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Interference reflection microscopy to visualize sub-diffraction limited objects in 3D

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The visualization of objects smaller than the diffraction limit has become a broad field, especially in life science. However, current technologies often require a fluorescent label and/or complicated optical setups. Here, we report the observation of single, non-labeled microtubules with interference reflection microscopy (IRM) using a light emitting diode for illumination. IRM can visualize sub-diffraction-limited objects, provides three-dimensional (3D) information with nanometer resolution in the axial direction, and a superior signal-to-noise ratio compared to differential interference contrast (DIC) microscopy. In comparison to other techniques such as DIC microscopy, IRM does not require any (expensive) polarizing optics, is independent of the object orientation, and can easily be implemented into conventional inverted microscopes. Also, IRM provides free access to the upper side of the sample and can be combined with other optical techniques such as fluorescence microscopy and optical tweezers.