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Time-lapsed, large-volume, high-resolution intravital imaging for tissue-wide analysis of single cell dynamics

David Entenberg [a,1,*], Jessica M. Pastoriza [b,1], Maja H. Oktay [a,c], Sonia Voiculescu [b], Yarong Wang [a], Maria Soledad Sosa [d], Julio Aguirre-Ghiso [e], John Condeelis [a]

[a] Anatomy and Structural Biology, Integrated Imaging Program, Gruss-Lipper Biophotonics Center, Einstein College of Medicine/Montefiore Medical Center, Bronx, NY, United States
[b] Department of Surgery, Einstein College of Medicine/Montefiore Medical Center, Bronx, NY, United States
[c] Department of Pathology, Einstein College of Medicine/Montefiore Medical Center, Bronx, NY, United States
[d] Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, United States
[e] Division of Hematology and Oncology, Department of Medicine, Department of Otolaryngology, Department of Oncological Sciences, Tisch Cancer Institute, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States

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ABSTRACT

Pathologists rely on microscopy to diagnose disease states in tissues and organs. They utilize both high-resolution, high-magnification images to interpret the staining and morphology of individual cells, as well as low-magnification overviews to give context and location to these cells. Intravital imaging is a powerful technique for studying cells and tissues in their native, live environment and can yield sub-cellular resolution images similar to those used by pathologists. However, technical limitations prevent the straightforward acquisition of low-magnification images during intravital imaging, and they are hence not typically captured. The serial acquisition, mosaicking, and stitching together of many high-resolution, high-magnification fields of view is a technique that overcomes these limitations in fixed and ex vivo tissues. The technique however, has not to date been widely applied to intravital imaging as movements caused by the living animal induce image distortions that are difficult to compensate for computationally. To address this, we have developed techniques for the stabilization of numerous tissues, including extremely compliant tissues, that have traditionally been extremely difficult to image. We present a novel combination of these stabilization techniques with mosaicked and stitched intravital imaging, resulting in a process we call Large-Volume High-Resolution Intravital Imaging (LVHR-IVI). The techniques we present are validated and make large volume intravital imaging accessible to any lab with a multiphoton microscope.

1. Introduction

In histopathologic analysis of fixed, stained tissues, low magnification images inform about the tissue wide structure and architecture, and are used to identify regions of the tissue for further analysis at high magnification. The high magnification analysis then reveals the identity of individual cells (based upon their staining and cellular and nuclear morphology), and the spatial relationships between them. While this switch between low and high magnification occurs repeatedly throughout histopathologic analysis, the low magnification perspective is most often not obtained in live tissue imaging, leading to a loss of tissue wide context.

The major reason for this underutilization of low magnification views arises from multiphoton microscopy's reliance upon high-magnification, high-numerical-aperture objective lenses for efficient and bright signal generation. Low magnification lenses typically do not generate enough multiphoton signal to create high quality images. An alternative approach that we have adopted from the field of digital pathology [1] is to acquire many high-magnification, high-resolution images in a mosaic pattern and stitch them together to produce a low-magnification image, a process we call Large-Volume, High-Resolution (LVHR) imaging.

Here we describe a method for combining LVHR imaging with intravital imaging to create Large-Volume High-Resolution Intravital Imaging or LVHR-IVI – obtaining large area, high-resolution imaging in living animals which combines the advantages of histopathologic analysis with the live dynamics of intravital

* Corresponding author.
E-mail address: david.entenberg@einstein.yu.edu (D. Entenberg).
[1] Co-First Authors.

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imaging. The method is composed of both a surgical technique for exposing and stabilizing the tissue of interest, and for the acquisition with multiphoton microscopy of multiple high-magnification tiles that are stitched together to form a large-area, low-magnification image. We also provide, as examples for the reader, designs for tissue stabilization tools, an example Fiji [2] macro, (based upon a published algorithm [3]), for automating the stitching of images over time and depth, and an example excel spreadsheet which can assist in generating and importing the numerous xy coordinate locations needed to control the microscope translation stage.

The novelty of this method lies in its combination of mosaicked-stitched imaging with intravital microscopy of live tissues. Here we provide a detailed description of the method and several examples of its utilization in a variety of tissues. To our knowledge, images utilizing this technique have not been previously reported in the published literature. Although we focus in this method on the imaging of compliant tissues, the procedure can be easily adapted to many other organs susceptible to physiologic motion–induced artifacts. These approaches do not require customized hardware or software, and are compatible with nearly all automated xy-stage equipped multiphoton imaging systems that are accessible to nearly all laboratories.

2. Materials and methods

2.1. Experimental design

Key to the success of LVHR-IVI in living animals is tissue stabilization. In our prior work we developed several techniques for stabilized intravital imaging using skin flap surgeries [4,5]. We have also developed surgical protocols for imaging mammary tumors [6] and lung tissues [7] using imaging windows (Fig. 1A). Our experience with these window surgeries has shown us that, when implanted correctly, tissues are well stabilized relative to the windows and any movement from the heartbeat or intercostal muscle contractions are transmitted to the entire window and displace the window and the tissue together as one. Thus, complete immobilization of the tissue relative to the objective lens can be achieved by immobilizing the window frame. This is accomplished by capturing the window in a tightly toleranced recess that is bored into a custom-made xy stage plate (Fig. 1B and Supplemental Fig. 1).

Using this concept as a basis for the design, we developed a method which is applicable to many different tissues, which includes compliant tissues such as the mammary fat-pad, lymph nodes, and liver which are particularly challenging as they are extremely compliant and easily transmit motion and vibrations from the animal's involuntary movements.

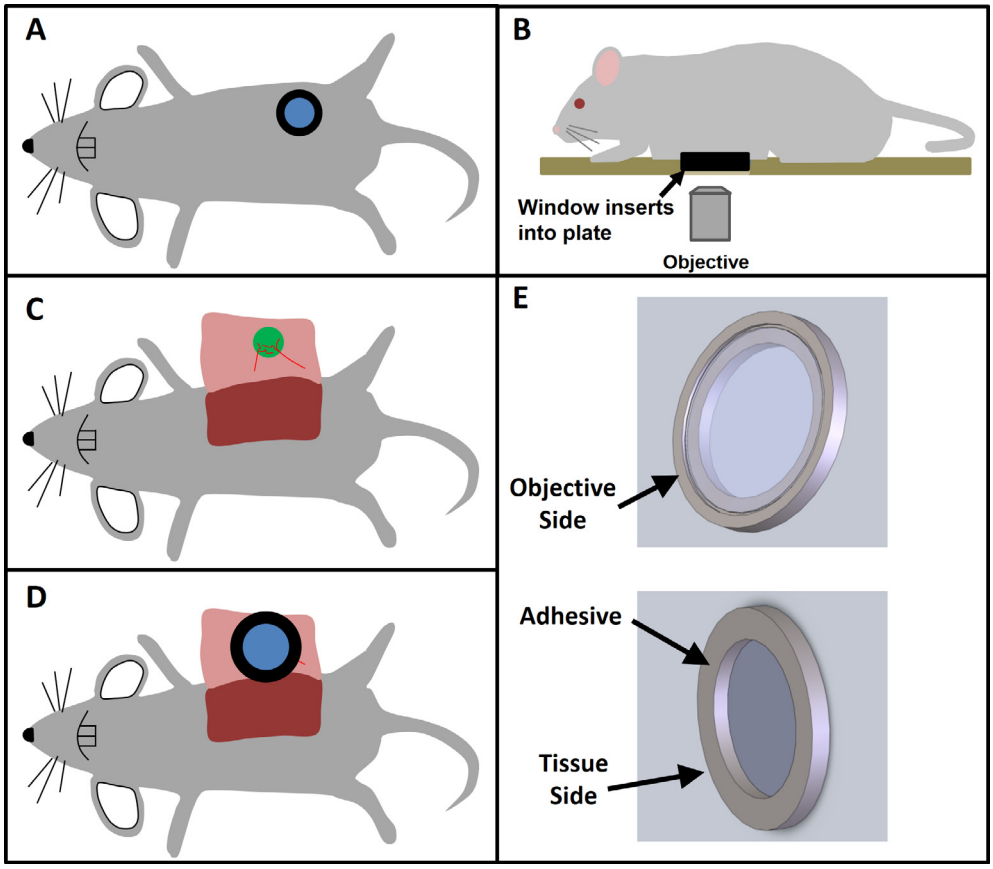

Fig. 1. Overview of surgical protocol. A) With implantable imaging windows such as the mammary, abdominal, or even lung imaging windows, the window itself provides a stable frame which keeps the tissue hydrated and immobilized relative to the glass. However, involuntary movements such as heart beat and respiration still cause movement of the tissue and window together. B) Inserting the window frame into a tight-fitting stage plate which is fixed relative to the objective lens completely immobilizes both the window frame and tissue. C) With skin flap surgeries, the tissue of interest (e.g. tumor, mammary fat pad, lymph node, salivary gland, etc.) is exposed by surgical incision through the skin. D) The conditions for stable imaging that are possible when using imaging windows can be recreated by attaching a cover-glass inset into a shallow window frame over the tissue of interest. Adhesive placed under the window frame, away from the tissue under study, immobilizes the tissue relative to the frame. The window frame can then be inserted as in B into a tight-fitting stage plate. E) 3D computer aided designs of the shallow window frame and cover-glass described in D.