Tools of the trade

Permeable dye-based fluorescent speckle microscopy for human cells

During cell division, the dynamic properties of microtubules are critical for the cell to assemble a functional mitotic spindle. Dynamics of spindle microtubules involve poleward flux, whereby tubulin subunits within microtubules are continuously translocated in the direction of their associated spindle poles. This process generates forces that drive chromosome movements and potentially account for the chromosome segregation fidelity. Microtubule poleward flux can be studied using photoactivation of fluorescently tagged tubulin. However, a limitation of this approach is that all of the microtubules within the irradiated area are photoactivated and thus the dynamic behaviour of individual microtubules cannot be discerned.

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Experiments on individual microtubules and microtubule population-specific poleward flux are mainly performed by using fluorescent speckle microscopy (FSM). In this method, a small concentration of fluorescently labelled tubulin is microinjected inside cells to create a non-uniform fluorescent signal on the microtubules. Owing to the stochastic association of labelled tubulin with the microtubule lattice, few clustered molecules of fluorescently labelled tubulin within resolution-limited areas can produce a speckled pattern, with each speckle labelling a single microtubule. The speckles can then be tracked in time to reveal dynamics of individual microtubules, even in a structure as complex as the spindle. FSM has been used in several model organisms, yet, studies of human cells have been challenging owing to difficulties of labelled tubulin delivery. We developed high-throughput FSM based on a cell-permeable dye, silicon rhodamine (SiR)-tubulin probe, which allows labelling of microtubules in human cells within minutes, with high specificity and low background fluorescence. The speckled signal was achieved by a sub-nanomolar concentration of the dye, which stochastically stains the microtubule lattice, coupled with high laser power and long exposure time as requirements for visualization. Owing to the short duration of the imaging, which is sufficient for studying highly dynamic processes, the required imaging settings showed no phototoxic effect. As the dye immediately permeates all cells within a sample, dozens of cells can be imaged simultaneously, allowing for a high-throughput multiple-cell approach. Moreover, the specific advantage of the permeable dye-based FSM lies in efficiency and non-invasiveness, as the microinjection can induce cell damage during penetration.

With this tool, we were able to analyse separately different populations of microtubules (kinetochore versus bridging microtubules), to better understand their contribution to the microtubule flux-driven chromosome alignment. Over the past decade, numerous cell-permeable dyes for microtubules have been commercialized, making the dye-based FSM method broadly available and easy to use. Overall, the applications of this simple FSM approach for microtubule cytoskeleton research are vast, opening the opportunities to mechanistically probe movement, assembly, and turnover of these polymers in human cells.

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Competing interests
The author declares no competing interests.

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