Editorial

Optical and genetic tools for *in vivo* single cell tracking

Multicellular organisms are composed of vast numbers of different molecules and cells that interact spatially and temporally. Classic post-mortem histological processing enables morphological and molecular characterization of cells, but knowledge derived from these studies is static and cannot reveal the natural dynamics of biological processes. A variety of *in vitro* and *ex vivo* methods are being used to decipher signaling pathways, explore and exploit biological processes, but cell properties may undergo substantial change when taken out of their native environment in the whole living organism. The best way to study life is to continuously observe biological processes in a living organism at a resolution high enough to pick out cellular behaviors and molecular signals underlying them. However, due to technological hurdles, this idea remained a coveted dream till recent years.

Leaps in research tools often occur at the interface of seemingly disparate disciplines. This holds true for the surprising but profound combination of microscopy with fluorescent proteins, leading to a new generation of imaging-based studies extending from the tracking of individual molecules within living cells to the observation of whole organisms. Optical microscopy has been used for intravital imaging for over a century. However, it is not until the discovery and widespread use of fluorescent proteins that intravital microscope undergoes exponential expansion in width and depth. Genetically encoded fluorescent protein-based probes/sensors/indicators enable the observation of cell morphology, status and function. Genetic labeling tools empower optical imaging and vice versa. The marriage of the two fields gives rise to a myriad of versatile and powerful techniques for *in vivo* interrogation of cellular dynamics at high resolution.

In a previous issue of the Methods for Single Cells series (de la Prida and Gozes, 2019), leading researchers in the field reviewed state-of-the-art approaches to study brain single cells from different perspectives, including genomics (Cembrowski, 2019; Gozes et al., 2019; Sauvage et al., 2019; van den Hurk and Bardy, 2019), electrophysiology (Cid and de la Prida, 2019; Valero and English, 2019), connectivity tracing (Schwarz and Remy, 2019), optophysiology (Katz et al., 2019; Pisano et al., 2019) and automation (Suk et al., 2019). The topic of this new Special Issue is right at the intersection of optical imaging and genetically encoded fluorescent probes. Here, leading researchers from this interdisciplinary field review cutting-edge methods to longitudinally visualize the migration, cell cycle, or activity of cells in whole animals to interrogate the biological processes underlying the whole animal physiology and behavior.

This special issue begins with a review by Li et al. (Li et al., 2021) on cell labeling methods, which by nature is the step prior to any imaging attempts. This review emphasizes genetically labeling cells with viral approaches for *in vivo* cell tracking, lineage tracing and neural circuits

mapping. It introduces the basic principle of viral vectors in cell tracking and highlights the examples of cell tracking in various research areas. The immediate next topic is on the probe with which cells are labeled with. We have two reviews on fluorescent probes. One is contributed by Heinrich et al. (Heinrich et al., 2021), which nicely summarizes the genetically encoded fluorescent sensors whose emission can be modified by light. They call these biosensors *photo-transformable probes*, including photoactivation, photoconversion or photoswitching probes, serving to highlight biological structures under *in vivo* imaging. Next, Tomura et al. (Tomura et al., 2021) provide the latest update on the development of fluorescence cell cycle indicators, which reveals the progression of cell cycles in labeled cells. They also introduce their work using photoconvertible proteins for tracking immune migration in live mice. These three reviews cover essential aspects of cell labeling for subsequent single-cell tracking, providing great examples of how optical sensors, combined with optical imaging, help to shed light on the finest details of biological processes.

We group optical imaging tools into one-photon-based and multiphoton-based imaging tools. For the study of translucent animals such as *Caenorhabditis elegans* or larval zebrafish, one-photon based imaging tools are suitable for whole-brain or even whole-animal imaging. Zhao et al. (Zhao et al., 2021) review recent single-cell tracking studies in the brain of larval zebrafish using light-sheet microscopy. They showcase the advantage of *in vivo* imaging of larval zebrafish with the help of fluorescent probes to delineate mechanisms underlying brain development and animal behavior. From the technological perspective, Zhang et al. (Zhang et al., 2021) provide a comprehensive review of light-field microscopy for fast brain imaging in freely moving animals. Traditional optical imaging technologies for *in vivo* neural activity recording are usually limited in throughput and cannot cover a large imaging volume at high speed. Light-field microscopy is a burgeoning technology that features a highly parallelized imaging collection mechanism and can enable the capture of highly dynamic processes in freely moving animals such as *Caenorhabditis elegans* or larval zebrafish.

For the study of highly scattering tissue, such as the mouse brain, one-photon based optical imaging is limited to the upper ~100 microns below the brain surface. This is where multiphoton microscopy beats the technology bottleneck. Liang and Walczak (Liang and Walczak, 2021) summarize the application of 2-photon microscopy for *in vivo* long-term single-cell tracking in the mouse and points out current challenges and opportunities for cell labeling and *in vivo* multiphoton-based imaging. They foresee a further expansion of this technology for the study of diseases, therapy and translational medicine.

Finally, for the study of neuronal activity in the brain, functional

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indicators such as calcium or voltage indicators, have been widely used, together with optical imaging tools, to delineate mechanisms underlying animal behaviors. While the previous Special Issue focused on targeting single cells, here Wenzel and Hamm (Wenzel and Hamm, 2021) provide a comprehensive step-by-step overview of theoretical, experimental, and analytical considerations for the identification and quantification of neuronal ensembles with high-resolution in vivo calcium imaging. Adam (Adam, 2021) reviews the recent progress in genetically encoded voltage sensor development and discusses the various aspects necessary to perform a successful ‘all-optical’ electrophysiology experiment in behaving, head-fixed animals. For deep brain areas such as paraventricular thalamus in the mouse brain, which are beyond the reach of direct illumination of excitation light, gradient-index (GRIN) lens is the way out for either one-photon or two-photon based imaging. Laing et al. (Laing et al., 2021) present an introduction on fluorescence microendoscopy and give an overview of the necessary components for performing in vivo optical calcium imaging, offering detailed guidance for GRIN lens-based in vivo brain imaging.

Overall, this is an exciting interdisciplinary field that is burgeoning over the past decade. Although this collection cannot be exhaustive, we hope that it will increase the interest of the scientific community in intravital single-cell tracking achieved through optical imaging and genetic labeling tools, whose impact goes beyond neuroscience. Tracking the function of single cells in their native growing environment is bound to advance therapies for diseases with a leap forward to precision medicine. Last but not least, we would like to thank the participating authors for their contributions, especially considering the immense challenges posed by the pandemic in the past year. It is hoped that the enclosed articles provide insights and inspirations for researchers who intend to practice in vivo single-cell tracking techniques.

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