Optical microscopy: High resolution and exact localization

Reference No: B78213

CHALLENGE

High-resolution imaging and precise localization of structures in biological samples are of great interest in science and research. Correlative microscopy combines two methods, optical fluorescence microscopy and for example electron microscopy (EM). This enables analyses with an improved resolution compared to conventional optical methods. The sample preparation is time-consuming, as separate samples need to be prepared for the different imaging techniques, and the resulting images need to be precisely superpositioned. Applying external markers can be challenging.

INNOVATION

The new approach is to replace the EM technique with optical correlative microscopy in super-resolution, whereas the localization precision is independent of the laser beam alignment. This results in structures to become visible at high resolution and greatly simplifies the analysis process. In addition, this method enables imaging of living organisms. Super-resolution methods such as stochastic optical reconstruction microscopy (STORM), photo-activated localization microscopy (PALM) or stimulated emission depletion microscopy (STED) show details on the nanoscale. Confocal imaging reaches a resolution of up to 215 nm FWHM (full width/half maximum) using excitation wavelengths in the near infrared range, and 2P-STED can resolve structures up to a few 10 nm.
The super-resolution microscopy technique significantly simplifies the positioning of the images of the high-resolution section with the lower-resolution. Only if the images correlate exactly valid conclusions can be derived from the generated images.
The newly developed 2C2P-STED technique physically compensates deviations in the beam path of the lasers during two-color two photon (2C2P) excitation. The overlap of the point-spread-functions in the focus defines the confocal excitation volume. This reduces the adjustment work and prevents errors in a multicolor confocal overview image. A localization precision in the range of +/- 30 nm can be achieved.
Furthermore the system clearly references the position of the subsequent high-resolution image, which is generated using 2P-STED (two-photon stimulated emission depletion) microscopy. With this technique, no additional laser source is required to generate the high-resolution STED images. Only two laser sources are sufficient for both - the 2C2P and 2P-STED image.

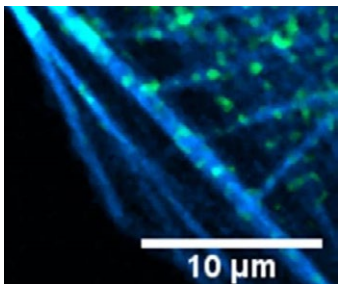

Figure 1: Actin network of a cell labeled with ATTO425 (blue) and Myosin labeled with ATTO594 (green). The Myosin can be localized at the actin filaments.

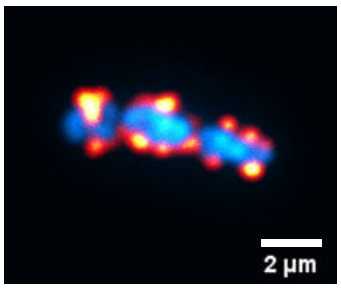

Figure 2: Streptococcus pneumoniae labeled with ATTO425 (blue) and pili filaments labeled with ATTO594 (red). The pili are attached at the bacteria surface.

COMMERCIAL OPPORTUNITIES

- Increase of usability by simplified handling and sample preparation
- High resolution combined with precise localization
- Setup requires only two lasers
- Intrinsic alignment of coherent image details
- High precision 3D live-cell imaging

DEVELOPMENT STATUS

Proof of concept: The method was tested successfully on a laboratory setup.

REFERENCES:

1 https://www.osapublishing.org/boe/fulltext.cfm?uri=boe-10-9-4516&id=416603

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