4D Traction Force Optical Coherence Microscopy for the Analysis of Single and Collective Cell Behavior

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Abstract

Traction force microscopy (TFM) is a family of optical techniques used to quantify **cell traction forces** (CTFs), which play an important role in many physiological and pathological processes.[1-7] 3D TFM is typically performed with confocal fluorescence microscopy, and can suffer from limited penetration depth, insufficient acquisition speeds, photobleaching, and phototoxicity. We recently developed **traction force optical coherence microscopy** (TF-OCM)[8,9] to overcome these limitations and enable 4D TFM for the study of both single cells and multi-cellular constructs embedded in scattering media. Here, we present prior and ongoing developments in our TF-OCM methods and their potential for enabling novel research in **mechanobiology**.

Methods

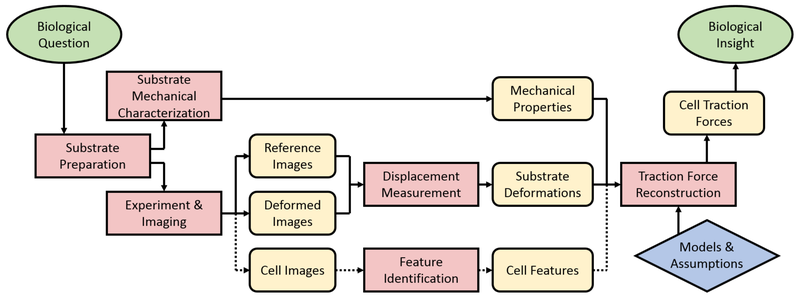
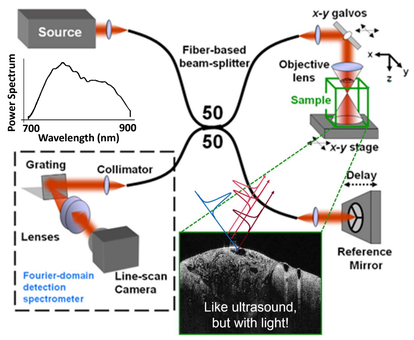


Fig. 1: Universal TFM workflow. Optical imaging records substrate structure in at least two states: a reference state (when CTFs are not present and the substrate is not deformed) and a deformed state (when the substrate is deformed by CTFs). Depending on the TFM method used, images may also provide measurements of the location and shape of cell collectives, individual cells, and/or sub-cellular features. These data are combined to quantify CTFs via numerical reconstruction.



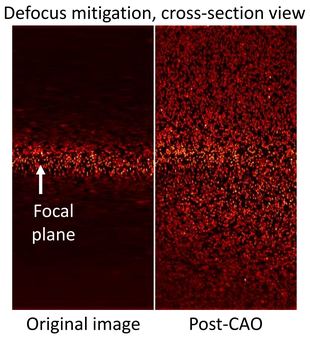


Fig. 2: Optical coherence microscopy (OCM) and computational adaptive optics (CAO) enable rapid volumetric imaging with cellular resolution. (a) OCM is a label-free, interferometric imaging modality which measures the magnitude and phase of backscattered light. A combination of near infrared illumination and parallelized signal acquisition along depth enable OCM to provide rapid 3D imaging in highly scattering media, without concern for photobleaching/phototoxicity. However, regions outside the focal plane are significantly degraded by defocus. (b) CAO leverages principles from synthetic aperture radar and digital holography to mitigate optical aberrations and defocus in post-processing. This dramatically increases the spatiotemporal throughput of OCM, providing cellular resolution over extended depth ranges with 'single-shot' acquisition[10,11].

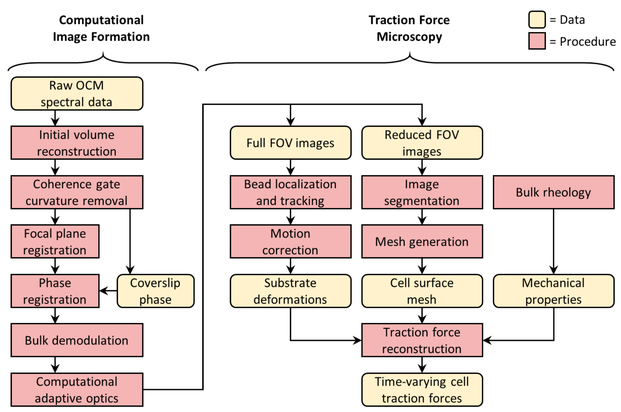


Fig. 3: Data processing workflow of TF-OCM. A novel computational image formation procedure (left column) was developed to maximize volumetric coverage, resolution, and signal-to-noise ratio of OCM images while minimizing geometric distortions[9]. The traction force microscopy module (right) reconstructs CTFs by solving an inverse problem via a Finite Element Method framework[9,12] (see Fig. 4).

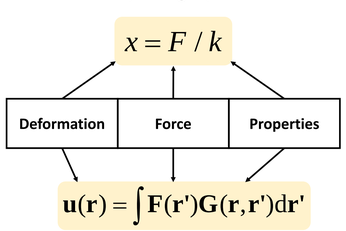


Fig. 4: Principle of CTF reconstruction. Custom FEM software[12] solves an inverse problem which reconstructs CTFs from measurements of substrate deformations and mechanical properties. Note that **u**, **F**, and **G**, refer to the 3D substrate displacement field, CTF field, and Green's tensor function, respectively. Currently, a linear elastic model is used. However, extensions to nonlinear and viscoelastic models may improve reconstruction accuracy in materials like collagen and fibrin.

TF-OCM for Single Cells

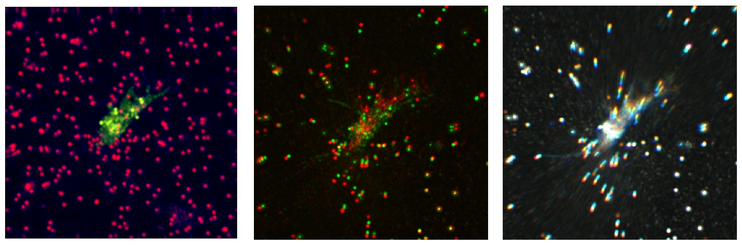


Fig. 5: Imaging of CTF-induced substrate deformations for 4D TF-OCM of single cells. Scattering beads (left, red channel) and cells (left, green channel) are embedded in a 3D hydrogel substrate. OCM captures CTF-induced deformations of the substrate by imaging bead motion between pairs of time points (center, color channels depict distinct time points). These deformations can be imaged repeatedly with minute-scale temporal sampling over experiments spanning hours to days (right, color channels depict distinct time points).

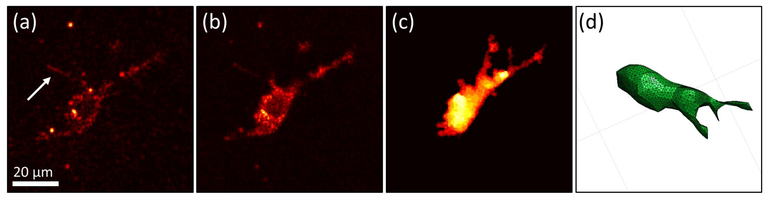


Fig. 6: Temporal speckle contrast enables automated image segmentation. Using *temporal* speckle fluctuations to suppress *spatial* speckle fluctuations, image segmentation is readily performed over tens to hundreds of volumetric images per experiment. Segmented images provide a record of time-varying cell morphology, and are converted into discrete meshes, which are then used for CTF reconstruction via the Finite Element Method (FEM)[9,12].

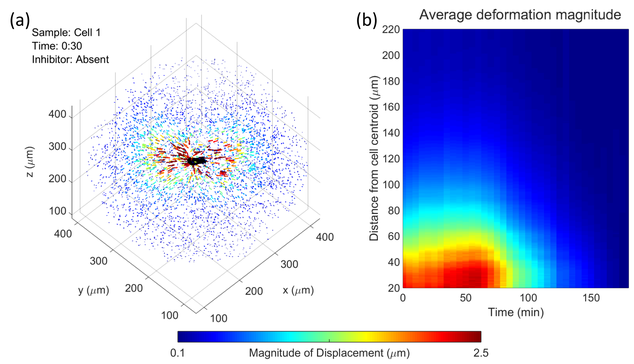


Fig. 7: 3D substrate deformations induced by an NIH-3T3 fibroblast in Matrigel. (a) Rendering of 3D bead displacements captured around the cell. (b) Distribution of average bead displacements as a function of time and distance from the cell centroid. A contractility inhibitor was added at time t=30 minutes. After a short delay, the bead displacements decay to zero as the cell relaxes[9].

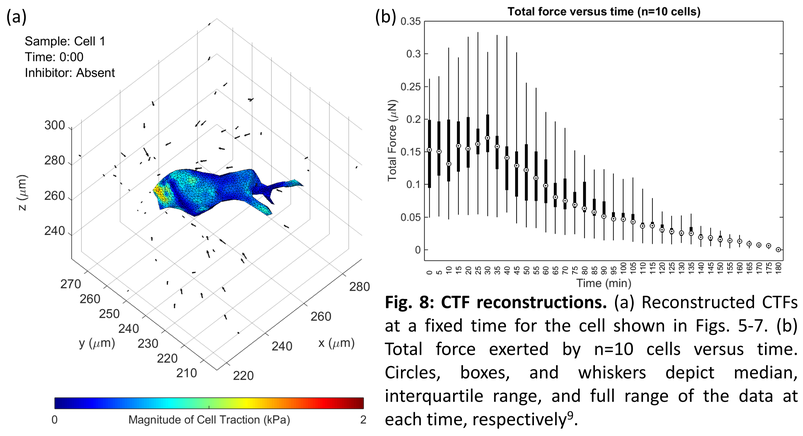


Fig. 8: CTF reconstructions. (a) Reconstructed CTFs at a fixed time for the cell shown in Figs. 5-7. (b) Total force exerted by n=10 cells versus time. Circles, boxes, and whiskers depict median, interquartile range, and full range of the data at each time, respectively[9].

TF-OCM for Multi-cellular Systems

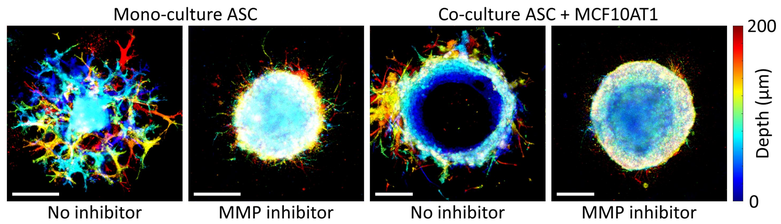


Fig. 9: Multi-day imaging of spheroid invasion dynamics. Label-free imaging with OCM enables continuous observation of spheroid growth and invasion within a collagen substrate. Temporal speckle contrast (as in Fig. 6) readily enables segmentation of cells from the background collagen substrate. The resulting generation of two synthetic 'channels' (corresponding to cells and collagen, respectively) is a novel capability for OCM imaging, and may enable TFM experiments that forgo the use of fluorescent labels and/or fiducial marker beads. Example renderings of the 'cell' channel for four spheroids are depicted above, from images acquired 48 hours after initial embedding. (ASC: adipose stromal cell. MMP: matrix metalloproteinase). Scale bars = 200 µm.

TF-OCM for Multi-cellular Systems

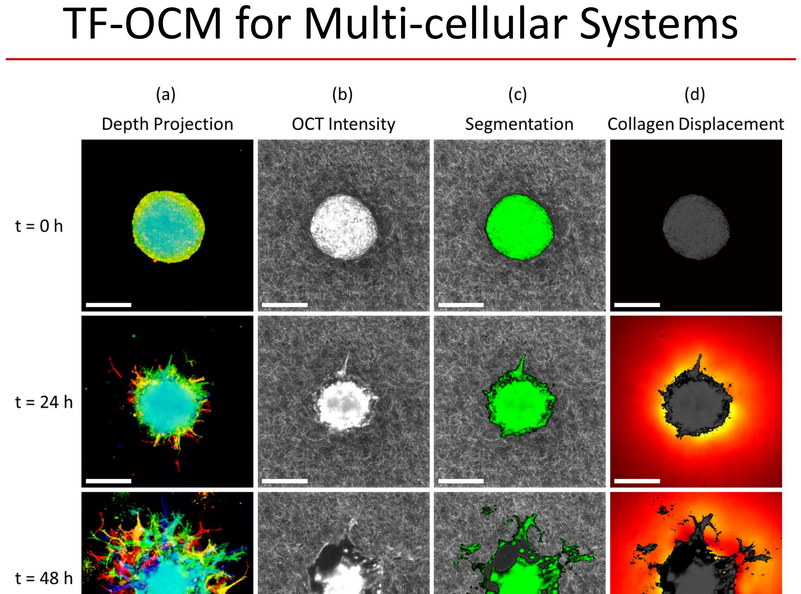


Fig. 10: Invasion of a mono-culture ASC spheroid into collagen. (a) *En face* visualization of the spheroid and invasive protrusions, using temporal speckle contrast to remove the appearance of collagen. (b) *En face* plane showing OCT intensity at a depth of 315 µm. (c) Local segmentation of cells (visible in green, obtained from the same data used to generate (a)). (d) Heat maps of the magnitude of the 3D collagen displacement vector field. Displacements were measured with respect to time t=0 hours. Scale bars = 200 µm.

Future Research

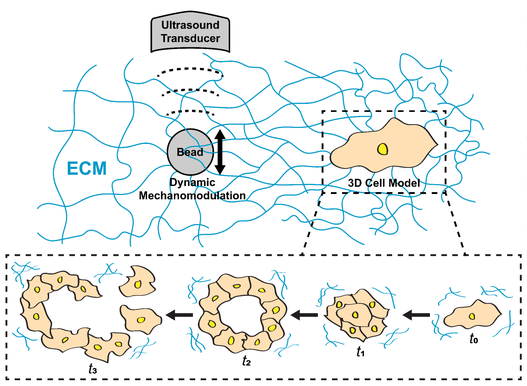


Fig. 11: 4D TF-OCM may enable novel investigations of cellular responses to mechanical cues. For example, we have postulated the use of ultrasound to apply dynamic mechano-stimulation to single cells and multi-cellular collectives via the acoustic manipulation of embedded beads (top). TF-OCM may be used to monitor both short- and long-term mechanical responses (CTFs, migration, etc.), and may provide insight into factors such as metabolism, signaling, and collective behavior (bottom).

Conclusions

- TF-OCM provides a new method to measure dynamic 3D cellular forces in scattering media by leveraging high-throughput volumetric imaging achieved with a combination of OCM and computed imaging methods
- Quantitative force reconstructions have been demonstrated in single-cell systems, and preliminary developments toward the study of multi-cellular systems are yielding promising results
- A complete implementation of TF-OCM for multi-cellular systems will provide new imaging and research capabilities for studying a variety of model systems of interest in mechanobiology

References

TFM + Mechanobiology

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TF-OCM

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CAO

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CTF Reconstruction

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