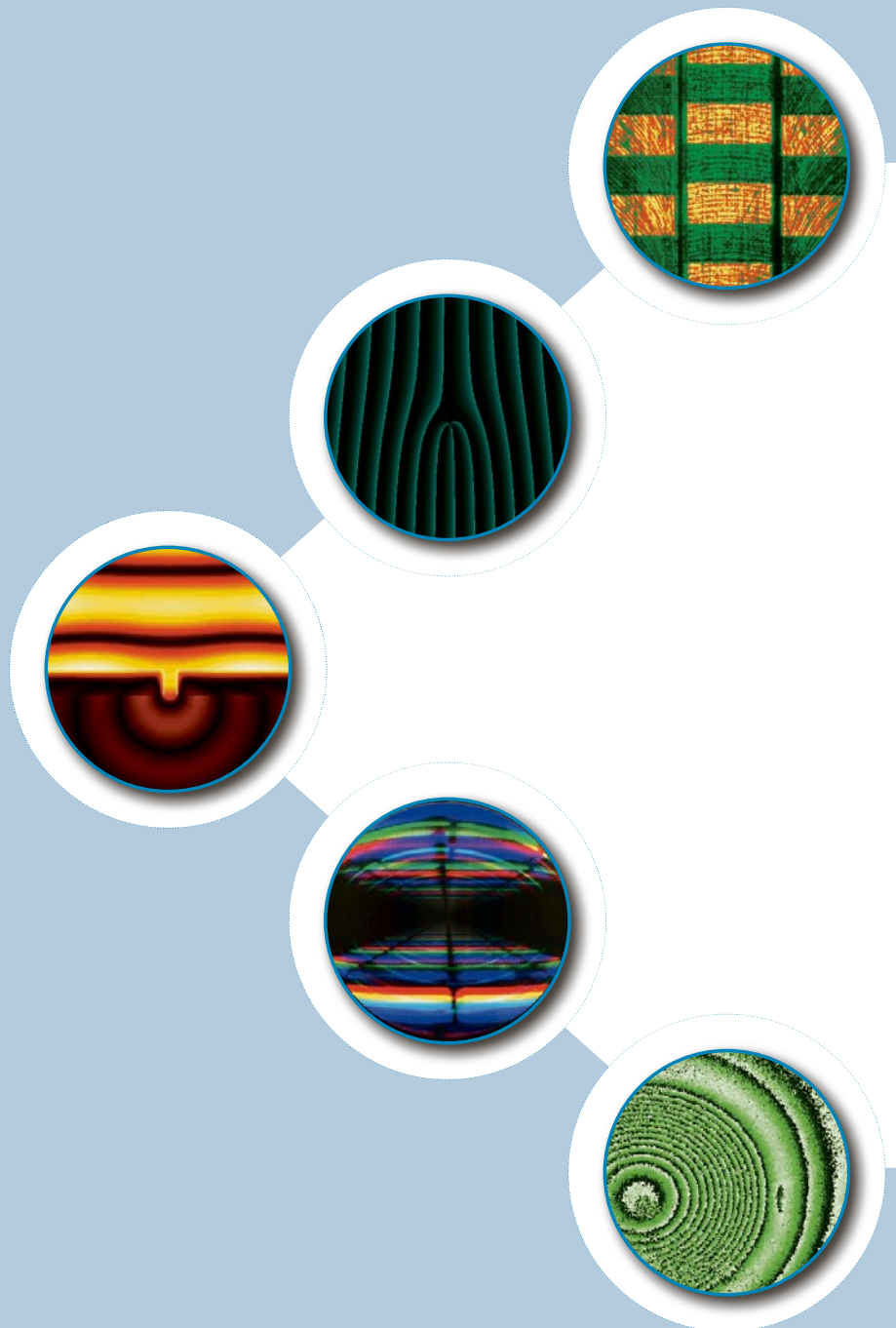




annual report
2007 / 2008

INSTITUT FÜR
TECHNISCHE OPTIK
UNIVERSITÄT STUTTGART



Universität Stuttgart

Active Optical Systems and Computational Imaging

High-resolution phase modulators for micromanipulation – new trapping approaches

Supported by: BMBF (FKZ 13N8809)

Project: "AZTEK"

High-resolution phase modulators for microscopic imaging

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Image based wavefront correction for wide field microscopy

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A Multi-Scale and Multi-Sensor Measurement System for Defect Detection:
a systematic approach for sensor fusion in optical metrology

Supported by: DFG (OS 111/18-2)

Project: "STRAMNANO"

High-resolution tomographic microinterferometry

Supported by: DFG (OS 111/20-1)

Project: "TOMI"

Optical computing using white light interferometry

High-resolution phase modulators for micromanipulation – new trapping approaches

S. Zwick, M. Warber, T. Haist, W. Osten

A major aim of the BMBF project AZTEK is the integration of a micromanipulation system, realized by a spatial light modulator (SLM) in a microscope built by Till Photonics.

The micromanipulation is based on optical tweezers, which operate on the basis of momentum transfer, when photons pass an object like a cell. Under appropriate circumstances (i.e. high numerical aperture, NIR trapping laser) microscopic objects like living cells can be trapped and moved in three dimensions. By applying a phase-only SLM (Holoeye Pluto-NIR) in the Fourier plane of the object plane and displaying a Fourier hologram, one can generate arbitrary light fields in the object plane, which opens a wide range of flexibility. Trap position and shape can be changed independently without any additional mechanics.

Within the project, an add-on module for a Zeiss Axiovert 200 was developed. New trapping methods have been tested, which take advantage of the flexibility of the holographic realization.

Figure 1 shows a yeast cell, which has been trapped by two independent spots. If one moves only one of the traps, the cell could be rotated in three dimensions. Although this is only shown laterally in this picture, three-dimensional manipulation is easily possible with holographic optical tweezers.

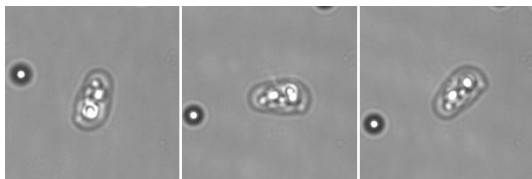


Fig. 1: Yeast cell trapped by two independent traps. By moving one of the traps, one can rotate the cell in three dimensions.

As mentioned before, conventional optical tweezers require high numerical aperture in order to realize stable three-dimensional trapping, as a high axial intensity gradient is needed. This leads to low-working distance and high intensity concentration, which may cause cell damage.

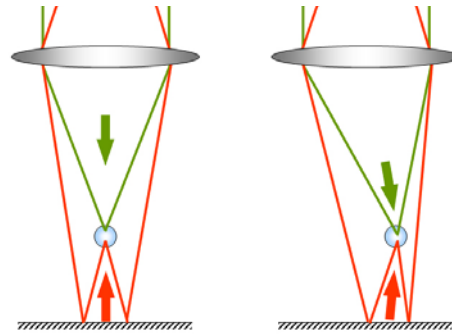


Fig. 2: Holographic twin traps are generated by creating two axially displaced traps via hologram and reflecting one at a dichroic object slide. The counter propagating scattering forces even out each other and stable axial trapping is possible even with low NA.

Holographic optical tweezers enable to realize holographic twin traps (see figure 2). Holographic twin traps are generated by creating two axially displaced traps via hologram and reflecting one at a dichroic object slide. The opposing scattering forces even out each other and stable axial trapping is possible even with low numerical aperture. To move the particle, both spots have to be moved in a synchronized way. Twin traps have been investigated theoretically. Also the principle has been demonstrated experimentally. They open the possibility of using low-NA microscope objectives, therefore increasing the working distance and reducing potential harm on living cells.

Supported by: BMBF (FKZ 13N8809)

Project: "AZTEK"

Acknowledgment: We would like to thank our project partners Holoeye Photonics AG and Till Photonics GmbH for the good cooperation.

References:

- [1] Zwick, S.; Haist, T.; Miyamoto, Y.; He, L.; Warber, M.; Hermerschmidt, A.; Osten, W. "Holographic twin traps", *J. Opt. A: Pure Appl. Opt.* 11 (2009) 034011.
- [2] Hermerschmidt, A.; Krüger, S.; Haist, T.; Zwick, S.; Warber, M.; Osten, W. "Holographic optical tweezers with real-time hologram calculation using a phase-only modulating LCOS-based SLM at 1064 nm", *Proc. SPIE* 6905 (2008).
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High-resolution phase modulators for microscopic imaging

S. Zwick, M. Warber, T. Haist, W. Osten

A further objective of the BMBF project AZTEK is the implementation of adaptive phase contrast imaging, realized with a spatial light modulator (SLM), in a microscope built by Till Photonics.

A lot of biological objects are phase objects and require phase contrast imaging methods. Most of these techniques (e.g., Zernike phase contrast) are based on optical filtering in the Fourier plane. Due to their static elements, these methods represent a trade-off, as ideal phase contrast imaging depends strongly on the object.

Therefore, we use a spatial phase-only modulator in the pupil plane for phase contrast filtering (figure 1). By changing the filter via software, we can easily adapt the filter to the object. Furthermore, it is possible to vary the different methods and their parameters in real time. As there are no mechanical changes, it is easily possible to combine those pictures digitally to benefit from the advantages of different methods and to improve the image quality.

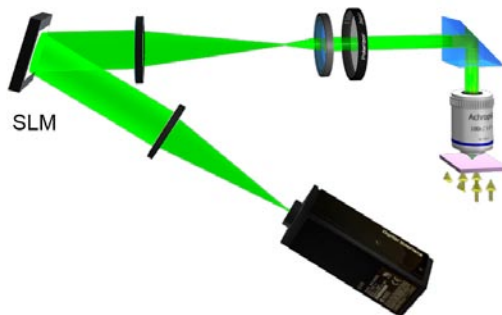


Fig. 1: Setup for adaptive phase contrast. The SLM, which is positioned in the Fourier plane of the object plane, displays a phase contrast filter depending on the applied phase contrast method.

Different phase contrast methods like Zernike phase contrast, dark field, spiral phase contrast, and differential interference contrast (DIC) have been implemented. Figure 2 shows cells of human

oral mucosa in bright field (a) without any filter applied, in positive (b) and negative (c) phase contrast, where the filter shifts the zeroth order by $\pi/2$ or $-\pi/2$ respectively by means of the SLM.

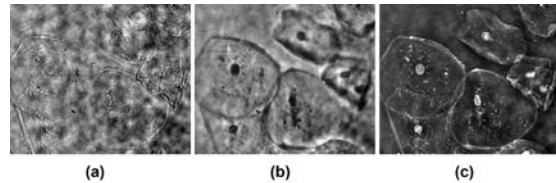


Fig. 2: Human oral mucosa cell imaged in bright field (a) without any filter applied, in positive (b) and negative (c) phase contrast, realized by displaying a Zernike filter by the SLM.

In addition, one can perform quantitative phase measurements by shifting the phase. Several experiments have been performed and good results were achieved for low-frequency objects. As the phase-shift of the phase-modulator strongly depends on the wavelength, the illumination is required to have a narrow bandwidth.

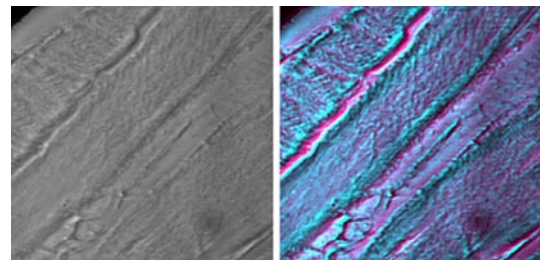


Fig. 3: Rabbit taste bud imaged by phase-shift DIC (left) and a superposition of phase-shift DIC and Zernike phase contrast displayed in different colour channels in addition with some image processing.

Figure 3 (left picture) shows a phase object imaged with phase-shift DIC, realized by SLM. Figure 3 (right picture) shows the same object imaged with Zernike phase contrast combined with subsequent image processing, where detail imaging could be improved.

Supported by: BMBF (FKZ 13N8809)

Project: "AZTEK"

Acknowledgment: We would like to thank our project partners Holoeye Photonics AG and Till Photonics GmbH for the good cooperation.

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Image based wavefront correction for wide field microscopy

M. Warber, S. Maier, J. Hafner, S. Zwick, T. Haist, W. Osten

We demonstrate a new method to measure and to correct the aberration of an optical system with a spatial light modulator (SLM). The advantage of that system is that we can implement it in our existing system for the BMBF project AZTEK without any additional parts. The Method of the System is related to the Shack-Hartmann principle but without microlenses. We measure the displacement but not of a small spot but of a picture.

In the setup we use the SLM, which is positioned in the Fourier plane of the object plane from the microscope objective lens see Figure 1.

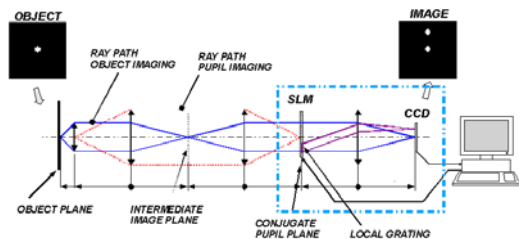


Fig. 1: A local grating written into the SLM leads to a shifted and bandpass-filtered copy of the spatially limited object.

Instead of micro lenses to get the local gradient of the aberrations we use a combination of small apertures with a grating written in the SLM and the tube lens, see Figure 1. As a result we achieve a shifted copy of the image on the CCD. Therefore we did not need additional optics or other parts. The amount of shift is proportional to the gradient of the aberrated wavefront. To accelerate the measurement, simultaneously eight apertures with different gratings were written in the SLM. To avoid the Nyquist problem the pupil is scanned randomly.

After the measurement we get a gradient map over the whole pupil, which is integrated using SVD (singular value decomposition) to obtain the wavefront (expressed by Zernike polynomials). It was solved with singular-value decomposition with the first 36 Zernike polynomials. To correct the aberrated image the calculated wavefront has to be inverted and written in the SLM.

Together with a carrier frequency we implemented the measurement and the correction system in LabView. With the Software we could optimize the measurement parameters like aperture size, number of measuring points, etc. With the optimized parameters we have a fully automated system to measure and correct the aberrations. The results are demonstrated in Figure 2.

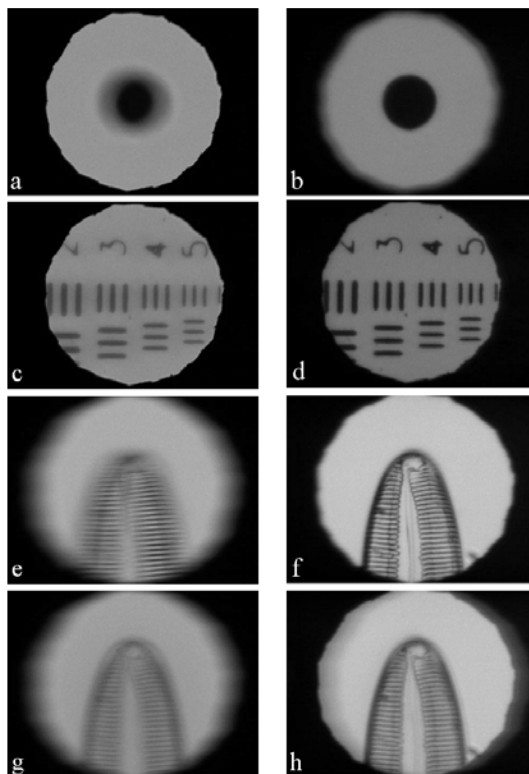


Fig. 2: Correction of different aberrations, (a, b) $10\mu\text{m}$ dot with defocus and correction, (c, d) USAF-Target with spherical aberration, (e, f) diatom with astigmatism, (g, h) diatom with combination of defocus, spherical aberration and astigmatism.

Because of contrast ratio and the diffraction limited resolution of the SLM it is not possible to use very small apertures. Therefore the measured gradient is an averaging over an area that is too large to detect small local aberrations. But the results show that for the most common (low order) aberrations in microscopy it is sufficient. The main advantage of the System is that one can measure and correct the system without additional parts and without a calibration object.

Supported by: BMBF (FKZ 13N8809)
Project: "AZTEK"

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- [1] Haist, T.; Hafner, J.; Warber, M.; Osten, W. "Scene-based wavefront correction with spatial light modulators", Proc. SPIE 7064, 70640M, 2008.

A Multi-Scale and Multi-Sensor Measurement System for Defect Detection: a systematic approach for sensor fusion in optical metrology

W. Lyda, A. Burla, T. Haist, W. Osten

Inspection of modern high quality components often requires measurement technologies with sub-micron resolution for surface characterization at wafer scale level. The limited space-bandwidth-product of optical sensors however enforces a conflict between large measurement field, high measurement resolution, and short measurement time. To balance this conflict an intelligent measurement strategy with multiple sensors fused in one system is utilized to characterise the surface at different scales.

The strategy pursues a multi-scale active exploration strategy, where coarse scale sampling provides an initial outline, followed by more detailed samples at higher resolution scales. Sensory and positioning data are processed step-by-step as they are acquired and merged using intelligent data fusion methods in order to find defects on the measured object and also to gradually improve the accuracy as more data becomes available. Task specific, coarse-scale indicator functions are used to select fine-scale features for further investigation (Fig. 1).

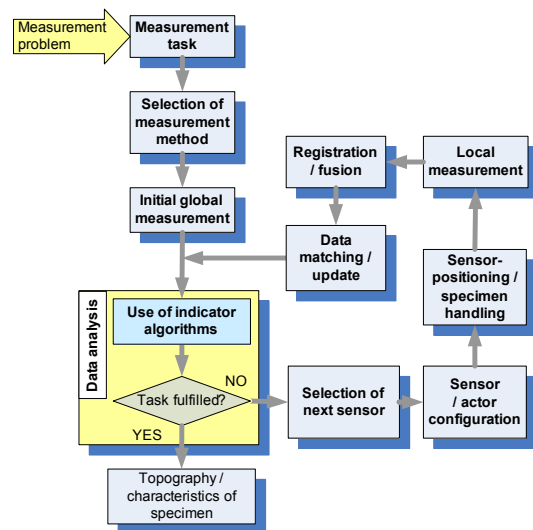


Fig. 1: Multi-scale measurement strategy.

This general design of an Automated Multi-Scale Measuring System (AMMS) was elaborated and realised in a prototype based on a modified Mahr MFU 100 armed with three different sensors for inspection of microlens arrays (Fig. 2). In the first scale, a video microscope is used to receive an initial outline of the sample followed by high resolution measurements with a confocal scanning microscope and a confocal point sensor. The lateral resolution of the sensor systems ranges from 13 μm over a field of 18 x 12 mm^2 with

the video microscope down to 0.6 μm with the confocal point sensor. In the middle scale the confocal microscope offers a variable lateral resolution from 10 μm down to 2 μm depending on the used front lens. This demonstrator was realised in cooperation with the Institute for System Dynamics (ISYS).

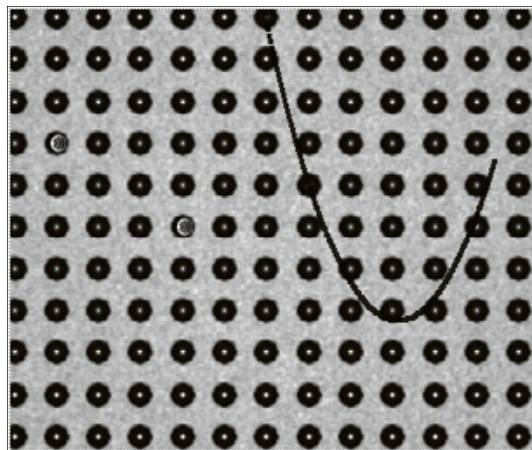


Fig. 2: Automated multi-scale measurement system based on a modified Mahr MFU 100.

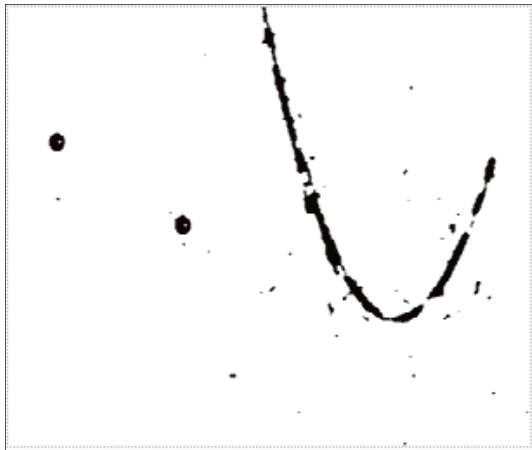
For the communication between different scales, indicators are used. These Indicators are deviations from the expected measurement results, giving a hint for an unresolved defect on the specimen in the actual sensor scale. For an exact classification of the possible defect, further measurements in finer scales are needed. Hence, in a step by step process the indicator functions provide the locations of the indicators for finer scale measurements. This effective method uses fine-scale sensors only when they are needed, balancing the conflict between measurement time, resolution and measurement field.

With three different sensors measuring a specimen for defects, different indicator detection functions are required to process the data at every sensor scale.

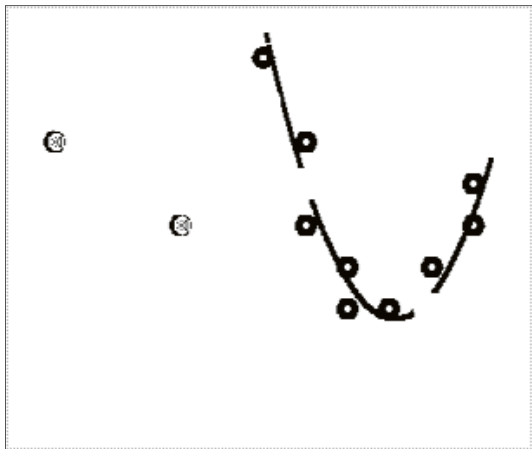
For the purpose of evaluating the AMMS-concept micro-lens arrays were used as measurement objects, due to the wide bandwidth of possible defects and defect sizes. Currently three distinct defect types are considered: 1) point-like defects, such as minute particles or dust speckle, 2) one-dimensional defects, including cracks, scratches and fine fibres,



a)



b)



c)

Fig. 3: **a)** shows the source image and **b)** and **c)** show the results of the indicator functions Fourier self filtering and Normalized correlation based filtering.

and 3) irregularities in the shape and size of the microlenses, including missing or partially missing microlenses. Several indicator algorithms were developed to accurately detect flaws and defects on the surface of the micro lens arrays. These algorithms were tested for reliability on microlenses with different surface and shape defects, simulated using synthetic data based on mathematical methods.

The indicator function algorithms include Fourier self-filtering, two-point statistical texture featuring, scratch detection and normalized cross correlation. These algorithms are parameterised according to the type of defect. Figure 3 shows the results of two algorithms, Fourier self filtering and correlation based filtering.

Future work is focused on the design of an expert system and the measurement reliability of the sensor systems. The expert system would suggest suitable sensors and image processing routines to better suit the task, based on the user specifications. Therefore, detailed information of the sensor measurement uncertainty and reliability of measurement data acquired from uncooperative surfaces is necessary.

*Supported by: DFG (OS 111/18-2)
Project: "STRAMNANO"*

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High-resolution tomographic microinterferometry

W.Gorski, W.Osten

Tomographic interferometry is based on a combination of multidirectional transmission interferometry and tomographic reconstruction algorithms. Usually it involves the multidirectional acquisition using rotation of a sample. The measurement result is the three-dimensional distribution of refractive index.

Previous work in this field proved that there are two main factors, which affect the final resolution of the 3D measurement. The first is diffraction, which appears at the border of materials with different refractive indices. An additional difficulty in this respect, may come from the structure of the object, the structure may itself be diffractive. The second factor limiting the resolution is mechanical error in the rotation, resulting in the radial run-out phenomenon. The aim of the DFG project "TOMI" was to improve the resolution of reconstruction of optical microobjects, particularly diffractive objects, applying improved algorithms and novel mechanical setup eliminating the rotation.

The successful reconstruction of complex diffractive structures, such as photonic crystal fiber or special structured fibers was achieved [1,2]. Two tomographic reconstruction algorithms were implemented, applied and results were compared (Fig. 1).

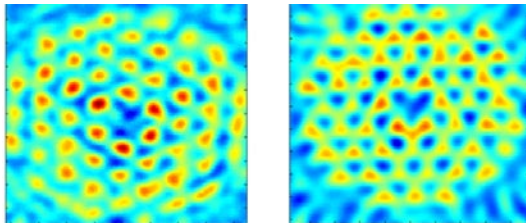


Fig. 1: The tomographic reconstruction of photonic crystal fiber, channels 3.6 μm diameter. Reconstruction using diffraction tomography (**left**) and inverse Radon transform (**right**) (single layer tomogram).

The reconstruction of a photonic crystal fiber with channel diameter 3,6 μm using the diffraction tomography algorithm delivered significantly better results than the classical inverse Radon Transform. However, when larger (factor two) fiber of the same design is considered the reconstructions obtained with those two methods deliver relatively similar results (Fig. 2).

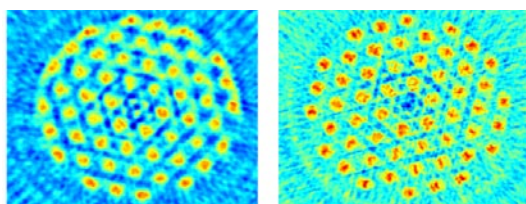


Fig. 2: The tomographic reconstruction of photonic crystal fiber, channels 8.175 μm diameter. Reconstruction using diffraction tomography (**left**) and inverse Radon transform (**right**).

This result is an important contribution to the discussion about the applicability of inverse Radon transform in optical tomography. The measurement of a Bragg fiber was aimed at preliminary quality control of a fiber laser components (Fig. 3).

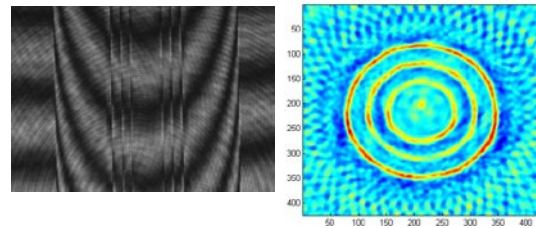


Fig. 3: The tomographic reconstruction of a Bragg fiber. An interferogram (**left**), a tomogram (**right**).

In the second stage of the project the specialized tomographic microinterferometer for optical microobjects measurement was designed and patented [3]. The innovative step was that the necessity of the rotation of the specimen was fully eliminated. Instead, small apertures were dynamically switched on using LCD modulators (Fig. 4). The light beams created with these subapertures are redirected using special element in the way, that the specimen is illuminated from many directions. The multidirectional data is registered on a single CCD camera chip. The new compact measurement system was built as a laboratory setup and the proof of principle was achieved.



Fig. 4: The measurement head of a compact tomographic microinterferometer.

Supported by: DFG (OS 111/20-1)
Project: "TOMI"

References:

- [1] Gorski, W.; Osten, W. "Tomographic imaging of photonic crystal fibers", *Opt.Let.*, Vol. 32, No. 14, 1977-1979 (2007).
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Optical computing using white light interferometry

T. Haist, W. Osten

White-light interferometry is a very powerful technique for measuring the topography of surfaces or for imaging layers within scattering media. Apart from these well-known approaches it can be also used for solving computational difficult problems. To this end, the problem to be solved is coded by optical path lengths and the superposition of all possible paths that a photon can travel is used for computing the solution. The solution itself is chosen by interference with the reference light. Several gedankenexperiments demonstrate how this method can be used for solving computational hard problems.

The core of the method consists of three steps:

1. Code the problem and its solutions by optical path lengths.
2. Use an optical system to generate a superposition of all possible solutions.
3. Find the correct solution by interference-based detection.

The method is strongly related to quantum computing, but works with purely classical waves. No quantum properties like entanglement are involved. Oltean proposed a pulse-based method for computing that is very similar to this interference-based technique. The main idea is the same as here, namely using superposition of all possible solutions which are coded by optical path lengths. Due to the so-called “coherent gain” that is exploited using interferometric detection, interference-based detection is superior for practical implementations.

The method can be used to solve NP-complete problems in linear time (e.g. the Ricochet Robot Problem). This advantage does not come for free. Due to photon statistics, one has an exponential increase in necessary energy with increasing problem

size. For the well known travelling salesman problem we estimate that with 1 W at $\lambda = 1 \mu\text{m}$ problems with 16 cities can be solved.

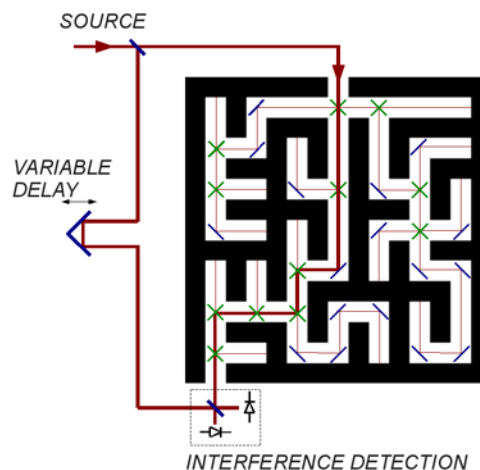


Fig. 1: Simple example for waveoptical computing: Solving the maze by N interferometric measurements if N denotes the number of junctions along the solution path. A photon entering the maze path of the interferometer travels through all possible paths simultaneously.

Apart from the solution of computational hard problems it is also possible to apply the method for the ultra-fast computation of more simple tasks. One important example is the computation of arithmetic expressions. We think that by using the interference it is possible to reduce the computation time and the latency of such computations – even with high precision arithmetic – to a very small delay in the range of some hundred of femtoseconds. The switches are located directly in front of the detectors and therefore the maximum speed that can be achieved by optical means would be reached. To achieve this speed together with high precision, a combination of white light interferometric computing and residue arithmetic is proposed.

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Impressum:

Publisher: Institut für Technische Optik (ITO)
Universität Stuttgart
Pfaffenwaldring 9
D - 70569 Stuttgart
www.uni-stuttgart.de/ito

Editor: Dipl.-Ing. (FH) Erich Steinbeißer steinbeisser@ito.uni-stuttgart.de
Dipl.-Des. Matthias Staufer, mamadesign.net (Graphic & Layout) mail@mamadesign.net

Printing: Breitschuh & Kock GmbH, Kiel

Print run: 400

ISBN 978-3-923560-62-2

