

# *The Image Processing and Analysis Cookbook*

*Companion to  
The Image Processing Toolkit  
(Version 3.0)*

*John C. Russ*

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## *How to use this guide*

Hands-on experimenting with the various algorithms discussed in *The Image Processing Handbook* (CRC Press, Boca Raton FL, Third Edition, 1998) and in workshops such as the Image Analysis Short Courses taught each May at North Carolina State University and each June at the Danish Technological Institute is an essential way to learn how the various processes work and when they should be used. It is important to develop the user's experience and judgment by the process of observing the effects of the various steps on different images. This leads to the ability to select appropriate techniques to extract the required information from images that require processing. The key seems to lie in becoming aware of what it is in the image that allows the viewer to "see" the desired information and separate it from its surroundings. Sometimes this involves differences in brightness, but in many cases it is color (hue) differences, texture differences, the presence of edge boundaries, or other subtle information. Once that identification is made, it is usually possible to find an appropriate computer algorithm, or a combination of them, that will isolate and measure the details of interest. There are a number of different measurements that can be made, and it is important to understand what they are and how the resulting data may be related to the structures of interest. These are the subjects of the book, the courses and of this tutorial.

A barrier to the hands-on practice that develops these skills lies in the wide variety of software packages (both commercial and free) that are available, and the fact that they run on quite different platforms (Unix, Mac, Windows, etc.). None of these programs is truly complete in all areas of algorithms and applications, and in addition they have very different user interface conventions for selecting the operations that are offered. The same process may exist with different names and means of selection in two different programs, while others may use the same name to describe quite different operations or measurements.

As a useful tutorial introduction to image processing and analysis that can be used on either MacOS or Windows platforms in completely identical environments, we have chosen Adobe Photoshop® as a host program. This program offers the same exact menus and naming conventions for functions on both platforms. In its basic form, Photoshop is not a comprehensive image processing and measurement package. Indeed, its principal thrust is toward imaging applications such as the graphic arts that are quite different from the quantitative measurement and interpretation processes discussed here. However, the program supports a wide variety of file formats and acquisition devices, handles details of image display and system access, and provides for the use of separately compiled "plug-in" programs that can be written to extend its functionality. We have taken advantage of this facility to create a set of plug-ins that give Photoshop a comprehensive set of the image processing and measurement operations found in quite high-end packages. In fact, some professional scientific image analysis programs support the same plug-in interface as defined by Adobe for Photoshop, and can also make use of these plug-ins to provide functionality that may otherwise be missing.

For those who do not have Adobe Photoshop, we include a fully functional alternative program on the Tool Kit CD. MicroFrontier's Digital Darkroom® is an inexpensive image processing program with identical user interface and appearance on MacOS and Windows platforms. It fully supports the Photoshop plug-in interface and therefore can use the Tool Kit plug-ins equally well. This tutorial is written primarily from the standpoint of the

Photoshop user, but with a few minor adjustments applies to Digital Darkroom as well. The principal difference is that plug-ins appear in Photoshop under the Filter menu, so that the menu selection is (e.g.) **Filter | IP•Process | Kirsch**. In Digital Darkroom the Filter menu has a submenu Plug-ins under which the Tool Kit plug-ins are listed, so that the same function would be selected as **Filter | Plug-ins | IP•Process | Kirsch**. In addition, Digital Darkroom does not have some of the built-in functions provided in Photoshop, such as displaying the image histogram, separating color channels, and various image adjustments. However, we have written plug-ins that provide these functions. The most common operation that differs between Photoshop and Digital Darkroom is duplicating an image. The Photoshop **Image | Duplicate** command is accomplished in Digital Darkroom by **Selection | All, Edit | Copy, File | New**, and choose "Fill with Clipboard" which will automatically set the image size and type. In the tutorial, there are a few sections where built-in Photoshop functions are discussed followed by the relevant similar operations using the Tool Kit plug-ins. For Digital Darkroom only the latter will be appropriate. Finally, Photoshop provides the capability to create Actions, which are sequences of operations that provide limited automation. Digital Darkroom does not have this capability.

The Tool Kit plug-ins do not really turn Photoshop or Digital Darkroom into a high-performance scientific image analysis program, although our experience suggests that many users of such programs also use Photoshop for various image presentation purposes and perhaps also for image acquisition. Photoshop and Digital Darkroom provide image acquisition from an extraordinarily broad range of devices - video and still cameras, flat bed and slide scanners, and so forth. Because of their virtual memory schemes and the fact that they are written using a general purpose program shell, Photoshop or Digital Darkroom with the plug-ins are not very fast programs nor ones that can be easily scripted or automated (Photoshop Actions can be used for limited automation of the functions, but interfacing hardware to control stages, etc., is not very practical). Nevertheless, the capabilities provided by these plug-ins are not limited in any way to tutorial use. They are faithful and complete implementations of the various methods described in *The Image Processing Handbook*, and in addition to the examples shown here can be used on your own images for additional training and familiarization.

It is expected that you will independently develop a basic facility with the Photoshop or Digital Darkroom programs themselves. Some of the built-in functions that are important for scientific image analysis are covered in this tutorial where they fit in functionally with the various plug-in routines. Basic operations such as opening and saving files, printing images, etc., are not covered here. Nor are those functions of the host programs that are primarily appropriate for graphic arts and other similar applications that lie beyond the scope of this tutorial (e.g., labeling images with text and markers). In addition to the documentation that is supplied by Adobe with the Photoshop software and the on-disk help files for Digital Darkroom, there are numerous other sources of support and information, including books and internet-based resources. For the latter, you can find a constantly updated list of sites on our web page

**<http://members.aol.com/ImagProcTK>**

This site also has information about updates to the plug-ins and tutorial, which can be downloaded and installed to use along with the ones you have installed from the CD-ROM.

The plug-ins discussed here are accessed by the program when they are placed in the "Plug-Ins" folder or directory belonging to Photoshop or Digital Darkroom, and appear under the "Filter" menu item. See the information in Appendix 1 describing installation for more details for either the MacOS or Windows version.

The use of the various image processing and analysis procedures provided by the plug-ins and by the built-in Photoshop and Digital Darkroom functions is organized by topic, following essentially the same sequence as in The Image Analysis Handbook, 3rd Edition (J. C. Russ, 1998, CRC Press, Boca Raton FL) for which the Image Processing Tool Kit is intended to be a companion. There are separate documents that describe and illustrate these procedures in detail (Part 1 through Part 8).

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The use of the various procedures is illustrated here using the images stored on the CD-ROM, although it is also possible (and recommended) to use your own images to better develop familiarity with the effects of the algorithms on images of particular interest. The images are stored as TIFF files on both the Macintosh and Windows partitions of the CD (with a few exceptions noted). Appendix 2 lists the images present (the names have been shortened to fit the Windows 8.3 naming convention). Most other image analysis programs can read this widely used format as well. General-purpose image format translators such as DeBabelizer (and numerous shareware programs) are widely available to convert other less common images formats to one that can be read, if it is required to handle your own images and the host program does not provide that capability. Photoshop can also open files using a "Raw" mode that allows you to open nearly any format provided that you know how the data are arranged in the file.

The tutorial shows the actual appearance of the images as the various operations are performed, as well as the menus, dialogs and results as they appear on the screen. The user interface that you see may vary slightly from these illustrations depending on your computer and operating system, but the essentials of the menus, dialogs and formatting remain the same. The menu illustrations correspond to Photoshop 5.0; in a few cases there are minor variations in placement of commands between these programs; for example, converting an RGB Color image to a

greyscale monochrome image is accomplished in Photoshop 3 by **Mode | Greyscale** and in Photoshop 4 and 5 by **Image | Mode | Greyscale** (the entire Mode menu in Photoshop 3 was made a submenu under the Image menu in Photoshop 4). In Digital Darkroom the same function is selected by **Image | 256 Greys**. A few of the procedures, such as manual drawing in layers for measurement, are dependent upon the layers capability of Photoshop 4 and 5. The History palette in Photoshop 5 is a wonderful tutorial tool (as well as being an important aid in other work and an unlimited Undo buffer), but is not emphasized in the examples shown. Using Undo/Redo to view the changes made by a processing step is strongly recommended; in Photoshop the keyboard short cut uses Command or Control-Z for both; in Digital Darkroom Command/Control-Z is undo and Command/Control-R is Redo. While the specific illustrations in the tutorial are for Photoshop 5, it is not expected that users having a basic familiarity with other host programs will encounter difficulties.

The Layers capability of Photoshop 4.0 and 5.0 is very useful in other ways, such as allowing you to keep the original image as the background, duplicate the layer for processing (perhaps several times for multi-step operations), and then adjust the visibility of the layers to see what the various operations have done. That capability is not shown in these examples, but the plugins operate correctly with layers and will process whichever layer is currently active.

By default, the plug-ins operate on the entire image. If a region has been selected (using Photoshop's marquee tool, freehand drawing tool, or magic wand) most of the plug-ins will operate only on the pixels within the selection (which may consist of several disconnected parts). There are exceptions, primarily in the measurement routines, because some of the operations require a rectangular image (either the entire image or a rectangular selection region). Most of the Fourier processing routines require a rectangular image whose dimensions in pixels are powers of 2 (128, 256, 512, 1024, etc.). The Setup 2nd Image routine always saves the pixels in a rectangular region that surrounds any irregular region that has been selected. In all these cases there are reminder or warning dialogs presented to the user.

### *Stereology*

Sections 7 and 8 of the tutorial are primarily concerned with image measurements. In many cases the data obtained from the images can be straightforwardly interpreted to yield the desired information, but this is not always the case. One particular situation in which care is needed is for images obtained from various kinds of microscopes (electron, light, etc.), in which the specimen may be a slice (thin section or cut surface) through a three-dimensional structure. The values measured on the two-dimensional image are related to the parameters of the three-dimensional structure, but some calculation and conversion is required. The same situation can arise in other, more macroscopic fields of application such as industrial quality control or medical X-rays or tomography.

The scientific and mathematical field that deals with the relationships between 2D images (either sections or projections) and the 3D images which they represent is Stereology. This tutorial does not deal with the mathematical or conceptual underpinnings of stereology, although many of the measurements performed in sections 7 and 8 are correct and appropriate for stereological use. As an aid to the user of the tutorial and the accompanying Image Processing Tool Kit, a separate set of files is provided on the CD containing the full text of a draft version of a forthcoming textbook on Stereology (Practical Stereology, Second Edition, Plenum Press). The ten chapters of that book including illustrations and references are in the form of Adobe Acrobat files (\*.pdf), the same as this tutorial, and can be viewed using the same reader software.

## *Compatibility*

There are many other programs that support Photoshop-compatible plug-ins. Most of these can use the Image Processing Tool Kit plug-ins. In some cases the plug-ins are not organized into sub-menus according to the function groups and names built into the "PiPL" resource that is included in the plug-in files. These programs recognize the plug-ins by their folder location and file type, but list them alphabetically by file name. You can change the file names as desired to create some order and organization in the menu (this will not affect the menu names in Photoshop, which uses the menu entry inside the resource in each plug-in file). Some of these programs also limit the total number of plug-ins that can be listed in the menus, so it may be necessary to choose which ones to place in the directory that the program scans.

Testing of the Image Processing Tool Kit plug-ins with a variety of programs that claim to support Photoshop plug-ins has produced the following observations (note in all cases the version levels that were tested - earlier or later versions may be different). We do NOT guarantee or warrant the plug-ins for use with any of these other programs, nor that our testing will keep up with changes and revisions in these programs. We would welcome additional information from users who may have tested them with other programs on either Mac or Windows platforms (email [DrJohnRuss@AOL.com](mailto:DrJohnRuss@AOL.com)).

**Photoshop** (Mac and Win) - All plug-ins work correctly in Photoshop 3.x, 4.x and 5.x under Mac System 7.x and 8.x and under Windows 95, 98 and NT 4.0. They should also run correctly under Photoshop 2.5 with Mac System 6.x (under Photoshop 2.5 the plug-ins are listed alphabetically rather than hierarchically), and under Photoshop 3 or 4 with Windows 3.1 and NT 3.51, although they have not been exhaustively tested in those environments. On the Mac, the plug-ins contain native code for both 680x0 and PPC processors; Photoshop 5 runs only on the PPC.

**Digital Darkroom** 1.0 and 1.2 (Mac and Win) - All plug-ins work correctly (version 1.2 is required for WinNT and recommended for all users). The files must reside in the Plug-ins folder within the DDStuff folder (on the Mac you can use an alias with that name to the actual folder containing the plug-ins). Digital Darkroom is included on the Tool Kit CD by special arrangement with the publisher.

**Canvas** 5 (Mac and Win) - All plug-ins work correctly. Set preferences to direct Canvas to the directory where your filters are stored (like Photoshop, the program correctly searches for plug-ins in subfolders and shows them in a hierarchical menu). Execution speed of some plug-ins is slower than in Photoshop possibly due to disk caching differences.

**DeBabelizer** (Mac and Win) - All plug-ins work correctly. Set preferences to the folder in which the Photoshop filters are stored. Sub-folders/sub-directories are NOT searched for plug-ins, but Mac aliases are followed. The menu lists plug-ins alphabetically under "Other."

**NIH Image** 1.62 (Mac) - All plug-ins work correctly except those that deal specifically with 24 bit color images (which are not passed to the plug-ins using the conventions of the Photoshop plug-in interface). Plug-ins must be placed in the program's Plug-ins folder (no aliases can be used) and are listed alphabetically in a single menu by their file names, not the menu names used by Photoshop. Only the 68K code is run (the faster PPC

code present in the plug-ins is ignored). The PC version of Image (Scion Image) does not at this time support plug-ins. Appendix 5 discusses NIH-Image use with the Tool Kit.

**Color-It** 3.0 and 3.2 (Mac) - All plug-ins work correctly. Requires filters (or aliases) to be placed in the program's own Plug-ins folder (no subfolders are searched). Plug-ins are listed alphabetically under "Other".

**ImageTool** 1.27 and 2.0 (Win) - Plug-ins for 8 bit grey scale images work correctly; they appear hierarchically under the Processing menu. The program requires Windows 95, 98 or NT4. It creates a new image window for each processing step and so does not support or require "undo" operations.

**Enhance** 3.0 (Mac) - Plug-ins for 8 bit grey scale images work correctly when they or their aliases are placed in the program's own Plug-ins folder. Plug-ins are listed alphabetically.

**PhotoDeluxe** (Mac and Win) - All plug-ins work correctly under Mac System 7.x, Windows 95 and NT. Because all images are treated as 24 bit color multilayer arrays the plug-ins operate more slowly than in Photoshop.

**Image Pro Plus** 4.0 (Win) - Plug-ins work correctly but the host program does not support all of the Photoshop interface conventions. Selections are not supported so all processing and measurement applies to the entire image. Call backs that display a progress bar and allow halting an operation with escape (Win) or command-period (Mac) do not function. The Image Pro Plus convention that features are white and background is black is the opposite of all other programs listed here; it may be helpful to invert images when using the Tool Kit plug-ins.

### *Hardcopy*

The total extent of the tutorial (this introduction, eight parts, plus appendices) is more than 350 pages. Most of the pages include graphics, and many have color images on them. For many users it will be most convenient to browse through these sections on the computer screen. For anyone wishing to print them, a few notes will save considerable frustration. The pages have been formatted to fill the width of a standard 640 pixel wide display, with very narrow margins. Printing these pages on standard US 8-1/2 x 11 inch letter size paper will result in clipping at the margins with most printers. The problem will be considerably worse with narrower European A4 letter size paper. For both, the best solution is to set the printer driver to reduction so that the document is printed at about 90% of its original size. This will allow it to fit comfortably on either size paper.

Prepare data for analysis by implement various data science concepts such as acquisition, cleaning and munging through R and Python. Build a predictive model and an exploratory model. Analyze the results of your model and create reports on the acquired data. Build various tree-based methods and Build random forest. About. As increasing amounts of data are generated each year, the need to analyze and create value out of it is more important than ever. Survival analysis and statistical inference are his main areas of research/interest, and he has published several research papers in peer-reviewed journals and also has authored two books on R: R Statistical Application Development by Example, Packt Publishing, and A Course in Statistics with R, Wiley.