MetaXpress® 6 Software Guide

Adjusting Image Display with Scaling and Look Up Tables

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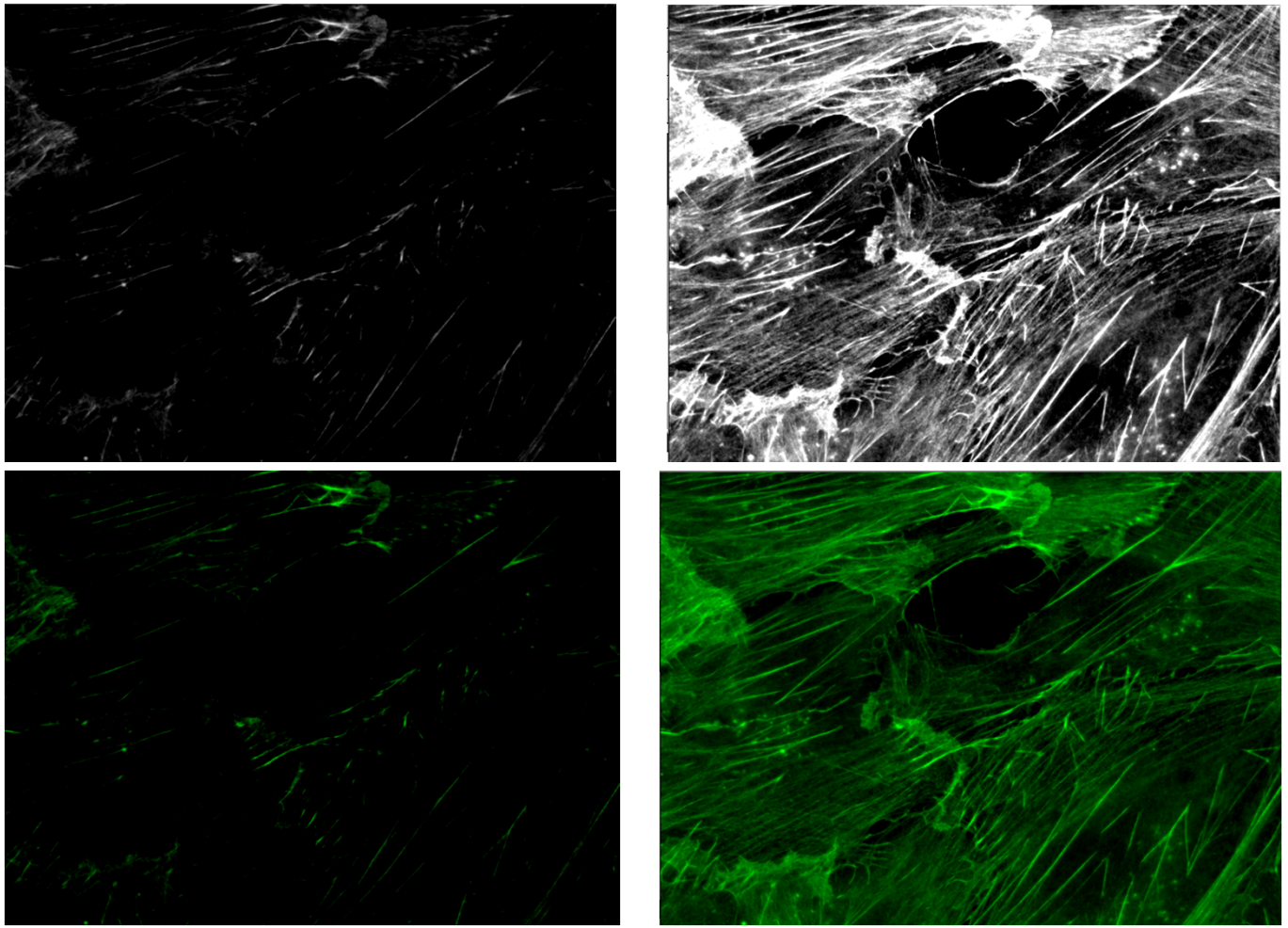
Chapter Purpose

The purpose of this chapter is to guide the user through adjusting image display using Scaling and Look Up Tables.

These functions only change how the image is displayed on the monitor and does not affect the raw pixel intensity data.

Scaling and LUT Changes Image Display

Below are different views of the same image: only the scaling (brightness and contrast) and the look up table (LUT) have been adjusted.

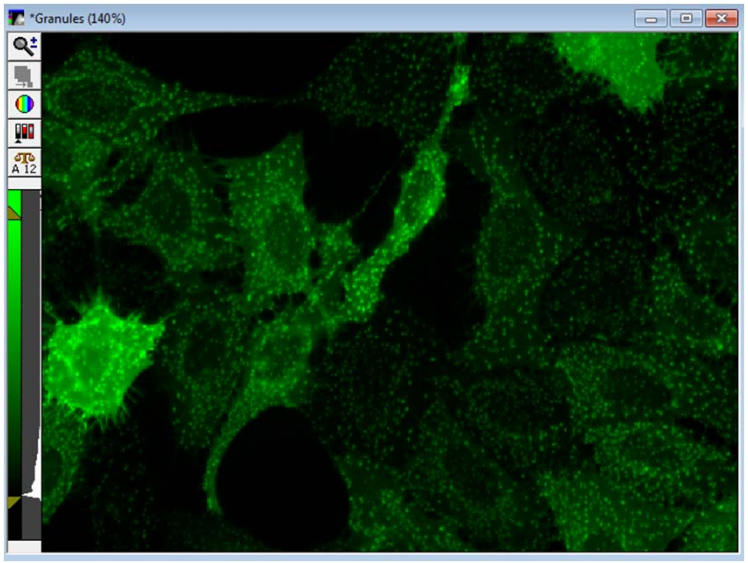


What is an Image?

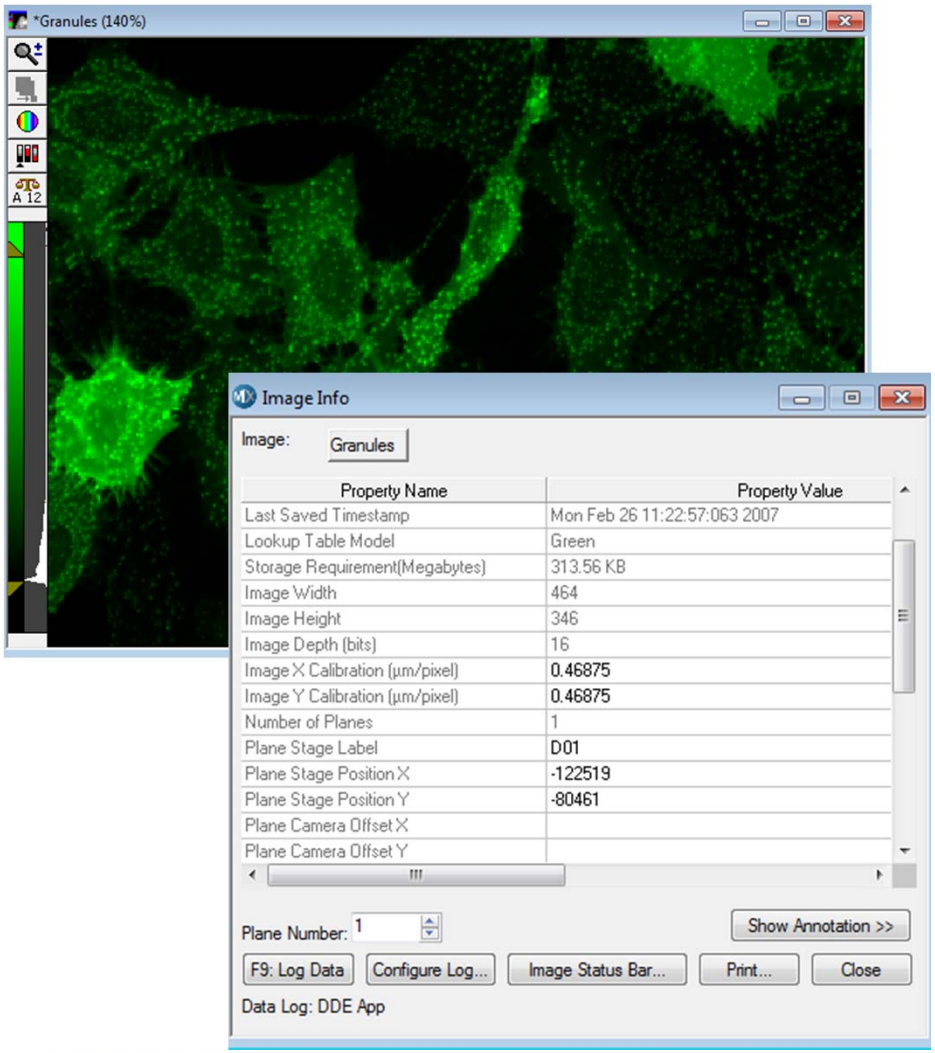
A digital image is a series of pixel intensities

- Two dimensional grid of pixels
- Each pixel contains an intensity value

494	494	522	509	477	507	520	501	497	513	480
487	516	498	481	486	507	521	513	497	499	509
491	496	512	498	492	521	497	494	509	490	513
498	523	499	520	499	496	514	493	505	509	497
521	530	508	495	495	510	483	501	514	493	523
495	509	501	495	484	524	490	513	509	521	516
498	519	503	512	495	515	490	510	510	502	506
511	510	491	521	508	514	505	496	495	493	495
520	523	501	527	493	508	486	494	484	467	495
496	498	513	488	518	506	484	490	493	509	484
505	489	503	501	494	493	483	497	503	480	503
488	504	508	514	516	506	502	513	481	486	510
514	509	515	514	493	500	504	496	496	533	495
487	515	497	524	496	499	492	502	487	500	495
500	504	526	490	510	517	498	509	498	492	516
505	492	490	531	492	511	484	500	506	488	505
520	493	525	518	495	507	500	514	505	519	512
540	522	521	518	516	497	503	512	504	498	506
530	527	518	527	506	529	497	512	529	512	519
547	543	515	531	510	517	541	501	524	507	552
591	537	544	572	550	528	538	546	534	551	576
609	582	615	605	571	599	591	598	621	617	647
557	632	586	559	562	571	617	597	635	642	724
543	585	623	566	560	554	539	546	568	575	634



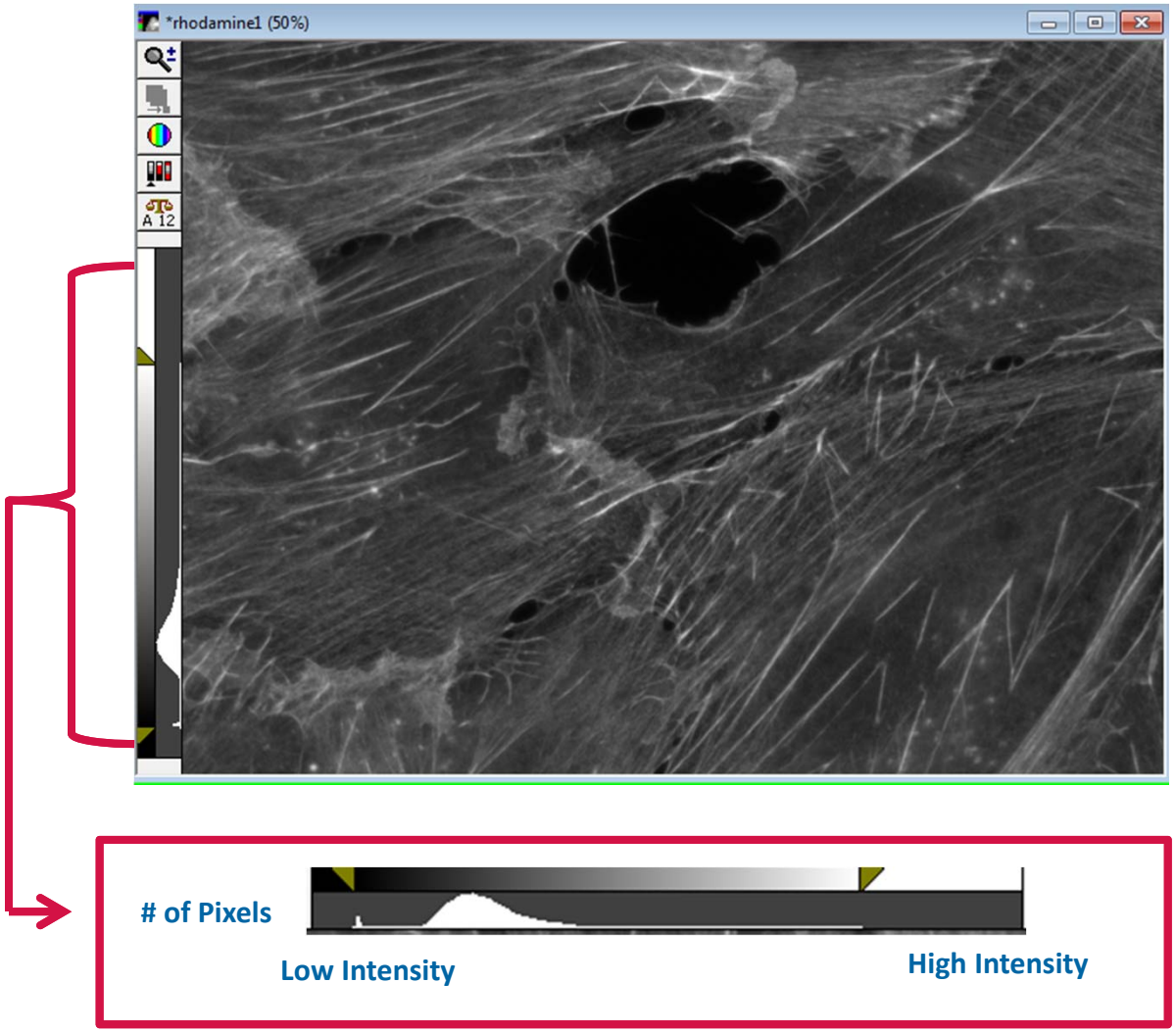
Displaying Image Information (Metadata)



- To view image info:
  - In the main menu, select **Edit > Image > Image Info**

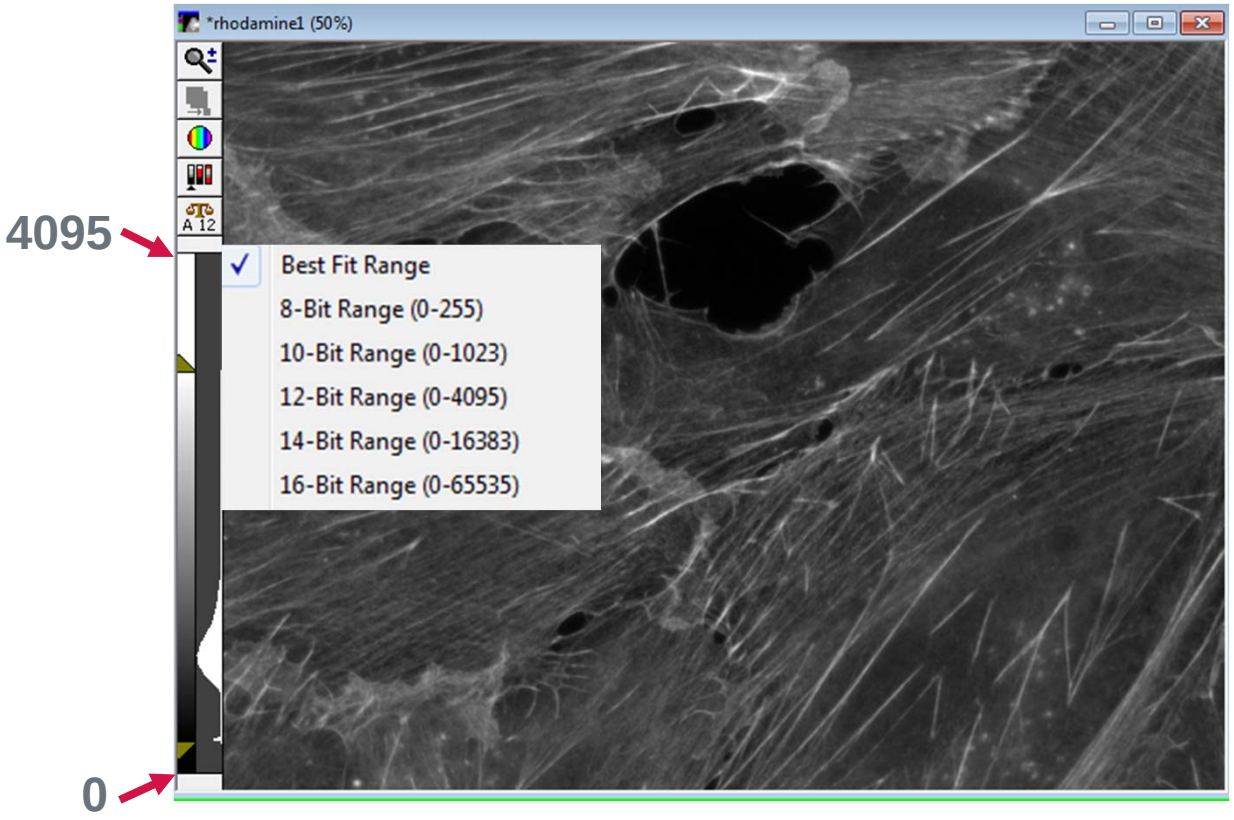
    OR
  - Click on the **Image Info** icon 
- In addition to pixel intensities, other information is stored with images when they are saved
- Additional information can be seen by clicking on the **Show Annotation>>** button

What and Where is the Image Histogram?



- Image histogram displays pixel intensity data
- Displayed vertically on left hand side of each image beneath the icons
- It is used as guide to:
  - Adjust image display or scaling (brightness and contrast)
  - See a quick view of the intensity data
  - Guide for setting image thresholds for segmentation

Displayed Range of Image Histogram



In the above image, the histogram range is from 0 to 4095 (a 12-bit image). This is indicated by the **12** in the **Scale** icon above the histogram 

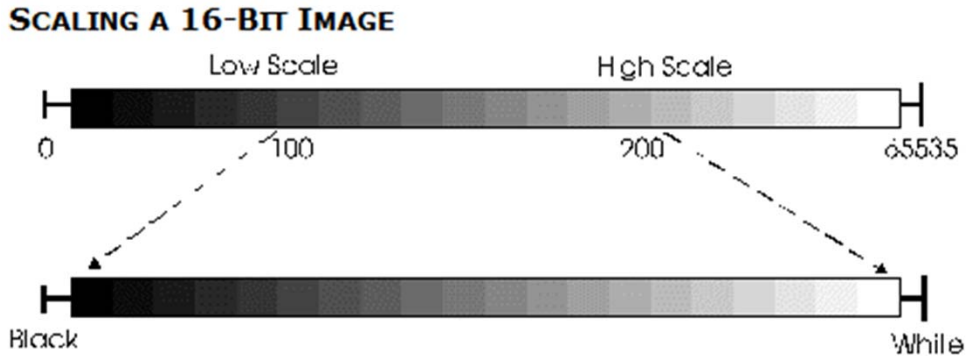
- The histogram scale can be changed for different bit-ranges by clicking on the Scale icon above the histogram
- Select **Best Fit Range** to automatically display the range of intensity values contained in the image

Why Adjust the Scale of an Image?

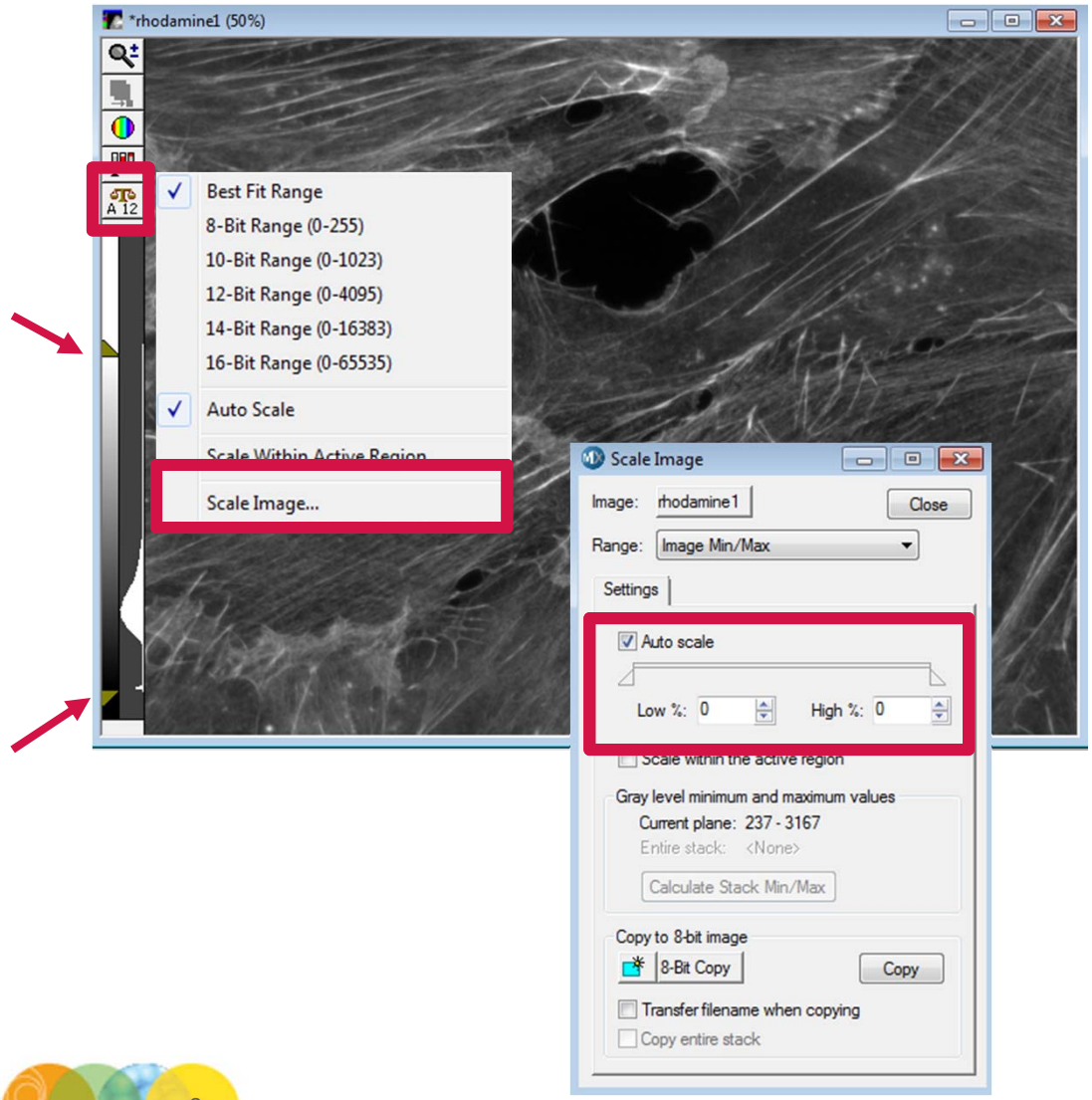
Images captured with ImageXpress systems are saved as 16-bit image file types (2^{16}), meaning an image can contain pixel intensity data up to 65,535 gray values. Depending on your system, your camera may be able to capture up to 12-bit or 16-bit intensity range of data.

Computer monitors can only display monochrome data (grayscale) in an 8-bit range (256 gray values) . Therefore we need a way to display intensity values up to 65,535 on a monitor with only 8-bit display.

The scale image tools effectively rescales the intensities in the original image, allowing you to see differences of grayscale values that might otherwise be impossible to view on your monitor.



How to Adjust the Scaling on an Image

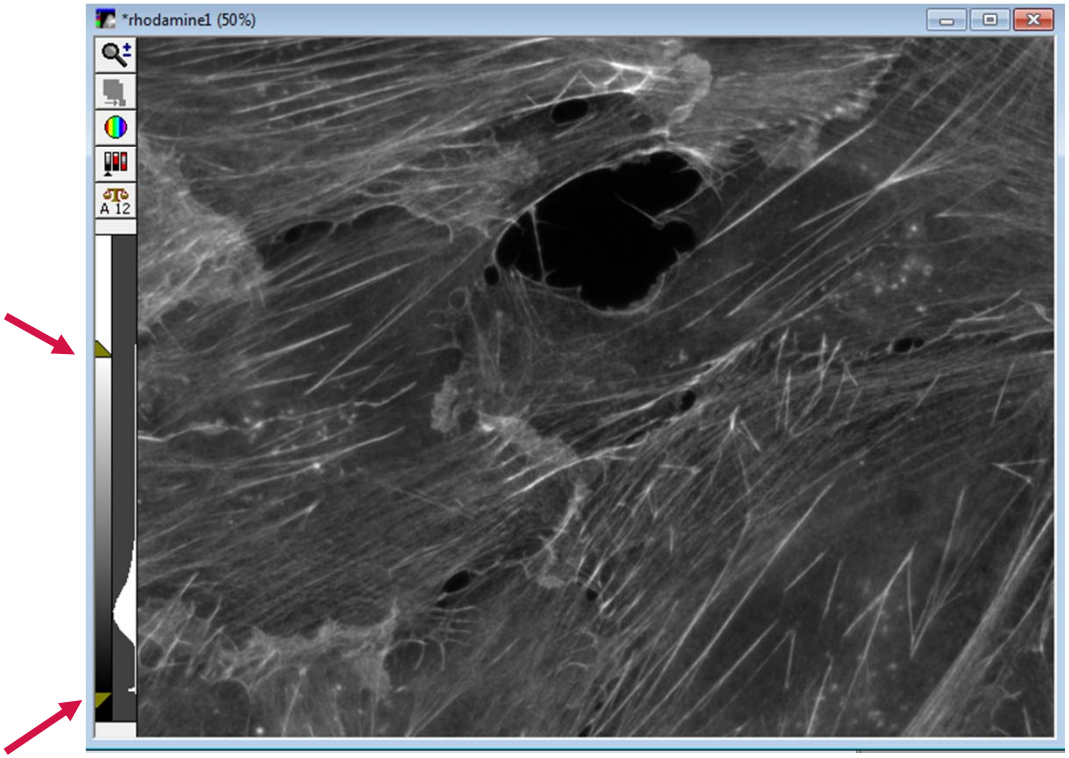


- In the main menu select **Edit > Display > Scale Image** or click on the **Scale** icon located on the left hand side of the image and select **Scale Image**

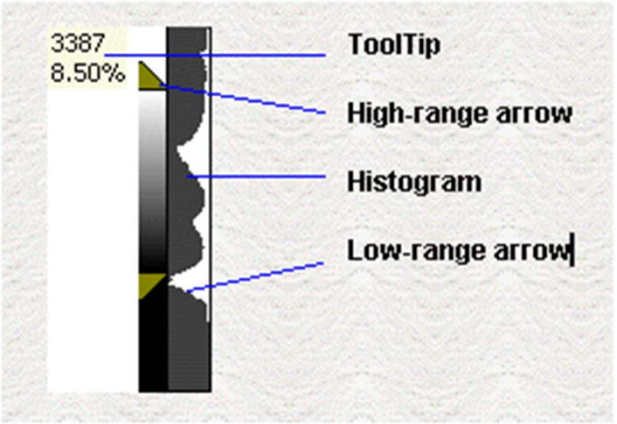
- Adjust the scale by:
  - Moving the high wedge to set the White (brighten signal)
  - Moving the low wedge to set the Black (darken background)

- Changes made here only adjust what is displayed and DOES NOT alter the data

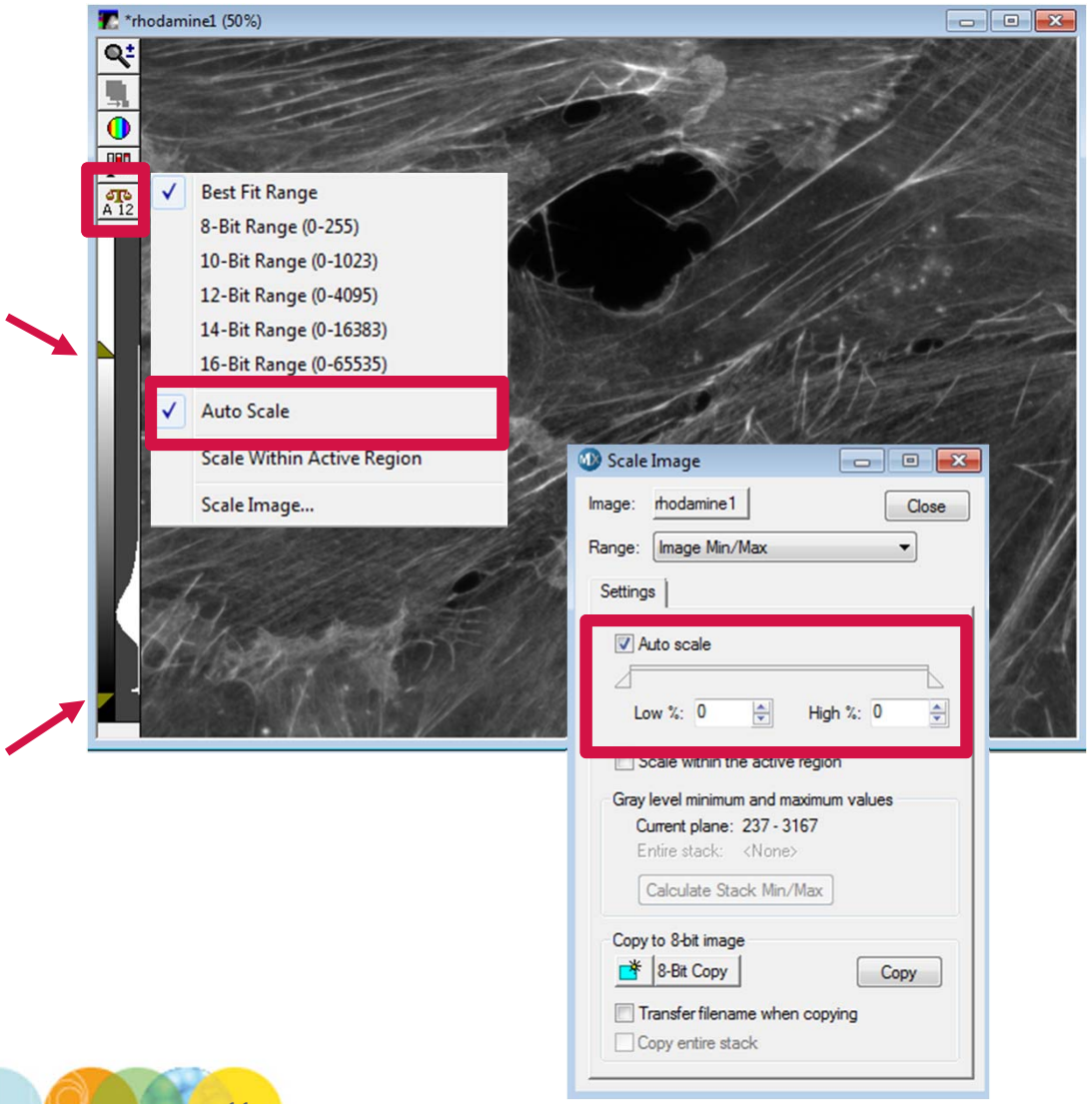
Viewing Pixel Intensity Values During Scaling



Point to the low / high wedge and a tool tip will appear displaying the values

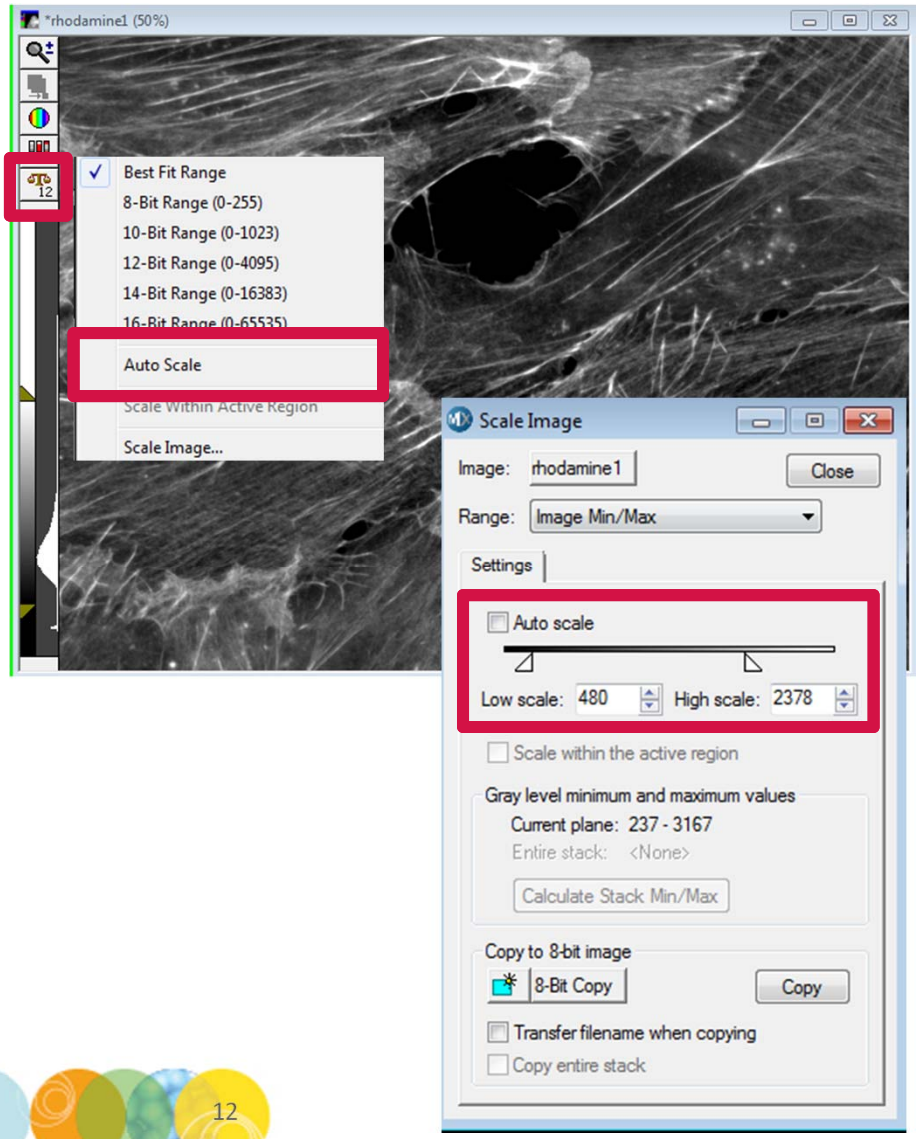


What is Auto Scale?



- Enabling **Auto Scale** limits the image display to the lowest and highest intensity in the image
- **Auto Scale** can be turned on or off from the **Scale Image** dialog or through the **Image Scale** icon. The **Image Scale** icon will display an "**A**" when turned on.
- Wedges can be adjusted to select % of pixels below and above wedges to display as black and white, respectively

Turning Auto Scale Off



- When **Auto Scale** is turned off, you can set the absolute low and high intensity values
  - Pixel intensities ≤ the low intensity value appear Black (or the darkest color of your look up table)
  - Pixel intensities ≥ the high intensity value are saturated (or the brightest color of your look up table)

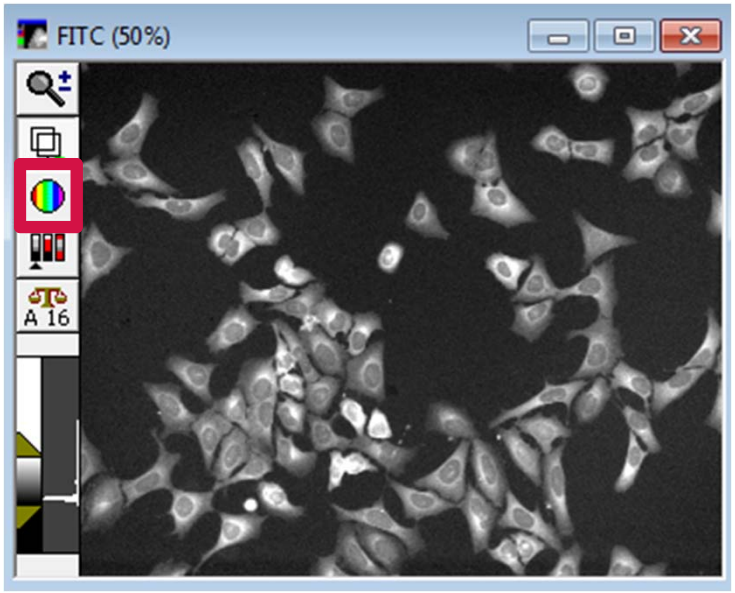
NOTE When Auto Scale is turned off and another image of the same wavelength is opened, absolute scaling will remain the same and allow you to compare intensities by eye

- To turn off **Auto Scale:**
  - Click on the **Scale** icon and click on **Auto Scale** to disable
  - Disable the check box next to **Auto Scale** on the **Scale Image** dialog
  - **Image Scale** icon will no longer display an "**A**"

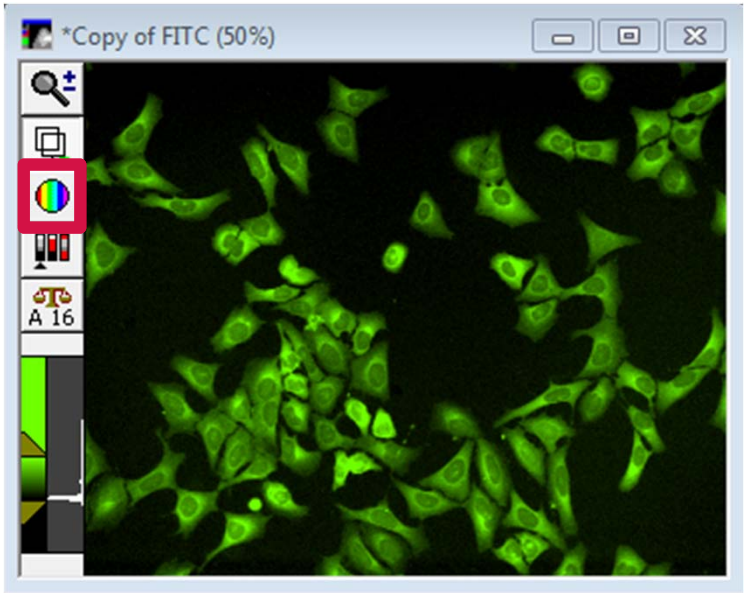
Changing Image Color: Look Up Table (LUT)

- The camera on the ImageXpress® Systems is monochrome and can only acquire different levels of gray

- An image Look Up Table is used to map monochrome intensities to color. For example, GFP images appear green rather than shades of gray



Monochrome LUT



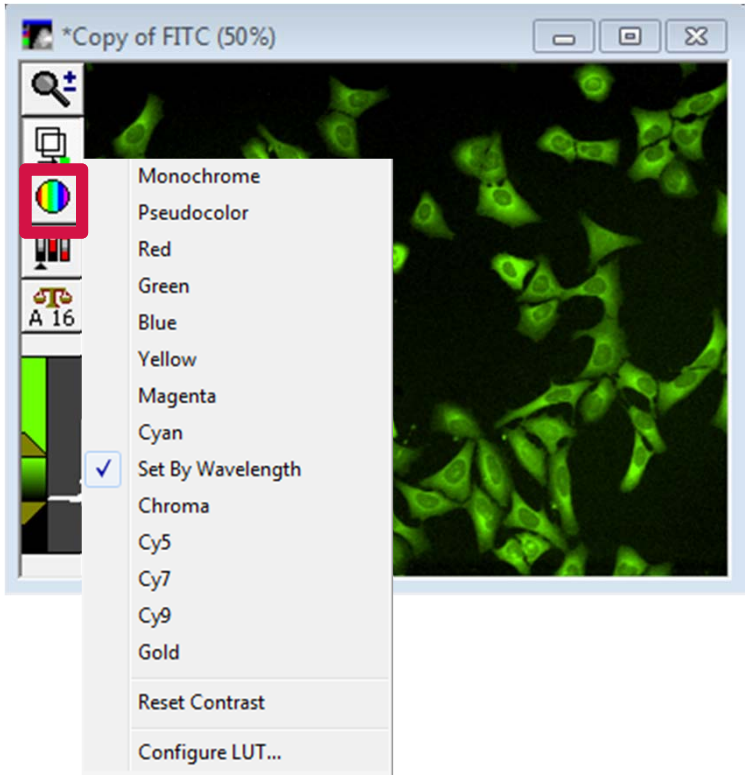
Green LUT

Changing Image Color: Look Up Table (LUT)

- Various LUTs are available including Monochrome, Pseudocolor, Red, Green, Blue, Yellow, Magenta, Cyan, Chroma, Cy5, Cy7, Cy9, Gold or Set By Wavelength.

- **Set By Wavelength** (default) uses a LUT to match the wavelength emission color.

LUT icon



Pseudocolor and Custom Colors

- **Pseudocolor** LUT displays differences in intensity within an image as colors where low intensity pixels appear dark blue (black) and highest intensity pixels are red or white.

- Users can also create a custom LUT using **Configure LUT** to map specific intensity ranges to color, such as saturated pixels.

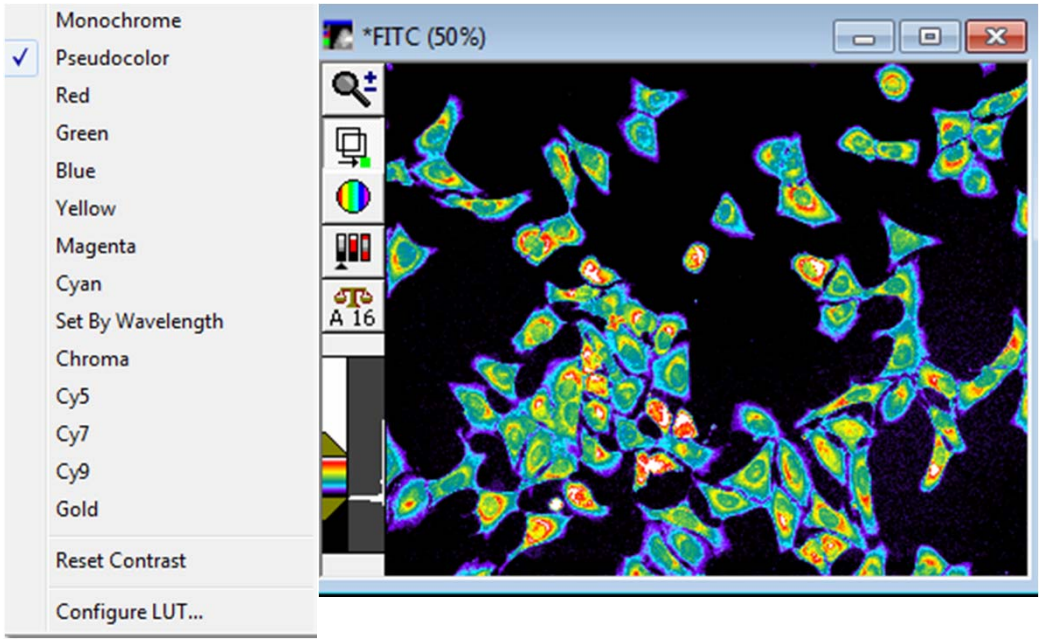
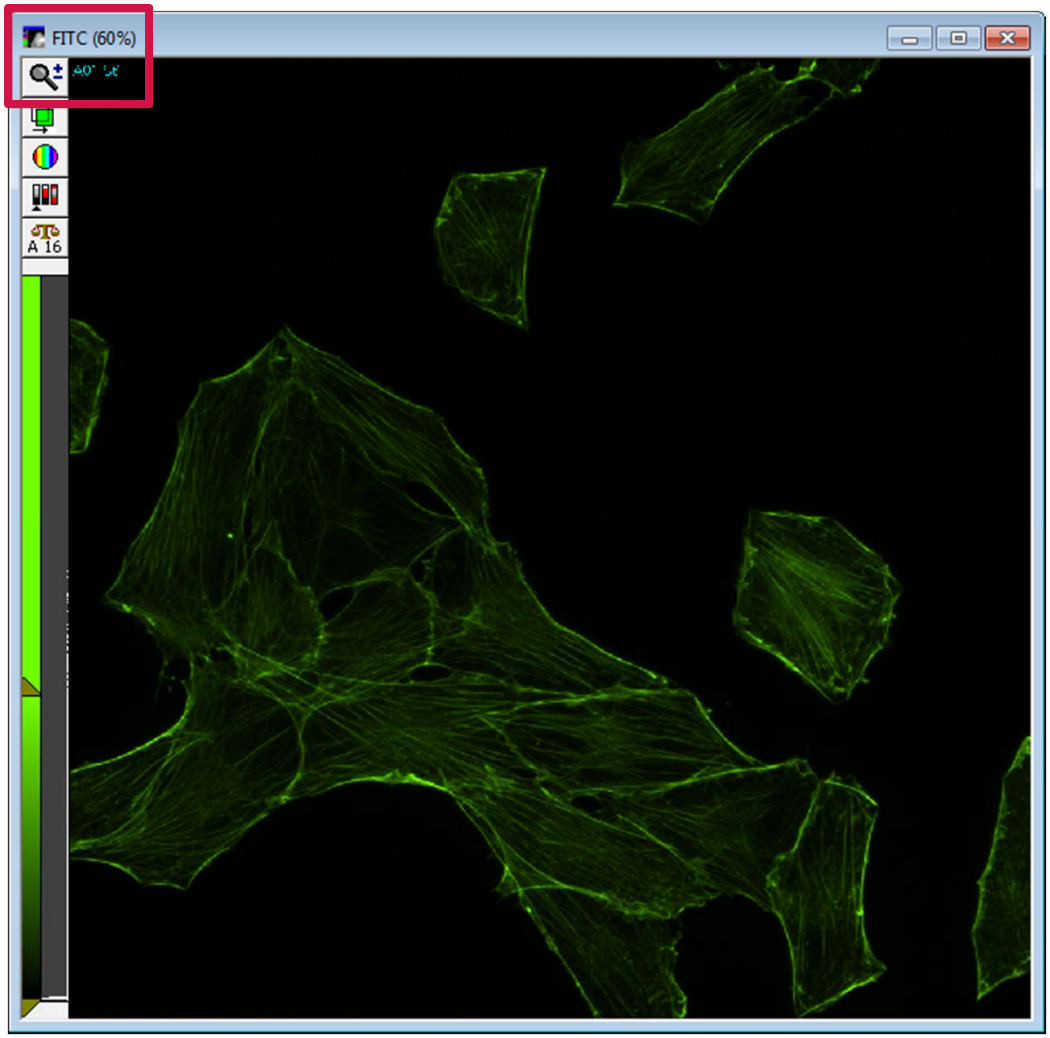


Image Zoom

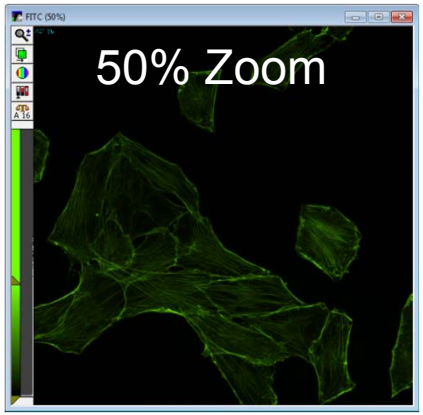


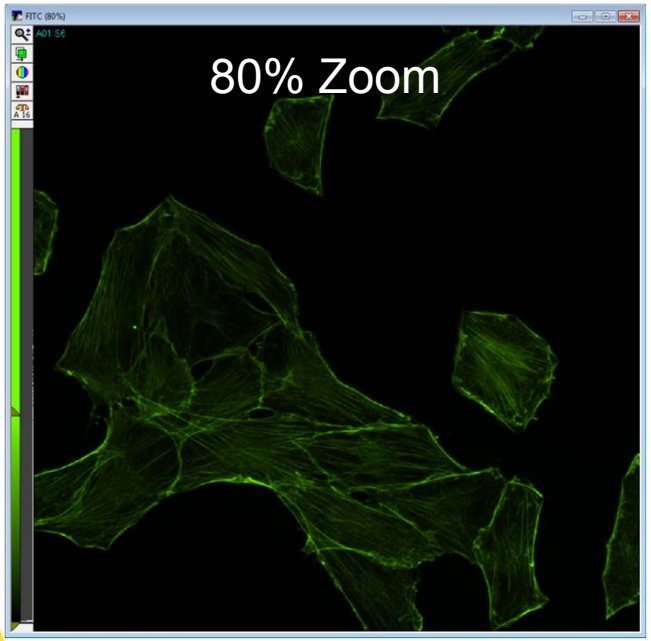
- The image zoom is indicated next to the image name. 100% Indicates an image displayed at full size.

- Image zoom can be adjusted in two ways:
  - Click on the image and use the mouse scroll wheel. This makes the image window larger or smaller.
  - Click on the **Zoom** icon  in the image tools section, then click and/or drag on an area of interest in the image. This zooms into the image, centering on the area you clicked, and does not change the size of the image window.

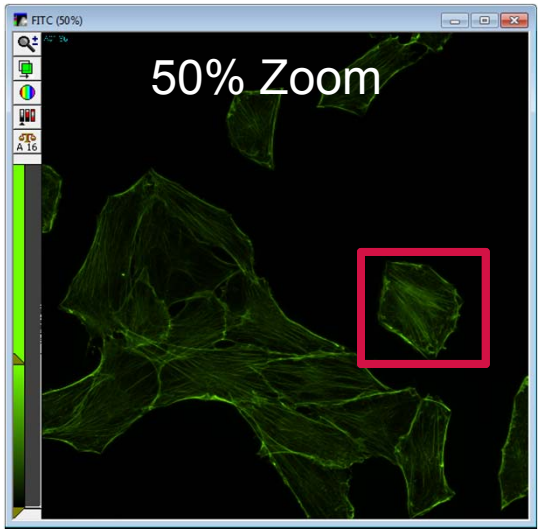
Image Zoom Methods

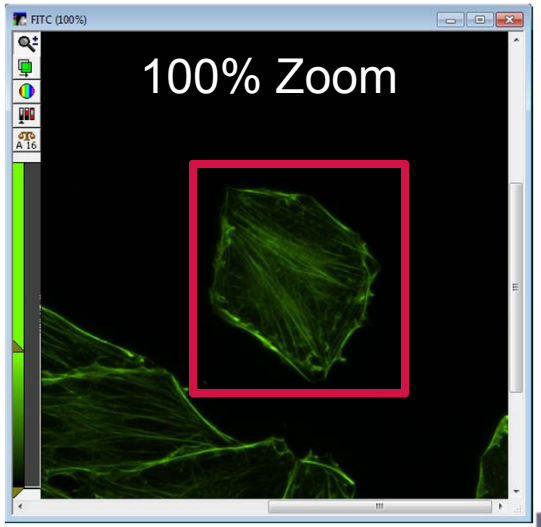
Using mouse scroll wheel



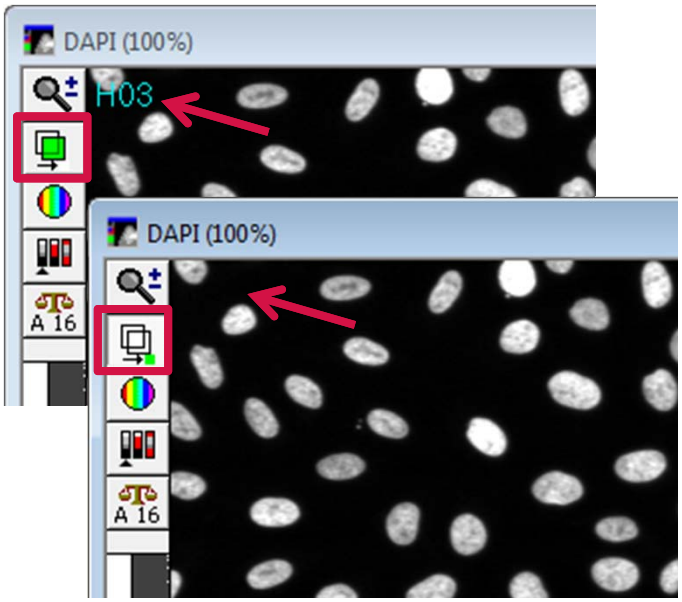


Using **Zoom** icon



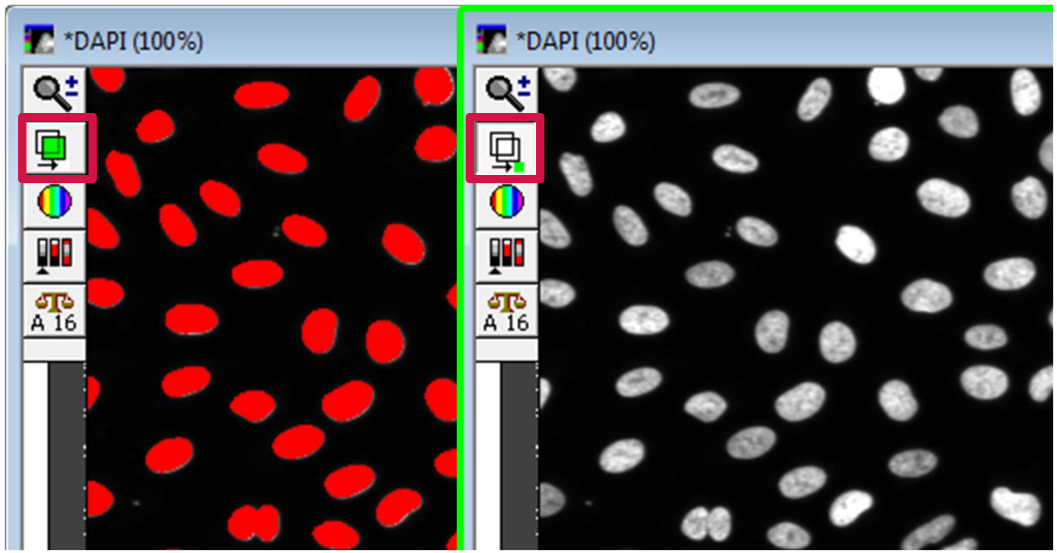


The Segmentation Overlay Icon



- The **Show/Hide Overlay** icon toggles display elements superimposed on the image
  - Well/Site name (upper left of image)
  - Analysis result value (bottom right of image)
  - Analysis segmentation overlay
- When toggled on, the icon displays green on white 
- When toggle off, the icon displays white on white 

NOTE The red overlay pictured here was generated using the Count Nuclei Application Module



Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments

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