

## Installing and Using the Adjust Image, Measure Features, and Stereology Panels

Panels are a new feature in Adobe Photoshop CS4. They make it possible to provide a high-level organization and automation that pulls together a variety of functions normally found in many different menus, including plugins. Those individual functions are still accessible in the usual ways, but the panel collects them together and provides a logical arrangement that can be especially useful for those who are not intimately familiar with the many capabilities that Photoshop and the Fovea Pro plug-ins offer.

In this initial offering, three panels (with the corresponding Photoshop actions) have been put together that should address a great many of the situations that typical users of Photoshop and Fovea Pro encounter, particularly beginning users. This may also be a useful learning tool for those who wish to examine the various functions in more detail, but to use them it is only necessary to click on the buttons in the panel.

Installing the panels involves several steps, some of which must be performed manually before launching Photoshop CS4. The Panels.zip package has been created and tested under Mac OS X 10.5.6. Unzip the package to find the following files, in addition to this Acrobat document.

- Adjust Image.mxp
- Measure Features.mxp
- Stereology.mxp
- IP-SelectTopLayer.jsx
- Panel Set.atn
- RankSharp.plugin
- AutoGamma.plugin
- Homomorphic.plugin

Each of these file types must be dealt with in a different but very specific way. The following steps are complicated, and must be followed with care, but after that the use of the new functionality is simple and automatic.

First, place the **RankSharp.plugin**, **AutoGamma.plugin** and **Homomorphic.plugin** files wherever you have installed the Fovea Pro plugins. In most cases that will be in the folder **Applications>Adobe Photoshop CS4>Plug-Ins>Filters>Fovea Pro**, but if you have performed a custom install, or previously installed Fovea Pro in an older version of Photoshop and have simply told Photoshop CS4 where to find that folder, you will need to place these files in the folder along with the other Fovea Pro plugins.

Second, place the **IP-SelectTopLayer.jsx** file in the folder **Applications>Adobe Photoshop CS4>Presets>Scripts** (this assumes you have accepted the default location for the Photoshop installation, otherwise you will have to find the correct folder). This is the location for scripts (written in Java) that Photoshop displays in its File->Scripts menu. This script is called automatically from the actions, which in turn are controlled by the buttons in the panel.

Next, double click on the **Adjust Image.mxp** file. That will launch the Adobe Extension Manager CS4 program, which will install the custom panel into Photoshop CS4 with no further action required (except for acknowledging the Adobe license agreement). Repeat this operation for the **Stereology.mxp** and **Measure Features.mxp** files. The result is the creation of three extension panels for Photoshop.

Now, launch Photoshop CS4. If the Actions palette is not visible, select Window->Actions to display it. Click on the icon in the upper right corner of the palette to access the drop-down menu for the palette, and select Load Actions. In the dialog that appears, find the **Panel Set.atn** file (it is probably still on your desktop, but it can be placed anywhere, including in the Adobe Photoshop CS4 folder). That will add the actions to the program. Once loaded, they will be present each time that Photoshop is run.

Finally, select Window->Extensions->Adjust Image, Stereology, and/or Measure Features to create and display the new Panels. Each panel can be kept open, or minimized by clicking on the << symbol in its header, combined with other panels by dragging the header, or closed and re-opened from the Window->Extension menu as desired.

## Adjust Image Panel

The **Adjust Image Panel** has 14 buttons, corresponding to 7 types of operations. In most applications, only a few of these will be required. In many cases, the fully automatic options can be used, but manual functions that allow for interactive adjustment of parameters are also provided. All of these functions first create a new layer containing the current image and operate on that layer, preserving whatever has been done previously.

**Color balance.** This is obviously not important if you are working with black and white images, or if the actual colors in the image are not important and it is only color differences that matter, for example to differentiate structures for later measurement. The **Automatic** method makes a neutral color assumption, which is often appropriate for real world images but may not be for images from stained tissue in a microscope, etc. This is equivalent to the Photoshop Image->Auto Color function. The **Manual** method allows customized adjustment of the individual red, green and blue color channels using the Photoshop Image->Adjustments->Curves dialog. You can either adjust the brightness alone, or the individual RGB channels, or use the eyedroppers to select neutral dark, light and midgrey points in the image.

**Nonuniform brightness.** If the image is nonuniformly bright because of off-center lighting, vignetting, etc., it can often be corrected by automatically fitting a function to either the **Brightest** or **Darkest** features present in the image, assuming that they are well distributed across the image area, and actually should all have the same brightness. These are equivalent to selecting the IP\*Adjust->AutoLevel plugin.

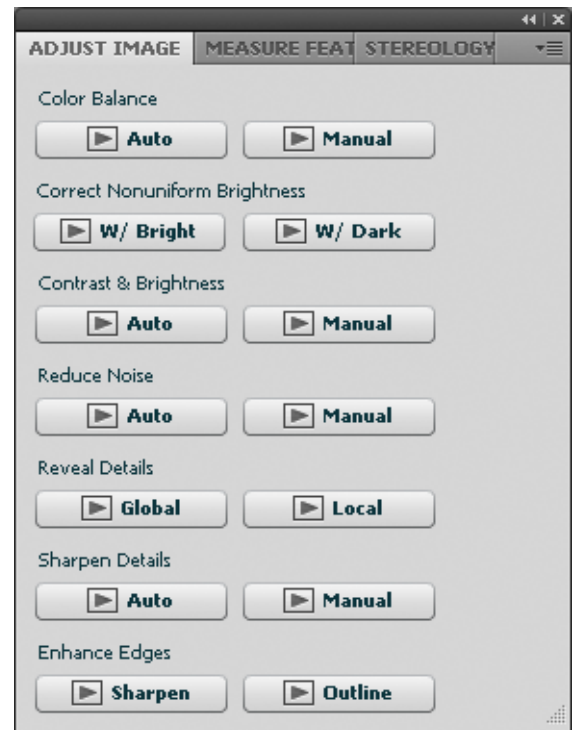
**Contrast & Brightness.** The **Manual** button brings up the Photoshop Adjust Levels dialog, in which the white and dark points, and medium gray slider, can be adjusted. The **Automatic** button maximizes contrast and selects an optimum gamma value for the image, using the IP\*Xtras->Auto Gamma plugin.

**Reduce Noise.** Median filters are the preferred tool for treating random speckle noise. The **Manual** button brings up the Photoshop median filter, while the **Automatic** button runs the IP\*Rank->Hybrid Median plugin, which better preserves edges, lines and corners. It is important to reduce noise before any of the enhancement operations below.

**Reveal Details.** Particularly in bright or dark areas of an image, fine details can be difficult to see. These tools decrease the overall contrast so that a greater amount of contrast is available for details. The **Global** button applies the IP\*Adjust->Homomorphic plugin to expand the contrast for details of all scales in the entire image, while the **Local** button applies the IP\*Process->Local Equalization plugin, which is best for increasing the contrast of very fine local details.

**Sharpen Details.** The visibility of fine details can be enhanced by increasing the local contrast. The **Manual** button applies the Photoshop Unsharp Mask routine, which can be interactively adjusted. The **Automatic** button uses the IP\*Xtras->Rank Sharpen plugin that avoids the "halo" effect introduced by the unsharp mask, and also deals better with steps in both the bright and dark portions of the image.

**Enhance Edges.** The steps and edges that define the boundaries of objects and structures are important for both visualization and measurement. The **Sharpen** button applies a maximum likelihood operator (IP\*Process->Sharpen Steps) to decrease the breadth of transitions and thus increase the sharpness of edges. The **Outline**



button brings up a selection of edge delineation functions (IP\*Process->Find Edges) which can be interactively selected.

## Measure Features Panel

The **Measure Features Panel** has 12 buttons in three groups. In most cases, if the image has not been previously converted to a binary (black and white) image in which the structure(s) to be measured are black on a white background, the first step will be to choose one of the buttons to perform this operation. This image may require processing to clean up feature edges, remove noise, fill holes etc., after which the desired measurements can be performed.

**Make Binary Image.** The **Threshold** button allows a single threshold value to be set to separate light from dark regions (IP\*Threshold->BiLevel). The **Slice** button provides two thresholds for the upper and lower brightness settings to define the regions of interest (IP\*Threshold->Threshold Levels). Both of these functions also have automatic tools, selected by default, for selecting threshold values. The **Region** button allows clicking on a point in the image whose color and brightness represent the areas to be selected, and setting tolerance values (IP\*Threshold->Color Tolerance). These routines first create a new layer containing the current image and operate on that layer, preserving whatever has been done previously.

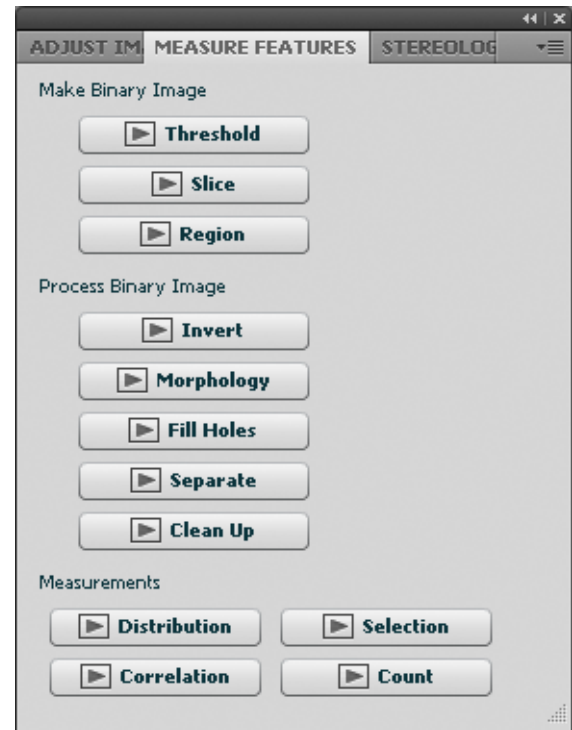
**Process Binary Image.** The **Invert** button (equivalent to the Photoshop Image->Adjustments->Invert) reverses black and white, since the background is sometimes easier to define than the structures of interest. The **Morphology** button allows opening or closing operations to be selected to smooth feature boundaries if required (IP\*Morphology->EDM Morphology). The **Fill Holes** button (IP\*Morphology->Fill Holes) fills any interior holes within features and the **Separate** button separates convex touching features (IP\*Morphology->Watershed). The **Clean Up** button allows specifying the size (in pixels) of small features to be removed, and optionally those touching the edges of the image (IP\*Measure Features->Reject Features). All of these routines first create a new layer containing the current image and operate on that layer, preserving whatever has been done previously.

**Measure Features.** The **Distribution** button displays a histogram of the measurement data for the features, for any selected size, shape, position or density parameter (IP\*Measure Features->Plot(Distribution)). The **Correlation** button displays a scatterplot relating any two measured values for each feature, to reveal correlations between them (IP\*Measure Features->Plot(Scatter)). The **Selection** button displays a histogram for any measurement value and allows setting limits to keep or remove features based on the measurement (IP\*Measure Features->Select Features); this button also generates a new layer containing the selected features. The **Count** button simply shows the number of separate black features present (IP\*Measure Features->Count).

## Stereology Panel

The **Stereology Panel** has 10 buttons in three groups. In most cases, if the image has not been previously converted to a binary (black and white) image in which the structure(s) to be measured are black on a white background, the first step will be to choose one of the buttons to perform this operation. Then the appropriate grid can be generated, and finally applied to perform the measurement.

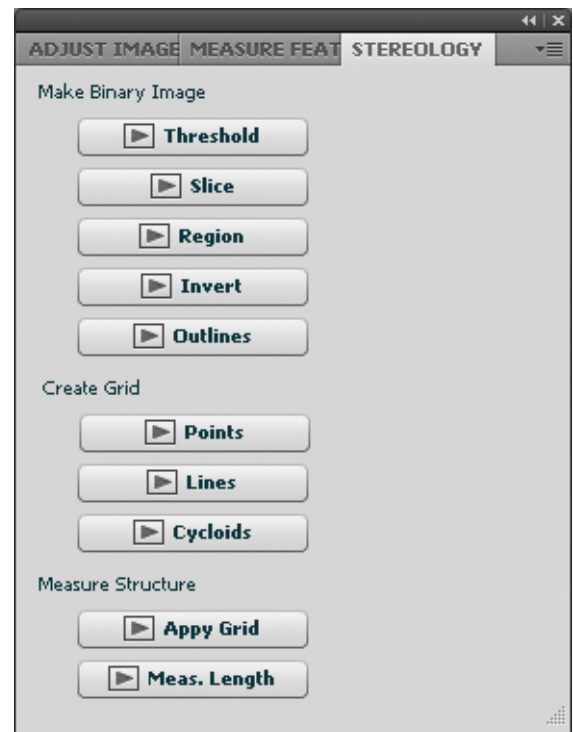
**Make Binary Image.** The **Threshold** button allows a single threshold value to be set to separate light from dark regions (IP\*Threshold->BiLevel). The **Slice** button provides two thresholds for the upper and lower brightness settings to define the regions of interest (IP\*Threshold->Threshold Levels). Both of these functions also have automatic tools for selecting threshold values. The **Region** button allows clicking on a point in the image whose



color and brightness represent the areas to be selected, and setting tolerance values (IP\*Threshold->Color Tolerance). The **Invert** button (Photoshop's Image->Adjustments>Invert routine) reverses black and white, since the background is sometimes easier to define than the structures of interest. The **Outline** button (IP\*Morphology->Outlines) may be used after these operations if the surface area of the structures is to be measured rather than the volume. All of these routines first create a new layer containing the current image and operate on that layer, preserving whatever has been done previously.

**Create Grid.** Three buttons are provided to generate **Point** grids (IP\*Lines and Points->Point Grids, for measuring volume) or line grids (for measuring surface area). The **Lines** button (IP\*Lines and Points->Line Grids) generates straight lines or circles, and the **Cycloids** button (IP\*Lines and Points->Cycloid Grid) generates cycloids. For all of these grids, dialogs are presented in which appropriate values for the grid spacings can be entered so that the structures are not oversampled. The number of points in a point grid, or the total length of the line grid, is shown and should be recorded. The grid is generated in a new window sized to match the original image.

**Measure Structure.** The **Apply Grid** button combines the binary image with the grid (using the IP\*Math->General Boolean plugin) and counts the number of "hits" (using the IP\*Measure Features->Count plugin), which is shown and can be used with the previously recorded data on the grid to calculate the volume or surface area of the structure. An overlay is also shown on the original image as a record of the grid and the location of the hits. The **Line Length** button is used without generating a grid, to measure the total length of the outlines generated by the Outline button under Make Binary Image. It uses the IP\*Measure Global->Total Line Length plugin and shows the total line length and image area, from which the surface area can be calculated.



The appropriate stereological calculations using these grids are:

Volume fraction = (Number of hits counted) / (Number of original grid points)

Surface area per unit volume =  $2 * (\text{Number of hits counted}) / (\text{Length of lines in the grid})$   
or

Surface area per unit volume =  $(4/\pi) * (\text{Length of outline}) / (\text{Total image area})$

Any comments about these particular panel functions, suggestions for additional ones, or thoughts about the utility of panels in general, would be welcome. Please send email to [DrJohnRuss@GMail.com](mailto:DrJohnRuss@GMail.com)