Targeting, resolving and quantifying cellular structures by single-molecule localization microscopy

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The second 'Single Molecule Localization Microscopy' symposium was held in August 2012 at the École Polytechnique Fédérale de Lausanne in Switzerland. During two and a half days, around 100 researchers from across the globe, and in disciplines spanning physics, biology, chemistry and computer science, gathered to discuss the developments in single-molecule super-resolution imaging and its applications to address important biological questions.

Introduction

The high-quality research presented at this meeting proved that the still young field of single-molecule super-resolution imaging has successfully entered the much-anticipated next stage in answering important questions in cell biology. The key motivation of this research field is the need to monitor cellular structures in intact cells, by using light microscopy with the best resolution. However, with conventional microscopes, this is limited to the observation of elements separated by more than approximately 200 nm, known as the diffraction limit in light microscopy. Several achievements to bypass this limit have been made, creating an unprecedented breakthrough in light microscopy. Among them, single-molecule-based techniques are certainly one of the most successful [1]. These techniques not only reach near-molecular spatial resolution in biological samples, but also have the advantage of being relatively simple in terms of the microscope hardware. As this research field has grown enormously in the past five years, it is anticipated that these young methodologies will become even more user-friendly and integrated in the near future. The dynamic nature of this field is reflected by the impressive number of published papers on this topic—30 published in *Nature Methods* alone since 2008—and a lively meeting in Lausanne.

The topics at the meeting included experimental approaches for multicolour, three-dimensional and live-cell super-resolution imaging, the tracking of single

biomolecules, quantitative single-molecule biology, highly specific labelling schemes and the development of new photoswitchable fluorescent probes. The meeting opened with an historical keynote lecture given by W.E. Moerner (Stanford U., USA), one of the pioneers in single-molecule imaging [2]. He reviewed the field from its beginnings, illustrating the journey from a single fluorophore detected with high-resolution spectroscopy at cryogenic temperatures, to single-molecule super-resolution imaging in living bacterial cells. He covered the key technological developments and fluorophore photophysics,

the advances in which have paved the way for the implementation of imaging in biological research [1].

The scientific contributions to the meeting can be subdivided into two main parts: technological developments, including new photoswitchable fluorescent probes, labelling strategies, optical configurations and data analysis, and applications of single-molecule super-resolution imaging to biological topics. Broad topics in cellular biology were covered, including receptor dynamics in live cells, the organization of virus particles, spatial organization of the bacterial cytoskeleton, transcription machineries and cell division machineries.

Technological developments

The session on technological developments was opened by Mark Bates (MPI for Biophysical Chemistry, Göttingen, Germany), who reviewed the fundamental properties of organic fluorophores. They have a photoswitchable fluorescence emission [3], which is a key feature of single-molecule localization-based super-resolution imaging. Jean-Baptiste Sibarita (CNRS/Bordeaux U., France) presented the combination of single-molecule tracking with photoactivatable fluorescent probes, and his findings in neurobiology on postsynaptic receptor organization [4]. The key advantage in combining single-molecule tracking with photoactivation is that a large pool of fluorophore-tagged biomolecules is available, from which only a few are

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stochastically switched into a fluorescent state on demand. This allows for the quantification of the localization and dynamics of biomolecules with excellent statistics on live samples. The next part of the session focused mainly on three-dimensional super-resolution imaging. Different approaches were introduced: Alipasha Vaziri (Vienna U., Austria) presented super-resolution imaging by using two-photon temporal focusing for fluorescent protein photoactivation. In this approach, axially confined light distributions enable the optical sectioning of thick samples and thus allow the generation of super-resolution images across multiple layers, as well as isotropic three-dimensional super-resolution images when combined with astigmatism [5]. Francesca Cella Zanachi (IIT, Genoa, Italy) tackled the same challenge by combining single-molecule localization microscopy with a light-sheet illumination scheme [6], in which a thin section of the sample is illuminated for minimal photodamage of sections above, below and in the reduced background. The technological session was closed by Bassam Hajj (Janelia Farm, USA), who presented a multi-focus microscopy technique based on the use of a diffraction grating, and Rafael Piestun (U. Colorado-Boulder, USA), who introduced the double-helix point spread function achieved by phase mask. Both techniques allow three-dimensional single-molecule localization with extended depth of field compared with standard biplane or astigmatism methods.

Advanced labelling strategies

The session on advanced labelling approaches focused on new developments to introduce photoswitchable fluorophores into biological samples. Jan Schmoranz (FU/FMP Berlin, Germany) presented his work on dual-colour single-molecule imaging by spectral demixing of carbocyanine fluorophores, an approach that intrinsically has no chromatic shift and allows robust co-localization of cellular structures. Helge Ewers (ETH, Zurich, Switzerland) presented a versatile experimental strategy that uses photoswitchable fluorophore-tagged nanobodies that are targeted with high affinity

to GFP [7]. This essentially allows any GFP-tagged protein to be imaged and can profit from existing libraries—for example, the GFP yeast library at U. California San Francisco, USA. The technique was applied by Charlotte Kaplan from the same laboratory to investigate septin structures in yeast. The approach can be readily extended to two-colour imaging by introducing a second nanobody that specifically binds to red fluorescent protein. In addition to proteins, it has always been a challenge to visualize DNA in the cellular context by using super-resolution imaging techniques. Cristina Flors (IMDEA Nanociencia, Madrid, Spain) presented her work on operating intercalator fluorophores as photoswitchable dyes, as well as DNA enzymatically labelled with organic fluorophores. Jonas Ries (EMBL, Heidelberg, Germany) presented an experimental strategy that builds on the reversible binding of intercalator fluorophores to DNA. By using this technique, the number of fluorophores bound to DNA ('active') can be controlled through the concentration of the fluorophore, and single-molecule localization imaging becomes possible. The session was closed with a presentation by Virgile Adam (IBS/CNRS, Grenoble, France), who presented his work on the design of photo-transformable fluorescent proteins optimized for brightness and photoswitching properties [8], and his efforts to understand the switching mechanism at the molecular level. The audience agreed that the development of new photoswitchable and photoconvertible fluorescent proteins is one of the important tasks in single-molecule super-resolution imaging.

Imaging cellular structures

On the second day, the applications of single-molecule localization-based super-resolution microscopy to biological targets were presented. The session was opened by Melike Lakadamyali (ICFO, Barcelona, Spain), who presented her work on probing cargo transport with correlated live-cell and super-resolution microscopy. Vesicle trajectories, obtained with single-particle tracking in living cells, are correlated with the three-dimensional architecture of the microtubule network obtained with super-resolution microscopy in fixed cells. This approach is used to study the decision-making of vesicles encountering microtubule crossings. Suliana Manley (EPFL, Lausanne, Switzerland) discussed DNA imaging by using a dye binding to the minor

groove of DNA (Picogreen) to visualize the dynamic structure of chromatin in live mammalian cells. She also presented work on single-molecule tracking of proteins by using bright, synthetic dyes both extracellularly and intracellularly. Finally, she opened a further topic in single-molecule-based super-resolution imaging, which was quantification by molecular counting of proteins in HIV particles. This was further addressed by Paolo Annibale from Aleksandra Radenovic's laboratory (EPFL, Lausanne, Switzerland), who investigated how reversible photoactivation of fluorescent proteins has an impact on quantification [9]. This effect is observed for many fluorescent proteins and leads to overcounting or the artefactual observation of clustering. Annibale presented routes for quantifying super-resolution data with two colours, as well as strategies to distinguish between apparent and real clustering. Mike Heilemann (Frankfurt U., Germany) presented work on quantifying nucleosomal proteins in yeast by using single-molecule photoactivation [10]. He further introduced a study on the organization of RNA polymerase in *Escherichia coli*, which unravelled clusters of polymerases that probably represent transcription sites of ribosomal RNA. Ann McEvoy (U. Berkeley, USA) presented structured illumination and localization microscopy images of the essential bacterial cell division protein FtsZ in both live and fixed *E. coli*. Maximilian Ulbrich (Freiburg U., Germany) presented his work on multimeric membrane receptors by using a combination of single-fluorophore photobleaching and single-molecule localization microscopy. The session was closed by a presentation from Jean-Bernard Fiche (CBS Montpellier, France), who investigated the localization and dynamics of the DNA translocase SpoIIIE during sporulation in live *Bacillus subtilis* cells. Common to all the presentations about biological applications was the important contribution of single-molecule super-resolution techniques: the ability to resolve cellular structures with a spatial resolution far better than the diffraction limit, as well as the ability to quantify small structures and even single biomolecules.

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In addition to the presentations, a selection of posters depicted topics from this rapidly growing research field. Two posters were awarded a prize, the first one going to André Lampe (FU/FMP Berlin, Germany) for his presentation on dual-colour super-resolution imaging through spectral demixing, and another to Julia Gunzenhäuser (EPFL, Lausanne, Switzerland) for her investigation of the stoichiometry and cluster morphology of the Gag protein, a major structural protein of HIV.

The meeting was highly appreciated by all participants. Entirely multidisciplinary but extremely focused on single-molecule-based super-resolution microscopy, it strongly favoured interactions between people working on similar and complementary research fields. The concept of inviting mainly young group leaders to share their experience contributed to the success of the meeting and enhanced

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the connectivity. 'Know-how' in setting up single-molecule super-resolution imaging platforms, in designing experiments and analysing data was shared, and new strategies to decipher relevant biological questions were developed.

Because of this tremendous success and feedback throughout, the meeting will be continued and extended to a European Meeting on 'Single-Molecule Localization Microscopy'.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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