MERFISHing for spatial context

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MERFISHing for spatial context

~~**(obviously open to change ☺ on the title)**~~

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Abstract

A new paper in Science by Zhuang and colleagues introduces multiplexed error-robust FISH (MERFISH). By combining a sequential hybridization strategy with a robust encoding scheme, MERFISH extends single-molecule imaging techniques to profile the copy number and localization patterns of thousands of genes, representing a major advance for spatial transcriptomics.

Recent applications of single cell genomic approaches have demonstrated that co-variation in gene expression can be used ~~to uncover, in an unbiased fashion,~~for unbiased discovery of putative cellular circuits and their regulators [1]. ~~.~~In performing such analyses, researchers have relied on the detection of coherent behaviors across several genes to counteract noise, both technical and biological, in any individual RNA measurement. This basic concern had limited the utility of RNA fluorescence in-situ hybridization (RNA-FISH)-based approaches, where spectral overlap ~~has~~ constrains~~ed~~ the number of RNAs that can be simultaneously assayed [2,3].

Recently, these barriers have been shattered by the development of several exciting approaches that rely on sequential rounds of hybridization and imaging. For example, using enzymatic digestion to remove previously bound probes, Cai and coworkers were able to reuse previously allocated color channels to image gene-specific probe sets and, thus, exponentially increase the number of RNAs they could assay in parallel [4]; the Nilsson [5] and Church [6] labs, meanwhile, were able to simultaneously determine the copy number of a few hundred or thousand genes, respectively, by directly reading individual bases with sequential in-situ hybridizations (in-situ RNA-Seq). Nevertheless, each of these pioneering approaches suffered~~s~~ from technical inefficiencies that limited detection (false negatives) and correct RNA calling,

Here, to directly address these two issues, Zhuang and colleagues present multiplexed error-robust FISH (MERFISH), a method that combines sequential hybridization with an error-robust RNA encoding scheme to dramatically improve sensitivity and accuracy [7]. In MERFISH, each RNA of interest is assigned a defining binary ‘word’ ~~that defines it~~of length *N*; for any given round of hybridization, a RNA should be fluorescently detected if its binary value is “1” and absent if its value is a “0”. Innovatively, to make their library robust, the team designed a 14-bit (N=14) binary dictionary in which each RNA-encoding

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‘word’ was separated by a hamming distance of 4 – meaning that 4 different errors would need~~ed~~ to occur to convert any one word into any other – and defined by a small number of positive detections (4). These modifications limit calling errors and false negatives due to inefficiencies in hybridization~~, respectively~~.

To implement their strategy in a cost-effective fashion, the authors developed a clever ~~oligonucleotide system that relies on two distinct sets of probes~~dual-labeling strategy with two distinct probe sets. In their design, a RNA is painted with a set of 192 target-specific ~~encoding~~ hybridization ~~probes that consist of target-RNA hybridization regions~~probes, collectively flanked ~~on each side~~ by four (of *N* possible) ‘encoding’ sequences~~a readout region.~~; ~~although any given oligonucleotide only has two readout sections, the entire set has 4 different ones (of N possible) corresponding to the 4 different detection events that define each ‘binary’ word/RNA. To profile RNA copy number, the encoding probe sets are first hybridized to cellular RNA in-situ and then~~ The identity of these is then~~the identity of each RNA is decoded~~ read out via *N* sequential rounds of hybridization with distinct~~*N* different~~ fluorescent readout probes targeting the encoding sequences, photobleaching between rounds ~~with~~ to erase previous detection. After reading, images are aligned with fiduciary marks, and binary strings are recorded and assigned to a specific RNA if the detected ‘word’ matches that RNA’s code or is only one error away.

By analyzing the occurrence of false-positives/negatives and comparing their measurements to conventional single molecule FISH (smFISH), the authors demonstrate that their method has a calling rate of ~80%. This means that MERFISH, as implemented, is sensitive enough to detect RNAs that are present at just one copy, though future work will better determine how RNA length, structure, and sequence degeneracy affect sensitivity.

~~By analyzing the occurrence of false-positives/negatives and comparing their measurements to conventional single molecule FISH (smFISH), the authors demonstrate that their method has a calling rate of ~80%. This means that MERFISH, as implemented, is sensitive enough to detect RNAs that are present at just one copy, though future work will better determine how RNA length, structure, and sequence degeneracy affect sensitivity. They~~Using ~~, used measurements from~~Applying MERFISH to profile human fibroblast cells using a 130-gene codeset, the team ~~to~~ identified~~y~~ a set of highly variable genes – including multiple heterogeneous ~~cellular~~ regulators of the cell cycle – and leveraged correlation patterns of expression across single cells to assign putative functions to poorly ~~correlated~~ characterized genes. Moreover, they identified two groups of genes with distinct patterns of intercellular localization. One group exhibited striking enrichment in the perinuclear region, while the other, representing~~ed~~ extracellular, membrane and other proteins, localized to the cell periphery.

Understanding the complex interplay between cellular environment, interactions, and decision-making is central to key questions in immunology; here, ~~, and~~ methods like MERFISH are poised to make important contributions. By a~~A~~nalyzing~~ing~~ the spatial distribution of cell types and activation states across a spatially patterned tissue, researchers ~~would serve as an invaluable tool for~~could study~~ing~~ infiltrating immune cells in the tumor microenvironment, immune compartmentalization in the spleen, ~~or~~ cell migration in the germinal center, or the spread of inflammation in the gut~~, disease-induced deficiencies, and more~~. Similarly, pr~~Pr~~obing correlations between a transcription factor and its targets as a function of position could reveal spatially-dependent

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regulatory circuitry previously masked in bulk measurements. Moreover, grouping genes by their intercellular localization could provide significant insights into the causes and consequences ~~for~~ of cell-cell interactions, for example, by identifying subsets of genes whose RNA expression patterns are ~~located close~~localized to particular cell-cell junctions.

MERFISH analysis requires a pre-designed codeset of probes which, in principle, limits ~~unbiased~~ the discovery process. A complementary approach, however, could be ~~used~~ to combine the unbiased aspects of sequencing-based profiling, with the targeted and spatially-resolved advantages of MERFISH. For example, high-throughput single cell transcriptomics could be leveraged to identify subsets of highly variable genes, enabling the informed design of a MERFISH codeset, and indeed new approaches are beginning to combine both imaging and sequencing data for single cell analysis [8,9]. In short ~~short~~, ~~space~~spatial context is ~~needed~~essential to understanding ~~how~~the links between a tissue's ~~biological~~ structure ~~informs function~~and molecular function, and MERFISH ~~provides~~represents a ~~giant~~powerful step forward for ~~deciphering~~decoding ~~this~~these relationships in health and disease.

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~~**Alex to write awesome last sentence summing it all up 😊**~~

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