

## Classic Spotlight: Seeing is believing- imaging single bacterial flagellar filaments in action

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Between the late 1970s and early 1980s it became clear that bacteria swim by rotating semi-rigid helical filaments. Structural studies had shown that the filaments of *Escherichia coli* and *Salmonella enterica* Typhimurium are hollow cylinders made of 11 protofilaments of the protein flagellin (3, 4, 7). The protofilaments could be short or long, depending on the conformation of the constituent flagellin subunits, and the ratio of short to long determining the overall shape of the helix. Protofilaments can switch between long and short forms in response to mechanical stress, giving rise to the so-called polymorphic transitions. In 1972 Brown and Berg showed that swimming *E. coli* cells alternate between periods of smooth swimming (runs) and short periods of changing direction (tumbles) (1). Tethering bacteria by a single flagellum revealed that the motors switch between counter-clockwise (CCW) and clockwise (CW) rotation, leading to the idea that CCW rotation causes a run and CW rotation a tumble (5). However, there was uncertainty about, for example, whether all of the ~6 *E. coli* flagella rotate CCW in a bundle and about how many flagella needed to switch to CW rotation for a tumble to occur.

Very bright illumination had allowed Pijper to image flagella on swimming cells as early as the 1930s. He described the helical flagellar bundles in a series of papers, but he incorrectly concluded that these bundles were an artefact of cell movement (8). This dark-field approach was however updated in the 1970s by Robert Macnab using a very bright short arc xenon or mercury lamp, rather than the South African sun. In the light of the structural studies and the data from tethered cells it was clear that he was visualizing bacterial flagellar filaments on swimming cells. The flagella rotated as a CCW bundle that pushed the cell forward, and the cells tumbled when the filaments rotated CW, changing both their helical wavelength and handedness (6). However, the required high intensity light needed to visualize the filaments resulted in a large flare from the cell body making it impossible to see the filaments close to the cell body. Although DIC microscopy reduced the problem, it only allowed imaging of filaments close to the glass surface, rather than all of the filaments on a free swimming cell (2).

The 2000 paper by Turner, Ryu and Berg changed all this (9). They found that amino-specific Alexa Fluor dyes brightly stained the flagellar filaments of *E. coli* and *Salmonella* but only lightly stained the cell body. Moreover, there were no major effects on cell behavior. This meant they could use a CCD camera on a standard fluorescence microscope to make movies of swimming cells, visualizing the behavior of the full-length filament as the cells ran and tumbled. They could thus accurately

observe different waveforms and polymorphic transitions and link these observations to the behavior of the cell body.

By recording the behavior of individual filaments leaving bundles and undergoing polymorphic transitions, they found most of the polymorphic forms predicted by the long vs short protofilament model. Their images settled the debate on how many motors had to switch to cause a tumble, clearly showing that not all filaments needed to be in a bundle for a run to occur, and only one or two needed to switch to CW to cause a tumble. They also showed that tumbles varied depending on the number of CW rotating flagella and the duration of the CW rotation. This led to a number of further elegant studies as new technologies developed, for example a 2007 J. Bacteriology paper from the Berg group used a faster camera which allowed accurate analysis of torque output and a detailed analysis of the behavior of individual filaments rotating within bundles (9).

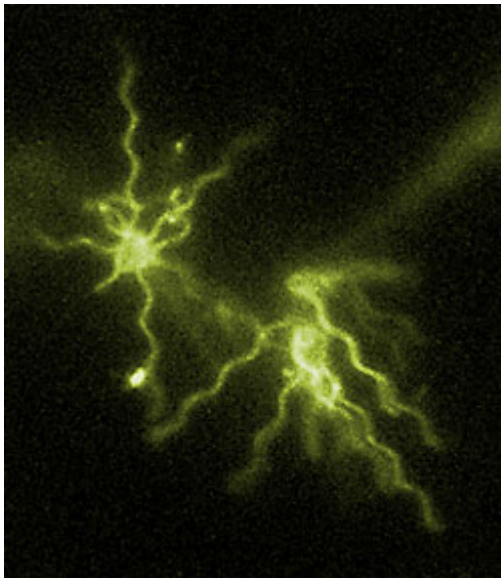
The year 2000 was before supplementary information came into vogue, but the videos used to extract the still pictures in the paper came from have always been freely available on the Berg website and 16 years later they have not been improved upon as a starting point for most lectures on bacterial motility. Clearly, movies illustrate, far better than words, the changes in filament shape caused by motor switching and the subsequent effect of swimming behavior (10). As it says in the final sentence of this elegant paper “The labelling technique is so simple, and the images are so vivid, even when seen with an ordinary light microscope, that the world of the flagellum is now more accessible”.

## References

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10. <http://www.rowland.harvard.edu/labs/bacteria/movies/ecoli.php>

## Figure legend

- 86 Snapshot of two *E. coli* cells labelled with Alexa Fluor dyes showing polymorphic  
87 transitions in individual flagellar filaments during a tumble.



88