

## Mechanisms controlling intraflagellar transport (IFT) train formation

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A 3D electron microscopy (EM) method called Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM) was used to reveal that intraflagellar transport (IFT) trains are localised on only 2 of the 9 doublet microtubules in the flagellum of *Trypanosoma brucei*. IFT trains are heterogeneous in length, raising the question of their identity.

Since cells were fixed, it was not possible to know in which direction trains were travelling (anterograde or retrograde). The diameter of the axoneme is 180 nm, so we turned to live imaging at high resolution using a strain expressing an mNeonGreen::IFT81 fusion. We have compared different imaging methods (SIM, TIRF, wide field) and the best approach providing sufficient temporal and spatial resolution turned out to be the use of a high numerical aperture objective (1.57) on a microscope equipped with a spinning disk. In these conditions, IFT is visible on only 2 tracks and takes place in both anterograde and retrograde direction on each of them.

Kymograph analysis was also performed on a kinesin mutant strain showing that IFT trains were less abundant and moved less fast. This mutant provides a new tool to improve the discrimination between anterograde and retrograde trains and shows a link between the number of trains and the abundance of the kinesin motor.

The aim of the project is now to develop a correlative light and electron microscopy method to combine structural and functional information to understand mechanisms controlling IFT train formation in *Trypanosoma brucei*.

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