CALIBER ID RS-G4

TECHNICAL APPLICATION NOTE

New technology for large area scans using resonance confocal microscopy

Introduction

Fluorescence imaging has long been a mainstay in biological research applications, encompassing an ever-growing array of techniques. One of the most commonly used is confocal imaging, a technique whereby out-of-focus light from a sample is removed, resulting in improved resolution and an increased signal-to-noise ratio. Traditional confocal microscopes are point-scanning microscopes, with which a diffraction-limited laser spot is scanned across a sample, and the emission light from each point in the sample is passed through a pinhole to remove the out-of-focus light. The final image is constructed point-by-point. Although this technique yields excellent images, it can cover only a small area of the sample and thus is limited in the size of the field of view it can cover.

Resonance-scanning confocal microscopes utilize a resonant-scanning galvanometer to focus the light to the sample by scanning the full aperture of the objective lens, which is swept across the field of view at high frequency. Collection of the light follows conventional confocal imaging with emission filters and a pinhole aperture to allow selective depth of image control. The scanning speed of resonance scanners can provide frame rates of several 10Hz, depending on the size of the field of view.

The major disadvantage of both standard confocal and resonant-scanning microscopes is that they are limited to imaging a relatively small region. Larger regions, while possible, take a prohibitively long time. The ability to only image a small field of view has become increasingly limiting to the biological researcher. With research progressing beyond the study of single cells, confocal imaging of larger groups of cells and whole tissues has become necessary, and techniques that allow the high-resolution confocal imaging of large sections of tissues are needed.

Figure 1. Caliber I.D. RS-G4.

The RS-G4 Features

The RS-G4 by Caliber I.D. is a purpose-built, large-format, resonant-scanning confocal microscope (**Figure 1**). It offers multiple laser lines (405/488/561/640/785) for single, sequential or simultaneous image acquisition of fluorescent labels.

A key advantage of the RS-G4 is its capability to collect high-quality confocal images of large biological samples (up to 80 x 120mm) in a fraction of the time that conventional confocal microscopes can achieve (**Figure 2**).

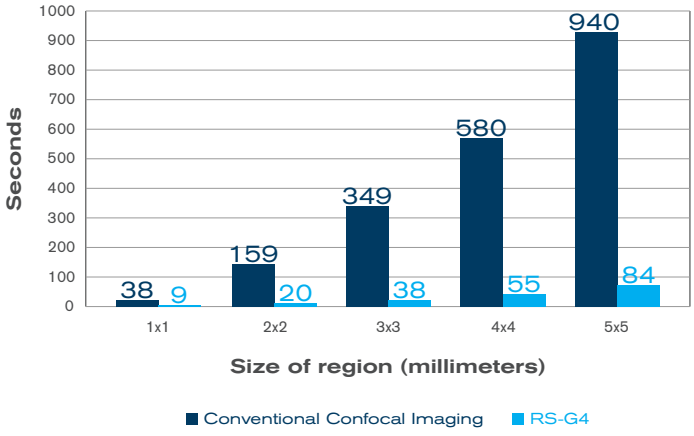

Figure 2. Comparison of the speed of conventional confocal imaging vs. the RS-G4

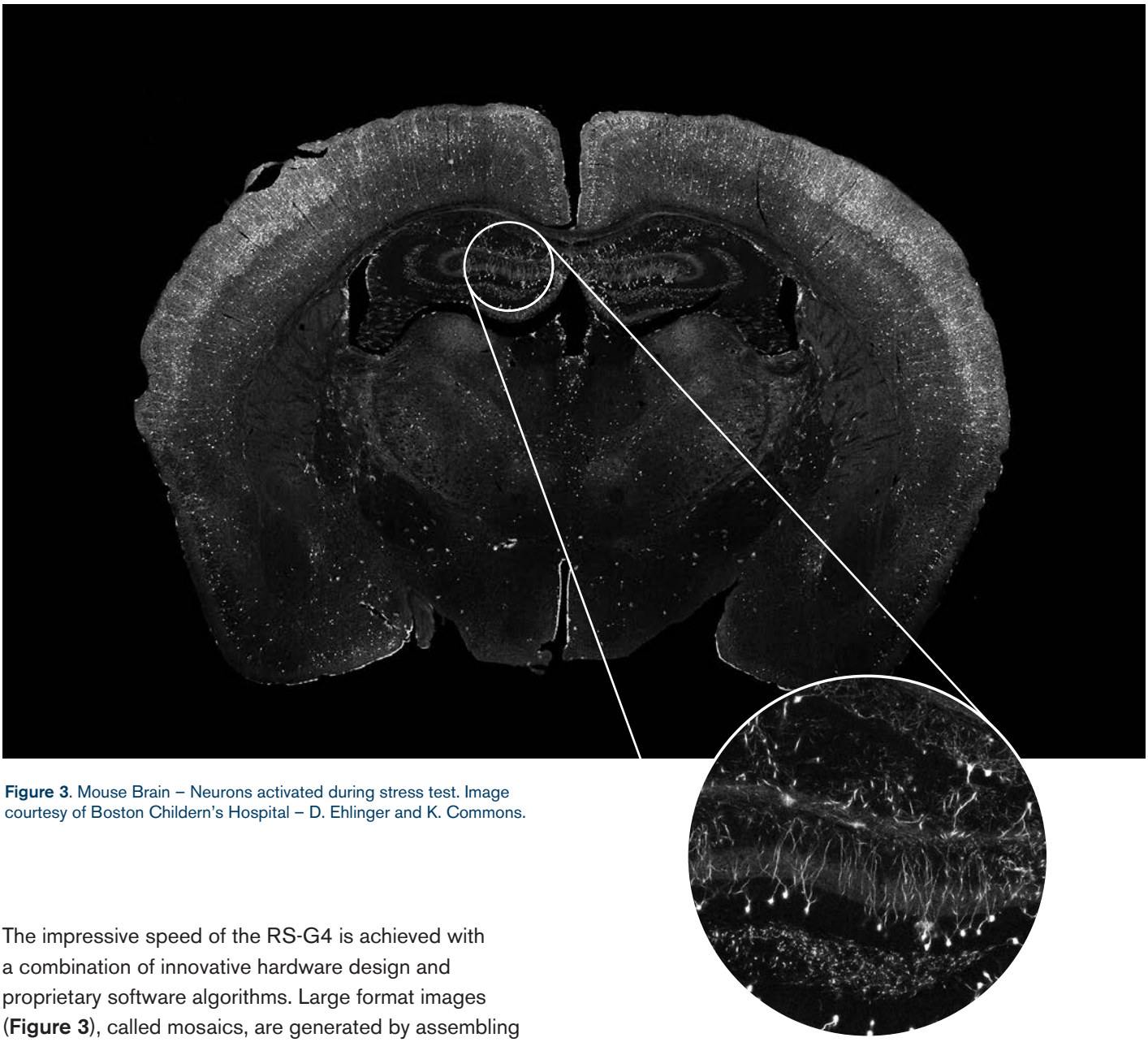

Figure 3. Mouse Brain – Neurons activated during stress test. Image courtesy of Boston Childern's Hospital – D. Ehlinger and K. Commons.

The impressive speed of the RS-G4 is achieved with a combination of innovative hardware design and proprietary software algorithms. Large format images (**Figure 3**), called mosaics, are generated by assembling a series of continuously acquired mosaic strips. Each mosaic strip is acquired along one axis of the sample, and a series of mosaic strips are acquired across the sample using a high-precision x-y stage to move the sample while the resonant scanner acquires the images. Using Caliber I.D.'s proprietary "image stitching" algorithm, RS-G4 assembles and aligns the multiple mosaic strips at pixel-level resolution to generate the complete large-format mosaic (**Figure 4**).

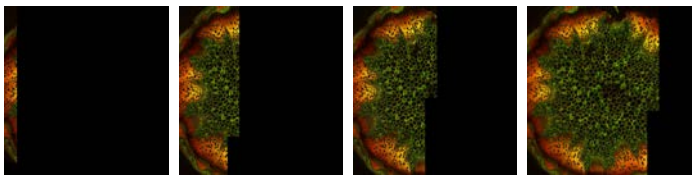

Figure 4. Strip mosaic movie - Camilla Leaf Stem. Image courtesy of The University of Chicago - C. Labno and V. Bindokas.

Applications

The ability to acquire high-quality confocal images of large regions has significant implications for a wide range of application areas. In neurological studies, for example, entire brain slices can now be imaged at high resolution, allowing for both gross morphological visualization as well as detailed cellular level characterizations (**Figure 3**). Whole organs, such as kidneys (**Figure 5**) can also be easily imaged. In developmental biology, whole model organisms such as Zebra fish and C. elegans can be imaged at high resolution, at different stages of the developmental cycle, providing insights into the developmental process not possible with standard low-resolution macroscopic imaging or high-resolution imaging of a smaller sub-regions of the organism.

Another application area for the RS-G4 is plant biology. Again, the ability to image both gross morphological structures, as well as the capacity to zoom into the same image to visualize high-resolution cellular structures, opens up avenues of studies not possible with current imaging methodologies.

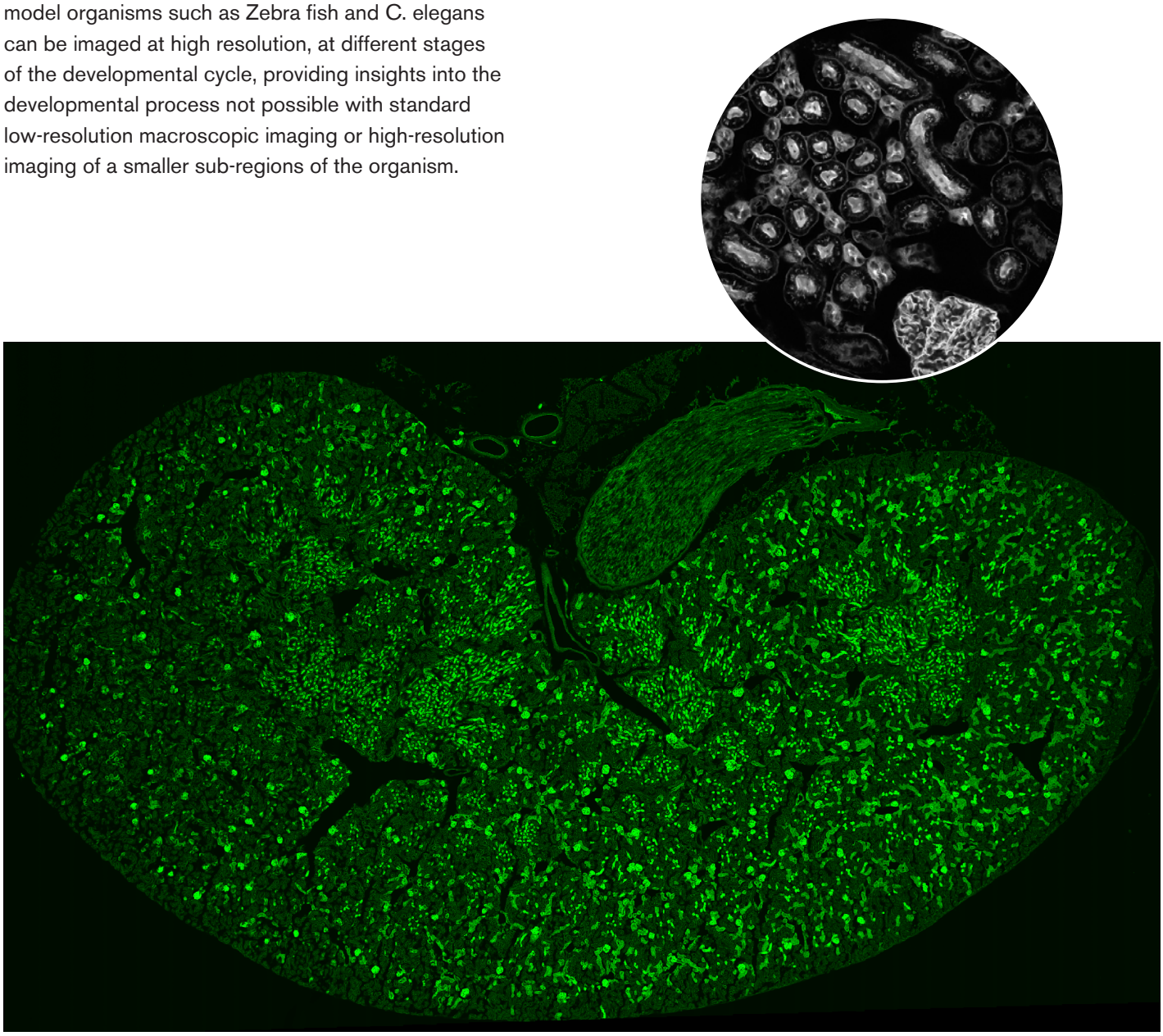

Figure 5. Mouse Kidney. Image courtesy of Caliber I.D.

The RS-G4 Advantage

Large-format, high-resolution confocal imaging of samples that can span up to several square centimeters presents specific computing challenges. Foremost is how to visualize such a large format, high-resolution data set. The RS-G4's proprietary software package has been specifically designed to overcome these challenges. With advanced memory management and image-visualization capabilities, the RS-G4 is well-suited to visualize such large data sets. For example, the user has the ability to visualize a large-area view of the sample at a lower resolution to identify large-scale structures and morphologies and then instantly zoom into a targeted area to view high-resolution structures within that region. As an example, morphogenesis can now be viewed at both the macro and micro scale with the same confocal clarity. Full correlation is available to interact from the macro view to zoom into the desired micro cellular detail for captured fluorescent images.

In addition to the ability to perform high-resolution fluorescence imaging with visible wavelength lasers, the RS-G4 provides an infrared laser (785nm) to enable in-vivo reflectance imaging of a biological sample with a penetration depth up to approximately 250 microns, depending on the cellular density. This provides new imaging capabilities for recording images inside juvenile or adult model specimen.

For many researchers, the field of view limitations of conventional confocal microscopy is proving to be a limiting factor in their studies. The RS-G4 delivers high-speed, large format confocal imaging without sacrificing image quality and resolution. For many labs, this will result in greater precision, clarity and efficiency.

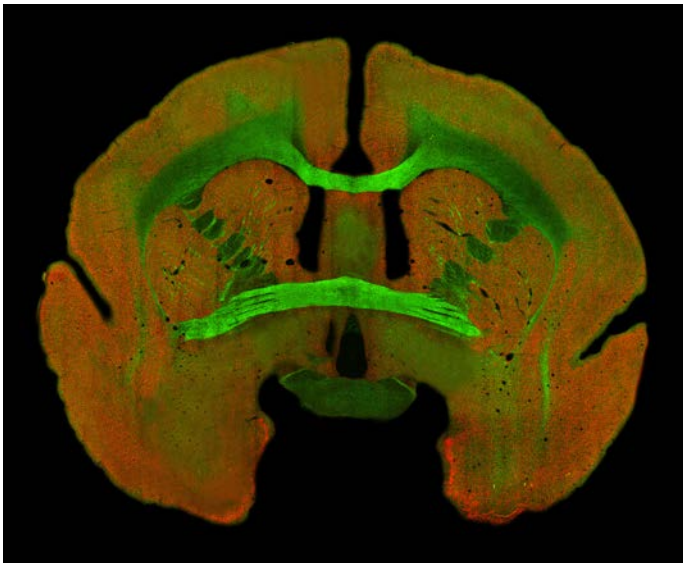

Figure 6. Marmoset Brain – Coronal section of marmoset brain, stained for rabies virus in green and neuronal somata in red. 20 x 20 mm area scan. Capture time for simultaneous imaging of both 488 and 635 nm channels equaled 4 minutes. Image courtesy of University of Pittsburgh Center for Biologic Imaging – A. Rose, P. Strick and S. Watkins.

> "The RS-G4 is a remarkably compact and fast confocal scanner. Very high quality tile stitching, and does very large sample areas is record time for multiple channels. Adjustable scan head angle opens access to image unusual materials. Easily changed objective. Easy to use software."

Vytas Bindokas, Ph.D.

Director, BSD Light Microscopy Core Facility
The University of Chicago

CALIBER I.D.

www.caliberid.com

Headquarters
Caliber Imaging & Diagnostics, Inc.
100 Burtt Road, Suite 203, Andover, MA 01810
Tel: (+1) 585-239-9800

Design and Manufacturing
Caliber Imaging & Diagnostics, Inc.
50 Methodist Hill Dr, suite 1000, Rochester, NY 14623

DOC_No.05626_B