# Quantitative single-cell analysis of RNA regulation at the single-molecule level

Fangyuan Ding, California Institute of Technology,
Division of Biology and Biological Engineering

**Abstract:**

Despite the central dogma of molecular biology, not all information in DNA is processed to RNA molecules. In eukaryotes, nascent transcripts (named pre-mRNA) are spliced to functional message RNA (named mRNA) by removing introns and including/excluding exons, a ubiquitous process named 'splicing'. However, it has remained unclear what functional roles such constitutive splicing could provide. To explore this issue, we asked how splicing affects the efficiency with which individual pre-mRNA transcripts are productively processed across different gene expression levels. We developed a quantitative single-molecule Fluorescent In-situ Hybridization (FISH)-based method to quantify splicing efficiency at the transcription active site in single cells. Using this method, we found both natural and synthetic genes exhibit an 'economy of scale' behavior in which splicing efficiency increased with transcription rate, rather than decreasing as expected for a standard enzymatic process. Correlations between splicing efficiency and spatial proximity to nuclear speckles could explain this counter-intuitive behavior. Functionally, economy of scale splicing represents a non-linear filter that amplifies the expression of genes when they are more strongly transcribed. These results indicate that constitutive splicing plays an active functional role in modulating gene expression.