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Mechanical Characterization of Glioblastoma Cell – Substrate Interaction by Fading Regularization Method

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1. Introduction

Glioblastoma multiform (GBM) is the most invasive and common malignant primary tumor of the brain. It has one of the worst 5-year survival rates among all human cancers. To better understand how GBM interacts with its environment, it is necessary to adopt a mechanical approach to the problem. The forces that a cell exerts on its environment, as well as the displacement fields of the cell and its environment, provide valuable data for analyzing invasion at the cellular level (del Alamo et al., 2013).

Measuring the displacement of a GBM cell is challenging due to the resolution at which the cell has to be observed and the way it is imaged by confocal microscopy associated with specific cell preparation.

In this context, we propose applying the fading regularization method (Caillé et al., 2019) to measurement computed by digital volume correlation. The benefit of this inverse method is that it reconstructs a complete displacement field and denoises the data.

2. Methods

2.1 Studied cells

The cells studied are labeled with fluorescent markers.

They were then placed onto a substrate composed of Corning® Matrigel® diluted in culture media. This gel has a Young's modulus of approximately 30Pa according to the supplier and is supposed linear elastic. We also added fluorescent beads, from ThermoScientific, with a diameter of 0.21 and 0.70 µm to this substrate to visualize it under a confocal fluorescence microscope.

2.2 Cell imaging

The cells are imaged in three dimensions using an Evident FV3000 confocal fluorescence microscope. The results shown in this paper are derived from a volume image with dimensions of 1024×1024×208 voxels for a recording time of approximately 30 minutes.

2.3 Digital Volume Correlation (DVC)

DVC is a method for computing displacement fields from two successive volume images: an initial reference image and a second, so-called "deformed" image. The method involves determining, for each voxel in the initial image, the mechanical transformation that occurred between the two steps. This is achieved by searching for a pattern common to both images using a pure translation transformation plus the first spatial gradient of that pure translation. This yields the three displacement fields along the x, y, and z axes (Valle et al., 2019; Franck et al., 2007). However, this method has a drawback: the reconstructed fields may contain incorrect fragments due to a lack of texture or a deterioration in the sought pattern. Consequently, the computed fields can be incomplete.

2.4 Fading Regularization Method (FRM)

The FRM was initially proposed to solve the Cauchy problem associated with the Laplace equation. (Cimetiere et al., 2000). The method was then developed for field reconstruction and boundary condition identification from partial elastic displacement field

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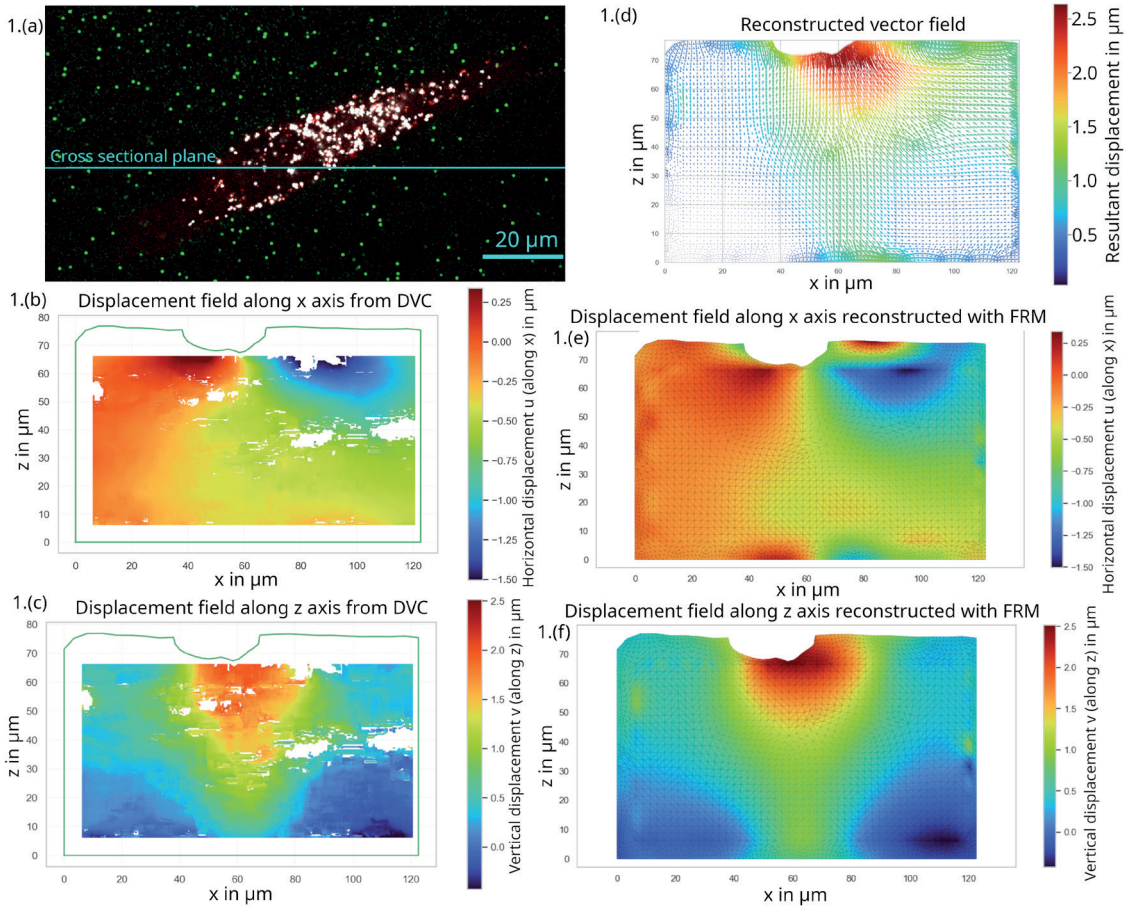

Figure 1. (a) XY slice of the cell from confocal fluorescence microscopy, (b-c) Displacement field along x and z axis from DVC results, (d) FRM solution displacement vector field, (e-f) Optimized displacement field along x and z axis found with the FRM.

Credit: Noé Bourgeois.

data (Caillé et al., 2019). This regularization approach is based on an iterative process whose convergence has been proven. It simplifies inverse problem by reformulating it as a sequence of optimization problems subject to equality constraints. At each iteration, the solution to the equilibrium equation that best approximates the partial data as well as the previous iteration is determined. The minimized functional consists of two terms: a relaxation term, which measures the deviation from the partial displacement field data, and a regularization term, which controls the gap between two successive iterations. Over the course of the iterations, this control term tends toward zero.

$$\mathcal{J}(U) = \|U^{k+1} - \phi_d\|^2_{\Omega_d} + c\|U^{k+1} - U^k\|^2_{\Omega}$$

With:

- U = Numerical displacement field
- ϕ_d= Experimental displacement field
- Ω_d= Experimental data domain
- Ω = Full domain

In this work, we use the method with finite element discretization, we use 6-node triangular elements.

The case studied here, the glioblastoma cell, represents a significant advancement for the method, with applications to fields and geometries derived from confocal microscopy imaging.

3. Results and discussion

Two volume images of a live U118 SH7+Cx43 cell attached to the substrate and exhibiting a wide spread (Fig1.(a)) were recorded by confocal microscopy. The displacements observed are solely due to the cell's natural cycle over a period of approximately 30 minutes;

no external forces were applied. We computed the 3D displacement fields across the entire substrate and then extracted a slice in the XZ plane passing through the center of the cell. We then removed the portions of the displacement field exhibiting outlier values resulting from the lack of beads in those areas (Fig1.(b-c)).

Displacement fields revealed mechanical effects caused by the cell, which pulls on either side of its axis of elongation.

We then apply the fading regularization method to reconstruct the partial displacement field from DVC over the entire domain following a linear elastic behavior law.

The completed displacement fields are shown below (Fig1.(d–f)).

The method successfully reconstructs a complete displacement field over the entire domain while denoising the accessible portion of the data.

4. Conclusions

The fading regularization method successfully processes experimental data at the cellular scale by reconstructing a complete, denoised 2D displacement field in accordance with the assumed laws of linear elasticity. This is promising, and we can envision developing it into a 3D application or even applying it to the cell itself using a nonlinear mechanical behavior law.

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Contributor Roles

NB: Conceptualization, Investigation, Methodology, Writing original draft; HZ: Conceptualization, Methodology, Writing – review & editing; AuG: Conceptualization, Investigation, Methodology, Validation; ND: Investigation, Ressources; MM: Supervision; SS: Conceptualization; ArG: Conceptualization, Supervision, Writing – review & editing; LC: Conceptualization, Supervision, Funding acquisition, Writing – review & editing;

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