

**Title:**

Track & trace: using dual-colour imaging to visualise transport in the beating flagella of *Leishmania mexicana*

**Abstract:**

Intraflagellar transport (IFT) is the bi-directional transport of proteins on IFT trains necessary for building flagella, including in *Leishmania mexicana*. *L. mexicana* promastigotes possess a 9+2 motile flagellum which is vital for normal life cycle progression. Since IFT discovery nearly three decades ago, IFT train movement has essentially exclusively been analysed in cells with flagella immobilised either mechanically or by mutations.

To visualise IFT train movement in actively beating flagella, we developed high framerate dual-colour fluorescence microscopy, using a triple mNeonGreen-tagged IFT protein (3×mNG::IFT172) and either red light phase contrast or mCherry-tagged flagellar proteins. IFT is visible in the green channel and the flagellum can be traced using the red channel, allowing simultaneous capture of flagellar bending and IFT train movement. Using this microscopy approach, we measured anterograde and retrograde IFT train speed in free moving flagella undergoing different beat types, in comparison to mechanically immobilised flagella.

IFT trains may experience steric hinderance from other axoneme-associated complexes and presumably compete for ATP with the motor proteins driving axoneme beating. Therefore, we also measured IFT train speeds in distal docking complex (dDC) 1, dDC2, dynein light chain 1, outer dynein arm beta, radial spoke protein 4/6 and paralysed flagella 16 deletion mutants with defective beats.

We showed that mechanical immobilisation of a motile flagellum to observe IFT puts it in a non-physiological state, resulting in slower trains with increased intermittent stalling than in free flagella. IFT train speed is independent of beat type- suggesting chemotaxis-based beat switching is not associated with flagellum length changes, although mechanical flagellum trapping may be. IFT train speed is also increased in mutants with defective flagellar motility. Together this data reveals the importance of visualising IFT in a free moving flagellum.