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**SCIENTIFIC AND MEDICAL IMAGING:
AN INTRODUCTION TO ADOBE PHOTOSHOP CS3**

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By

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Image File Formats:

TIFF - Tag Image File Format (TIFF)

The TIFF file format is the undisputed leader when best quality is required. TIFF is very commonly used in commercial printing or professional environments.

JPEG - Joint Photographic Experts Group

JPG files are NOT the best choice to be the master copy of your image. However JPG cannot be beat for emailing photographs to friends, and for web page use due to its small size.

PDF - Adobe Acrobat Portable Document Format

Industry standard file format for distribution to multi-recipients across multiple platforms.

PSD - Adobe Photoshop Document

Resolution:

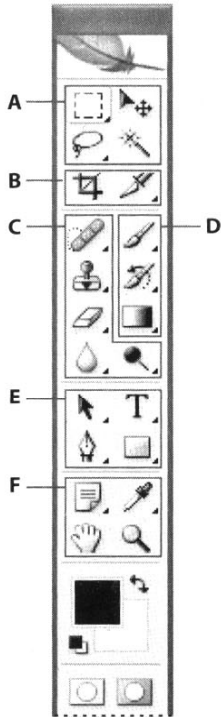
Refers to the sharpness and clarity of an image.

Image Formats

(JPEG vs TIFF)

JPEG		TIFF	
Pros	Cons	Pros	Cons
<p>Smaller File Size: JPEG uses lossy compression to reduce file size making use on the Internet or creating backup CDs less hassle.</p> <p>Supported by most software and photo sharing websites.</p>	<p>Lossy Compression: Lossy means with data loss. JPEG compression does discard some image data based on the amount of compression used.</p> <p>High Quality but not the absolute best.</p> <p>Not as good a choice for editing: JPEG files use lossy compression. If you plan to edit a photo and then re-save it you will lose some quality each time.</p>	<p>No Compression: Our TIFF files do not get compressed. This means 100% of the data captured during scanning is retained.</p> <p>Absolute best quality!</p> <p>Better if you plan to edit: Because TIFF files don't use compression they do not degrade in quality each time the photo is edited.</p>	<p>Large File Size: TIFF files are much larger than JPEGs making them harder to upload or email.</p> <p>Not supported by most photo sharing websites but is supported by most software.</p>

Toolbox overview



A Selection tools

- **Rectangular Marquee (M)**
- Elliptical Marquee (M)
- ⋮ Single Column Marquee (M)
- ≡ Single Row Marquee (M)
- **Move (V)**
- **Lasso (L)**
- ⬮ Polygonal Lasso (L)
- ⬮ Magnetic Lasso (L)
- **Magic Wand (W)**

B Crop and slice tools

- **Crop (C)**
- **Slice (K)**
- Slice Select

C Retouching tools

- **Spot Healing Brush (J)**
- Healing Brush (J)
- Patch (J)
- Red Eye (J)
- **Clone Stamp (S)**
- Pattern Stamp (S)
- **Eraser (E)**
- Background Eraser (E)
- Magic Eraser (E)
- **Blur (R)**
- Sharpen (R)
- Smudge (R)
- **Dodge (O)**
- Burn (O)
- Sponge (O)

D Painting tools

- **Brush (B)**
- Pencil (B)
- Color Replacement (B)
- **History Brush (Y)**
- Art History Brush (Y)
- **Gradient (G)**
- Paint Bucket (G)

E Drawing and type tools

- **Path Selection (A)**
- Direct Selection (A)
- **Pen (P)**
- Freeform Pen (P)
- Add Anchor Point (P)
- Delete Anchor Point (P)
- Convert Anchor Point (P)
- **Horizontal Type (T)**
- Vertical Type (T)
- Horizontal Type Mask (T)
- Vertical Type Mask (T)
- **Rectangle (U)**
- Rounded Rectangle (U)
- Ellipse (U)
- Polygon (U)
- Line (U)
- Custom Shape (U)

F Annotation, measuring, and navigation tools

- **Notes (N)**
- Audio Annotation (N)
- **Eyedropper (I)**
- Color Sampler (I)
- Measure (I)
- **Hand (H)**
- **Zoom (Z)**

G ImageReady only tools

- **Rectangle Image Map (P)**
- Circle Image Map (P)
- Polygon Image Map (P)
- **Image Map Select (J)**
- **Tab Rectangle (R)**
- Pill Rectangle (R)
- **Toggle Image Map Visibility (A)**
- **Toggle Slices Visibility (Q)**
- **Preview Document (Y)**
- **Preview in Browser (cmd+option+P)**

Reference Guide

Acquiring and Scanning Images

5 Steps to Follow When Acquiring Digital Images:

- White Balance (Color Images Only: Brightfield)
- Set Koehler illumination on microscope (Brightfield)
- Correct for Uneven Illumination in the Field (especially at low magnification: <10x)
- Avoid Over-Saturation (fluorescence primarily)
- Image- or Frame-Average (when possible for fluorescent or dim images)

File Size Settings when Scanning

Color Scans without text	6-8 megabytes
Color Scans with text	~10 megabytes
Grayscale without text	2-3 megabytes
Grayscale with text	4-6 megabytes
Binary	Ignore file size and set to 1200- 2400dpi (the smaller the text, the larger the dpi).

Setting up Monitor

1. Using controls on your monitor itself, adjust brightness so that it is at the halfway point, and contrast so that it is all the way up (as contrasty as possible). Keep ambient lighting LOW.
2. If not done already, set up computer screen for color and contrast using Adobe Gamma:

Windows:

1. Click on Start.
2. Select Settings/Control Panel.
3. Double click Adobe Gamma.
4. Choose Step by Step Wizard (See steps at right for details).
5. When setting gamma, choose 2.2 (Europe: 1.8).

Mac:

1. Click on Apple symbol (upper left).
2. Highlight Control Panels/Adobe Gamma.
3. Choose Assistant.
4. When setting gamma, choose 2.2 (Europe 1.8).

Troubleshooting (Wrong Layer, Text Layer, Wrong Mode)

"Cannot complete request because the file layer is locked" or "Cannot complete because area is empty." You are on the wrong layer or on the background layer (in 6x, 7x).

Computer crashes often in Photoshop. "Scratch disks are full." In Preferences, choose primary & secondary disks with lots of hard disk space (3 times the size of the file). Defragment these disks regularly.

Crop tool magnetizes to the edge. Use Marquee tool, instead (4x, 5x) or under View uncheck Snap To > Document Bounds.

Crossed line over circle icon appears. Menu selections are grayed out. You are probably on a text layer: select the layer of interest. It can also be a result of the image being 16-bit or Indexed Color: change the Mode (Image > Mode > RGB Color or 8-bit)

I used a function (such as Levels), but nothing happens. You have accidentally drawn a tiny selection with the Marquee tool (control/command key + D deselects) or you are on the wrong layer.

Line tool doesn't make a line. Make sure the foreground color in the toolbar is not the same color as what you are drawing over. Make another layer in 4x, 5x (a bug in these programs). In 6x, 7x, render the layer (Layer > Rasterize).

"No pixels are more than 50% selected. The selection edges will not be visible." You are on an empty layer: choose layer of interest; or you have feathered your edges, or chosen a Fuzziness value in Color Range, too radically.

Setting Preferences

- Preferences > General > Transparency & Gamut: Set Grid Size to None.
- > Display & Cursors: Set Painting Cursors to Brush Size.
- > Plug-Ins & Scratch Disks: Set 2nd if you have additional hard drive or partition.
- Preferences > Color Settings: Uncheck Ask When Opening to undo color mismatch prompt.
- View > Show Rulers or Rulers.

Setting up Info Box

Click on arrowhead and select Palette Options.

Set First Color Readout to Grayscale.

Set Second Color Readout to Actual Color

K: Previous black ink amount / Current black ink amount.

Common Steps for Single Images (Typical)

<p>Mode: Mode may have to be changed to Grayscale or RGB Color; 16-bit to 8-bit. Image>Mode.</p> <p>Levels: Image>Adjust(ments)>Levels. Adjust contrast with white and black sliders; middle slider if image is not evaluated by light to dark values (as in gels, blots and images intended for densitometry). Move Output black slider so that K value in Info box reads at >90% (usually ~85%); white Output slider is at 250 - 254 (unless the image is a graph or drawing).</p> <p>Scanned images: Align. Drag down guideline (View>Show Rulers: drag from Rulers). Image>Rotate Canvas>Arbitrary. Rotate or Flip: Image>Rotate Canvas>Flip or 180/90 CW CCW.</p> <p>Crop: Use crop tool. Under View uncheck Snap To>Document Bounds (version 7x).</p> <p>Dust & Scratches: Filter>Noise>Dust & Scratches. Keep Radius at 1 or 2. Use Eyedropper tool for larger areas; Healing tool in 7x. Choose fuzzy-edged Brush (in menu for 6x, 7x; open Windows > Brushes in 4x, 5x). Be ethical! Consult with your advisor.</p> <p>Sharpen: Filter>Sharpen>Unsharp Mask. Move Amount slider; keep Radius between 1 & 2.</p> <p>Color Correction: Image>Adjust(ments)>Levels. Click white eyedropper tool on white background for Brightfield images or use Auto Color (Image > Adjustments > Auto Color in 6x, 7x).</p> <p>Color Adjustments: Image>Adjust(ments)>Hue & Saturation. Start by desaturating offensive colors. Shift blue to cyan for fluorescent images.</p> <p>Color Matching 2 or more images. Use Color Matching protocol. Balance backgrounds first. Use location marker (Eyedropper Tool + Shift key) to mark similar points on 2 images in the Info Box. Use Curves (Image > Adjust(ments) > Curves) & pull down or across from top to match RGB values.</p> <p>Color Adding (fluorescence): Image>Adjust(ments)>Curves. Select color from Channel OTHER than desired color. Pull down line from top to bottom quadrant; blue should be pulled further to add yellow to green or red (be sure gradient at bottom of dialogue box is clicked on to be dark on the left).</p> <p>Adding White Space to Images for Lettering (gels, etc.): Image > Canvas Size. Put in new values. Be sure background at bottom of toolbar is white.</p>	<p>Common Shortcuts</p> <p>Zoom: Control +, Control -, AppleKey + & -</p> <p>Navigate: Spacebar</p> <p>Move Tool: Control/Apple Key (when on Marquee tool)</p> <p>F7: To reveal Layers Box</p> <p>F8: To reveal Info Box</p> <p>Copy: Control + C key</p> <p>Paste: Control + V key</p>
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Common Steps for Making Multi-Image Plates & Working With More Than One Image

<p>Merge Images (Fluorescence: Colocalization): Open red & green image. Select All on red image, cut, close, don't save. Paste on green image. In Layers box, select Screen (vs. Normal) from drop down list.</p> <p>Make New Layout Page: File > New. Typically image dimensions are best determined by making a letter-sized page (8.2 X 10.7 -allowing for margins) at 300dpi. Copy and Paste, or drag images onto this page using Move tool (or Marquee tool + control/command key). Be sure that images are flattened (Layers > Flatten) before moving, or that Layers are locked with lock icon.</p> <p>Scale Images: Edit > Transform, hold down Shift key while re-sizing by clicking on a corner. If images are not on a Layer, these will have to be selected with the Marquee tool first. Images can be scaled similarly by paying attention to scaling percentage in the Info Box.</p> <p>Lining up Images, Making borders: Drag down/across guidelines from Ruler Area. Make borders between by zooming in to create 10-20 pixel borders (Preferences > Units & Rulers > Units: Pixels). Under View > Snap or Snap To Guides should be checked.</p> <p>Lettering: Click on T tool in 6x, 7x. Double click in 4x, 5x. Settings for tool are in Character Palette on menu (6x, 7x). Click check mark or return to Marquee tool to finalize (6x, 7x). Move lettering by moving cursor outside lettering area. Edit lettering by dragging over lettering to highlight first. Each new click of the Text tool makes a new layer!</p> <p>Changing Orientation of Type: Edit > Transform > Rotate. Drag from a corner. Hold down Shift key for increments.</p> <p>Arrows/Scale Bars: Select Line tool on Toolbar (double click on it in 4x, 5x). Click on arrowhead on menu or select Arrowheads in Palette (4x, 5x). Width is 400% or so, Length 600% or so, Concavity 0. Weight is set for thickness of line in pixels. Scale Bars: Draw line (sans arrowheads) while holding down Shift key & checking Info Box for desired length in pixels.</p> <p>Boxes, Circles: Make new Layer in Layers bos. Choose Marquee, hold down for Ellipse. Draw rectangle (for box or circle hold down Shift key). Edit > Stroke. Set Width to desired thickness.</p>	<p>In Image Size dialogue box: Check Resample when going to: Publisher, Photographic Printers. Uncheck Resample when going to: PowerPoint, Ink Jet, Laser Jet, Slide Writer. Crop Layout, then set desired Width and Height.</p> <p>Saving Files JPEG: PowerPoint, Word. PSD: Photoshop for Re-Editing Purposes. TIFF: For Publisher. PDF: For Distribution/Grants.</p>
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Showing Colocalization

When making an image which shows colocalization, it is best to start with the red colorized image. Often red is dim compared to the blue and green, and so the latter two may need adjustments to their opacity.

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1. Open your first file, the red colored image. Then open the green colored image.
2. Under Select on the menu, choose All to select all of the green image. Under Edit choose Copy. Under File choose Close. Under Edit choose Paste to paste the green image onto the red image, thus creating a layer.
3. Open Layers dialogue box under Window on the menu, if this dialogue box is not already open. Click arrowhead for drop down list at top left (it reads Normal) to select Screen. Screen is a function normally used in the printing industry to give equal percentages of 4 layers for CMYK. Where you have colocalization, you should see yellow. If the green is too bright compared to the red (which often happens), then you can adjust the intensity of the green by changing the Opacity slider. Obviously, this should only be done to the degree that the relative intensities of both the red colored image and the green colored image appear to have the same brightness. Often the green image is never reduced by more than 15 percent.
4. If three or four images need to be colocalized, repeat what you did with the green colored image (Select All, Copy, Close, Paste, choose Screen from drop down in the Layers box).

You might also want to change contrast of each layer in case there is too much background color on any of the layers, or in case any of these layers are too dark AFTER running this method.

Free Image Processing and Analysis Programs

ImageJ and Image Tools are free open source programs that allows users to acquire, display, edit, analyze, process, compress, and save images. Both have an extensive array of plug-ins that can be downloaded from the links listed below.

ImageJ (Produced by Wayne Rasband of the NIH)

<http://rsb.info.nih.gov/ij/> (ImageJ Installation file)

<http://rsb.info.nih.gov/ij/plugins/index.html> (Plug-in Index for ImageJ)

Image Tools (Produced by The University of Texas Health Science Center at San Antonio)

<http://ddsdx.uthscsa.edu/dig/itdesc.html#What>

Wright Cell Imaging Facility, Toronto Western Research Institute

<http://www.uhnresearch.ca/facilities/wcif/imagej/>

Currently available Plug-ins for ImageJ (Partial List)

Line Analyzer	Image Correlator	Area Calculator	Particle Remover
Measure Angles	Batch Statistics	Cell Counter	Multi Cell Outliner
Color Comparison	Radial Profile Plot	Microscope Scale	Sync Measure 3D
Colocalization	Texture Analysis	Cell Outliner	RGB Profiler
Colocalization Finder	Color Profiler	MR Urography	RGB Stack Merge
FRETcalc - FRET by acceptor photobleaching			
UCSD Confocal Microscopy Plugins			
FRET and Colocalization Analyzer			

How to determine band intensity of Western blot?

Start With a High-Quality Original

Before working with Photo Shop, before you begin the scanning process, it's important to make sure that the original print, negative, or transparency (slide) is of good quality. While image manipulation programs, like PhotoShop, can correct for flaws in the original (for example, its tone, brightness, etc.), you cannot bring out elements that don't exist in the original image. Also, a high-quality original means less work for you later.

First thing you have to do is get a GOOD exposure of your gel. This means you DON'T overexpose. Rule of thumb is you have to be able to see through the bands (i.e. put it up against a piece of paper and see if you can read some text through it. Do not make a jpeg of your film, jpegs contain far less image data than tiff files.

Secondly, ALL the bands you want to compare have to be on the same film. The quantification is all relative towards one band you put as 1 (or 100%).

- 1) In PS, load you your image
- 2) Then do, Image>Adjust>Invert (this way, you invert the scale)
- 3) Draw a box, which covers ONE band using the Marquee Tool (do the biggest band first, as the box HAS to be the same size all way through the analysis. This way to don't have to take the box size into consideration)
- 4) Then do, Image>Histogram and write down the Mean
- 5) Then drag the box to the next band and repeat
- 6) Finally, drag the box to measure the Mean from the Background, and subtract the number from all the others.
- 7) Divide all the numbers with your reference number for the one set as 1 (or 100%)

This is a VERY rough way of doing it, but does give you some hints on the relative increase/decrease of expression. Alternatively, you can use ImageJ software to conduct your analysis.

Modified from Ref. 4

Ethics of Retouching Photos in Science

Photo Manipulation

In 2006, the Journal of Cell Biology gained publicity for instituting tests to detect photo manipulation in papers that were being considered for publication. This was in response to the increased usage of programs by scientists such as Photoshop, which facilitate photo manipulation. Since then more publishers, including the Nature Publishing Group are instituting similar test and require authors to minimize and specify the extent of photo manipulation when a manuscript is submitted for publication

Although the type of manipulations that are allowed can depend greatly on the type of experiment that is presented and also differ from one journal to another, in general the following manipulations are not allowed:

- splicing together different images to represent a single experiment
- changing brightness and contrast of only a part of the image
- any change that conceals information, even when it is considered to be a specific, which includes:
 - changing brightness and contrast to leave only the most intense signal
 - using clone tools to hide information
 - showing only a very small part of the photograph so that additional information is not visible

And more in general, most journals nowadays urge authors to use photo manipulation with restraint and great care.

Links of Interest:

- **Adobe Digital Imaging Solutions for Scientist and Healthcare Professionals**
<http://www.adobe.com/digitalimag/science.html>
- **Photoshop for Science, Medicine & Industry Notes**
<http://rawlight.com/quickphotoshop.html>
- **Online Adobe Photoshop Training**
<http://www.trainingtools.com/online/photoshop6/>
- **How to Guard Against Fraud**
<http://www.the-scientist.com/article/display/23156/>

Additional Documents Available On-line (<http://www.med.uottawa.ca/ruthslack/adobe.html>)

- **Adobe Photoshop CS2 in Scientific and Medical Imaging**
Optimum strategies for Using Adobe Photoshop CS2 in Scientific and Medical Imaging
- **Matching Colors**
Imaging corrections (matching colors) amongst several images
- **Making TIFF Files from Drawing, Word Processing, Powerpoint and Graphing Programs**
Provides a guide on how to make high resolution TIFF files from commonly used programs such as Powerpoint and Excel and import then into Photoshop
- **RGB to CMYK Format**
Converting images in RGB format to CMYK for publication
- **Image Stitching**
Stitch several images together using Photoshop

References:

- 1) <http://www.adobe.com/>
- 2) Adobe Photoshop CS2 Classroom in a Book for Windows (2005)
- 3) Jerry Sedgewick (2003) Quick Photoshop for Science: A Guide to Digital Imaging
- 4) <http://www.protocolonline.org/forums/lofiversion/index.php?t5062.html>
- 5) <http://jcb.org>
- 6) <http://www.nature.com/nature/authors/submissions/images/index.html>

Adobe Studio on Adobe Photoshop CS2


Enhance scientific and medical images

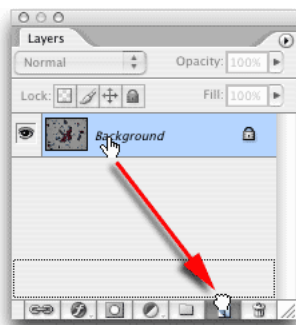
Light, shadow and detail interact in wild and mysterious ways in microscopic photography, posing special challenges for the researcher and educator. With Adobe® Photoshop® CS2, it's a snap to enhance the detail in any image, whether it's destined for publication or the classroom.

Create layers to protect your original image

With Photoshop CS2, you can use layers to enhance your scientific images while keeping the original data intact. You can do this in two simple ways—by duplicating the original data layer and working only with the duplicate layers, and by using adjustment layers, which lie above the data and modify only how that data appears to the viewer. Adjustment layers can also simply be hidden or deleted to reveal the original image.


1 Duplicate the original background image.

When you first open a captured microscopic image, it contains a single layer labeled Background. Drag the layer to the New Layer  button at the bottom of the Layers palette, or choose Layer > Duplicate Layer. The image is duplicated in a new layer labeled Background Copy.



To duplicate any layer, drag it to the New Layer button in the Layers palette.

2 Hide the original layer.


Click the eye icon  next to the original background layer to hide the background, preserving your original image data in the Photoshop document. You can reveal this layer again at any time by clicking the empty eye icon box.

3 Give the duplicate layer a more meaningful name.

Double-click the label Background Copy and type a descriptive name, such as Cytology Slide 1. Press Enter or Return to save the new name. The layer is now highlighted to indicate it remains selected. You are now ready to bring out all of the detail you can get from the image.

Use a Levels adjustment layer to bring out the detail

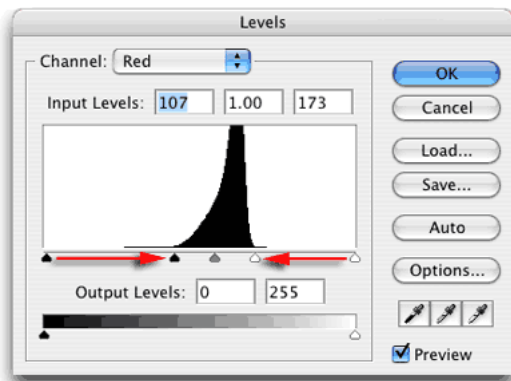
1 Create the adjustment layer.

With the Cytology Slide 1 layer still selected, click the Create Adjustment Layer  button and choose Levels from the pop-up menu, or choose Layer > New Adjustment Layer > Levels. The Levels command is an easy yet powerful tool for enhancing detail, giving much finer results than you can achieve with simple brightness or contrast adjustments.

2 Evaluate and adjust the histogram for the red color channel.

The histogram at the center of the Levels dialog box shows the relative number of pixels in the shadows (at the left of the histogram), midtones (middle), and highlights (right). Although you can adjust all color channels (RGB) in the image at once, here we will adjust each color channel individually, for greater precision. Adjusting the levels for each channel separately also allows you to adjust the color of the image, because you are modifying the relative amounts of red, green, and blue. Adjusting all channels simultaneously maintains the original color.

Choose Red from the Channel menu and make sure that Preview is selected, so that you see the effects of your changes as you experiment with different settings. Drag the shadows input slider (the black triangle below the histogram) to the right, until you reach the left edge of the distribution curve. Next, drag the highlights input slider (the white triangle) to the left, until you reach the right edge of the distribution curve. You can also drag the midtones slider (the gray triangle) to the right or left to adjust contrast in the midtones.

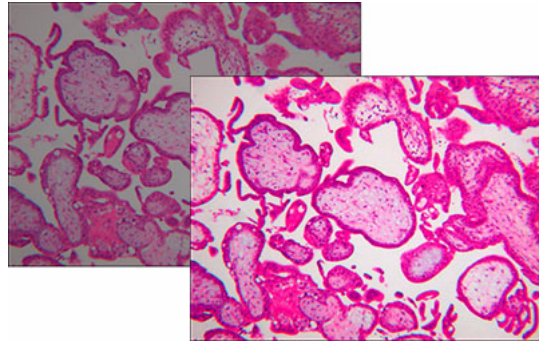


Drag the shadow and highlights controls in the Levels adjustment dialog box to precisely control the brightness and contrast in an image.

3 Evaluate and adjust the remaining color channels.

Choose Green from the Channel menu and repeat step 2, then choose Blue and repeat step 2 once more. When you are finished, click OK to apply your changes.

Look at the Layers palette to see the adjustment layer you just created above the Cytology Slide 1 layer. You can turn off the adjustment temporarily by clicking the eye icon next to the adjustment layer, or delete the adjustment layer by dragging it to the Delete button . You can also double-click the adjustment layer to make further modifications, and all without permanently altering the pixel data in the layer below.



Before (left) and after (right) applying a Levels adjustment layer.

Discriminate cell contents with a Color Balance adjustment layer

A Levels adjustment brings out the detail in a slide by improving the contrast between highlights and shadows, but for images of stained cells, adjusting the color balance is an effective way to help distinguish individual features. Here we will start with a confocal image with little interior detail, and use a Color Balance adjustment layer to bring out detail in the shadows.

1 Duplicate the background layer.

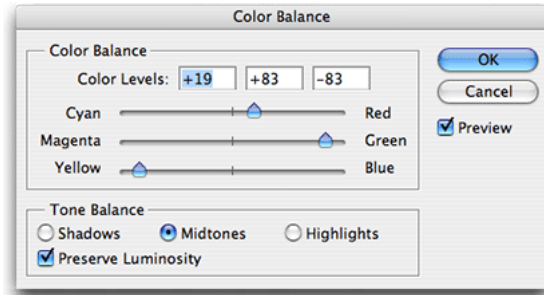
Drag the background layer to the New Layer button. The new layer is selected in the Layers palette, ready for editing.

2 Create a Color Balance adjustment layer.

Click the Create Adjustment Layer button and choose Color Balance from the menu. Alternatively, you can choose Layer > New Adjustment Layer > Color Balance.

3 Adjust the color balance for maximum detail.

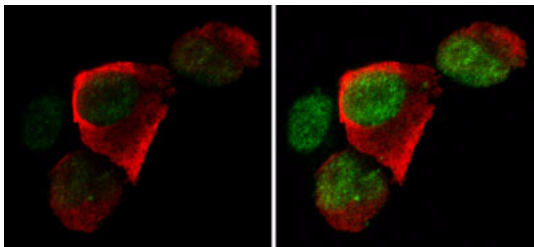
First, enable the Preview check box to see the potential effect of your changes. Then, under Tone Balance, select Midtones and adjust the Cyan, Magenta, and Yellow sliders to accentuate cell detail. If necessary, you can select Highlights and/or Shadows to discretely adjust the detail in these ranges.



A Color Balance adjustment layer easily brings out details in stained microscopic images.

4 Apply your changes.

When you achieve a color balance that brings out as much detail as possible, click OK to apply the adjustment layer.



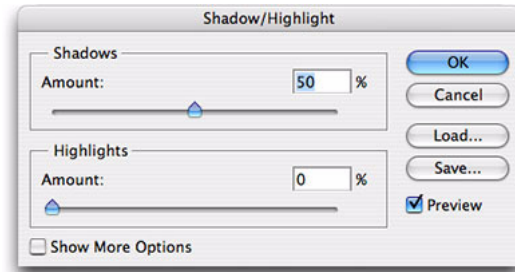
Before (left) and after (right) applying a Color Balance adjustment layer.

Brighten shadow detail with the Shadow/Highlights adjustment

Brightening an image can bring out detail in the shadows, but it can also clip detail in the highlights. The Shadow/Highlight adjustment prevents clipping, by correcting only those areas that need adjustment.

1 Open and duplicate an image.

Open an image with properly exposed highlights but overly dark shadows. Duplicate the image by dragging the background layer to the New Layer button, then choose Image > Adjustment > Shadow/Highlight.



The Shadow/Highlight adjustment brings more detail out of shadow or highlight regions in an image without affecting other areas.

2 Evaluate the shadow adjustment.

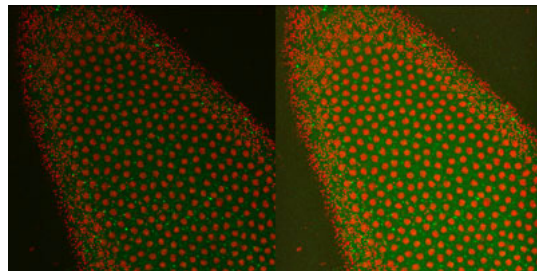
You should see improvement in the shadow detail as soon as the Shadow/Highlight dialog box appears. If the default adjustment doesn't show enough detail in the shadows, drag the Shadows slider to the right.

3 Evaluate the highlights.

If you need to dim the highlights to see more detail in those regions, drag the Highlights slider to the right.

4 Fine tune your changes, if needed.

As you make your adjustments, notice that you can adjust the shadow detail without affecting the highlights, and vice versa. If you need finer control over the adjustment, enable the Show More Options check box to show settings for tonal width and radius, color correction and midtone contrast.



Cells before (left) and after (right) enhancing with Shadow/Highlight.

Use Smart Sharpen to correct blurred detail

New in Photoshop CS2 is the Smart Sharpen filter, an advanced yet easy-to-use way to enhance detail that is often blurred in microscopic photography. Smart Sharpen improves upon the Unsharp Mask filter, giving you greater control over different kinds of blurring, and discrete control of sharpening in different tonal ranges. Use it to sharpen images quickly and efficiently, without the halo artifacts associated with other sharpening methods.

1 Open and duplicate an image.

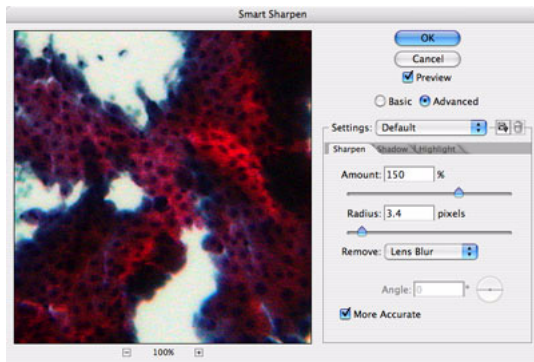
Open an image with insufficient detail and drag its background layer to the New Layer button in the Layers palette. Then choose Filter > Sharpen > Smart Sharpen.

2 Adjust Smart Sharpen settings.

Choose Lens Blur from the Remove menu and enter 2.0 or 3.0 in the Radius text box. Then drag the Amount slider to the right until you are satisfied with the amount of sharpening.

3 Make advanced adjustments in shadows and highlights if necessary.

If the blur is predominantly in the shadows or highlights, click the Advanced radio button. Use the Shadow and Highlight tabs to selectively fade the sharpening effect on shadows and highlights, respectively.



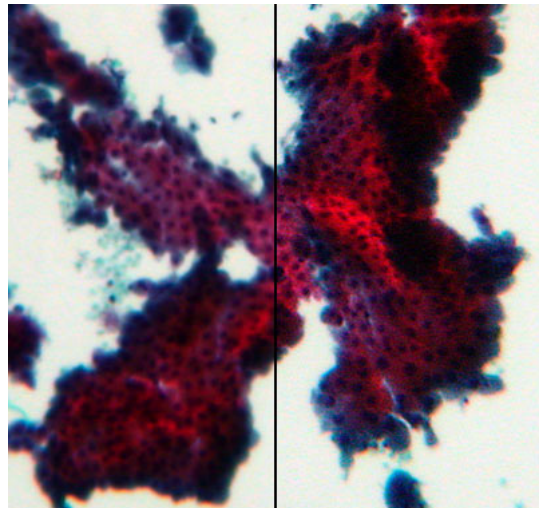
Smart Sharpen provides advanced sharpening of images, with fewer artifacts and discrete adjustments for shadows and highlights areas.

4 Preview and save your changes.

Evaluate your changes and adjust them until you are satisfied with the sharpening. Enable the More Accurate check box for the most precise sharpening. Click OK to apply the adjustment.

5 Save your settings for future use.

If you have a group of slides requiring the same kind of correction, you can correct one slide, save your settings, and then apply the saved settings to other slides in the group. Click the Save Settings button and type in a name to save the current settings in the Smart Sharpen dialog box. Once saved, they will appear under that name in the Settings menu, and can be applied with a single click.



Cells as seen through the microscope (left) and after processing with Smart Sharpen (right).

Coordinate colors in a series of slides

After you adjust the detail and color balance in a series of slides, you may end up with a jumble of discordant colors that won't be attractive in a presentation. The Match Color command quickly and easily coordinates colors across images, for a unified, professional presentation.