Microscope (Speckle-)Interferometry System for Biomedical Applications

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A microscope (speckle-)interferometry system has been realized which permits the non destructive and full field analysis of displacements and micro-motions of cellular tissue and living cells. For an automated analysis it is investigated if the modulation of the speckle interferograms can be applied for an automated mask generation for the unwrapping process of the difference phase and for the reconstruction of the object structure.

1 Introduction

For the analysis of surface displacements and optical path length changes in cellular range a microscope (speckle-)interferometry system has been realized. Thereby, (speckle-)interferometry enables a non destructive and marker free full field measurement of biological specimens. For a fast and objective analysis of the measurement data an automated data processing is of particular advantage. For this reason, investigations have been carried out if the modulation of (speckle-)interferograms can be applied as a quality parameter for the selection of interferogram data that is suitable for further evaluation, e. g. phase unwrapping and for the reconstruction of the object structure.

2 Experimental Methods

Setup

Fig. 1 depicts the experimental setup of the microscope (speckle-)interferometry system. The coherent light of a frequency doubled Nd:YAG laser (λ= 532 nm) is separated in an object wave and a reference wave. The object illumination can performed either with incident light or in transmission arrangement for the investigation of transparent probes. The investigated sample is imaged by a microscope lens onto a CCD-sensor (Sony XCD X700). The light of the reference wave is guided directly over a beam splitter cube onto the CCD sensor to interfere with the (back-)scattered light of the object wave. The adjustment of the speckle size and the suppression of scattered light for investigations in transmission arrangement is performed by an aperture behind the microscope lens.

Phase difference determination and application of the modulation

The quantitative detection of surface displacements and optical path length changes is performed by a spatial phase shifting method [1]. For this purpose, the phase difference mod 2π is determined pixelwise from neighboring CCD-pixels of the recorded (speckle-)interferograms. Furthermore, the modulation distributions of the (speckle-) interferograms are calculated [2]. In transmission arrangement the modulation is applied to reconstruct the object structure from the spatial phase shifted interferograms. For incident light arrangement a mask for the phase difference is generated by application of a threshold value for the modulation. For further evaluation, the mask is applied for automated unwrapping of the phase difference.

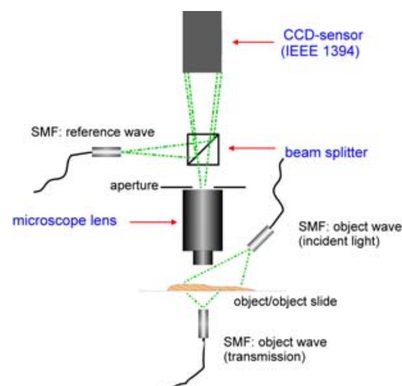

Abb. 1 *Setup for a microscope (speckle-)interferometer. Light source: frequency doubled cw-Nd:YAG laser (λ = 532 nm); CCD sensor: Sony XCD X700 (IEEE 1394 standard); SMF: single mode fibers for object illumination and reference wave; the illumination of the sample can be performed with incident light or in transmission arrangement; AP: aperture for regulation of the speckle size and suppression of scattered light.*

By taking into account the geometry of the optical arrangement the surface displacement or the optical path length changes can be determined.

3 Results

Fig. 2 shows results from investigations on the interferometer's lateral resolution. For this purpose, an USAF 1951 Test Chart (transmission type) is imaged by a 40x magnifying immersion microscope lens (NA 0.8). Fig. 2(a) depicts the interferogram. In Fig. 2(b) the contrast enhanced reconstruction of the object structure by means of

the modulation is shown. A lateral resolution of 2.1 µm is obtained.

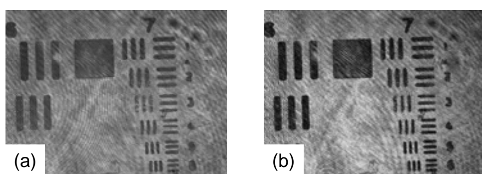

***Fig. 2:** (a) Interferogram and (b) reconstructed object structure γ of an USAF 1951 Test Chart (transmission type). A lateral resolution of 2.1 µm is obtained.*

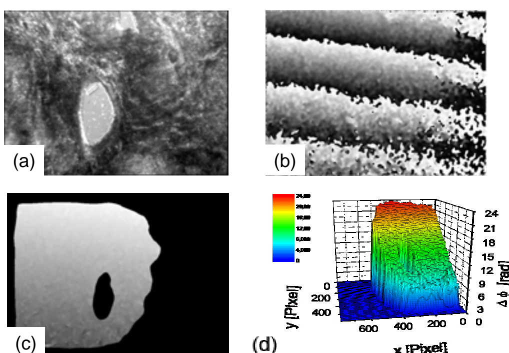

***Fig. 3:** Results obtained by application of a 10x magnifying microscope lens (NA 0.25). (a) White light image of the investigated area of a tumorous ovary fixed on an object slide; (b) smoothed phase difference distribution mod 2π provoked by tilting the probe; (c) unwrapped phase difference distribution superposed with the generated mask; (d) pseudo-3d-plot of (c).*

Fig 3 shows results of investigations by application of a 10x magnifying microscope lens (NA 0.25). A tumorous ovary fixed on a object slide has been investigated with incident light. Fig. 3(a) shows the white light image of the investigated area. In Fig. 3(b) the smoothed difference phase mod 2π provoked by tilting the probe is depicted. The unwrapping process of the phase difference is supported by a mask generated by means of the modulation. Therefore, areas with low reflectivity are masked out (see Fig. 3(c) and 3(d)).

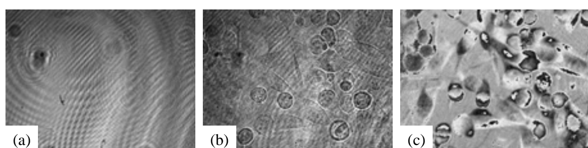

***Fig. 4** Results of investigations on living tumorous human liver cells (HepG2) in culture medium (transmission arrangement, 40x magnifying immersion microscope lens); (a) interferogram, (b) reconstructed object structure γ,* (c) phase difference distribution mod 2π.

In Fig. 4 results of investigations on living tumorous human liver cells (HepG2) in culture medium (transmission arrangement, 40x immersion microscope lens) are shown. Fig. 4(a) shows the interferogram. In Fig. 4(b) the contrast enhanced reconstructed object structure γ is depicted. In Fig. 4(c) the corresponding phase difference mod 2π after 30 min is presented visualizing microchanges of the sample.

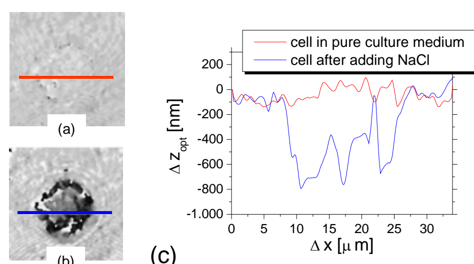

***Fig. 5**: (a) phase difference distribution mod 2π in pure culture medium and (b) after adding NaCl solution. (c) cross section representing the change of the optical path length Δz_{opt} (a) red line (cell in pure culture medium), (b) blue line (cell in culture medium after adding NaCl solution).*

Fig. 5(a) shows the phase difference distribution mod 2π for a single cell in pure culture medium and (b) after adding NaCl solution to the culture medium. The cross sections representing the change of the optical path length Δz_{opt} (red line: cell in pure culture medium, blue line: cell in culture medium after adding NaCl solution) demonstrate that the addition of NaCl solution to the culture medium provokes a contraction of the cells.

4 Discussion and Conclusion

A microscope (speckle-)interferometry system for the detection of surface displacements and optical path length changes has been realized. The results of investigations on biological probes for incident light arrangement show that the modulation of the (speckle-)interferograms is an objective parameter for mask generation for the phase unwrapping process of phase difference data. Furthermore, for investigation in transmission arrangement, a contrast enhanced reconstruction of the object structure by means of the modulation is possible. In conclusion, the modulation of the (speckle-)interferograms is a versatile parameter for the automated analysis of displacements and micro-motions of cellular tissue and living cells.

5 Acknowledgement

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Literature

[1] B. Kemper, J. Kandulla, D. Dirksen, G. von Bally: *Optimization of spatial phase shifting in endoscopic electronic speckle pattern interferometry*, Optics Communications, **217,** 151-160 (2003).

[2] S. Knoche, B. Kemper, J. Kandulla, W. Avenhaus, G. von Bally: *Investigations on Automated Fringe Analysis in Endoscopic Electronic Speckle Pattern Interferometry and Application on Biological Objects*, OWLS VII, in press.