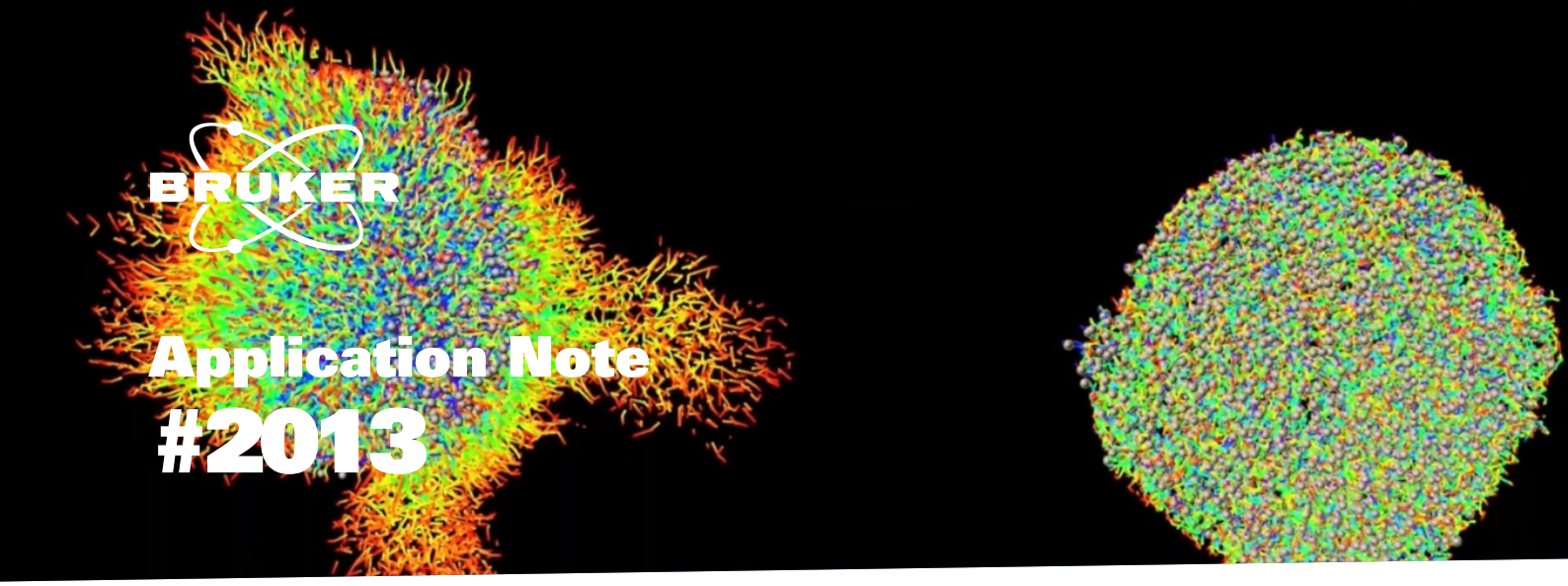

Webinar Recap: Modelling 3D Tissue Flow Using Forces and Tissue Mechanics

Using light-sheet microscopy, samples can be imaged fast, with low phototoxicity, and with subcellular resolution. The multi-view setup of Bruker's Luxendo MuVi SPIM allows for 3D imaging of fast dynamics simultaneously from different views. Thus, the microscope system is able to provide insights into global tissue dynamics, such as in spheroids and embryos. This light-sheet webinar exemplifies how combining state-of-the-art imaging, modeling with biophysics, and testing models with biological experiments can provide new insights into a variety of fields, here shown by cancer metastasis and embryonic gastrulation.

Investigating Tissue Flow Forces and Patterns

In this webinar, Professor Dr. Timo Betz from the University of Göttingen presents how mechanical forces in combination with viscoelastic tissue properties underlie tissue flow. Using 3D mapping of tissue flow in cancer spheroids and zebrafish embryos, he presents how to establish models for these experiments, as well as how to, measure, and predict forces in the tissue driving these flows.

Understanding Pressures in Spheroids

It is known that tumors have variable pressure within them, can exert pressure on their surroundings, and change morphology based on surrounding extracellular matrix (ECM). To study biophysics in cancer spheroids, the group used HeLa spheroids with tagged nuclei embedded in collagen (Figure 1).

Betz's lab studied the role of tissue stiffness by altering the collagen concentration in the agarose that was used for embedding the spheroids, and by taking timelapse images every 15 minutes over 12 hours. When tissue stiffness was high at 2.5 mg/ml collagen, no tissue invasion was seen. When reducing the collagen concentration to 0.5 mg/ml, they saw bursts of cells forming from the spheroids (Figure 2) [1]. This was controversial to existing literature, which suggested metastasis on stiff tissues, and led them to further investigate the reasons for these observations.

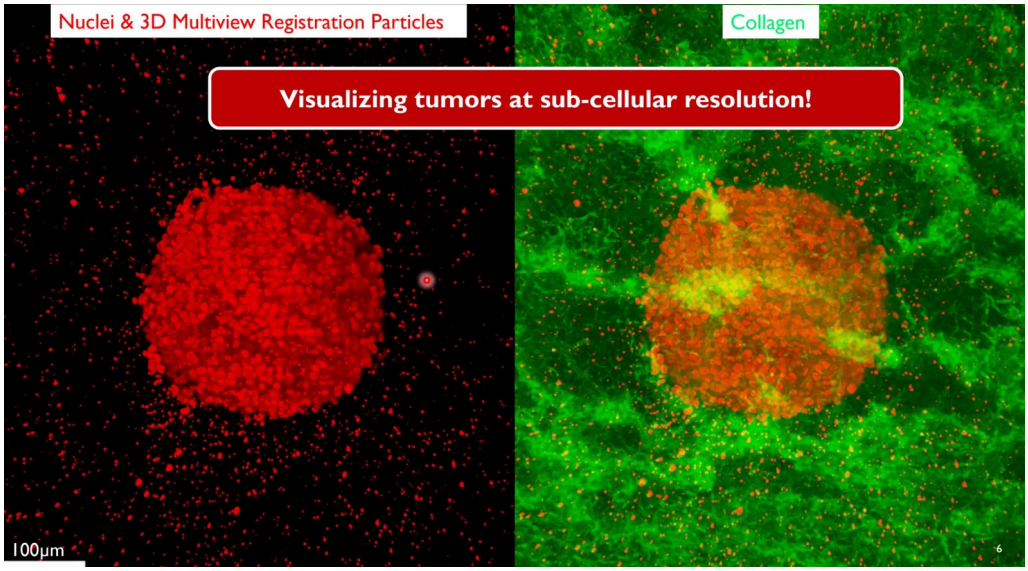

FIGURE 1

Left: Red HeLa spheroid nuclei surrounded by beads for multi-view bead-based registration. Right: Same image as left but overlaid with collagen (green).

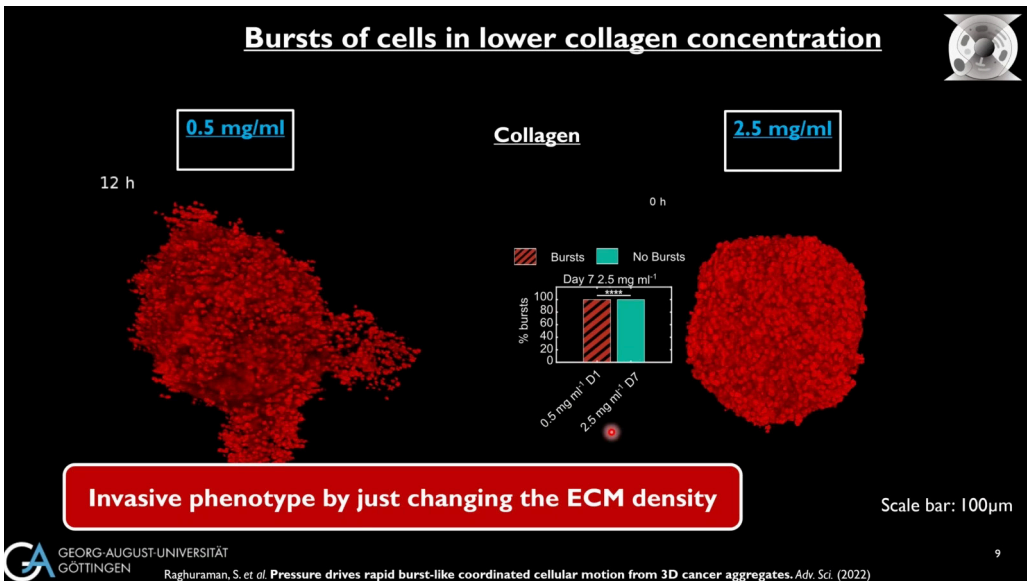

FIGURE 2

Bursts of cells were seen in lower collagen concentration.

Understanding Differences in Migration in Spheroids

To understand the principles of cell migration, they next extracted the mean squared displacement from single-cell tracking (Figure 3) to conclude if migration was random, directed, or constrained. They found that migration in 2.5 mg/ml collagen was diffusive, while migration in 0.5 mg/ml collagen peaked at 8 hours and could be described by a "super-diffusive peak pattern."

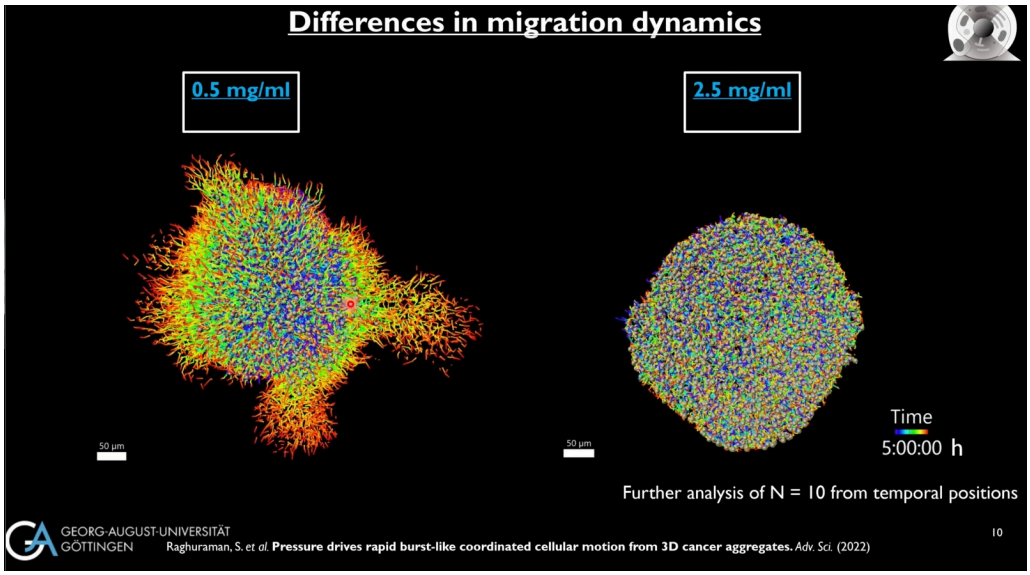

FIGURE 3

Single-cell tracking of spheroid nuclei allowed insights into differences in migration dynamics.

To answer the question of whether the cells underwent collective migration, they looked at motional correlation as a function of distance in migration (i.e., correlation length). They found that, to a certain extent, cells started to move in a coordinated manner. This was independent of cell-cell adhesions as E-Cadherin inhibition did not inhibit bursts, making it distinct from classical collective migration.

To study cell volumes during bursting, they used nuclei information to produce Voronoi cell approximation. This showed that in the low collagen the cell volume increased, and that cell swelling led to a pressure that facilitated the bursts. To measure the pressures, they developed a system (Figure 4) where deformation of a hydrogel by the spheroid was used as a measure of pressure. They found that the elastic gel deforms as the cell aggregate of the spheroid pushed down from pressure.

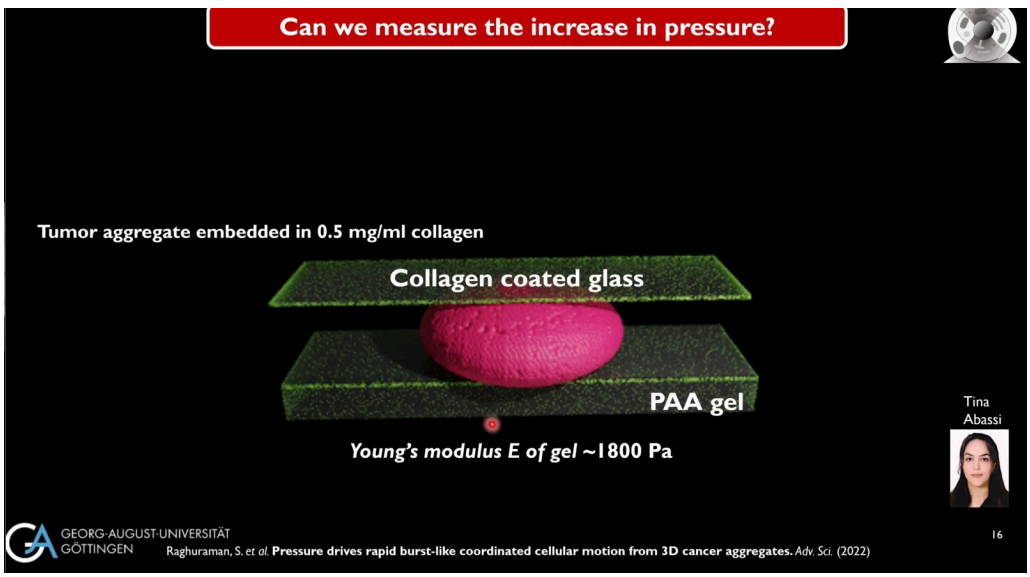

FIGURE 4

To measure pressures exuded by the spheroid, they developed a system where the spheroid (magenta) was placed between a collagen-coated glass and a polyacrylamide gel (PAA). Deformation of the PAA was used to measure pressure.

Identifying the Mechanism of Spheroid Bursts

The group next looked at integrin, which is known to interact with collagen to produce pulling forces. After integrin inhibition with the mAb-13 antibody, they found that bursts were no longer occurring. They investigated the role of integrin pulling on the environment, which then led to phosphorylation of the myosin light chain. The group proposed that the cells are unable to perform mechanotransduction due to low collagen density in the surrounding tissue, then undergo cell swelling as a response to being in a sub-optimal environment and that bursts form in the direction of least mechanical resistance. Together, their finding suggests that bursts depend on cell-ECM interaction and actomyosin-dependent contractility, which leads to changes in cell health.

Explaining Tissue Motion with Physics, Showcased by Zebrafish Gastrulation

The second project presented in the webinar considered biophysics on a more global level by, investigating the biophysics of tissue migration during zebrafish development. The first research question addressed whether epiboly, a process where cells on top of the cell yolk start a downward migration to encompass the yolk, was similar to wetting, i.e., a liquid moving over a surface (Figure 5).

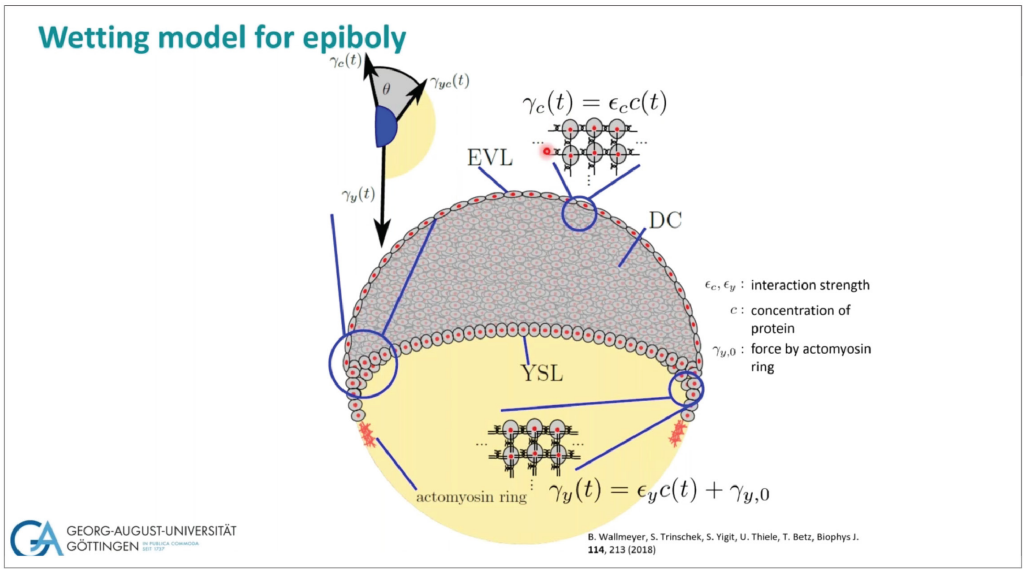

FIGURE 5

The Betz group developed a model epiboly, derived from the principles of a liquid wetting a surface.

They measured the contact angle on the tissue level as a function of time, using multi-view light-sheet microscopy on zebrafish with tagged nuclei. This showed that over time, the angle would get smaller. When modeling this, and fitting the model to the data, they found good agreement to the experimental data[2].

Describing Embryogenic Development as a Liquid?

They next studied tissue dynamics using red nuclei markers, and sparse green deformable hydrogel particles. Using the *Tracking with Gaussian Mixture Models (TGMM)* software, they segmented and analyzed individual cells[3]. Solving the hydrodynamic model using neural networks to understand the forces in the tissue, they were able to successfully model the downward migration seen during epiboly. Surprisingly, the model also correctly predicted a partial internal upward migration, which is seen in the embryos during invagination.

Next, they used the sparse green deformable hydrogel particles as elastic tension sensors. As these beads are deformable, their shape was used to infer forces in the surrounding tissue. Using spherical harmonics provided an analytical approach to describe bead surfaces and thus tissue forces, which they called Bead Deformation Analysis (BDA; Figure 6).

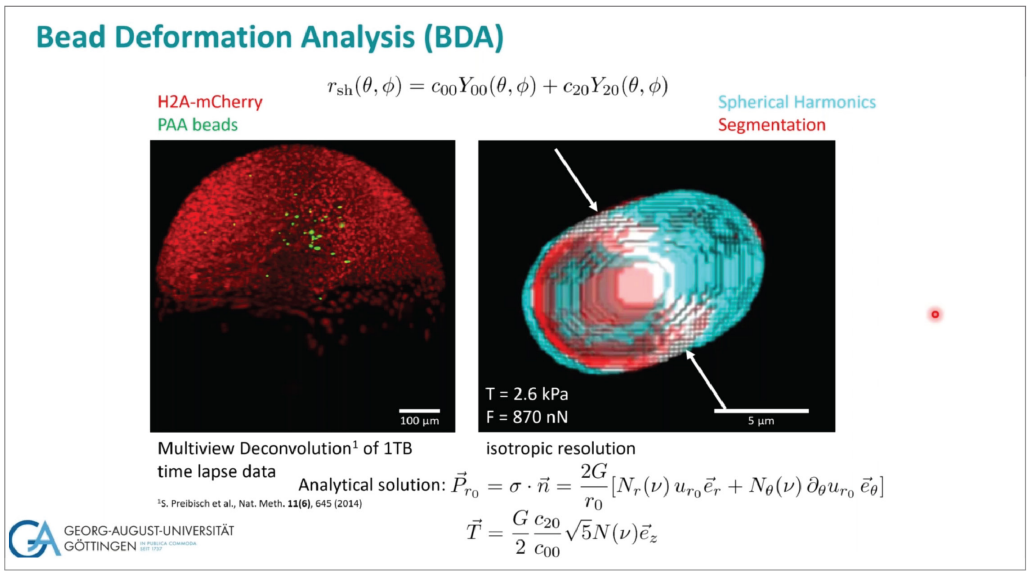

FIGURE 6

Using spherical harmonics to describe hydrogel bead surfaces the group was able to describe tissue forces (forces depicted as white arrows in the right image).

Using Velocity Fields for Registration

To address the limitation that embryos are aligned in different directions during imaging, the group next wanted to bring the data into one spatial coordinate system by image registration. Here, they used the biological processes of epiboly (downward migration) and convergence (migration to the future body axis) as proxies for velocities that were then employed to align the embryos at the thickened dorsal region (called "shield"). Again, they used a biological experiment to check their model and found that their registration overlapped with the expression of a dorsal protein.

Stabilizing the Position of Gastrulation by Converging Flow?

To study what stabilized gastrulation, whether it was proteins or biophysics, they wanted to trigger flow via ablation. Here, the MuVi SPIM equipped with a photomanipulation (PM) module was used for a global scale characterization and laser ablation. After successfully developing an ablation protocol, they found that after photomanipulation, epiboly was still robust, and this suggests that biophysics was not critical to body axis formation.

Conclusion

This webinar showcased that combining state-of-the-art imaging, modeling with biophysics, and testing models with biological experiments provide novel insights into tissue flow. Professor Dr. Betz demonstrated that on cancer spheroids and zebrafish gastrulation.

Further Reading

1. S. Raghuraman et al., "Pressure Drives Rapid Burst-Like Coordinated Cellular Motion from 3D Cancer Aggregates," *Advanced Science*, vol. 9, no. 6, p. 2104808, 2022, doi: 10.1002/advs.202104808.

2. B. Wallmeyer, S. Trinschek, S. Yigit, U. Thiele, and T. Betz, "Collective Cell Migration in Embryogenesis Follows the Laws of Wetting," *Biophys J*, vol. 114, no. 1, pp. 213–222, Jan. 2018, doi: 10.1016/j.bpj.2017.11.011.

3. F. Amat et al., "Fast, accurate reconstruction of cell lineages from large-scale fluorescence microscopy data," *Nat Methods*, vol. 11, no. 9, Art. no. 9, Sep. 2014, doi: 10.1038/nmeth.3036.

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