Andre M O Gomes, Andrea C De Oliveira, Valeria Levi, Rafael B Goncalves, Umesh Katpally, Thomas J Smith, and Enrico Gratton.

Real time 3D tracking of virus particles in live cells.

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Abstract

During the last year, we developed a new technique to track particles in three dimensions using a 2-photon microscope. The next step involves developing and testing the technique for tracking fluorescent particles in living cells. As a test system, we used relatively large particles, such as viruses. Detailed mechanisms of infection remain unclear for most animal viruses probably because of experimental difficulties. Tracking single particles in living cells eliminates the need for large amounts of viral material in order to have a good signal-to-noise ratio. Here we describe the possibility to track virus particles in living cells with nanometer accuracy and millisecond time resolution. The model utilized was the Human Rhinovirus (HRV14). Rhinoviruses are small RNA viruses, members of Picornaviridae family. Virus capsids were labeled with FITC and incubated with HeLa-H1 cells at 4oC to allow attachment to the membrane. Then the temperature was raised to 37oC and the particles were tracked during cell entry. Our data show that particles move in centripetal trajectories with steps of fast movement towards the center of the cell followed by periods of constrained lateral motion. The fluorescence intensity is recorded during the experiment and the intensity traces shown an oscillation pattern suggesting fast conformational changes of the virus particles. In order to relate the trajectories with the infected cell structure we labeled the membrane with Laurdan and z-stacks of the cell were obtained. The proposed methods of analysis of molecular trajectories can open a new field for the study of complex systems and ... [truncated at 250 words]