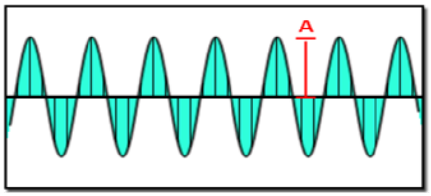
MICROSCOPY GLOSSARY

Absorption: *Blocking or reduction of one or more wavelengths of light. Used to block certain colours of light – colour filters, or all colours of light,* ***Neutral Density (ND)*** *filters.*

Airy Disk:

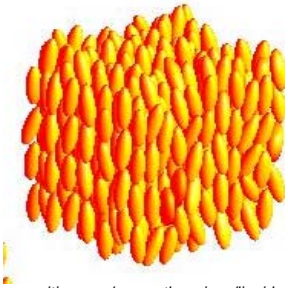
Amplitude: *Height of peaks or valleys in the light waves in the electromagnetic field.*



http://micro.magnet.fsu.edu/primer/lightandcolor/electromagintro.html

Amplitude Objects: *Objects that change the amplitude of different wavelengths of light as it is transmitted through the object. For example, samples that are stained, e.g. H&E slides.*

Anisotropic Material: *Optical properties are dependent on direction (ordered crystal).*



http://www.geocities.com/prasanth_p_jose/liquid_crystal.jpg

Aperture Diaphragm: *is built into the condenser at the front focal plane and influences image quality.*

Binary Image: *Digital image with two possible values for each pixel either 0 or 1.*

http://en.wikipedia.org/wiki/Binary_image

Birefringence (double refraction): *Decomposition of a ray of light into two rays (ordinary and extraordinary rays) when it passes through certain types of materials.*

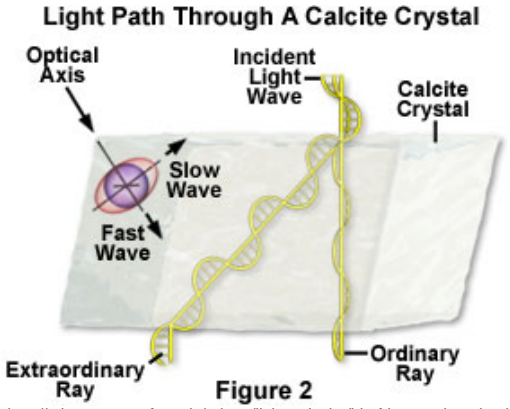
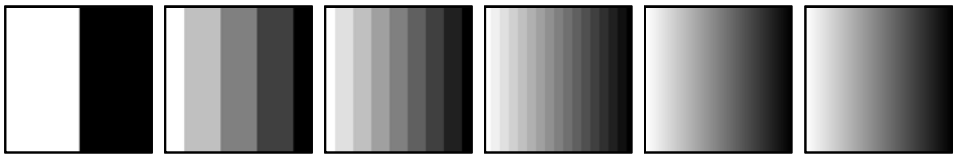


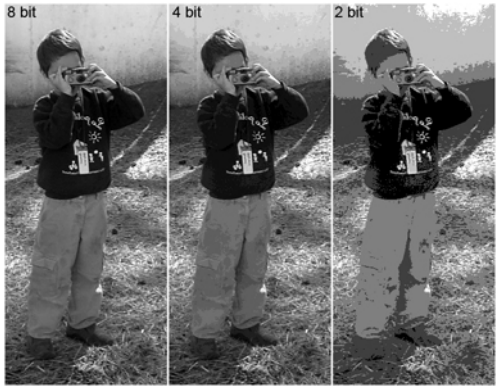
Figure 2

http://micro.magnet.fsu.edu/primer/lightandcolor/birefringenceintro.html

Bit depth: *Power of two which gives the number of possible intensity levels within an image* $2^{(\text{bit depth})}$ *(e.g.* $2^8 = 256$*). A higher bit depth give more dynamic range within an image making a system more sensitive with the ability to distinguish subtle variations in intensities within an image.*



1 bit	2 bits	3 bits	4 bits	6 bits	8 bits
2 levels	4 levels	8 levels	16 levels	64 levels	256 levels



12 bit: *4096 levels (0-4095)*
16 bit: *65536 levels (0-65535)*

Binning: *Adding together data from multiple pixels within a pixel array to increase signal to noise and camera acquisition time while also decreasing image resolution and digital image size. Binning can also be performed post acquisition in image processing software.*

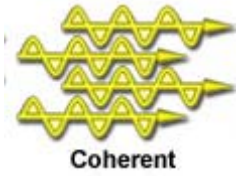
BMP: *Bitmap file format. Uncompressed map of the bit information for each pixel in an image.*

Brightness: *Intensity level for displaying pixels in an image. Increasing the brightness will increase the amount of light used to display pixels of every valve.*

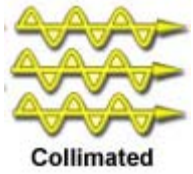
Clipping: *When images are saturated or the offset (background) is set too black so that the "true" intensity data is cut off.*

CMYK: *Images made up of a composite of cyan, magenta, yellow, and key (black) in order to generate colour and depth of colour. The black is usually used for printing to avoid using excess amounts of the CMY to print black.*

Coherent Light: *Waves of a given wavelength that have the same phase relationship.*



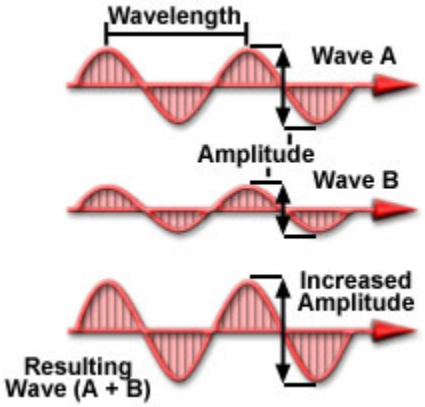
Collimated: *Waves having the same path of propagation, not convergent or divergent, but not necessarily the same wave-length, phase or polarization.*



Conjugate Planes: *A set of planes in an image forming system that are optically linked and whose images are superimposed at the detector/eye.*

Condenser: *Provides even illumination across the field of view for a wide range of magnifications.*

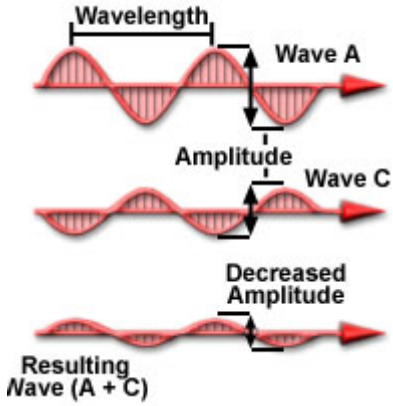
Constructive Interference: *Addition of two or more waves that are in phase resulting in a wave that has a higher amplitude.*



http://micro.magnet.fsu.edu/primer/lightandcolor/interferenceintro.html

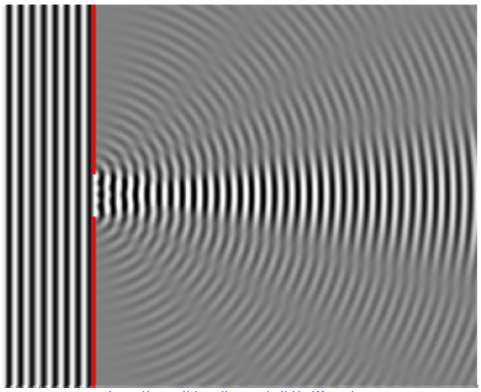
Contrast: *A measure of the different between bright and dark pixel intensities. The higher the contrast the larger the difference in brightness between dark and bright pixels. It can be expressed as a ratio of (difference in brightness)/(average brightness) between adjacent regions in the image.*

Destructive Interference: *Addition of two or more waves that are not in phase resulting in a wave that has a lower amplitude.*



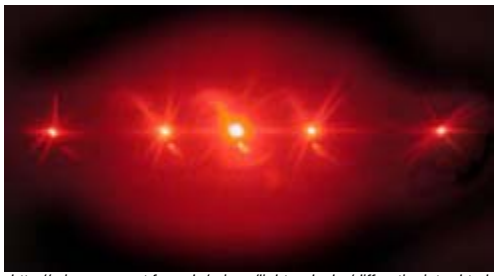
http://micro.magnet.fsu.edu/primer/lightandcolor/interferenceintro.html

Diffraction: *The bending of light as it passes a corner, an edge, or through an opening or slit that is physically the same size or smaller than the wavelength of the light.*



http://en.wikipedia.org/wiki/Diffraction

Diffraction Pattern: *Light pattern produced due to the constructive and destructive interference of bending light beams after passing through a slit or aperture physically the same size or smaller than the wavelength of the light.*



http://micro.magnet.fsu.edu/primer/lightandcolor/diffractionintro.html

Diffuser: *Used to spread out or scatter lamp light to ensure a uniform field of illumination.*

Dilation: *Geometric convolution that enlarges the boundaries of objects. Frequently used for image segmentation. Below dark area is dilated by convolution.*

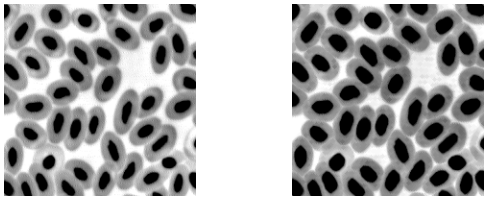
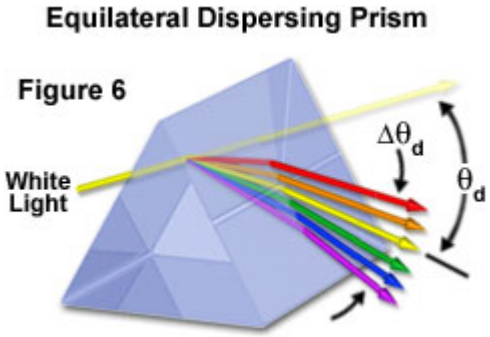


Image from Molecular Devices

Dispersion: *Wavelength dependent deviation in the angle of light refraction.*



http://micro.magnet.fsu.edu/primer/lightandcolor/prismsandbeamsplitters.html

Divergent Light: *Waves that propagate along different paths diverging from one another.*

DPI: *Digital image or printer resolution in* ***D****ots* ***P****er* ***I****nch*

MetaMorph

20 dpi ***72 dpi*** ***180 dpi***

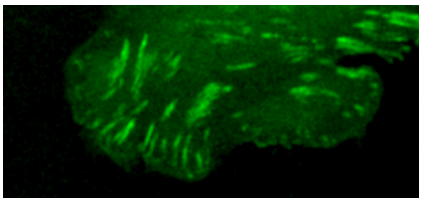
Take Care: Programs like Photoshop can introduce image artifacts

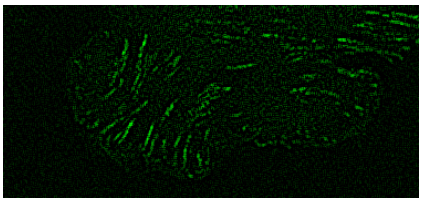
20 dpi ***72 dpi*** ***180 dpi***

Dynamic Data Exchange (DDE): ***D****ynamic* ***D****ata* ***E****xchange, is a protocol for exchanging data between two programs, such as between the image analysis system and a spreadsheet.*

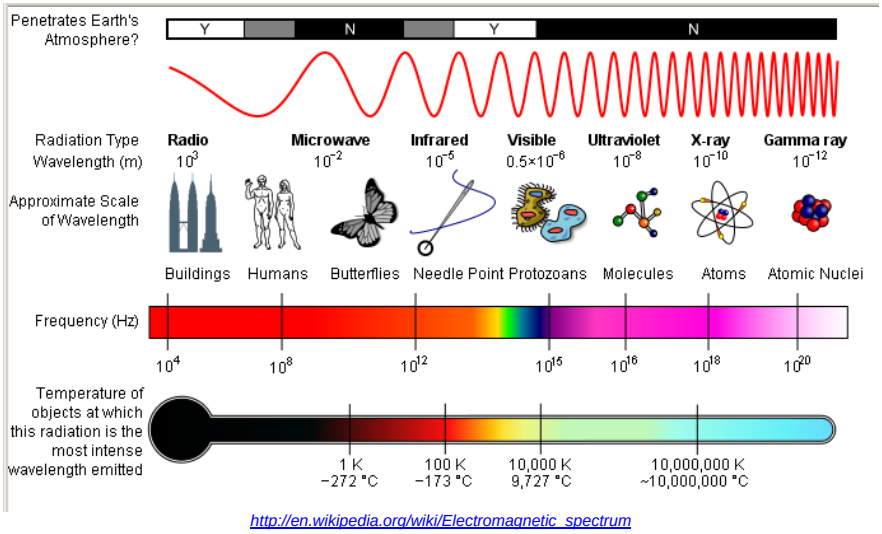
Dynamic Range: *The highest intensity measurement possible by the sensor divided by the smallest possible signal or the noise level ("noise floor") of the sensor.*

Edge Detect: *Detects areas within a digital image where the intensity of adjacent pixels shifts abruptly.*





Electromagnetic Spectrum:



http://en.wikipedia.org/wiki/Electromagnetic_spectrum

Erosion: *A geometric convolution that shrinks the boundaries of objects. Frequently used for image segmentation. Below dark area is eroded by convolution.*

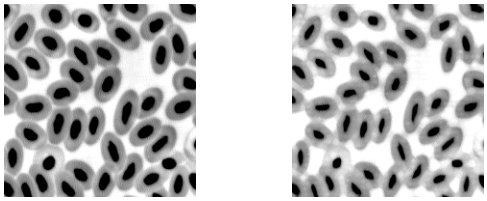


Image from Molecular Devices

Eyepiece Lens (ocular lens): ***L****ens on a microscope that is closest to the eye. Magnifies the real image at the back focal plane and projects it on the retina of the observer.*

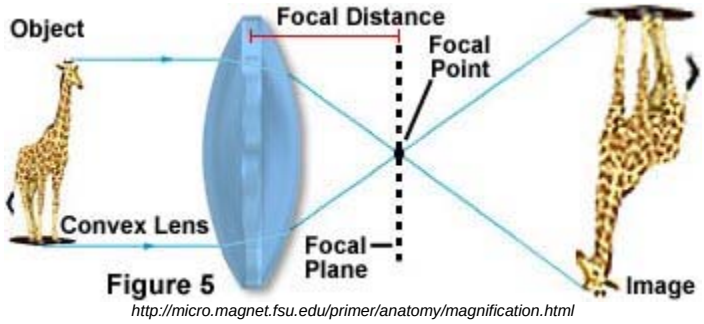
Fast Fourier Transform (FFT): *A command used to remove or enhance patterns of periodic "noise" in an image.*

Field Diaphragm: *Used to centre the condenser and to select only the central portion of light from the lamp or to collimate the light.*

Focal Distance (also called Focal Length): *Distance between the centre of the convex lens and the* ***focal plane****.*

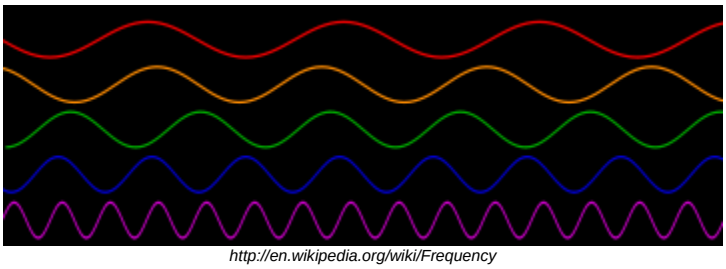
Focal Point: *Point behind a convex lens where light from an object is brought into focus.*

Focal Plane: *Plane in which the* ***focal point*** *lies.*



http://micro.magnet.fsu.edu/primer/anatomy/magnification.html

Frequency (n): *The number of sinusoidal cycles (complete wavelengths) that pass a given point per second (n = c/λ), where c = speed of light and λ = wavelength), usually expressed in quantities of hertz (Hz) or cycles per second (cps) (1 Hz=1 cps). Long wavelength (red) is low frequency and short wavelength (violet) is high frequency.*



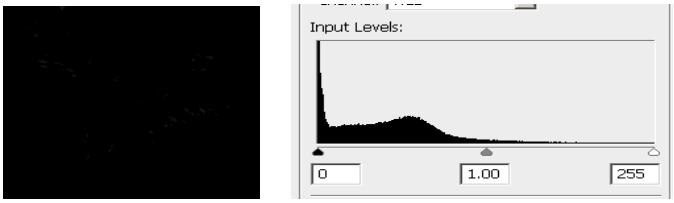
http://en.wikipedia.org/wiki/Frequency

Gamma Factor: *Non-linear display of intensity data to increase contrast in either dark or light regions of images.*

GIF: G*raphics* ***I****nterchange* ***F****ormat, an 8-bit bitmap image format introduced by CompuServe in 1987. Commonly used on the web but not high enough dynamic range for colour printing.*

Grayscale: *pixels contain only intensity information (variable levels of gray between black (minimum) and white (maximum)) and no colour information. The term is synonymous with black and white or* ***monochromatic*** *images.*

Histogram: *For digital images the histogram is typically a plot of intensity versus number of pixels. Each bar has a height proportional to the number of pixels measuring that intensity value.*



Histogram Stretch: *Spreading intensity data over whole dynamic range of image to increase contrast.*

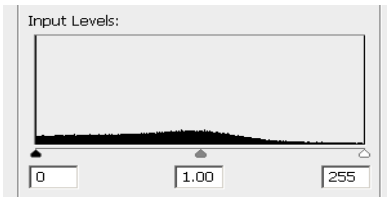


Image: *Rectangular array of pixels containing intensity and sometimes colour information for given positions within a sample.*

Image Acquistion Software: *Software package with all the necessary drivers to control instrumentation (cameras, shutters, filter wheels, moving microscope parts) for acquiring images.*

Image Analysis: *Any operation that generates numerical data from an image.*

Image Headers: *Information within a digital image file containing such information as magnification, pixel size, exposure time. Headers differ dependent on image type and software platform the image was collected or saved with.*

Image Processing: *The use of processing functions to improve the visual display of an image.*

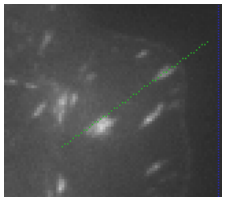
Interference: *The addition of two or more waves resulting in a new wave pattern.*

Jpg: *Compressed image format (10-20X) created by the **J**oint **P**hotographic **E**xperts **G**roup in 1992. Works best on photographs and paintings of realistic scenes with smooth variations of tone and color. is not as well suited for line drawings and other textual or iconic graphics, where the sharp contrasts between adjacent pixels cause noticeable artifacts.*

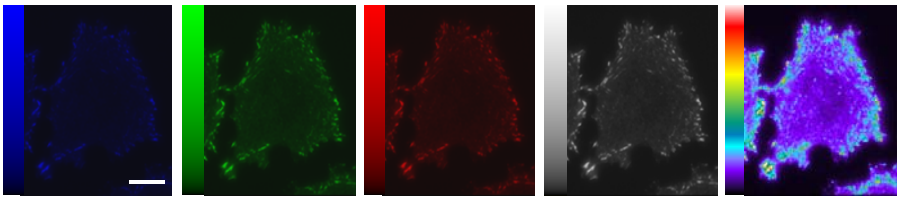
Köhler Illumination: *First described in 1893 by August Köhler. Uses collector lenses to focus the image of the lamp filament at the back focal plane of the objective to create even illumination over the specimen field of view without seeing the filament image.*

Kernel: *A matrix of numbers used for processing an image. The kernel is passed over each pixel in the image. The pixel under the kernel is replaced by the sum of each element in the kernel times the intensity value of the pixel in the same relative position to the pixel being replaced.*

Kymograph: *A display of the time versus intensity along a line within images from a time series.*



Look-up table (LUT): *A theoretical table of values that specifies the conversion of the grayscale value of each pixel in an image into another gray value or color for image display.*



Lossless: *Refers to a form of image compression in which the original image information is perfectly preserved. An example of a lossless image format is GIF.*

Lossy: *Refers to a form of image compression in which some of the original image information is removed to make the compressed file smaller. This information is regenerated during playback so that the image "appears" unaltered. An example of a lossy image format is JPEG. These images should NOT be used for image analysis.*

Low pass filter: *A filter that passes low frequency signals but attenuates high frequency signals. Usually used to "smooth" or "blur" images to remove noise.*

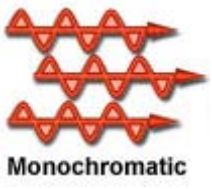
Isotropic Material: *same optical properties in all directions, homogeneous material.*

http://www.geocities.com/prasanth_p_jose/liquid_crystal.jpg

Magnify: *Enlarging something only in appearance, not in physical size.*

Mask: *A binary image of a pattern used to control the retention or elimination of portions of an image by performing a logical operation between the image and the mask.*

Monochromatic: *Light of one wavelength or colour.*



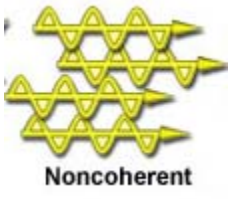
Morphometry: *The measure of shape. It is used to find count, differentiate, and quantify object within an image.*

Multi-Dimensional Imaging: *Image collection over more than three dimensions. Commonly this means multiple stage positions, multiple wavelengths, multiple Z-positions, and/or multiple time points.*

Metadata: *Information about processes that have been applied to an image (filters, background subtracting) that can be carried along with modified images throughout the analysis chain of events.*

Noise: *Signal sensed by the detector even in the absence of light. Sometimes call* ***"dark current"****. Increases with more sensitive detectors (e.g. higher PMT gain) and temperature.*

Non-Coherent Light: *Light source that displays a variety of phase relations for different wavelengths.*



Non-polarized Light: *Light waves going in all directions.*

Objective Lens: *Lens closest to the object being viewed. Collects light transmitted through or reflected from the object and brings it into focus creating an image.*

Offline Software: *Software package purely for image processing and analysis, NOT image acquisition.*

Optical Density:

Polarized Light (Linear): *Isolation or selection of light from one direction or electromagnetic plane.*

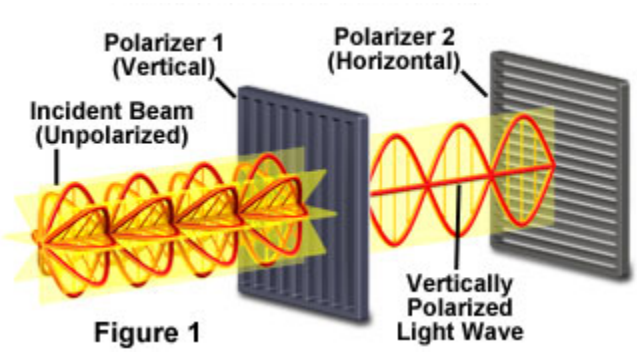
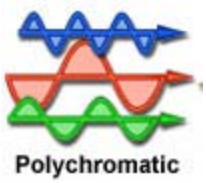


Figure 1

http://micro.magnet.fsu.edu/primer/lightandcolor/polarizedlightintro.html

Polychromatic: *Light made up of many wavelengths or colours.*



Phase Objects: *transparent objects that do not absorb light, but instead, produce a phase change of light passing through them.*

Pixel Array: *x, y array of pixel intensity data that taken together composed a digital image*

Pixel: *Picture element, the fundamental spatial unit that makes up an image defined by the x, y and z spatial locations as well as intensity.*

PNG: *A bitmap image format that employs lossless data compression.*

Point Spread Function (PSF):

RAW: *Raw image data as it comes off the sensor with no compression.*

Reflection: *Bouncing of light off a reflective surface with the absence of* ***absorption****.*

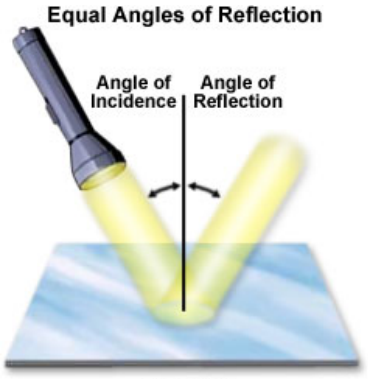


Figure 2

http://micro.magnet.fsu.edu/primer/lightandcolor/reflectionintro.html

Refraction: *Bending of light due to a change in speed as it passes, at an angle, from one material to another. These two materials must have different* ***optical densities*** *for refraction to occur.*

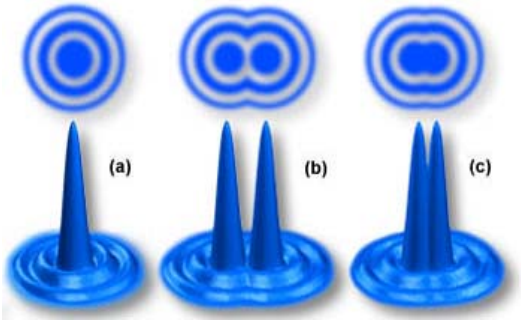
Refractive Index (RI): *Measure of how much the speed of light is reduced in a medium relative to the speed in a vacuum. The RI in a vacuum is set to 1.0 and all other measures are made relative to this.*

Material	*Refractive Index*
Air	*1.0003*
Water	*1.333*
Glycerin	*1.473*
Immersion Oil	*1.515*
Glass (Crown)	*1.520*
Glass (Flint)	*1.656*
Zircon	*1.920*
Diamond	*2.417*
Lead Sulfide	*3.910*

http://micro.magnet.fsu.edu/primer/lightandcolor/refractionintro.html

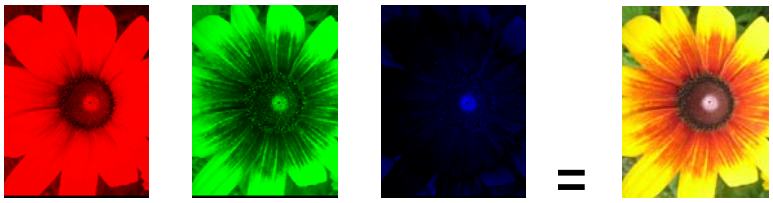
Registration: *The degree of accuracy with which one image is positioned into alignment with another.*

Resolution: *The smallest distance between two points on a specimen that can still be distinguished as two separate entities. Higher numerical aperture lenses have higher resolution. Shorter wavelengths of light give higher resolution.*



http://micro.magnet.fsu.edu/primer/anatomy/numaperture.html

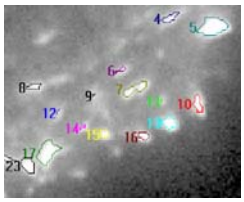
RGB - 24 bit colour: *composite of three 8 bit single colour images, 8 bit red (256 colours), 8 bit green (256 colours), 8 bit blue (256 colours) total of 256x256x256 = 16.8 million colours (true colour)*



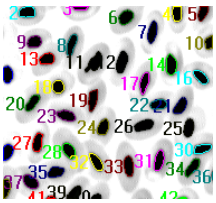
ROI: R*egion* ***O****f* ***I****nterest.*

Scatter: *Combination of many light-matter interactions sending light off in a variety of directions.*

Segmentation: *Partitioning an image into multiple regions. Pixels are assigned as part of an object or part of the background and to be excluded from analysis. This process generally uses the intensity of the pixel and thresholding.*



Focal adhesions



Frog egg nuclei

Shading correction: *A process which reduces the amount of shading in an image. This typically involves dividing the acquired image, pixel-by-pixel, by a "shading image" that is taken with the same settings as the data images but in the absence of a specimen.*

Shape Factor: *Measure of how round a 2D object is with 1 being a perfect circle.*

Sharpening: *Optical sharpness is defined by the quality of the microscope lenses and the imaging sensor. Software sharpness is a digital process for making edges within an image more clear to the human eye. Sharpening does not actually improve image quality.*

Signal-to-noise ratio (SNR, S/N): *ratio of the level of signal to the level of noise in the image. Ratio of the mean pixel intensity to the standard deviation in the intensity. Higher ratios give better quality images.*

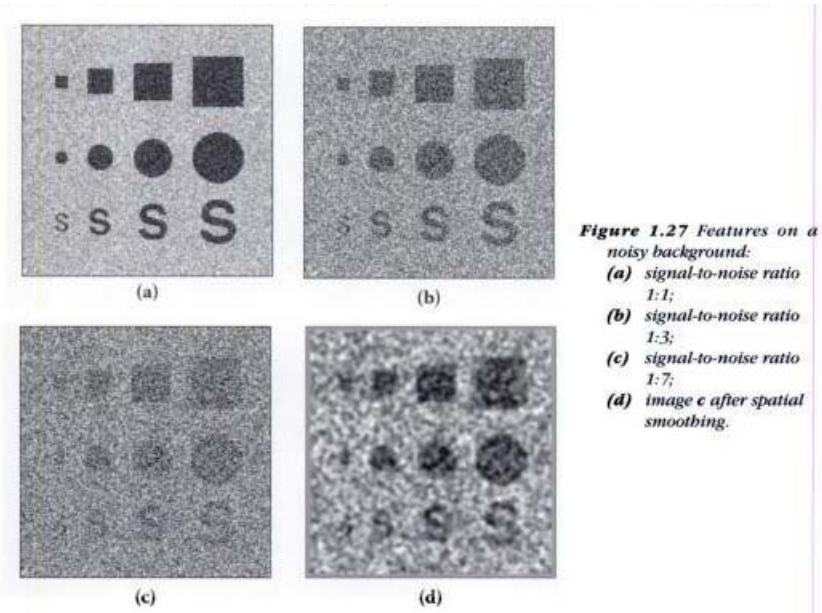


Figure 1.27 *Features on a noisy background:*
(a) signal-to-noise ratio 1:1;
(b) signal-to-noise ratio 1:3;
(c) signal-to-noise ratio 1:7;
(d) image c after spatial smoothing.

John C. Russ, The Image Processing Handbook, Fifth Edition

Sphericity: *Measure of how round an object is. Set to one for a sphere.*

Threshold: *Set intensity at a given value to distinguish objects brighter or darken then this value.*

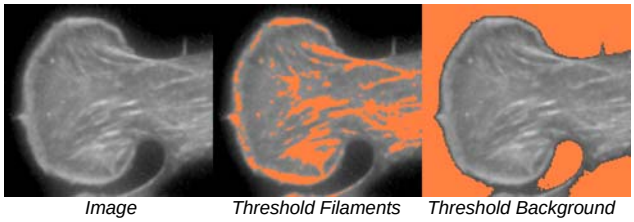
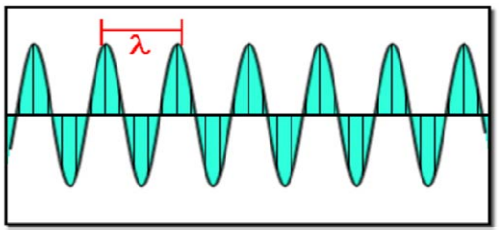


Image *Threshold Filaments* *Threshold Background*

TIFF (TIF): T*agged* ***I****mage* ***F****ile* ***F****ormat. Highly compatible between software platforms and information is not lost on compression but image files can be large.*

Virtual Image: *An image in which the outgoing rays from a point on the object never actually intersect at a point. The virtual image appears at a distance from the actual object.*

Wavelength (λ): *Distance between two successive peaks (or valleys) in the light wave electromagnetic field.*



http://micro.magnet.fsu.edu/primer/lightandcolor/electromagintro.html