Structured Illumination and the Analysis of Single Molecules in Cells

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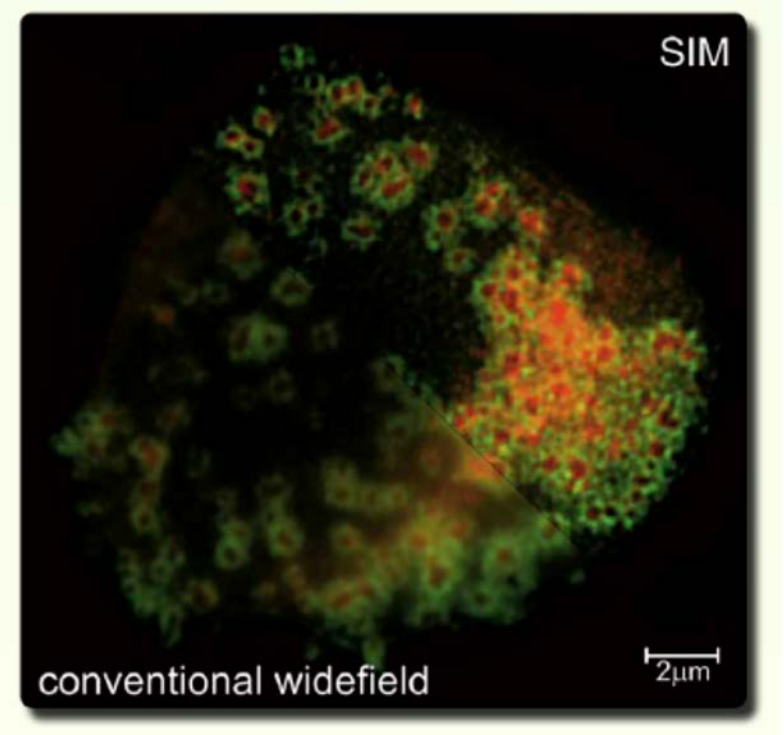
In the past decade revolutionary advances have been made in the field of microscopy imaging. One such method is based on transforming conventionally unresolvable details into measurable patterns with the help of an effect most people have already personally experienced: the Moiré effect. If two fine periodic patterns overlap, coarse patterns emerge. This is typically seen on a finely weaved curtain folding back onto itself. Another example is fast moving coarse patterns on both fences of a bridge above a motorway, when approaching it with the car. The microscopy method of **structured illumination** utilizes this effect by projecting a fine grating onto the sample and imaging the resulting coarser Moiré patterns containing the information about invisibly fine sample detail. With the help of computer reconstruction based on several such Moiré images, a high-resolution image of the sample can then be assembled.

Another way to obtain a high-resolution map of the sample is to utilize the blinking behavior inherent in most molecules, used to stain the sample. Recent methodological advances (Cox et al., Nature Methods 9, 195-200, 2012) enable us to create **pointillist high-resolution maps** of molecular locations in a living biological sample, even if in each of the required many individual images, these molecules are not individually discernible. Examples will be shown as a film of a cell at 30 millionths of millimeter resolution and 6 seconds between the individual movie frames.



Podosomes of a cell image with structured illumination (SIM). f-actin shown in red and vinculin in green. Image courtesy of Marie Walde, Gareth Jones and James Moneypenny.