

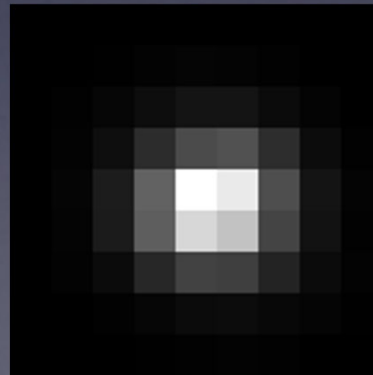
High Throughput Imaging



Nico Stuurman
UCSF Microscopy Course
4/28/2010

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

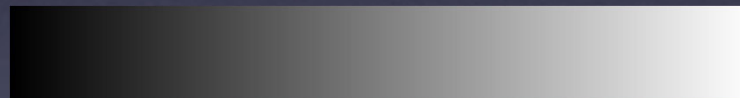
4

16



8

256



1-byte

12

4096



2-bytes

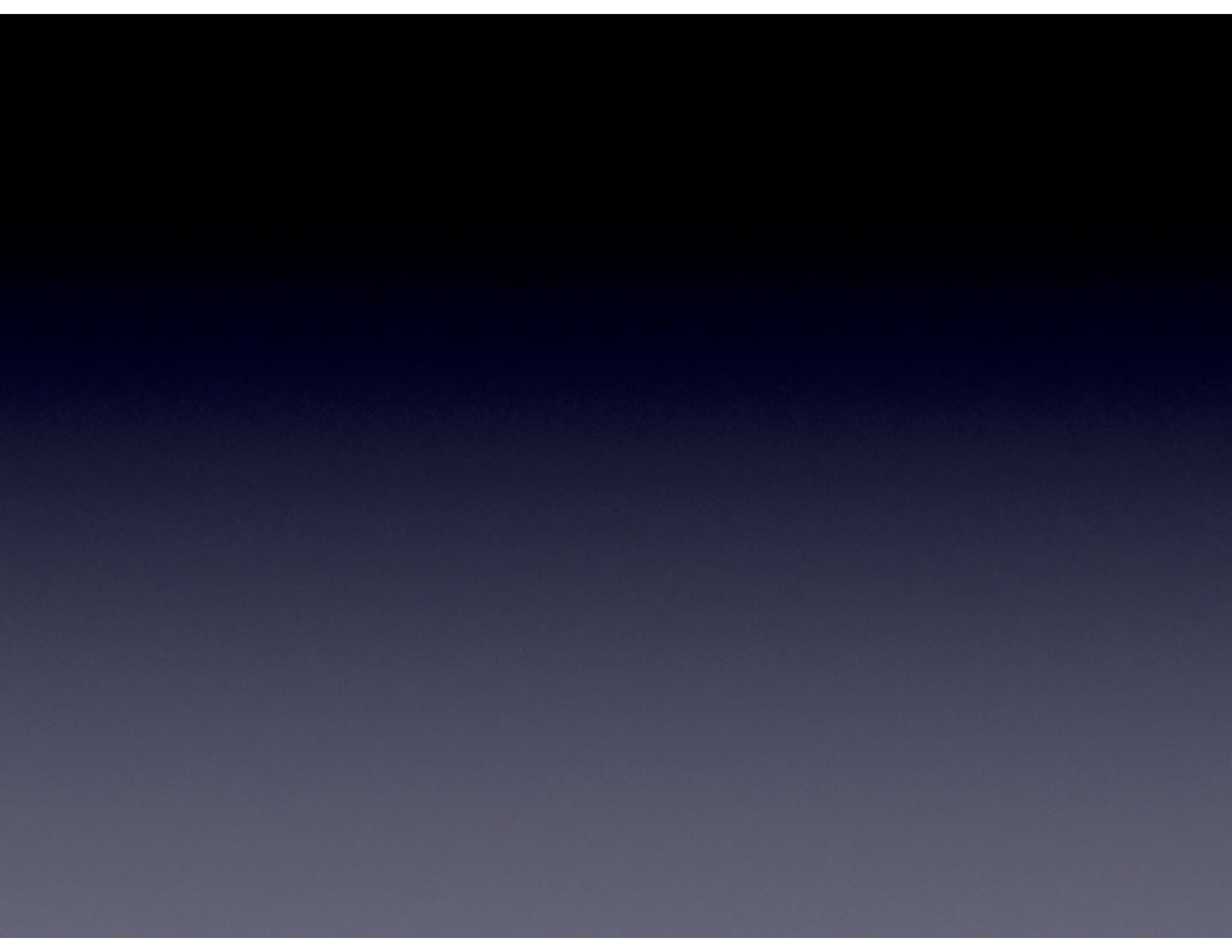
16

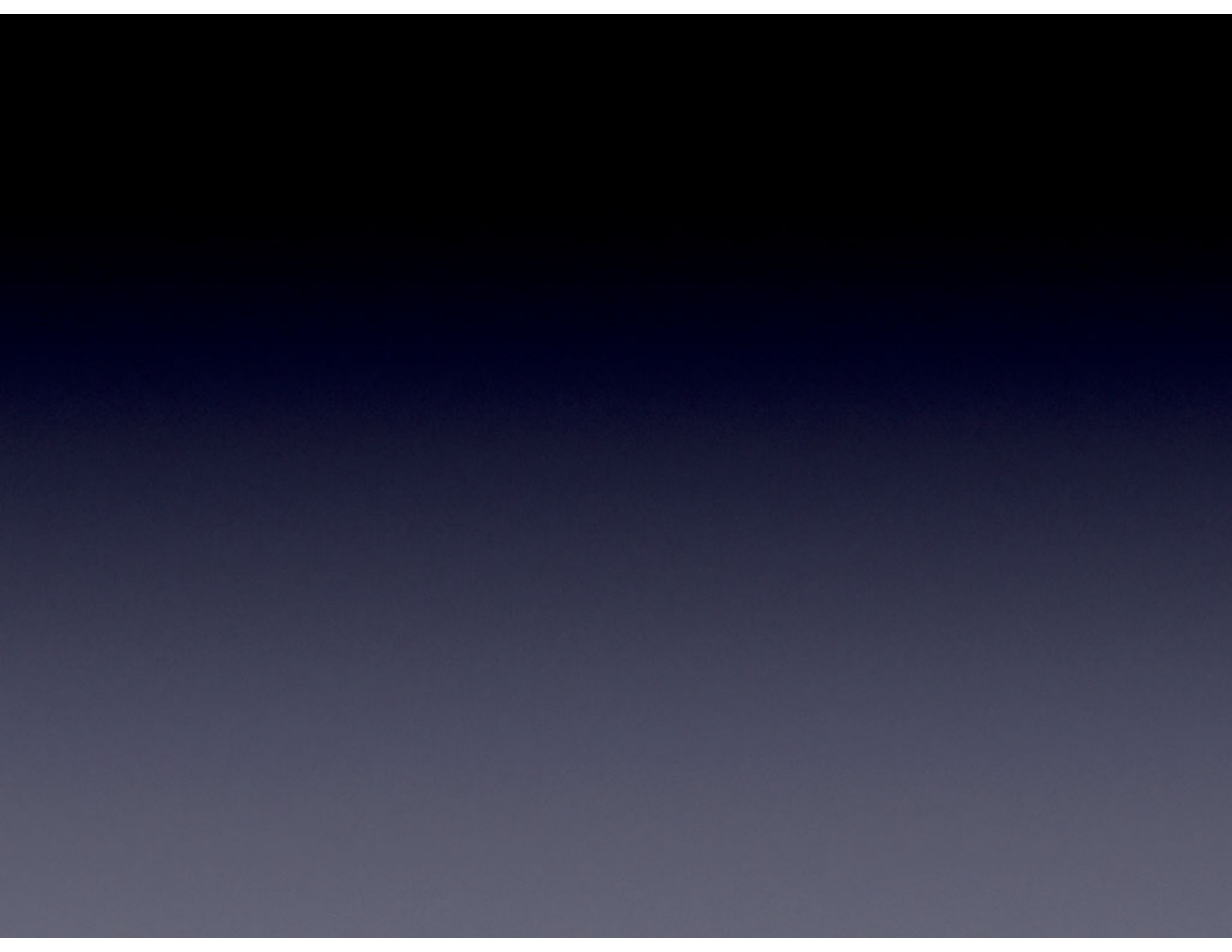
65536



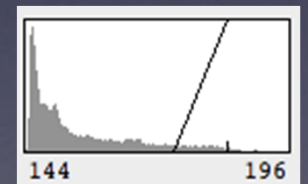
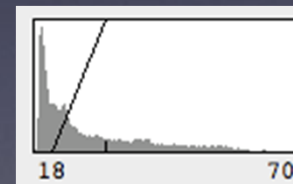
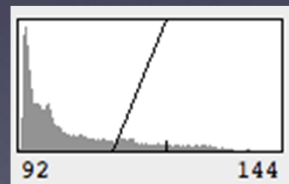
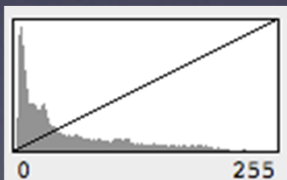
Bit-depth and resolution





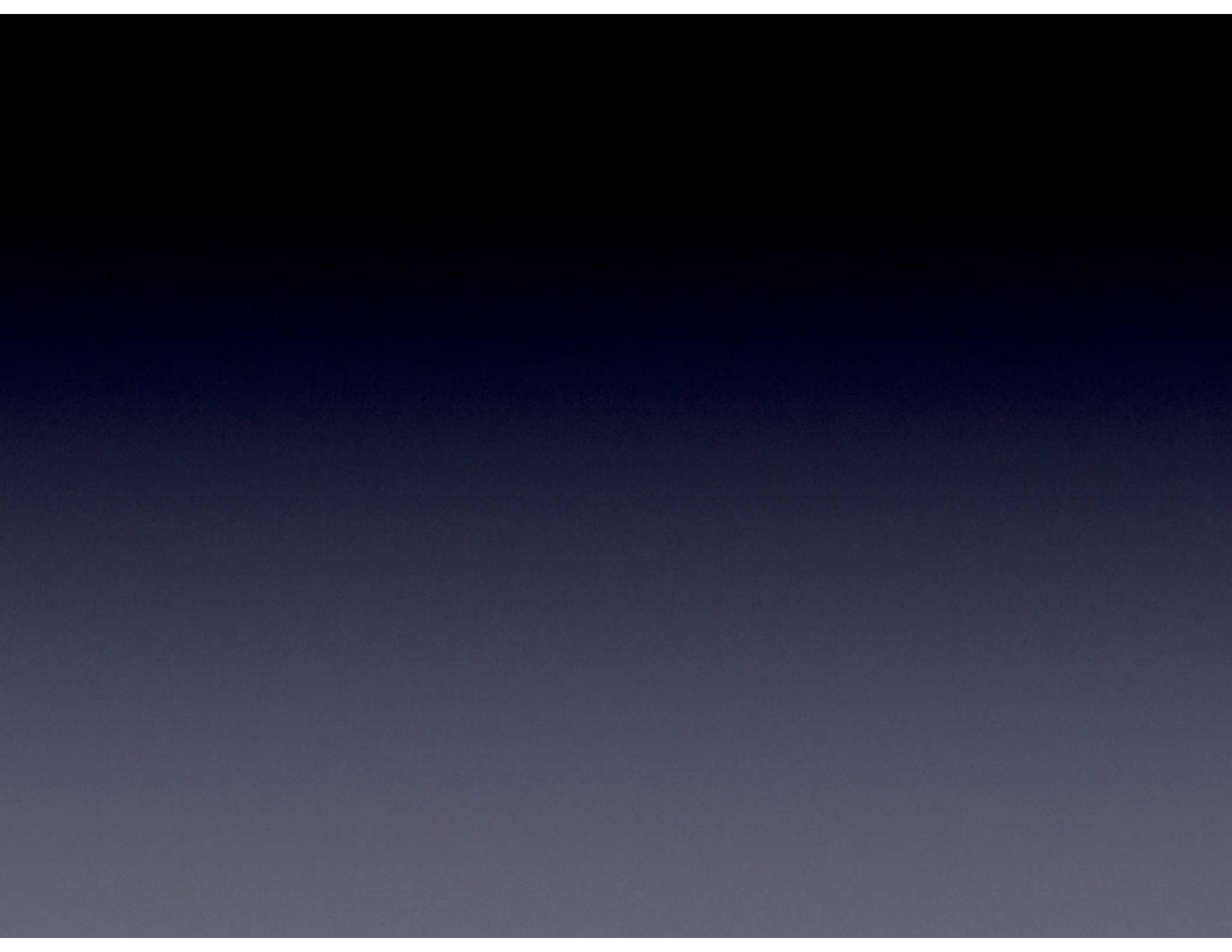


Mapping values onto display: Brightness/contrast

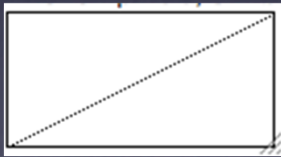


Slope = contrast

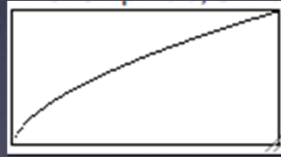
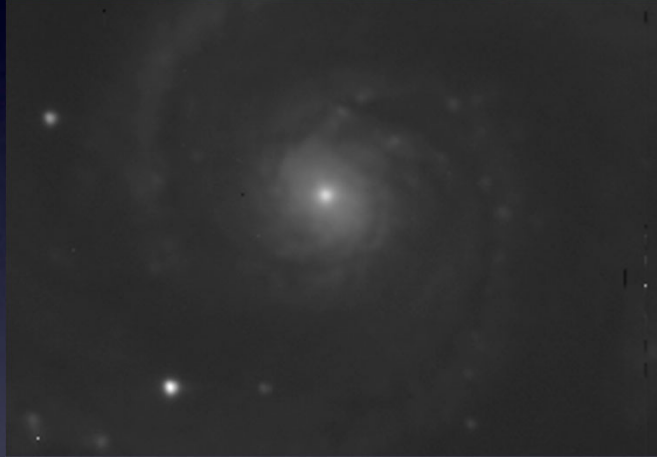
Brightness



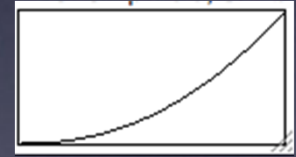
Gamma adjustment



1



0.6



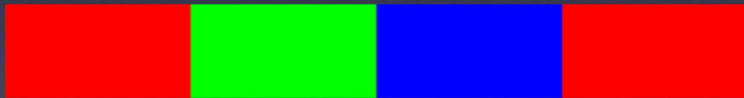
2.2

What are acceptable image manipulations?

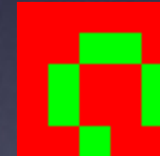
- JCB has the best guidelines
 - <http://jcb.rupress.org/content/172/1/9.full>
 - <http://jcb.rupress.org/content/166/1/11.full>
- Brightness and contrast adjustments ok, so long as done over whole image and don't obscure or eliminate background
- Nonlinear adjustments (like gamma) must be disclosed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

Lookup Tables (LUTs)

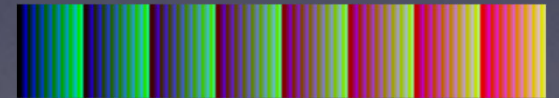
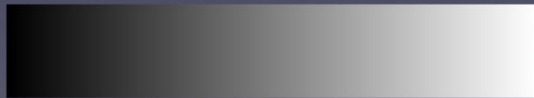
0 1 2 3



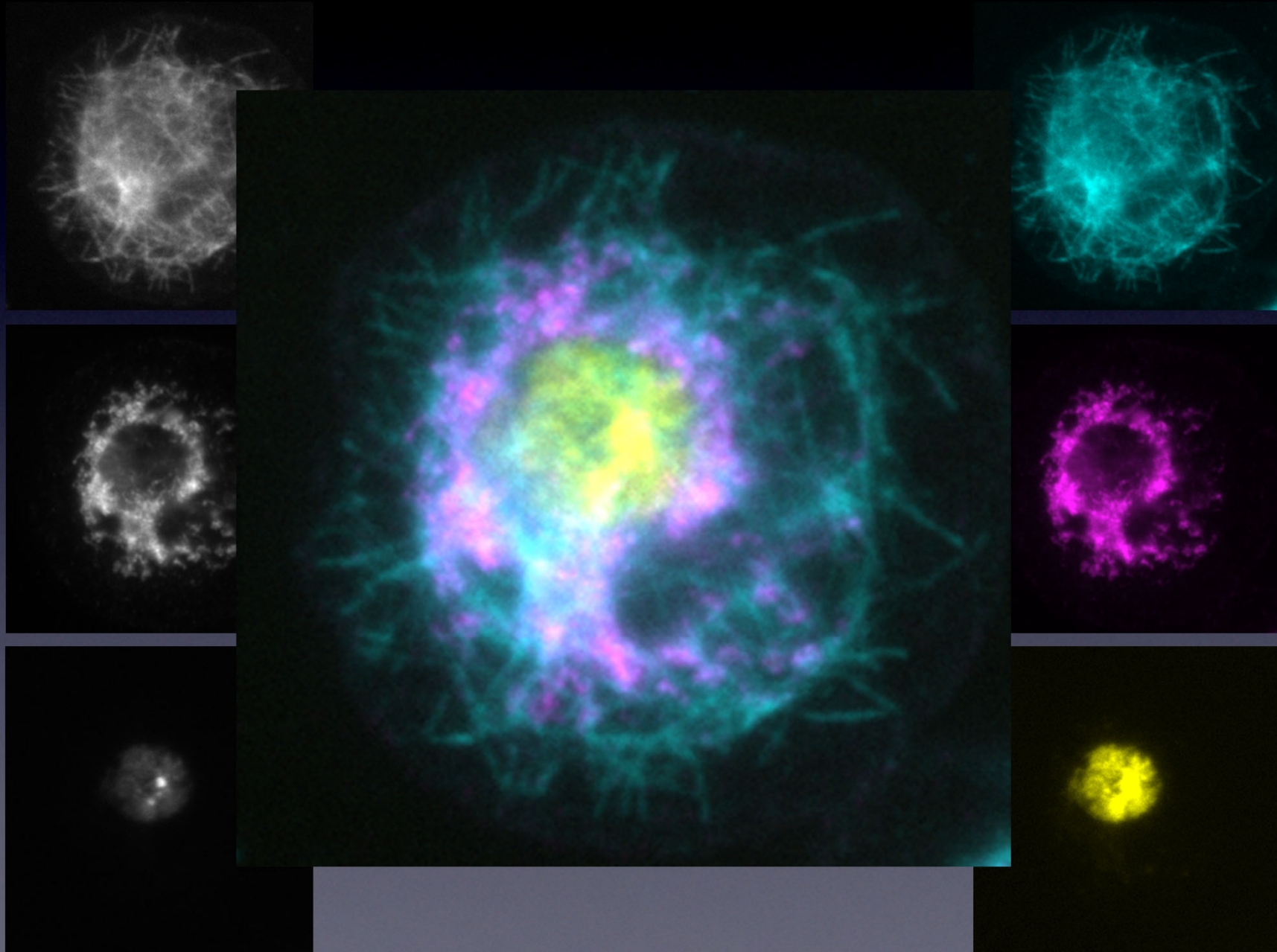
0	0	0	0	0
0	0	1	1	0
0	1	3	3	1
0	1	3	3	1
0	0	1	0	0



Lookup Tables (LUTs)



Lookup Tables (LUTs)



Color Images



Either:

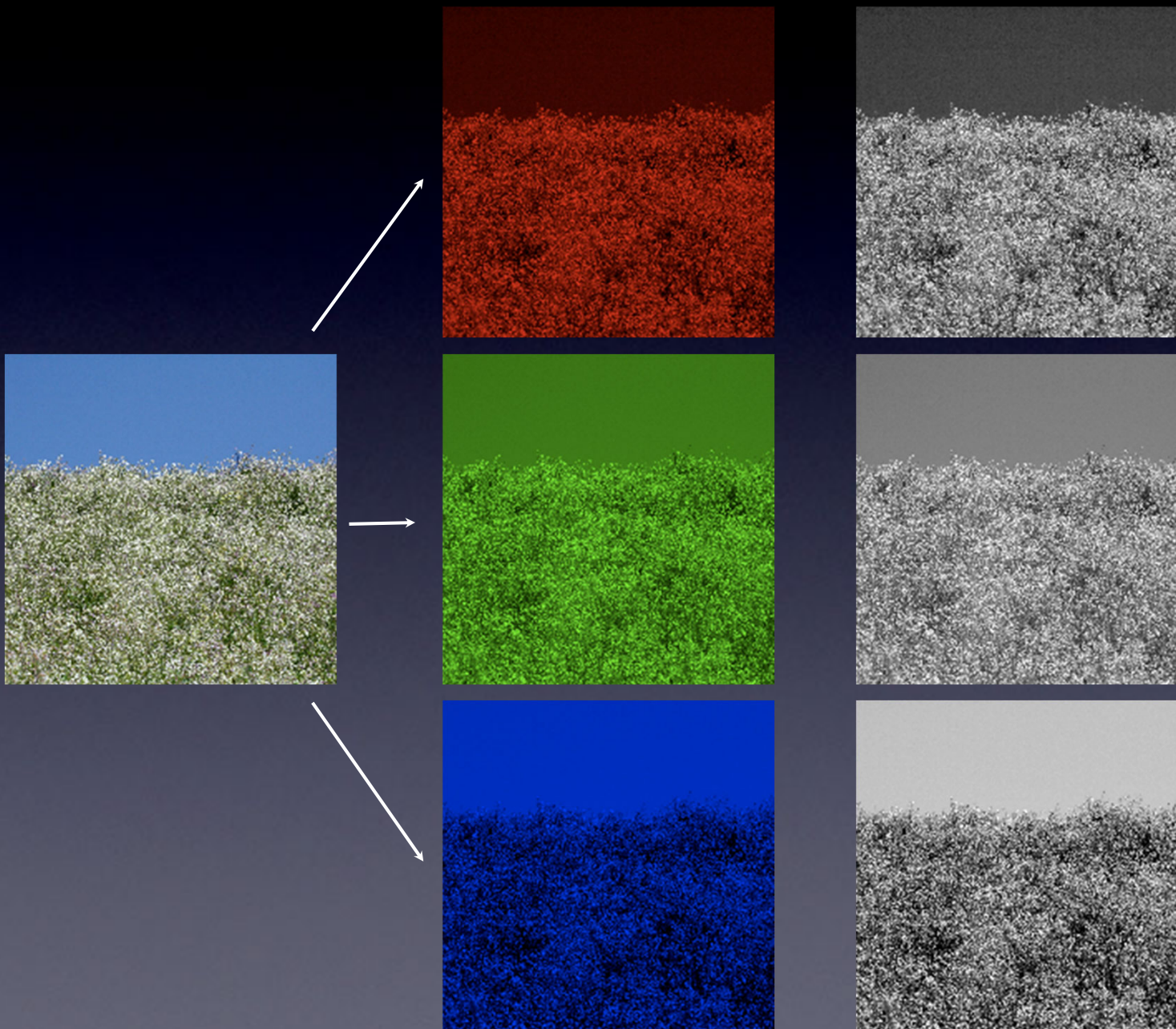
255 209 139	0 89 93	93 255 231	255 0 0
0 0 0	255 0 0	134 0 185	93 90 0
0 39 185	0 255 255	214 255 0	137 0 255
93 90 0	255 0 0	0 0 0	249 185 255

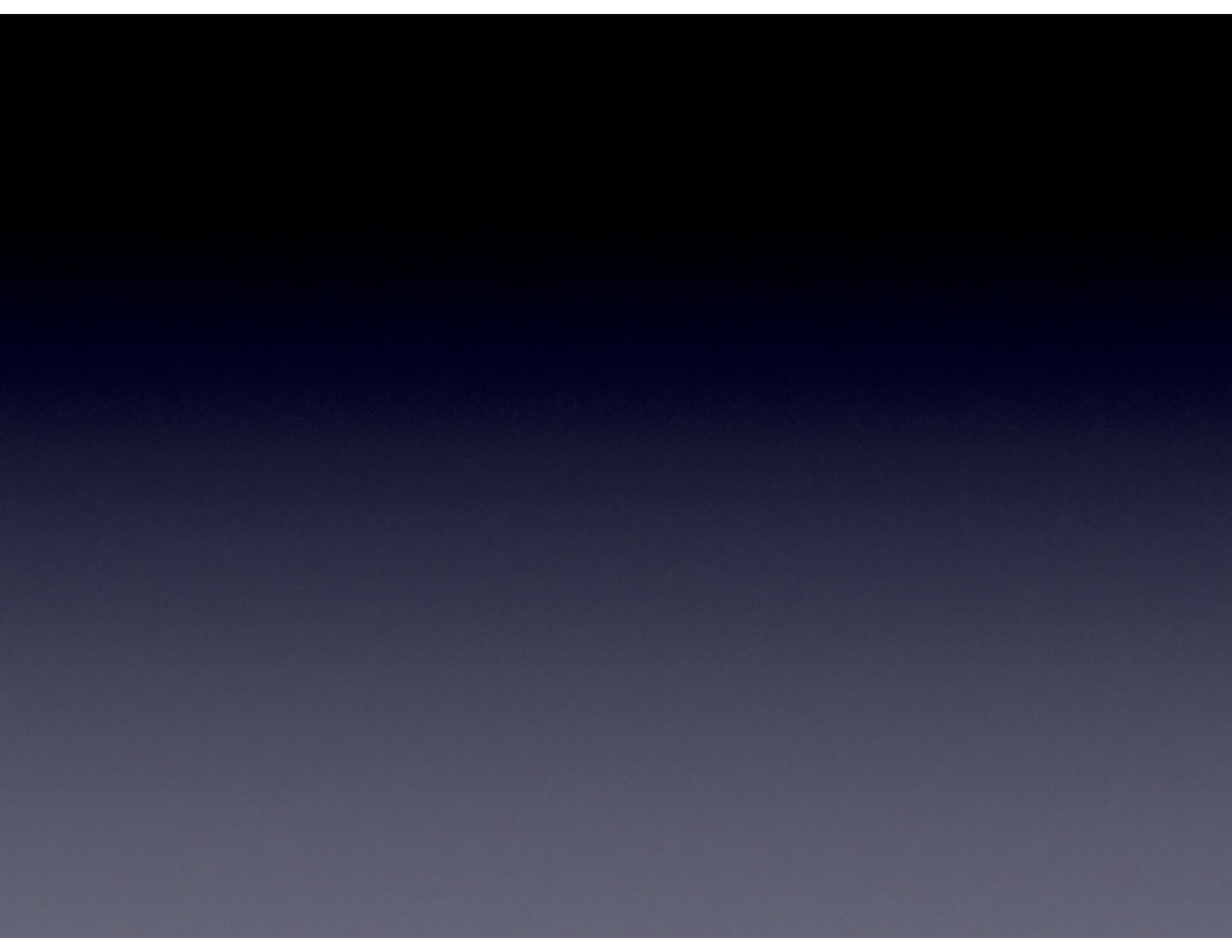
Or:

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





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!

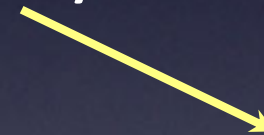
Lossless versus Lossy



None (raw)

Run-length encoding

Dictionary approaches, etc..



Discards data not
essential for visual
appearance

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

File Formats

Desired:

- Widely used
- No compression (or losless)
- 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 losless compression, 8-bit only)

Software Tools

Acquisition + Analysis

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

Micro-Manager

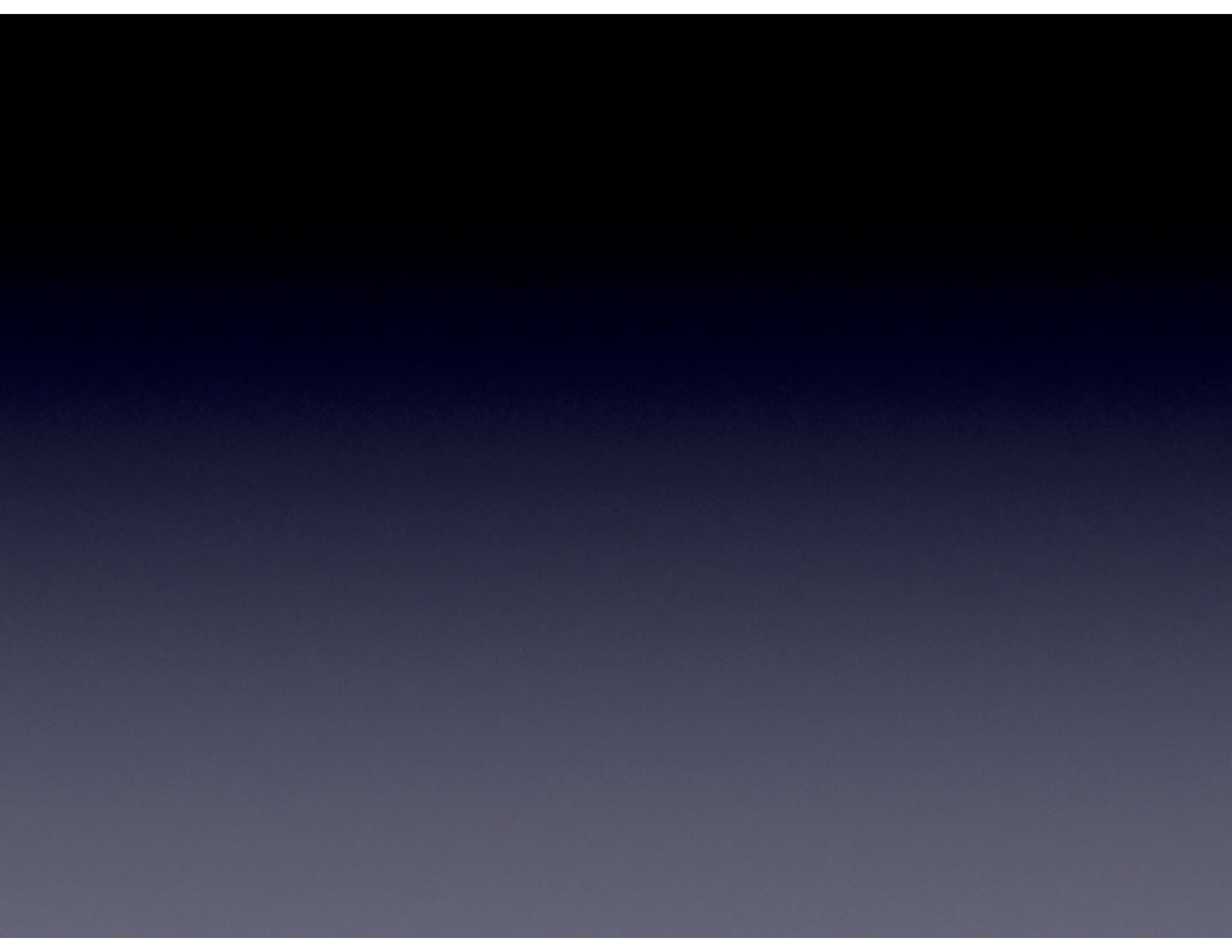
<http://micro-manager.org>

Presentation

- Photoshop
- Gimp

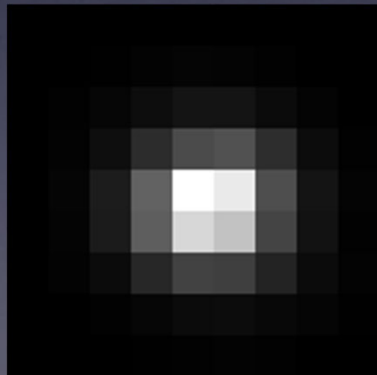
Analysis

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



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



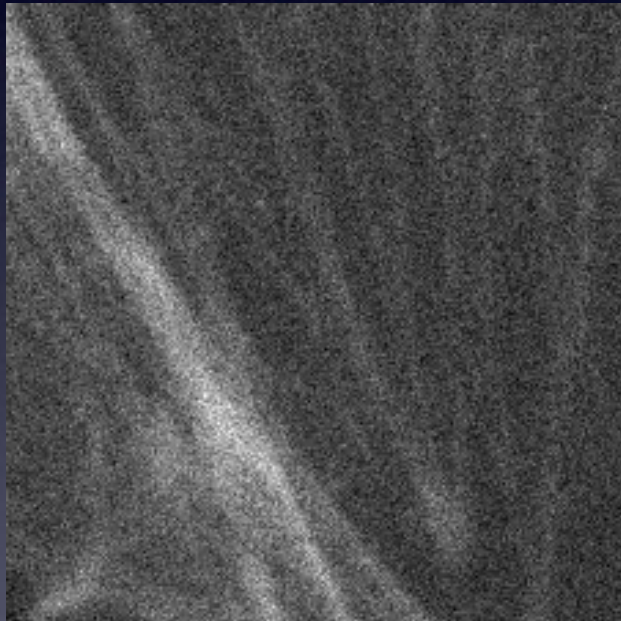
Measured PSF



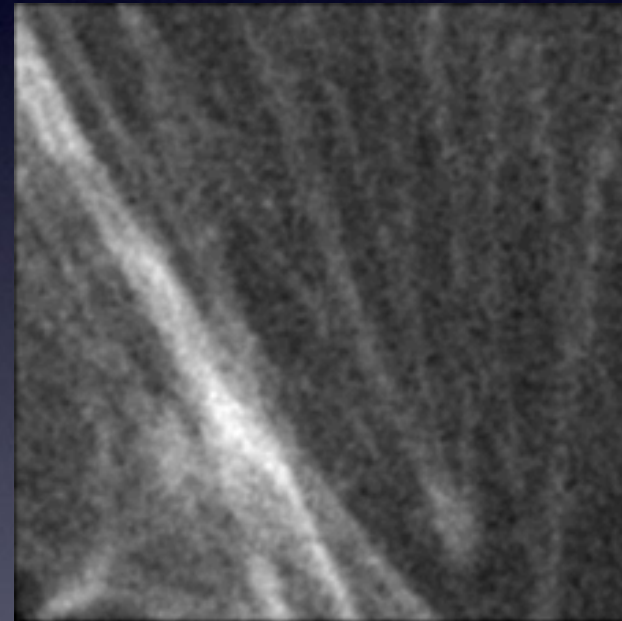
Gaussian Filter

Why smooth?

Averages redundancy and suppresses noise



10 photons/pixel average
5 e- read noise



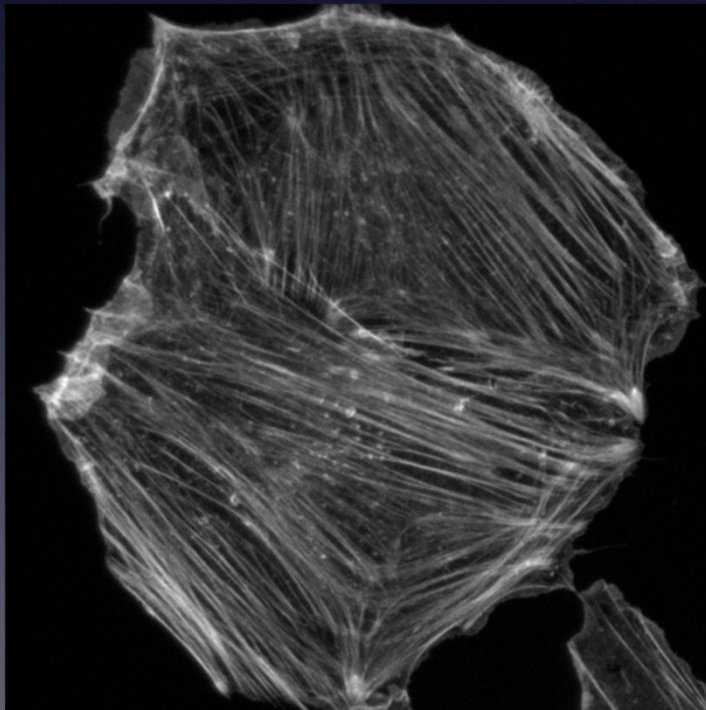
Gaussian smoothing filter,
 $\sigma = 1$ pixel

Other Filters

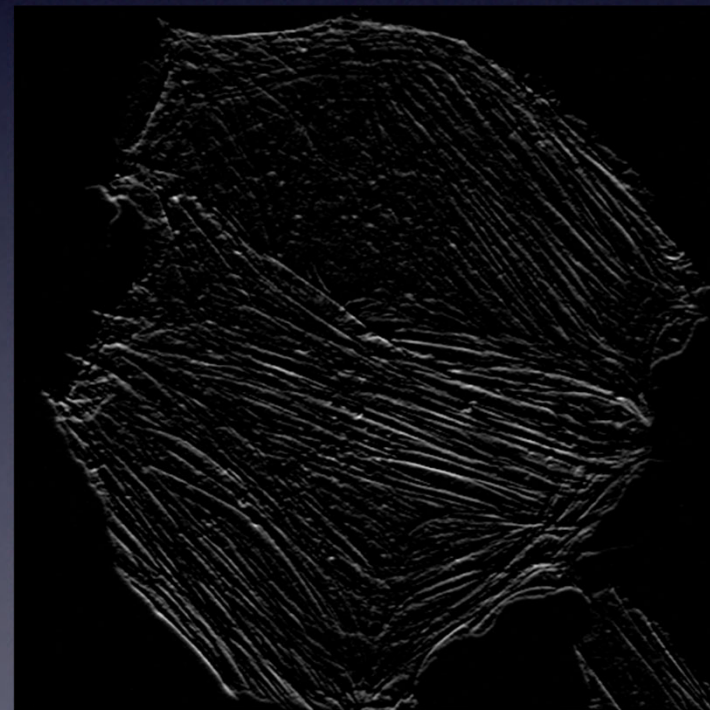
Edge Detection

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

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



Original

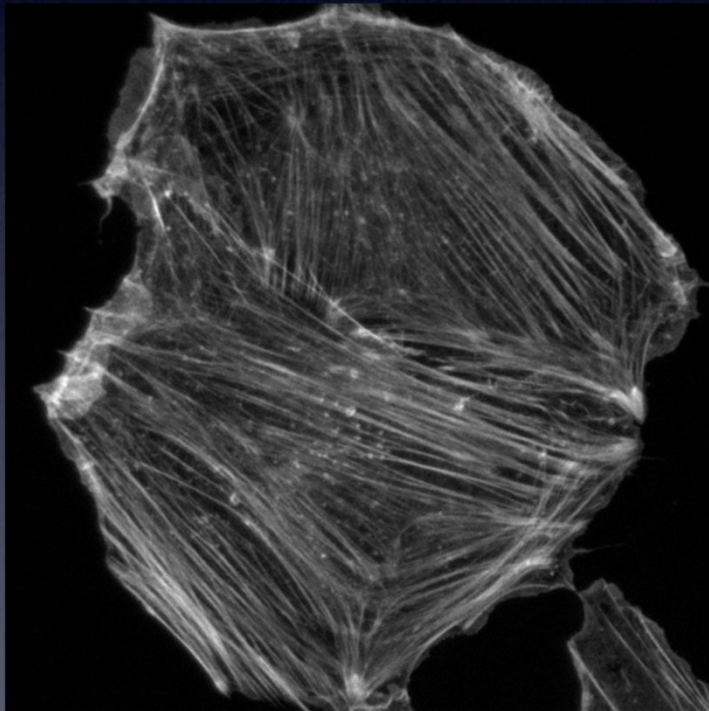


Horizontal edge detection

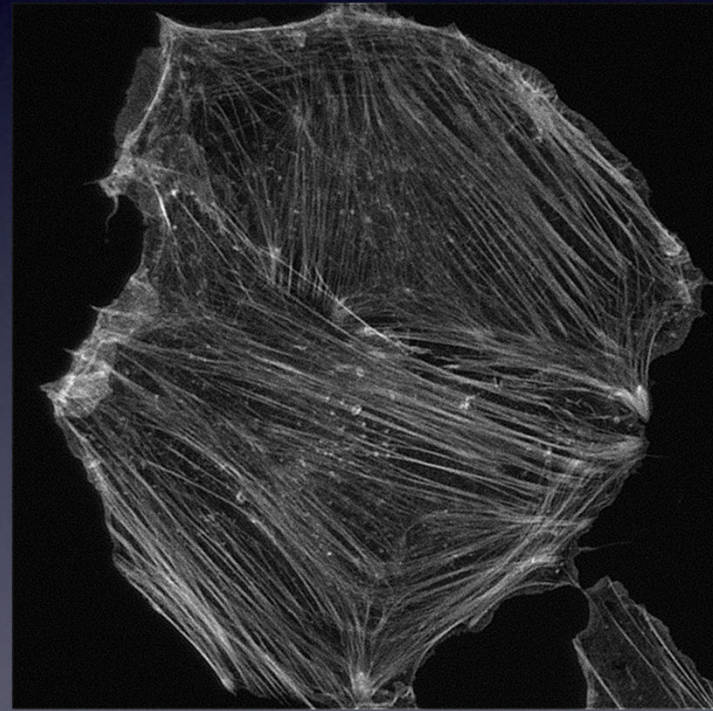
Other Filters

Unsharp Masking

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



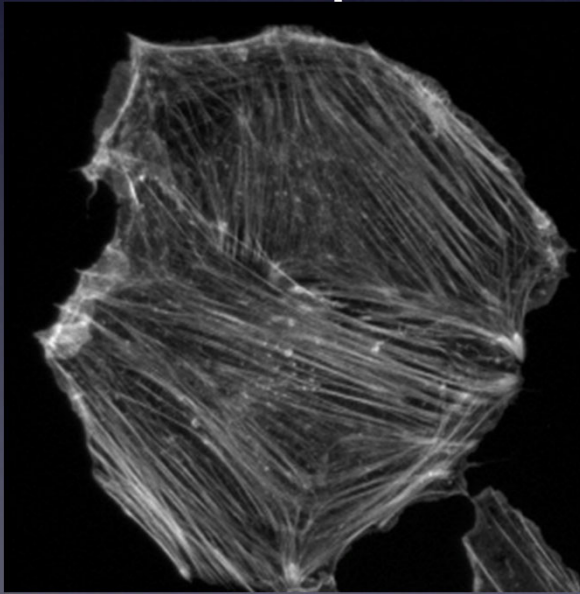
Original



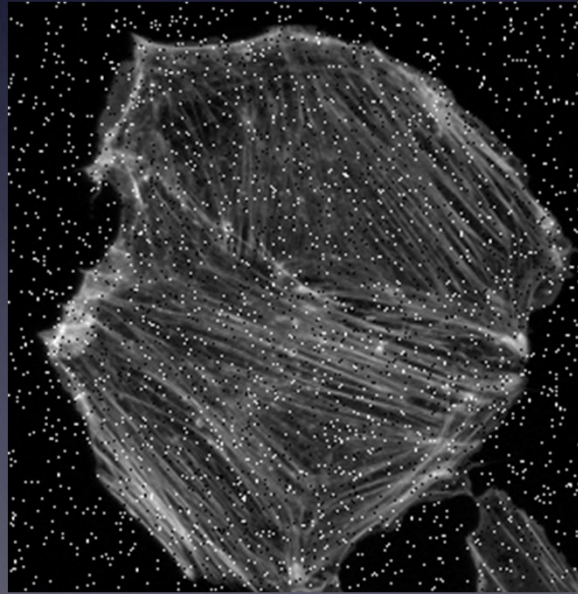
Unsharp masked

Non-linear Filters

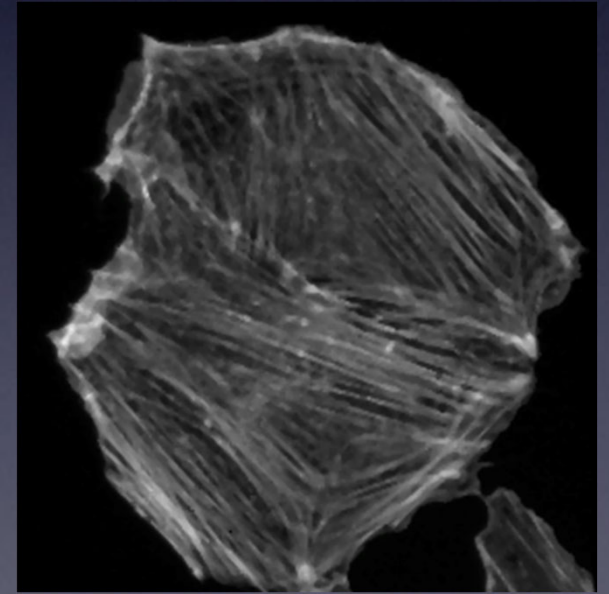
- Replace central pixel with min, max, median
- Median filter is a good noise filter, at the expense of resolution



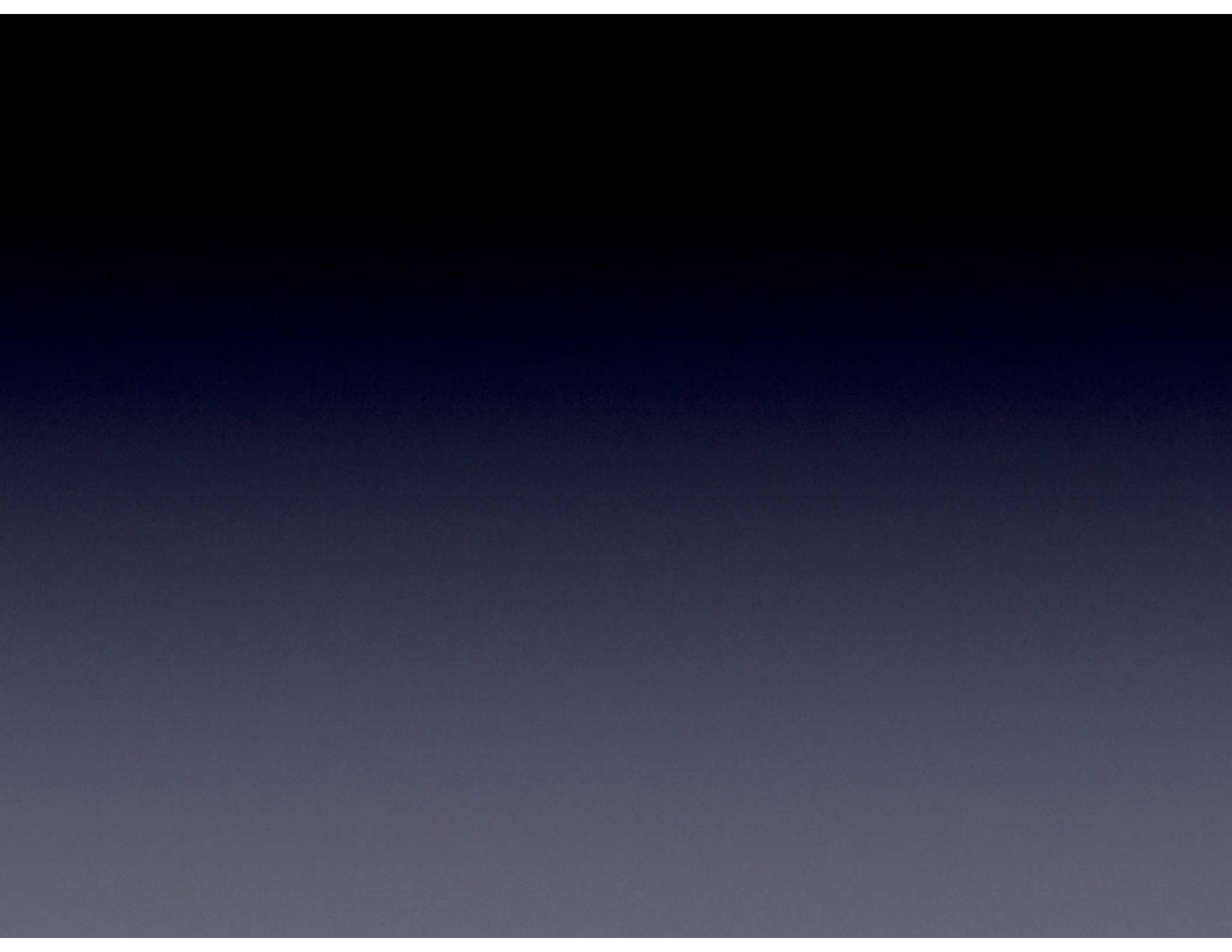
Original

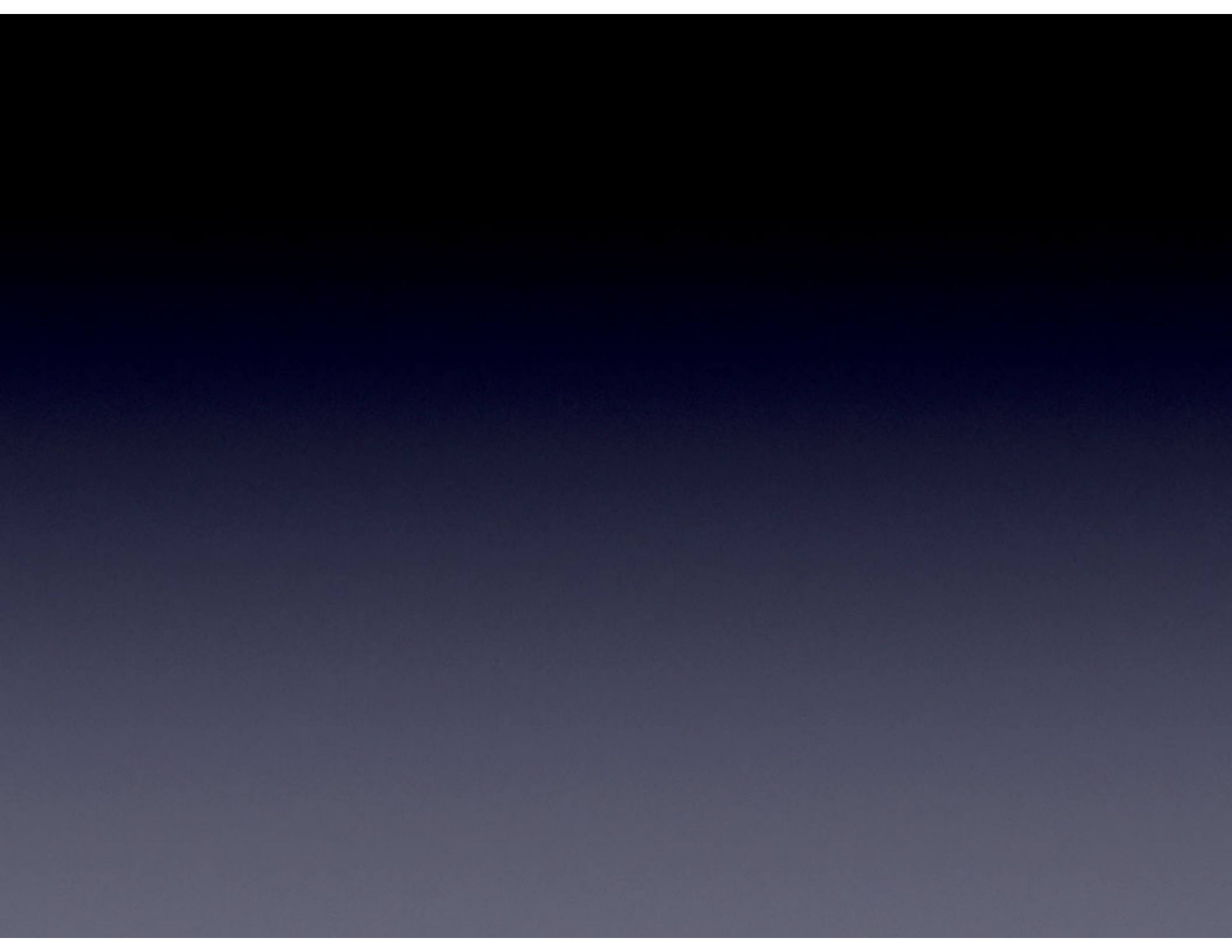


Artificial Noise



Median Filter





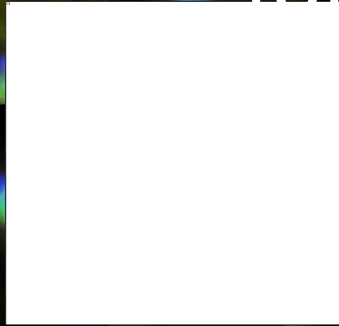
High-throughput Imaging

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



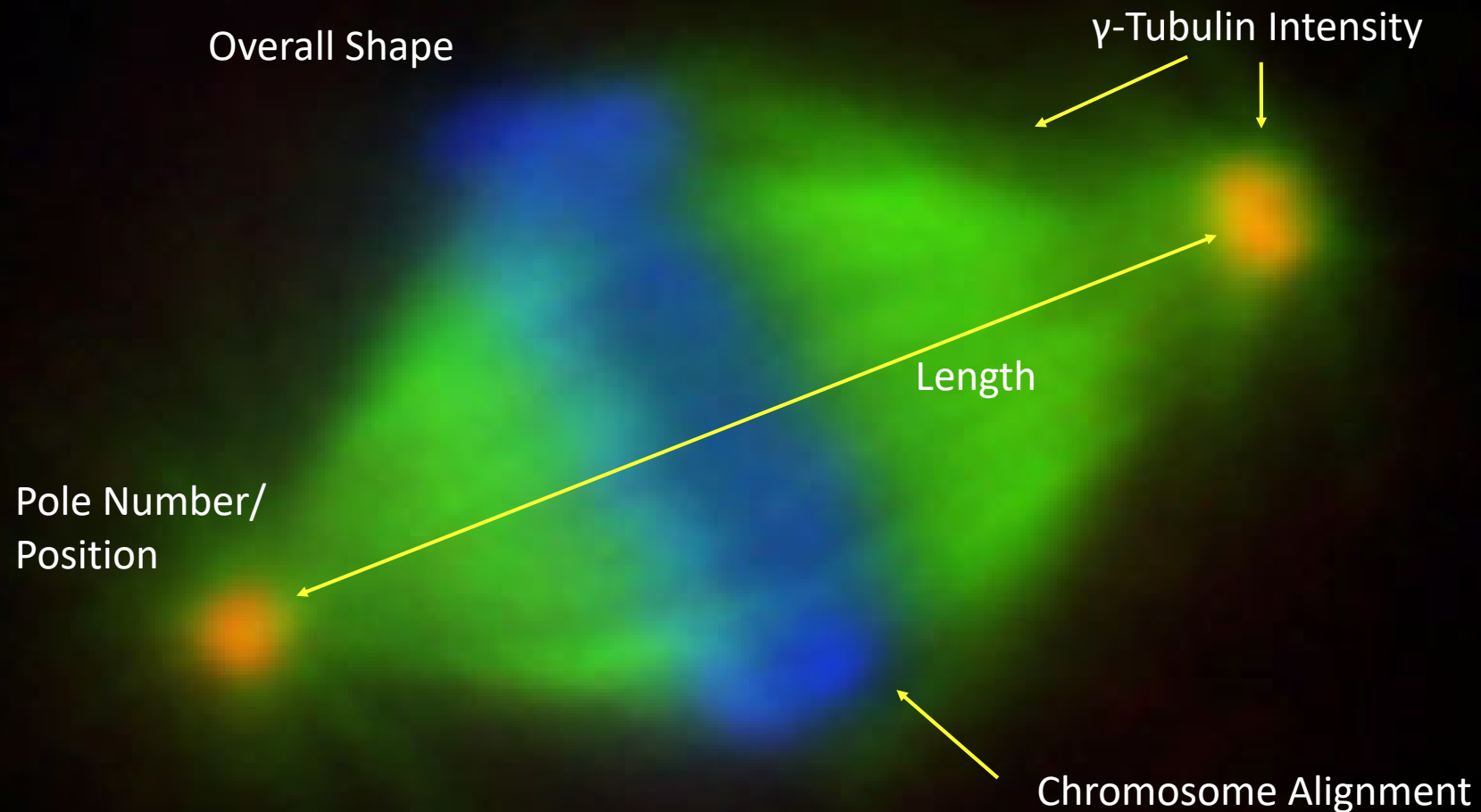
Gohta Goshima, Nico Stuurman, Nan Zhang, Sarah Goodwin (UCSF)

Roy Wollman,
Jon Scholey (UC Davis)



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

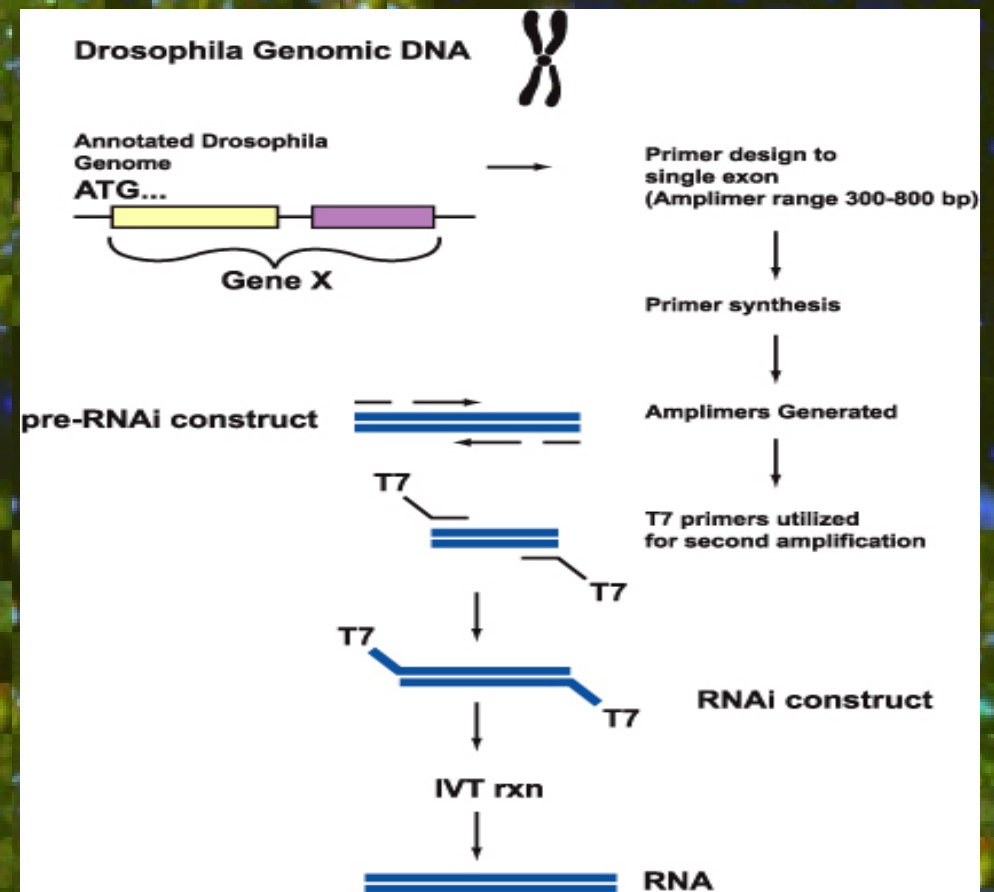
Image-Based Approach for Identifying Spindle Defects Generated by RNAi



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

High-throughput RNAi Screen

1. Full Fly Genome dsRNA Library:



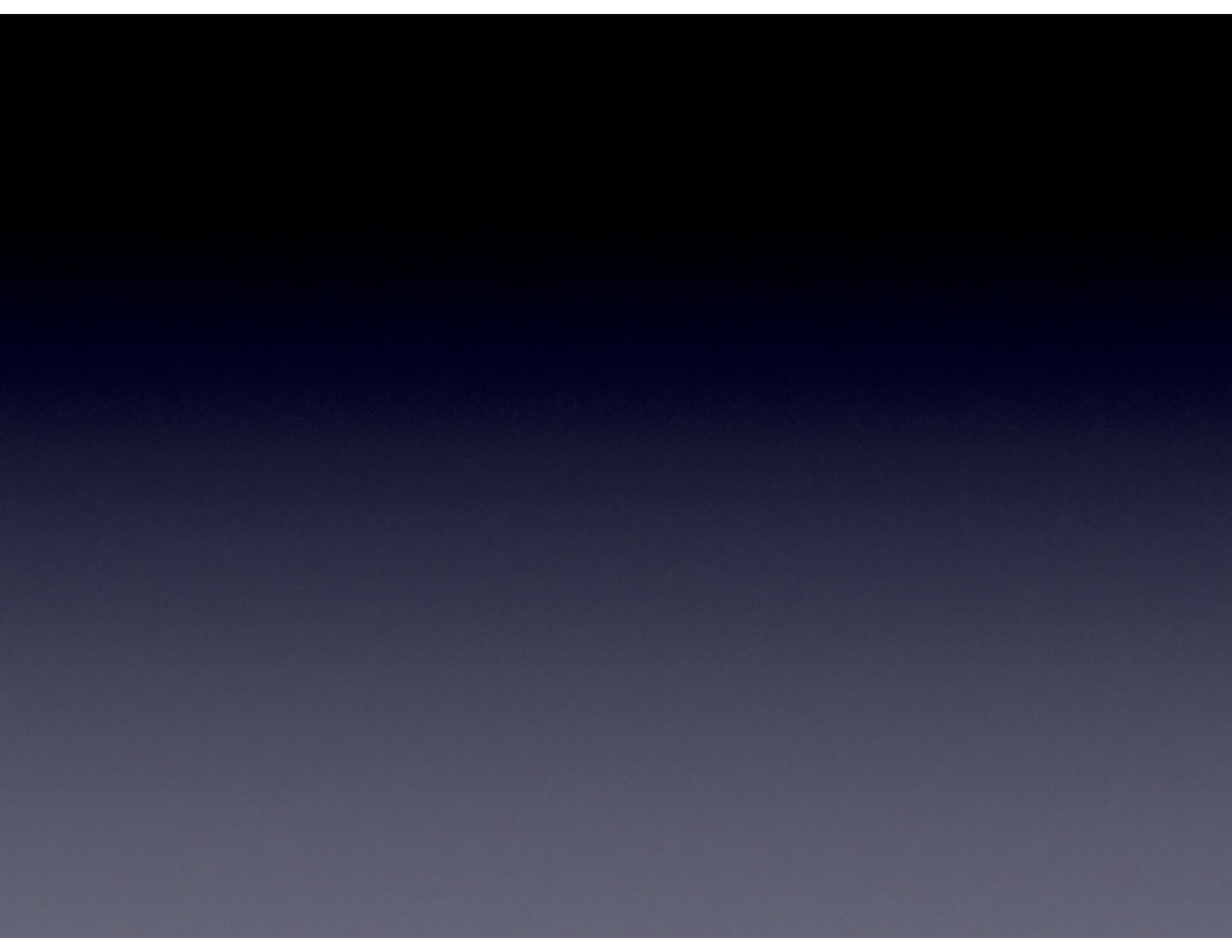
High-throughput RNAi Screen

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

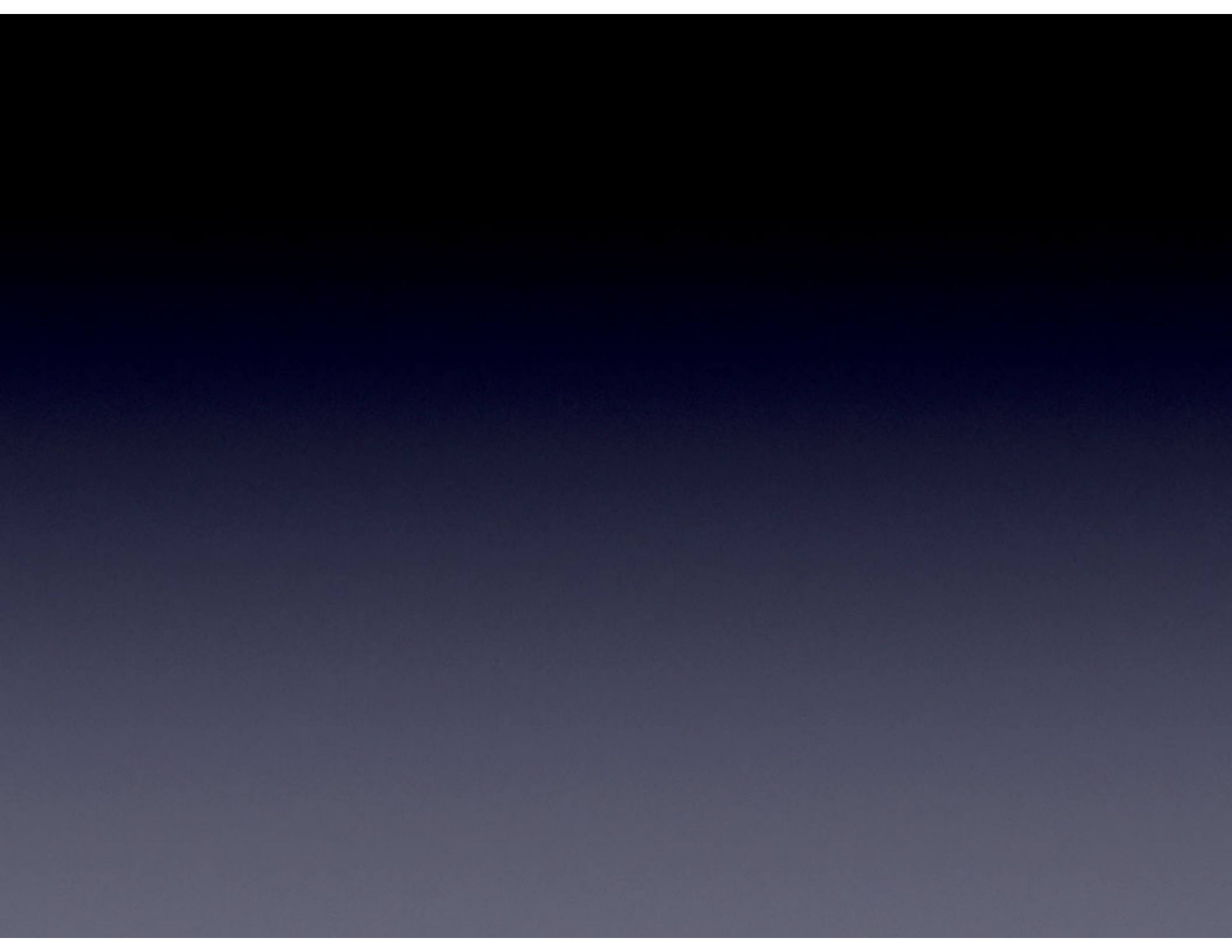


High-throughput RNAi Screen

3. High-throughput Microscopy Images

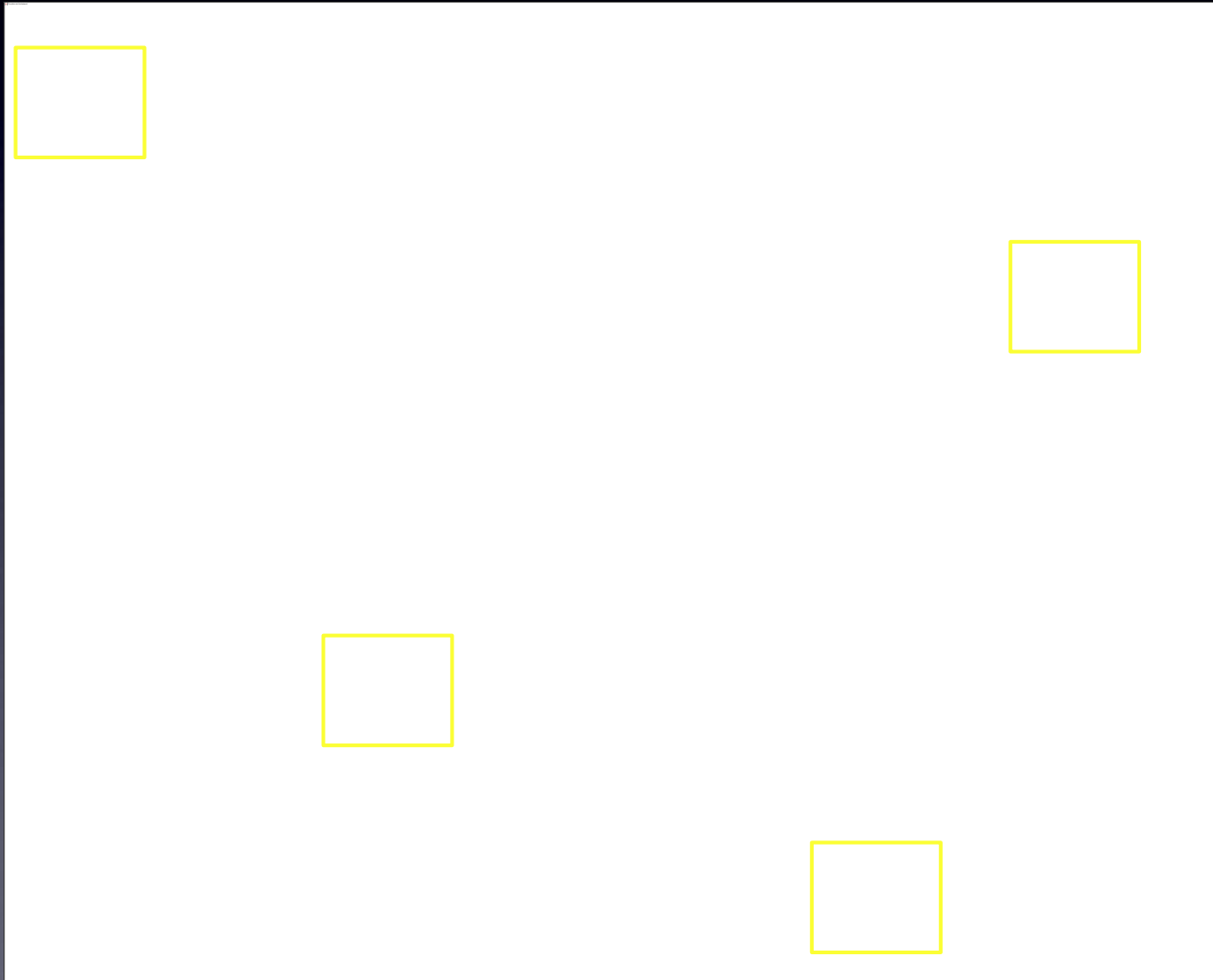


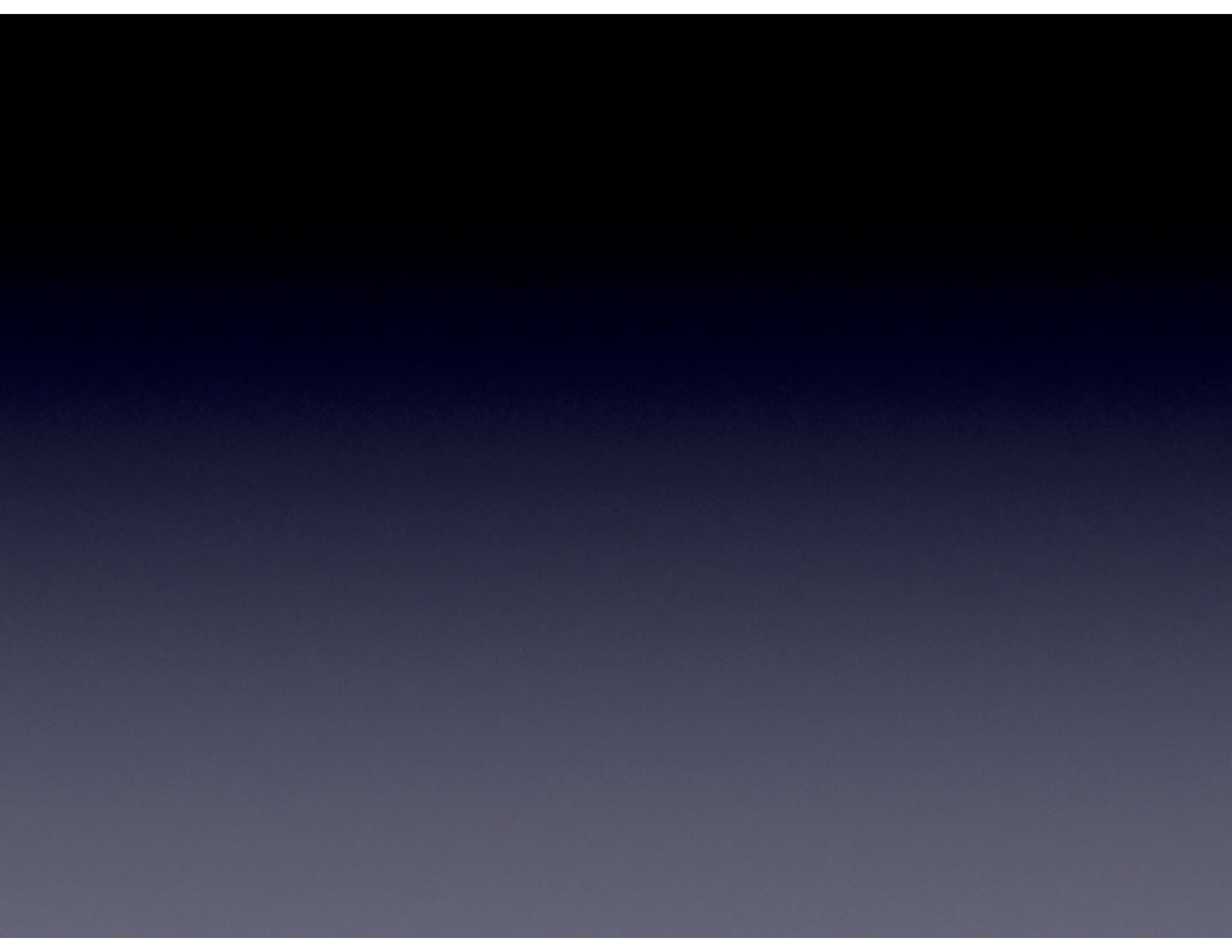
30-70 sites
8-bit BMP!
~25GB/plate
4TB total



High-throughput RNAi Screen

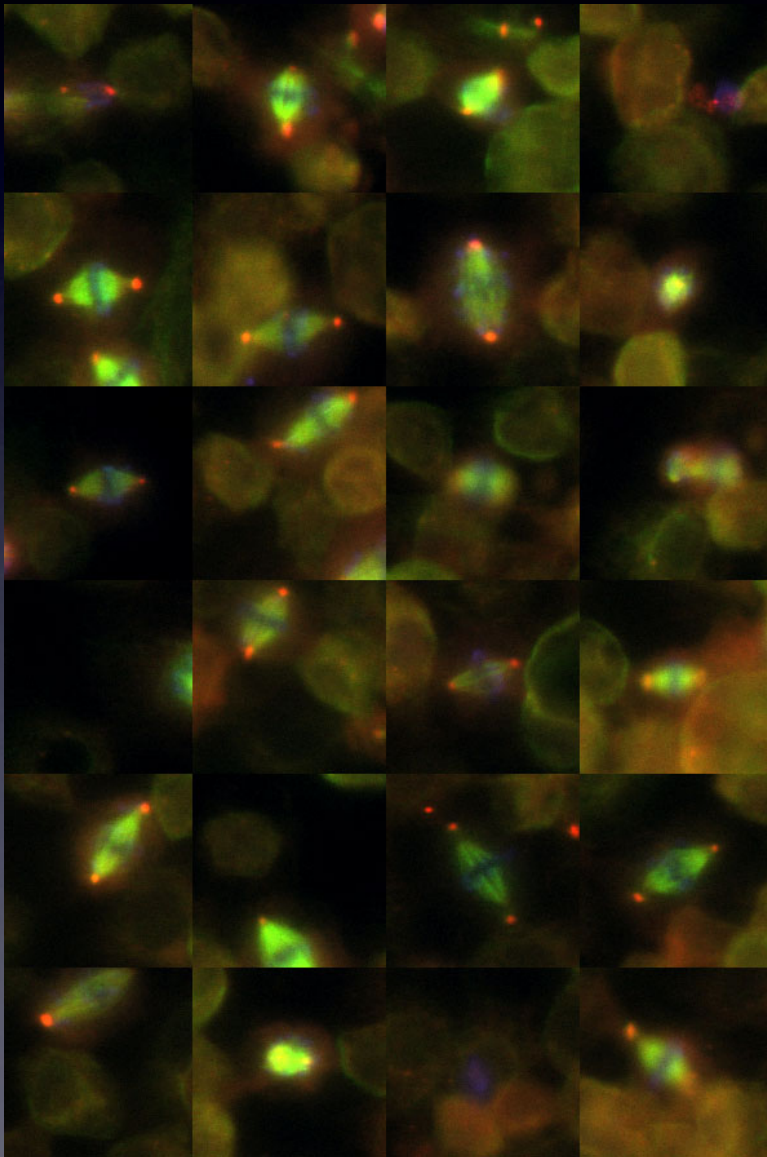
4. Automatic detection of metaphase cells



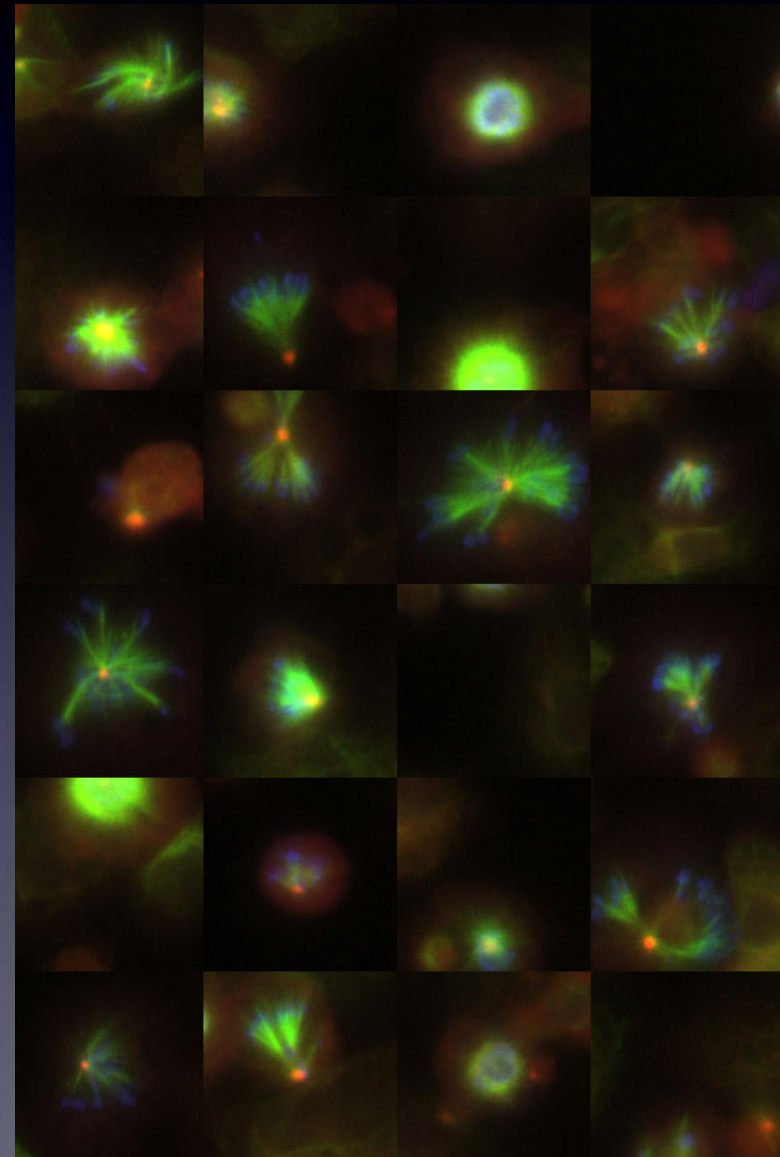


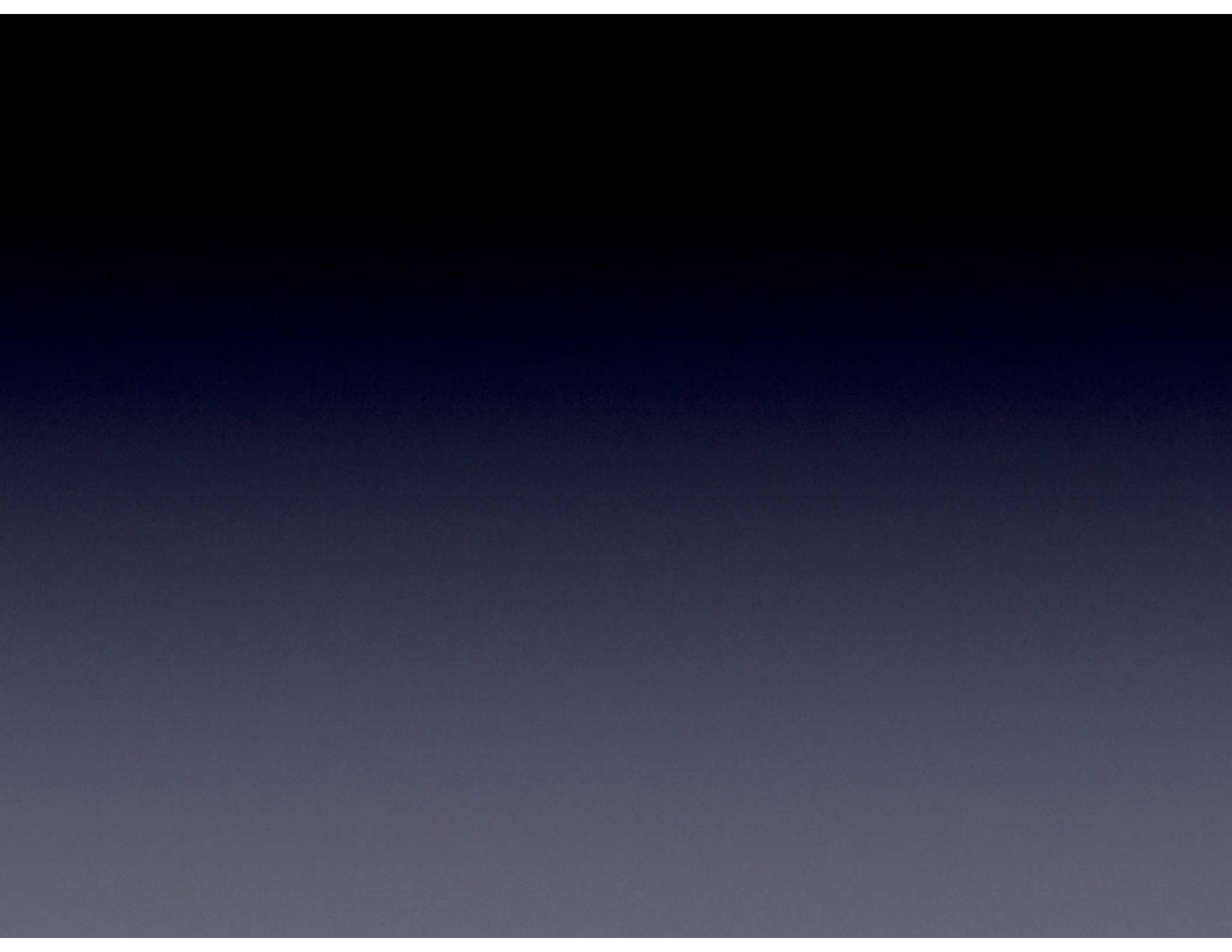
Example

Control



Monopolar spindle (Kinesin-5/Klp61F)





Workflow

Automated microscope

valecrunch.ucsf.edu

Database

EyectorixUI

Matlab Analysis

valebup2.ucsf.edu

Matlab Analysis

Backup

valelab.ucsf.edu

8-bit conversion - storage

phplabware - PostgreSQL



Vale Lab Screen Data Base

(<http://rna.i.ucsf.edu>)

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 long spindles

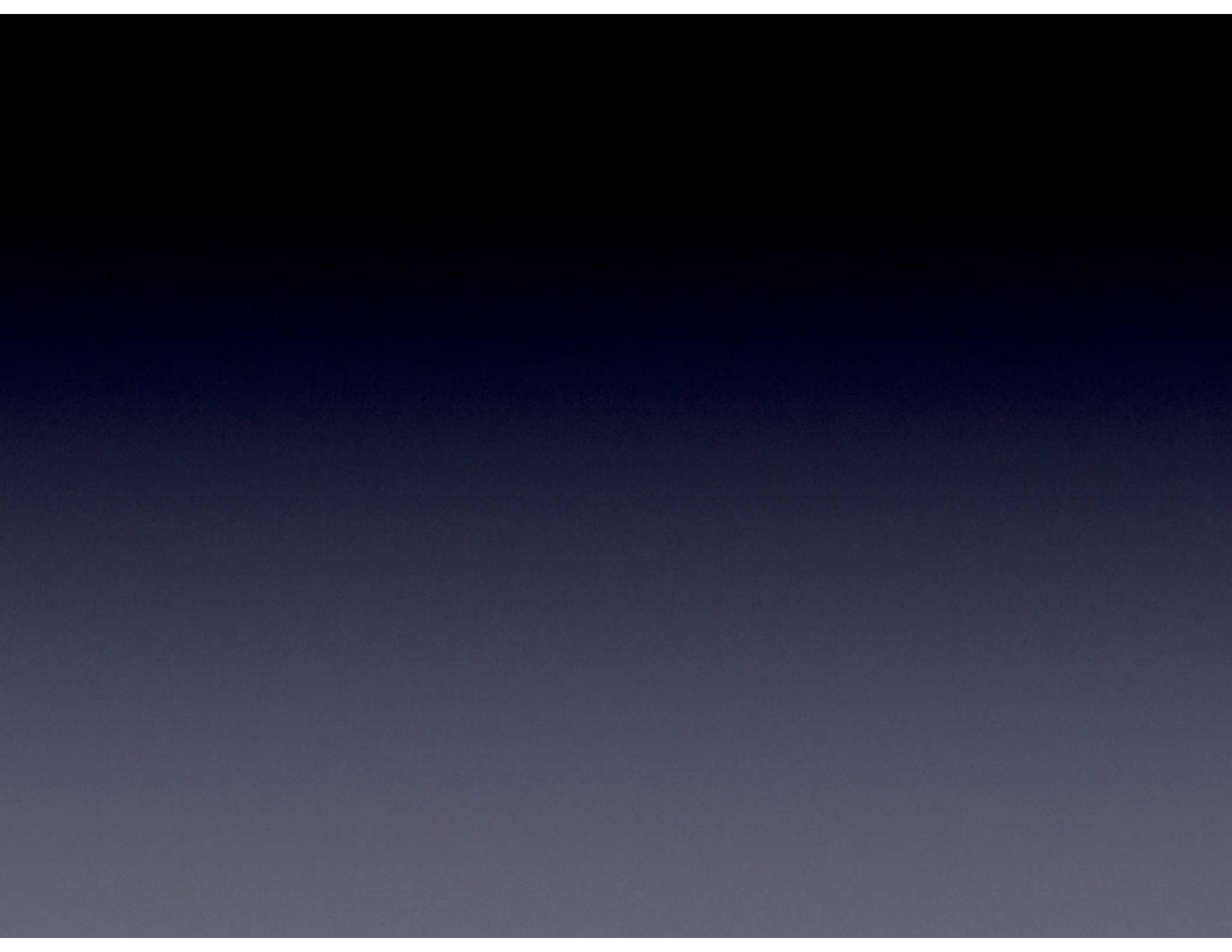
Search Show All

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 - Clear 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																	
prc																	
inter																	
prot																	
								119.71	9.43%								
								96.02	9.79%								
								140.21	8.47%								
								144.57	8.23%								
								73.62	7.21%								
								72.67	4.88%								
								88.19	10.88%								
								98.67	7.55%								
								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%								
								76.07	11.86%								
								77.60	7.46%								
								81.07	7.40%								
								82.71	6.30%	No	No				1 2		
								95.49	9.04%	No	No				1 2 3		

Link to dsRNA information

Link to phenotype gallery

Done valelab.ucsf.edu





Acknowledgements/Reference

S

Kurt Thorn

Gohta Goshima

Roy Wollman

Yi Guo

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)