Operations Guide

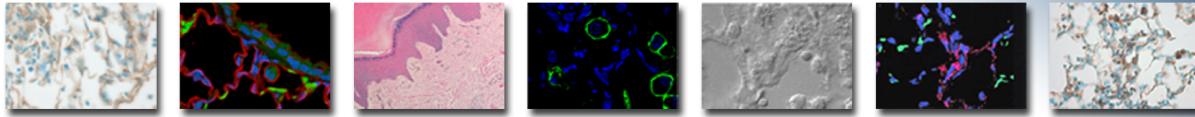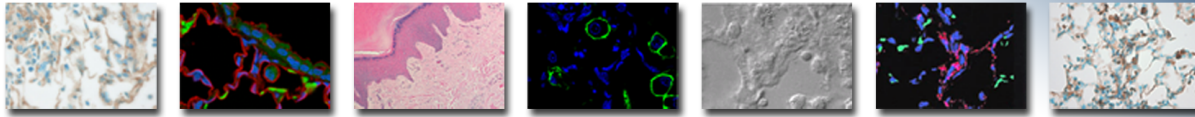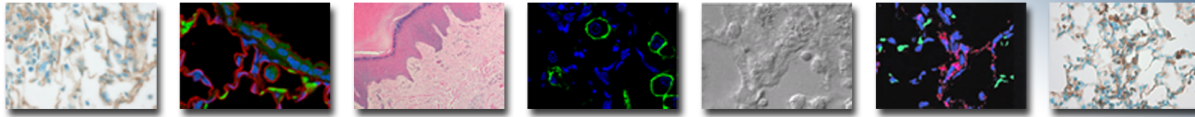**Nikon**

ECLIPSE

T*i*-E

Contents

- **STARTUP**
- **MICROSCOPE CONTROLS**
- **CAMERA CONTROLS**
- **SOFTWARE CONTROLS**
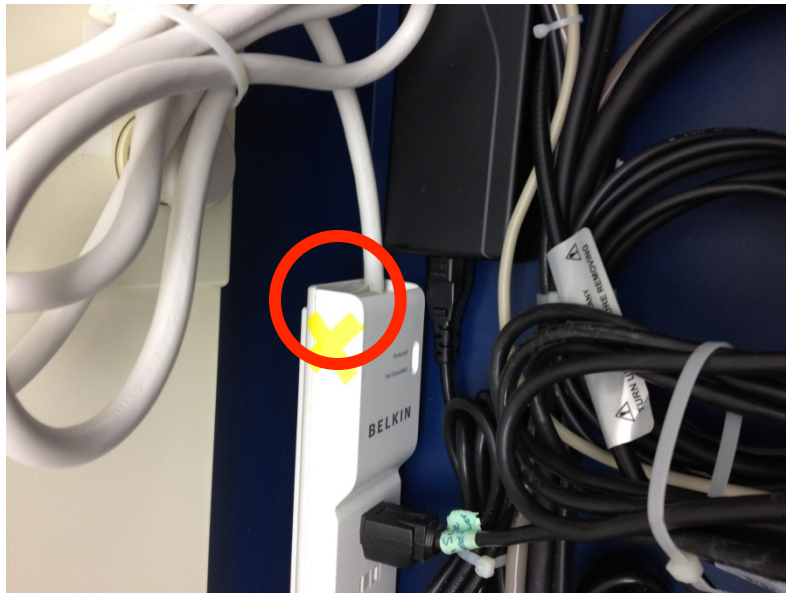- **EXPOSURE AND CONTRAST**
- **MONOCHROME IMAGE HANDLING**

Nikon Eclipse Ti-E Operations Guide

STARTUP

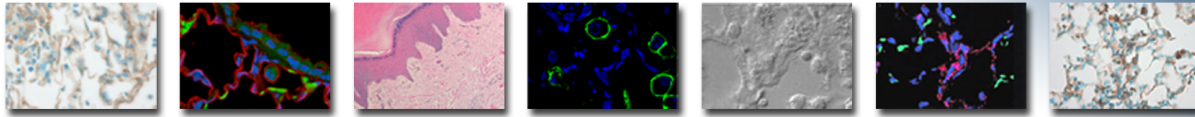# Startup – Powering Up

Microscope System

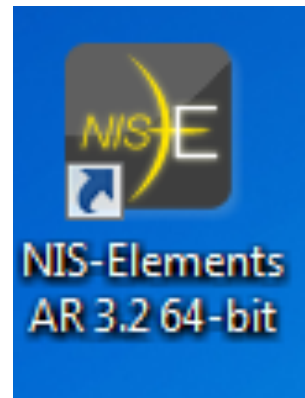Power strip behind left of microscope

Camera

Next to PC

Note: Please give microscope and camera at least 1 minute to initialize before starting software
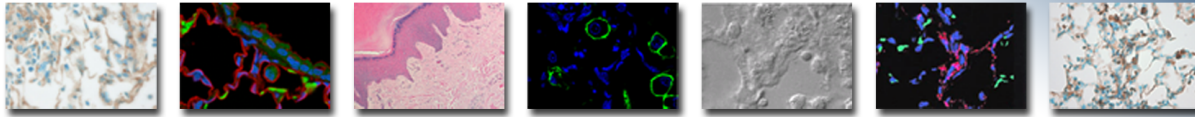Please do not touch any other buttons.

Startup – Opening Software

After logging into computer, Double left click on the icon, or right click on and select "Open"

Note: The software will look for all microscope components when it starts up – please make sure everything is on and initialized before starting software.
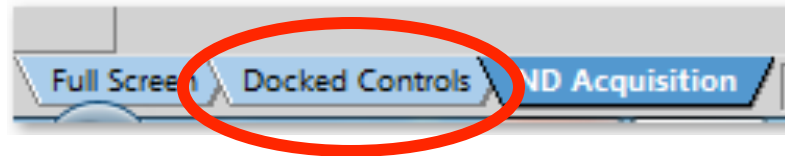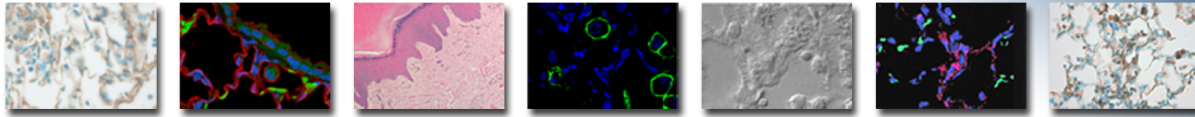
Startup – Loading Software Defaults

Before pushing any buttons in the software, please push the "Restore All" button on the left hand tool bar.

Also, this button can be pushed any time during a session if the screen or any part of the software is acting abnormal.
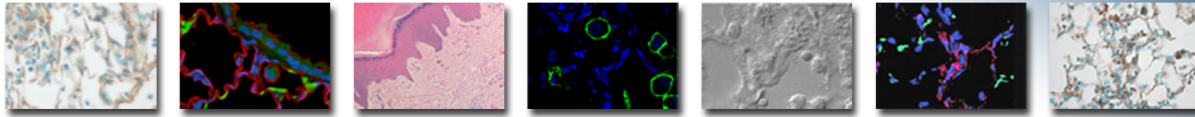
Startup – Software Panes

Software has three main display screens:

"Docked Controls" - shows all control panels. This is the primary display that should be used.

"Full Screen" – displays open work surface of software without any control panels. This gives more open space for image handling.

"ND Acquisition" – provides control box for multi-dimension imaging – see section on ND Acquisition
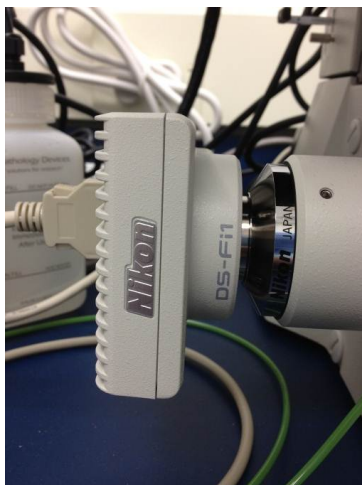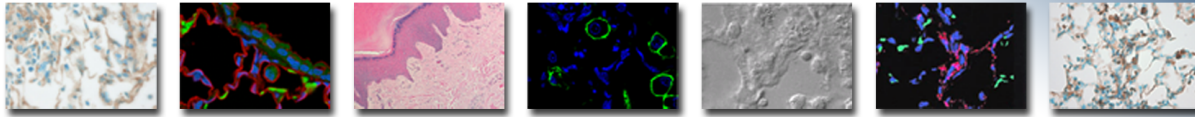
Startup – Camera Selection

Qi1 Monochrome Camera
- <u>Default camera on software startup</u>
- Produces pseudo colored images for the different fluorescence channels.
- This camera is best for fluorescence and phase contrast imaging

Fi1 Color Camera
- Produces true color images
- Should only be used for brightfield imaging with samples that have real color information – non-fluorescent stains
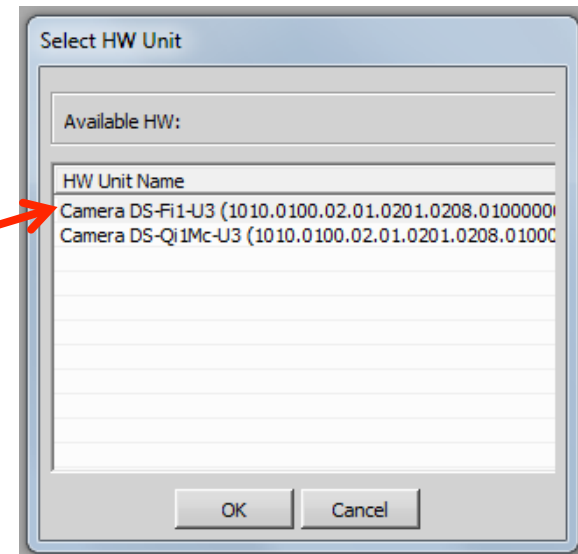
Startup – Camera Selection

If using the Qi1 Monochrome Camera, you don't need to make any camera selection.
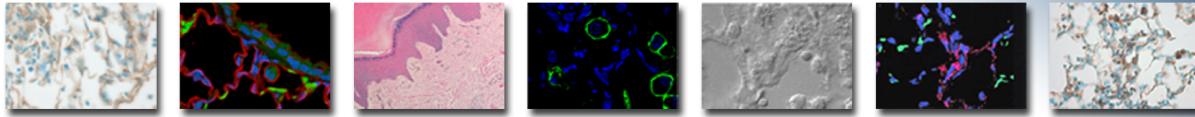
To switch from the **Qi1 Monochrome** camera to the **Fi1 Color** camera, push the switch button on the left hand tool bar.

The following dialog box will appear – select **"Camera DS-Fi1-U3"** and hit OK

Select HW Unit

Available HW:

HW Unit Name
Camera DS-Fi1-U3 (1010.0100.02.01.0201.0208.01000000
Camera DS-Qi1Mc-U3 (1010.0100.02.01.0201.0208.01000

OK   Cancel

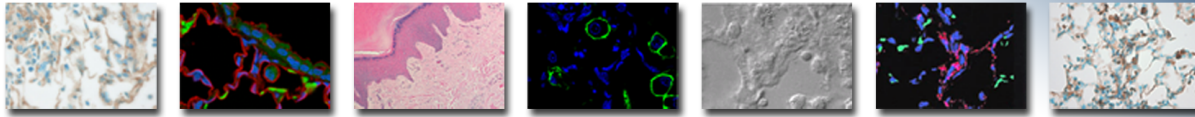Note: it will take about 20 seconds for the change to occur

Startup – Toggle Camera

To ensure that the software sets up properly it is best to hit the **"Restore All"** button again when changing between cameras.

During your imaging session you may use the switch button to toggle back and forth between the cameras.

After the first switch, you will not see the camera dialog box again, it will just choose the opposite camera every time the button is pushed.
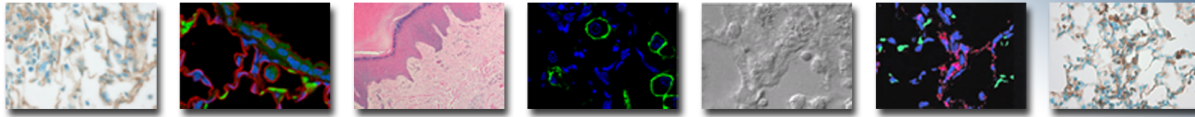
Startup – Image Bit Depth

The Qi1 Monochrome Camera will produce 12 bit images.
These images can be saved out in their 12 bit state or converted to 8 bit RGB for viewing with any software program.

Or you can set the software to save out all images as 16 bit by going to: "Edit" on the main menu bar and then select "Options".
In the General screen under Options, select the "TIFF Options" button and select "Save 10, 12, 14 bit images as 16 bit". This must be done for every software session.
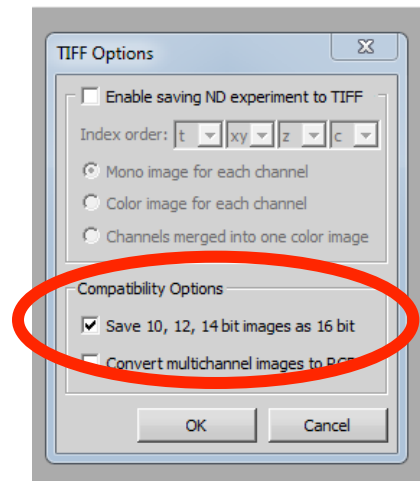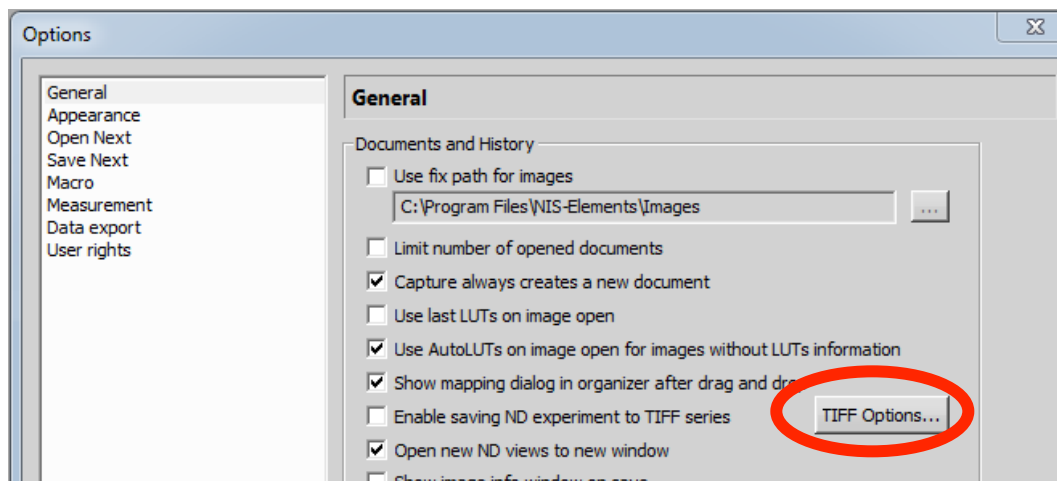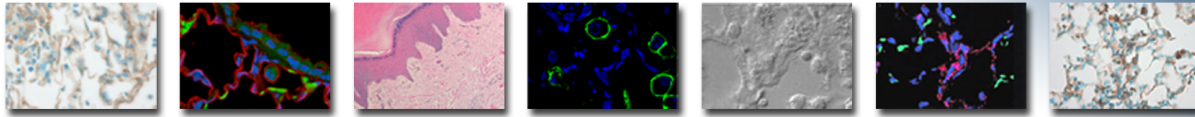
Startup – Optical Configurations
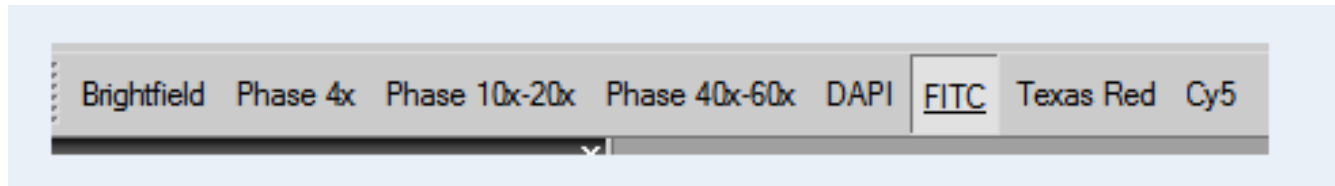
Optical Configuration *(OC)* buttons are located at top of screen, just under the main menu option line:

- **OC's** are your main point of communication with the microscope system.

- Selecting an **OC** will put the microscope in the condition needed to do the indicated type of imaging

- Select an **OC** by left clicking on its name – its name will depress – see "FITC" above
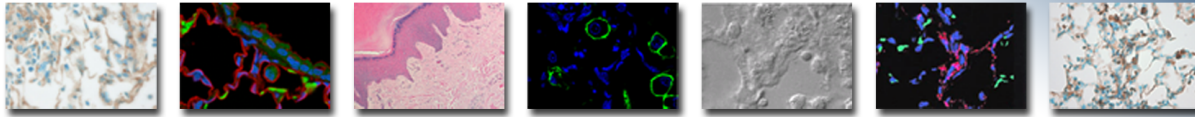
Startup – Optical Configurations
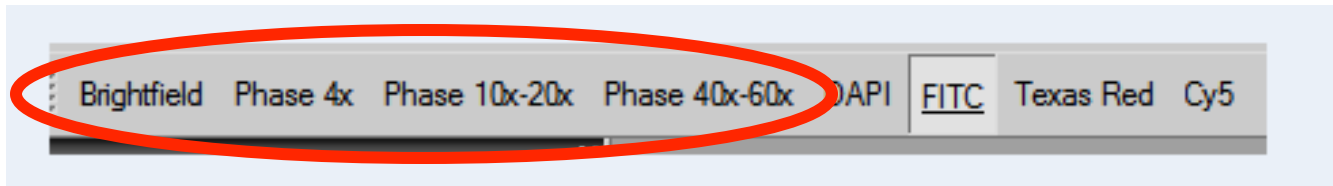
Brightfield and Phase Contrast OC's:

- Automatically turn on the halogen light above

- Automatically put the condenser in the correct position for each

- Note: there is only one "Brightfield OC for all lenses, while there are multiple "Phase" OC's for the different lenses
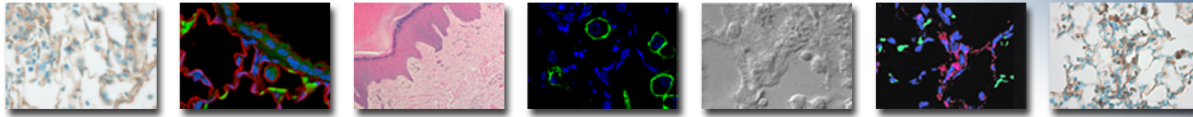
Startup – Optical Configurations

Brightfield   Phase 4x   Phase 10x-20x   Phase 40x-60x   DAPI   FITC   Texas Red   Cy5
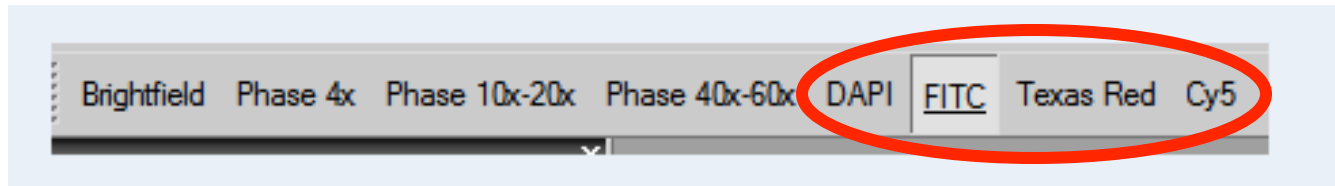
Fluorescence OC's:

- Automatically turn **off** the halogen light above

- Automatically put the correct fluorescence filter cube in place

- When viewing by eye, you will open/close the fluorescence shutter as needed

- When using the camera, the fluorescence shutter will open/close automatically synchronized with the camera buttons, and when in monochrome mode, the image will be pseudo colored appropriately (eg FITC is green)

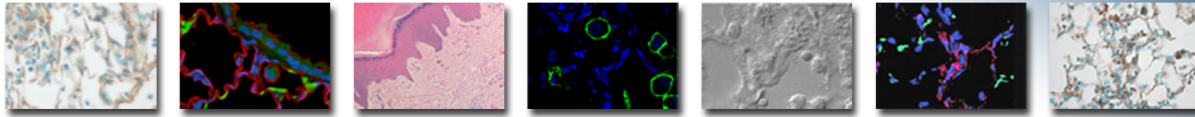# Startup – Objective Selection
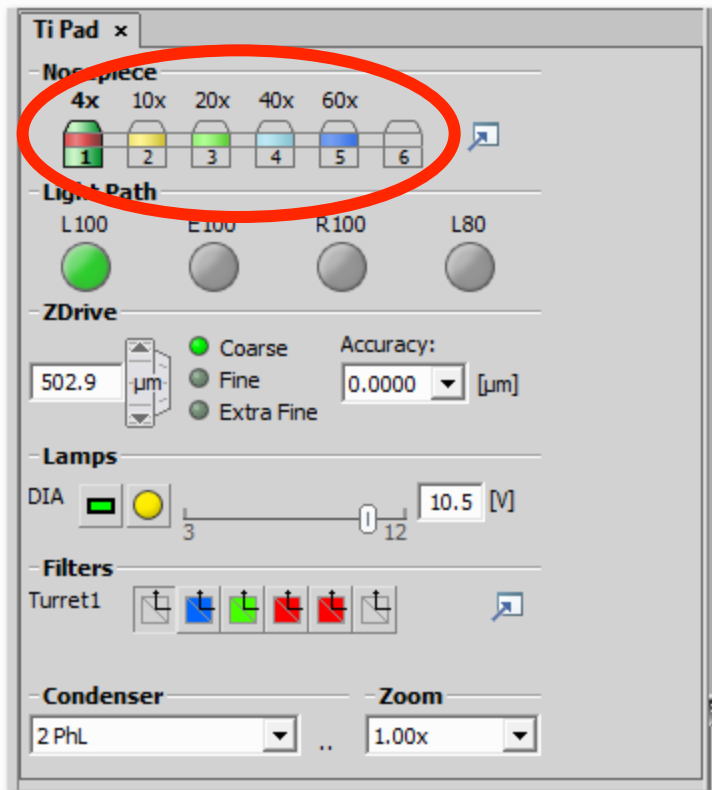
The Ti microscope control pad is located in the left control panel of the software screen.

Use the objective lens icons to select the lens you want to use.

The objective lens in place will be highlighted in the display

NOTE: Objective Lens Selection is the only control that should be used in this panel. Everything else will be controlled the Optical Configuration (OC) buttons.
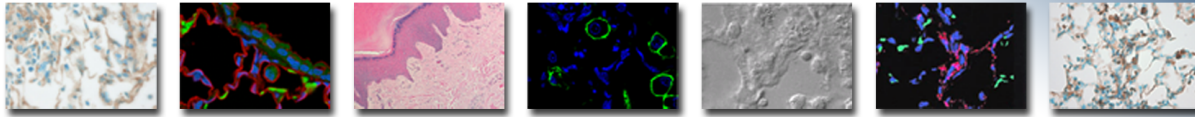
Startup – Ocular Selection

Any time you wish to view with your eyes, push the "eye" button on the left hand toolbar.
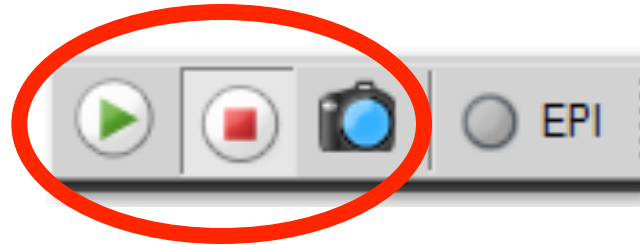
Use the OC buttons to change the microscope state as needed.

Use the "Epi" button on the top left toolbar to open/close the fluorescence shutter as needed.
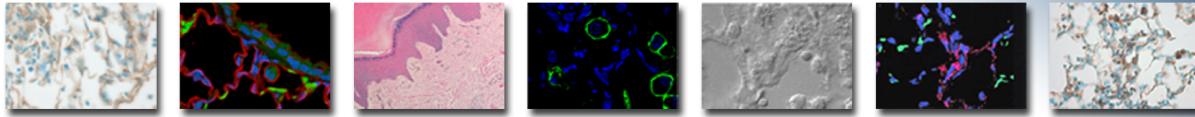
Startup – Camera Controls

Green Arrow – starts live video image on the screen – opens shutter for fluorescence OC's.

Red Square - stops live video image – closes the shutter for fluorescence OC's.
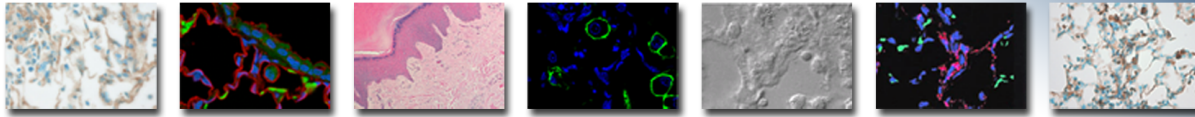
Camera - creates a new image in the software work space – opens/closes shutter for fluorescence OC's.

When you push Green Arrow or Camera buttons the software will automatically switch from eyepieces to the appropriate camera port.
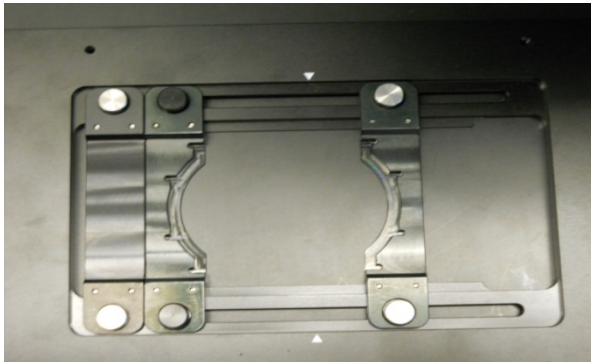
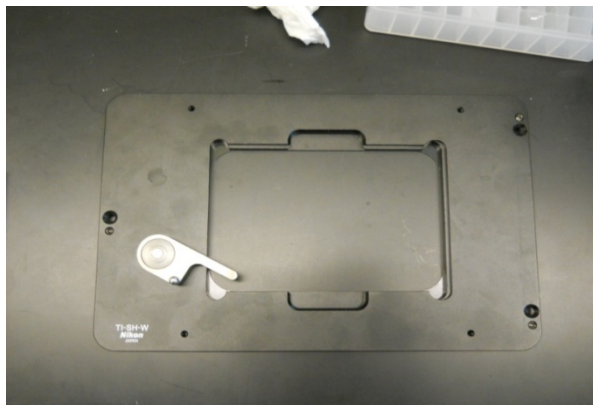**Nikon Eclipse Ti-E Operations Guide**

MICROSCOPE CONTROLS

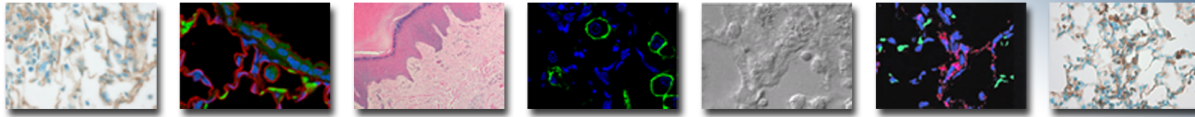# Microscope Controls – Sample Holders

Sample Holders for Stage – either one will drop into the open frame of the motor XY stage:

Universal Holder for dishes and slides – slide arms to snug up against your sample – center the sample on the white arrows on top and bottom of insert

Multi-well plate holder – spring arm keep plate tight in place

Microscope Controls – Stage Movement

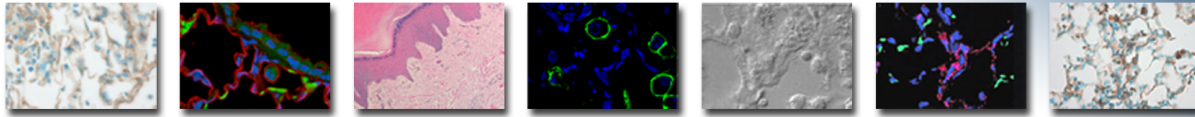**XY Joystick:**
Controls movement of XY stage

Twist just the top of knob left or right to go from coarse to fine to extra fine motion

"Z Speed":
Sets how speed of focusing

Focus drive knob:
Controls microscope focus

Do Not Use "Constant Speed" button

Microscope Controls – Correction Collar

The 20x, 40x and 60x objectives have a correction collar, which allows the user to adjust the lens for the thickness of the plastic/glass of the specimen vessel.
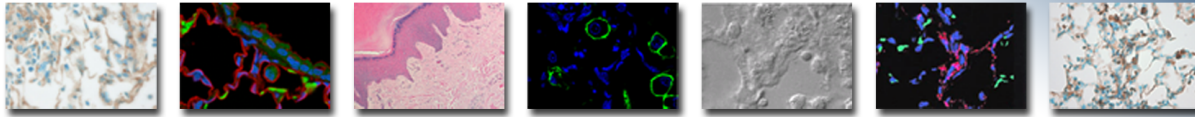
The collar numbers are in mm units:

0.17 is a standard cover slip
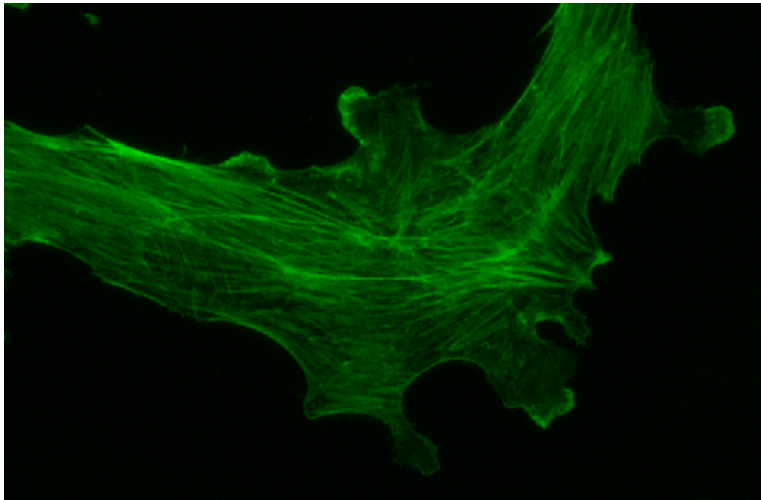
1.2 is a standard TC vessel

1-2 marks under 1.2 is a standard slide thickness

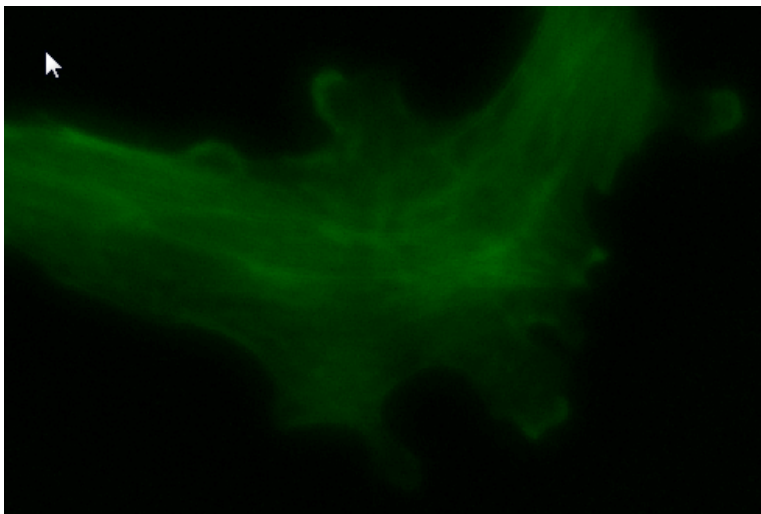Adjust the collar relative to the fixed reference mark to set for thickness.

In practice you will likely turn the collar a bit and then refocus the microscope in repeated successions to hunt for the ideal setting.
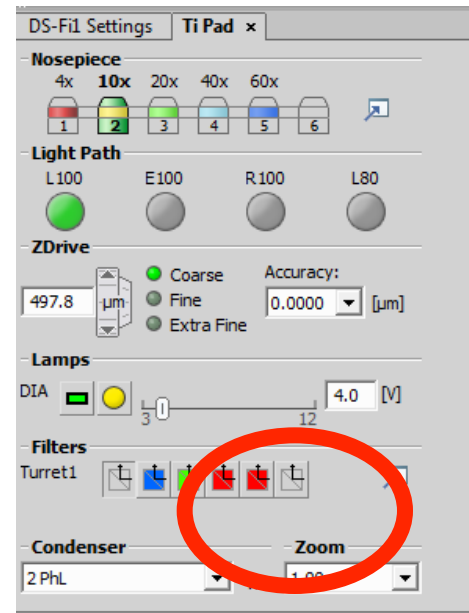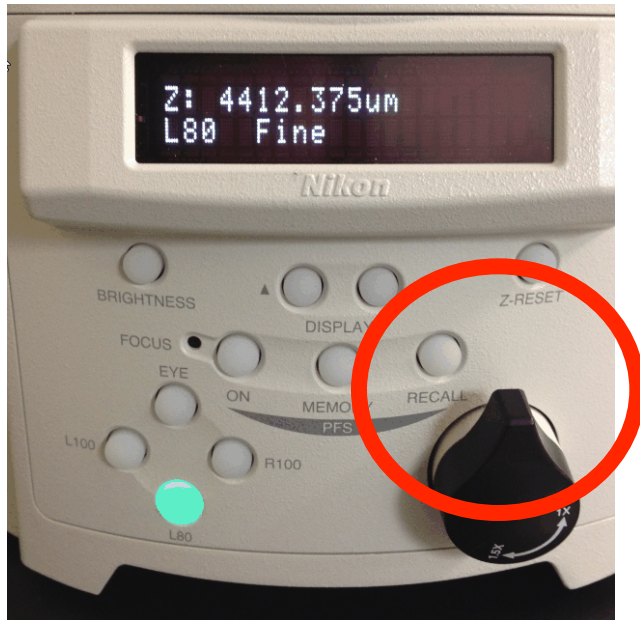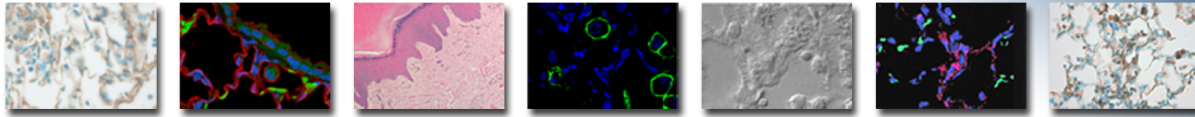
Microscope Controls – Correction Collar

Correction collar setting will produce a sharp image if correct

And a blurry image if incorrect. It will look out of focus and there will be no way to get it into focus.
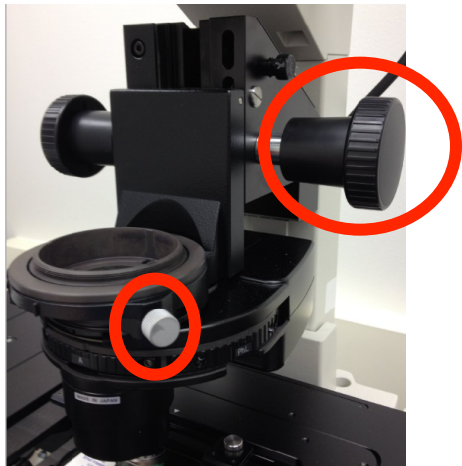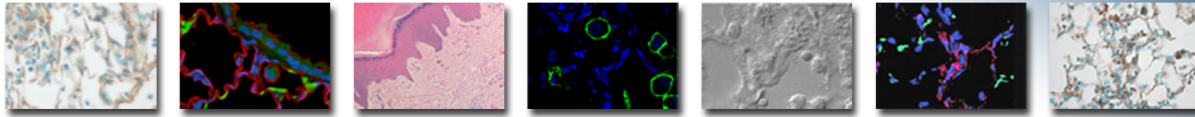
Microscope Controls – Zoom Knob

1x is standard – can be set to 1.5x for more magnification at all lenses
Note: if you set this to 1.5x you must also change the zoom setting in the software to match for proper image calibration.
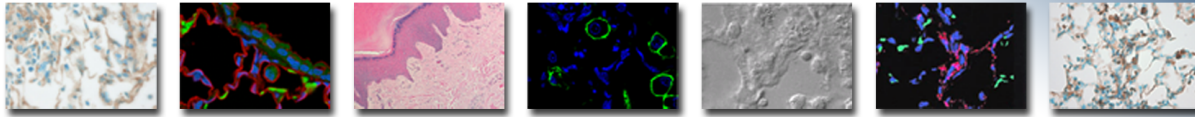
Microscope Controls – Condenser

Condenser Height Knobs – leave condenser at bottom of travel

Condenser Centering Knobs – do not adjust

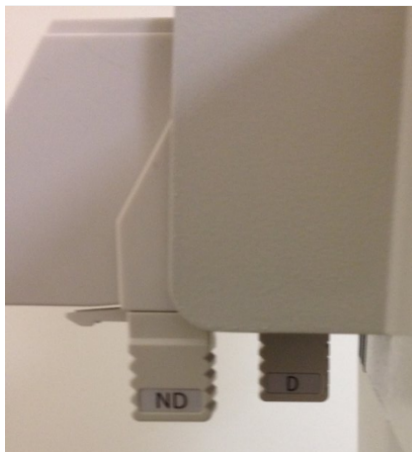Transmitted Light Field Diaphragm – leave fully open (fully to right)
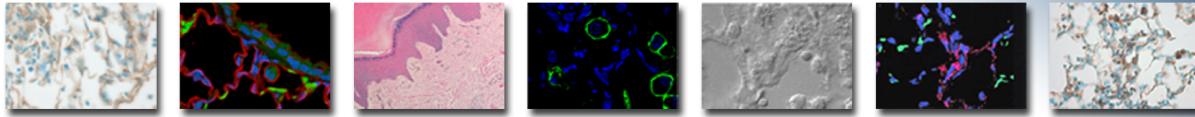
Microscope Controls – Filters

NCB Filter pushed in

GIF Filter pulled out

D Filter pushed in

ND Filter pulled out

Microscope Controls – Filters

Right Side

"Filter" is empty – do not use

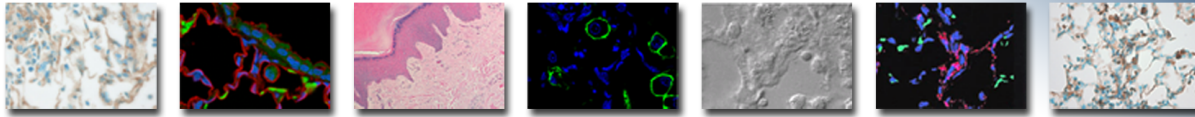"ND4 and ND8" – cut down the fluorescence light intensity. Can be pushed in as needed.

"AS" Slider Arm – should ALWAYS be pulled out (toward you)

Left Side

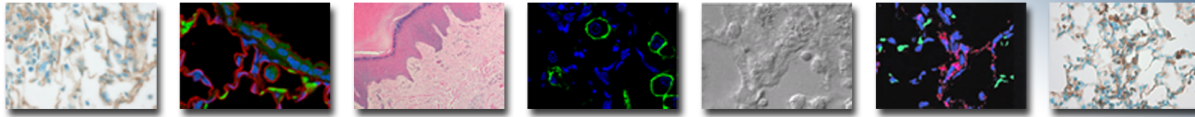"F" Slider Arm – should ALWAYS be pulled out
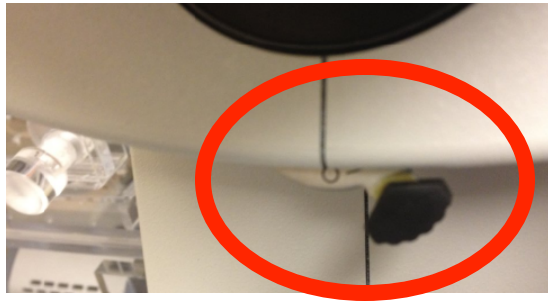
Microscope Controls – Manual Shutter

Manual Fluorescence Light Shutter – the light source has a built-in motorized shutter. This manual shutter should remain in the open "O" position.

Right side or microscope, under the objective lenses.
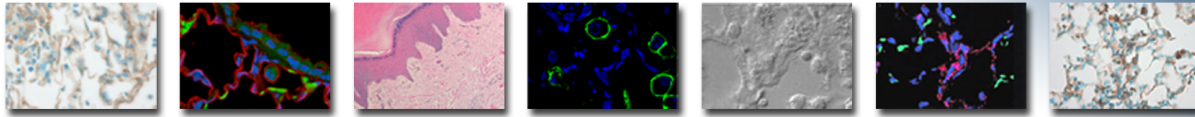
Microscope Controls – Binocular Head

Under the eyepieces:

This lever should be set to "O"

This slider should be pushed "IN"
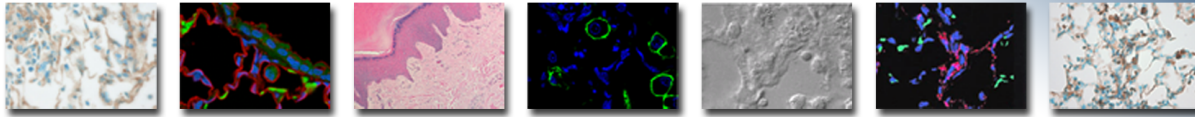
The "B" knob should not be adjusted.

Microscope Controls – Main Body

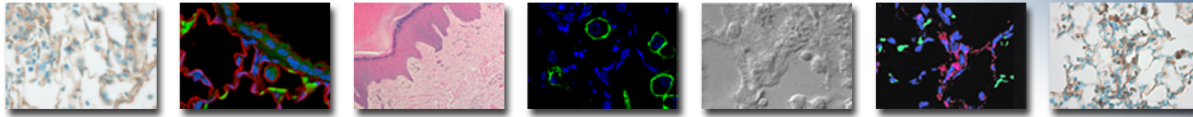There is no need to touch any of the buttons on the stand of the microscope.

All of the functions of the microscope stand should be controlled by software "Optical Configurations" (OC's).There is a focus knob control on the stage XY joystick. But, you can also use the focus buttons on the microscope stand if desired.

Nikon Eclipse Ti-E Operations Guide
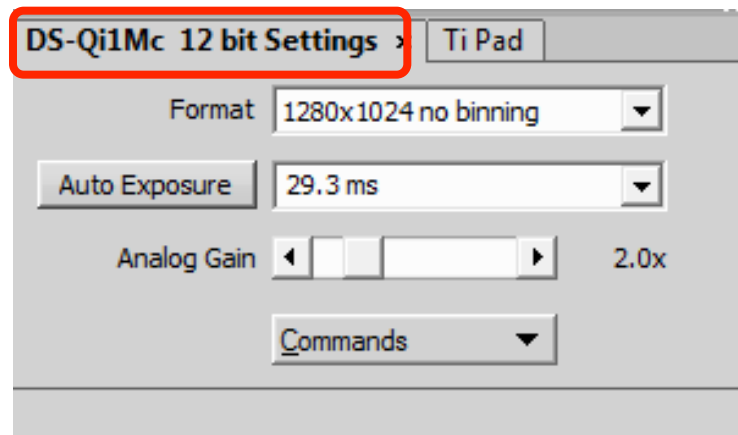
CAMERA CONTROLS

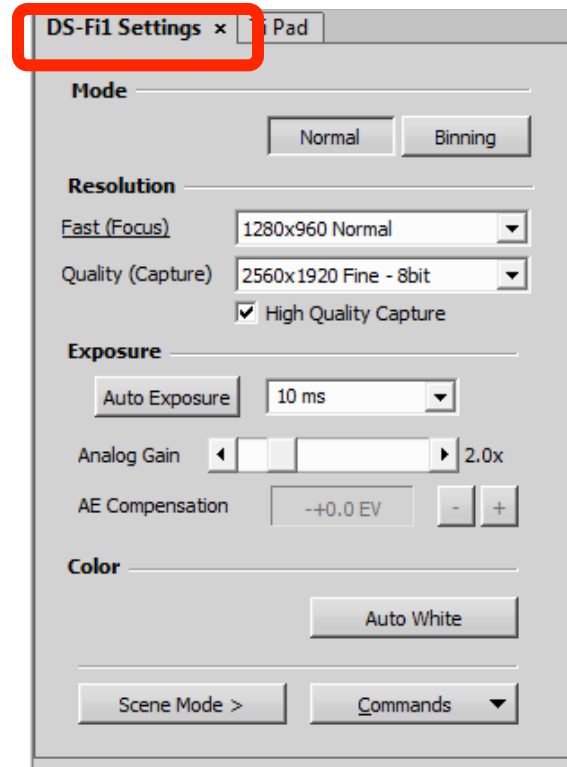# Controls Controls – Camera Settings

Camera Settings Box appears in the upper left corner of the software workspace and changes as the camera is changed
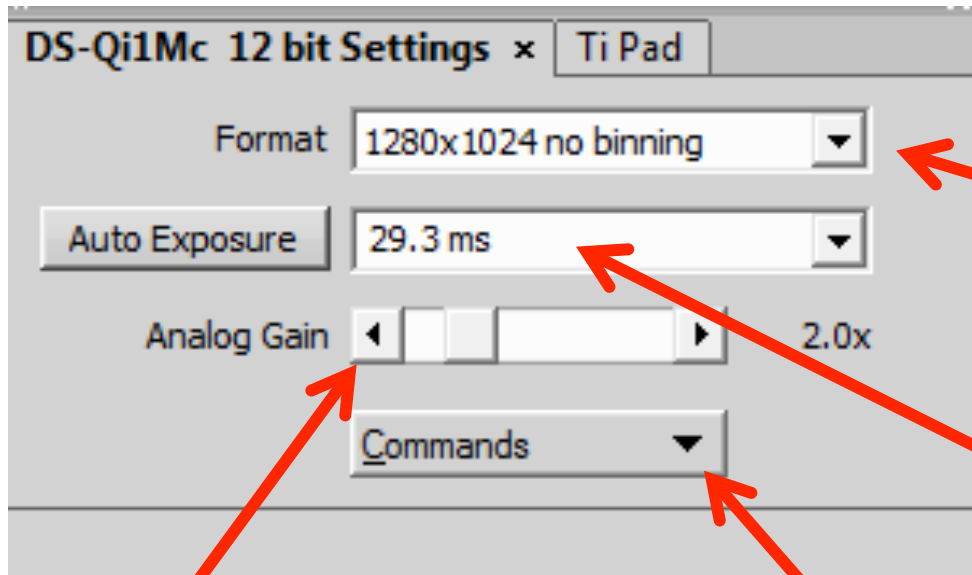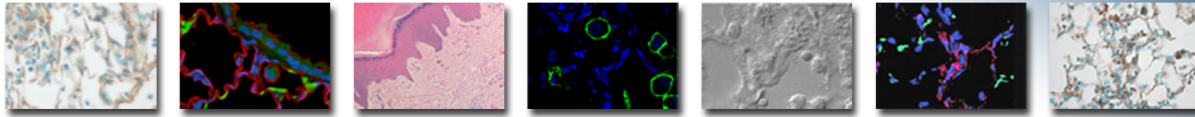
Note title of camera at top

Qi1 Monochrome

Fi1 Color

Controls Controls – Qi1 Settings

DS-Qi1Mc 12 bit Settings × | Ti Pad

Format | 1280x1024 no binning ▼

Auto Exposure | 29.3 ms ▼

Analog Gain | ◄ [   ] ► | 2.0x

Commands ▼

Format:
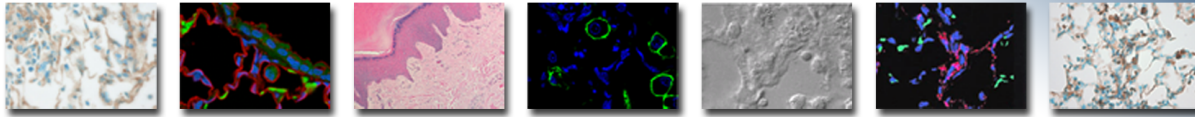**No Binning –** highest resolution images.
**Binning –** lower resolution but higher sensitivity

Exposure Setting:
Auto Exposure – push once and it sets exposure for that image
Manual Exposure – select from pull down list or type in an exposure time

Note: Do not use Commands menu

Analog Gain:
Use slider bar to increase brightness in image for any given exposure time
By increasing gain you can use shorter exposures, but noise is introduced in image

Controls Controls – Fi1 Settings
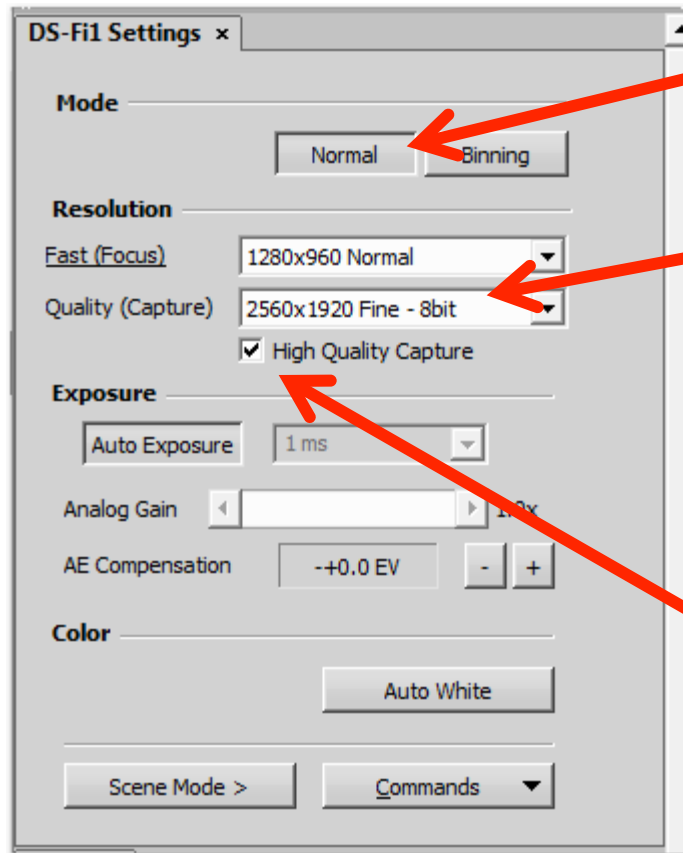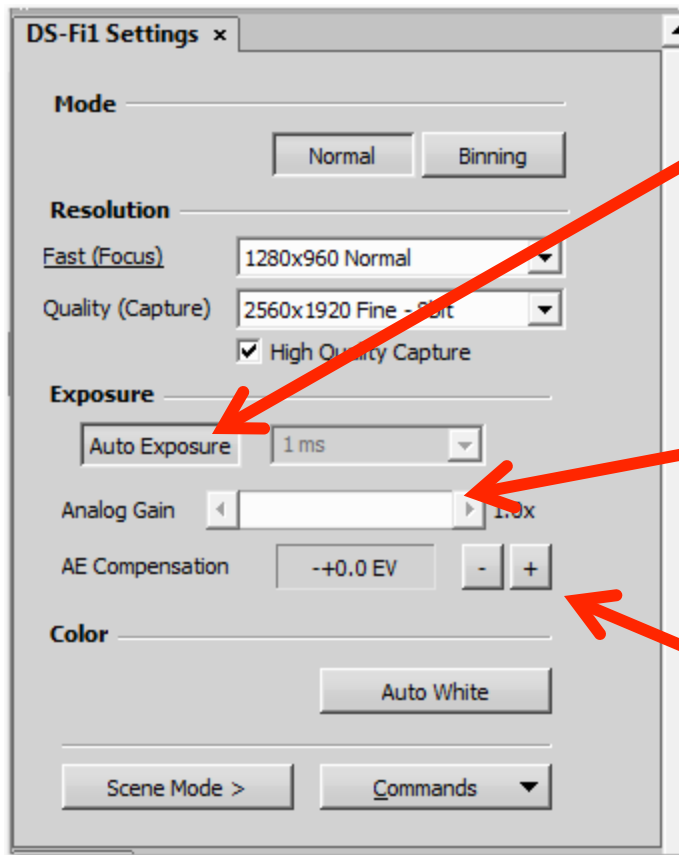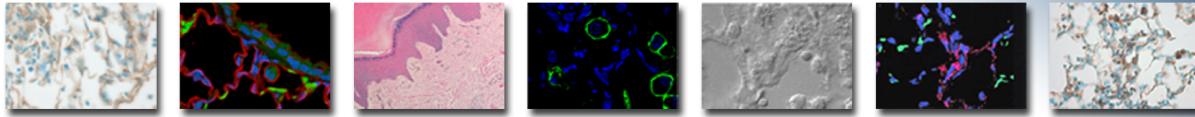

Mode:
Always use "Normal"

Resolution:
Fast – for live focus image
Quality – for captured image

Note: using lower resolution for live image makes live image go faster

High Quality Capture:
Always leave this checked
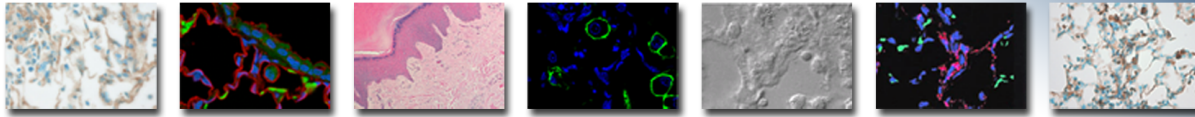
Controls Controls – Fi1 Settings

Push "Auto Exposure" button to let software determine best exposure.

This is recommended setting.

"Analog Gain" is automatically set by software as well when using Auto Exposure

"AE Compensation" can be used to push software to increase or decrease its exposure setting

Controls Controls – Fi1 Settings

Unselect "Auto Exposure" and choose exposure time from pull down list.

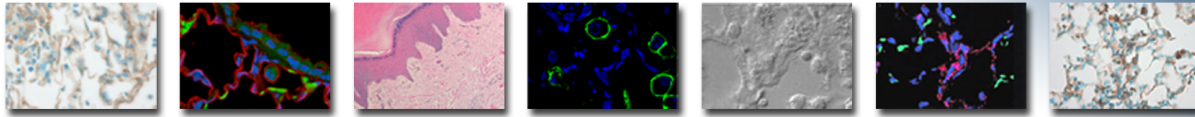"Analog Gain" can be set to fine adjust the intensity in the image.

Controls Controls – Fi1 Settings

"Auto White" will set white balance so camera can produce proper color.

Go to empty area of slide and push Auto White to set white balance.

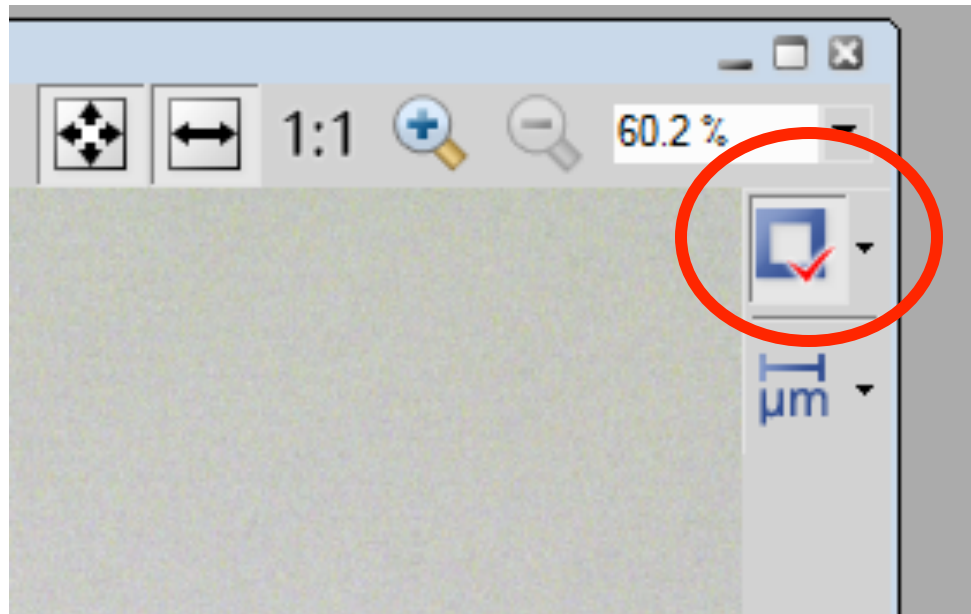Do Not use "Scene Mode" or "Commands" controls.
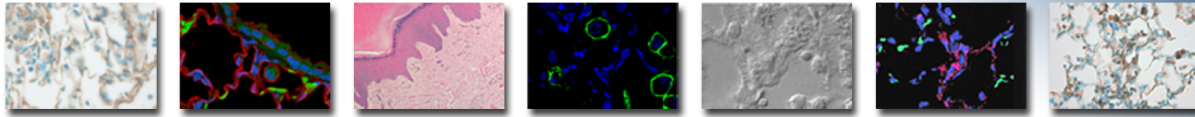
Nikon Eclipse Ti-E Operations Guide

SOFTWARE CONTROLS

Software Controls – White Balance

If you did not white balance ahead of acquisition, you can activate the white balance probe – located on the upper right of the image window - size it, and move it to a blank area in the existing field to perform white balance.
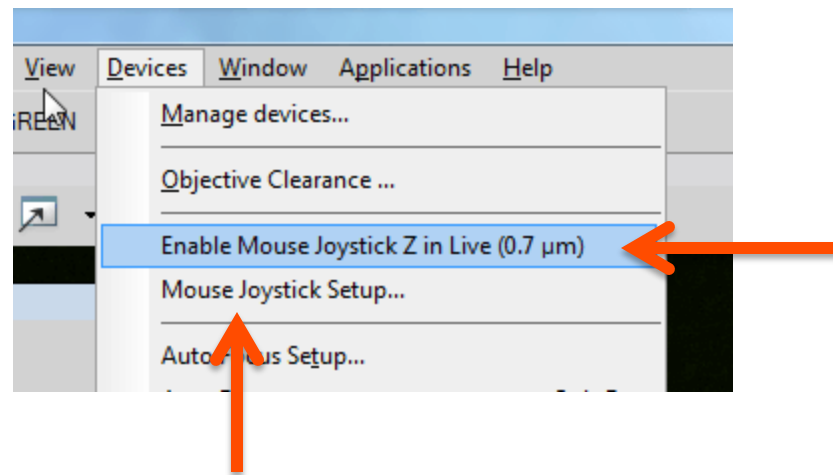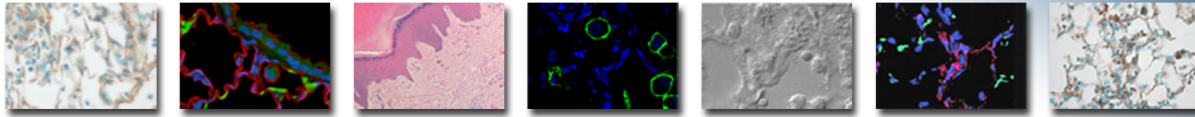
Software Controls – Live Mouse XY

At the top of the "Live" image window is a button to use the Mouse XY. Push this button in and you can use the mouse to move the stage in XY by left clicking with the mouse and dragging the image in the direction you want to move it. This stays active until you push it off again.
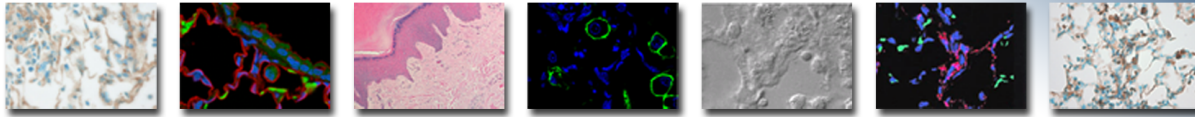
Software Controls – Live Focuse

The mouse wheel can be used to focus the microscope in live mode. It will stay selected until someone turns it off. When active, left click the mouse in the live image window once, and then turn the mouse wheel to focus up/down
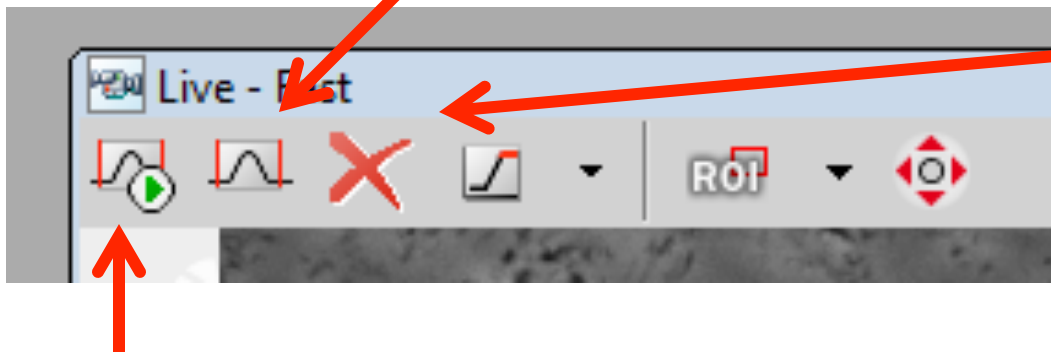

Note: Mouse Joystick Setup allows you to set the Z step size of each mouse wheel click

Software Controls – Auto Contrast

Auto Adjust Contrast – sets contrast to best viewing setting – more on contrast settings in following pages

Red "X" resets contrast to null

Keep Auto Adjust - It is common in the live image window to use the "keep auto adjust" which will continuously adjust the contrast for changing conditions in the live image window, which can be helpful when scanning around and changing between phase contrast and different filter cubes.
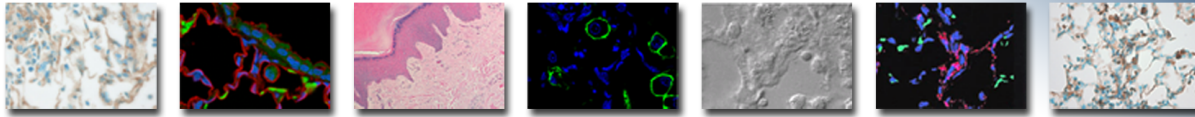
Software Controls – Other Window Controls

Pixel Saturation Indicator – when pushed it will show overexposed pixels in color you select from a pull down menu found with the black arrow

ROI controls activates a sub-region box – pull down arrow let's you start this feature and position box. Pushing ROI button turns sub-region imaging on/off
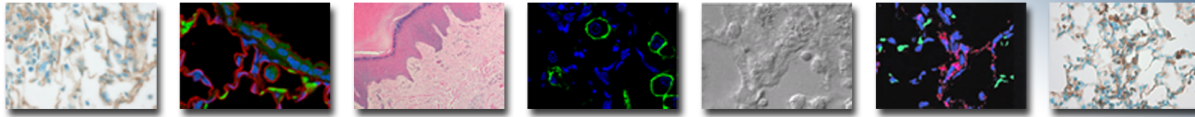
Software Controls – Scale Bar

Every Image has a scale bar button on the upper right hand side.

Push it to activate scale bar in the image.

Once active on the screen, the scale bar can be grabbed with the mouse and moved around the screen to location you desire.

Software Controls – Scale Bar Burn

Right click the mouse on the scale bar and you get two options:

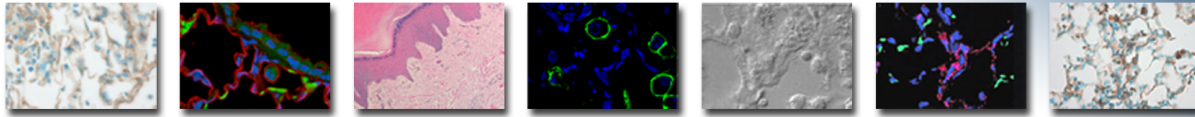**"Burn"** – which permanently stamps the bar into the image.

If the image is not already an 8-bit RGB it will now be converted to that format.

Always save out your original image before burning the scale bar, which should then be saved out as a copy of the image.

You may want to go back to the original without a scale bar. Also, initially it may take some trial to get the scale bar properties to your liking.

"Change scale properties" – set the size, color, font, etc.

Note that you can let the software auto size the length of the scale bar or uncheck and select from a pull down of different sizes.
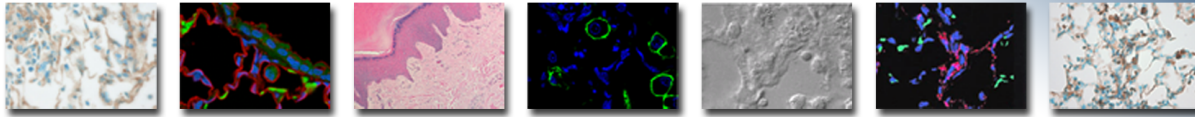
Software Controls – Autofocus

If the focus is in close range, and when changing objective lenses, you can use the software autofocus feature to critically focus:

AF1 – Primary Coarse Autofocus

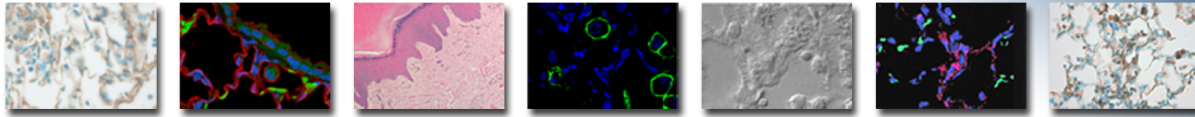AF2 – Secondary Fine Autofocus

Software Controls – Other Controls

Arrow – return to normal mouse arrow

Magnifying Glass – Click on this control to get magnifier icon on mouse. Left click activates zoom window within any image window.
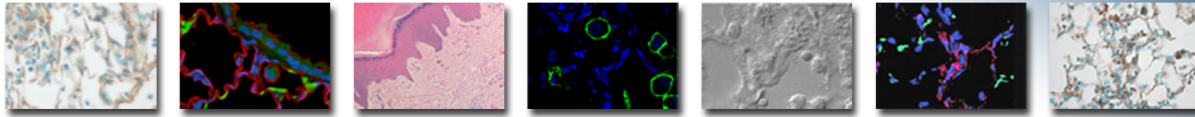
Close All – closes all open images, with a dialog prompt to be sure you want to discard all open images.

Convert Image to 8-bit RGB – converts any image to 8-bit RGB color image - discussed in detail in "Exposure and Contrast" section.

Restore All

AF1

AF2

RGB

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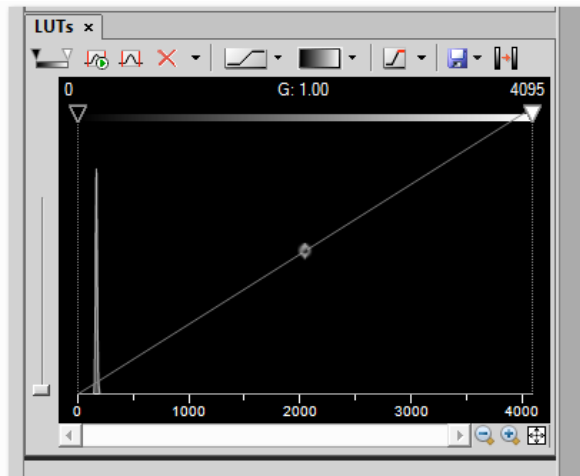
EXPOSURE AND CONTRAST
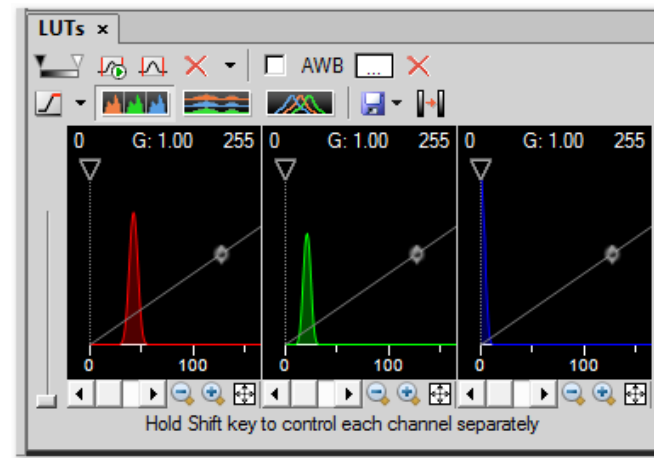
Exposure and Contrast – LUT's

The best possible image is one that fills the dynamic range of the camera without overloading any pixels and causing saturation.
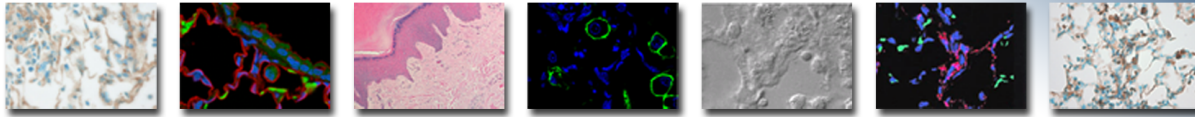
The "LUTs" window in the bottom left corner of the software window shows a graphic representation of the dynamic of the camera and the intensity of the image.

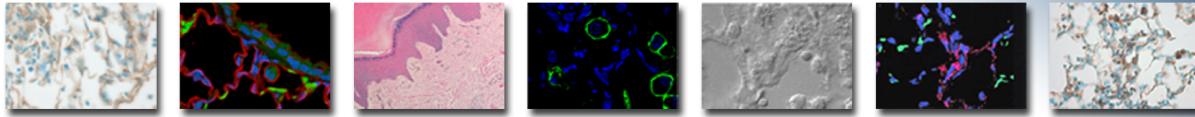Monochrome Image   Color Image

Exposure and Contrast – Methods for Use

1) Use "Auto Exposure" button in camera control box – software will determine the longest possible exposure before saturation occurs.

2) Adjust exposure manually and view histogram in LUT's window to fill dynamic range of camera to your liking.

Manual exposure adjustment is usually preferable for fluorescence imaging where auto exposure will may you an unnecessarily long exposure.
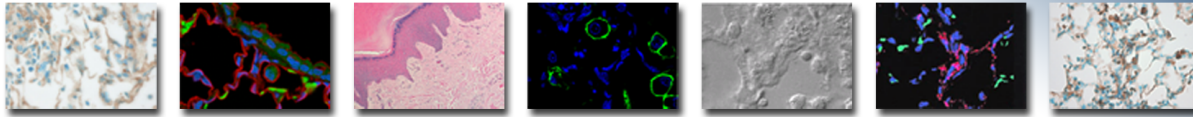
Exposure and Contrast – Saturation

The toolbar at the top of the live image window has a saturation indicator button. Pushing this will show any overexposed areas in the image.

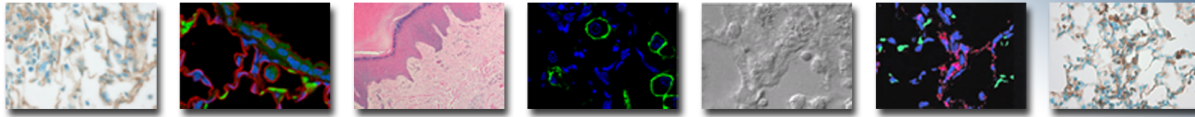The black arrow next to it will show a list of options to change the color of the indicator, etc.

Exposure and Contrast – Adjusting Contrast

It is not necessary to only use exposure to get the best and brightest possible image. It is often desirable to use a shorter exposure and then use the software contrast controls to adjust the image brightness to your liking.

The black bar is the contrast setting.
The best setting is generally just at the left edge of the image histogram.
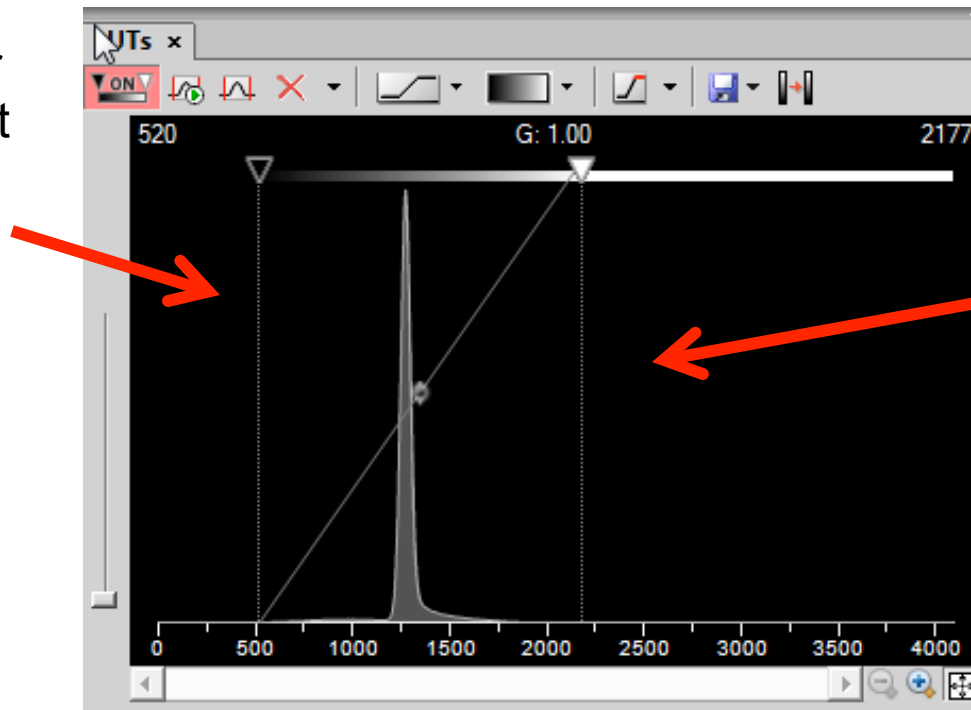
The white bar is the brightness setting.
The best setting is generally just at the very right edge of the image histogram.

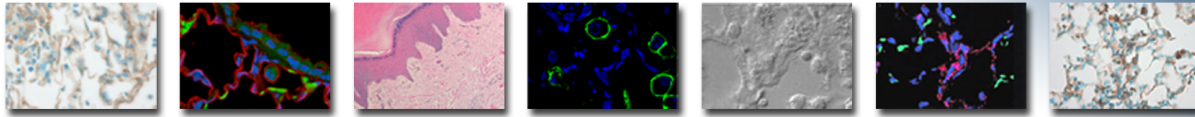# Exposure and Contrast – Adjusting Contrast

The auto adjust button will set the brightness bar at the right edge of the image histogram.

Red "X" button will reset the contrast to non-adjusted

You can use the mouse to drag the contrast bar up over from the left to adjust the background darkness.

Use the mouse to drag the brightness bar if you want to manually adjust brightness
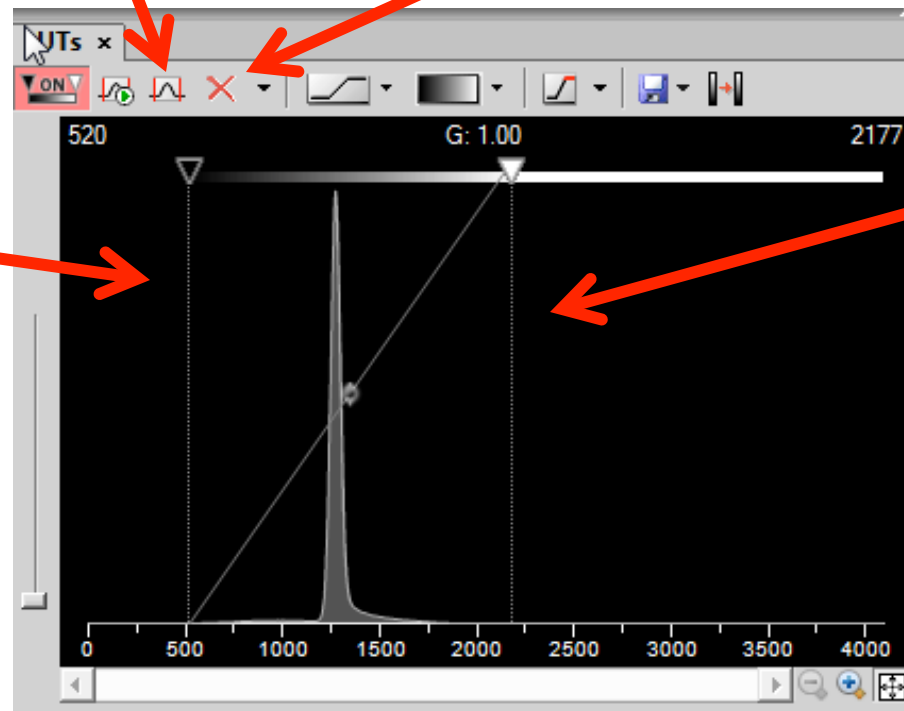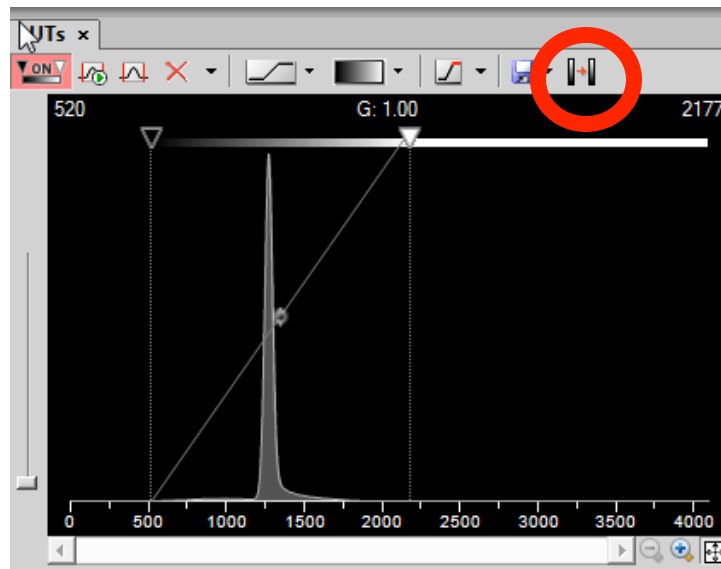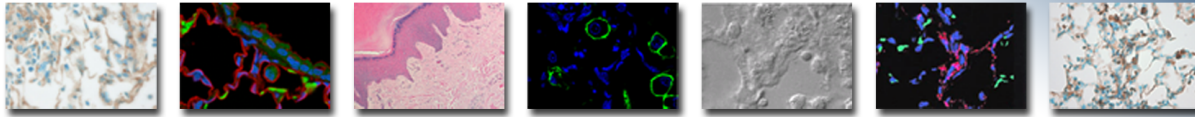
Exposure and Contrast – Adjusting Contrast

Adjusting image contrast in Elements does not change the image data, just the way it looks on the screen.

If you want to permanently fix the contrast for viewing in other programs as well, push the "Apply LUTs" button.

A warning message will indicate you are about to permanently change the image.

Note: It is best to save a raw original before applying LUT changes
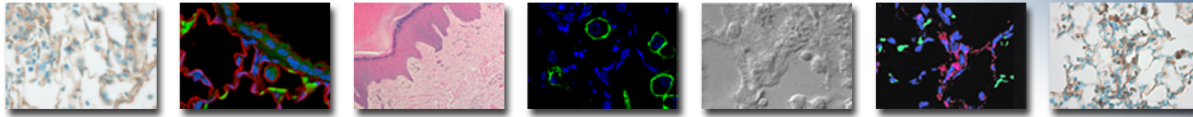
Exposure and Contrast – Saving Image Contrast

Adjusting image contrast in Elements does not change the image data, just the way it looks on the screen.

If you want to permanently fix the contrast for viewing in other programs as well, push the "Apply LUTs" button.

A warning message will indicate you are about to permanently change the image.

You can save an LUT setting and then later reapply it to another image using the "Save LUTs" button.
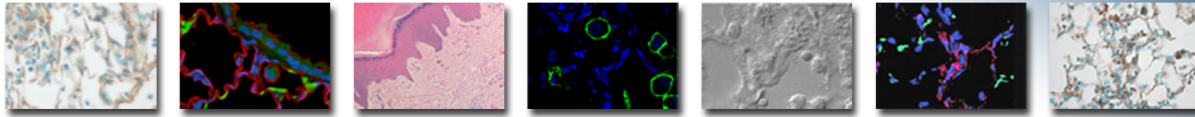
Exposure and Contrast – RGB Contrast

Real color images from the Fi1 color camera will have 3 look up tables – Red, Green, and Blue

Sliding the contrast bars will move all colors equally to maintain color balance. Holding shift key while sliding a color bar will adjust only that color, and color balance will change.
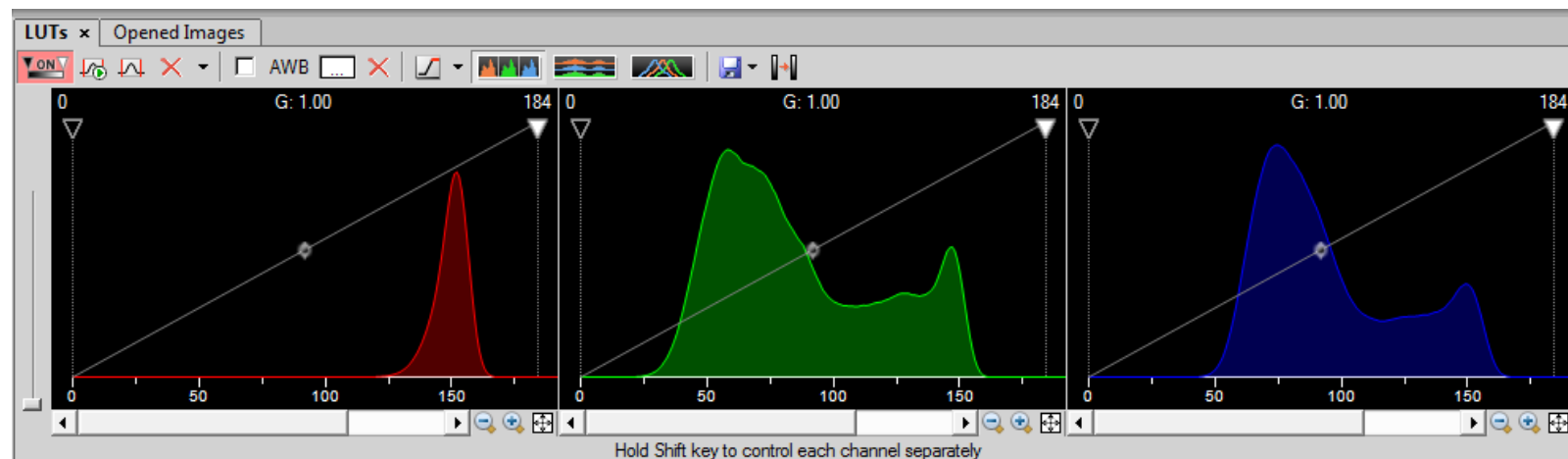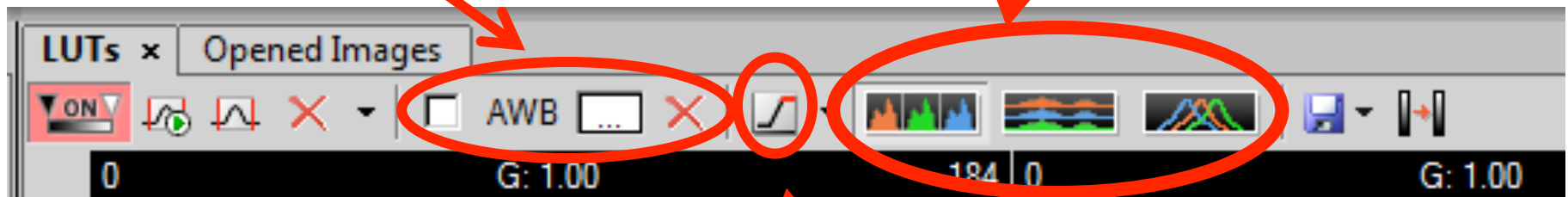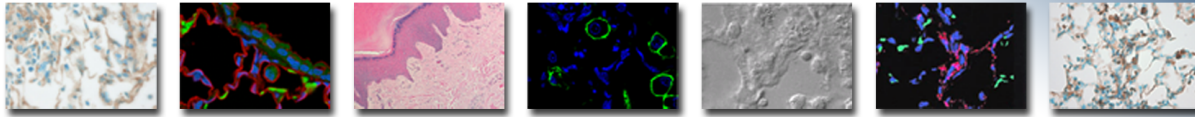
Exposure and Contrast – RGB Contrast

AWB – for post capture
white balance adjustments
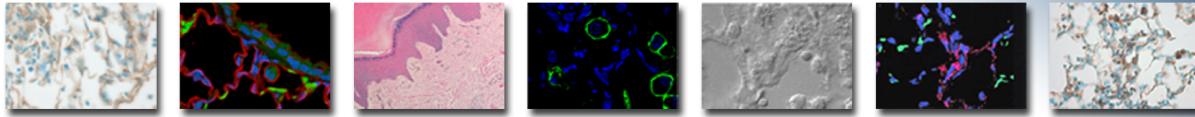
Change orientation of LUT boxes

Pixel Saturation Indicator – will
color any pixels in the image that
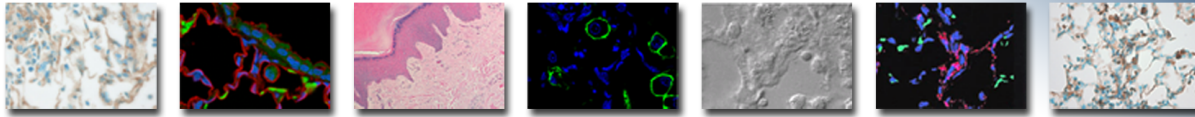are overexposed

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MONOCHROME IMAGE HANDLING

Monochrome Image – Pseudo Color

By default Elements will display a monochrome image in the pseudo color dictated by the Optical Configuration. To turn off this aid and view images in true monochrome, right click in the image window and unselect "View Channel in Color"
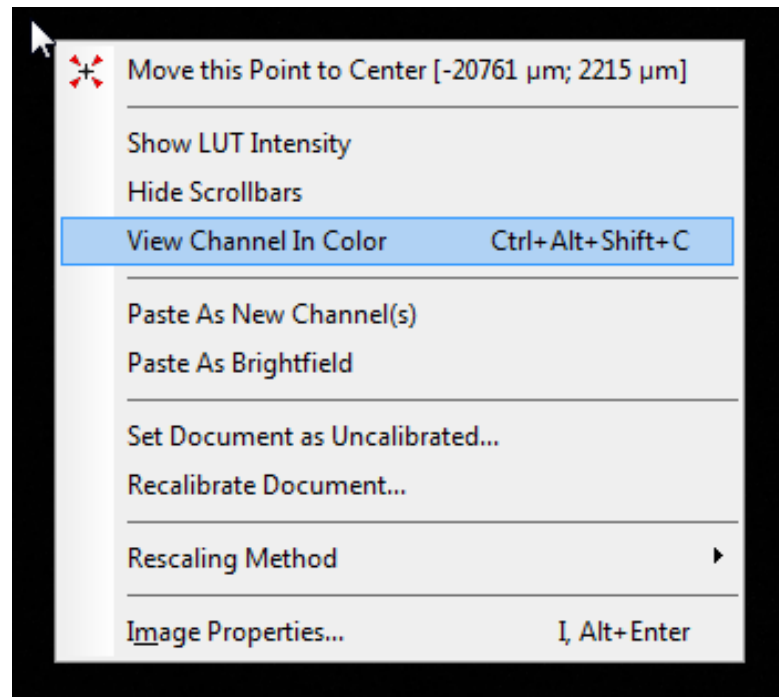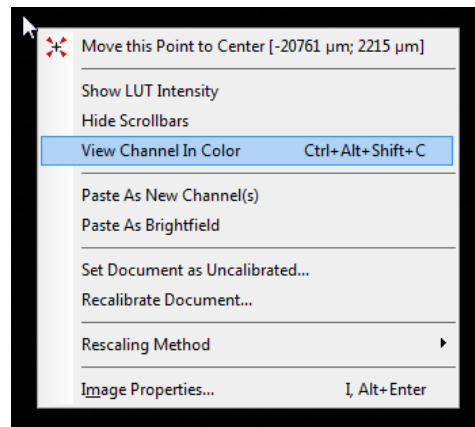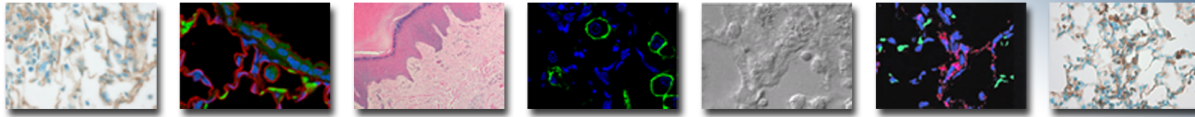
Monochrome Image – Pseudo Color

The pseudo color you see in Elements software will not follow the image if you try to just save it and open in another program. See the section that follows later on converting images to 8-bit RGB.
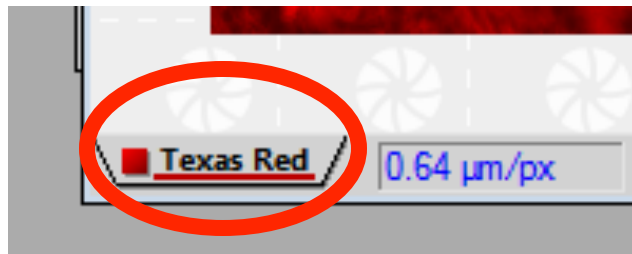
Note: Any single pseudo color image must have "View Channel in Color" active before it can be made into a color 8-bit RGB image.
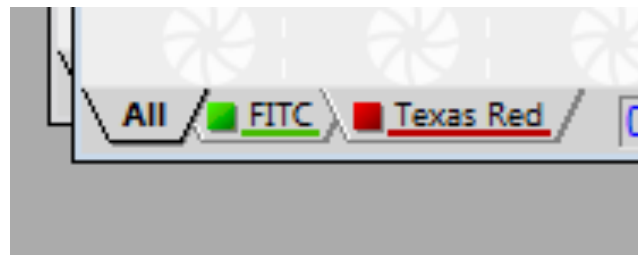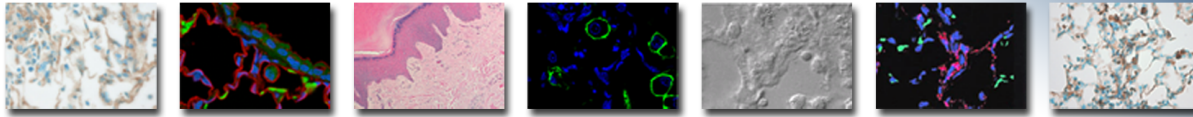
Monochrome Image – Overlay

To make a multi-color image from single pseudo color images you can overlay them with each other – including overlaying phase contrast with fluorescence.

To overlay, use the mouse to left click on the **_name tab_** of the image you are moving, and then holding down the mouse drag and drop it into the **_image space_** of the other image.
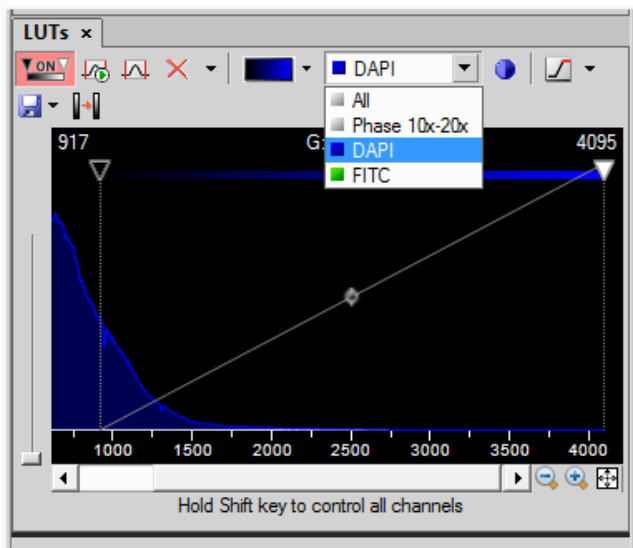

The result image will now have multiple name tabs – "All" which will show all colors, and then a tab for each color that will just show that individual color in the image window.
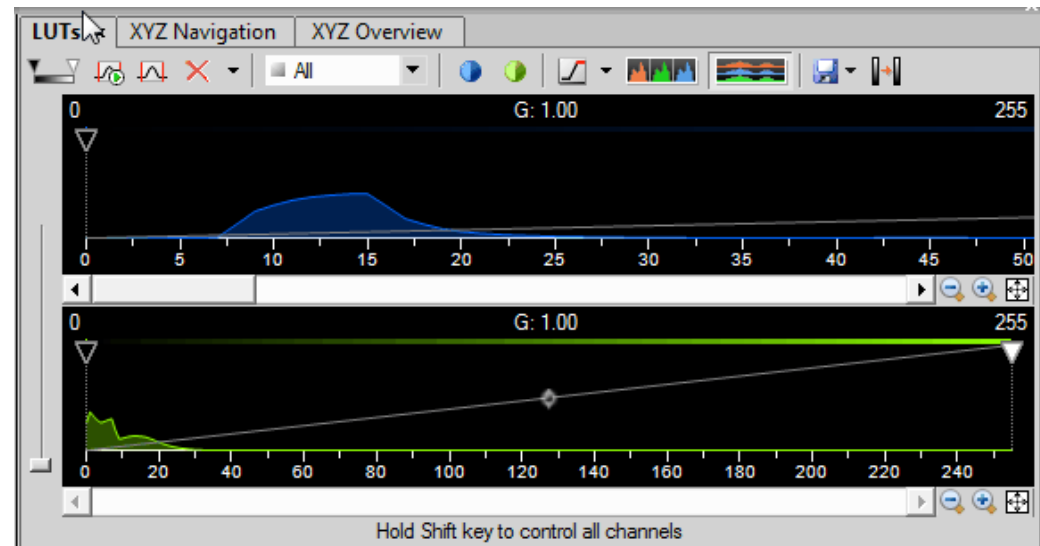
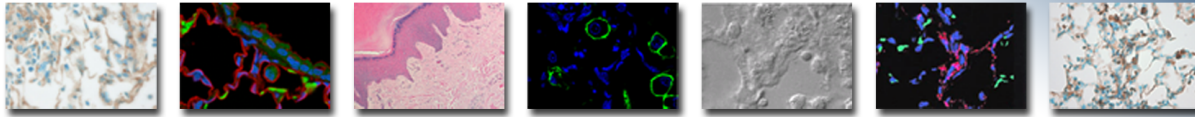# Monochrome Image – Pseudo Color Multi Channel Image Contrast

When you overlay multiple monochrome images, you will get multiple contrast windows for each color of your overlay. Colors can then be adjusted separately to balance the look of the image.

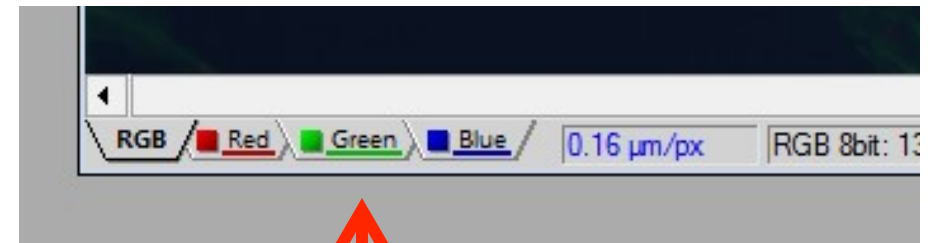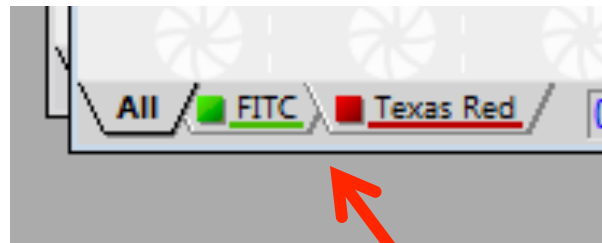Single Channel View   Multi-Channel View

Monochrome Image – Pseudo Color to RGB

To take images out of Elements and display them in other programs, you can convert to an 8-bit RGB image.

Press the quick button on the left hand side of the screen to make the conversion.

It is prudent to save the original image before making the 8-bit RGB copy so you have a raw file.

Note: The image name tab will change to RGB tabs.