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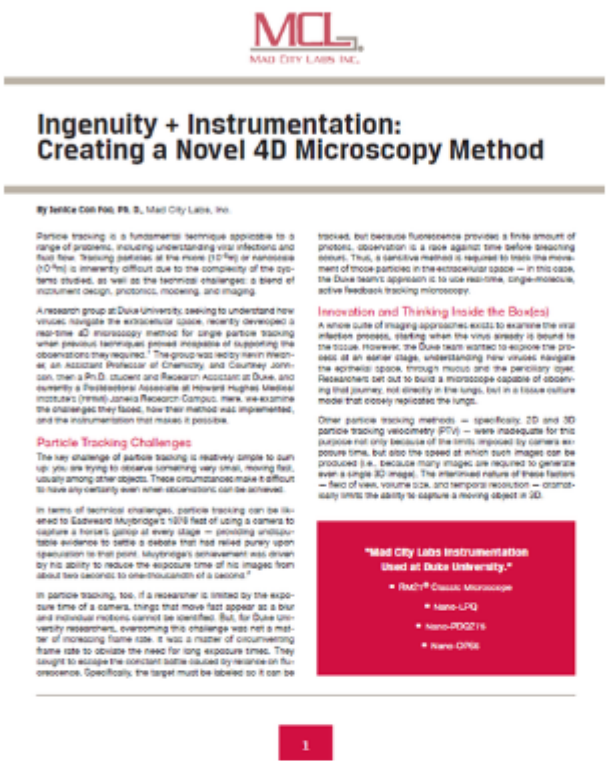

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Ingenuity + Instrumentation: Creating a Novel 4D Microscopy Method

By Janice Coen Foo, Ph. D., Mad City Labs, Inc.

Particle tracking is a fundamental technique applicable to a range of problems, including understanding viral infections and fluid flow. Tracking particles at the micro (10⁻⁶m) or nanoscale (10⁻⁹m) is inherently difficult due to the complexity of the systems studied, as well as the technical challenges: a blend of instrument design, photonics, modeling, and imaging.

A research group at Duke University, seeking to understand how viruses navigate the extracellular space, recently developed a real-time 4D microscopy method for single particle tracking when previous techniques proved incapable of supporting the observations they required.[1] The group was led by Hanin Ihnaini, an Assistant Professor of Chemistry, and Courtney Johnson, then a Ph.D. student and Research Assistant at Duke, and currently a Postdoctoral Associate at Howard Hughes Medical Institute's (HHMI) Janelia Research Campus. Here, we examine the challenges they faced, how their method was implemented, and the instrumentation that makes it possible.

Particle Tracking Challenges

The key challenge of particle tracking is relatively simple to sum up: you are trying to observe something very small, moving fast, usually among other objects. These circumstances make it difficult to have any certainty even when observations can be achieved.

In terms of technical challenges, particle tracking can be likened to Eadweard Muybridge's 1878 feat of using a camera to capture a horse's gallop at every stage — providing undisputable evidence to settle a debate that had relied purely upon speculation to that point. Muybridge's achievement was driven by his ability to reduce the exposure time of his images from about two seconds to one-thousandth of a second.[2]

In particle tracking, too, if a researcher is limited by the exposure time of a camera, things that move fast appear as a blur and individual motions cannot be identified. But, for Duke University researchers, overcoming this challenge was not a matter of increasing frame rate. It was a matter of circumventing frame rate to obviate the need for long exposure times. They sought to escape the constant battle caused by reliance on fluorescence. Specifically, the target must be labeled so it can be tracked, but because fluorescence provides a finite amount of photons, observation is a race against time before bleaching occurs. Thus, a sensitive method is required to track the movement of those particles in the extracellular space — in this case, the Duke team's approach is to use real-time, single-molecule, active feedback tracking microscopy.

Innovation and Thinking Inside the Box(es)

A whole suite of imaging approaches exists to examine the viral infection process, starting when the virus already is bound to the tissue. However, the Duke team wanted to explore the process at an earlier stage, understanding how viruses navigate the epithelial space, through mucus and the periciliary layer. Researchers set out to build a microscope capable of observing that journey, not directly in the lungs, but in a tissue culture model that closely replicates the lungs.

Other particle tracking methods — specifically, 2D and 3D particle tracking velocimetry (PTV) — were inadequate for this purpose not only because of the limits imposed by camera exposure time, but also the speed at which such images can be produced (i.e., because many images are required to generate even a single 3D image). The interlinked nature of these factors — field of view, volume size, and temporal resolution — dramatically limits the ability to capture a moving object in 3D.

"Mad City Labs Instrumentation Used at Duke University."
- RM21® Classic Microscope
- Nano-LPQ
- Nano-PDQ316
- Nano-DT68

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Ingenuity + Instrumentation: Creating a Novel 4D Microscopy Method

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