

MICROSCOPY


Tech Pulse




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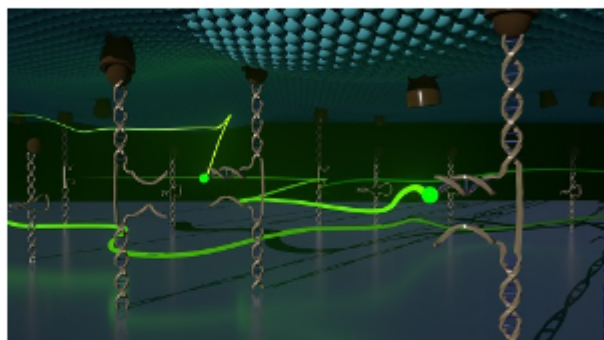


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'Firefly' Imaging Technique Sheds Light on Molecular Forces

Using tools made of luminescent DNA, researchers at Emory University have visualized the mechanical forces of cells at the molecular level.



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PROMOTED CONTENT



Mad City Labs Inc.

Understanding Virus Mechanisms – One Particle at a Time

Single-molecule microscopy techniques facilitate direct study of molecular mechanisms, enabling leaps in understanding surrounding how viruses assemble, disassemble, and interact with their hosts. In this researcher profile, we describe how Prof. Tijana Ivanovic is working to understand cell entry mechanisms and the relationship between virus particle structure/organization and the early steps of infection.

Understanding Virus Mechanisms – One Particle At A Time

UNDERSTANDING VIRUS MECHANISMS: HOW VIRUSES ASSEMBLE, DISASSEMBLE, AND INTERACT WITH THEIR HOSTS.

Tijana Ivanovic, Ph.D., is an Assistant Professor of Biochemistry at Emory University (Atlanta, GA) and Principal Investigator for the Ivanovic Lab, whose current research focuses on diverse virus pathogens and nanotechnology-based systems. "We try to dissect cell entry mechanisms and describe the relationship between virus particle structure/organization and the early steps of infection, including virus particle attachment to primary surface antibodies or membrane receptors," Ivanovic says of her laboratory's research. "Once a virus is inside a cell, all hell breaks loose and the most complex tasks are introduced. Since the virus enters the cell, we have a convenient spot to prevent virus infection. This simply needs to be understood from the virus particle entry and the interaction with the host."

Ivanovic obtained her undergraduate degree for studying HIV cell entry as a postdoctoral fellow at Emory University's CDC Research Center. One year later, she moved to the lab of Mad City Labs, Ph.D., at Harvard Medical School, learning about membrane penetration. Ivanovic returned at Harvard Medical School as a postdoctoral fellow under Robert C. Mackay, Ph.D., at which point she started exploring more deeply biological and single-particle virus imaging. "The integration of all these various experiences led to novel imaging tools in my own lab. It's a very interdisciplinary group. We apply biological experiments, as well as cutting-edge imaging and analysis by physical approaches," Ivanovic says.

Tools and Techniques
Working on nanoscale, the Ivanovic Lab team developed a way to study its assembly in single-molecule microscopy.

"We can watch a fluorescently-labeled particle come together and see how it changes. Not for envelope viruses, we used the mechanism of membrane fusion," Ivanovic says.

Among the laboratory tools used for this purpose are a set of Mad City Labs (MCL) microscopes that incorporate the Microscopy TIRF (Total Internal Reflection Fluorescence) technique and high-resolution nanomanipulation. These micro-

scopes' ability to provide strong signal-to-noise ratios over multiple viewpoints (providing real-time imaging is vital to the study of these viral processes. Additionally, the microscope comes equipped for sub-nanometer positioning, which is critical in single-molecule applications, where objects of interest often are separated by only a few nanometers.

"We built our instrumentation around Mad City Labs' platforms and use the blades™™ to move small objects by color so we can send each channel to different parts of our CCD camera," Ivanovic explains. "For example, in one channel, we can get information on the particle size, in another channel, we can watch the process of membrane fusion."

Using this instrumentation, Ivanovic Lab researchers dissect the mechanisms of membrane fusion for enveloped viruses, as well as membrane penetration for non-enveloped viruses. They can watch individual virus particles undergo different steps in the process of cell entry, observing (in real-time) that are otherwise "frozen out" in traditional experiments.

"This platform allows us to learn in real-time imaging. We can not only dissect and see interactivity of a process, we can – in the same time, for example – quantify certain structural features for our virus particles or quantify how they're interacting with antibodies or receptors," Ivanovic explains. "We can look at a population of virus particles and identify if it is piezoelectric – meaning particles may vary in shape and size. If we have a fluorescently-labeled virus particle in a very tight focus with particle size, you can get information about particle size at a very precise level, watching how size affects these processes."

Further, the Ivanovic Lab's instrumentation allows researchers to record movies of these processes over time, allowing for more granular study. The researchers can identify interactions and gaps that based on nature the system measures (such as membrane fusion, size, glycoprotein density, or how many inhibitors/antibodies they have bound to them).

In addition to observing viral cell entry mechanisms, Ivanovic Lab researchers use their TIRF system to study virus receptors.

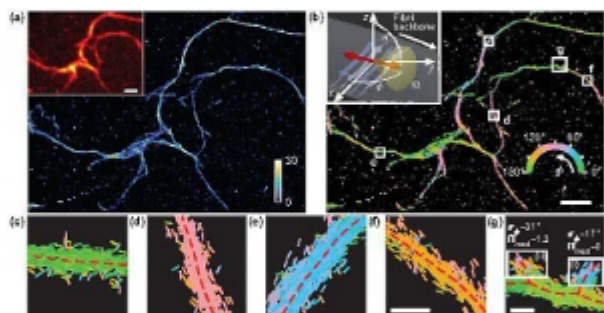
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Methodology Aids Microscopic Study of Fibrils in Molecules

Researchers from Washington University in St. Louis have found a mechanism to efficiently and accurately measure the point spread functions (PSFs) of the position of molecules, called variance upper bound (VUB). They believe this will someday aid scientists and clinicians as they look to understand what is happening at the molecular level in the body.

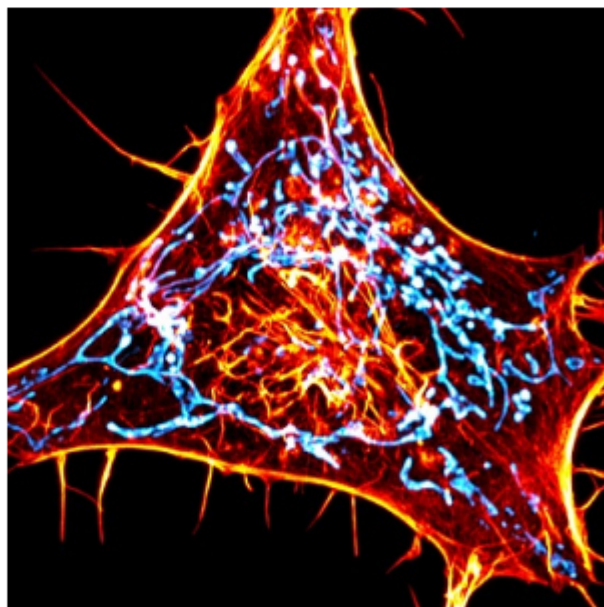
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Imaging Method Enables Research on Cellular Protein

Researchers at the Salk Institute have developed a new imaging method that allows them to monitor actin, a small subset of skeleton-like filaments within cells. The method has enabled research on how actin mediates an important function: helping mitochondria divide in two.

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Raman Spectroscopy Pinpoints Viral RNA in Single Cells

A new technique for identifying and quantifying viral RNA in living cells, based on surface-enhanced Raman spectroscopy (SERS) and developed by scientists at Rutgers University, can detect minor changes in RNA sequences that could give viruses an edge or make some people "superspreaders."

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