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Biological Study Using 3-D Tissue Cytometry

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Abstract: 3-D tissue cytometry has been successfully developed to optimize biological specimen throughput allowing the characterization of cell-cell, cell-tissue interaction to be quantified in 3-D tissue by combining high speed TPM, automated x-y specimen stage, and precision specimen sectioning mechanism. 3-D tissue cytometry is applied in two biomedical applications.

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1. Introduction

3-D tissue cytometry has been developed with two-photon microscopy (TPM) that is capable of in situ 3-D imaging of tissue up to a volume of several mm³ with subcellular resolution [1] (refer to figure 1). This high throughput tissue cytometry achieves an imaging rate of 2 mm³/hour. The differences in the cellular behavior in their native tissue setting and in artificial environment are relevant in the interpretation of clinical and basic research results. There is a pressing need for the development of new cytometric tools that can effectively characterize cells in their native tissue state. Investigating the behavior of cells in intact tissues offers several advantages. This thesis presents the technological development of high throughput 3-D tissue cytometry and its applications in biomedicine. 3-D tissue cytometry is applied in two biomedical applications.

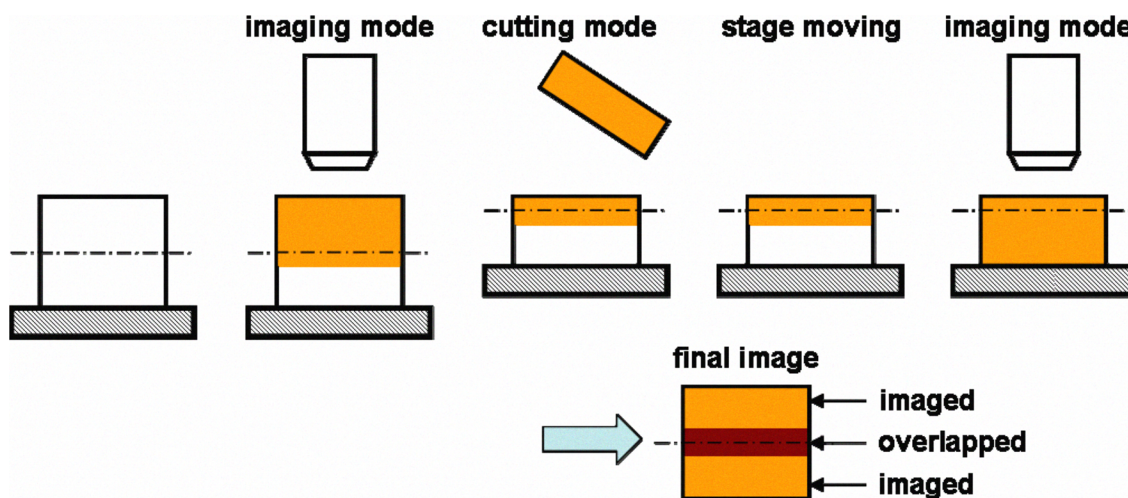


Figure 1. 3-D tissue cytometry imaging procedure

2. Multi-scale study of mouse tongue musculature using 3-D tissue cytometry

We investigated the muscle architecture of whole mouse tongues. We have shown that force generation and deformation of the tongue can be characterized by “fiber tracks” observed in tongues using high field MRI. However, the morphological origin of these tracks has not been fully qualified on the cellular level and we seek to correlate these tracks with structures observed using 3-D tissue cytometry. In Diffusion Spectrum Imaging (DSI), preferential water diffusion paths in the tissue has been shown to be correlated with physiological motion of the tongue. On the other hand, fiber tracts found from TPM with imaging analysis is from the second harmonic signals of collagen. We demonstrated the mesoscale architectural linkage between the microscopic and tissue scales validating the hypothesis that the fiber tracks observed from DSI is consistent with the distribution of myocyte fibers in the tissue (refer to figure 2).

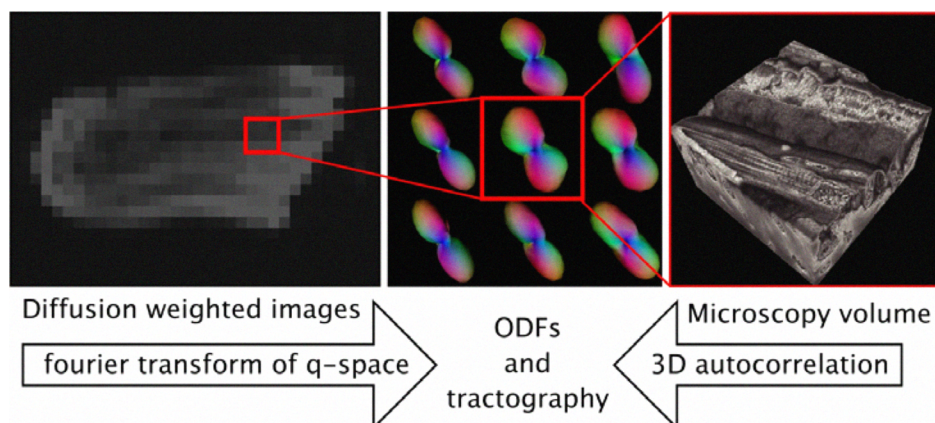


Figure 2. Mesoscale comparison of fiber orientation obtained from DSI and TPM

3. Three dimensional cardiac architecture determined by 3-D tissue cytometry

Cardiac architecture is inherently three-dimensional, yet most characterizations rely on two-dimensional histological slices or dissociated cells which remove the native geometry of the heart. Because understanding the structure of the heart is important to characterizing hypertrophy and other disarrays, we developed a method for labeling the intact heart without dissociation and imaging large volumes while preserving the three-dimensional structure.

We report the use of intravital staining and two-photon microtomy to image intact heart volumes (several hundred microns, cubed). After data acquisition, the sections are assembled using image processing tools and both qualitative and quantitative information are extracted. By examining the reconstructed cardiac blocks, one can observe end-to-end adjacent cardiac myocytes (cardiac strands) changing cross-sectional geometries, merging and separating from other strands. Quantitatively, representative cross-sectional areas typically used for determining hypertrophy omit the three-dimensional component; we show that taking orientation into account can significantly alter the comparisons being made. Using fast-fourier analysis, we determine and analyze the gross organization of cardiac strands in three-dimensions. We show that cardiac strands from alpha-crystallin mutant mice exhibit greater cross-sectional area but do not consistently exhibit disorganization.

These results demonstrate that intravital staining combined with the two-photon microtome and post-processing can acquire three-dimensional structural information in intact hearts. By characterizing cardiac structure in three-dimensions we are able to determine that the alpha-crystallin mutation leads to hypertrophy with cross-sectional area increases, but not necessarily via changes fiber orientation distribution.

4. Conclusions

The utility of these 3-D tissue cytometry were demonstrated by two biomedical studies. We demonstrated the use of the two-photon 3-D tissue cytometry in the study of musculature of mouse tongue. We have investigated the three dimensional cardiac architecture. 3-D imaging of specimen on the size scale of cubic centimeters allow the study of whole organ physiology with cellular and molecular resolution. Another important area where the in situ tissue properties of cells are of critical importance is in the field of cancer research. 3-D tissue cytometry will be used to investigate cancer studies.

4. References

[1] Timothy Ragan, Jeremy D. Sylvan, Ki Hean Kim, Hayden Huang, Karsten Bahlmann, Richard T. Lee, and Peter T. C. So, High-resolution whole organ imaging using two-photon tissue cytometry *J. Biomed. Opt.* 12, 014015 (2007)