MetaMorph®

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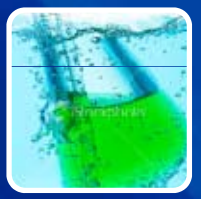
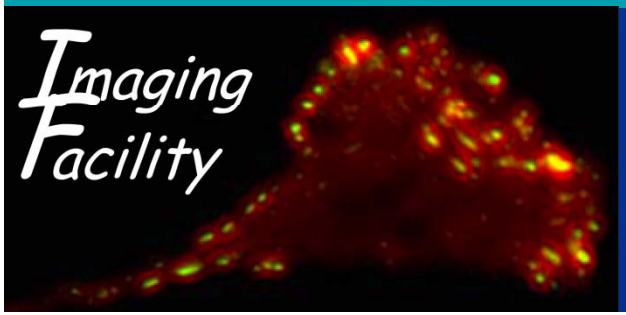



Fundamentals of MetaMorph Workshop



Claire Brown and Vincent Pelletier
Imaging Facility and Quorum Technologies
October 25 – 26 , 2010

Quorum

Agenda

- Images & Image Display
- Calibration
- Working With Stacks
- Image Processing
- Image Analysis
- Presenting Images
- Journals

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Goals

- Familiarize you with MetaMorph's tools and where to find them
- Provide a basic understanding of each tool's use
- Not covering every button and setting in every function

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How to get help

1. Phone

   1-800-635-5577

   1. Press 3 for Tech Support
   2. Press 2 for Cellular Imaging
   3. Press 2 for MetaMorph

2. Email

   Support.dtn@moldev.com

3. Web

   http://support.meta.moleculardevices.com/

   http://www.meta.moleculardevices.com/authorize/

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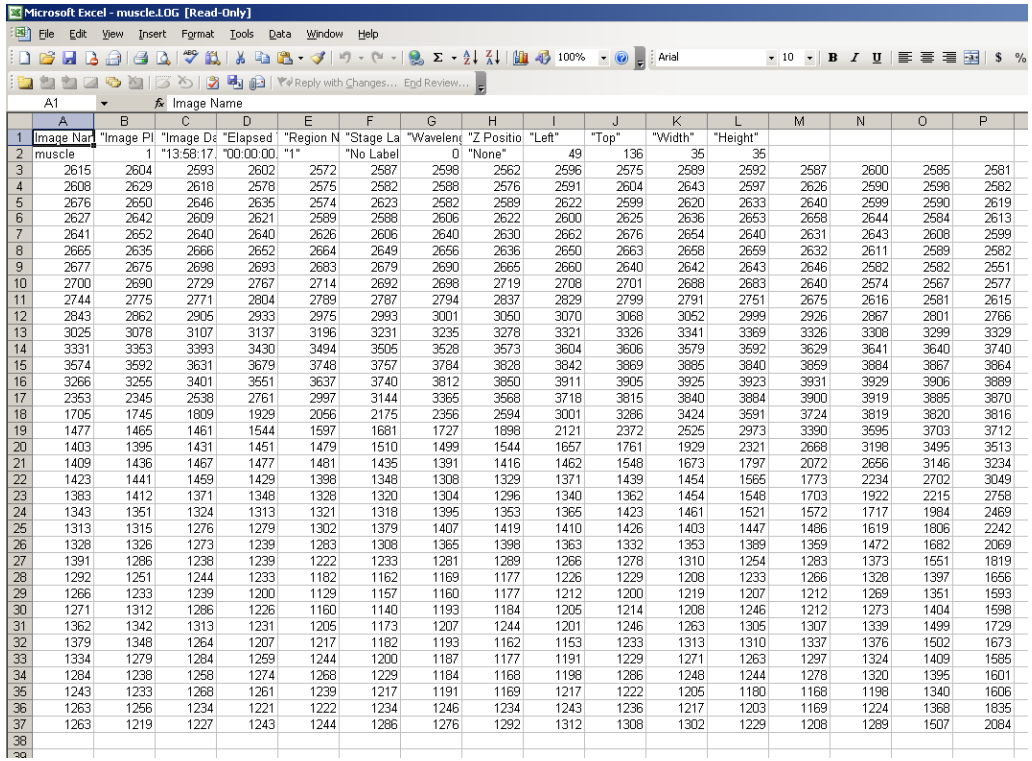
Image Tools & Display

Image Display



Microsoft Excel - muscle.LOG [Read-Only]

File Edit View Insert Format Tools Data Window Help

A1 Image Name

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33	1334	1279	1284	1259	1244	1200	1187	1177	1191	1229	1271	1263	1297	1324	1409	1585
34	1284	1238	1258	1274	1268	1229	1184	1168	1198	1286	1248	1244	1278	1320	1395	1601
35	1243	1233	1268	1261	1239	1217	1191	1169	1217	1222	1205	1180	1168	1198	1340	1606
36	1263	1256	1234	1221	1222	1234	1246	1234	1243	1236	1217	1203	1169	1224	1368	1835
37	1263	1219	1227	1243	1244	1286	1276	1292	1312	1308	1302	1229	1208	1289	1507	2084
38																

- Can you guess what this image looks like?
- Our perception is trained to interpret light intensity from the eye to images
- Our perception is not adapted well to convert numbers to brightness in an image

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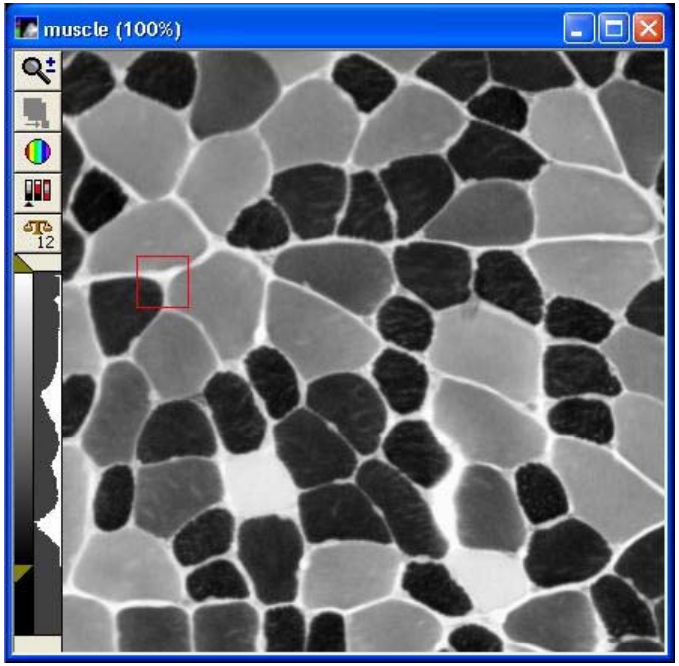
Image Display



- MetaMorph uses a mapping of intensities to produce an image
  - Contrast
- Definition of an image
  - Two dimensional grid of pixels
  - Each pixel contains an intensity value
- Other information in images
  - Annotation and properties

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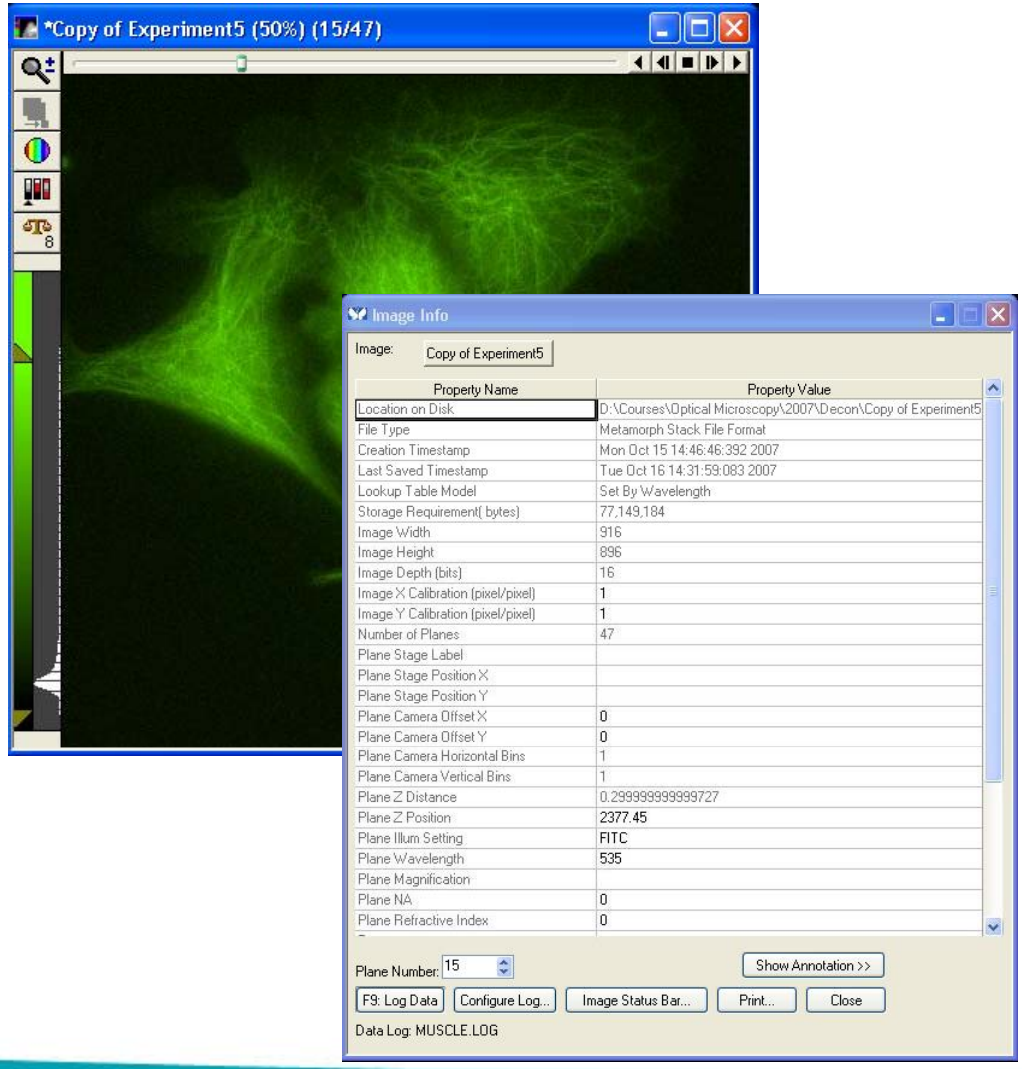
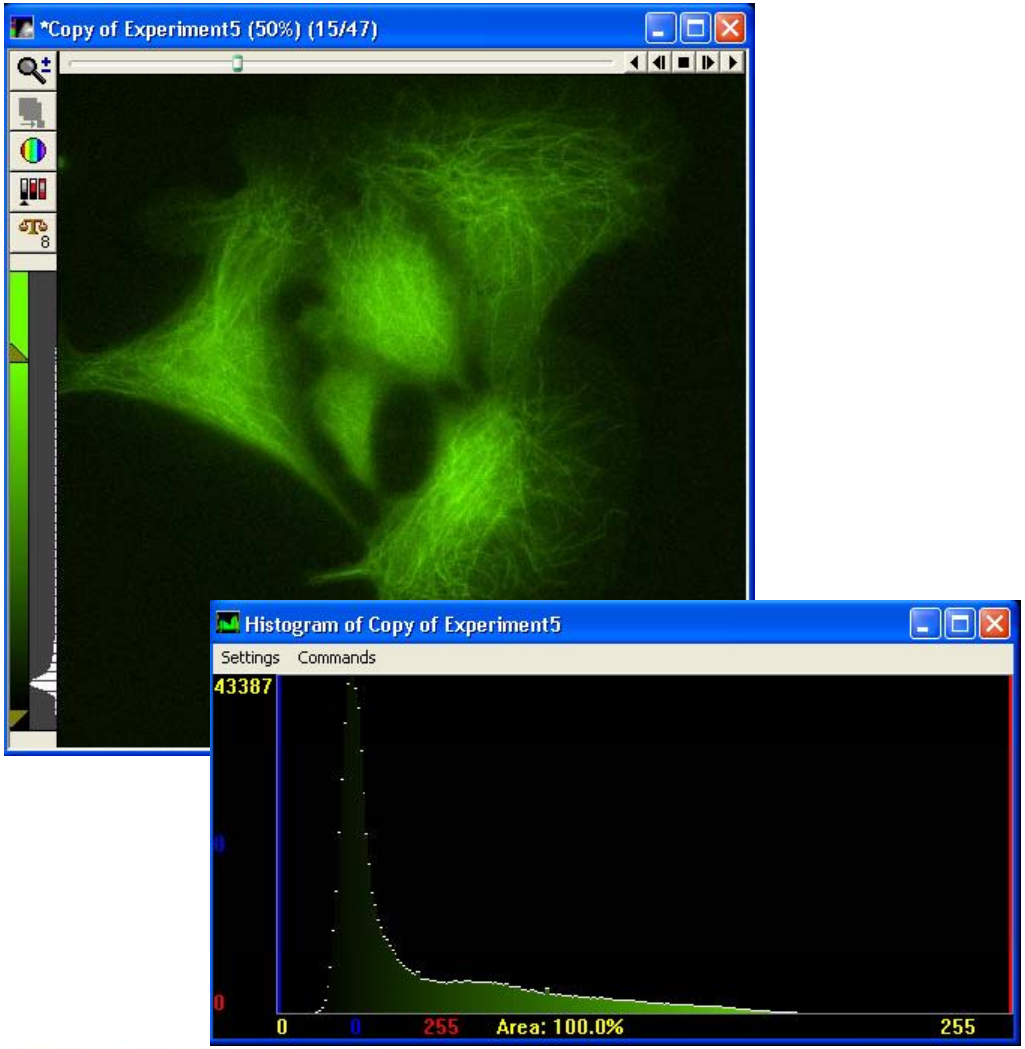
Image Info



- Edit Menu
- Besides the pixel data, other information is stored with images when they are saved

Image Histogram



- Measure Menu
- A graph of the distribution of the number of pixels at each intensity in the image
- Why do we care about this histogram?
- Tells us how effectively we are utilizing the detector

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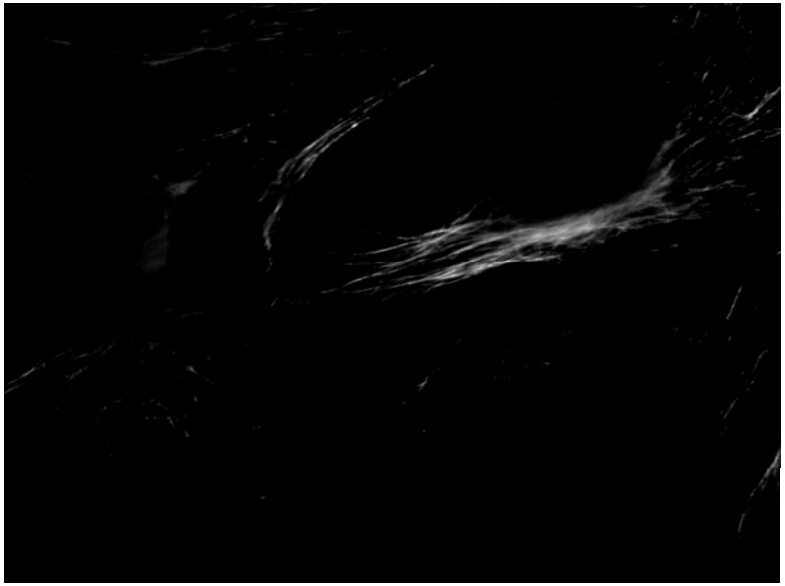
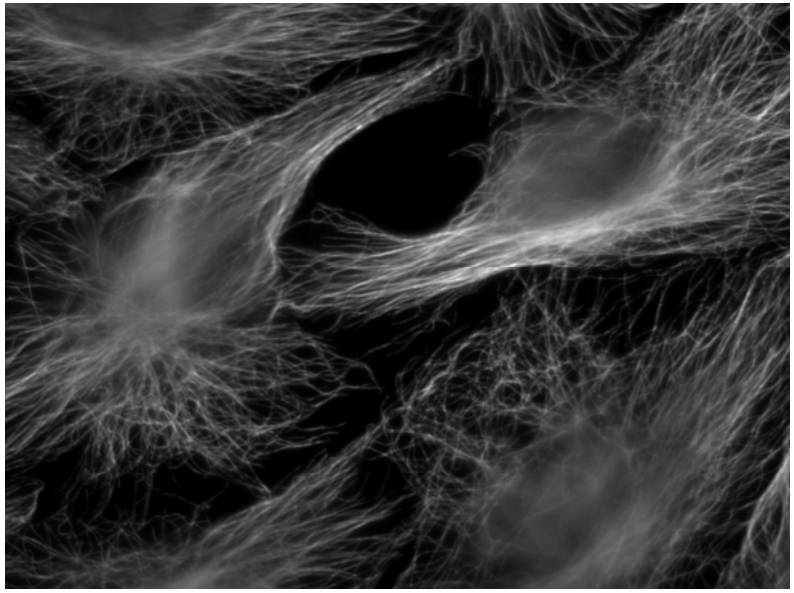
What's wrong here?

- These two images are of the same field of view





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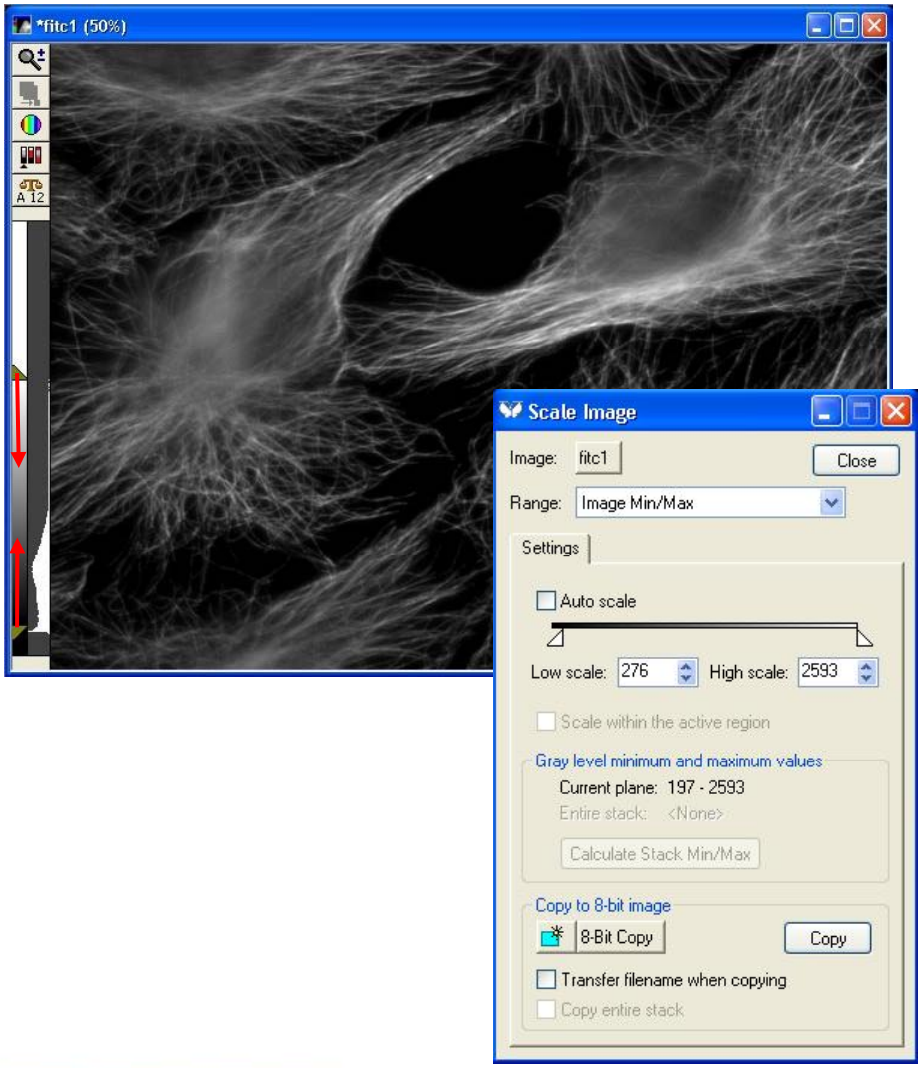
Scale Image



- Display Menu
- Use the High Wedge to set the White
- Use the Low Wedge to set the Black
- Changes display, does **NOT** alter the data, only how it appears to our eyes

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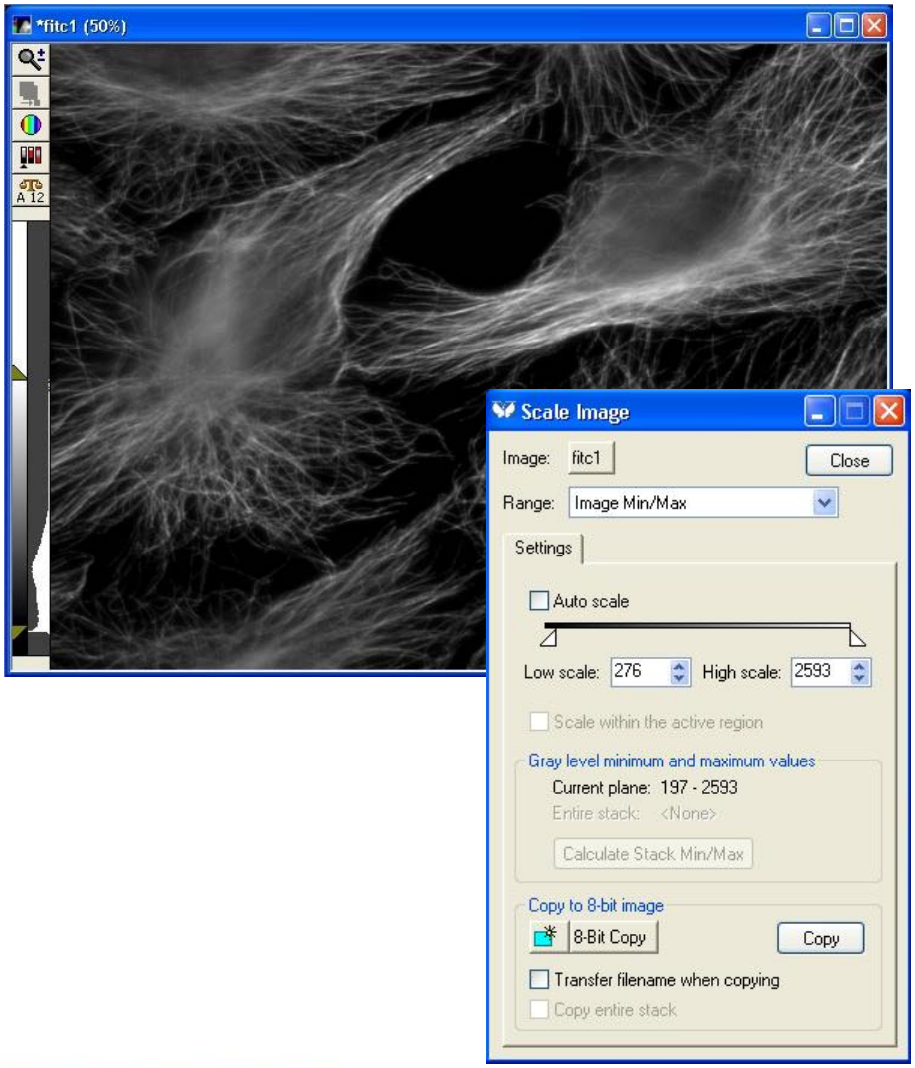
Scale Image



- Pixels equal and above the High intensity value are White
- Pixels equal and below the low intensity value are Black
- Changes display, does **NOT** alter the data, only how it appears to our eyes

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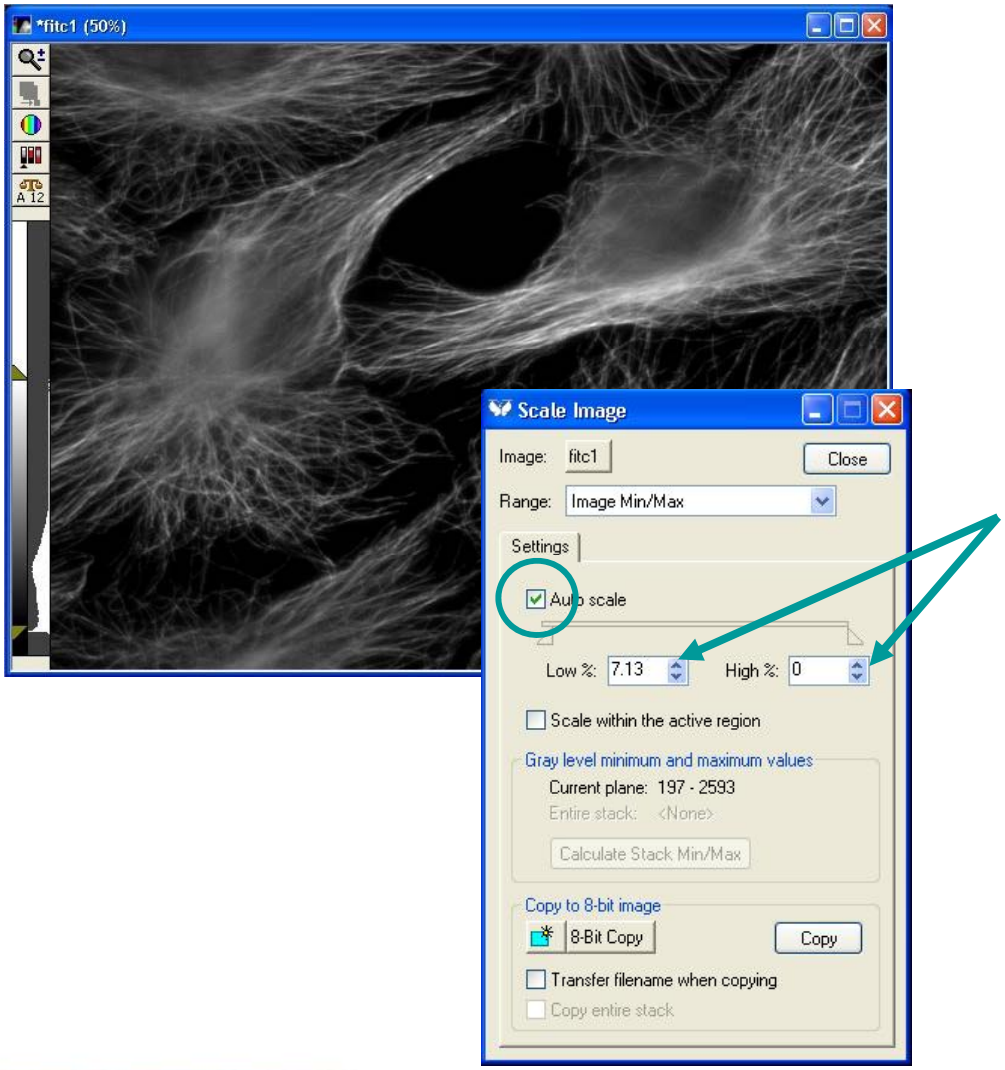
Scale Image



- AutoScale
- Automatically finds the lowest and highest intensity in the image and sets the scaling there
- Wedges can then be changed to select % of pixels below and above wedges

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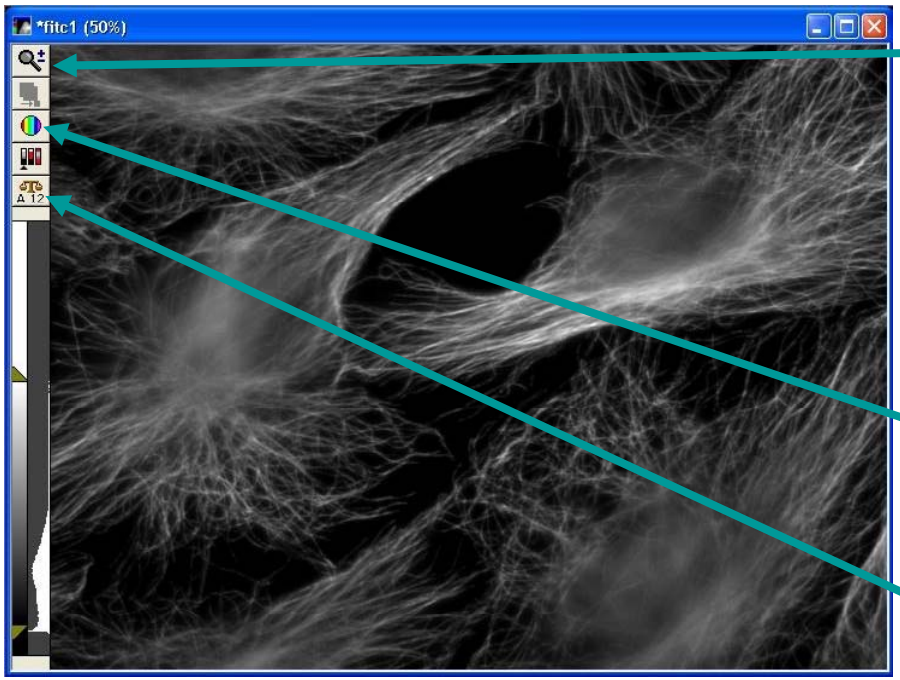
Image Window Tools



- Zoom – makes the image larger / smaller
  - You can also use the wheel on the mouse
- Look Up Table
- Scale Image

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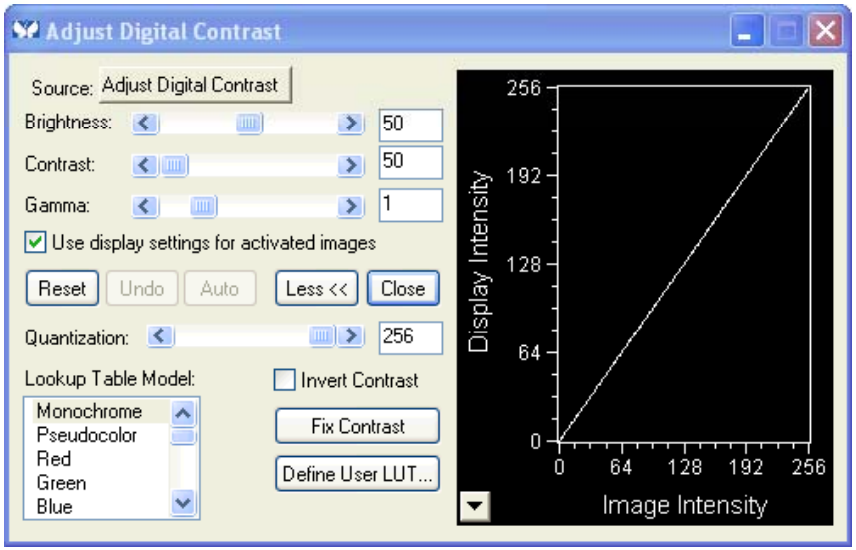
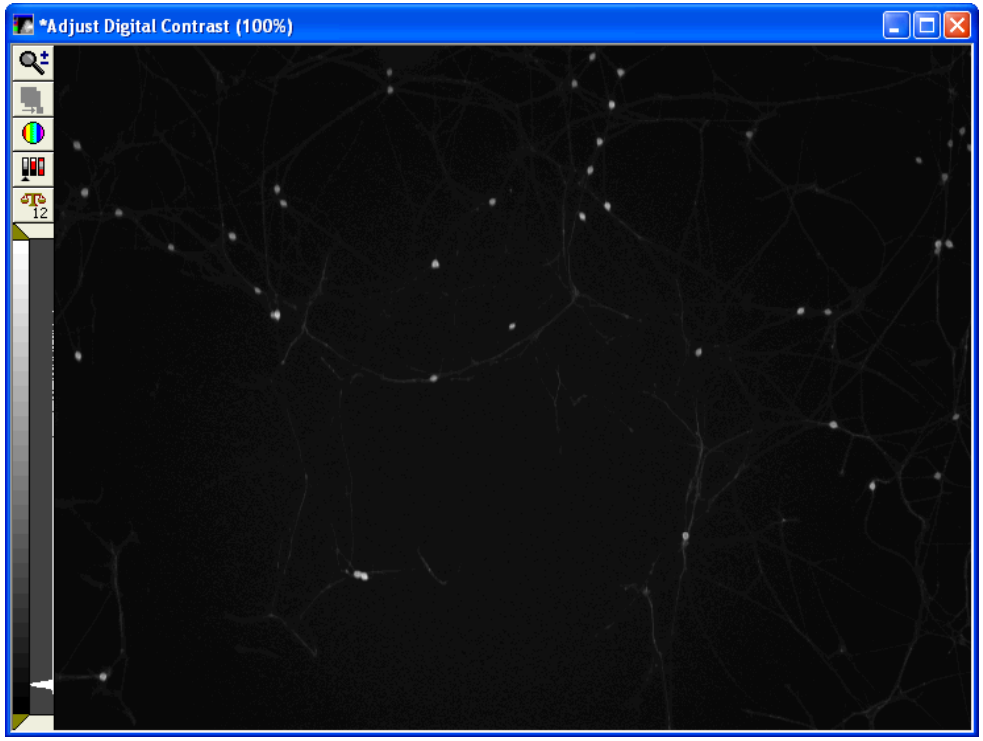
Adjust Digital Contrast





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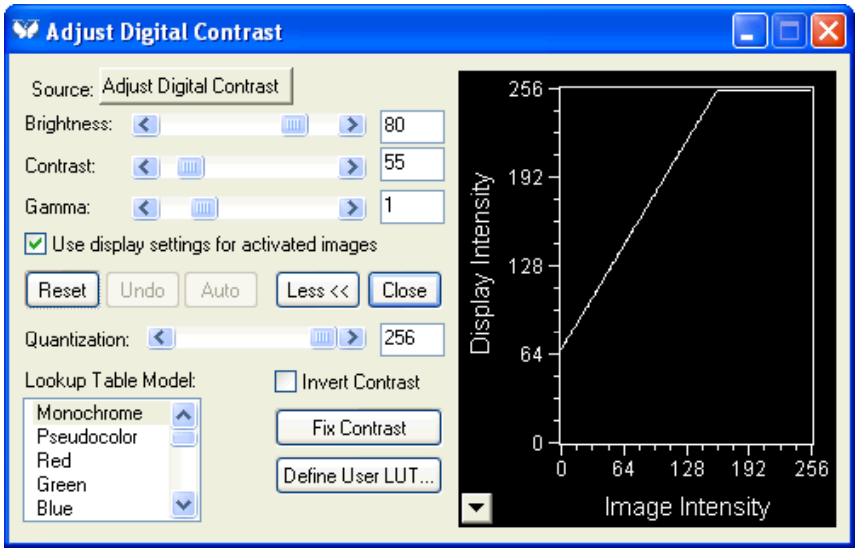
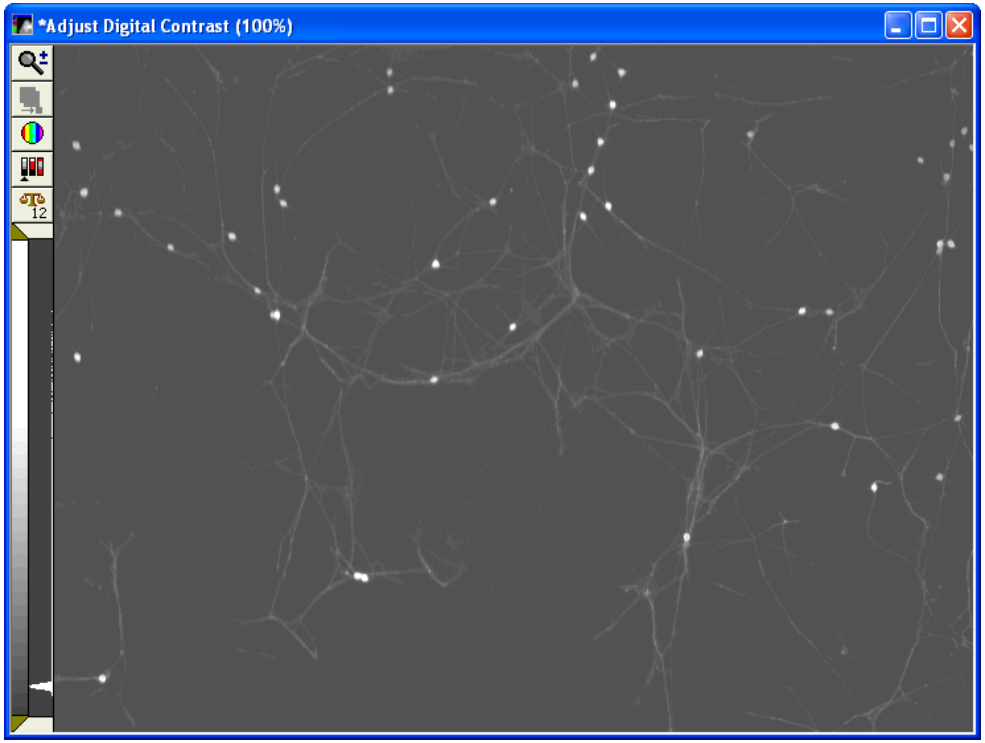
Adjust Digital Contrast





- Adjust brightness and contrast.

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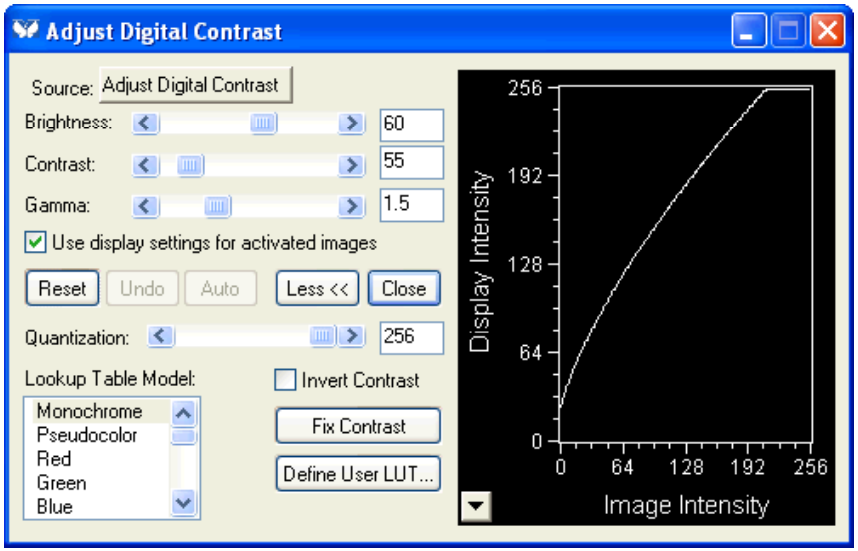
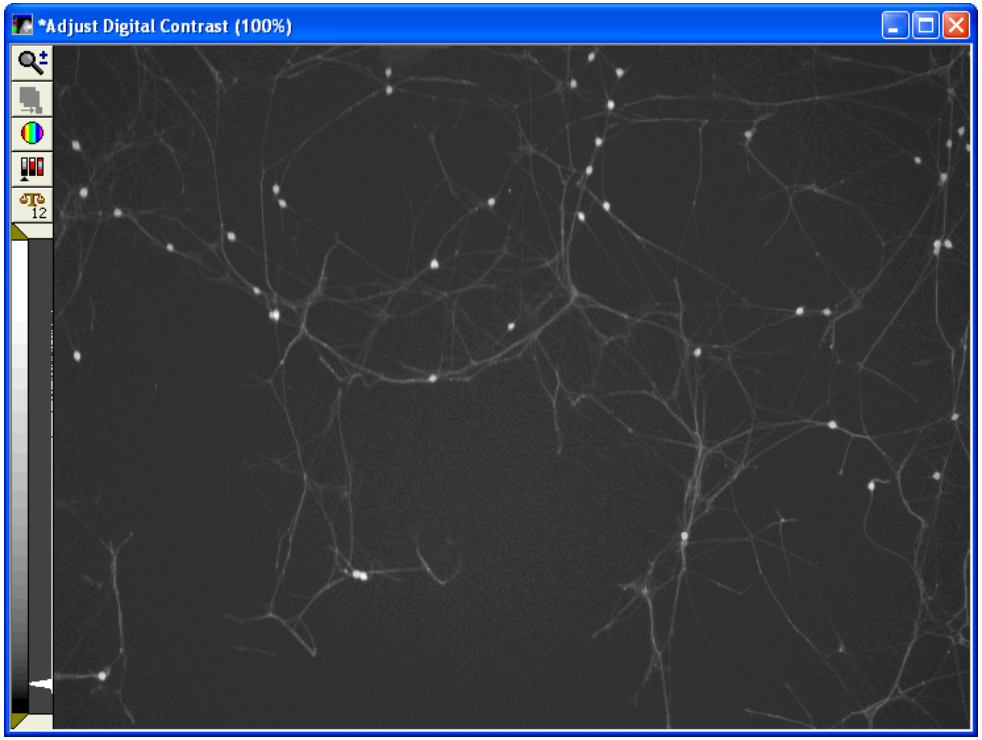
Adjust Digital Contrast





- Adjust brightness and contrast.
- Gamma adjustment.

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Color Image

- What's the difference between monochrome and color images?
- How can you tell?
- Why should you care?

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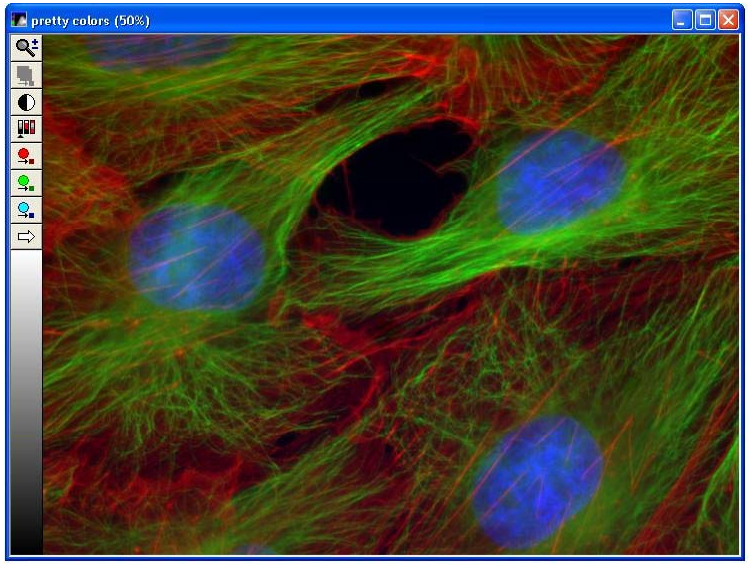
Color Image



- No Scale Image tool
- Pick Color Channel
- Three intensities per pixel instead of one
- Easier to get images into other programs
- Create color images with overlays for presentation

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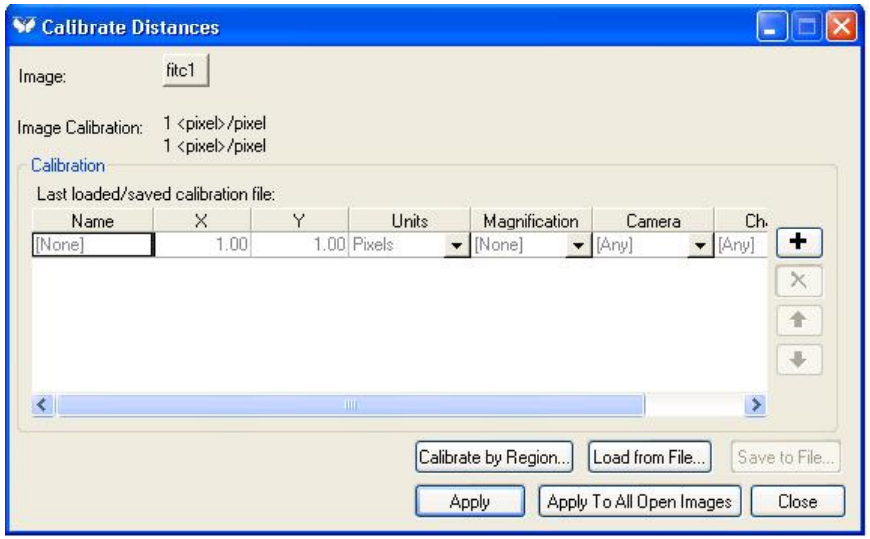
Calibrate Distance



- Measure Menu
- Set the relationship of pixel size to a real physical dimension
- Each pixel is how many microns
- Calibrations typically setup for each magnification

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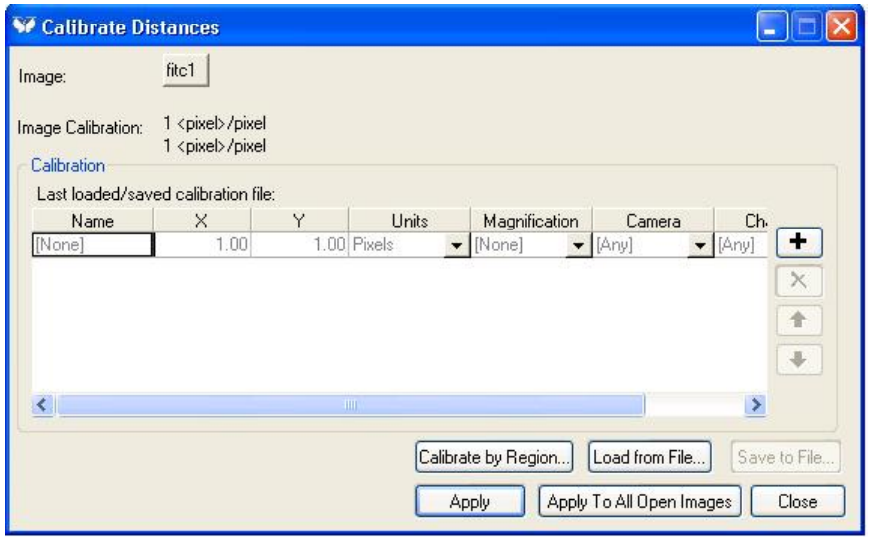
Calibrate Distance



- List of calibrations available in table
- Edit changes in the table for existing calibrations
- Save calibrations to a file
- Define new calibrations and measure with regions

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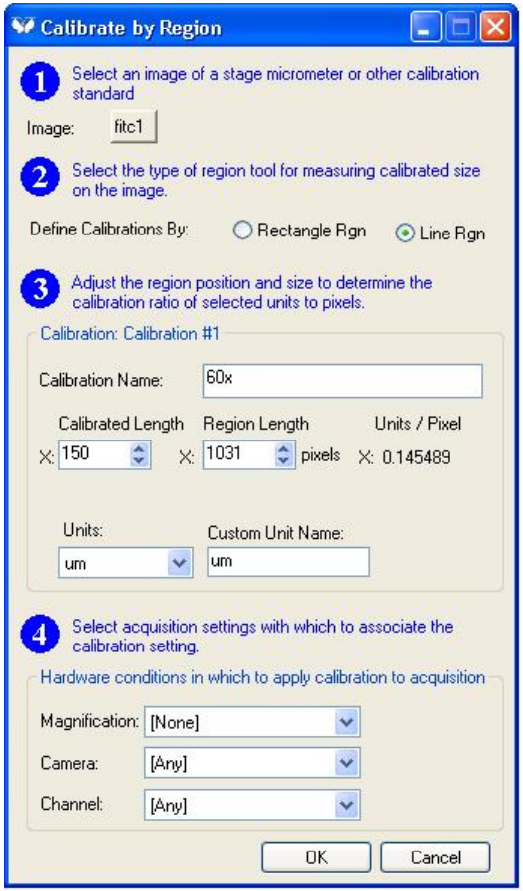
Calibrate Distance



- Calibrate by region, image of stage micrometer
- Select region type and resize region across the image to known marker dimensions
- Enter the name (typically the magnification) and calibrated length
- Associate the calibration to magnification and acquisition hardware

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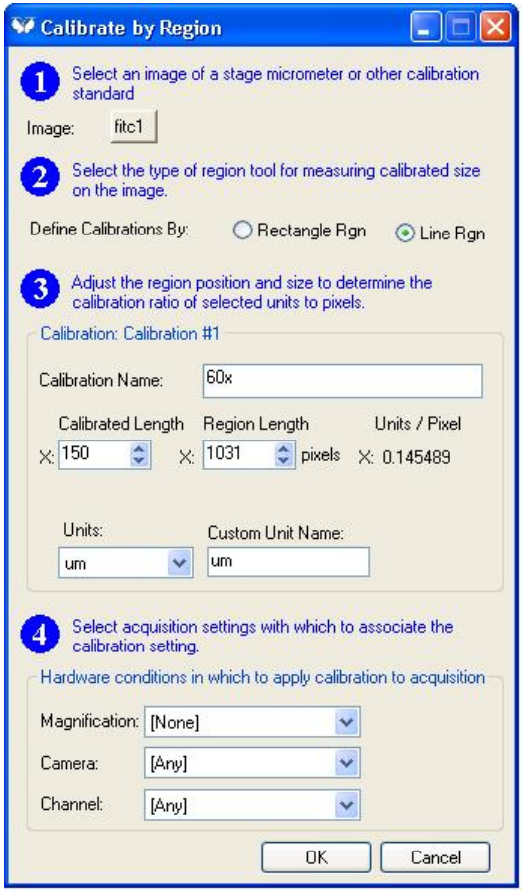
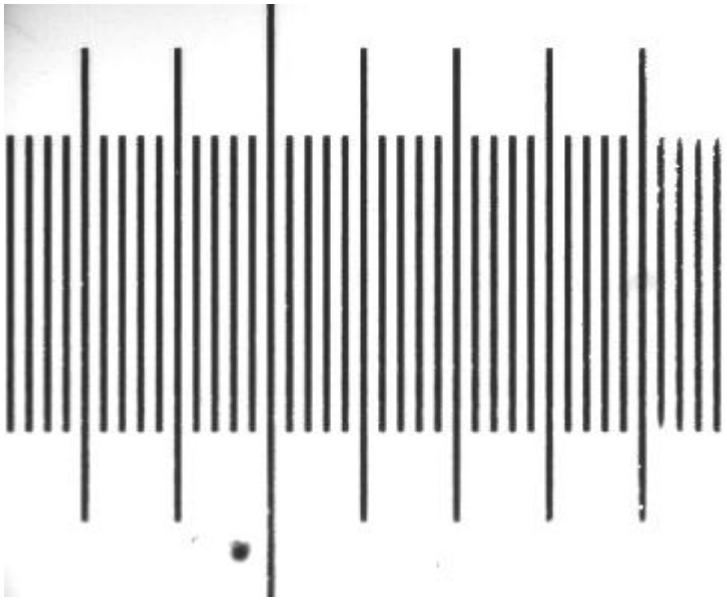
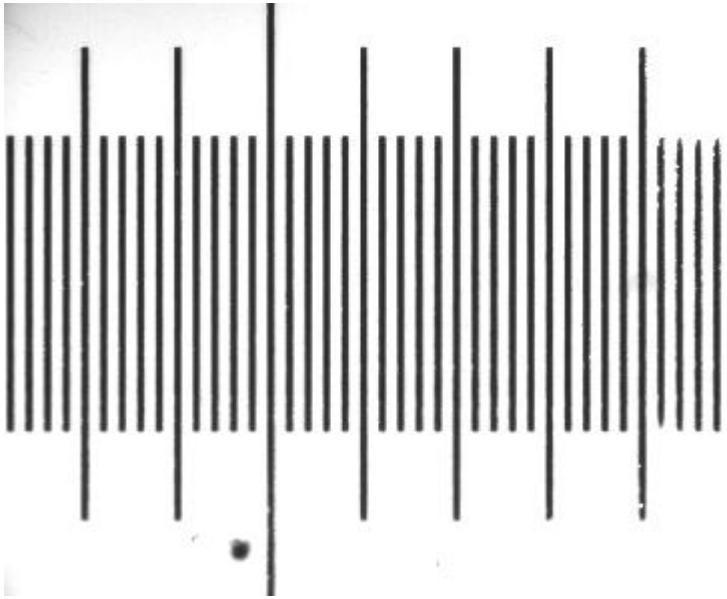
Calibrate Distance





Calibrate Distance



- Quick estimate of the calibration in your images?
- Size of pixels on camera / total system magnification
- Example:
  - 6.45 µm per pixel / 60x objective (no tube lens magnification) = ~0.1 µm per pixel

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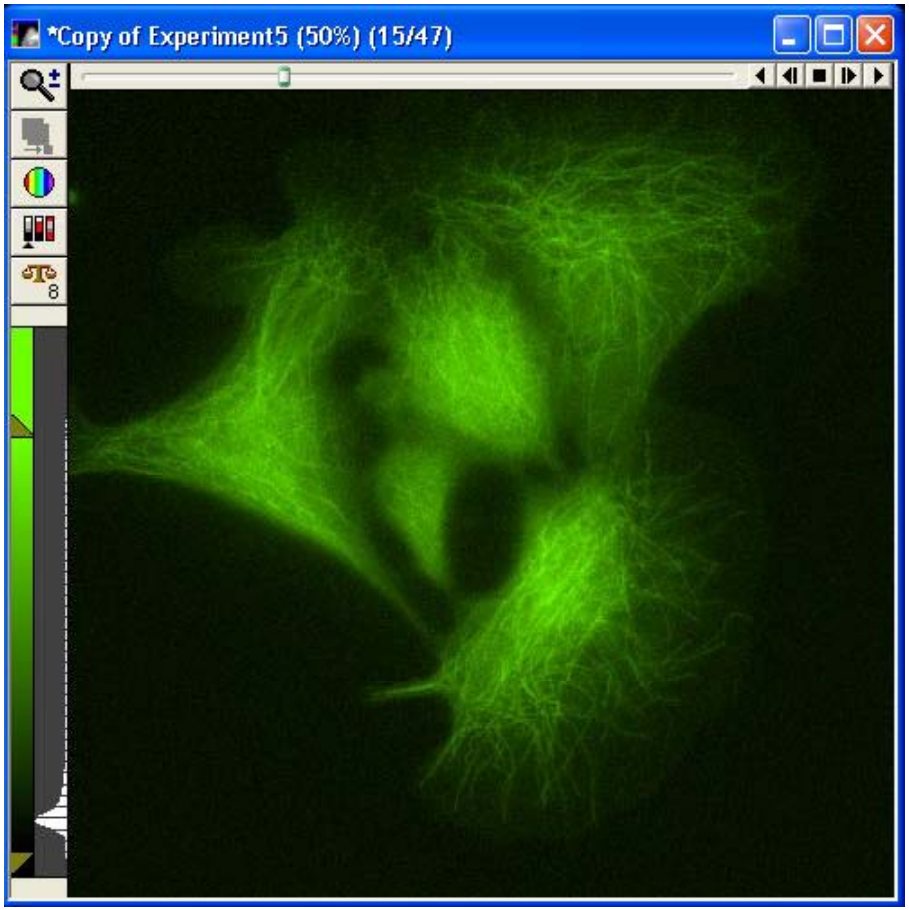
Working with Stacks

Stacks



- What are stacks?
- A set of images organized in a series, displayed one image at a time in an image window
- Time Series
- Z Series
- Wavelength Series
- Many people think of stacks as movies

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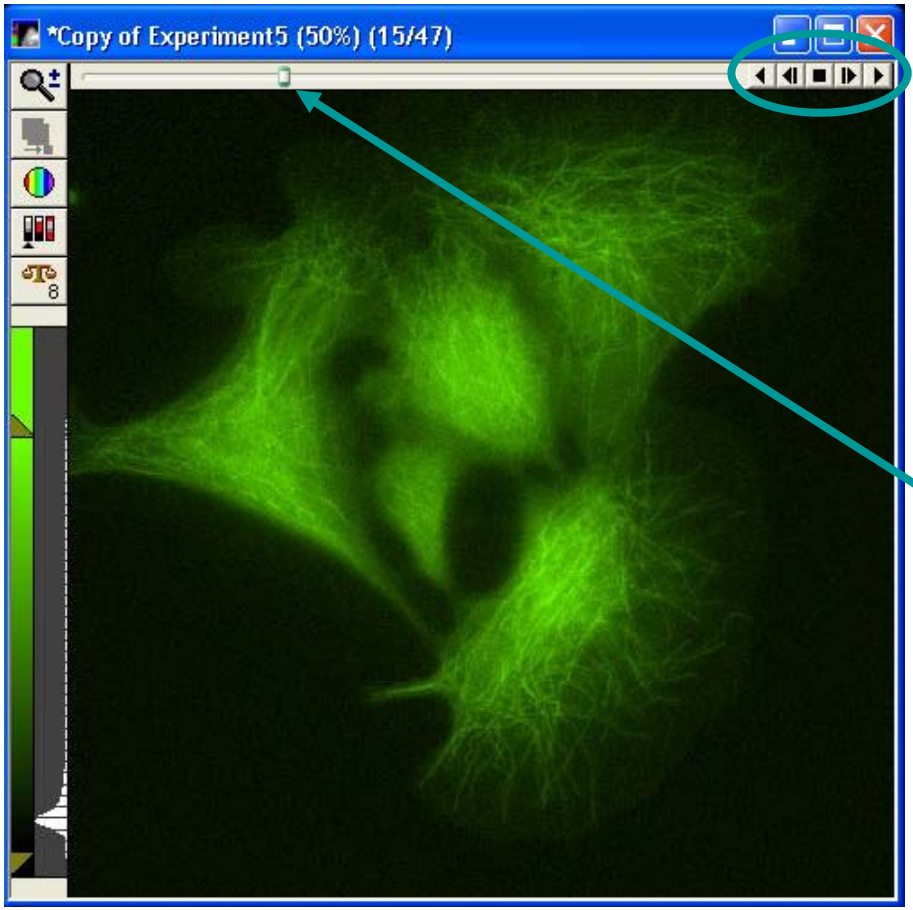
Stacks



- Usually saved in one file
  - 2.0 – 7.1 an .stk file
  - 7.5 or greater a multi-image tiff
- Stack Player controls
- “Thumb” for picking a single plane
- Play Preferences on Stack Menu

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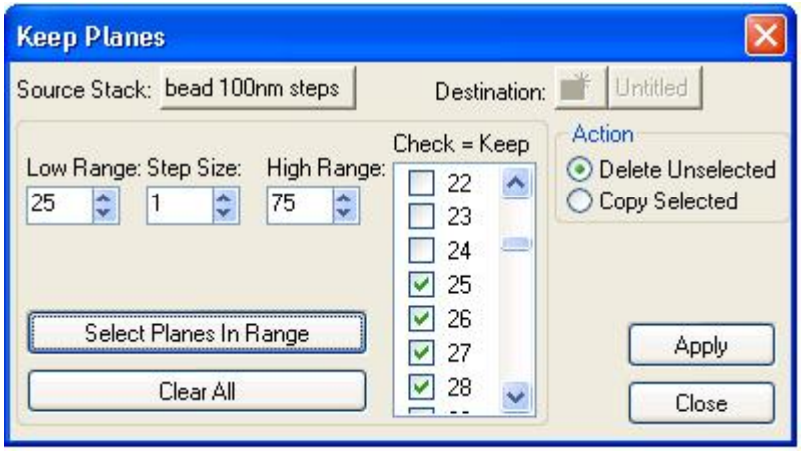
Keep Planes



- Stack Menu
- Used to copy a portion or crop a stack
- Can sub-sample a data set with the Step Size setting

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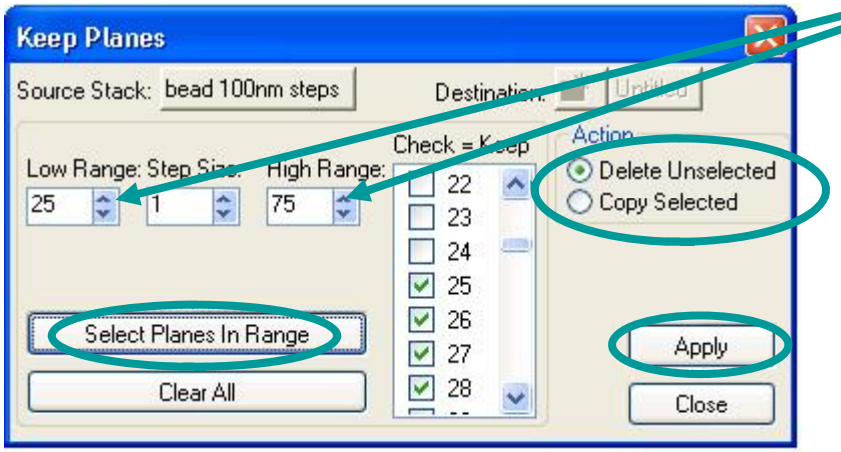
Keep Planes



- Specify a range of planes to select
- Press Select Planes in range
- Scroll list to see check marks
- Pick the action
- Click Apply

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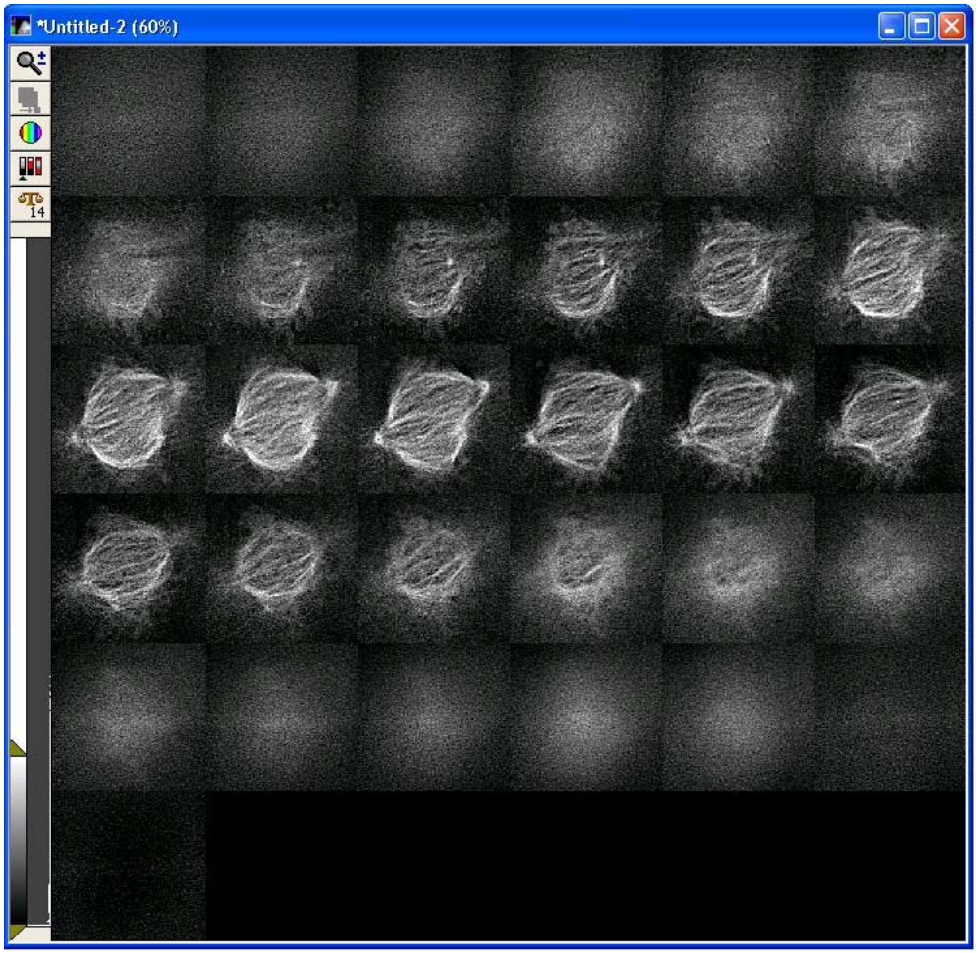
Montage



- Stack Menu
- Arrange planes side by side to create a single image of the stack
- Rows and columns can be configured
- Result can be zoomed or stitched

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Montage

- Select the fill order
- Select the rows and columns if you don't like the defaults
- Zoom the image if necessary
- Stitch the result if source is an X,Y Stage Scan
- Press OK

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Exercises

Part 1

1) Image Display
2) Calibrate Distance
3) Working with Stacks

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Image Processing

Image Filtering

- Filtering helps prepare images for segmentation and/or analysis
- Filtering accomplishes:
  - Noise removal
  - Correction of uneven background
  - Elimination of image details other than the objects of interest
- No image filter will work perfectly on all images

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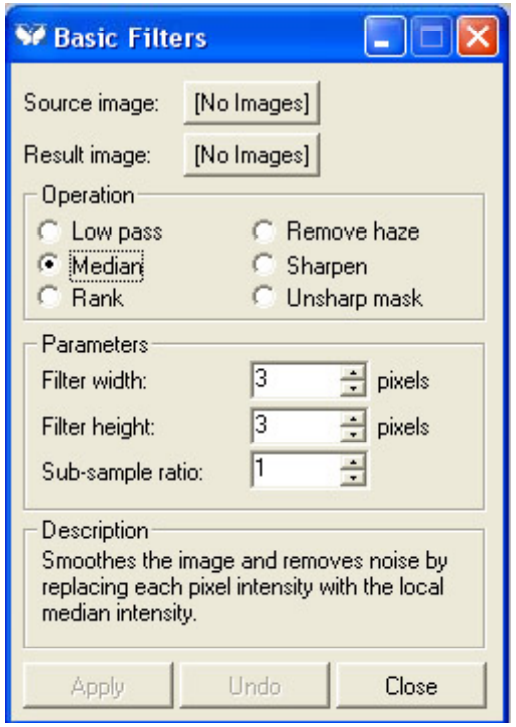
Basic Filters



- Process Menu
- Weighted filtering and rank filtering
- Examining each pixel and its neighborhood of adjacent pixels
- The size of the neighborhood is called the “kernel”

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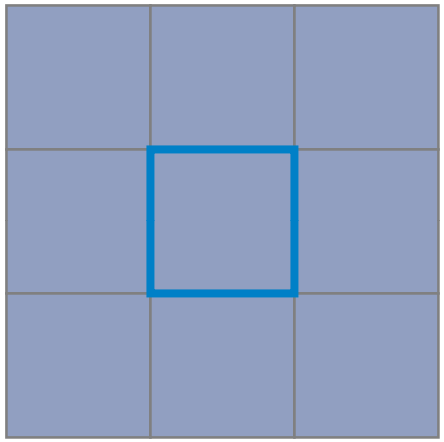
Basic Filters



- The center of the kernel defines the pixel location being processed
- Larger size kernels enhance the effect of any specific filter
- Kernels are placed at each pixel in the image and the filter operation is performed
- Watch for edge effects

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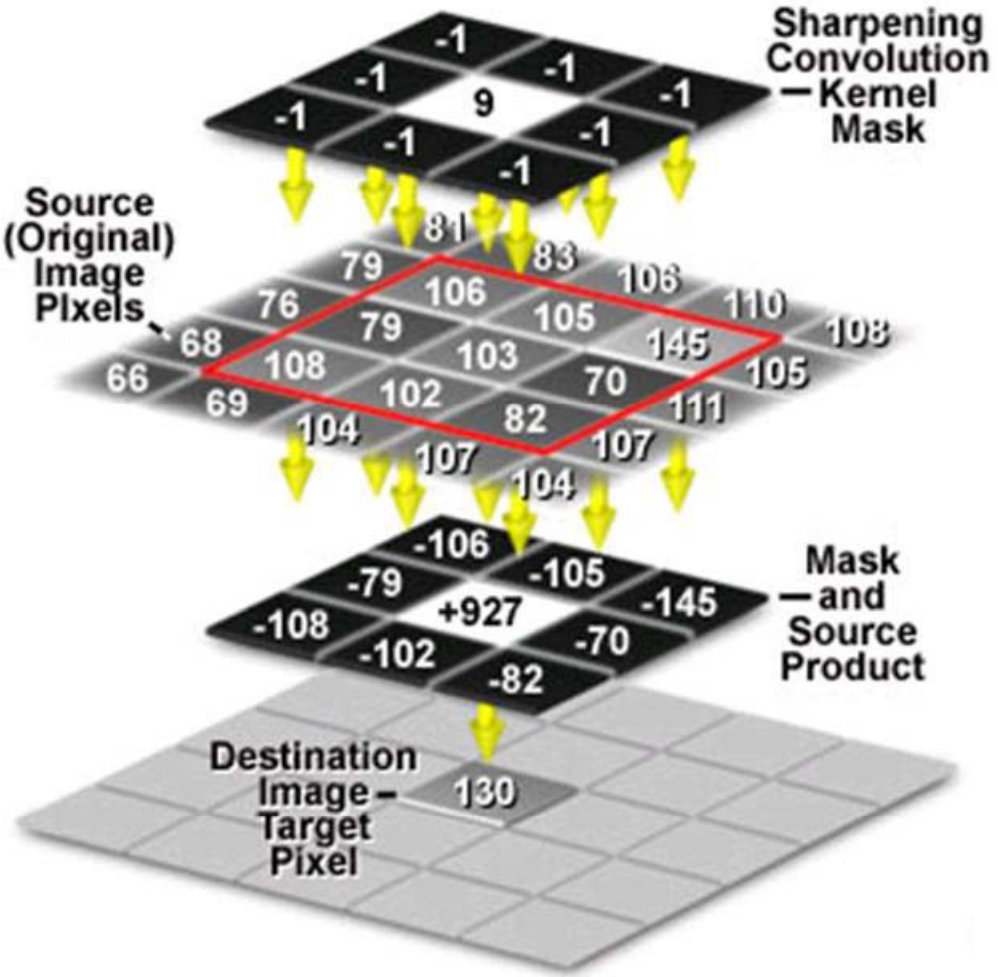
Basic Filters

1. *Kernel or mask with an odd number of pixels (3x3, 5x5, 7x7) is applied to each target pixel in the image.*

2. *The image pixels centered on the target pixel are multiplied by the kernel.*

3. *The pixels are added together and the central pixel is given that value.*



http://micro.magnet.fsu.edu/primer/digitalimaging/imageprocessingintro.html

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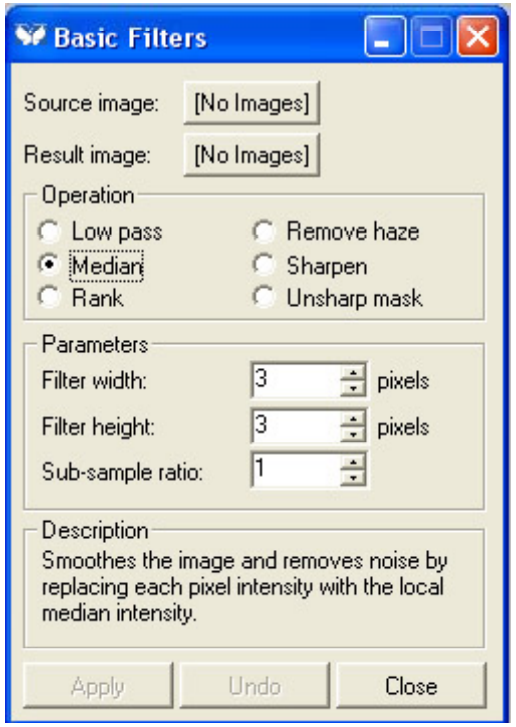
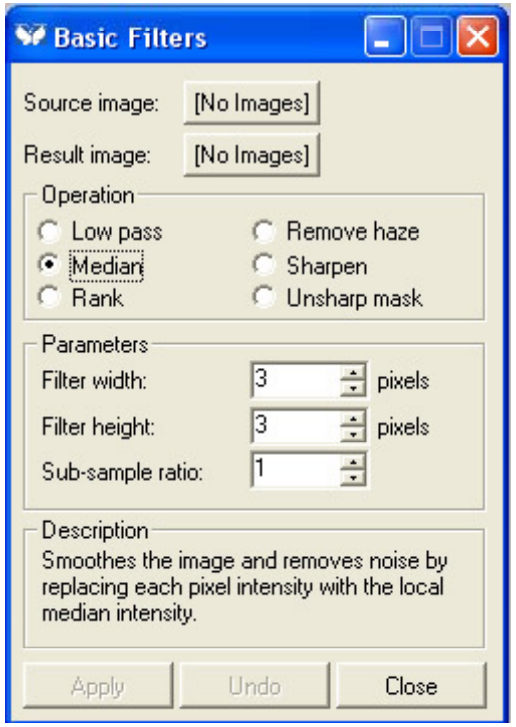
Basic Filters



- **Low Pass**
  - suppresses high frequencies by replacing each pixel intensity with the local average intensity
  - blurs edges
- **Sharpen**
  - enhances edges by applying a high pass filter
  - multiplication of each kernel's value with pixels and summing results

Molecular Devices

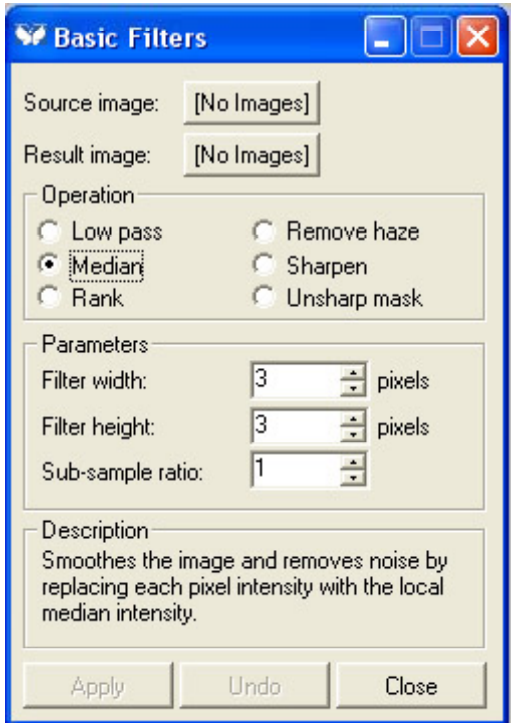
Basic Filters



- **Median**
  - removes noise by replacing each pixel intensity with the local median intensity
  - good at noise removal while preserving edges
- **Rank**
  - removes noise by replacing each pixel intensity with a local rank statistic intensity such as minimum, maximum, or median

Molecular Devices

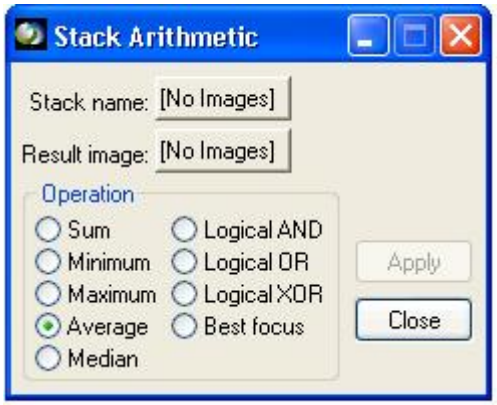
Basic Filters



- **Low Pass**: suppresses high frequencies by replacing each pixel intensity with the local average intensity
- **Median**: removes noise by replacing each pixel intensity with the local median intensity
- **Rank**: removes noise by replacing each pixel intensity with a local rank statistic intensity such as minimum, maximum or median
- **Remove haze**: subtracts out of focus blur estimated from neighboring planes
- **Sharpen**: enhances detail by applying a high pass filter
- **Unsharp mask**: subtracts out of focus blur estimated by applying a low pass filter

Molecular Devices

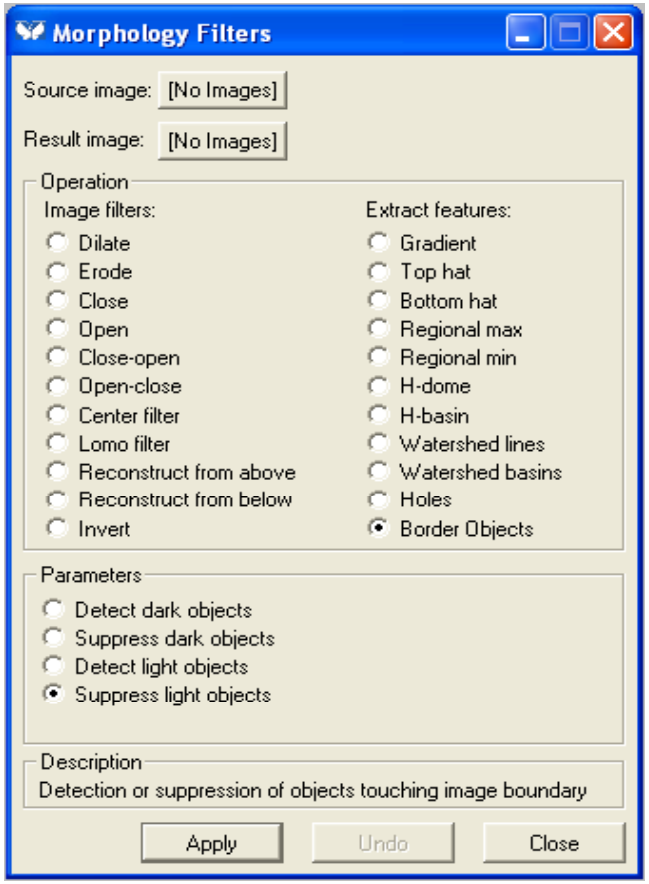
Stack Arithmetic



- Stack Arithmetic
- Compress the data from an entire stack into one image
- Can be used:
  - to improve signal to noise
  - compress stack of images into single plane
  - show the path of motion in a single plane

Molecular Devices

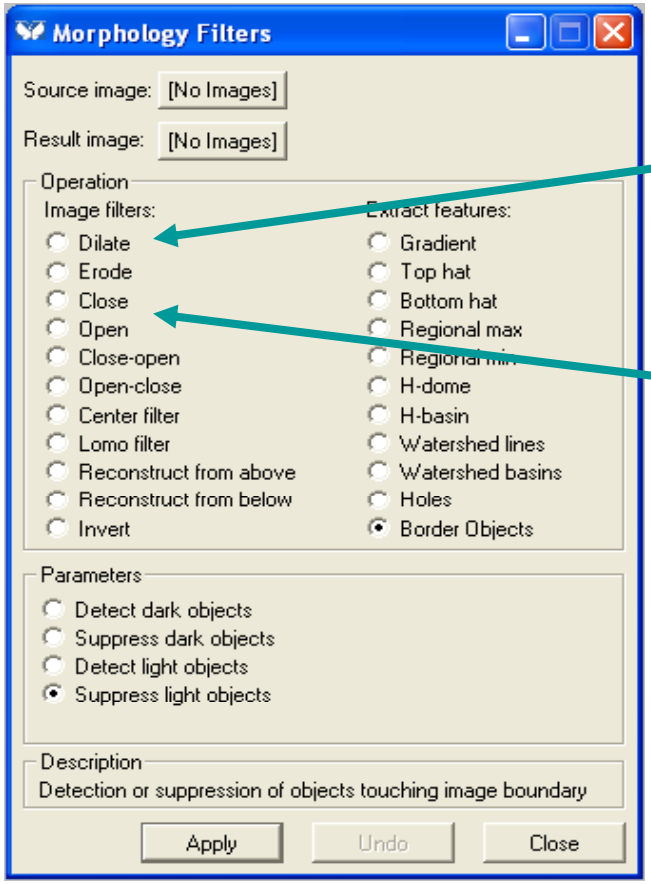
Morphology Filters



- Process Menu
- Spatial and intensity-based filtering
- Settings for basic shape, size, and area
- Most intuitive results on binary images, but can operate on grayscale images

Molecular Devices

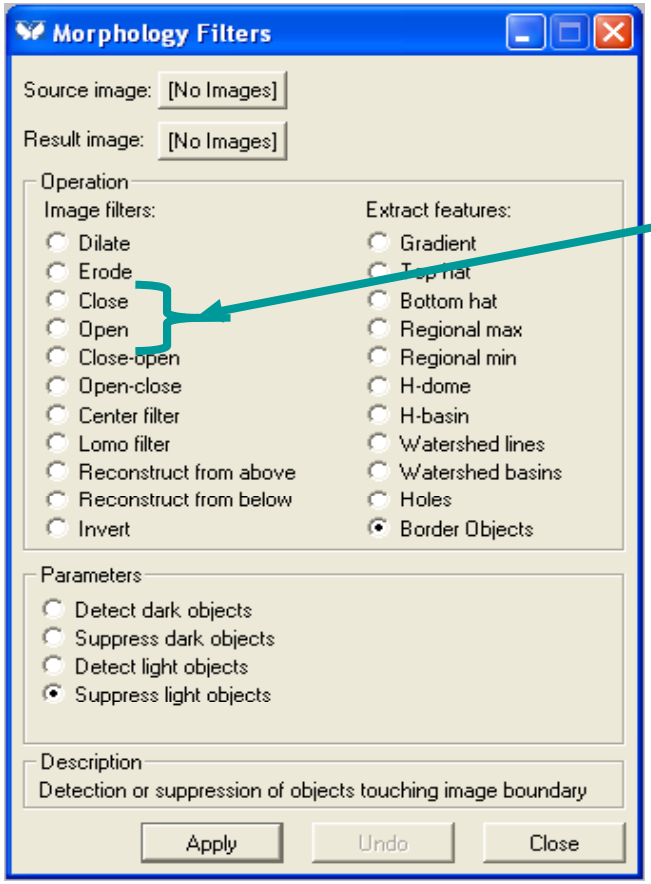
Morphology Filters



- Dilate – grow bright things
- Erode – shrink bright things
- Close – dilate then erode
- Open – erode then dilate
- Open and Close are equivalent to where does the shape fit

Molecular Devices

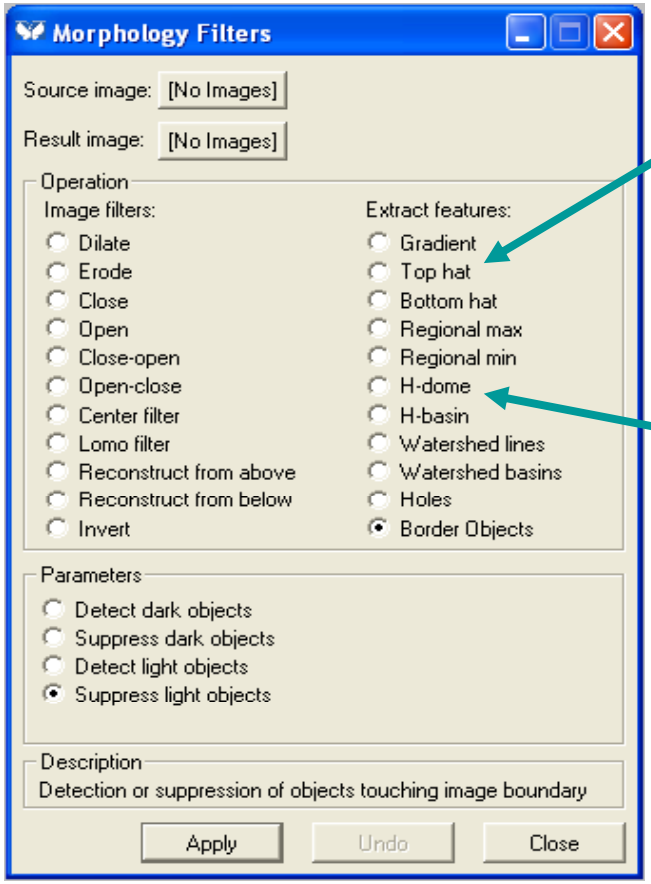
Morphology Filters



- Close to fill small black holes in white
- Open filters out small white in a black background

Molecular Devices

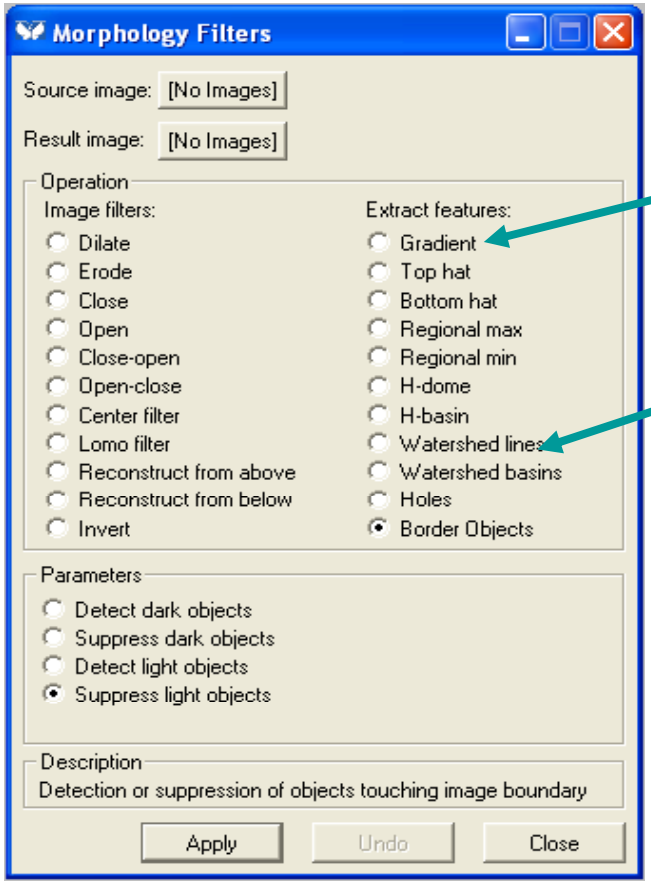
Morphology Filters



- Top Hat – Residue of Open Filter
  - Keeps the things that the open filter would have removed
  - Top Hat to find small features
- Bottom Hat – Residue of Close
- H-Dome – Local intensity peaks
- H-Basin – Local intensity troughs
- Top Hat to find small features
- H-Dome to flatten an uneven background

Molecular Devices

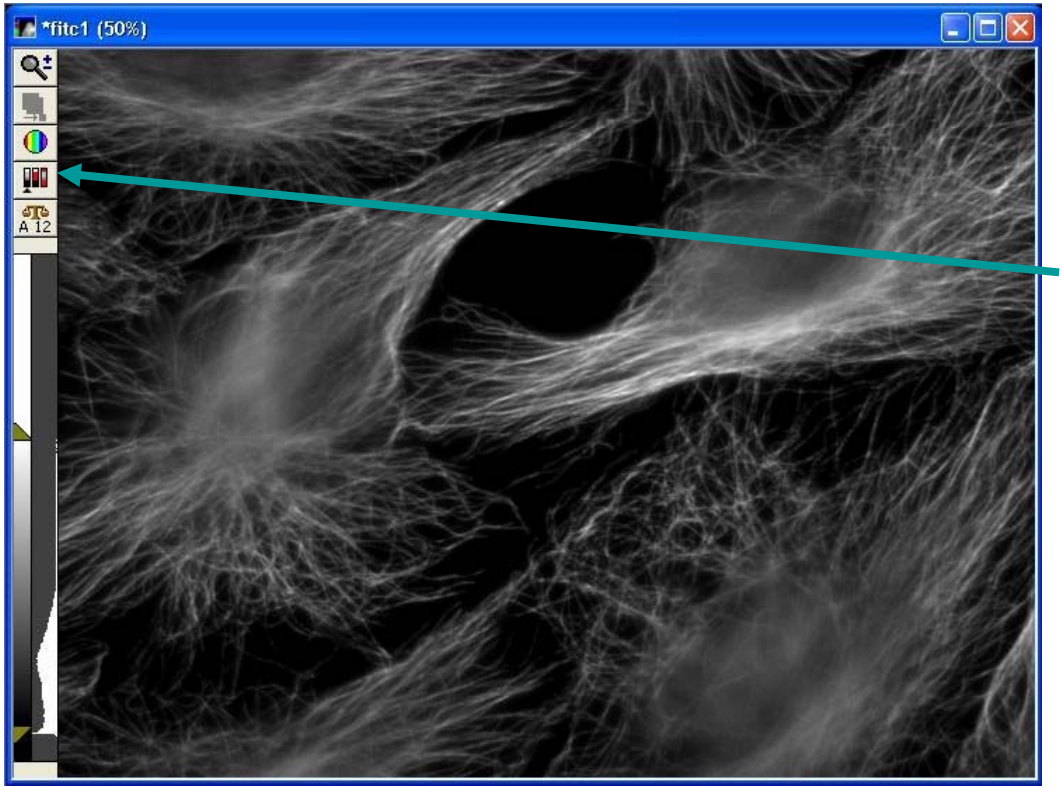
Morphology Filters



- Gradient – Difference between Dilate and Erode
- Watershed Lines – Helps with splitting touching features

Molecular Devices

Segmentation



- Differentiate between what to and what not to measure

- Regions of Interest
- Thresholding
- Morphology
- Morphometry
- Application Modules

Molecular Devices

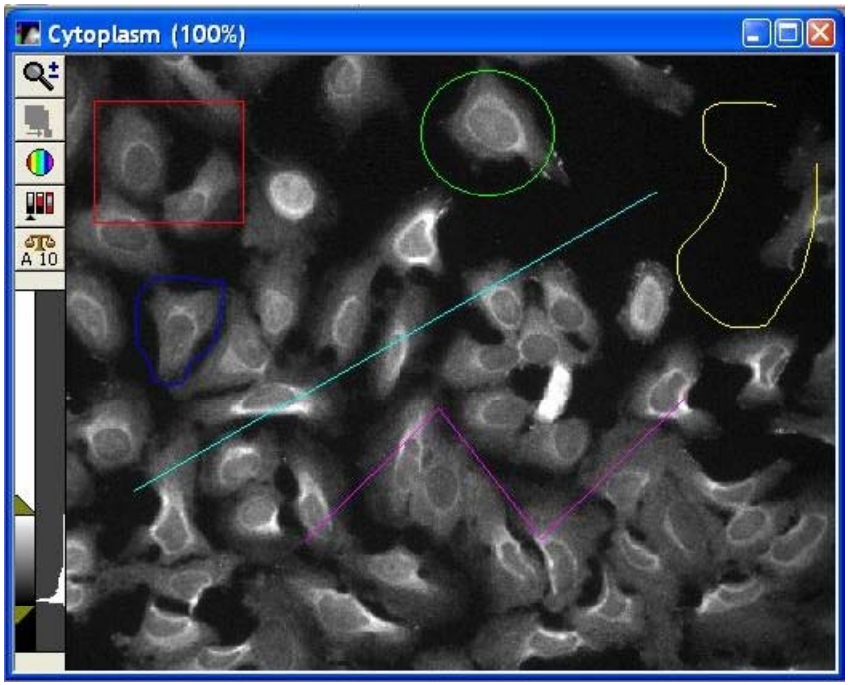
Regions of Interest

- Regions Menu

- Rectangle
- Ellipse
- Polygon
- Line
- Multi-Line
- Multi-Line Trace
- Region Properties

Molecular Devices

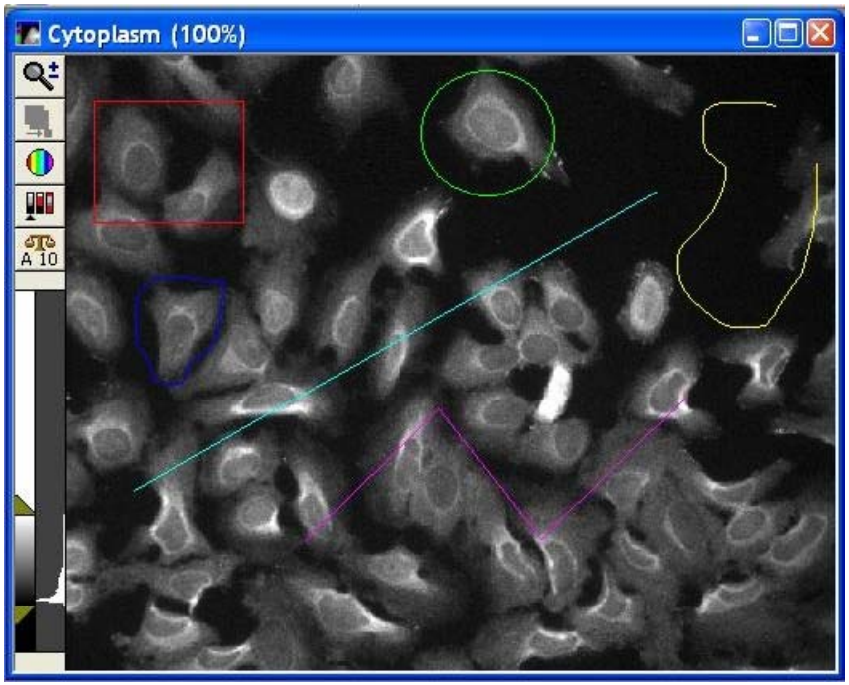
Regions of Interest



- Active region
- Region tooltips for position and size (in calibrated units)
- Some filtering tools operate in active region

Molecular Devices

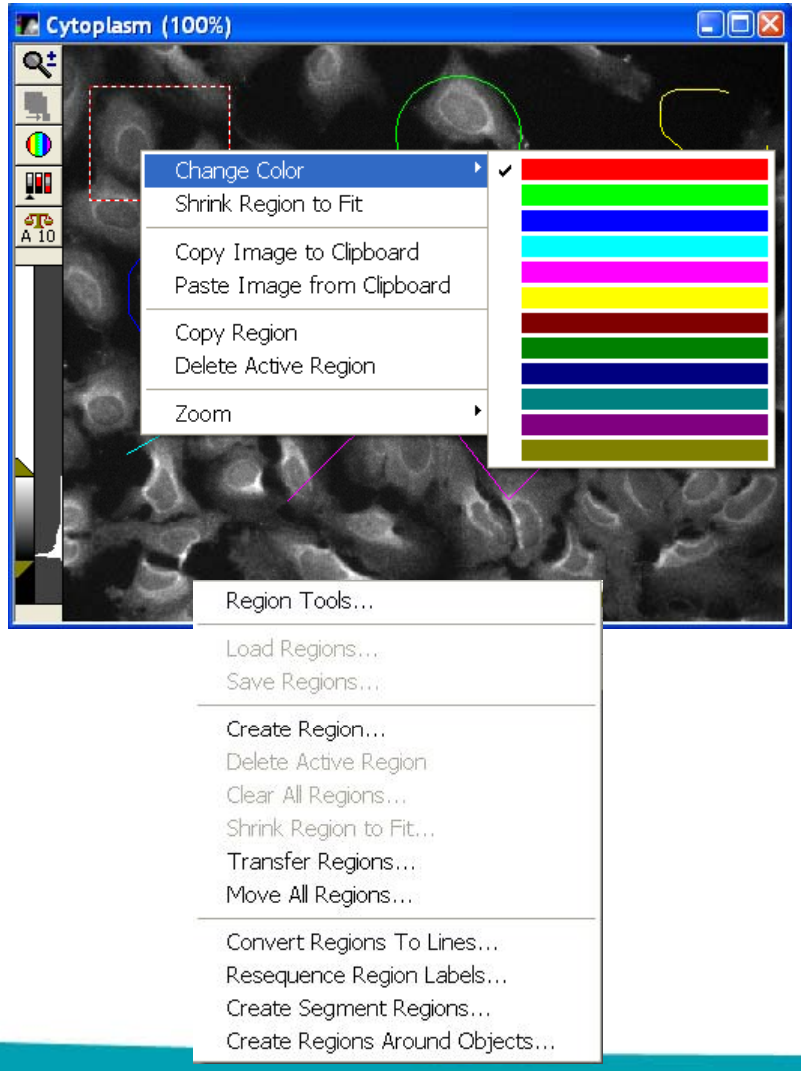
Regions of Interest



- Double-click on line regions to edit positions of points
- Use Rectangle region and Ctrl-D to crop an image or Ctrl-Shift-D to crop a stack
- Tooltips are displayed when regions are being created

Molecular Devices

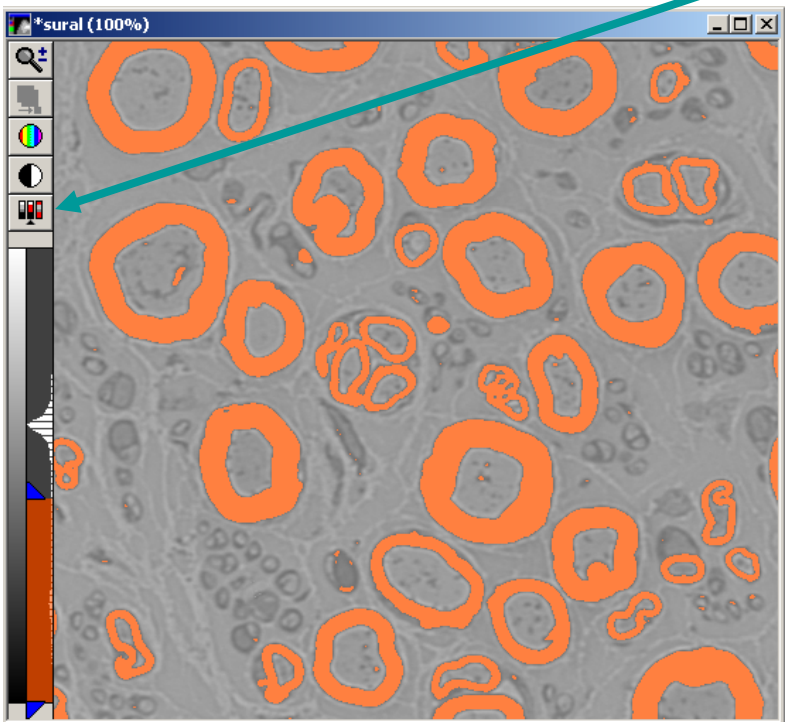
Regions of Interest



- Right-click menu: copy, paste, delete regions
- Save and load regions to a file
- Transfer regions to another image
- Create Region function

Molecular Devices

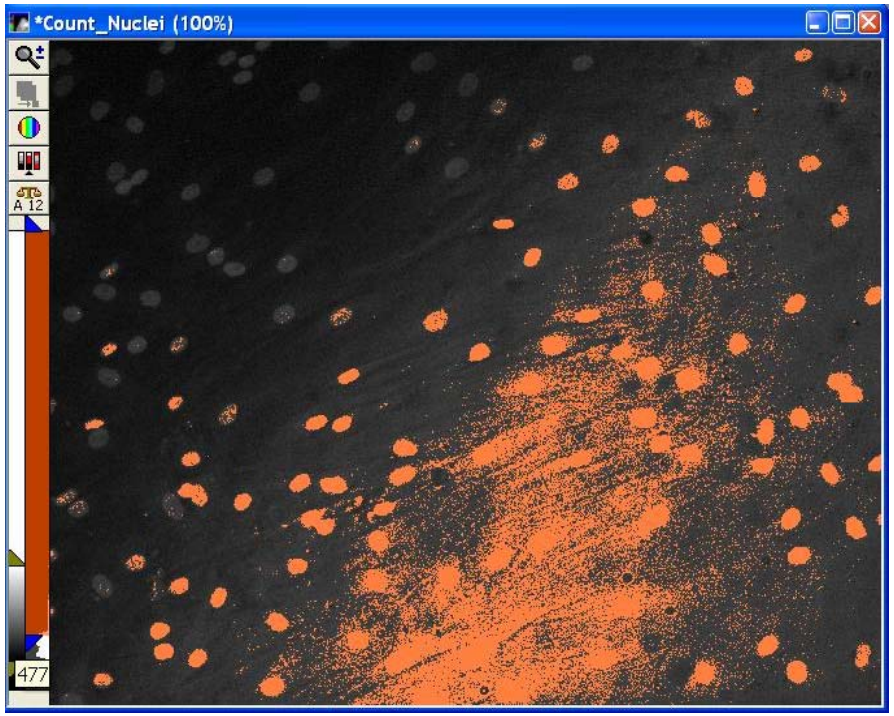
Thresholding



- Measure Menu and on image window toolbar
- Select pixels in image based on a range of intensities
- Three states
  - Off
  - Inclusive Threshold
  - Exclusive Threshold
- Subjective to user

Molecular Devices

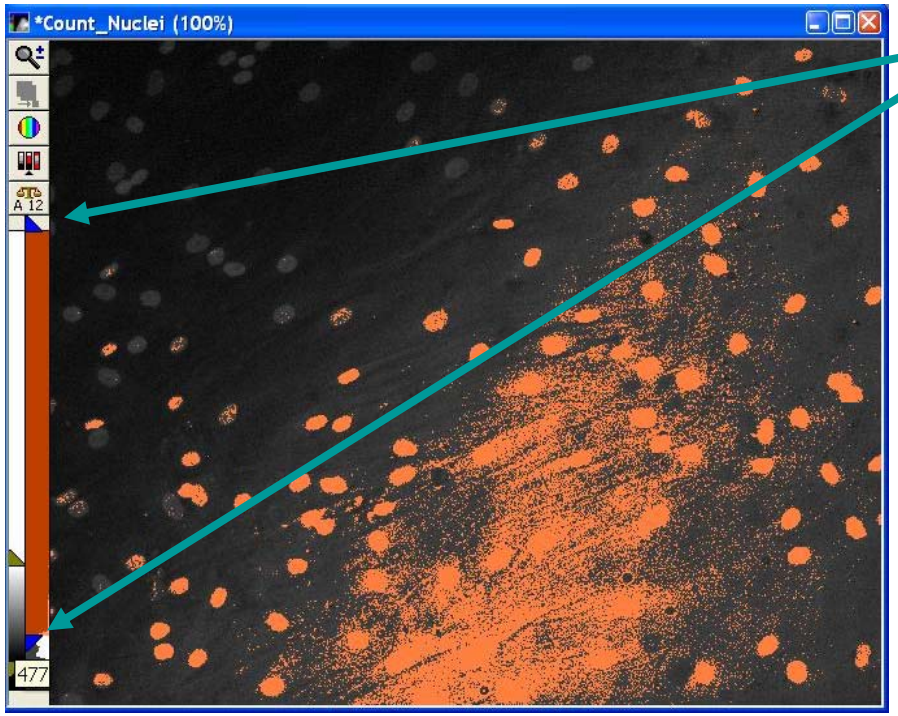
Thresholding



- Colored overlay visible on image
- Even illumination/background necessary for good thresholding
- Pixels are either thresholded or not so binary images can be created
- A prerequisite step for some analysis functions

Molecular Devices

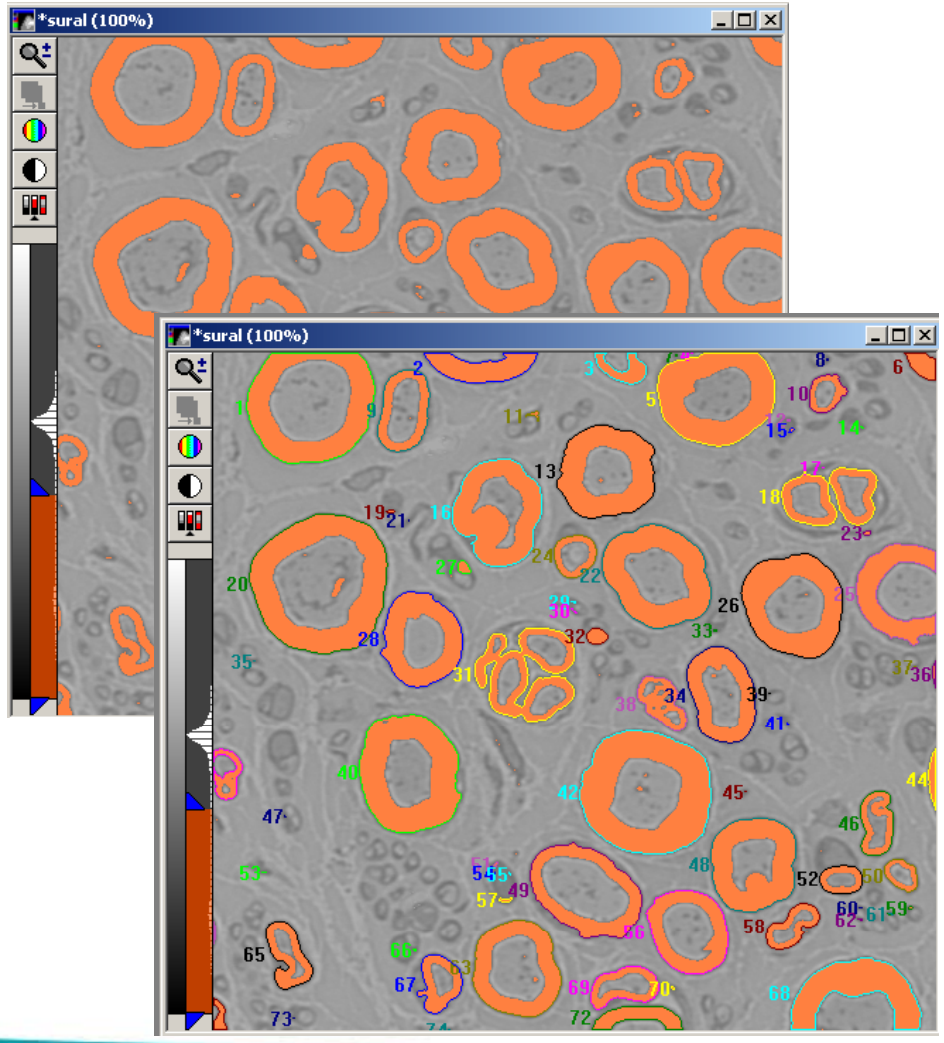
Thresholding



- Blue Wedges on image windows can be used to adjust threshold
- Autothreshold to find either bright or dark objects in image
- Preference to select between two different algorithms

Molecular Devices

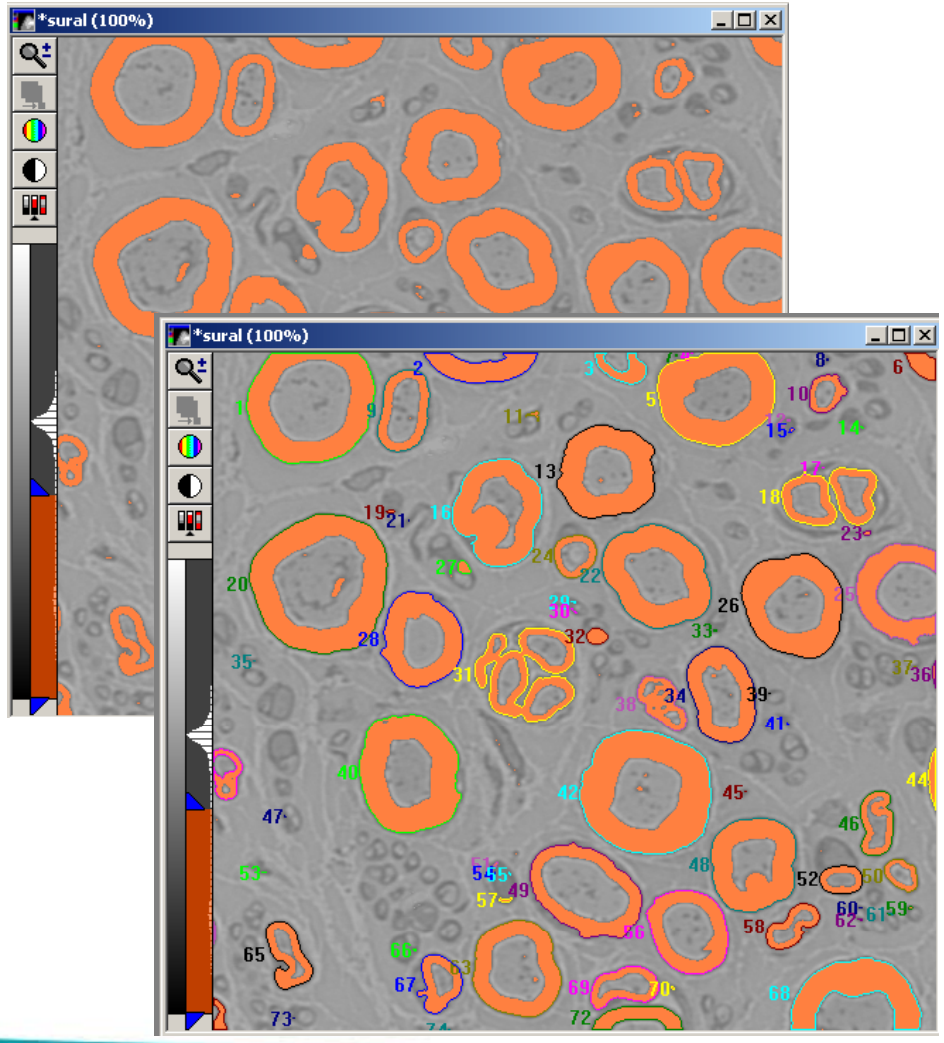
ROI + Thresholding



- You can combine using Regions of Interest and Thresholding
- Threshold Image
- From the Regions menu select Create Regions around Objects

Molecular Devices

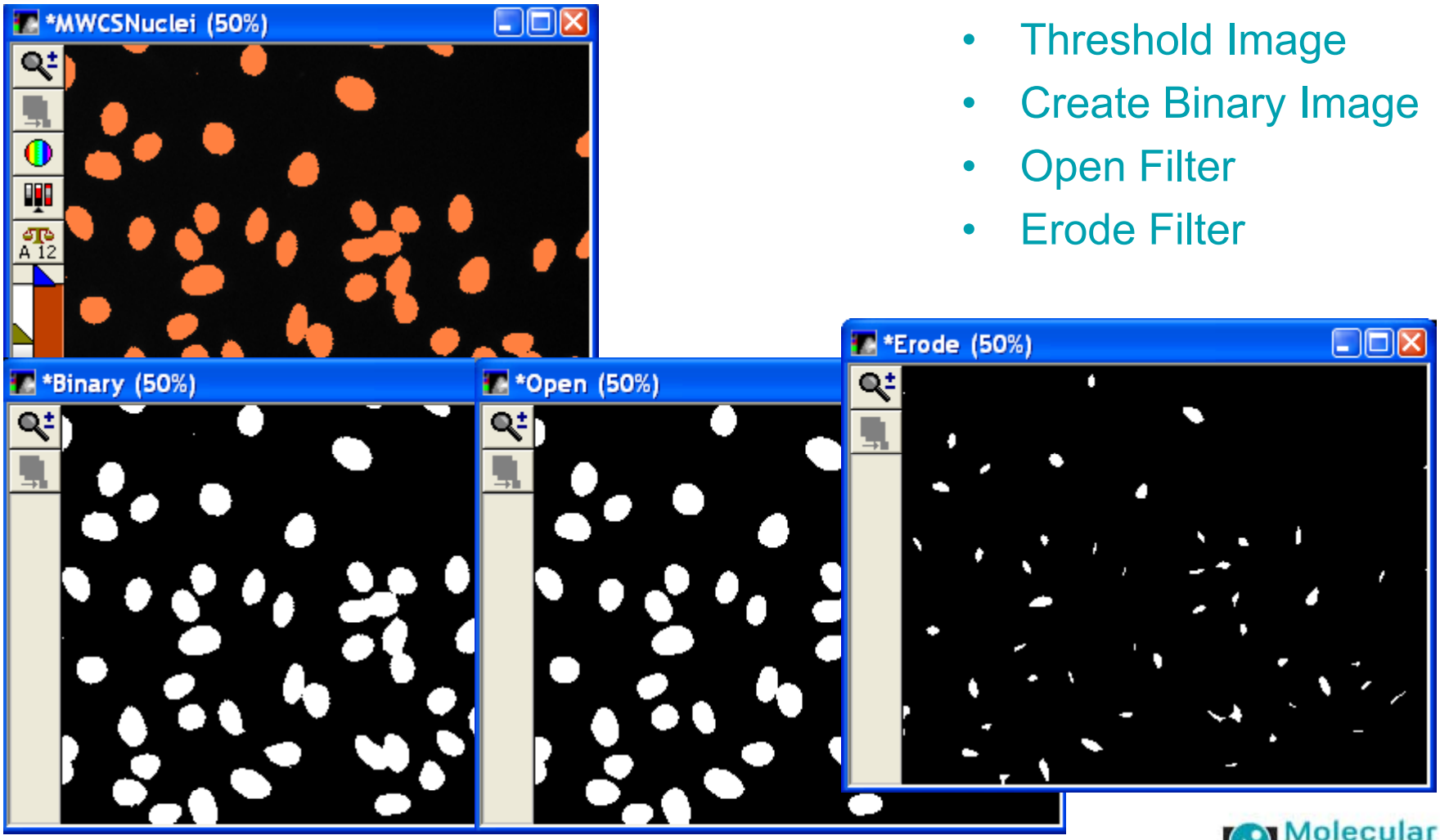
ROI + Thresholding



- Each discrete object will be traced with a region
- Must be at least two adjacent pixels to create a region

Molecular Devices

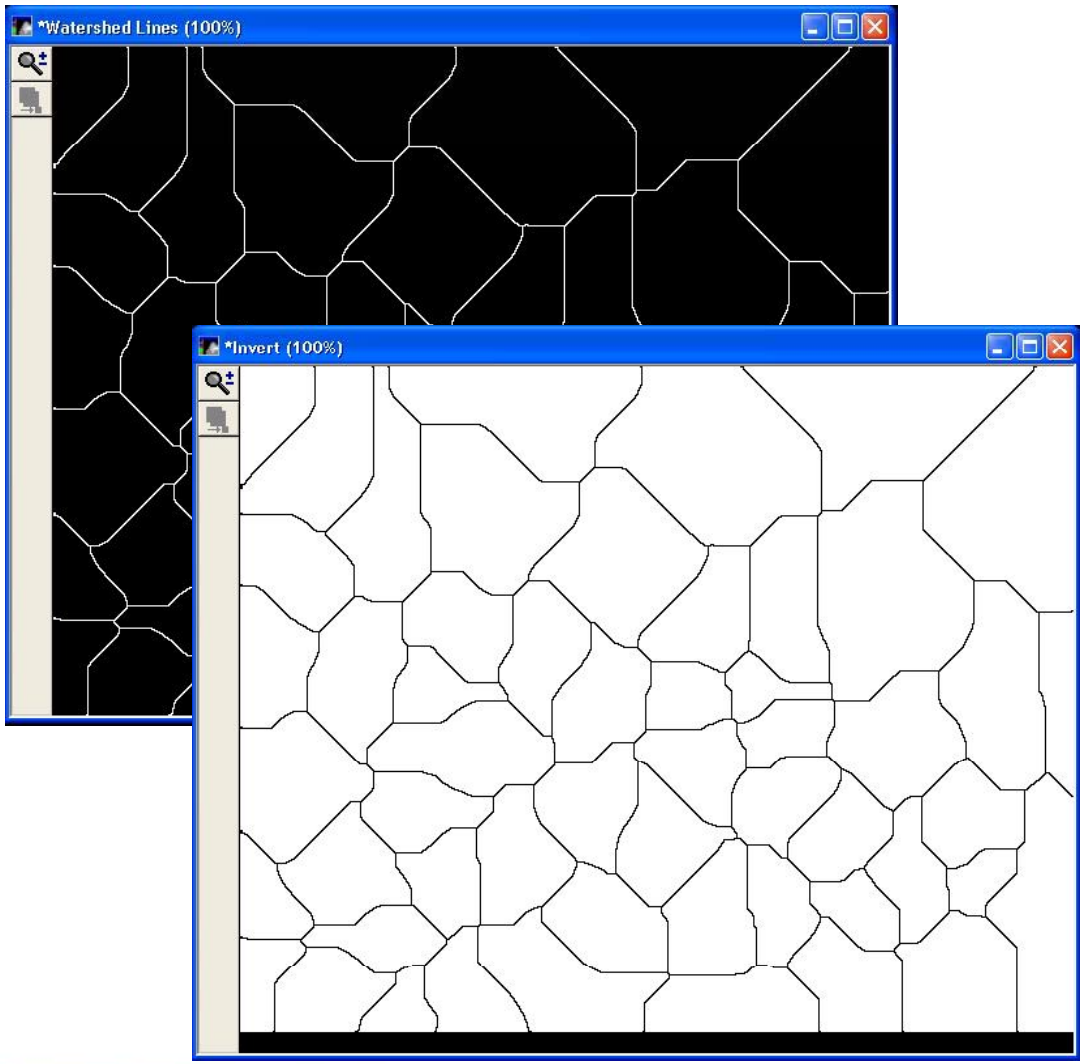
Combining Tools



- Threshold Image
- Create Binary Image
- Open Filter
- Erode Filter

Molecular Devices

Combining Tools

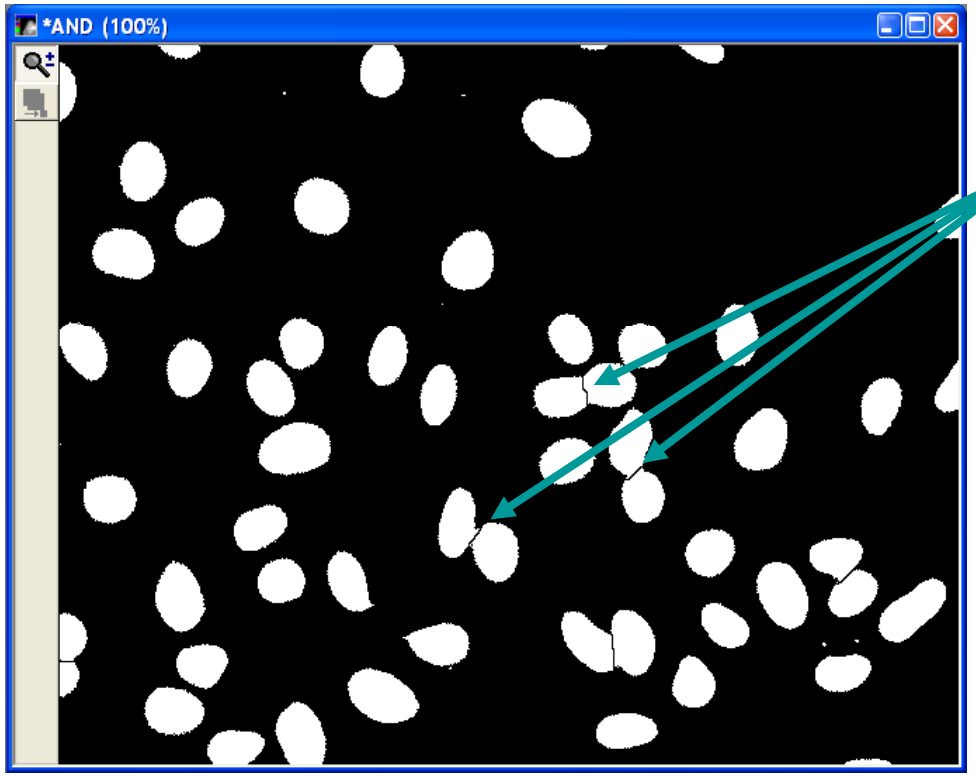


- Threshold Image
- Create Binary Image
- Open Filter
- Erode Filter

- Watershed Lines Filter (using Binary and Erode images)
- Invert Watershed
- Logical AND between Binary and Invert Result

Molecular Devices

Combining Tools



- Notice some of the new lines separating the nuclei
- Use the Arithmetic function to create a final image using the original image and this filtered image to get the original image intensities combined with the Watershed Segmentation applied

Molecular Devices

Exercises

Part 2

1) Filtering
2) Segmentation
3) Thresholding and Measuring Regions

Molecular Devices

MetaMorph®

Molecular Devices

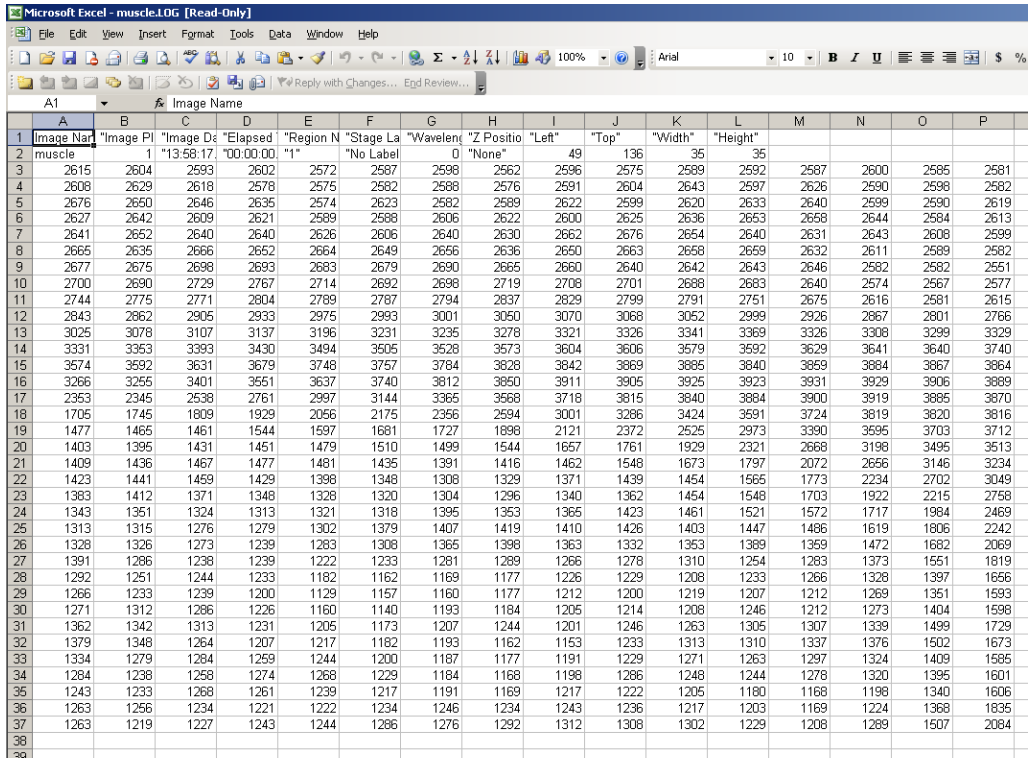
Image Analysis

Image Analysis

- What questions can MetaMorph answer about images?
  - How Many
  - How Bright
  - How Big
  - How Long
  - How Far
  - How much Overlap
- Quantify Results

Molecular Devices

Image Analysis


Microsoft Excel - muscle.LOG [Read-Only]

File Edit View Insert Format Tools Data Window Help

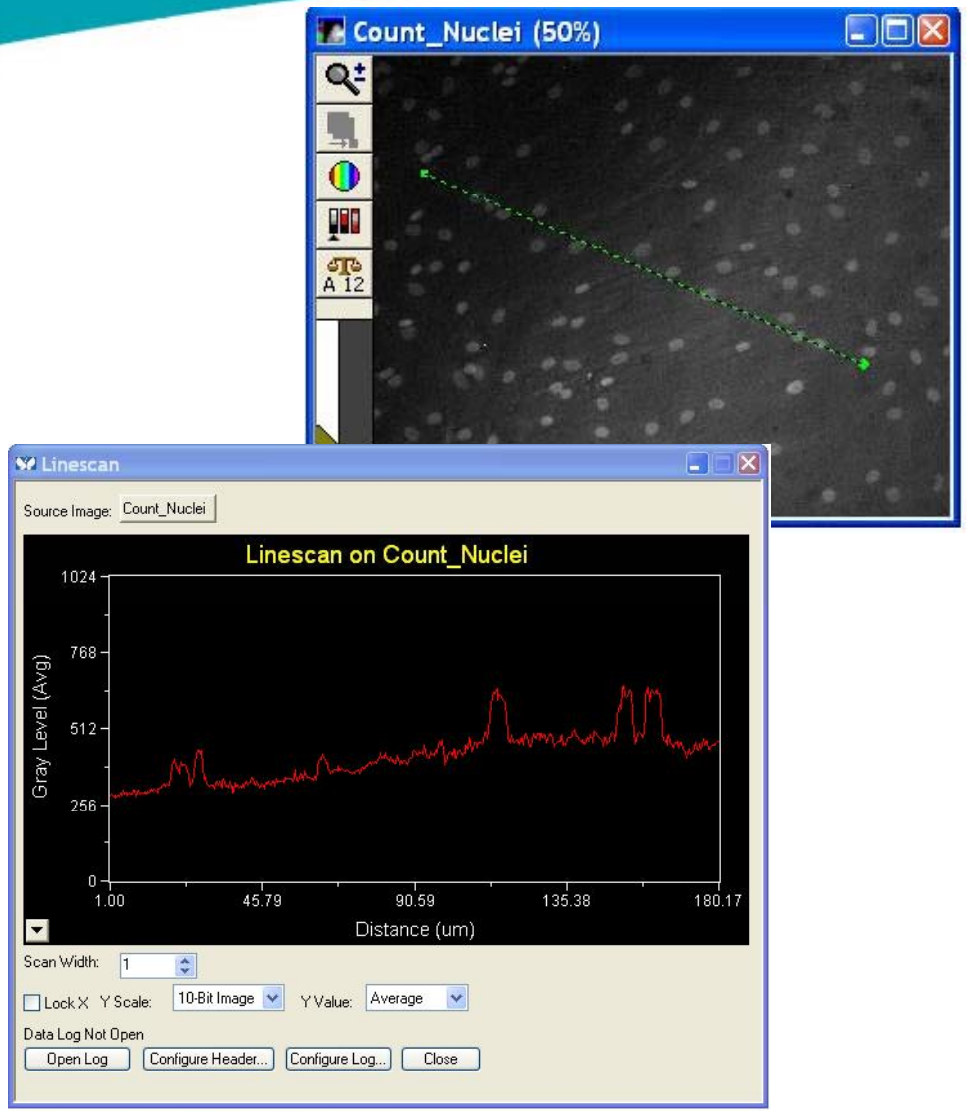
A1 Image Name

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Image Nar	"Image Pl	"Image Da	"Elapsed	"Region N	"Stage La	"Wavelen	"Z Positio	"Left"	"Top"	"Width"	"Height"				
2	muscle	1	"13:58:17	"00:00:00	"1"	"No Label	0	"None"	49	136	35	35				
3	2615	2604	2593	2602	2572	2587	2598	2562	2596	2575	2589	2592	2587	2600	2585	2581
4	2608	2629	2618	2578	2575	2582	2588	2576	2591	2604	2643	2597	2626	2590	2598	2582
5	2676	2650	2646	2635	2574	2623	2582	2589	2622	2599	2620	2633	2640	2599	2590	2619
6	2627	2642	2609	2621	2589	2588	2606	2622	2600	2625	2636	2653	2658	2644	2584	2613
7	2641	2652	2640	2640	2626	2606	2640	2630	2662	2676	2654	2640	2631	2643	2608	2599
8	2665	2635	2666	2652	2664	2649	2656	2636	2650	2663	2658	2659	2632	2611	2589	2582
9	2677	2675	2698	2693	2683	2679	2690	2665	2660	2640	2642	2643	2646	2582	2582	2551
10	2700	2690	2729	2767	2714	2692	2698	2719	2708	2701	2688	2683	2640	2574	2567	2577
11	2744	2775	2771	2804	2789	2787	2794	2837	2829	2799	2791	2751	2675	2616	2581	2615
12	2843	2862	2905	2933	2975	2993	3001	3050	3070	3068	3052	2999	2926	2867	2801	2766
13	3025	3078	3107	3137	3196	3231	3235	3278	3321	3326	3341	3369	3326	3308	3299	3329
14	3331	3353	3393	3430	3494	3505	3528	3573	3604	3606	3579	3592	3629	3641	3640	3740
15	3574	3592	3631	3679	3748	3757	3784	3828	3842	3869	3885	3840	3859	3884	3867	3864
16	3266	3255	3401	3551	3637	3740	3812	3850	3911	3905	3925	3923	3931	3929	3906	3889
17	2353	2345	2538	2761	2997	3144	3365	3568	3718	3815	3840	3884	3900	3919	3885	3870
18	1705	1745	1809	1929	2056	2175	2356	2594	3001	3286	3424	3591	3724	3819	3820	3816
19	1477	1465	1461	1544	1597	1681	1727	1898	2121	2372	2525	2973	3390	3695	3703	3712
20	1403	1395	1431	1451	1479	1510	1499	1544	1657	1761	1929	2321	2668	3198	3495	3513
21	1409	1436	1467	1477	1481	1435	1391	1416	1462	1548	1673	1797	2072	2656	3146	3234
22	1423	1441	1459	1429	1398	1348	1308	1329	1371	1439	1454	1565	1773	2234	2702	3049
23	1383	1412	1371	1348	1328	1320	1304	1296	1340	1362	1454	1548	1703	1922	2215	2758
24	1343	1351	1324	1313	1321	1318	1395	1353	1365	1423	1461	1521	1572	1717	1984	2469
25	1313	1315	1276	1279	1302	1379	1407	1419	1410	1426	1403	1447	1486	1619	1806	2242
26	1328	1326	1273	1239	1283	1308	1365	1398	1363	1332	1353	1389	1359	1472	1682	2069
27	1391	1286	1238	1239	1222	1233	1281	1289	1266	1278	1310	1254	1283	1373	1551	1819
28	1292	1251	1244	1233	1182	1162	1169	1177	1226	1229	1208	1233	1266	1328	1397	1656
29	1266	1233	1239	1200	1129	1157	1160	1177	1212	1200	1219	1207	1212	1269	1351	1593
30	1271	1312	1286	1226	1160	1140	1193	1184	1205	1214	1208	1246	1212	1273	1404	1598
31	1362	1342	1313	1231	1205	1173	1207	1244	1201	1246	1263	1305	1307	1339	1499	1729
32	1379	1348	1264	1207	1217	1182	1193	1162	1153	1233	1313	1310	1337	1376	1502	1673
33	1334	1279	1284	1259	1244	1200	1187	1177	1191	1229	1271	1263	1297	1324	1409	1585
34	1284	1238	1258	1274	1268	1229	1184	1168	1198	1286	1248	1244	1278	1320	1395	1601
35	1243	1233	1268	1261	1239	1217	1191	1169	1217	1222	1205	1180	1168	1198	1340	1606
36	1263	1256	1234	1221	1222	1234	1246	1234	1243	1236	1217	1203	1169	1224	1368	1835
37	1263	1219	1227	1243	1244	1286	1276	1292	1312	1308	1302	1229	1208	1289	1507	2084
38																

- All Analysis function can send data to a text file or Microsoft Excel; also known as “Logging”
- Simplest measurement is a pixel intensity with the mouse

Molecular Devices

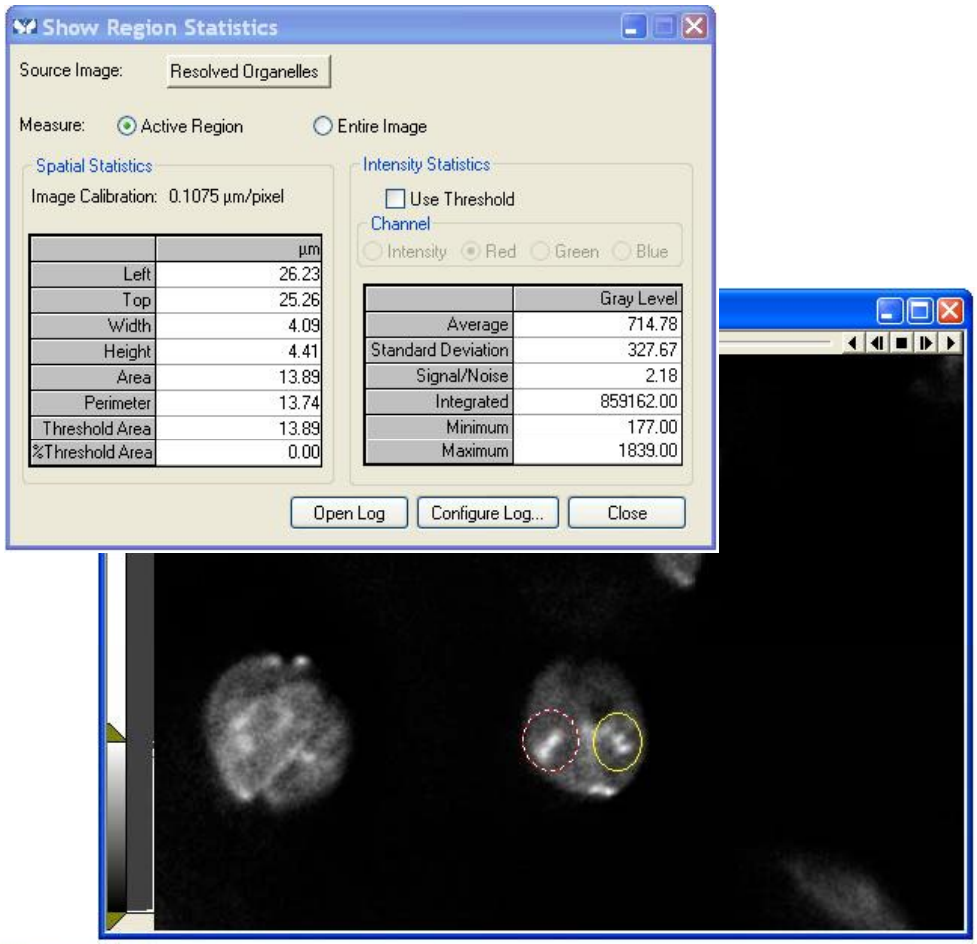
Line Scan



- Measure Menu
- Intensity profile along a line region
- Results shown in graph
- Good for checking the flatness of the background
- One line at a time

Molecular Devices

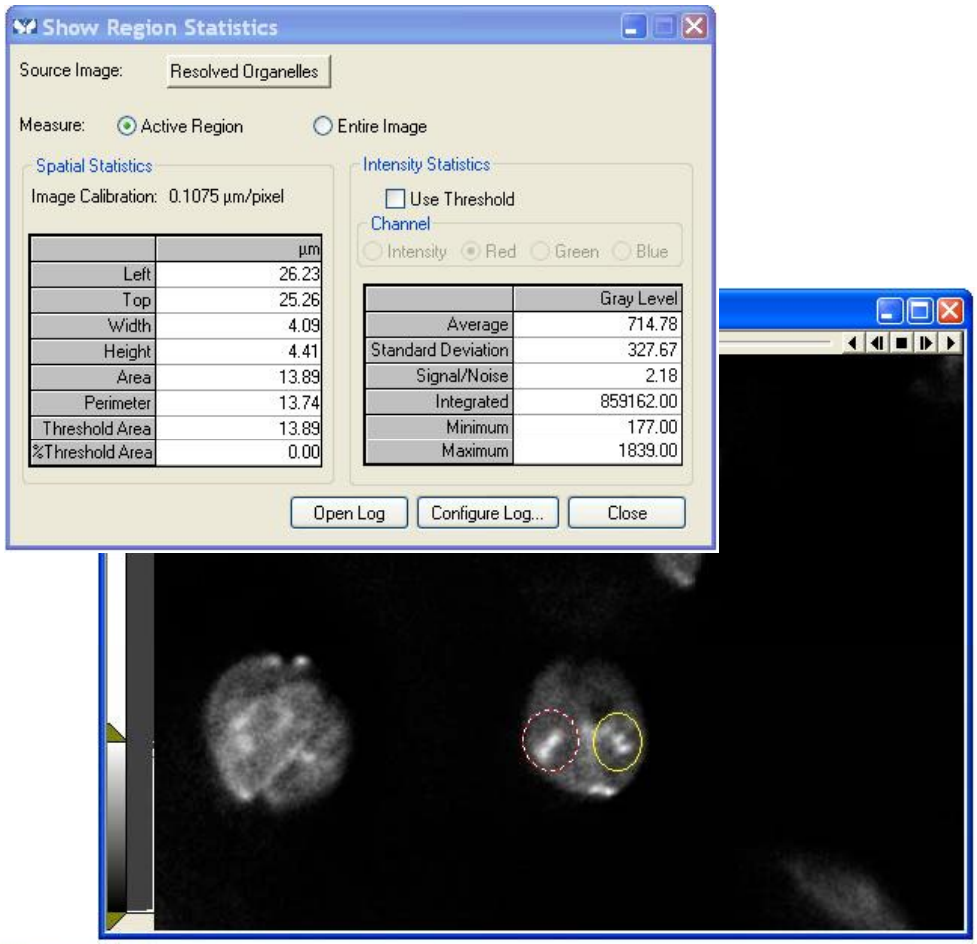
Show Region Statistics



- Measure Menu
- Measurements of a single region
- Measurements on a single image
- Intensity and spatial measurements

Molecular Devices

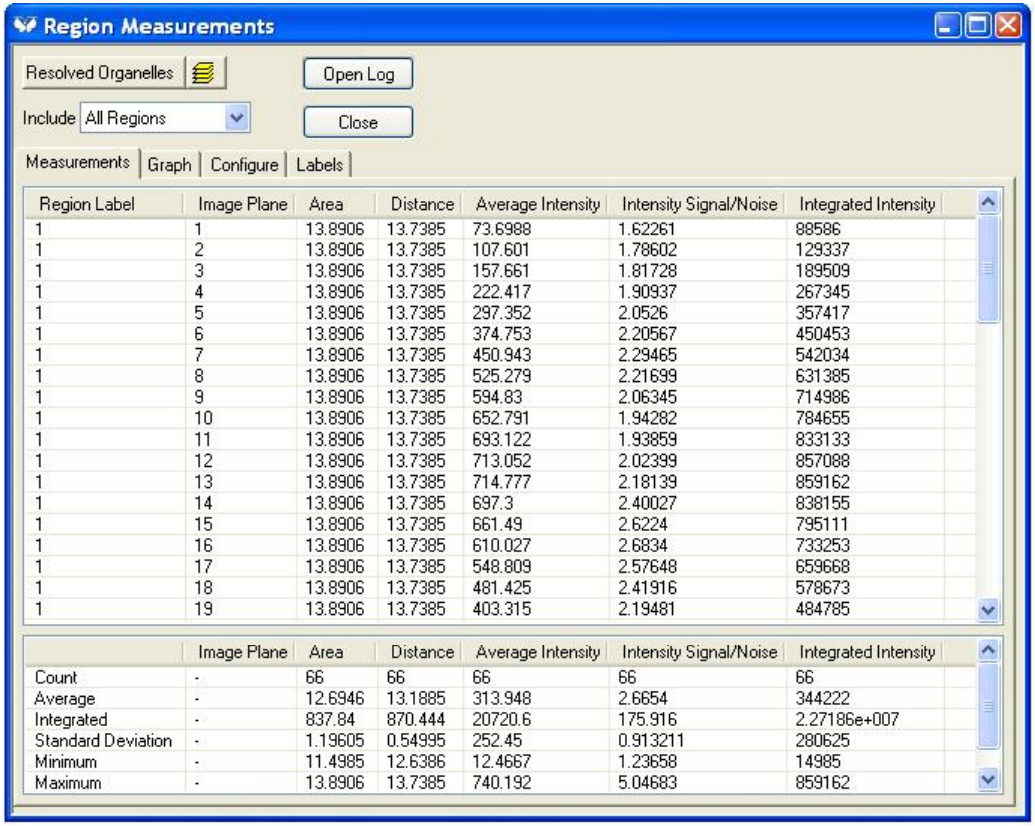
Show Region Statistics



- Pick the active region or the entire image
- Check Use Threshold to measure on thresholded pixels in the region
- Updates even if measuring on the Live Image

Molecular Devices

Region Measurements



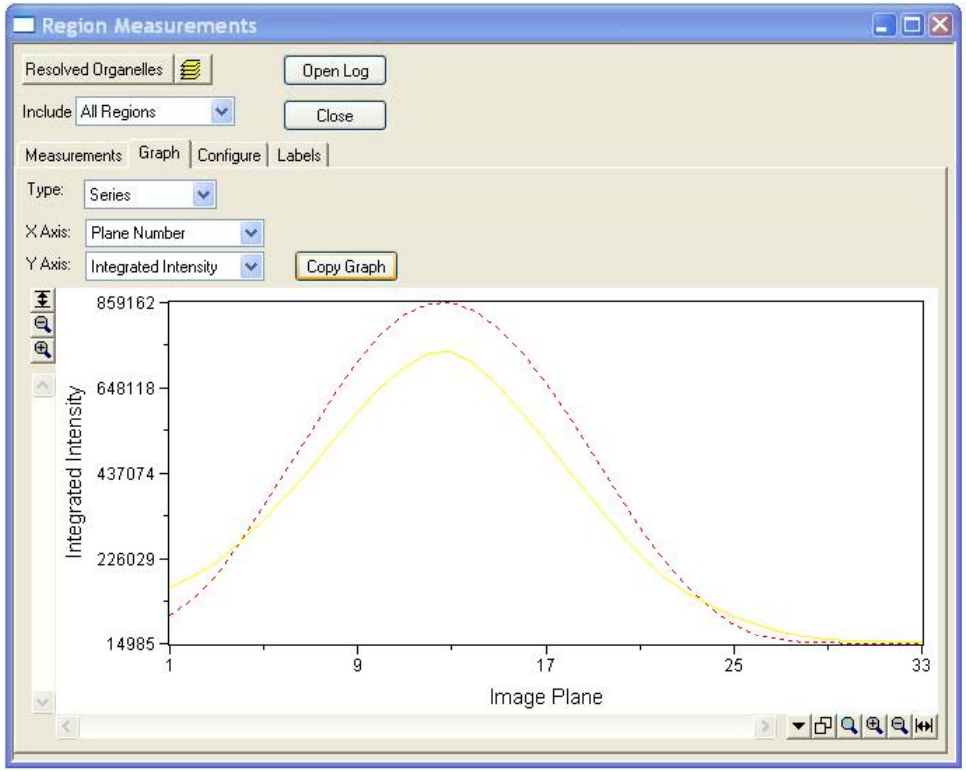
Region Label	Image Plane	Area	Distance	Average Intensity	Intensity Signal/Noise	Integrated Intensity
1	1	13.8906	13.7385	73.6988	1.62261	88586
1	2	13.8906	13.7385	107.601	1.78602	129337
1	3	13.8906	13.7385	157.661	1.81728	189509
1	4	13.8906	13.7385	222.417	1.90937	267345
1	5	13.8906	13.7385	297.352	2.0526	357417
1	6	13.8906	13.7385	374.753	2.20567	450453
1	7	13.8906	13.7385	450.943	2.29465	542034
1	8	13.8906	13.7385	525.279	2.21699	631385
1	9	13.8906	13.7385	594.83	2.06345	714986
1	10	13.8906	13.7385	652.791	1.94282	784655
1	11	13.8906	13.7385	693.122	1.93859	833133
1	12	13.8906	13.7385	713.052	2.02399	857088
1	13	13.8906	13.7385	714.777	2.18139	859162
1	14	13.8906	13.7385	697.3	2.40027	838155
1	15	13.8906	13.7385	661.49	2.6224	795111
1	16	13.8906	13.7385	610.027	2.6834	733253
1	17	13.8906	13.7385	548.809	2.57648	659668
1	18	13.8906	13.7385	481.425	2.41916	578673
1	19	13.8906	13.7385	403.315	2.19481	484785

	Image Plane	Area	Distance	Average Intensity	Intensity Signal/Noise	Integrated Intensity
Count	-	66	66	66	66	66
Average	-	12.6946	13.1885	313.948	2.6654	344222
Integrated	-	837.84	870.444	20720.6	175.916	2.27186e+007
Standard Deviation	-	1.19605	0.54995	252.45	0.913211	280625
Minimum	-	11.4985	12.6386	12.4667	1.23658	14985
Maximum	-	13.8906	13.7385	740.192	5.04683	859162

- Measure Menu
- Measure many regions all at once
- Measure single plane or all planes in a stack
- Data in table or graph
- Summary data available

Molecular Devices

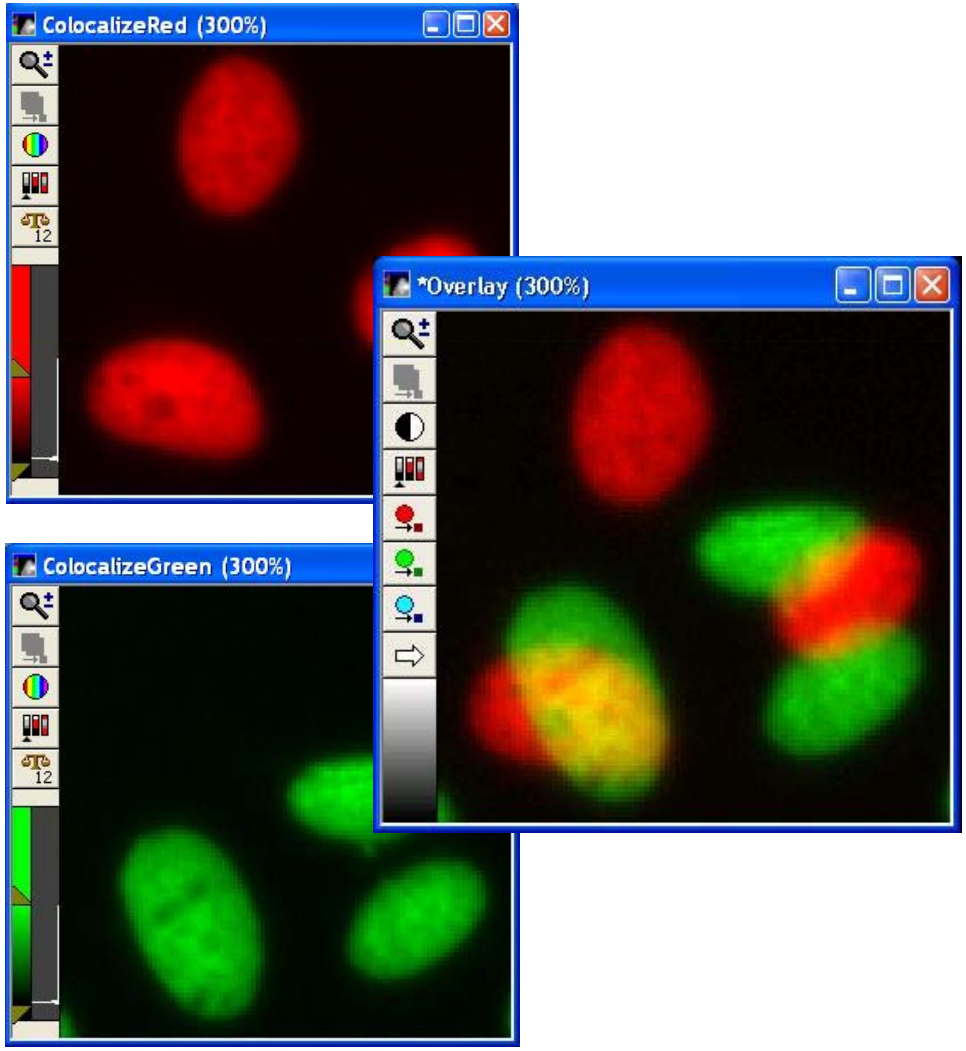
Region Measurements



- Graph measurements for all the planes in a stack
- Pick the X axis as Plane Number, Elapsed Time, Z, or Wavelength
- Y axis is the available list of measurements enabled

Molecular Devices

Colocalization

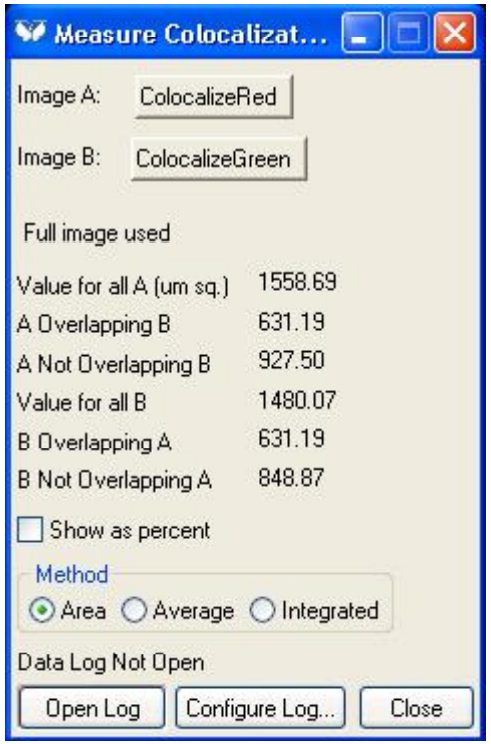


- What is colocalization? Assume we are overlaying two separate images
  - Provides quantitative data regarding the amount of overlap of two fluorescent probes
  - Aggregate measurements

red & green probes colocalized

Molecular Devices

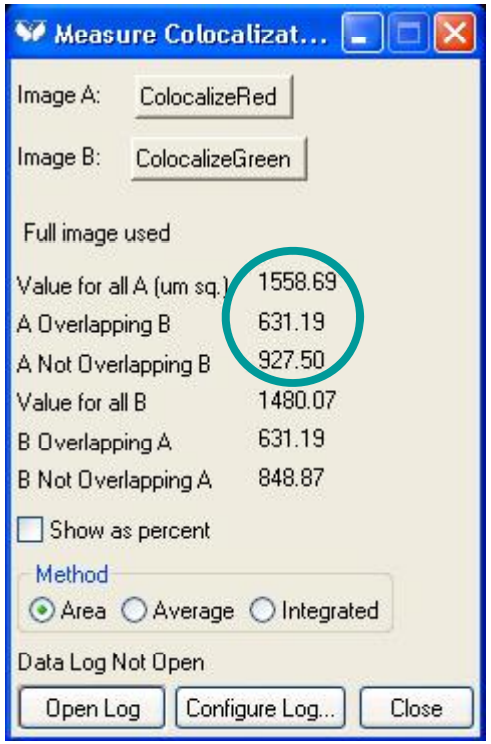
Measure Colocalization

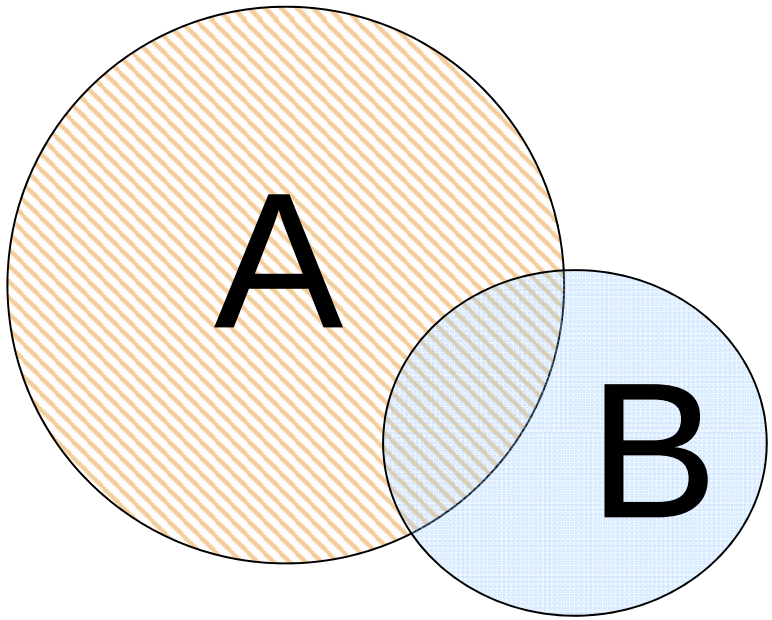


- Apps Menu
- Threshold images
- Measures the amount of overlap in wavelengths

Molecular Devices

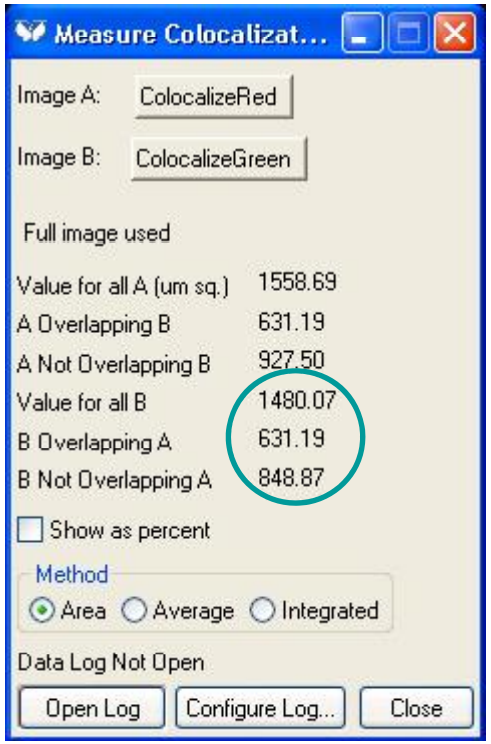
Measure Colocalization

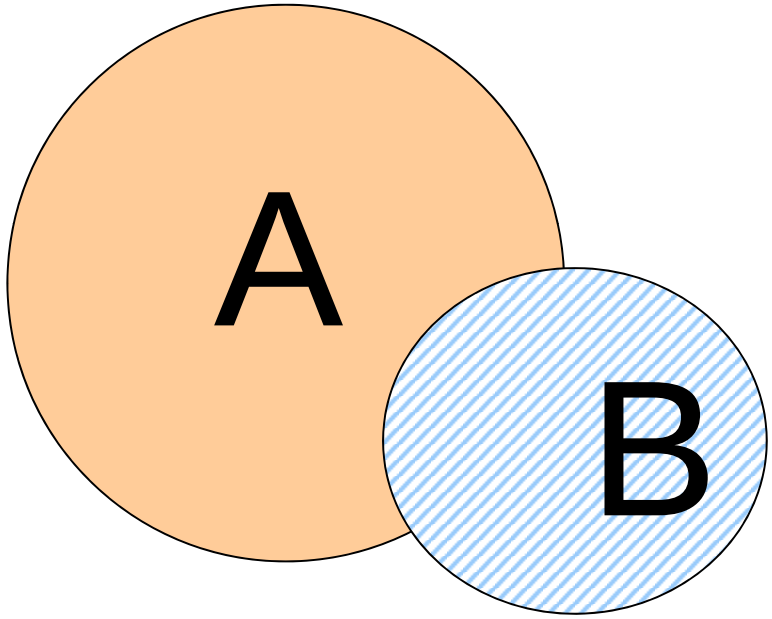




Molecular Devices

Measure Colocalization



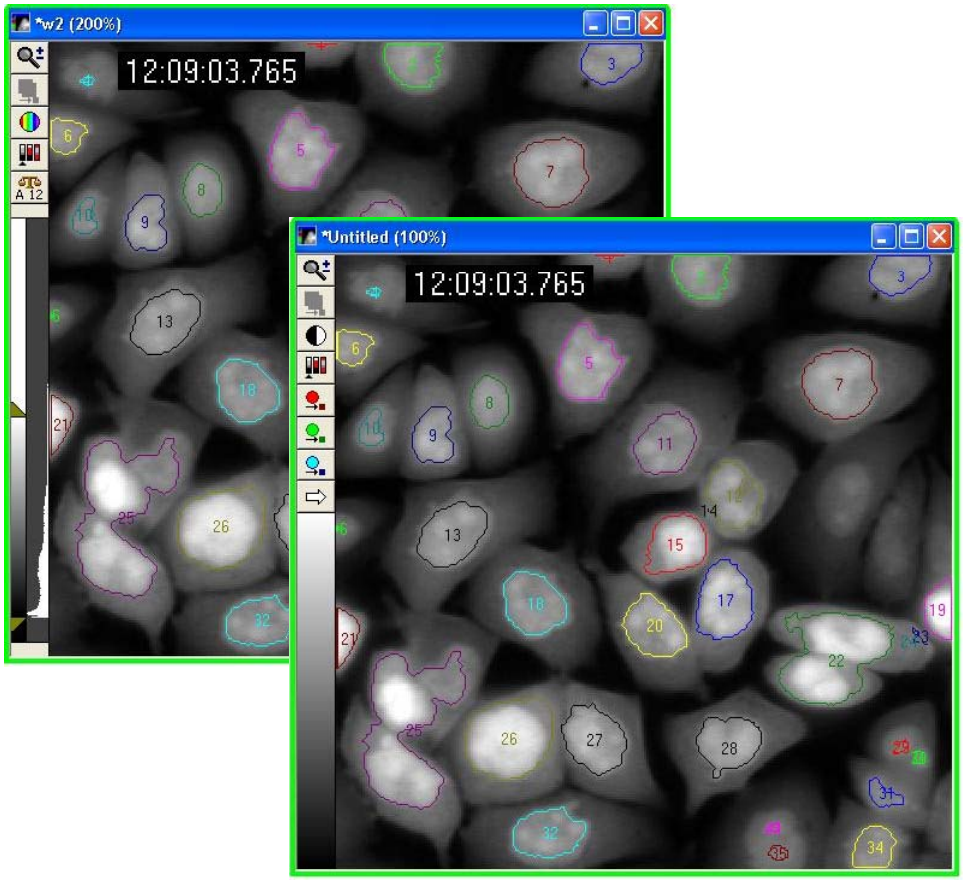


MetaMorph®

Molecular Devices

Presentation of Images

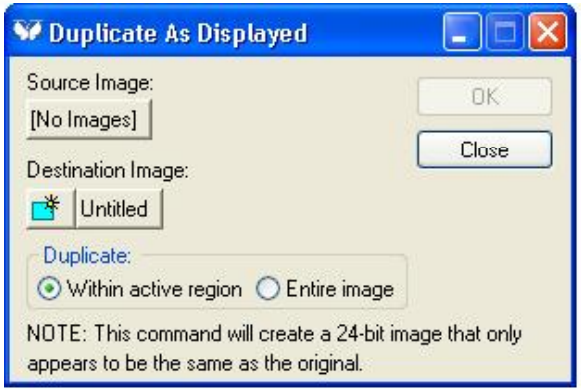
Duplicate as Displayed

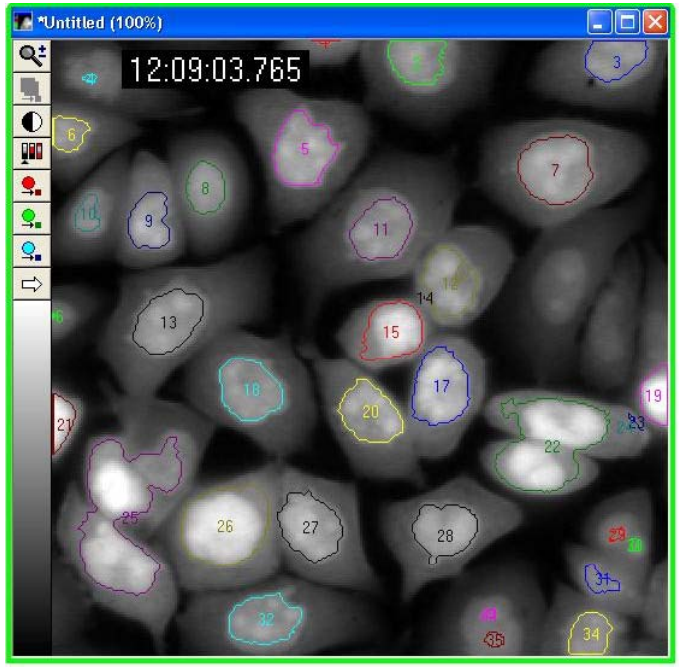


- Want to show regions and other overlays on image
- Create a “snapshot” of the image and all the items on it
- The new image can be saved and opened in other applications

Molecular Devices

Duplicate as Displayed

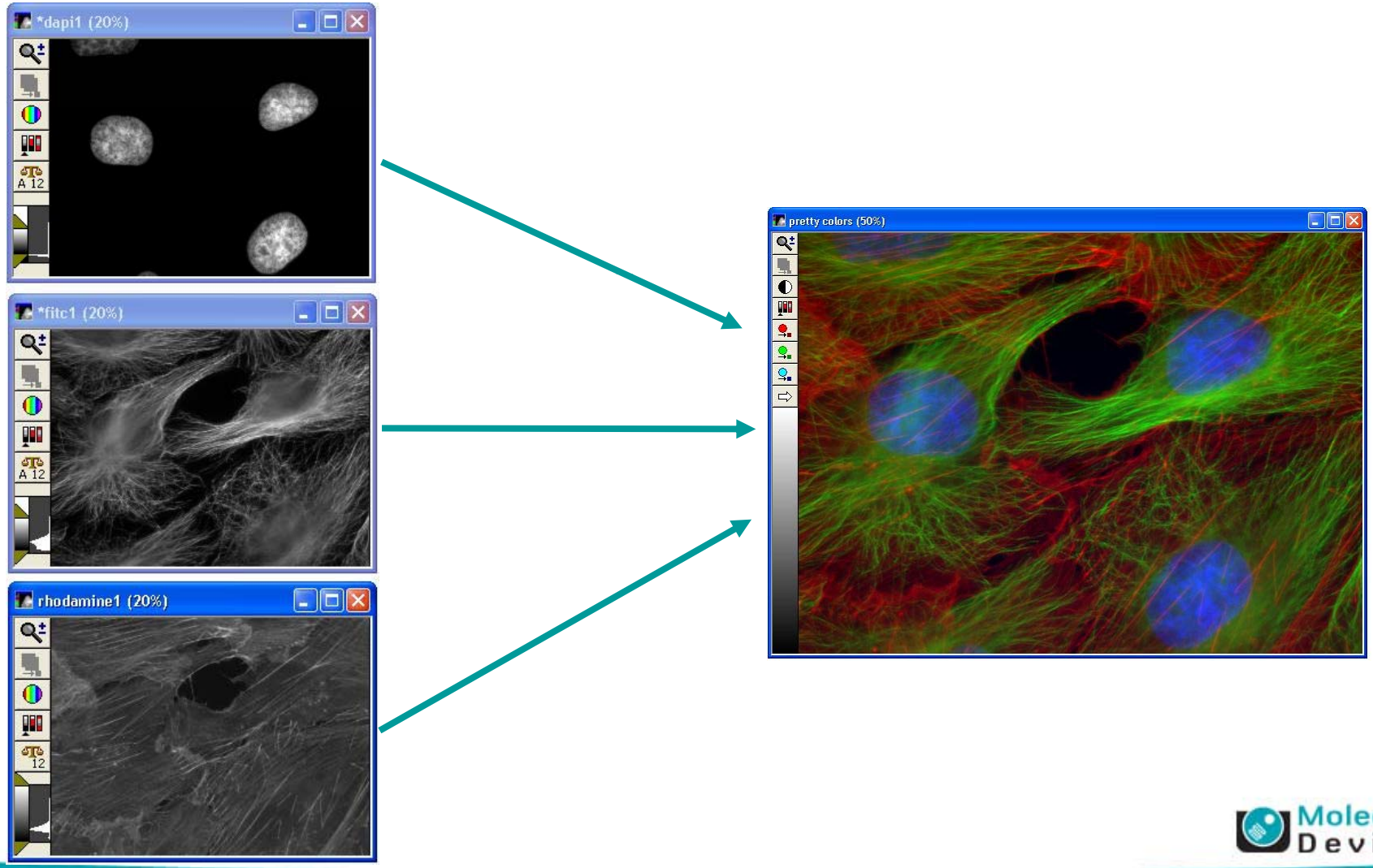




- Edit – Duplicate – Duplicate as Displayed
- Dropin
- Representative of the original data, but not identical

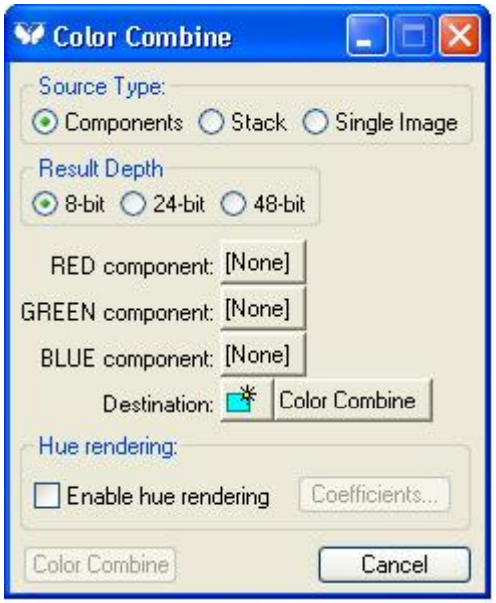
Molecular Devices

Color Combine



Molecular Devices

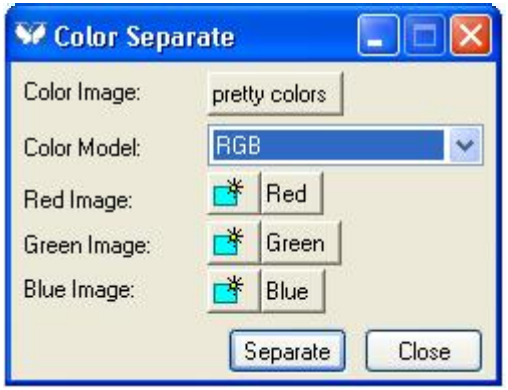
Color Combine

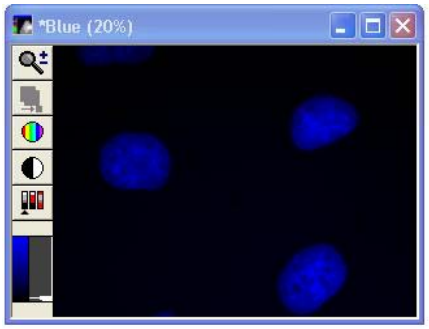


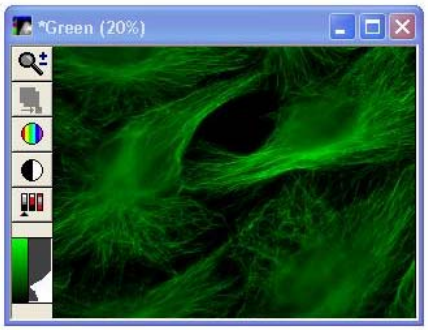
- Display Menu
- Combine one to three images together to make a color result
- Only does red, green, and blue
- Does not handle transmitted light images (brightfield, DIC, phase contrast)

Molecular Devices

Color Separate



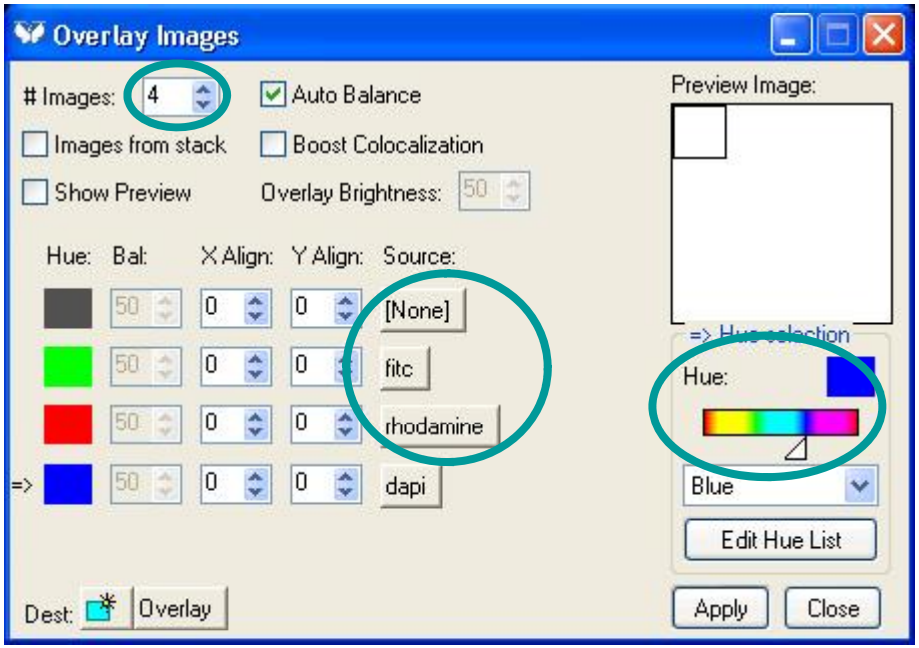




- Display Menu
- Separates dolor images into their component amounts

Molecular Devices

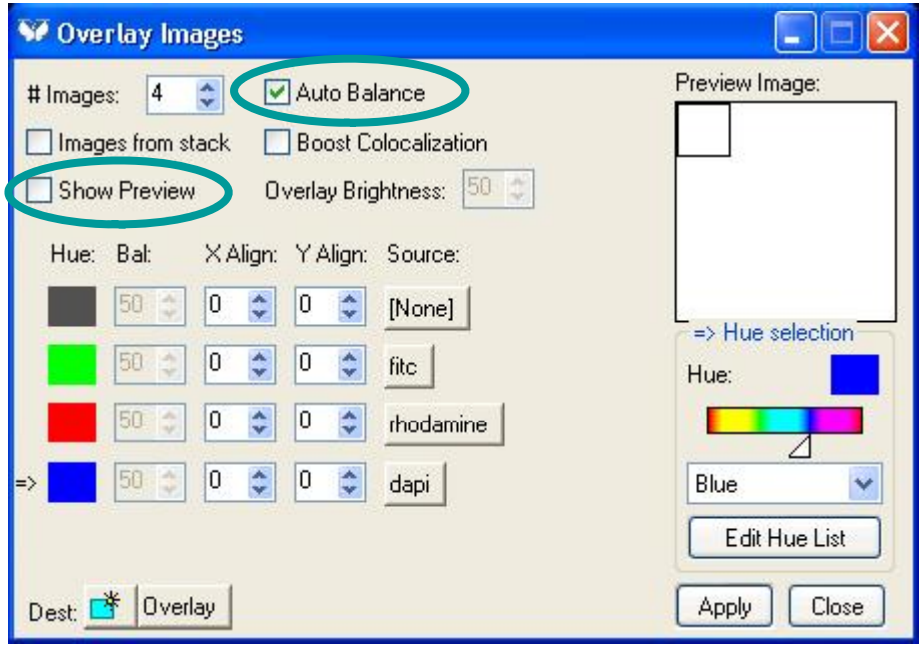
Overlay Images



- Display Menu
- Create a color image combining/overlaying up to 7 different images together
- Select 5 predefined colors or use the hue selection
- Pick the images corresponding to each hue

Molecular Devices

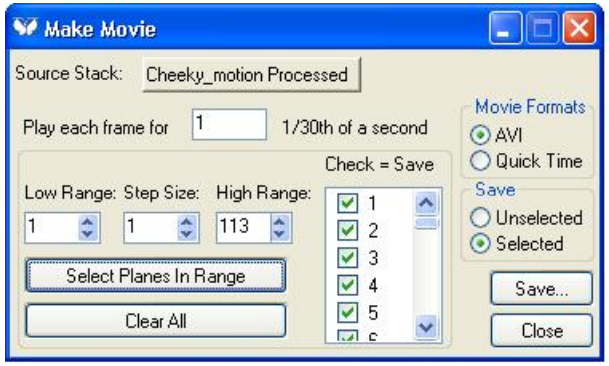
Overlay Images

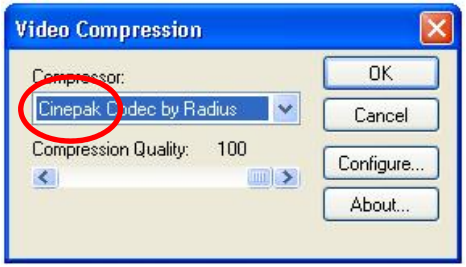


- Automatic balancing
- Adjusting scaling in images also effects result
- Preview result

Molecular Devices

Make Movie





- Stack Menu
- Make an AVI or Quick Time move
- Duration of each frame; e.g., 30 - 30ths = 1 second in movie
- Pick a file name and location
- Select a compression Codec

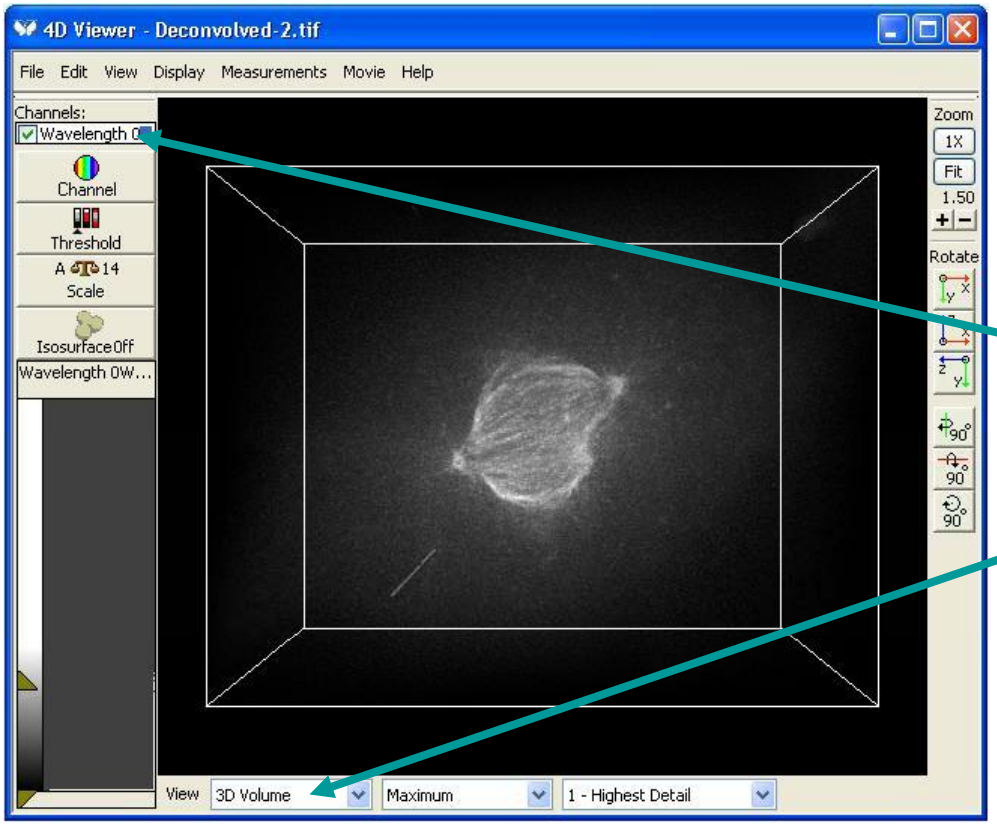
Molecular Devices

MetaMorph®

Molecular Devices

4D Viewer

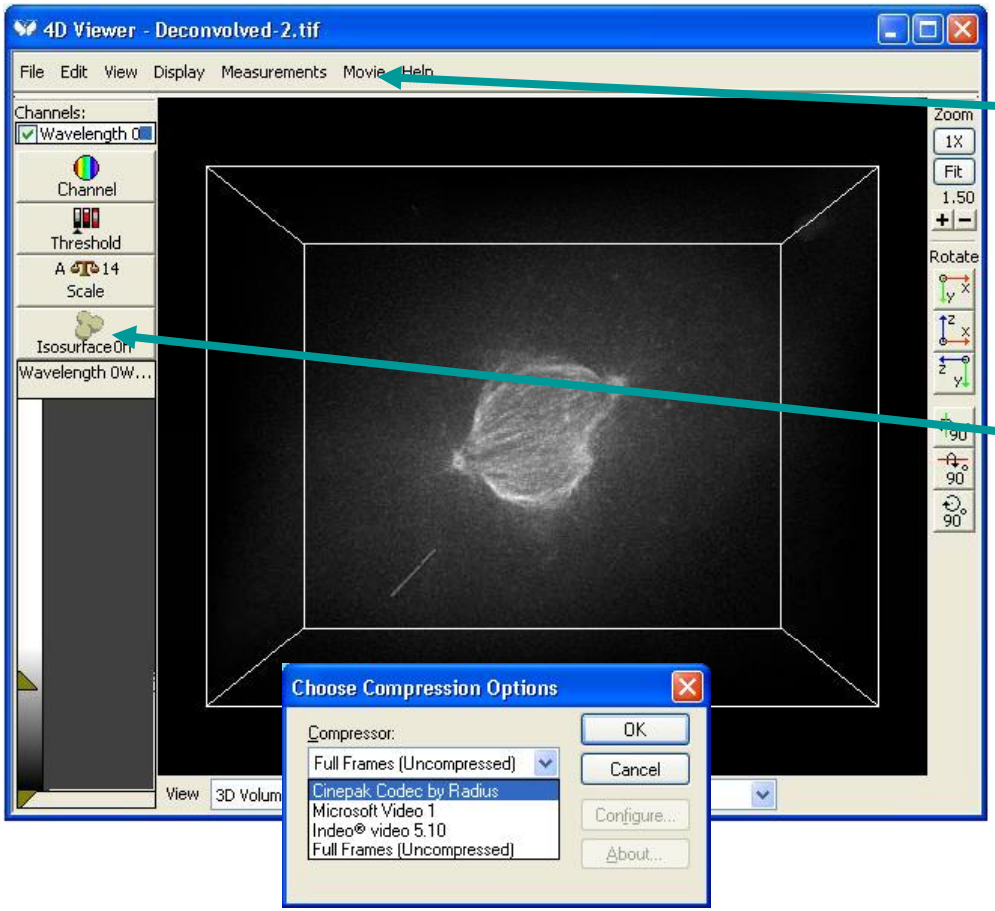
4D Viewer



- File Menu
- 2D and 3D Display
- Different wavelengths overlayed simultaneously
- Original and volume displays available
- Similar controls as regular image window

Molecular Devices

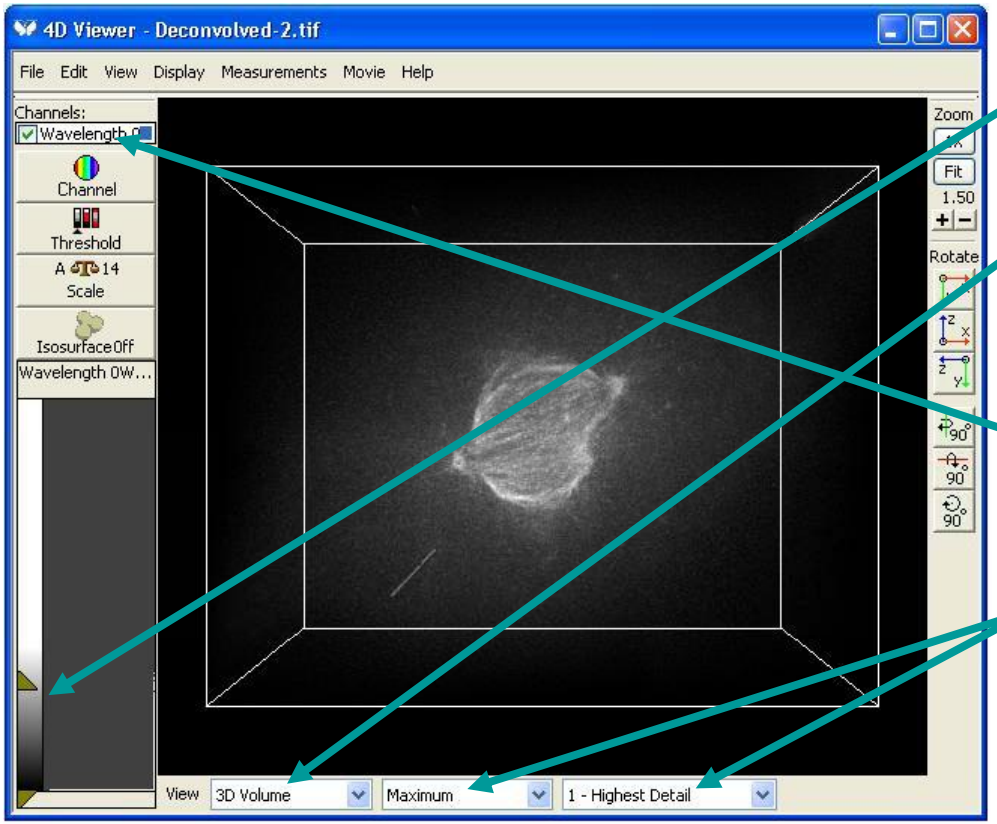
4D Viewer



- Use the mouse to rotate and spin the 3D projection
- Make movies of rotations, time series or both in 3D
  - Select a Codec - Cinepak
- Surface projection to identify discrete volumes and make measurements
  - Volume size and intensity
  - Distances
  - Colocalization

Molecular Devices

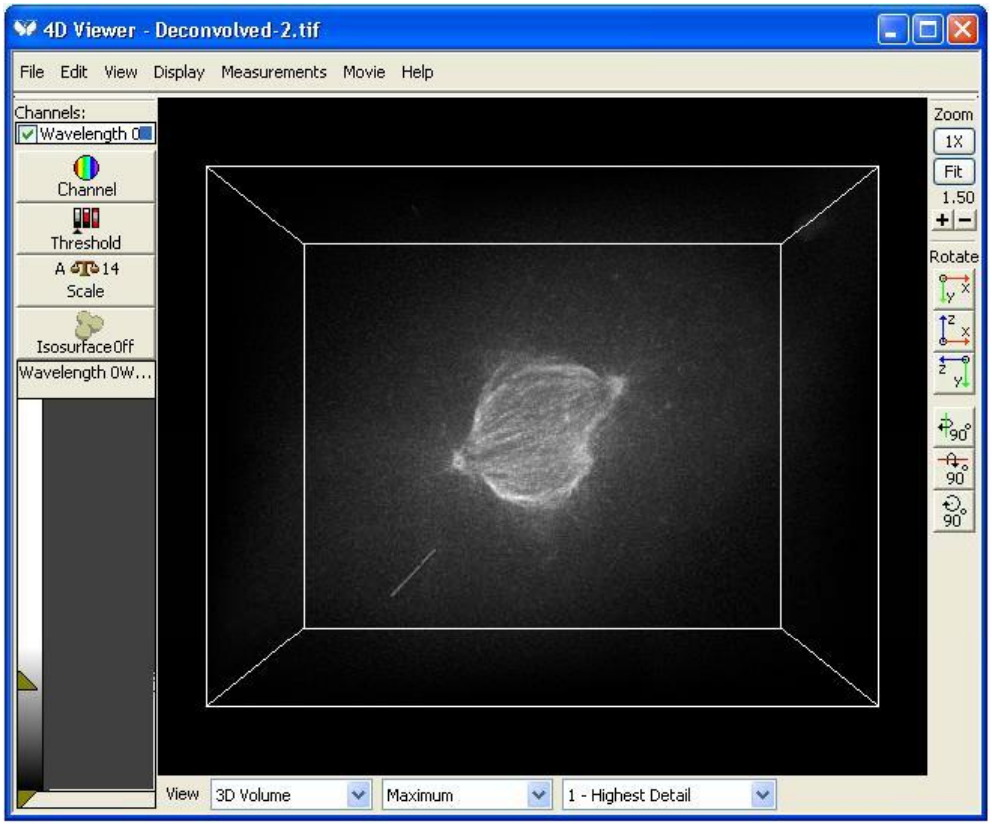
4D Viewer



- Scaling
- Switch between 2D and 3D views
- Select wavelengths
- Select rendering option and level of detail

Molecular Devices

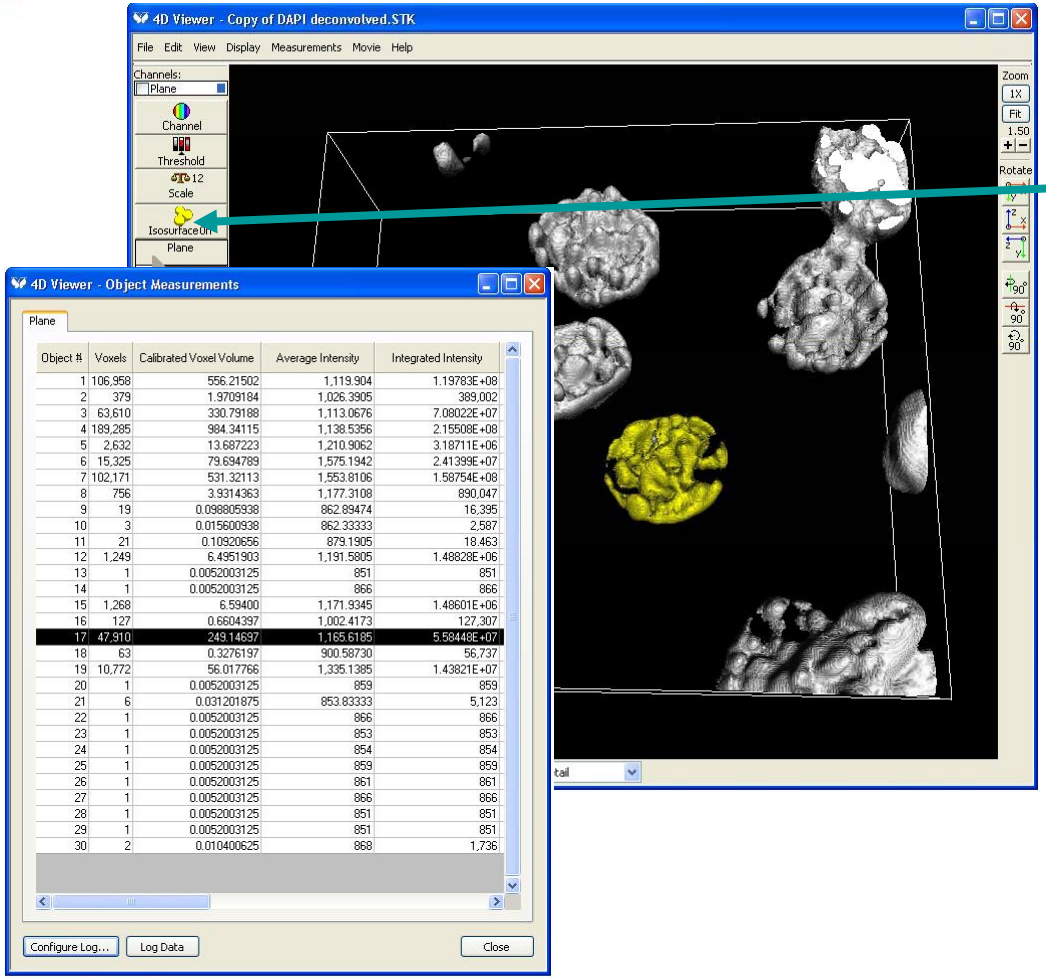
4D Viewer



- Set the X & Y calibration
  - Or set the Z calibration from Edit menu
- "High performance" display card required to see finest detail
- Large data sets are problematic
- As much physical RAM as possible for best performance

Molecular Devices

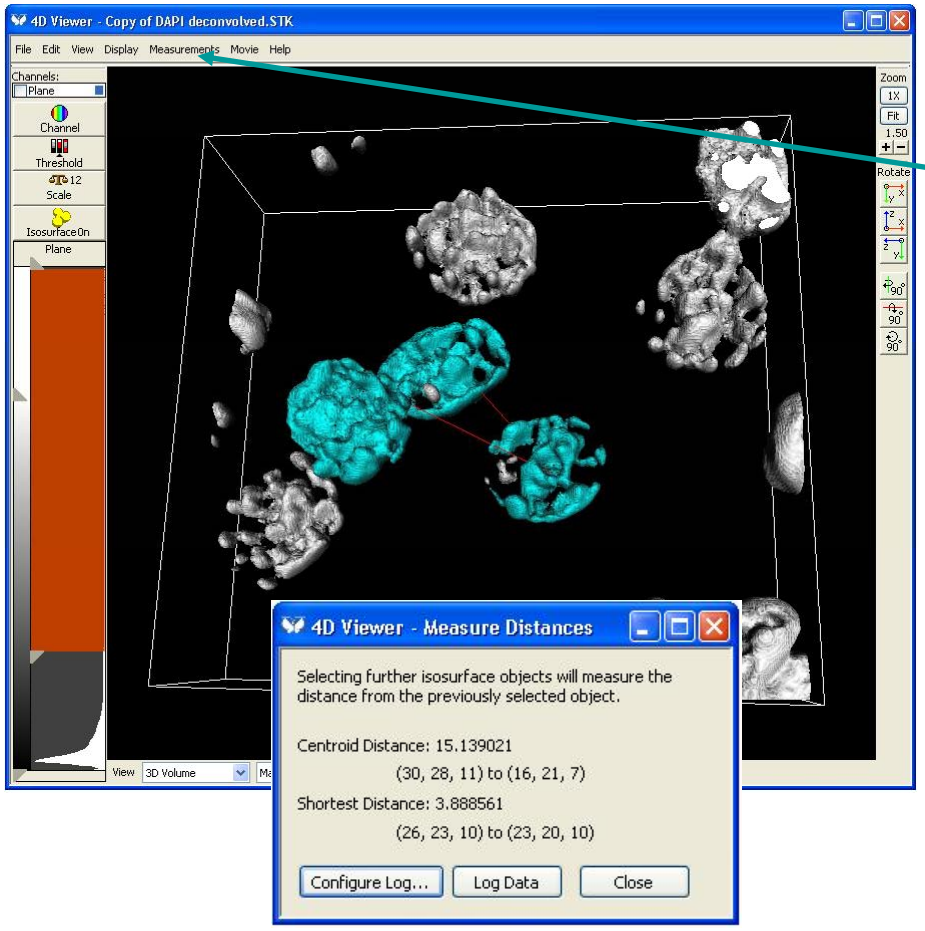
4D Viewer



- IsoSurface enabled
- Select individual objects with the mouse, double-click
- Object volume measurements available

Molecular Devices

4D Viewer



- Distance measurements
- Colocalization available when multiple wavelengths are in the same data set

Molecular Devices

Exercises

Part 3

1) Colour Separate & Colour Combine
2) Basic Co-localization
3) 4D Viewer

Molecular Devices

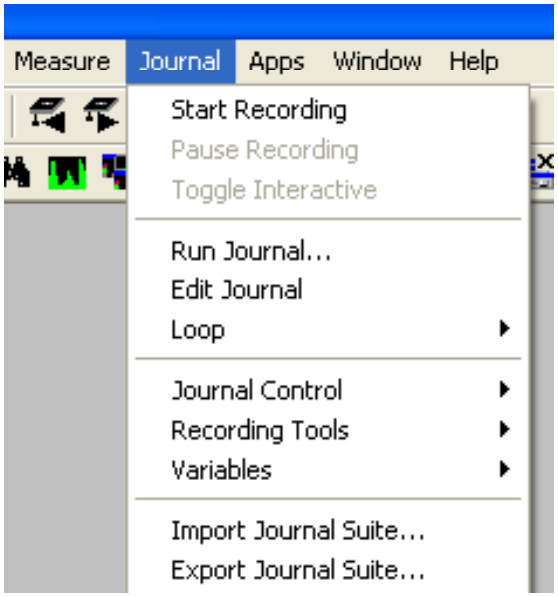
MetaMorph®

Molecular Devices

Introduction to Journaling

MetaMorph Journals

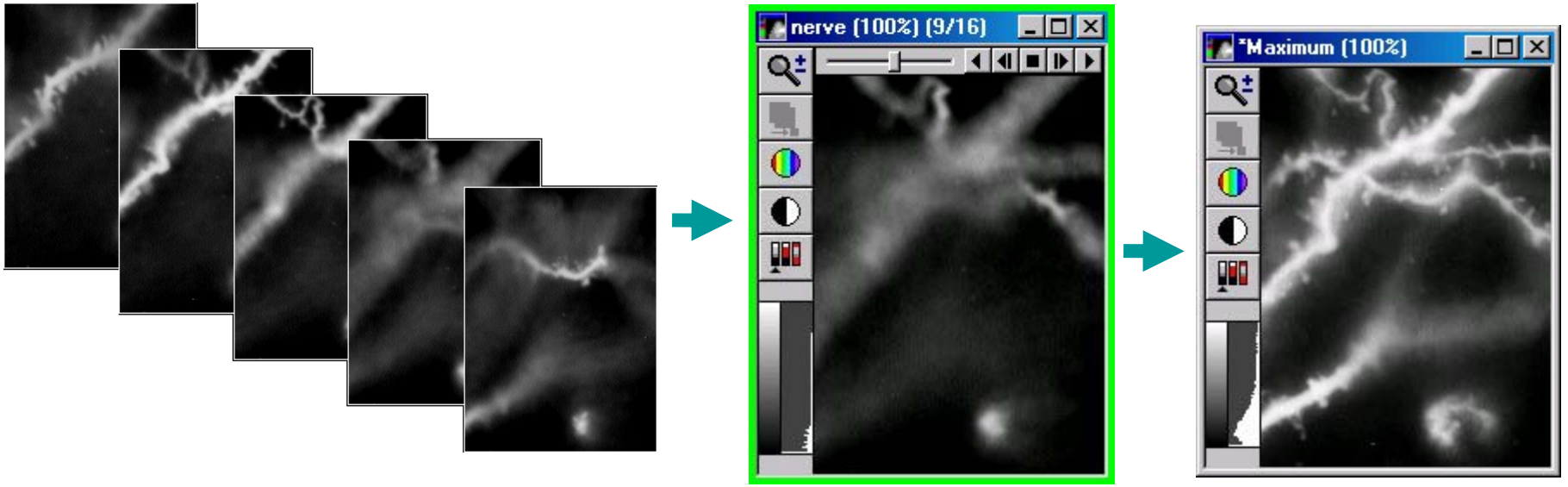
- What are journals?
  - Specific sequence of steps
  - Macros that automate repetitive tasks
- Journals can be created via:
  - Recording a series of mouse clicks
  - Using the Journal Editor
- Journals can be run from:
  - The Journal Menu
  - Within another journal
  - Specific dialogs
  - Taskbars
  - Toolbars
- The Journal Editor
  - Used to edit existing journals or create new journals



Molecular Devices

An Example: Maximum Projection

- A Maximum Projection
  - Collapses a volume of fluorescence thru-focus slices into a single image
  - A very useful tool for anyone visualizing fluorescence structures in 3D
  - Fast and easy to do



Molecular Devices

An Example: Maximum Projection

- The Goal
  - Make it an easy “one button” task to make a Maximum Projection
- The Method
  - Record the Maximum command into a Journal
  - Create a taskbar
  - Put the journal on the taskbar

Molecular Devices

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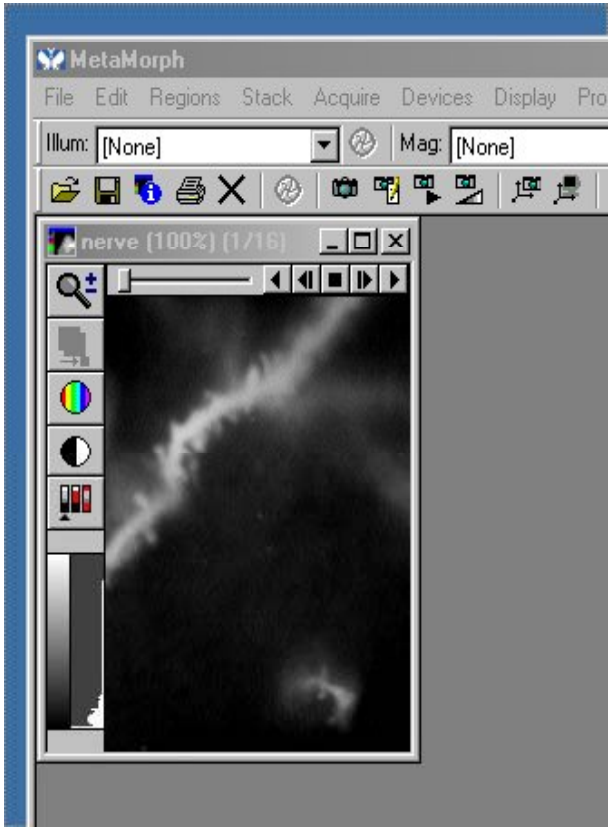
Molecular Devices

Recording the Journal

Example: Maximum Projection

Step 1: Open an image

- Choose Open (File Menu)
- Open the nerve.stk sample image



Note: The Open is not recorded into the journal. Instead we are opening a sample image stack (nerve, in this case) before making the journal. The journal we make will work on any grayscale stack that is loaded in MetaMorph.

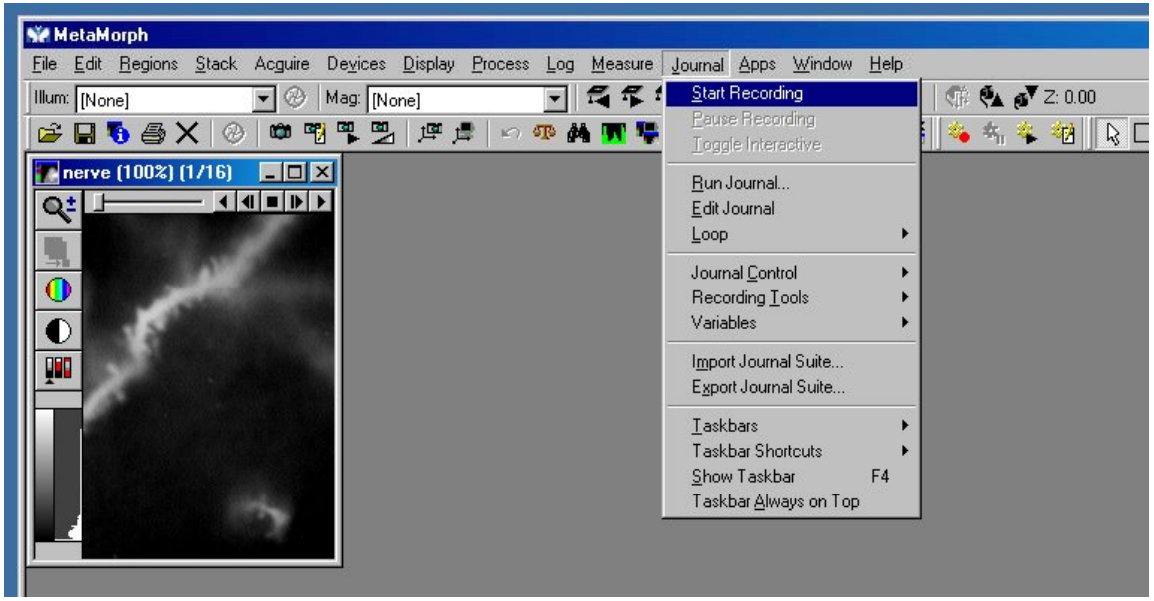
Molecular Devices

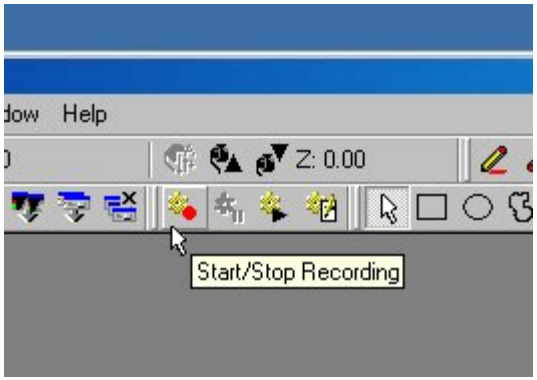
Step 2: Start recording

- Choose Start Recording (Journal Menu)

or

- Press the Start/Stop Recording icon on the Journal toolbar

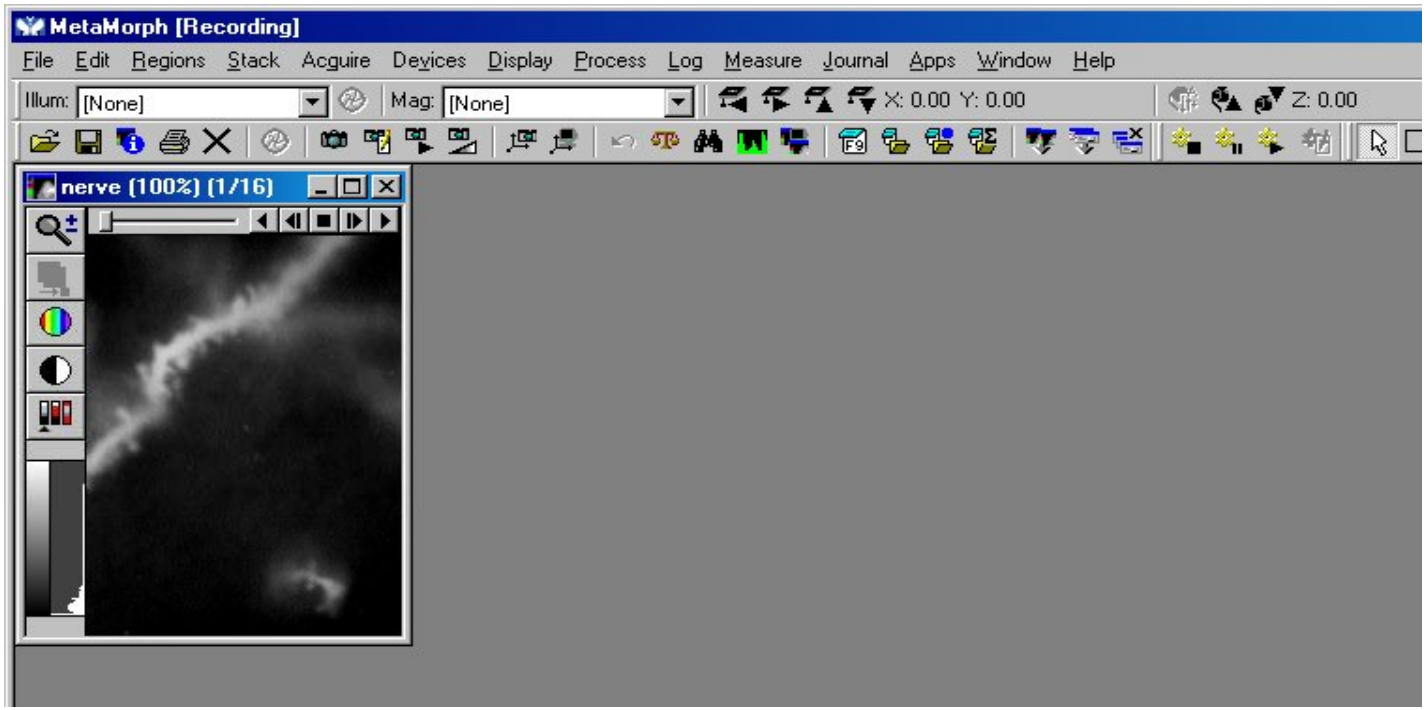




Molecular Devices

Step 3: Verify that recording is on

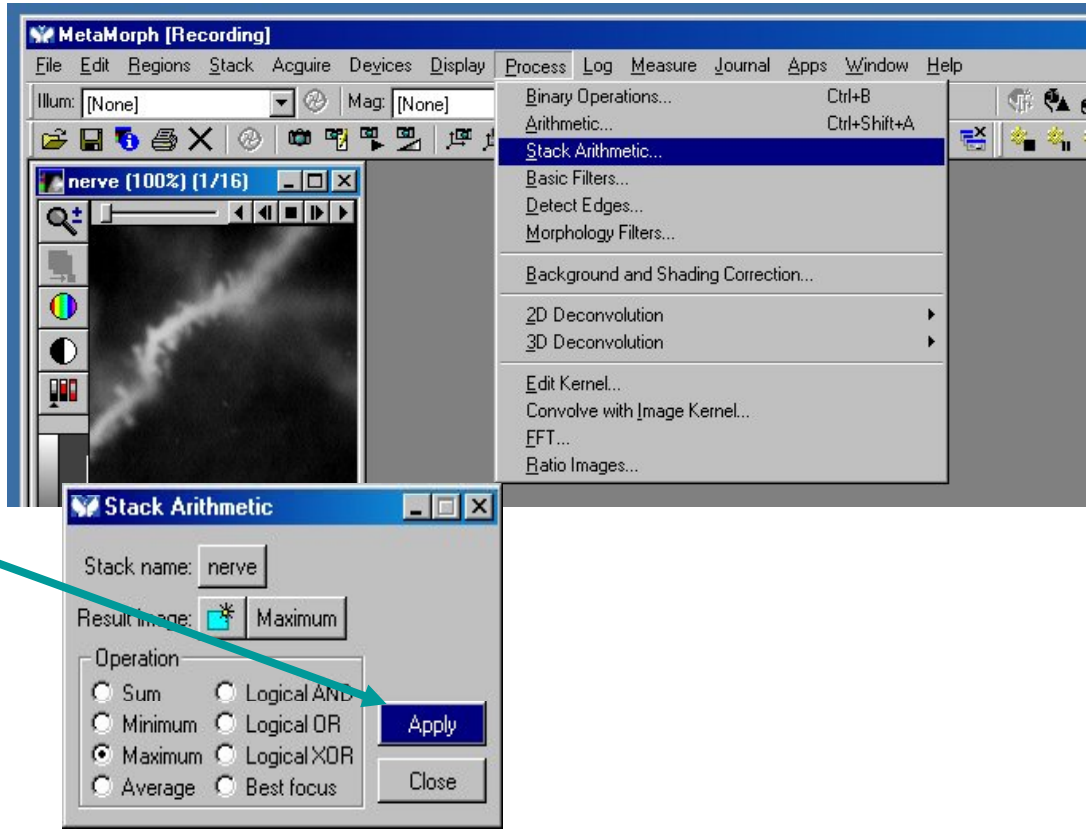
- You can verify that recording is on by:
  - The phrase “[Recording]” appears in the title bar
  - The “Start/Stop Recording” icon changes from  to 



Molecular Devices

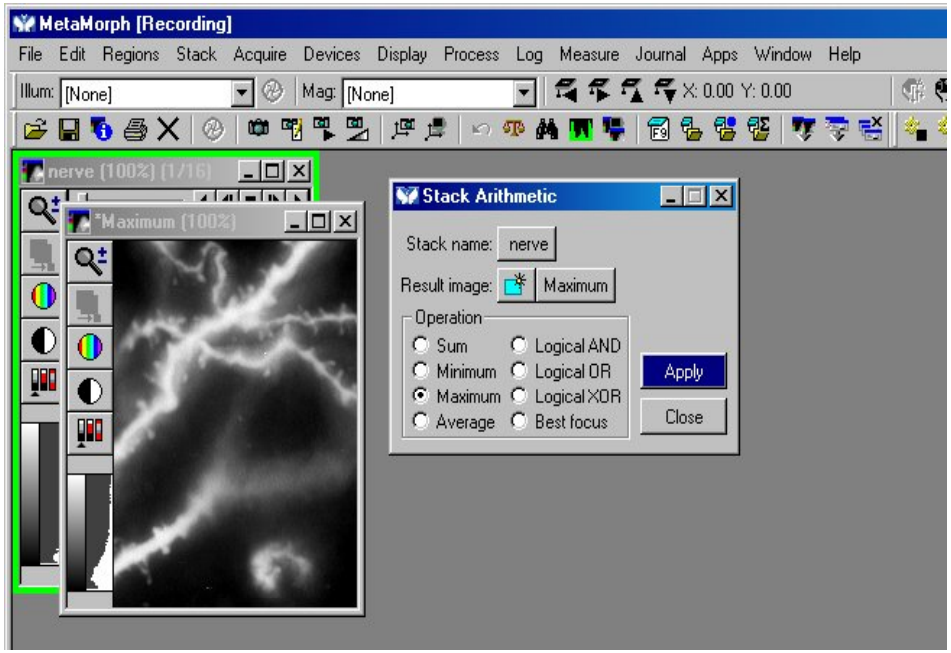
Step 4: Choose the command(s) to record

- Choose Stack Arithmetic (Process Menu)
- The Stack Arithmetic dialog will open
- While recording a journal, any button that records its action to the journal will be highlighted in blue
- With Stack Arithmetic, the Apply button is highlighted in blue
- Set the parameters (in this case choose the stack name to work on, “nerve”, and the operation, “Maximum”)
- Press Apply



Step 5: Results of the command(s) chosen

- After pressing Apply, a new result image called Maximum will be created and displayed



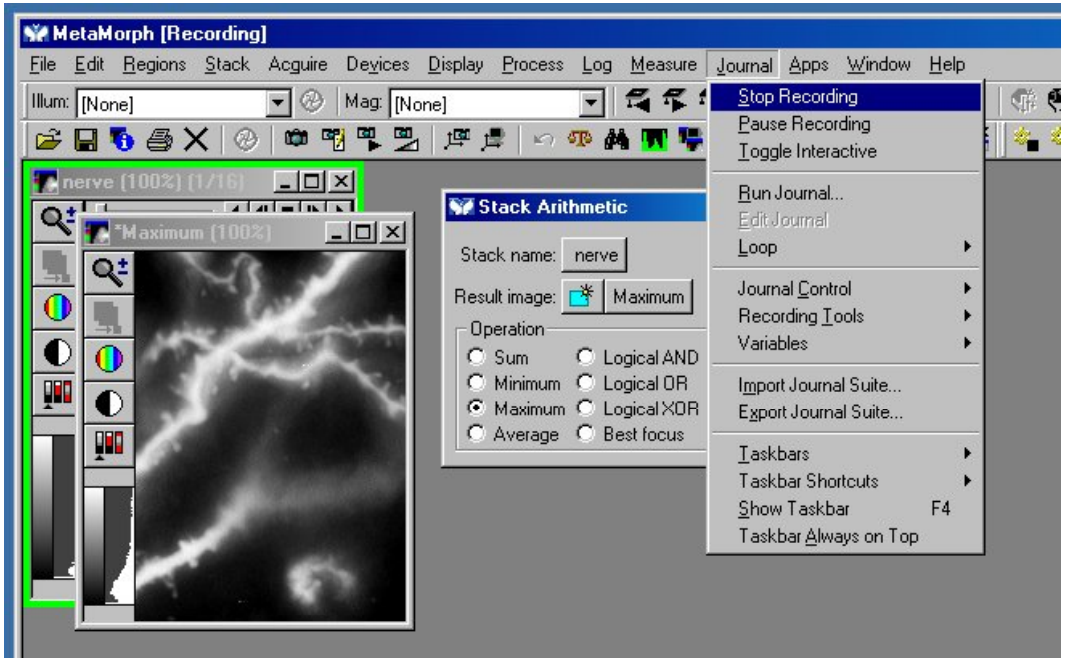
Molecular Devices

Step 6: Stop recording

- Choose Stop Recording (Journal Menu)

  or

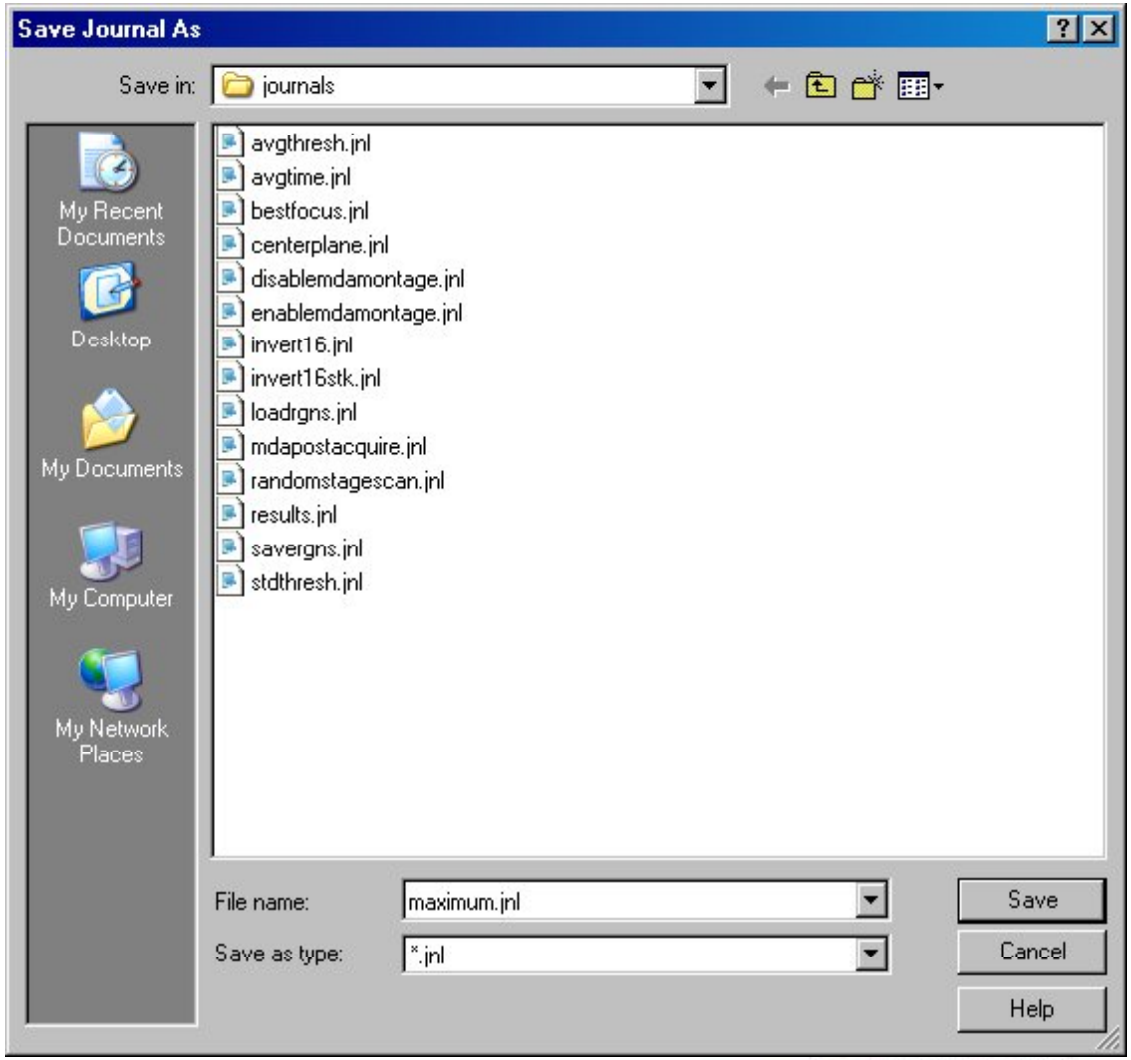
- Press the black  “Start/Stop Recording” icon in the Journal toolbar



Molecular Devices

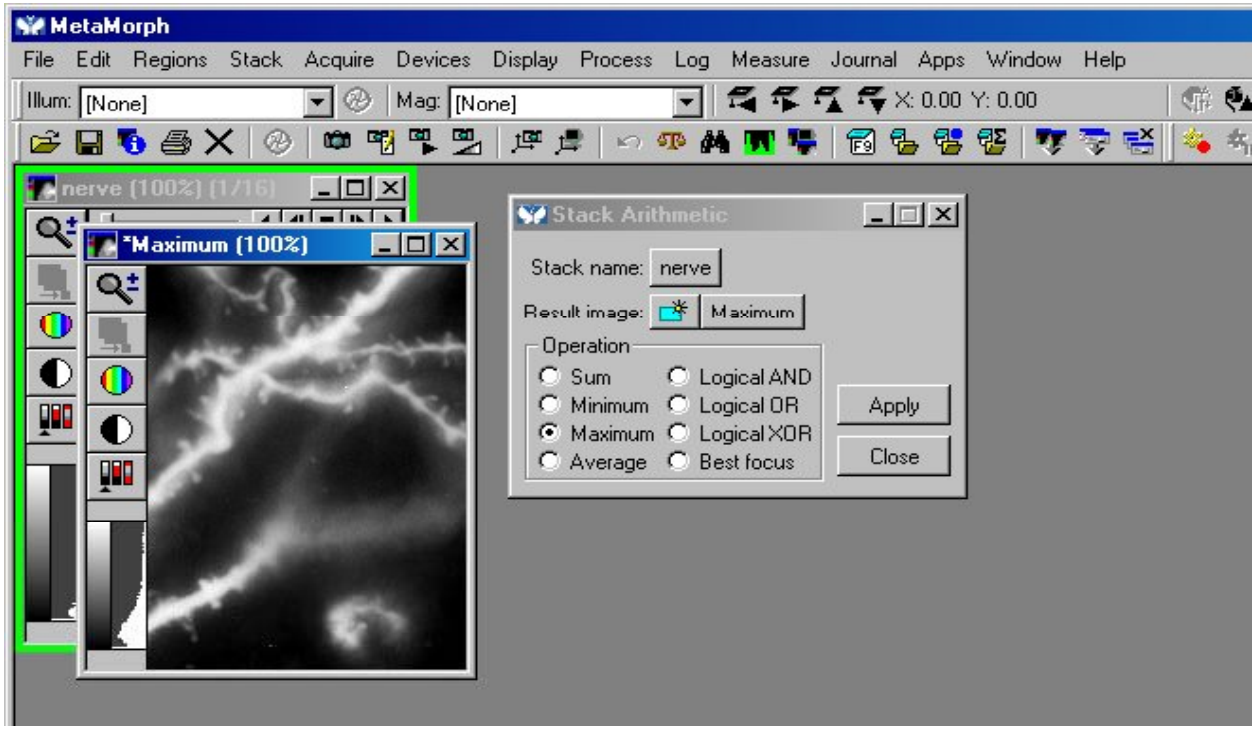
Step 7: Name the Journal

- When you stop recording you will be asked to save the journal
- Choose a name (in this example choose “maximum” since the journal is computing the stack maximum)
- Press Save once you have entered the name



Step 8: Verify that recording is off

- You can verify that Recording is Off by:
  - The phrase “[Recording]” no longer appears in the title bar
  - The “Start/Stop Recording” icon changes back to 



Molecular Devices

How to run the Journal

- There are several ways to run the journal
  - Run Journal (Journal menu)
  - Run Journal icon on the Journal toolbar
  - From a taskbar

- Using the menu or icon is generally useful only for journals that are run occasionally, as you have to select the journal by name from the file list

- Using a taskbar is the most convenient method as you can create a “one button” solution where clicking the button (whose name you can choose) runs the journal
- The first 10 buttons on a taskbar also have a “hotkey” associated with them – so in addition to “one button” the journal can be run with one key-press

Molecular Devices

MetaMorph®

Molecular Devices

Creating the Taskbar

Example: Maximum Projection

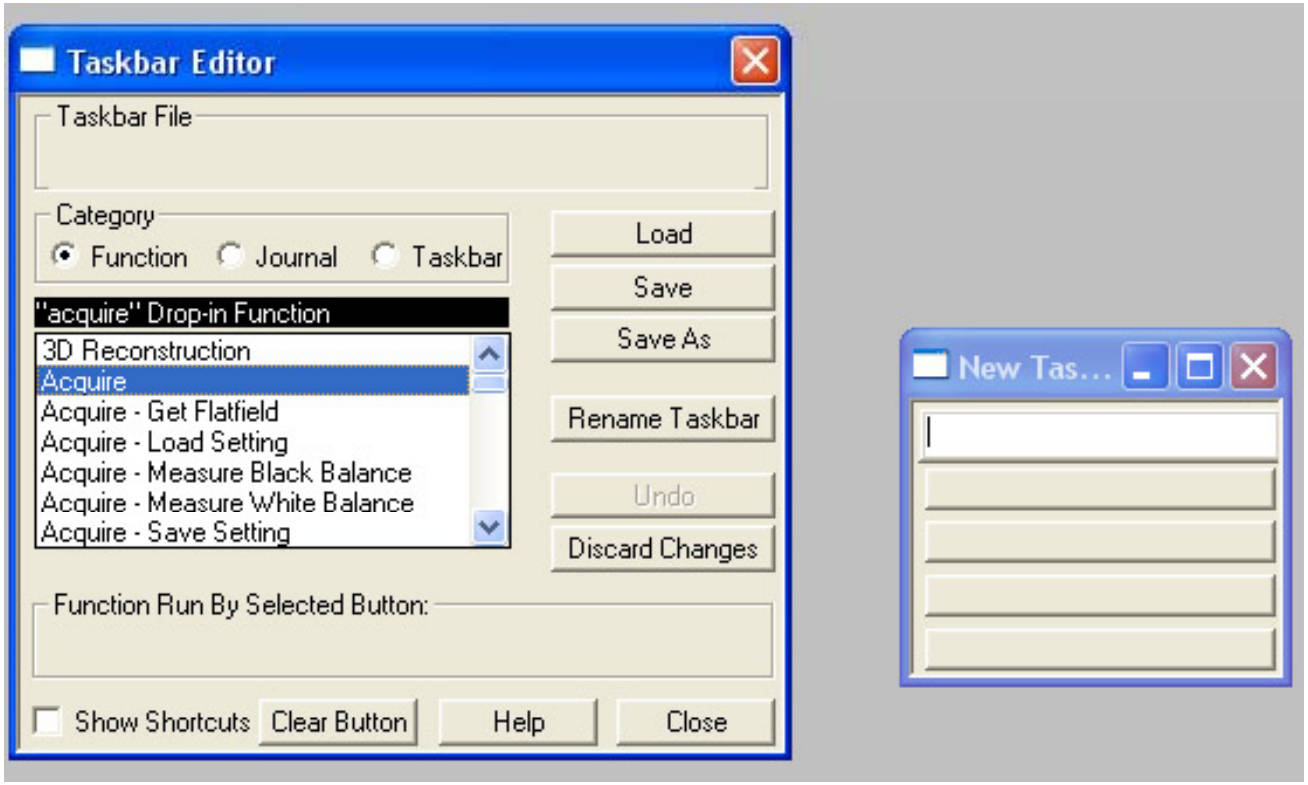
Taskbars

- What is a taskbar?
  - A taskbar gives “one-click” access to journals, functions, and other taskbars
  - A taskbar is customizable
  - Taskbars can be resized to modify the width of the buttons, change the number of columns, and change the number of rows
  - The active taskbar is saved when you exit MetaMorph
  - When you start MetaMorph the next time, the taskbar you were last using will be displayed again
  - Only one taskbar can be used at a time
- Creating a taskbar
  - Journal Menu > Taskbars > Create Taskbar
  - The Taskbar Editor dialog box will open

Molecular Devices

Creating a taskbar: step-by-step

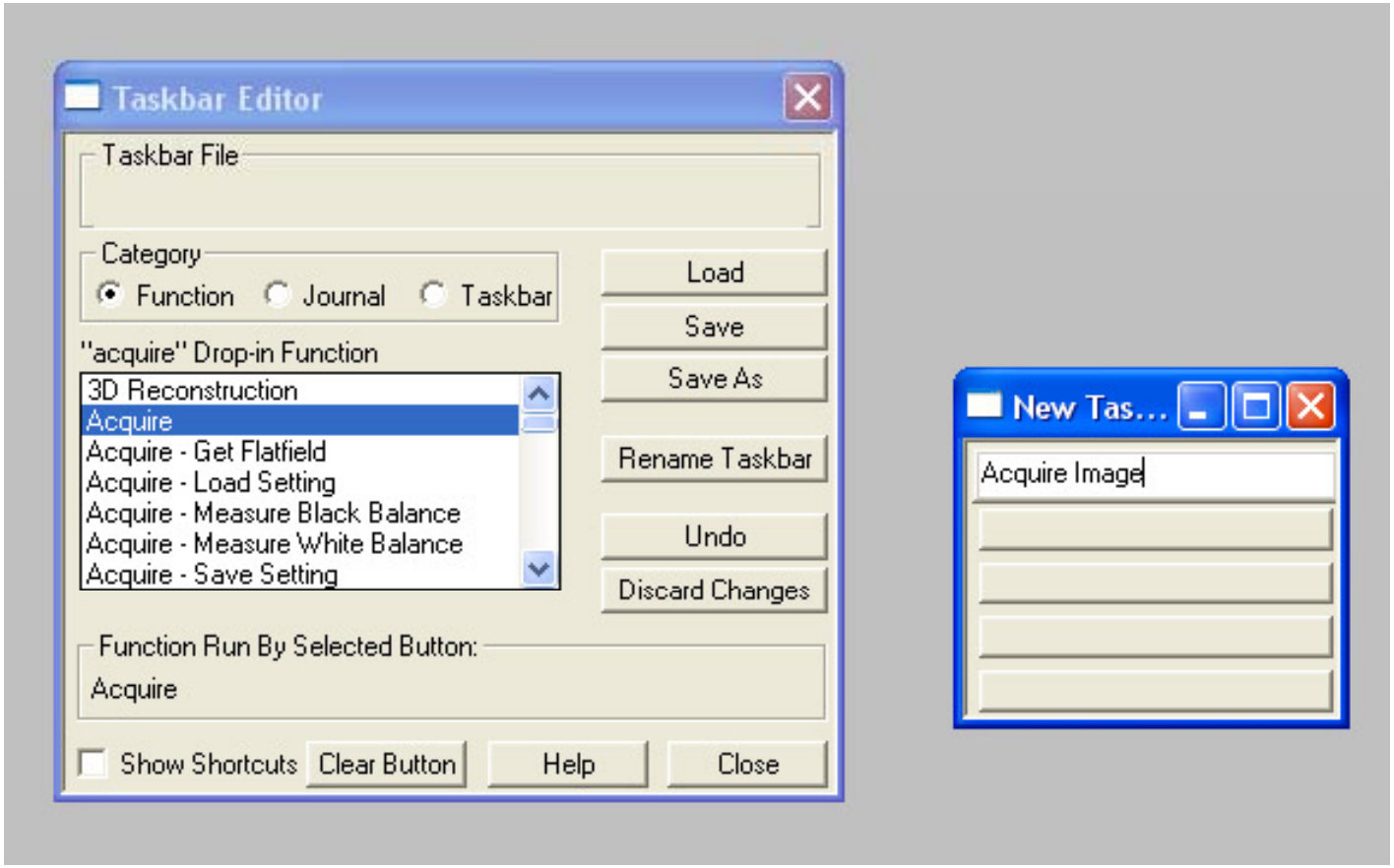
- Drag items from the list in the Taskbar Editor dialog box onto the new taskbar
- There are three categories of items:
  - MetaMorph functions, journals, or other taskbars



Molecular Devices

Creating a taskbar: step-by-step

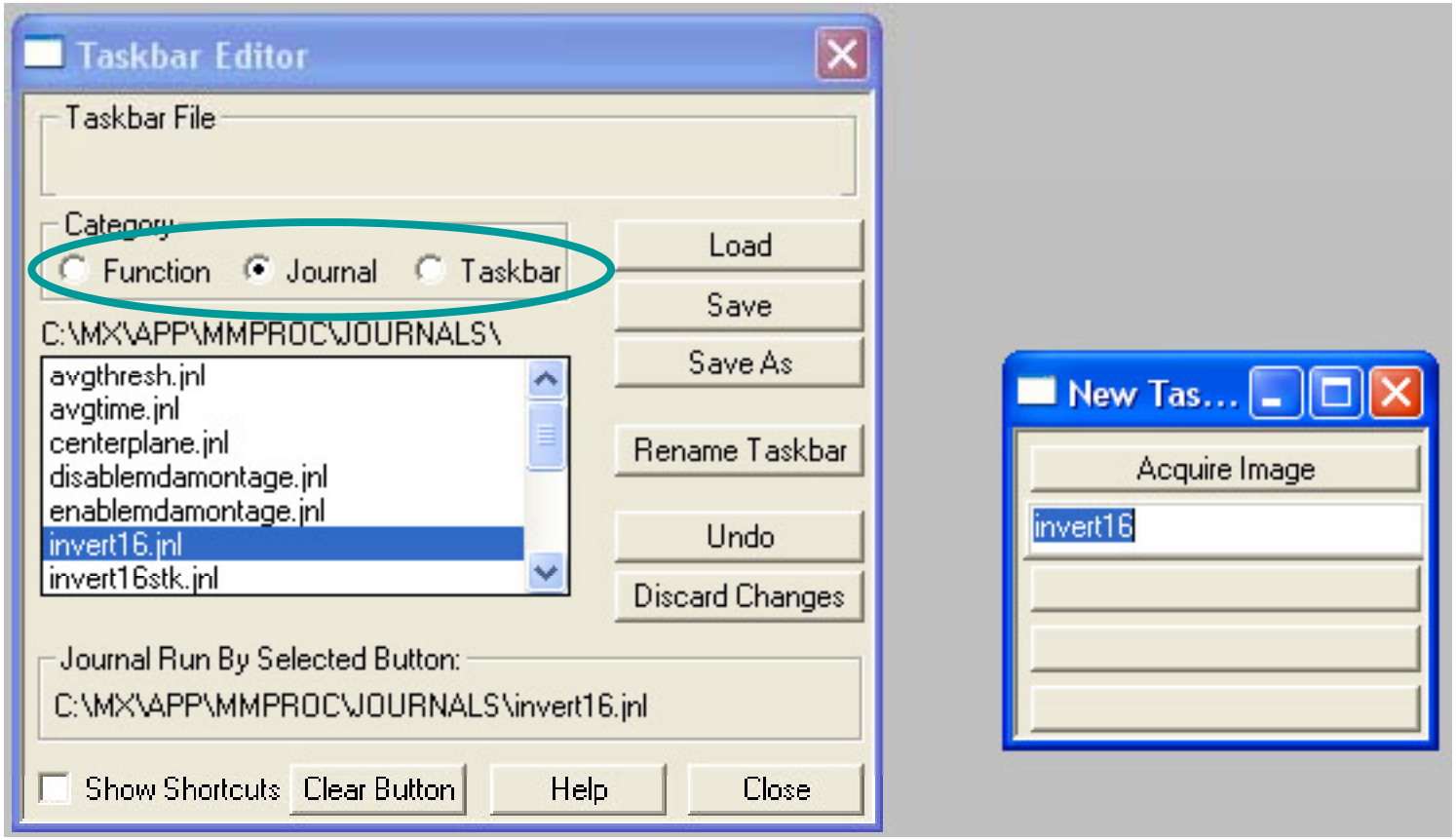
- Renaming the taskbar button



Molecular Devices

Creating a taskbar: step-by-step

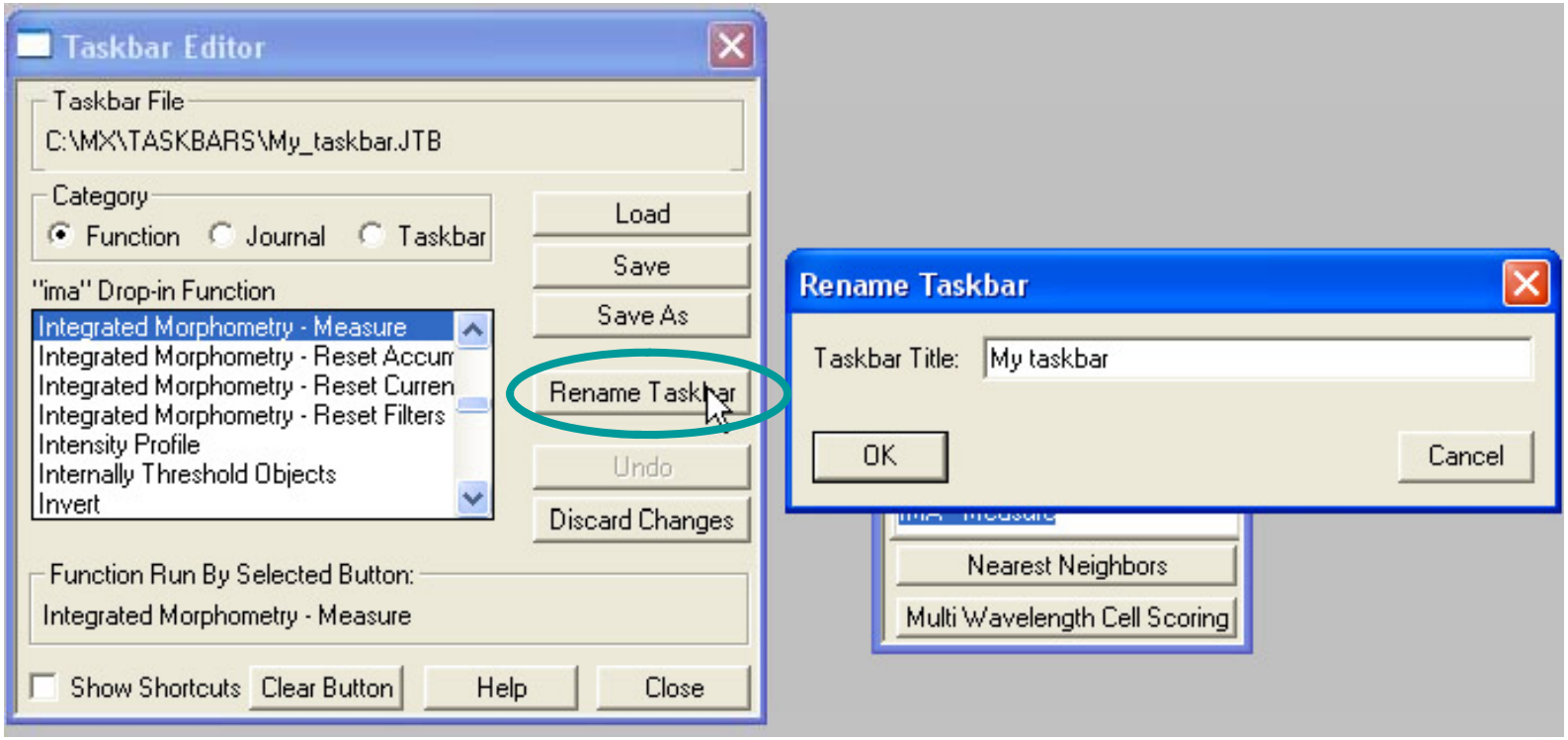
- Click on one of the Category radio buttons to select whether to put Menu Functions, Journals, or other Taskbars on the Taskbar



Molecular Devices

Creating a taskbar: step-by-step

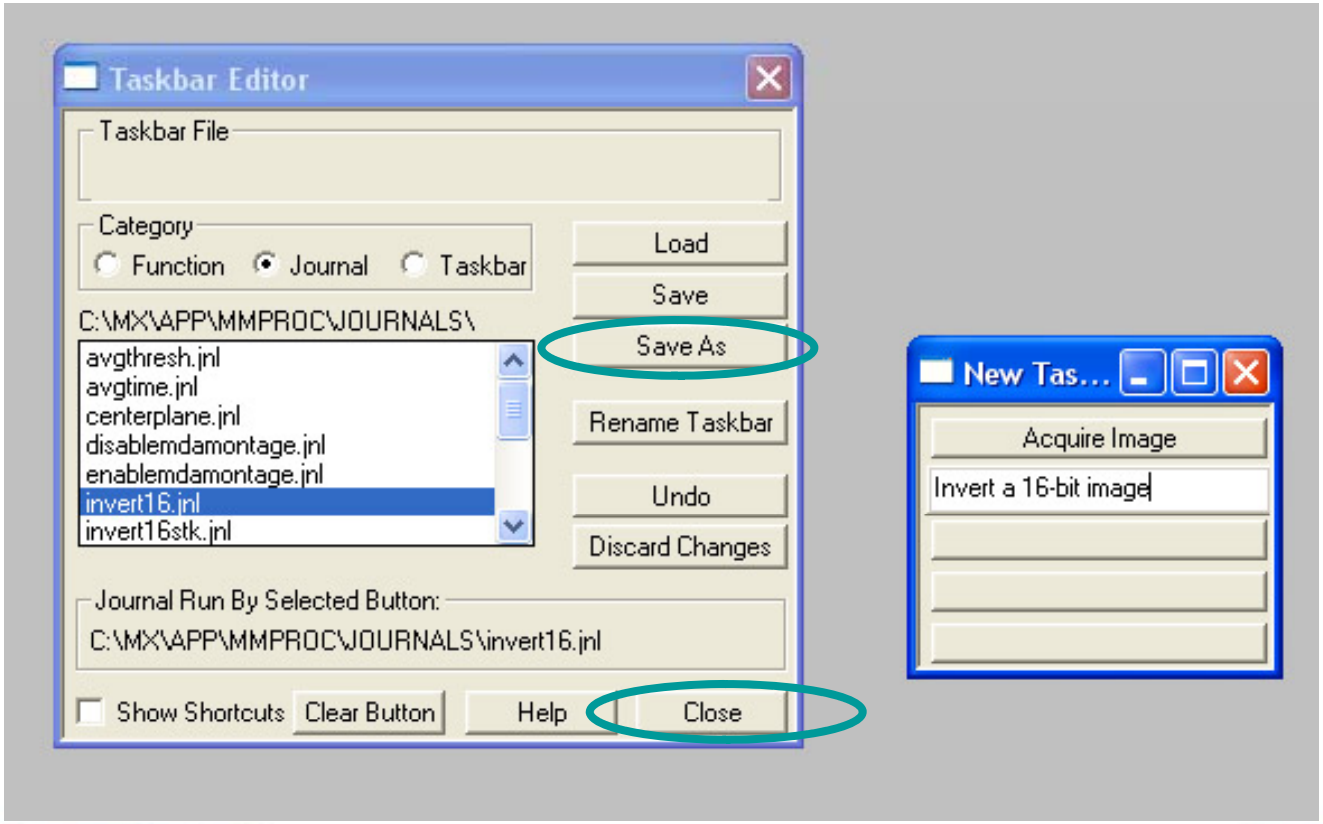
- Click the Rename Taskbar button to rename your taskbar
- The Rename Taskbar dialog box opens
- Once you have typed a Taskbar Title, click OK



Molecular Devices

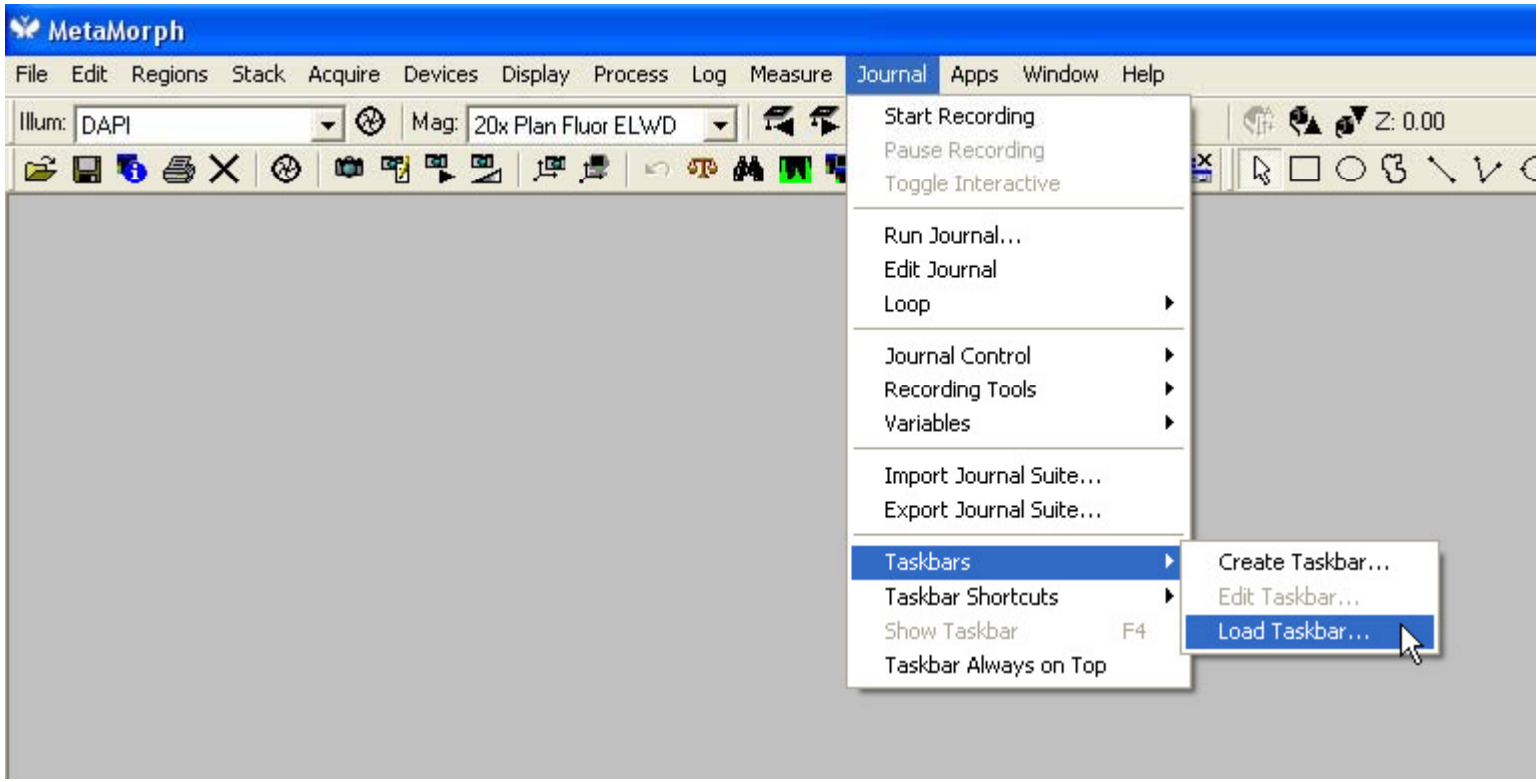
Creating a taskbar: step-by-step

- Once the taskbar is created, click “Save As” to save your taskbar
- Name your taskbar and click OK
- Click Close on the Taskbar Editor to close the dialog box



Creating a taskbar: step-by-step

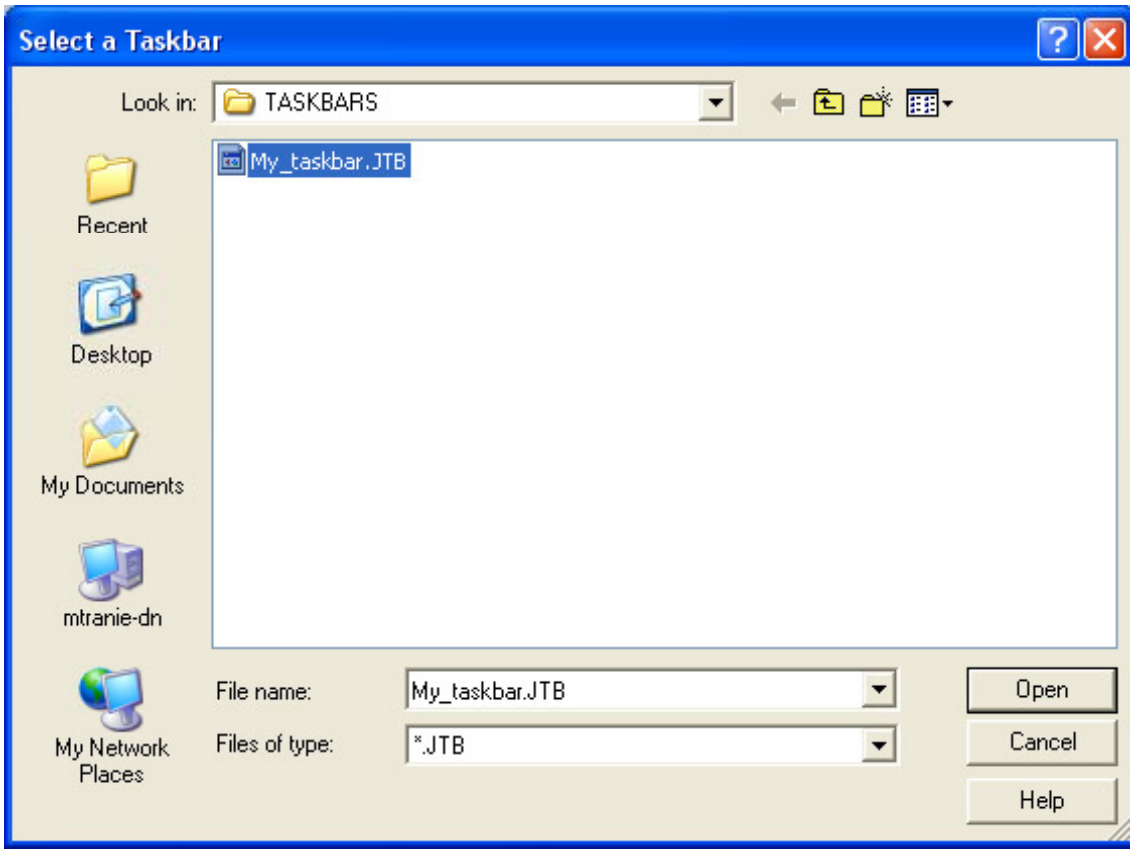
- To load the taskbar use Journal menu > Taskbars > Load Taskbar



Molecular Devices

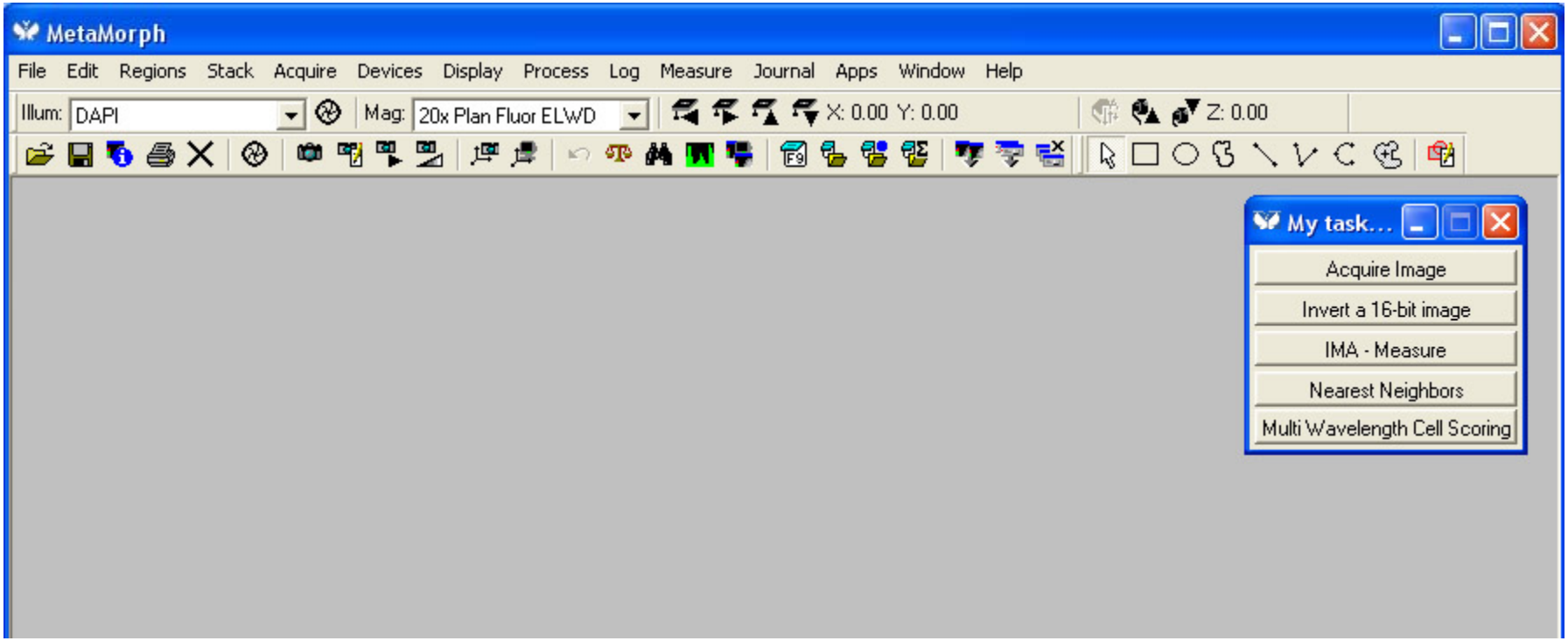
Creating a taskbar: step-by-step

- Select the taskbar to open



Molecular Devices

Creating a taskbar: step-by-step



Molecular Devices