A Streamline Approach for Assembly, Reconstruction, Visualization, and Management of Mesoscopic Molecular Tissue Imaging Data

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Introduction

Cryo-Fluorescence Tomography (CFT) is a novel technique for 3-Dimensional Molecular Tissue Imaging (MTI). Imaging at a mesoscopic scale creates an increased data management issue in comparison to more traditional in vivo fluorescent imaging techniques. While the volume of the samples that are imaged are the same as that in various in vivo techniques, CFT results in an increased amount of data density that requires robust reconstruction and visualization tools.

Methods

To improve workflow of CFT data collection two applications have been developed. One application is dedicated to Reconstruction or large data formats. While the second application is dedicated to Visualization of large data sets.

Reconstruction

The reconstruction tool (Figures 1 and 2) is specifically designed to enable fast processing of raw data. In order to optimize workflow, the full raw dataset is loaded into memory. With a user friendly interface, the user can preview and apply reconstruction algorithms which include: landmark alignment, histogram balancing and fluorescent sharping. The Applications user friendly layout streamlines the workflow by giving the user the following features when data is loaded:

- View Settings –allows for zooming.
- Preview Box- Allows you to see how changes will appear.
- Hide Settings Button.
- White light Viewer.
- Fluorescent Viewer.
- Slide Viewer –allows you to slide through image stack.
- Discard Button.
- Run Recon Button

After raw data has been evaluated, it is submitted for reconstruction. During reconstruction corrections are applied. Data is easily aligned and corrected. Corrections include:

- White Light balance
- Fluorescent signal normalization
- Subsurface fluorescence correction

Data is output in a format that allows for large data visualization. The output data format allows a user to view the data in 2D and/or 3D, depending on the user's workflow and preference.

Figure 1

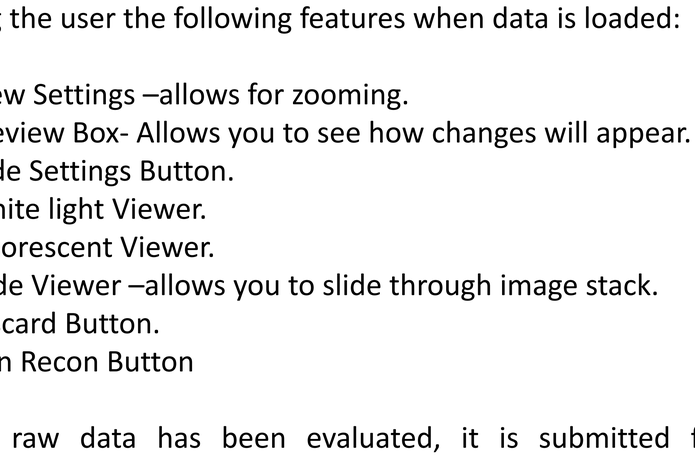

Figure 1: Data is visualized simultaneously in white light and fluorescence. The user can quickly view the data stack to manually QC data. The is able to remove data that may have image artifacts or was collected in error. A streaming log as well as a progress bar communicate to the user what operations are happening in the software.

Figure 2

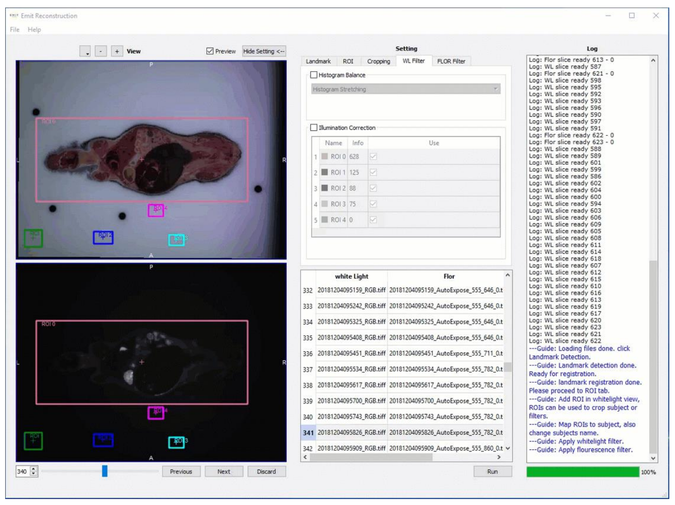

Figure 2: Wight light correction and color balance are easily applied by using the ROI tools with in the software. A region of interest can be selected for output data. This allows subsections of the collected data to be processed and visualized without the need to reconstruct the entire collected field of view.

Visualization

VivID™ is a large data viewer designed specifically for mesoscopic MTI. The platform enables visualization of white light and fluorescence volumetric data by using an optimized bricked level of detail data representation. With a simplified and friendly interface, a user is able to interact with data in both 2D and 3D. The user can apply and modify various color palettes to generate volume renders and flythrough movies.

Key Features in the software tool (Figures 3 and 4):

- Reset Button – Resets views to how they were loaded.
- Volume Render - Gives option to choose method of volume render and ability to rotate along fixed axis.
- SliceViewer – allows user to navigate 2D views with high precision. LOD is the level of detail with which the 2D images are displayed. Each decrease results in an image downsized by a factor of 2.
- Transfer Function – Sets RGB and alpha values for viewing images. Save can be used to save user specified configurations as palettes. Palettes can be loaded with Load.
- Progress Viewer – Volume render will decrease LOD during motion to prevent lag and go to higher LOD when still. Shows what LOD volume is at and how much is loading.

Figure 3

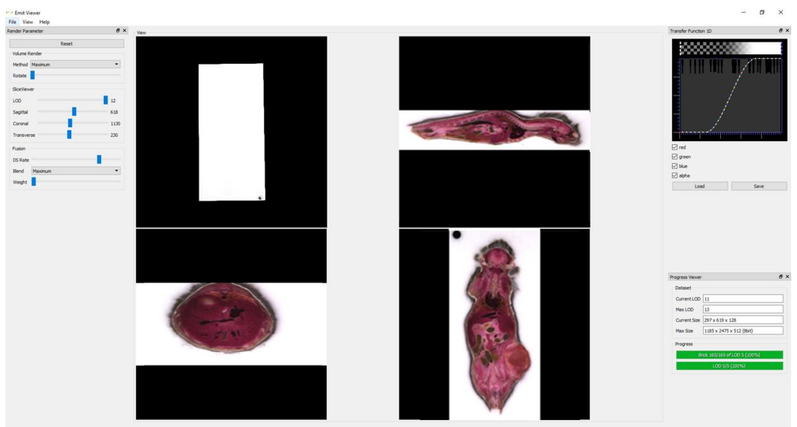

Figure 4

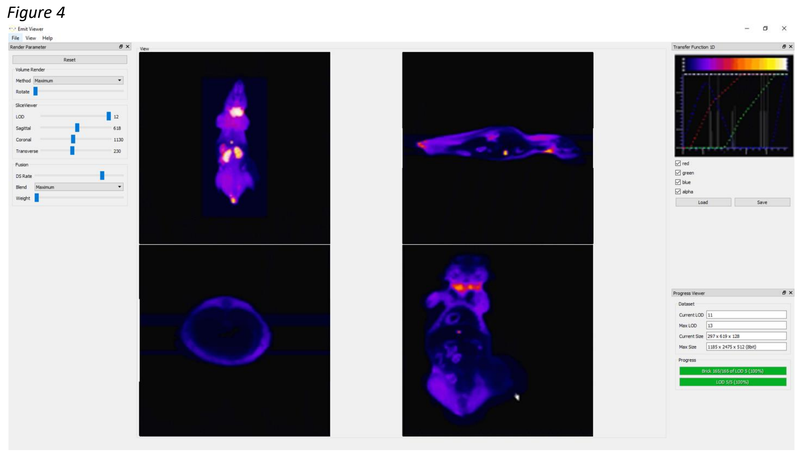

Figure 3 and 4: VivID™ allows for users to view date from the white light channel and the fluorescent channel. A variety of color pallets cant be selected to represent the fluorescent data.

Figure 5

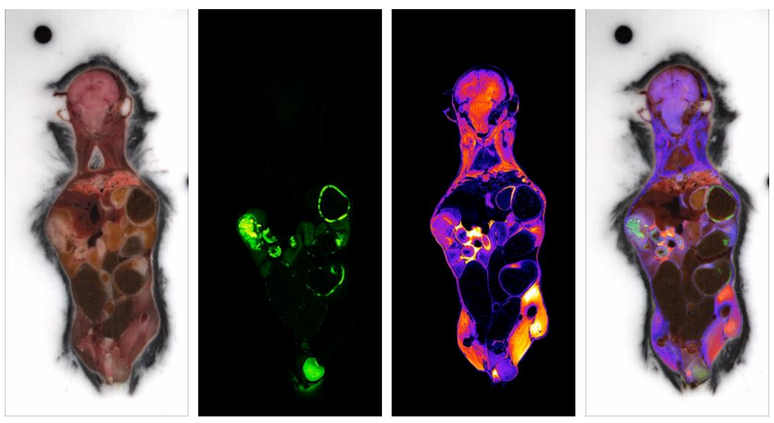

Figure 5: Representative data output from VivID™ Data collection consisted of a white light image as well two fluorescent image channels. Coronal images are represented here as the data was collected with the animals embedded in the prone position. The white light collection (A), GFP (B), TdTomato (C), and a composite overlay was generated.

Results

By using the CFT reconstruction platform and VivID™ (The large data viewer) data representations are easily saved into formats that can be imported into presentations and reports (Figure 5). The software uses a progressive rendering algorithm that takes into account the computer hardware to optimize visualization of data. The algorithm enables the utilization across a wide range of computer configurations, from a basic 32bit system to high-end workstation.

Conclusion

Dedicated applications for reconstruction and visualization have created a workflow that is easy and optimized. While techniques like CFT have existed in the past in various academic research environments, optimization of data management was needed to make this technique mainstream. By creating software that is stable and robust researchers able to access data, present and share results much faster in comparison to previous methods.

Cryo-Fluorescence Tomography

Cry-Fluorescence Tomography (CFT) is an emerging molecular imaging technique where a specimen is prepared with exogenous or endogenous fluorescence, fresh frozen or paraffin embedded, and mounted into a slicing instrument. The specimen is serially sectioned, and for each slide a white light and at least one fluorescence image is taken.

The technique allows for co-registration between a wide variety of imaging technologies that vary in resolution and sensitivity. The reconstruction and 3D rendering into isotropic voxels allows easy registration and visualization with 3D in vivo imaging modalities. CFT offers a range of resolutions. The flexible platform design allows for the researcher to interrogate anything from tissue samples to entire animals at a variety or resolutions.

About Invicro

Invicro was founded with a mission of improving the role and function of imaging in translational drug discovery and development. To this effort Invicro offers a suite of services and software with applications ranging from tissue to human, from target identification to Phase IV trials across the entire electromagnetic spectrum of imaging techniques.

About Emit

Emit Imaging enables discovery by bridging the large gap between in vivo imaging and pathology. Our technology uniquely enables high resolution imaging combined with a whole body comprehensive 3D data set. This technology can dramatically increase the value of each sample in a multi modal study.

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Work was done in collaboration with: