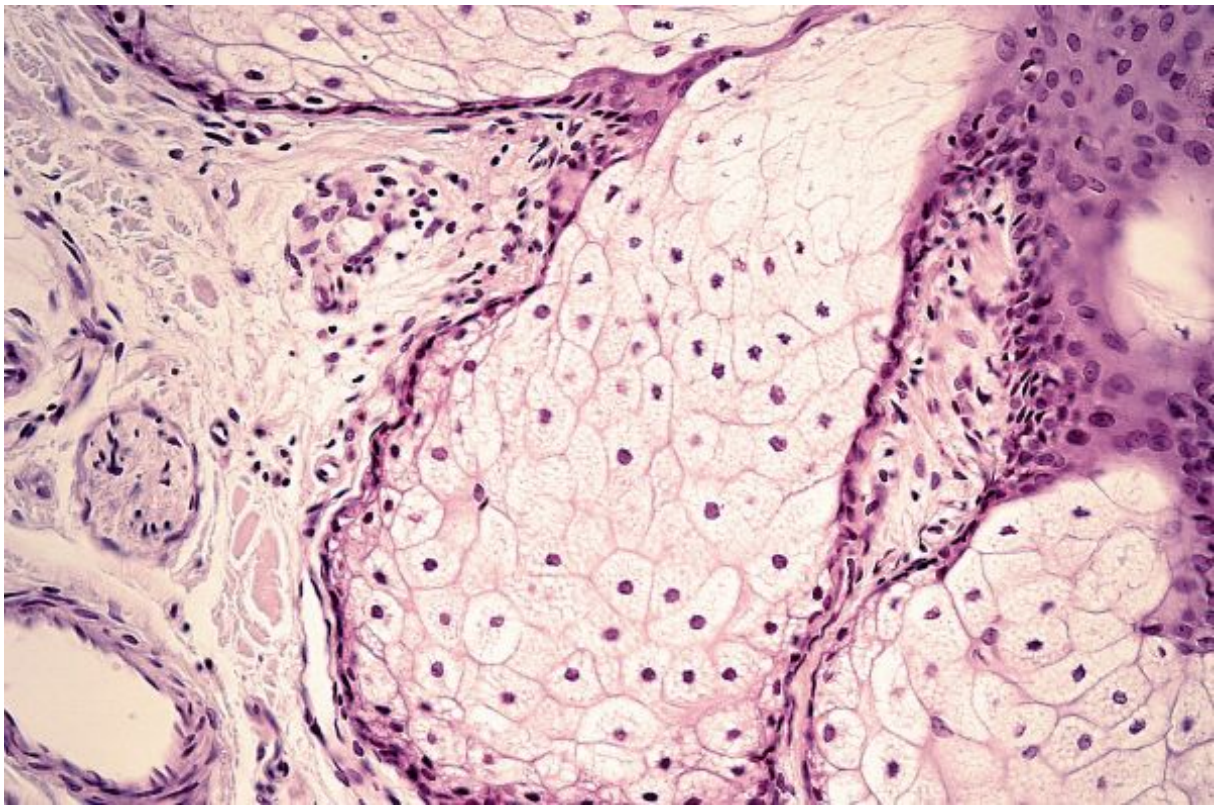


Global contest puts tools for single-molecule motion analysis to the test

Inside living cells, molecules are constantly on the move - binding, diffusing, interacting. Comprehending these processes is essential to deeply understand the cell's behavior. An international competition where ICFO has actively participated has now delivered a systematic comparison of analytical methods for single-molecule motion analysis, highlighting both the current strengths and the pressing challenges in this rapidly advancing field. The results have been recently published in Nature Communications.

July 28, 2025

In the intricate world inside living cells, molecular motion reveals crucial clues about how cells function, communicate, and sometimes fail. But extracting meaningful insights from these complex molecular trajectories is a formidable challenge, one that has spurred a global

race to develop better analytical tools.
Now, an international team of scientists led by Dr. Gorka Munoz-Gil from the University of Innsbruck, Prof. Dr. Giovanni Volpe at the University of Gothenburg, and Prof. Dr. Carlo Manzo at the University of Vic, with the collaboration of ICFO researchers, **Gabriel Fernandez-Fernandez**, **Dr. Borja Requena**, and **ICREA Prof. Maciej Lewenstein**, has organized a competition to evaluate these tools systematically. Their findings, just published in Nature Communications, offer an unprecedented assessment of the strengths and weaknesses of existing approaches.

Putting methods to the test

Single-molecule imaging has become an essential technique in modern cell biology and biophysics. By tracking individual molecules in live cells, researchers can study fundamental processes like protein interactions, transport mechanisms, and molecular crowding. However, analyzing the resulting data-whether in the form of particle trajectories or raw video-requires sophisticated computational methods. Most of these approaches are based on machine learning algorithms that are continuously refined to better detect patterns, classify motion types, and extract meaningful parameters from noisy experimental data.
To address the lack of objective benchmarks, the team designed a competition, the 2nd AnDi challenge, using a software library that simulates realistic experimental data. These simulations incorporated widely used diffusion and interaction models under conditions mimicking those in actual experiments. Research groups from around the world applied their best tools to analyze the same dataset. Based on their results, the competing teams-and thus the methods they devised-were ranked.

Current strengths and challenges

i¿½The competition revealed clear progress in some areas, but also highlighted significa t limitations in others,i¿½ summarizes Dr. Gorka Munoz-Gil, researcher at the Universit of Innsbruck and leading author of the article. i¿½It's important to have a quantitative pict re of how well-or poorly-current methods perform across a range of realistic scenari s.i¿½ Beyond ranking existing tools, the competition aims to drive innovation. By identifyi g where methods fall short, the researchers hope to encourage the development of new ap roaches that can more accurately decipher the noisy, heterogeneous world of molecular

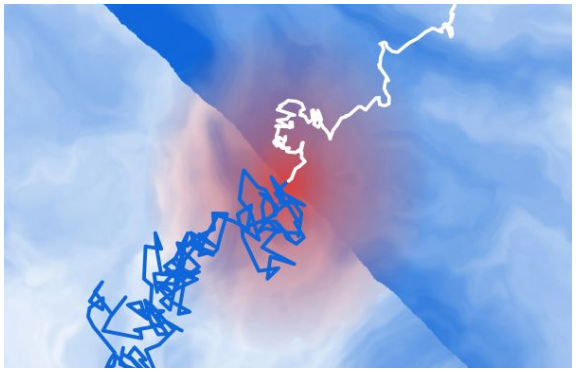
Guidance for the community

The results offer practical guidance for experimentalists seeking the right tools for their studies. i¿½We want to help researchers navigate the growing landscape of analytic l methods and choose the ones best suited for their datai¿½, says Prof. Dr. Carlo Manzo, se ior author of the article from the University of
ic. This is not the first time the community has taken up the challenge. The first edition of

the AnDi Challenge, focusing on anomalous diffusion, provided critical insights that shaped the field's development. The second edition expands this effort, focusing now on mo
ion changes and biologically relevant experimental conditi
ns. With the rapid advances in imaging technologies and data acquisition, the need for reli
ble analysis methods has never been greater. Initiatives like this competition provide an essen
ial roadmap for researchers, software developers, and experimentalists alike - helping to en
ure that the molecular stories captured under the microscope are accurately and fully captu
ed. The freely available software lays the groundwork for future projects and invites
the community to help drive further innovat

Reference:

Munoz-Gil, G., Bachimanchi, H., Pineda, J. et al. Quantitative evaluation of methods to analyze motion changes in single-particle experiments. Nat Commun 16, 6749 (2025).
DOI: https://doi.org/10.1038/s41467-025-61949-x



Trajectory of a biological particle moving across two distinct regions of space, each with different physical properties that alter how the particle diffuses. Credit: Gorka Munoz-Gil, University of Innsbruck.