Through the Looking Glass: Imaging Animal Models of Human Diseases

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Abstract

The mammalian immune system is a highly complex organ that detects and eliminates infection caused by pathogens. Pathogens, in return, must undergo and escape immune surveillance of the host in order to succeed in infection spread. For this, they recruit great number of strategies to survive and multiply, and those are usually highly dynamic and interactive events that occur inside a mammalian organism. While in vitro and in vivo experiments provide strong evidence for how pathogens interact with, or evade the immune system of the host, visualizing these processes in both space and time allows for a more refined understanding of the infection spread and its outcome. In recording ongoing, rather than static, biological processes inside a living tissue, multiphoton (MP) microscopy offers major advantages with respect to confocal or epifluorescent microscopy by combining speed, high-resolution, deep penetration and minimal photo-damage. Intraviatal microscopy (IVM) allows imaging live animals at their cellular level, recorded during ongoing physiological processes. However, IVM usually requires anesthesia and surgery. Even then, some compartments of immune system such as the lung are difficult for the microscope to access. Ex vivo microscopy of tissues or whole organs overcomes many of these difficulties by immobilization, better access to the area of interest, and availability of immunostaining. In an ideal biomedical study, both techniques become indispensable when applied together. To recruit both techniques we have used a custom-built inverted confocal DIVE (Deep In Vivo Explorer) and Stellaris 8 microscopes (Leica Microsystems) each equipped with dual MP lasers (Spectra Physics), and imaged various animal models of infectious diseases. In addition to mouse lymph nodes, we have established the ability to visualize liver, spleen, brain, gut mucosa, and lung from model animals. By employing both in vivo an ex vivo techniques, we can visualize in great detail infection spread and immune responses to viruses, bacteria and pathogenic protozoa. We have established high speed and high-resolution imaging of live Staphylococcus aureus (1, 2), Mycobacterium tuberculosis (3, 4), Leishmania major (5, 6), Vaccinia virus (7, 8), SIV (unpublished) and SARS-CoV-2 (Fig 1) viruses among other pathogens, for up to continuous 6 hours and over the course of days. This reveals new potential targets for therapeutical intervention and effective vaccine development in human patients.
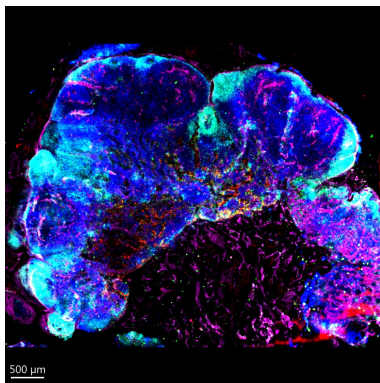
Figure 1. SARS-CoV-2 infected pulmonary lymph node of a Rhesus macaque.

Live-tissue multiphoton microscopy reveals architecture of SARS-CoV-2 infected monkey lymph node. Fluorescent reporters: Blue, CD8; Cyan, CD20; Yellow, MR macrophages; Red, lymph node medullary macrophages; Magenta, Laminin.

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