

## Investigation of Dengue virus infection in live cells using Single Particle Tracking

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Dengue (DENV) is a positive sense RNA virus of the Flaviviridae family with four known serotypes (DENV1,2,3,4) which cause dengue fever. The cellular infectious pathway of DENV and how the different strains behave in response to cellular and environmental perturbations can help to better understand the mechanisms involved in infection so as to aid in developing techniques to reduce or disable infectivity of virions. The cellular infectious pathway has not been fully studied in-vivo, and Single Particle Tracking (SPT) is a powerful tool which enables us to follow individual virus particles inside live cells in real-time to probe the effects of such perturbations on virus behavior and infectivity.

The most straight forward SPT application uses 2D imaging, and can be conducted in widefield or using TIRF (tracking in 2D close to the sample cover slip, cell membrane and bilayers), HILO illumination (2D tracking inside live system in a highly inclined thin light sheet) schemes or by using confocal microscopy. By using HILO illumination scheme on a TIRF microscope, we were able to track fluorescently labeled DENV2(NGC) heat treated at 37 °C (physiological body temp) and 40 °C (high fever temp) prior to infection carried out at 37 °C. When compared to tracks of endosome markers Rab5 and Rab7 in the same environment, it was evident that DENV2 showed similar velocities to that of Rab markers, while co-localization of DENV2 with Rab markers was observed by confocal time-lapse imaging.<sup>1</sup>

In this work, SPT has been extended to 3 dimensions by placing a weak cylindrical lens ( $f = 10000$  mm, SCX-50.8-5000.0-C, CVI Melles Griot) in the detection path of the SPIM microscope to introduce axial astigmatism in the image for 3D localization. The PSF is circular at focus, and shows ellipsoidal shape above and below focus, with its major axis shifting by 90° depending on the direction of movement (Sturm's conoid)<sup>2,3</sup> A feedback loop is employed to keep the particle of interest at focus at each frame, where the PSF shape is analyzed on-the-fly to determine the required sample Piezo movement by use of a calibration curve and a user friendly home written Java plug-in (Micromanager 1.4).

This SPT work enabled us to follow single virus inside live cells, and has made it possible to analyze the movements and responses of individual virus particles to different cellular and environmental perturbations, making it a promising technique for the future study of virus infectious pathways.

### References:

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