Webinar Q&A Responses: Preclinical imaging with fluorescence *in vivo* endomicroscopy

Questions and answers from the January 15, 2019 webinar titled "Preclinical imaging with fluorescence *in vivo* endomicroscopy"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address.

1. **What is the diameter of the probe, and is probe diameter related to the resolution of the image, mimicking different objectives?**

   The diameter of the FIVE2 probe is fixed - 4mm. Compared to classical benchtop microscopy where the objectives and numerical aperture define the magnification and resolution, this system has a scanning mechanism with resolution of 0.5µm.

2. **Can I see blood flow?**

   Yes. The FIVE2 system is versatile and it can image any sample that fluoresces at 488nm. Due to its ability of continuous capture, one can not only monitor the blood flow, but also perform various analysis for microvasculature and cellular direction. Please refer to our website for further details.

3. **Can one visualize 2 fluorochromes at same time?**

   Yes. The FIVE2 is equipped with 8 preinstalled and 4 customizable filters. These filters facilitate spectral differentiation of fluorophores, allowing you to perform dual color imaging.

4. **I wanted to expose intestinal cells with a 405nm laser, can we do this with the FIVE2 (ViewnVivo) and is it safe to do so?**

   Yes. The instrument uses a 2R laser and it completely biosafe, for the animal as well human operators. It goes without saying, however, that you should not stare directly into the laser for a long period of time.

5. **What is the image capture or sampling rate?**

   The speed of capture depends on the image type and one can go up to 3.5 fps.

6. **Does this technology have a filter for Na flu (Sodium Fluorescein)?**

   Sodium fluorescein has an excitation wavelength between 475-490 nm and an emission wavelength of 510-520 nm, making it one of the most ideal fluorophores for FIVE2. Please refer to the product brochure for specifications.

7. **If the tip of the laser inside the brain becomes cloudy, does that means that the images will not be clear?**

   The answer depends on the composition of fluid. The fluid between the sample and the probe doesn't impact significantly if there's no fluorophore in the liquid. However, this fluid may absorb some of the illumination laser and you might have to increase laser power accordingly.

8. **What is the resolution at 400µm depth?**

   Resolution is not affected by the optical depth. In other words, the resolution is similar at 100µm depth as it is at 400µm depth. However, depending on the sample, the SNR will get distorted, which may result in lower resolution.

9. **What is the magnification and numerical aperture of the system?**

   The term magnification originates from traditional microscopy where objects were magnified using a system of lenses and viewed with an eyepiece. With digital imaging, the magnification from the object to the displayed image depends on the size that the image is displayed (i.e. the size of the monitor, electronic zoom etc.). A more useful parameter with digital imaging is the Field of View (FOV), as FOV does not change when the image is displayed on monitors of different sizes. The FOV refers to the size of the area that is represented in the image, or in other words the area of tissue that is scanned to collect the image. In the case of the FIVE2, the FOV is 475µm x 475µm when imaging in a 1:1 aspect ratio. The field of view varies particularly in the y-axis when imaging using other aspect ratios.

10. **How deep can the probe image within the tissue?**

    Theoretically, one can go 400µm deep in the tissue. However, the practical imaging depth depends on the tissue type.

11. How is the FIVE2 different from a regular confocal microscope?

The FIVE2 is a miniaturized portable confocal endomicroscope that is quick and easy to set up. The system has been designed to be installed on a trolley if required, enabling the system to be moved from lab to lab with ease. High quality images can be obtained holding the probe by hand, mounting it in our multi-axis micrometer adjustable imaging stage or fitted to the manipulators on a stereotactic frame. Instead of sectioning tissues to view on a slide, FIVE2 (ViewnVivo) can image directly from a tissue. The flexibility of the system's probes enables researchers to perform *in vivo* imaging from any angle. If you can touch the tissue with the probe, you can image it.

12. What is the difference between a point scanning confocal probe and a fibre bundle probe?

Fibre bundle probes employ a bundle of several thousand optical fibres. Laser light is scanned across the proximal end of the fibre bundle, which results in the laser light only travelling through the core of only 1 fibre at any instant in time. When the distal tip of the bundle is placed in contact with the tissue, each individual fibre makes a discrete fluorescence intensity measurement, and by collecting the signal from each fibre in the bundle, an image is formed. While fibre bundles allow for very small diameter probes with no moving parts at the distal end, they sacrifice resolution and do not allow variable imaging depth – an essential feature of confocal microscopy. Resolution of fibre bundles is limited due to the small number of fibres in the bundle (compare to the analogue scanning of a point scanning confocal system), and the spacing between the cores of the fibre.

13. What is the optical resolution and field of view of the FIVE2?

The FIVE2 system has a lateral resolution of 0.55µm, and an axial resolution (optical slice thickness) of 5.1µm. The field of view is 475µm x 475µm when imaging in a 1:1 aspect ratio. The field of view varies particularly in the y-axis when imaging using other aspect ratios.

14. Does the endomicroscope probe need to touch the tissue of interest?

The endomicroscope is designed to press gently on the sample to be imaged, and then move the focal plane in the probe to locate the region of interest. This gentle pressure provides imaging stability and "wet contact" with the sample provides a good optical interface. It is possible to image without touching the probe to the sample. But imaging in this configuration will be very prone to movement artefact, as any microscopic movement between the probe and the sample will be very obvious.

15. **Is it possible to image very active tissue that moves a lot (e.g. a beating heart)?**

    Where tissue movement is minimal, obtaining motion artifact-free images is easy. The tip of the endomicroscope is placed in gentle contact with the tissue meaning that the probe and tissue can move together resulting in minimal relative movement between the two surfaces. Tissues that are very mobile are more difficult to image, but in many instances, careful probe positioning can enable imaging of tissue that is actively moving.

16. **Does contact pressure affect imaging depth?**

    The endomicroscope only needs to touch the tissue lightly when imaging. It is not necessary to apply pressure, and varying pressure does not affect imaging depth. The imaging depth is varied by the operator via a foot pedal or mouse control that moves the optics within the distal tip of the FIVE2 probe.