Integrating 2D/3D Time-Lapse Microscopy Images to Quantify Angiogneic Sprout Formation

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***Abstract*—We develop algorithms to automatically process heterogeneous images from angiogenesis experiments to track migrating cells and sprout formation. Our approach jointly combines 3D cell tracking and 2D vessel tracking in the spatial and temporal domain using Bayesian filtering.**

I. Introduction

Angiogenesis is the process of generating a vascular network from an existing blood vessel. A population of endothelial cells residing in a blood vessel (lumen) can sprout out and create a new vascular network when exposed to growth factors. In micro-fluidic 3-D angiogenic sprouting experiments [1], two types of images are obtained at discrete time steps using confocal microscopy: a) three-dimensional fluorescent images of stained cell nuclei and b) bright field images of the gel matrix. These two sources of images provide supplementary information as the outline of the lumen formed in the matrix by the migrating cells can be seen in the images of the gel. A significant amount of data can be produced from these experiments particularly from time-lapse confocal images. Automated image analysis is therefore a more favorable alternative to manual tracking for a more efficient and accurate data assessment.

We present an approach to simultaneous cell and vascular sprout tracking; fusing nuclei images with gel matrix images. Our methodology can optimally (a) combine data from heterogeneous images and known biological models, (b) track cell movements despite a low sampling rate of 3D nuclei position and (c) detect changes in vascular geometry such as branching. Although we have developed our methodology specifically for angiogenic sprouting, our framework can be generalized and applied to other applications which involve interactions between migrating cells and the surrounding gel.

II. Integrated Tracking System

In sprout formation, tip-cells lead the forming sprout, while stalk cells trail behind, supporting the newly formed sprout. Tip cells are characterized by rich filopodia protrusions, which can be seen from the sprout outline. We segment 3D nuclei centroid locations and 2D sprout profile from the images. We classify cells to tips and stalk cells and the sprout profile to filopodia and lumens.

We track a joint state representation of tip/stalk cells and lumen and filopodia, using the concepts of Simultaneous Localization and Mapping (SLAM) [2]. The Bayesian filtering framework developed augments both the cell and lumen/filopodia parameters to the same state vector, allowing mathematically consistent simultaneous observation updates from both channels. We incorporate biological models into our cell and sprout tracking algorithms [3].

III. Results And Conclusion

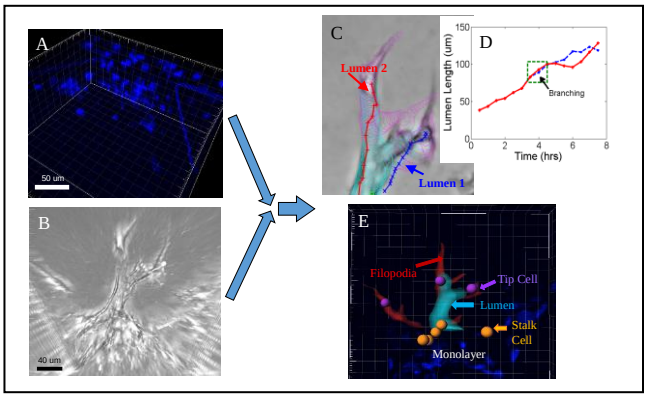

Figure 1. We combined time-lapse fluorescent images of stained nuclei (A) with bright filed images (B), which provide 2D information of the sprout. In (C), we show the final lumen, filopodia and cell trajectories. During the time lapse, a stalk cell (red '+'), following a tip cell (blue 'x'), changed its phenotype. It became another tip cell and a branch in the sprout was formed. We quantified the lumen elongation change over time in (D) and detected when branching occurs. In (E), we visualize lumens (cyan tube) and filpodias (red tubes). We also classify cells to tip cells (purple spheres) and stalk cells (orange spheres).

In Figure 1, we demonstrate the advantages of jointly tracking sprout and nuclei information. We obtain lumen growth details, cell trajectory statistics and detect vascular geometry changes. Our outcome is software to track and visualize lumen formation, filopodia extension and retraction, and nuclei locations and tip/stalk phenotype at each time step.

References

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