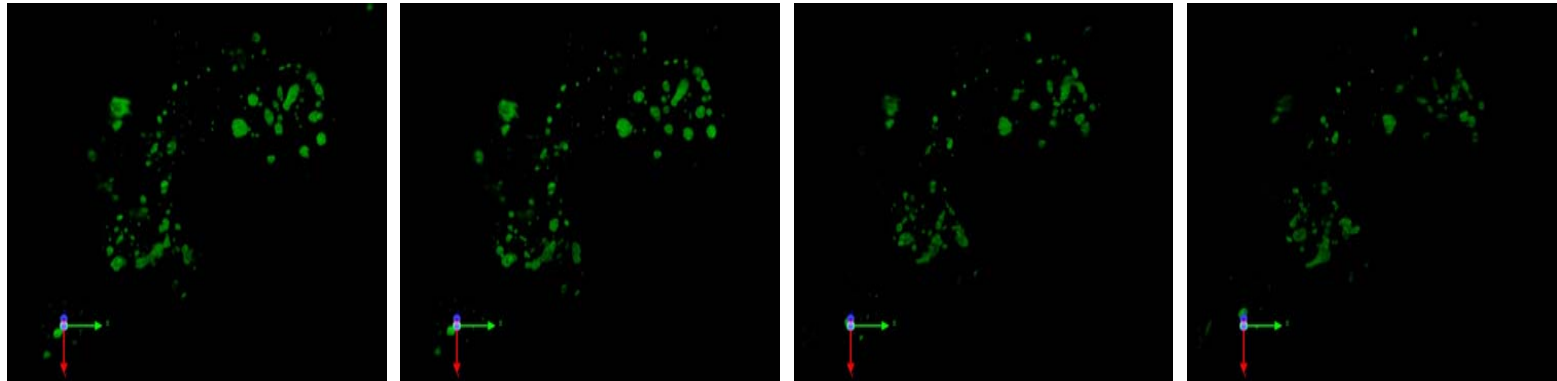




## Time-lapse microscopy analysis of Dvl-2 cellular distribution



Improvision Volocity 3DM time-lapse analysis of HA-Dvl-2-EGFP transient ectopic expression in HEK293 cells

Dr Matthew Smalley and colleagues from the Institute of Cancer Research in London, the University College London and the Cardiff School of Biosciences, are interested in the Wnt signalling pathway which controls many events during the embryogenesis. At the cellular level it regulates morphology, proliferation, motility and cell fate. Also, inappropriate activation of the Wnt pathway has been shown to play a key role in human tumorigenesis. The researchers are particularly interested in an intracellular mediator of the Wnt signalling pathway called Dishevelled (Dvl-2).

The mechanism of Dishevelled function in the Wnt pathway remains unclear and previous experiments seem to indicate that its cellular localization in cytoplasmic puncta may play a key role in its signal transduction function. In order to test this hypothesis, 3D time-lapse fluorescence microscopy analysis of Dvl-2 cellular distribution was performed using MDCK cells microinjected with EGFP-tagged Dvl-2 proteins. Data was collected using an inverted microscope controlled by the *Volocity Grid Confocal* acquisition system which generated confocal quality 3D images by using the OptiGrid structured light technology.

With its advanced high speed parallel processing and video streaming architecture, *Volocity Acquisition* allowed the capture of 17 Z-stacks of 45 images. Each stack was acquired in just 34 seconds. Once the data was acquired, Dr Smalley and his colleagues were interested in analyzing the movement of Dvl-2 cytoplasmic puncta in the absence and in the presence of Wnt signal. Using *Volocity Classification* to track over 104 Dvl-2 puncta, measurements such as velocity and meandering index were automatically generated. These results indicated that the puncta travelled at  $0.024 \mu\text{m/s}$  with a meandering index of around 0.5, suggesting that the movement is random rather than directional. In the presence of a Wnt signal, analysis of the data showed no difference in the movement of the Dvl-2 puncta. The images above are individual frames from a movie created with the *Movie Sequencer* in *Volocity Visualization*.

One of the aim of this experiment was to investigate the nature of the 'vesicular' distribution of Dvl-2 proteins. Time-lapse analysis indicates that the puncta structures travel relatively slowly and randomly which suggests that the Dvl-2 puncta are protein aggregates rather than vesicles.