HIGH-SPEED PANORAMIC LIGHT-SHEET MICROSCOPY TO UNDERSTAND ENDODERMAL CELL DYNAMICS

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In long-term high-speed light sheet microscopy Terabytes of data accumulate within a few hours, limiting its applicability in fields that demand a high sample count such as developmental biology. We designed an imaging system that combines SPIM (Selective Plane Illumination Microscopy) with real-time image processing, dedicated for rapid imaging of entire zebrafish embryos. This approach extracts the desired information during image acquisition and reduces the amount of data by a factor of 200, facilitating data storage, visualization and further analysis.

Multi-view acquisition makes SPIM an ideal tool for in vivo imaging of millimeter-sized samples, which cannot be imaged entirely from a single side. Combined with a fast rotational motor, a classical 2-lens SPIM setup with one illumination and one detection lens can provide multiple views in a few seconds [1]. We built a 4-lens SPIM setup consisting of four identical objective lenses. The two detection lenses image their common focal plane onto two sCMOS cameras, capturing two opposite views in one scan of the sample through the light sheet [2]. The result is a high-resolution 3D dataset of an entire zebrafish embryo. However, storing and post-processing of the enormous amounts of data that accumulate during time-lapse acquisition limits the number of experiments. Therefore, real-time processing of the data is inevitable for quantitative developmental biology studies.

We found that image transformations tailored to the shape of the sample can be performed in real time to efficiently compress the data stream from the microscope. The shape of an early zebrafish embryo can be approximated by a sphere. On its surface, the endodermal cells form a single cell layer. We exploited this spherical geometry to generate a radial maximum intensity projection. Taking insights from cartography, a 2D map of this data is created to visualize the entire tissue in a single image. Our radial projections are computed in real time during acquisition without saving any raw image data, which not only reduces the amount of data from Terabytes to Gigabytes but also offers visualization and evaluation of cell migration in the context of whole embryos. A panoramic view of the entire embryo is obtained within ten seconds. Further, cell segmentation and flow analysis reveal characteristic migration patterns of the early endoderm that can be quantitatively compared between embryos. This novel approach enables us to simultaneously visualize and correlate single cell dynamics and processes at the level of whole tissues.

References