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High-Resolution Ex Vivo Tissue Clearing, Lightsheet Imaging, and Data Analysis to Support Macromolecular Drug and Biomarker Distribution in Whole Organs and Tumors

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Ex vivo tissue distribution studies of macromolecular drugs, targets, and biomarkers are crucial for enhancing the understanding of relationships between pharmacokinetics, distribution to the site of action, and pharmacodynamics. Traditional studies have been typically limited to methods requiring tissue homogenization, which lose information on spatial heterogeneity and structural complexity of whole organs and tumors. Alternatively, high resolution spatial information can be elucidated by imaging techniques such as confocal and two photon microscopies; however, due to optical constraints, these imaging methods can only be applied for analysis of thin tissue sections or images up to a limited depth beneath an organ's surface. Thus, obtaining a holistic three-dimensional (3D) view of spatial information in an intact organ or tumor at a cellular or subcellular resolution is not feasible.

In this work we explored the application of tissue clearing, immunolabeling, and lightsheet microscopy techniques to enable the assessment of *ex vivo* distribution of macromolecular drugs and biomarkers in intact whole organs and tumors [1]. Multiplex lightsheet datasets allow simultaneous visualization of multiple markers across any anatomical plane and quantification of features and objects of interest in whole organs [2,3], or tumors [4]. Here we describe recent applications of this 3D imaging technology and analysis workflows applied to support the preclinical development of drugs across multiple therapeutic areas. Specifically, we developed an analysis pipeline to characterize drug and vascular distribution in preclinical tumors at a high resolution that utilizes machine-learning algorithms to improve accuracy of image segmentation [5] and high-performance computing to enable analysis of ~ 1Tb data in under 2 hours. We also investigated beta-amyloid plaque pathology in an Alzheimer's disease mouse model, showcasing the ability to visualize metrics in the context of a brain atlas and comparison of plaque distribution across brain regions using a commercial tool. Overall, we provide key insights on onboarding and applying tissue clearing and lightsheet microscopy techniques and how 3D histology can support drug discovery.

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