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Studying spatial gradients of signaling proteins in mitotic spindles with time-integrated multipoint moment analysis DOOGIE OH, DANIEL NEEDLEMAN, Harvard University — The organization of the mitotic spindle is orchestrated by the activities of multiple signaling proteins, such as the GTPase Ran. It has been proposed that the Ran pathway produces a cascade of events which gives rise to spatial gradients in the behavior of soluble proteins, which in turn produce spatial gradients in microtubule behaviors important for spindle assembly. Previous experiments have directly demonstrated the existence of gradients around the spindle in the upstream components of the Ran pathway, but it is still unclear if there are significant gradients in the downstream soluble components in this pathway. We recently developed a method, TIMMA, time-integrated multipoint moment analysis, a multipoint form of fluorescence fluctuation spectroscopy capable of quantitatively measuring the concentration, diffusion coefficient, and molecular brightness of soluble proteins throughout live cells. We are using TIMMA to characterize the behaviors of the upstream and downstream components of the Ran pathway in live mitotic cell to test the validity of the Ran gradient model.

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