Mach-Zehnder Interference Microscopy
The measurement of the refractive index of microscopic objects conveys a multitude of important material-specific and structural information. In the context of material science, for example, the evaluation of both the refractive index and the dispersion relation enable the qualification of micro and crystal optical components.

A core issue regarding the observation of thin, transparent objects through a conventional light microscope is posed by their low image contrast. An interference microscope applied to live cell imaging resolves this issue without any reactive agents or dyes: This principle converts a cell’s refractive index into contrast, yielding a simple method to quantify cell properties by optical means using visible light. Such measurements allow, for example, the evaluation of a cell’s dry mass. In summary, the approach opens up many novel applications in biology as well as medical diagnostics.

To obtain the interference contrast of a phase object, light passes through the object. The contrast originates from the extent to which the object shifts the phase of the transmitted light. The superposition of the resulting object wave with a coherent reference wave visualizes this phase contrast. This highly sensitive process demands a complete separation of the object and reference wave’s beams as well as an exact wave optical tuning of the optical components. With perfect beam alignment, white light illumination creates an interferogram, resulting in characteristic interference colors which directly correspond to the object’s refractive index.

A key design concept for the dual beam interference microscope stems from the Mach Zehnder optical setup of mirrors and beam splitter. This setup for the observation and measurement of transmitted light places the desired object in one of the interferometer’s arms. A challenge is posed by the alignment of the two independent light beams as compared the Michelson interferometer setup, which can function solely with a single set of high-tech optical components. The dual nature of the Mach Zehnder interferometer setup demands the highest precision in both the selection of the twin optical components and the tuning of the beam paths. Modern optical construction and automation concepts inherited from state-of-the-art laser technology satisfy these higher demands at relatively low cost and thus guarantee both high precision and simple calibration.

All phase objects from biological samples like cells to transparent materials like glass or plastic create contrast follow the same principle: the material’s thickness and its refractive index correspond to an optical »thickness«. Depending on this thickness, the object wave is shifted with respect to the reference wave. The interference of these two light waves in the microscope amplifies this shift. An object’s local difference in refractive index and/or thickness leads to a distortion in light pattern of the interferogram, which appears as a color shift as seen, for example, on an optical fiber in the image on the left, below. Note, that the color as well as its shift do not result from image processing, but are a direct output of the interferometer. Here, a phase shifting algorithm can represent, for example, the spatial distribution of the refractive index across the picture from a series of images.
Stain-free Life Cell Imaging

The advancement of modern biotechnology, genetics and pharmaceutical research largely depends on the microscopic investigation of living tissue. To obtain the necessary contrast in light microscopy nowadays, a diverse and complex toolbox of chemical methods exists to stain specific cell components. These cell compartments are selectively linked to dye molecules for the observation with fluorescence excitation. However, all staining procedures more or less interfere with the cell’s metabolism: These invasive processes can lead to unwanted effects ranging from differing gene expression patterns over changes in protein kinetics to phototoxicity: the light induced disruption of cell metabolism. Interference contrast microscopy, on the other hand, does not need to add any compounds to visualize cells, thus allowing for undisturbed long-term observations of cell physiology.

The refractive index can measure the density distribution within the cell and between cell populations. Like this, the expression of membrane proteins and changes in membrane potential can be observed without the application of dyes.

Material Science and Analysis

Interference microscopy also offers the possibility to measure the refractive index distribution in thin and transparent materials like glass, plastics and liquids. The refractive index and the dispersion relation depend on material specific properties such as, for example:
- Mass density
- Concentration of heavy ions
- Size and orientation of molecules
- Coordination of chemical bonds

In the context of quality management, high-throughput assays could scan for inhomogeneities in glass or for the aggregation of crystallites in transparent plastic films in real time. A potential application could also be the characterization and measurement of optical aberrations in microoptics.

Developmental Goals

A cooperation with the department for Technical Optical Systems TOS of the RWTH Aachen University aims at the construction of a Mach Zehnder interference microscope, which automatically aligns to a specific measurement configuration, thus enabling an automation of the measurement process itself. For applications in the context of cell biology, this system will be coupled with an upright fluorescence illumination. For special applications in material science the system could be enhanced by the ability to measure polarization contrast.

Contact

Prof. Dr. P. Loosen
Phone +49 241 8906-186
peter.loosen@ilt.fraunhofer.de

Dipl.-Phys. D. M. Mahlmann
Phone +49 241 8906-172
daniel.mahlmann@ilt.fraunhofer.de
At present, the Fraunhofer-Gesellschaft maintains more than 80 research units, including 56 Fraunhofer Institutes, at 40 different locations in Germany. The majority of the 13,000 staff are qualified scientists and engineers, who work with an annual research budget of 1.3 billion euros. Of this sum, more than 1 billion euros is generated through contract research. Fraunhofer’s experts develop technical and organizational solutions to the problems set by industrial contractors up to the installation of complete systems.

The Fraunhofer Institute for Laser Technology ILT develops methods and systems for laser material processing and laser measuring technology within the framework of R&D contracts. The know-how of 260 employees as well as state-of-the-art technology are made available to the contractor.

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The RWTH Aachen University Chairs for Laser Technology LLT and the Technology of Optical Systems TOS, along with the teaching and research department for the non-linear dynamics of laser production methods NLD, represent an outstanding cluster of expertise in the field of optical technologies. This permits an effective treatment of basic and application-related research topics. The close cooperation with the Fraunhofer Institute for Laser Technology ILT not only permits industrial contract research on the basis of sound fundamental knowledge, but also provides new stimuli for the advanced development of optical methods, components and systems. The synergy of infrastructure and know-how is put to active use under a single roof.

Subject to alterations in specifications and other technical information. 04/2008.