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4D ALIGNMENT ANALYSIS PROTOCOL V.2

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We use this protocol and it's working

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Abstract

This protocol describes a 4D analysis workflow for reconstructing and quantifying the alignment of chromosomes and NDC80-GEM foci in the spindle over time using Imaris software.

Guidelines

1. Open time-lapse image datasets in Imaris software (Bitplane).
2. Reconstruct and segment chromosomes and NDC80-GEM foci in 3D using the automatic thresholding function.
3. Manually validate all segmented surfaces according to fluorescent signals and record it.
4. Manually estimate the spindle axis at each timepoint based on chromosome distribution and record it using the camera manager function.
5. Define the spindle center as the center of mass of the chromosomes.
6. For timepoints before the spindle axis is established (e.g., 2 hours after NEBD), apply the spindle axis determined at 3 hours after NEBD.
7. In the alignment analysis, manually exclude NDC80-GEM clusters located outside the spindle.



Protocol

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- 7 In the alignment analysis, manually exclude NDC80-GEM clusters located outside the spindle.