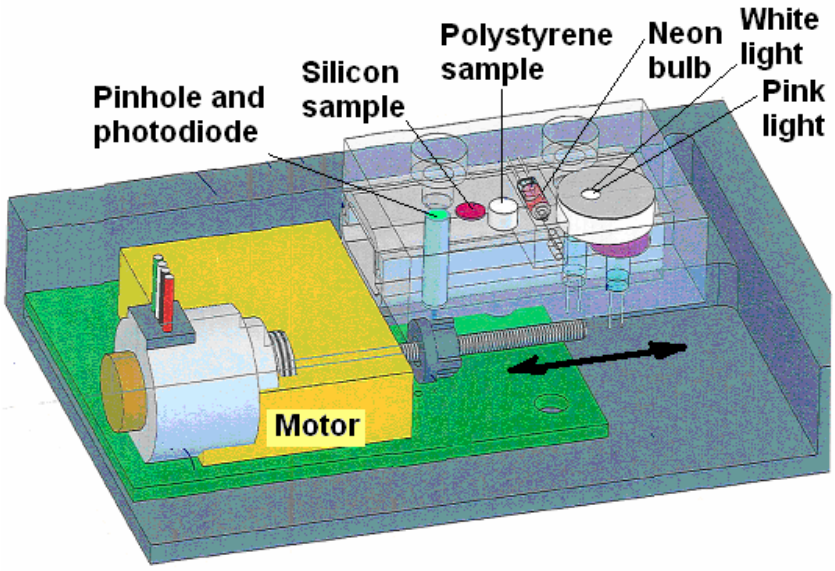
DXR Glossary

Brightfield Microscope illumination where the incident light travels down the center of the objective and collected light travels back along the outer edge of the objective. Visually the area around the sample will appear very bright and the sample will appear dark. This technique does not typically provide high contrast differentiation, so often sample with little contrast difference will be hard to see. However, this technique does provide true color of the sample.

Calibration Because the DXR is a dispersive spectrometer it requires calibration of the x or wavelength axis. The DXR performs this calibration by using a neon bulb (which has known emission lines) and polystyrene (which has well known wavenumber peak locations). The neon bulb and polystyrene are located inside the customer alignment tool and are moved into position by the OMNIC software during the calibration routine. The calibration routine also performs a white light correction in order to eliminate the instrument response and correct relative band intensities. A white light source is located inside the align tool. A pink light is used for the white light correction for the 780 laser.

DXR Raman Microscope Customer Alignment Tool



CCD Charge Couple Device detector – a two dimensional array of pixels, each approximately 26 microns in size, used in dispersive Raman spectroscopy. The useful wavelength range cuts off around 1050 nm. These detectors offer very high sensitivity.

Confocal Microscopy A microscopy approach that allows non-destructive depth analysis of a sample by simply focusing the scope objective focal point to different depths in the sample. The technique involves placing a 25 micron aperture in the collected light path and then adjusting the z-axis of the scope stage until the focus is at the depth desired. Typically, it involves focusing on the surface of the sample, where the visual image is crisp, and then using the micron marks on the z-axis fine adjust knob to sequentially step deeper into the sample.

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Dark Current The residual background current of a CCD that accumulates even when there is no light falling on the CCD. This current is constant and is a function of the CCD. Cooling the CCD reduces this current, but does not completely eliminate it. A "Background" collect measures the dark current by collecting data under the conditions of the experiment, with the spectrograph shutter closed. The response is then subtracted from all subsequent data. A new background must be collected anytime the collection time or the aperture size is changed. The system automatically prompts for this.

Darkfield A contrast technique in which the incident light travels down the outer rim of the objective and the collected light travels back up the center of the objective. This causes the incident light to be reflected off of the sample at a very high angle that does not get collected by the objective, so only light that is scattered by the sample features is collected. Visually, the background is dark and the sample is light. This technique is very good for seeing samples of low contrast, but since scattered light is susceptible to diffraction, the colors are not true.

Depth-of-Field The vertical distance in the sample through which features are simultaneously in focus. As defined for confocal microscopy, this is similar to a resolution term, identifying the "thickness" of the sampled depth in the experiment. By using small apertures and high magnification objectives, confocal Raman provides information from different depths within the sample, without contributions from above or below the focal point. Depth-of-field refers to how thick the sampled region is in the z-axis. The thinner (or smaller the depth of field) the less the amount contaminated information that comes from above or below the desired sampling point.

Edge Filter Edge filters are designed to filter the Rayleigh scatter in Raman Spectroscopy. The only difference is that the edge filter is only designed to acquire close to the laser line on the Stokes side of the laser line, which is longer wavelengths (shorter wavenumbers). An edge filter cuts out all anti-stokes energy.

Fluorescence background Some samples emit photons at longer wavelengths when hit by visible light producing an elevated baseline which obscures the Raman spectral features. Fluorescence can be minimized by using a lower energy (longer wavelength) laser. Fluorescence may also be minimized by photo-bleaching.

Grating Dispersing element used in dispersive Raman spectroscopy, that spatially separates incoming light into the different wavelength components. The grating is composed of a series of parallel "lines" that set up interference patterns preferentially casting the individual wavelengths to different locations in the output plane. The resolution of the grating is a function of the number of lines/mm, with more giving higher spectral resolution. Gratings are also manufactured to have efficient throughput over specific wavelength ranges.

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Laser Energy Related to the frequency of the laser by the equation $E = h\nu$, where h is a constant. The energy of the laser is *completely independent* of laser power, and has a significant influence on whether fluorescence occurs from a given sample. The only way to change the laser energy is to switch to a different wavelength laser.

Laser Frequency(ν) Related to laser energy by the equation $E = h\nu$, where h is a constant. Also related to wavelength as $\lambda = 1/\nu$. The frequency of the laser is *completely independent* of laser power, and has a significant influence on whether fluorescence occurs from a given sample. The only way to change the laser frequency is to switch to a different wavelength laser.

Laser Power The flux density of output photons from the laser. This parameter determines how many photons the laser will emit of the specified wavelength. The power can be adjusted without affecting the laser energy or laser wavelength (frequency). The parameter often determines whether a sample will heat up or will be thermally destroyed, since a sample that absorbs specified laser wavelength strongly will heat up proportionally to the number of photons (power) that hit it at once. The intensity of the Raman scatter is also approximately linearly dependant on power, so it is recommended to use the lowest power setting possible when analyzing unknowns and then slowly increase the power until a spectrum of desired quality is obtained or until there is evidence of heating.

Laser Wavelength (λ) Related to frequency by the equation $\lambda = 1/\nu$ and also related to laser energy by the equation $E = h\nu$, where h is a constant. The wavelength of the laser is *completely independent* of laser power, and has a significant influence on whether fluorescence occurs from a given sample. The only way to change the laser wavelength is to switch to a different laser.

Long Working Distance (LWD) Objectives Microscope objectives that are designed with the focal point of the objective further from the objective lens while maintaining high magnification. Standard objectives, such as 50x and 100x have working distances of only a few millimeters, making it difficult to measure irregular samples and leaving the objective lens susceptible to vapors that may emit from a heated sample and settle on the objective surface. LWD objectives have much longer working distance, but to achieve this, the Numerical Aperture (NA) is lower, so the collection efficiency is not as high. LWD objectives are needed for analysis using well plates.

Numerical Aperture (NA) Defines the efficiency of the objective at collecting the light coming from the sample. The NA defines the largest angle off of normal (directly perpendicular to the flat surface of the sample) of light that the objective is able to collect. The higher the NA, the more efficient, however this usually comes at the expense of working distance or objective lens diameter.

Optical Density A measure of the efficiency of a filter to filter a given wavelength of light. The higher the optical density at a given wavelength, the more photons of that wavelength that can be collected. Typical OD values for the Rayleigh filters are between 6 and 10.

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Photo-bleaching A method used to minimize sample fluorescence caused by sample impurities. The sample is exposed to the Raman laser for several minutes in order to burn off the impurities and minimize the fluorescence which obscures the Raman spectral features.

Pixel The individual light sensitive elements on a CCD array. Each pixel can be considered an independent detector element.

Polymorphs Different crystalline forms of the same compound. This in an important distinction, particularly for pharmaceutical synthesis, since the different forms can affect dissolution, reactivity or other characteristics of drug delivery. The different forms are also recognized as different chemical entities that can be independently covered by patent.

Rayleigh Scatter The dominant scattering event when electrons in a molecule are excited to a virtual state by a laser. This scatter is exactly the same wavelength as the laser, therefore provides no vibrational information about the sample. It is 10^4 to 10^6 more intense than Stokes or Anti-Stokes scatter so must be efficiently filtered.

Read Noise The small amount of noise introduced each time the energy collected by a pixel is transferred to the A/D converter of the CCD device. This noise is mainly introduced when the charge in the shift registry is "read", not during pixel-to-pixel transfer or binning. The magnitude of this noise is very small, typically a few electrons, and only becomes an issue when very weak Raman signals are to be measured. To minimize read noise, it is necessary to bin pixels on chip.

Shot Noise Random statistical noise at the detector. There is a square root relationship between noise and useful signal, so increasing (or decreasing) the signal being measured has a smaller effect on the shot noise. In CCDs, there is shot noise associated with both the photon generated signal and the dark current, however the shot noise associated with the signal is usually dominant except when measuring extremely weak Raman signals.

Spatial Resolution In microscopy, the ability to differentiate a single feature from closely spaced background surrounding the sample. The higher the spatial resolution, the smaller the sample can be before it is indistinguishable from its surroundings.

Spectral Resolution The ability to distinguish two highly overlapped spectral peaks. The higher the spectral resolution, the more overlapped the two peaks can be and still be resolved. Typically, the degree of resolution is measured by the depth of the valley between the two peaks, with the deeper valley corresponding to higher resolution.

Stokes Scatter The low energy Raman shift that results from an electron residing in the ground vibrational level, being excited to a virtual state and then relaxing back to an upper vibrational level. This is the Raman energy that we collect with the DXR.

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White Light Correction The process of measuring the broadband radiation produced by a white light source with known emission properties. In the DXR, the light itself is mounted inside the customer alignment tool. This known emission is modeled and the resulting equation applied to collected spectra to remove instrument specific effects. These instrumental effects are associated with the optical components and detector response. This is only necessary if the data is to be compared with data from other spectrometers or in some quantitative analyses and library searching.