Optimum Strategies for Using Adobe Photoshop CS3 Extended in Biomedical Imaging

Digital images play a key role in the work of medical professionals, whether in a diagnostic workflow, communication of findings, or patient education. The industry standard in digital imaging, Adobe Photoshop[®] Creative Suite[®] 3 offers an affordable, easy-to-use comprehensive solution to handling the vast majority of native image formats. Now it comes in two editions: Photoshop CS3 and the enhanced Photoshop CS3 Extended, designed to meet the diverse needs of a wide range of professionals. For physicians and other healthcare professionals, Photoshop CS3 Extended helps improve patient care with powerful tools for documenting treatment, visualizing potential outcomes, and publishing the results.

Adobe Photoshop CS3 Extended adds the highest quality imaging toolset and broadest range of digital imaging capabilities specifically for physicians and biomedical professionals, in a wide range of fields: radiology, pathology, ophthalmology, surgery, cosmetic surgery, dermatology, cardiology, dentistry, and more. These time-saving tools help medical professionals clearly and accurately extract and communicate information contained in images, securely manage volumes of image data, and work more efficiently and effectively with digital images.

Photoshop CS3 Extended for biomedical professionals

The use of Photoshop-enhanced digital images is as broad, varied, and individual as medical practices and research studies. Physicians, dentists, and other medical professionals can strengthen communications with patients by presenting proposed procedures alongside beforeand-after images, and share findings with peers. Scientists can optimize images for both visual appreciation and further automated analysis. Photoshop CS3 Extended features especially useful to biomedical professionals include:

- Support for the specialized DICOM (Digital Imaging and Communication in Medicine) format, the most common standard for receiving medical scans, enables Photoshop CS3 Extended to open files from medical imaging devices such as CT scans, X-rays, and other radiological images.
- Measurement, counting, and analysis tools facilitate performing high-quality qualitative analysis, including in perspective with the Vanishing Point tool. For example, dermatologists could use the Count tool to show skin changes by quantifying and tracking lesions over time. Dimensions, size, and intensity results can be exported for additional analysis.
- Time-saving compositing and image overlay tools allow stacking multiple images into a single Smart Object with a similar frame of reference, and then filtering common areas, for example, to track and distinguish the progress of disease or a treatment regimen.
- Enhanced animation features for presentation include the ability to clone objects from one point in time to another, and import frames as layers. Physicians can combine a series of still images, such as of a wound healing, and export it to common motion-image formats, such as QuickTime, MPEG, and FLV.
- Integration of the MATLAB format, a high-level technical computing language allows grabbing image data directly from Photoshop CS3 Extended, using MATLAB to run different image processing routines, and then returning the image data to Photoshop Extended to view the results.
- Painting and using layers in 32-bit/HDR files now is possible. Both Photoshop CS3 and CS3 Extended support the enhanced Merge to HDR, as well as basic HDR editing using Levels.

Photoshop CS3 Extended offers a complete solution for biomedical professionals from image capture to the widest variety of output options for final images. This paper details Photoshop CS3 Extended features of particular benefit to medical professionals, and describes some biomedical digital imaging workflows.



TABLE OF CONTENTS

- 1 Photoshop CS3 Extended for biomedical professionals
- 2 Meeting the imaging needs of the biomedical community
- 3 Working with DICOM images
- 4 Image standards and file format compatibility
- 4 Using an efficient workflow
- 5 Maintaining image integrity
- 5 Editing nondestructively
- 6 Enhancing communications and presentations
- 6 Publishing and printing findings
- 6 The medical and scientific image workflow
- 16 Resources

Meeting the imaging needs of the biomedical community

As medical professionals and others discover new features and functionality in Photoshop, they widen the path to exciting new uses and discoveries, and enrich the well-established network of resources to learn and develop expertise. Medical professionals have special requirements for the digital images they work with. These professionals need to:

- Work efficiently with a variety of devices, technology, and file formats and sizes, including DICOM (Digital Imaging and Communication in Medicine).
- Use a workflow that allows easily combining images from a variety of sources, while matching resolution and image size.
- Maintain and track the integrity of images while working collaboratively with many others.
- Edit images nondestructively.
- Present, share, and collaborate on their work easily and meaningfully.

Note: It's important to remember that Photoshop CS3 Extended is not an FDA-approved tool, which means it cannot be used for diagnosis or therapy.

Here are just a few of the ways that Photoshop CS3 Extended meets the imaging needs of the biomedical community:

- Radiologists now can accurately replicate what they perceive in DICOM files in the reading room, using the full power of Photoshop CS3 Extended and its support of 16- and 32- bit images. They can improve images for presentation and publications with Photoshop Extended's ability to import DICOM files and use its many tools and procedures to optimize images. Controls include conventional grayscale adjustments of windowing within the CS3 Extended DICOM import interface, and the option to keep patient information anonymous.
- In patient care, a physician's skillful use of images can improve patients' understanding of their condition by helping them visualize it and engage them actively in their therapy. For example, using images, a doctor can compare pathology to the expected normal anatomy.
- With the capability to composite images and register them properly, physicians and other biomedical professionals working with autoradiography, histological samples, and other images can gain additional insight and confirm hypotheses.
- Surgeons can construct information that relates to a patient's condition, combine it with standard anatomical texts, and—by adjusting coloration, orientation, and size—facilitate communication.
- With patient approval of disclosure, physicians can transmit images to others for referrals and consulting. Patients can share and accurately reference images with their loved ones, improving everyone's understanding and support.
- Health care professionals performing reconstructive procedures can use Photoshop-enhanced images to demonstrate possible outcomes, create realistic expectations for the work, and show a range of options. Incorporating these details and potential outcomes, professionals can improve patient care with a portfolio of possibilities as well as create documentation needed for third-party insurance claims.
- Using Photoshop CS3 Extended analytical tools, dentists can communicate to patients reconstruction or smile design. By calibrating measurements of the difference between the actual tooth dimension and the proposed size, dentists can create the actual amount of the tooth redesign.
- Biomedical researchers now can apply tools enhanced in Photoshop CS3 Extended to JPEG and TIFF files, and use the Analysis menu and tools in CS3 Extended to conduct basic analysis.
- Physicians and researchers who want to publish or print their findings for colleagues and others, can prepare images and enhance them for improved print visualization. Tools in Photoshop CS3 Extended help authors stay competitive in their publish-or-perish disciplines.

Working with DICOM images

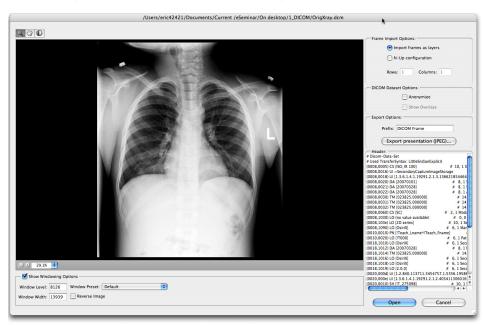
Radiologists and other professionals in medical imaging can now import DICOM files directly into Photoshop CS3 Extended, and enhance them for output with various Photoshop CS3 Extended tools and procedures. The DICOM file format supports a multitude of nuclear and magnetic image types including nuclear medicine, ultrasound, endoscopy, and magnetic imaging, captured as X-rays, MRI, PET (positron emission tomography), and CT (computed tomography), among others. As its acceptance as a medical-image standard grows, the DICOM format brings greater transparency and interoperability to the medical field, and may someday support output of all medical images, both radiological and bright-field (pathology images).

On importing DICOM files into Photoshop CS3 Extended, radiologists can enhance them in the Import window, using the windowing and level adjustments in the DICOM viewer as well as the built-in conventional grayscale adjustment controls. Patient and imaging information appended to DICOM files can be displayed or private patient data can be removed.

After editing in Photoshop CS3 Extended, images can be exported back into the DICOM file format, retaining only what that file standard supports; or they can be saved in many other file formats that allow additional editing, such as PSD or TIFF. The latter formats retain the high-bit definition acquired in the original image.

Photoshop CS3 Extended tools and features can be applied to the full bit-depth DICOM image, either globally or to selected regions, for example to:

- Apply curves to adjust DICOM images with precision and repeatability, and save the defined curve as a preset for application to other images.
- Apply levels to adjust brightness, contrast, or tone.
- Annotate an image with text and vector shape tools, or with audio comments.
- Animate images. DICOM images can have multiple frames. Animation features in Photoshop, using either the Animation or Layers palette, allow quickly scrubbing through dozens or even hundreds of files, to show different aspects of an image's subject.
- Composite multiple frames into a single image. This matrix of the images displays all of the images simultaneously and could be exported as a single image.
- Analyze DICOM images and measure their anatomical structures using Photoshop CS3 Extended analysis tools. Convert two-dimensional anatomical structures into selections and compare regions of image density within a single image or sets of images.



Import DICOM files using its special import interface.

3

Image standards and file format compatibility

Medical images come from various sources, including microscopic and radiological devices, computerized scanning equipment (CAT, PET, MRI), and specialized camera equipment. Images can be in a variety of formats, and range from monochromatic to 32-bit, high dynamic-range (HDR) color. Photoshop CS3 Extended supports multiple formats, which makes it easier to handle varied source data from medical and scientific devices and specialized cameras and streamlines integration of images into the medical workflow.

Besides the DICOM and MATLAB formats, Photoshop CS3 Extended can read a wide variety of image formats, including Photoshop PSD and PSB, GIF, JPEG, EPS, PICT, Pixar, PNG, Portable Bit Map, Scitext CT, Targa, TIFF, Cineon, PCX, BMP, Radiance, Wireless Bitmap, DNG; and most Camera Raw formats, such as CRW and NEF. You can also add more specialized image format support with plug-ins, many of which can be located by searching the partners database at <u>www.adobe.com</u>.

If an image requires additional work, it's best to save the file in the PSD format. For an image that will be moved to an image analysis program, save it in the TIFF format without layers. Save images in the JPEG format for emails, web pages, and presentation, when space is a consideration. When archiving an image, choose a file format that maintains the most image information, remains accessible even with changes in standards and equipment, and that allows retrieving images and continuing to edit them after an indefinite period of time.

The vast majority of Photoshop CS3 Extended features are now available for 16-bit color images; features also support 32-bit, floating-point, HDR capabilities. Combined with a maximum image size of 300,000 x 300,000 pixels and the newer PSB Large Document Format, Photoshop can handle the most complex images, including those obtained by high-resolution medical and satellite devices and cameras. When creating composite images, either manually or using the Photomerge* feature to stitch tiled images together, the maximum pixel dimensions of a Photoshop CS3 file enable compositing up to 900 transmission electron microscopy (TEM) negatives into one image. The capabilities of computer hardware now mainly limit image size.

Using an efficient workflow

Some of the many ways Photoshop CS3 Extended can improve a professional's medical practice or scientific research include its support for a comprehensive workflow that starts with an image capture device and ends with final images in the widest variety of output options.

Workspace optimization—Photoshop CS3 Extended includes workspaces optimized for particular tasks, which can be customized, saved, and shared. Built-in workspaces include preconfigured setups to aid in automation, image analysis, color and tonal correction, and printing, and proofing.

Automation of repetitive processes—Most specialists have their own particular set of image processes, whether that means a preferred method of color balancing microscopic images, or a desired final format, size, and resolution for images destined for publication. Photoshop CS3 Extended provides various ways to automate repetitive processes:

- Automation processes, such as the Image Processor, can quickly convert images into a new format. Photoshop automation includes creating web pages of images, running Image Statistics on two or more images, and determining operational outcomes of averaging, summing, minimums, maximums, among other routines.
- The Action feature allows easily recording a series of actions that can be run at any time, to reduce time spent on monotonous tasks in a workflow.
- Extensive scripting support allows creating complex automation scripts for ongoing use, such as to facilitate batch-processing of multiple images, common in biomedical workflows.
- A wide variety of third-party plug-ins, many designed specifically for biomedical use, are available from various Internet and user sources. These automate very useful functions such as importing multiple images and straightening and cropping them or quickly creating contact sheets of images for reference.

4

Maintaining image integrity

Maintaining the accuracy of an image and its environment without bias or fraud is crucial in biomedical fields. Any editing necessarily must avoid altering a professional's conclusions. It's best to make adjustments to an image as a whole rather than to selected parts; use only those filters and adjustments that alter the entire image at once without affecting the visual relationships between objects in the images; or use nondestructive editing features, like Smart Filters and adjustment layers.

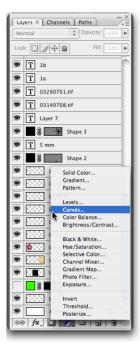
Various tools in Photoshop facilitate tracking changes to ensure image authenticity, including:

- The History Log tracks changes made to an image so that they can be replicated on another series of images, and maintains an audit trail that can provide evidence that the images have not been materially altered. An audit trail is particularly important to forensic scientists, who are generally barred from making any changes that could affect the use of imagery as evidence in legal proceedings, and to medical specialists, who may make treatment decisions or base diagnoses on the images.
- Camera Raw image support preserves digital images in an unadulterated state, with image improvements recorded in an associated file as metadata accessible in Bridge 2.0 and Photoshop CS3 Extended.
- Filters such as Levels, Color Balance, Smart Sharpen, and Match Color, enhance the ability to discern detail in scientific images and, thus image analysis, without skewing its results.
- Retouching tools such as the Spot Healing brush highlight and clarify areas of interest. For example, an educator could highlight a particular cell structure for a class by removing other visible features of the image, to leave only the cell or structure of interest.

Editing nondestructively

It's important to edit biomedical images with accuracy in mind. Introducing bias or fraud, or making permanent changes that affect the accuracy of the object being studied or portrayed, invalidate the image. Various features in Photoshop CS3 Extended allow enhancing the image without changing it permanently, such as Smart Objects, the new Smart Filters, and adjustment layers—special layers that contain a filter or effect that is applied to the layers beneath it. These features also allow testing variables side-by-side while tracking every adjustment.

Layers also are useful to add notations and adjustments or reveal the original nonenhanced image at any time; easily reverse any changes by simply deleting their layers; and to construct composites and montages. Keep track of adjustments using the History palette.



The multilayered Photoshop file format allows you to isolate labels, callouts, and enhancements from the image data, which is preserved on the original locked background layer.

Enhancing communications and presentations

Photoshop CS3 Extended features particularly suited to biomedical presentations include the new Quick Selection tool for "painting" selections and the Refine Edges feature for highlighting selection edges. Other features allow creating large-format images and composites, enhancing images and facilitating reviews:

- Photomerge allows constructing high-resolution, large field-of-view montages, for a view of the complete image.
- Zoomify allows creating an interactive image with zooming capabilities that can be used with any browser. Users can pan in or out of these high-resolution images to see more detail. The basic-size image downloads in the same time as an equivalent size JPEG file. Photoshop exports the files as JPEG and HTML files that can be uploaded to the web.
- Images can be animated or enhanced with notes or audio for shared reviews or collaboration.
- Adobe Connect, built in to Bridge, supports live web-based conferences or long-distance learning; for more information, see www.adobe.com/products/connect/.



An Adobe Connect session is an effective way to conduct long distanced learning. Connect makes it easy to interact with Photoshop enhancements, shown here on importing a DICOM image during a teaching session.

Publishing and printing findings

Clear, informative images are key to compelling presentations, lectures, and journal articles, as well as imperative in the biomedical profession that requires publication for communication, advancement of ideas, and professional recognition. For publication, whether in print or online, Photoshop CS3 Extended includes a host of tools for optimizing images for publications, lectures and conferences; and excels at basic image processing tasks that published images require, such as cropping, resizing, and adjusting contrast.

In addition, Photoshop CS3 Extended lets authors polish their work and add impact by adding graphics and text annotations to images to highlight regions or interest, adding color to blackand-white images, selectively brightening a particular area of an image, and adjusting color balance to reduce the color cast of red or orange from operating room lights. Authors can easily animate a sequence of still images and export them in image formats such as QuickTime, MPEG, or FLV. Final images can be saved in a variety of common formats—JPEG, GIF, PNG, depending on the images' intended use and publication.

6

The medical and scientific image workflow

Whether their work is medical imaging, cosmetic applications, or scientific investigations, physicians, dentists, and researchers all rely on images to do their work. Photoshop CS3 Extended provides many features to enhance these biomedical images. Regardless of field, Photoshop workflows typically follow five steps.

1. Image acquisition

Photoshop CS3 Extended is an important part of the imaging workflow even when images have been created using a combination of proprietary hardware and software. In many cases, these systems export their images in a universal file type. Transfer raw images from network devices or removable media onto your local hard drive by simply dragging image files from one Adobe Bridge 2.0 window to another, or from the source folder in the Bridge main window to the destination folder in the Folders palette.

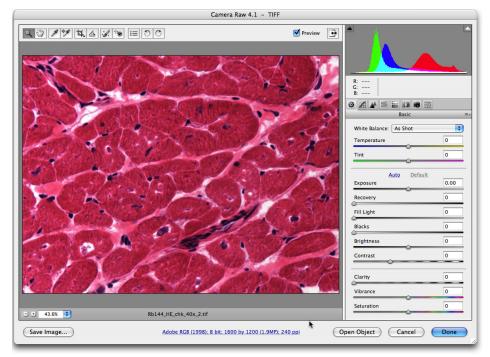
To supply more visible data to work with, for best results, consider acquiring images at higher resolution, higher real magnification, or larger (physical) area beyond the target boundaries; or consider over-sampling. Photoshop CS3 Extended easily handles the larger files. Details in the subject area appear sharper and more vivid. A large field of view ensures visibility of the surrounding and background areas, which puts the subject into context. The subject can then be cropped in Photoshop CS3 Extended, to focus on aspects of the image without having to retake the photo.

Acquisition formats—Images can be acquired directly from scanners or cameras, in the Camera Raw, JPEG, GIF, or other file formats. For image capture, Photoshop supports the Twain plug-in, which allows controlling the parameters of image acquisition by scanners and other devices. Many image acquisition devices such as digital cameras and scanners have a TWAIN component that allows importing images directly into Photoshop. Using the supplied TWAIN interface, scanners and scientific cameras settings can be tailored to the correct exposure conditions.

For research scientists, the TIFF file format is a standard that preserves the image data reliably. Radiologists depend on the DICOM standard, now supported natively in Photoshop CS3 Extended. The DICOM import function gives physicians precise control over individual image frames as well as entire image stacks. Images can be acquired using the TWAIN plug-in to import the image directly into Photoshop, or the file can be scanned using third-party software and then opened in Photoshop.

Camera Raw 4.0—Both Photoshop and Bridge 2.0 include a comprehensive set of tools to process RAW files. New with Photoshop CS3 Extended, Camera RAW tools and workflows can be applied to TIFF and JPEG files. Opening them in the Raw Import dialog box allows modifying them without applying changes permanently, until the images are opened in Photoshop. (To open TIFF and JPEG images in the Raw Import dialog box, set the appropriate preferences (File > Preferences > File Handling (Windows) or Photoshop > Preferences > File Handling (Mac OS).) You can also use the Camera Raw dialog box to process one image out of multiple exposures of the same shot, and then apply the settings to all of the other shots.

The Raw 4.1 enhancement includes extensive controls for making global enhancements, such as adjusting white balance, exposure, contrast, sharpness, tone curves, and much more. Several enhancements to the Camera Raw dialog box allow even more precise image adjustments. You can now adjust curves parametrically (rather than at one point): moving the control sliders shifts entire regions of the curve to the new parameters. Plus, White Balance controls allow adjusting clarity and vibrance handles to "punch up" an image.



Use Camera Raw features to edit JPG and TIFF files

Conversion of files to other formats—To convert files to a new format, you can select the source files in Bridge and open them in Photoshop CS3 Extended. You can select File > Scripts > Image Processor to set up a conversion in a few seconds, and create new files in PSD, TIFF, or JPEG formats. The PSD and TIFF formats are best for image editing. For direct export or transporting images via the Internet without further editing, the "lossy" JPEG format creates smaller files, but at the expense of image quality. One of the main advantages of file conversion at capture is that it creates an entirely new image and preserves the original unchanged. For example, an original series of TIFF images can be batch-converted to PSD format for editing in Photoshop, while the original image files remain stored on a CD or DVD. If the originals are Camera Raw files, they are preserved because Photoshop CS3 Extended always processes them into new Photoshop documents for editing.

Acquisition of high dynamic-range images —Bridge and Photoshop CS3 Extended both can handle 32-bit HDR images captured by a few cameras and some high-level medical devices. Photoshop CS3 Extended also can merge a series of bracketed images from a conventional camera into a single 32-bit HDR image, a facility that might be of interest to some disciplines, such as forensics and astronomy.

Acquiring at a higher resolution when possible has several advantages. First, Photoshop CS3 Extended takes advantage of hardware containing Intel processors like the Intel® Corel® 2 Duo Processor now used in new Apple CPUs. Working with larger images is less time-consuming, and allows researchers to inspect images at greater magnification to see additional details.

Images that contain 32 bits of color data yield better color. The support of High Dynamic Range also provides images that represent the full extent of brightness and darkness the eye can appreciate. Radiologists will be able to more faithfully present X-rays in publications with the subtlety that was reserved for their radiological reading rooms. Photoshop CS3 Extended support for high bit-depth images preserves fine detail, which is important to scientists who need to track small changes in their subject matter. Image patterns are better portrayed and minute details are not obscured by neighboring areas.

2. Organization and management of images

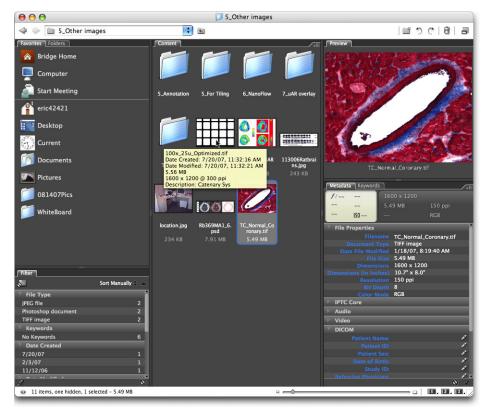
After collection, the investigator reviews the images and assesses them, evaluating them according to labor or expert standards. Adobe Bridge 2.0 helps organize your image library and lets you interact with research files by letting you rank, select, and reject images, and create image sets by forming stacks. It assists in record keeping with the ability to filter images by their associated metadata contained in the EXIF file, which is the data stored with an image when captured by a digital camera. Automatically included in the EXIF data is time and date of

capture as well as camera settings such as ISO. Bridge also includes fields where the users can add copyright information and text details.

You can change selected images globally in Bridge, ensuring that the entire image is modified with the same parameters; or open them in Photoshop CS3 Extended to edit selections and apply Photoshop-specific controls (for example, to add layers, apply Smart Filters, conduct analyses, or add text).

The many features in Bridge 2.0 are ideal solutions to the challenges of scientific imaging—the need to manage a proliferation of images; locate previous images quickly and compare them to more recent ones, or collect them for presentation or publication; and easily access image details.

Bridge offers multiple views and scalable image thumbnails for simple image browsing and detailed previewing. It also has a robust search facility that can locate specific images based on name, date, and metadata carried within the files. Its multiple-window capabilities enable you to drag images from one folder to another, and the Metadata and Keyword palettes provide instant access to image information, including shutter speed, aperture, and the date images were captured. The Metadata palette can also display the complete editing history of the file, providing both an audit trail and the ability to replicate a desired enhancement process.



Bridge 2.0 provides a central location for organizing and maintaining images sets and other digital media files.

Also use Bridge to batch-process and convert files to such formats as TIFF and JPEG. For example, you can batch-rename files captured from devices and cameras that automatically generated filenames without meaningful descriptions. Simply select a group of new files, choose Tools > Batch Rename in the Tools menu of Bridge, and quickly assign more meaningful file names to each group of images.

3. Basic image correction

Typical adjustments include setting the desired image resolution, straightening or adjusting the orientation of the image and rotating it if needed, and cropping unneeded parts of the image. It may be necessary to sharpen part of the image; adjust the tone, brightness, or contrast in the image; correct the color; or remove noise.

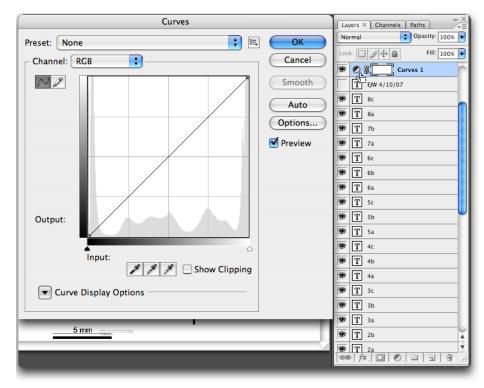
Note: Always work on a copy of your original image. A corollary of this is to save your original image in an unalterable form. For optimal security, save a duplicate off site.

These Photoshop CS3 Extended features are particularly helpful in a scientific context.

Levels —The Levels adjustment is ideal for enhancing most scientific images that need it. A histogram of a typical microscopic image would reveal that most pixel values fall in a narrow band. The Levels adjustment manages the dynamic range of the image by remapping the values currently assigned to the pixels. To enhance image contrast significantly without losing any image datea, simply drag the shadow and highlights input sliders from the left and right in toward the middle, and stop at the beginning and end of the histogram curve. You can use the Levels adjustment on an entire image or discretely on its individual color channels for even finer modification. Apply a level as an adjustment layer, separate from the layer containing the actual image, to modify or remove its effects at any time. To apply a level, select Layers > New Adjustment Layer > Levels or Image > Adjustments > Levels.

Color balance—Color balancing is often used to remove a color cast from an image. To balance color, drag the slider control toward the color to add or away from the color to remove. The Color Balance adjustment is also effective at enhancing detail within the image. To apply color balancing, choose Layers > New Adjustment Layer > Color Balance or Image > Adjustments > Color Balance.

Curves—The Curves feature provides a more objective and repeatable method to remove a color cast than using the Color Balance tool. It's also useful for making more precise tonal corrections than the Levels feature, by adjusting the white and black point. To make Curves adjustments, choose Layers > New Adjustment Layer > Curves or Image Adjustments > Curves.



Use a Curves adjustment layer to remove a color cast or adjust the tone.

Correction of blurring and chromatic aberrations—The Smart Sharpen filter (Filters > Sharpen > Smart Sharpen) corrects different kinds of blurring and enables separate control of sharpening in the image highlights or shadows. In contrast to the Unsharp Mask feature, the advanced algorithms of Smart Sharpen apply a much greater correction before edge halo artifacts appear. This is best suited for images that are only used for perception and not for image analysis. For similar results, you can use the Chromatic Aberration sliders of the Lens Correction filter (Filters > Distort > Lens Correction).

Noise reduction —Color noise is an issue in all digital images, but especially with those images captured using high ISO settings. Previous methods of correcting color noise caused blurring. The new Reduce Noise filter applies an advanced noise reduction that leaves image detail sharp. To use this filter, choose Filters > Noise > Reduce Noise.

4. Image enhancement

In science and medicine there are two divergent purposes for modifying images. The first is to adjust the image for human perception, useful for presentation of images and analysis by manual means. The second is to clarify complex and vague situations that human perception can handle, but that would stymie automated analysis systems. Where researchers require taking accurate and repetitive measurements, enhancement techniques modify images for easier discrimination by software.

What follows are descriptions of some of the main enhancement features of Photoshop CS3 Extended that are helpful in a biomedical context.

Image merge—Photomerge allows constructing high-resolution, large field-of-view montages, for a view of the complete image. Example applications include the faithful portrayal of directly observed histological results, using a composite image to compare serial tissue sections stained using different histological methods.

Photomerge automatically assembles component images and includes advanced blending options for more realistic, seamless montages. Automatic or manual adjustments smooth out lighting differences at image joins and correct the perspective for extreme panoramas. The Reposition Only feature prevents stretching and distortion of the images, so that measurements remain accurate. These tiled images display the entire object or area, thus distinguishing regions-ofinterest better because they aren't based simply on what fits in a frame or viewfinder. Either save the final image as a complete composite or with the component images on separate layers for advanced blending.

To use this feature, choose File > Automate > Photomerge in Photoshop, or Tools > Photomerge in Adobe Bridge.

Smart Objects—Smart Objects facilitate assembling assets for presentation and publication. Special layers that contain a copy of the original data for the layer, Smart Objects can be scaled, rotated, or warped nondestructively and without resampling the resolution. Duplicating an existing Smart Object updates all instances when one instance of the Smart Object is replaced or updated. Users are actually editing a preview of the original file rather than the original itself. This feature lets scientists repeatedly scale images down and up over the course of arranging objects for a book, paper, or presentation slide, without losing quality from the repeated transformation and while retaining the original pixel data.

A Smart Object can be Adobe Illustrator[®] artwork or two or more Photoshop layers. A Camera RAW file can be placed as a Smart Object, thus allowing scaling and rescaling the file as much as desired (as long as the maximum noninterpolated file size in the Camera RAW dialog box is not exceeded). To use Smart Objects commands, choose Layer > Smart Objects.

Smart Filters—New in CS3, Smart Filters allow modifying an image in a nondestructive way, similar to applying Smart Objects or adjustment layers. Using Smart Filters, professionals can develop innovative methods easier and quicker than before when they would have to rely on multiple undo's. Smart Filters can be adjusted, rearranged in order, turned off and on, and deleted. They can be applied only to a Smart Object layer; so the layer itself can no longer be edited, just its Smart Filter effects. You can apply any filter (including third-party filters)—except for Extract, Liquify, Pattern Maker, and Vanishing Point—as a Smart Filter. To use Smart Filters, select the layer to which you will apply the filter and choose Filter > Convert for Smart Filters; then choose Filter > [filter name] to apply a filter.

Note: In general, Smart Filters and regular filters are appropriate for presentations and nonanalytic work. Keep in mind that most scientific journals do not allow use of filters or other image editing that substantially changes the original.

Text and vector shape tools for labeling and demonstration—Adding vector and type layers, which can remain fully editable, is useful for labeling image details for presentation and publication without affecting the image data. For example, label individual features with text and a call-out arrow pointing from the label to the area of interest, or draw a circle around the area to highlight it for the reader.

To allow the same image to be used in all contexts, keep text and vector shape layers separate and hidden, using the eye icon in the Layers palette. Select type and shape tools in the Tools palette,

and their options in the Options bar. For example, select the Line tool and then use the Custom menu in the Options bar to automatically add arrowheads to a line's beginning, end, or both while drawing the line.



With a vector tool selected in the Tools palette, use the Options bar to control shapes, including adding arrowheads to lines drawn for image callouts.

Edit History Log—The Edit History Log provides a lasting record of how images have been manipulated in Photoshop CS3 Extended, giving the researcher a way to check modifications done on images, as well as replicate enhancements that have worked well in the past. You can store the log in a master text file that contains a list of all edits of all files, and in each individual image file. Storing the edit history within the image provides an extra level of security—if the external history log is lost, the internal log remains—and also allows the history to be examined in Bridge in the Metadata palette.

To read the history log for an image, choose File > File Info in Photoshop CS3 Extended when the image is open and active, or from the File menu in Bridge when the image is selected. The Edit History Log is enabled in the General panel of Photoshop Preferences, and can be set to store detailed history, concise history, or only sessions, which is a list of times the image was opened and closed. When archiving an image, save the History file created along with it.

	Metadata Keywords									
	File Source	:		Digital Camera	4					
	Make	1		NIKON						
	Model	:		E995						
▼ Edit History										
			0	0 File immunohistochemistry b x20						
	objective lens.psd opened Open Porto:Users:avery:Documents:Freelance:Adobe:IComm:Sci- MedWhitePaper:SourceGraphics:immunohistochemistry b x20 objective lens.psd									
	2005-04-24T13:59:32-0 objective lens.psd saved Save As: Photoshop In: Porto:Users:avery:Desk With Lower Case			10 File immunohistochemistry b x20 p:						
	Hide Annotation 0 Delete Annotation Delete current Annotation Levels 1 Layer Make adjustment layer Us		g	: adjustment layer						
	Type: levels Adjustment: levels adjustr levels adjustment Channel: composite chanr Input: 60, 179			nt list						
	mput. 00, 179									
	Select layer "Background"	W	ľ	thout Make Visible						
	Color Balance 1 Layer Make adjustment layer Us Type: color balance Shadow Levels: 0, 0, 0	in	g	: adjustment layer						
	Midtone Levels: 0, 9, 0									
	Highlight Levels: 0, 0, 0 With Preserve Luminosity									
	2005-04-24T14:01:15-0 objective lens.psd saved	7:	0	0 File immunohistochemistry b x20	Ţ					
					0 1					

The Edit History Log records all editing; it can be stored both inside the image and externally on your hard drive.

Brush, patch, and cloning tools—Brush tools radically alter image pixels, so they are only suitable—but invaluable—for educational purposes. For example, removing dust and scratches from a scan would alter image data, and would be unacceptable in some contexts. The Smart Healing Brush replaces a targeted area with pixels from the surrounding area. The Healing Brush and Patch Tool are similar, except that the user manually adjusts the source area, and in the case of the Patch Tool, selects areas rather than paints them. All of these tools blend the replaced pixels with the texture and shading of the original; the replacement is less obvious than with the Clone Stamp, which simply copies complete pixels from the source point and paints them onto the target area. These tools have two main uses in scientific imagery: to emphasize a region of interest or to correct visual flaws. For example, in an image taken from a slide containing many cells and other

structures, you could quickly remove everything except the one cell in the foreground that is of particular interest.

5. Image Analysis

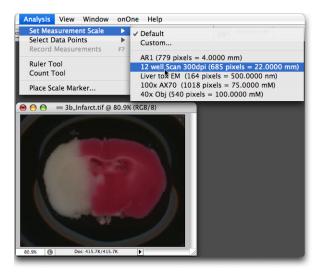
New in Photoshop CS3 Extended, the Analysis menu and its tools allow acquiring data and conducting basic analysis. Analysis of digital imagery has been one of the biggest challenges facing the scientific community. The software packages dedicated to quantitative analysis are difficult to learn and use, and extremely expensive.

Photoshop CS3 Extended analysis capabilities are built into the image processing program that is its market leader. You can rely on the software to conduct some basic measurements and easily incorporate them into your workflow. After precisely selecting parts of images with tools like the Magic Wand and Magnetic Lasso, you can perform image analysis and comparison with the Measurement tool, Histogram palette, or Info palette.

Analysis tools —The Analysis menu in Photoshop CS3 Extended allows collecting data easily from within Photoshop, conducting basic analysis, and exporting data (including histogram data) to spreadsheet programs for statistical analysis. As well, this feature allows comparing and contrasting differences in images that have been objectively modified. A calibration tool uses an external standard to calibrate pixels to a selected size. You can save, reuse, and share pixel settings, and thus collect empirical data.

Analysis menu items are arranged in a workflow order. To use these analysis tools, first set and save a calibration; then collect and display data using the Measurement Log; if desired, export recorded data for additional uses, as follows:

1. Set the measurement scale (for example, 512 pixels = 30 cm) using the Set Custom Calibration dialog box to attribute the number of pixels to a standard; for example, use the Ruler tool to mark a known distance, and then enter the known length and units. If you'll use the calibration for other images, also save it as a preset to assign it to the current image. You can select the preset and assign it to any subsequent image.



Easily create calibration standards with the Analysis menu.

- 2. Select the data points to record (Analysis > Select Data Points).
- 3. Select what to measure:
- Make a selection to acquire basic data types—Area, Perimeter, Circularity, and Integrated Density—for relatively simple image analysis.
- To measure manually, use the Ruler tool to measure straight linear distances or angles. To create the angle to be measure, hold down the Alt (Windows) or Option (Mac OS) key to display a second line.
- Use the Count tool to manually count items that are more visually ambiguous: for example, counting nuclei in one cell type while avoiding nuclei in other cell types.
- 3. Collect the values using the Record Measurements feature.

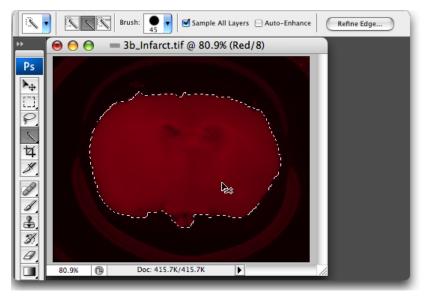
- 4. When your image is ready for output, use the Place Scale Marker command to insert a properly calibrated line mark and associated text on a separate image layer.
- 5. To export values as a text file for further analysis, select a data set or specific rows and columns in the Measurement log, and then export them.

	Se	lect Data Points						
D. Cor E E E E E E E E E E E E E E E E E E E	t: Ms Brain Section ata Points mmon Label Date and Time Document Source Sale Sale Antor Sale Sale Sale Sale Sale Antor Count Area Permeter Circularity Select All ton (Timeline) Measurements	Leselect All	Save	OK ancel Preset) te Preset	80.9%	 3b_Infarct.tif @ 80.9 40.9 0 	% (Red/8)	
	Label	Date and Time	Area	Perimeter	Integrated Density	Scale	Scale Units	Scale Facto
0001	Measurement 2	7/31/07 3:45:32 PM	58.580272	30.884404	4675961.000000	12 well Scan 300dpi (685 pixels =	mm	31.1363
			×.					

Photoshop CS3 Extended displays image analysis results in a table that can be easily exported.

Annotating data—The Analysis menu also lets you automatically place a scale bar annotation, for easily adding basic information to an image. Photoshop CS3 Extended has various features for annotating an image or creating infographic. For example, add text, labels, vector shapes, and other highlighting, such as to highlight regions; draw an irregular shape with vector tools to demonstrate a rough edged region of interest; add circles to mark subject material in an image; or draw lines and arrows to connect text or point out targets. You can also use these vector tools to create a grid overlay to aid in counting and other analysis.

Selection tools—Photoshop CS3 Extended builds on the strengths of Photoshop selection capabilities with two new selection features, the new Quick Selection tool and the enhanced Refine Edges dialog box. Complementing the myriad of Photoshop selection features, Quick Selection works by "painting" a selection within defined edges, Refine Edges then lets you view the edge several ways and optimize it using slider adjustments. Once you've made a selection, Photoshop CS3 Extended can record the measurement points previously selected using the Analysis menu.



Select the region of interest by setting the Quick Selection tool brush to an appropriate size and then click to make the selection automatically.

💿 🔿 😑 = 3b_Infarct.tif @ 80.9% (Red/8)	T	Refine Ec	lge	
	Radius:	10.5	рх	ОК
	Contrast:	17	%	Cancel Default
N 21	_ <u> </u>		_	🗹 Preview
	Smooth:	19		9
	Feather:	0.0	рх	
	Contract/Expand:	+39	%	
80.9% (B) Doc: 415.7K/415.7K	- ·	0	+	
	l 🕹 🕹	æ	හ	
	Description			
	Press P to toggle the previo through the preview mode:	ew of the edge s, and X to tem	refinement porarily vie	ts. Press F to cycle aw the image.
		_	_	_

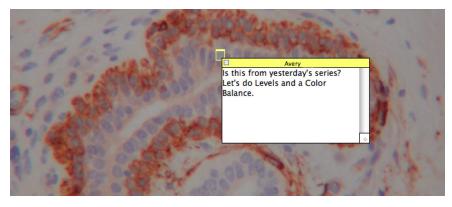
Finesse the exact selection border to limit what's included.

Image stacks —An image stack combines a group of images with a similar frame of reference, but differences of quality or content across the set. Once combined in a stack, multiple images can be processed nondestructively to produce a composite view that eliminates unwanted content or noise. For example, physicians can overlay multiple images of the same region taken at different times to more efficiently track and distinguish the progress of disease or a treatment regimen. Microscopists could use the new stack mode to run various algorithms on an image set, such as finding the maximum, mean, range, and various other variables, and then generate an averaged background more suitable for analysis and presentation.

6. Collaboration and output

Photoshop CS3 Extended makes it easy to share images and information between researchers and practitioners, with features including security, easy conversion to the open, universal PDF format, text and audio annotation, layers, animation of image files, and color management.

Adobe PDF—Any Photoshop image can be saved to an Adobe PDF file, making it easy to collaborate with colleagues around the world. PDF preserves multiple layers. Text and audio annotations in a Photoshop image stay embedded when the image is exported to an Adobe PDF file. You can e-mail the PDF file to a colleague, who can view the image and notes, and listen to any audio annotations using the free and easily available Adobe Reader* (from www.adobe.com). Recipients who have Adobe Acrobat* 8 Professional or Adobe Reader 7 or 8, can add their own comments to the PDF document and return it to the originator for ongoing collaboration. Where security is a concern, Photoshop CS3 Extended allows applying password-based security to any PDF file it creates to control the ability to read, print, and modify its contents.



Use the Notes tool to easily attach notes to any image. Save the file as an Adobe PDF file to make the notes comments in Adobe Acrobat or Adobe Reader 7 or 8.

Layers—In image enhancement, layers keep original image data unmodified and separate components of a composite image. But each layer can also contain a complete and separate image. For example, to share a series of three slides that show the beginning, midpoint, and end

of a process, place each image on a separate layer in the same image file. Colleagues viewing the file click the eye icon next to each layer to hide and reveal them.

Layer comps—Layer comps provide a way to store the location and visibility of all of the layers in an image at once, which makes it a powerful way to create small presentations or alternate versions of a presentation slide. For example, store a microscope slide on one layer, with callouts and labels for three different features in the slide each stored on its own layer; then define each layer comp as a combination of the slide layer, one callout layer, and one text label. Change the visible layer comp with one click, and then focus individually on each feature in the slide.

Animation—Animating layers or frames allows showing different aspects of an image. Or use adjustments layers to modify DICOM image frames making up an animation, and then export them as MOV format files or in other formats.

Animation (Timeline) × Measurement Log						Layers × Channels Paths	^â
0:00:04:00 (2.00 fps)	00	02:00f	0.00f	06:00f	08:00f	Normal * Opacity	
👌 Comments						Lock: 🖸 🌶 🖨 🛛 Fill	: 100% ►
Tayer 1						E Layer 1	
🖉 📄 🐨 Altered Video							_
O Position							
👌 Opacity							
🖄 Style							
🖄 Global Lighting							
aa ai - a i⊳	· 22 Ø					14	
				🖬 Untitled-	1 @ 100% (Gray	(1	
			K		3		1 3 3 4

Built-in animation tools in Photoshop CS3 Extended can be used on many file types.

Color management—You can embed color profiles in many Photoshop CS3 Extended formats, including in exported PDF files, to color manage files and thus enable others to see your images in the colors you expect and to print and publish images reliably. Color fidelity of images is critical for observing scientific subjects. For best results, calibrate your computer system and displays. Consider using Adobe Bridge 2.0 to synchronize color settings across Creative Suite 3 applications.

Resources

Visit these sites for more information on digital imaging and tools for the biomedical community.

Adobe web pages with testimonials, white papers and success stories by members of the biomedical community: <u>www.adobe.com/products/photoshop/photoshopextended/medical</u>

Adobe web pages with testimonials, white papers and success stories by members of the scientific community: <u>www.adobe.com/products/photoshop/photoshopextended/science</u>

National Association of Photoshop Professionals, the major association of Photoshop users providing training through its website and conferences: <u>www.photoshopuser.com</u>

PubMed, a searchable database for scientific research papers: www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed

Reindeer Graphics, the maker of Photoshop image analysis plug-ins Fovea Pro and Image Processing Toolkit: <u>www.reindeergraphics.com</u>

Dave Clunie's Medical Image Format Site, with DICOM information and links to other imaging resources: <u>www.dclunie.com</u>

Eric J. Wexler In my Mind's Eye—A blog on the biological images analysis and Photoshop CS3 Extended features and their applications: <u>www.ericjwexler.blogspot.com/</u>