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Induced cellular deformation as a standardized measurement method based on modified Micro-ESPI

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Summary

During the course of their lifetime cells are exposed to different kinds of mechanical stress. These mechanical effects have a strong influence on a single cell, aggregated cells, their structure and metabolism especially in case of immunologic reaction similar to a chemical stimuli.Aim of this work is to introduce a modified electronic speckle pattern interferometry (ESPI) setup to this field of science to validate induced deformations in certain biological samples contact-free and thus close to their natural conditions.In the long term it is the aim to develop a “set” of these reference values and to use them in term of preliminary analysis for medical treatment.In conclusion this could lead to complete new approach in cell based analysis.

Introduction

During the course of their lifetime cells are exposed to different kinds of mechanical stress. These mechanical effects have a strong influence on a single cell, aggregated cells, their structure and metabolism especially in case of an immunologic reaction similar to a chemical stimuli. One important factor for the mechanical characteristics of a “cell” is for example the cytoskeleton. In the past time it has been proven that in most cases a changing of the mechanical behavior of a complete cell or its single components occurs due to a change of the integrity which can be ascribed to a mutation or malfunction of a biological function or even vitality. The understanding of these mechanisms gives a deeper insight into the metabolism of the cell especially in case immunologic reaction. Different researches on that matter lead to a better understanding of cell intern mechanisms and helped the development of new approaches on different types of therapy.

Aim of this work is to introduce the electronic speckle pattern interferometry (ESPI) to this field of science. By using the ESPI it is for example possible to validate induced deformations in certain biological samples contact-free and thus close to their natural conditions. Based on this advantage is would be possible to generate a parameters for the mechanical properties of cells and also their vitality. In conclusion this could lead to complete new approach in cell based analysis.

Approach

Before performing the first analysis using the Micro ESPI System on different types of cells a special mount system was needed. This system had to be capable of containing the used adhesion medium and a certain volume of reaction fluids including the excitation substance itself. Therefore two different designs one for each used objective (Long Distance Objective and Immersions Objective) were developed (Figure 1). Both designs were manufactured using a CNC portal milling machine from “HaaseComputertechnik” and consist of biocompatible Polycarbonate. Polycarbonate is autoclavable and therefore suitable for the use in cellculture technology.In preparation of each experiment HeLa cells (cervix carcinoma cells) were seeded in a concentration of $3*10^4$ cell/ml and incubated for 24h at 37°C and 5% CO_2. As adhesion medium a 10x10mm dimensioned piece of a silicon wafer was used. After placing the adhesion medium under the objective the intensities of the reference- and object beam were adjusted and the complete measurement setup was calibrated. To start the experiment an untreated cell was selected as reference state and the deformation was induced using an excitation reagent. After the completion of thecellular reactionordeformation a measurement image was generatedand analyzed and the static out-of-plane deformationwas recorded.

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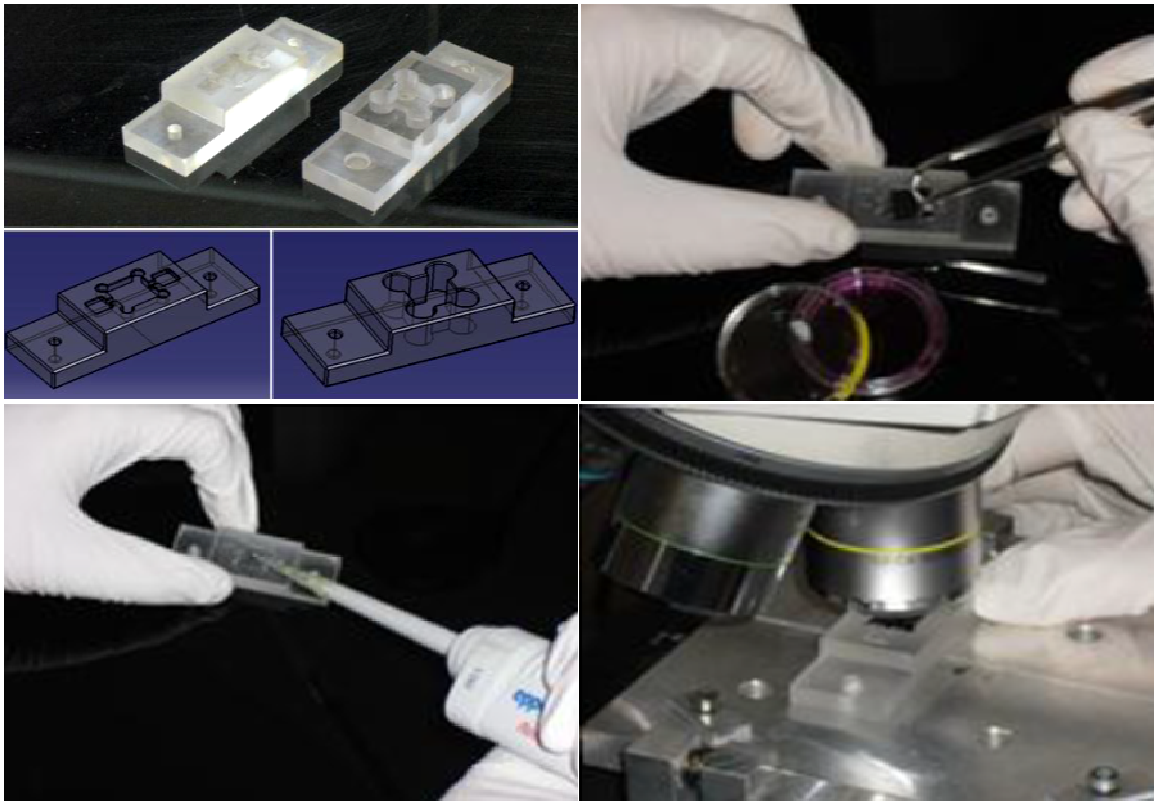


Figure 1: Preparation of the specimen before the measurement; both mount systems (upper left), placement of the adhesion medium (upper right), add of buffer (lower left) and in the direct measuring range (lower right)

Deformation Reaction

For the developmentofthismethod the usedHeLacells werestimulate to perform a specific cellmovementor deformationthrough varioussubstances. As excitation substances Trypsin / EDTA (celldetaches from theadhesion medium), DMSO (necrotic reaction) and Ceramid-6 (leads to an apoptosis reaction) were used and preheated to 37°C.

For the first experiments to measure the detachment of the HeLa cells from the adhesion medium Trypsin / EDTA was used in a 1:1 ratio against the provided isotonic buffer (PBS). At this point the adhesion medium was completely covered with PBS. Directly after the Trypsin / EDTA was added the reference state was recorded and the measurement started. In case of the necrotic reaction 20 µl of a 4% DMSO solution was used to induce the aspired deformation. Similar to the previous experiment the adhesion medium was completely covered with PBS. To measure the cellular behavior during an apoptosis reaction a solution of Cermaid-6 (c= 200µM/ml) was used. This solution was addeddirectlyonto theadhesion mediumwithout any buffer.

0Results

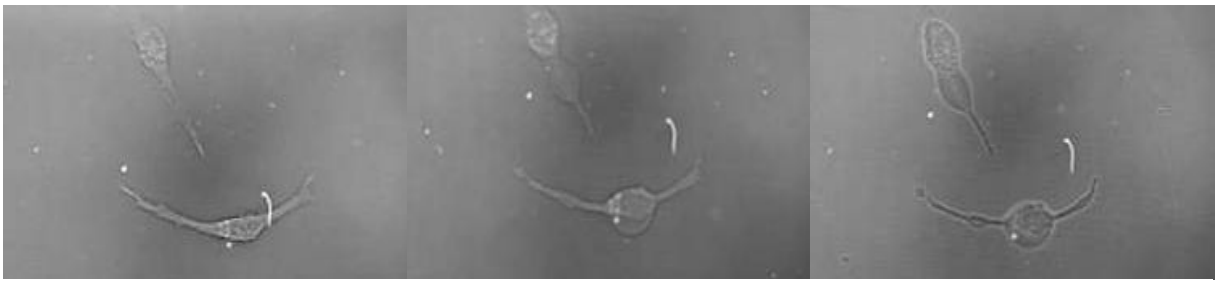
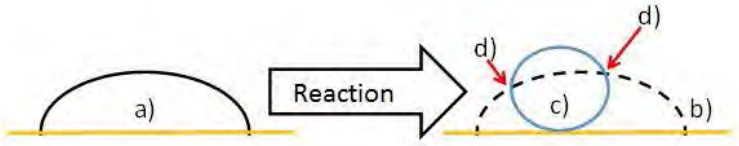


Figure 2: Necrotic deformation of HeLa cell due to osmotic and thermic shock (5 minutes measurement time under white light microscope 200times magnification)

By using Trypsin / EDTA it was possible to induce reproducible the aspired cellular deformation. Furthermore a certain edge blur was detected at the transition between these two states in the final result image. These occurredbecause nomeasurablesurfacemodificationwas detected at this specific part of the cell (see figure 3). Also an additional deformation was detected and whose originsmay lie inthesurfacemovementsof theliquid layer. One possible explanation is, that maybe due to the energy input from the laser a temperature gradient was created and therefore a flow in the liquid occurred



which was finally recorded as a surface deformation.However was it possible to visualize both states (reference state and the detached cell) with the Micro ESPI System.Hereby the complete reaction from the originalcell (still attached to the adhesion medium) to theroundedcell (completely detached cell) was recorded in each image. The results of one exemplarymeasurement are shown in figure 4.

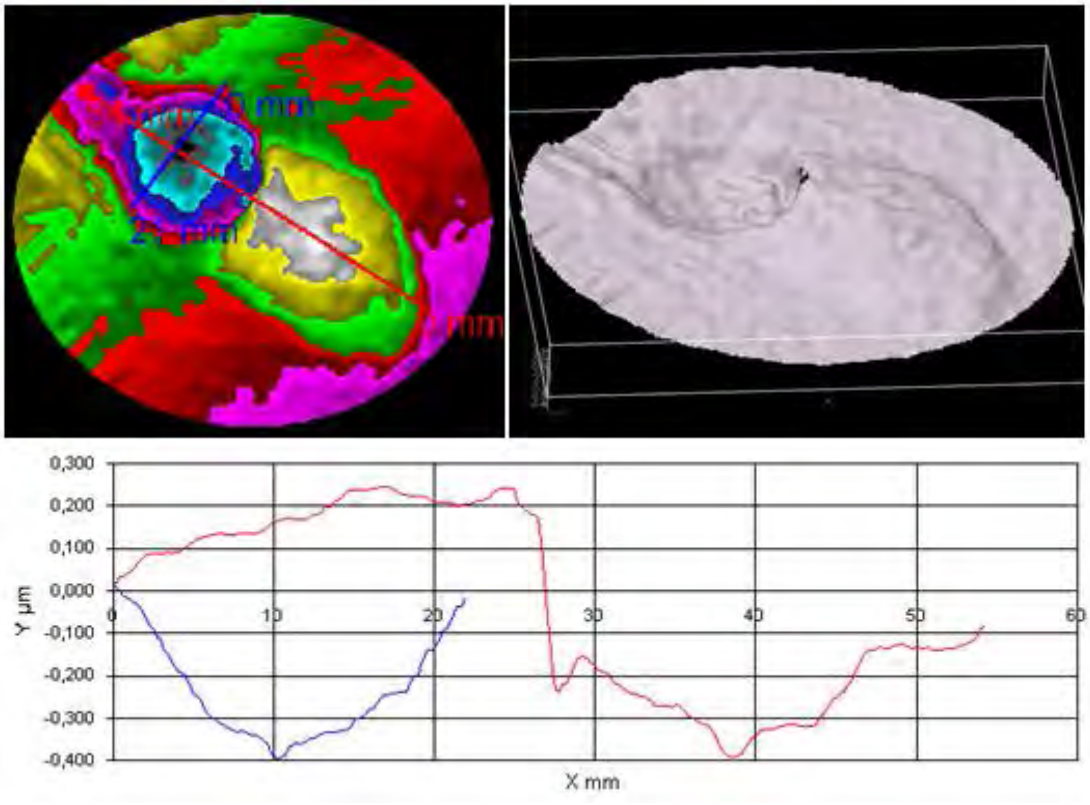
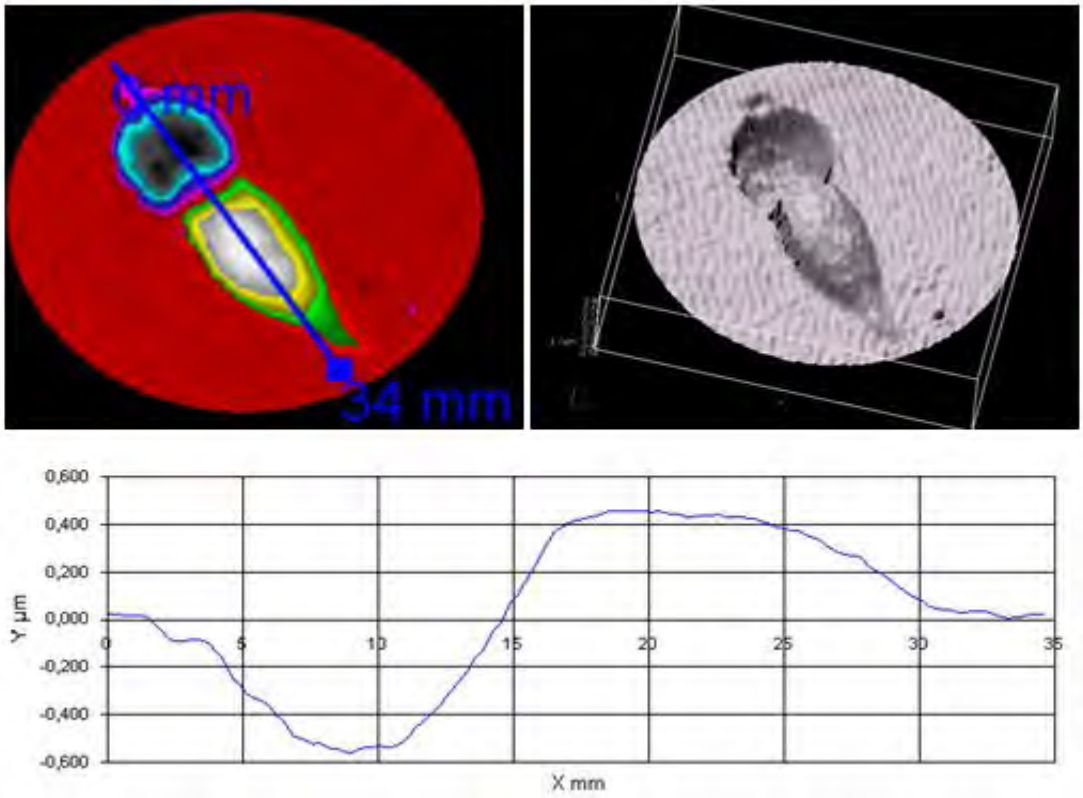


Figure 4: Results of the Trypsin / EDTA treatedHeLacells (long distance); result phase image (upper left), visualized 3D deformation (upper right) and surface trace lines (lower image)

In the second experimental approach it was possible to record the complex processof an induced cellular necrosisof aHeLacell. Using the Micro ESPI System the complete necrotic reaction was visualized with a very high accuracy (figure 5). As expected the cell membrane was perforated by the DMSO which enabled the surrounding liquid to stream into the cell and therefore caused the lysis of the cell. Similar to the previous experiments also an edge blur between the original cell and the necrotic cell was detected.



The next approachfor thegeneral applicability of thismethod of measurement was the usage of Ceramid-6 as stimulation substance to induce individualapoptoticstates. A cleartransition fromapoptosistosecondarynecrosis was detected using the Micro ESPI System and is shown in the phase image (figure 6). This was indicated by the merging of thecharacteristicmembranepinchesof apoptosisto largersections.

By meansof the experiments that were performedin this worka first versionof a generalmeasurement approach for theinvestigationof cellulardeformationsusingtheMicro ESPI was created. As shown this technique is already capable of detecting simple and also complex cellular

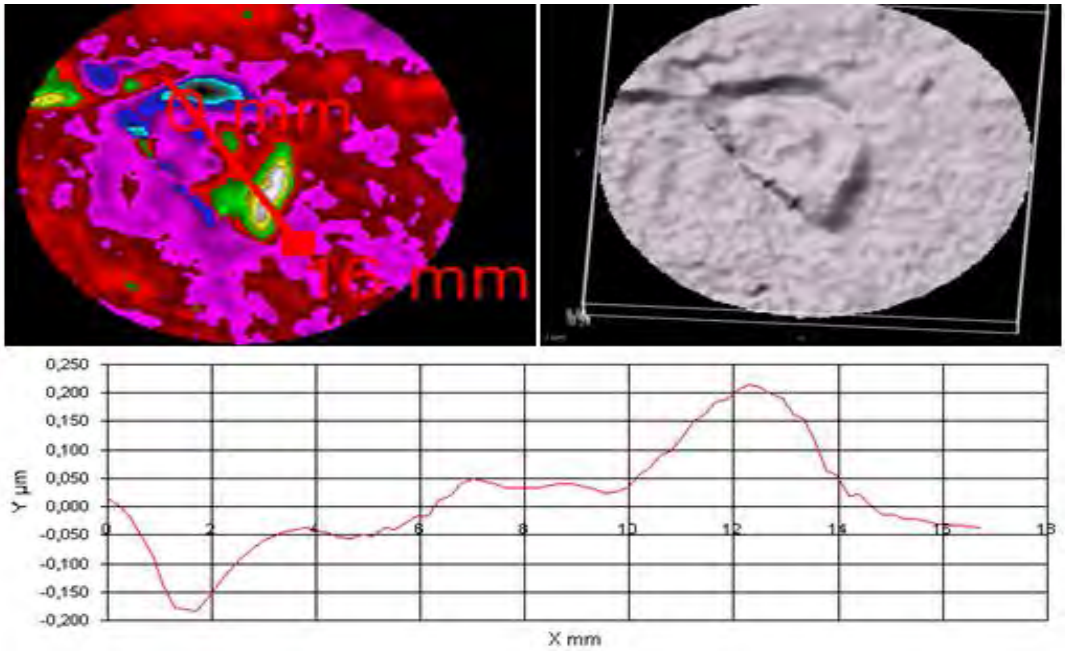


Figure 6: Results of the Cermid-6 treated HeLa cells (long distance); result phase image (upper left), visualized 3D deformation (upper right) and surface trace lines (lower image)

movements or deformation. Other advantages of this method like its capability of a nondestructive and invitro measurement without the need for a complex modification were demonstrated in this work. Thus this presented approach is suitable to analyze different specimen and a wide range ofmorphologicalcellular processesin a relative stress-free environment. This developed method includes a basic approachfor the treatment of biological samples, the general structureof the experimentalmeasuring device and an example of a complete calibration andmeasurement cycle at a self-selected sample. Additionally the measurement method also includes the evaluation ofgeneratedtest resultsand their interpretation.

Outlook

However the used setup and therefore including the developed measurement method are currently inan experimental stage andthus still the subjectof a continuousdevelopment process. Both showat several pointspotential for modifications. One possible approach would be the implementation of an external control system to handle the environmentalparameters like for example the temperature and the concentration of particles within thedirectmeasuring range. This possible modification would increase the stability during a measurement and thus also increase the quality of themeasurements itself.

Another approach could include the modification of the hardware and the complete measurement setup to a partial automated system. This would facilitate the adjustment and maintenance of the Micro ESPI System and maybe even decrease the possibility of a human error.

Additionally it should be pointed out that the in this work used setup was mainly configured to analyze and measure deformations of macroscopic specimen. The original setup was strongly modified but sadly some minor problems dealing with the used measurement software (for example the lowest selectable dimension for a trace line is in the range of mm) still needs to be fixed.

The developed measurement method is capable to detect and analyze simple cellular deformation like the detachment from an adhesion medium or more complex reactions like necrotic and apoptosis reactions. Further additional cellular reactions are possible and would increase the bandwidth of the method. Possible application for this combined approach of Micro ESPI System and the developed method could be found in case of vitality analysis or drug screening. The long-termgoal of this workis to establishtheMicro ESPI as a new “standard” toolin the fields of biotechnologyand biomedicine.

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