DATA-DRIVEN 3D TRACTION FORCE MICROSCOPY IN FIBRILLAR HYDROGELS

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Introduction

Many diseases are driven by or have a direct effect on cell mechanical interactions with the extracellular matrix (ECM)[1,2]. Traction Force Microscopy (TFM) is the most common methodology to measure cell-matrix mechanical interactions within in vitro cultures. Collagen hydrogels are typically used to mimic the three-dimensional ECM. Classic 3D TFM considers the ECM as a continuum medium with homogeneous mechanical properties. However, local remodeling of the fibrillar network by cells leads to changes in the ECM mechanical properties near the cell that cannot be captured by continuum models and the length scale at which forces can be recovered is limited. In this work, we present a novel 3D data-driven TFM workflow based on a discrete fiber model that incorporates geometrical information from the imaged collagen network. Instead of solely relying on one constitutive model that describes the global behavior of the ECM, we accommodate the mechanical behavior of the collagen hydrogel to the geometric information of the fiber network extracted from a microscopy image. Moreover, we combined it with our previously presented nonlinear inverse method to accurately compute cellular forces[3,4]. As a result, we present a methodology that takes into account a more realistic representation of the cell's mechanical microenvironment to further improve cell force recovery in collagen hydrogels.

Methods

Data-driven fibrous matrix generation. We developed a synthetic fibrous matrix generator, in which fibers of a given diameter and stiffness are discretized using nonlinear beam elements. The architecture of these synthetic matrices was defined by segmenting and skeletonizing fibers from second harmonic generation (SHG) images of real collagen hydrogels (Fig. 1A). Model parameters were obtained from fitting stress-strain curves obtained by means of shear rheology.

In silico ground truth simulations. First, we segmented a real confocal microscopy image of a cell that was embedded in a collagen hydrogel. Then, we embedded the relaxed state of the cell geometry in the synthetically generated matrix and we prescribed a 7 µm displacement at the closest point to the protrusion tip and obtained a ground truth matrix displacement field and a ground truth nodal force of around 3.5 pN (Fig. 1B). To analyze the performance of our traction recovery methods we added different levels of white Gaussian noise to the ground truth displacements. We tested the traction recovery accuracy of two different methods: a forward method, which computes forces directly from the measured (noisy) displacements, and our physics-based inverse method (PBIM)[3], which imposes equilibrium of forces in the hydrogel domain.

Results

Fig.1C shows that the forward method leads to higher forces in other nodes of the cell surface that are not the tip node. This effect becomes more prominent with increasing noise, for which the magnitude of the forces is overestimated (~3 times higher force magnitude). PBIM is more robust against noise in this discrete-fiber framework since the node of maximum force corresponds to the cell's tip for all the cases while preserving vector magnitudes and directions close to those of the GT. Moreover, it recovered forces more accurately than the forward method with errors below 20% versus errors of up to 80%, respectively.

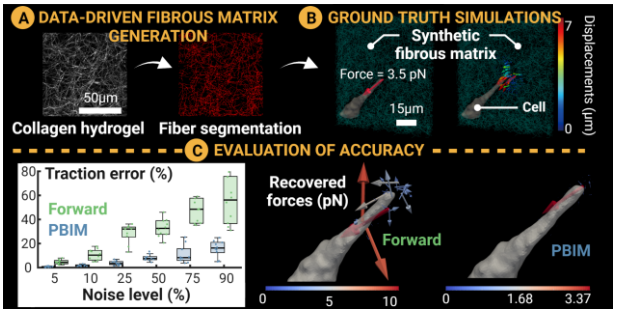

Figure 1: Summary of the work.

Discussion

In this work, we presented a novel data-driven 3D TFM approach and validated its accuracy and viability by means of in silico ground truth simulations. These preliminary results lay the foundations of our future work, which will focus on applying this framework to real experiments with cells to obtain multiscale cell force information at length scales closer to the length scale of mechanotransduction.

References

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Acknowledgements

Financial support: JBF (FWO:1259223N), HVO (KUL:IBOF-21-083), AAF & JSH (PID2021-126051OB-C42), GHK & IM (Convergence programme Syn-Cells for Health).