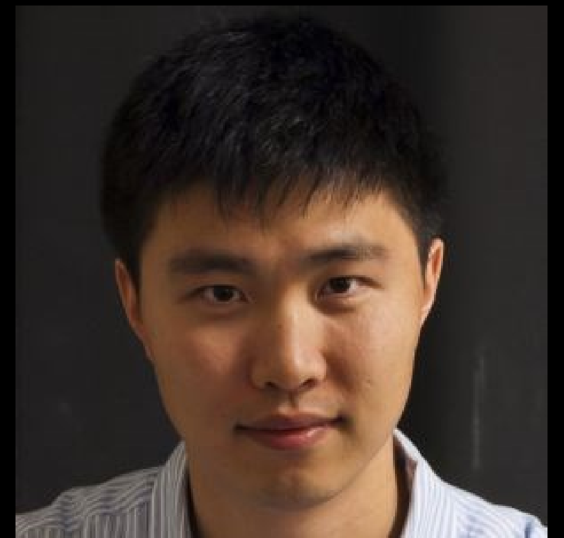
Yamate Evening Seminar

September 8th, 2016, 16:00-17:00

Large meeting room, 2nd floor, Yamate 3rd Bldg.

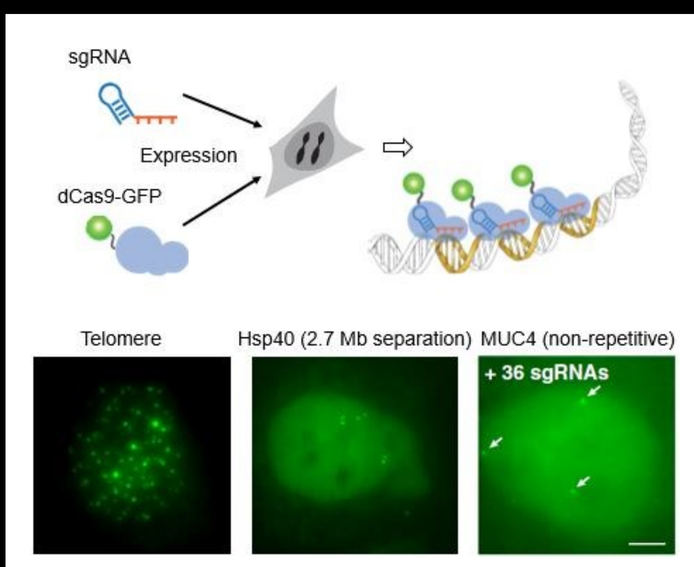
Prof. Bo Huang
(UCSF)

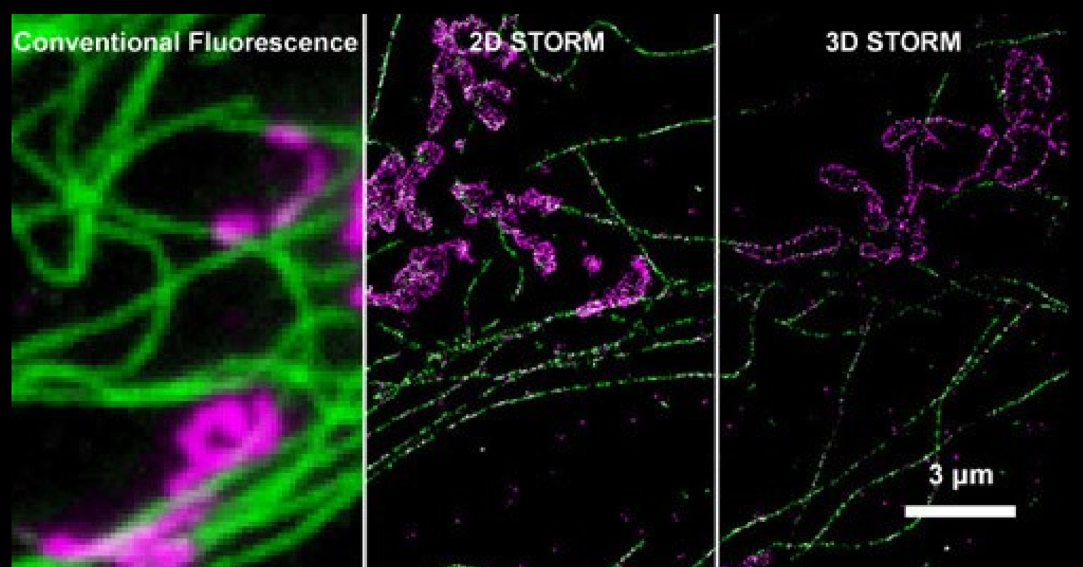


Imaging the Genome in Living Cells

The spatial architecture and temporal dynamics of the genome play critical roles in regulating its function. However, visualizing endogenous DNA sequences in living cells remain challenging due to the lack of imaging tools. We have developed such an imaging tool by repurposing the CRISPR system, previously engineered for RNA-guided gene editing and regulation. Utilizing fluorescent protein-tagged endonuclease-deficient Cas9 proteins and structurally-optimized sgRNAs, we have demonstrated robust imaging of both repetitive and non-repetitive DNA sequences in mammalian cell nucleus. The target flexibility of the CRISPR system allows us to simultaneously track multiple genomic loci in different colors, helping to elucidate chromosome structure change during the cell cycle. Our study defines a new class of genome imaging tool and highlights its potential to visualize genomic organization in living cells and how it regulates gene expression.

To systematically study the products of gene expression in a native cellular background, we sought to create libraries of cell lines expressing proteins tagged with a functional sequence at their endogenous loci. Taking advantage of the split GFP11/1-10 system and CRISPR/Cas9-mediated gene editing, we describe a scalable method for the robust, scarless, and specific tagging of endogenous human genes with GFP. Our approach requires no molecular cloning and allows a large number of cell lines to be processed in parallel. We demonstrate the scalability of our method by successfully labeling 30 human genes for fluorescence microscopy. Our method paves the way for the large-scale generation of endogenously tagged human cell lines for the proteome-wide analysis of protein localization and interaction networks in a native cellular context.





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