Automated 5-D Analysis of Cell Migration and Interaction from Multi-spectral Multi-dimensional Image Series

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This paper presents an automated method to analyze fluorescently labeled thymocytes and dendritic cells (DC) in mouse thymic cortex from 5-D (x, y, z, t, λ) images acquired by two-photon laser scanning microscopy (TPLSM). Importantly, we study migration patterns and dynamic phenomenon of motile thymocytes and quantify thymocyte contacts with stationary DCs. Prior study [1], based on manual inspection and measurements, was labor intensive, time costly, and error-prone. To overcome these limitations and set the stage for large-scale quantitative multi-spectral multi-dimensional studies of cell dynamics and interactions, software was developed for efficient inspection, validation and corrective editing. Results from our automated analysis agreed with manually generated measurements to within 7%.

Our method entails four steps as shown in **Fig. 1**. First, two-photon laser scanning microscopy was performed at mouse thymic cortex over 20~40 minutes at 37-second intervals, with a resolution 164×164×40 μms or 256×256×21 voxels at 8 bits at each time point. **Fig. 1-A** shows sample images of DCs and wild type thymocytes at two successive time points. Second, after un-mixing spectral overlapping across GFP and YFP channels, cells were delineated via mean-shift clustering [2], as displayed in **Fig. 1-B**. Segmentation outputs were used to measure geometric features of each thymocyte, such as centroid, volume, and radius. Third, thymocytes were traced over time using multiple hypothesis tracking [3]. Tracked cells were consistently numbered and color-coded in **Fig. 1-C**. Finally, a rich set of dynamic features (e.g. speed, displacement, and directionality index), were computed from tracking results to quantify motility patterns of thymocytes, and duration of thymocyte-DC contacts undergoing signal selection events (**Fig. 1-D**).

To ensure the validity of machine-generated outputs, we developed a Matlab-based tool (**Fig. 1-E**) to automatically evaluate and high-light statistical outliers for further inspection and editing.

References

1. Robey, EA, Bousso, P., Immunological Reviews, 195:51-57.
2. Comaniciu, D., *et. al.*, IEEE Trans. on PAMI, 24: 5, pp: 603-619, 2002.
3. Al-Kofahi, O., *et. al.*, Cell Cycle, 5: 3, pp: 327-335, 2006.
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Figure 1. Schematic of steps involved in 5-D automatic study of cell dynamics and interactions.