Visualizing viruses: new research pinpoints tiny invaders

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In the war against infectious disease, identifying the culprit is half the battle. Now, research professor Shaopeng Wang and his colleagues from the Biodesign Institute at Arizona State University, describe a new method for visualizing individual virus particles. Their research opens the door to a more detailed understanding of these minute pathogens, and may further the study of a broad range of micro- and nanoscale phenomena.

The group's findings appear in the August 23rd *Proceedings of the National Academy of Science,* advanced online issue.

Detection and identification of infectious invaders are critical for efforts to diagnose, prevent, and control these skillful pathogens. In the current study, individual H1N1 influenza virus particles, along with the somewhat larger HCMV virus were visually detected through a label-free method for the first time, using a high-resolution technology known as surface plasmon resonance microscopy.

In addition to identifying single virus particles, the technique permits the study of surface binding of viruses to specific antibodies. Critically, it also enables measurement of particle mass, with a detection limit rivaling conventional methods by three to four orders of magnitude. The work was conducted under the supervision of Nongjian (NJ) Tao, director of the Biodesign Institute's Center for Bioelectronics and Biosensors.

Various methods have been applied for the detection of virus particles, Tao notes, citing a number of exotic techniques used to hunt single viruses or more often, to statistically evaluate groups of particles. Often, fluorescent dyes are affixed to molecules for the purpose of visualization, though such techniques come at a price. "The label may cause a change in the function of the molecule," Tao says, further stressing that labeled methods do not permit the direct observation of intrinsic physical characteristics (e.g., mass) of the viruses, displaying instead, only the synthetically labeled sites.

In the current study, surface plasmon resonance microscopy is used to examine affinity interactions of viruses and their associated antibodies, producing the first label-free images of individual viruses. As Wang observes, "optical imaging of this kind can detect a virus in its native state, in aqueous solution." Previously, resolution of such minute particles had to rely on electron microscopy, where samples must be fixed and detection carried out under a vacuum.

Surface plasmon resonance occurs when polarized light strikes a biochip coated with a thin metallic layer. Given the right conditions of wavelength, polarization and incident angle, free electrons (or plasma) at the surface of the chip absorb incident photons, converting them into surface plasmon waves, which propagate across the surface in a manner similar to waves in water.

When molecules such as virus particles interact on the surface of the chip, they can disrupt these subtle plasmon waves, causing a measurable change in light reflectivity. Normally, these wave disruptions are averaged over the entire surface, though this conventional approach registers noise as well as the detected particles, which only occupy a small area of the total chip surface.

In the current study, the group demonstrated for the first time that it is

possible to image and detect individual H1N1 viral particles with label-free surface plasmonics technique in real time. This technique allowed an averaging of the signal only in the area where the virus particles are present, dramatically improving the accuracy of measurement.

In order to be certain that the observed visual signals were indeed those of the H1N1 virus particles binding to their associated antibodies, the team conducted three separate experiments. In the first case, a permanent binding of viral particles to the unadorned gold-coated chip was observed. Next, the experiment was repeated after applying polyethylene glycol (PEG) to the gold surface, which acts to block non-specific absorption. In this case, none of the virus particles bound to the surface, but instead, wandered freely, obeying the random behavior known as Brownian motion.

Finally, virus particles were observed on a chip functionalized with H1N1 antibodies with PEG applied. The virus particle displayed reversible binding with their antibody counterparts, dissociating in the manner characteristic of virus-antibody pairs. "In this way, we could be certain the detection is actually the binding of the H1N1 particles to the antibodies," Tao says. "That's the trick we use to prove that we can specifically detect a target virus, not other molecules or substance in the solution, which will also produce a signal. " Additional confirmation came from using HCMV virus particles, which failed to bind with the H1N1-specific antibodies.

As lead author Wang notes, a further benefit of this exquisitely sensitive technique is that it permits the measurement of viral mass. The mass can be inferred from the intensity of the optical signal, which in turn is proportional to the degree to which the particle disturbs the surface plasmon wave. The group's technique allows a mass detection limit down to 1attogram—one quadrillionth of a gram. "We have tried to push label-free optical imaging well beyond conventional limits," Wang says,

adding that the method allows the observation and characterization of tiny biological entities in their natural state.

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