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Molecular mapping of developmental disease progression using HiTS-FAST

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Three-dimensional molecular mapping of RNA expression within intact biological tissue is allowing for new insight into the relationship structure and function. Current RNA quantification approaches are depth-limited to less than 200 μm by the requirement for single-molecule read out of sequential barcoded RNA fluorescence in-situ hybridization (RNA-FISH) or hydrogel embedded in-situ RNA sequencing. An alternative approach to single-molecule readout is to sacrifice intra-cellular localization by using amplified RNA-FISH to detect RNA expression on a per cell basis. This strategy has the potential to extend three-dimensional molecular mapping of RNA expression to cubic millimeters or centimeters of tissue. Building on our previous work on autofocusing, inertia-free light sheet fluorescence microscopy for cleared tissue, we designed a platform for rapid multiplexed molecular interrogation of cleared tissue samples. The High Throughput Scalable Fluorescence Assay for Spatial Transcriptomics (HiTS-FAST) platform combines closed-loop feedback to maintain co-planar alignment of the exciting light sheet and optical detection plane in thick samples, programmable fluidics for sample clearing and sequential multiplex labeling, and third generation single-molecule hairpin chain reaction (smHCR v3.0) to reliably label RNA expression millimeters deep in cleared tissue samples. I will present our hardware and software implementation to maintain co-planarity, automated sample handling, and present initial results from multiplexed labeling of both RNA and protein in healthy and developmentally disrupted whole rat lungs.

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