SCIAN-Drop & SCIAN-Force: Quantification of Membrane Deformation via Image Analysis of Biocompatible Fluorocarbon MicroDroplets

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ABSTRACT

Introduction: Cancer is one of the leading causes of death worldwide, with metastasis as its main driver of mortality. An essential and under-explored factor are the mechanical properties that influence tumor cells during metastatic dissemination. While various techniques exist to measure cellular forces, many are invasive and require direct manipulation, which limits the assessment of migration processes. An innovative, non-invasive and viable approach for this question is the use of ***Biocompatible fluorocarbon MicroDroplets*** **(BMD)** as force sensors. Under mechanical stress induced by forces of moving cells, BMD surface deformations can be observed, acquired and quantified with fluorescence microscopy and posterior image analysis.

Material & Methods: We developed two software tools for BMD quantitation, (i) *SCIAN-Drop* measures interfacial tension of fluorocarbon oil using the *'Pendant Drop'* method, and (ii) *SCIAN-Force* calculates curvatures and stress over 3D BMD surfaces. Both software were validated by synthetic images and applied to real experiments involving the use of BMD in a transwell 3D migration assay with human melanoma cells.

Results: *SCIAN-Drop* effectively calculates interfacial oil tension in a calibration setup, while *SCIAN-Force* calculates mechanical stresses on surface reconstructions of cell-droplet migration 3D images. Our developed tools present a validated method for quantifying mechanical forces during cell migration, both in terms of robustness, accuracy and user experience.

Discussion: We developed and implemented two easy-to-use tools to calculate membrane forces in a non-invasive manner. Future work includes enhancing algorithm accuracy and refining software user experience to further make our tools available to the scientific community.

MATERIALS & METHODS

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Problem: Quantifying the deformations of a BMD during the migration of tumor cells under *in vivo* conditions, enabling the measurement of mechanical forces exerted by the cells with minimal intervention in the migratory process (Figure 1).

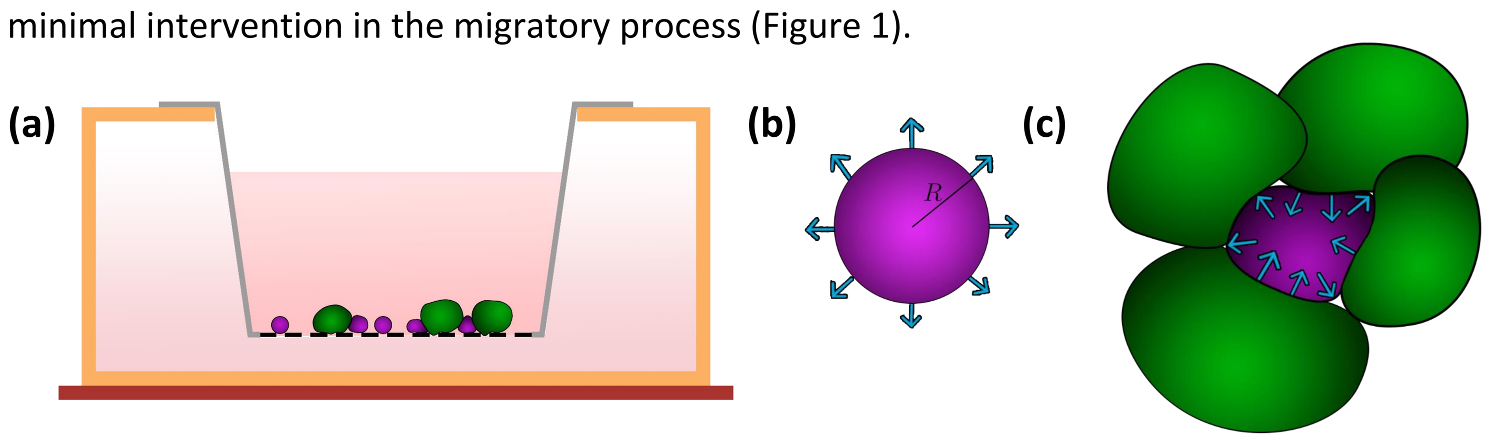

Figure 1. (a) Experimental setup in which cells, attempting to cross the transwell membrane, impact and deform the BMD; **(b)** BMD in isotropic state, exhibiting a spherical and uniform shape; and **(c)** cells applying mechanical stress on the BMD, generating an anisotropic state characterized by surface deformations.

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Approach: The anisotropic normal stress $\delta\sigma(q)$ affecting the BMD at a point q on its surface can be described by Equation 1 [1].

$$\delta\sigma(q) = 2\gamma\left(H(q) - \frac{1}{R}\right)$$

Equation 1. γ is the interfacial tension between the BMD and the surrounding medium, R is the radius of the droplet in its isotropic state, and $H(q)$ is the mean curvature at point q on the surface.

Based on this equation, we developed **SCIAN-Force** software to compute the initial radius of the BMD and its surface mean curvature, and **SCIAN-Drop** to determine the interfacial tension between the fluorocarbon oil and the medium using the *'Pendant Drop'* method.

The Pendant Drop method measures the interfacial tension between two liquids by analyzing the shape of a drop suspended from a needle in a surrounding medium [2]. Using image capture, the Bond number β is determined to quantify the interfacial tension using the Equation 2.

$$\beta = \frac{\Delta\rho g R_0^2}{\gamma}$$

Equation 2. γ is the interfacial tension, R_0 the radius of curvature at the drop apex, g the gravitational acceleration, and $\Delta\rho$ the density difference.

Calculating the Bond number directly is not possible, so it was necessary to solve the differential equation (Equation 3) [3] describing the drop shape, and to estimate β (using Least Square algorithm) in terms of a new variable $\sigma = D_S/D_E$, obtainable from the drop shape (Figure 2).

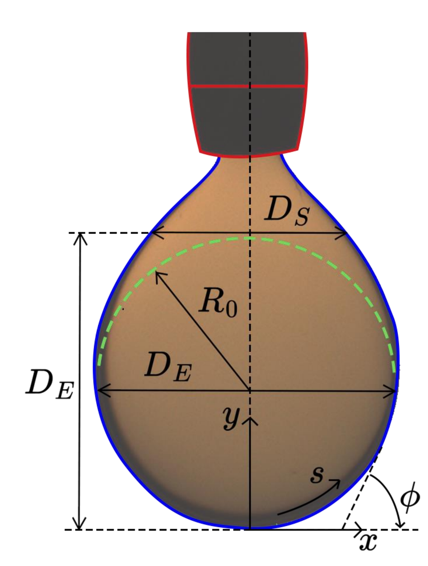

Figure 2. Pendant drop profile, represented by the coordinates (x, y, ϕ), and the arc length s.

$$\frac{\partial\phi}{\partial S} = 2 - \beta Y - \frac{\sin(\phi)}{X} \qquad \frac{\partial X}{\partial S} = \cos(\phi) \qquad \frac{\partial Y}{\partial S} = \sin(\phi)$$

Equation 3. Differential equation. Uppercase variables scaled by R_0.

While the interfacial tension measurement from SCIAN-Drop is a key input for analyzing stress on BMDs, a 3D representation of the BMD surface is still required for its calculation. This was obtained from experimental images captured with a **Spinning Disk microscope (1408 x 898 x 79 voxels; voxel size: 0.163 x 0.163 x 0.500 μm³)**, which were manually segmented and 3D-reconstructed using SCIAN-Soft/IDL to characterize deformations.

The 3D reconstruction produces a triangular mesh of the surface (Figure 3), with the availability of numerous algorithms. Assuming the volume of the BMD remains constant before and after deformation, the volume was calculated, and the isotropic radius R was determined using the sphere volume equation, representing the original shape. Also, the **Rusinkiewicz algorithm** [4] and **Laplace operator** [5] calculations were employed to estimate the mean curvature H across the BMD surface, enabling to quantify the stress.

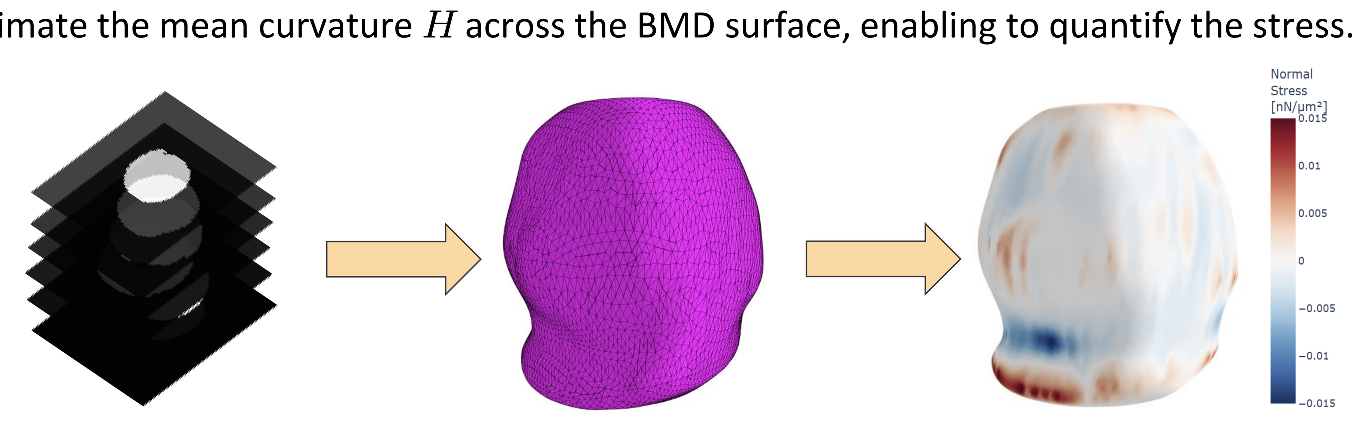

Figure 3. Surface mesh reconstruction overview. **Left:** Segmented binary image stack from a sample BMD. **Center:** BMD triangular surface mesh. **Right:** triangular mesh colored by anisotropic normal stress values.

RESULTS & DISCUSSION

3

Main Contributions: (i) SCIAN-Drop measures the interfacial tension between two fluids using the *'Pendant Drop'* method, featuring an intuitive, portable, and user-friendly interface (see Figure 4). The software was tested under various experimental conditions, with consistent measurements, in terms of precision and sensitivity, to changes in conditions (Figure 5).

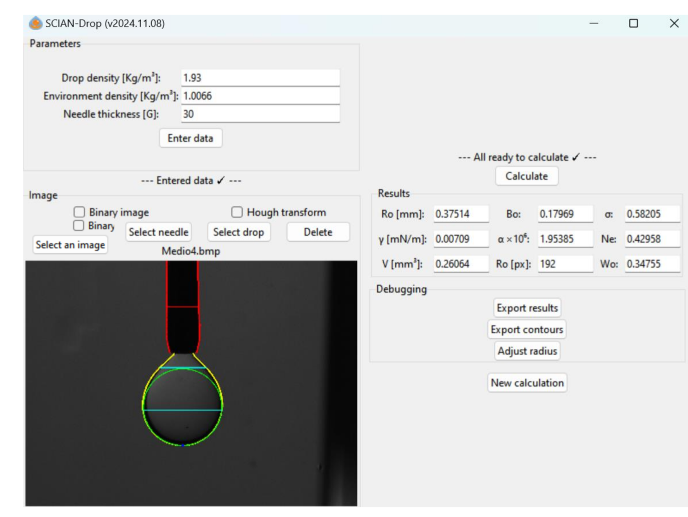

Figure 4. SCIAN-Drop interface with guided prompts. The interface offers options for specifying experimental paremeter values (top panel) and input image (bottom panel), together with interactive segmentation options for needle and droplet shapes.

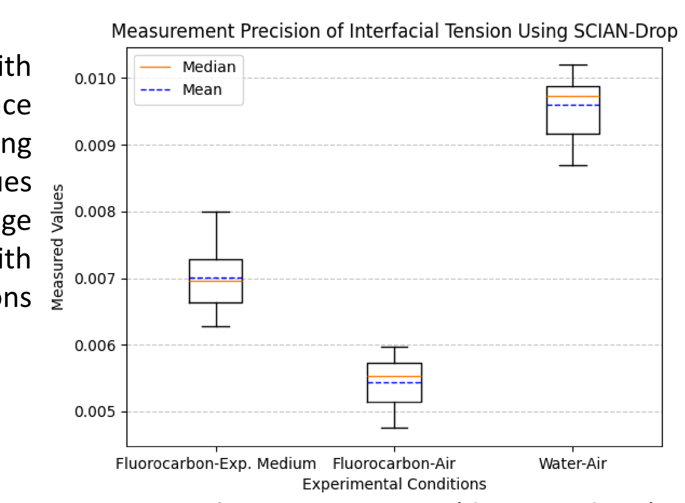

Figure 5. SCIAN-Drop measurements in three experiments (drop–medium) with N = 14, N = 13, and N = 9 (scaled values), respectively.

(ii) SCIAN-Force quantifies anisotropic forces on 3D objects. Its interface is user-friendly, straightforward, and supports various settings for optimal visualization (see Figure 6). It is designed for BMD surfaces deformed by migrating, cells and has been validated with synthetic objects such as spheres and ellipsoids (Figures 7 & 8).

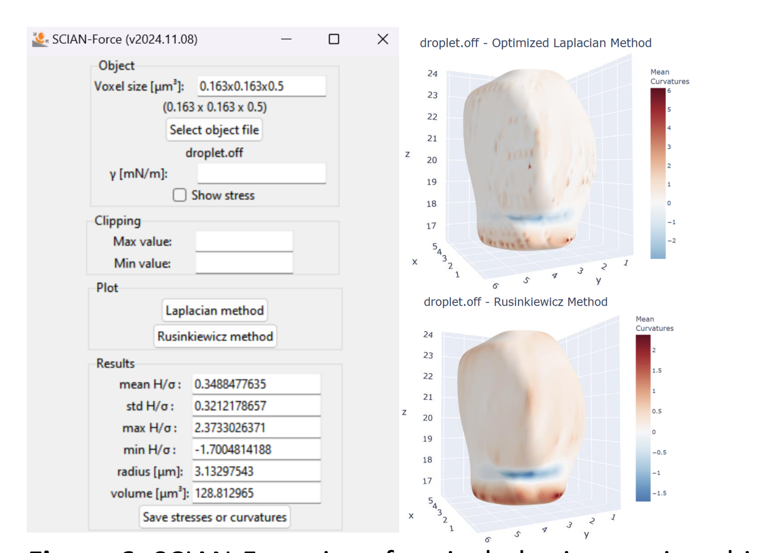

Figure 6. SCIAN-Force interface includes interactive object visualization and retrieval of multiple data outputs.

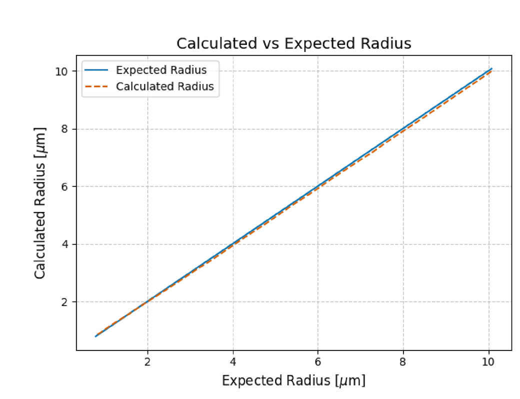

Figure 7. Radii measured from synthetic spheres of varying sizes. Voxel size: 0.163 x 0.163 x 0.500 μm³

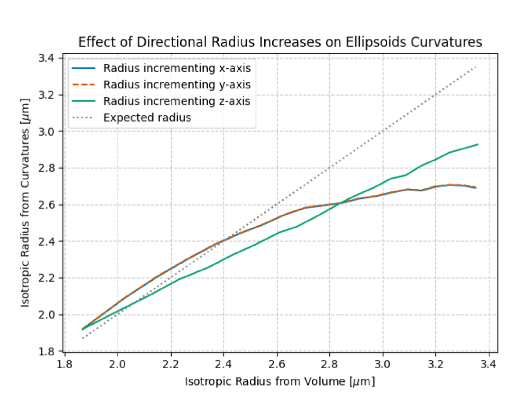

Figure 8. Radii measured from synthetic ellipsoids. Voxel size: 0.163 x 0.163 x 0.500 μm³

SCIAN-Drop and SCIAN-Force together enable the quantification of mechanical forces exerted by migrating tumor cells on BMD surfaces under *in vivo* conditions. This **integrated method** (Figure 9), theoretically supported and validated through tests, provides precise, error-bounded measurements of BMD deformations.

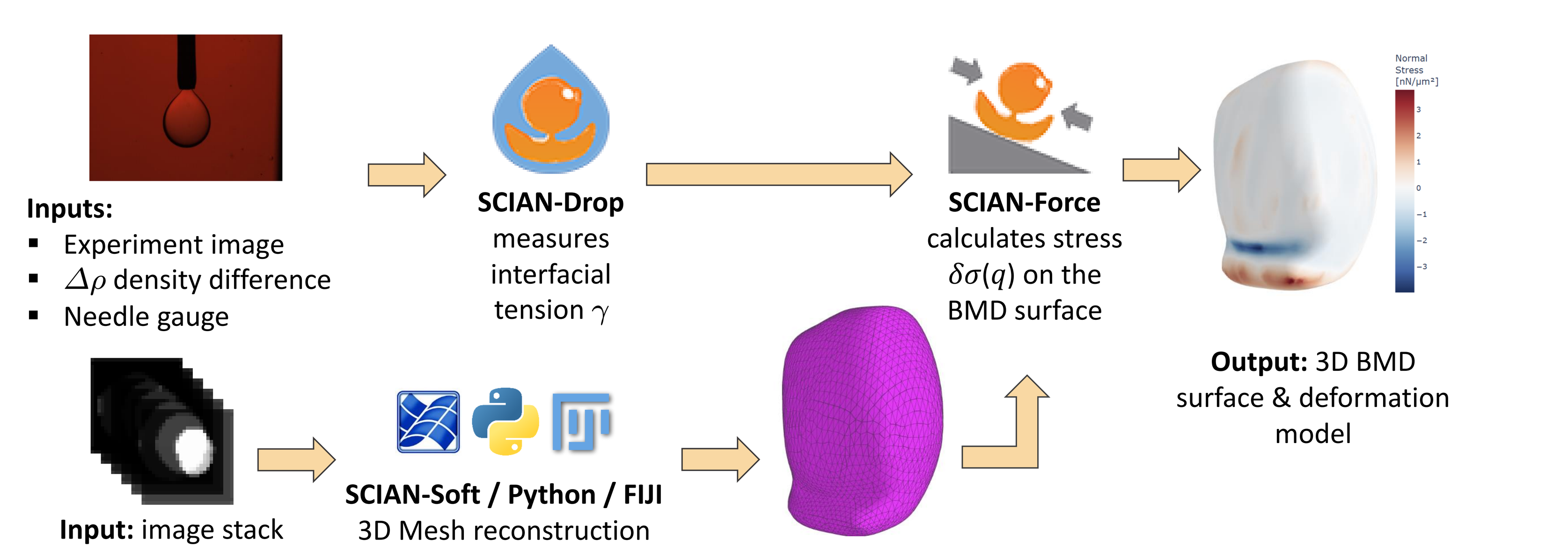

Figure 9. Process for quantifying the deformations of a BMD after the experiments. **SCIAN-Drop** measures the interfacial tension γ and **SCIAN-Force** uses the 3D reconstruction and γ to calculate $\delta\sigma(q)$ on the BMD surface.

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Applications: This method is currently being applied to the study of tumor progression and metastasis using BMD experiments (Figure 10). Additionally, it can be extended to similar conditions to measure deformations or determining of unknown interfacial tensions.

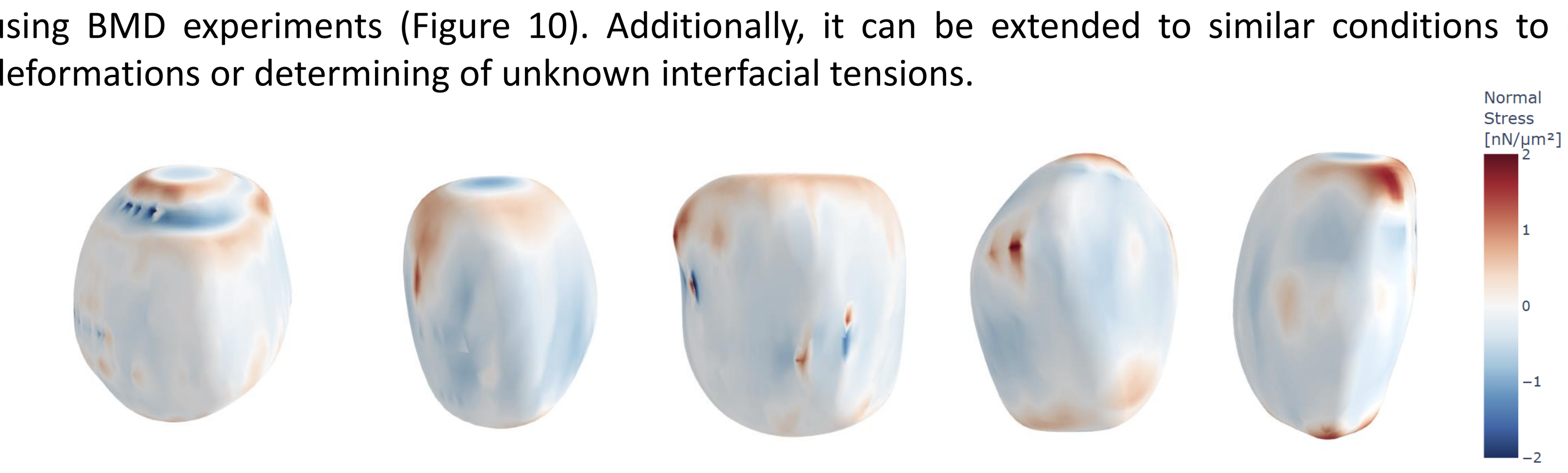

Figure 10. Examples obtained with the designed method of droplets deformed by migrating cells.

https://github.com/L3N73J4M4N/SCIAN-Drop
https://github.com/L3N73J4M4N/SCIAN-Force

SCIAN-Drop Repository
SCIAN-Force Repository

CONCLUDING REMARKS & OUTLOOK

Quantification of the deformations of a BMD during tumor cell migration: Achieved by SCIAN-Force and SCIAN-Drop, using the Pendant Drop method for interfacial stress and curvature calculations for stress.

Future user improvements: Integrate more automated processing steps in SCIAN-Drop, reducing manual intervention. Optimize visualization and interactivity in SCIAN-Force for a more efficient user experience.

Software extensions: Refinements in SCIAN-Drop will improve surface extraction and radius determination for greater accuracy. Further, ongoing tests with SCIAN-Force will refine results, to account for voxel resolution variability. SCIAN-Force will integrate surface reconstruction and mesh refinement to streamline workflows and enhance 3D stress and strain analyses.

REFERENCES

[1] Càmpas O. *et al* (2014). Nat Methods, doi:10.1038/nmeth.2761
[2] Berry J. *et al.* (2015). Elsevier Inc., doi:10/fjgbgf
[3] Hansen F. K., (1990). Elsevier Inc., doi:10.1016/j.jcis.2015.05.012
[4] Rusinkiewicz, S. (2004). IEEE, doi: 10.1109/TDPVT.2004.1335277
[5] Mayer M. *et al.* (2003). Mathematics and Visualization, doi: 10/gg8xjg

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