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

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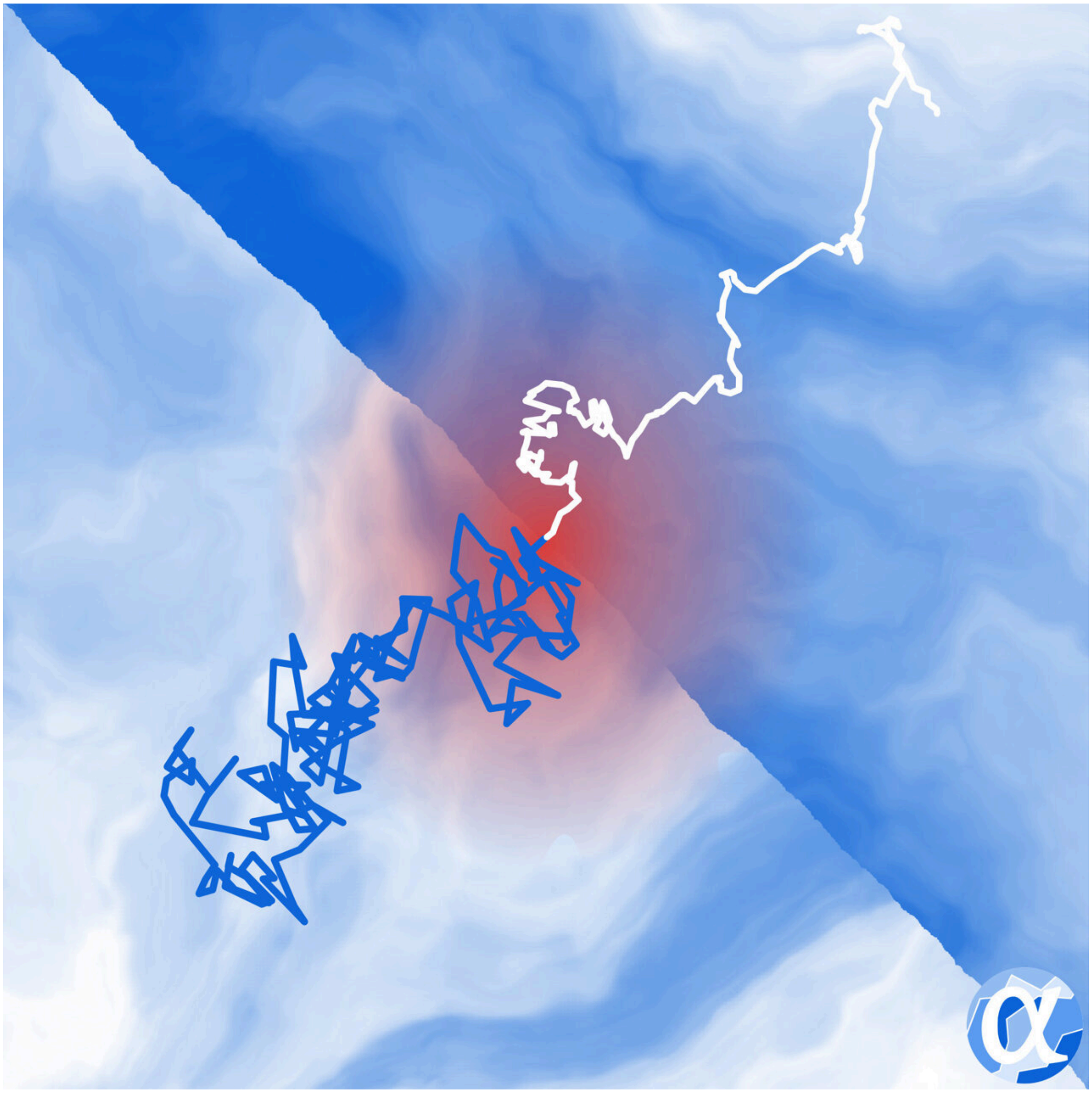

The trajectory of a biological particle moving across two distinct regions of space, each with different physical properties that alter how the particle diffuses. As it crosses the boundary, its behavior changes from subdiffusion (blue region), where movement is constrained, to superdiffusion (white region), where motion becomes more directed and rapid. Credit: University of Innsbruck

Inside living cells, molecules are constantly on the move—binding, diffusing, interacting. An international competition, the 2nd AnDi Challenge, 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.

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 Gorka Muñoz-Gil from the Department of Theoretical Physics at the University of Innsbruck (Austria), Giovanni Volpe at the University of Gothenburg (Sweden), and Carlo Manzo at the University of Vic (Spain) has organized a competition to evaluate these tools systematically.

Their findings, 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

"The competition revealed clear progress in some areas, but also highlighted significant limitations in others," summarizes Gorka Muñoz-Gil. "It's important to have a quantitative picture of how well—or poorly—current methods perform across a range of realistic scenarios."

Beyond ranking existing tools, the competition aims to drive innovation. By identifying where methods fall short, the researchers hope to encourage the development of new approaches that can more accurately decipher the noisy, heterogeneous world of molecular motion.

Guidance for the community

The results offer practical guidance for experimentalists seeking the right tools for their studies. "We want to help researchers navigate the growing landscape of analytical methods and choose the ones best suited for their data," says Carlo Manzo from the University of Vic.

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 motion changes and biologically relevant experimental conditions.

With the rapid advances in imaging technologies and data acquisition, the need for reliable analysis methods has never been greater. Initiatives like this competition provide an essential roadmap for researchers, software developers, and experimentalists alike—helping to ensure that the molecular stories captured under the microscope are accurately and fully captured.

The freely available software lays the groundwork for future projects and invites the community to help drive further innovation.

More information: Gorka Muñoz-Gil et al, Quantitative evaluation of methods to analyze motion changes in single-particle experiments, *Nature Communications* (2025). DOI: 10.1038/s41467-025-61949-x

Provided by University of Innsbruck

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