Automated Volume Imaging using Two Photon Microscopy

Aagam Shah

Institute for Computational Medicine

Johns Hopkins University

Test the Metabolic Sink Hypothesis

- In ischemia followed by reperfusion, production of ROS followed by opening of mitochondrial IMAC channel and oscillatory ROS release causes oscillatory collapse of mito membrane potential (Ψ_m).
- Reduced ATP production increases open probability of $I_{K, ATP}$ channel and hyperpolarizes myocyte membrane potential.
- This creates time varying regions of conduction block, known as metabolic sinks
- Hypothesis - metabolic sinks may be arrythmogenic.”

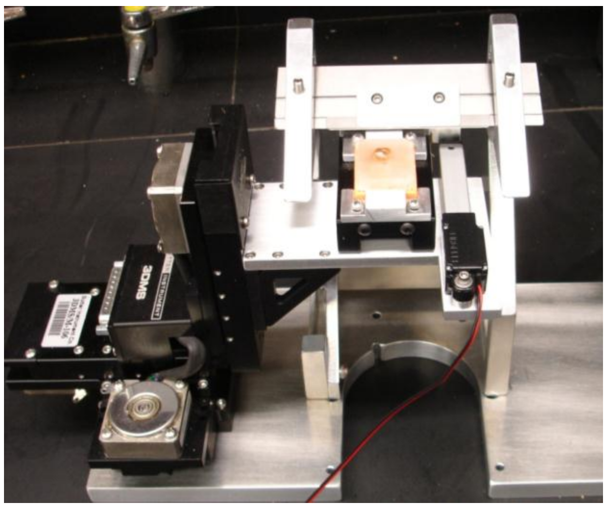
Approach – Ischemia -> Reperfusion

- Heart excised and perfused on Langendorf apparatus
- Induce global ischemia by suspending perfusion for 30 mins
- Upon reperfusion, mitochondria labeled with fluorescent indicator MitoTracker Red (fluoresces at red wavelength for polarized mitochondria, no or reduced fluorescence upon collapse of $\Psi_{m)}$
- Identify regions in which mitochondrial Ψ_m has collapsed based on presence/absence of red fluorescence

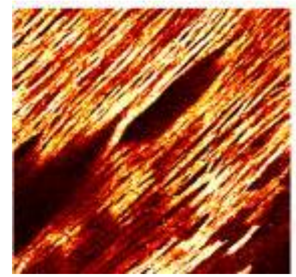
Approach (2)

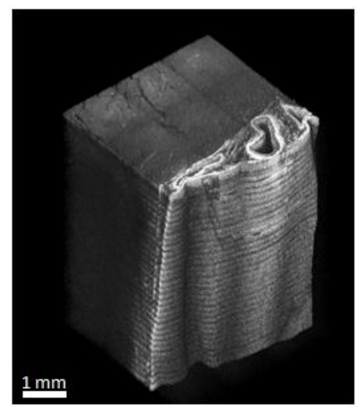
- To identify metabolic sinks, image normal and labeled hearts at high spatial resolution with two photon microscope and automated microtome stage
- Reconstruct acquired dataset into image volume.
- Perform statistical analysis to label voxels within metabolic sinks.
- Need to interactively visualize these large datasets at multiple spatial scales to explore properties of metabolic sinks.

Aim: Image, Visualize, Segment, Map



- Imaging with 10X or 20X objective





- Image volume reconstruction, High resolution visualization

- Label voxels based on fluorescence
- Map regions across multiple spatial scale

Procedure

- Acquire mice, rat, guinea pig hearts – label with fluorescent markers to highlight different features
- Fix, embed the hearts.
- Using Zeiss 2-photon microscope, automated stage and microtome blade, acquire optical and mechanical sections of the volume, in the form of tiles.
- Reconstruct (correct, align, stitch) these tiles to create a cross section of the myocardium (“slice”)

Example

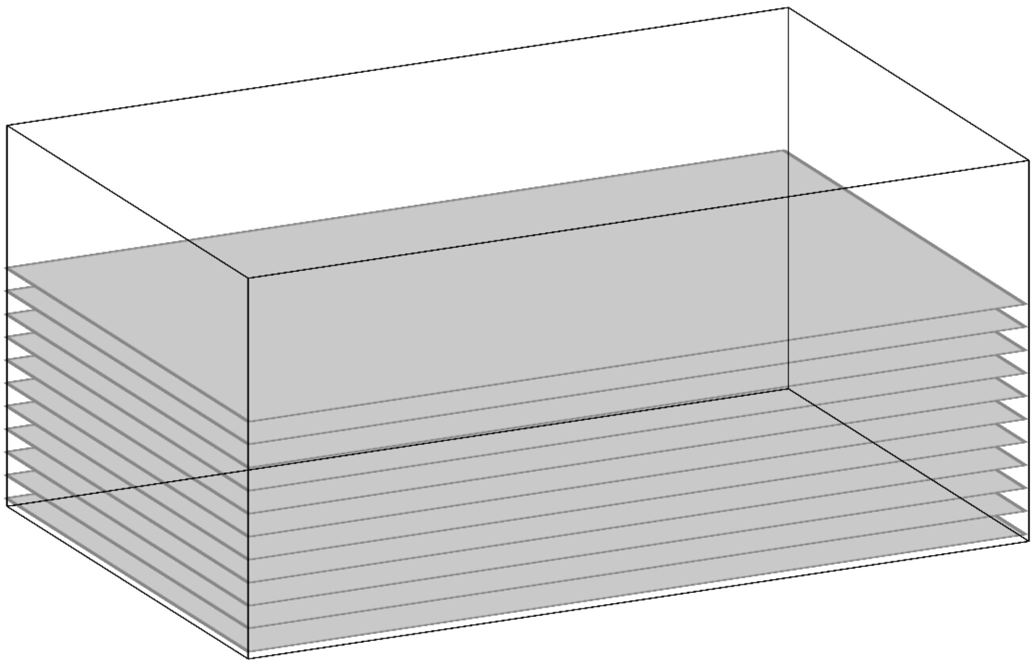
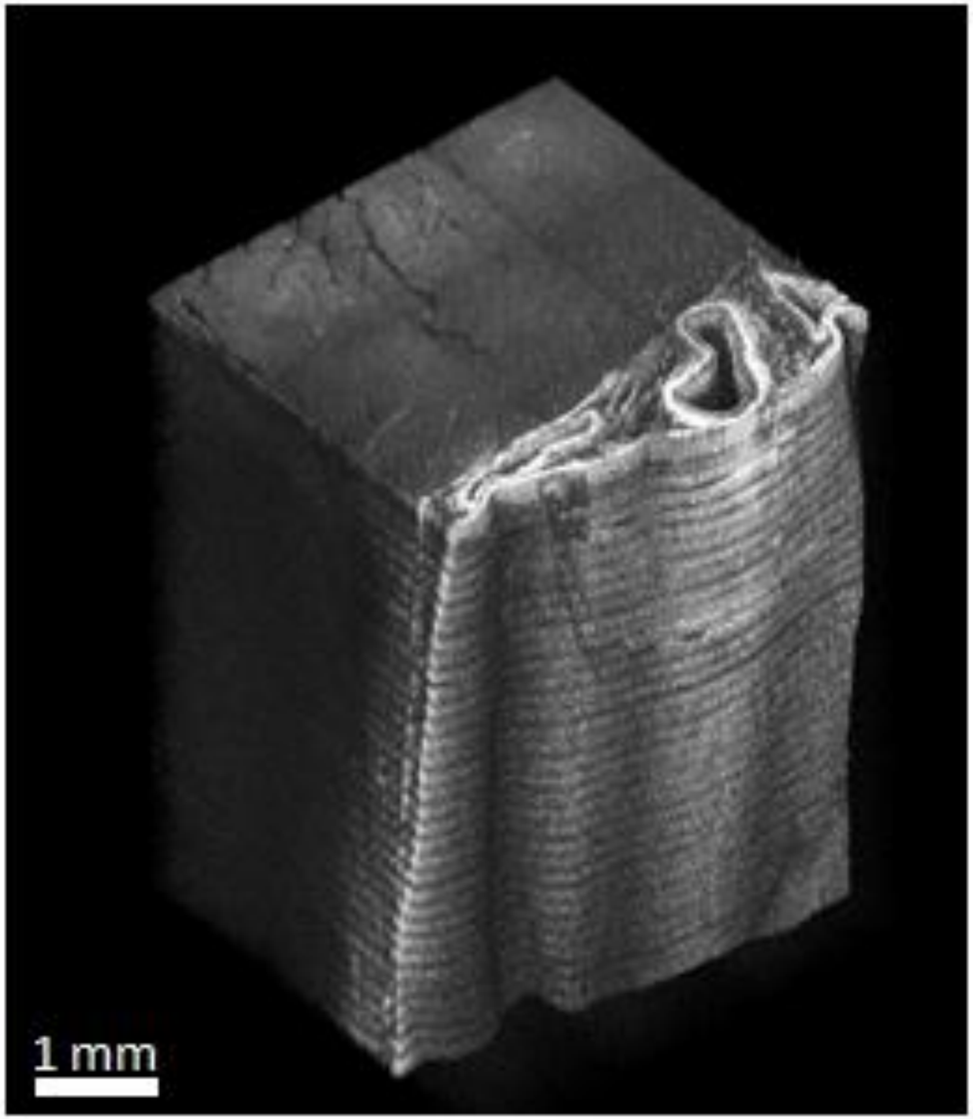
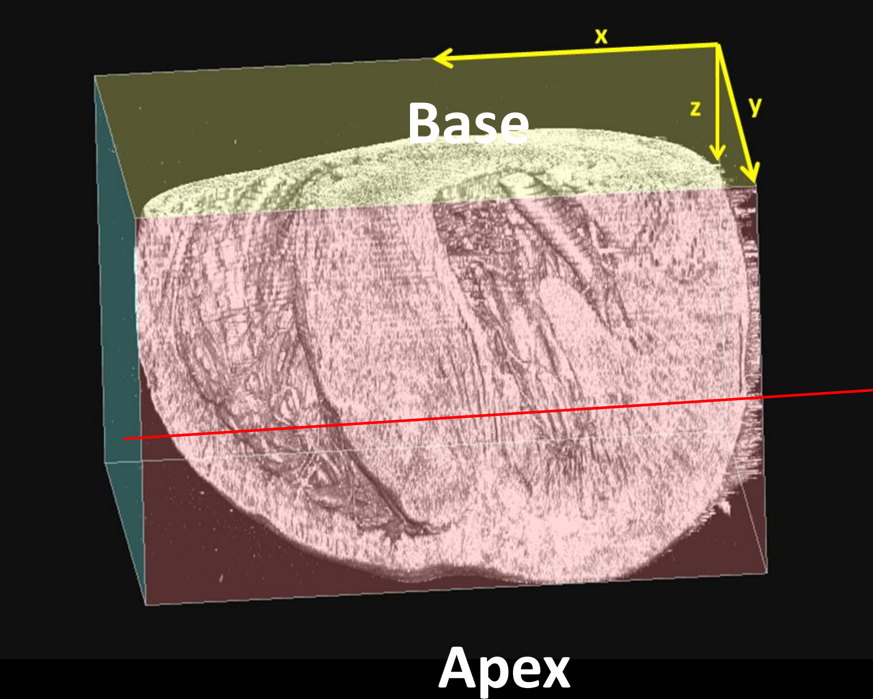
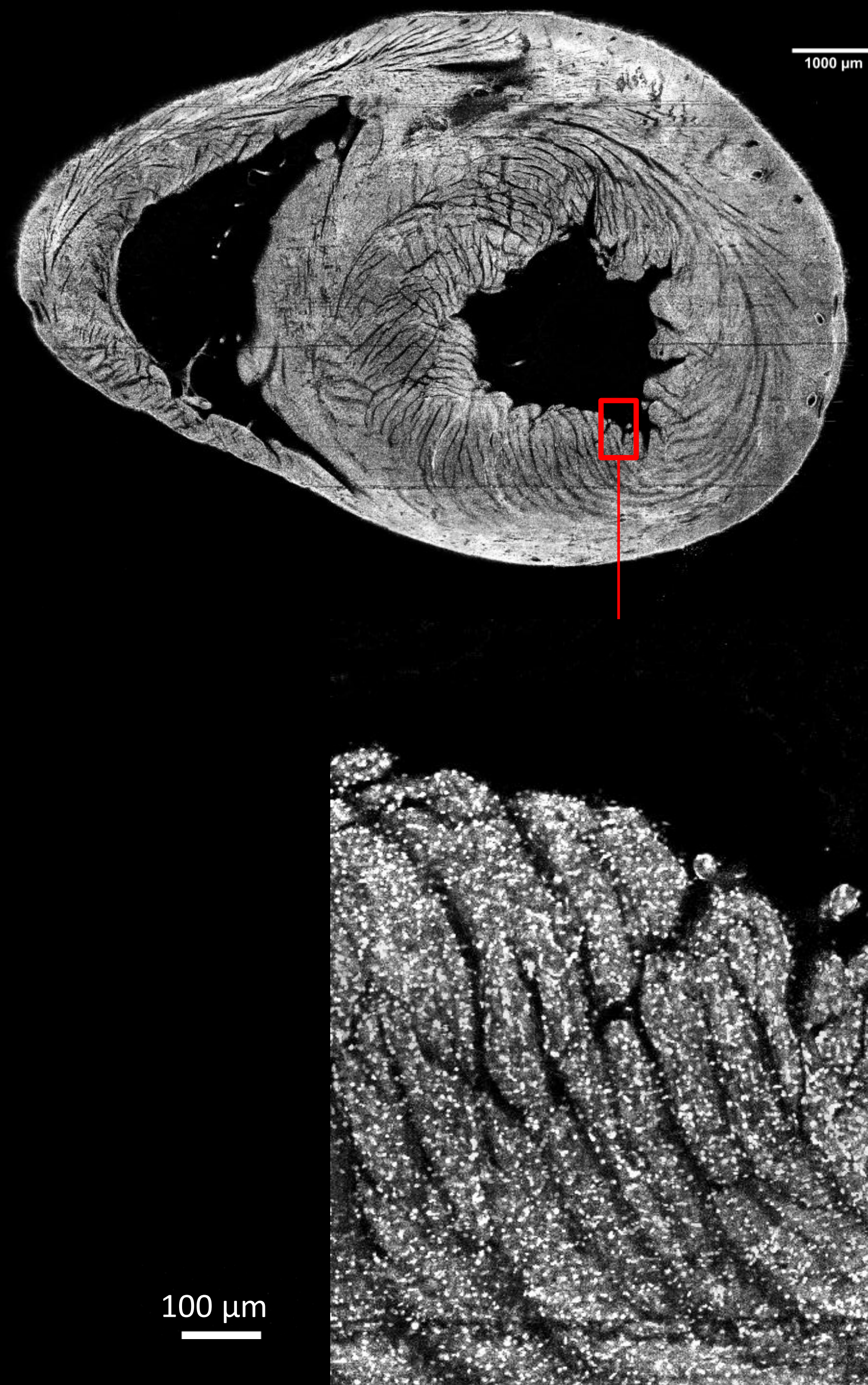


Image Volume Rendering. Each plane is a “slice”



Slice orthogonal to the z-direction





X-Y Resolution – 1.6 µm
Z Resolution – 10 µm

Characteristics of the dataset

<u>Guinea Pig (Mouse) heart image dataset</u>

- 20 X lens
- **0.8 µm (desired resolution)**
- 512 x 512, 8 bit images
- 30% overlap in Z
- 800,000 (300,000) images
- 400 (280) GB

- 10 X lens
- 1.6 µm
- 512 x 512, 8 bit images
- 30 % overlap in Z
- 200,000 (80,000) images
- 140 (70) GB

Final images, uncompressed, including overlaps (for alignment).

Imaging Requirements

- Interactive Visualization of the entire Image volume
- Multi-Resolution Visualization à la Google Earth.
- Labeling – Must be able to extend visualization software to perform voxel labeling based on statistical analysis of fluorescence properties.

FluoRender

Advantages

- Use of Gamma Function as Transfer Function
- Clipping Planes, Rotation of the clipping box
- Movie Recording, Automated Rotation.

Disadvantages

- There appears to be a loss of spatial resolution (can't view image volume at the fundamental resolution of dataset)
- Not possible to hold the entire dataset in memory.

Are we correct about these things?

ImageVis3D

Advantages

- Great Volume Rendering
- 1D and 2D Transfer Functions
- Can visualize large datasets.

Disadvantages

- Conversion to ‘.uvf’ format is slow
- Inability(?) to select a sub volume for viewing at full resolution

Are we correct about these things?

Questions

- What is the best visualization tool meeting our requirements ?
- What is the best desk-side workstation configuration for this tool ?
- Will we be able to modify the source code to add statistical analysis of he image data ?

Acknowledgements
Robert Kazmierski, Dr. Raimond Winslow

General Question

Are you aware of any good approaches to in-plane tile registration and alignment (arrows show examples of mis-alignment)?