Serial Two-Photon Plus (STP^2) for Medical Device Application: 3D Analysis of Ablated Pig Liver and Kidney

Introduction

Radiofrequency catheter ablation is a therapeutic method that utilizes programmed electrical stimulation to alter or destroy cells causing irregular parenchymal activity. Efficacy and safety are both determined by the ablated tissue volume and the impact of treatment on target and off-target cell populations. However, existing approaches, such as standard histology, only provide limited visualization of the ablated region or, in the case of *in vivo* CT imaging, sacrifice resolution of microscopic features. To address this gap, we have collaborated with CBSET (Lexington, MA) to develop a novel Serial Two-Photon Plus (STP^2) pipeline to visualize and quantify both the 3D ablated tissue and its cellular features across treated porcine liver and kidney samples.

TissueVision, Inc. (TVI) uses STP^2 technology to generate 3D volumetric datasets of whole tissue samples with sub-micron resolution. This label-free modality leverages endogenous tissue autofluorescence and second harmonic generation (SHG) imaging to capture both general morphology and the dense collagen fiber network within tissue (**Figure 1**). Using a porcine model prepared by CBSET, control and ablation conditions from liver and kidney samples were processed using the STP^2 imaging platform. 3D modeling and quantitative analysis of the ablation region of interest (ROI) allows for targeted evaluation of spatial and depth-dependent variations in collagen micro-morphology due to treatment.

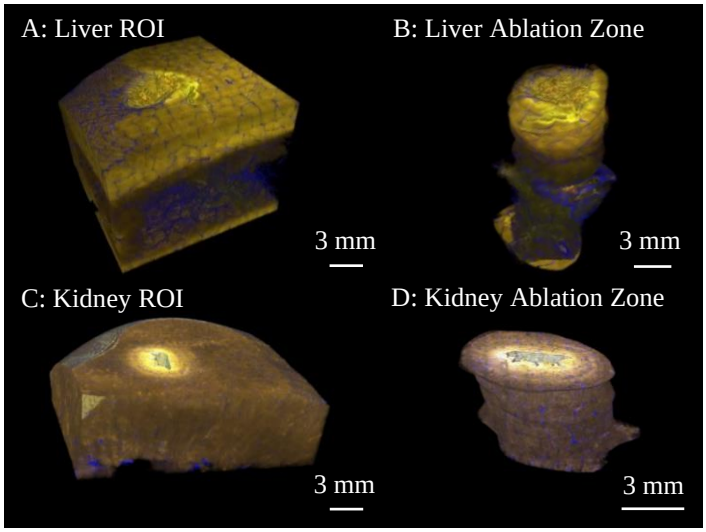

Figure 1. Identifying the 3D ablation zones.
Left: Full 3D render of (a) liver and (c) kidney samples showing autofluorescence (red, green) and SHG collagen (blue).
Right: 3D render of ablation area from (b) liver and (d) kidney samples digitally segmented from whole tissue.

STP^2 Insight

TissueVision's STP^2 imaging platform provides fully automated sectioning and 3D imaging of tissue samples. Porcine liver and kidney samples from CBSET were treated *ex vivo* with an ablation pen at a low power or unpowered (control) setting for 20 seconds. Samples were sectioned serially at 100 µm using a TissueCyte 2000™ system to collect images and preserve the sectioned tissue slices. With an excitation wavelength of 780 nm, autofluorescence pertaining to the ablated tissue was readily detected in all channels. Additional imaging at 920 nm revealed not only the autofluorescence of anatomical features in the ablation region of interest (ROI), but also the micro-morphology of the collagen network detected through second harmonic generation (SHG). **(Figure 2)**.

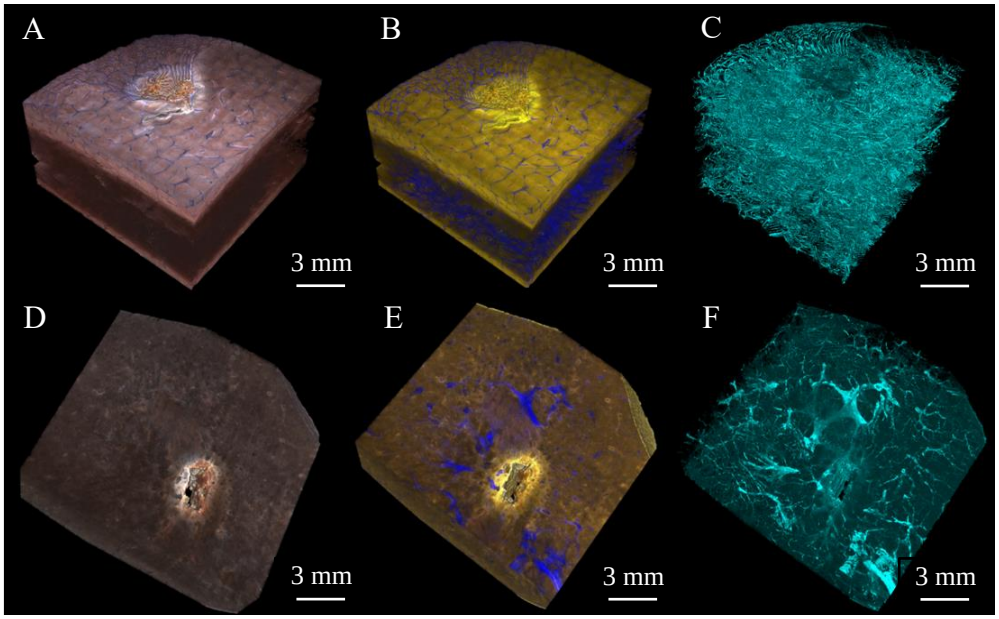

Figure 2. Characterizing tissue micro-morphology.
Top: 3D renders of ablated liver sample, including (a) three-channel data imaged at 780nm, (b) three-channel data imaged at 920nm, and (c) SHG channel only (920nm).
Bottom: 3D renders of ablated kidney sample, including (d) three-channel data imaged at 780nm, (e) three-channel data imaged at 920nm, and (f) SHG channel only (920 nm).

Annotation and 3D modeling of the ablation probe ROI allows volumetric high-throughput quantitative analysis of treated tissue. TVI's automated image analysis pipelines can compute informative metrics on ablation volume, collagen density and organization, and signal variation as a function of probe power, exposure time, and spatial distance. Evaluation of the collagen morphology throughout the 3D sample indicates altered collagen content in areas of high ablation **(Figure 3)**.

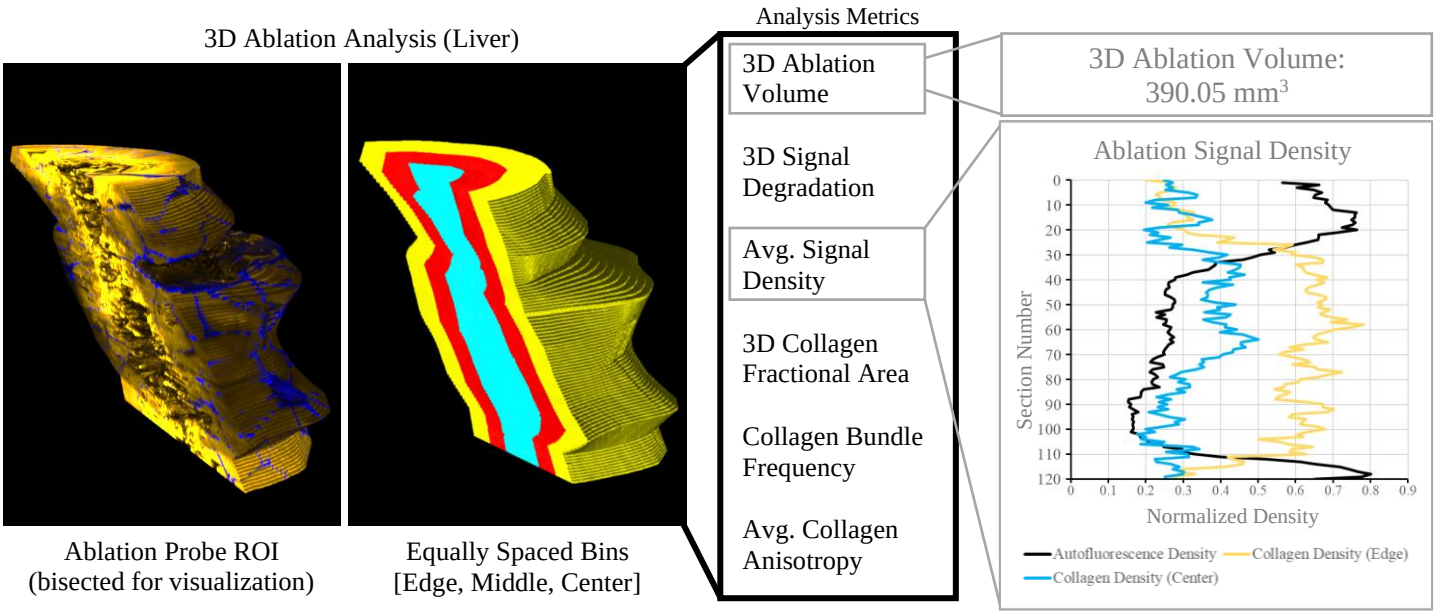

Figure 3. Analyzing micro-morphological ablation effects.
Left: Annotation of the ablation region enables 3D modeling of treatment area and localized analysis at defined distances from probe.
Middle: Automated analysis platforms compute key metrics characterizing extent of ablation damage and tissue organization. Metrics are computed for each 3D spatial bin and evaluate the morphological changes within high-resolution 2D image sections like traditional histological analysis.
Right: High-throughput analysis reveals that collagen content varies according to the amount of ablated tissue and distance from probe. This effect would be obscured in traditional approaches which consider images at a single depth or exclude variation between ablation center and edge regions.

The STP^2 pipeline produces high-throughput preclinical ablation data with enhanced sensitivity and precision, providing the ability to quantify changes in the total volume space of an ablated area for a variety of tissue types. Computational results provide informative 3D context, exposing local variations in tissue morphology not captured by the limited sampling of traditional histology. Yet, the flexibility of *ex vivo* STP^2 imaging still allows comparison with secondary analysis and *in vivo* data through its collection of preserved sections, offering a translatable approach for exploratory evaluation of preclinical test conditions.

Experimental

Tissues: Kidney and liver samples were sacrificed and harvested from porcine tissue by CBSET. Samples were drop fixed with 10% NBF, collected, and stored in 0.1% sodium azide in PBS before embedding.

Embedding: Tissue samples were embedded in a stabilizing polymer matrix to allow for whole tissue processing on the TissueCyte™ systems.

Sectioning: The tissue was serially sectioned with 100µm thickness and preserved for additional secondary analysis.

STP^2 Imaging: Whole tissue tomographic imaging was captured using the TissueCyte™ system. General tissue morphology was captured via autofluorescence signatures, and collagen signatures were detected with second harmonic generation (SHG) imaging.

Software Analysis: Targeted analysis platforms were developed to perform 3D modeling and compute quantitative metrics of tissue pathology and structure on high-resolution volumetric datasets at scale.

Benefits

- STP^2 technology creates 3D submicron resolution datasets
- Captures target sections for secondary analysis
- Approaches projects with GLP-like documentation with quality control review
- Utilizes fast online data viewer for high resolution image accessibility at https://www.tissuevision.io/

Keywords

Autofluorescence, ablation, collagen, imaging, liver, kidney, 3D, serial, microscopy, serial two-photon plus (STP^2), second harmonic generation (SHG), bioinformatics, lesion, quantitative analysis, volumetric analysis, collagen bundle organization, clinical translation, medical device.

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