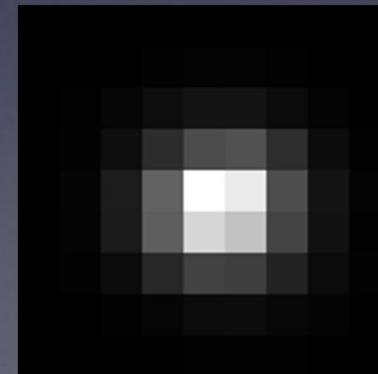


High Throughput Imaging

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 | 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



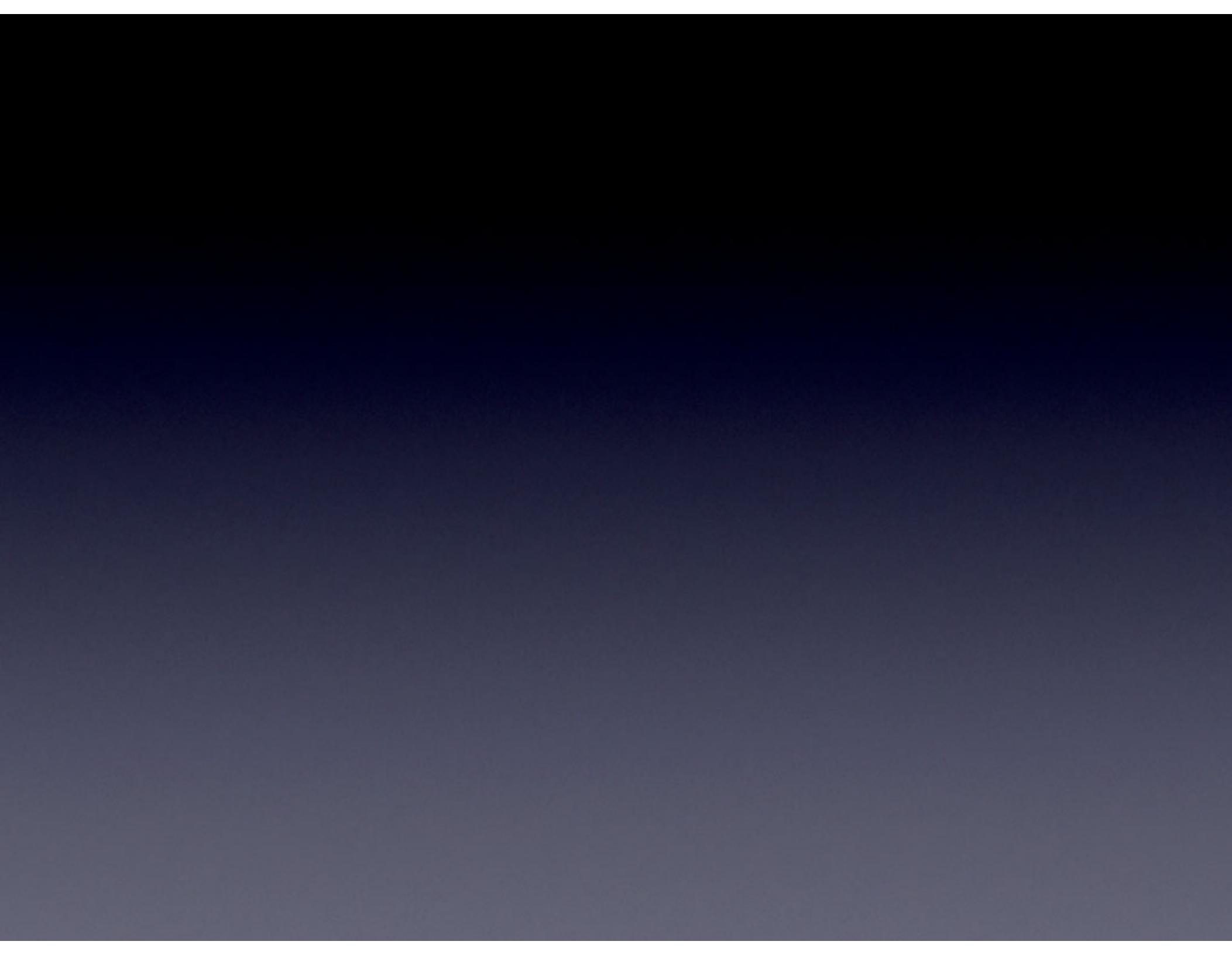
4-bit

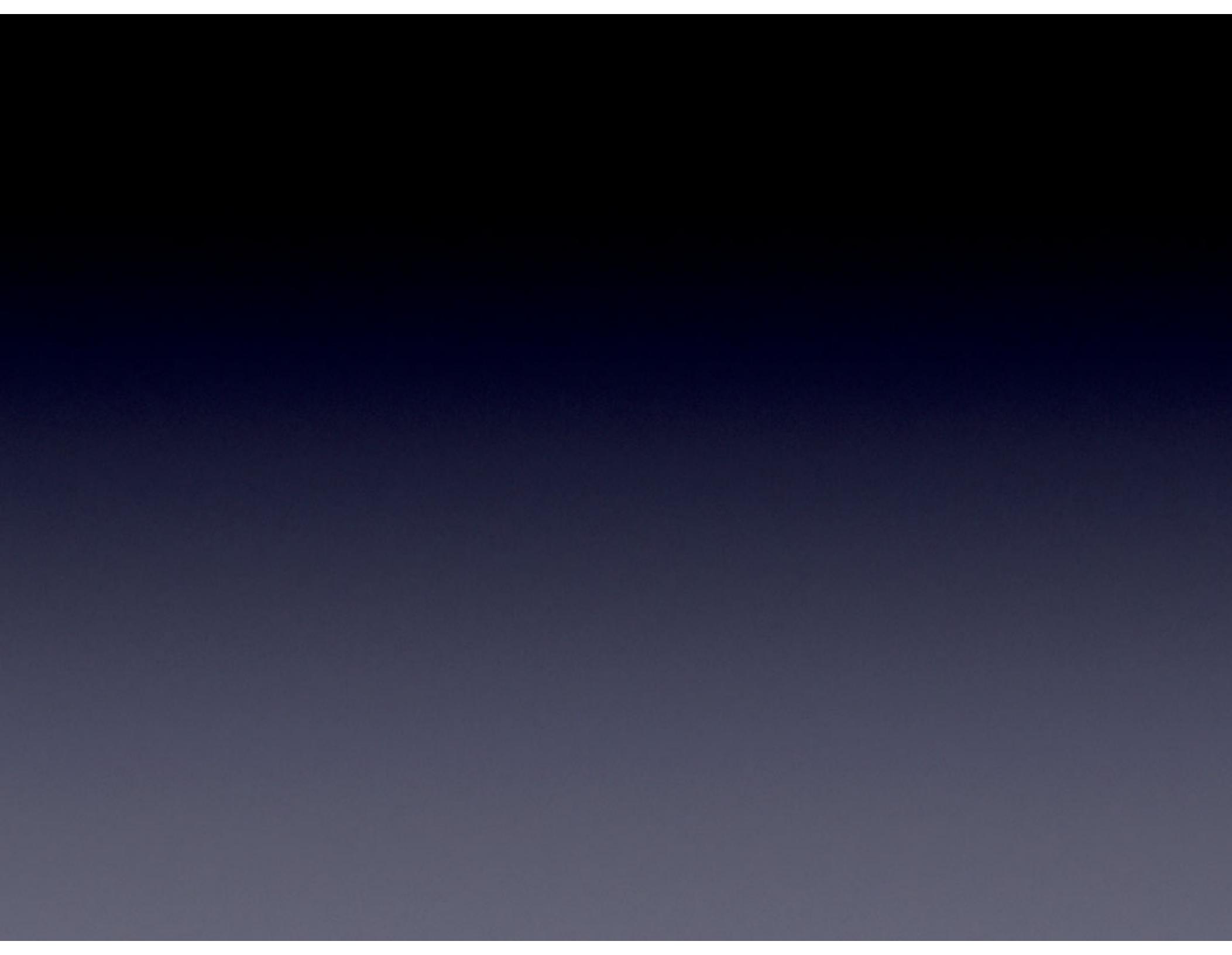


6-bit

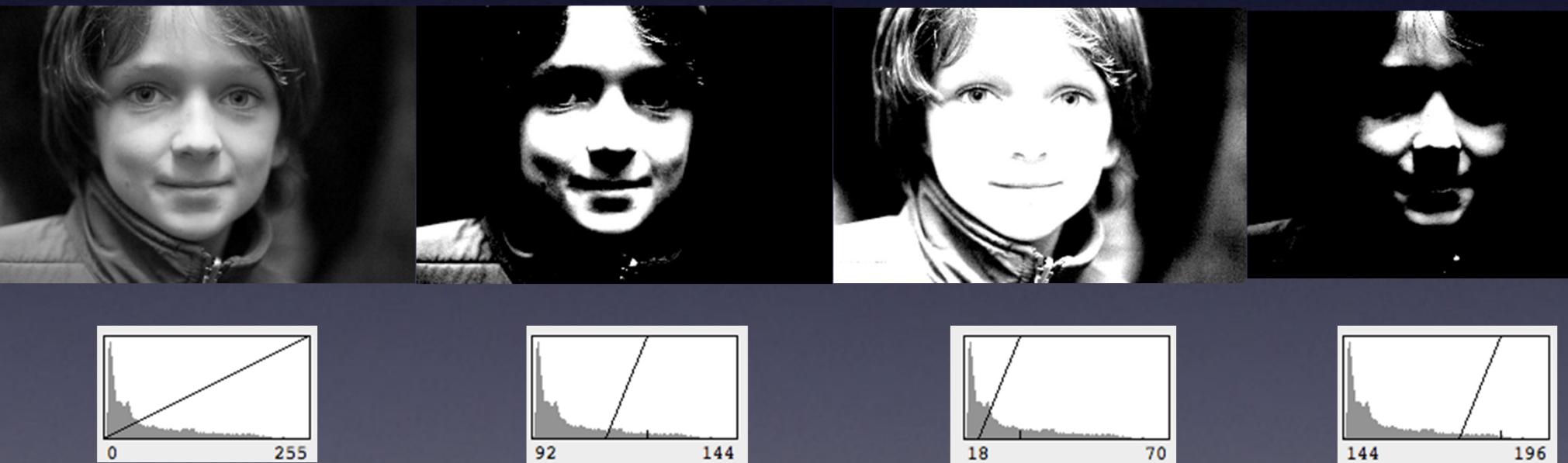


2-bit



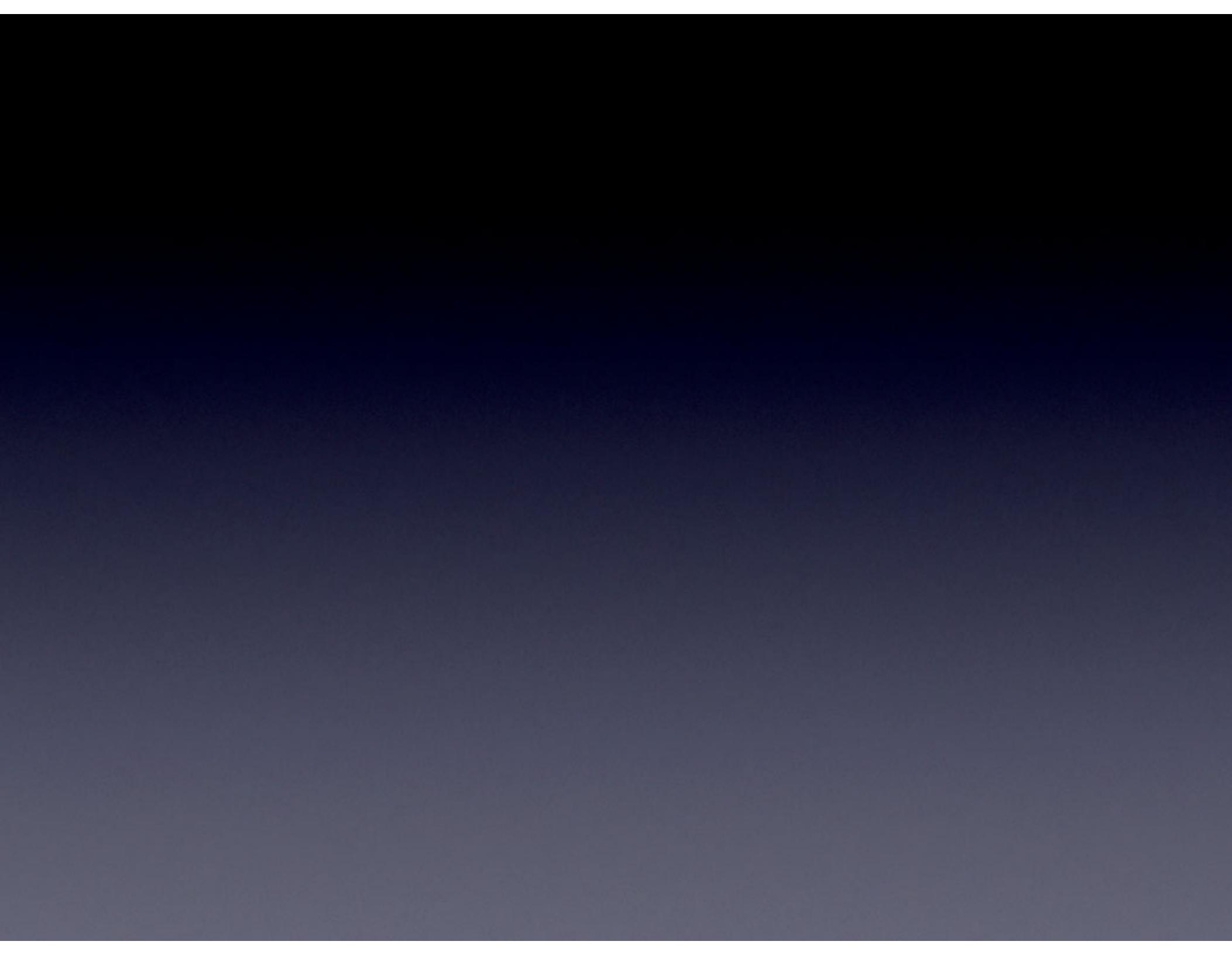


Mapping values onto display: Brightness/contrast

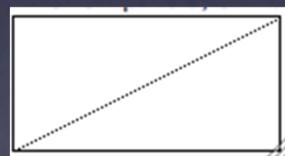
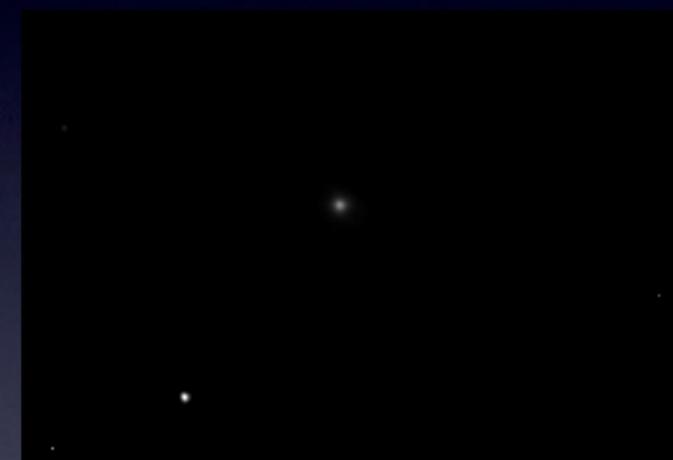
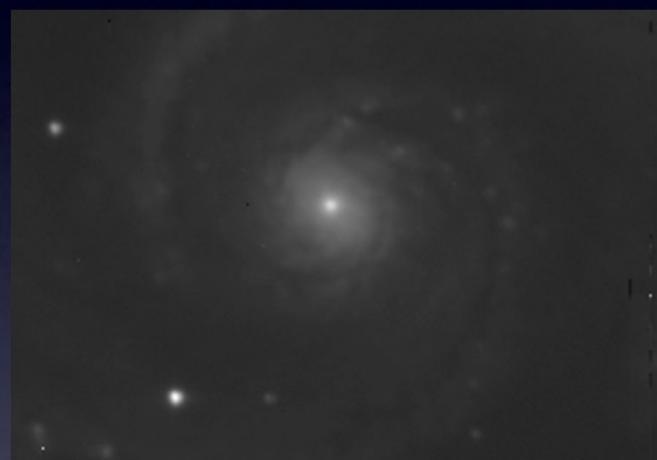


Slope = contrast

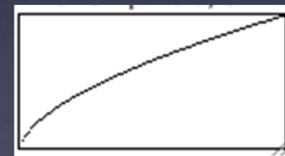
Brightness



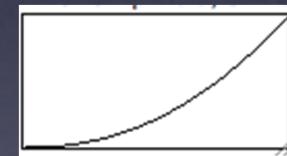
Gamma adjustment



1



0.6



2.2

What are acceptable image manipulations?

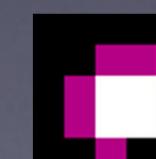
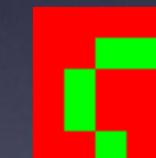
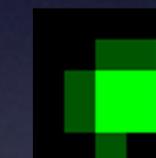
- JCB has the best guidelines
 - <http://jcb.rupress.org/content/172/1/9.full>
 - <http://jcb.rupress.org/content/166/1/11.full>
- Brightness and contrast adjustments ok, so long as done over whole image and don't obscure or eliminate background
- Nonlinear adjustments (like gamma) must be disclosed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

Lookup Tables (LUTs)

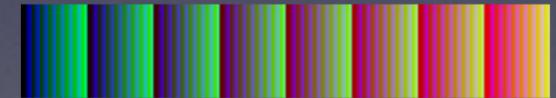
0 1 2 3



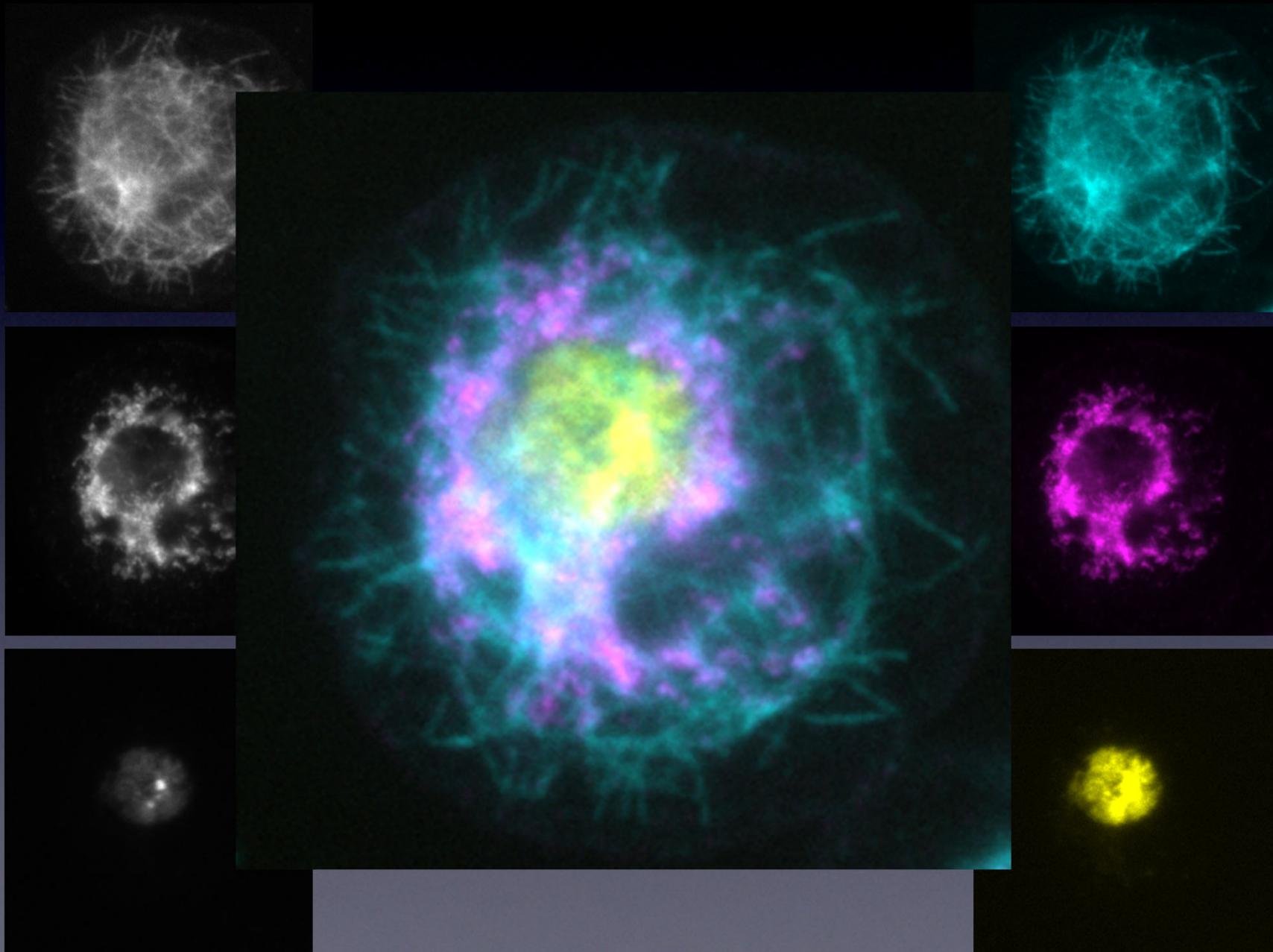
| | | | | |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 0 |
| 0 | 1 | 3 | 3 | 1 |
| 0 | 1 | 3 | 3 | 1 |
| 0 | 0 | 1 | 0 | 0 |



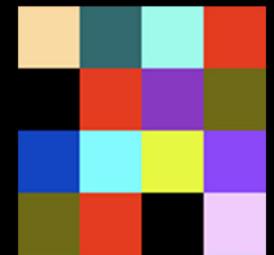
Lookup Tables (LUTs)



Lookup Tables (LUTs)



Color Images



Either:

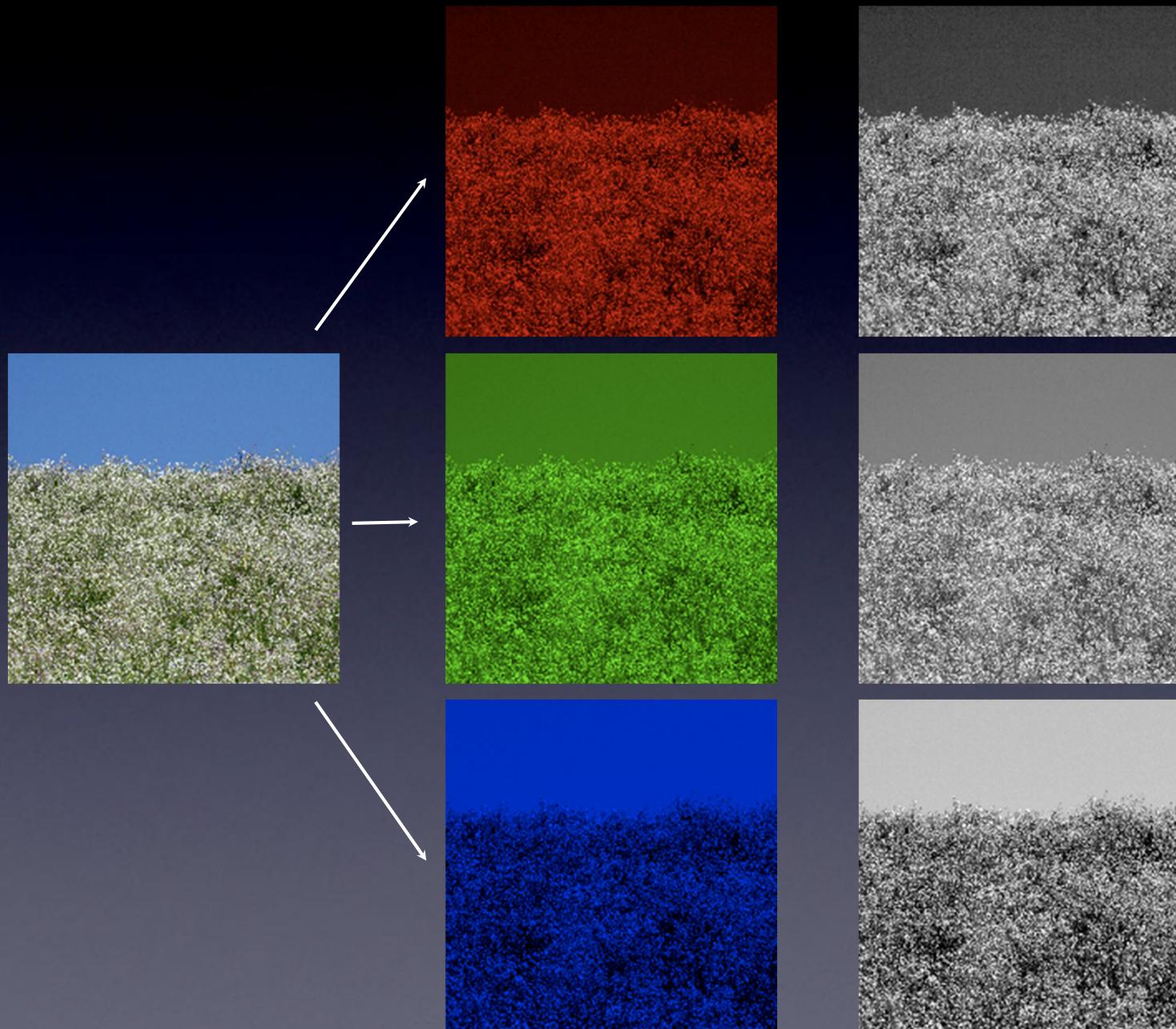
| | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|
| 255 | 209 | 139 | 0 | 89 | 93 | 93 | 255 | 231 | 255 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 255 | 0 | 0 | 134 | 0 | 185 | 93 | 90 | 0 | 255 | 0 | 0 |
| 0 | 39 | 185 | 0 | 255 | 255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

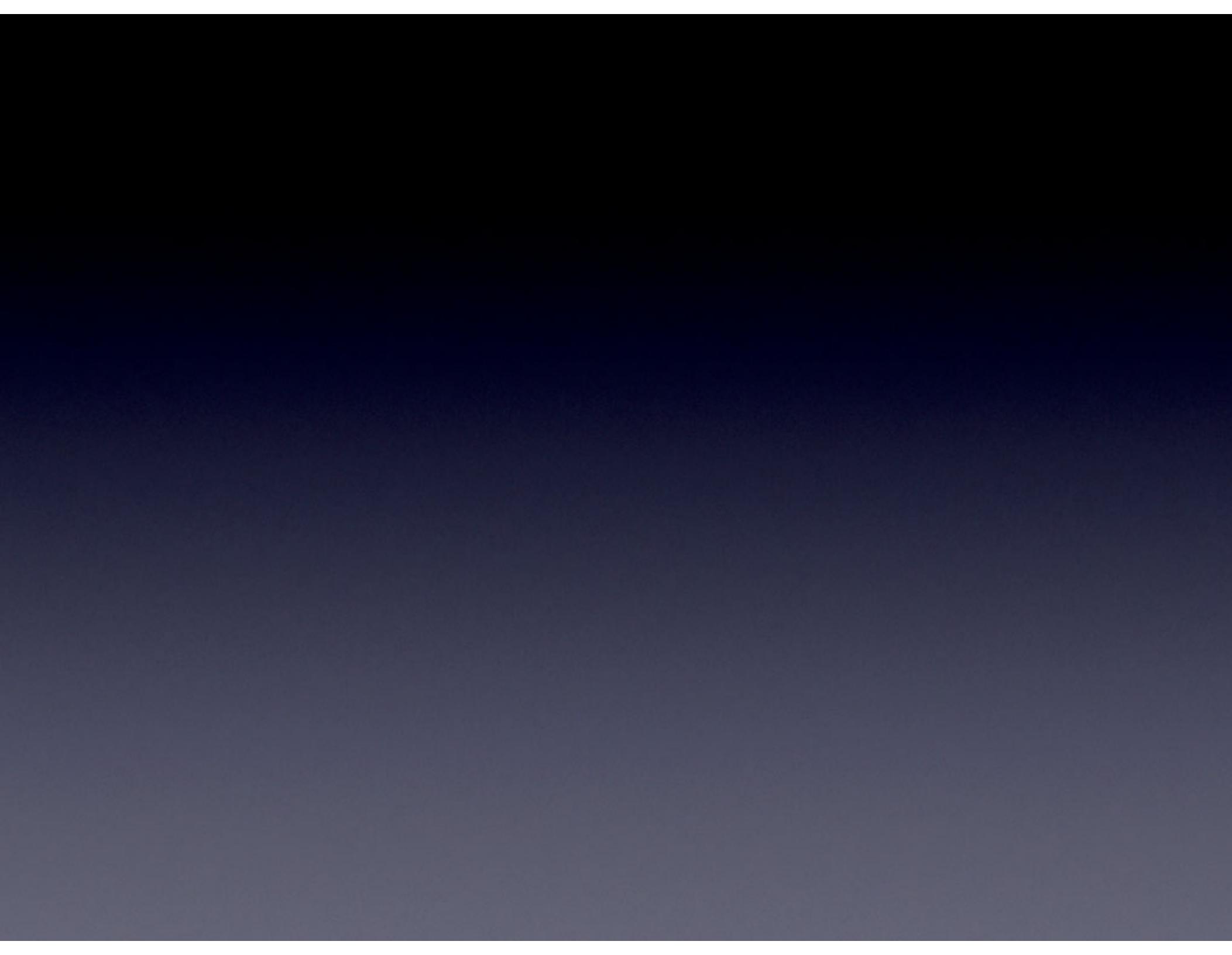
Or:

| | | | |
|-----|-----|-----|-----|
| 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!

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

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

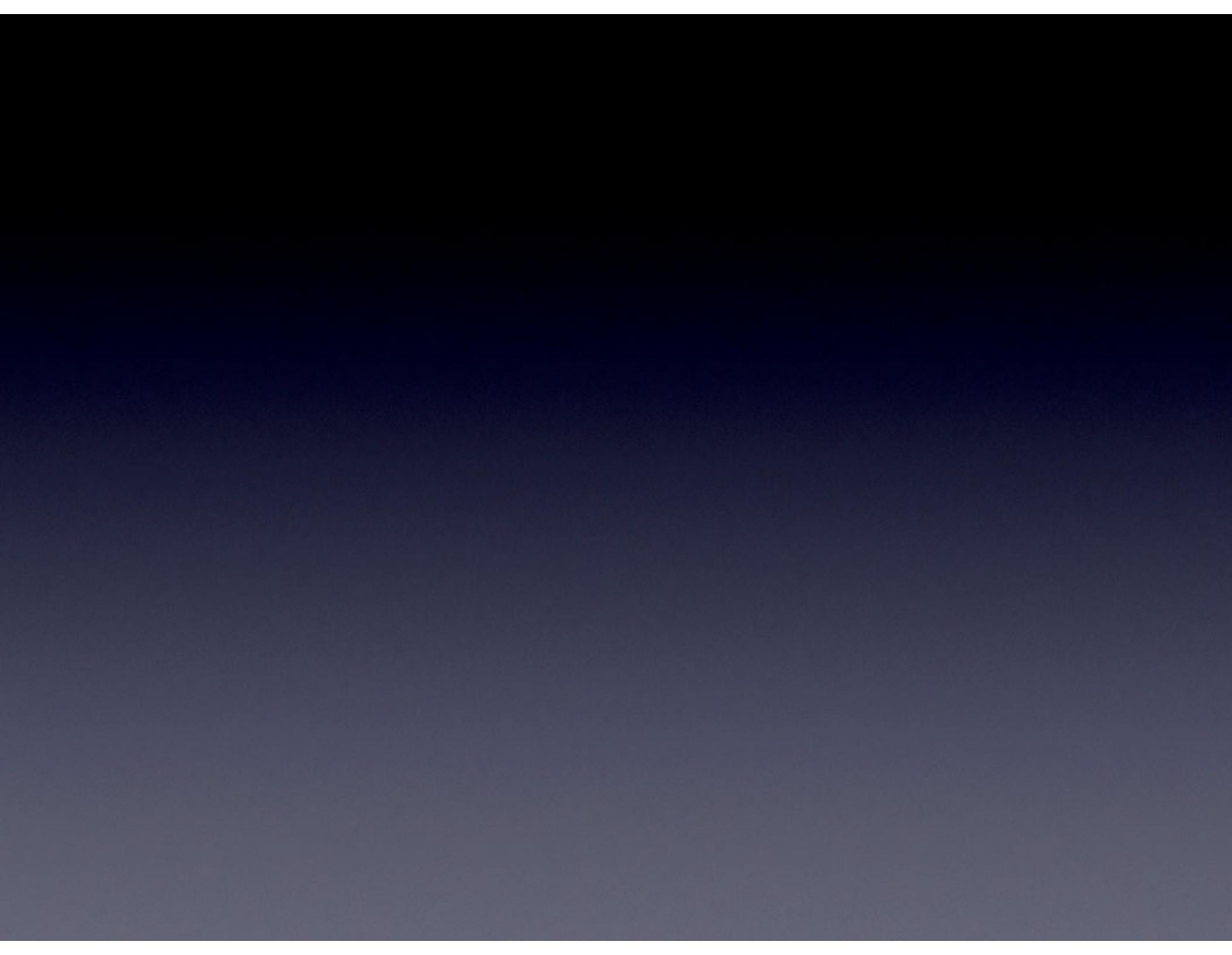
<http://micro-manager.org>

Presentation

- Photoshop
- Gimp

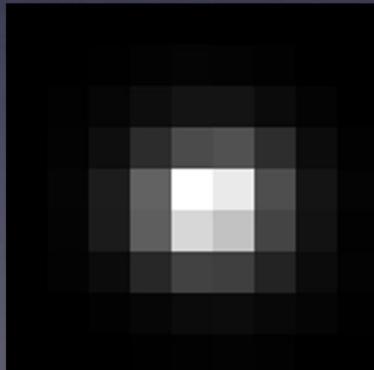
Analysis

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

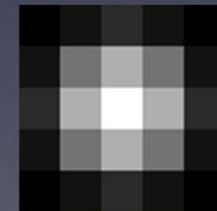


Why smooth?

- 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



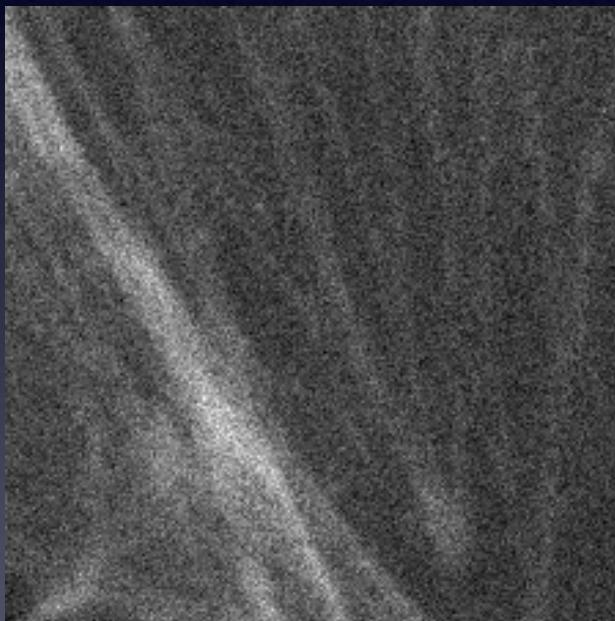
Measured PSF



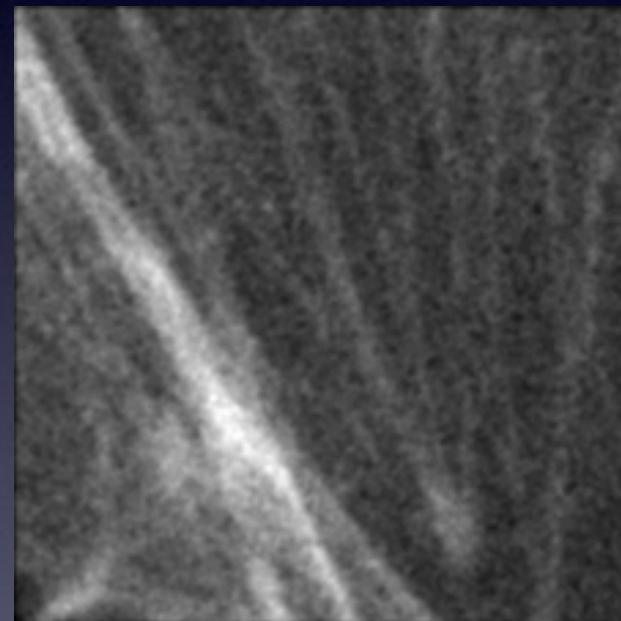
Gaussian Filter

Why smooth?

Averages redundancy and suppresses noise



10 photons/pixel average
5 e- read noise



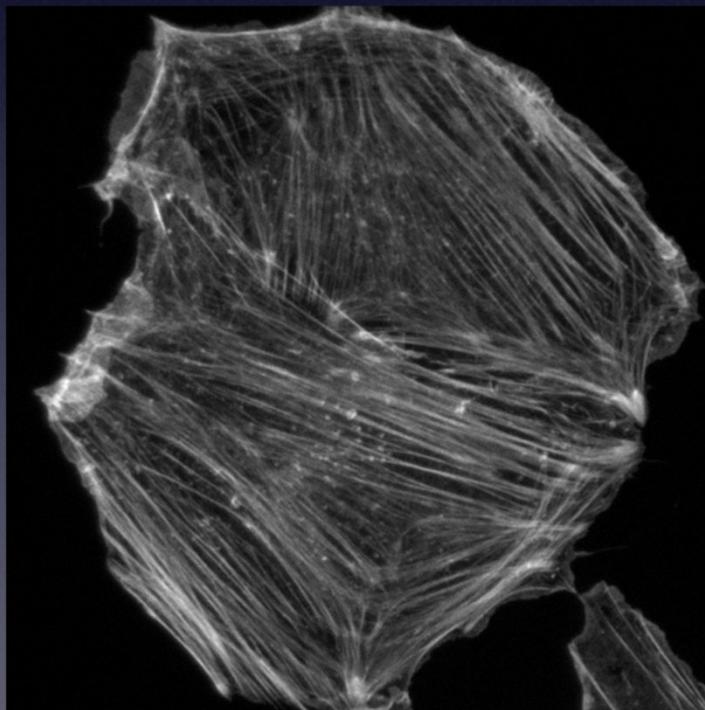
Gaussian smoothing filter,
 $\sigma = 1$ pixel

Other Filters

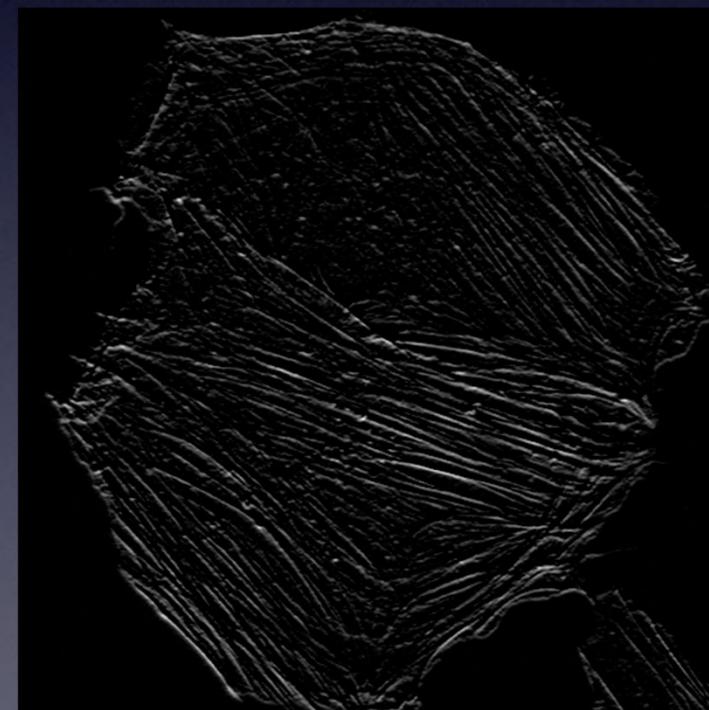
Edge Detection

| | | |
|----|----|----|
| 1 | 1 | 1 |
| 0 | 0 | 0 |
| -1 | -1 | -1 |

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



Original

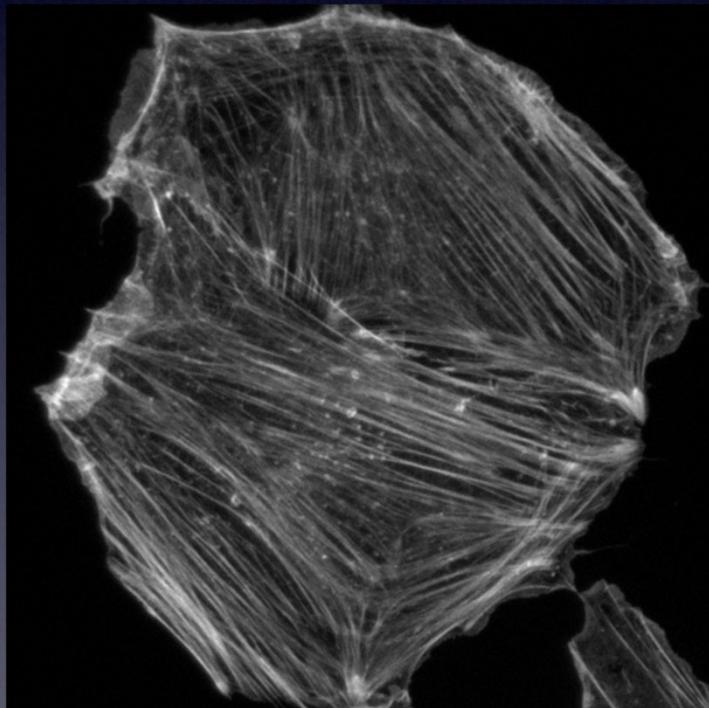


Horizontal edge detection

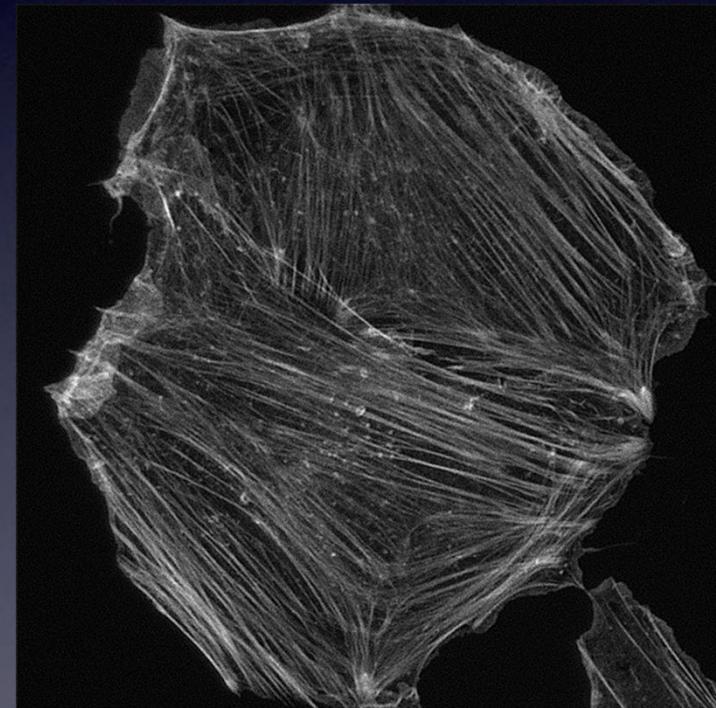
Other Filters

Unsharp Masking

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



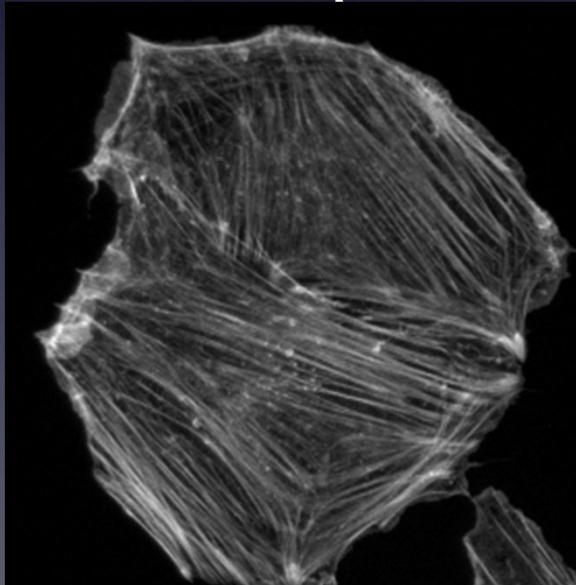
Original



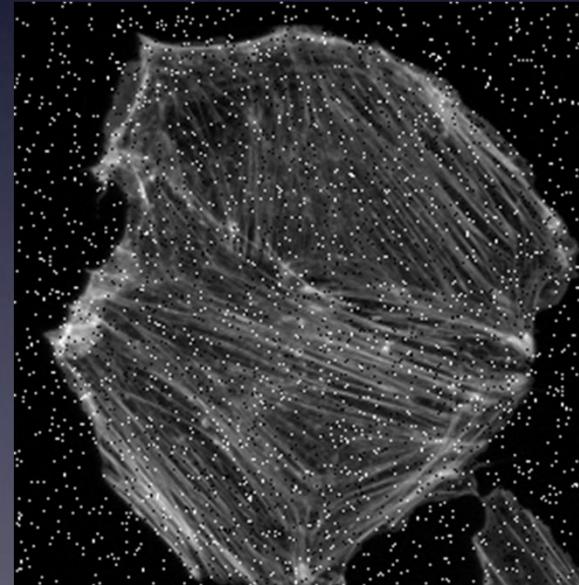
Unsharp masked

Non-linear Filters

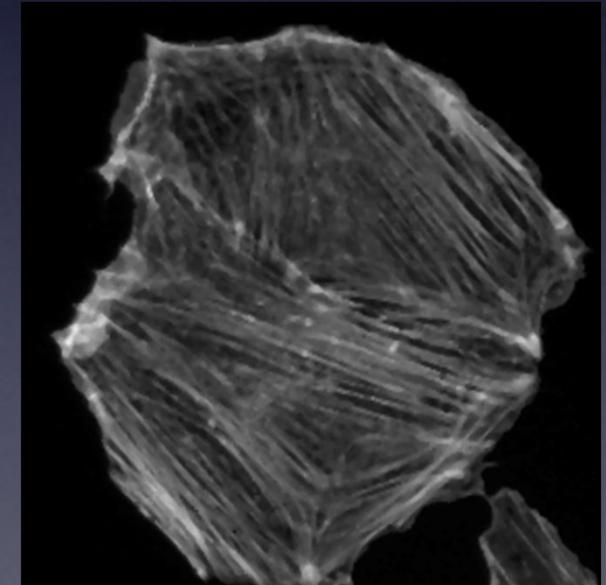
- Replace central pixel with min, max, median
- Median filter is a good noise filter, at the expense of resolution



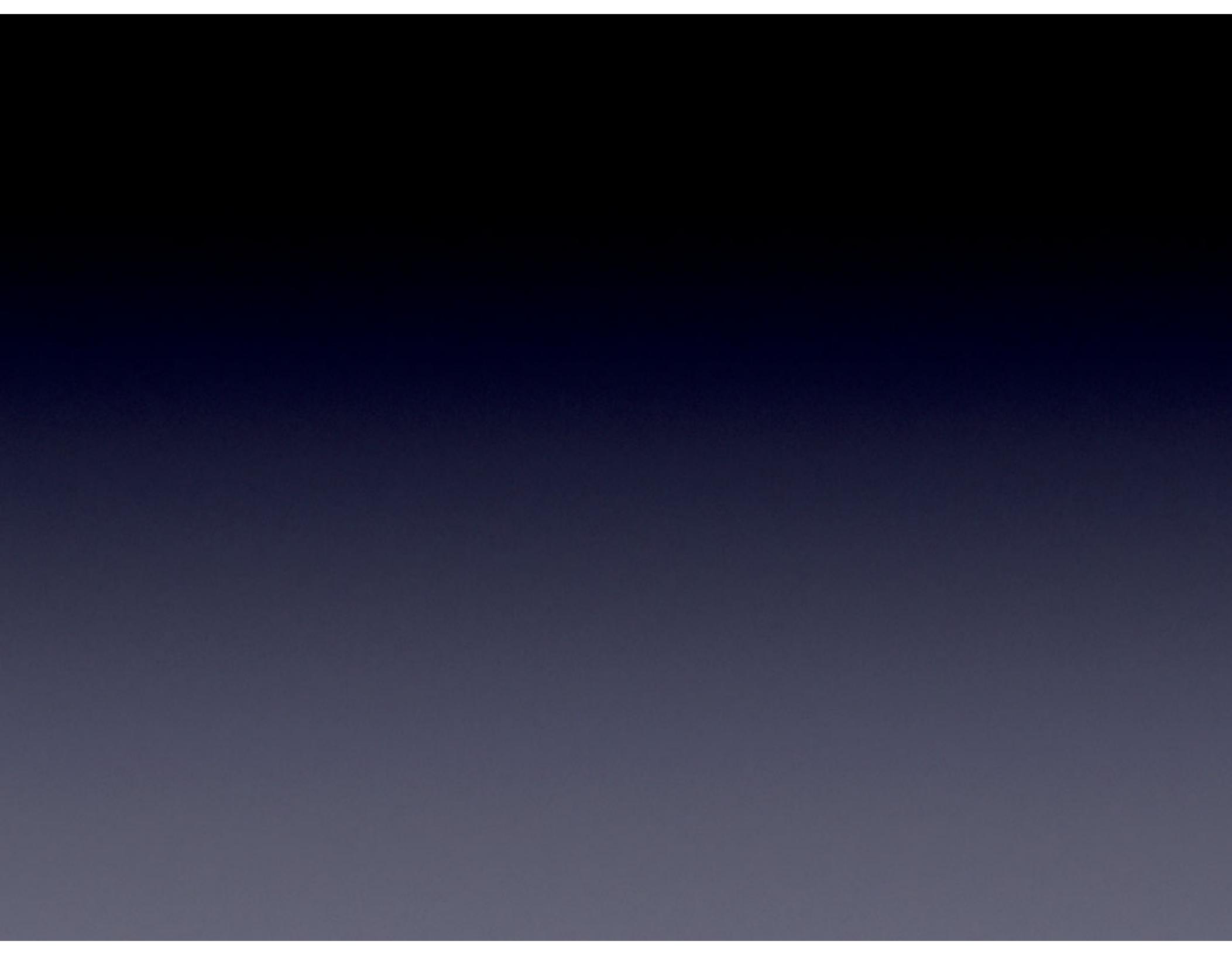
Original

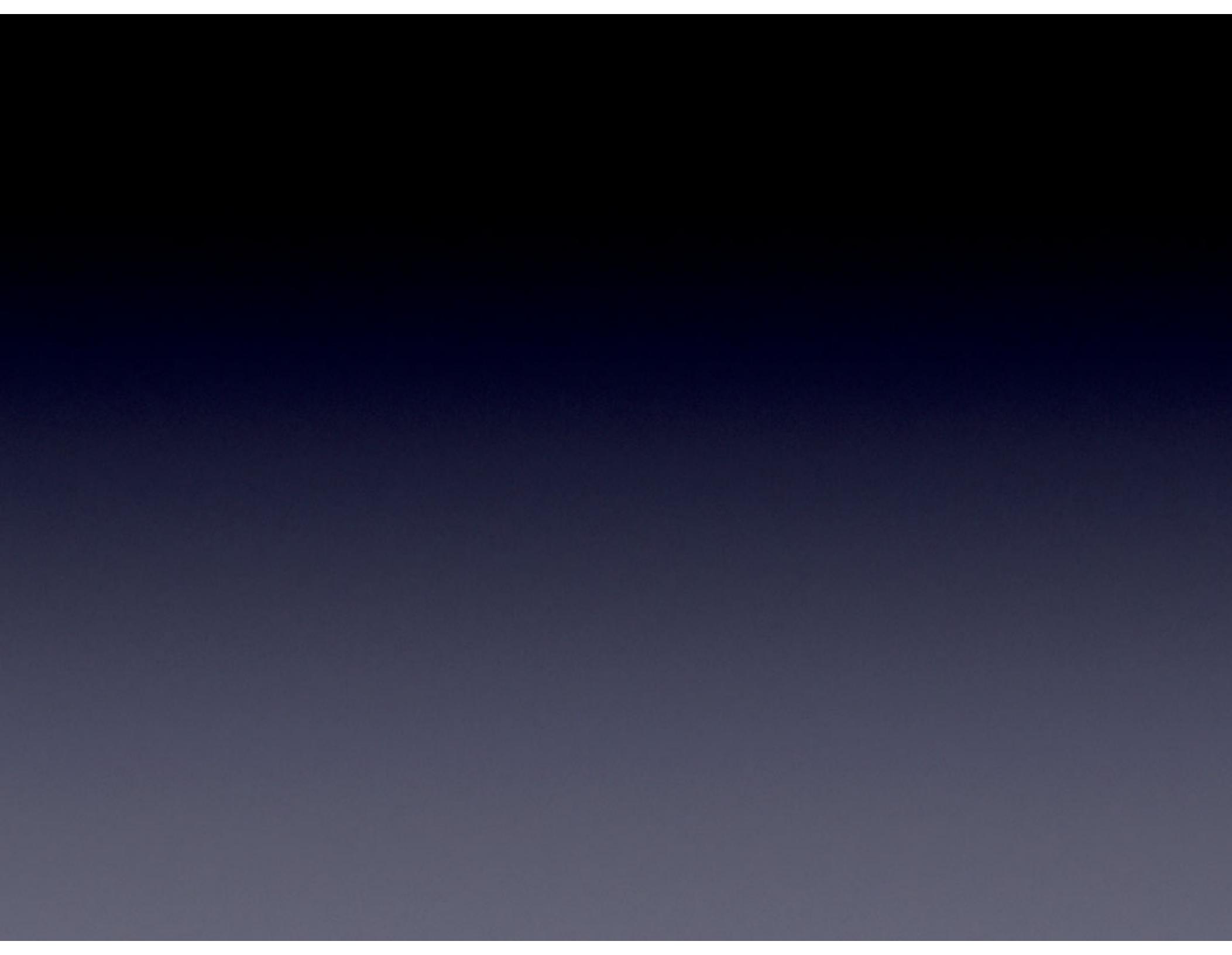


Artificial Noise



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



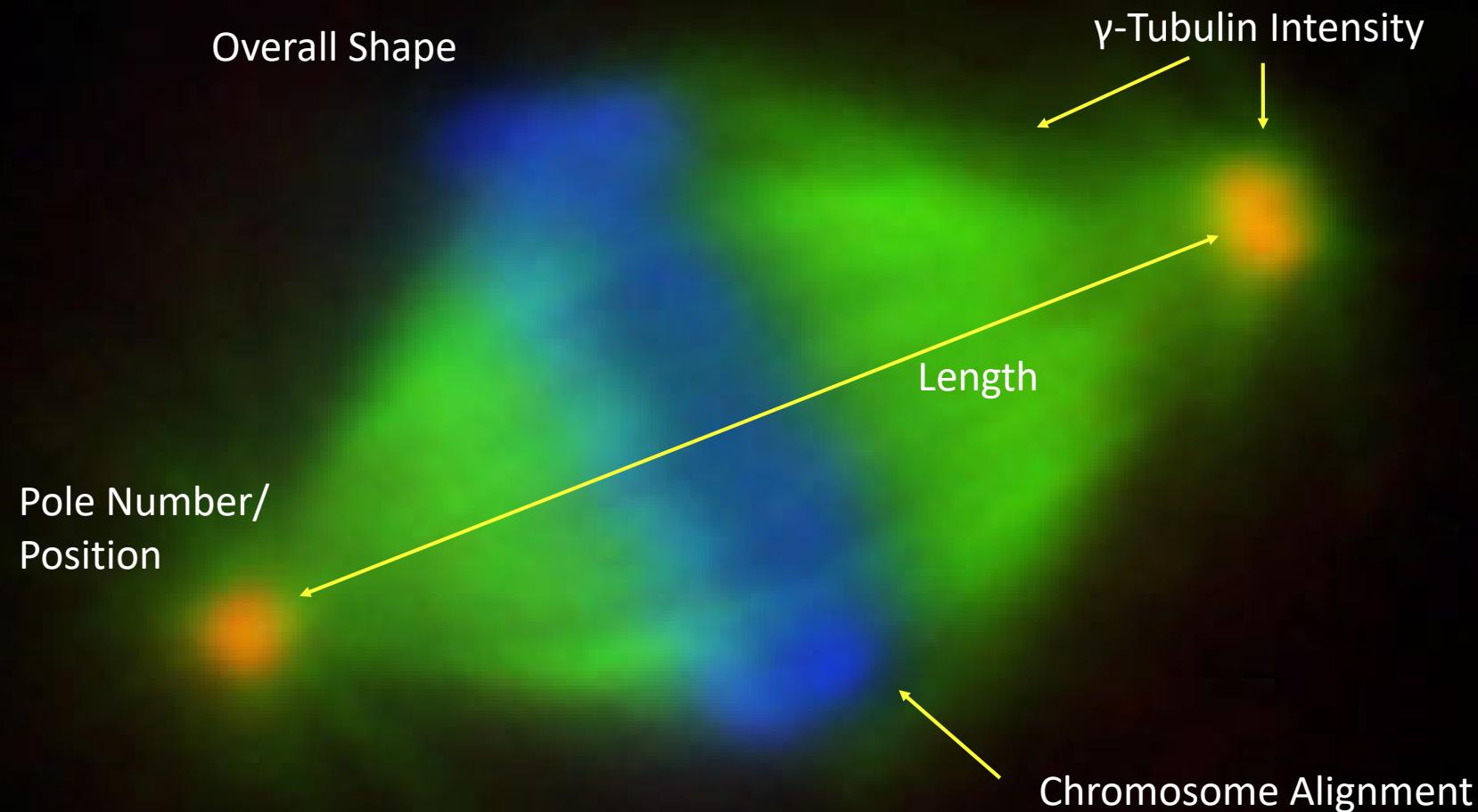
Gohta Goshima, Nico Stuurman, Nan Zhang, Sarah Goodwin (UCSF)

Roy Wollman,
Jon Scholey (UC Davis)



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

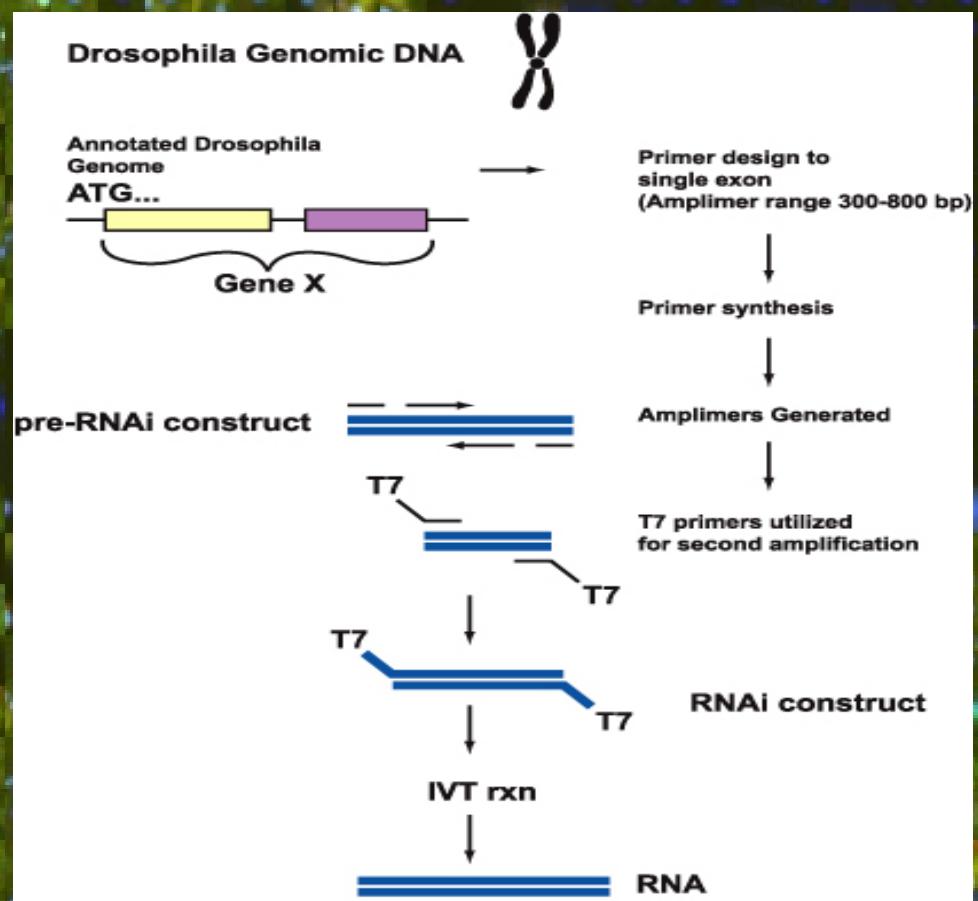
Image-Based Approach for Identifying Spindle Defects Generated by RNAi



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

High-throughput RNAi Screen

1. Full Fly Genome
dsRNA Library:



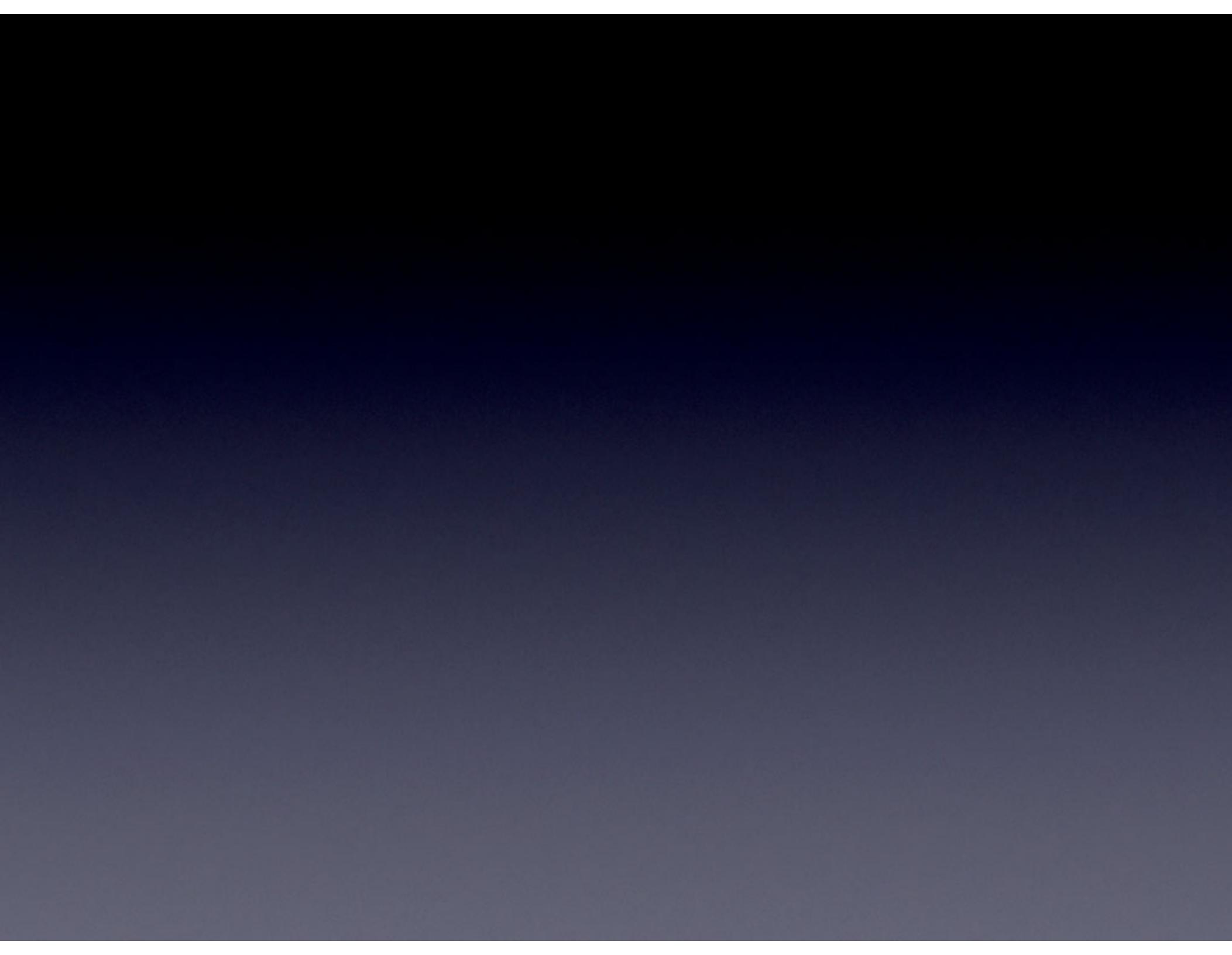
High-throughput RNAi Screen

2. Treat S2 Cells with dsRNA
for 4 days

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



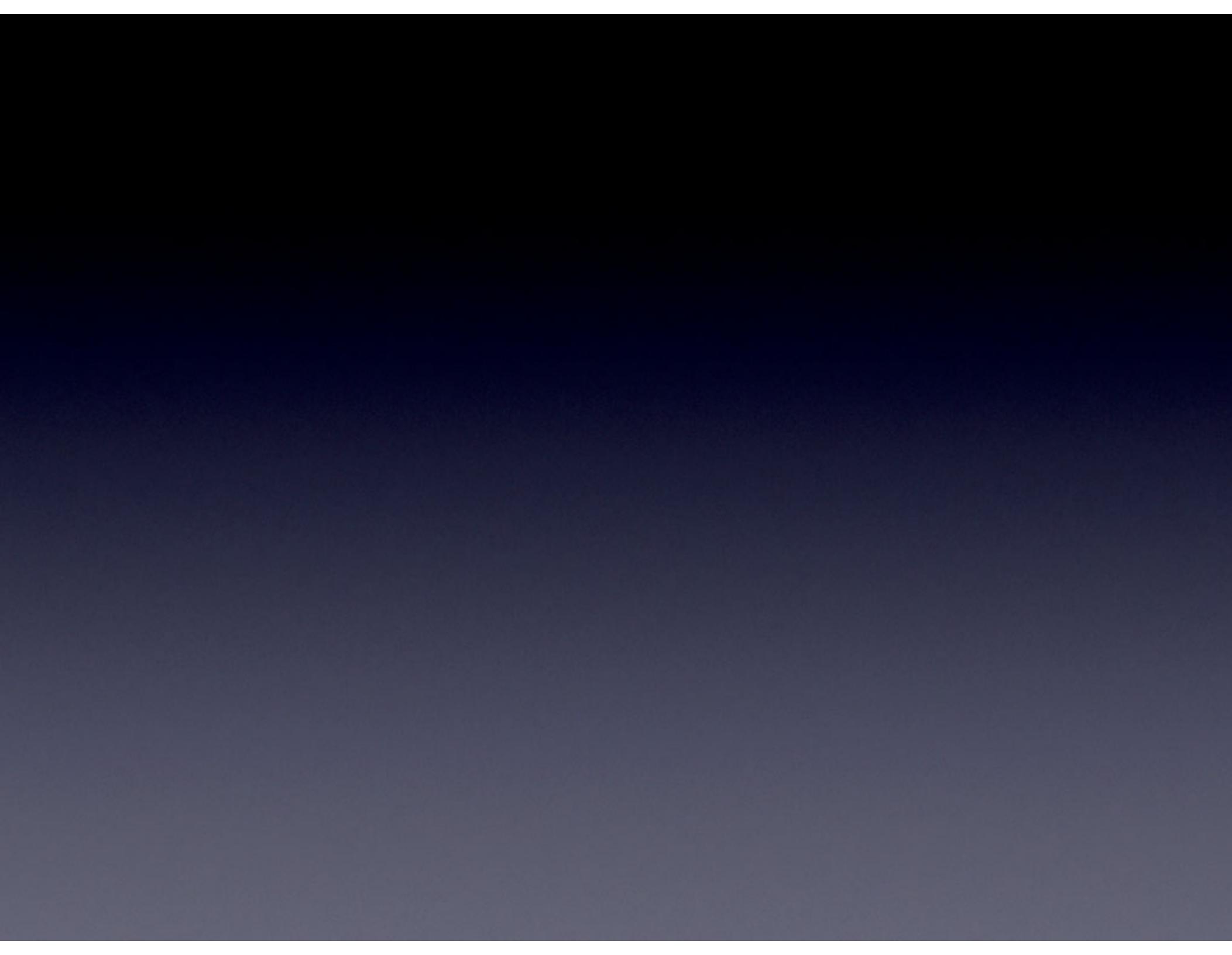
+ APC dsRNA to
induce metaphase arrest



High-throughput RNAi Screen

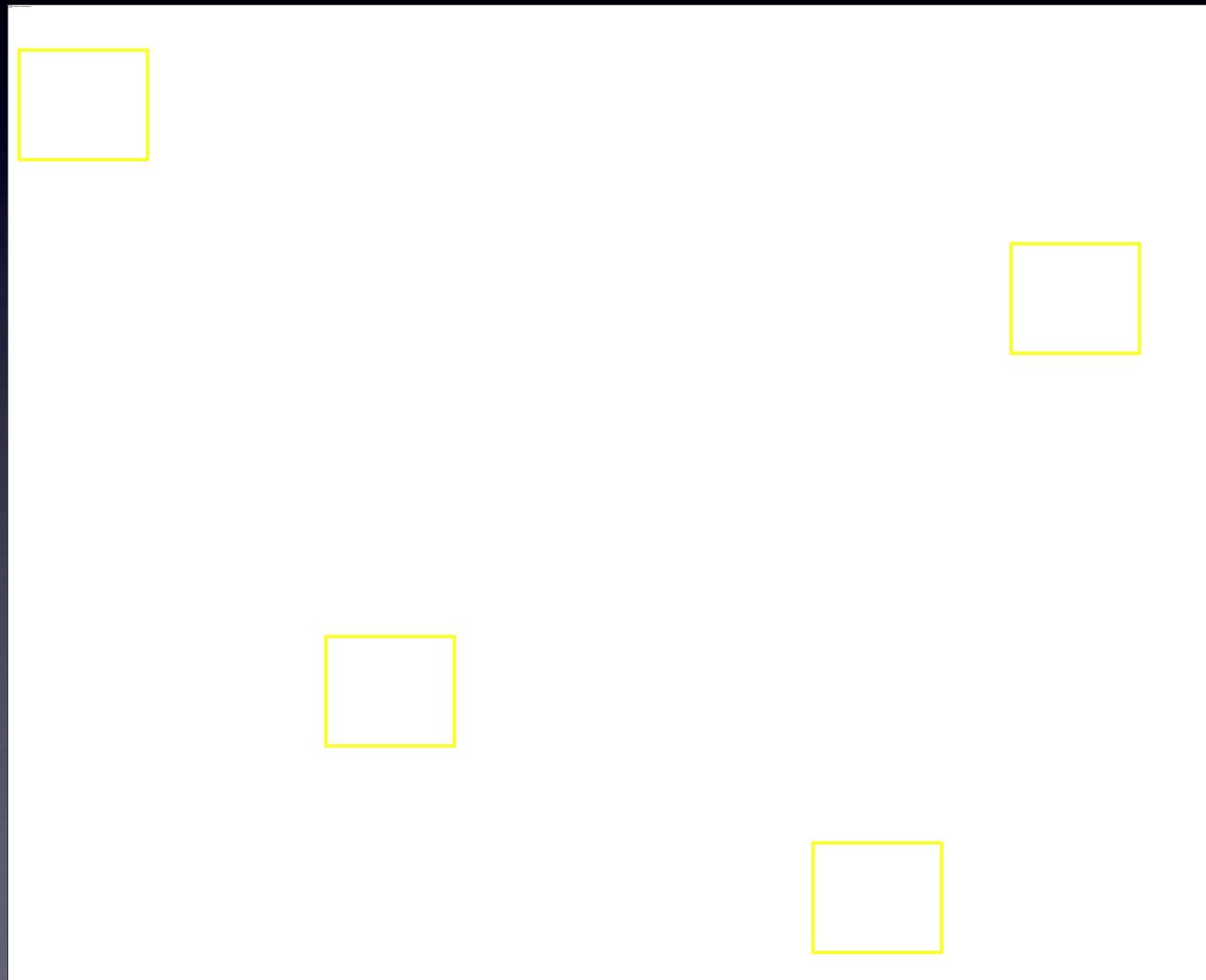
3. High-throughput Microscopy Images

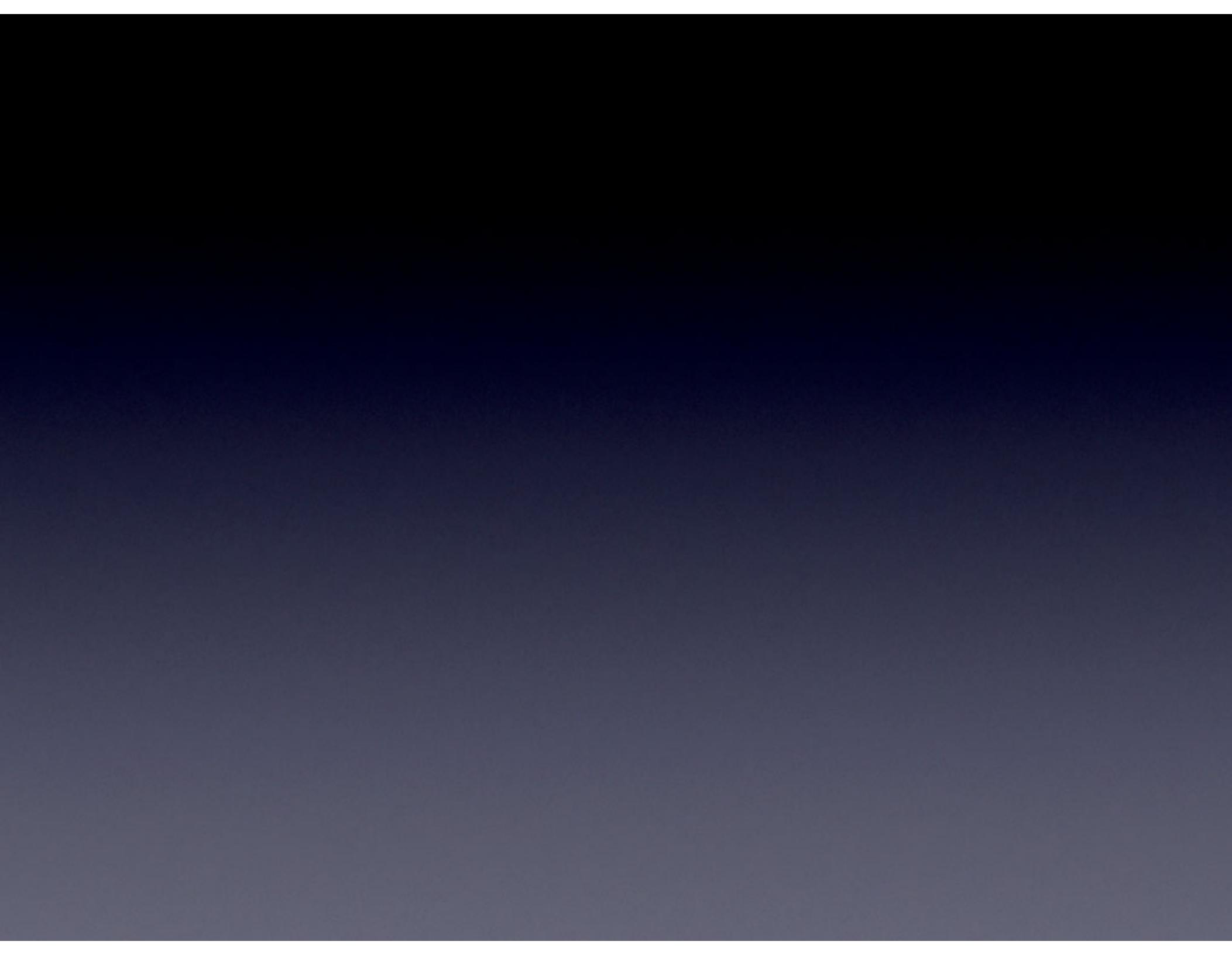
30-70 sites
8-bit BMP!
~25GB/plate
4TB total



High-throughput RNAi Screen

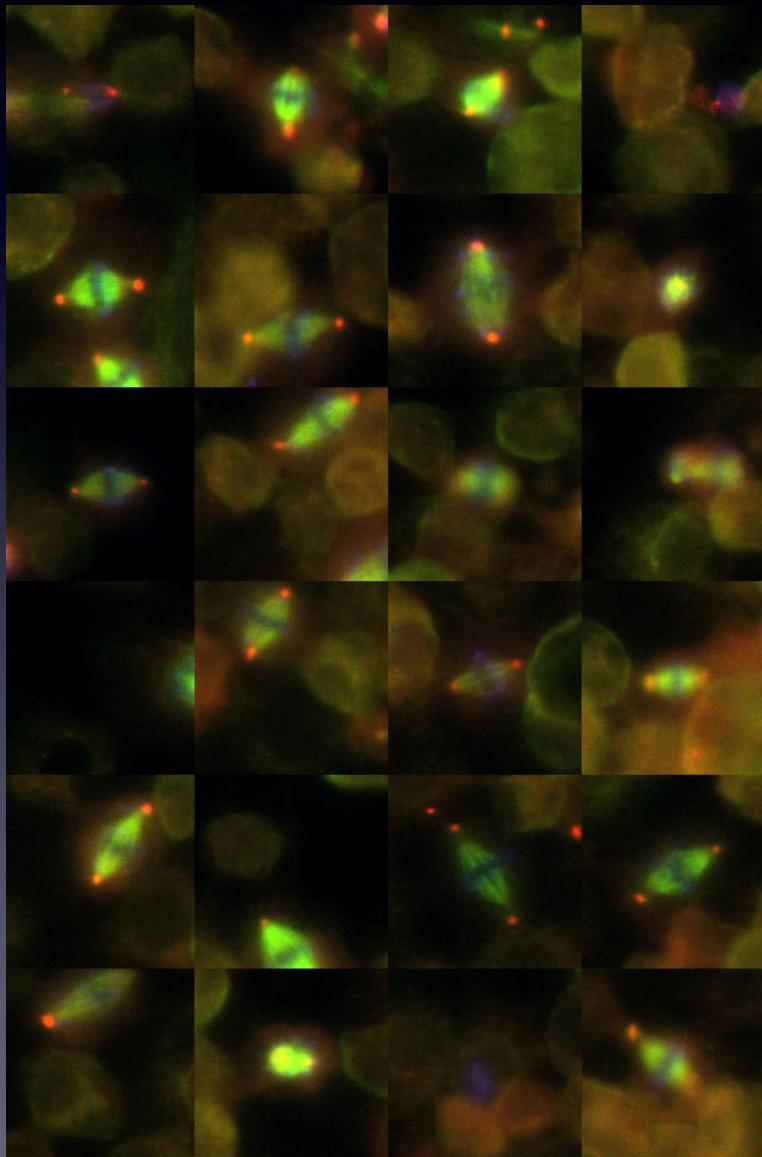
4. Automatic detection of metaphase cells



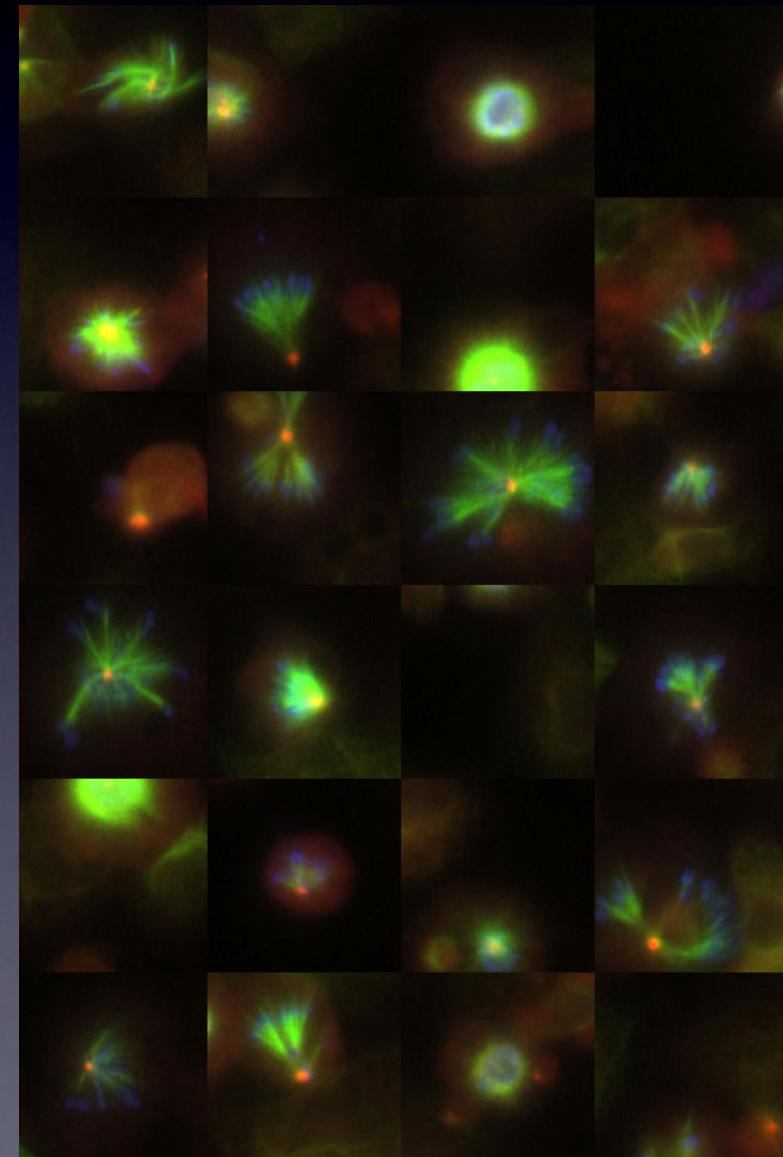


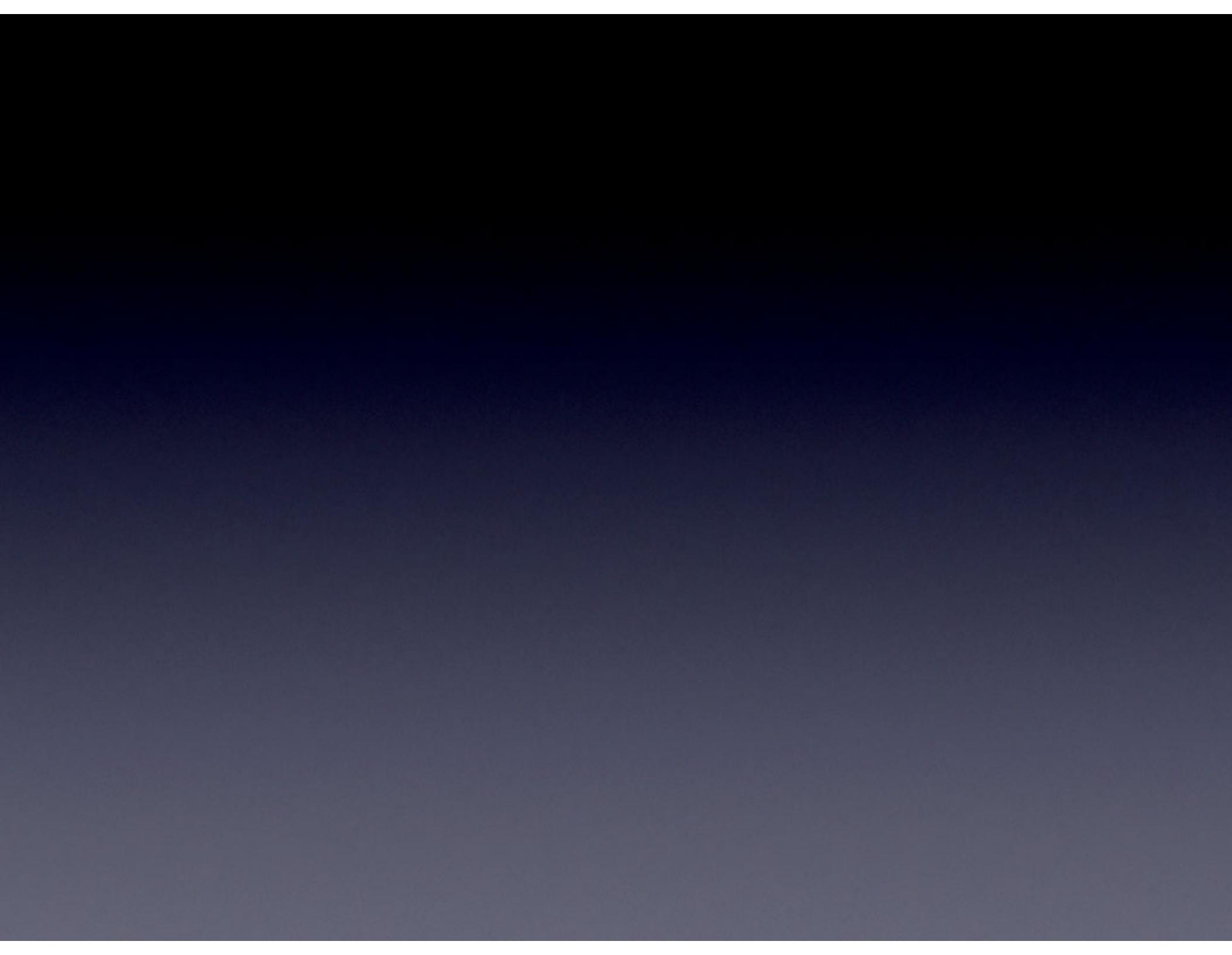
Example

Control



Monopolar spindle (Kinesin-5/Klp61F)





Workflow

Automated microscope

valecrunch.ucsf.edu

Database

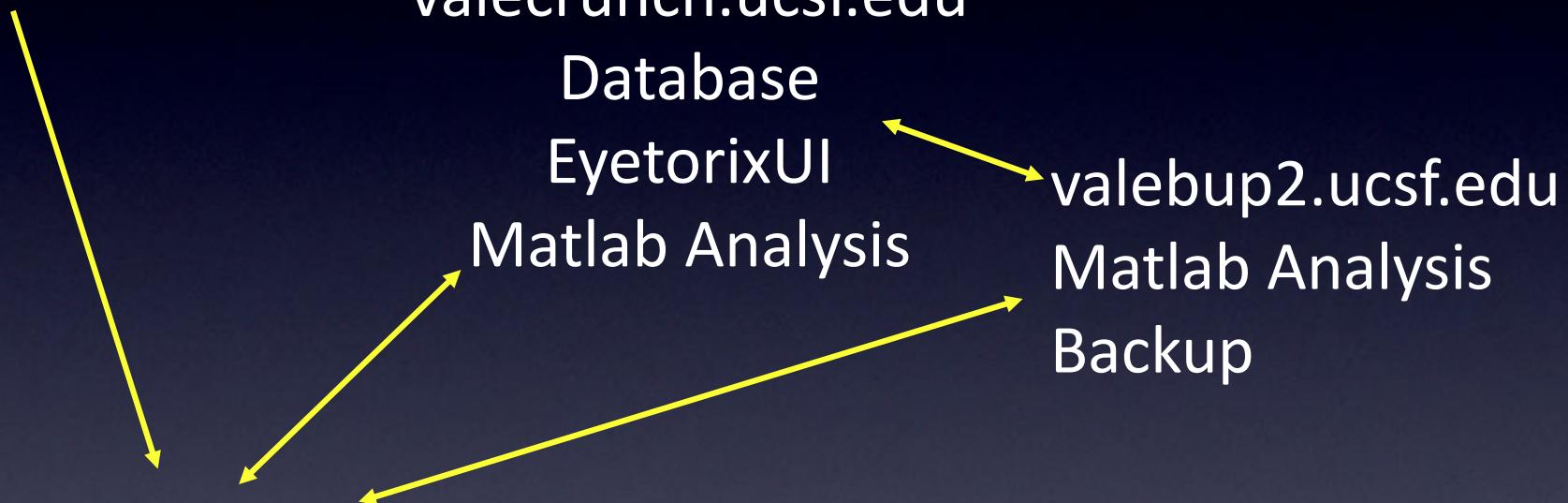
EyetorixUI

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: Gohta Edit views

Import Data

95 Records found. Showing 1 through 95.

96 Records per page

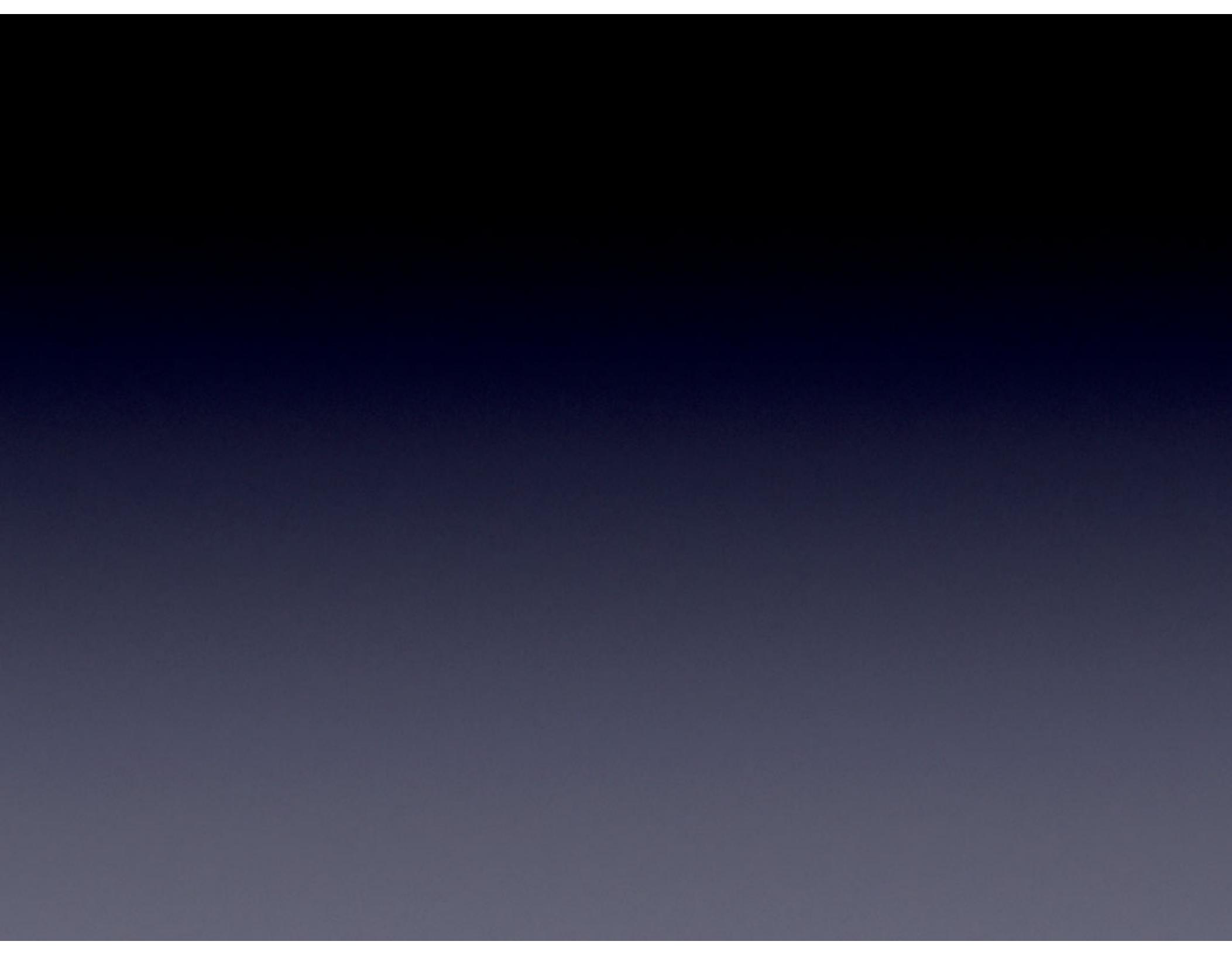
Search Show All

| △ 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 | | |
| | 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 | 129 | A | 7 | probe info | | 96.02 | 9.79% | N | | | | | | | |
| lethal | | | | | | | | 140.21 | 8.47% | N | | | | | | | |
| | | | | | | | | 144.57 | 8.23% | N | | | | | | | |
| | | | | | | | | 73.62 | 7.21% | N | | | | | | | |
| | | | | | | | | 72.67 | 4.88% | N | | | | | | | |
| | | | | | | | | 88.19 | 10.88% | N | | | | | | | |
| | | | | | | | | 98.67 | 7.55% | N | | | | | | | |
| | | | | | | | | 91.90 | 8.89% | N | | | | | | | |
| | | | | | | | | 97.05 | 8.59% | N | | | | | | | |
| | | | | | | | | 101.95 | 9.90% | N | | | | | | | |
| | | | | | | | | 99.76 | 8.88% | N | | | | | | | |
| | | | | | | | | 71.93 | 7.55% | N | | | | | | | |
| | | | | | | | | 87.88 | 8.51% | N | | | | | | | |
| | | | | | | | | 74.88 | 9.70% | N | | | | | | | |
| | | | | | | | | 76.07 | 11.86% | N | | | | | | | |
| | | | | | | | | 77.60 | 7.46% | N | | | | | | | |
| Suppressor of variegation 3-7 | Su(var)3-7 | 8599 | 129 | C | 2 | probe info | | 81.07 | 7.40% | N | | | | | 1 2 | | |
| | CG15888 | 15888 | 129 | C | 3 | probe info | | 82.71 | 6.30% | No | No | | | | | | |
| | CG15897 | 15897 | 129 | C | 4 | probe info | | 95.49 | 9.04% | No | No | | | | 1 2 3 | | |

Link to phenotype gallery

Link to dsRNA information

Done valelab.ucsf.edu





Acknowledgements/References

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)*