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MERFISHing for spatial context

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'word' was separated by a hamming distance of 4 – meaning that 4 different errors would need 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: 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 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 used measurements from Applying MERFISH to profile human fibroblast cells using a 130-gene codeset, the team identified 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 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 analyzing the spatial distribution of cell types and activation states across a spatially patterned tissue, researchers would serve as an invaluable tool for could studying 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, probing 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, 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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