FOCUSING ON RAPIDLY DEVELOPING TECHNIQUES

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Cover legend: Through a convergence revolution, a fluorescently labeled human cell with nucleus and cell boundary outlined enables the super-resolved tracking of single RNA molecules over time, with the ultimate goal of understanding RNA biology from first principles.

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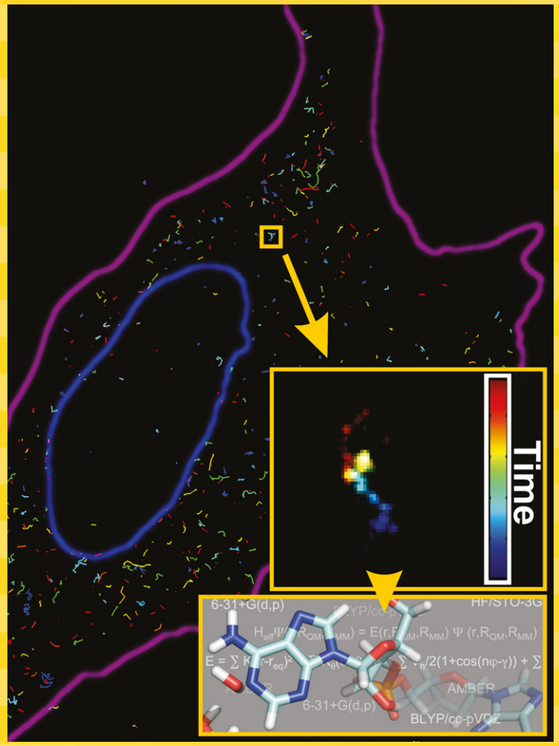
Convergence of Science and Technology: Fluorescent Resolution of Single RNA Molecules

Editor
Nils G. Walter



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Introduction to "Convergence of Science and Technology: Fluorescent Resolution of Single RNA Molecules"

As Phillip Sharp, Tyler Jacks and Susan Hockfield of MIT posit in a 2016 report [1]: "The life sciences are in the midst of a revolution… The Convergence Revolution promises to enhance quality of life worldwide … as a result of the sharing of methods and ideas by chemists, physicists, computer scientists, engineers, mathematicians, and life scientists across multiple fields and industries. It is the integration of insights and approaches from historically distinct scientific and technological disciplines." Perhaps nowhere is this both more apparent and more needed than in taking advantage of the rapid expansion of the RNA biosciences universe and the resulting opportunities for translating discoveries into precision medicines [2]. Converging technological advances, ranging from single-cell RNA sequencing to single-molecule particle tracking at super-resolution, are laying the foundation for reaching an entirely new level of insight into cellular physiology that eluded us just a few years ago [3]. Beyond the emerging catalogues of cellular contents, mechanistic studies of the diverse modes of action of RNAs and their interaction partners are handing us the keys for accelerating progress toward shaping our own destiny. The better we understand the scope and limitations of these new tools, the better we will be equipped to wield them effectively.

This thematic issue represents a naturally limited sampling of the single-molecule and super-resolution fluorescence microscopy tools emerging to highlight RNAs and their dynamic properties inside and outside the cell. Clearly, such a rapidly moving field in the biosciences constrains the availability of authors so that the current sampling is primarily meant to inspire debate and ideas for further development and expansion.

First, Walter and colleagues provide a practical guide to a recently developed method, termed single-molecule recognition through equilibrium Poisson sampling (SiMREPS), for the amplification-free detection of ultra-low quantities of nucleic acid disease biomarkers found in human biofluids that provides both single-molecule sensitivity and single-base selectivity by monitoring the repetitive binding of fluorescent probes to the immobilized targets [4]. The authors demonstrate how this kinetic fingerprinting filters out signals arising from nonspecific probe binding, yielding virtually zero background, while achieving an apparent single-base discrimination factor of over 3 million.

Next, Hoskins and colleagues analyze conceptually similar single-molecule colocalization data, emerging from studies of spliceosome assembly pathways *in vitro*, with maximum likelihood methods to fit the resulting dwell time distributions without the need for binning [5]. They discuss critical aspects of analyzing these distributions with histograms and pitfalls that can be encountered if improperly binned histograms are used, then offer assistance through automated analysis through a freely available software package.

Third, Hengesbach and colleagues explore how to utilize in truly collaborative manner NMR spectroscopy to resolve RNA base pairing patterns and single-molecule FRET (smFRET) microscopy to map long-range structural dynamics of RNA [6]. They provide detailed protocols aimed at facilitating the use and integration of NMR and smFRET to yield complementary data. Their approach achieves novel insights into the structural dynamics of an adenine-responsive riboswitch.

Next, Cissé and colleagues present a CRISPR/Cas9-based gene editing approach to insert an MS2 labeling cassette with selectable marker into the untranslated region of any coding gene [7]. This tool enables tagging of the mRNA of, for example, a transcription factor in mouse embryonic stem cells. Combined with quantitative fluorescence microscopy, the number of nascent transcripts at the nuclear locus and the fraction of cells expressing the gene can be determined, leading to an unprecedented digital view of cellular biology.

Fifth, Kubitscheck and colleagues adapt a sparse labelling strategy initially used to track mRNA for the fluorescent labeling of the small ribosomal subunit [8]. Using single-particle tracking *in vivo*, they observe single subunits in both the nucleolus and nucleoplasm, identifying states of distinct diffusion coefficients. A surprisingly significant fraction of ribosomal subunits become immobilized in the nucleoplasm, which is not observed for inert control molecules.

And last but not least, Yang and colleagues take the next logical step by implementing live-cell single-particle tracking for the nuclear export of mRNA [9]. Leveraging the tool of single-point edge-excitation sub-diffraction (SPEED) microscopy that combines high-speed microscopy with a 2D-to-3D transformation algorithm, they map the speed and route of mRNAs through the nuclear pore complex and evaluate the reliability and accuracy of SPEED microscopy.

Taken together, the editor hopes that this issue of Methods may prove valuable to researchers in the RNA field and beyond as the convergence of science and technology becomes ever more pervasive, leading to a bright future for human health through the fluorescent resolution of single RNA molecules.

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