Quantification of dynamic behavior of microtubules in living cells using novel computational methods

Alphan Altinok, Erkan Kiris, Emre Sargin, Leslie Wilson, Stuart C. Feinstein, B.S. Manjunath, Kenneth Rose.

Neuroscience Research Institute, Center for BioImage Informatics Dept. of Electrical and Computer Engineering, and Dept. of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106

Studies of MT dynamic instability in living cells require MT tip tracking, data analysis and calculation of dynamics statistics. Generally, these tasks are performed manually. Investigators manually follow the MT plus-end, *-the tip-*, position throughout entire videos in a frame by frame manner. The length of a MT is approximated by the Euclidean distance between the MT tip and an arbitrarily selected, fixed position along the MT body. Although growing and shortening lengths and rates are of primary interest, additional parameters could also be of biological significance, such as MT curvature and MT directionality. As an alternative to manual tip tracking, we have developed computational methods that track entire MT bodies, thereby generating not only more accurate growth and shortening data but also generating curvature and directionality data. Additionally, the entire process is much less time-consuming and laborious relative to manual methods. We quantify the change of curvature (Δ Curvature) from MT body over time as follows. Initially, a set of curvature values are calculated along the MT body on the first frame. Then, the set of curvature values are calculated repeatedly throughout the frames of the image sequence to measure the changes of MT body shape over time. Smaller curvature values indicate a linear shape and larger values indicate a circular shape of the body. Finally, curvature change over time is calculated from the curvature series. The direction of MT growth is quantified by the deflection angle of the MT tip from the main axis. On each frame of the image sequence, the MT axis is determined by approximating it with a line segment starting from the tip. Between two consecutive frames, the angle formed between the line segments is measured. This change in direction is computed as the deflection of the MT tip from the axis over time. Higher values indicate more deflection in positive or negative direction. Furthermore, we can now analyze the dynamic behavior of multiple MTs in selected regions of an image sequence. The dynamics statistics can be calculated as correlations between MTs as opposed to examining individual MTs. For example, growth and shortening patterns of neighboring MTs can be correlated with the growth and shortening patterns of selected MTs. These novel statistics are computable by tracking the full MT bodies over time. The tracking and analysis tool is available from the Center for Bioimage Informatics (Univ. of California, Santa Barbara).

Grants:
NIH Grant R01CA57291
NIH Grant R01NS13570
NIH Grant R01NS35010
NSF ITR 0331697