Collagen Fiber Displacements around Single Cells and Multicellular Sprouts

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***Introduction*:** Cell-matrix mechanical interactions play a key role in a variety of physiological processes. Recently, the quantification of cellular tractions by means of traction force microscopy (TFM) has been extended to 3D cell cultures. Cell traction-induced matrix deformations are mainly obtained from the displacements of a large number of fiducial markers (fluorescent beads), which could alter the matrix mechanical properties. Here, we show for two different 3D culture systems how full field displacements induced by cells embedded in a fibrillar matrix like collagen type I can be obtained without the need for fluorescent beads.
***Materials and Methods*:** Human fetal lung fibroblasts (MRC-5) were embedded as single cells in collagen type I hydrogel and allowed to contract the matrix for 18 hours. Human umbilical vein endothelial cells (HUVEC) were seeded on top of a collagen gel and induced with pro-angiogenic factors to form multicellular sprouts. As control for the calculation of the matrix deformations, 200 nm fluorescent beads were attached to the collagen fibers. Second harmonic generation (SHG) and laser-scanning confocal microscopy were used to acquire Z-stacks of label-free collagen fibers and fluorescent beads, respectively, during the live imaging before and after cytochalasin induced relaxation of the cells. The calculation of the matrix deformations was formulated as a B-spline –based 3D non-rigid image registration process that warps the image of the stressed gel to match the image of the gel after relaxation. The calculation of these displacements was independently performed from both fiber and bead images.
***Results and discussion*:** Fibroblasts showed a strong contractile behavior and relaxation of the cells resulted in an elaborate pattern around the cells with numerous fiber displacements towards the cell centroid (left figure). HUVEC sprouts induced smaller deformations and the relaxation of the sprouts resulted in more subtle fiber displacements (right figure). Additionally, we observed that the recovered displacements from the label-free fiber images were equivalent or more detailed than the ones obtained from bead images.
***Conclusion*:** Our methodology allows a marker-free mapping of 3D collagen matrix displacements around both single cells and multicellular sprouts. Additional experiments are currently ongoing to more quantitatively compare SHG-based and bead-based displacement fields.
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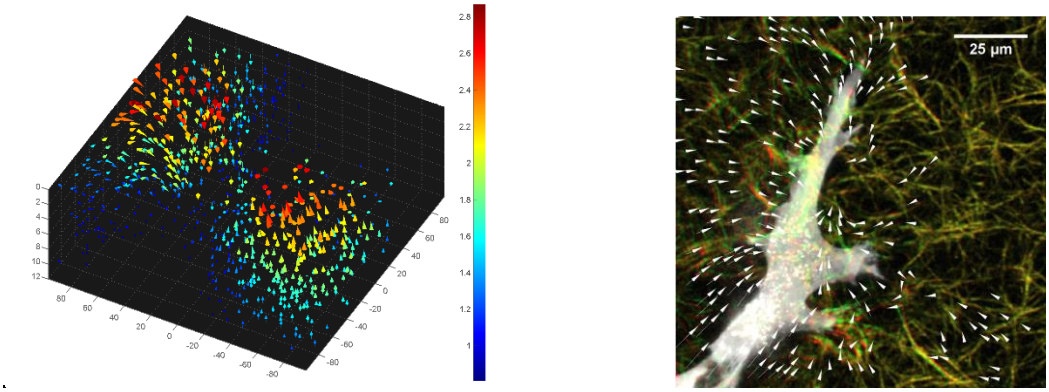

Figure 1: Displacement field (in µm) around MRC-5 cell in collagen (left) and displacement directions (arrows) of collagen fibers (green =stressed, red=relaxed) around HUVEC tip cell (white)

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