Visualizing embryo development in Virtual Reality

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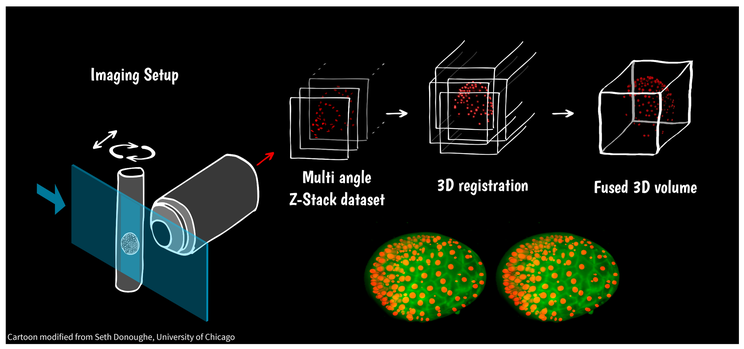
LightSheet Microscopy

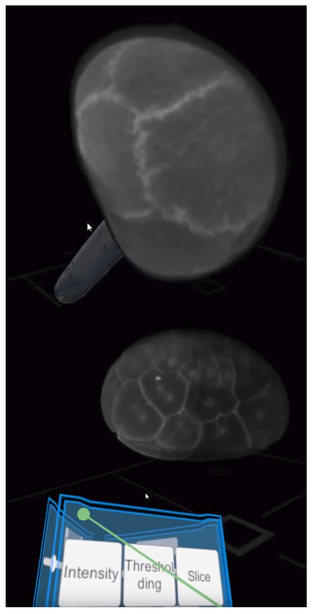
LightSheet microscopy (also called SPIM: Selective plane illumination microscopy) is a modern microscopy technique invented in 2005[1] that consists of generating a planar illumination field. The field is aligned and focused with the collection objective such that only the fluorophores in the plane in focus will be excited. The emitted light therefore comes from a single plane (see schematic) and the contrast is similar to confocal microscopy. Moreover, due to the imaging of an entire plane per acquisition (as opposed to a single point in confocal) this technique is very fast. Finally, by rotating the sample and imaging it from different angles, it is possible to fuse the different image stacks into an isomorphic 3D microscopy volume. Due to those advantages, it has been used extensively to image developing embryos which undergo rapid cell movement, division and morphogenesis in 3D.

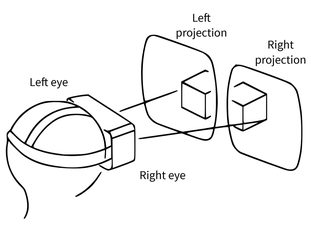
Here, I present results from images collected on *Parhyale hawaiensis* embryos at Janelia Research Campus thanks to the support of Anastasios Pavlopoulos and the generous lending of dual objective SPIM scopes by the Keller Lab[2]. Data from 4 embryos were collected in total, across 50 hours of development and starting at the 16 cell stage. Below is a stereoscopic rendering of the embryo at 30 hours post-fertilization (hpf). The cell membranes were tagged with Lyn-GFP and the nuclei with H2B-RFP, both fusion proteins expressed in the embryo by injecting mRNA at the 1 cell stage. Embryos were processed using FIJI, and the rendering performed with Blender.

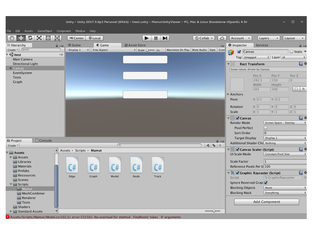


Virtual Reality

Virtual reality (VR) is an interactive computer-generated experience taking place within a simulated environment, which incorporates mainly auditory and visual feedback, as well as other types of sensory feedback including haptic. This immersive environment can be similar to the real world or it can be fantastical, creating an experience that is not possible in ordinary physical reality such as observing, entering or holding an embryo magnified 1000 times. LightSheet microscopy generates 3D images, making it a challenge to visualize these data on a 2D screen. However, VR is capable of immersing the user in a 3D environment. There exist commercialy available solutions, but due to the lack of available open source free software to my knowledge to visualize such datasets, I developed a Virtual Environment using the game engine Unity3D[3] to visualize and manipulate 4D microscopy datasets. In this environment, one can observe a 3D sample through time and perform basic image manipulations including modifying contrast, slicing, etc.
Currently, I am working on adding manual nuclei tracking capabilities along with tracks observation and manipulation. Ultimately, the goal is to release the code as an open source software.



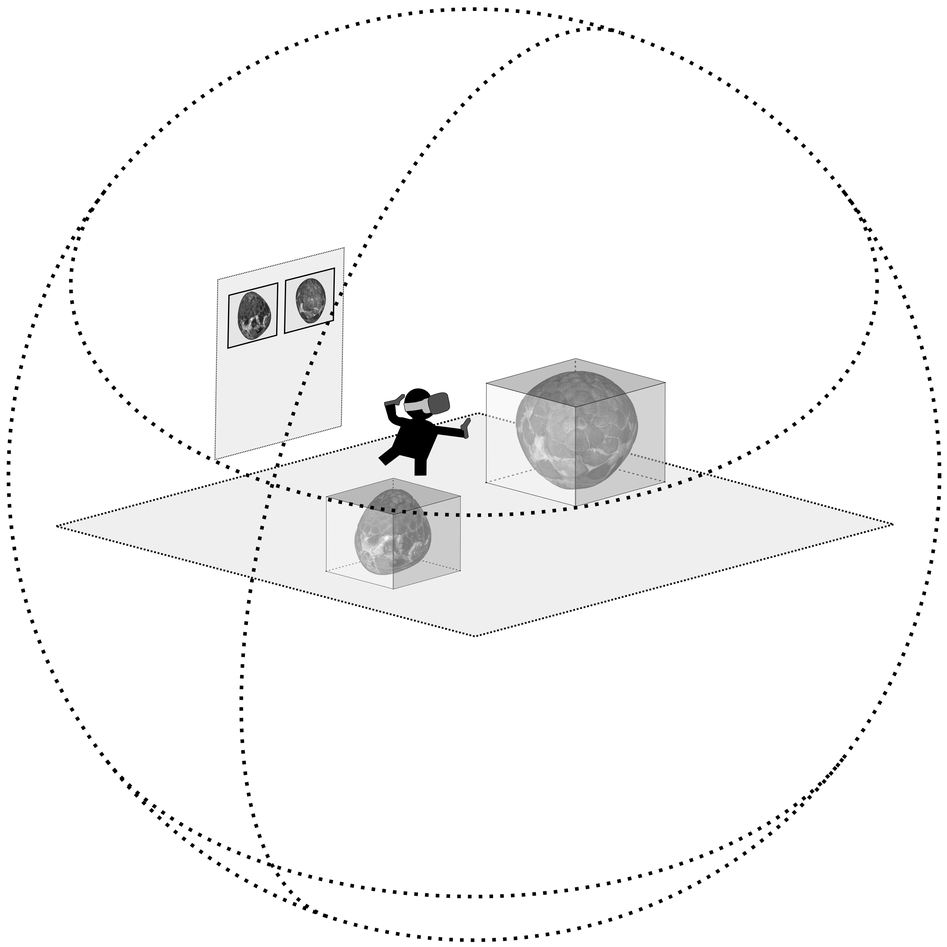




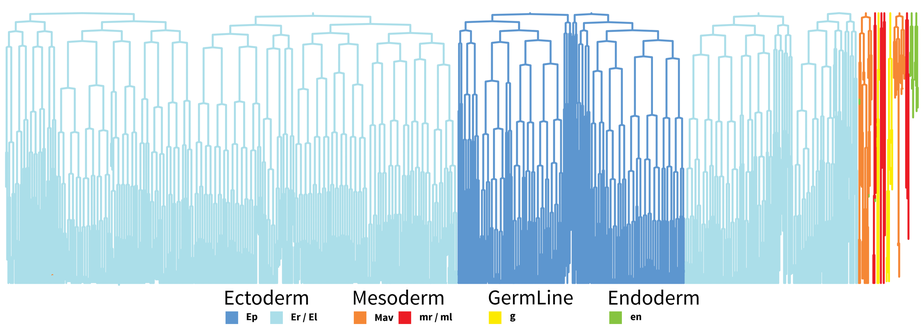
Left: Schematic of the principle underlying VR glasses. Each eye receives a different rendered image mimicking real 3D perception. Top: Examples of the virtual environement rendering the membrane tag Lyn-GFP in a *Paryhale hawaiensis* embryo. Screenshot of the Unity3D game engine editor.

Abstract

Embryos come in many shapes and sizes, but in all cases, they form a three-dimensional structure. With the advent of new microscopy technology such as LightSheet, it is now possible to record the development of an embryo from all angles in high resolution, generating a 3D + time dataset. However, visualizing such datasets is usually limited by the need to create two-dimensional projections on a computer screen. Thankfully, the development of consumer-accessible Virtual Reality (VR) now allows researchers to “immerse” themselves in 3D or 4D data, and thus, to visualize such datasets in a three-dimensional world. Here, I present a new VR tool I developed to visualize and manipulate LightSheet microscopy datasets, along with some new insights into the development of the amphipod crustacean *Paryhale hawaiensis*.



Lineage Tree

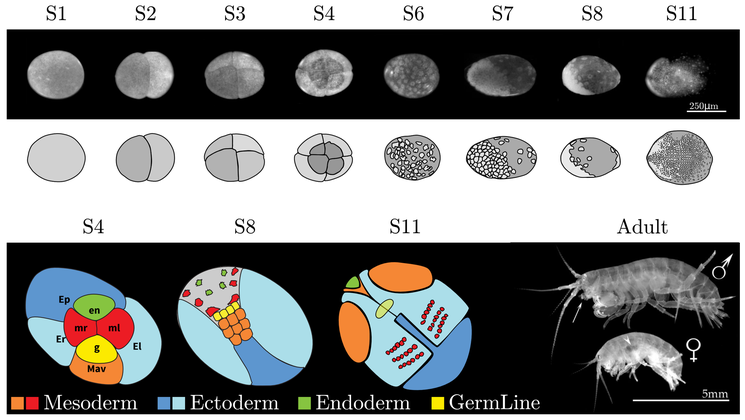


Using the tracking information obtained from 1 embryo (see Results section), one can follow cell lineages through time. Above is a tree representation of the cell lineages of a single *P. hawaiensis* embryo visualized in a LightSheet time lapse starting at the the 16 cell stages over 50 hours of development. Interestingly, we can observe that the lineages have drastically different rates of divisions. For example, the ectoderm lineages divide much more rapidly than the mesoderm lineages. Cells descending from ml and mr only contribute to ~2% of the cells at 60hpf. Finally, many more cells are not represented here as they cannot be detected during gastrulation.

Come Try it

Experience the development of an embryo in virtual reality during poster session hours!

Parhyale hawaiensis



Parhyale hawaiensis is an amphipod crustacean first described on the coast of Hawaii. Embryogenesis lasts for 10 days at 28C. Above are selected stages of embryogenesis showing the first cleaveages (S1-S4), gastrulation (S8) and the formation of the embryonic rudiment, called the germ band (S11). [4]
The schematics in color show the known fate map. Cells are commited to a germ layer at the 8 cell stage. Color-coding of the germ layers applies to the entire poster.
The following features make this animal an excellent model organism to study cell fate determination:

Holoblastic cleavages allowing for single cell isolation

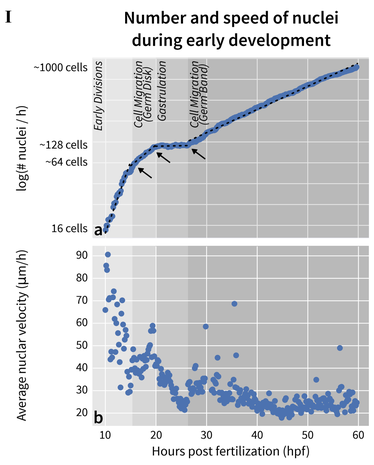
Low number of cells (~1500) at the germ band stage

Germ layer commitment at the 8 cell stage

Well annotated genome

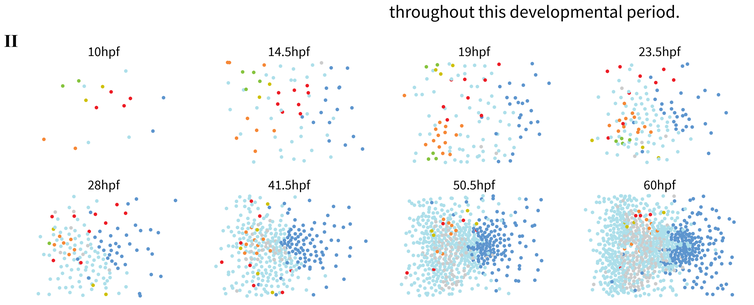
Ease of culture

Results



Dotted lines: linear regression of the segment with $p<10^{-5}$. Black arrows: inflection point in the rate of division. Data extracted from tracking of a single *P. hawaiensis* embryo over 300 time points.

The nuclei in one embryo were tracked automatically using Ilastik[5] and tracks were corrected in Mamut[6]. In I.a, using the tracking information, we calculated the number of nuclei over time (assuming no cell death) to look at the rate of division. Interestingly, we noticed that average cell cycle length varied across developmental time in a way that could be correlated with different phases of cell behavior during embryogenesis.. First, cells divide rapidly from the 16 cell stage to the 64 cell stage (cell number doubling time (dt) = 1.06h). Second, the rate of division more than halves from the 64 cell stage to the 128 cell stage (dt=2.7h). During this phase cells start to migrate towards the posterior pole of the embryo. Third, during gastrulation mitosis stops almost completely (dt=22.5h). Finally, after gastrulation to the elongation og the germ band the divisions resume at a lower rate during the formation and elongation of the germ band (dt=5.5h). Interestingly as shown on I.b, this corresponds to varying average nuclear velocities. On panel II, using the 3D coordinates of the nuclei, we performed a spherical projection onto a 2D surface. While more analysis are needed it seems that the teritories of cells are defined early and stay the same throughout this developmental period.



2D spherical projection of tracked nuclei of one embryo of *P. hawaiensis*. A sphere was fitted on the nuclei positions and the their positions projected onto it. Finally, the angles where projected onto a 2D plane. Color coding is as per 8 cell stage schematic above.

[1] Huisken, J.; Swoger, J.; Del Bene, F.; Wittbrodt, J.; Stelzer, E. H. "Optical sectioning deep inside live embryos by selective plane illumination microscopy". Science. 2004 Aug 13;305(5686):1007-9. DOI: 10.1126/science.1100035
[2] Tomer, R.; Khairy, K.; Amat, F.; Keller, P. J. “Quantitative high-speed imaging of entire developing embryos with simultaneous multiview light-sheet microscopy”. Nat Methods. 2012 Jun 3;9(7):755-63. DOI: 10.1038/nmeth.2062.
[3] https://unity3d.com/
[4] Browne, W. E.; Price, A. L.; Gerberding, M. & Patel, N. H. “Stages of embryonic development in the amphipod crustacean,Parhyale hawaiensis”. Genesis. 2005 Jul;42(3):124-49. DOI: 10.1002/gene.20145
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[6] Wolff, C.; Tinevez, JY. & Pietzsch, T. et al. "Multi-view light-sheet imaging and tracking with the MaMuT software reveals the cell lineage of a direct developing arthropod limb.", eLife. 2018 Mar 29;7 DOI: 10.7554/eLife.34410

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