Novel localisation microscopy technique for high density & artefact free images

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Technology description

Super-resolution and localization fluorescence microscopy techniques have attracted considerable attention in the past decade in particular including the Nobel Prize in Chemistry, 2014.
Academics at King’ s College London have developed a novel method based on multiscale temporal filtering to enable high density, artefact free and multi colour localisation microscopy.
Localisation microscopy is a type of super-resolution microscopy that allows images to be taken with resolution better than the diffraction limit. While a popular technique, localisation microscopy suffers from several key limitations. First, the speed is very limited because thousands of frames must be acquired to produce a single super-resolution image. Second, it is fairly frequent for artefacts from the analysis to be present in the image, and detecting and removing these is time consuming and requires some degree of expertise. Third, multicolour images often suffer from chromatic aberration, and correcting this will degrade the resolution.
Previous methods to address these problems have either simply subtracted frames (BALM), or used global correlation information for the whole time series (SOFI, SRRF, 3B). This meant that either the resolution was limited or the reconstruction took a long time to calculate.

Key features
Speed- Faster than any other high density method compared to several of the most recent and popular.
Time- The preprocessing takes a few seconds and the final processing depends on the factor of temporal filtering.
No artificial structure or artificial sharpening as its failure mode- The failure causes a more blurred version of the underlying structure which means that the results are reliable, and reflective of the actual resolution obtained
Agnostic to the final analysis method- Improved results when used in conjunction with other low and high density analysis methods which means that whatever the preferred analysis method within a lab, our pre-processing method could be used
3D- Works on 3D data without producing artefacts, & at a higher density than other algorithms.
Preserves intensity informationwhen used in conjunction with single frame fitting methods (unlike other methods such as SOFI and SRRF).
Allows check with any data analysis problems- There is currently no method which allows users to check if there has been a problem with the data analysis. Therefore there is no way of warning users if their images may contain artefacts.

Advantages

Further advantages with regard to multicolour imaging:
- Allows two colour imaging to be carried out without using an image splitter or double conjugation of fluorophores
- Allows use of more than one fluorophores with similar emission spectra (for example Alexa 647 and Atto 647, two of the highest performance fluorophores)
- Faster compared to other methods such as sequential DNA-PAINT labelling as both colours are imaged simultaneously

Institution

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