TOWARDS DIGITAL REPRESENTATION OF DROSOPHILA EMBRYOGENESIS

Preibisch, S.[1]*, Ejsmont, R.*[1]*, Rohlfing, T.*[2]*, Tomancak, P.*[1]

[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[2]SRI International, Neuroscience Program, Menlo Park, CA 94025, USA

ABSTRACT

Animal development can be described as a complex, three-dimensional cellular system that changes dramatically across time as a consequence of cell proliferation, differentiation and movement. Using *Drosophila* embryogenesis as a model we are developing molecular, imaging and image analysis techniques to record an entire developmental system at cellular resolution.

We image *Drosophila* embryos expressing fluorescent markers *in toto* using Single Plane Illumination Microscopy (SPIM). SPIM offers the unique ability to image large living biological specimens in their entirety by acquiring image stacks from multiple angles while also providing high temporal resolution necessary for following dynamic developmental processes. We have developed an image analysis pipeline that efficiently processes long-term time-lapse multi-view SPIM data by aligning the different angles with high precision for a single time point and propagating the alignment parameters throughout the time series. The registered views are fused using an approach that evaluates the image content in each view.

Index Terms— SPIM microscopy, 3D image analysis

1. INTRODUCTION

Developing biological systems are complex, three-dimensional (3D) cellular assemblies that change dramatically across time as a consequence of cell proliferation, growth, differentiation, movement and death. These concerted cellular behaviors in development are encoded in the genome and tissue specific gene expression is the most direct manifestation of the developmental program. Several decades of molecular genetic analysis provided an impressive understanding of gene regulatory networks in development. It has been shown that cell autonomous regulatory events are integrated with inputs resulting from cell-to-cell communications are constrained by developmental history and tissue architecture of the developing embryo and form a complex network that drives cells to their developmental fate. It is clear that in order to fully understand how genomic information transforms into animal development, we need to study the system as a whole. Of course, we currently do not possess the ability to follow all components of the gene regulatory network simultaneously on the molecular level. However, emerging reverse genetic and microscopic approaches allow us to follow developmental events and gene expression regulation in the entire organism on the cellular level.

Developmental processes, and the role of gene expression regulation in them, have been studied for decades using sophisticated microscopy techniques. It is standard in developmental biology to draw conclusions about genes' expression pattern from subjective assessments of two-dimensional images of fixed and stained biological specimens. The resolution of these images is usually insufficient to distinguish individual cells in the entire 3D specimen. In order to achieve cellular resolution the microscopy focuses on only a small part of the developing system. The temporal dynamics of changes in gene expression patterns are captured by a sparse sampling of different developmental times in different specimens and the relationship between patterns of gene expression and cellular behavior is often neglected.

To fully represent the developmental system with current and emerging technologies means to digitize development by following every cell in an entire developing system from the zygote to the mature embryo, and to assign gene expression levels to every cell, at every time, and, ultimately, for every gene.

We focus on *Drosophila* embryonic development because its developmental anatomy is well described and gene regulatory networks are well understood. Genome–wide *in situ* surveys provide a complete overview of the types of gene expression regulation [1]. Importantly, the development of the *Drosophila* embryo occurs within a relatively small and stable 3D volume, which provides significant benefits for whole animal imaging.

Single Plane Illumination Microscopy (SPIM) offers a number of key advantages over other imaging technologies that are also capable of optical sectioning such as confocal microscopes [2]. In SPIM, optical sectioning is achieved by focusing the excitation laser into a thin light sheet, which penetrates the living sample embedded that is in an agarose gel and suspended by gravity in a water–filled chamber (Figure 1). The objective lens is arranged perpendicular to the axis of illumination and the laser illuminates only the imaged plane

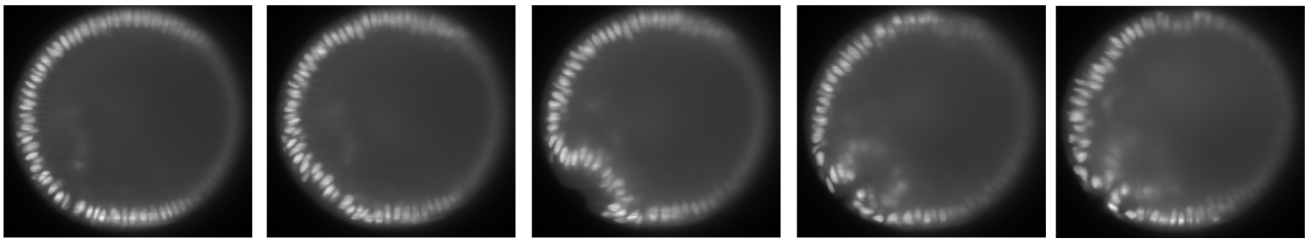

Fig. 2. **SPIM captures dynamic developmental processes.** Slices from single view SPIM image stack of *Drosophila* embryo undergoing gastrulation. The entire specimen volume was scanned from four different angles every five minutes. Note that even the fastest developmental event during embryogenesis, the gastrulation movement, is recorded with sufficient temporal resolution.

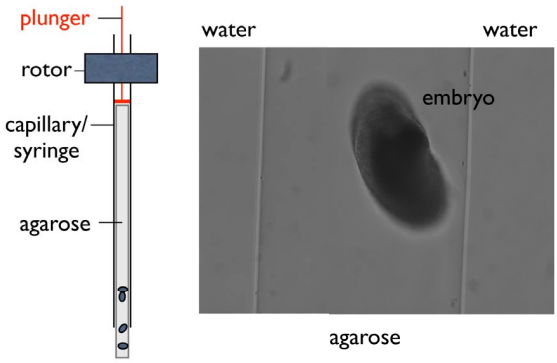

Fig. 1. **New sample mounting paradigm in SPIM microscopy.** *Left:* Schematic drawing of SPIM sample holder. The specimen, for example (*Drosophila* embryo), is embedded in agarose gel that is pushed out of a glass capillary or syringe by a wire plunger and into a water filled sample chamber (*Right*).

of the specimen. This minimizes photo-bleaching and laser damage to the living samples and allows very long time-lapse recordings. The images are captured by a CCD camera enabling very fast acquisition rate important for capturing dynamic developmental events in living embryos (Figure 2). The SPIM instrument can, in principle, achieve an isotropic, high resolution along x, y and z axis, thus allowing for the first time to image large 3D specimens in their entirety. In order to achieve an isotropic resolution uniformly across the sample's 3D volume, it is necessary to rotate the sample and record image stacks from different angles (usually 8 to 12).

We describe herein an application of SPIM microscopy to generate multi-view, long-term, time-lapse recordings of *Drosophila* embryogenesis marked with ubiquitous fluorescent cellular markers resulting in *in toto* representation of the living system. We present an efficient image analysis approach to register and fuse multi-view SPIM data across large time-lapse recordings.

2. MATERIALS AND METHODS

For all live recordings presented in this paper we used *Drosophila melanogaster* embryos expressing NLS-GFP (NLS = Nuclear Localization Signal; GFP = Green Fluorescent Protein) transgene that marks all nuclei in the specimen. The embryos were imaged with a prototype of SPIM microscope developed by Carl Zeiss Microimaging GmbH using a 20×/0.5 Achroplan objective. We acquired 4 and 8 angles for each time-point in the extended time-lapse recording, spaced by 90° and 45°, respectively.

3. RESULTS

To achieve isotropic image resolution for large 3D biological specimen, SPIM image stacks acquired from different angles (views) must be aligned with one another ('registered') and then fused to form a single 3D volume. The registration and fusion is confounded by degradation of the signal due to scattering of light along the illumination and detection axes (Figure 3), as well as by the limited overlap between individual views of the 3D volume in large scattering specimens such as Drosophila embryos. For inter-view alignment we developed a registration algorithm that identifies the rotation axis common to all views by iterative optimization of FFT-based phase correlation between adjacent views. Registered views are combined using content-based image fusion method that limits the incorporation of degraded signal into the fused image. For fast processing of long-term time lapse recordings the parameters of the rotation axis identified for one time point are propagated to adjacent time-points.

3.1. SPIM Image Registration

For the acquisition of each view, the glass capillary containing the specimen is rotated around its center and translated, so that the specimen is in the field of view of the objective. We

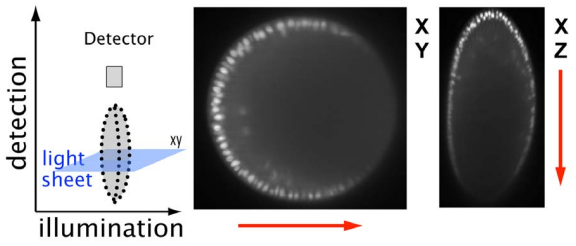

Fig. 3. **Signal degradation in SPIM.** *Left*: Schematic representation of the SPIM imaging set-up with illumination axis perpendicular to the detection axis. *Right*: Individual slices from a single view SPIM stack show the signal degradation (red arrows) along the x and y axes due to attenuation of the illumination laser light sheet and along the x and z axes due to scattering of emission fluorescence within the specimen.

exploit these design properties by defining a SPIM–specific transformation model, which prevents the registration scheme from converging to solutions that are physically impossible. This restriction furthermore substantially limits the number of possible transformations [3]. The transformation model, therefore, consists of (a) the individual position of each view in a global coordinate space (b) the known rotation of each view around a rotation axis and (c) the orientation of this axis, which is invariant but unknown. Instead of 6 transformation parameters for each view (3 translations + 3 rotation angles) we end up with 3 parameters for each view plus 2 parameters which define the common rotation axis for all views.

In order to determine these parameters, we maximize the alignment quality of a translation-only registration between all the views for different hypotheses of the orientation of the rotation axis. This approach yields the correct rotation axis as the alignment quality measure peaks when the rotational offsets between the views are minimal. The rotation axis orientation is continuously updated using a hill-climbing scheme similar to the algorithm proposed by [4], in combination with a multi-resolution approach to speed up the computation. The translation-only registration is efficiently implemented using the FFT-based phase correlation method [5]. Unlike gradient-based methods the FFT-based approach cannot get trapped in local minima, as it is a global approach that examines all possible translations between two views in one operation. This is crucial to find the correct alignment. In order to define the quality of a certain rotation axis we compute the translations between all adjacent views and average their normalized cross correlation coefficients over all image pairs. A volume registered from two adjacent views using this approach is shown in Figure 4.

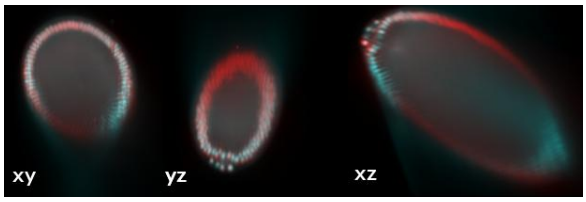

Fig. 4. **Two registered adjacent views.** The images show 3 slices through the volume of two registered SPIM views, coded in red and cyan. Note that perfectly overlapping views result in gray pixels. In areas where the content of the views overlaps one can clearly see how the registration matches the views down to single nuclei. However, in other regions of the embryo the views are very different and illustrate the difficulty of the registration task.

3.2. SPIM Image Fusion

The algorithm for fusion of the registered views has to ensure that only high–quality data from each view contributes to the final isotropic 3D image. Following the idea presented by Goshtashby [6] we locally evaluate the information content in each view and use it as a weighting factors for the contribution of the respective area to the final image [3].

3.3. SPIM Long-Term Time Lapse Processing

Long-term time lapse, multi-view SPIM recordings generate staggeringly huge amounts of raw image data. Typical 12 hour (130 time-points), 8 view, single color SPIM recordings consist of more then 100,000 images and occupy ca. 80GB of disk space, even in compressed state. Each individual time-point (8 stacks), is typically larger than 4 GB at full resolution, and the computation time for registration and fusion on an 4-core Intel Xeon 5160 (2.66 Ghz) system with 24GB of RAM takes roughly 8-10 hours. Processing of such time-lapse recordings using the aforementioned registration approach would be prohibitively slow, but parallelization of the task is very expensive and therefore not suitable for routine use.

To allow reasonably fast processing of long–term time lapses we apply the computationally expensive, unconstrained initial alignment only to a single time point and subsequently propagate the transformation parameters to the adjacent time-points. Such propagation is possible because the rotation parameters remain relatively constant across the time–series and can be seen as an initial, software–based calibration of the imaging system. The registration algorithm converges significantly faster in most cases when launched with initial transformation parameters derived from an adjacent time–point (see Table 1). Sequential, accelerated processing of massive time-lapse dataset has the additional benefit, that it can be started while the microscope is still acquiring

Time Point	Rotation Axis Vector (x, y, z)	Registration Time
TL100	$(1.0, 0.02031, 0.00908)$	50 Min
TL101	$(1.0, 0.02070, 0.00946)$	12 Min
TL102	$(1.0, 0.01934, 0.01060)$	45 Min
TL103	$(1.0, 0.01934, 0.01158)$	14 Min
TL104	$(1.0, 0.01964, 0.01238)$	19 Min
TL105	$(1.0, 0.01955, 0.01272)$	9 Min

Table 1. Direction of rotation axis after registration of six consecutive time points of a two angle SPIM time lapse recording. The registration of the fist time point was unconstrained; for the following time points the registration was initialized with the previously determined rotation axis. Note the substantial speed–up of the registration time, except for time point TL102, in which the rotation axis had changed significantly relative to the used step size.

later data and thus enables examination of processed data immediately after their acquisition.

4. DISCUSSION

We have developed a SPIM image processing pipeline that is capable of combining long-term time lapse multi-view SPIM image stacks into isotropic 4D data sets. So far, we have evaluated the precision of the registration process by visual inspection of nuclei overlapping between adjacent views (Figure 4). Quantitative evaluation of the registration accuracy is not possbile with the currently available datasets. We plan to achieve objective and precise measurements of the registration error by comparing the center positions of sub-resolution fluorescent beads that will be included in future SPIM recordings.

The algorithm can be further accelerated substantially by outsourcing the processing of the compute–intensive operations (FFT based registration) to broadly available graphics hardware using the NVidia CUDA API[1].

Generating complete isotropic recordings of developing embryos is the first step towards realizing the daunting task of digitizing development. We have developed reverse genetic strategies to monitor cellular behavior and gene expression patterns in the context of the intact, living embryo, which are compatible with high–resolution SPIM imaging. To analyze the 4D SPIM microscopic recordings of embryonic development, we will establish an image analysis pipeline able to assign gene expression levels to individual cells, follow cells in time and space and compare topological, cellular level models of different embryo recordings to one another.

When successful, this approach will allow the study of the interplay between global cellular behaviors and gene expression regulation events during development. By mapping different experiments to a common reference system we will be able to evaluate the variability of gene expression and developmental processes among different individuals, strains, or species, and to rigorously compare wild–type and mutant embryos. Overall we aspire to create a new paradigm of how developing biological system are described and studied: *in toto*, at cellular spatial resolution and with high temporal resolution. The collected data will serve as a foundation for computer modeling of development.

[1] `http://www.nvidia.com/cuda`

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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