

# Microscopy

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**Microscopy** is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye). There are three well-known branches of microscopy: optical, electron, and scanning probe microscopy.

Optical and electron microscopy involve the diffraction, reflection, or refraction of electromagnetic radiation/electron beams interacting with the specimen, and the collection of the scattered radiation or another signal in order to create an image. This process may be carried out by wide-field irradiation of the sample (for example standard light microscopy and transmission electron microscopy) or by scanning of a fine beam over the sample (for example confocal laser scanning microscopy and scanning electron microscopy). Scanning probe microscopy involves the interaction of a scanning probe with the surface of the object of interest. The development of microscopy revolutionized biology, gave rise to the field of histology and so remains an essential technique in the life and physical sciences.



Scanning electron microscope image of pollen



Microscopic examination in a biochemical laboratory

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## Optical microscopy

Optical or light microscopy involves passing visible light transmitted through or reflected from the sample through a single or multiple lenses to allow a magnified view of the sample.

<sup>[1]</sup> The resulting image can be detected directly by the eye, imaged on a photographic plate or captured digitally. The single lens with its attachments, or the system of lenses and imaging equipment, along with the appropriate lighting equipment, sample stage and support, makes up the basic light microscope. The most recent development is the digital microscope, which uses a CCD camera to focus on the exhibit of interest. The image is shown on a computer screen, so eye-pieces are unnecessary.

## Limitations

Limitations of standard optical microscopy (bright field microscopy) lie in three areas;

- This technique can only image dark or strongly refracting objects effectively.
- Diffraction limits resolution to approximately 0.2 micrometres (*see: microscope*). This limits the practical magnification limit to  $\sim 1500\times$ .
- Out of focus light from points outside the focal plane reduces image clarity.

Live cells in particular generally lack sufficient contrast to be studied successfully, since the internal structures of the cell are colourless and transparent. The most common way to increase contrast is to stain the different structures with selective dyes, but this often involves killing and fixing the sample. Staining may also introduce artifacts, apparent structural details that are caused by the processing of the specimen and are thus not legitimate features of the specimen. In general, these techniques make use of differences in the refractive index of cell structures. It is comparable to looking through a glass window: you (bright field microscopy) don't see the glass but merely the dirt on the glass. There is a difference, as glass is a denser material, and this creates a difference in phase of the light passing through. The human eye is not sensitive to this difference in phase, but clever optical solutions have been thought out to change this difference in phase into a difference in amplitude (light intensity).

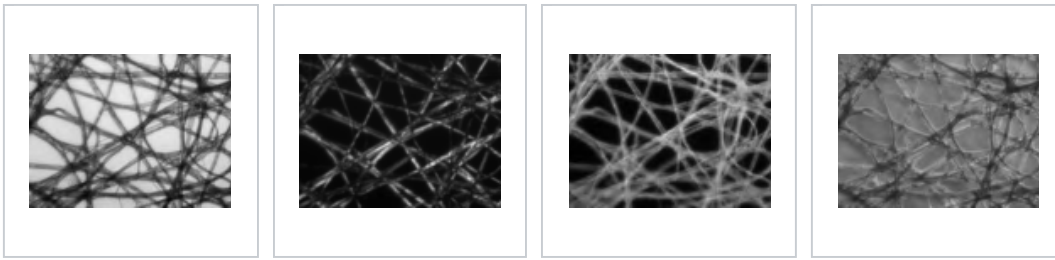
## Techniques

In order to improve specimen contrast or highlight certain structures in a sample special techniques must be used. A huge selection of microscopy techniques are available to increase contrast or label a sample.

**Four examples of transillumination techniques used to generate contrast in a sample of tissue paper.  $1.559\ \mu\text{m}/\text{pixel}$ .**



Stereo microscope



Bright field illumination, sample contrast comes from absorbance of light in the sample.

Cross-polarized light illumination, sample contrast comes from rotation of polarized light through the sample.

Dark field illumination, sample contrast comes from light scattered by the sample.

Phase contrast illumination, sample contrast comes from interference of different path lengths of light through the sample.

## Bright field

Bright field microscopy is the simplest of all the light microscopy techniques. Sample illumination is via transmitted white light, i.e. illuminated from below and observed from above. Limitations include low contrast of most biological samples and low apparent resolution due to the blur of out of focus material. The simplicity of the technique and the minimal sample preparation required are significant advantages.

## Oblique illumination

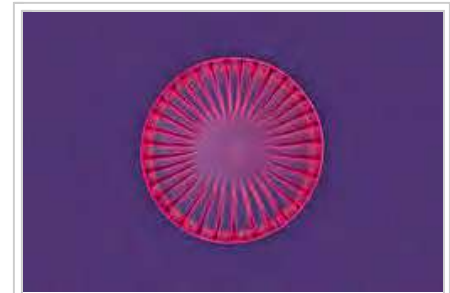
The use of oblique (from the side) illumination gives the image a 3-dimensional appearance and can highlight otherwise invisible features. A more recent technique based on this method is *Hoffmann's modulation contrast*, a system found on inverted microscopes for use in cell culture. Oblique illumination suffers from the same limitations as bright field microscopy (low contrast of many biological samples; low apparent resolution due to out of focus objects).

## Dark field

Dark field microscopy is a technique for improving the contrast of unstained, transparent specimens.<sup>[2]</sup> Dark field illumination uses a carefully aligned light source to minimize the quantity of directly transmitted (unscattered) light entering the image plane, collecting only the light scattered by the sample. Dark field can dramatically improve image contrast –

especially of transparent objects – while requiring little equipment setup or sample preparation. However, the technique suffers from low light intensity in final image of many biological samples, and continues to be affected by low apparent resolution.

*Rheinberg illumination* is a special variant of dark field illumination in which transparent, colored filters are inserted just before the condenser so that light rays at high aperture are differently colored than those at low aperture (i.e. the background to the specimen may be blue while the object appears self-luminous red). Other color combinations are possible but their effectiveness is quite variable.<sup>[3]</sup>



A diatom under Rheinberg illumination

### Dispersion staining

Dispersion staining is an optical technique that results in a colored image of a colorless object. This is an optical staining technique and requires no stains or dyes to produce a color effect. There are five different microscope configurations used in the broader technique of dispersion staining. They include brightfield Becke line, oblique, darkfield, phase contrast, and objective stop dispersion staining.

### Phase contrast

*In electron microscopy: Phase-contrast imaging*

More sophisticated techniques will show proportional differences in optical density. **Phase contrast** is a widely used technique that shows differences in refractive index as difference in contrast. It was developed by the Dutch physicist Frits Zernike in the 1930s (for which he was awarded the Nobel Prize in 1953). The nucleus in a cell for example will show up darkly against the surrounding cytoplasm. Contrast is excellent; however it is not for use with thick objects. Frequently, a halo is formed even around small objects, which obscures detail. The system consists of a circular annulus in the condenser, which produces a cone of light. This cone is superimposed on a similar sized ring within the phase-objective. Every objective has a different size ring, so for every objective another condenser setting has to be chosen. The ring in the objective has special optical properties: it, first of all, reduces the direct light in intensity, but more importantly, it creates an artificial phase difference of about a quarter wavelength. As the physical properties of this direct light have changed, interference with the diffracted light occurs, resulting in the phase contrast image. One disadvantage of phase-contrast microscopy is halo formation (halo-light ring).

## Differential interference contrast

Superior and much more expensive is the use of **interference contrast**. Differences in optical density will show up as differences in relief. A nucleus within a cell will actually show up as a globule in the most often used **differential interference contrast** system according to Georges Nomarski. However, it has to be kept in mind that this is an *optical effect*, and the relief does not necessarily resemble the true shape. Contrast is very good and the condenser aperture can be used fully open, thereby reducing the depth of field and maximizing resolution.

The system consists of a special prism (Nomarski prism, Wollaston prism) in the condenser that splits light in an ordinary and an extraordinary beam. The spatial difference between the two beams is minimal (less than the maximum resolution of the objective). After passage through the specimen, the beams are reunited by a similar prism in the objective.



Phase-contrast light micrograph of hyaline cartilage showing chondrocytes and organelles, lacunae and extracellular matrix.

In a homogeneous specimen, there is no difference between the two beams, and no contrast is being generated. However, near a refractive boundary (say a nucleus within the cytoplasm), the difference between the ordinary and the extraordinary beam will generate a relief in the image. Differential interference contrast requires a polarized light source to function; two polarizing filters have to be fitted in the light path, one below the condenser (the polarizer), and the other above the objective (the analyzer).

Note: In cases where the optical design of a microscope produces an appreciable lateral separation of the two beams we have the case of classical interference microscopy, which does not result in relief images, but can nevertheless be used for the quantitative determination of mass-thicknesses of microscopic objects.

## Interference reflection

An additional technique using interference is **interference reflection microscopy** (also known as reflected interference contrast, or RIC). It relies on cell adhesion to the slide to produce an interference signal. If there is no cell attached to the glass, there will be no interference.

Interference reflection microscopy can be obtained by using the same elements used by DIC, but without the prisms. Also, the light that is being detected is reflected and not transmitted as it is when DIC is employed.

## Fluorescence

When certain compounds are illuminated with high energy light, they emit light of a lower frequency. This effect is known as fluorescence. Often specimens show their characteristic autofluorescence image, based on their chemical makeup.

This method is of critical importance in the modern life sciences, as it can be extremely sensitive, allowing the detection of single molecules. Many different fluorescent dyes can be used to stain different structures or chemical compounds. One particularly powerful method is the combination of antibodies coupled to a fluorophore as in immunostaining. Examples of commonly used fluorophores are fluorescein or rhodamine.

The antibodies can be tailor-made for a chemical compound. For example, one strategy often in use is the artificial production of proteins, based on the genetic code (DNA). These proteins can then be used to immunize rabbits, forming antibodies which bind to the protein. The antibodies are then coupled chemically to a fluorophore and used to trace the proteins in the cells under study.

Highly efficient fluorescent proteins such as the green fluorescent protein (GFP) have been developed using the molecular biology technique of gene fusion, a process that links the expression of the fluorescent compound to that of the target protein. This combined fluorescent protein is, in general, non-toxic to the organism and rarely interferes with the function of the protein under study. Genetically modified cells or organisms directly express the fluorescently tagged proteins, which enables the study of the function of the original protein in vivo.



Images may also contain artifacts. This is a confocal laser scanning fluorescence micrograph of thale cress anther (part of stamen). The picture shows among other things a nice red flowing collar-like structure just below the anther. However, an intact thale cress stamen does not have such collar, this is a fixation artifact: the stamen has been cut below the picture frame, and epidermis (upper layer of cells) of stamen stalk has peeled off, forming a non-characteristic structure. Photo: Heiti Paves from Tallinn University of Technology.

Growth of protein crystals results in both protein and salt crystals. Both are colorless and microscopic. Recovery of the protein crystals requires imaging which can be done by the intrinsic fluorescence of the protein or by using transmission microscopy. Both methods require an ultraviolet microscope as protein absorbs light at 280 nm. Protein will also fluorescence at approximately 353 nm when excited with 280 nm light.<sup>[4]</sup>

Since fluorescence emission differs in wavelength (color) from the excitation light, an ideal fluorescent image shows only the structure of interest that was labeled with the fluorescent dye. This high specificity led to the widespread use of fluorescence light microscopy in biomedical research. Different fluorescent dyes can be used to stain different biological structures, which can then be detected simultaneously, while still being specific due to the individual color of the dye.

To block the excitation light from reaching the observer or the detector, filter sets of high quality are needed. These typically consist of an excitation filter selecting the range of excitation wavelengths, a dichroic mirror, and an emission filter blocking the excitation light. Most fluorescence microscopes are operated in the Epi-illumination mode (illumination and detection from one side of the sample) to further decrease the amount of excitation light entering the detector.

An example of fluorescence microscopy today is two-photon or multi-photon imaging. Two photon imaging allows imaging of living tissues up to a very high depth by enabling greater excitation light penetration and reduced background emission signal.

See also: total internal reflection fluorescence microscope Neuroscience

## **Confocal**

Confocal microscopy uses a scanning point of light and a pinhole to prevent out of focus light from reaching the detector. Compared to full sample illumination, confocal microscopy gives slightly higher resolution, and significantly improves optical sectioning. Confocal microscopy is, therefore, commonly used where 3D structure is important.

## **Single plane illumination microscopy and light sheet fluorescence microscopy**

Using a plane of light formed by focusing light through a cylindrical lens at a narrow angle or by scanning a line of light in a plane perpendicular to the axis of objective, high resolution optical sections can be taken.<sup>[5][6][7]</sup> Single plane illumination, or light sheet illumination, is also accomplished using beam shaping techniques incorporating multiple-prism beam expanders.<sup>[8][9]</sup> The images are captured by CCDs. These variants allow very fast and high signal to noise ratio image capture.



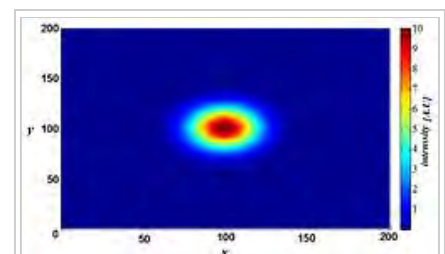
## Wide-field multiphoton microscopy

Wide-field multiphoton microscopy<sup>[10][11][12][13]</sup> refers to an optical non-linear imaging technique tailored for ultrafast imaging in which a large area of the object is illuminated and imaged without the need for scanning. High intensities are required to induce non-linear optical processes such as two-photon fluorescence or second harmonic generation. In scanning multiphoton microscopes the high intensities are achieved by tightly focusing the light, and the image is obtained by stage- or beam-scanning the sample. In **wide-field multiphoton microscopy** the high intensities are best achieved using an optically amplified pulsed laser source to attain a large field of view ( $\sim 100\ \mu\text{m}$ ).<sup>[10][11][12]</sup> The image in this case is obtained as a single frame with a CCD without the need of scanning, making the technique particularly useful to visualize dynamic processes simultaneously across the object of interest. With wide-field multiphoton microscopy the frame rate can be increased up to a 1000-fold compared to multiphoton scanning microscopy.<sup>[11]</sup>

## Deconvolution

Fluorescence microscopy is a powerful technique to show specifically labeled structures within a complex environment and to provide three-dimensional information of biological structures. However, this information is blurred by the fact that, upon illumination, all fluorescently labeled structures emit light, irrespective of whether they are in focus or not. So an image of a certain structure is always blurred by the contribution of light from structures that are out of focus. This phenomenon results in a loss of contrast especially when using objectives with a high resolving power, typically oil immersion objectives with a high numerical aperture.

However, blurring is not caused by random processes, such as light scattering, but can be well defined by the optical properties of the image formation in the microscope imaging system. If one considers a small fluorescent light source (essentially a bright spot), light coming from this spot spreads out further from our perspective as the spot becomes more out of focus. Under ideal conditions, this produces an "hourglass" shape of this point source in the third (axial) dimension. This shape is called the point spread function (PSF) of the microscope imaging system. Since any fluorescence image is made up of a large number of such small fluorescent light sources, the image is said to be "convolved by the point spread function". The mathematically modeled PSF of a terahertz laser pulsed imaging system is shown on the right.



Mathematically modeled Point Spread Function of a pulsed THz laser imaging system.<sup>[14]</sup>

The output of an imaging system can be described using the equation:

$$s(x, y) = PSF(x, y) * o(x, y) + n$$

Where  $n$  is the additive noise.<sup>[15]</sup> Knowing this point spread function<sup>[16]</sup> means that it is possible to reverse this process to a certain extent by computer-based methods commonly known as deconvolution microscopy.<sup>[17]</sup> There are various algorithms available for 2D or 3D deconvolution. They can be roughly classified in *nonrestorative* and *restorative* methods. While the nonrestorative methods can improve contrast by removing out-of-focus light from focal planes, only the restorative methods can actually reassign light to its proper place of origin. Processing fluorescent images in this manner can be an advantage over directly acquiring images without out-of-focus light, such as images from confocal microscopy, because light signals otherwise eliminated become useful information. For 3D deconvolution, one typically provides a series of images taken from different focal planes (called a Z-stack) plus the knowledge of the PSF, which can be derived either experimentally or theoretically from knowing all contributing parameters of the microscope.

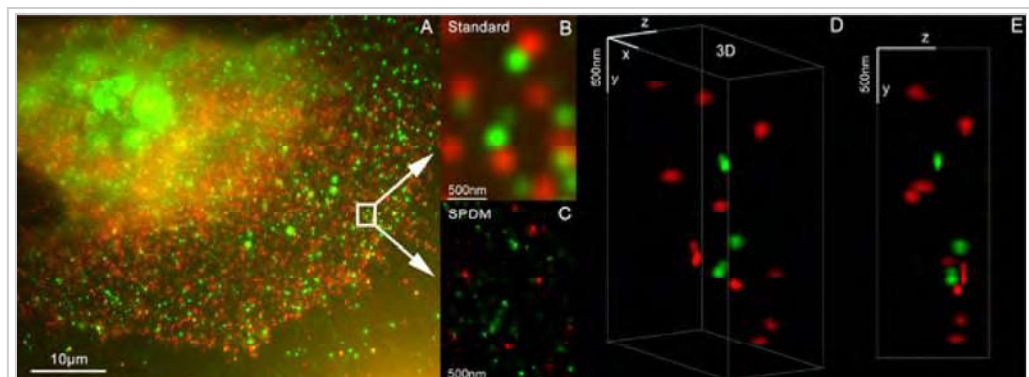
## Sub-diffraction techniques

A multitude of super-resolution microscopy techniques have been developed in recent times which circumvent the diffraction barrier.

This is mostly achieved by imaging a sufficiently static sample multiple times and either modifying the excitation light or observing stochastic changes in the image.

Knowledge of and chemical control over fluorophore photophysics is at the core of these techniques, by which resolutions of  $\sim 20$  nanometers are regularly obtained.<sup>[18][19]</sup>

## Serial time-encoded amplified microscopy



Example of super-resolution microscopy. Image of Her3 and Her2, target of the breast cancer drug Trastuzumab, within a cancer cell.

Serial time encoded amplified microscopy (STEAM) is an imaging method that provides ultrafast shutter speed and frame rate, by using optical image amplification to circumvent the fundamental trade-off between sensitivity and speed, and a single-pixel photodetector to eliminate the need for a detector array and readout time limitations <sup>[20]</sup> The method is at least 1000 times faster than the state-of-the-art CCD and CMOS cameras. Consequently, it is potentially useful for a broad range of scientific, industrial, and biomedical applications that require high image acquisition rates, including real-time diagnosis and evaluation of shockwaves, microfluidics, MEMS, and laser surgery.

## Extensions

Most modern instruments provide simple solutions for micro-photography and image recording electronically. However such capabilities are not always present and the more experienced microscopist will, in many cases, still prefer a hand drawn image to a photograph. This is because a microscopist with knowledge of the subject can accurately convert a three-dimensional image into a precise two-dimensional drawing. In a photograph or other image capture system however, only one thin plane is ever in good focus.

The creation of careful and accurate micrographs requires a microscopical technique using a monocular eyepiece. It is essential that both eyes are open and that the eye that is not observing down the microscope is instead concentrated on a sheet of paper on the bench besides the microscope. With practice, and without moving the head or eyes, it is possible to accurately record the observed details by tracing round the observed shapes by simultaneously "seeing" the pencil point in the microscopical image.

Practicing this technique also establishes good general microscopical technique. It is always less tiring to observe with the microscope focused so that the image is seen at infinity and with both eyes open at all times.

## Other enhancements

Microspectroscopy:spectroscopy with a microscope

## X-ray

As resolution depends on the wavelength of the light. Electron microscopy has been developed since the 1930s that use electron beams instead of light. Because of the much smaller wavelength of the electron beam, resolution is far higher.

Though less common, X-ray microscopy has also been developed since the late 1940s. The resolution of X-ray microscopy lies between that of light microscopy and electron microscopy.

## Electron microscopy

Until the invention of sub-diffraction microscopy, the wavelength of the light limited the resolution of traditional microscopy to around 0.2 micrometers. In order to gain higher resolution, the use of an electron beam with a far smaller wavelength is used in electron microscopes.

- Transmission electron microscopy (TEM) is quite similar to the compound light microscope, by sending an electron beam through a very thin slice of the specimen. The resolution limit in 2005 was around 0.05 nanometer and has not increased appreciably since that time.
- Scanning electron microscopy (SEM) visualizes details on the surfaces of specimens and gives a very nice 3D view. It gives results much like those of the stereo light microscope. The best resolution for SEM in 2011 was 0.4 nanometer.

Electron microscopes equipped for X-ray spectroscopy can provide qualitative and quantitative elemental analysis.

## Scanning probe microscopy

This is a sub-diffraction technique. Examples of scanning probe microscopes are the atomic force microscope (AFM), the Scanning tunneling microscope, the photonic force microscope and the recurrence tracking microscope. All such methods use the physical contact of a solid probe tip to scan the surface of an object, which is supposed to be almost flat.

### Ultrasonic force

Ultrasonic Force Microscopy (UFM) has been developed in order to improve the details and image contrast on "flat" areas of interest where AFM images are limited in contrast. The combination of AFM-UFM allows a near field acoustic microscopic image to be generated. The AFM tip is used to detect the ultrasonic waves and overcomes the limitation of wavelength that occurs in acoustic microscopy. By using the elastic changes under the AFM tip, an image of much greater detail than the AFM topography can be generated.

Ultrasonic force microscopy allows the local mapping of elasticity in atomic force microscopy by the application of ultrasonic vibration to the cantilever or sample. In an attempt to analyze the results of ultrasonic force microscopy in a quantitative fashion, a force-distance curve measurement is done with ultrasonic vibration applied to the cantilever base, and the results are compared with a model of the cantilever dynamics and tip-sample interaction based on the finite-difference technique.

## Ultraviolet microscopy

Ultraviolet microscopes have two main purposes. The first is to utilize the shorter wavelength of ultraviolet electromagnetic energy to improve the image resolution beyond that of the diffraction limit of standard optical microscopes. This technique is used for non-destructive inspection of devices with very small features such as those found in modern semiconductors. The second application for UV microscopes is contrast enhancement where the response of individual samples is enhanced, relative to their surrounding, due to the interaction of light with the molecules within the sample itself. One example is in the growth of protein crystals. Protein crystals are formed in salt solutions. As salt and protein crystals are both formed in the growth process, and both are commonly transparent to the human eye, they cannot be differentiated with a standard optical microscope. As the tryptophan of protein absorbs light at 280 nm, imaging with a UV microscope with 280 nm bandpass filters makes it simple to differentiate between the two types of crystals. The protein crystals appear dark while the salt crystals are transparent.

## Infrared microscopy

The term *infrared microscopy* refers to microscopy performed at infrared wavelengths. In the typical instrument configuration a Fourier Transform Infrared Spectrometer (FTIR) is combined with an optical microscope and an infrared detector. The infrared detector can be a single point detector, a linear array or a 2D focal plane array. The FTIR provides the ability to perform chemical analysis via infrared spectroscopy and the microscope and point or array detector enable this chemical analysis to be spatially resolved, i.e. performed at different regions of the sample. As such, the technique is also called infrared microspectroscopy <sup>[21]</sup> [1] (<http://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a5609.pub2/abstract>) (an alternative architecture involves the combination of a tuneable infrared light source and single point detector on a flying objective). This technique is frequently used for infrared chemical imaging, where the image contrast is determined by the response of individual sample regions to particular IR wavelengths selected by the user, usually specific IR absorption bands and associated molecular resonances . A key limitation of conventional infrared microspectroscopy is that the spatial resolution is diffraction-limited. Specifically the spatial resolution is limited to a figure related to the wavelength of the light. For practical IR microscopes, the spatial resolution is limited to 1-3X the wavelength, depending on the specific technique and instrument used. For mid-IR wavelengths, this sets a practical spatial resolution limit of ~3-30  $\mu\text{m}$ .

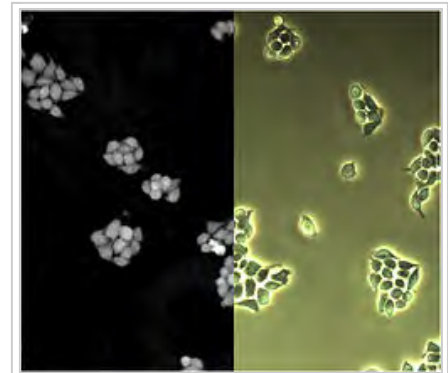
IR versions of sub-diffraction microscopy (see above) also exist <sup>[21]</sup> [2] (<http://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a5609.pub2/abstract>). These include IR NSOM,<sup>[22]</sup> photothermal microspectroscopy, and atomic force microscope based infrared spectroscopy (AFM-IR).

## Digital holographic microscopy

In digital holographic microscopy (DHM), interfering wave fronts from a coherent (monochromatic) light-source are recorded on a sensor. The image is digitally reconstructed by a computer from the recorded hologram. Besides the ordinary bright field image, a phase shift image is created.

DHM can operate both in reflection and transmission mode. In reflection mode, the phase shift image provides a relative distance measurement and thus represents a topography map of the reflecting surface. In transmission mode, the phase shift image provides a label-free quantitative measurement of the optical thickness of the specimen. Phase shift images of biological cells are very similar to images of stained cells and have successfully been analyzed by high content analysis software.

A unique feature of DHM is the ability to adjust focus after the image is recorded, since all focus planes are recorded simultaneously by the hologram. This feature makes it possible to image moving particles in a volume or to rapidly scan a surface. Another attractive feature is DHM's ability to use low cost optics by correcting optical aberrations by software.



Human cells imaged by DHM phase shift (left) and phase contrast microscopy (right).

## Digital pathology (virtual microscopy)

Digital pathology is an image-based information environment enabled by computer technology that allows for the management of information generated from a digital slide. Digital pathology is enabled in part by virtual microscopy, which is the practice of converting glass slides into digital slides that can be viewed, managed, and analyzed.

## Laser microscopy

Laser microscopy is a rapidly growing field that uses laser illumination sources in various forms of microscopy.<sup>[23]</sup> For instance, laser microscopy focused on biological applications uses ultrashort pulse lasers, in a number of techniques labeled as nonlinear microscopy, saturation microscopy, and two-photon excitation microscopy.<sup>[24]</sup>

High-intensity, short-pulse laboratory x-ray lasers have been under development for several years. When this technology comes to fruition, it will be possible to obtain magnified three-dimensional images of elementary biological structures in the living state at a precisely defined instant. For optimum contrast between water and protein and for best sensitivity and resolution, the laser should be tuned near the nitrogen line at about 0.3 nanometers. Resolution will be limited mainly by the hydrodynamic expansion that occurs while the necessary number of photons is being registered.<sup>[25]</sup> Thus, while the specimen is destroyed by the exposure, its configuration can be captured before it explodes.<sup>[26][27][28][29][30][31]</sup>

Scientists have been working on practical designs and prototypes for x-ray holographic microscopes, despite the prolonged development of the appropriate laser.

<sup>[32][33][34][35][36][37][38][39]</sup>

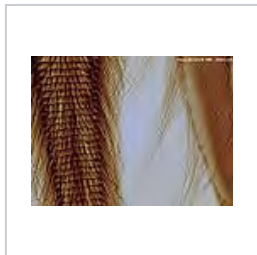
## Amateur microscopy

*Amateur Microscopy* is the investigation and observation of biological and non-biological specimens for recreational purposes. Collectors of minerals, insects, seashells, and plants may use microscopes as tools to uncover features that help them classify their collected items. Other amateurs may be interested in observing the life found in pond water and of other samples. Microscopes may also prove useful for the water quality assessment for people that keep a home aquarium. Photographic documentation and drawing of the microscopic images are additional tasks that augment the spectrum of tasks of the amateur. There are even competitions for photomicrograph art. Participants of this pastime may either use commercially prepared microscopic slides or engage in the task of specimen preparation.

While microscopy is a central tool in the documentation of biological specimens, it is, in general, insufficient to justify the description of a new species based on microscopic investigations alone. Often genetic and biochemical tests are necessary to confirm the discovery of a new species. A laboratory and access to academic literature is a necessity, which is specialized and, in general, not available to amateurs. There is, however, one huge advantage that amateurs have above professionals: time to explore their surroundings. Often, advanced amateurs team up with professionals to validate their findings and (possibly) describe new species.

In the late 1800s, amateur microscopy became a popular hobby in the United States and Europe. Several 'professional amateurs' were being paid for their sampling trips and microscopic explorations by philanthropists, to keep them amused on the Sunday afternoon (e.g., the diatom specialist A. Grunow, being paid by (among others) a Belgian industrialist). Professor John Phin published "Practical Hints on the Selection and Use of the Microscope (Second Edition, 1878)," and was also the editor of the "American Journal of Microscopy."

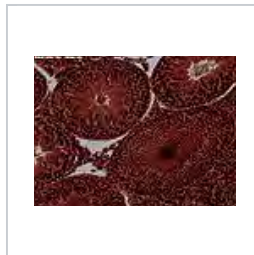
### Examples of amateur microscopy images:



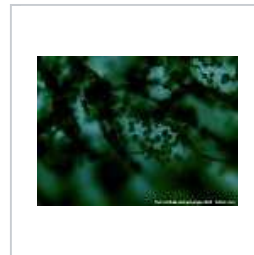
"house bee"  
Mouth 100X



Rice Stem cs  
400X



Rabbit Testis  
100X



Fern Prothallium  
400X

## See also

- Acronyms in microscopy
- Digital microscope
- Digital pathology
- Imaging cyler microscopy
- Interferometric microscopy
- Köhler illumination
- List of microscopists
- Microdensitometer
- Timeline of microscope technology
- Two-photon excitation microscopy
- USB microscope

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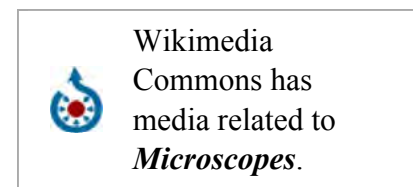
## Further reading

- Pluta, Maksymilian (1988). *Advanced Light Microscopy vol. 1 Principles and Basic Properties*. Elsevier. ISBN 978-0-444-98939-0.
- Pluta, Maksymilian (1989). *Advanced Light Microscopy vol. 2 Specialised Methods*. Elsevier. ISBN 978-0-444-98918-5.
- Bradbury, S.; Bracegirdle, B. (1998). *Introduction to Light Microscopy*. BIOS Scientific Publishers. ISBN 978-0-387-91515-9.
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- Cremer, C; Cremer, T (1978). "Considerations on a laser-scanning-microscope with high resolution and depth of field" (PDF). *Microscopica acta*. **81** (1): 31–44. PMID 713859. Theoretical basis of super resolution 4Pi microscopy & design of a confocal laser scanning fluorescence microscope
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## External links

### General

- Microscopy glossary (<http://www.3dham.com/microgallery/glossary.htm>), Common terms used in amateur light microscopy.
- Nikon MicroscopyU (<http://www.microscopyu.com>) Extensive information on light microscopy
- Olympus Microscopy (<http://www.olympusmicro.com/index.html>) Microscopy Resource center
- Andor Microscopy Techniques ([http://www.andor.com/microscopy\\_systems/techniques/](http://www.andor.com/microscopy_systems/techniques/)) - Various techniques used in microscopy.



- Carl Zeiss "Microscopy from the very beginning" (<http://www.zeiss.de/C1256B5E0047FF3F?Open>), a step by step tutorial into the basics of microscopy.
- Microscopy in Detail (<http://www.biologie.uni-hamburg.de/b-online/e03/03.htm>) - A resource with many illustrations elaborating the most common microscopy techniques
- WITec SNOM System (<http://witec.de/products/snom/>) - NSOM/SNOM and Hybrid Microscopy techniques in combination with AFM, RAMAN, Confocal, Dark-field, DIC & Fluorescence Microscopy techniques.
- Manawatu Microscopy (<http://confocal-manawatu.pbworks.com/>) - first known collaboration environment for Microscopy and Image Analysis.
- Audio microscope glossary (<http://www.histology-world.com/microscope/audiomicroscope/audiomicroscope.htm>)

## Techniques

- Ratio-metric Imaging Applications For Microscopes (<http://www.pti-nj.com/EasyRatio/EasyRatioPro-Applications.html>) Examples of Ratiometric Imaging Work on a Microscope
- Interactive Fluorescence Dye and Filter Database (<https://www.micro-shop.zeiss.com/?s=2525647761b33&l=en&p=us&f=f>) Carl Zeiss Interactive Fluorescence Dye and Filter Database.
- Analysis tools for lifetime and spectral imaging (<http://www.spechron.com>) Analysis tools for lifetime and spectral imaging.
- New approaches to microscopy (<http://spie.org/x112606.xml>) Eric Betzig: Beyond the Nobel Prize—New approaches to microscopy.

## Organizations

- Royal Microscopical Society (<http://www.rms.org.uk/>) (RMS)
- Microscopy Society of America (<http://www.microscopy.org/>) (MSA)
- European Microscopy Society (<http://www.eurmicsoc.org/>) (EMS)
- Non-membership International online organisation (<http://www.microscopy-uk.org.uk/>) (Mic-UK)
- Quekett Microscopical Club (<http://www.quekett.org/>) (QMC)

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