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Deformation Analysis of Biological Modelsystems

1.INTRODUCTION

In the course of their lifetime cells are exposed to different kinds of mechanical stress. They have several methods to resist these forces. The understanding of these mechanisms gives a deeper insight into the metabolism of the cell. Researches on that matter lead for an example to the development and new approaches on different types of therapy. Aim of this work is to introduce a new optical technique to this field of Life science, the electronic speckle pattern interferometry. Primarily from the field of material science, this technique has the ability to visualise deformation in the scale of micrometer and below. Our working group developed a measurement setup based on ESPI with which we are able to analyze Microsystems in its different states of deformation and vibration (Jia *et al.* 1994). Main advantage of this technique is that we can test the system “as received” without any previous preparation. Outcome of this was the question if we were able to use ESPI with an extreme high magnification on complete plane samples. These samples show no significant roughness and can’t be even roughen. Furthermore biological samples have to be kept in liquid solution during the experiments to measure “in-vivo” which means under nearly natural conditions. Within this work these problems could be solved and a three-dimensional deformation could be investigated.

2. CELLS

Living cells in a human body are constantly being stressed and strained. These mechanical forces have a strong influence on the cells. Depending on the direction and power of these mechanical stress cells have different mechanisms to withstand. One important key role plays cytoskeleton. This polymer consists of several semi flexible proteins and shows viscous elastic properties which differ from common synthetic (Georges *et al*. 2004). Beside the cytoskeleton the other cell compartments like cell core (Rowat *et al.* 2006), (Gruenbaum *et al*. 2005) and the cell membrane (Mitchison *et al*. 1954) play also a significant role for the stress resistance. Several researches gave proof, that changes in the mechanical characteristics such as deformation behaviour are connected to changes of the cell physiology (Lim *et al*. 2006) and are often caused by changes in biological functions.

3. TECHNOLOGY

The Electronic Speckle Pattern Interferometry (ESPI) is a laser based technique which is able to visualize static and dynamic displacements. Leendertz and his co-workers first showed it's applicability for the contactless determination of deformations (Leendertz *et al.* 1970). Usually located in the field of engineering technology (Wang et al. 1998), (Jin et al. 1998) this technology is able to measure displacements with a high precision. The specimen normally are meso- or macroscopic in size with lateral dimensions typically ranging from centimetres to some meters. Basic principle of ESPI is the creation of a so called speckle pattern. The component under investigation must have an optically rough surface so that when it is illuminated by an expanded laser beam. Each asperity is origin of a new elementary wave. These waves interfere with each other and result is a pattern of changing intensity maxima and minima (Bauer *et al.* 1991), the image formed is a subjective speckle pattern. A second light field, known as the reference beam, is derived from the same laser beam and is superimposed with the first field. Both fields interfere with each other and result is a speckle pattern. The speckle pattern is scattered from a finite area of the object, and its phase, amplitude and intensity, which are all random, are directly related to the microstructure of that area in the object. Therefore a speckle pattern can be understood as some sort of “fingerprint” of the surface at a certain state. If the object is displaced or deformed, the distance between object and image will change, and hence the phase of the image speckle pattern will change. To visualise this effect, the image and reference beams are combined on a video camera and recorded. In general to measure a displacement two speckle pictures are taken (two pictures and two states) and the difference between both is mathematical calculated. Result is a visualization of the surface deformation between both states.

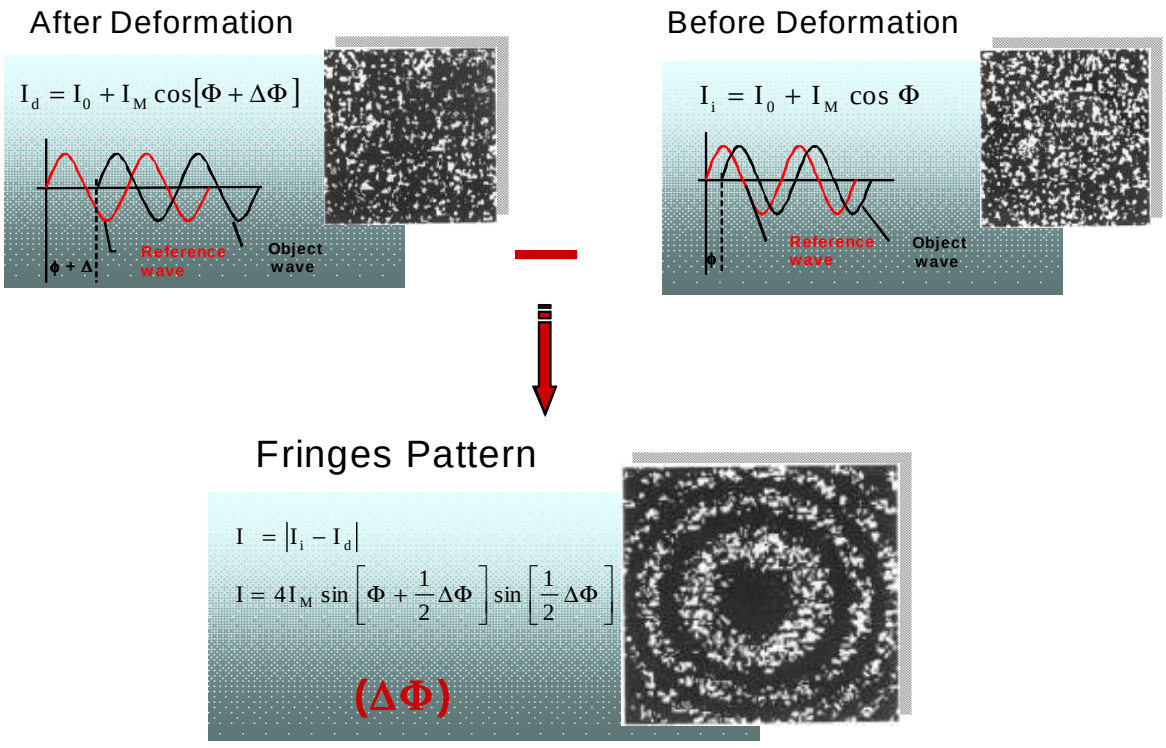

Figure 1: creation of a speckle pattern, two speckle patterns taken each in one state of the deformation (above) and computer addid calculation of difference between both, so called finges pattern (below)

4. EXPERIMENTAL RESULTS

4.1 Drop of Glycerine

To verify our measurement system several so called reference experiments has been realized. Aim was to gain information about the accuracy of our system. As first example, the vibration behaviour of a drop of glycerine was measured via ESPI. Figure 2 show the results of this experiment. As Reference

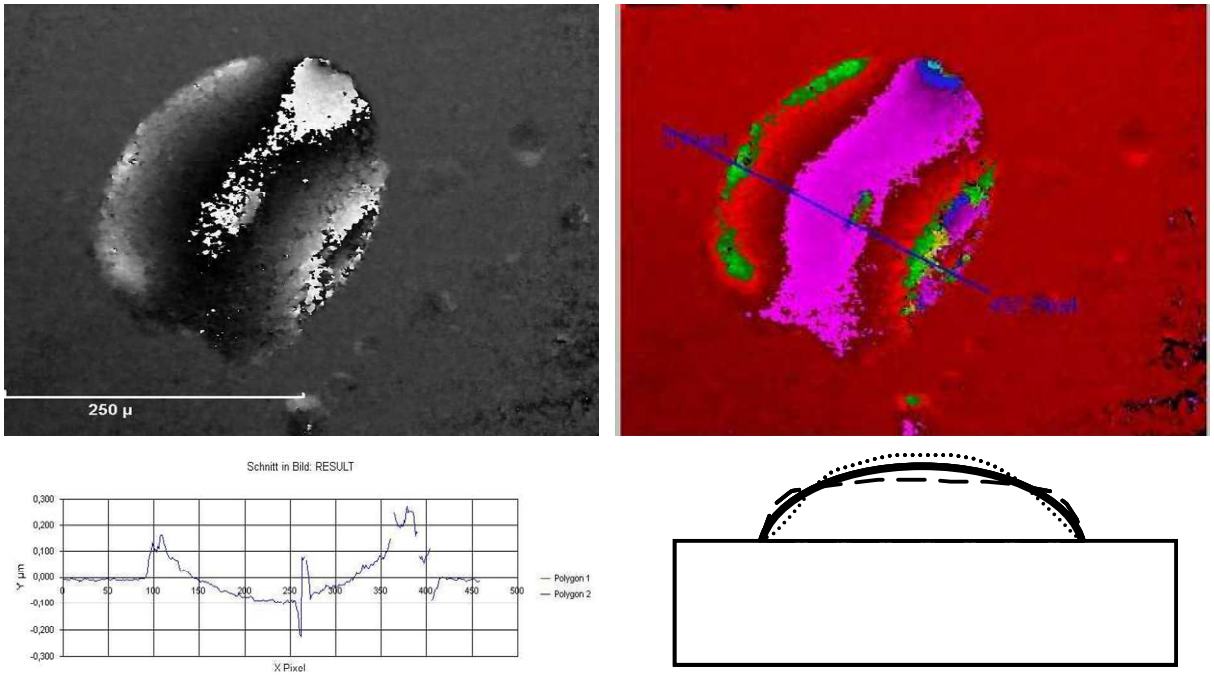

Figure 2: Single drop of glycerin on a glas plate, excited via a piezo with 56 kHz, phasepicture of the drop (left), in false color display (right) and model for the vibration behavior (below)

an image of an not excited drop was used. The model for the vibration behaviour of the glycerine drop shows a snapshot of a so called standing wave. At the edges the surface increases and decreases in the middle. In this experiment the maximum of displacement could be measured around 100 – 200 nm. Difference between the shown vibration behaviour and an idealised sinus wave is caused due to a) the shape of the glycerine drop (thin at its edges and thick in the middle) and b) the accuracy of measurement when using ESPI on transparent samples.

4.2 Forisom

Aim of our work is to establish ESPI as a tool in the field of life science. On this account we selected as next sample a protein complex, a so called “Forisom”. This protein complex has all optical characteristics as a living cell (transparent, in liquid solution, smooth surface and just some micro meters in its dimension) and furthermore can be excited to elongation via an electric impulse. Figure 3 shows a Forisom through the optic of a microscope and also a dataset of the three dimensional deformation measured via ESPI. For the first time it was possible to visualize a biological micro object in 3D during its deformation. This experiment proofs, ESPI can successfully be used on biological samples. In a second experiment the relaxation of the Forisom was also detected via ESPI. Furthermore single protein strains inside of the Forisom could be visualized during this experiment (figure 4).

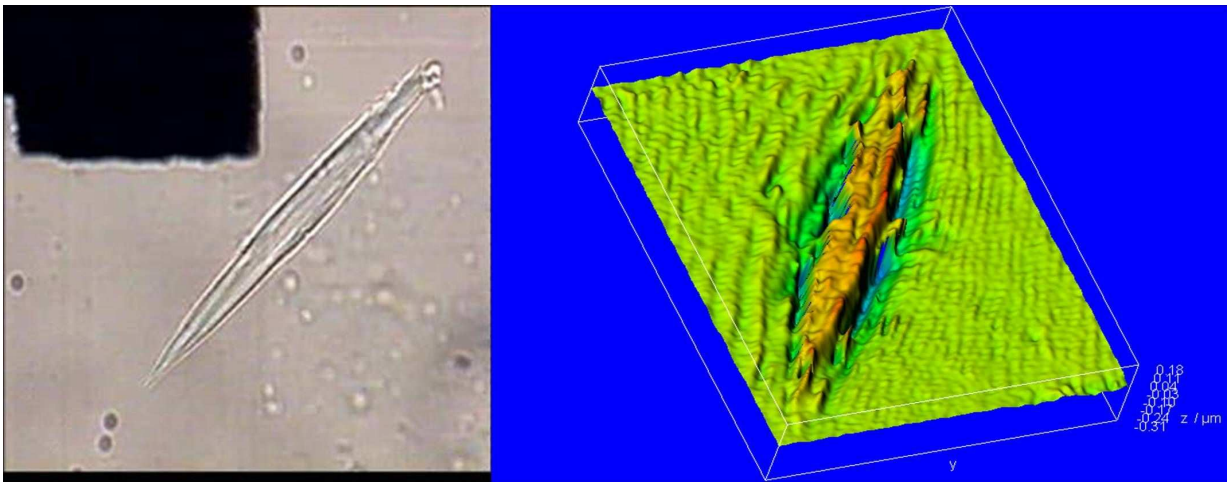

Figure 3: Forisom, 30 µm length and 5 µm width, photo (left) and 3d deformation visualized via ESPI (right)

To minimize the influence of artifacts a last speckle pattern of the forisom was recorded and analysed via ESPI after the full relaxation of the forisom. As expected no surface deformation could be detected (figure 5).

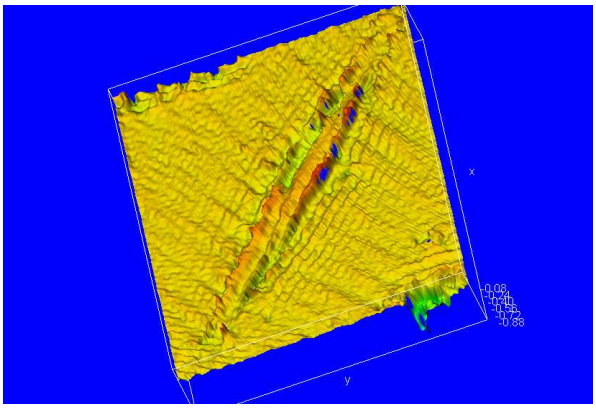

Figure 4: Forisom measured during relaxation, single protein strain (1µm width) are visible in 3D-Model and can be measured via ESPI

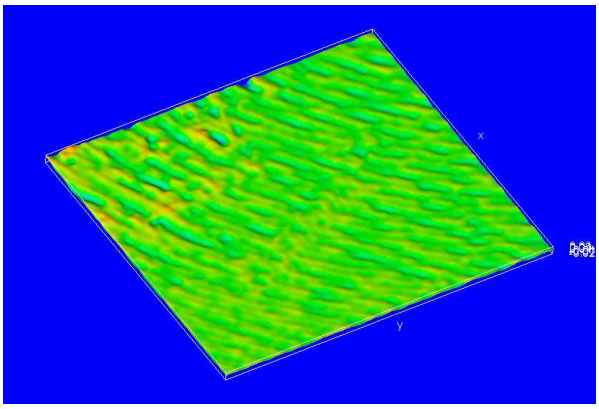

Figure 5: Correlationexperiment to determine the accuracy of Visualisation of surface deformation and zero point, no significant surface deformation could be detected

4.3 Deformation of a cell

In literature several techniques to deform biological samples are already known and verified (Lim *et al.* 2006). In our first series of experiments we wanted to deform cells in an inhomogeneous electric field (Guido *et al*. 2006). By using an ITO etched electrode array we were able to deform the used HL-60 cells depending of the electric field strength. In figure 6 two electrodes and the used cells are shown. For the measurement the cell solution was put over the electrodes. When switching on the electric field movement and attachment of the cells at the electrodes could be monitored. Via modulation of the electric field we were able to measure the shape change of the cells and visualize this with ESPI.

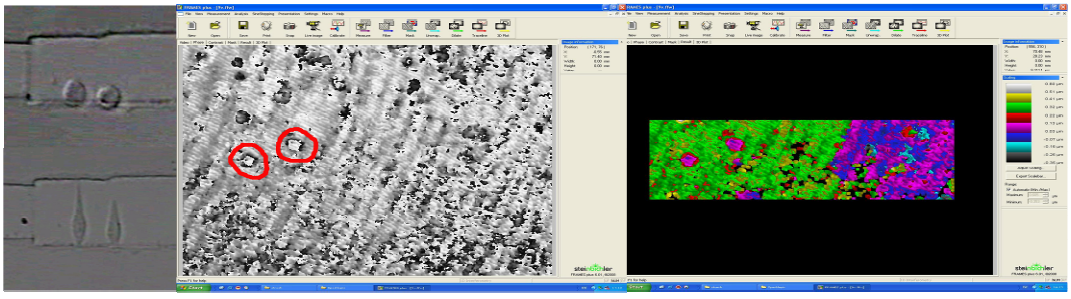

Figure 6: deformation of HL-60 cells in an electric field (left), contrast image (middel) and demodulated phase picture (right)

5. OUTLOOK

In this work we shown the ability to detect and visualize the deformation of biological samples with our ESPI setup. Occurring problems could be solved and for the first time three-dimensional deformation of could be visualized via ESPI. In the future we want to modify our system to gain information from inside the cell and single cell compartments. Later on we want to correlate the experimental data with the biological background of the cell and create a vitality test system, which is mainly based on physical parameter.

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