Introduction to Photoshop

Images may be processed for presentation purposes or for subsequent analysis

Programs such as Photoshop and ImageJ, among others, are used to minimize defects in otherwise correctly obtained images. Images may also have structures enhanced or diminished for analysis.

"Image processing should never add anything to the image. In general, the role of the various processing operations is to remove or reduce one portion of the image contents so that others may be better seen or measured."

~ John Russ

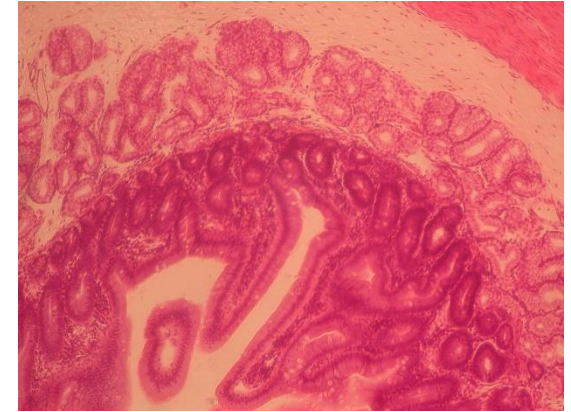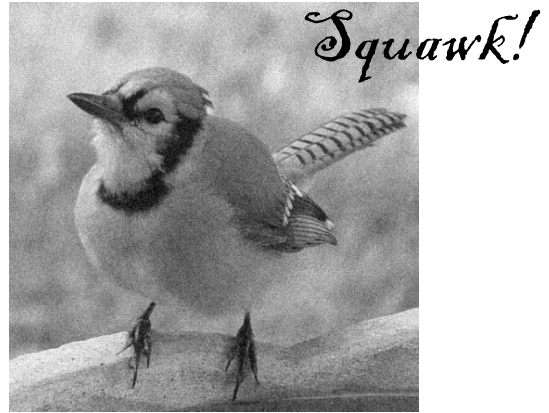FYI

Background Information Only

Image Defect Examples

Uneven background illumination

Color imbalances

Noise & scratches

Contrast issues

Photoshop Workspace

Tool attributes

Palettes in reserve

[Set number of undos in Preferences]

HISTORY

LAYERS

Tool bar

Palette

Photoshop Toolbar

Selection

Crop

Eraser

Scroll & mag.

Default

Move

Paint

Dodge & burn

Text

Line (arrow)

Measure

Swap colors
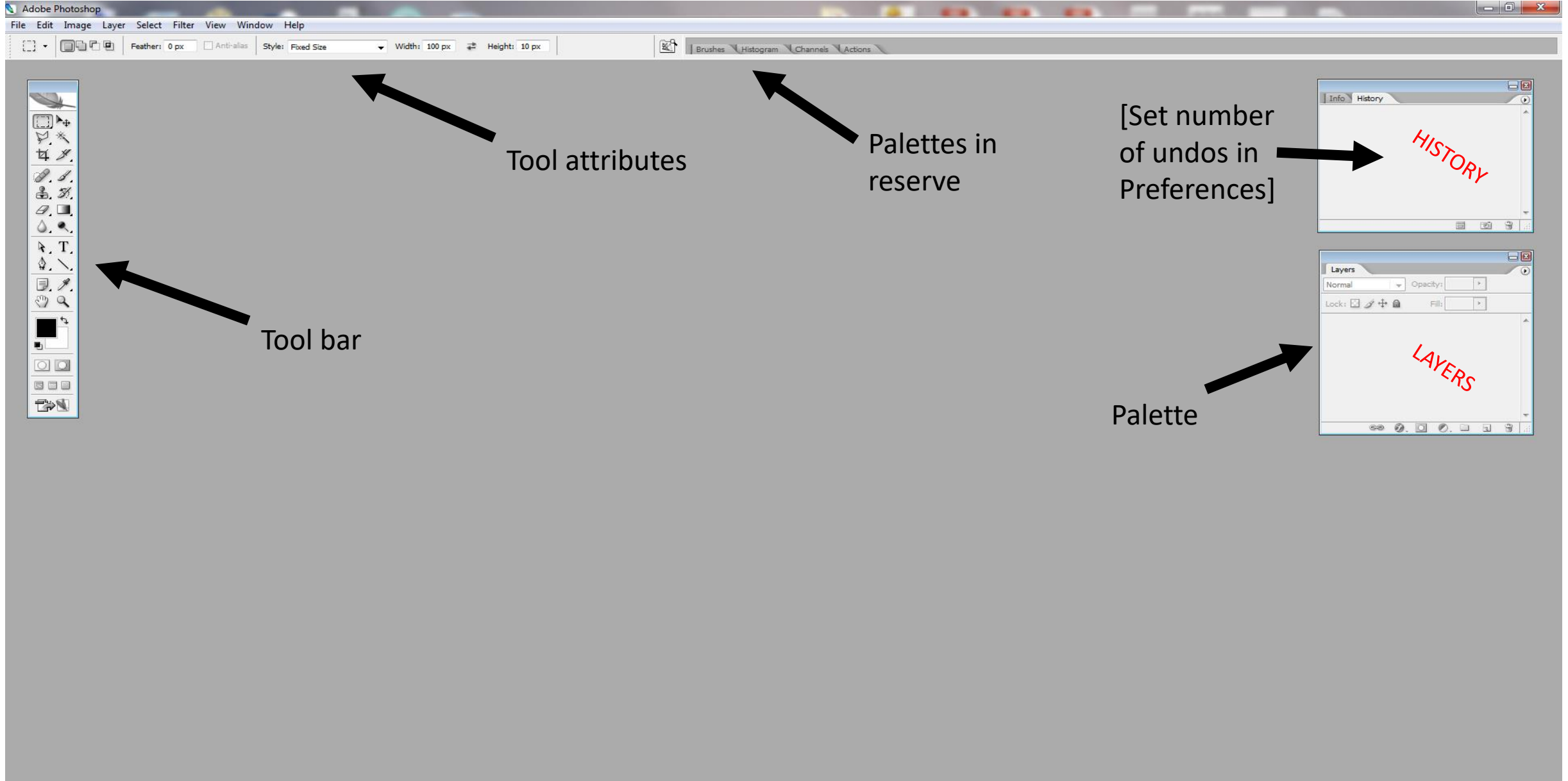
Foreground & background color
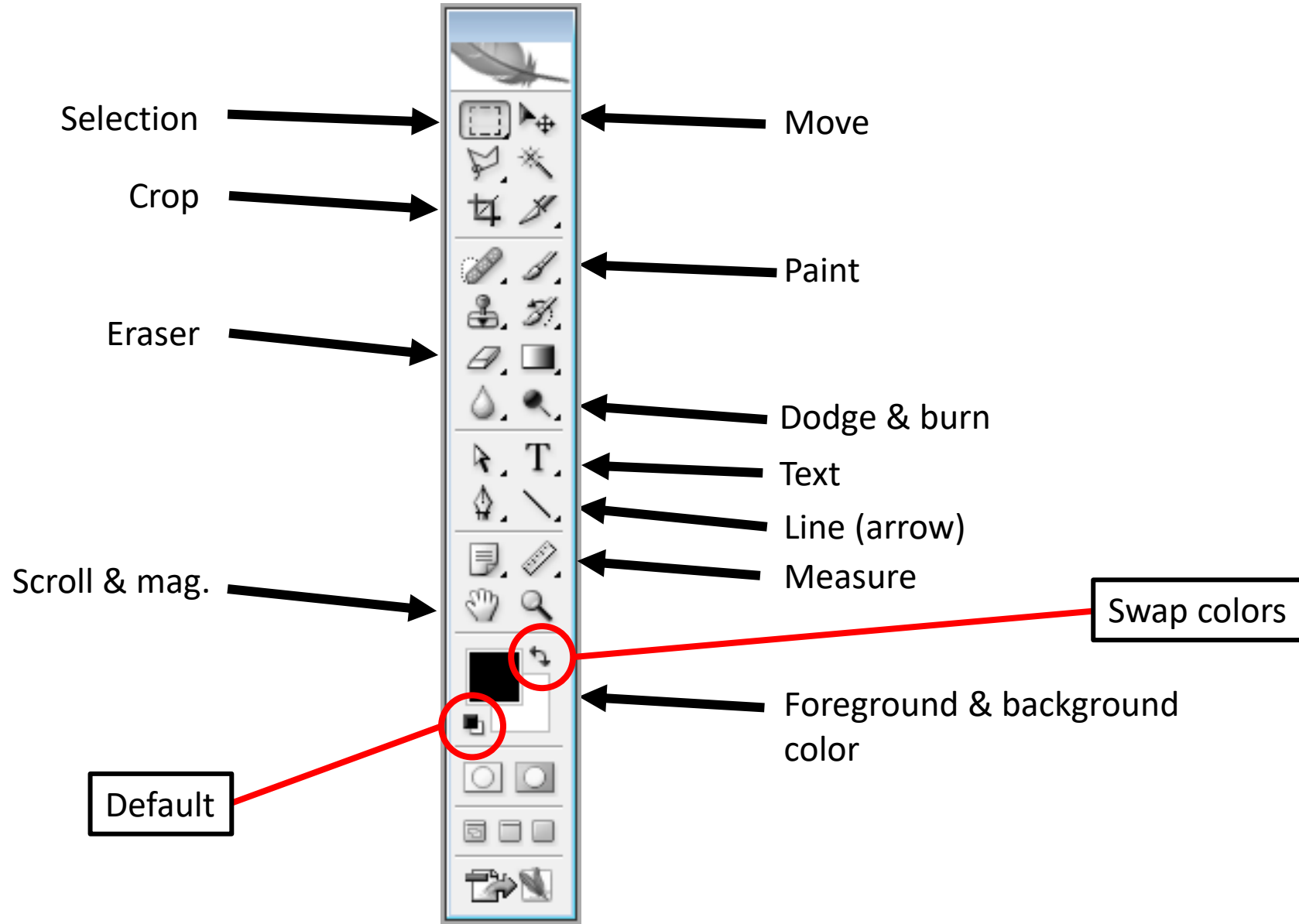
Photoshop Toolbar (cont.)

Tools with black arrowheads in the lower right corner have related but hidden tools

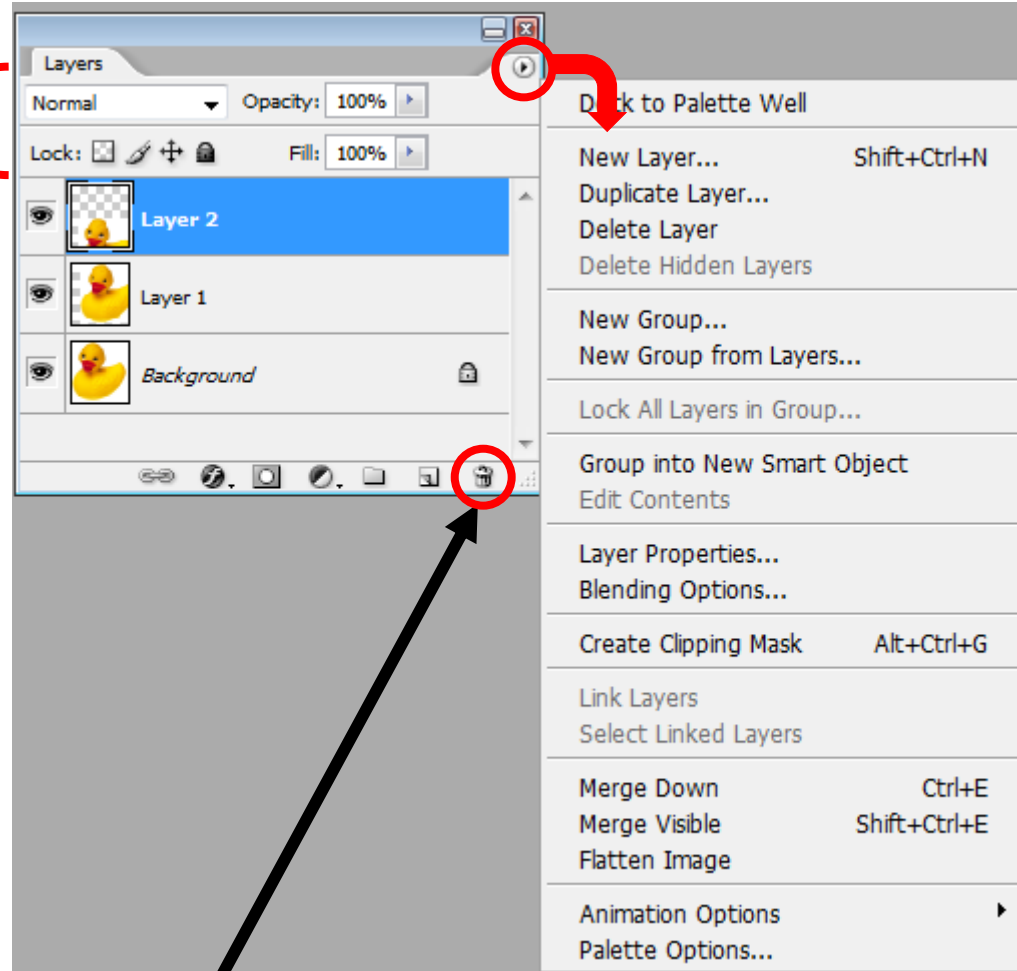|			
	☐	☐ Rectangular Marquee Tool	M
		○ Elliptical Marquee Tool	M
		═══ Single Row Marquee Tool	
		▌ Single Column Marquee Tool	

Click with mouse to change colors

Color Picker

Select foreground color:

OK
Cancel
Color Libraries

H: 0 °   L: 0
S: 0 %   a: 0
B: 0 %   b: 0

R: 0   C: 63 %
G: 0   M: 52 %
B: 0   Y: 51 %
         K: 100 %

000000

☐ Only Web Colors

Palette Parts

Palette options →

Menu options (drop down box) →

Trash

Layers

Normal   Opacity: 100%
Lock: Fill: 100%

Layer 2
Layer 1
Background

Dock to Palette Well

New Layer...   Shift+Ctrl+N
Duplicate Layer...
Delete Layer
Delete Hidden Layers

New Group...
New Group from Layers...

Lock All Layers in Group...

Group into New Smart Object
Edit Contents

Layer Properties...
Blending Options...

Create Clipping Mask   Alt+Ctrl+G

Link Layers
Select Linked Layers

Merge Down   Ctrl+E
Merge Visible   Shift+Ctrl+E
Flatten Image

Animation Options
Palette Options...

Frequently Used Photoshop Menu Items

Adobe Photoshop

File   Edit   Image   Layer   Select   Filter   View   Window   Help
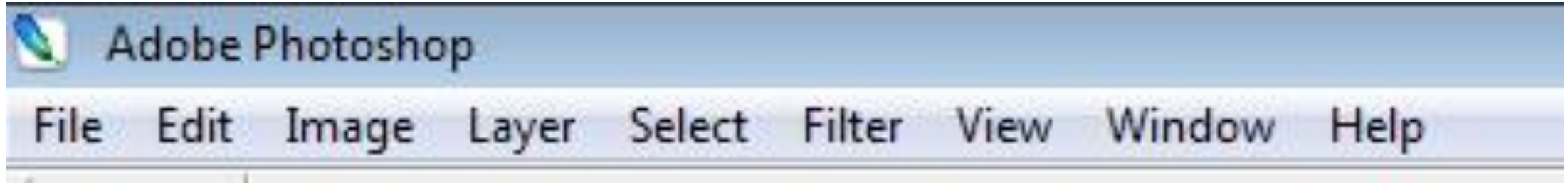
Fill & transform

Color modes & Image adjust; Image size

Median, smooth & sharpen

Rulers & guides

Palettes

Photoshop 'Image' Menu

Photoshop
Image | Layer | Select | Filter | View | Window | Help

Mode	▶	Bitmap
Adjustments	▶	Grayscale *
		Duotone
Duplicate...*		Indexed Color...*
Apply Image...		✔ RGB Color *
Calculations...		CMYK Color *
		Lab Color *
Image Size...*	Alt+Ctrl+I	Multichannel
Canvas Size...	Alt+Ctrl+C	✔ 8 Bits/Channel
Pixel Aspect Ratio	▶	16 Bits/Channel
Rotate Canvas	▶	32 Bits/Channel
Crop		
Trim...		Color Table...
Reveal All		
Variables	▶	
Apply Data Set...		
Trap...		

Photoshop
Image | Layer | Select | Filter | View | Window | Help

Mode	▶	☐ Show Transform Controls	
Adjustments	▶	Levels... *	Ctrl+L
		Auto Levels	Shift+Ctrl+L
Duplicate...		Auto Contrast	Alt+Shift+Ctrl+L
Apply Image...		Auto Color	Shift+Ctrl+B
Calculations...		Curves... *	Ctrl+M
		Color Balance... *	Ctrl+B
Image Size...	Alt+Ctrl+I	Brightness/Contrast... *	
Canvas Size...	Alt+Ctrl+C		
Pixel Aspect Ratio	▶	Hue/Saturation..*	Ctrl+U
Rotate Canvas	▶	Desaturate *	Shift+Ctrl+U
Crop		Match Color...	
Trim...		Replace Color...	
Reveal All		Selective Color...	
		Channel Mixer...	
Variables	▶	Gradient Map...	
Apply Data Set...		Photo Filter...	
		Shadow/Highlight...	
Trap...		Exposure...	
		Invert *	Ctrl+I
		Equalize *	
		Threshold...	
		Posterize...	
		Variations...	

* - Items discussed in lecture

Pixels

❑Pixel = picture element

❑Smallest unit of a digital image

❑Properties
• Usually square
• Location
• Dimension (if calibrated)
• Color or brightness value

Tile mosaic

Resampling

Resampling refers to:
- combining pixels into a larger 'super-pixel'
- subdividing a pixel into smaller units
- **Important!!: Note that the pixel dimensions (nm, μm, etc.) will change**

1 unit

4 units
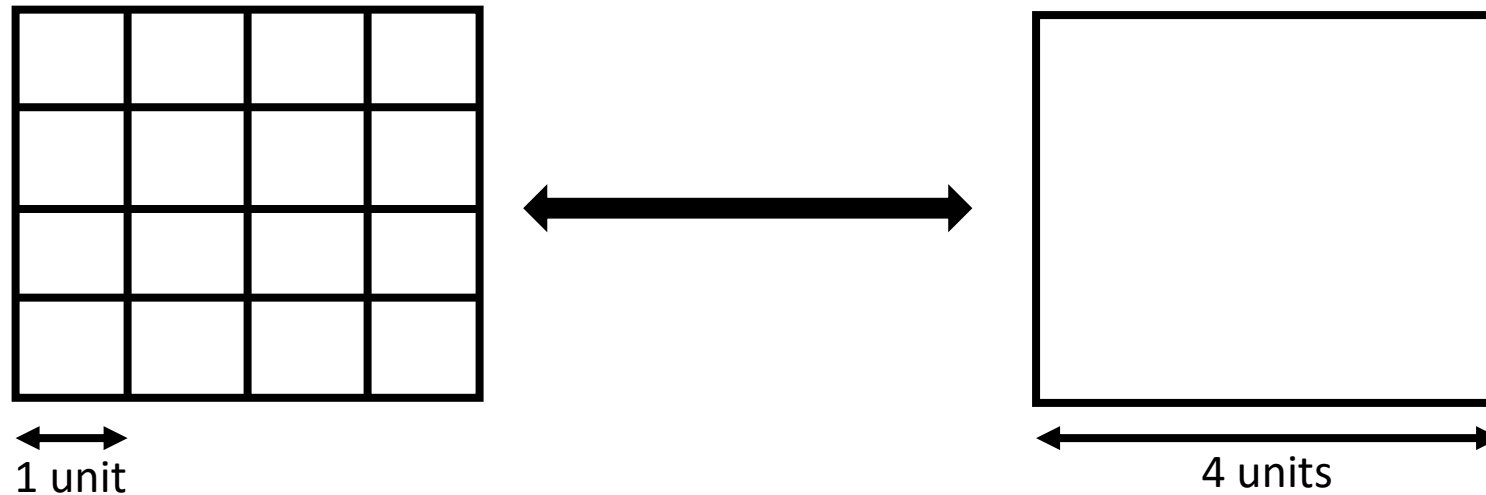
A related concept is **binning** during image collection to:
- Increase collection speed
- Increase signal-to-noise ratio
- Decrease file size
     [Reduced spatial resolution]

Resolution is set during image collection and cannot be increased by subdividing a pixel into smaller units later.

- BP

Resampling images

Image > Image Size

Resample off

Resample on
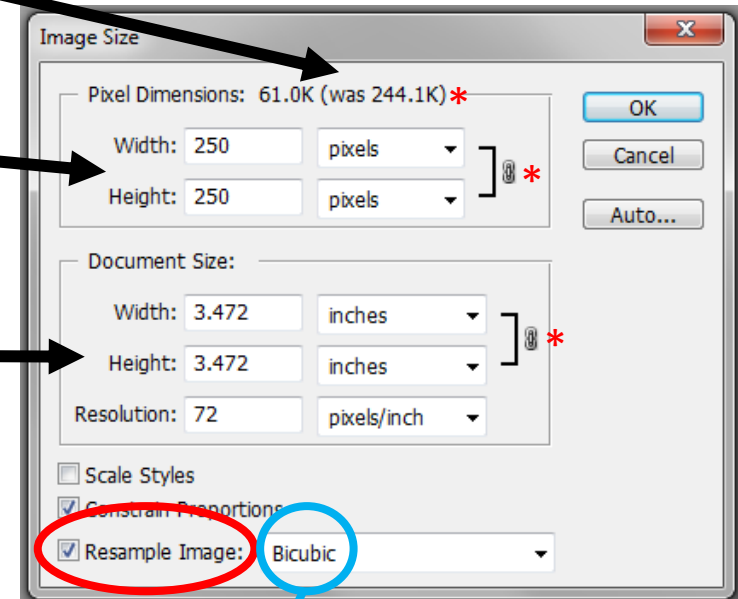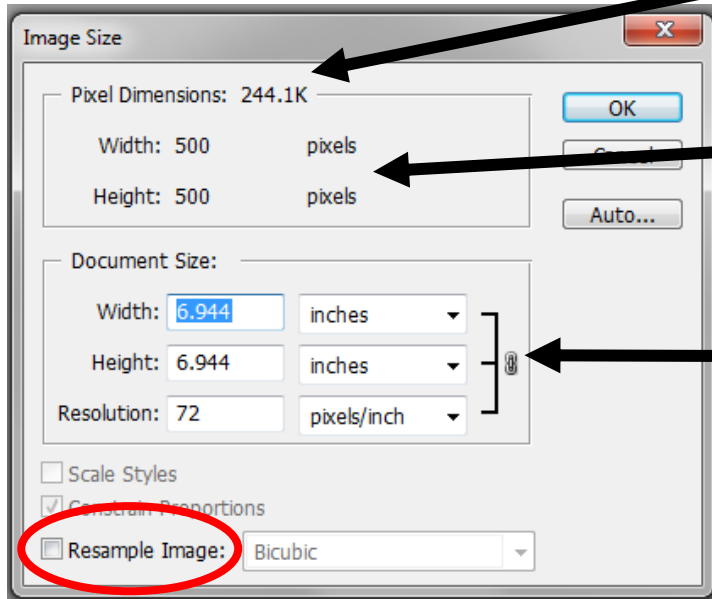
File size

Image **pixel** dimensions

Image **print** dimensions

Image Size

Pixel Dimensions: 244.1K

Width: 500 pixels
Height: 500 pixels

OK

Auto...

Document Size:

Width: 6.944 inches
Height: 6.944 inches
Resolution: 72 pixels/inch

Scale Styles
Constrain Proportions
Resample Image: Bicubic

Image Size

Pixel Dimensions: 61.0K (was 244.1K)*

Width: 250 pixels
Height: 250 pixels

OK
Cancel
Auto...

Document Size:

Width: 3.472 inches
Height: 3.472 inches
Resolution: 72 pixels/inch

Scale Styles
Constrain Proportions
Resample Image: Bicubic

Anti-alias filter reduces stair-stepping artifacts

Optical resolution describes the ability of an optical system to distinguish between two closely spaced points. It depends on the alignment of the illumination system, quality of the objective lens and collection system, and the suitability of the specimen and its preparation. It is ultimately limited by diffraction.

Image (pixel) resolution is often considered the pixel count in a recorded digital image. An image with a high pixel count <u>does not</u> necessarily imply a high lateral resolution.

Layers

Layers can be thought of as individual images placed onto a canvas. Each layer is discrete and can be moved and edited independent of the others. This also applies to text and arrows.

- Layers can be rearranged, created, copied, deleted or hidden.
- Layers can interact with other layers by changing their blending options and opacity.
- Layers can be grouped, linked or merged together.
- Layers can be filtered or enhanced independently.

Blending & opacity

Lock layer

Show/hide

Create, delete, etc.

Selected layer

Layer locked
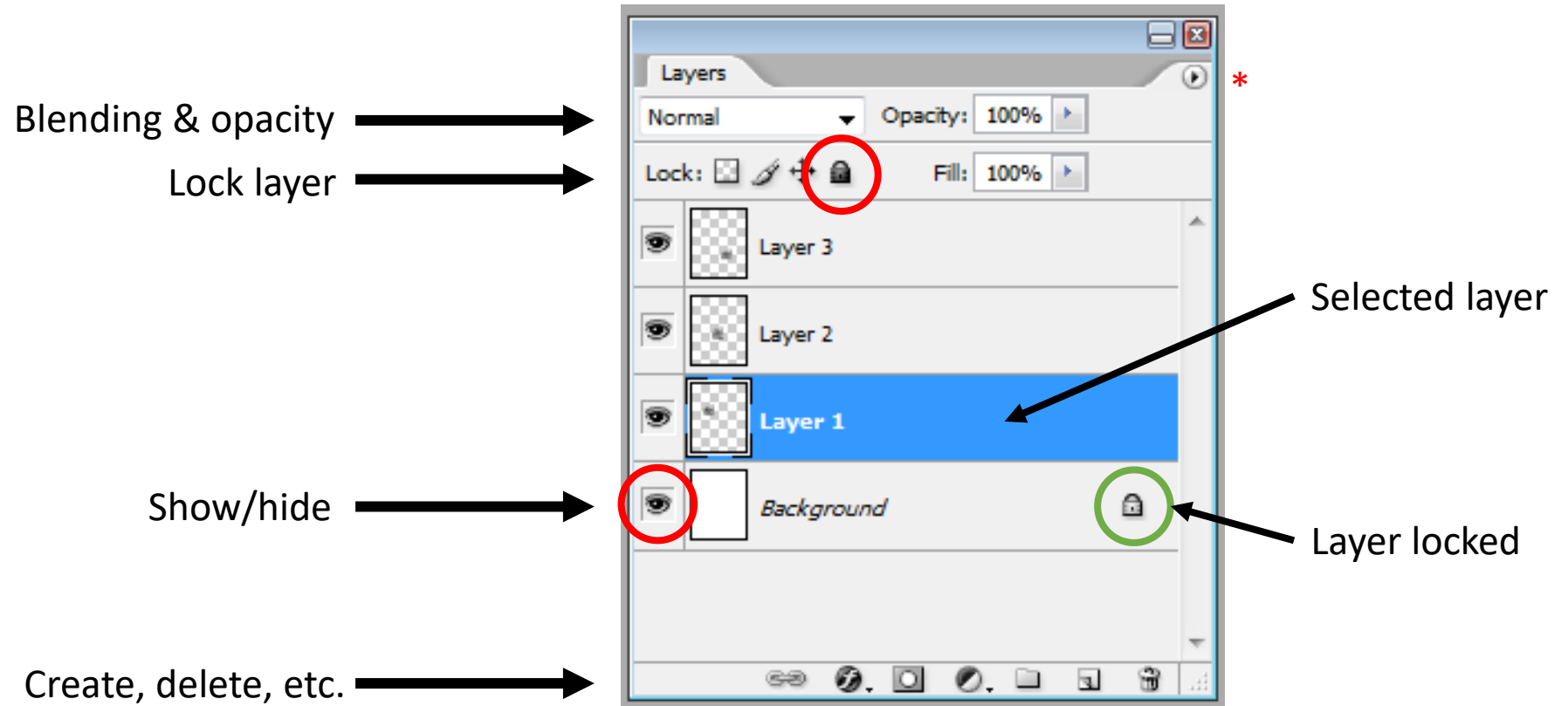
Lets Build a Plate

File > New (define parameters)
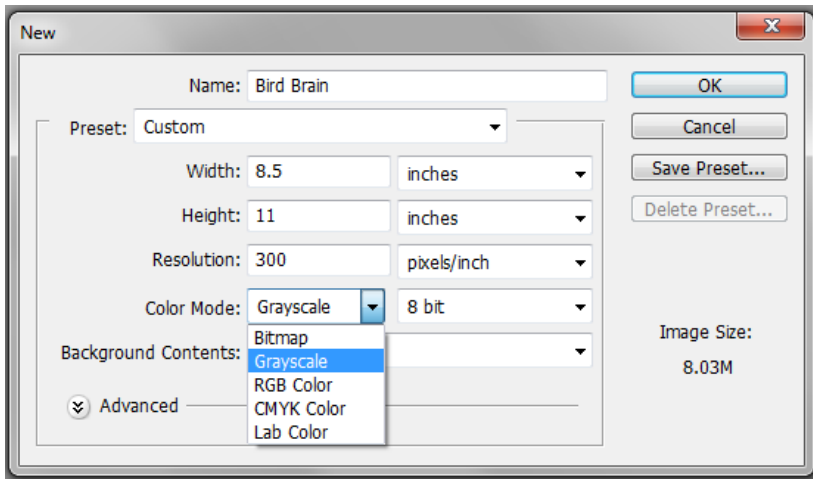
View > Rulers (if not visible)
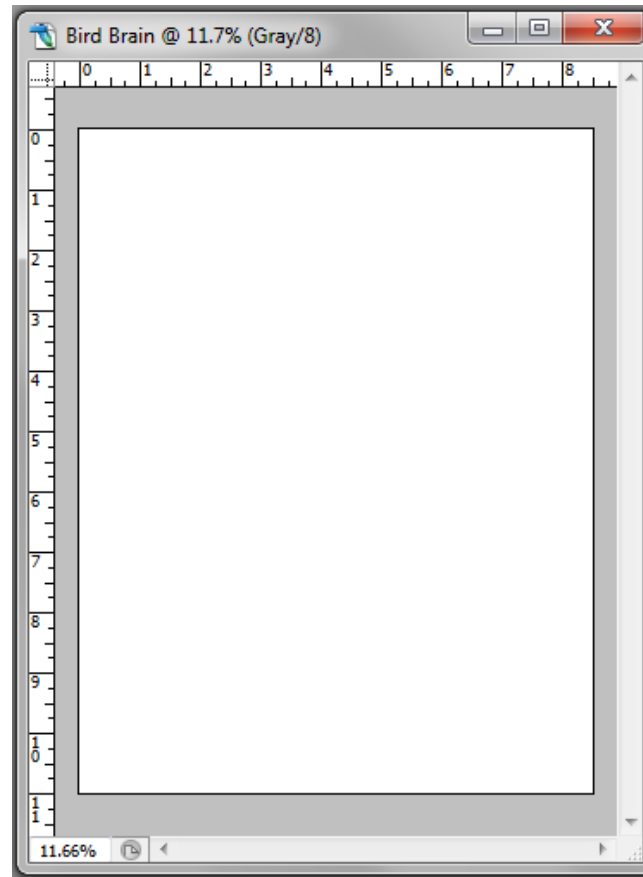
Right click in ruler to change units
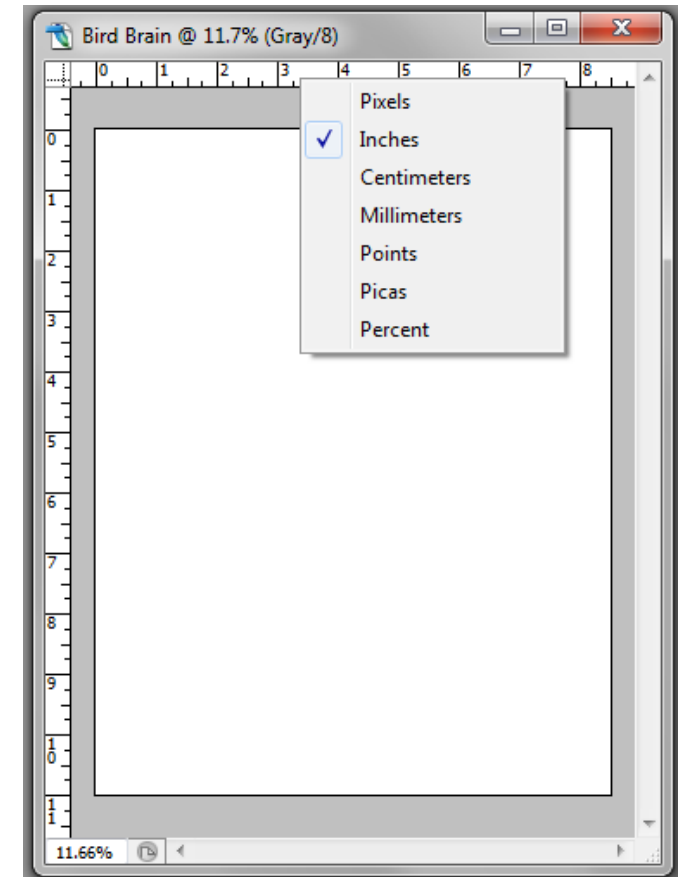
Select 'Move' tool

- Drag guides from the ruler
- Hover tool to reposition
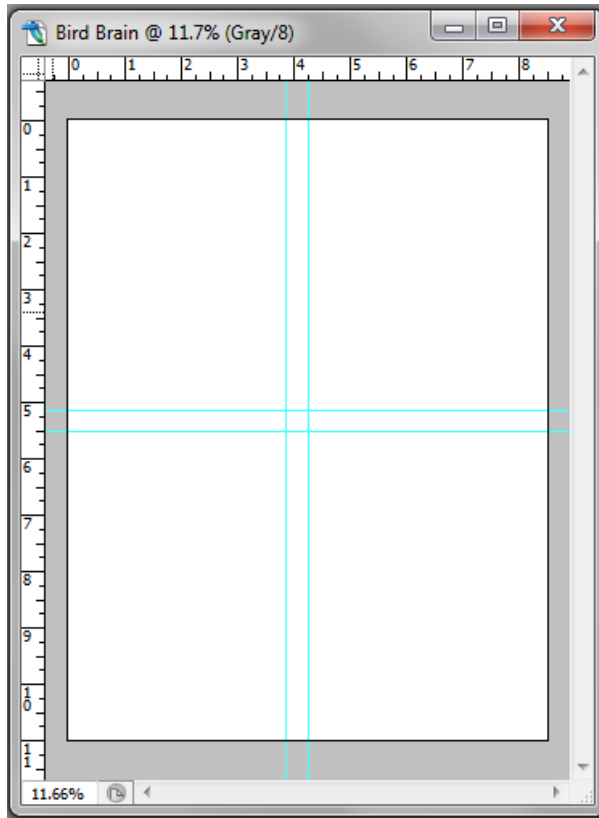
Drag image onto plate with tool
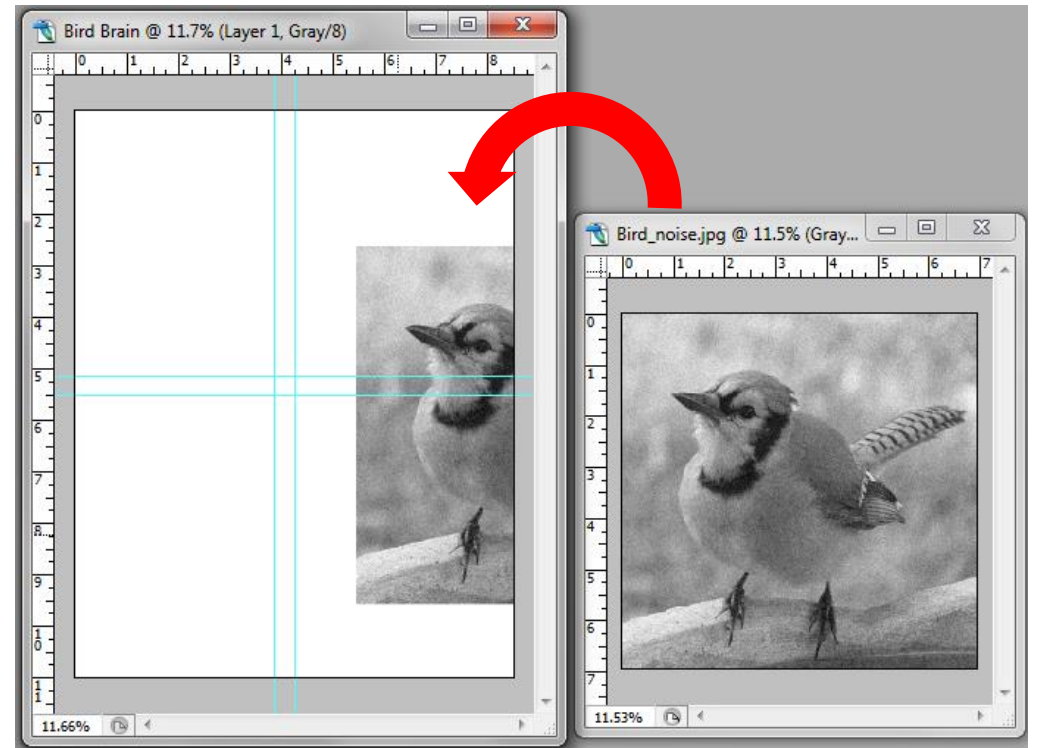
Edit > Free Transform (**CTRL+T**)

Hold **Shift** and drag a corner handle to fit image
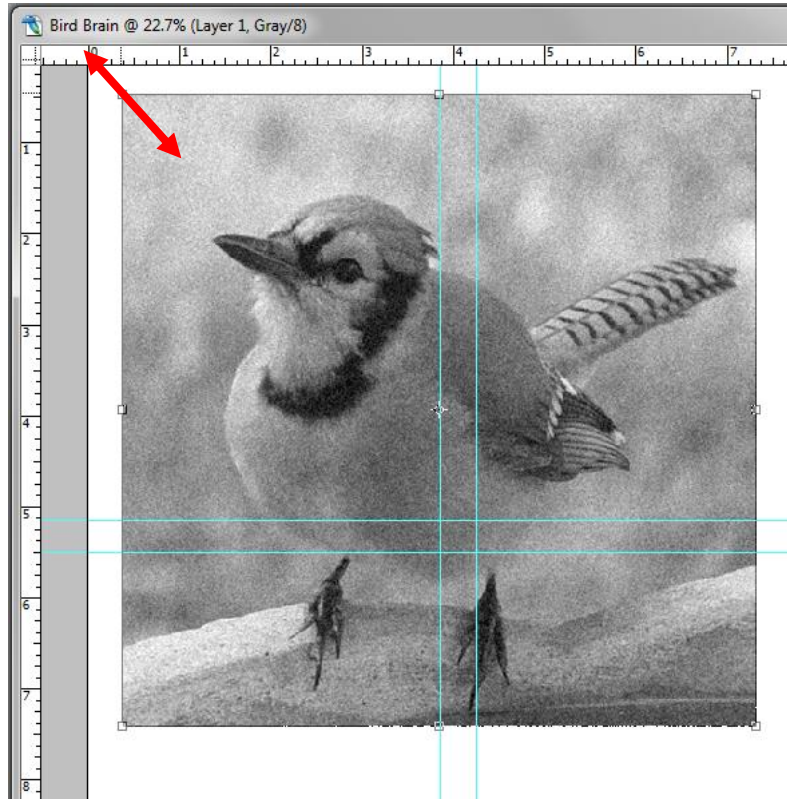
Press **Enter** or select **Apply** icon when done
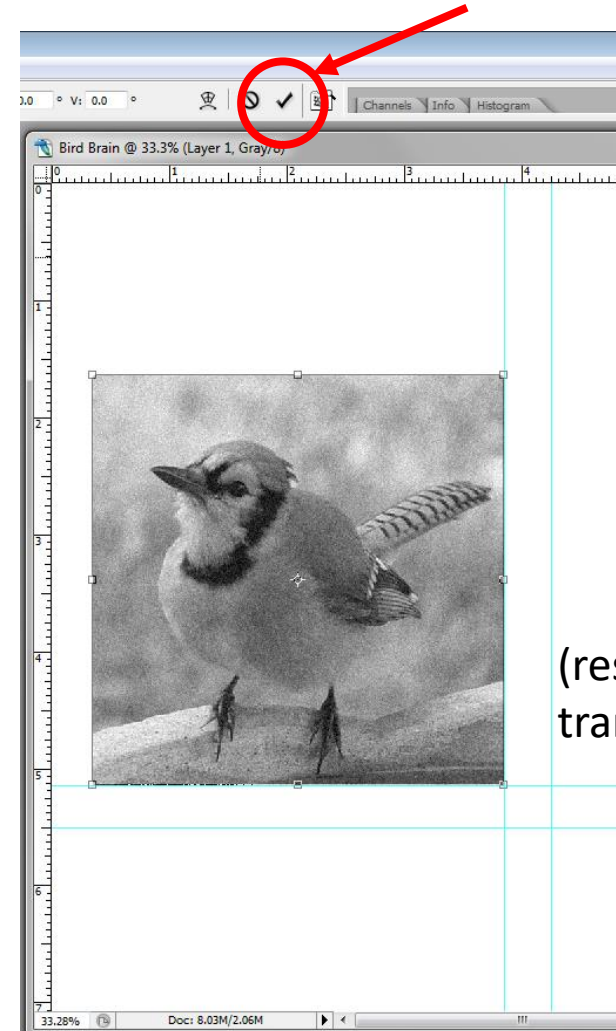
(resampled image after transform)

Free Transform

+ SHIFT
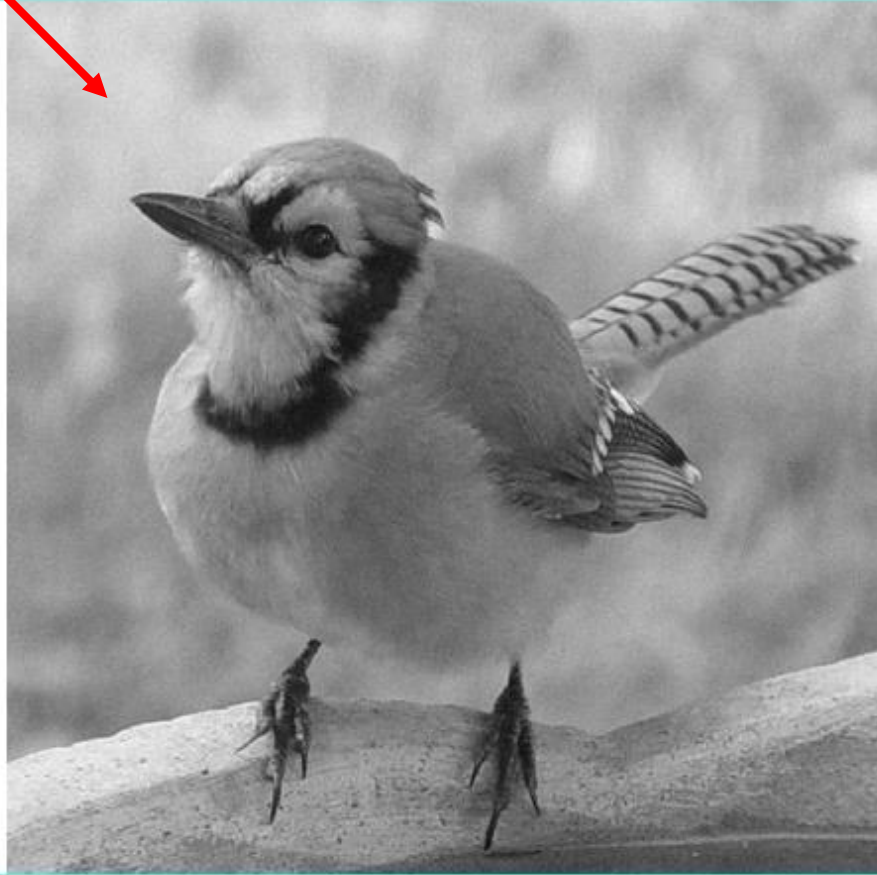
Image properly transformed by holding **Shift** and dragging from corner handle
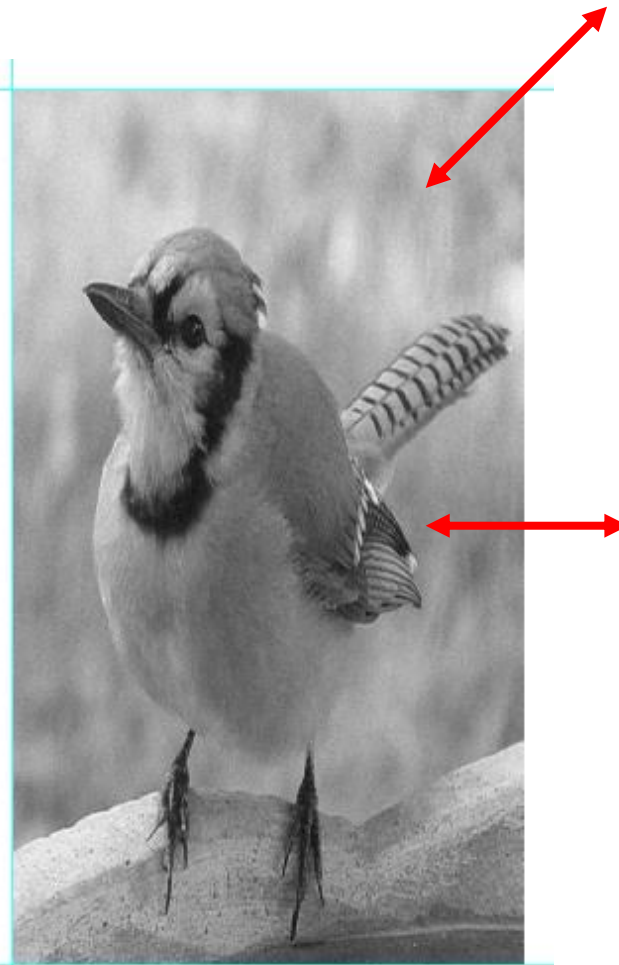
Image improperly transformed by not holding **Shift** or dragging from a side handle

Hold **CTRL+ALT** and drag out several copies
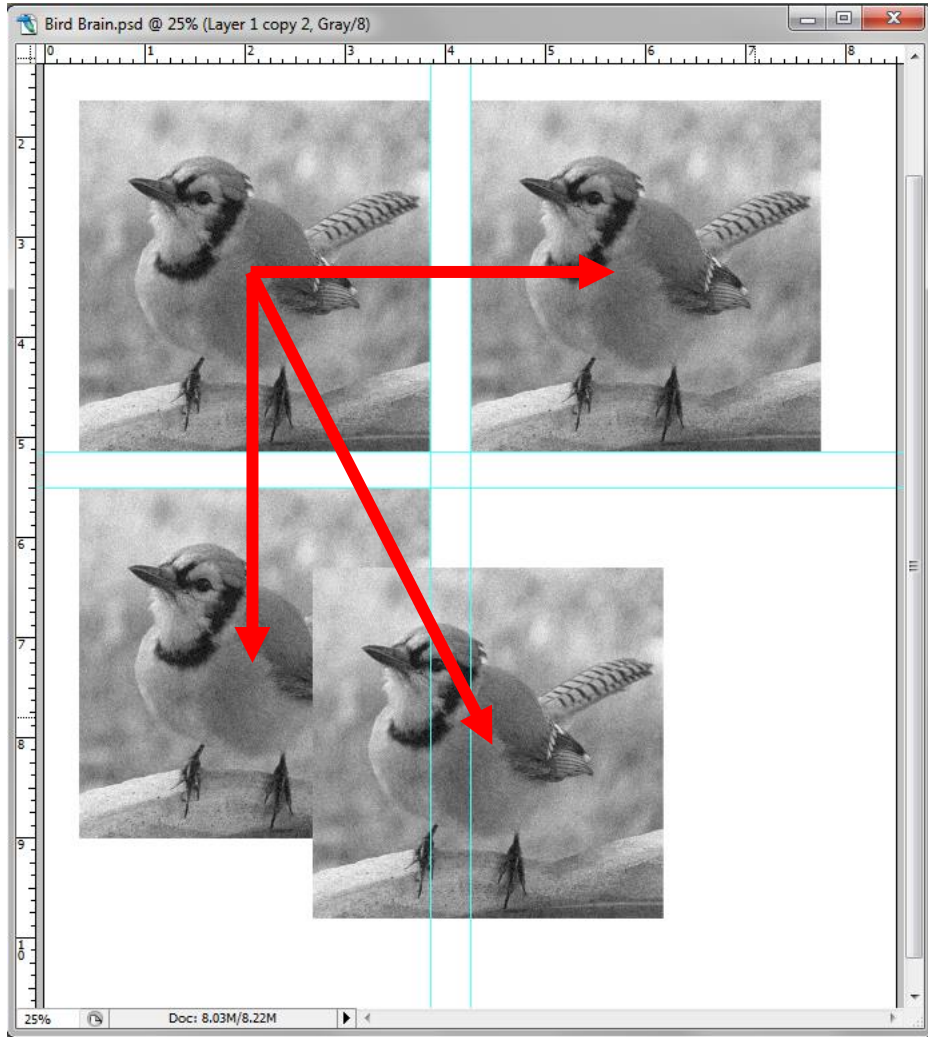
Add text, arrows, etc.
OOPS! Too late for scale bars

Text

Arrow
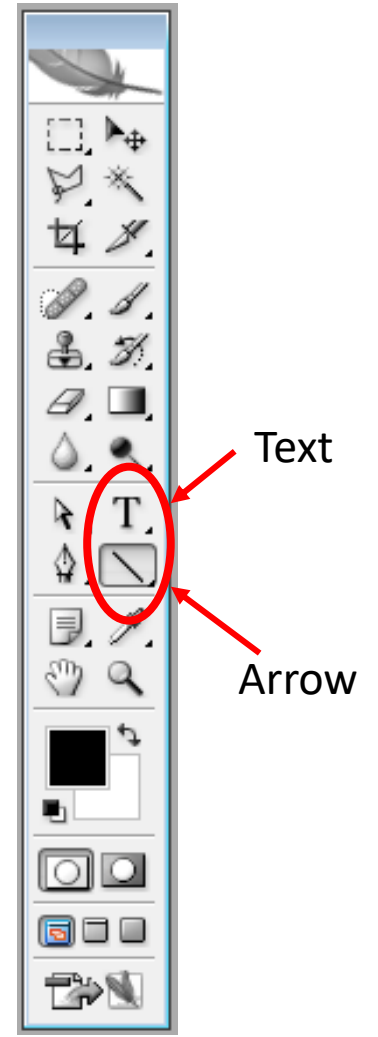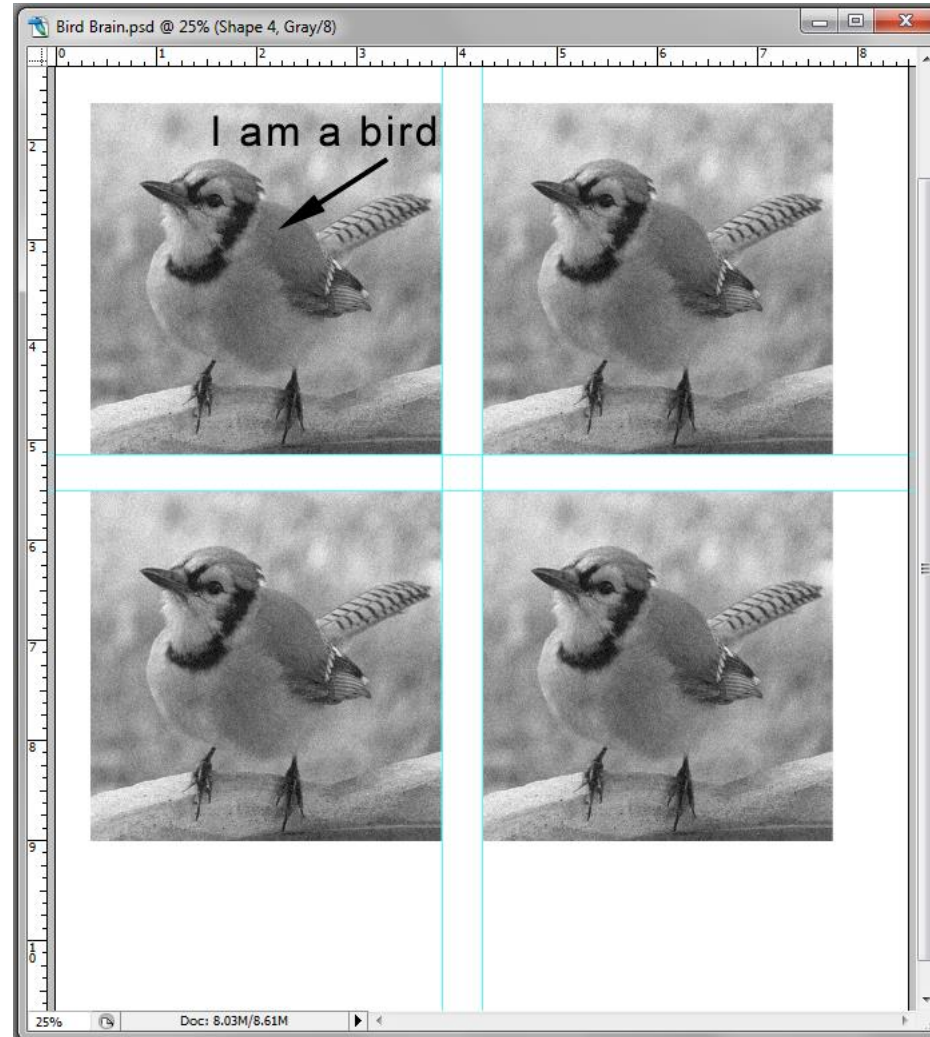
Crop image if desired

Crop

* **View > Clear Guides**

*To flatten layers use:
Layers > Flatten
-Or-
Palette drop-down

Save Image

- Use ***.PSD** format to preserve layers
- Use ***.TIF** format to prevent compression artifacts

Contrast

- Contrast is the difference in brightness or color that makes an object distinctive from other objects and the background in the same field of view.

- The maximum contrast of an image (i.e. the darkest to brightest) is the contrast ratio or dynamic range.

- Contrast is frequently expressed in terms of bit depth. A bit depth of 8 is common and gives us 2^8 or 256 levels of brightness from black to white with 254 shades of intervening gray. Color images such as the RGB mode would have 8 bits per channel or a bit depth of 24 (2^{24} = 16.8 million colors). Other common values collected by cameras include 10, 12 and 16 bits per channel.

- When collecting images it is preferred to acquire, as much as possible, the full dynamic range to ensure separation of small variations in brightness.

Modifying Contrast

Contrast & Brightness

Image > Adjustments > Brightness/Contrast
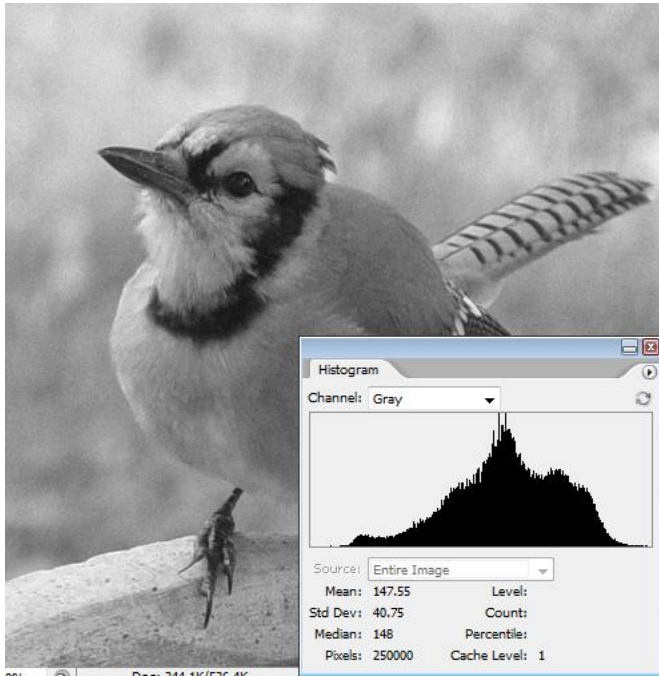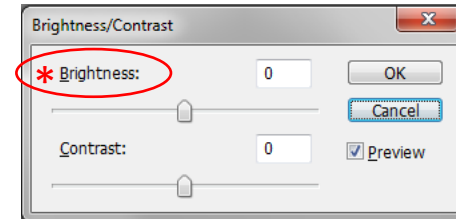
Original
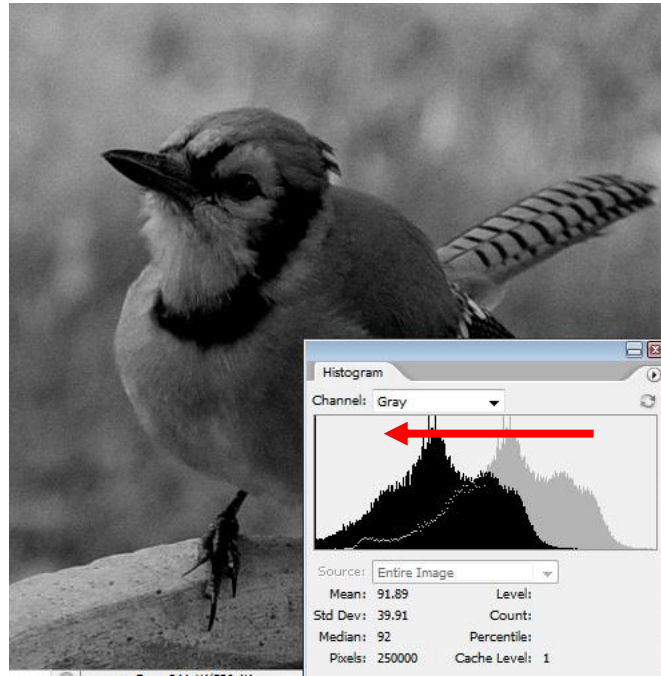
Darker
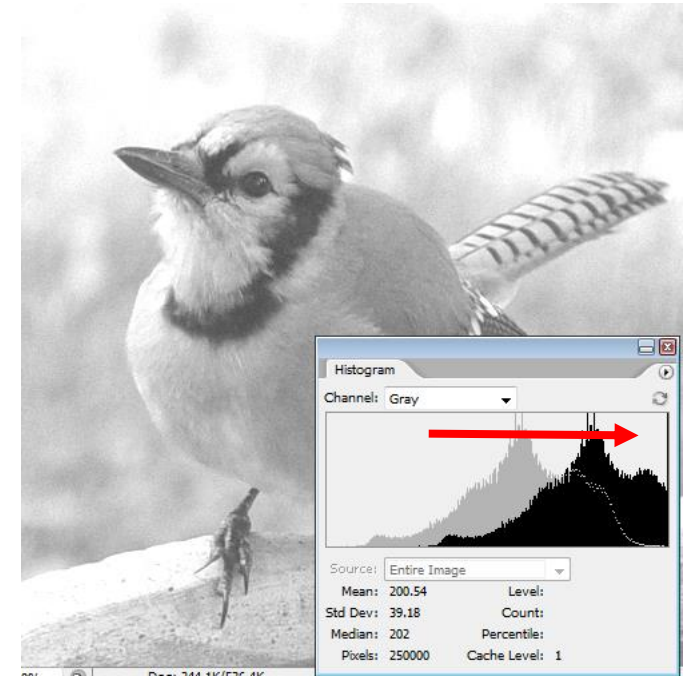
Brighter

We have decreased the dynamic range; data loss at extremes. Not recommended.

Contrast & Brightness (cont.)
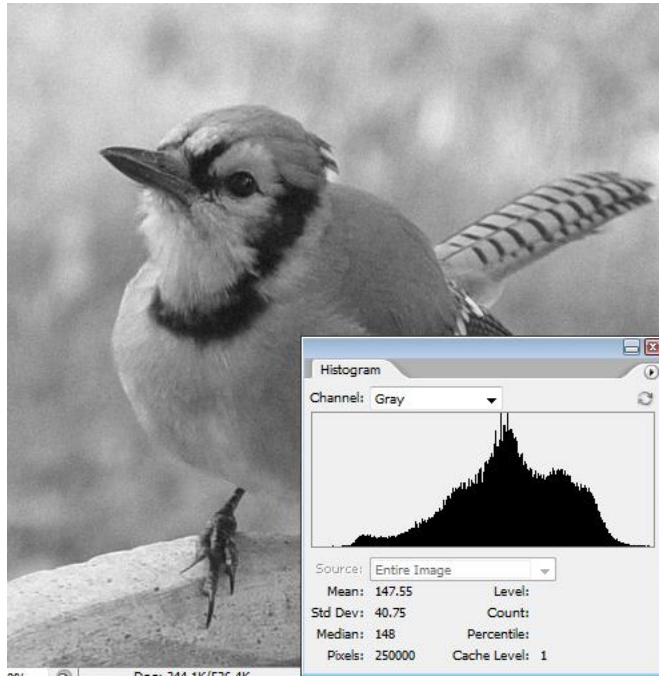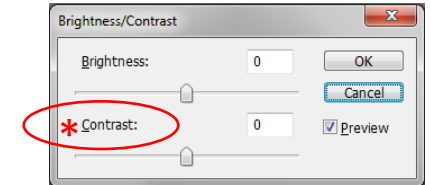
Original
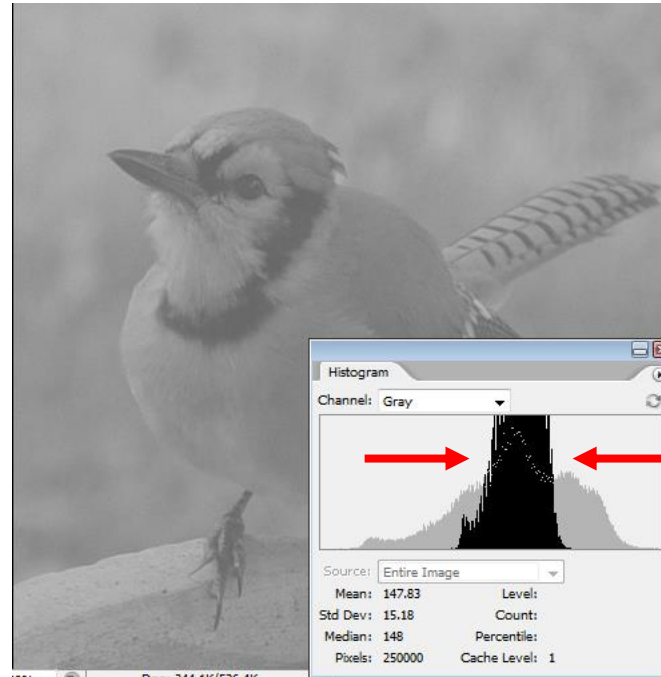
Low contrast
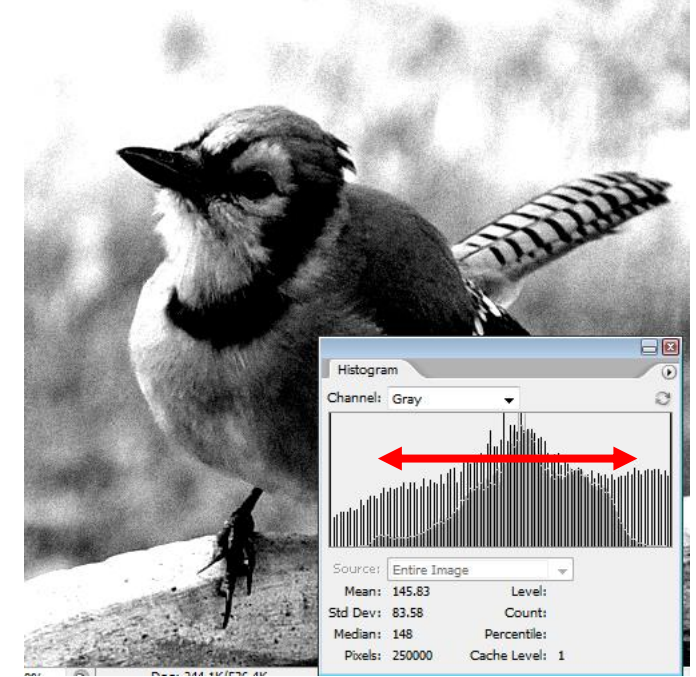
High contrast

- Low contrast - we have decreased the dynamic range
- High contrast - we have shifted the pixel values toward the extremes, eventually becoming a binary image.
- <u>Again this is not recommended</u>

Dodge & Burn (in the darkroom)
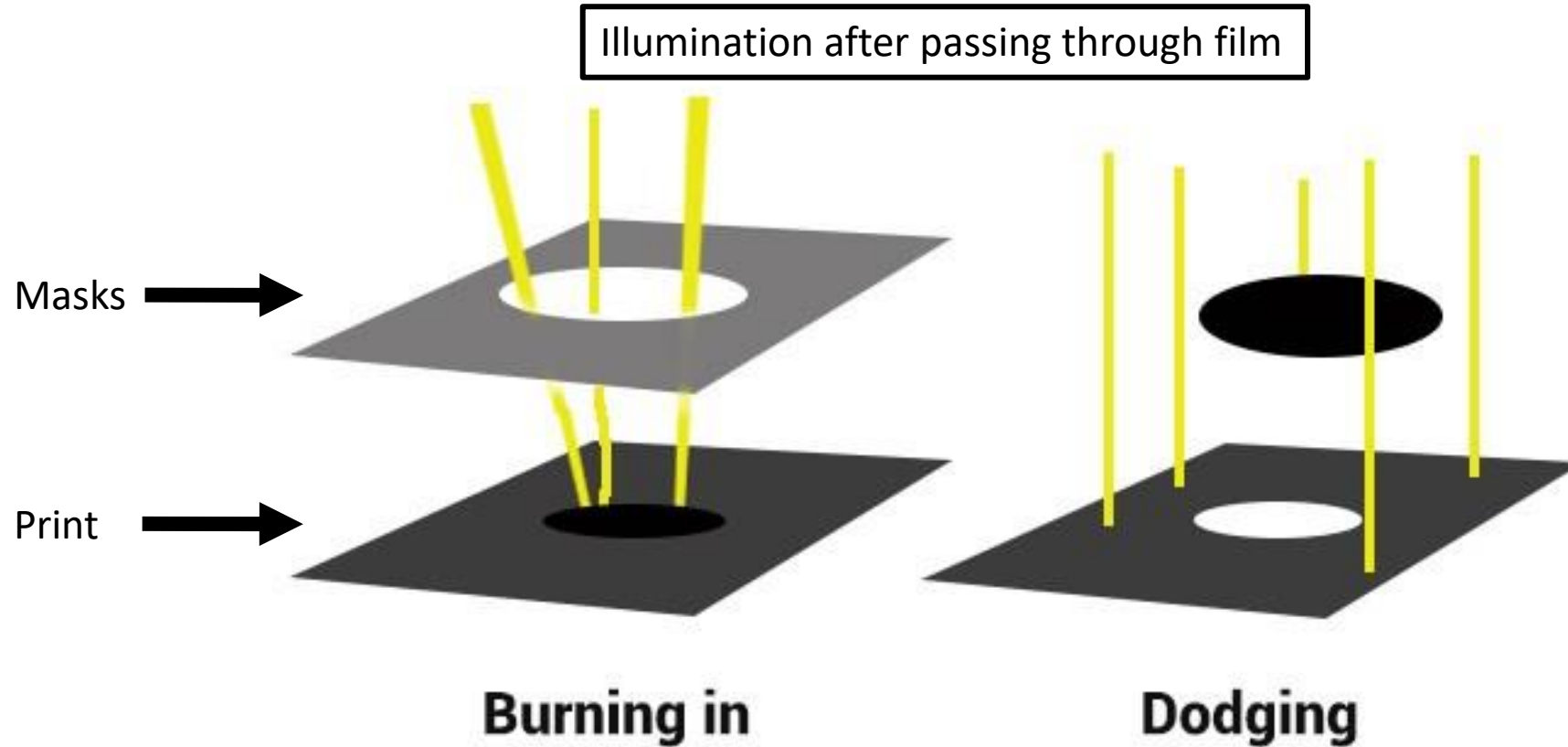
Illumination after passing through film

Masks →

Print →

Burning in

Dodging

Dodge Tool
Burn Tool
Sponge Tool
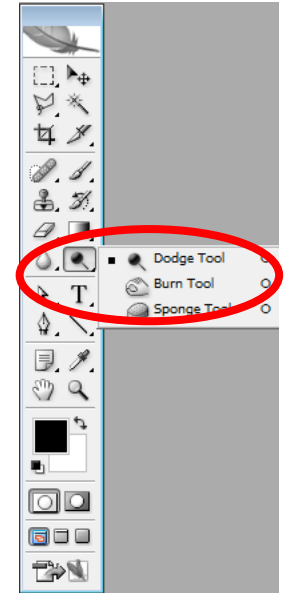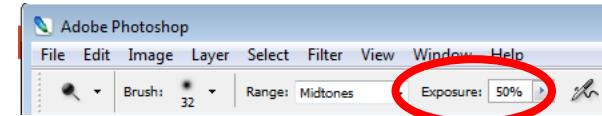
Dodge and **Burn** are used to change the exposure on localized areas of a print

Dodge & Burn (cont.)
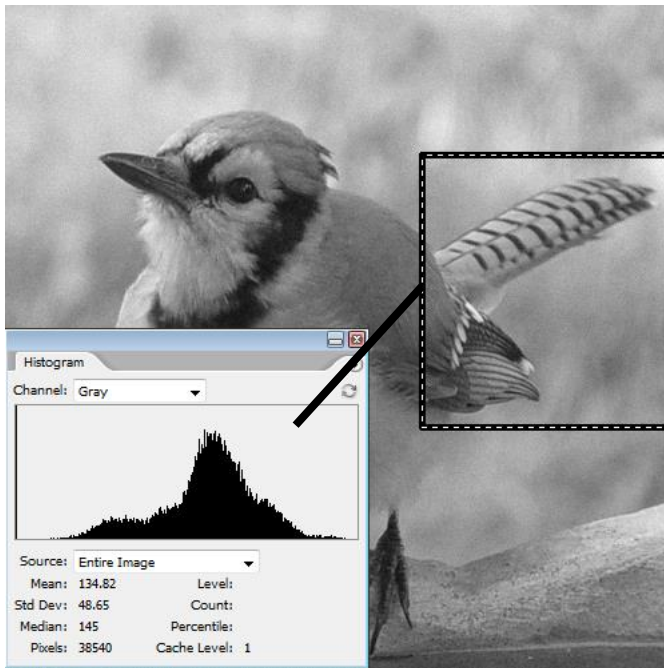
Original
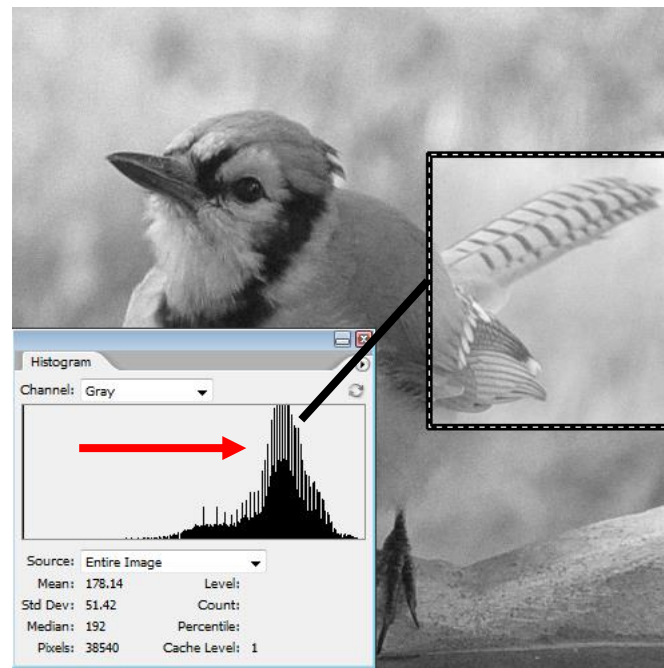
Dodge tool (75%)
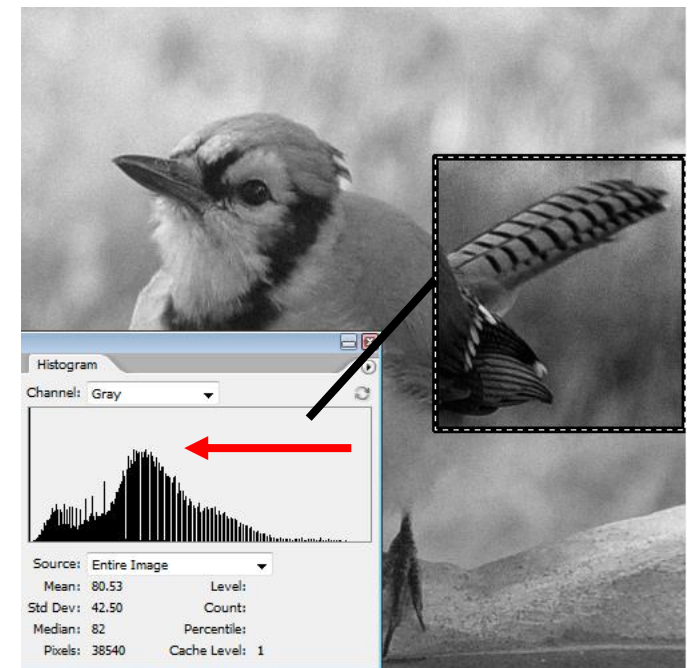
Burn tool (75%)

- Some loss of dynamic range
- Histograms shift toward the extremes
- Not for the faint of heart

Levels
[Contrast enhancement, contrast stretch, histogram stretch …]

Image > Adjustments > Levels
       (CTRL + L)

Original
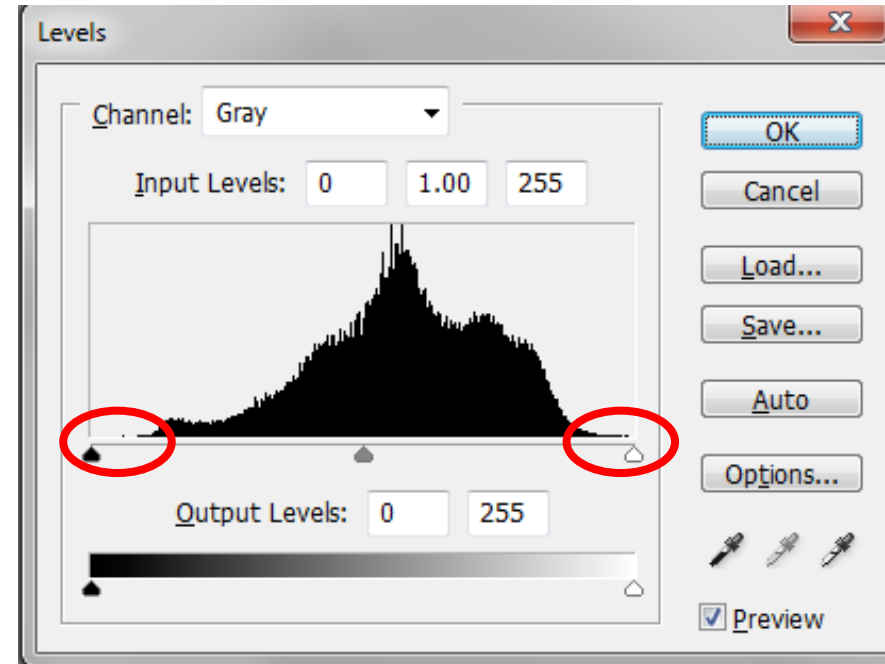
Histogram just short of utilizing full 8-bit

Levels (cont.)

After leveling
(Compare to original)
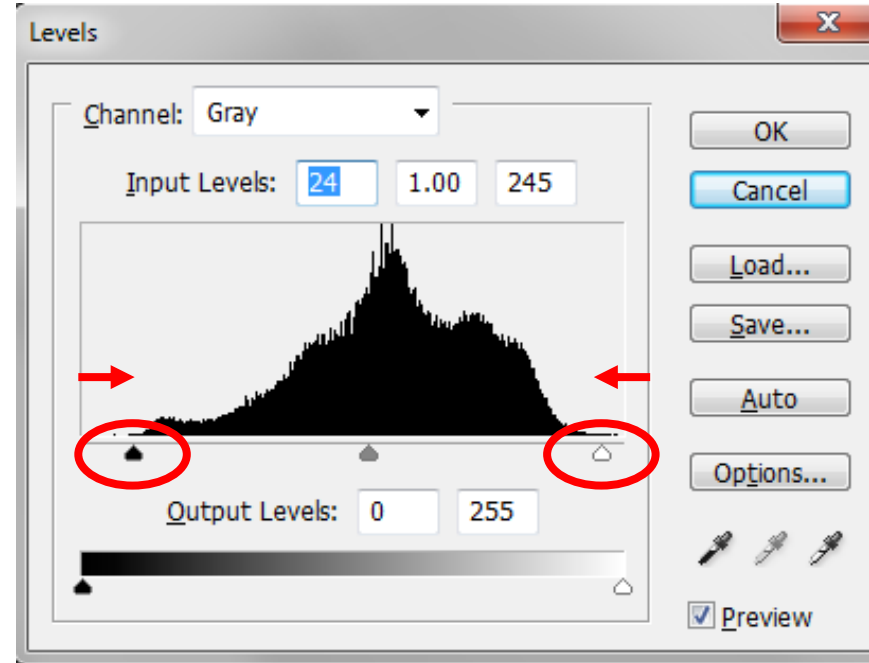
Move outer sliders to bracket histogram and perhaps a little more

[Auto functions usually oversaturate, or 'clip', the extremes by at least 0.1%]
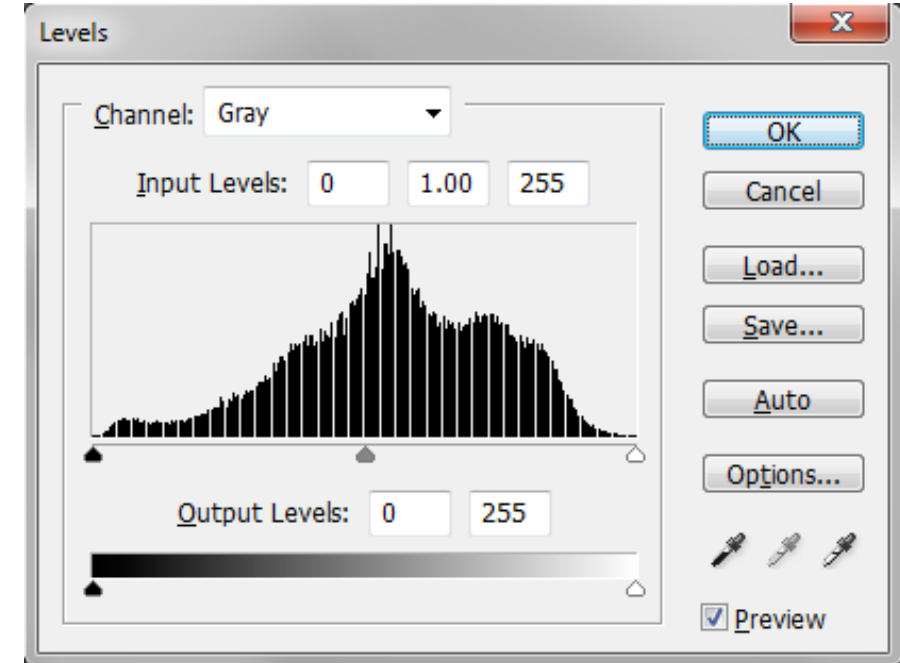
Resulting histogram utilizing full 8-bit. Notice gaps in histogram – tell tale sign of enhancement

Gamma

FYI

A film's response to light is shown on the D – $\log_{10}E$ curve, where D is the optical density and E is the exposure
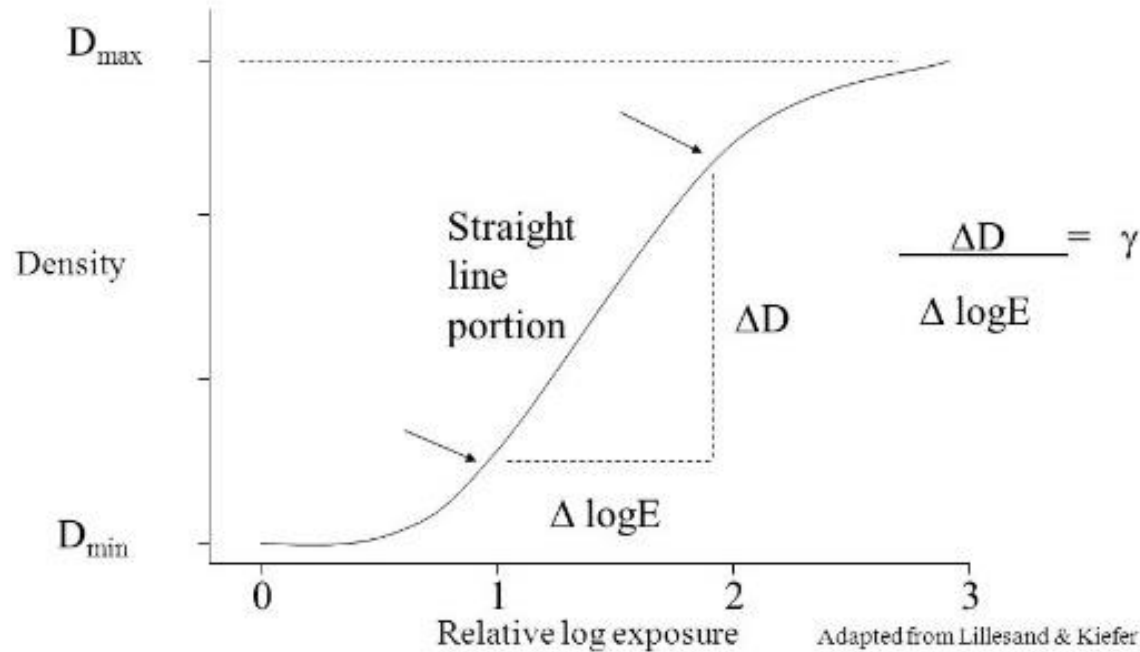
- **Gamma** is defined as the slope of the linear portion of the curve and is itself a logarithmic function

- A steep slope allows for greater changes in D from small changes in E resulting in a higher contrast image (greater dynamic range)

$$\frac{\Delta D}{\Delta \log E} = \gamma$$

Gamma Adjustment in Image Analysis Programs

FYI

Gamma 0.5

255

0

OUTPUT

0   **INPUT**   255
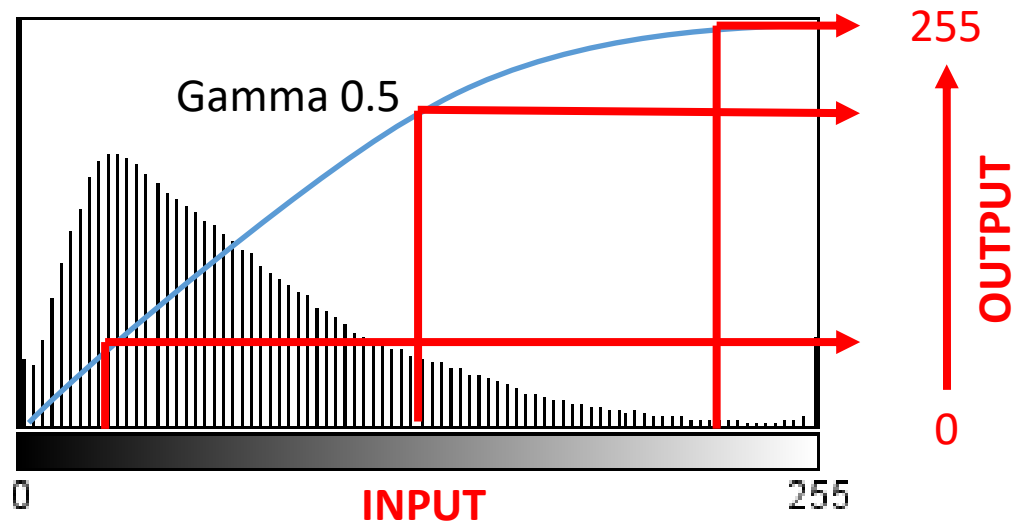
Count: 1048576   Min: 0
Mean: 29.279   Max: 255
StdDev: 48.369   Mode: 0 (622057)

- Image analysis programs typically show γ as a logarithmic curve

- Pixel intensities are changed to a new value depending on where it lies relative to the curve

- Pixel order is retained

Resulting histogram

0   255
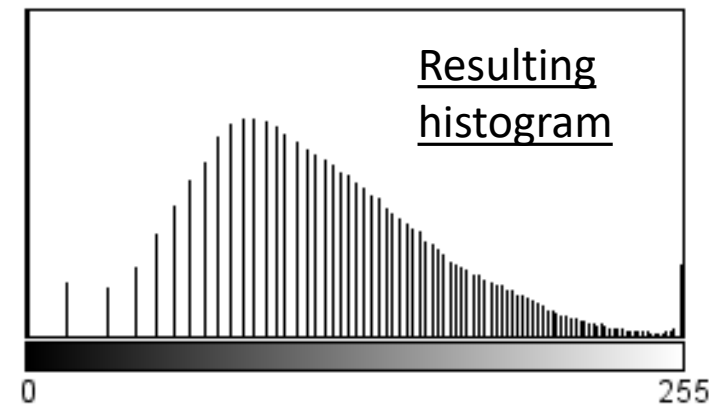
Count: 1048576   Min: 0
Mean: 51.379   Max: 255
StdDev: 69.073   Mode: 0 (622057)

[Note: A curve is sometimes referred to as a **transfer function**]

Gamma Adjustment in Photoshop

Open Levels (**CTRL+L**) in the menu

After γ applied

[compress]

[0.50]
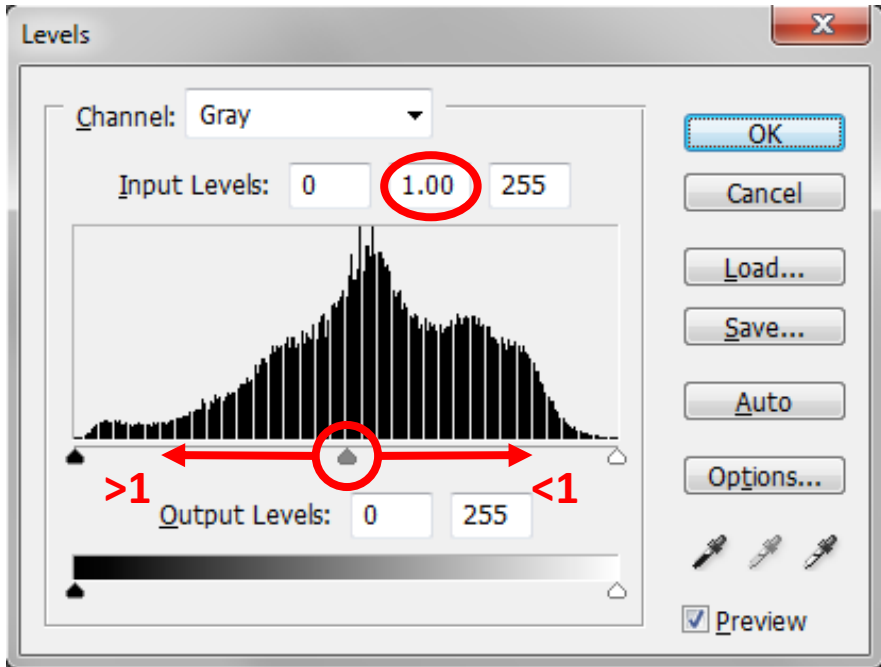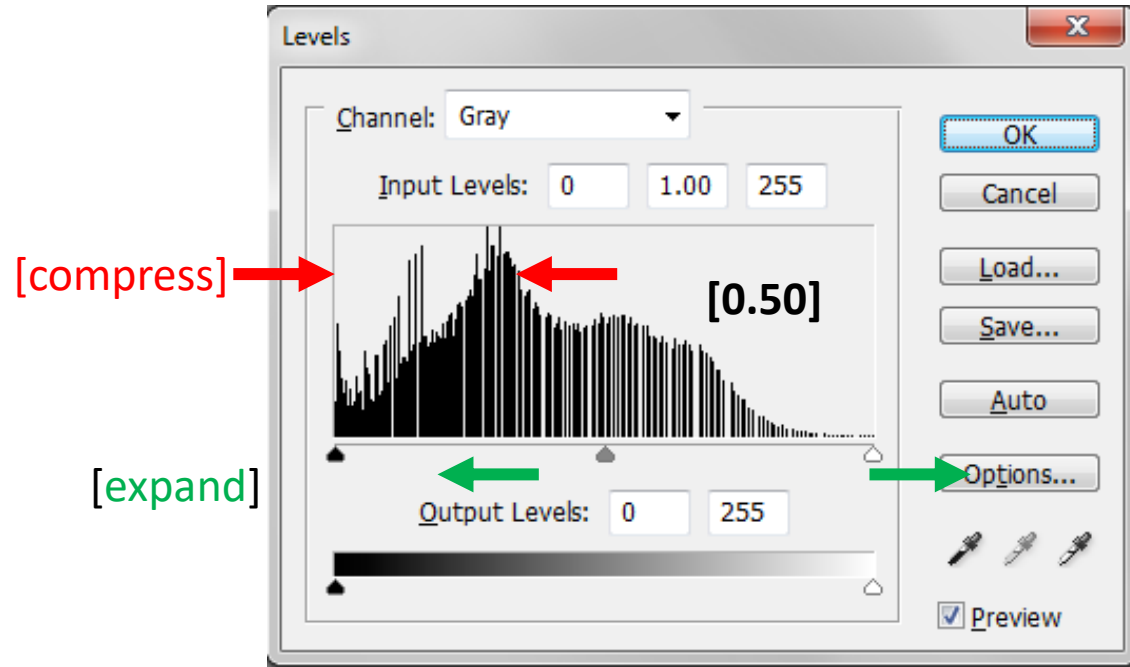
[expand]

- Gamma is the center slider. The default value is '**1**'

- Pixel order is retained

- Maintains dynamic range if not applied too heavily

- Contrast expansion in part of the histogram, the rest is compressed

- No data loss!!!

- Original image can be restored by applying the inverse (e.g. γ = 2.0)

Gamma Adjustment in Photoshop (cont.)
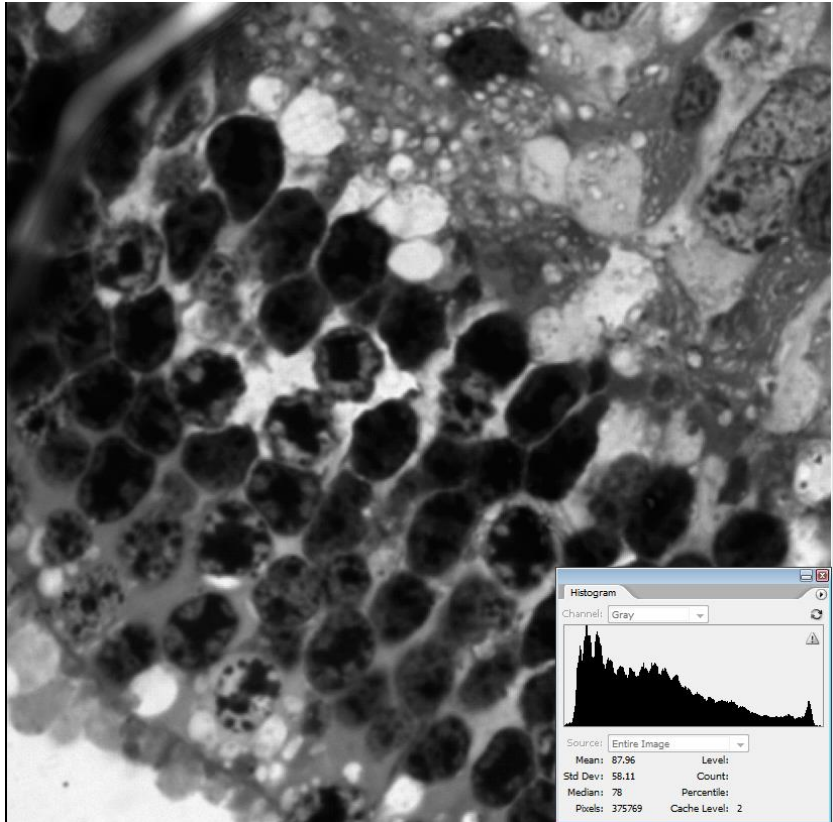
Gamma 1.8

Original
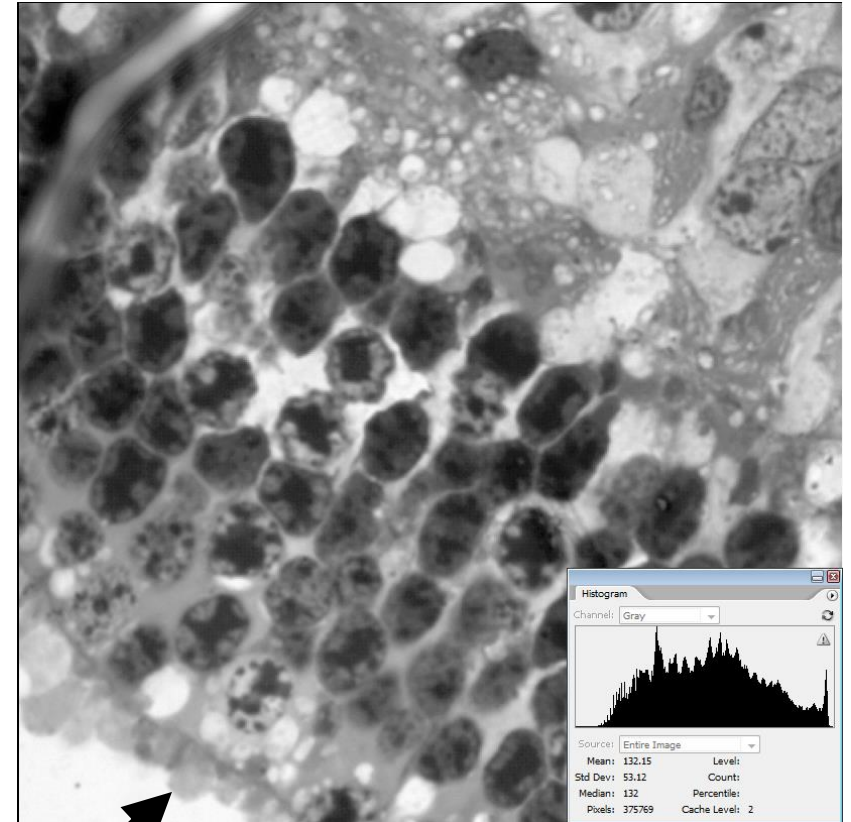
- **Contrast expanded in dark regions to reveal fine detail**
- **Contrast compressed in lighter regions**
- **No information loss!!**

Equalization

FYI

- Logarithms are not the only functions used to manipulate the contrast of an image.

- Gaussian, exponential and power functions can also be used.

- Equalization attempts to more evenly distribute the brightness values.

- Data clustered in peaks (small brightness variations) will spread out, while 'valleys' will compress.

Image > Adjustments > Equalization

Image Inversion

Because human vision responds approximately logarithmically to brightness, it is often easier to discern details in the brighter areas of the original image when inverted (negative image).
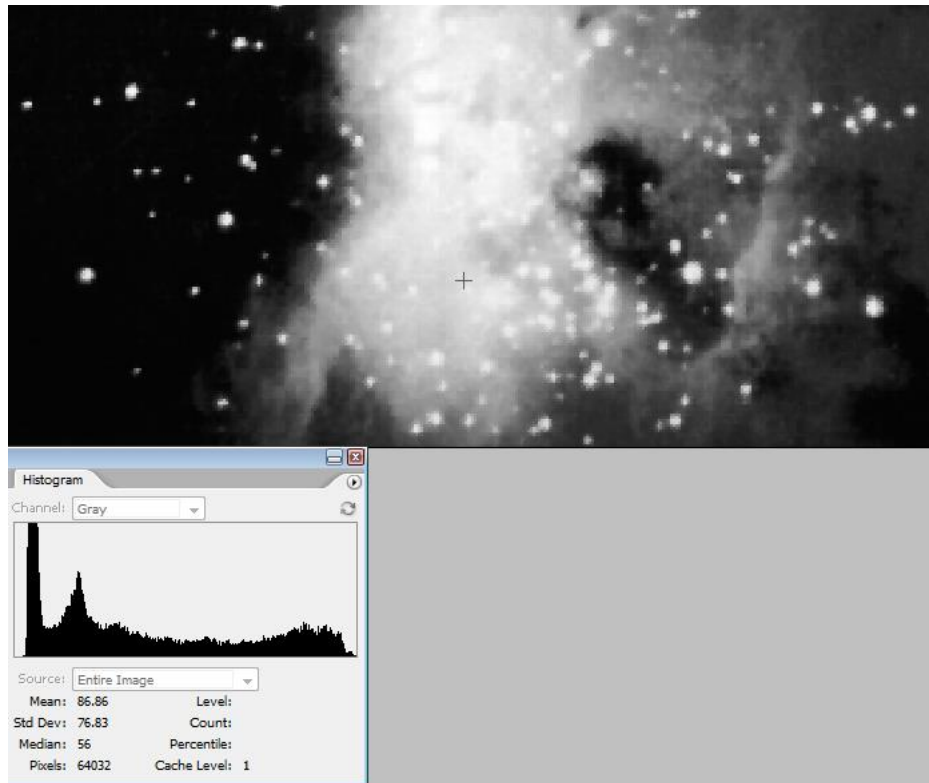
Image > Adjustments > Invert
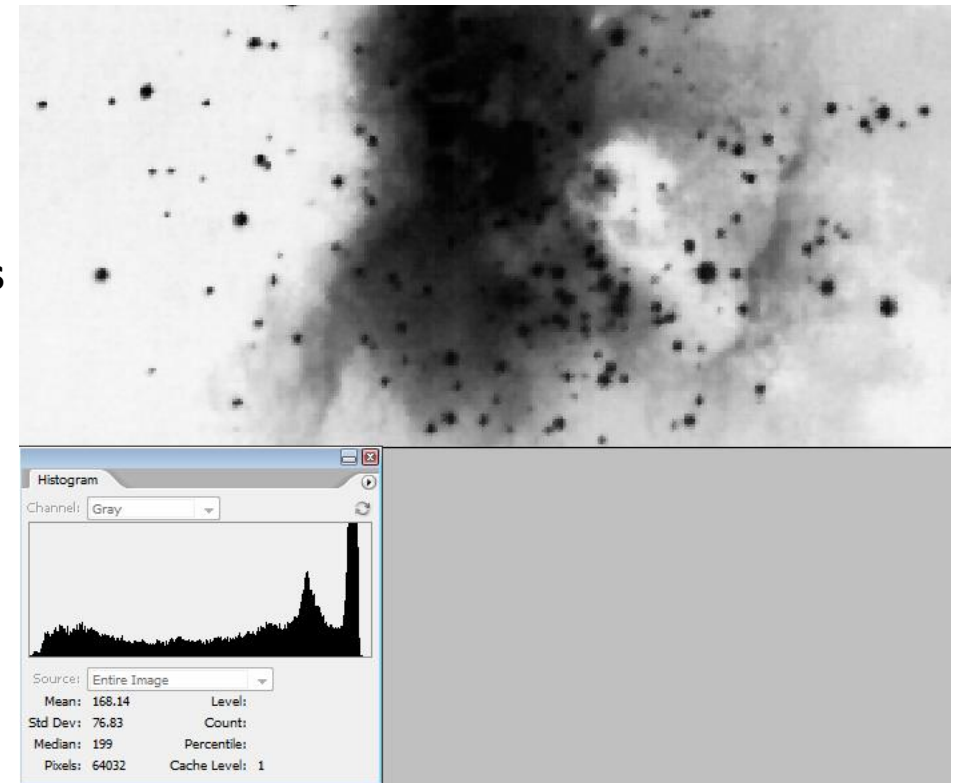
Levels, including gamma, is the most commonly used and accepted method of contrast enhancement!

Digital Filters

- Digital filtering is a technique for modifying or enhancing an image.

- You can enhance, deemphasize or remove a feature.

- Filters are commonly used for edge detection, removing noise and to blur/sharpen an image.

- It is not uncommon to apply a 'small' filter several times (iteration) to reduce artifacts that a single 'large' filter might introduce.

Digital Filters

FYI

Square kernels

'Circular' kernal

- A digital filter is a matrix of numbers

- This matrix is referred to as a **kernel**

- The filter is usually square but can be 'circular' if zeroes are placed in the corners

- The filter performs math on the original image to produce a new one

- The numbers in the matrix **weight** the variables in the equation

- If the filter uses a math function it is sometimes referred to as a **convolution**. Otherwise it is a **ranking** filter

Filters (cont.)

Center element of the kernel is placed over the source pixel. The source pixel is then replaced with a weighted sum of itself and nearby pixels.

Source pixel

Convolution kernel (emboss)

New pixel value (destination pixel)

(4 x 0)
(0 x 0)
(0 x 0)
(0 x 0)
(0 x 1)
(0 x 1)
(0 x 0)
(0 x 1)
+ (-4 x 2)
-8
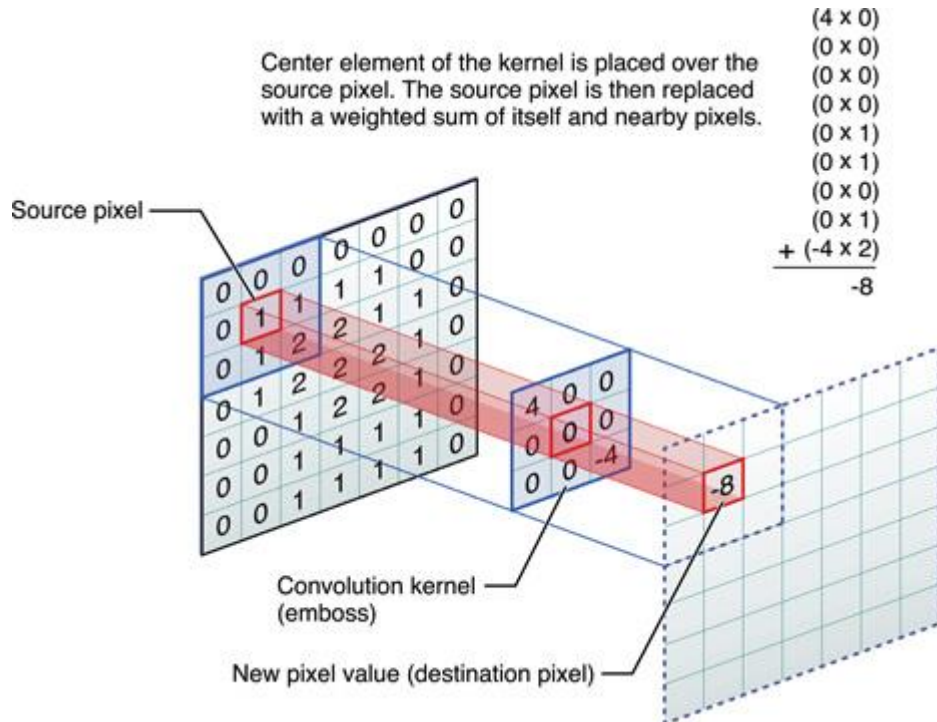
- The filter is scanned over each pixel

- The central pixel is compared to its neighbors

- Math is performed using the weights in the matrix

- The central pixel is output to a new image that is constructed

- The next pixel is scanned and compared to its neighbors and so on until the new image is complete

Median (Noise) Filter

Filter > Noise > Median

Original with salt/pepper noise
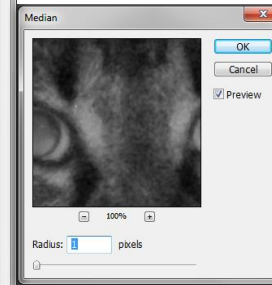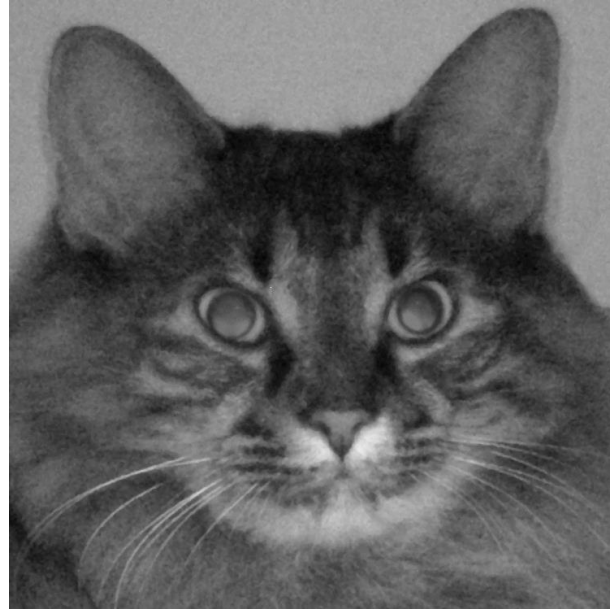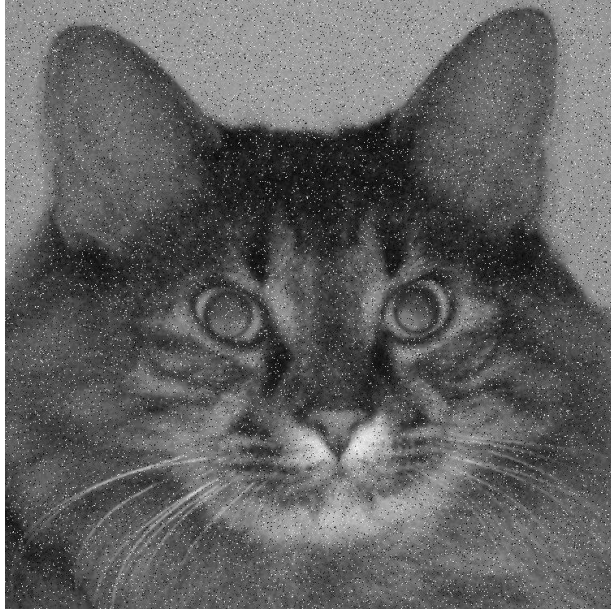
{12, 12, 13, 14, 21, 23, 23, 23, 23, 29, 100}

median

Noise?

- Median filter uses the **median** value of the neighborhood to replace the central pixel

- Since noise tends to be an outlier value it should eventually be discarded

- Considered a **ranking** filter

- Fine detail can be lost

- Edges are preserved

- Large regions can take on the same brightness

Smoothing Filter

Filter > Blur > Gaussian Blur

3D Gaussian

2D Gaussian

Gaussian Blur

OK
Cancel
☑ Preview

Radius: 3.3 pixels

1 standard deviation

- Can reduce random noise

- Can blur and displace edges, and reduce contrast

- Mimics an out-of-focus condition
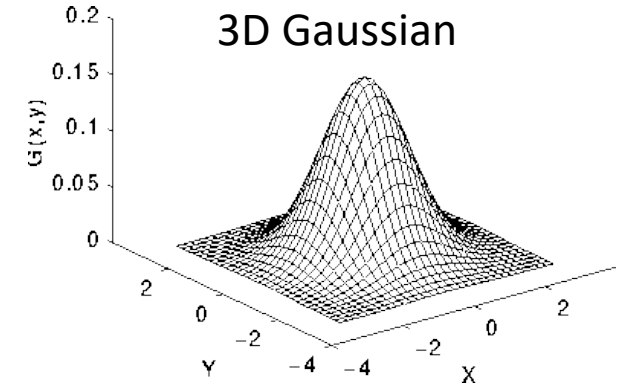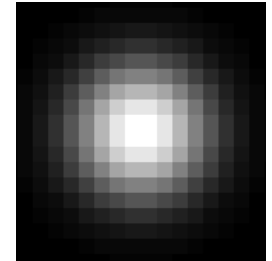
Unsharp Mask Filter (in the darkroom)

FYI

γ

Original film negative

Negative of original and slightly out-of-focus film

Sharpened print image

Unsharp Mask (cont.)

Filter > Sharpen > Unsharp Mask
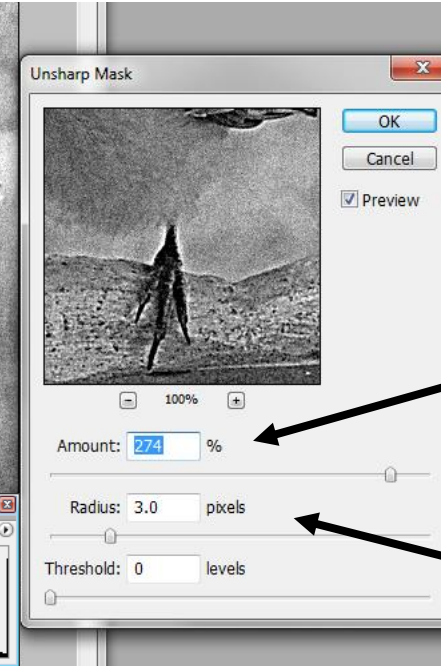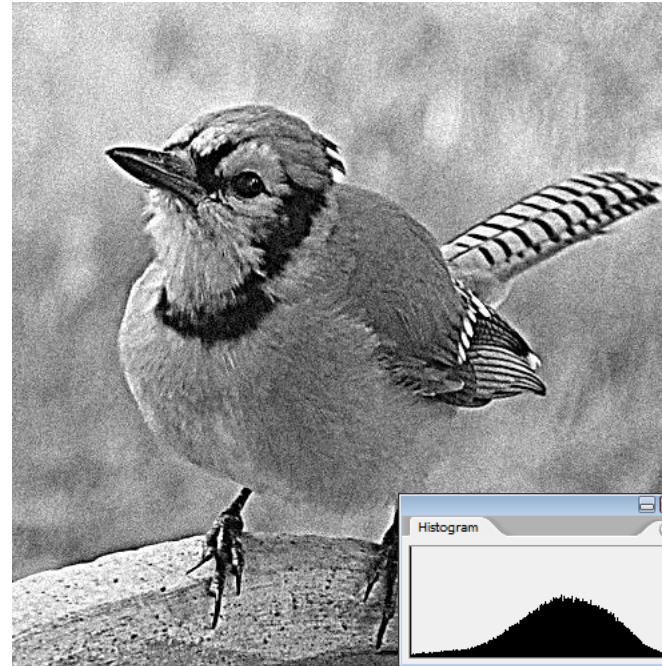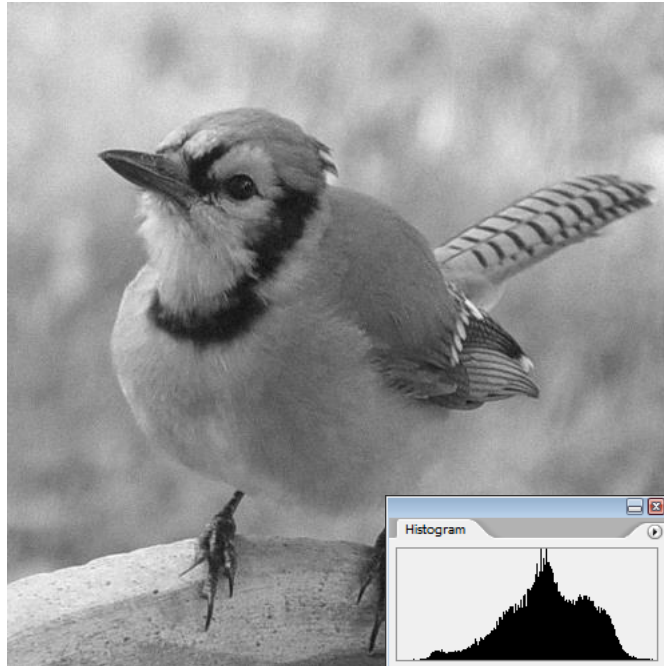
Strength or weight of kernel

1 std. dev.

Photoshop recommends:
- Amount 150% to 200%
- Radius 1-2 pixels

- Produces an increase in local contrast near points, lines and edges

- Minimizes noise enhancement

- Note artifacts: Graininess, halos and some edge shift

Scale Bars

- **Open image**
- **View > Rulers if the rulers are not visible**
- **Right click on ruler to select 'pixels'**
- **Layer > New > Layer**
- **Calculate length of scale bar in pixels**

- **Use the 'Rectangular Marquee Tool'**
- **Change tool option to 'Fixed Size'**
- **Enter scale bar dimensions**
- **Click on image (make sure new layer is selected)**
- **Edit > Fill with desired color**
- **Layer > Flatten Image**

Scale Bars (cont.)
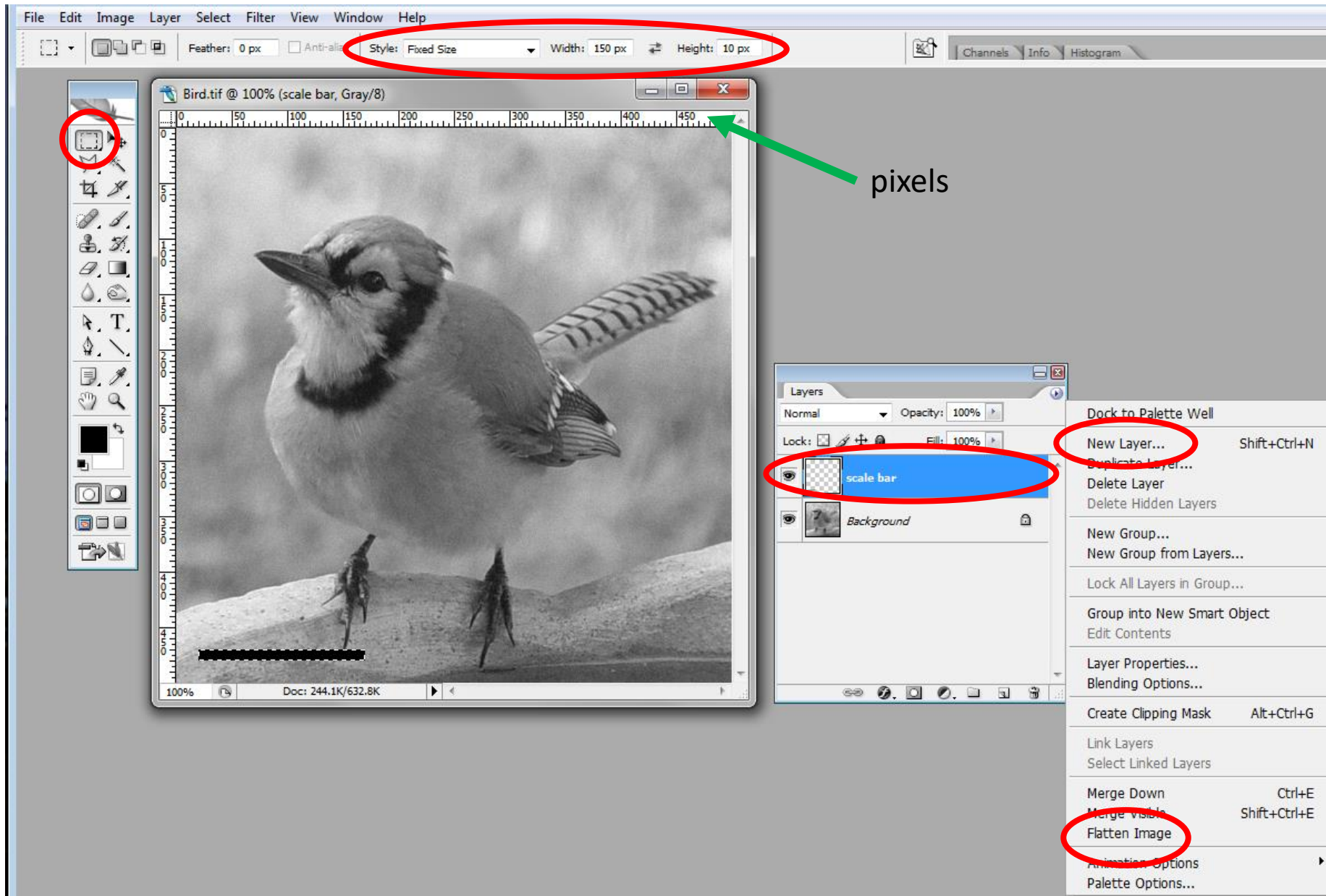
Scale Bars (cont.)

Original image with correct scale bar

Same scale bar applied **after** resampling image

- Apply scale bars before resampling images!!

Arrows
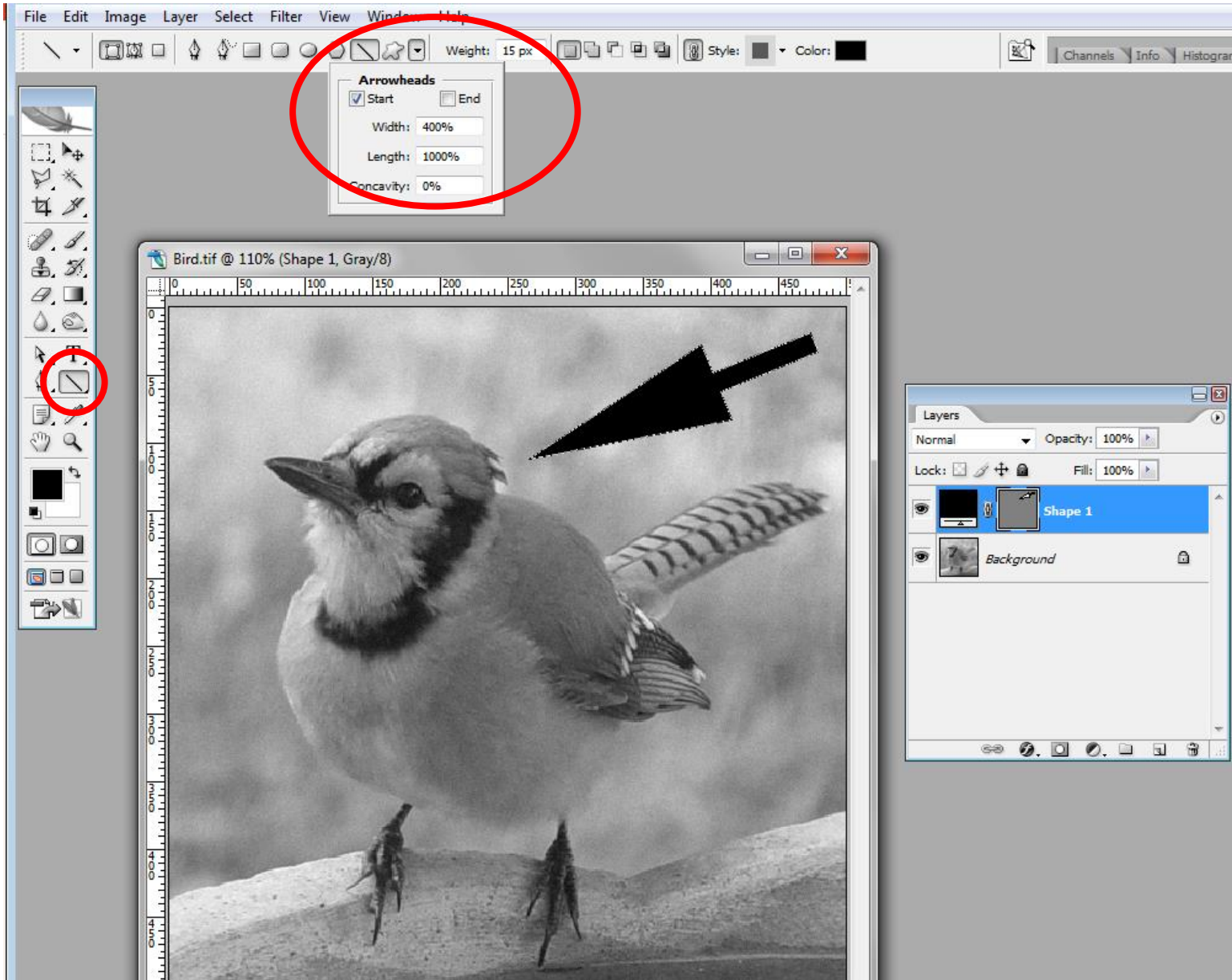
- Use the '**Line**' tool
- Tool options:
  1. **weight** is stem thickness
  2. arrowhead **%** based on weight

Text

Cancel/apply

Color Modes

Different color modes:

- RGB mode (millions of colors)
- CMYK mode (four-printed colors)
- Index mode (256 colors)
- Grayscale mode (256 grays)
- Bitmap mode (2 colors)

- RGB (Red/Green/Blue) and Grayscale are the most commonly used

- CMYK (Cyan/Magenta/Yellow/Black) used by printers

- Index color is an older mode used to save file space (8-bit color)

- Do not confuse grayscale conversion with desaturation

To confuse matters further, images using pixels are considered **bitmap** (or **raster**) vs.
vector based images that use scalable Bézier curves (a Powerpoint shape is such a curve)

Color Modes (cont.)

Grayscale
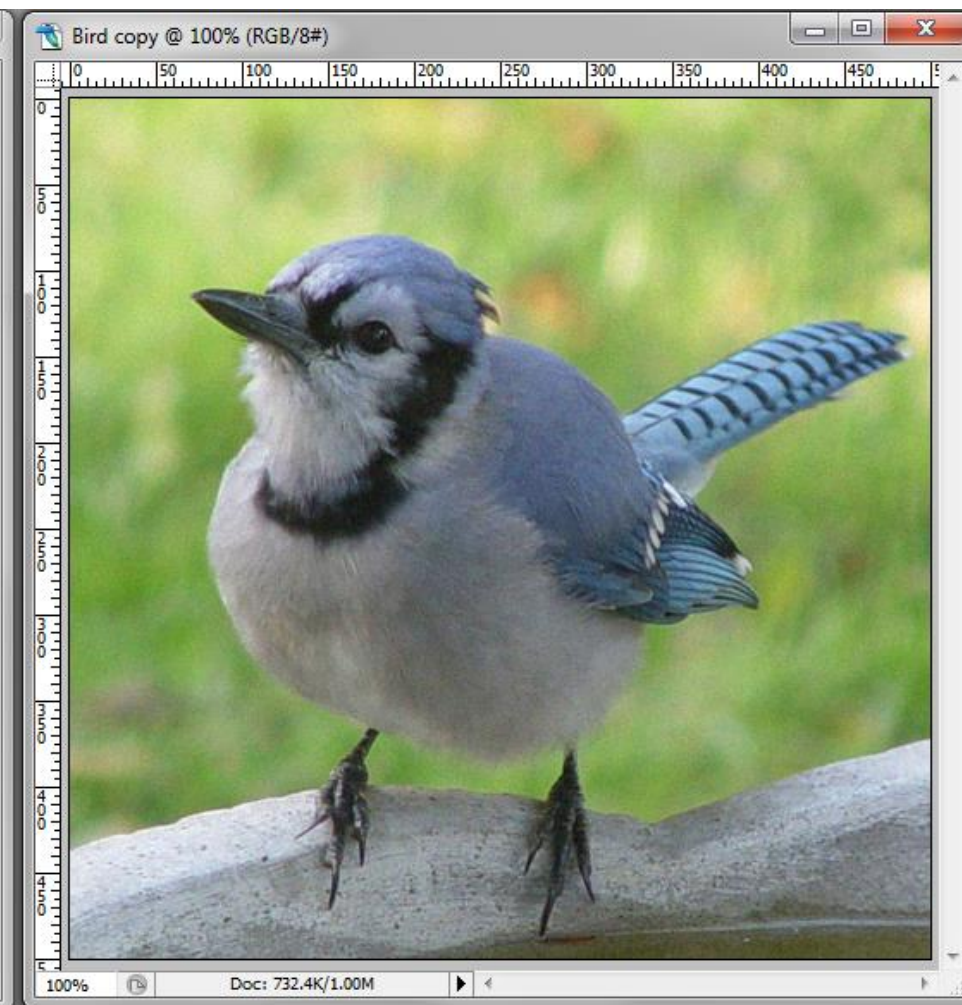
RGB color

Grayscale vs. Desaturation

FYI

Sometimes we want to change a color image to gray tones
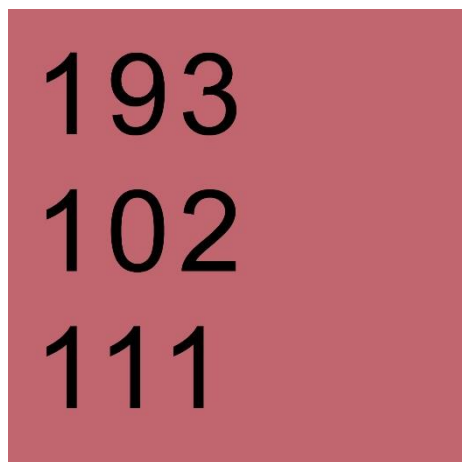
193 102 111	147	130
Original color	Desaturation	Grayscale
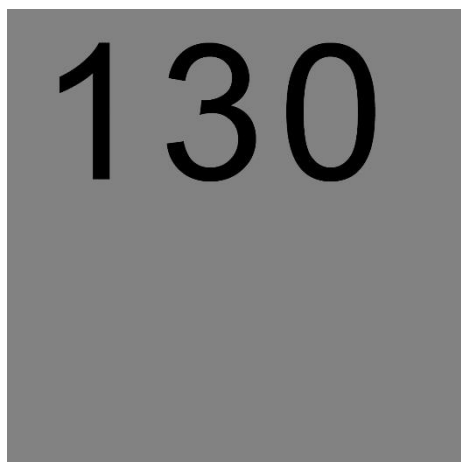
Desaturation is usually defined as the average of the red, green and blue components:
(red + green + blue)/3

Image > Adjustments > Desaturate

Grayscale is usually weighted to better fit human vision, and is the preferred method:
0.3% red + 0.6% green + 0.1% blue

Image > Mode > Grayscale

Color Wheel

Primary colors: red, green, blue

Complementary colors: magenta, cyan, yellow

- **Hue** = color
- **Saturation** = intensity of the color, or how much gray is mixed in
- **Brightness** = amount of black or white mixed with the color

Brightness is the axis coming out of the plane of the page
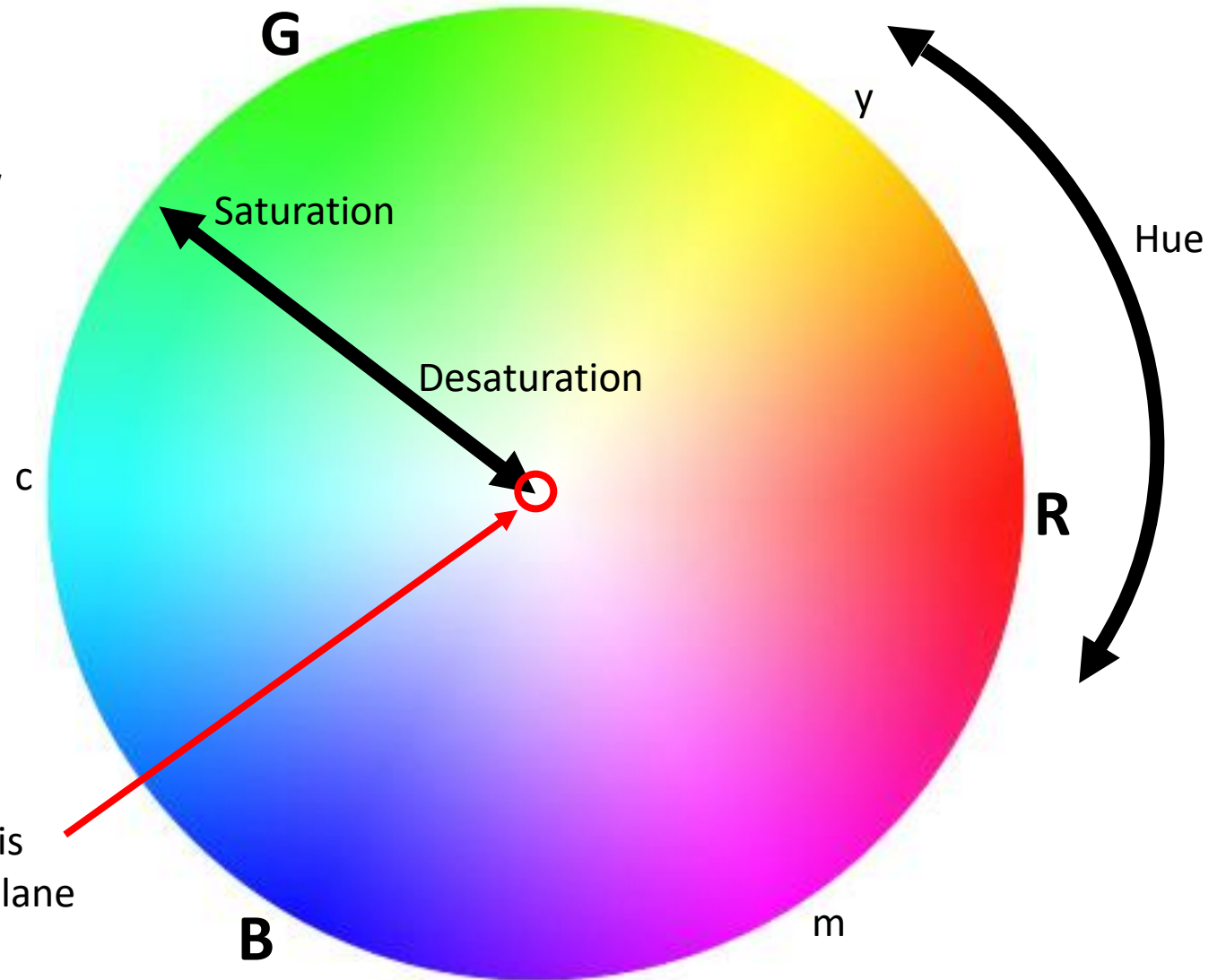
Color Balance

Image > Adjustments > Color balance
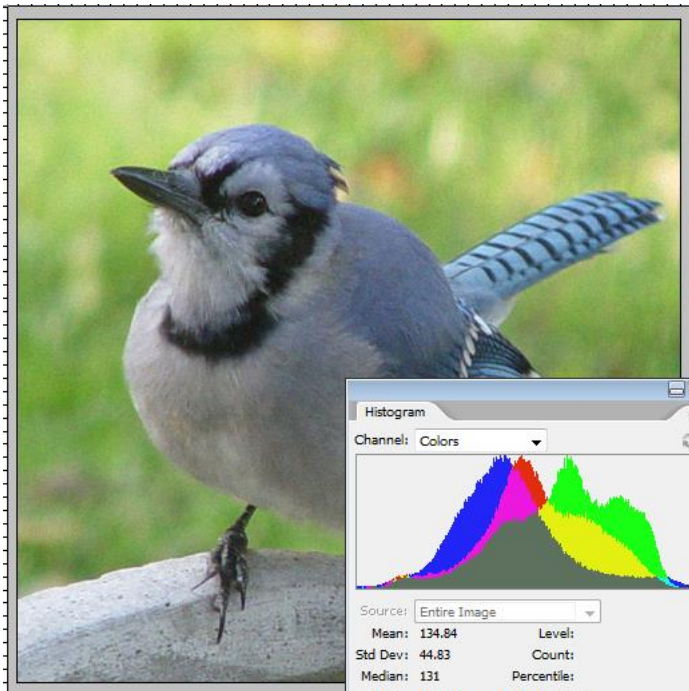
Original
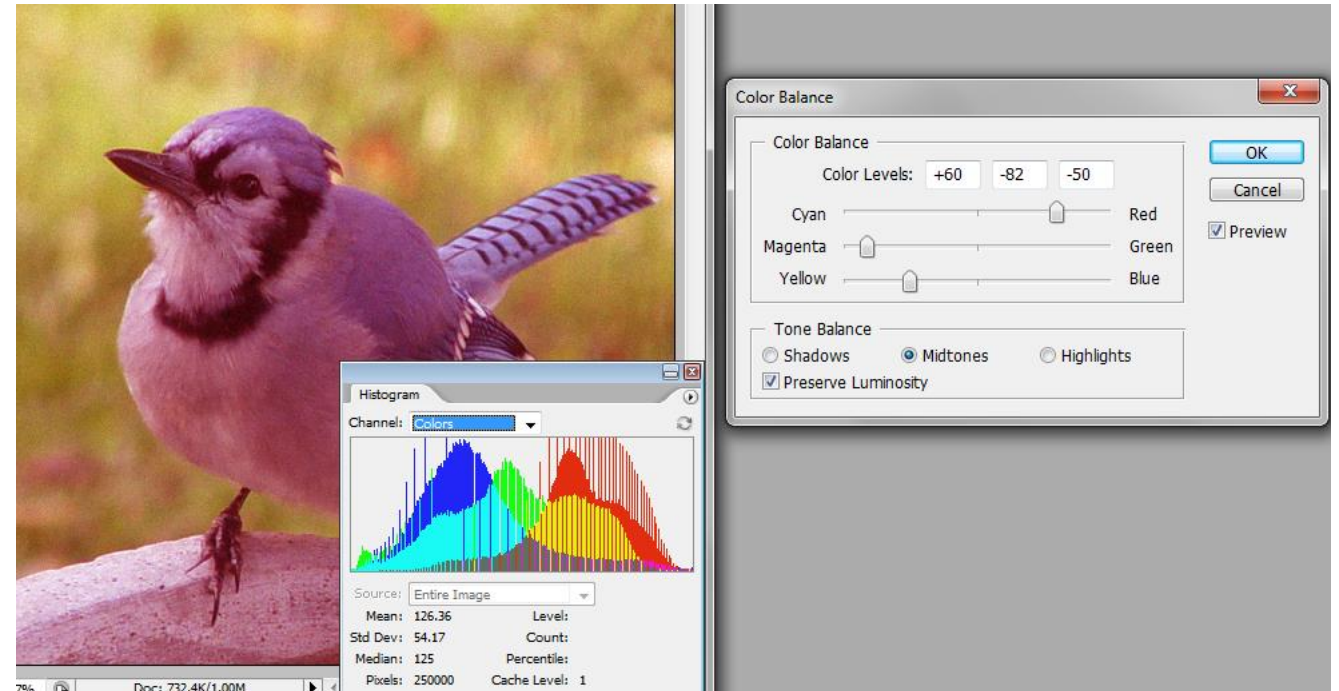
- Primary colors opposite complementary colors
- Frequently seen in printer software
- Color artifacts and brightness shifts

Hue and saturation

Image > Adjustments > Hue/Saturation
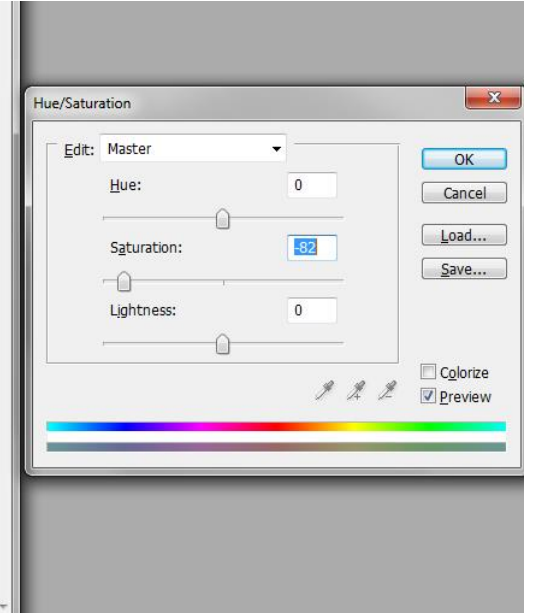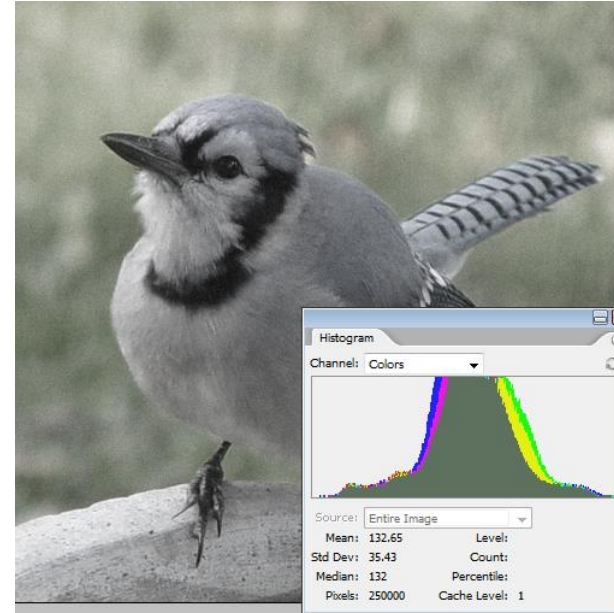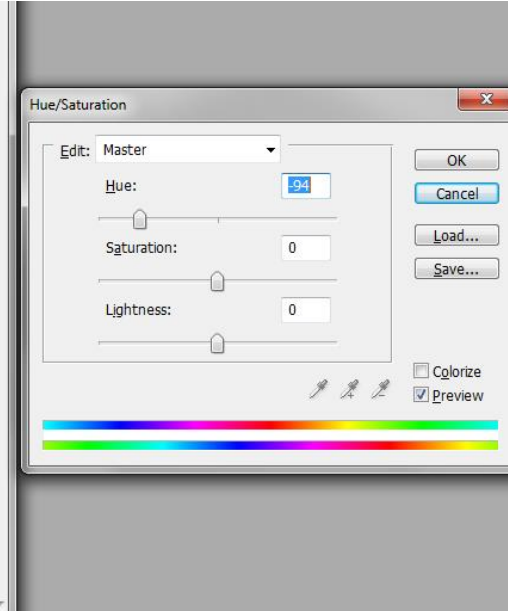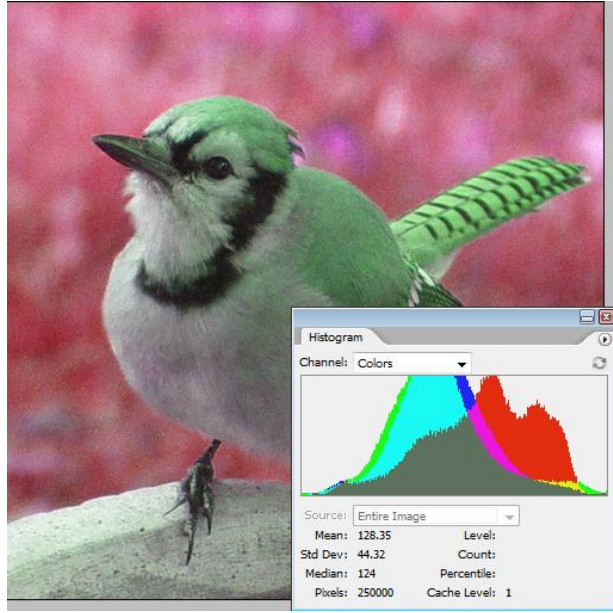
Hue adjustment (color artifacts)
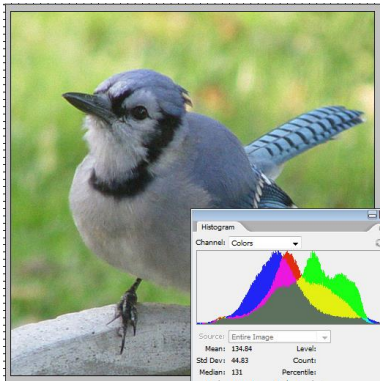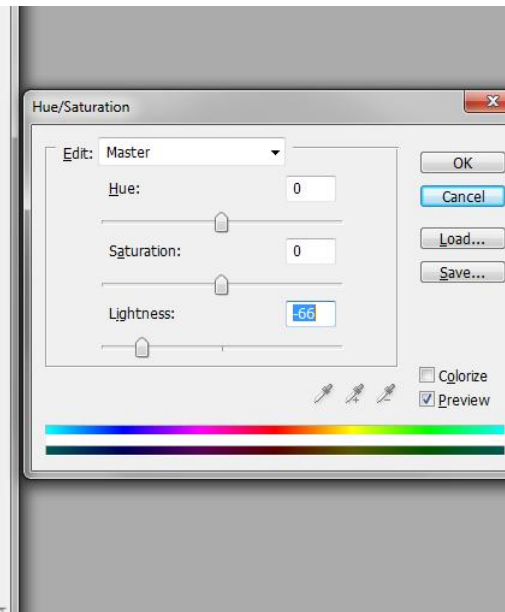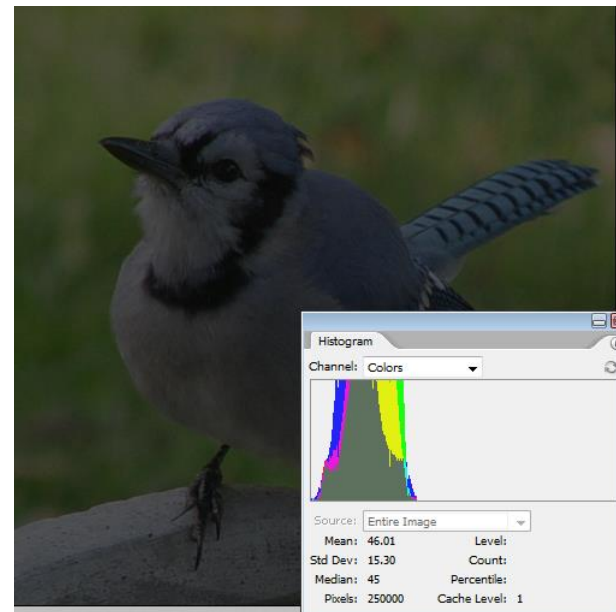
Desaturation (loss of color information)

original

Darker (loss of dynamic range)

Curves

Image > Adjustments > Curves   [CTRL + M]

The '**Curves'** command acts like '**Levels'** but gives you more flexibility than just black/white point and gamma
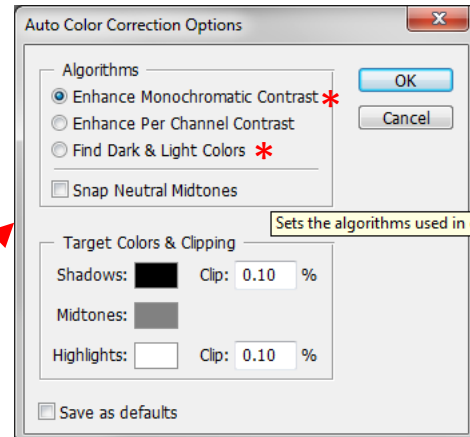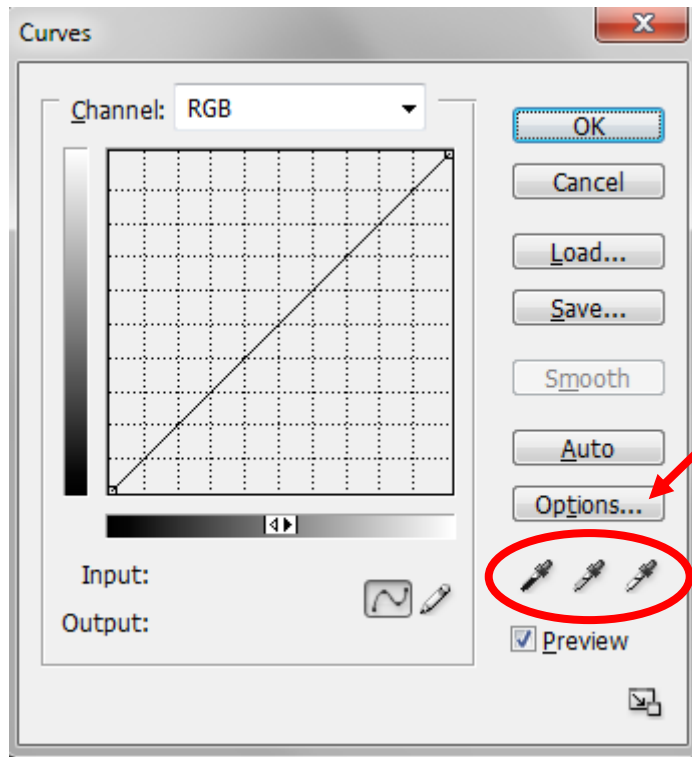
The '**options'** sub-palette acts like Auto-Levels with a clip at the extremes of the histogram

Black, neutral gray, and white pickers (you just have to decide what is black/gray/white in the image)

Curves (cont.)

FYI
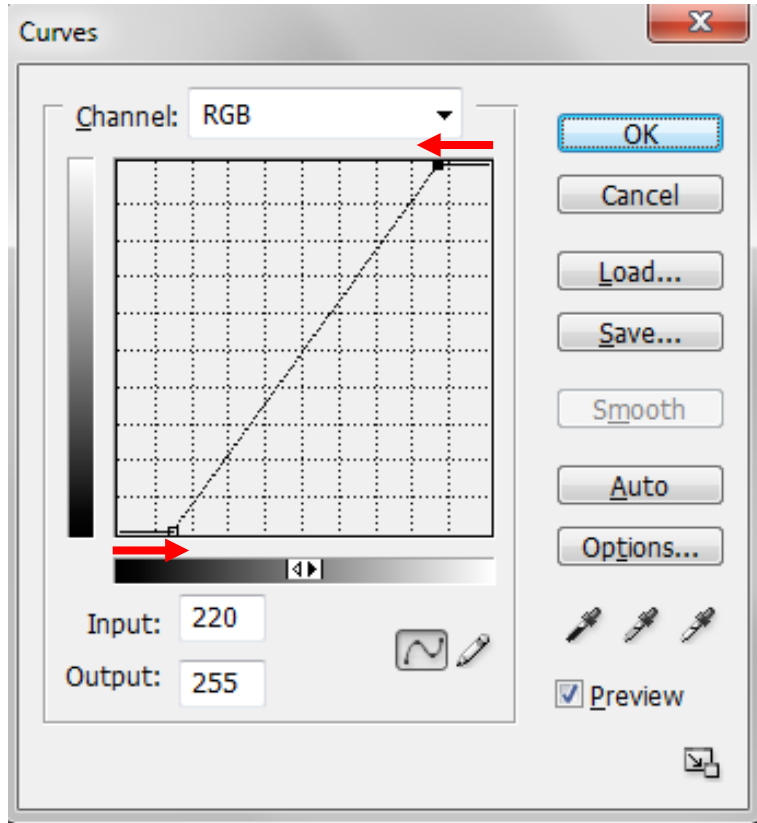
Points can be moved to manually set the white and black points

Points can be added and moved to make complex curves
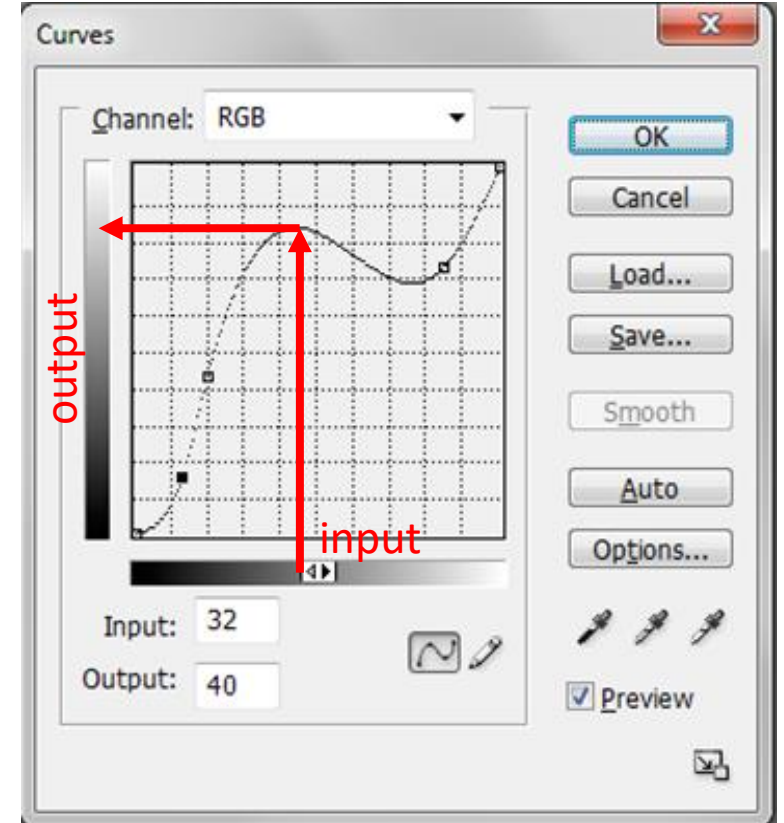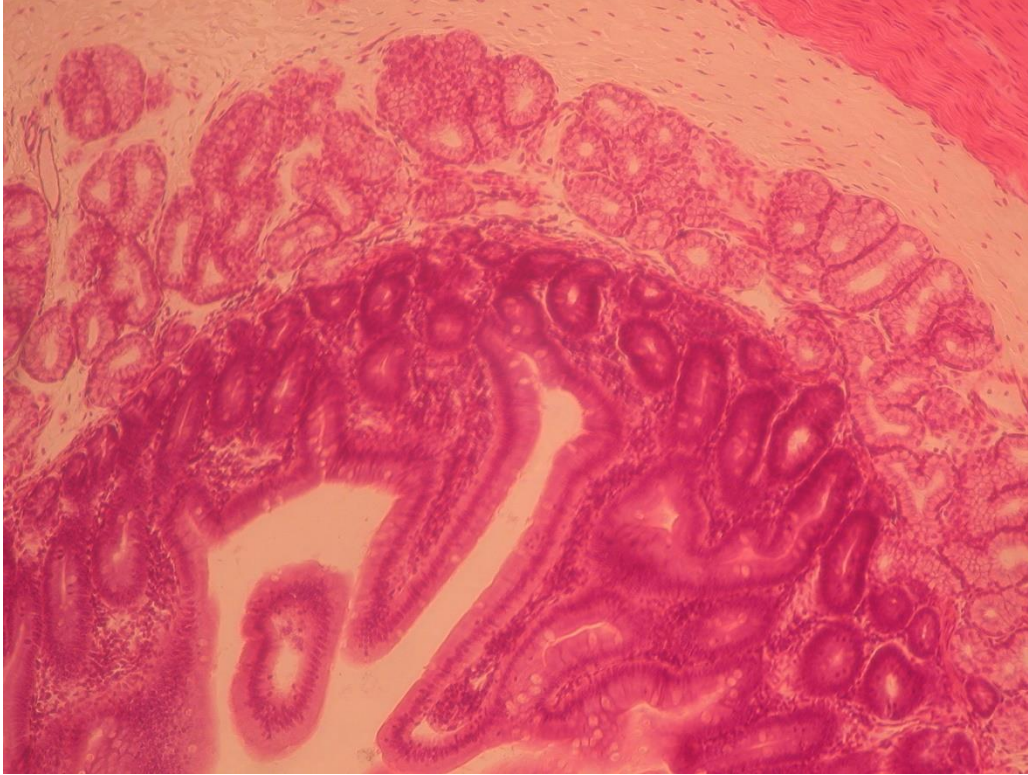
- In this image the red is too predominant

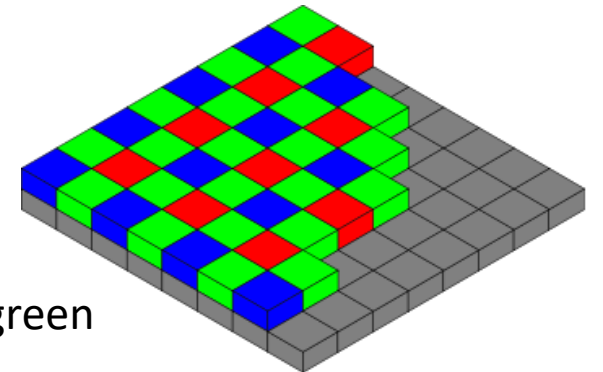- This should have been corrected at the microscope
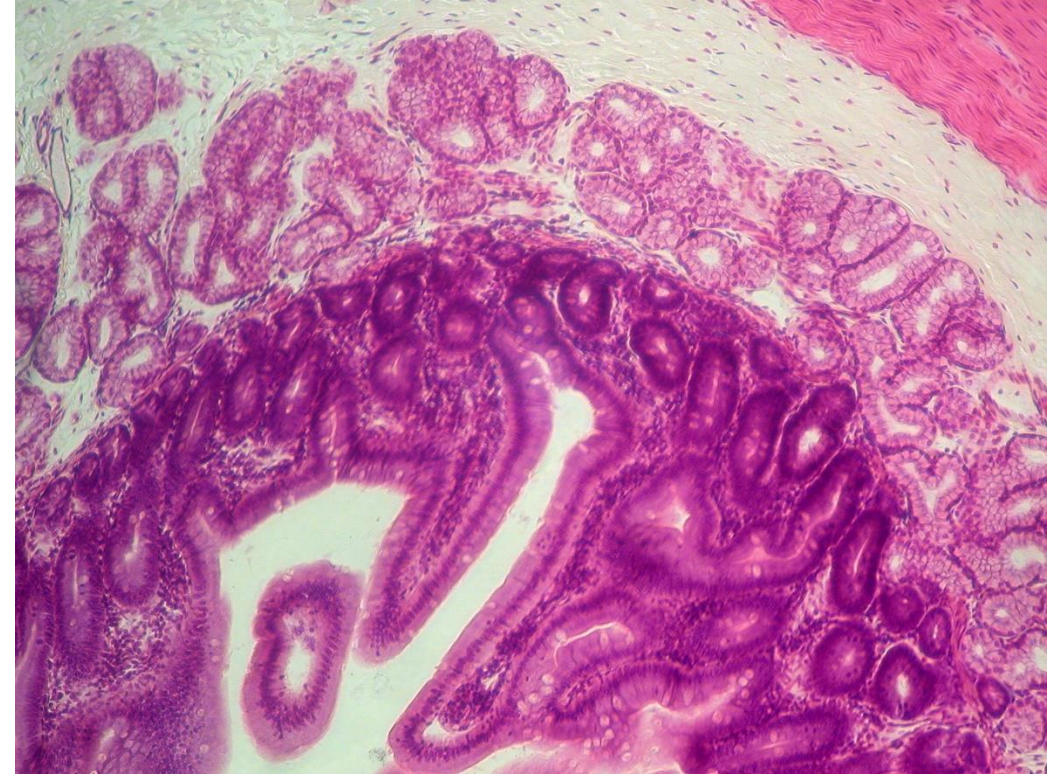
- Even with **Curves** the image is difficult to correct

- Empty areas still have a color cast

- Note the uneven background in the upper left

RGB Color Mode

RGB Color Space
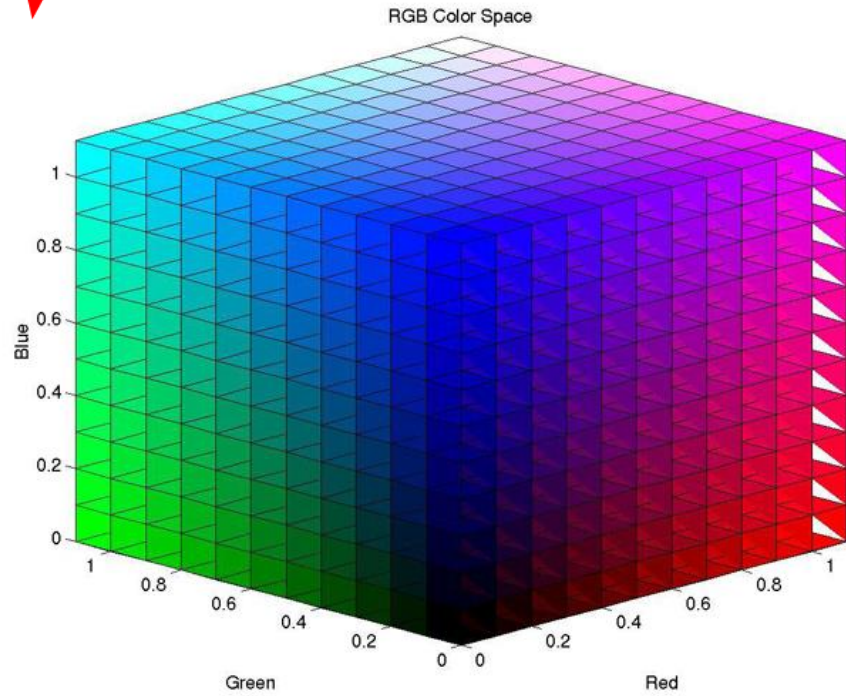
The RGB mode is one of the most commonly used for image collection

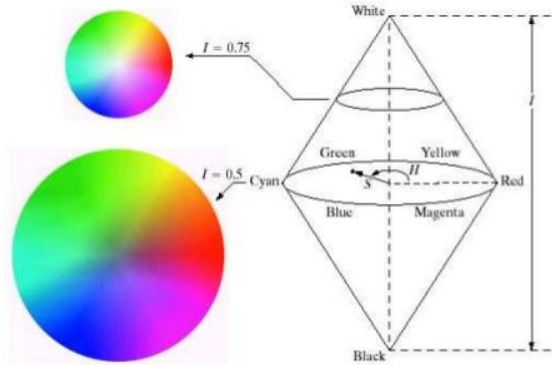The Bayer filter on CCD cameras rely on red, 2x green and blue

FYI

HSI & Lab Color Modes

Color images present some problems for image processing and analysis
For this reason sometimes a color mode is chosen that has a brightness component

FYI

Hue, Saturation, Intensity

HSI Color Model (cont.)

Intensity is given by a position on the vertical axis.

L*a*b* model

White L*
Yellow +b*
Green -a*
Red +a*
Blue -b*
Black

- Computer vision model

- Similar models include HSB, HSL & HSV

- Intensity component is White/Black vertical axis

- Hue expressed in degrees
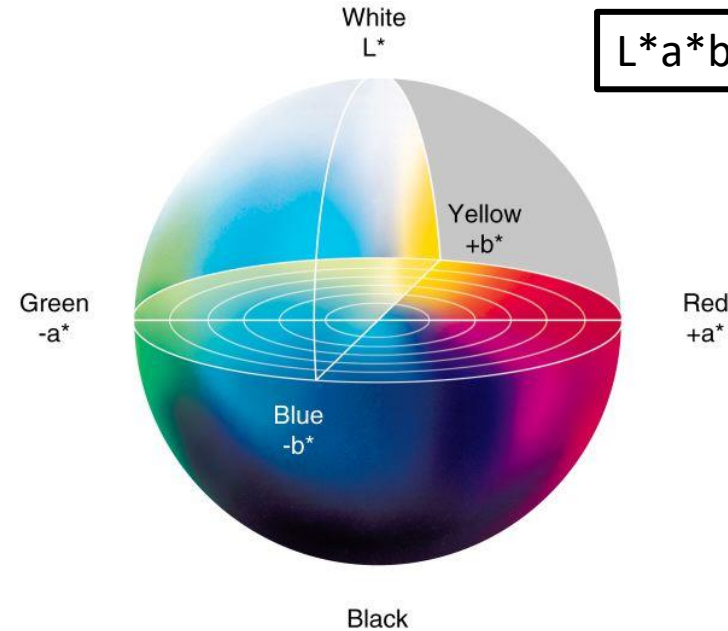
- Computationally simpler model

- This mode is in Photoshop

- Lightness component is White/Black vertical axis

- a = green to red; b = blue to yellow

FYI

Image > Mode > Lab Color
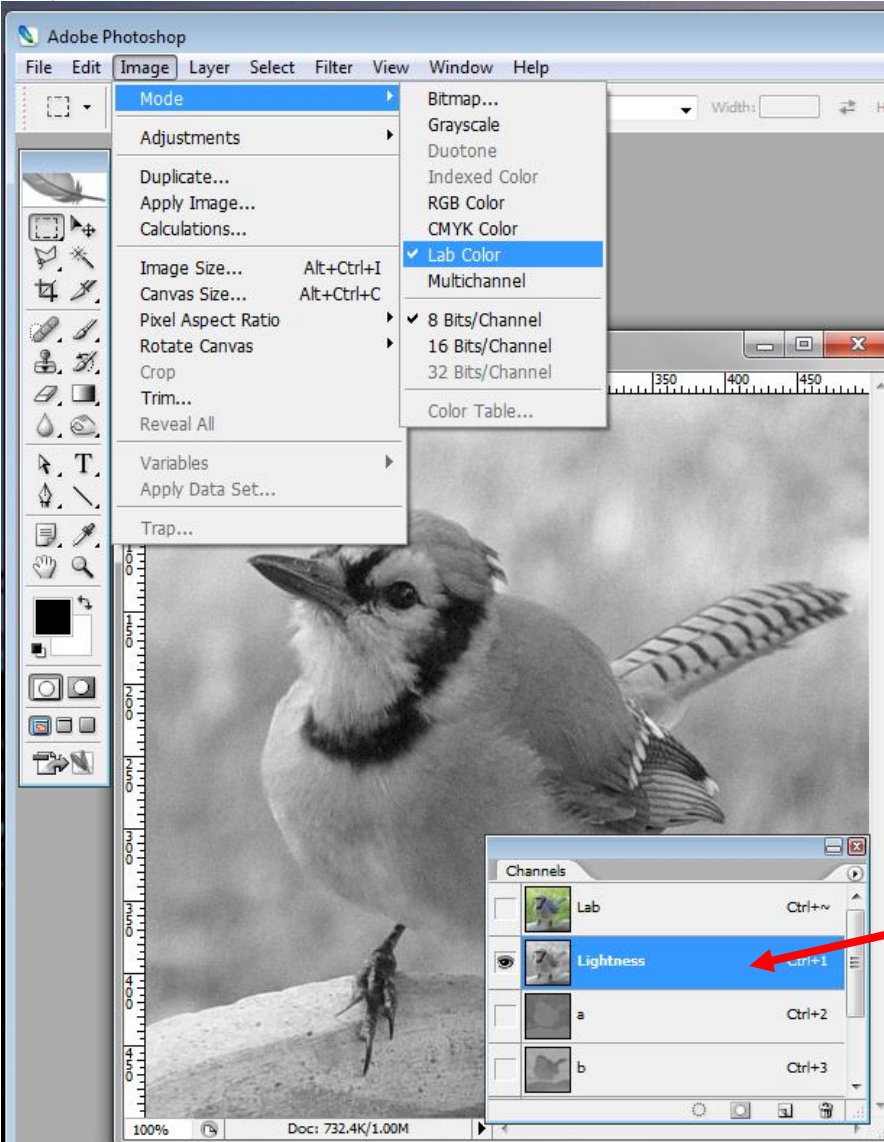
This image can be processed without affecting the color component

When done, the 'a' & 'b' components can be turned back on and the image converted back to **RGB Color**

Only L-component selected

['a' & 'b' turned off using 👁 icon]

Unsharp Mask on Color Image

Original
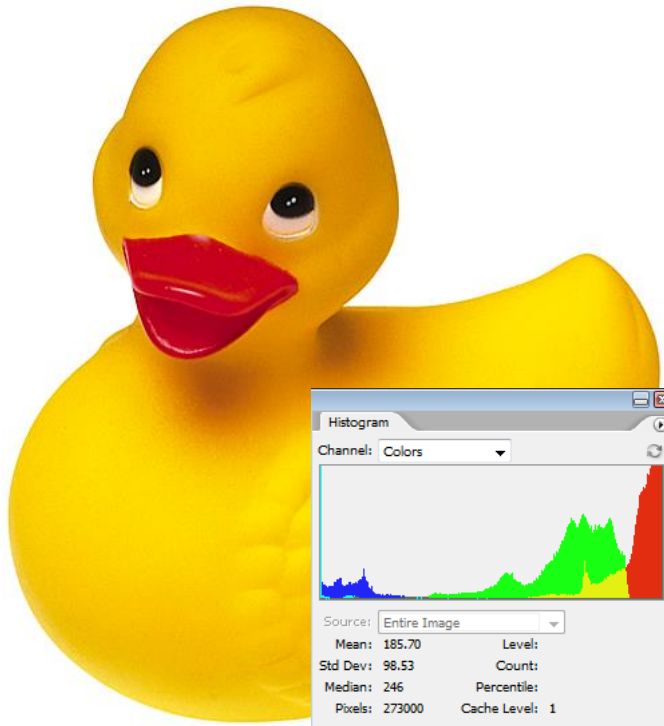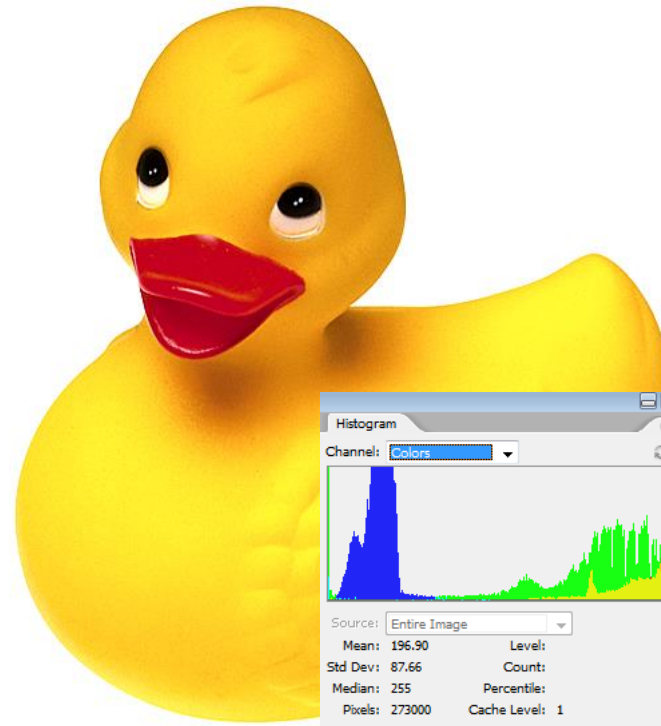
Applied to L-component of L*a*b* only and color added back
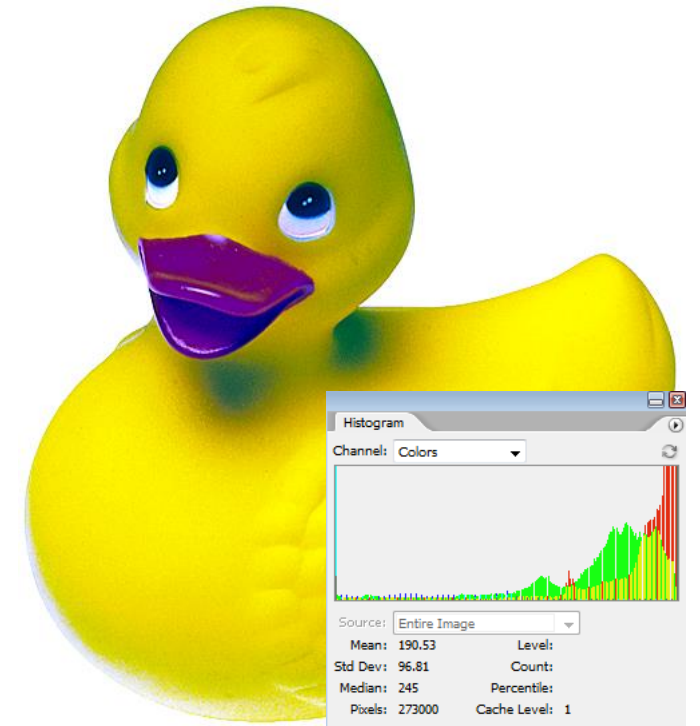
Applied to entire RGB image

Note color artifacts

Contrast Enhancement (true or genuine colors)
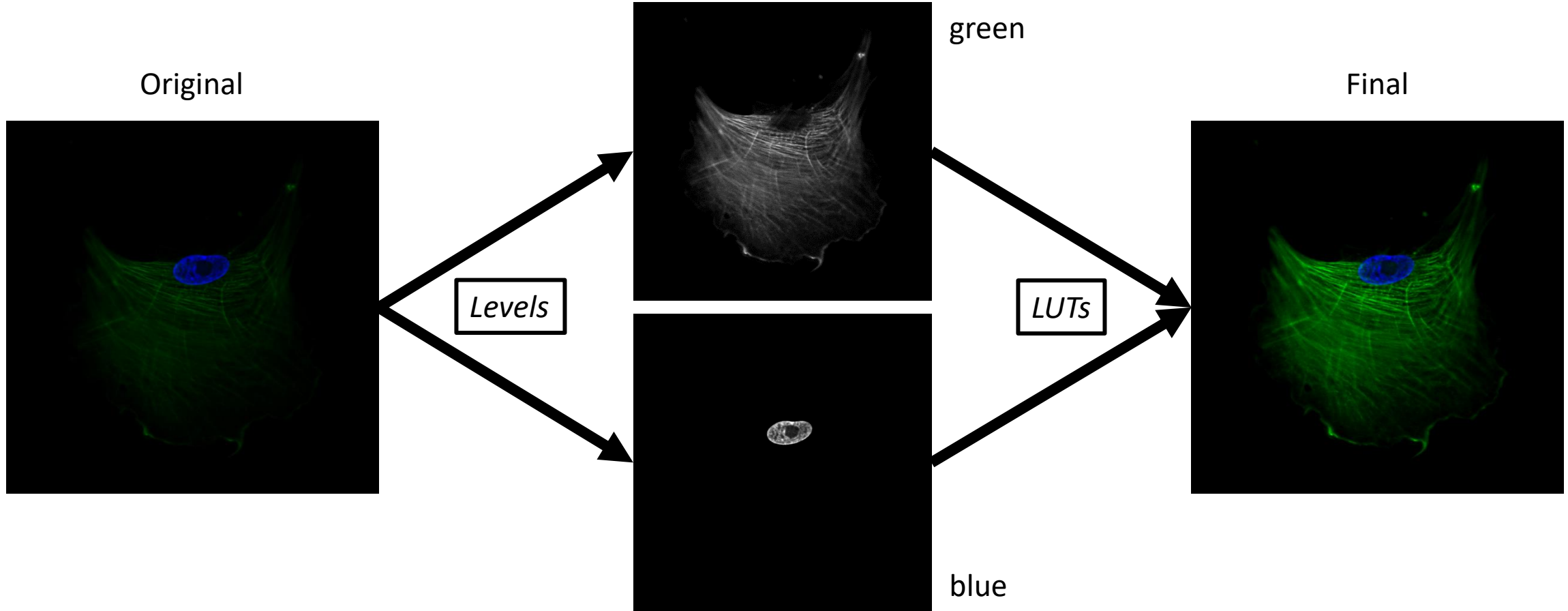## e.g. Light Microscope

Original

L-channel of L*a*b*
color mode only

Each individual color
of RGB mode adjusted

For this type of image be careful when treating the colors as independent variables

Contrast Enhancement (color composite or pseudocolor)
## e.g. Confocal Microscope

Original

green

Levels

LUTs

blue

Final

These types of images are independent grayscales that are combined and colored from a look-up-table (LUT)
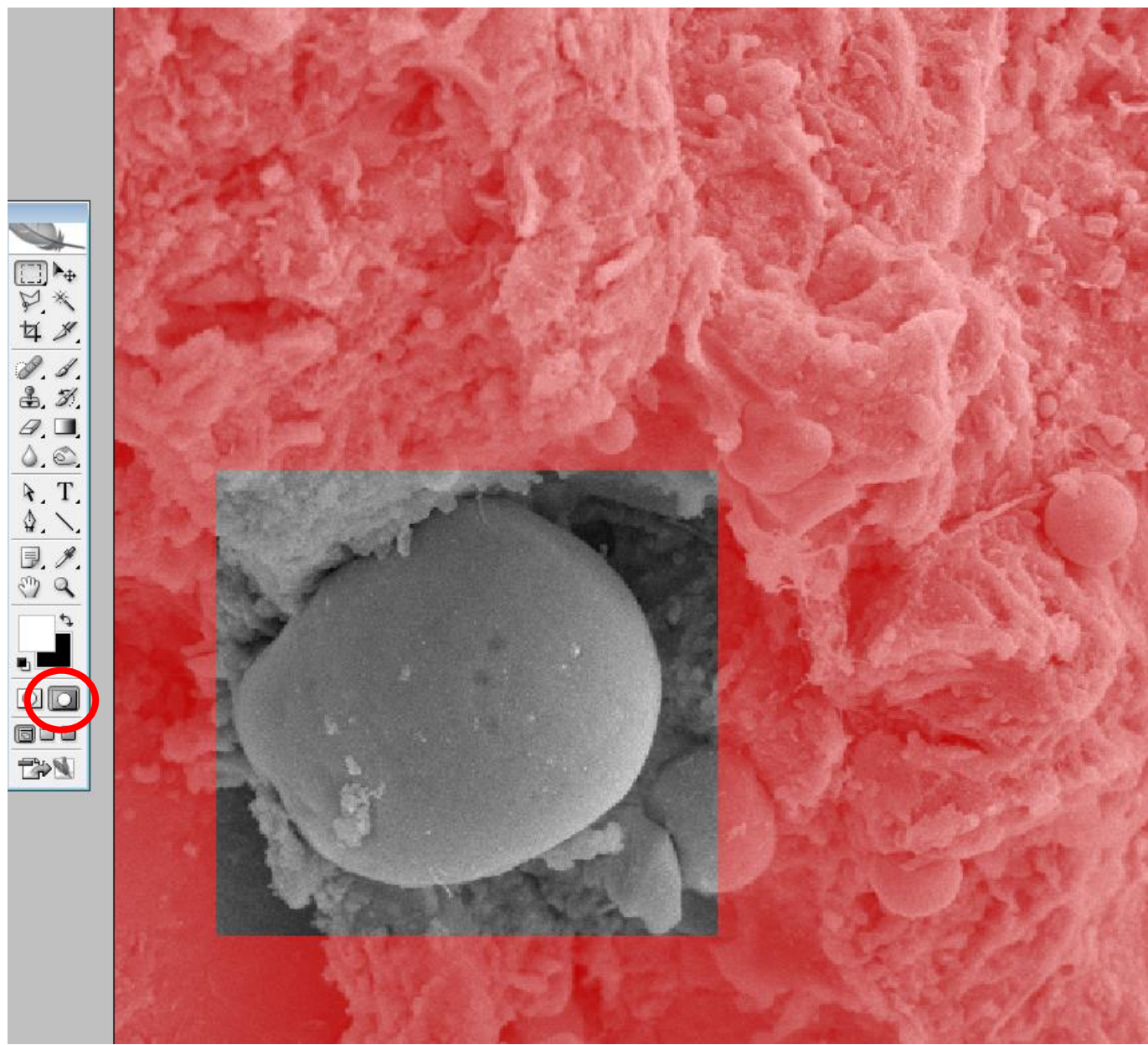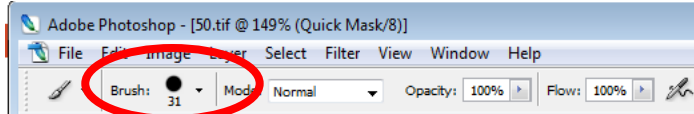
False Coloring an SEM or TEM Image

- We will use the **'red acetate'** method to make a selection. Anything that is **<u>red</u>** will not be selected.

- Open image and convert to RGB

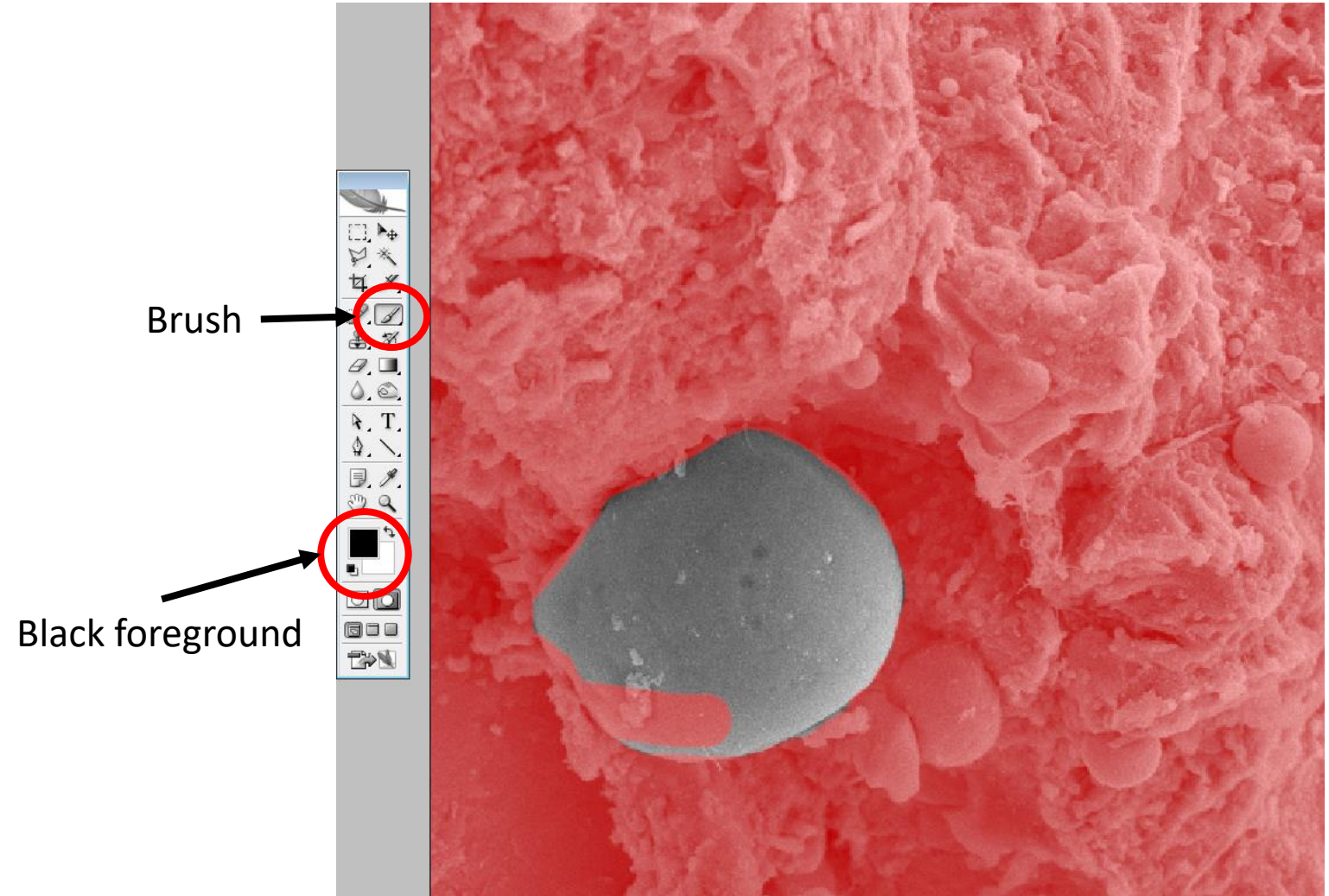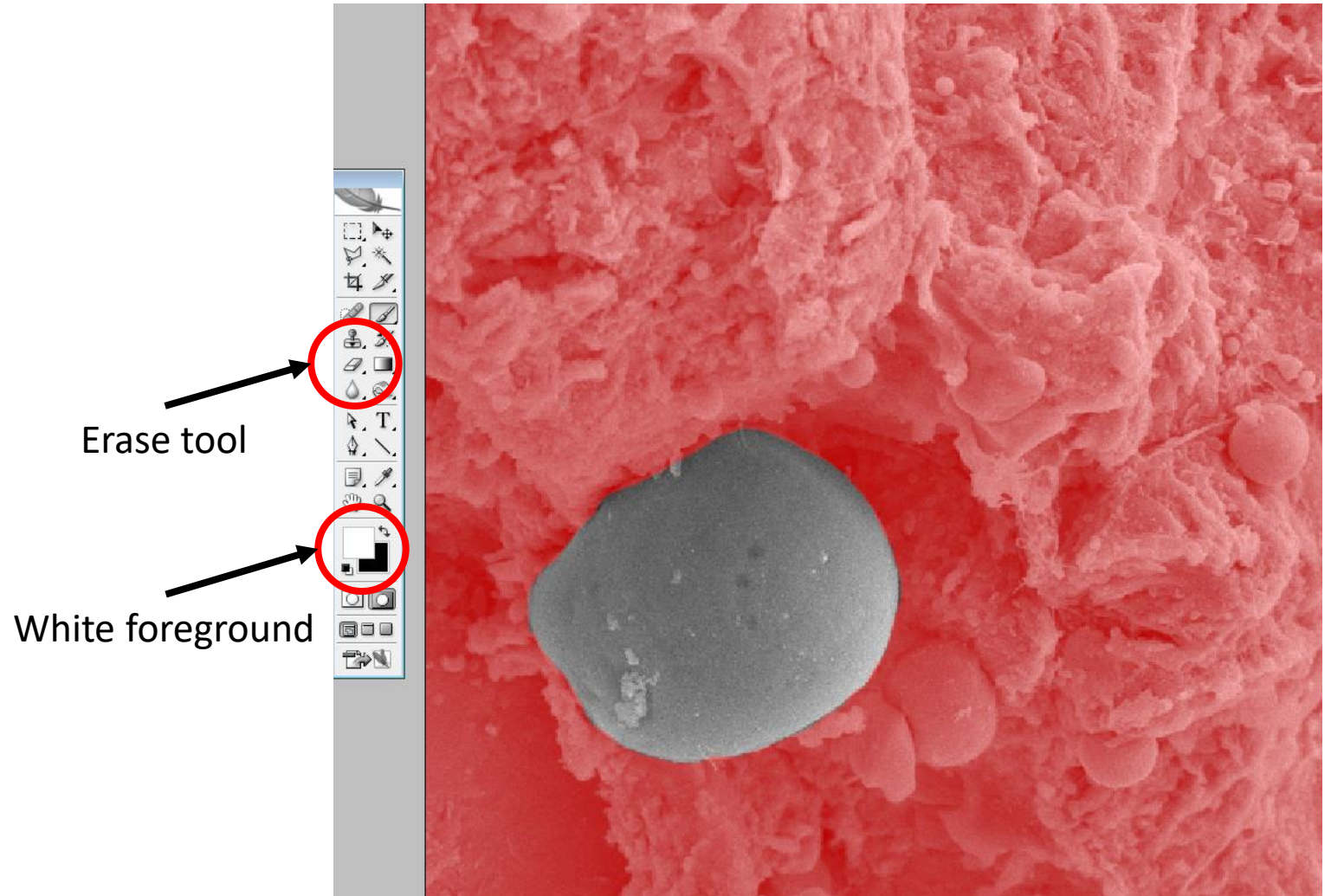- Draw a selection around an area of interest
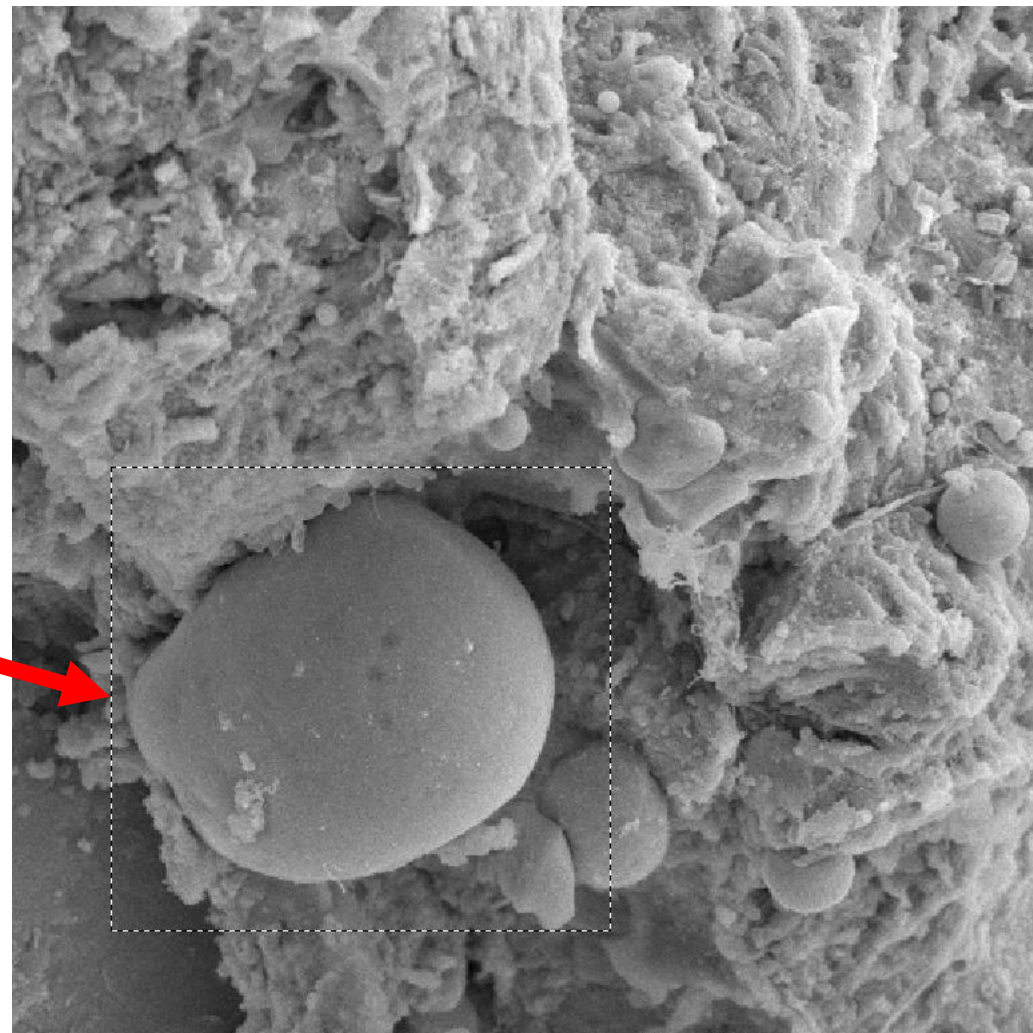
FYI

Enter 'Quick Mask Mode'

FYI

Brush size

Brush

Black foreground

- Use **Brush** tool to paint around area of interest

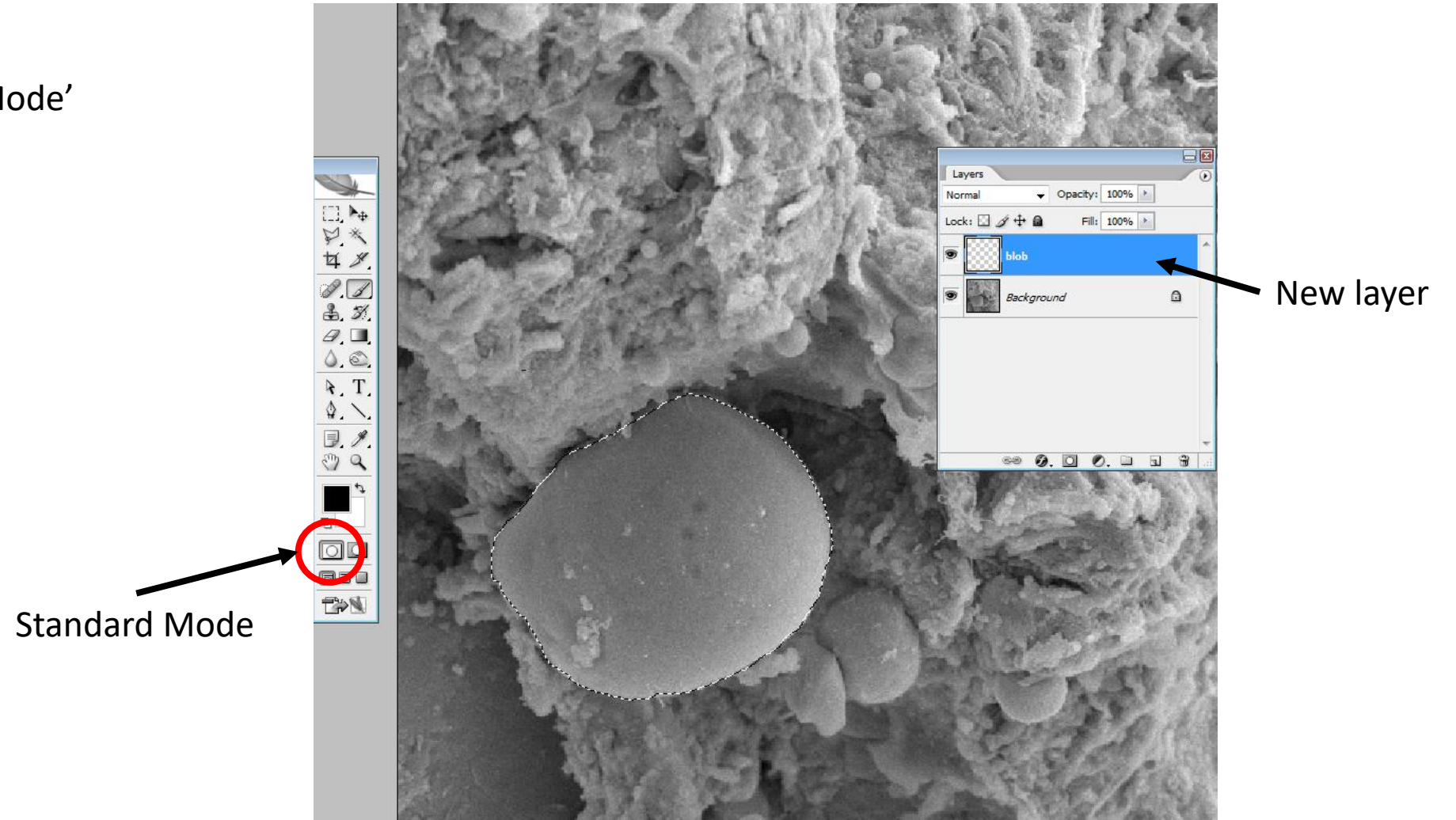- Adjust brush size as required

- Do not worry if you make a mistake

FYI

- Use Brush with white foreground to correct any mistakes
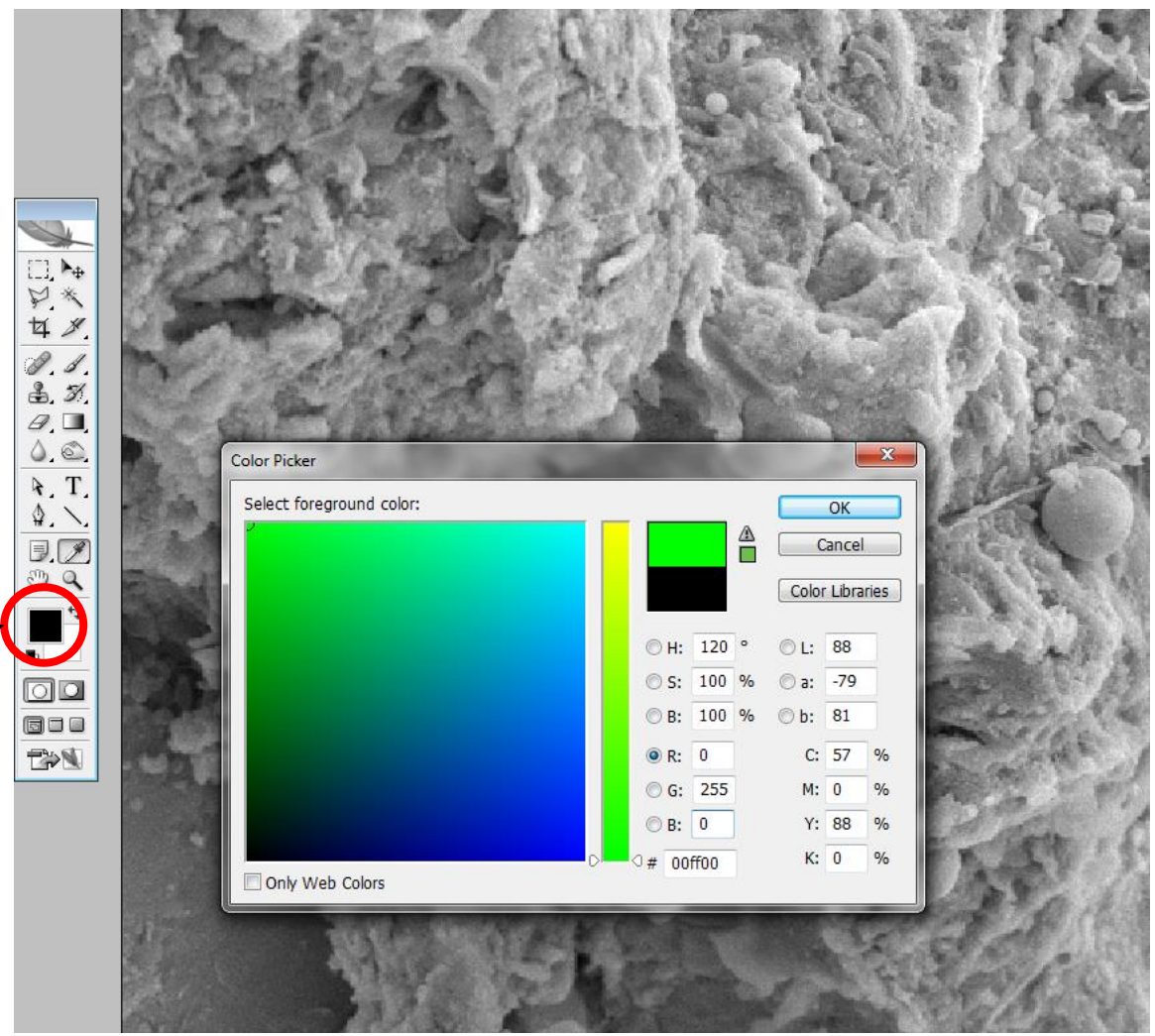
- You could also use the Erase tool

Erase tool

White foreground

- Return to 'Standard Mode'

- Create a new layer

New layer

Standard Mode

FYI
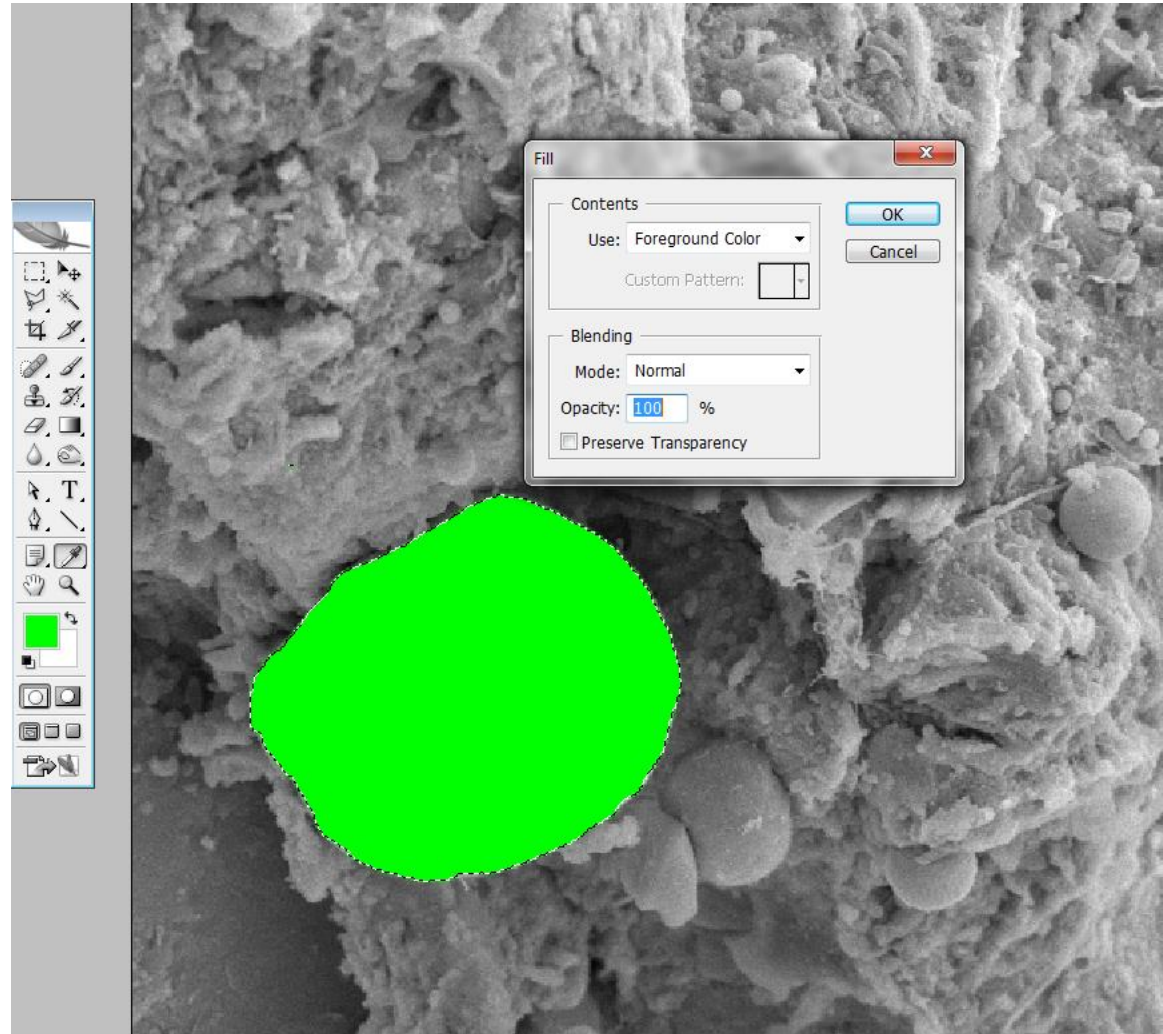
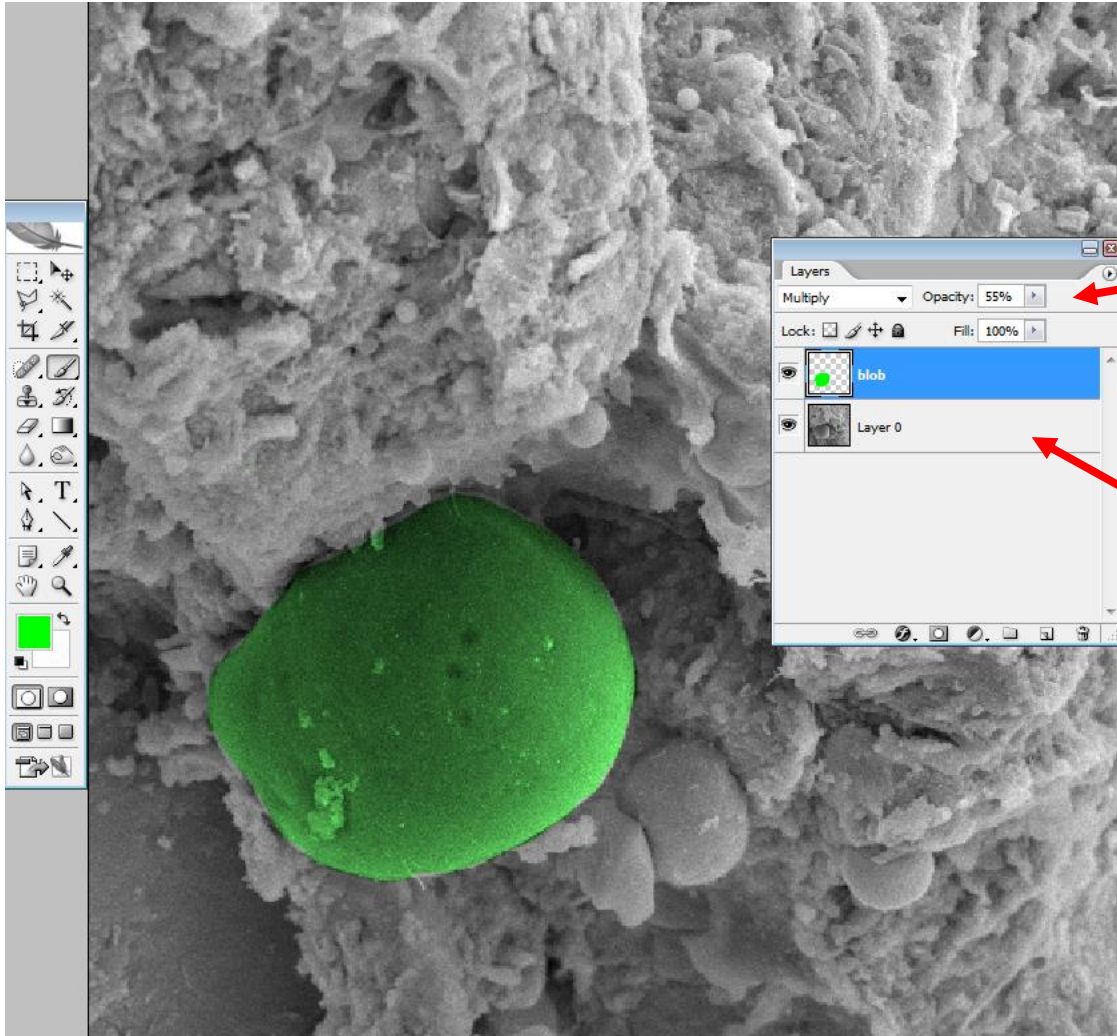- Click on 'Foreground Color' and choose a color

Foreground Color

- **Edit > Fill**

- Use: Foreground Color

Blending and opacity changed

Layer **0** unlocked (by double clicking)
and 'Levels' performed on selection.

- Optionally lock the layer to prevent changes

- Deselect the area and do another if you want

- Finish by flattening image and saving as *.tif