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Immobilization in single-particle tracking: Escape from the point spread function of the microscope

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A key idea in single-particle tracking (SPT) analysis is that the pure unhindered random walk is the null hypothesis. To claim that a diffusing tracer particle is doing something, one must evaluate, informally or formally, the probability that similar behavior would occur by chance in a pure random walk of the same duration. One biophysically important behavior of a tracer is immobilization. Immobilization is usually defined in terms of noise, specifically the observed scatter of positions of a tracer physically immobilized on the slide. But the minimal operational definition of immobilization is escape from the effective point spread function (PSF), not the raw optical PSF but the optical PSF as narrowed by the SPT localization algorithm. A prominent feature of the PSF is the asymmetry between radial and axial resolution. Standard SPT algorithms narrow the effective PSF in the radial direction but not in the axial direction; increasing the axial resolution requires modifying the optics. The criterion for immobilization thus becomes, does the tracer remain in the effective PSF for a longer time than expected by chance?

We use Monte Carlo calculations to examine the minimal criterion and the effect of the radial-axial asymmetry on the criterion. The PSF is modeled as a wall corresponding to a prescribed radial-axial intensity contour. The dwell time is a well-defined first passage problem for a random walk. A tracer starts inside the wall, and carries out a random walk until it first reaches the wall. Wall geometries examined include circles, ellipses, generalized ellipses, and more complicated contours from the numerical solution for the PSF. Dwell times are obtained from analytical and Monte Carlo calculations. For tracers initially uniform, the probability density function is monotonically decreasing, power-law for short times and exponential for large times. For tracers initially at the origin, the distribution is unimodal. It resembles a log-normal distribution but is not. The shapes of the distributions are not strongly dependent on wall shape.