in vivo Confocal Microscopy

Exquisite resolution with fluorophore imaging in living animals
Scintica Instrumentation and Invicro are collaborating to market their services for the provision of in vivo confocal microscopy equipment and image analysis. Scintica instrumentation is the exclusive North American distributor for the Optiscan ViewnVivo confocal microscopy system. The ViewnVivo B30 is the latest miniaturized fluorescence endomicroscope from Optiscan, optimized for real-time Preclinical Research in vivo imaging in animal models. Invicro has extensive experience in image analysis in all of the common imaging modalities and brings their experience with sophisticated algorithms to the analysis of in vivo confocal microscopy.

The ViewnVivo system enables acquisition of images from multiple organs in living animals. While fluorophores can be administered in vivo and the confocal microscopy images acquired ex vivo this is no substitute for acquiring the images in living tissue where dynamic changes can be captured. In addition, for selected organs, imaging can be performed on a longitudinal basis, allowing changes over time to be documented at the cellular level.

Images have been acquired in both normal rats and mice and in a range of abnormal conditions. The instrumentation can readily be used in larger species. Following are examples of normal and abnormal images from various organ systems and a sample of the image analysis methods that can be used to extract quantitative data from the images. All images series comparing normal and abnormal were acquired with the same parameters. Fluorophores used were fluorescein, acriflavine, fluorescein isothiocyanate – Dextran 150 (FITC) and annexin V.

Liver

Following a laparotomy, the tip of the probe is placed on the surface of the liver and images collected at a preselected depth, or at a variety of depths to obtain a perspective of changes within the organ. Abnormal images were obtained from animals that had been treated with a single dose of thioacetamide, which is an established model of acute liver necrosis. Imaging occurred at 24 hours post administration of the thioacetamide. Figure 1 demonstrates an example of an image from a normal animal and the liver at 24 hours post thioacetamide treatment.

Swelling of the liver cells results in an overall narrowing of the blood containing sinusoids. This can be quantified using image analysis routines. In the
example below the images were pre-processed using a 3-pixel median filter. The liver sinusoids were segmented from the cells using an adaptive histogram equalization followed by an adaptive thresholding. As this resulted in a segmentation that was slightly too liberal, this was followed by an image erosion of 2 pixels. Finally, any topologically connected region smaller than 15 pixels was considered to be part of the noise and removed. A histogram of the size of the sinusoid regions was calculated for both the normal and abnormal liver images and confirms the reduction in size of the sinusoids, and thus increase in size of the cells.

Annexin V is a fluorophore that specifically binds to apoptotic tissue following intravenous administration. Figure 4 illustrates an abnormal liver showing accumulation of the fluorophore in apoptotic cells. An image of normal tissue would show no fluorescence.

Kidney

Similar to the liver, images are obtained at a laparotomy with the probe tip being placed on the surface of the organ. A small incision in the kidney capsule allows greater depth of penetration into the parenchyma. The folic acid model of acute renal disease was used for the abnormal images. In this disease model early pathology associated with necrosis of the renal tubule cells is present at 24 hours post administration, at which time the images were collected. Figure 5 demonstrates normal and abnormal kidney tissue. These images were acquired following the intravenous administration of fluorescein with topical administration of acriflavine. The fluorescein is excreted via the kidney and fills the lumen of the kidney tubules, while the acriflavine stains the nuclei of tubular cells.
The following two methods were explored in order to quantify changes in the tubule structure in a 2D image. The images were pre-processed using a square median filter with a kernel size of 7 pixels.

The images (Figure 5) were segmented using adaptive histogram equalization and adaptive thresholding to separate the foreground tubules from the background. To avoid false positives, this segmentation was combined with an exclusionary background region that was determined using a two-class one-value threshold followed by a topological close operation. To avoid false negatives, topologically connected background regions of solidity above 0.6 were added to the foreground region.

A distance-to-background transform was calculated on the foreground tubules regions. This distance-to-background was considered as a proxy for the measurement of tubule thickness. However, since sampling of the entire distance transform does not represent the cross-sectional thickness of any tubule, a skeletonization of the foreground tubule region was also performed. The distance-to-background was reported within this skeletonization as an additional, but more representative, proxy for tubule thickness (Figure 6).

A histogram was calculated for the distance-to-background both within the tubule region and within only the skeletonized region for the normal and abnormal regions (Figure 7). The histograms demonstrate that both methods indicate a difference in tubule diameter. The implication of this is that there is obstruction to tubular urine flow as a result of the pathology and either of these methods could be used to monitor changes in the extent of pathology over time.

**FIGURE 5:** Images of normal (top) and abnormal (bottom) rat kidney. Note that the tubules appear to be larger and more dilated in the image of the abnormal kidney.

**FIGURE 6:** Illustration of the segmentation process for abnormal (top row) and normal (bottom row) using the thresholding method and the distance to background method.
Colon

In mice the colon can be imaged in the same way as the other organs, the tip of the probe being placed on the surface of the colon. In rats greater than approximately 250g body weight the probe can be passed into the rectum and the surface of the colon visualized. For the colon, the disease model used was the dextran sulfate sodium (DSS) model. The DSS is administered in the drinking water and by the fifth day following the start of the medication clinical signs of colitis are present. Normal and abnormal images of the rat colon are presented in Figure 8 that were acquired after the intravenous administration of FITC. The images demonstrate increased vascularity, as primarily evidenced by the larger size of the vessels in the center of the crypts. Additionally, in the abnormal image, the normal architecture has been lost and the cells are larger and more variable in shape, all consistent with colitis.

Vascularity

Blood vessels can be identified with several of the common fluorophores when given intravenously and images are collected immediately following the infusion. FITC is ideal for this purpose and clearly demonstrates the content of the vessels. Figure 9 demonstrates the vascular structures in the wall of the mouse colon.
Following fluorescein administration and collection of a dynamic series of images, blood flow within the vessel can be quantified. Additionally, the outlines of the red blood cells can be identified (Figure 10).

*FIGURE 9:* Demonstration of blood vessels of various sizes in the outer wall of the colon.

*FIGURE 10:* Intravascular fluorescein outlines red blood cells in a vessel in the rat brain (Top). The red arrows in the bottom image indicates the direction of flow. A gif of the bottom figure that illustrates the blood flow can be downloaded from https://boston.ipacs.invicro.com/publiclink/dl/b49d832f9438c7a5. The time between frames in the animation is 1.34 seconds.
By placing image patches of 51 pixel edge length which are then registered between temporally adjacent images using normalized cross correlation the flow rate within the patch can be calculated. These data can be presented either as a map corresponding to each image (Figure 11 center, a gif of the dynamic image can be downloaded from https://boston.ipacs.invicro.com/publiclink/dl/251e587a28964da7) or it can be presented as a histogram (Figure 11 bottom, a gif can be downloaded from https://boston.ipacs.invicro.com/publiclink/dl/6a06b70991f101dd)

The images presented to the right are a small sample of what can be achieved. When a specific research question needs to be answered, protocols can be designed to suit the need. Similarly, image analysis routines can be tailored to provide specific quantitative data outputs from the images, either static or dynamic.

**FIGURE 11:** Raw image (top) with corresponding flow map (center) and histogram of speed (bottom)
Invicro is the leading global diagnostic core lab services provider from discovery to preclinical to translational to late-phase clinical trials, offering the most comprehensive medical imaging capabilities to support drug research and development. As part of the Konica Minolta precision medicine group, Invicro offers quantitative biomarker assays from gene-to-protein-to-cell-to-organ, using multi-imaging modalities that support a breadth of therapeutic areas including, but not limited to oncology, neurology, cardiovascular, and musculoskeletal. With headquarters in Boston, MA, Invicro has offices, laboratories and clinics around the world, from coast-to-coast within the United States, to Europe and Asia that support leading pharmaceutical and biotechnology companies and top research universities. Visit www.invicro.com for more information.

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Unique brands carried by Scintica include the ViewnVivo miniaturized fluorescence endomicroscope system from Optiscan Imaging, optimized for real-time in vivo imaging in live animal models. Our team consists of scientists, applications experts, engineers and sales professionals from a cross section of backgrounds, who excel at simplifying transactions and ensuring that scientists have the best equipment for achieving research excellence.