Nanoscopy with Focused Light
(Breaking the Light Barrier)

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In 1873, Ernst Abbe discovered that the spatial resolution of lens-based optical microscopy is limited to about 200 nanometers due to diffraction of light waves at the microscope focus. This so-called "diffraction limit" has been the practical resolution limit of optical microscopy ever since. In this lecture we will discuss concepts that circumvent the resolution-limiting role of diffraction by using light to manipulate the internal (quantum) states of the molecules making up the sample.

The first demonstration of this concept, Stimulated Emission Depletion (STED) microscopy, uses a laser beam focused into a doughnut-shaped pattern with a dark region at the center. Molecules outside this dark region are driven into a state that is essentially transparent to a second, overlapping laser beam, while those within the dark region absorb light from the second laser and emit fluorescence, which is then detected by the microscope. The effective size of the doughnut hole, and thus the spatial resolution of the microscope, can be arbitrarily reduced in size by increasing the intensity of the doughnut laser.

The concept underlying STED microscopy can be expanded by employing a variety of molecules that can be switched the between transparent and fluorescent states via different photophysical mechanisms. Examples include photoswitchable organic compounds and fluorescent proteins that undergo a photoinduced conformational change that controls their optical activity. Some photoswitchable molecules have two stable conformations, and this optical bistability enables switching at low intensity values, which in turn allows for non-destructive imaging of relatively delicate biological samples with nanometer-scale resolution. Organic chemists continue to play a key role in developing new photoswitchable markers, including fluorescent proteins, and we will discuss recent work showing how optical 'nanoscopy' can be used to solve fundamental problems in biology.