Confocal microscope

Photo of a confocal microscope in a darkened room. A more contrasted confocal microscope photo can be found for example here [1]

Confocal microscope is a type of light microscope.

When observing a real preparation, the thickness of which is not negligible (the ideal preparation for light microscopy has a thickness approaching zero), the observation is distorted by rays coming from the material above and below the focused plane. This adverse effect is prevented precisely by the use of a confocal microscope. In addition to this advantage, the confocal microscope provides the possibility to reconstruct 3D models of the specimen.

History

The idea of a confocal microscope comes from Marvin Minsky, who patented it already in 1957. At that time, however, it remained unresponsive, as Minsky did not find a suitable light source for the construction of a functional device. Ten years later **M. Petráň *and* *M. Hadravský*** from the Medical Faculty of the Charles University in Pilsen patented a confocal microscope based on a rotating Nipkow disk. With this device (known in the professional literature as Tandem Scanning Confocal Microscope), high-quality optical sections were obtained for the first time through a thick specimen, namely brain tissue. However, the tandem confocal microscope has not been widely used in practice. The era of confocal microscopy only begins in the late 1970s, when the first reliable confocal microscope with a swept laser beam was constructed.

Principle

Principle of confocal microscope The lights source for a confocal microscope is laser (ultraviolet, infrared or visible spectrum), which illuminates the specimen through a point (confocal) aperture and objective. The reflected light (or emitted fluorescent radiation if it is fluorescence confocal microscopy) then passes through the same objective. The rays pass through the dichroic mirror and continue to the spot diaphragm, where light from other planes is filtered out. Finally, the beams enter the photomultiplier (https://cs.wikipedia.org/wiki/Foton%C3%A1sobi%C4%8D) where they are amplified and detected.

It follows from the arrangement of the microscope that in one step we obtain information about only one point - to obtain an image of the entire plane, it is necessary to create a series of images.

According to the rasterization mechanism, we distinguish:

- **confocal microscope with a sweeping laser beam (CLSM, LSCM)** - rastering takes place by moving the beam with the help of an aperture (placed between the dichroic mirror and the objective) gradually to all points of the plane (similar principle to moving along the television screen), approximate speed : 3 frames/sec
- **confocal microscope based on a rotating Nipkow disk'** - predecessor of the swept laser beam, speed up to 60 frames/s

If a sufficient number of planes are scanned, it is possible to use a computer to assemble a 3D model of the specimen.

Usage

A confocal microscope is used in the study of surface properties of materials, evaluation of hardness tests of metals and plastic materials, measurement of the height of structural elements on semiconductor chips. In biology, it is used in the **study of the spatial structures of cells'** (e.g. cytoskeletonu). Advantageously, confocal microscopes can be used to study the architecture of neuronal networks in brain tissue when it is stained using Golgi's method. It is also possible to study intracellular **ion concentrations**, measure **membrane potential** and intracellular **pH**. When using immunofluorescence methods, it is possible to study, for example, the distribution of receptors in membranes. In cytology and cytogenetics, the confocal microscope is used to study the ``topology of the cell nucleus *(the internal arrangement of chromatinu).*

Links

- Optical Microscope
- Electron microscope

References

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