Aug 13, 2025 Version 1

4D ALIGNMENT ANALYSIS PROTOCOL V.1

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvodrmzg4o/v1

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Designing protein-base...

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Protocol Citation: Yuanzhuo Zhou, Kohei Asai, Hirohisa Kyogoku, Tomoya S. Kitajima 2025. 4D ALIGNMENT ANALYSIS PROTOCOL. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvodrmzg4o/v1

Protocol status: Working
We use this protocol and it's working

Created: August 12, 2025

Last Modified: August 13, 2025

Protocol Integer ID: 224588

Keywords: alignment of chromosome, 4d analysis, alignment, chromosome, gem foci, imaris software, 4d, using imaris software, analysis protocol, spindle over time, spindle, analysis protocol this protocol

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

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.

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Acknowledgements

KITAJIMA Lab