

Resolving single molecules in two and three dimensions: how far can we go beyond Rayleigh's limit?

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Classical resolution criteria such as Rayleigh's criterion, although extensively used, are well known to be based on heuristic notions that are incompatible with modern microscopy techniques. Not surprisingly, recent 2D imaging experiments have shown that distances well below Rayleigh's limit can be resolved between single molecules, thereby illustrating the inadequacy of classical resolution criteria for quantitative imaging approaches. Recently, we derived a new resolution measure that overcomes the limitations of Rayleigh's criterion and provides a quantitative measure of the ability to resolve two closely spaced single molecules in 2D [1]. The new result predicts that there is no resolution limit but that the resolvability depends on the number of detected photons from the single molecules. Further, it was experimentally verified that distances as small as 12 nm can be measured through a regular optical microscope with accuracy as predicted by the new resolution measure [1]. We have also derived a new 3D resolution measure for optical microscopes, which showed that separation distances well below the ~ 1 micron limitation imposed by the classical 3D resolution criterion can be resolved [2].

Previously, we had developed a novel imaging modality called multifocal plane microscopy (MUM) which enables simultaneous imaging of multiple planes within a cell-sample [3]. We had also shown that MUM provides superior depth discrimination when capability compared to a standard microscope, which in turn paves the way for high resolution 3D single molecule tracking within a live cell environment [5-6].

In the context of the 3D resolution problem, image data captured at the multiple focal planes in a MUM setup provide additional information pertaining to the distance between the molecules. Based on calculations of the Fisher information matrix, we expect that with MUM it is possible to resolve distances well below the classical 3D resolution limit. Here, we present a detailed analysis on the 3D resolution capabilities of a two plane MUM setup and identify the circumstances in which the MUM setup provides superior resolution when compared to a standard microscope.

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