Laboratory for Nanobiology

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What are we after?

Nature's molecular-scale design principles

Our lab leads an interdisciplinary research program that aims to **innovate and engineer novel technologies** like **single molecule detection**, **quantitative genomics** and **microfluidics** with focus on **infectious diseases.**

Generating quantitative understanding of RNA virus growth and host-pathogen interaction using super-resolution microscopy

PI: Rahul Roy (ChemE/BSSE/MBU)

Broad question: How does a virus hijack the cell to aid its growth?

Primary Objectives of the project:

A. *Development of a super-resolution imaging platform based on a Reflected Light Sheet Microscope for high-speed and high resolution imaging of single viruses in single cells.*
   Imaging single (or few) molecules in single cells is challenging due to several technical limitations but is necessary to understand virus infections as they start from single viruses (comprising of single RNA genome). We will develop a microscope capable of capturing movies of single virus infection and events leading upto 'killing' of the cell by the virus progeny. Automated data acquisition and image analysis will require key innovations here.

B. *Developing a RNA tag based RNA reporter virus for measuring real-time dynamics of virus replication and test feasibility of quantifying viral RNA by single RNA counting.*
   A tandem repeat (24X) of Broccoli tag introduced on the DNA clone (at one of UTR regions) will be in vitro transcribed and transfected into the cells. Replication will be quantified by live cell imaging at various stages of infection. Validation of the RNA imaging will be accomplished by viral RNA FISH. This construct will be useful for assessing spatial localization of viral RNA in cells as well as virus RNA packaging and virus export.

C. *Imaging of viral and host protein localization using immunofluorescence based super resolution imaging*
   A small orthogonal peptide* would be introduced as a fusion to virus proteins in the virus DNA clone. After testing infectious virus generation, organization and spatial distribution of virus proteins will be mapped. These reagents will also help in pull-down of host cofactors at a later stage. Co-localisation of viral RNA (RNA-FISH) with virus or host proteins in super resolution will help identify proteins involved in virus growth.

Prerequisites

The main prerequisite for this project is a fascination to understand complex systems quantitatively and willingness to engineer new imaging and analysis tools to study biological systems. Previous experience with optical systems and microscopy, data acquisition and instrumentation, image and signal processing, programming languages like MATLAB, C++, Python, molecular biology and cell culture will be advantageous. Tentative List of courses to be taken at IISc: Optical microscopy/ Spectroscopy, Image and Signal processing/ Virology and Systems Biology. Nature of work will include wet-lab work, optical microscopy, data analysis algorithms, micro/nano fabrication and basic computational modeling.

Challenge / Goals

While much of our knowledge in biology comes from ensemble experiments, many biological phenomena like virus RNA infection and growth occur one molecule at a time. However, mechanistic understanding of the process inside the cells is complicated as it is inherently stochastic because it initiates from a small number of single stranded (ss)RNA molecules (sometimes just one) during its initial phases and generates several heterogeneous intermediates during its growth.

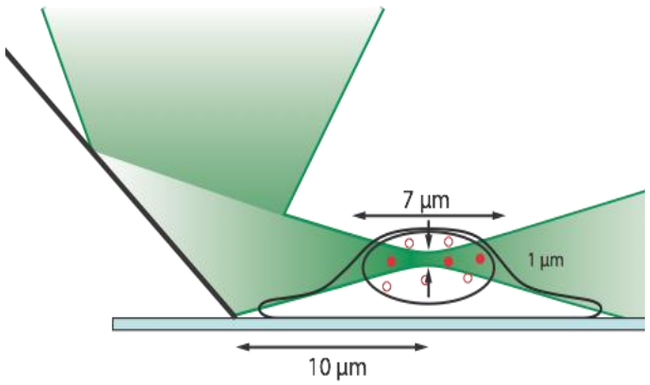
Hypothesis: Viruses hijack both the cellular machinery and architecture to aid their growth.

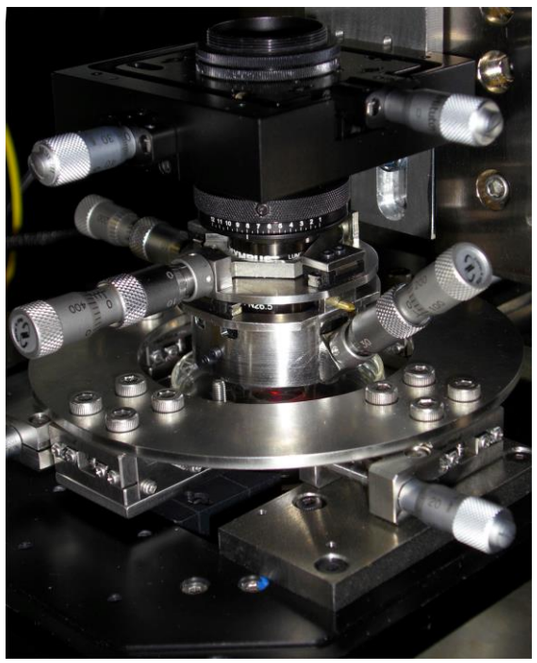
Challenge: Spatio-temporal changes in levels and organization of virus growth inside single cells are difficult to study due to limits on detection sensitivity and resolution.

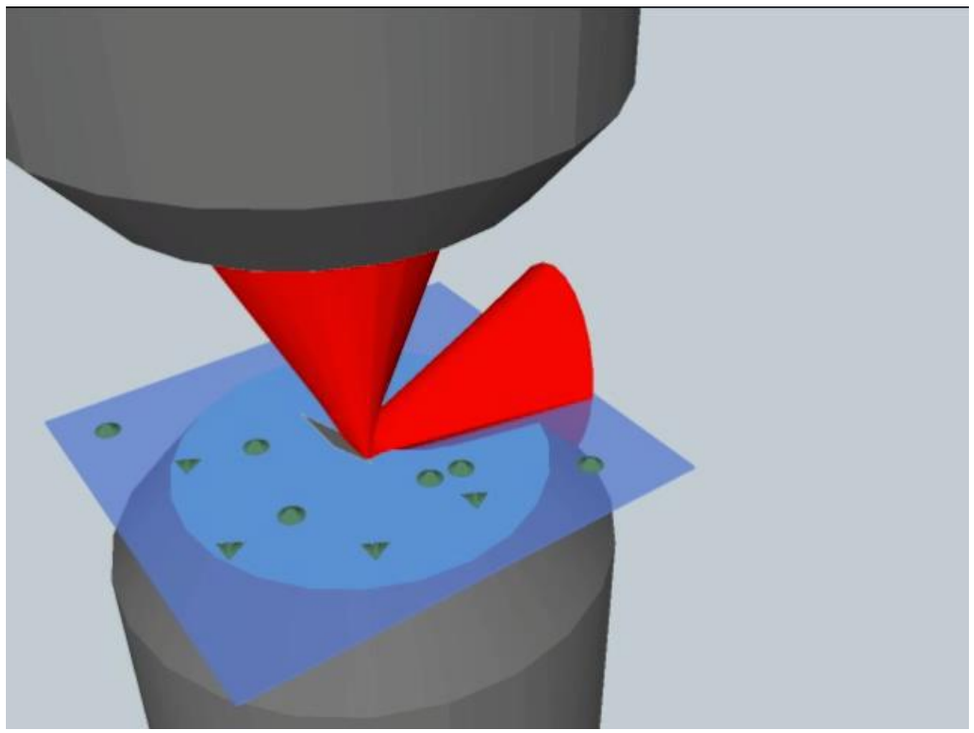
GOAL: To develop super-resolution microscopy platform for measure virus levels in single cells in real-time.

Method/ Technique

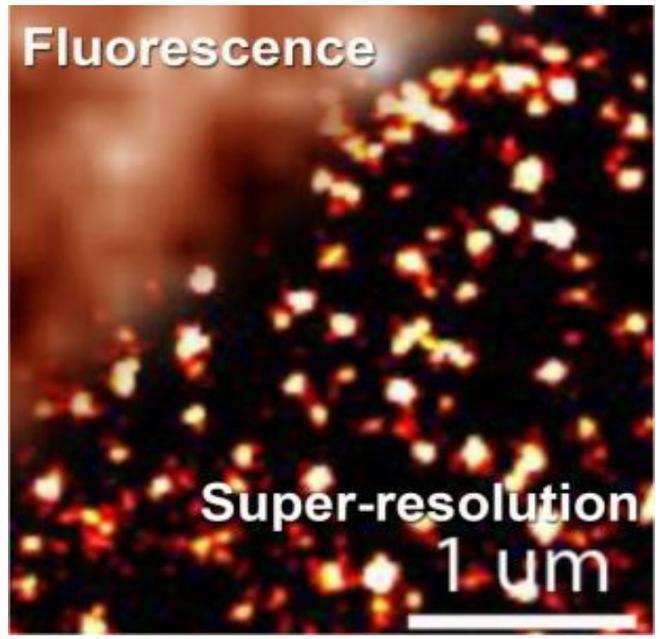
High resolution single molecule imaging in single live cell







Reflected light sheet super-resolution microscopy
(microscope capable of detecting single molecules deep in living cells)



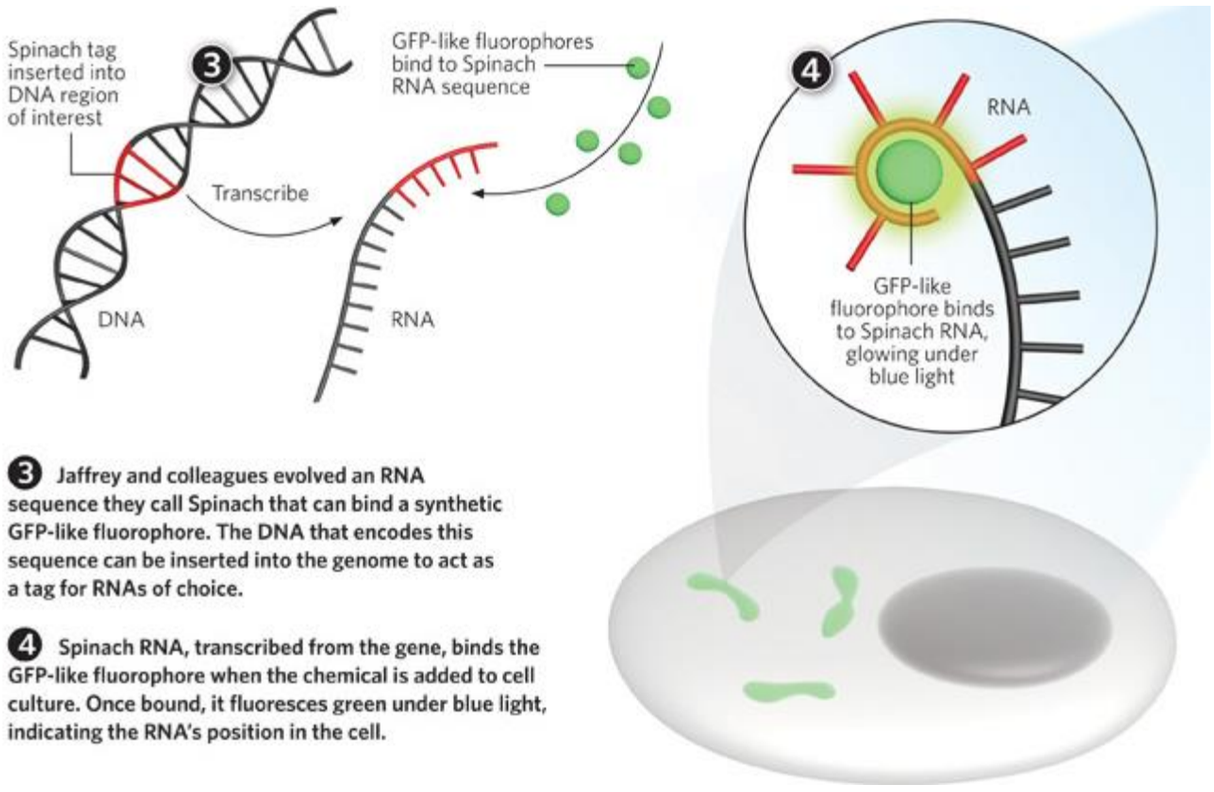
Distribution of RNA Polymerase II, a key cellular protein, inside a section of the human cell nucleus as revealed by super-resolution microscopy.

Gebhardt, Suter, Roy, et. al., Nat Met 2013
Zhou, et. al., PNAS 2014
Roy, Current Science 2014

Method/ Technique

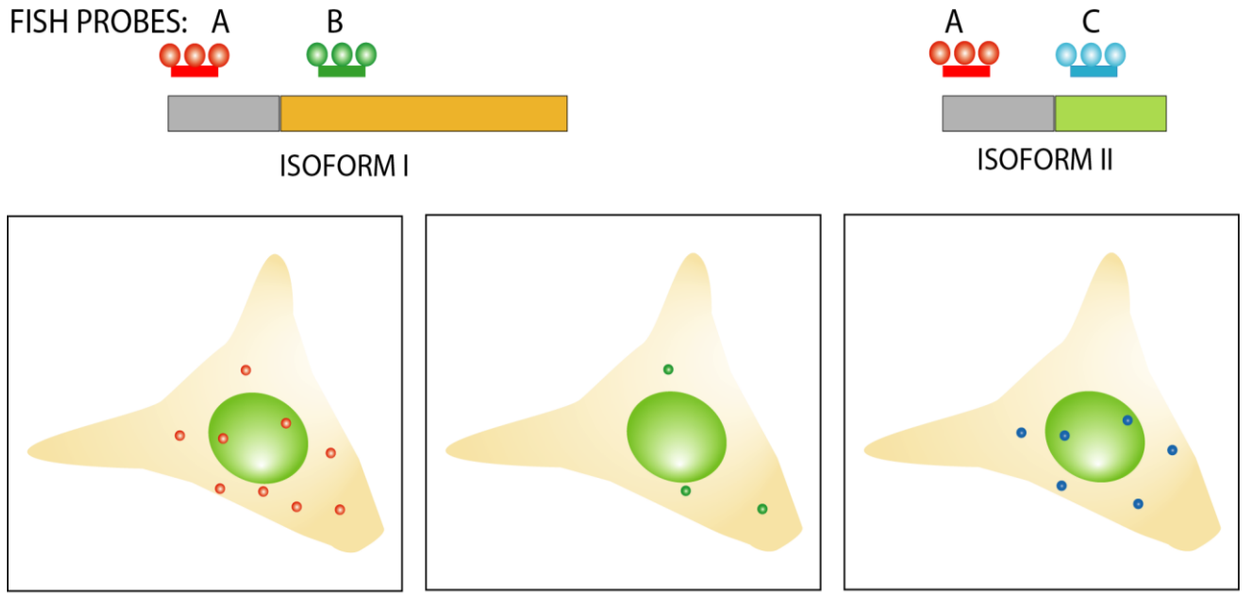
Methods to image single virus RNA molecules

Fluorescent RNA- tags



A RNA tag based RNA reporter virus for measuring real-time dynamics of virus replication will be developed.

RNA- FISH



Multiplexed RNA-FISH method: Variants of an virus RNA molecule cannot be distinguished by single color probe. However, using a combination of multiple colors, two virus RNA in different states can be distinguished as depicted. Under super-resolution microscopy, single mRNA molecules will be visible as localized fluorescent puncta. By co-localizing the multiple colored probes (each image for each individual color) with SSRM in the same cell, we can then distinguish each of the variants and count their absolute numbers down to a single RNA molecule.

Paige, et. al. Science 2011
Tyagi, Nature Methods 2009