# MicroImage – Image Analysis Software

What is Image Processing?

An image is a visual representation of an object or group of objects. Image processing manipulates information within an image to make it more useful. Digital image processing is a specific type of image processing performed with a computer.

Image Digitization

The digitization process divides an image into a horizontal grid, or array, of very small regions called "picture elements," or "pixels".

In the computer, the image is represented by this digital grid, or bitmap. Each pixel in the bitmap is identified by its position in the grid, as referenced by its row (x) and column (y) number. By convention, pixels are referenced from the upper-left position of the bitmap, which is considered position 0,0 (row 0, column 0).
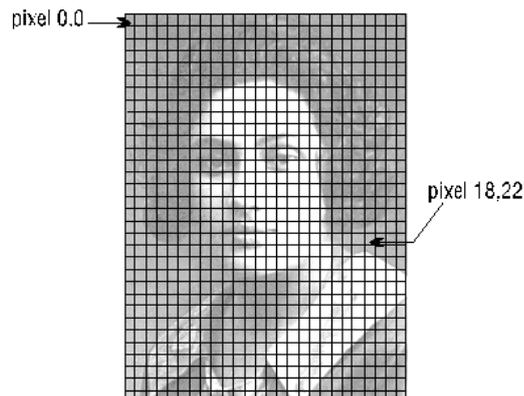
pixel bitmap

Note - For illustrative purposes, the pixels in the drawing above are shown much larger than their actual size. A pixel usually represents a very small region within an image, often 1/300th of an inch square, or less.

When a source image, such as a photograph, is digitized, it is examined in grid fashion. That is, each pixel in the image is individually sampled, and its brightness is measured and quantified. This measurement results in a value for the pixel, usually an integer, which represents the brightness or darkness of the image at that point. This value is stored in the corresponding pixel of the computer's image bitmap.

When the image is digitized, the width and height of the array are chosen and fixed. Together, the bitmap's pixel width and height are known as its spatial resolution.

Pixel Depth

Depending upon the capability of the measuring hardware and the complexity of the image, anywhere from 1 to 32 bits might be used to store each pixel value.

Pixel values for line art images, which contain only black and white information, can be easily represented by a single bit: 0 = black, 1 = white.

However, a photographic-like image contains much more information. It takes 24 bits to represent all the possible colors that might occur in a true color image. Given 24 bits, over 16 million colors, far more than the human eye can differentiate, can be represented.

an example of a simple line drawing that can easily be represented with a single bit per pixel

The number of bits used to represent the pixel values in an image is referred to as its pixel depth, or bits-per-pixel (BPP). The number of bits per pixel used to represent each pixel value determines the image's class.

Image Class

While the bit depth (BPP) tells us how many unique colors an image can possess, it does not tell us what colors are actually contained within the image. Color interpretation is determined by bit depth and one of several conventions, which Micro Image refers to as Image Class. The following classes are supported by Micro Image:

- Gray Scale 8
- Gray Scale 12
- Gray Scale 16
- Floating Point (Gray Scale 32)
- RGB 24 (True Color)
- RGB 36
- RGB 48
- Palette

Bilevel

A Bilevel image stores image data with 1 bit-per-pixel (1 BPP). Each pixel is interpreted as either completely black (0) or completely white (1). Bilevel images are commonly referred to as either "Black and White" images, "Line Art" or "Halftones."

Gray Scale

Gray Scale pixel values represent a level of grayness or brightness, ranging from completely black to completely white. This class is sometimes referred to as "monochrome."

In an 8-bit Gray Scale image, a pixel with a value of 0 is completely black, and a pixel with a value of 255 is completely white. A value of 127 represents a gray color exactly halfway between black and white (medium-gray), and a pixel value of 64 has a gray color halfway between medium-gray and black.

Although Gray Scale images with bit depths of 2, 4, 6, 12, 16 and 32 exist, 8 BPP Gray Scale images are the most common. This is for two reasons: 1) its 1-byte-per-pixel size makes it easy to manipulate with a computer, and 2) it can faithfully represent any gray scale image because it provides 256 distinct levels of gray (the human eye can distinguish less than 200 gray levels).

Micro Image supports the following gray scale bit depths:

NAME	BPP	DESCRIPTION
Gray Scale	8	The most common gray scale format in use. Used by most popular monochrome image formats (e.g., TIFF, PCX).Intensity values are represented with 8-bit integers. Provides 256 (0 - 255) levels of gray.
Gray Scale 12	12	A gray scale format generated by many specialized imaging systems. Intensity values are represented with 12-bit integers. Provides 4096 (0 - 4095) levels of gray.
Gray Scale 16	16	A gray scale format generated by many specialized imaging systems. Intensity values are represented with 16-bit integers. Provides 65,536 (0 - 65,535) levels of gray.
Floating Point	32	A gray scale format that is not native to any device or image format, but is useful for certain arithmetic and filtering operations. Intensity values are represented with 32-bit floating point values. Provides a virtually unlimited number of gray levels.

The Gray Scale 12, Gray Scale 16, and Floating Point classes are used for specialized operations and applications. Gray Scale 12 and Gray Scale 16 images are often generated by specialized imaging equipment such as infrared cameras and medical imaging devices.

RGB

The RGB image class uses the most straightforward way of representing color images. RGB stands for "Red, Green and Blue," the three primary colors of light. From the development of color photography and color television we have learned that any color can be represented as a mixture of varying levels of pure red, green and blue light. RGB-24 is referred to as True Color.

In a True Color bitmap, each pixel contains a 24-bit value, called an RGB "triplet" or "chunk." This RGB-triple is made up of three separate 8-bit samples. Each sample represents the level of brightness of its respective color channel: Red, Green or Blue.

These brightness values represent levels within a 256-level scale, just as they do in a Gray Scale image. The first sample is a level of Red, ranging from 0 (black) to 255 (brightest red).

The second sample is interpreted as a level of green, and the third sample is the level of blue. Equal levels of Red, Green and Blue always generate a level of gray.

Micro Image version 4.0 supports 36- and 48-bit color images. The storage for the classes is similar to the method used to store the 24-bit images: triplets of 16-bit words (16-bit red, followed by 16-bit green, and 16-bit blue, followed by the triplet for the next pixel).

The two classes are different only in the maximum range for intensity-related values (4095 for RGB-36 versus 65535 for RGB-48). Although Micro Image supports True Color 36 and True Color 48 images for analysis purposes, most popular file formats do not support these image classes.

Palette

The Palette image class uses 8 bits-per-pixel to store color information. Palette format is a convenient and efficient way to store images that have fewer than 256 colors. It requires far less storage space than encoding an image in True Color (RGB-triple) form.

Unlike all other image classes, the pixel value in a Palette image does not represent a brightness value. Instead, the value within the pixel is an index (pointer) to an entry in the image's "palette." The palette, which is stored with the image, is a 256-entry table of RGB values. Each entry in the table contains the 24-bit RGB value for that particular index. In a Palette image, you could change the color of all pixels with a certain value, say 10, by simply redefining the contents of entry 10 in the palette.

It is important to understand that the values in a Palette image have no significance in terms of intensity; i.e., you cannot assume that a pixel value of 0 means black, a value of 255 means white, or that low values are dark and high values are bright. Because of this, Palette images should not be used for intensity-driven calculations, such as filtering, or intensity analysis operations such as histogram and line analysis. If you want to perform such operations on a Palette image, you should first convert it to a continuous-tone image class such as True Color (RGB-24) or Gray Scale.

Modify the Intensity Index

One way to enhance an image is to change the way intensity values are interpreted. For example, if your image was very dark overall, you could boost all the values by a certain amount. You might boost all values by 20 points, or flatten a range of intensities to a single value (e.g., set all intensities from 75 through 150 to 127).

The following intensity manipulation tools are provided by Micro Image.

Brightness

Brightness is a term used to describe the overall amount of light in an image.

When brightness is increased, you increase the value of every pixel in the image, moving each pixel closer to 255, or white. When brightness is decreased, you reduce the value in each pixel, moving it closer to 0, or black.

Contrast

Contrast is a term used to denote the degree of difference between the brightest and darkest components in an image.

An image has poor contrast if it contains only harsh black and white transitions, or contains pixel values within a narrow range (an image whose values ranged from 100 to 140 would have poor contrast). An image has good contrast if it is composed of a wide range of brightness values from black to white. The amount of the intensity scale used by an image is called its "dynamic range." An image with good contrast will have good dynamic range.

During a contrast operation, each pixel value is scaled by a contrast value, which serves to redistribute the intensities over a wider or narrower range. Increasing the contrast spreads the pixel values across a wider range, while decreasing contrast squeezes the values into a narrower range.

Gamma Correction

Gamma correction is a specialized form of contrast enhancement that is designed to enhance contrast in the very dark or very light areas of an image. It does this by changing the midtone values, particularly those at the low end, without affecting the highlight (255) and shadow (0) points.

Gamma correction can be used to improve the appearance of an image, or to compensate for differences in the way different input and output devices respond to an image.

The Gamma control modifies an image by applying standard, nonlinear gamma curves to the intensity scale. A gamma value of 1 is equivalent to the identity curve, which has no effect on the image. An increase in the gamma value (setting it to a value greater than 1) will generally lighten an image and increase the contrast in its darker areas. A decrease in the gamma value (setting it to a value less than 1) will generally darken the image and emphasize contrast in the lighter areas. The figure below illustrates the effect of the gamma curves on pixel values 0 through 255.
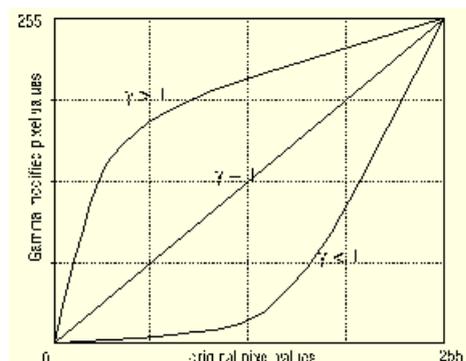

Gamma Correction Curve

Histograms

Histograms measure and illustrate in graph form, the brightness and contrast characteristics of an image. Histogram data can be created and viewed for data gathering and analytical purposes, or can be manipulated for image enhancement purposes.

As you can see in the example below, the X-axis in a histogram represents the intensity scale (0 to 255 in this example), and the Y-axis measures the number of pixels in the image possessing that value.
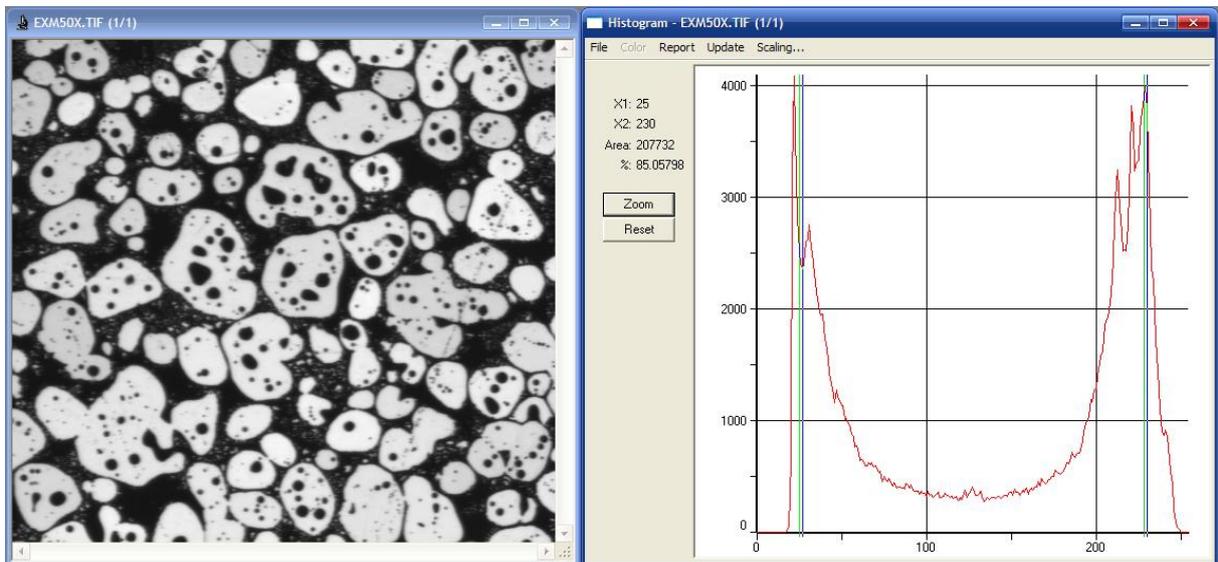
When you are working with Gray Scale 8 images, the X-axis represents gray values 0 through 255. When working with True Color images, you can choose to measure either the image's combined luminosity or its separate color channels (e.g., Red or Green or Blue, Hue or Saturation or Intensity...).

A histogram will show you what kind of brightness/contrast deficiencies exist in an image. Images with low contrast will have histograms that are clustered around a very narrow portion of the color range. The position of the cluster will indicate whether the image is too dark, too light, or simply too gray.

Brightness, contrast and gamma (BCG Controls) adjustments modify the shape of a histogram. Contrast operations affect the width of a histogram; compressing it when it is decreased, and stretching it when it is increased.

Intensity Analysis

Intensity analysis operations let you collect data from your image based upon the intensity values it contains. Micro Image provides the following intensity analysis tools:

- Histogram Analysis
- Line Profile Analysis
- Bitmap Analysis.

Histogram analysis lets you create a histogram of an image or AOI. Line Profile analysis lets you plot the intensity values along a given line. Bitmap analysis allows you to display the values of individual pixels in a bitmapped image.

Although the analysis tools actually measure the intensity levels within your image, you can calibrate Micro Image to express these values in units relevant to your application. For example, by setting Micro Image's Std. Optical Density option on the Intensity Calibration

dialog box, the intensity scale can be calibrated to standard optical density values used by most transmitted light experiments. You could also calibrate the intensity scale to indicate temperature, or protein content, for example.

Histogram Analysis

Histograms measure and illustrate in graph form, the intensity characteristics of an image. They can be used for data gathering purposes to measure the area associated with a specific intensity value.

In a histogram, the X-axis represents the intensity scale (as defined by the current intensity calibration), and the Y-axis measures the spatial value (as defined by the current spatial calibration). A satellite image that had been calibrated to express intensity as temperature, would show temperature along the X-axis, and area (perhaps in square miles) along the Y-axis

Line Profile Analysis

Line profile analysis allows you to collect the actual index values along a line that you define. A profile plot shows the pixel positions of the line along the X-axis, and, on the Y-axis, measures the index value for each position along the line.

In a line profile, the X-axis represents the spatial scale (as defined by the spatial calibration), and the Y-axis measures the intensity value (as defined by the current intensity calibration). A DNA gel that had been calibrated to express intensity as density, would show density along the Y-axis.

Bitmap Analysis

Bitmap Analysis Bitmap Analysis command on the Measure menu is used to view the pixel values of the active window (or AOI) in numeric format. These values can be saved to an ASCII file for later use with an external program, or copied to the Clipboard and pasted into another application (a 3D plotting package, for example).

Measuring and Counting

One of Micro Image's most powerful capabilities is its ability to let you perform spatial measurements upon your image, manually or automatically. There are two basic ways to perform measurements within Micro Image:

- **Manually measuring single objects**. Using the Measurements command in the Measure menu, you can measure the length of lines or polylines that you define, the area of polygons that you define, and the angles of arcs that you define. The Measurements command also lets you automatically trace and measure the edge of an object or feature in your image. You can measure distances between any two features, and perform tolerance testing.

- **Automatically counting and measuring multiple objects**. Using the tools on the Measure menu, you can collect multiple measurements of multiple objects within a single image. If your objective is to count the number of cells in a sample, and measure the area, roundness or perimeter of these cells, this is the tool to use. Once the objects have been counted and measured, you can use the Count/Size window's Measure menu options to automatically sort and classify the objects by any of the measured characteristics. You can also visualize the classified data by plotting it on a scattergram, or pseudo-coloring the counted objects by class.

Calibration

Measurement operations are performed in terms of image pixel positions, e.g., the length of a measurement is determined by the number of pixels along the line, the area of an outlined object is determined by the number of pixels within the outline, and so forth.

Micro Image's pixel-level measurements can be scaled to fit any coordinate system. This allows you to obtain measurements which are reported in terms meaningful to your application. For example, you can calibrate the measurement scale so that one pixel is equal to one foot, or so that five pixels are equal to one foot (or inch, or mile, or micron). Fractional values are allowed. Micro Image will express your measurements in terms of that unit. Additionally, if your image contains a measurable object of the unit length, you can calibrate your scale directly from that object.

Frequency Filtering

Image interference that presents itself as a regular pattern across an image can be especially difficult to remove using a spatial approach. The best way to eliminate such periodic or coherent noise is by converting the image to a set of frequencies, and editing out the frequencies causing the problem.

Converting an image to its frequency domain is called a Fourier Transform (named for its inventor, Joseph Fourier), and is performed in Micro Image using the FFT (Fast Fourier Transform) command on the Process menu.