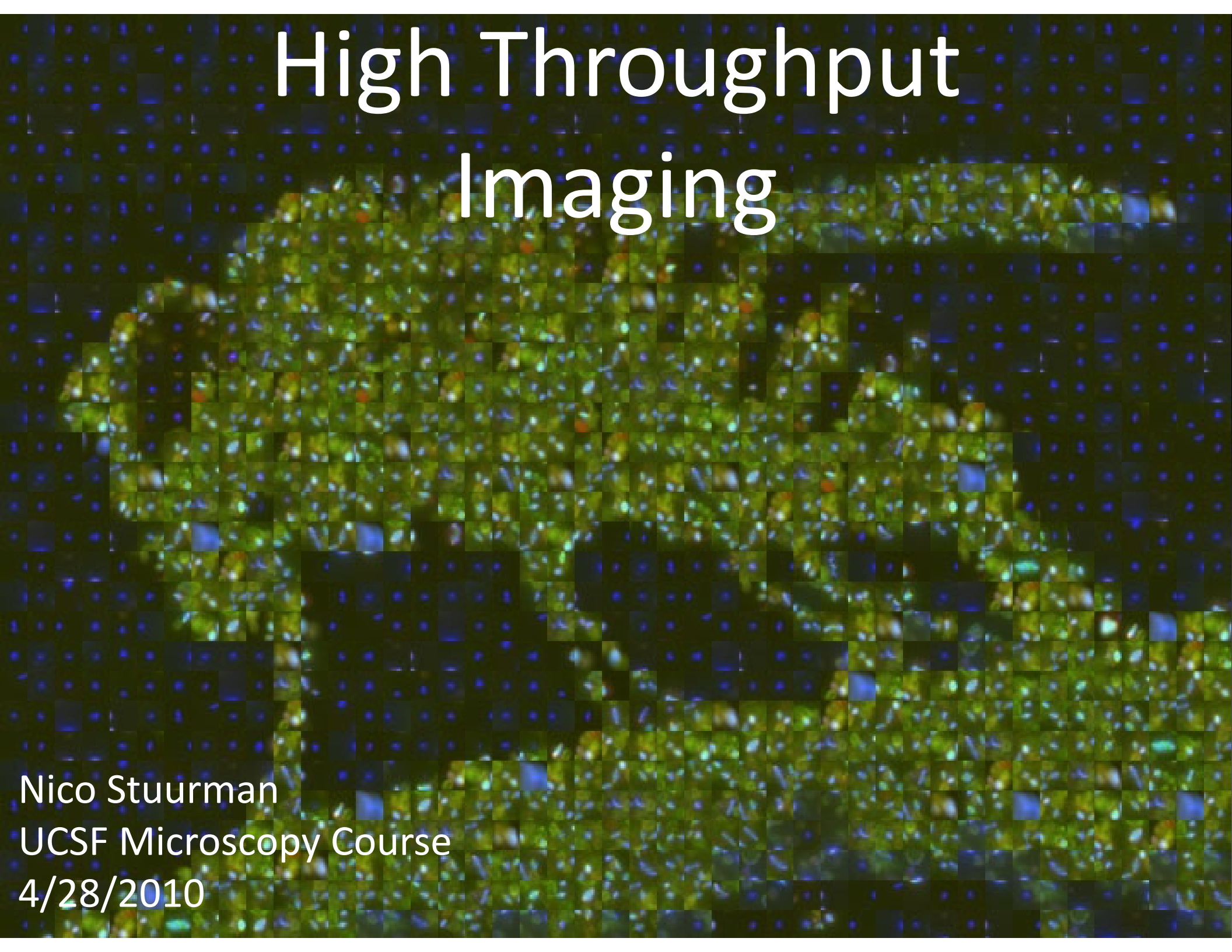
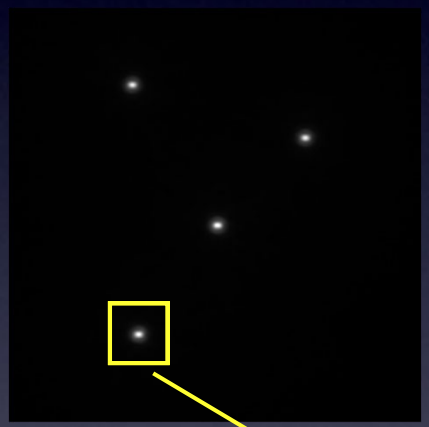
# High Throughput Imaging

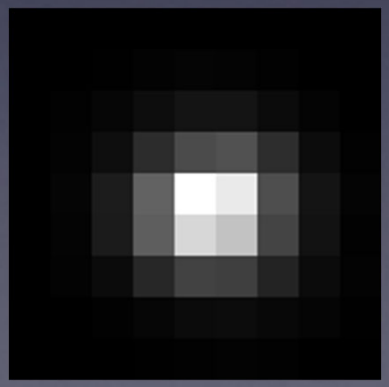


Nico Stuurman
UCSF Microscopy Course
4/28/2010

# What is a digital Image?

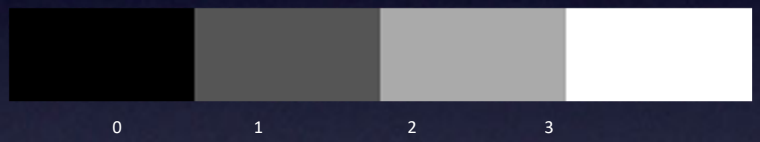
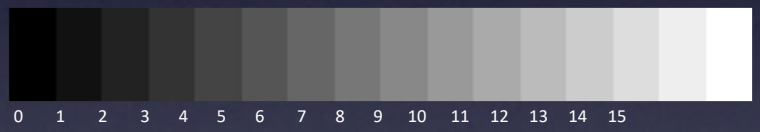
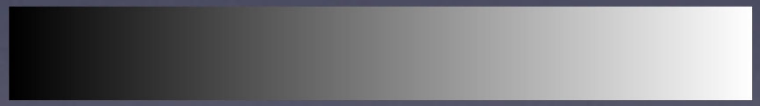
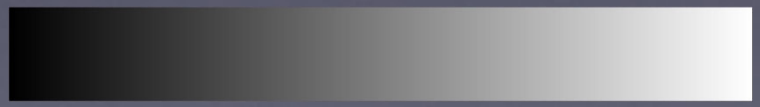
Many measurements of photon flux





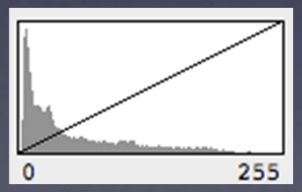
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 3 | 5 | 4 | 2 | 0 | 0 |
| 0 | 2 | 6 | 13 | 20 | 20 | 11 | 4 | 0 |
| 0 | 3 | 14 | 44 | 75 | 81 | 45 | 12 | 2 |
| 0 | 5 | 28 | 98 | 255 | 234 | 78 | 20 | 4 |
| 0 | 4 | 27 | 94 | 215 | 194 | 68 | 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 |

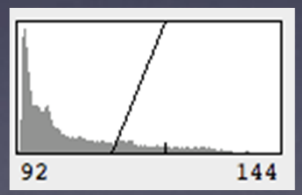
# Bit depth and dynamic range

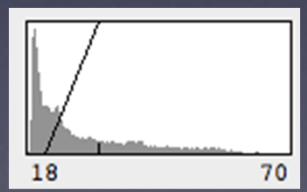
| Nr. bits | range | | |
|---|---|---|---|
| 1 | 2 | | Binary Image |
| 2 | 4 |   | Grayscale Images |
| 4 | 16 |   | |
| 8 | 256 | | 1-byte |
| 12 | 4096 |  | 2-bytes |
| 16 | 65536 |  | |

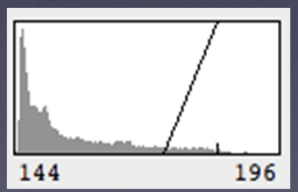
# Bit-depth and resolution

# Mapping values onto display: Brightness/contrast



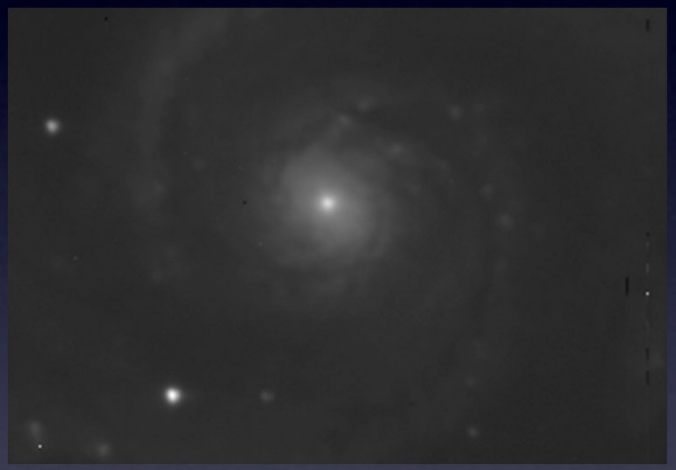



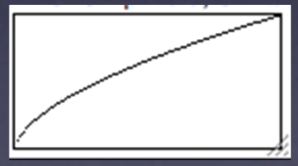

Slope = contrast

Brightness

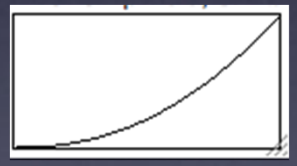
# Gamma adjustment

1





0.6



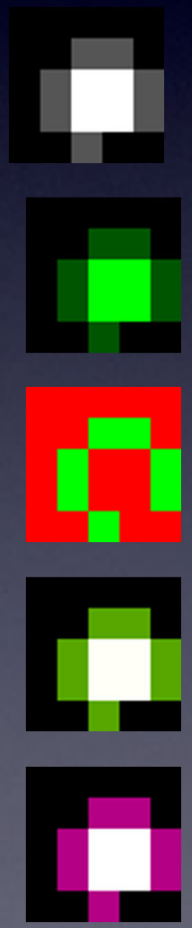
2.2

# What are acceptable image manipulations?

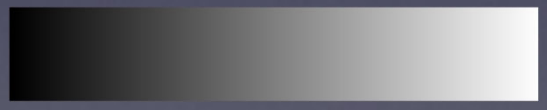
- JCB has the best guidelines
  - http://jcb.rupress.org/content/172/1/9.full
  - http://jcb.rupress.org/content/166/1/11.full
- Brightness and contrast adjustments ok, so long as done over whole image and don't obscure or eliminate background
- Nonlinear adjustments (like gamma) must be disclosed
-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

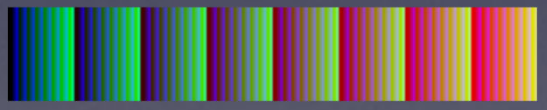
# Lookup Tables (LUTs)

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

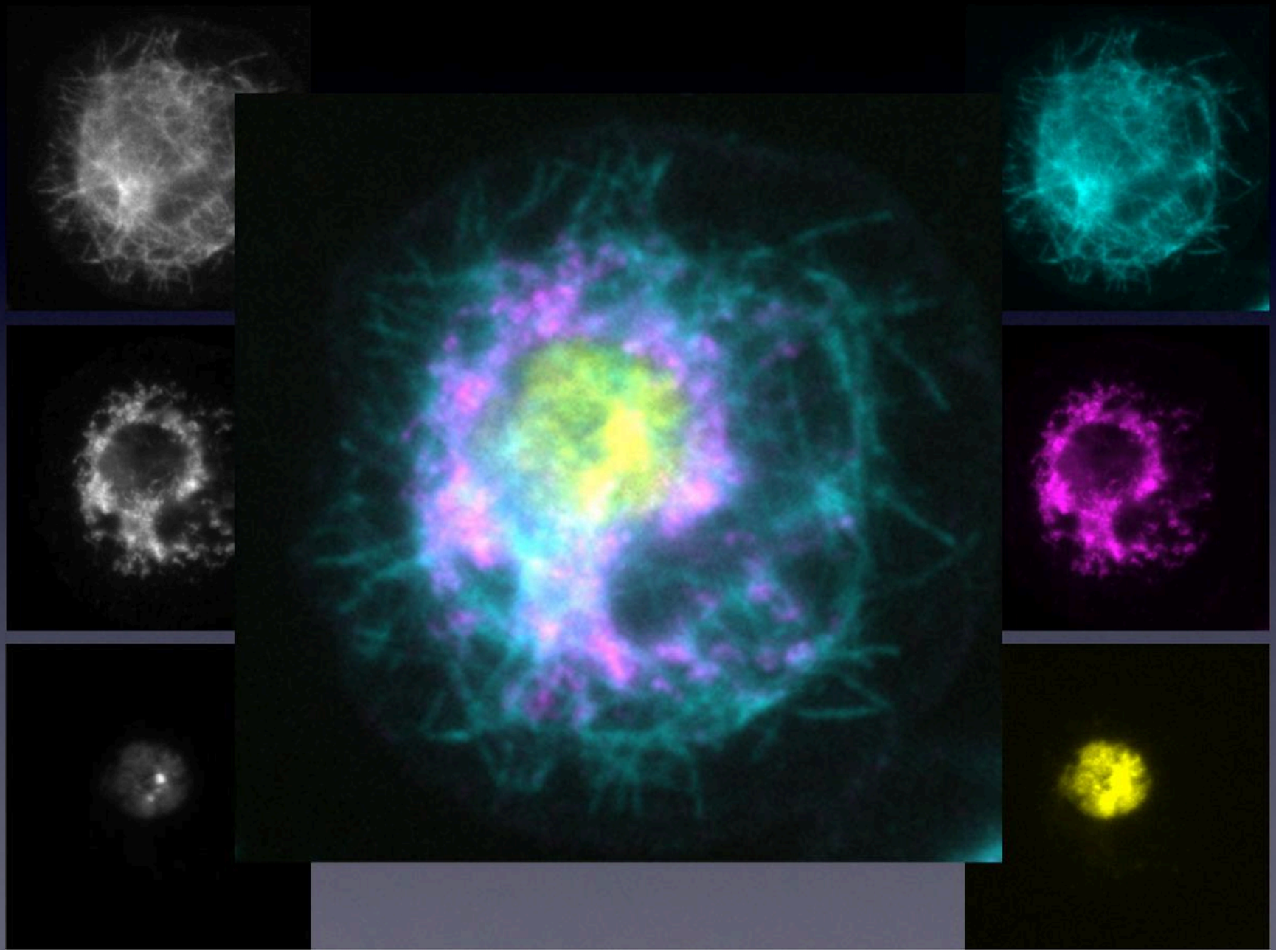


# Lookup Tables (LUTs)

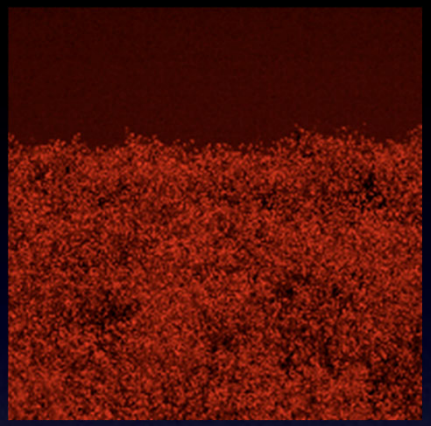


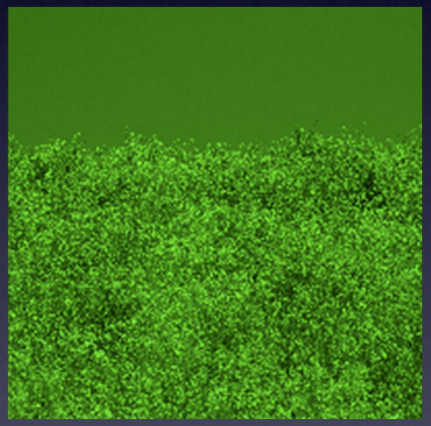


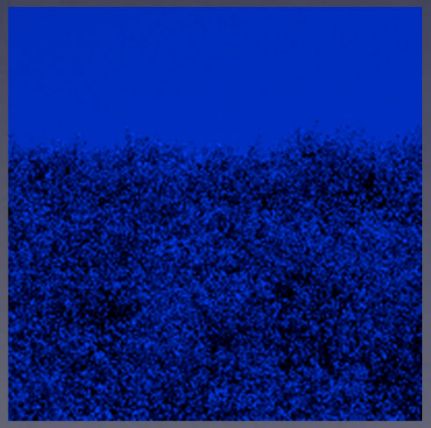
# Lookup Tables (LUTs)

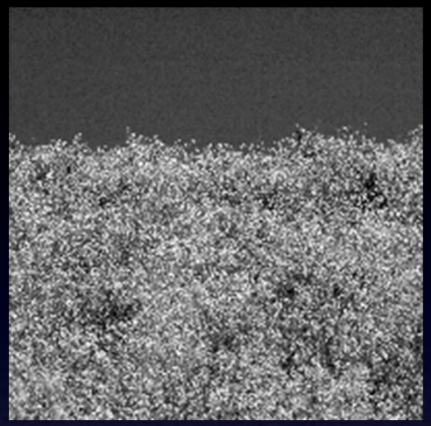


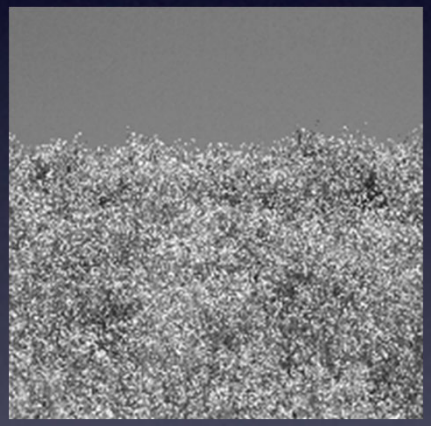
# Color Images











## 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:

1392x1040x2 = 2.8MB

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

2.8 * 3 * 15 * 200 = 25.2GB

→ Compression

Compression → Losless versus Lossy

Original data can be restored

Loose original data!

Losless versus Lossy →

None (raw)

Run-length encoding

Dictionary approaches, etc..

Losless versus Lossy →

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

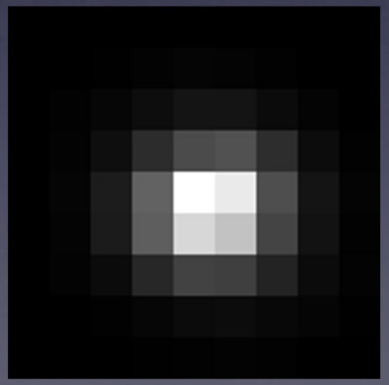
- Photoshop
- Gimp

## Analysis

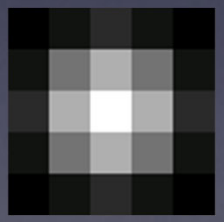
- Matlab
- IDL
- ImageJ (free, many plugins) http://rsb.info.nih.gov/ij/
- Priism (Agard/Sedat labs) http://msg.ucsf.edu/IVE/
- CellProfiler http://cellprofiler.org

# 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



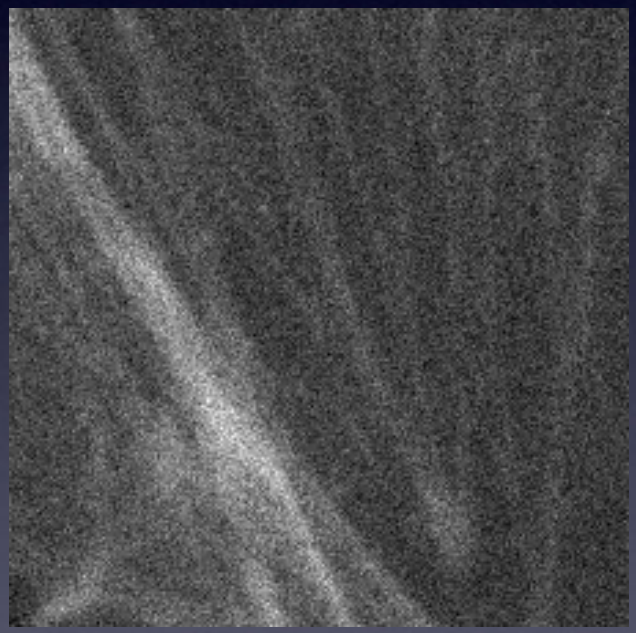
Measured PSF



Gaussian Filter

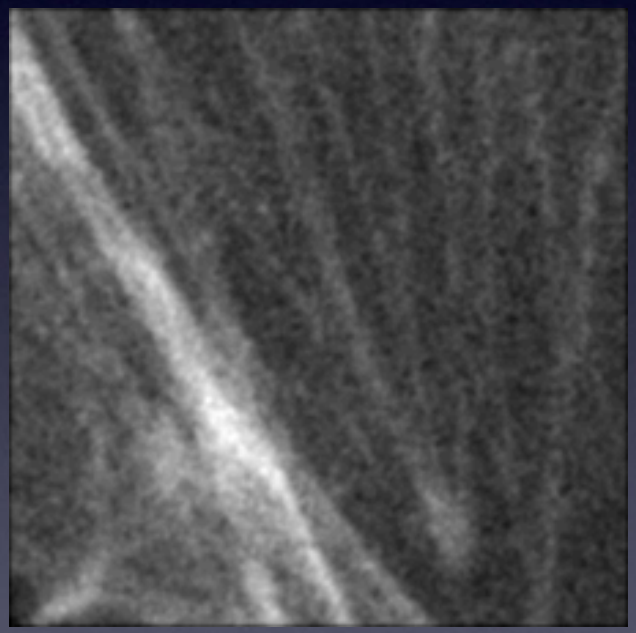
# Why smooth?

- Averages redundancy and suppresses noise



10 photons/pixel average

5 e- read noise



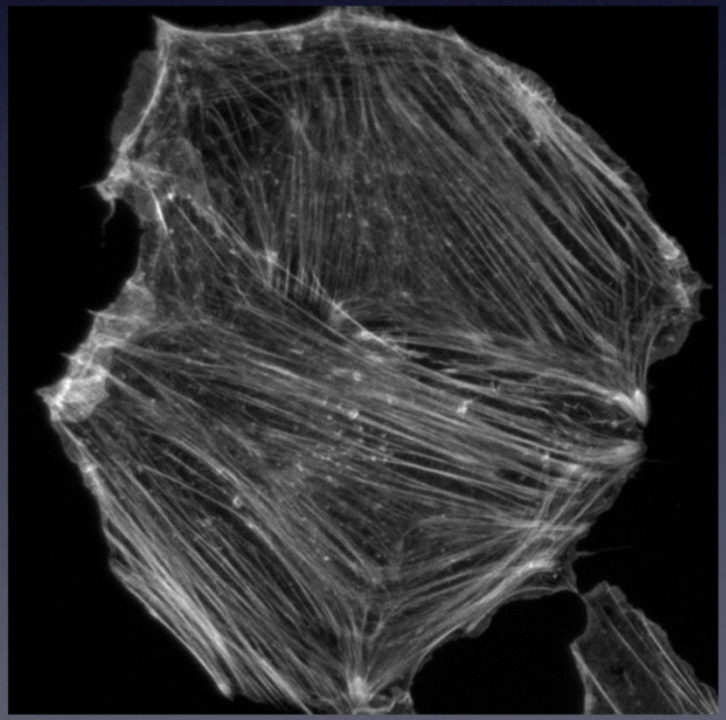
Gaussian smoothing filter,
$\sigma$ = 1 pixel

# Other Filters

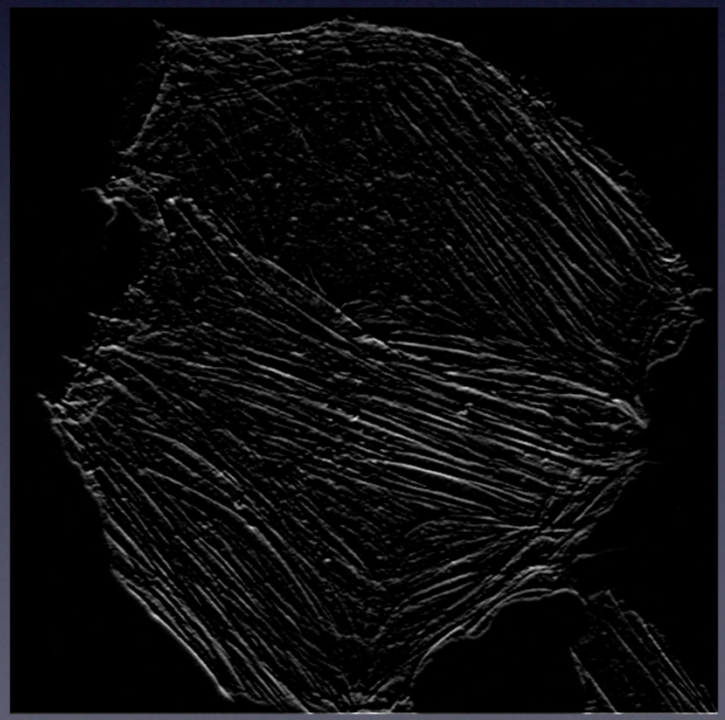
Edge Detection

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

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



Original

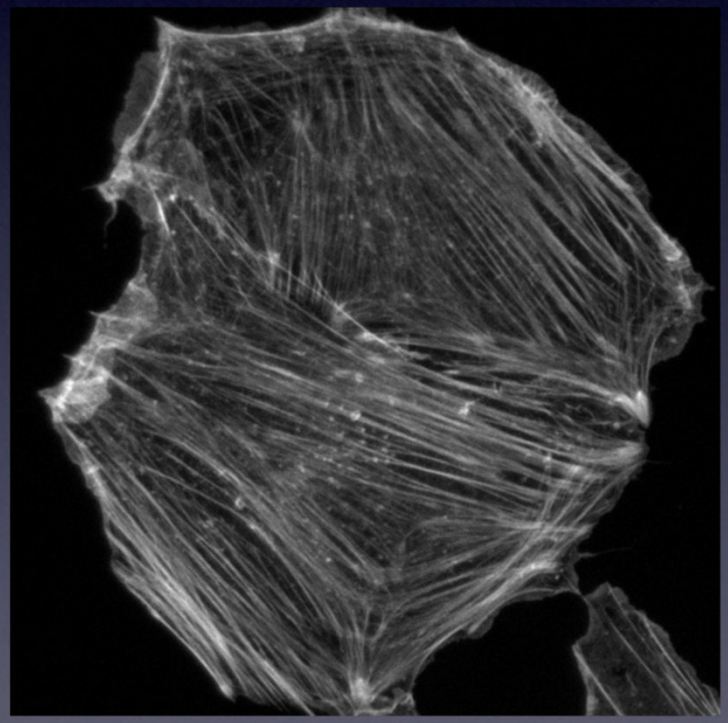


Horizontal edge detection

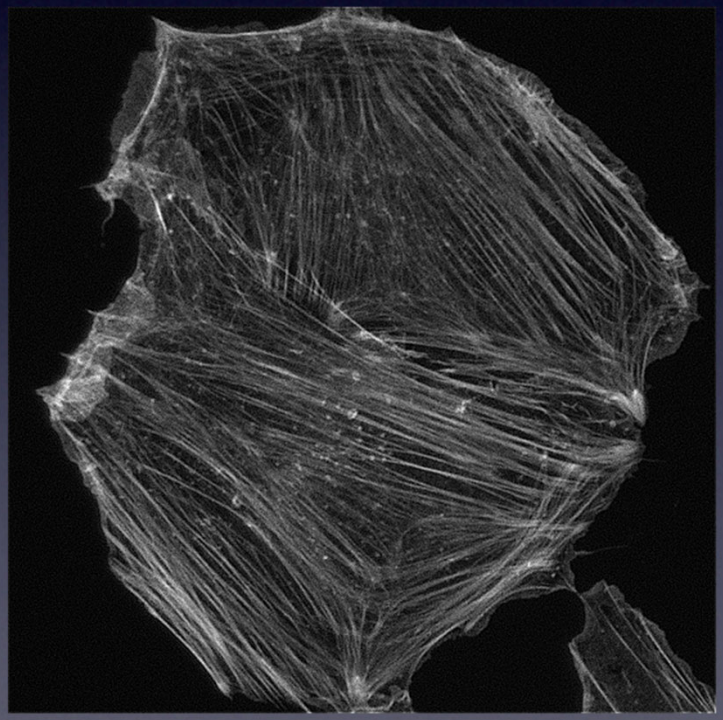
# Other Filters

## Unsharp Masking

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



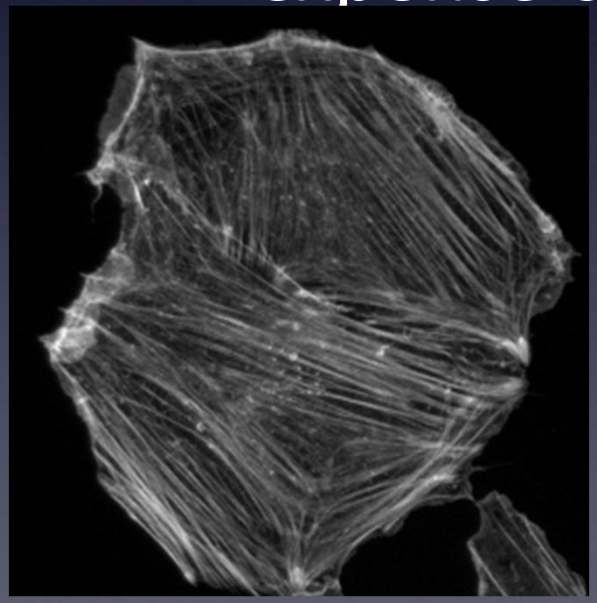
Original



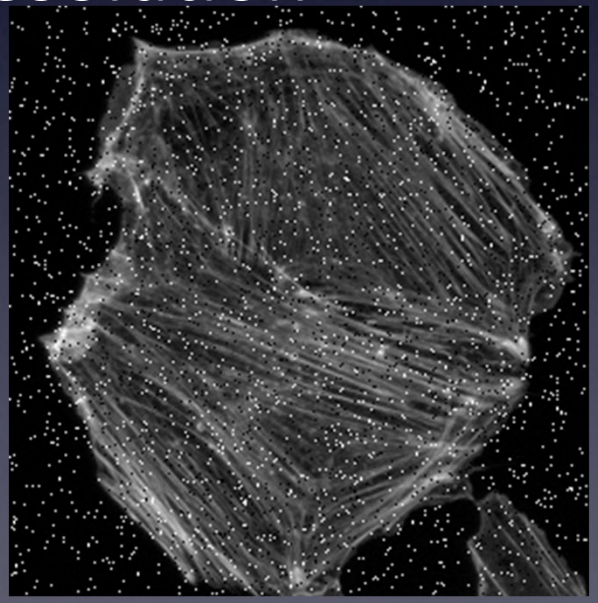
Unsharp masked

# Non-linear Filters

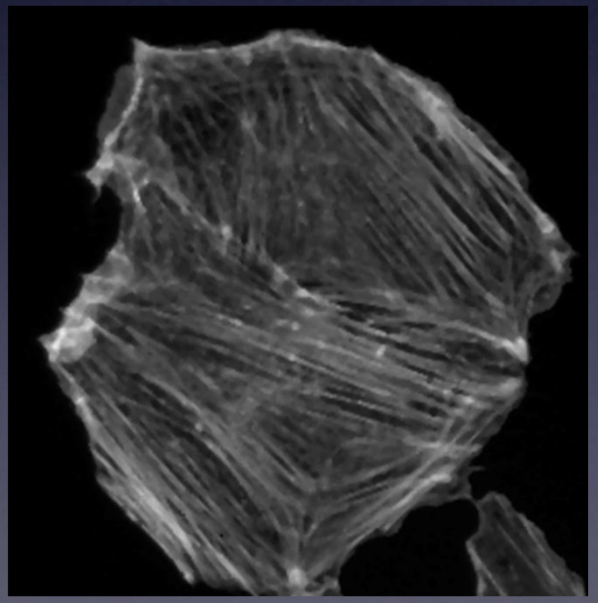
- Replace central pixel with min, max, median
- Median filter is a good noise filter, at the expense of resolution



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

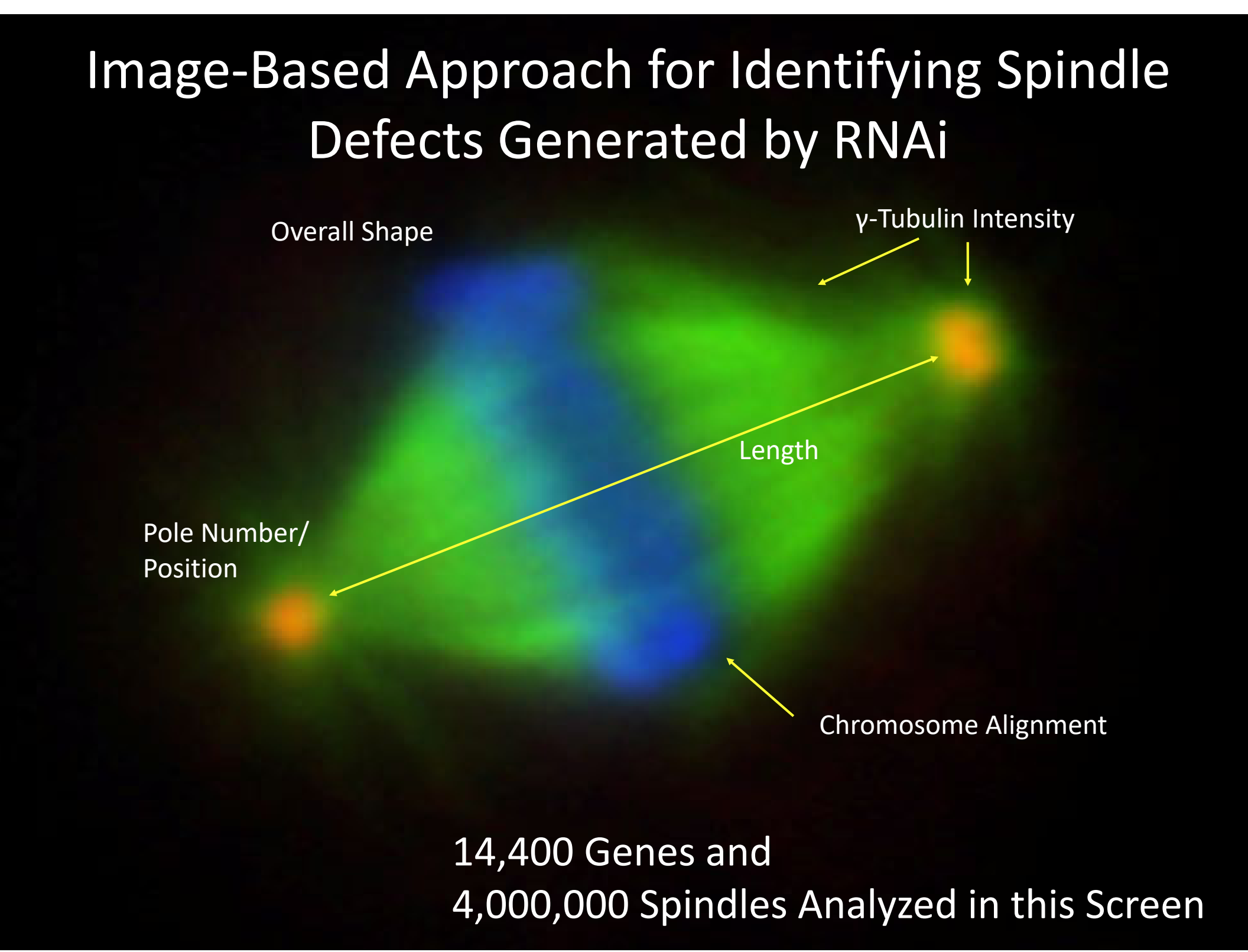
Gohta Goshima, Nico Stuurman, Nan Zhang, Sarah Goodwin (UCSF)

Roy Wollman,
Jon Scholey (UC Davis)

Goshima et al. Science, 417 316 (2007)

Wollman and Stuurman. J. Cell Sc. 120 3715 (2007)

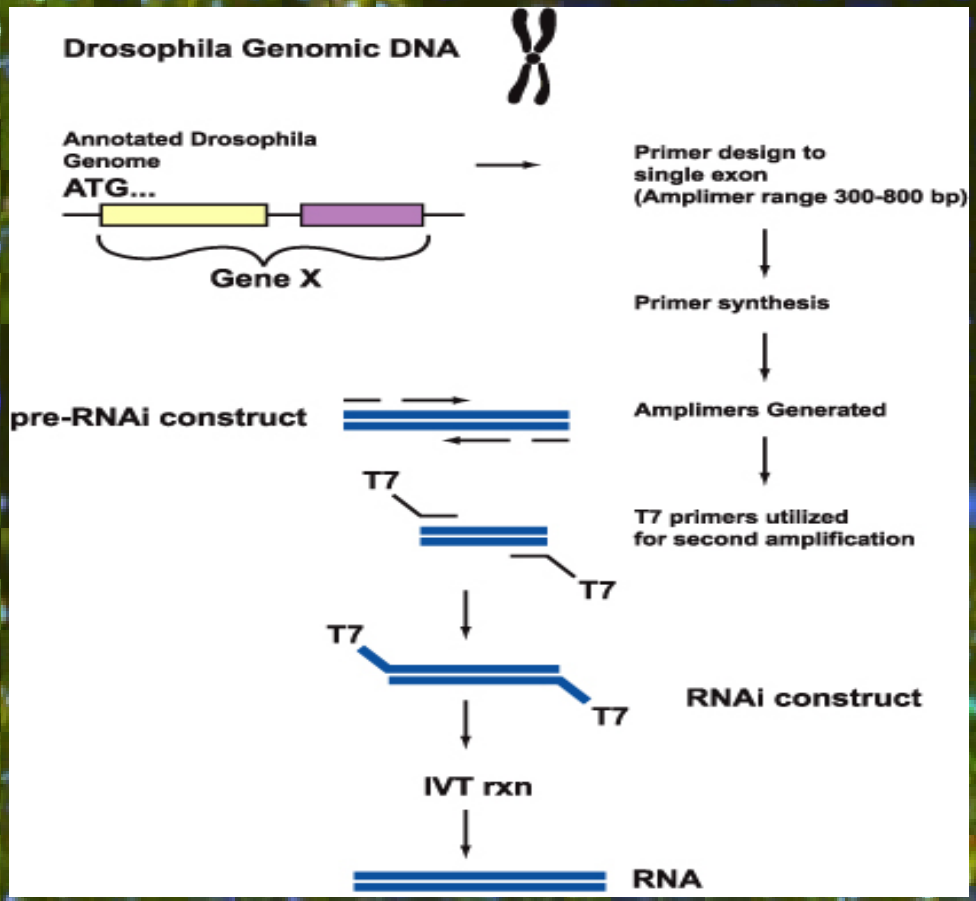
# Image-Based Approach for Identifying Spindle Defects Generated by RNAi




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

# High-throughput RNAi Screen

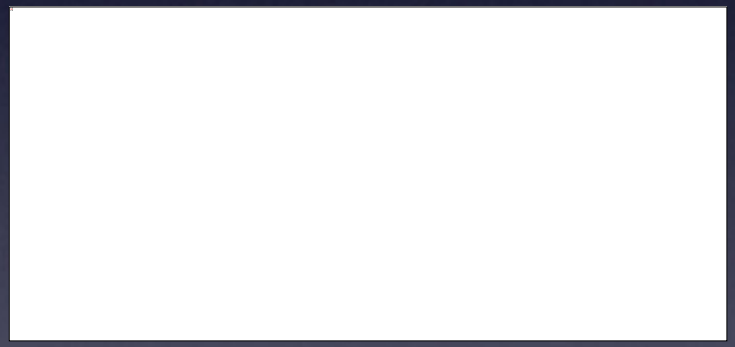
1. Full Fly Genome dsRNA Library:



# 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

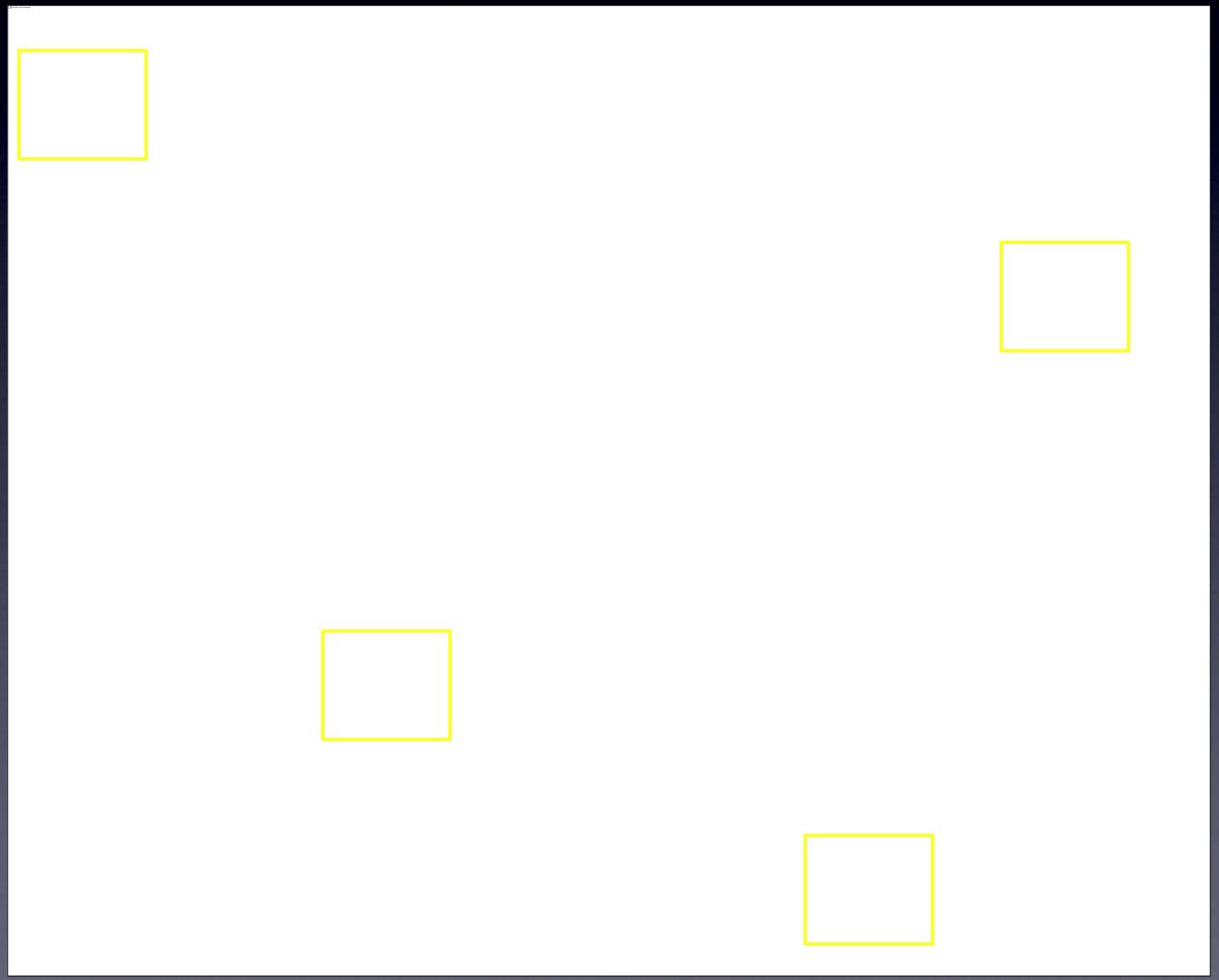
# High-throughput RNAi Screen

3. High-throughput Microscopy Images

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

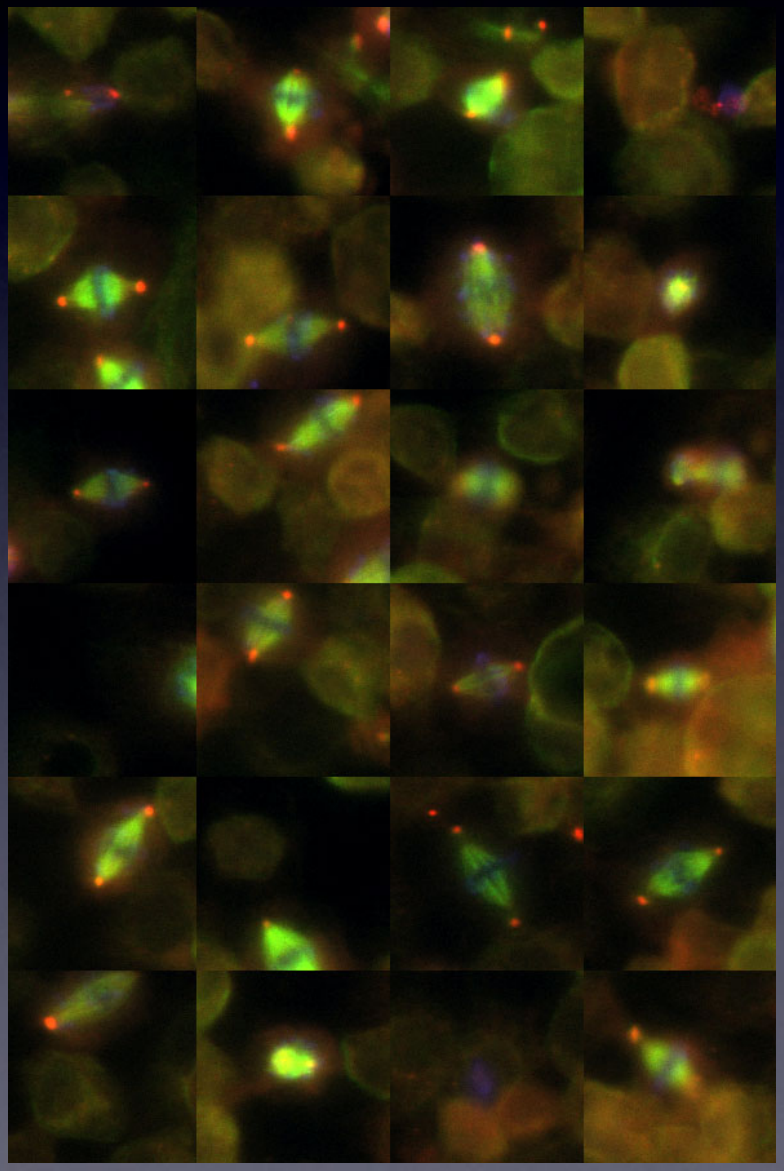
# High-throughput RNAi Screen

4. Automatic detection of metaphase cells

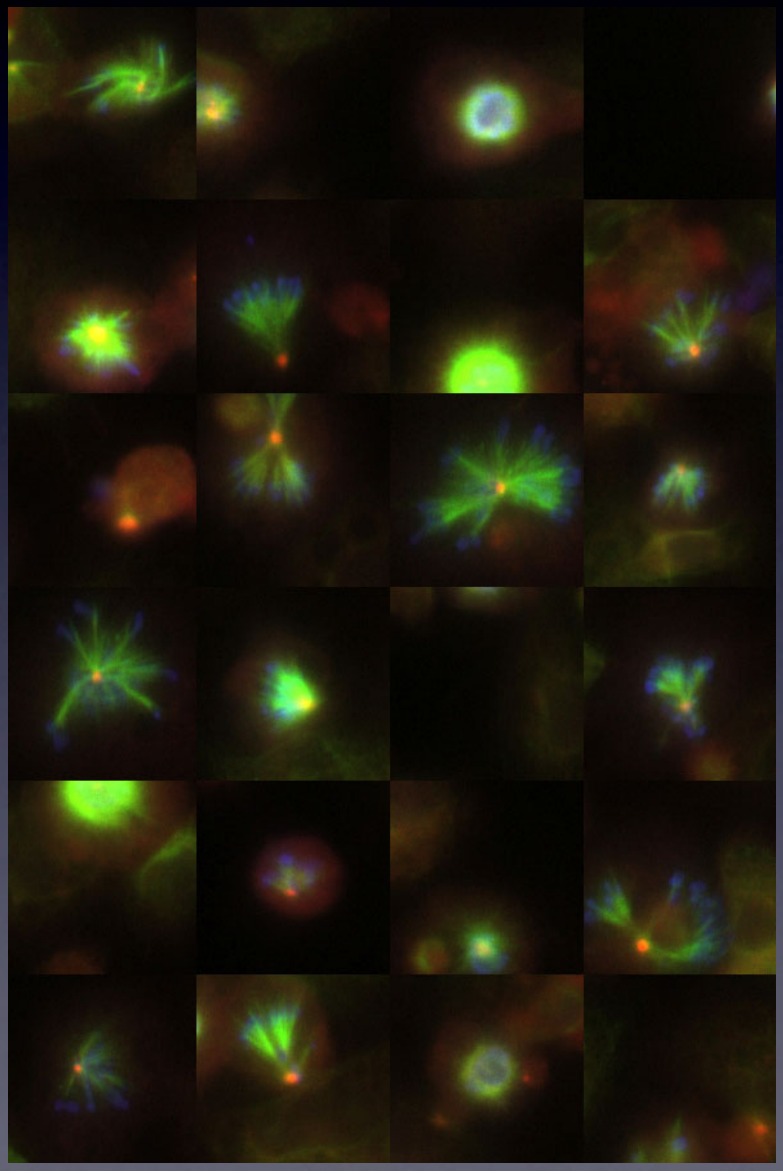


# Example

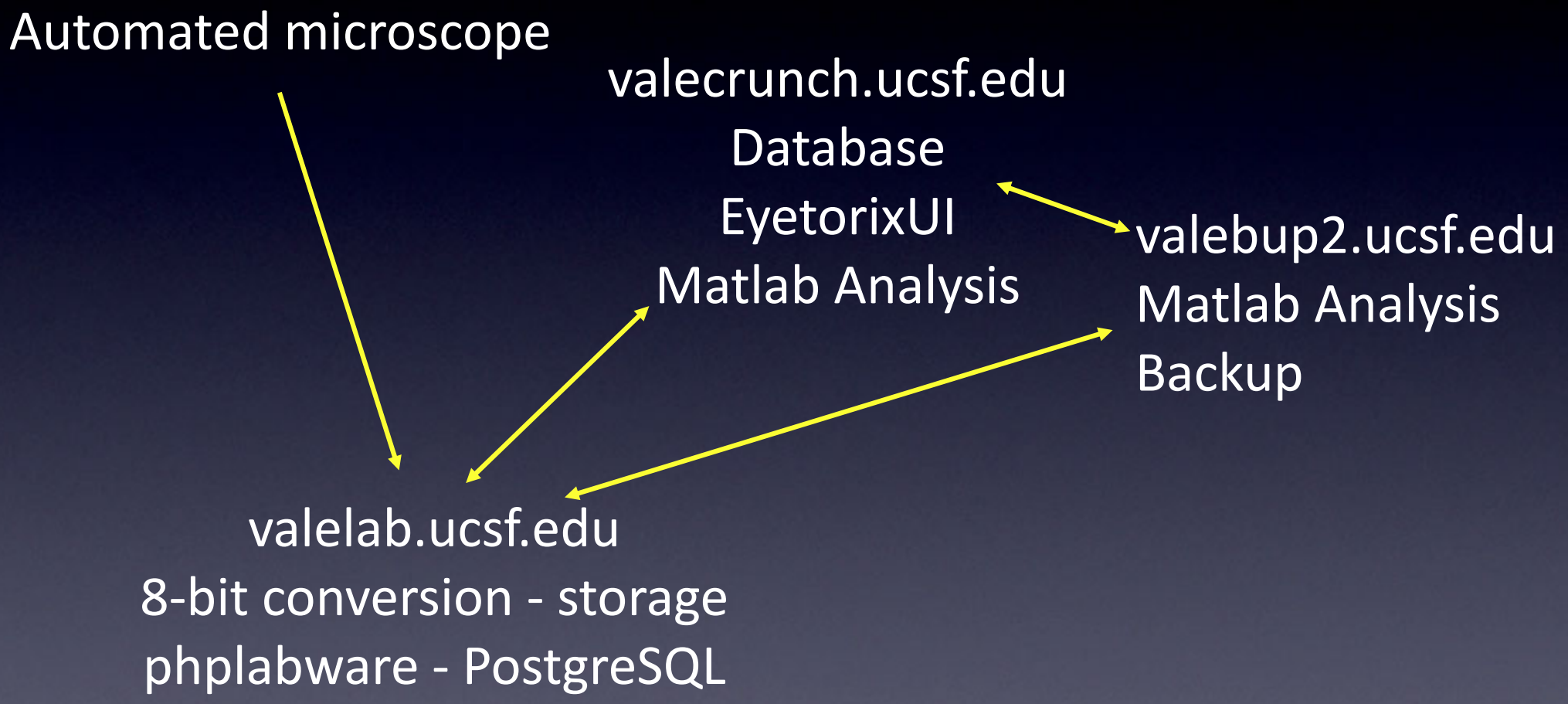
Control



Monopolar spindle (Kinesin-5/Klp61F)



# Workflow


Automated microscope
valecrunch.ucsf.edu
Database
EyetorixUI
Matlab Analysis
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=&cr

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<br>Long spindle Misalign<br>Misalign - Clear | long spindles<br>high circ2num | Large spindle - Clear<br>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% | | | | | | | | |
| yel… | | | | | | | | 144.57 | 8.23% | | | | | | | | |
| | | | | | | | | 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

# 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)