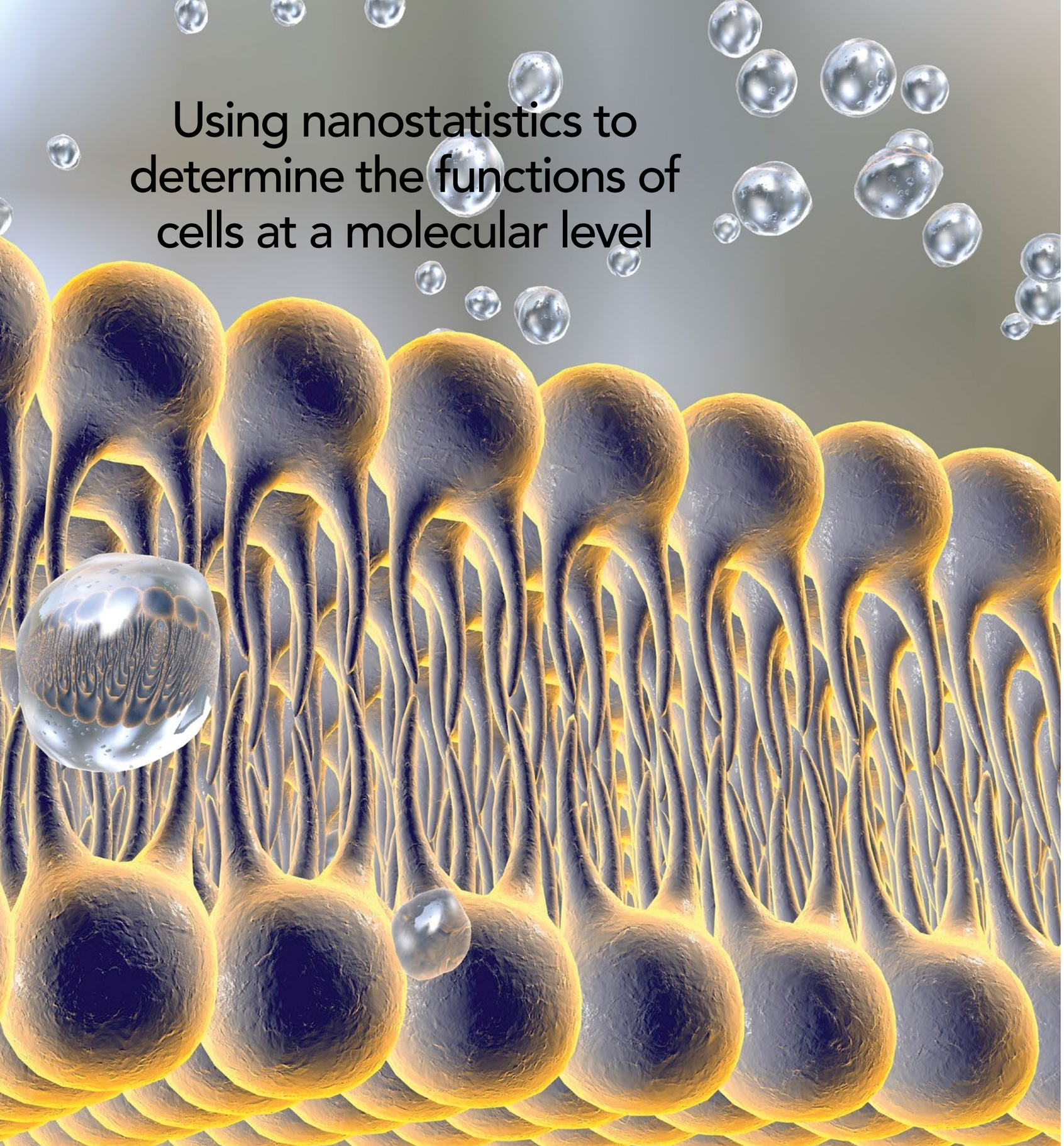
Using nanostatistics to determine the functions of cells at a molecular level



Dr Axel Munk from the University of Göttingen and the Max Planck Institute for Biophysical Chemistry focuses on nanostatistics and the development of methods that allow researchers to analyse data and recover objects from a series of indirect and random measurements. His work is at the cutting edge of statistical inverse problems with potent applications in biophysics, such as the signal extraction of electrophysiological data for understanding protein–membrane interactions, cell and molecular biology, and optical nanoscale microscopy. Moreover, Dr Munk's work has proved to be of significant assistance to forensics and security by modelling the growth of fingerprints, improving matching for adolescents.

What exactly is a statistical inverse problem? To understand the importance of Dr Munk's work, we must first answer this question. In principle, the nature of statistical inverse problems revolves around the notion of inverse recovery: *I give you the answer, can you tell me the question*? Therefore, statistical inverse problems fundamentally involve backward reconstruction such as recovering structures from parts of the body in tomographic scans or protein structures in a compartment of a cell from spectroscopy or optical microscopy. In all these cases, the nature of the initial observations is, however, random by default. Hence, in a statistical inverse problem a random number of letters of the answer are wrong in addition. *I give you an incomplete answer, can you still tell me the question?* This renders solving these problems as particular difficult and tricky.

STATISTICAL INVERSE PROBLEMS

Therefore, it is essential to develop computational, statistical and mathematical methods that have the ability to extract as much information as possible from the initial random data. This therefore requires the causal factors that produced the given set of random observations to be quantified. This is exactly what Dr Munk's work in the recently founded Felix Bernstein Institute for Mathematical Statistics in the Biosciences at the University of Göttingen and the Max Planck Institute for Biophysical Chemistry (Germany) has managed to achieve.

FUNCTIONAL PRINCIPLES OF CELLS AT A MOLECULAR LEVEL

Molecular biology, biophysics and biomedicine all investigate activities in various systems and compartments of cells down to a molecular scale. Hence, they all study biological macromolecules as a collective result of atomic-resolution structural characterisations and subcellular-scale observations. The implementation of a distinct set of computational methods and algorithms can provide researchers with significant information regarding the spatial and temporal distribution of specific molecules within a compartment of a cell. This will, in turn, allow for the precise estimation of the number of molecules present at a given location (where) and at a given time (when). It is here that the nature of statistical inverse problems at the nanoscale (the millionth part of a millimetre) becomes of utmost importance.

STATISTICAL METHODS FOR OPTICAL NANOSCALE MICROSCOPY

The development of superresolution fluorescence microscopy has allowed for the substitution of conventional light microscopes that have a very limited resolution. Therefore, superresolution microscopy can visualise the structures and the dynamics of biomolecules down to nanoscale levels with an unprecedented ▸

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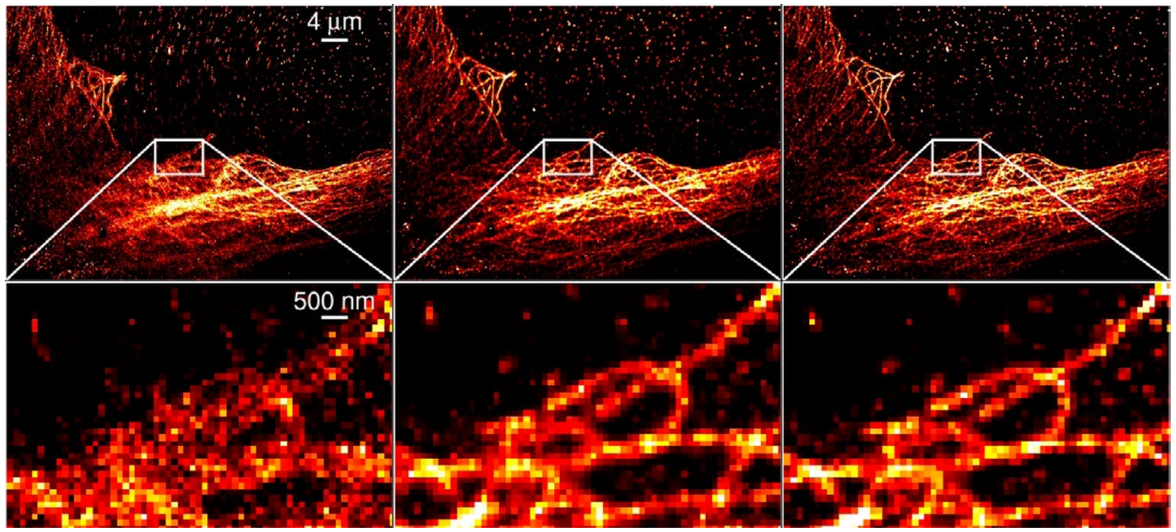

Statistical alignment of a sequence of 35,000 recordings of a beta tubulin network in Hela cells imaged with single marker switching optical nanoscopy. Total recording time is several minutes. During the measurement process the tubulin network moves in an unknown way. Left: Superposition of all recordings is blurred by this movement. Middle: registered and corrected with a fiducial marker (an artificially included bright shining fluorophore, which can be tracked). Right: Drift correction by a purely statistical method developed in Munk's group, which does not require this marker. (Hartmann et al. 2016, Drift estimation in sparse sequential dynamic imaging: with application to nanoscale fluorescence microscopy. J. Royal Statist. Society, Ser. B, 78(3), 563–587.)

resolution – higher than the resolution limit set by the diffraction of light. However, a major problem of superresolution microscopy is that the intrinsic random nature of the measurements – where discrete quantum effects are predominant – is rendering conventional solution strategies for statistical inverse problems ineffective. Surprisingly, and owing to the resolution being extremely small, current statistical laws become untrue and conventional statistical techniques do not allow effective image deconvolution and reconstruction.

OVERCOMING THE PROBLEM OF CONVENTIONAL STATISTICAL LAWS AND METHODS

Dr Munk and his group have employed alternative strategies and specific statistical modelling that focus on the underlying random mechanisms. For instance, in superresolution fluorescence microscopy, his research team, in collaboration with the group of Prof Stefan Hell, has found a statistical way to map the distribution of molecules by exploiting the fact that a single molecule emits only a single photon at a given time. Hence, the light from multiple photons arriving at the same time can, indeed, allow us to quantify how many molecules are present in a specific recording volume. Consequently, stimulated emission depletion (STED) microscopy can provide us with the exact distribution of molecules with subdiffraction resolution. Therefore, researchers are now able to extract a much higher amount of information from such measurements – with given statistical guarantees – by exploiting the discrete physical mechanisms of fluorescent molecules and light, as well as their distributions in time and space.

STATISTICAL METHODS FOR PROTEIN-MEMBRANE INTERACTIONS

Moreover, Dr Munk and his team emphasise the analysis of ion channels – channels that regulate all transport processes in the cell membrane – requiring the implementation of statistical methods that can be used to model and evaluate electrophysiological data. These occur when measuring pore-forming membrane proteins, one of the most important elements in human physiology because they control the flow of ions – gating – across the cell membrane, thus regulating signal transduction, energy conversion, and transporting.

Dr Munk and his team emphasise the analysis of ion channels and the implementation of statistical methods that can be used to model and evaluate electrophysiological data at small temporal scales

Therefore, pore forming membranes can be thought of as the regulators that trigger physiological functions, such as a normal heart beat, but also far more complex ones such as walking. This is why ion channels are potential drug targets for central nervous system disorders.

Multiscale ion channel analysis, as developed in Munk's group, propels the detection of gating characteristics of events on various temporal scales. Dr Munk's team have been implementing statistical methods that allow for the identification of real gating events – gating dynamics of these channels – with high precision. More specifically, ion channel recordings are subjected to multiresolution statistics, where events are automatically distinguished according to their length and conductivity. The signal is, thus, determined by the time points at which events (such as the opening and closing of a channel) occur, and by the conductance in between. However, events to a different conductance level may happen faster than sampled. Therefore, and by means of Jump-Segmentation by MUltiResolution Filter (J-SMURF), the signal at the jump points is segmented and we can effectively determine whether an event was missed or not – even for low signal-to-noise ratio.

The statistical guarantees stemming from this process are unique and have allowed Dr Munk's team in collaboration with Claudia Steinems lab at Göttingen to demonstrate that, for example, a chemically modified variant of a gramicidin A channel can, indeed, exhibit subgating events.

Therefore, such an approach is bound to provide a greater understanding regarding the spatial and temporal organization of membrane components and their function.

Dr Munk and his team at the University of Göttingen and in the Max Planck Institute for Biophysical Chemistry are currently performing pioneering research in the field of nanostatistics. Looking to the future, developing methods capable of extracting data and recovering objects from a series of indirect or random measurements at the nanoscale has the capacity to provide the scientific community with a greater insight into the exciting fields of optical nanoscale microscopy and protein–membrane interactions, among others.

Q&A

Your work has many applications across different fields – do you often collaborate with scientists from non-mathematical backgrounds?

Yes, I have collaborated mainly with researchers from natural science (medicine/lifescience, biology, physics, chemistry), but also occasionally from agricultural and forest science, even philosophy. In addition with several industrial/public sector partners.

Does this type of collaboration have any particular challenges?

Each collaboration requires a reasonably good understanding of the particular subject, which, in turn, requires an effort to learn the specific background to some extent. Intense communication with partners is extremely important: fun but also demanding. In general, statistical and computational methods cannot simply be transferred from one area to another; this always requires good subject knowledge. However, there are common statistical and computational principles which allow a unifying look at apparently different problems.

Your work into gating characteristics has enabled us to detect these across a variety of temporal scales – can you give examples of these different scales?

A small temporal scale is below a millisecond; a large one parts of a second.

Where do you hope your work will go in the next five years?

Towards development of statistical methodology at a broader range for unravelling nanoscale structures across different temporal and spatial scales, establishing unifying principles and models. We are just at its beginning. As the technical progress of measurement technologies for understanding molecular function at the nanoscale is progressing so rapidly, lab scientists will require more and more sophisticated nanostatistics to evaluate their complicated data and to extract as much information as possible from this.

Intense communication with partners is extremely important: fun but also demanding

Detail

RESEARCH OBJECTIVES

Dr Munk is a statistician whose research is fundamental to statistical inverse problems and extracts relevant information from complex biological data. He develops specific statistical methods to do this, controlling the level of statistical error for relevant recoveries obtained from the data.

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- Prof Tim Salditt
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BIO

After completing his PhD at the University of Göttingen back in 1994, Professor Dr Axel Munk completed a DFG research fellowship in Philadelphia and Cornell before working as a Research Assistant at Bochum University. He later went on to work as a professor for numerous universities before starting at the University of Göttingen in 2002. Since 2009, he has been the Felix-Bernstein Chair for Mathematical Statistics and since 2010 he has also worked at the Max-Planck-Institute for Biophysical Chemistry. In 2014 he founded the Felix-Bernstein Institute for Mathematical Statistics in the Biosciences.

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