More than 25 years ago, low temperature experiments aimed at establishing the ultimate limits to optical storage in solids led to the first optical detection and spectroscopy of a single molecule in the condensed phase. At this unexplored ultimate limit, many surprises occurred where single molecules showed both spontaneous changes (blinking) and light-driven control of emission, properties that were also observed in 1997 at room temperature with single green fluorescent protein variants. In 2006, the diffraction limit in microscopy of about 250 nm was overcome using single molecules as tiny beacons, much like fireflies in the night. Essential to this is the combination of single-molecule fluorescence imaging combined with active control of the emitting concentration and sequential localization of single fluorophores decorating a structure.

Super-resolution microscopy has opened up a new frontier in which nanoscale biological and non-biological structures and behavior can be observed with resolutions down to 20-40 nm and below. Examples range from protein superstructures in bacteria to details of the shapes of amyloid fibrils and much more. Current research addresses ways to extract more information from each single molecule such as 3D position and orientation, and both of these can be obtained by proper point-spread function engineering of a wide-field microscope.