

**Module 1:** Single-Cell Optical Microscopy Experiments and Image  
“Fluorescent Labeling Techniques used in single-cell Research. Part 2”

**Lecturer:** Linda Forero-Quintero

**e-mail:** [linda.forero\\_quintero@colostate.edu](mailto:linda.forero_quintero@colostate.edu)

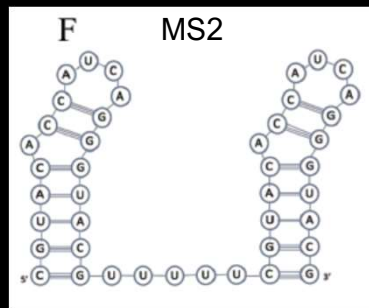
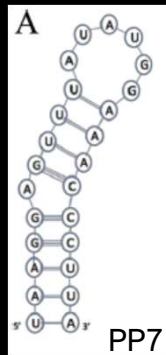


# Fluorescent Labeling Techniques used in single-cell Research

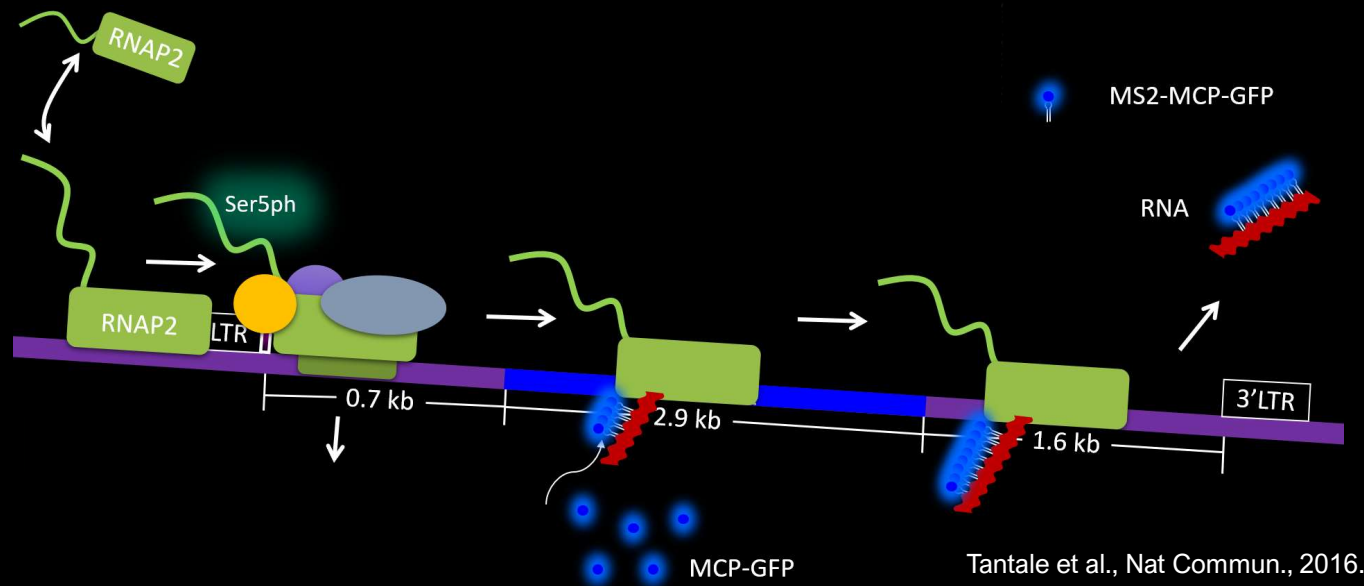
## Outline-Part 2:

1. Labeling Techniques Employed in live cells:
  - a. To visualize transcription
  - b. To visualize translation-Nascent Tracking Chain Probes
2. Label-free Methods:
  - a. Phase Imaging/diffraction tomography
3. Sources

- **MS2 (Bertand et al. 1998) or PP7 (Larson et al. 2011) tagging** are aptamers-based approaches to label RNA. This technique takes advantage of the natural interaction of MS2 or PP7 bacteriophages coat proteins (MCP or PCP) with a stem-loop structure from the phage genome.



Taken from Gemmill et al. 2020

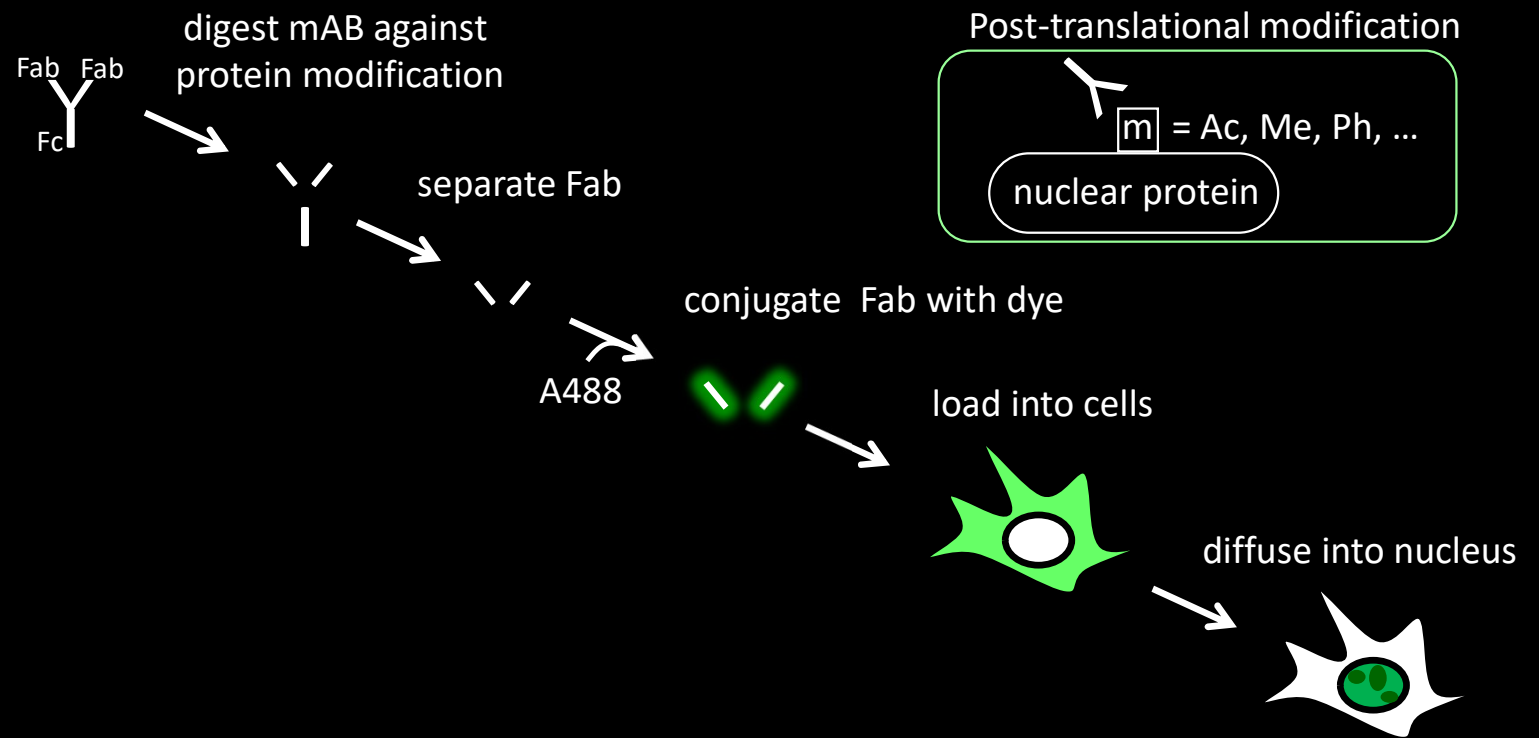


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➤ **FabLEM** are fragmented antibodies designed to target endogenous post-translational modifications in live cells.

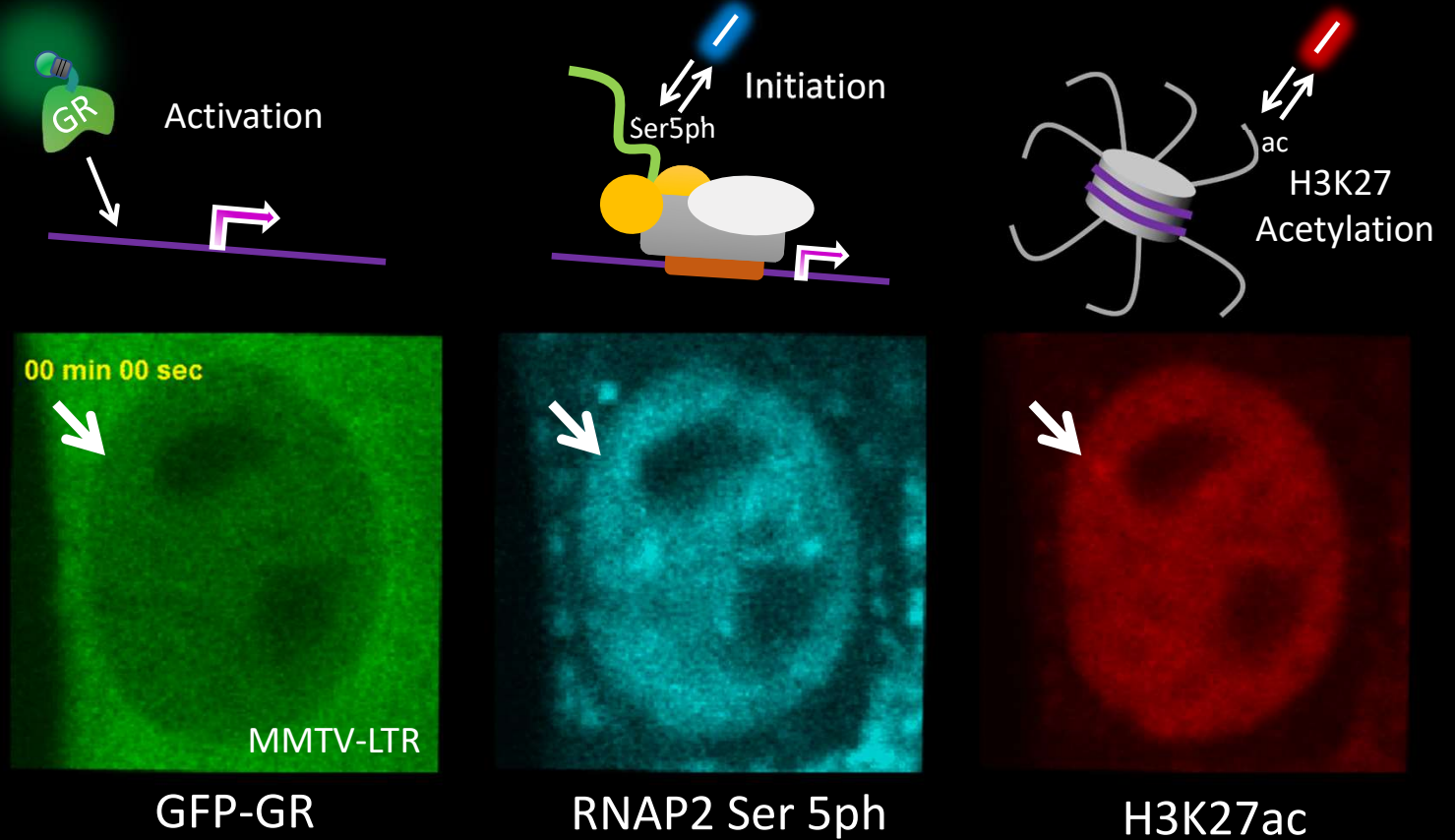


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## Example of imaging modifications using FabLEM at a tandem gene array



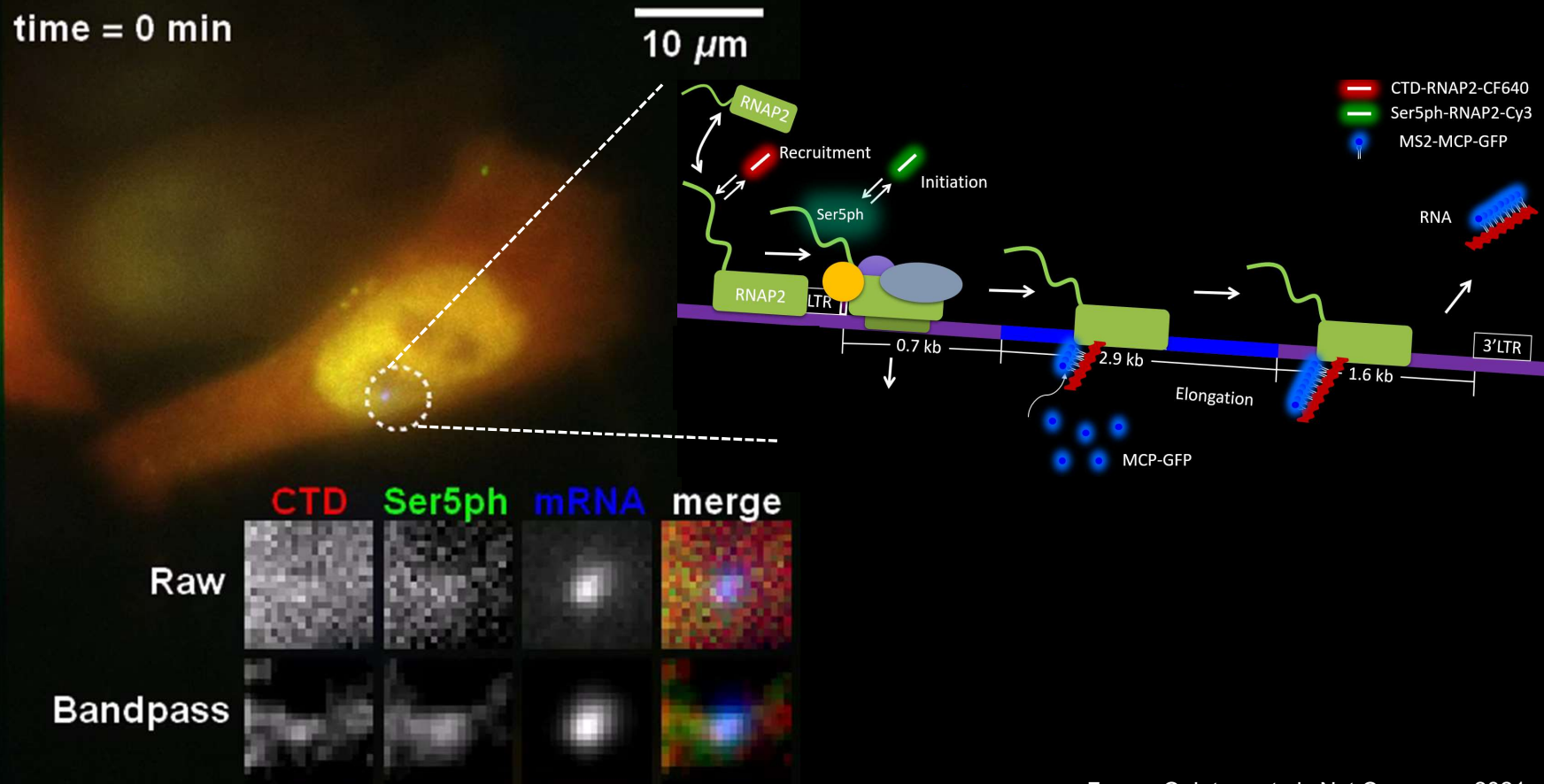
MMTV-LTR: Mouse Mammary tumor virus-Long Terminal Repeat  
 GR: Glucocorticoid Receptor  
 Stasevich et al, Nature 516, 272-275 (2014)

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## Example combing MS2 labeling and FabLEM to visualize RNAP2 phosphorylation dynamics at a single-copy gene

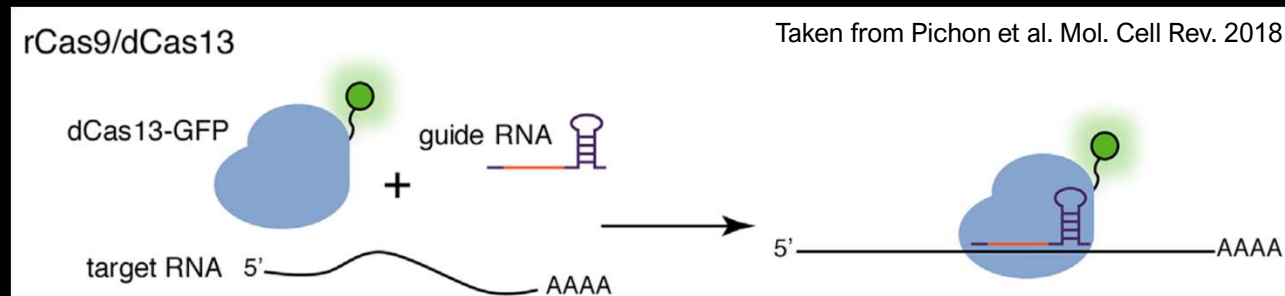


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- **dCas9 labeling** reproposes the CRISPR/Cas9 system (Nobel Prize in Chemistry 2020) to bind and image RNA. The catalytically inactive Cas enzyme is fused to a fluorescent protein and binds target RNA in the presence of a guide RNA.



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### 1. Labeling Techniques

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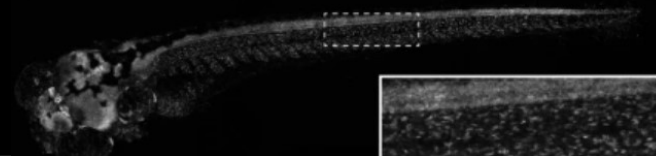
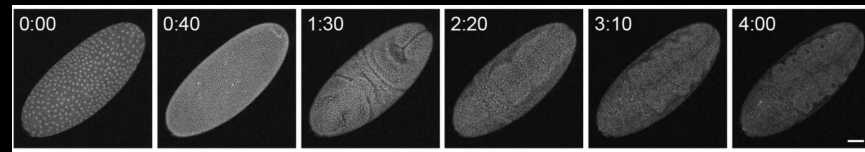
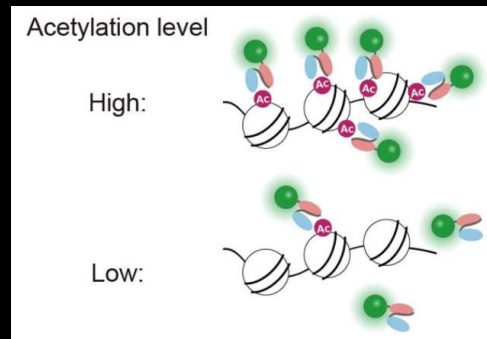
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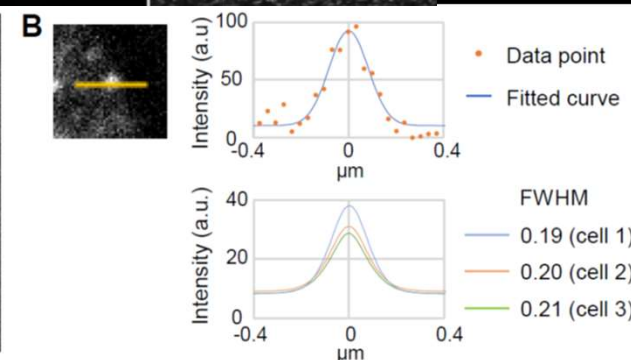
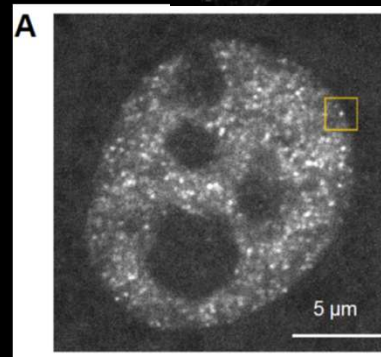
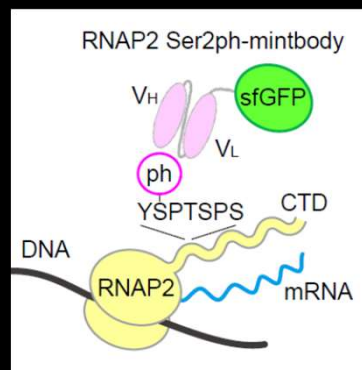
- a. Phase Imaging/diffraction tomography

### 3. Sources

- **Genetically encoded modification-specific intracellular antibody (mintbody) probes** are designed against a specific modification (such as H3K9ac or RNAP2-Ser2ph). To generate a mintbody, the coding sequence of several antibodies heavy and light chains specific against the desired modifications are cloned and tagged with a fluorescent protein (e.g. sfGFP) and then transfected into the desired cells.



Sato et al., Sci. Rep, 2013



Uchino et al., pre-print bioRxiv, 2021

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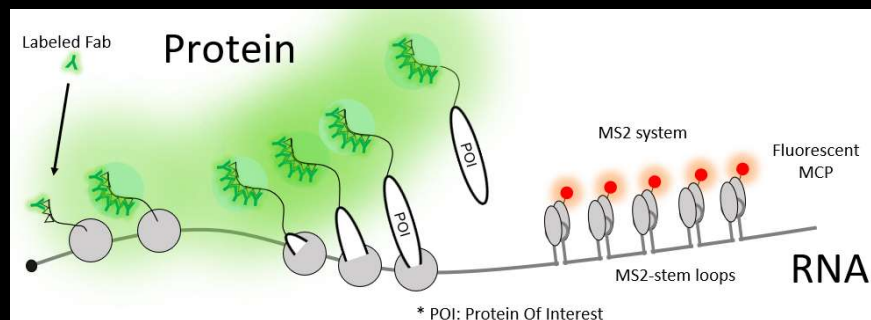
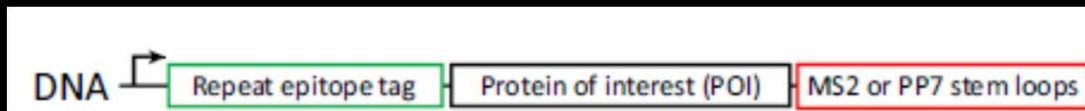
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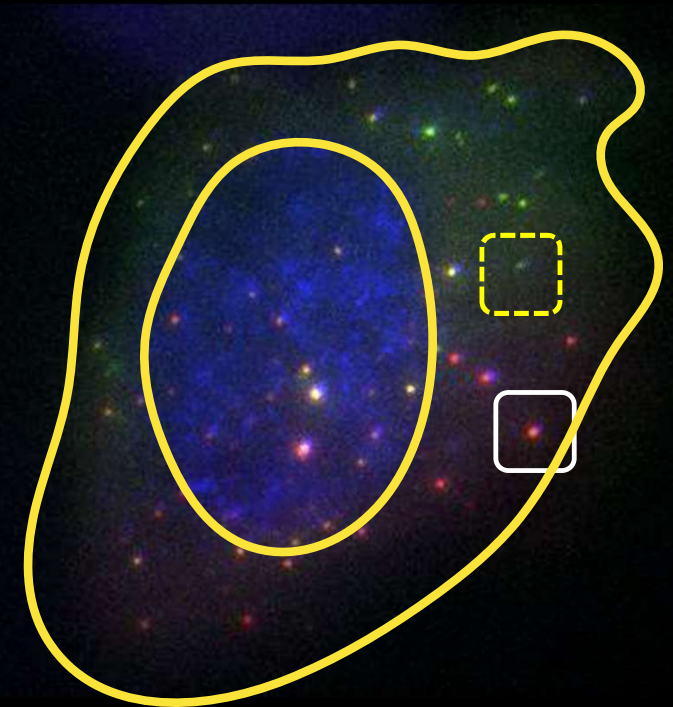
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- **Nascent Chain Tracking (NCT)** allows of single-mRNA nascent peptide translation.



Taken from Lyon and Stasevich. Trends in Genetics Rev., 2017

- RNA can be labeled in live cells using MS2/MCP system.
- Peptides can be labeled with multiple fluorescent antibody fragments.
- Quantify Nascent protein translation from a single mRNA.

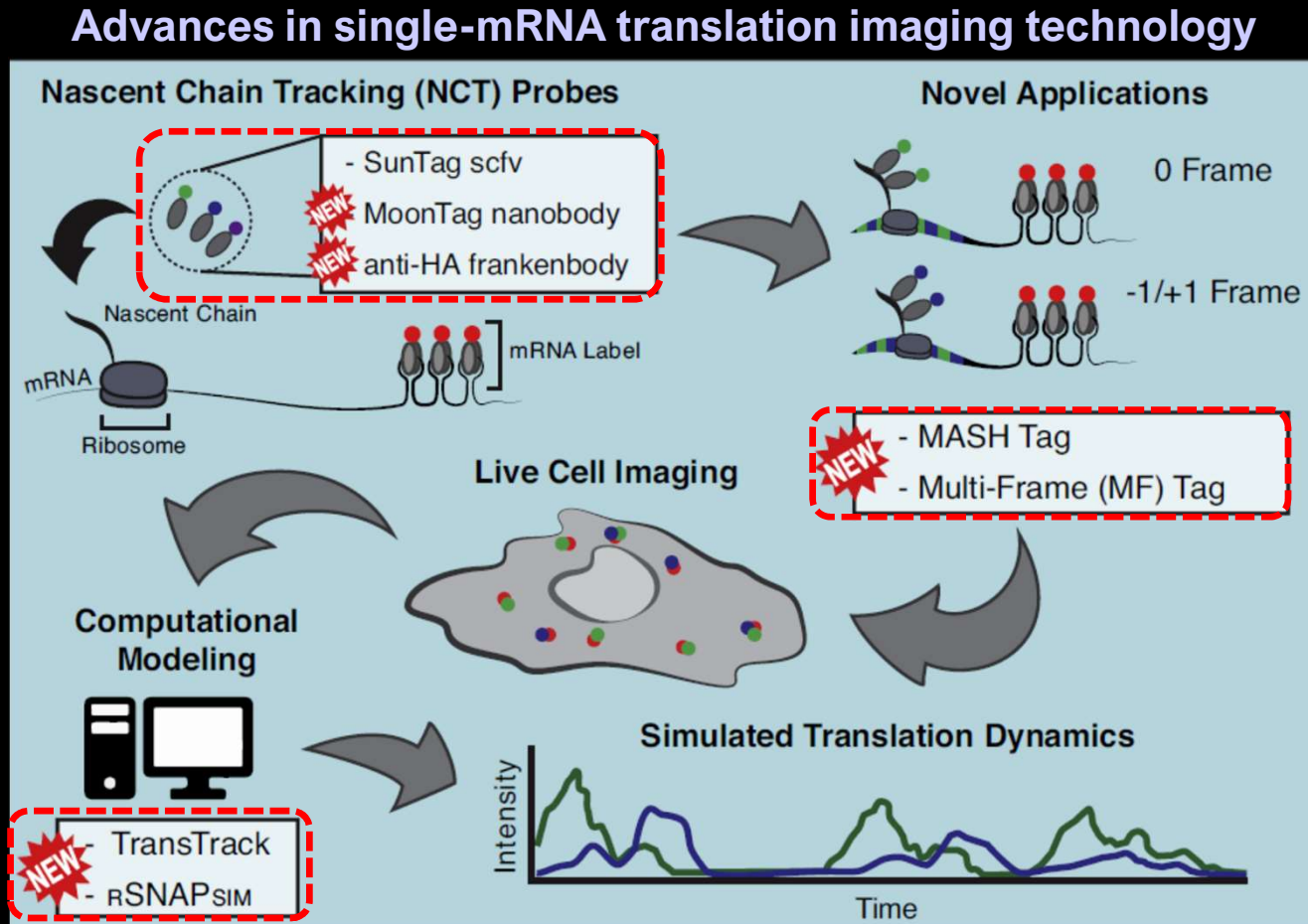


Morisaki et al., Science, 2016



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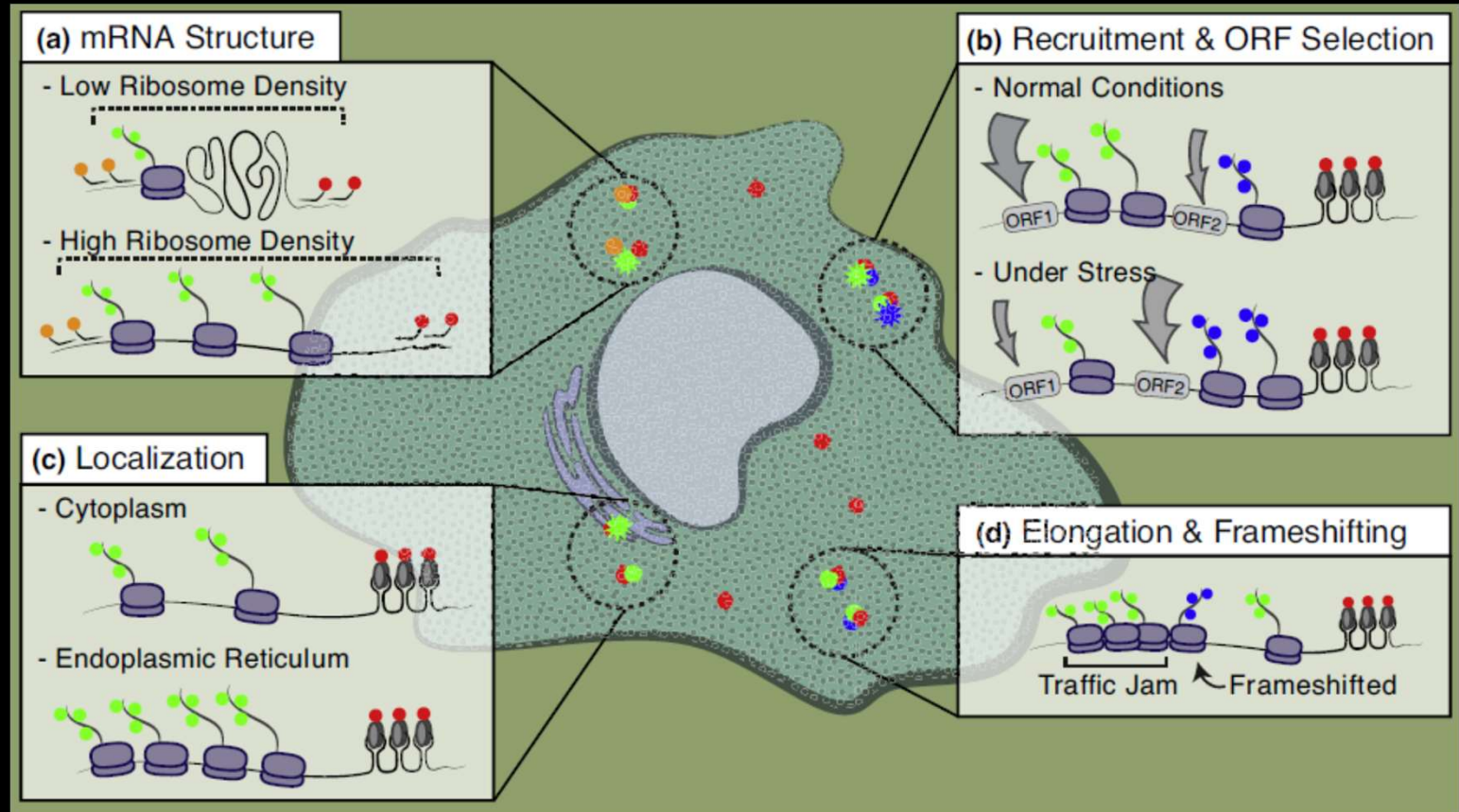
Taken from Cialek et al. Current Opinion in Genetics & Development. 2020

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## Recent applications of NCT to study active translation dynamics



Taken from Cialek et al. Current Opinion in Genetics & Development. 2020

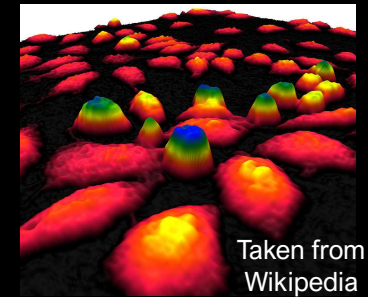
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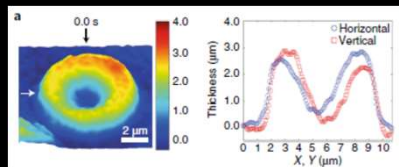
➤ **Quantitative phase imaging (QPI)** is an emerging valuable tool to visualize cells and tissues without using fluorescent labels. QPI quantifies the phase shift that occurs when light waves pass through a more optically dense object by combining qualities found in microscopy, holography and light scattering techniques.

### Some applications

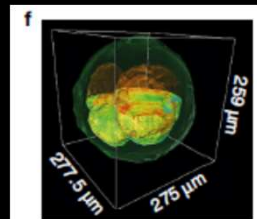


Taken from Wikipedia

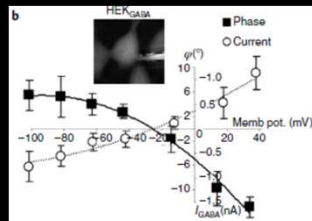
### Basic Science



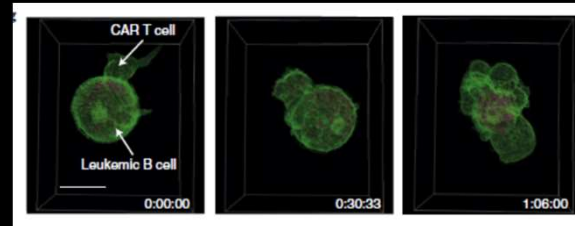
RBC structure



Bovine embryos over several days

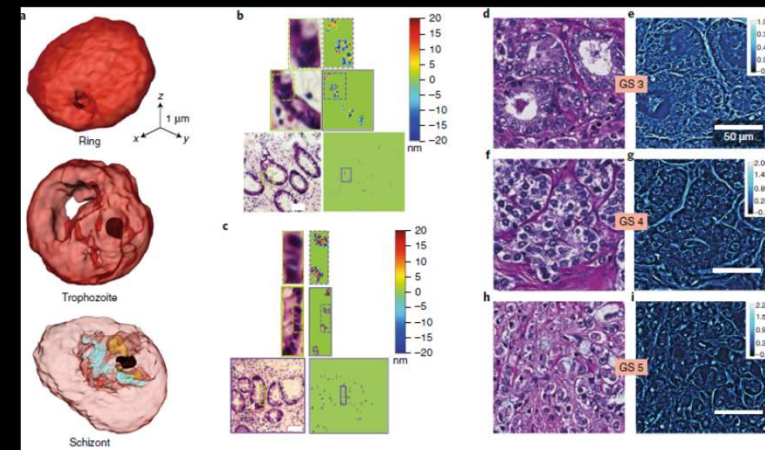


Neuronal Network Activity



3D imaging of a chimeric antigen receptor T cell killing a target cancer cell

### Medical



Blood Screening & photodynamic anticancer activity

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1. Bertrand et al. *Mol Cell*. 1998 Oct;2(4):437-45.
2. Larson et al. *Science*. 2011 Apr 22;332(6028):475-8.
3. Gemmill et al. *Biochemistry and Cell Biology*. **98**(1): 31-41.
4. Katjana Tantale et al., *Nat Commun* **7**, 12248 (2016).
5. Hayashi-Takanaka et al., *Nucleic Acids Res*. 2011 Aug;39(15):6475-88.
6. Stasevich et al., *Nature*. 2014 Dec 11;516(7530):272-5.
7. Forero-Quintero et al., *Nat Commun* **12**, 3158 (2021).
8. Pichon et al., *Mol Cell*. 2018 Aug 2;71(3):468-480.
9. Ochiai et al., *Nucleic Acids Res*. 2015 Oct 30;43(19):e127.
10. Sato et al., *Sci Rep* **3**, 2436 (2013).
11. Uchino et al., pre-print bioRxiv, 2021
12. Morisaki et al., *Science* **17**, Jun 2016:Vol. 352, Issue 6292, pp. 1425-1429
13. Cialek et al. *Curr. Opin. Genet. Dev.*, vol 61, April 2020, Pages 75-82
14. Park et al. *Nature Photon* **12**, 578–589 (2018).