

1 CONFOCAL CHROMATIC

1.1 Presentation

Confocal microscopy may be derived from conventional microscopy by double spatial filtering. The first filter ensures that, at a given instant, a single point of the field is illuminated; the second one ensures that light returned from the object may reach the detector only if it originates from this point.

This configuration is depicted in Fig 1a, where the point source W is imaged by the objective lens L on the object point M. Backscattered light passes back through objective lens L and is then directed towards the detector by a beamsplitter. The pinhole P, located at the image of M given by L, plays an essential role in this configuration as it stops light coming from all points except M, in particular points located on the optical axis, above or below M (fig.1b).

When (x,y) scanning is added to the confocal configuration, one obtains the image of a plane located at a precise distance from the objective L, with no interference from points lying outside this plane. This property is called "optical sectioning" and it constitutes the main advantage of confocal microscopy. An additional advantage resides in the excellent lateral resolution, about 30% better than that obtained with conventional microscopy.

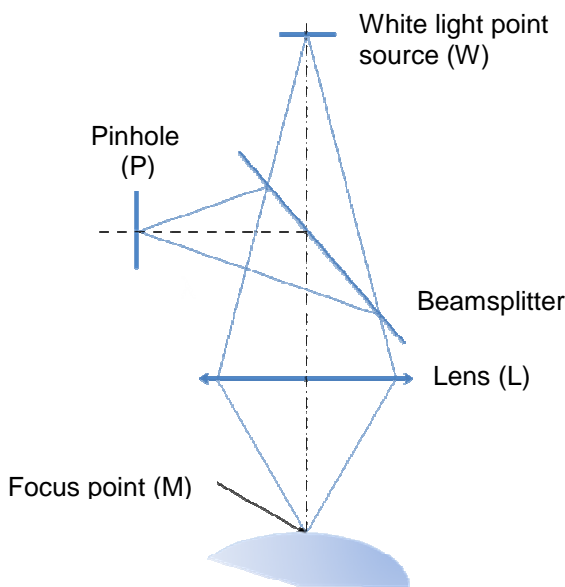


Fig 1a : confocal configuration

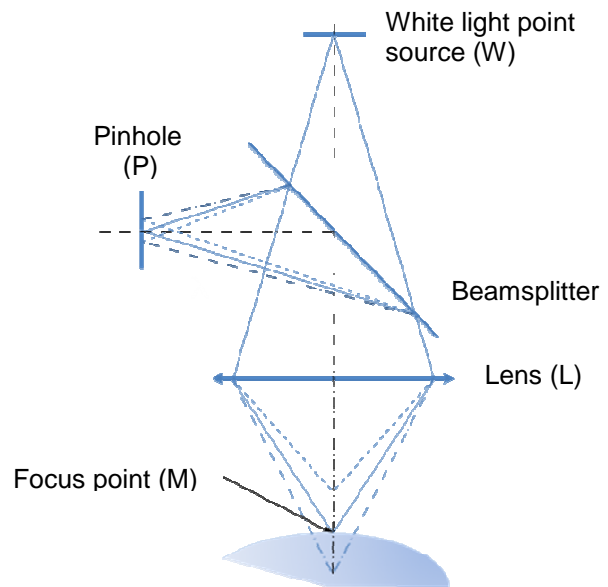


Fig 1b : optical sectioning with confocal configuration

The microtopography of a sample consists on measuring the altitude (z coordinate) of each point of the surface on the sample. This may be achieved by dynamic focusing, in other terms, by moving some mechanical part along the z axis. Non-temporal coding of the measurement space is an alternative approach which eliminates the need for moving parts.

Practically, a white light source is imaged by an objective lens with extended axial chromatism on a series of monochromatic point images in the measurement space. When the measured sample intercepts the measurement space at point M, a single of the monochromatic point images is focalized at M. Due to the confocal configuration, only the wavelength λ_M will pass through the spatial filter with high efficiency, all other wavelengths will be out of focus.

Suppose now that the object is constituted of a several transparent or semi-transparent thin layers. Each interface between adjacent layers reflects light at a different wavelength, and the spectrum of the detected

light is composed of a series of spectral peaks. In other terms, all the interfaces may be detected and their positions measured simultaneously.

The next step is decoding of the collected light in order to extract the information about the z-coordinate of the point M. This may be realized by spectral analysis. After its passage through the pinhole, the light beam is directed towards a diffraction grating which deviates each wavelength at a different direction. If a line CCD intercepts the light coming out of the diffraction grating, the position of the intensity maximum on the CCD signal is directly correlated with the position (Z-coordinate) of point M.

- The principle of confocal imaging yields an excellent spatial resolution regardless of ambient illumination,
- The chromatic coding ensures that measurement is insensitive to reflectivity variations in the sample and allows to work with all types of materials, transparent and opaque, specular or scattering, polished or not, with no need to treat the sample prior to measurement.
- The use of a white light source and not of a coherent source (laser) eliminates completely all the difficulties associated with speckle.

In the measurement space altitude is coded spectrally using the axial chromatism of the objective lens. For conventional optical systems, axial chromatism is considered as an aberration and optical engineers work hard to compensate it. Chromatism results from dispersion (the variation of the refractive index as a function of wavelength) of the lenses used in optical systems. When the optical characteristics of an objective lens depend on wavelength, the image of a point source emitting white light is generally a continuum of monochromatic image points distributed along the optical axis (fig.2).

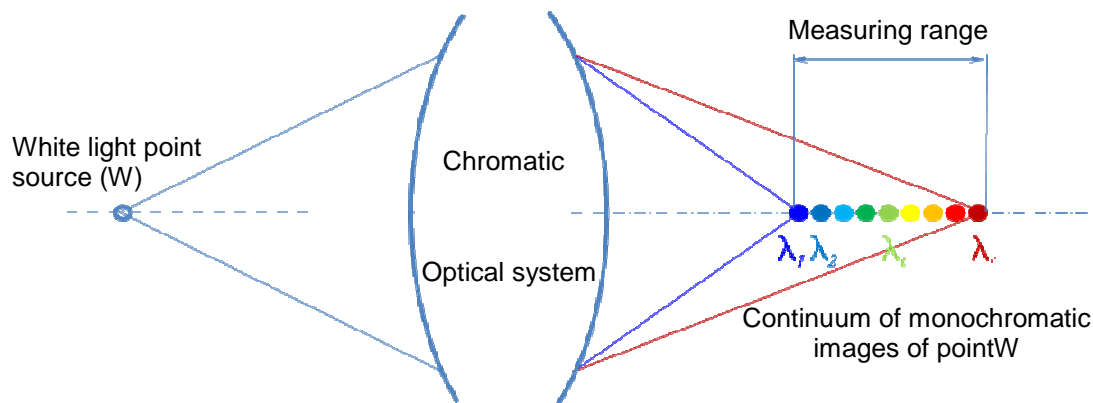


Fig 2 : principle of chromatic coding

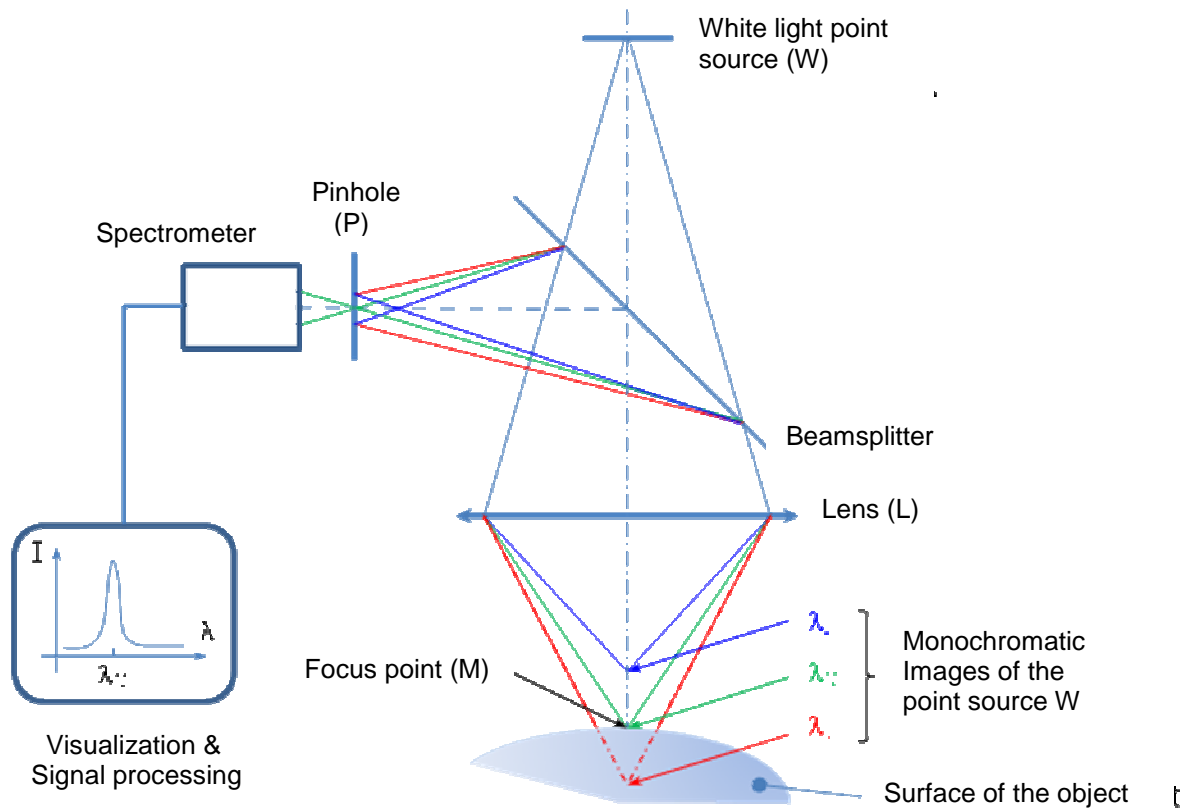


Fig 3 : layout of confocal microscopy with extended z-axis field