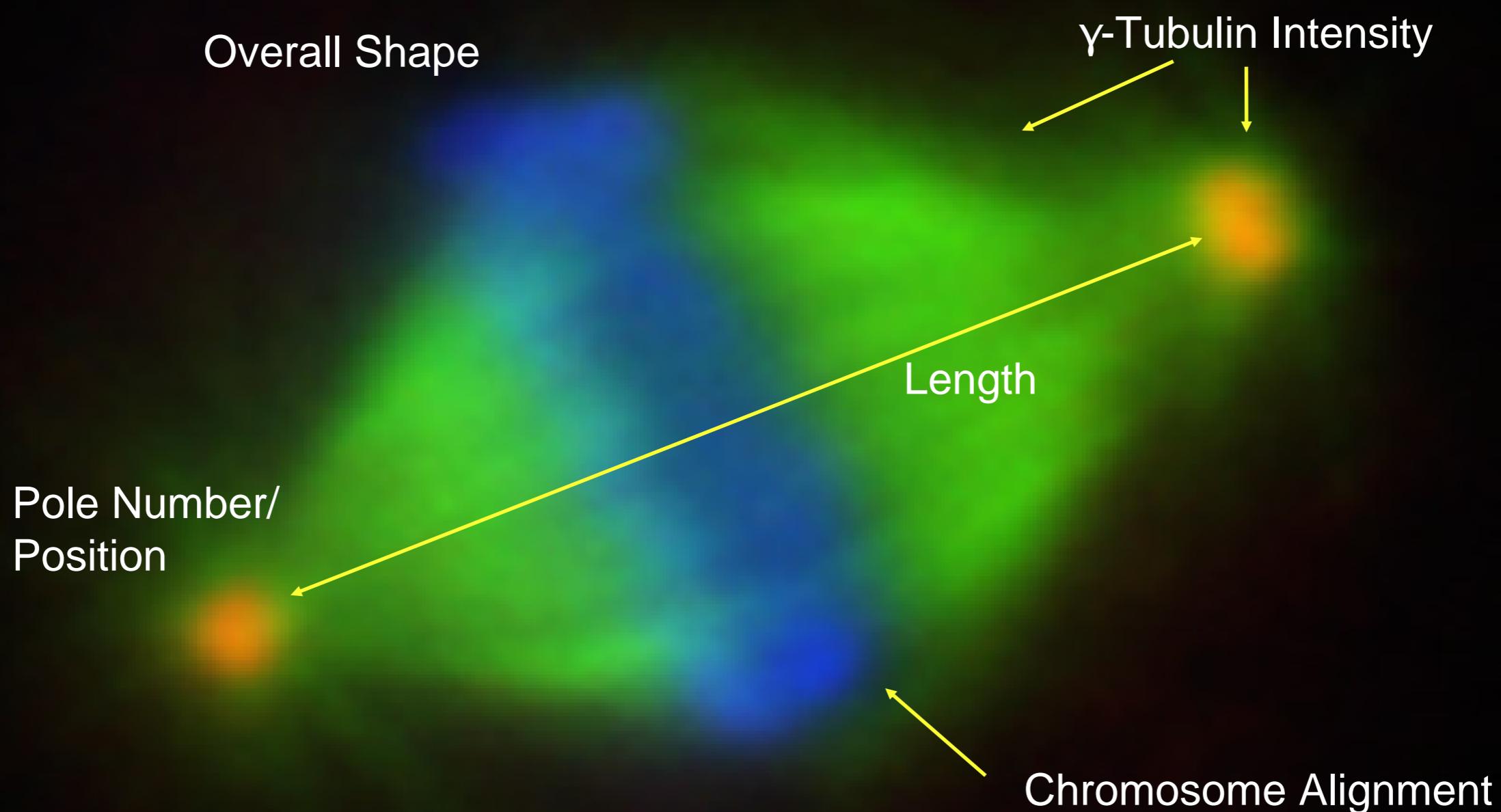


Gohta Goshima, Nico Stuurman, Nan Zhang, Sarah Goodwin (UCSF)



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

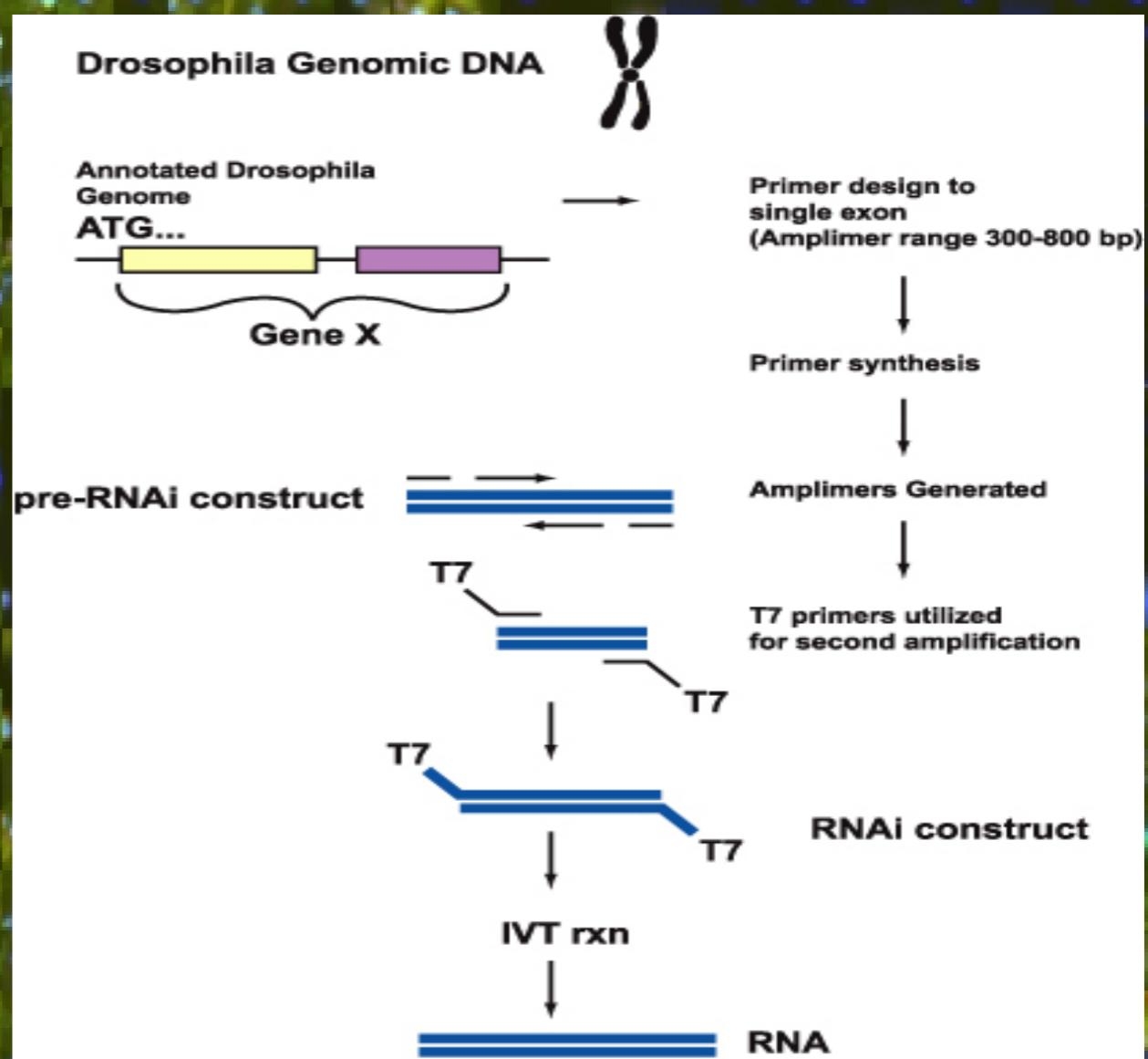
# Image-Based Approach for Identifying Spindle Defects Generated by RNAi



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 Content Screening

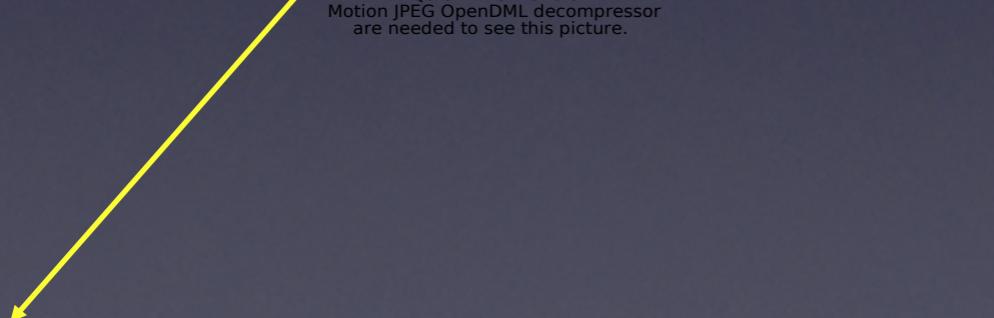
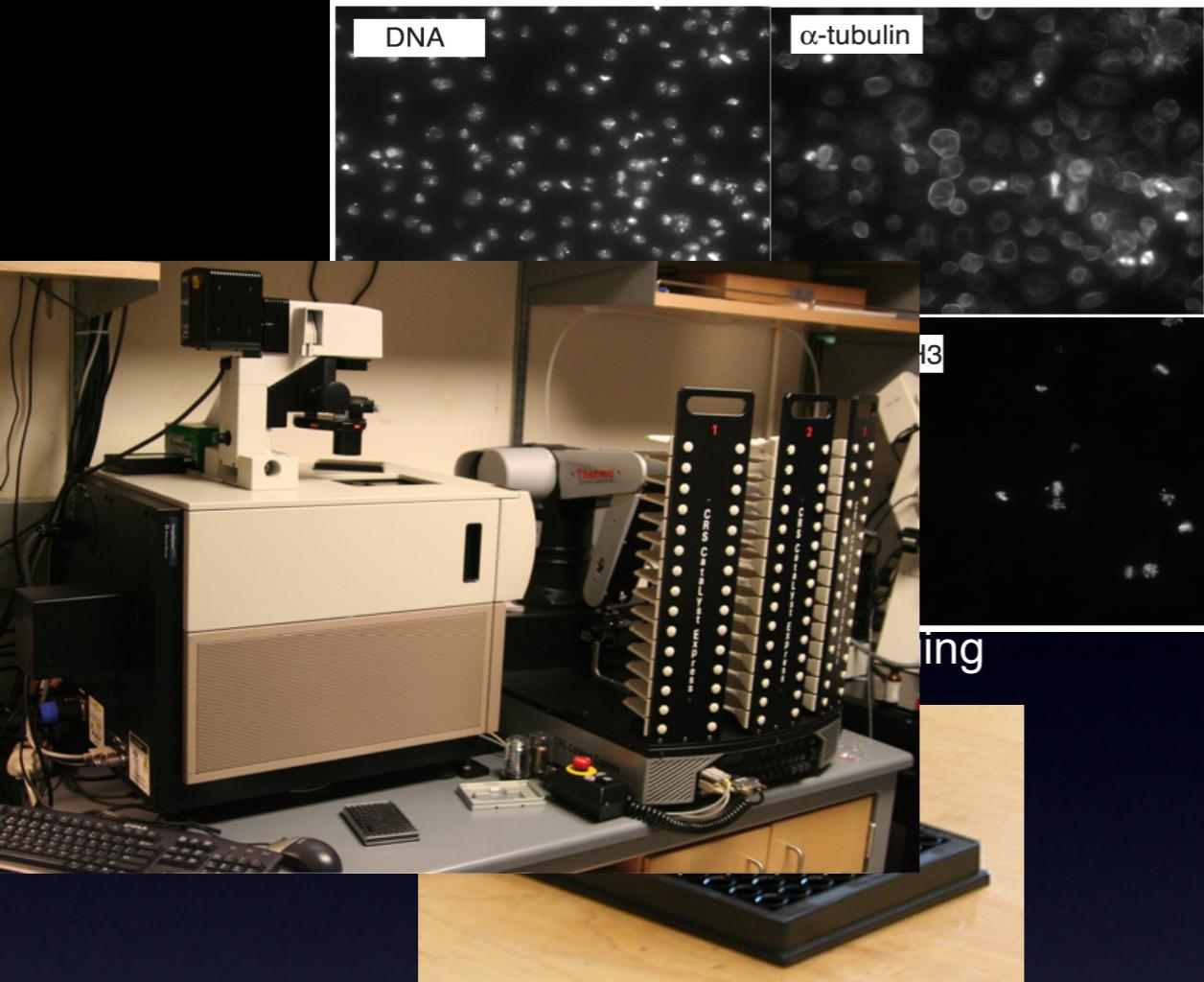
High Content  
Microscopy

## Autofocus

- Image-based
- Reflection-based

Fix & Stain  
and Image

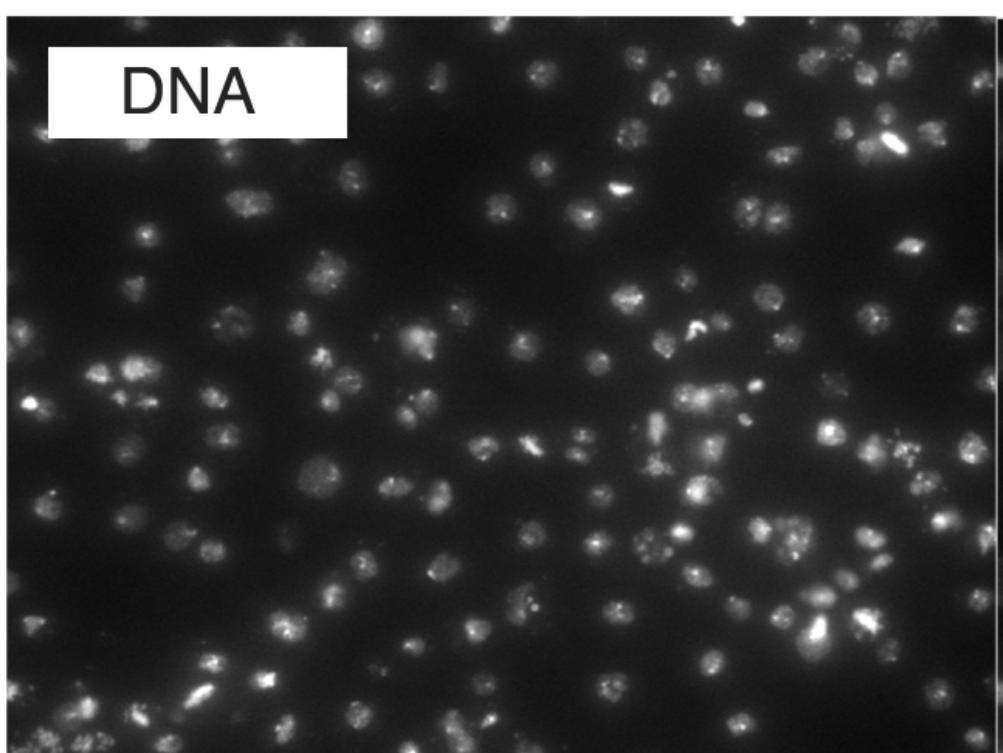
QuickTime™ and a  
Motion JPEG OpenDML decompressor  
are needed to see this picture.



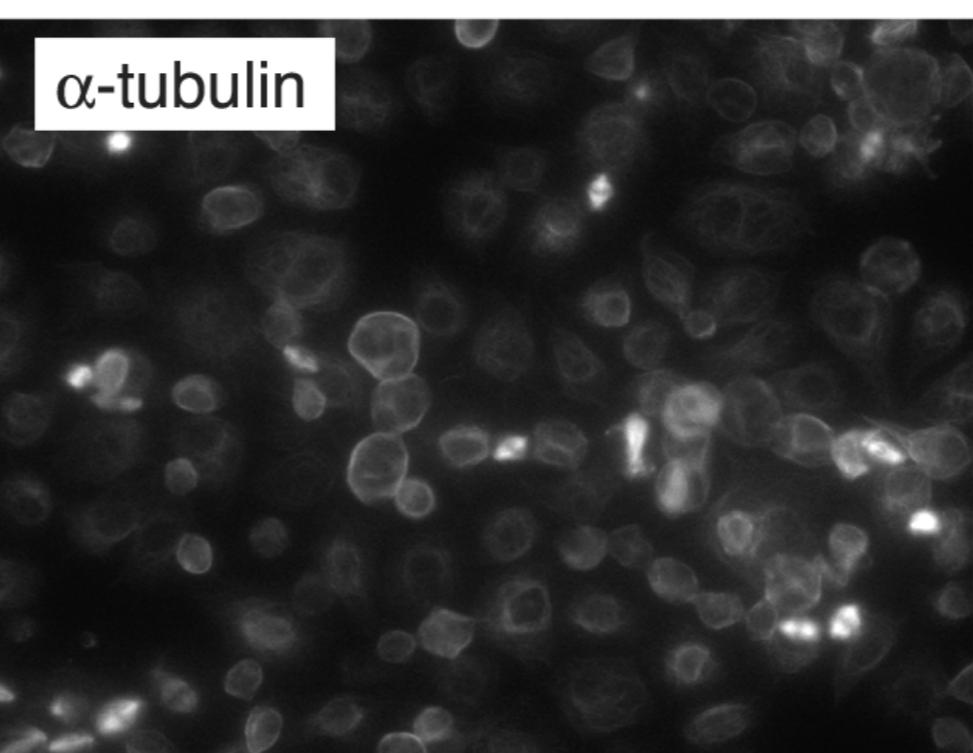
# High-throughput RNAi Screen

## 3. High-throughput Microscopy Images

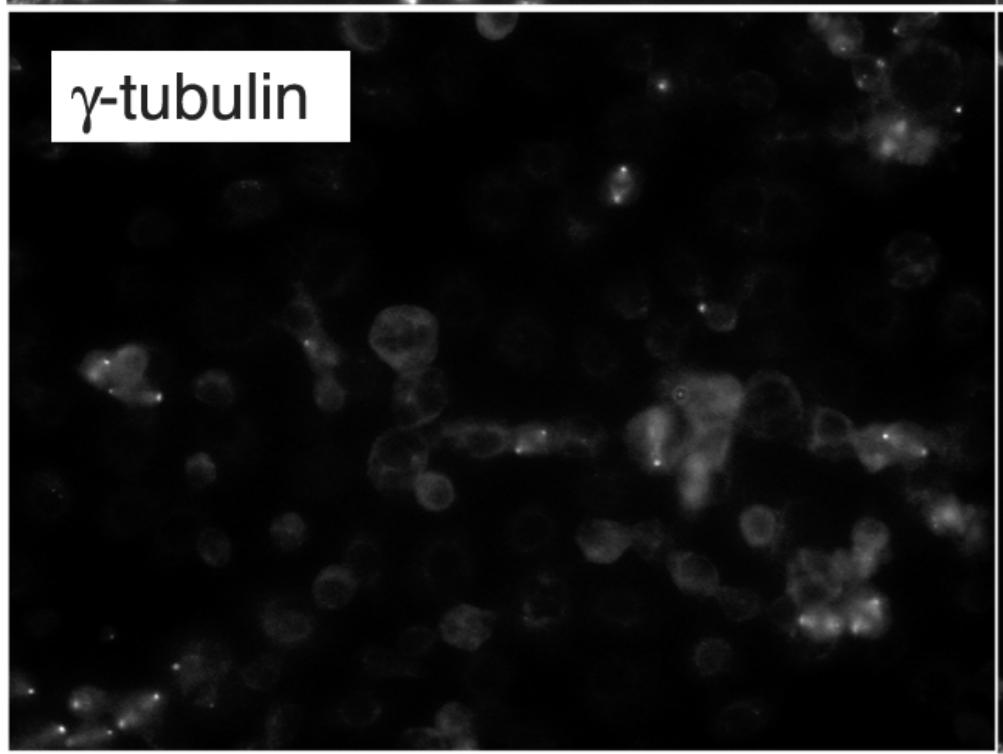
DNA



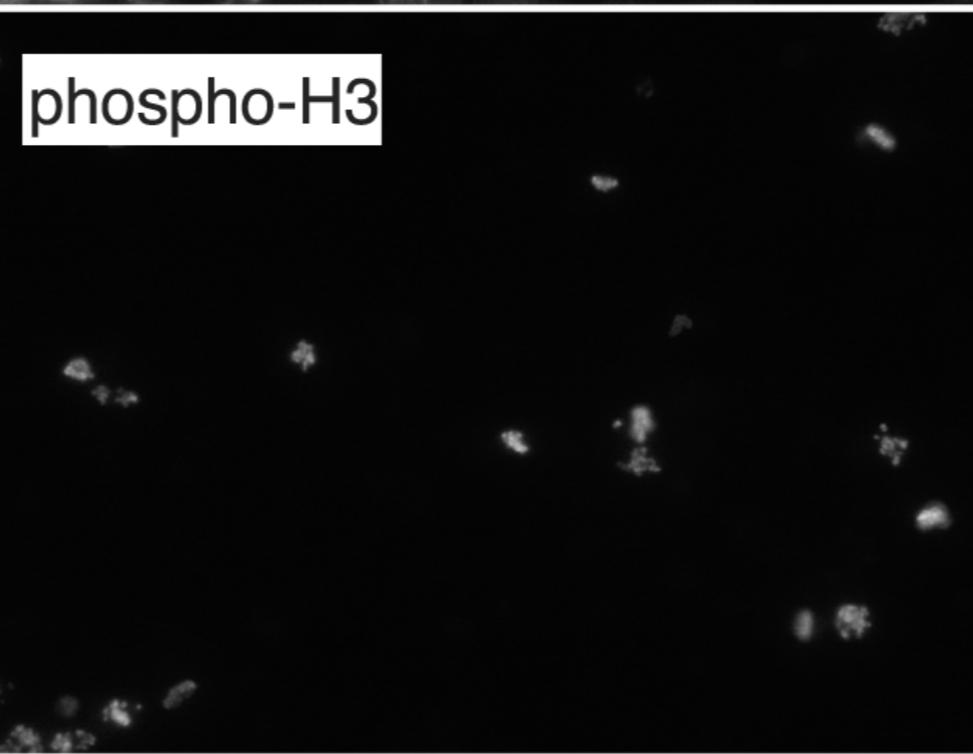
$\alpha$ -tubulin



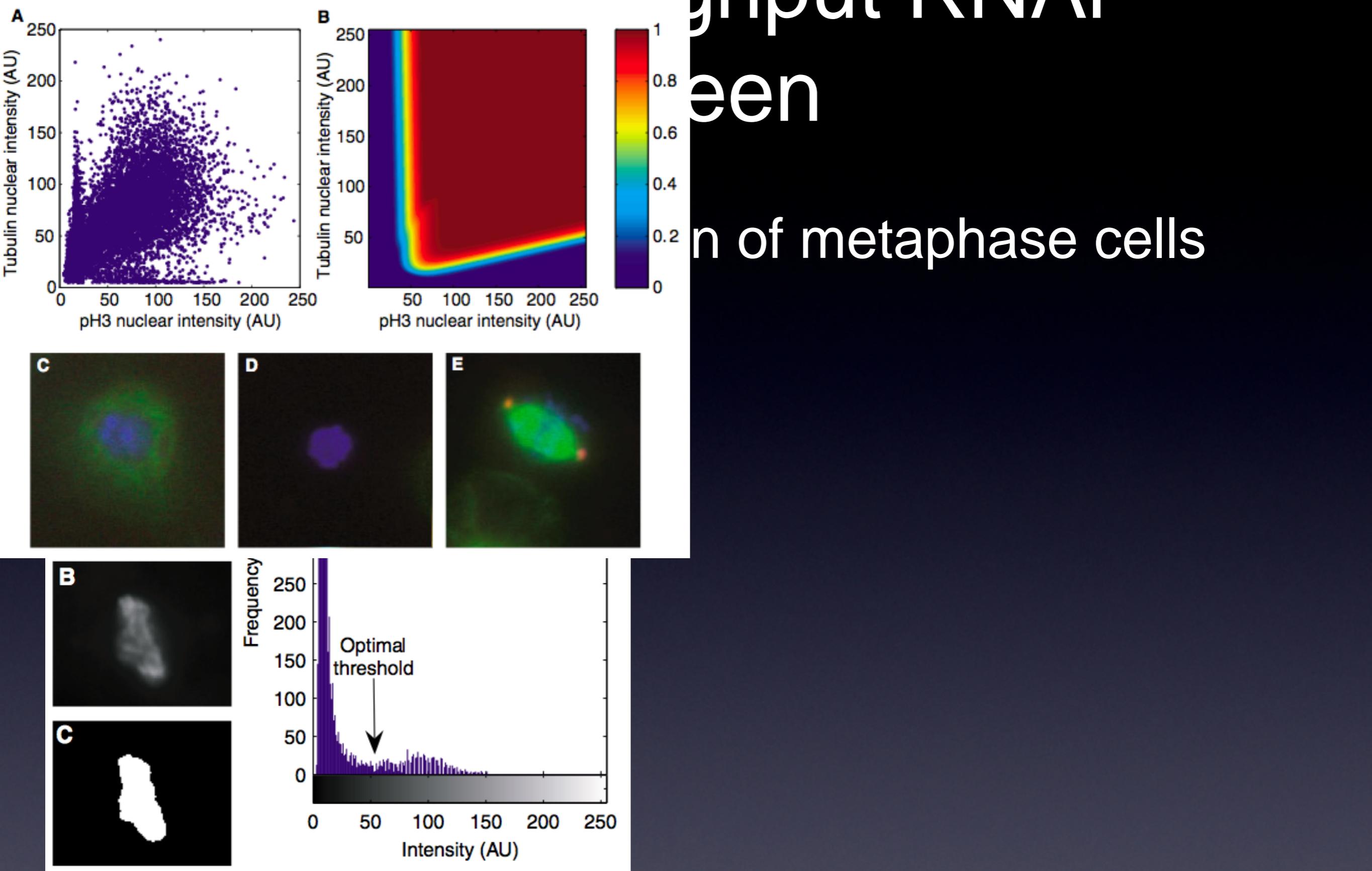
$\gamma$ -tubulin



phospho-H3



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

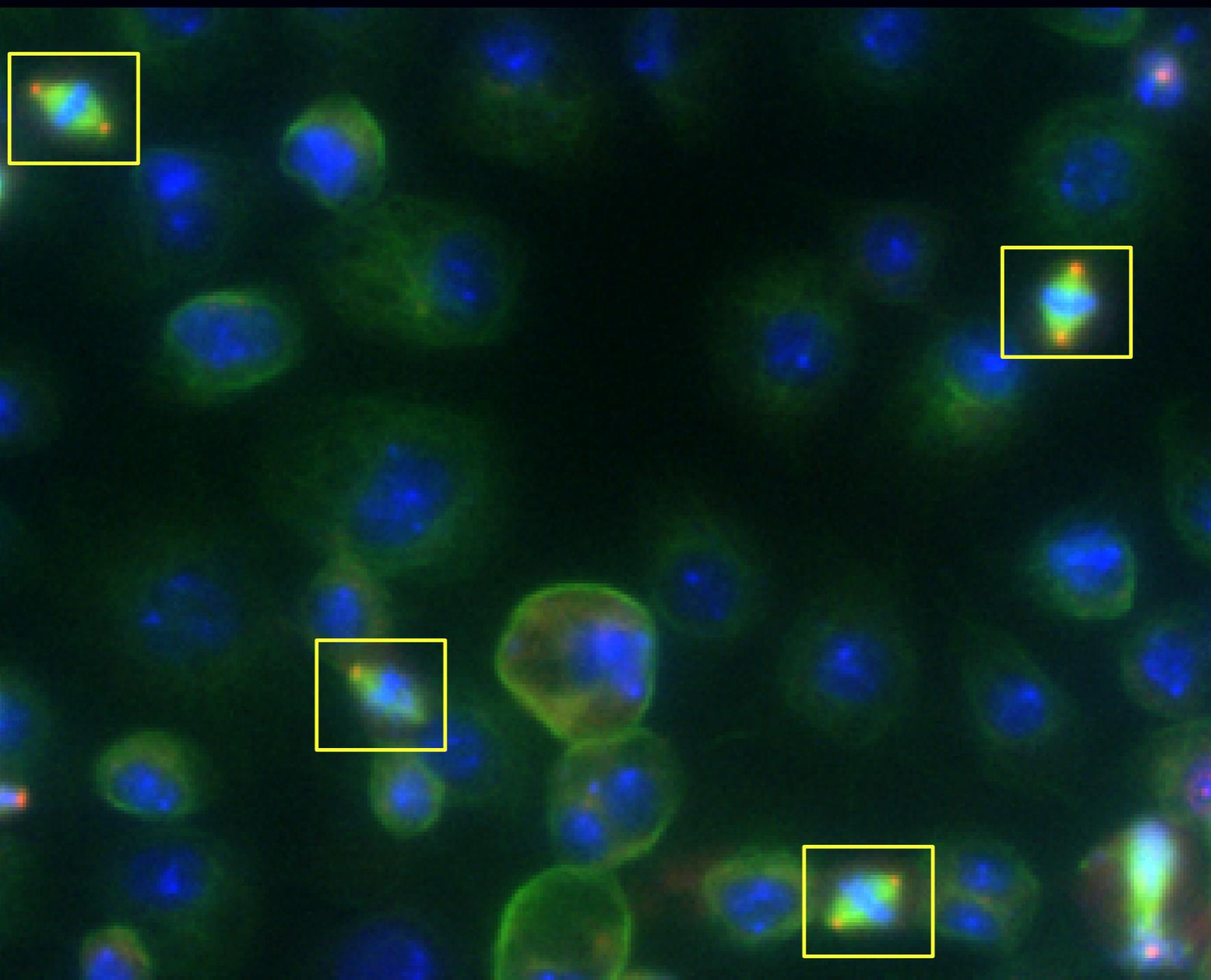


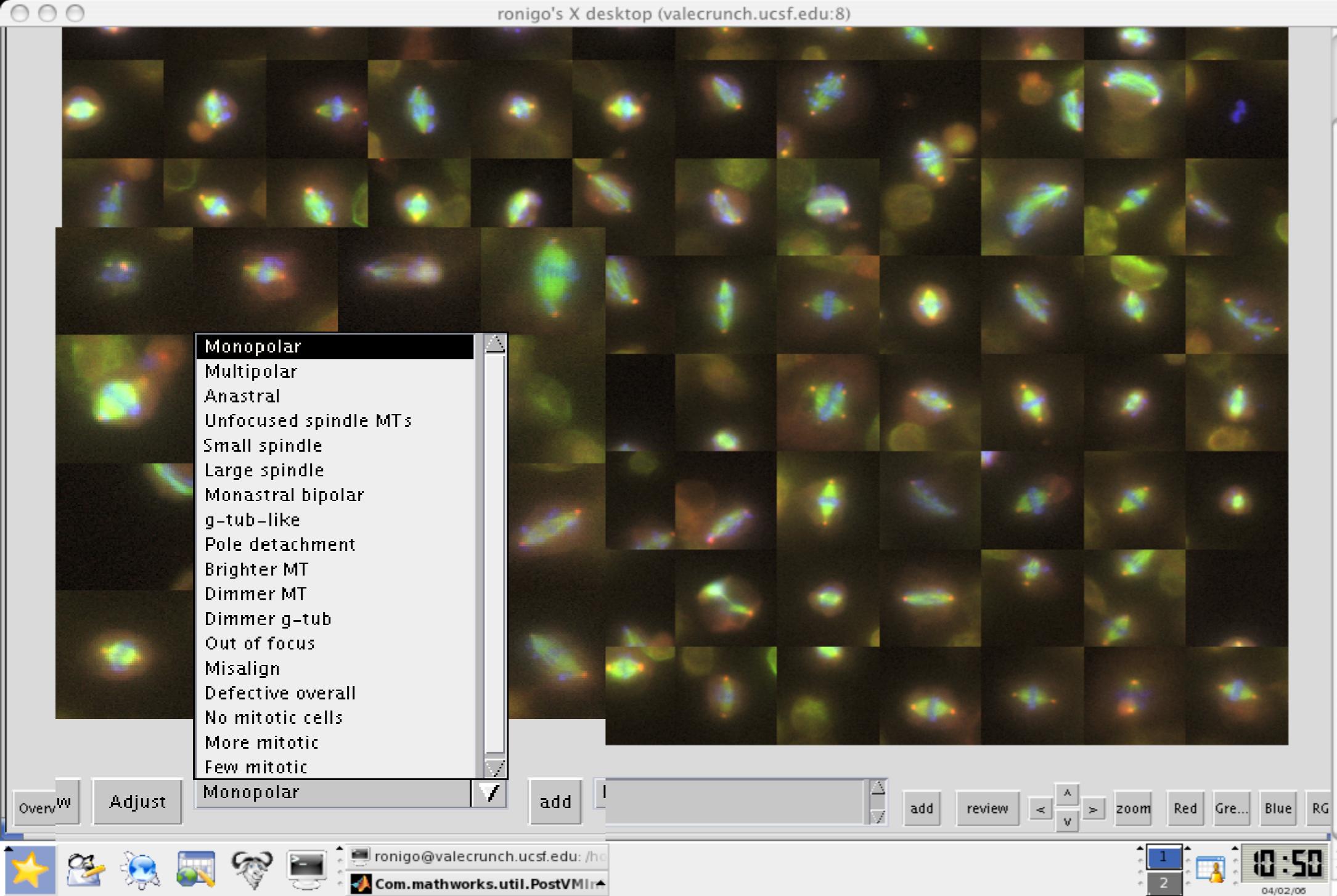
Automatic segmentation using Otsu's method

Classification using a trained neural network

# High throughput RNAi Screen

## 4. Automatic detection of metaphase cells

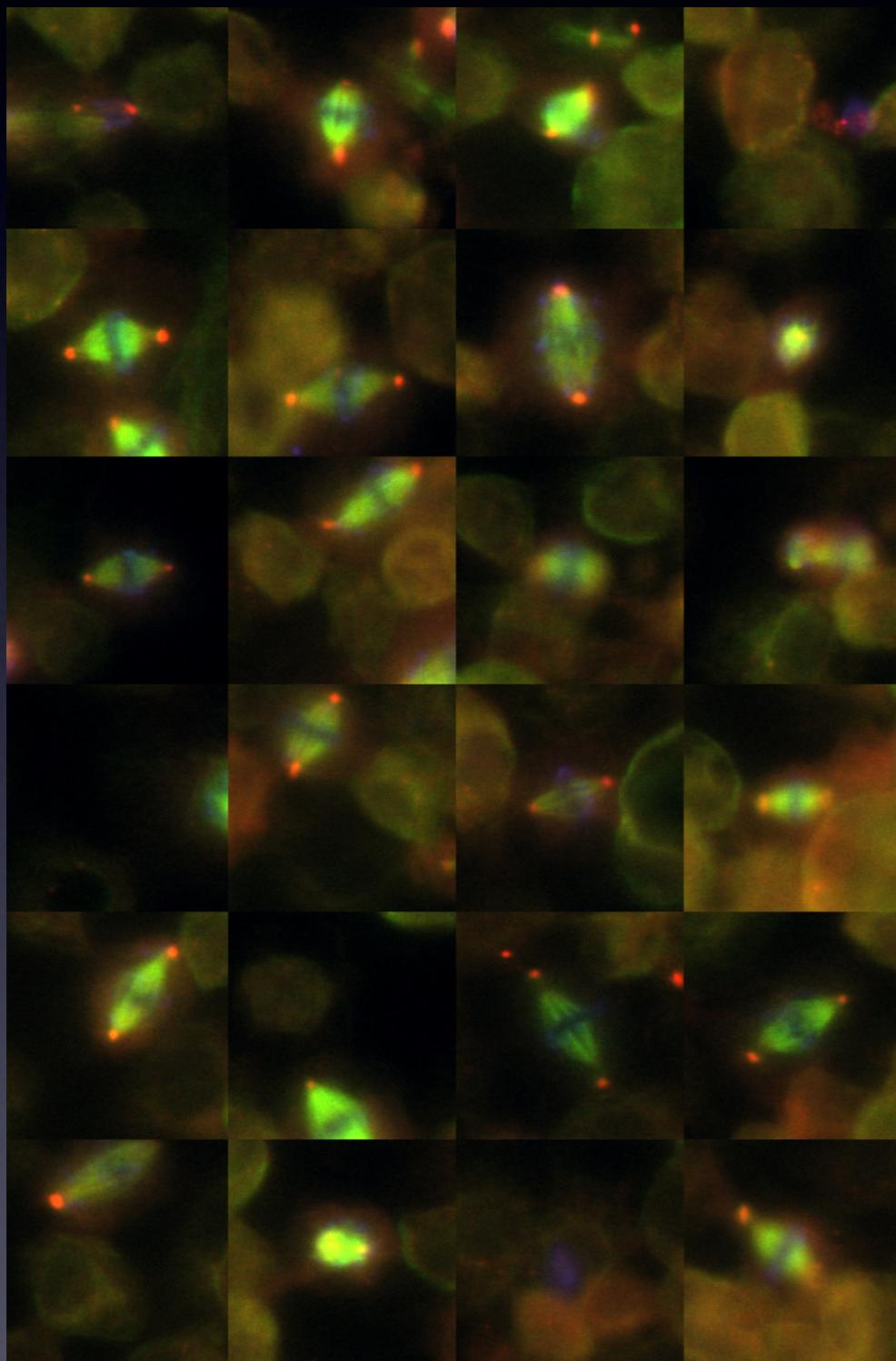




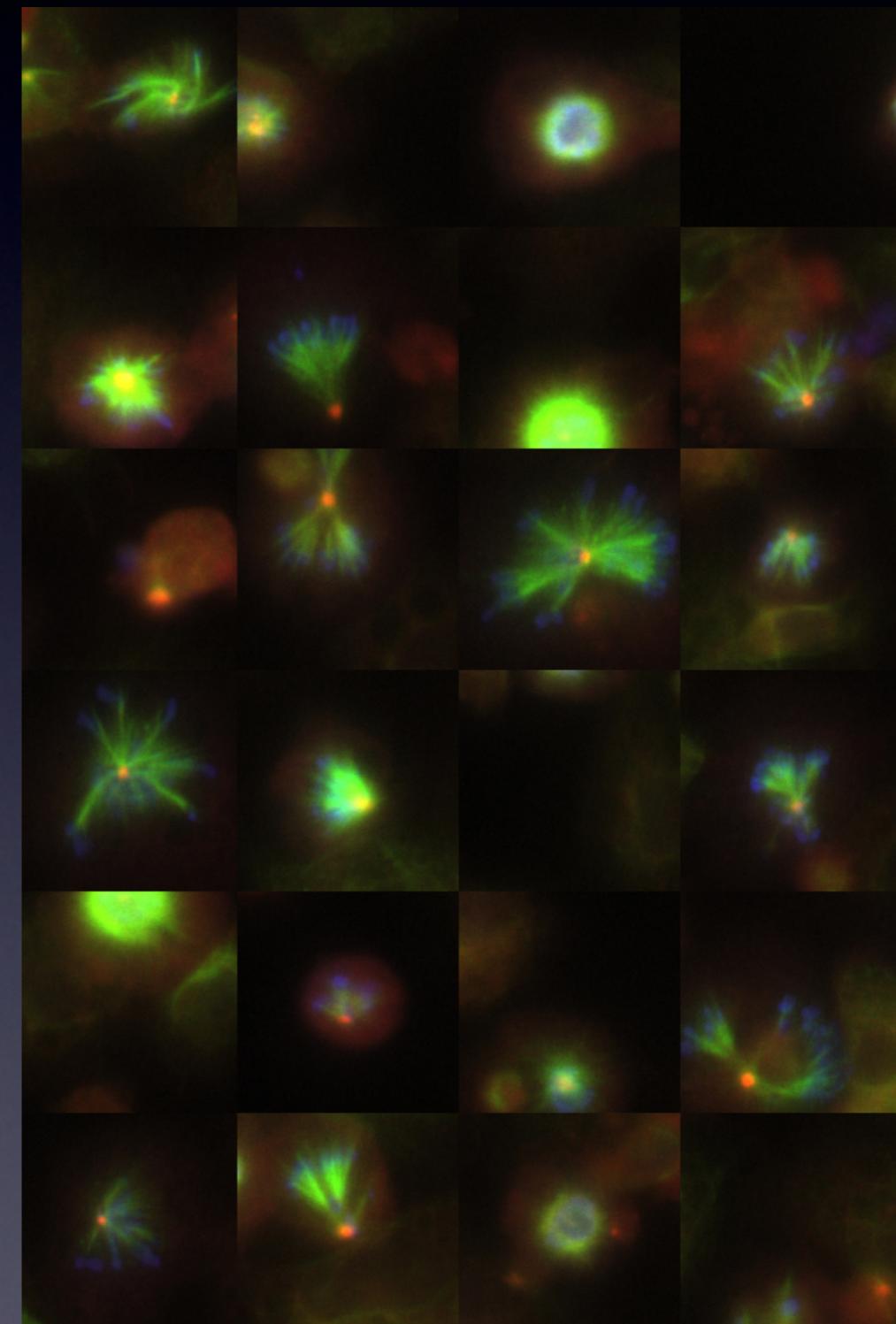
Side-by-side comparison of spindles made visual phenotype screening possible!

# Example

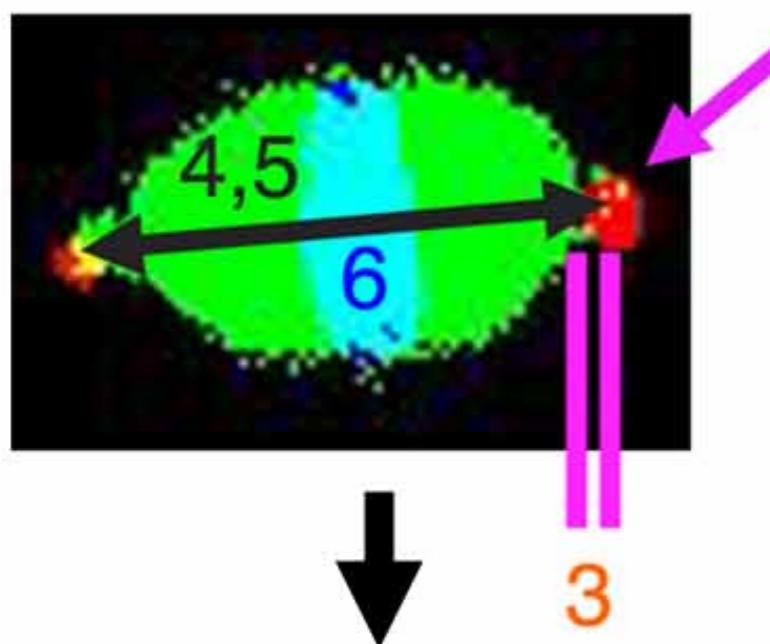
Control



Monopolar spindle (*Kinesin-5/Klp61F*)



## Image segmentation

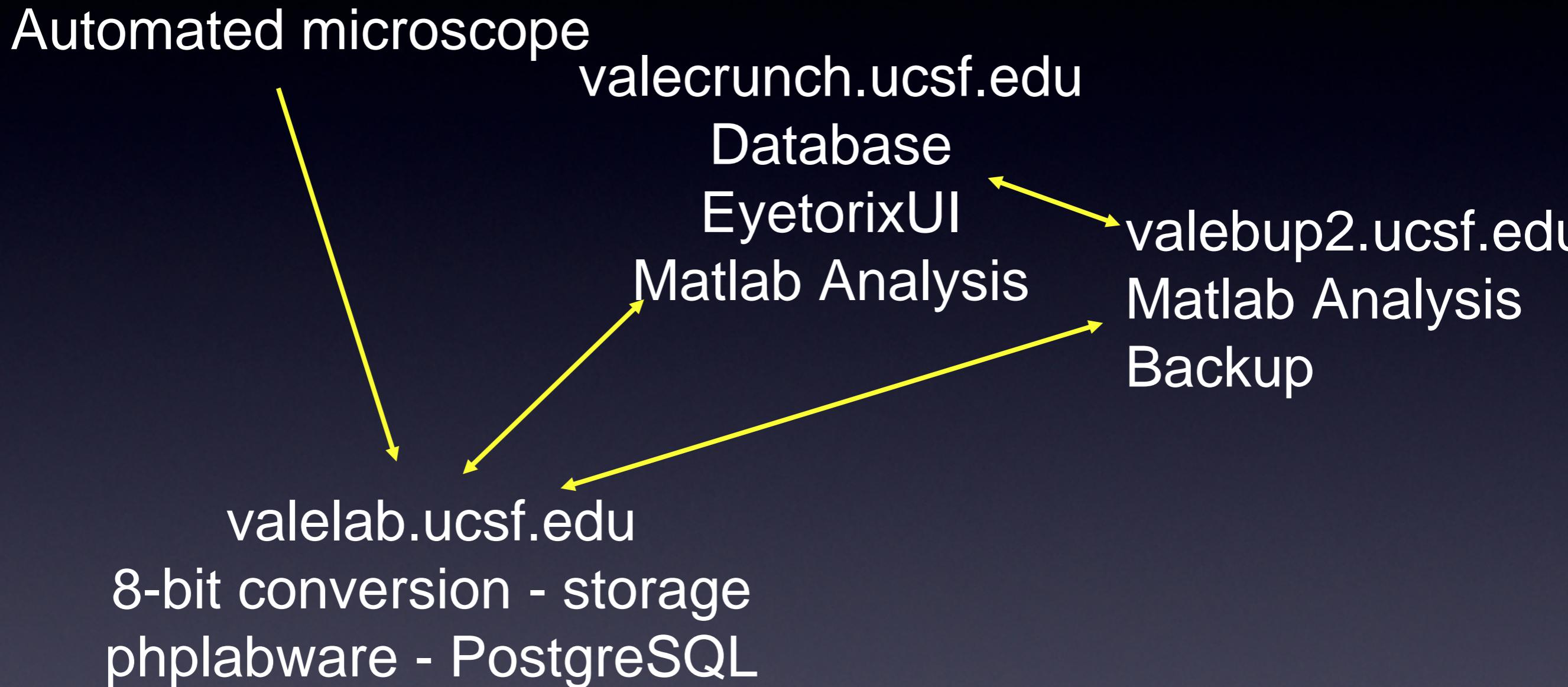


## Analysis 2: Segmentation and Computational Analyses

1. Monopolar
2. Multipolar
3. Pole detachment
  - frequency
  - mean distance
4. Long spindle
5. Short spindle
6. Misalignment
  - Chromatin area size
  - # of chromatin masses
  - Circumference/area ratio
7. Large  $\gamma$ -tubulin area
8. Dim  $\gamma$ -tubulin

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

# Workflow



# Vale Lab Screen Data Base

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

PhpLabware: MitoSpindleScreen

August 1, 2006, 7:59 pm

Report: ---Reports--- Send to: screen file [Edit reports](#)

View: [Gohtha](#) [Edit views](#)

Import Data

95 Records found. Showing 1 through 95.

96 Records per page

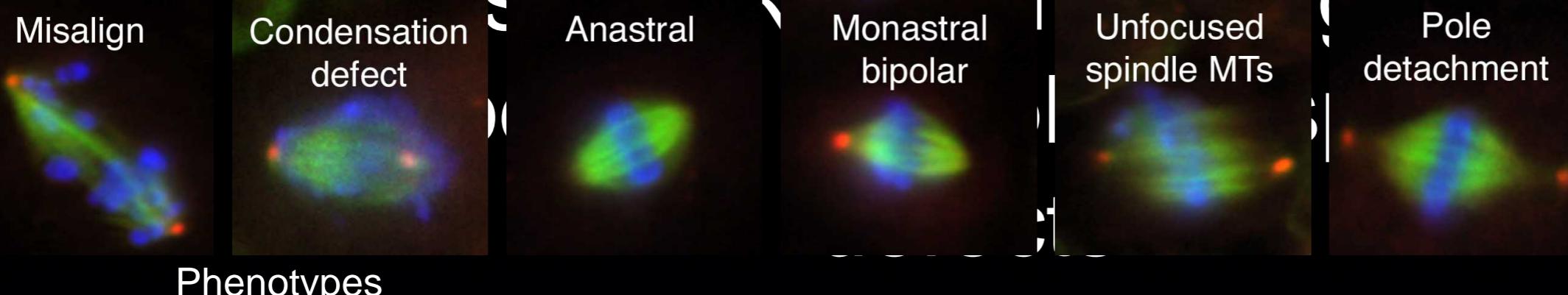
Search [Show All](#)

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		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
	CG14391	14391	129	A	2	probe info		94.43	8.50%	No	No				1 2 3		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
	CG14394	14394	129	A	3	probe info		97.69	10.97%	No	No				1 2 3 4		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
	beat-Vc	14390	129	A	4	probe info		91.29	5.27%	No	No				1 2		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
	beat-Va	10134	129	A	5	probe info		68.64	6.00%	No	No				1 2		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
	beat-Vb	31298	129	A	6	probe info		108.02	7.14%	No	No				1 2 3		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
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		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
	CG14384	14384	129	A	8	probe info		96.90	5.95%	No	No				1 2		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
polyA-binding prc <sup>000</sup> inter: prot																	<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
yellow																	<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
lethal (R)																	<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
	Ravus	15009	129	C	1	probe info		96.02	9.79%								<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
								140.21	8.47%								<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>
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								95.10	9.04%	No	No				1 2 3		<a href="#">Edit</a> <a href="#">Delete</a> <a href="#">X</a>

Link to phenotype gallery

Link to dsRNA information

Done valelab.ucsf.edu



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)



# Acknowledgements/References

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