On and off again: Tools and models for single molecule fluorescence imaging of actin and tropomyosin

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The cytoskeleton of cells is controlled and regulated by actin filaments. These filaments govern a multitude of different functions. It is thought that much of this functional specialisation is *via* the binding of different tropomyosin isoforms which additionally regulate the binding of other actin-binding proteins to the filaments.

In vitro fluorescence imaging has developed as an important tool in the observation of the kinetics of the polymerisation of actin filaments and interactions with actin-binding proteins. Clever biochemical manipulations have allowed the imaging of filaments tethered by seeds attached at one end and suspended in flow along microfluidic channels. These experiments have allowed the observation of binding and dissociation events within a single filament. Much of this activity occurs well below the resolution of the imaging systems however. Nonetheless we show that it is possible to derive useful data to dissect the possible modes of binding and dissociation of the actin and tropomyosin isoforms.

Image analysis and modelling tools have been developed which facilitate the extraction of information from these data and which explore the possible interactions between the proteins. These models also facilitate the extraction of data for the individual filaments, straighten curved filaments and determine binding and dissociation rates.