Kilohertz-3D-super-resolution imaging of shape changing helical bacteria using scattered laser light

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The helical bacterium spiroplasma melliferum is a wall-less bacterium with a minimal set of genes, just sufficient for independent life and self-reproduction. Hence, they are of extreme structural simplicity and are among the smallest cells in the world (~200nm thin, 3-5µm long). However, they infect various plants and insects and thereby do tremendous harm to agriculture industry. Their motility, defined by helicity changes, kinking and propelling is very complex, and enables propagation in complex environments.

By scanning a highly focused laser beam across a trapped bacterium, we show how to record 3D movies of the living cell at a frame rate of 800 Hz and faster. Tiny phase changes of the laser light, which are induced specifically at each slope of the bacterium can be measured by back-focal plane interferometry. Hence, no staining is required and acquisition times play no role because of high-signal to noise ratios.

Changes in bacterial shape correspond to the accumulation of mechanical energy, which serve a new way to characterize the health state of a bacterium. Due to the high image contrast, frame-rate and spatial resolution, it is possible to distinguish smallest shape changes of the bacterium generated either by ATP-hydrolysis or by thermal noise. As an application, we analyze the response in mechanics and energetics of a single bacterium to externally induced stress until it dies.

Figure: A 3D image sequence of a whorled 200nm thin helical bacterium measured at 800 Hz.