



Multi channel localization and tracking of T cells in 4D

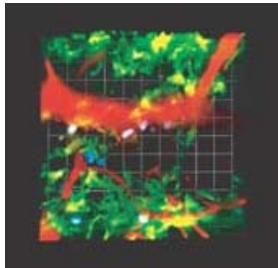


Fig 1: Volocity 3D rendering of two-photon confocal images. Red: HEV; Green: non specific T cells ; Blue: specific T cells
Grid: 30 x 30 μ m

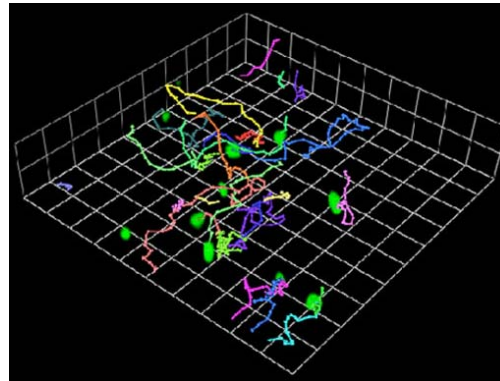


Fig 2: Volocity tracking of T cells in 4D in the lymph node ([QuickTime movie](#))

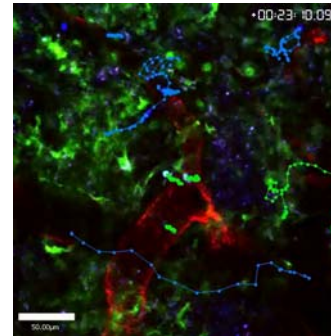
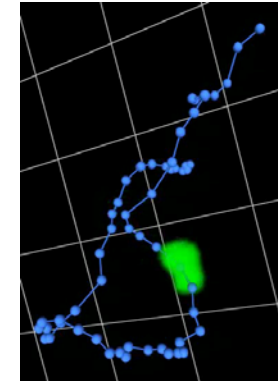


Fig 3: [QuickTime movie](#) showing arrest of antigen-specific T cells near HEVs



[QuickTime movie:](#)
Volocity single T cell track in 4D

Dr Guy Shakhar from Dr Michael Dustin's Lab at the Department of Molecular Pathology at the Skirball Institute of Biomolecular Medicine in NYU Medical Centre, works on the Immune Response. The focus of the lab is to understand T cell activation through immunological synapse. By using *in vivo* imaging of T cell migration and activation in mice lymph nodes, Dr Shakhar and his colleagues study the interaction between dendritic cells (DCs) and T cells which plays a key role in *in vivo* immunity development and is therefore important in understanding immunity-linked pathologies.

In vivo, T cells develop into tolerant or priming cells depending on their interaction with antigen-presenting-DCs in lymph nodes. In order to examine tolerance and immunity in live mice, Dr Shakhar used **Volocity** for localization and tracking analysis.

To determine whether T cells are differentially distributed in lymph nodes in conditions of tolerance and immunity, the fate of antigen-specific T cells and non specific T cells, both expressing EGFP, was examined in lymph nodes after exposure to antigen, using **Volocity Visualization. Volocity Classification** was used to measure the distances travelled by T cells moving from high endothelial venules (HEVs) into lymph nodes. Results showed that T cells that recognize a specific antigen are restricted to the area around HEVs in the early phases of tolerogenic and immunogenic responses. Fig 1 shows a 3D reconstruction of two-photon confocal images showing specific and non-specific T cells near an HEV in a lymph node.

After localizing T cells in lymph nodes, the researchers aimed to compare T cell-DC interactions in tolerance and priming conditions *in vivo*. For both conditions, Dr Shakhar tracked cells in 3D over time and quantified T cell mobility in terms of velocity, immobility and directionality. Tissues of 50x400x400 μ m volume were scanned using a multiphoton microscope with a 3 μ m Z-step and 30 seconds intervals. Time lapse sequences were imported into **Volocity. Volocity Classification** allowed the researchers to automatically calculate cell speeds and the coordinates of each cell. In total 2100 cells were tracked from 62 imaging fields in 29 mice (example of tracks Fig 2). QuickTime movies were also generated with the Volocity Movie Sequencer (Fig 3).

Volocity enabled the researchers to show that specific T cells slowed down and demonstrated confined motion after encountering antigen, with many arresting in the vicinity of HEVs, whereas non-specific T cells maintained their speed and directionality. The early arrest near HEVs was identical in tolerance and priming conditions, but antigen specific T cells regained mobility more rapidly in conditions of tolerance than in priming conditions.