

TIME RESOLVED ACOUSTIC MICROSCOPY OF SINGLE CELLS

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1. INTRODUCTION

The local elastic properties of living cells are important to the understanding of cellular processes such as volume regulation, attachment, migration, division or contraction. Since a mechanical wave i.e. sound interacts with the elastic properties it is possible to image and measure these properties directly with ultrasound. Using focused high frequency ultrasound with center frequency of 1 GHz a lateral resolution of $1\mu\text{m}$ is possible. Therefore in combination with optical microscopy the full spectrum of optical and elastic properties is accessible.

2. METHODS

Direct digitisation of the high frequency echo signal allows the separation of cell front echo and substratum echo on which the cells are grown. To form a 2D image the acoustic lens is scanned over the cell and the changes of the substratum echo amplitude are coded as greyscale. Contrast in the images arises mainly from the local absorption of sound in the cell which is related to the local polymerisation state.

For quantitative measurements longer integration times are needed. Therefore the lens is moved to a point of interested and the local density, the sound speed, the absorption coefficient and the local cell height are derived from the front echo of the cell and the substrate echo. To account for the PSF of the acoustic lens the measurements are taken at different focus positions. The theoretical acoustic response function of the cell is calculated and a correction factor for the acoustic parameters is derived from the response function.

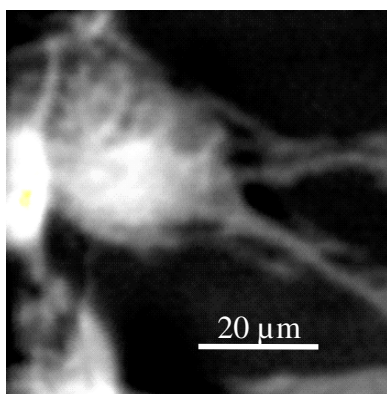


Figure 1: Acoustic image from C2C12 myoblast taken at 1GHz. A connected network of white lines show the cytoskeleton.

3. MATERIALS

Different cell types were grown on rimmed cover slides. The cells were stained for cell nucleus, mitochondria and f-actin using fluorescence stains. Acoustic images were taken with 1GHz center frequency and lateral resolution of $0.16\mu\text{m}$ with the focus set $1\mu\text{m}$ above the substrate. Synchronous fluorescence images were taken with a 40x objective and a Hamamatsu ccd digital camera.

4. RESULTS

Different features in the acoustic images could be identified as the actin filaments of the cytoskeleton the nucleus and the nucleolus by comparing fluorescence and acoustical images. Quantitative measurements showed an increase of sound speed for the actin filaments compared to the surrounding cytosol. In summary acoustic Microscopy has proven to be a valuable tool for investigation of elastic properties of single living cells.