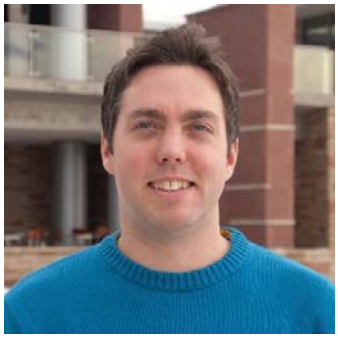
# Seminar

**Time & Date:** Friday June 28 16:00-

**Place:** Biosystem Building 2F Seminar Room

**Title:**
Multi-color imaging of non-canonical translation dynamics with single molecule resolution in living cells

**Speaker:** Timothy J. Stasevich, Ph.D.



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My lab is developing technology to image with high spatiotemporal resolution the dynamics of single mRNA translation in living cells. In this talk, I'll describe recent multicolor extensions of our technology to image non-canonical translation. First, I'll describe how complementary repeat epitopes can be placed in overlapping translation reading frames to monitor single RNA translation frameshifting [1]. Application to HIV-1 reveals frameshifting occurs only on a subset of RNA in stimulated bursts that last for many rounds of translation. Mathematical modeling of the data provides new insight into how frameshifting occurs at the single molecule level, implicating ribosomal traffic jams in maintaining the frameshifting state, even when global translation initiation is inhibited. Second, I'll describe unpublished work examining IRES-mediated translation. By inserting complementary epitopes into a bicistronic reporter, cap and IRES mediated translation from a single RNA can be simultaneously monitored in real-time. Using this sensor, we find the EMCV IRES recruits approximately one ribosome for every three ribosomes recruited to the cap. Upon cellular stress, however, this balance dramatically shifts in favor of the IRES. Interestingly, even in unstressed conditions, we find evidence for crosstalk between IRES and cap-mediated translation, implying ribosome recycling from cap to IRES often occurs. Finally, I'll discuss new genetically-encoded imaging reagents we created to facilitate "live-cell immunofluorescence" of small epitope tags like HA [2] and FLAG. These reagents are cheap and easy to use and should therefore make multicolor imaging of single RNA translation more widespread.

1. K. Lyon, L. U. Aguilera, T. Morisaki, B. Munsky, and T. J. Stasevich, Live-Cell Single RNA Imaging Reveals Bursts of Translational Frameshifting, Molecular Cell 75, 1-12, 2019 https://doi.org/10.1016/j.molcel.2019.05.002
2. N. Zhao, K. Kamijo, P. D. Fox, H. Oda, T. Morisaki, Y. Sato, H. Kimura, T. J. Stasevich, A genetically encoded probe for imaging nascent and mature HA-tagged proteins in vivo, Nature Communications 2019 (in press, preprint on biorxiv: doi: https://doi.org/10.1101/474668).

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