

## Localization Light Emitting Molecules of Unknown Orientation (P-899)

### Keywords

- Method of three-dimensional localization of light emitting marker entities of unknown orientation in a sample
- Fluorescence super-resolution
- Single molecule localization accuracy

### Abstract

Many light emitting entities routinely used, in cell biological settings show a dipole orientation. If the exact  $z$  position of the light emitting entity is unknown, using simple two-dimensional Gaussian fit algorithms to the light intensity distribution of a range of emitted light, may lead to calculation errors of the  $x$ - and  $y$ -position of several 10nm. The invention discloses a method for calculation of the exact spatial position and orientation of a light emitting in a sample.

### Development Stage

The method has been verified experimentally as well as theoretically (see reference below).

### The Technology

First the virtual  $x$ - and  $y$ -positions and of a light emitting entity are determined for several plans parallel to the focus plan. Thereafter the absolute and/or relative total or peak emitted light intensities in the images of the marker entity are accounted for in determining the  $z$ -position and spatial orientation of the marker (see figure).

### Applications and Commercial Opportunity

DKFZ is looking for a licensee for further development and commercialisation this technology for samples in high resolution microscopes particularly FPALM, PALM, STORM and PALMIRA.

### Inventors

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### Intellectual Property

Priority filing was October 9, 2009 as EP09172670.3. An international PCT was published as [WO2011042493](#). The US patent [US 8,559,021](#) has been granted; the European patent equivalent [EP2486438](#) is pending.

### Further Information

No other public information is currently available, but further information (speaking with the inventor) is available under a signed Confidential Disclosure Agreement (CDA).

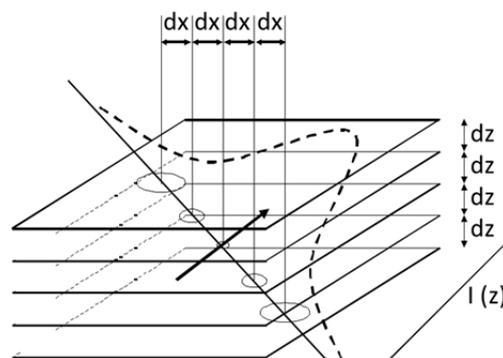
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### Reference:

"Molecular orientation affects localization accuracy in super-resolution far-field fluorescence microscopy." by Johann Engelhardt *et al.* in [Nano Lett. 2011 Jan 12;11\(1\):209-13](#)

### Figure:



The figure shows the peak emitted light intensity  $I(z)$  and the relative  $x$ - and  $y$ -position of a dipole in images depending on the  $z$ -position of the focal plane. The exact spatial orientation of the light emitting entity can be calculated using all these information.