Spatial Gene Expression Mapping in a Model of Human Brain Development with Multiplexed RNA Fluorescence In Situ Hybridization

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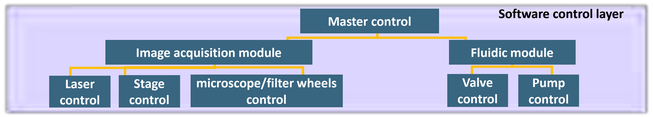
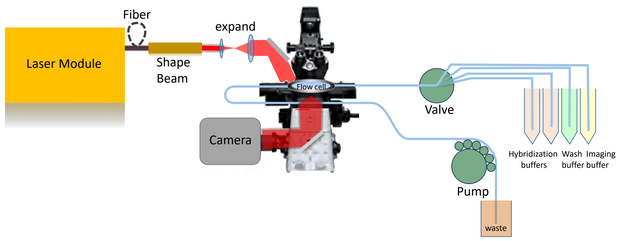
INTRODUCTION

- Multiplexed RNA FISH (mFISH) is a spatial omics technique for quantifying gene expression in single cells within tissue architecture at subcellular resolution.
- Human brain organoids are an accessible iPSC-derived model of the developing human brain. Since their cellular architecture and markers are relatively well characterized, they constitute an ideal system for validating mFISH.

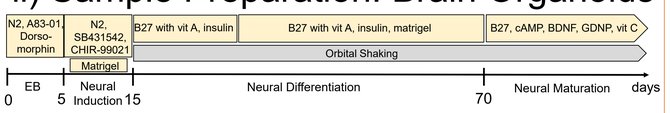
METHODS

i) Imaging Platform

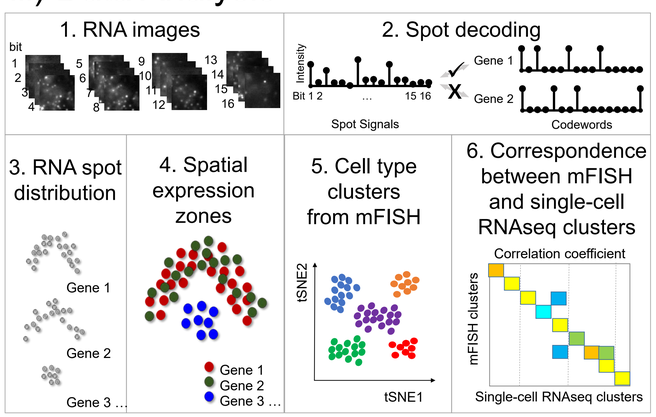




ii) Sample Preparation: Brain Organoids



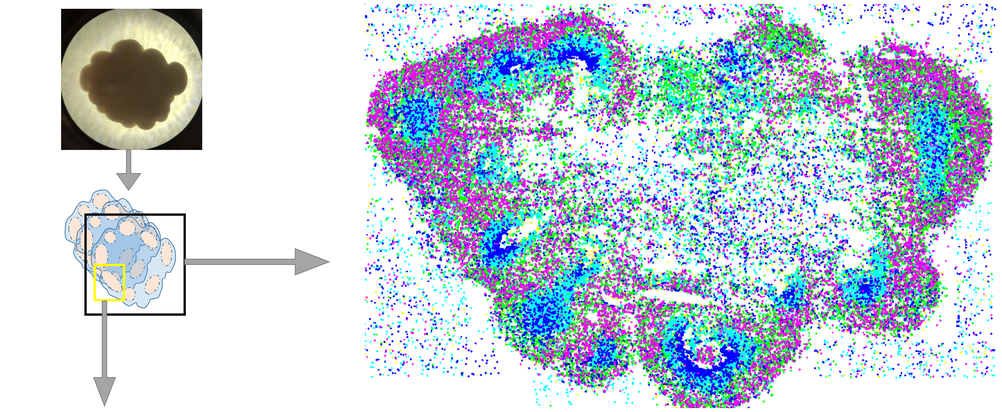
iii) Data Analysis



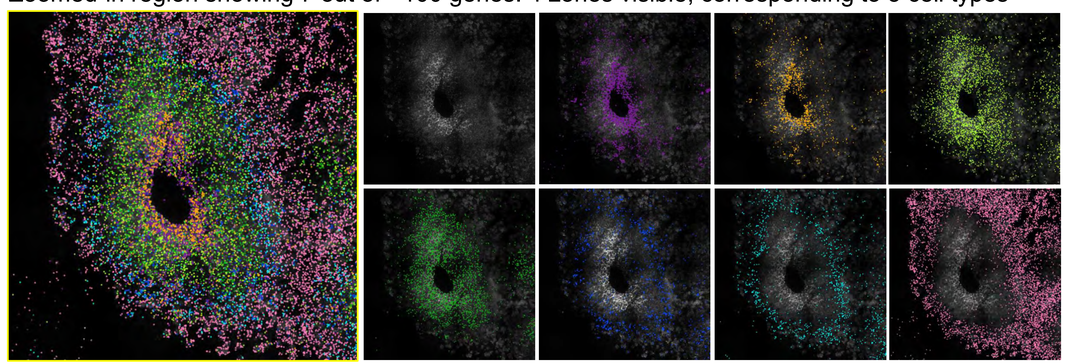
RESULTS

RNA distribution maps

Complete section showing 5 out of ~100 genes

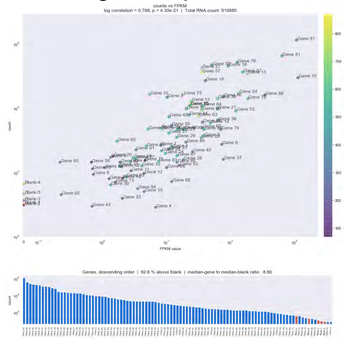


Zoomed-in region showing 7 out of ~100 genes. 4 zones visible, corresponding to 5 cell types

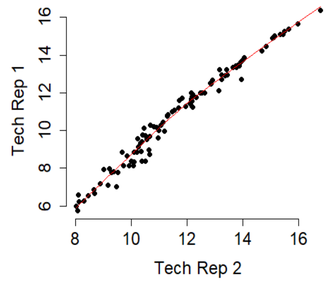


Technical Validation

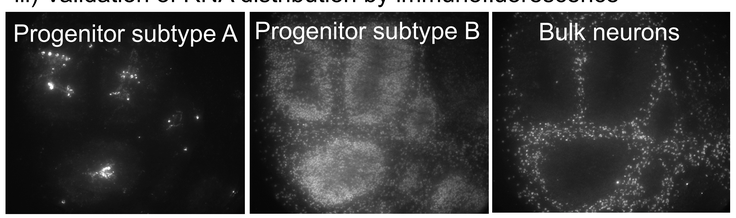
i) Correlation with bulk gene expression



ii) Technical replicates (adjacent tissue sections)

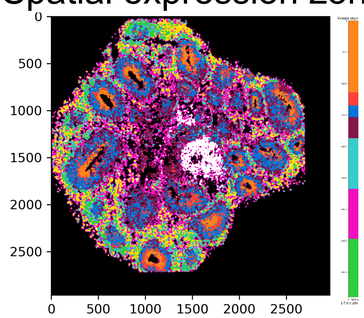


iii) Validation of RNA distribution by immunofluorescence

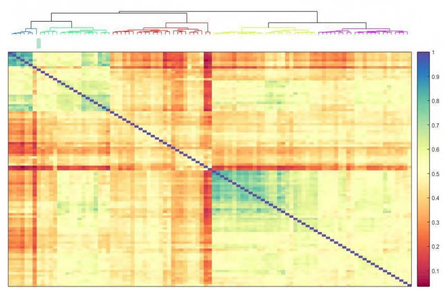


- RNA image for one bit
- Dividing cells
- Progenitor subtype A
- Progenitor subtype B
- Progenitor subtype B
- Progenitor subtype C
- Progenitor subtype D
- Bulk neurons

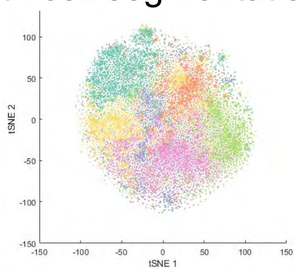
Spatial expression zones



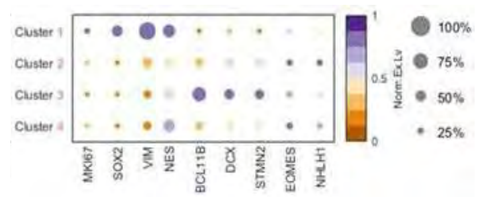
Marker gene clusters



Cell types from mFISH (with cell segmentation)



Differential gene expression (for selected clusters)



CONCLUSION

We have developed a subcellular-resolution, multi-color imaging platform for mFISH and used it to characterize the spatial distribution of transcripts from ~140 genes in brain organoids at single-molecule (subcellular) resolution. Our results faithfully recapitulate the known tissue architecture and cell types within the developing human brain, and thus validate the performance of our mFISH platform. We propose that this experimental system could support novel mechanistic analyses of neurodevelopmental disorders in a 3D model of the human brain.

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