A Novel 3D Imaging Pipeline for Analyzing Radiofrequency Catheter Ablation in Preclinical Animal Models

Gianna Ferron[1], Anthony Knesis[1], Neil Patel[1], Yookyung Jung[1], Stefan Linehan[1], Tim Ragan[1], Jay Budrewicz[2], David Parrillo[2], Rami Tzafriri[2]
[1]TissueVision, Inc., Newton, MA; [2]CBSET, Inc., Lexington, MA

Abstract ID: 4415
Poster Number: P286

Purpose

- 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 only provide limited visualization of the ablated region or sacrifice resolution of microscopic features.
- To address this gap, we have collaborated with CBSET 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.

Methods

Tissue Preparation

Porcine liver and kidney samples were treated *ex vivo* with an ablation pen at three timepoints (0, 20, and 40 seconds) using a model prepared by CBSET. Following ablation, samples were fixed with 10% neutral buffered formalin (NBF) for twenty-four (24) hours, then stored in 1x PBS and 0.1% sodium azide. Samples were embedded in an agarose block and polymerized in an embedding matrix to provide stability for whole-organ processing with the STP^2 imaging pipeline **(Figure 1)**.

STP^2 Imaging

Porcine 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 signatures pertaining to the ablated tissue were readily detected in all channels. Additional imaging at 920 nm revealed not only the autofluorescence profiles of anatomical features in the ablation region of interest (channel 1; channel 2), but also the micro-morphology of the collagen network detected through second harmonic generation (SHG) in channel 3 (emission spectrum: 442-478 nm) **(Figure 2)**.

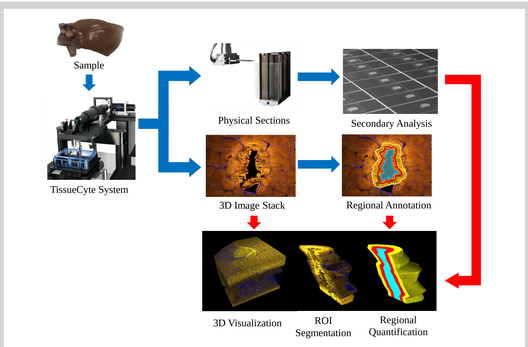

Figure 1. TissueVision's Serial Two-Photon Plus (STP^2) pipeline.

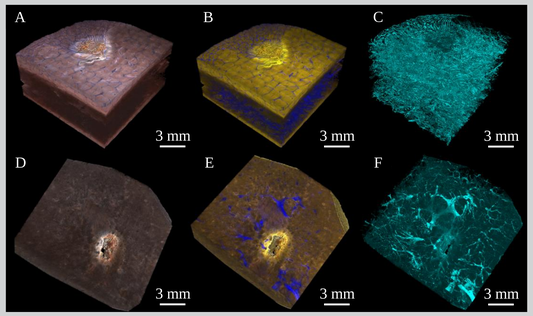

Figure 2. Characterizing tissue micro-morphology.
3D renders of ablated porcine samples show gross anatomy and cellular features from autofluorescence (red; green) and SHG (blue).
(a) 3D ablated liver sample imaged at 780 nm excitation wavelength.
(b) Liver sample imaged at 920 nm.
(c) Liver sample imaged at 920 nm, SHG channel only.
(d) 3D ablated kidney sample imaged at 780nm excitation wavelength.
(e) Kidney sample imaged at 920nm.
(f) Kidney sample imaged at 920 nm, SHG channel only.

Results

3D Ablation Annotation

Analysis pipelines were developed to process and extract the damaged area of each sample from the background through a semi-automated process. The ablation region of interest (ROI) was annotated for a subset of sections and interpolated over the full dataset to generate a full 3D volume of the probe area in treated samples. The extent of the ablation area within each section was qualitatively determined according to the observed increase in autofluorescence signal compared to surrounding nominal tissue. The resulting high resolution 3D models demonstrated successful isolation of the ablated tissue volume and allowed qualitative evaluation of the damage exhibited within both liver and kidney samples. **(Figure 3)**.

3D Quantitative Analysis

Annotation of the ablation probe ROI allows high-throughput volumetric quantitative analysis of treated tissue. Our automated analysis pipelines compute informative metrics on ablation volume and collagen morphology, including signal density, fiber bundle alignment, and fiber bundle aggregation. Analysis of the autofluorescence signal intensity and its variation as a function of probe power, exposure time, and spatial distance was also developed. The ablation annotation was divided into three (3) equally-spaced bins to enable localized spatial analysis in 3D at defined distances from the probe **(Figure 4)**. The ablation volume was quantified for each spatial bin and physical section and summarized for the total 3D volume **(Figure 5)**. Metrics were also computed to compare changes in the density of autofluorescence and collagen signals throughout the sample. Quantitative analysis revealed depth dependent and spatially dependent variations in collagen content in areas of high ablation **(Figure 6)**.

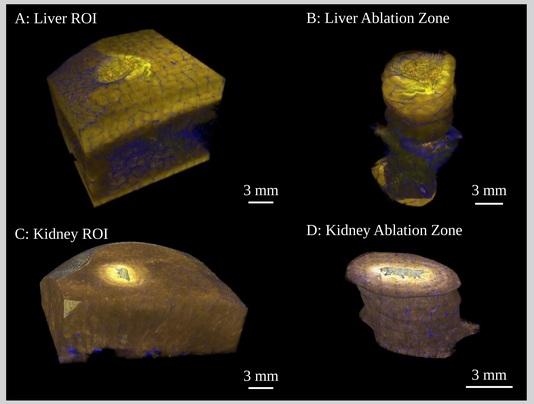

Figure 3. Identifying the 3D ablation zones.
STP^2 datasets show features in autofluorescence (red; green) and SHG (blue).
(a) 3D liver sample; (b) 3D annotation of the liver ablation area.
(c) 3D kidney sample; (d) 3D annotation of the kidney ablation area.

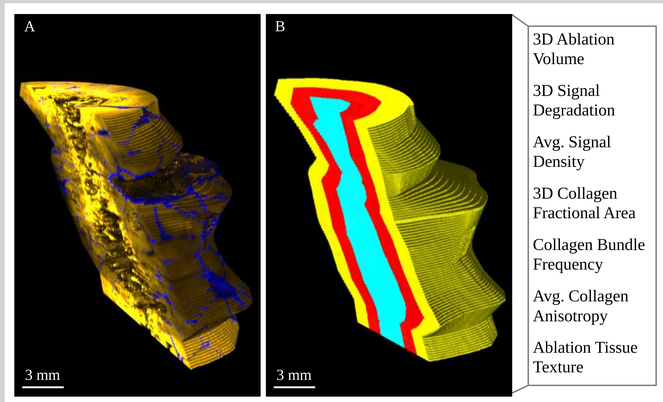

Figure 4. 3D modeling of the ablation zone for analysis.
(a) 3D annotation of the liver ablation area (bisected to show probe insertion area).
(b) Labelled bins computed from the 3D annotation, dividing ablation into three equally-spaced regions (edge, middle, center). Metrics developed for ablation analysis are applied to each bin individually to capture spatial variation in tissue properties.

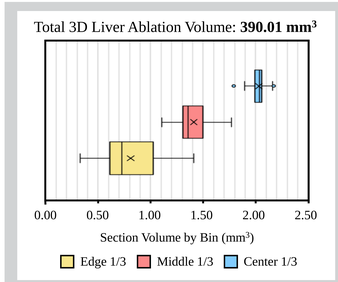

Figure 5. 3D ablation volume for full liver sample and bins.

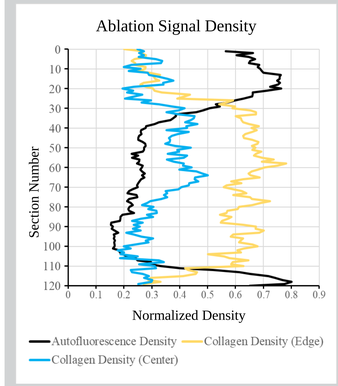

Figure 6. Analysis of ablation effects on collagen.
Quantitative analysis can compare the effects of ablation on both autofluorescence within the sample and collagen content within the center and edge bins. Collagen density was observed to decrease in areas of high ablation or in close proximity to the probe.

Conclusions

- Liver and kidney ablation samples were imaged and visualized in 3D using a novel STP^2 imaging pipeline, revealing autofluorescence-based and collagen-specific changes due to tissue damage.
- The tissue samples and ablation ROI were visualized and annotated in 3D, providing informative volumetric context while preserving features at the cellular level.
- Quantitative analysis of the ablation area exposed spatial and regional differences in tissue properties that cannot be 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.
- The STP^2 pipeline produces translational high-throughput preclinical ablation data with enhanced sensitivity and precision, providing the ability to quantify changes in volume and micro-morphology for a variety of timepoints, treatment conditions, and tissue types.