# *Roadmap Guide to Image Analysis*

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## 0. Overview of Image Processing and Analysis

### A. Relationships

***Image Processing*** operates on images and results in images, with changes intended to improve the visibility of features, or to make the images better for printing or transmission, or to facilitate subsequent analysis. ***Image Analysis*** is the process of obtaining numerical data from images. This is usually accomplished by a combination of measurement and processing operations. The data may subsequently be analyzed statistically, or used to generate graphs or other visualizations.

### B. Work flow

The procedures in the following sections are generally applied in the order shown, as appropriate to a given image and final purpose (i.e., skip those steps that are not required in a particular application, but work from the top down). For instance, for a grey scale image you would skip the steps that pertain to color images; for images with good, uniform contrast you would skip steps that correct nonuniform illumination, adjust contrast, or increase local contrast; etc. For each topic, examples will be shown that will help explain the procedures and show results on a wide variety of images. Of course, original images should be archived and all processing and measurement steps documented.

The process of image analysis - obtaining meaningful numeric information from images - typically requires several steps performed in sequence. Many software programs contain at least the basic tools, ranging from NIH-Image, through Photoshop with Fovea Pro, to expensive solutions such as Image Pro Plus (which also control hardware), but it is important for the user to understand what each technique does and when to use them. The following roadmap offers a condensed guide to the most common procedures. In the great majority of typical situations, this roadmap should cover the required steps.

The emphasis in this presentation is on when and why to apply various techniques to images. For a deeper explanation of how the various algorithms function, please refer to John Russ "The Image Processing Handbook," 5th edition, CRC Press, Boca Raton FL, 2006 (isbn 0-8493-7254-2). Algorithms discussed there and shown here are programmed into Fovea Pro, from Reindeer Graphics <http://ReindeerGraphics.com>, as plug-in modules for Adobe Photoshop (both PC and Mac) and compatible programs (which include Photoshop Elements, Paint Shop Pro and Image Pro Plus). The CD with those plugins also contains a detailed hands-on step-by-step tutorial on the use of the routines, and many of the images used here.

## 1. The program environment

### A. Image acquisition, storage and printing

*Devices (cameras, scanners, etc.)*

Understand the type of camera (color, monochrome, video, digital, tube-type, CCD, CMOS, etc.) or scanner (single pass, three pass, bit depth, reflective or transparency, etc.) and the characteristic types of artefacts that each produces (noise - both periodic and random, real resolution - as opposed to the number of stored pixels, color response, etc.)

*File types*

TIFF files are a good choice on most computer platforms and for most software packages. Adobe PSD is also cross-platform and accommodates multiple layers. Avoid absolutely (!)

any type of lossy compression (JPEG, wavelet, fractal) since you cannot be sure what details will be lost or changed that might be important in subsequent analysis.

*Printing hardcopy*

Dye-sub, ink jet, and laser printers may be useful. Printer type, ink and paper affect image quality (balance against the uses for prints: draft, report, publication, poster, etc.). Correct color rendition requires ICC profiles for all output devices (screen and printer). The dynamic range of prints is less than that of the screen display, which is less than that of film.

*Storage*

Writing image files to CD-R, or storing on network RAID drives are popular tools, but you need a good database program and some discipline to make this work. QBIC (Query by image content) tools that can find an image by comparison or example are becoming practical but most image "cataloging" tools rely on a tiny thumbnail and human recognition.

**B. Basic image display and manipulation**

*Managing multiple image windows*

The "frontmost" or selected image window is usually the one being processed or edited. Some operations require two images (e.g., subtraction), and different programs offer various methods to designate how this is done. Most programs provide "Undo" capability, some spawn new copies with each operation.

*Selection using ROI and wand tools*

Various means of selecting regions of interest (rectangular or circular regions, arbitrary outlines, multiple disjoint areas, selection with a "wand" or region growing tool) may be available for interactive (manual) measurement or to restrict processing regions.

*Drawing on and labeling images*

Tools for adding text, lines and other labels are often very useful, with selection of colors, fonts, etc., as required. Keeping the text, etc., separate from the original image pixels is usually a good idea, preserving image details and also allowing for better quality graphics.

**2. Correction of image defects**

**A. Color images**

*Is color correction required?*

If the true colors in the image are important, it is possible to make either manual or automatic corrections, but it helps to have an image of a reference standard.

*Color filtering and separation to improve contrast*

If color information is important to distinguish different structures, then applying color filters, or separating the image into color channels (RGB or HSI) may improve the contrast and simplify selection. Processing in HSI or LAB space avoids problems introduced when RGB channels are used. Merging multiple channels to produce a color image is only possible for 3 channels at a time.

*Reduction from color to greyscale*

Color can be removed from an image in several ways: reducing saturation to zero, calculating the monochrome intensity or luminance, selecting a color channel, or calculating an optimum grey scale axis by principal components. Principal components analysis finds the set of axes in color space that correspond to the most significant combinations of pixel values in the various channels, and is useful for clarifying the structures in subtle color images and identifying the phases present in complex multichannel data sets.

**B. Noisy images**

*Random speckle noise*

Random noise is commonly removed with either Gaussian smoothing (an optimum low pass filter) or median filtering. The latter is almost always preferred, as it retains edge sharpness and position. The neighborhood size (ideally a circular region for isotropic noise) controls the size of noise removed (and the processing time).

*Shot noise (black and/or white spots) removal*

Dropout pixels (common in some types of microscopy such as interference microscopes, or from dust on a scanned negative) are typically filled in with a median filter.

*Periodic noise removal*

Filtering in Fourier transform space is very efficient and can often be automated, as a way to remove electronic interference, halftone patterns from printing, vibration, some types of scan line noise, etc.

*Scan line noise removal*

Even/odd scan line noise from video cameras, and some scan line artefacts from AFM, can be eliminated with a median filter using a tailored non-isotropic neighborhood.

**C. Nonuniform image illumination**

*Is a measured background image available?*

A background image records variations in illumination, optical vignetting, etc.

*Is background visible throughout the image?*

Automatic leveling based on fitting a polynomial to selected regions, or automatically to either a bright or dark background is fast and applies to gradual brightness variations typical of nonuniform illumination.

*Are the features small in one dimension?*

Rank-based leveling removes the features and allows creation of a background for leveling.

*When to subtract or divide by the background*

This depends on whether the acquisition device is linear (e.g., CCD cameras) or logarithmic (e.g., film, vidicon tubes). Or, just try both and use whichever gives level results.

*Correcting varying contrast across n image*

This is particularly applicable to sections of varying thickness, and is an extension of the polynomial fitting approach.

**D. Expanding image contrast**

*Linear expansion for grey scale and color images*

Maximizing the contrast by setting dark and bright limits using the image histogram stretches the brightness over the full display range.

*Non-linear adjustments (gamma, equalization)*

Nonlinear functions selectively expand contrast in one part of the grey scale range by contracting contrast elsewhere. This can also be used to compensate for the characteristics of the acquisition device.

*Too great a dynamic range*

Even in 8 bit images, and especially with a greater camera dynamic range, it is not possible to view or print images that cover the full range without clipping, while preserving the local detail. A range compression method known as homomorphic filtering produces superior results.

*Negative images*

Human vision is logarithmic, and can detect brightness variations better sometimes in the negative image.

**E. Distorted or foreshortened images**

*Making pixels square*

Particularly with video cameras and analog to digital converters, adjustment is needed to make dimensions the same vertically and horizontally.

*Perspective distortion (non perpendicular viewpoint)*

The light microscope has a shallow depth of field and usually the viewpoint is perpendicular to the sample, but in the electron microscope it is common to have tilted specimens which results in trapezoidal distortion. This must be corrected to permit meaningful measurements and even to facilitate proper image processing.

**F. Focus problems**

*Shallow depth of field*

Multiple images obtained by moving the lens relative to the sample can be combined by keeping the in-focus pixels from each (defined by having the greatest local variance).

*Deconvolution of blurred focus*

Dividing by the Fourier transform of the system point-spread function can dramatically improve image resolution. The PSF can be measured, calculated, or estimated interactively. The effects of image noise are limited by apodization or the introduction of a Wiener constant.

**G. Tiling large images**

*Shift and align multiple fields of view*

Assuming perfect stage motion and no image distortion, stitching multiple fields together is easy. In most real cases auto alignment and local scale adjustments are needed and require 10-20% overlap between fields.

**3. Enhancement of image detail**

**A. Poor local contrast and faint boundaries or detail**

*Local equalization*

Increasing the local contrast within a moving neighborhood is a powerful non-linear tool for improving the visibility of detail. Adaptive equalization and variance equalization also provide noise rejection and preservation of boundaries. Homomorphic range adjustment is particularly useful for images with a wide dynamic range (e.g. medical x-rays, electron diffraction patterns).

*Sharpening (high pass filters)*

Classical sharpening operations (e.g., the Laplacian) increase the local contrast at edges. They are equivalent to FFT-based high-pass filters. Directional derivatives and other neighborhood convolutions are applied in specific cases.

*Unsharp mask and difference of Gaussians*

The basic unsharp mask operation is a powerful tool derived from long-established photographic darkroom technique. Sharpening methods increase the visibility of noise, so the Difference of Gaussians technique, equivalent to a band-pass filter in Fourier space, should be used for noisy images.

Just as the median filter is a ranking operation for noise removal that is analogous to, but often better than, a Gaussian smooth, so too can rank operations be used for detail enhancement. Subtracting the result of a median filter or grey scale opening or closing from the original image leaves just the detail that was removed by the ranking operation. As for the unsharp mask, this detail may optionally be added back to the original.

*Feature selection based on size*

The Top Hat filter is closely related to the Difference of Gaussians, but based on ranking rather than smoothing. It can be used to select bright or dark objects for retention or removal. This is also very useful for locating peaks ("spikes") in FFT power spectra.

*Pseudo-color (Cluts), pseudo-3D, and other display tools*

The use of false- or pseudo-color look-up tables makes small brightness differences visually evident. Rendering images as though they were physical surfaces also assists in recognizing and interpreting detail.

**B. Are feature edges important?**

*Edge enhancement with derivative operators*

Locating edges in images has been a major area of algorithm development because of the importance of edges in human vision, and for measurement. A variety of methods range from the simple Sobel to more advanced techniques like the Frei and Chen use multiple linear (or convolution) operators. Nonlinear (e.g., using the difference between the brightest and darkest pixels in a small neighborhood)) and statistical operators (e.g., the local variance) are also useful.

*Increasing edge sharpness and region uniformity*

Maximum likelihood techniques can assign doubtful pixels to regions and create abrupt transitions that facilitate thresholding and segmentation.

**C. Converting texture and directionality to grey scale or color differences**

In many images, structures are discernible visually based on textural rather than brightness of color differences. Processing tools such as the Sobel direction operator or a wide variety of local texture measurements based on entropy, fractal dimensions, statistical properties, etc., can convert these variations to brightness differences for thresholding.

**D. Fourier-space processing**

*Isolating and measuring periodic structures or signals*

The FFT power spectrum can facilitate the selection of regular structures in the image and their measurement. Besides its use for removing periodic noise from images (e.g., electronic interference or halftone printing moiré patterns), this facilitates averaging and measuring repetitive structures (e.g., TEM images of atomic lattices).

*Location of specific features*

Cross-correlation with a target image is a powerful object finder. Autocorrelation measures the representative size of structure present in a complex image. Cross-correlation is also used to match points, e.g., for alignment or stereo pair parallax measurement.

**E. Detecting image differences**

*Alignment*

Alignment of images - both shift and rotation - consisting of either different color channels, serial sections, etc., may be performed manually or automatically, either based on the image contents or on discrete fiducial marks.

*Subtraction and ratioing*

Combining multiple images by subtraction or ratioing, and displaying the results in RGB or HSI color channels, is an effective way to eliminate lighting variations on curved surfaces, thickness variations in sections, variations in stain concentration, etc. It also makes it easy to display differences that might otherwise be overlooked.

**4. Thresholding of image features**

**A. Thresholding using the histogram**

*Manual settings*

Interactive setting of thresholds can be used to produce binary and contour images. This is where most image measurement errors arise, because of inconsistent human judgment.

*Automatic methods*

A wide range of automatic methods for thresholding are available, many originally developed for reading printed text on paper. Selecting the appropriate technique is based on prior knowledge a that there are just two phases present, or that boundaries should be smooth).

*Select a color range by example, or with the histogram*

In either RGB or HSI space, a range of colors can be specified to select structures for binarization. In most real cases, this involves more than a simple Boolean combination of individual color channels.

*Contour lines (iso-brightness lines)*

These lines are continuous, used to measure smoothness of boundaries, create contour maps.

**B. Marking features manually**

*Region growing*

Rather than histogram-based methods, a “wand” approach allows human interaction to select features for measurement.

*Manual lines, points, etc*

Most systems provide some mechanism for the user to mark lines, points, circles, or other features onto the image which are counted and/or measured, when it is impractical to process the image to permit thresholding and automatic measurements.

**5. Binary image processing**

**A. Removing extraneous lines, points or other features**

*Erosion/dilation with appropriate coefficients to remove lines or points*

The use of combinations of erosion, dilation, opening and closing permit selective correction of binary image detail when thresholding leaves artefacts behind.

*EDM based opening to remove small features or protrusions*

Erosion + dilation based on the Euclidean distance map is much faster and more isotropic than traditional pixel-based techniques.

*EDM based closing to fill in gaps*

Dilation and erosion using the Euclidean distance map fills in gaps while retaining the shape of complex features.

**B. Separating features that touch**

The watershed, based on the EDM, is a powerful tool for separating touching convex shapes.

**C. Combining multiple images of the same area to select features**

*Boolean logic to apply multiple criteria*

For different colors, or images thresholded and processed differently, Boolean logic can combine the information and isolate the structures of interest. The basic Boolean functions are AND, OR, Exclusive-OR and NOT, which can be combined in many ways.

*Using markers to select objects*

Unlike the conventional pixel-based logic, this method selects entire features based on markers in a second image. In Boolean logic based on entire features ("Feature-AND") the order of images is important, unlike the conventional pixel-based Boolean operators, which commute (A AND B = B AND A). Feature selection by markers can also be used to implement the stereological disector, by swapping the foreground/background colors so that matched features are erased and unmatched ones are kept.

*Region outlines as selection criteria*

Outlines and boundaries are important markers to find adjacent features.

**D. Feature skeletons provide important shape characterization**

*Grain boundary, cell wall, and fiber images*

Broad thresholded lines can be thinned to single pixel width. Grain boundaries and cell walls form tesselations without end points, so pruning of branches with ends is an appropriate clean-up method. Removal or measurement of short branches based on length also provides a powerful tool. For overlapped fiber images, the number of fibers can be determined as half the number of end points, and the mean length as the total length divided by number

*Measuring total skeleton length, number of ends, number of branches*

**E. Using the Euclidean Distance Map**

*Distances from boundaries or objects*

Assigning values from the EDM to features enables the selection or measurement of feature position relative to irregular boundaries. Sampling the EDM with the skeleton provides width measurement for irregular structures.

**6. Measurements**

**A. Calibration**

*Calibrating image dimensions*

This is typically done by acquiring an image of a stage micrometer or some standard object.

*Calibrating density/greyscale values*

This requires density standards, which are readily available at macro scales but hard to make for microscopes. Recalibration is needed often because of variation in line voltages, lamp aging, optical variations, camera instabilities, etc.

**B. Intensity Profiles**

Profiles of intensity, calibrated density, or color information often provide efficient ways to extract data for analysis.

**C. Stereological measurements**

Relationships between three-dimensional structure and two-dimensional images make it easy to correctly measure metric properties such as volume fraction, surface area, length, etc., as well as topological properties such as number and connectivity. Modern stereology emphasizes the best ways to perform sampling and sectioning to avoid bias. Most stereological techniques utilize appropriate grids superimposed on images and counting of intersections, which can often be automated.

**D. Counting features**

Some features may be eliminated based on size or shape (e.g., dirt). It is also necessary to correct for edge-touching features (which cannot be measured), since large features are more likely than small ones to be affected.

**E. Measuring features**

Feature measurements can be grouped into four categories:

- Size (area, length, breadth, perimeter, etc.)
- Shape (topology, dimensionless ratios, fractal dimension)
- Intensity (density, color)
- Location (absolute coordinates or distances from other objects)

Measurement data can be analyzed statistically, used for feature classification, etc.

**7. Automation and batch processing using Actions**

Once a suitable procedure has been worked out for a particular application - type of image and desired information - it can usually be automated for routine use.

**For further information** *(shameless self-promotion)*

1. **Read a good book** - for example J. C. Russ, **The Image Processing Handbook**, 5th edition, 2006, CRC Press. Available from Amazon.com.
2. **Go on-line** - for example there are many step-by-step tutorial examples shown for the topics above at **ReindeerGraphics.com**. The Florida State Univ. tutorial website has extensive interactive Java applets illustrating the principles <http://microscopy.fsu.edu/primer/digitalimaging/russ/index.html>.
3. **Get a software package and practice** - for example, Fovea Pro comes with many example images and an extensive tutorial that is both a valuable reference tool and also provides a complete college-level course in image analysis. Go to **ReindeerGraphics.com**
4. **Take a course** – for example, the 3- or 4-day hands-on workshops that I teach at the University of Missouri and elsewhere (see the listing of upcoming workshops on my website <DrJohnRuss.com>).
5. **Just do it** - you can’t learn this stuff by reading about it. You need to work through images yourself - ideally your own images - and learn by trying them out just what the various processing and measurement tools do. And there is no greater driving force than needing to solve a specific problem.