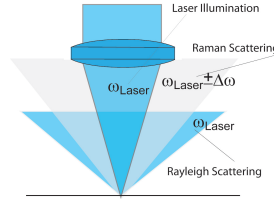
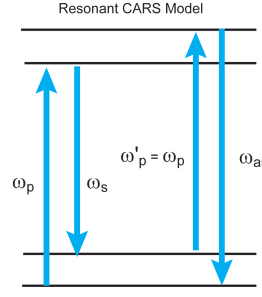
Raman and CARS Microscopy

Raman microscopy (also called chemical-imaging microscopy) is a technique based on Raman scattering, and it evaluates the vibrational properties of a sample. It is used to study minerals, crystals, plastics, and chemical reactions.



It uses a laser beam focused on a solid object. Most of the illuminating light is scattered, reflected, and transmitted (in various proportions) and, in principle, preserves the parameters of the illumination beam (the frequency is the same as the illumination). However, a small portion of light is subject to a shift in frequency. The difference between illumination and the scattering frequencies bears information about the molecular composition of the sample: $\omega_{Raman} = \omega_{Laser} \pm \Delta\omega$.

Raman scattering is very weak and demands high-powered sources and sensitive detectors, and it must be examined with spectroscopy techniques.



Coherent anti-stokes Raman scattering (CARS) microscopy overcomes the problem of weak signals associated with Raman imaging. It uses a pump laser and a tunable Stokes laser to stimulate a sample with a four-wave mixing process. The fields of both lasers [pump field $E(\omega_p)$, Stokes field $E(\omega_s)$, and probe field $E'(\omega'_p)$] interact with the sample and produce an anti-Stokes field [$E(\omega_{as})$] with frequency ω_{as}, so $\omega_{as} = 2\omega_p - \omega_s$.

CARS can work as a resonant process providing a signal if the vibrational structure of the sample matches its frequency. Therefore, it has to provide phase matching so that l_c (the coherence length) is greater than $\pi/|\Delta\mathbf{k}| = \pi/|\mathbf{k}_{as} - (2\mathbf{k}_p - \mathbf{k}_s)|$. $\Delta\mathbf{k}$ is a phase mismatch. CARS is orders of magnitude stronger than Raman scattering, it does not require any external contrast, it provides good sectioning ability, and it can be configured into forward-transmission and reflectance modes.

SPIM

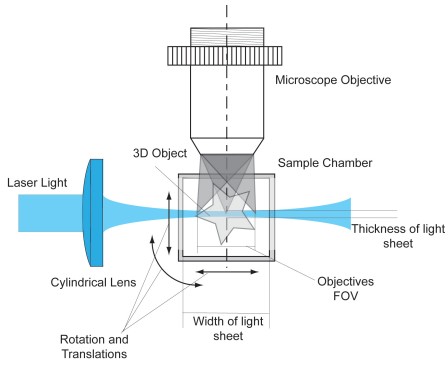
Selective plane illumination microscopy (SPIM) is dedicated to the high-resolution imaging of large 3D samples. It is based on three principles:

- The sample is illuminated with a **light sheet**, which is obtained with cylindrical optics. The light sheet is a beam focused for one direction and collimated for another direction. This way, the thin and wide light sheet can pass through the object of interest (see figure below).
- The sample is imaged in the direction perpendicular to the illumination.
- The sample is rotated around its axis of gravity and linearly translated into axial and lateral directions.

Data is recorded on a CCD camera for multiple object orientations and can be reconstructed into single 3D distribution. Both scattered and fluorescent light can be used for imaging.

The maximum resolution of the technique is limited by the longitudinal resolution of the optical system (for a high numerical aperture, objectives can obtain micron-level values). The maximum volume imaged is limited by the working distance of a microscope and can be as small as tens of microns or exceed several millimeters. The object is placed in an air, oil, or water chamber.

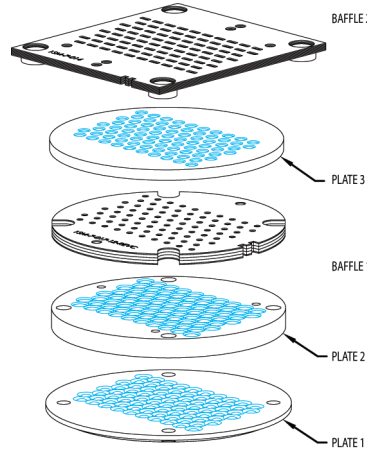
The technique is primarily used for bio-imaging, ranging from small organisms to individual cells.



Array Microscopy

An **array microscope** is a solution to the trade-off between field of view and lateral resolution. In the array microscope, a miniature microscope objective is replicated tens of times. The result is an imaging system with a field of view that can be increased in steps of an individual objective's field of view, independent of numerical aperture and resolution. An array microscope is useful for applications that require fast imaging of large areas at a high level of detail. Compared to conventional microscope optics for the same purpose, an array microscope can complete the same task several times faster due to its parallel imaging format.

An example implementation of the array-microscope optics is shown in the figure. This array microscope is used for imaging glass slides bearing tissue (histology) or cells (cytology). In that case, there are a total of 80 identical miniature 7×/0.6 microscope objectives. The summed field of view measures 18 mm. Each objective consists of three lens elements. The lens surfaces are patterned on three plates that are stacked to form the final array of microscopes. The plates measure 25 mm in diameter. Plate 1 is near the object, at a working distance of 400 µm. Between plates 2 and 3, there is a baffle plate. A second baffle is located between the third lens and the image plane. The purpose of the baffles is to eliminate cross talk and image overlap between objectives in the array.



The small size of each microscope objective and the need to avoid image overlap jointly dictate a low magnification. Therefore, the array microscope works best in combination with an image sensor divided into small pixels (e.g., 3.3 µm).

Focusing the array microscope is achieved by an up/down translation and two rotations: a pitch and a roll.