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Highly-multiplexed smFISH of sectioned zebrafish embryos

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The zebrafish embryo is a well-established and powerful in vivo system for both imaging and high-throughput genomics, yet applying cutting-edge approaches for spatial transcriptomics remains a challenge due to protocols, panels, and pipelines being generally focused on larger mammalian tissues. To meet this challenge, we developed an approach to allow higher-throuput microtome sectioning of multiple, spatially aligned, zebrafish embryos. The approach can be adapted to different developmental stages, spatial orientations, and throughput scenarios. We have performed highly multiplexed single-molecule RNA FISH using the 10x Genomics Xenium platform with a 300-gene custom probe panel on >30 serial sections of multiple spatially-aligned 24hpf zebrafish embryo tails. The custom gene panel was chosen mostly based on cell-type marker genes from our own 10x Genomics single-cell ATAC and RNA multiome sequencing data (also on 24hpf tails) and the Xenium experiment was highly successful in detecting tissue-specific expression patterns of the chosen genes with single-molecule resolution. DAPI and ribosomal RNA-based cell-body stains contributed to a seemingly quality cell-segmentation performed by the on-board Xenium software, allowing single-cell resolved clustering and annotation from the resulting cell-by-gene matrix. The resulting data presents several challenges for processing and analysis, with the immediate goal being visualization and quantification of differences along the entire ~800um embryonic trunk of six embryos (three replicates each in two different biological conditions) in a single experiment.

Presenter: LACADIE, Scott

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