RNA Imaging with Multiplexed Error Robust Fluorescence in situ Hybridization

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Abstract

Quantitative measurements of both the copy number and spatial distribution of large fractions of the transcriptome in single-cells could revolutionize our understanding of a variety of cellular and tissue behaviors in both healthy and diseased states. Single-molecule Fluorescence In Situ Hybridization (smFISH)—an approach where individual RNAs are labeled with fluorescent probes and imaged in their native cellular and tissue context—provides both the copy number and spatial context of RNAs but has been limited in the number of RNA species that can be measured simultaneously. Here we describe Multiplexed Error Robust Fluorescence In Situ Hybridization (MERFISH), a massively parallelized form of smFISH that can image and identify hundreds to thousands of different RNA species simultaneously with high accuracy in individual cells in their native spatial context. We provide detailed protocols on all aspects of MERFISH, including probe design, data collection, and data analysis to allow interested laboratories to perform MERFISH measurements themselves.

smFISH is a powerful technique because it allows the quantitative measurement of the exact copy number and spatial distribution of individual mRNAs within single cells in intact tissues. MERFISH extends these powerful abilities to the transcriptome scale by massively multiplexing smFISH measurements by encoding individual RNA species with error-robust barcodes and reading out the barcodes via a series of smFISH measurements. We anticipate this method to be relatively easily adopted by other laboratories and the techniques described in this collection should facilitate these efforts as will the supporting protocols and code that can be found at:

http://zhuang.harvard.edu/merfish/.

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Collection

PROTOCOLS

1. RNA Imaging with MERFISH - Design of Oligonucleotide Probes

CONTACT: Max Brown

2. RNA Imaging with MERFISH - Probe Construction

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3. RNA Imaging with MERFISH - Sample Preparation and Staining

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4. RNA Imaging with MERFISH - Imaging

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5. RNA Imaging with MERFISH - Data Analysis

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