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3D Single-molecule Super-resolution Light Sheet Imaging Throughout Mammalian Cells

Dr. Gustavsson joined the faculty at Rice University in the summer of 2020 as a CPRIT Scholar and the Norman Hackerman-Welch Young Investigator Chair. Dr. Gustavsson received her PhD in Physics from the University of Gothenburg, Sweden, in 2015. Her work focused on studying dynamic responses in single cells by combining and optimizing techniques such as fluorescence microscopy, optical tweezers, and microfluidics. Upon completion of her graduate work, Dr. Gustavsson joined the group of Nobel Laureate W. E. Moerner at Stanford University as a Postdoctoral Fellow in 2015. Her research focused on the development and application of 3D single-molecule super-resolution microscopy for cellular imaging, and included the implementation of light sheet illumination for optical sectioning of mammalian cells. Dr. Gustavsson's work has been recognized with multiple honors, awards, and fellowships, most notably the *FEBS Journal Richard Perham Prize for Young Scientists* in 2012, the 3-year *Swedish Research Council International Postdoc Fellowship* in 2016, the *PicoQuant Young Investigator Award* in 2018, the *NIH K99/R00 Pathway to Independence Award* in 2019, and the *CPRIT Recruitment of First-Time Tenure-Track Faculty Members Award* in 2020.

Abstract: To obtain a complete picture of subcellular structures, cells must be imaged with high resolution in all three dimensions (3D). In this talk, I will present tilted light sheet microscopy with 3D point spread functions (TILT3D) (1, 2). This imaging platform combines tilted light sheet illumination with engineered long axial range point spread functions (PSFs) for low-background, 3D super-localization of single molecules as well as 3D super-resolution imaging in thick cells. Light sheet illumination is a clever approach to improve imaging in thick cells, where the sample is excited by a thin sheet of light orthogonal to the detection axis. The light sheet selectively illuminates molecules close to the image plane, which greatly reduces the problems of out-of-focus background fluorescence, photobleaching, and photodamage. The powerful yet simple approach of PSF engineering allows for scan-free wide-field 3D detection of single-molecules over several μm per slice. TILT3D is built upon a standard inverted microscope and has minimal custom parts.

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The result is simple and flexible 3D super-resolution imaging with tens of nm localization precision throughout thick mammalian cells.

The performance of TILT3D was validated by nanoscale imaging of several cellular structures, including mitochondria and the entire nuclear lamina (1). In this talk, I will also demonstrate how TILT3D allowed us to map the molecular distribution of sugars in the glycocalyx in cancer cells (3). The mammalian glycocalyx is a heavily glycosylated extramembrane compartment found on nearly every cell. It is the first component of a cell to interact with the environment and therefore plays an important role in cell-cell and cell-matrix interactions critical to embryonic development, immune cell trafficking, cancer progression, and many other normal and pathological processes. By directly imaging the distribution of sugars and the thickness of the glycocalyx, we determined the effect on the glycocalyx nanoscale organization and structure during cancer progression. To further demonstrate the versatility of this imaging platform, I will present our recent discovery of previously unknown nanoscale fibrillary protein arrangements in the inversin compartment in primary cilia (4). The primary cilium is a signaling organelle that projects from the surfaces of specific cell types, including many human tissues. Primary cilia contain a membraneless sub-compartment called the inversin compartment to which four proteins are known to localize. The function of the inversin compartment is unknown, but it appears to be critical for normal development, including left–right asymmetry and renal tissue homeostasis. We applied our imaging platform to map the 3D nanoscale distribution and organization of the inversin compartment proteins, which, together with genetic dissection of the protein–protein binding relationships, allowed us to develop a new structural model of this compartment.

TILT3D is a versatile imaging tool for biophysical and biomedical research which allows for studies of molecular mechanisms and interactions in a wide range of conditions related to normal cellular function and disease progression. We think that TILT3D in the future will become an important tool not only for 3D super-resolution imaging, but also for live whole-cell 3D single-molecule tracking (5).