3D Visualization of the Cardiac Conduction System through GFP Cryo-Fluorescence Tomography (CFT) Imaging

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Introduction

The cardiac conduction system (CCS) comprises a heterogeneous network of cells that orchestrate the generation and propagation of electrical impulses to coordinate the heartbeat. Disorders of the CCS have been implicated as the site of initiation of many inherited and acquired forms of cardiac arrhythmias, heart block, and sudden cardiac arrest. Major gaps exist in our understanding of the mechanisms responsible for the formation, function and dysfunction of the CCS. This is due, in part, to an inability to visualize and interrogate these rare (1% of cells in the heart) and complex conducting structures. Previous work has used X-ray Computed Tomography, MRI, and fluorescence imaging facilitated by mouse models expressing fluorescent proteins to visualize the CCS. CT and MRI lack the resolution for rodents and collecting 3D images with GFP is difficult due to the limited penetration of blue/green light. Tissue clearance techniques enable visualization of the GFP cells in intact tissues (Figure 1B) but requires extensive processing times on the order of months. Cryo-Fluorescence tomography (CFT) is serial slicing with off-the-block anatomical and fluorescence imaging and is capable of imaging a whole animal into a 3D dataset. This technique can also image isolated tumors either from rodent models, or human patients, at higher resolution. CFT has a unique ability to provide both high resolution anatomical images along with very high sensitivity and specificity. Sections can also be transferred to slide for further histology and co registration back to the whole sample data set. In this study, we utilize CFT in several tumor models to track tumor microenvironment and heterogeneity, metastatic spread, and expression of specific markers in excised human tumors.

Methods

Mouse hearts were collected from P21 Cntn2-EGFP BAC transgenics and a knockin model of human mutation I-P on residue 183 of the Nkx2-5 gene. Hearts imaged with whole mount fluorescence imaging (Figure 3) were cut open to visualize the left and right ventricular conduction system using a Zeiss M2Bio microscope. Hearts for light sheet imaging were prepared with CLARITY tissue clearing technique (Figure 4). Hearts were frozen and then mounted to the CFT bed with O.C.T. CFT data was collected by serial slicing and imaging the block face at each slice for anatomical and fluorescence images with narrow (11nm) bands to minimize autofluorescence. The data had a resolution of 7 microns in plane, and 10 micron slice thickness and includes white light and fluorescence channels in 3D.

Results

Whole mount Fluorescence (figure 3) images enable visualization of the CCS, but certain aspects are obscured due to cuts to expose the lumen or behind tissue. Light sheet microscopy provides good visualization of the cell tracts (figure 4) while providing limited anatomical landmarks post-tissue clearing. CFT provides high resolution anatomical and fluorescence images (Figure 2) and is able to identify all major components of the CCS (Figure 3,4) including the sinoatrial node, atrioventricular node, Bundle of His, and the left and right Purkinje fiber network.

Figure 1: Cryo Fluorescence Tomography System comprised of an imaging chamber on top of a cryo sectioning system. Optical magnification allows the system to vary the FOV and resolution.

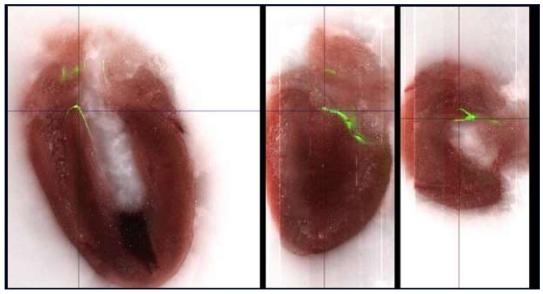


Figure 2: Sagittal, coronal and axial views of the 3D data set. White light anatomical image clearly gives reference locations for fibers that show up in the green channel.

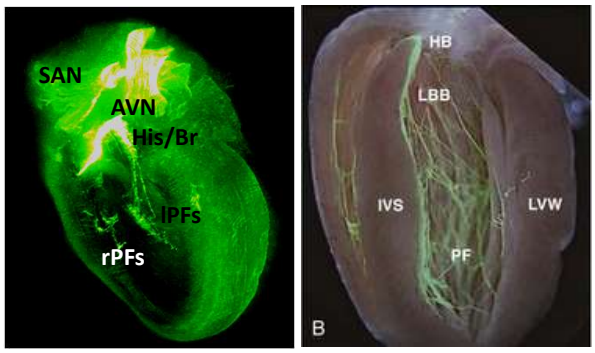
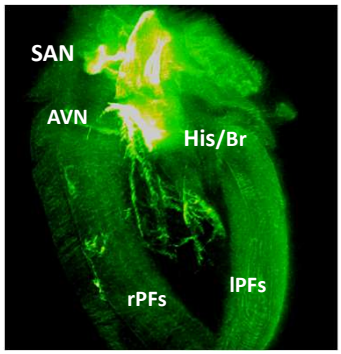


Figure 3: CFT (left) gives full visualization of the fibers, some of which might be obscured in a 3D whole mount fluorescence photograph (right). All major conductive structures are visible; sinoatrial node (SAN), atrioventricular node (AVN), Bundle of His (His/Br), and the left (lPFs) and right Purkinje fiber network (rPFs).

Conclusion

CFT provides high resolution anatomical and fluorescence images of all major components of the CCS including the sinoatrial node, atrioventricular node, Bundle of His, and the left and right Purkinje fiber network. CFT can provide a fast and comprehensive visualization of CCS in the native three-dimensional environment. CFT allows for the most accurate representation of the CCS and its position WRT the cardiac tissue due to the 3D color anatomical reference. The tissue also undergoes less morphological changes which creates a better spatial representation of the network.



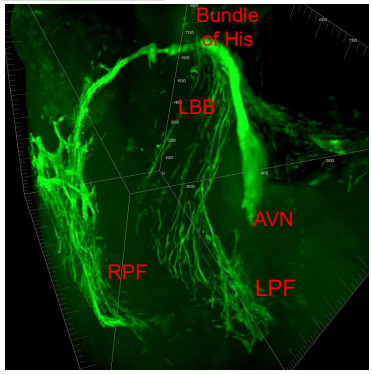


Figure 4: Light sheet microscopy of cleared tissue (bottom) provides very good visualization of conductive structures, but preparation of the tissue requires months. CFT (top) can image tissue in a matter of hours and can provide similar visualization of all major conductive structures are visible; sinoatrial node (SAN), atrioventricular node (AVN), Bundle of His (His/Br), and the left (lPFs) and right Purkinje fiber network (rPFs).

Cryo-Fluorescence Tomography

CFT has proven to be a powerful technique for cardiology, neurology, pharmacology and oncology investigations. This technique can characterize the 3D bio-distribution and localization of gene expression, antibody drug conjugates, or diagnostic antibodies, across an entire sample. Imaging expression of GFP is particularly helpful because in vivo imaging of green fluorescence is not possible in deep tissue like the heart, and imaging large amounts of tissue with traditional microscopy is very time consuming.

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Work was done in collaboration with:
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