How to measure separations and angles between intramolecular uorescent markers

Mortensen, Kim; Sung, Jongmin; Spudich, James A.; Flyvbjerg, Henrik

Publication date:
2018

Document Version
Early version, also known as pre-print

From: meetings@cshl.edu
Subject: CSHL Meeting Abstract Submission
Date: 8 June 2018 at 23.23
To: kim.mortensen@nanotech.dtu.dk

Dear Kim Mortensen,

Thank you for submitting your abstract for the 2018 Cold Spring Harbor meeting: Single Biomolecules .

You have indicated the following presentation preference: Talk or Poster. Approximately four (4) weeks ahead of the meeting, you will be notified via email of the status of your abstract. A full list of talks And posters will be available on our website at that time. We will contact you as soon as the organizers have made their decisions. This usually takes about a month. A full list of talks and posters will also be available at our website:

Abstract Status.

For your reference, your abstract appears below. We look forward To your participation!.

Sincerely,
CSHL Meetings & Courses Program

How to measure separations and angles between intramolecular fluorescent markers

Kim I Mortensen[1,2], Jongmin Sung[2,3], James A Spudich[2], Henrik Flyvbjerg[1]
[1]Technical University of Denmark, Department of Micro- and Nanotechnology, Kgs. Lyngby, Denmark, [2]Stanford University School of Medicine, Department of Biochemistry, Stanford, CA, [3]Stanford University, Department of Applied Physics, Stanford, CA

We demonstrate a novel, yet simple tool for the study of structure and function of biomolecules by extending two-colour co-localization microscopy to fluorescent molecules with fixed orientations and in intra-molecular proximity. From each colour-separated microscope image in a time-lapse movie and using only simple means, we simultaneously determine both the relative (x,y)-separation of the fluorophores and their individual orientations in space with accuracy and precision. The positions and orientations of two domains of the same molecule are thus time-resolved. Using short double-stranded DNA molecules internally labelled with two fixed fluorophores, we demonstrate the accuracy and precision of our method using the known structure of double-stranded DNA as a benchmark, resolve 10-base-pair differences in fluorophore separations, and determine the unique 3D orientation of each DNA molecule, thereby establishing short, double-labelled DNA molecules as probes of 3D orientation of anything to which one can attach them firmly.