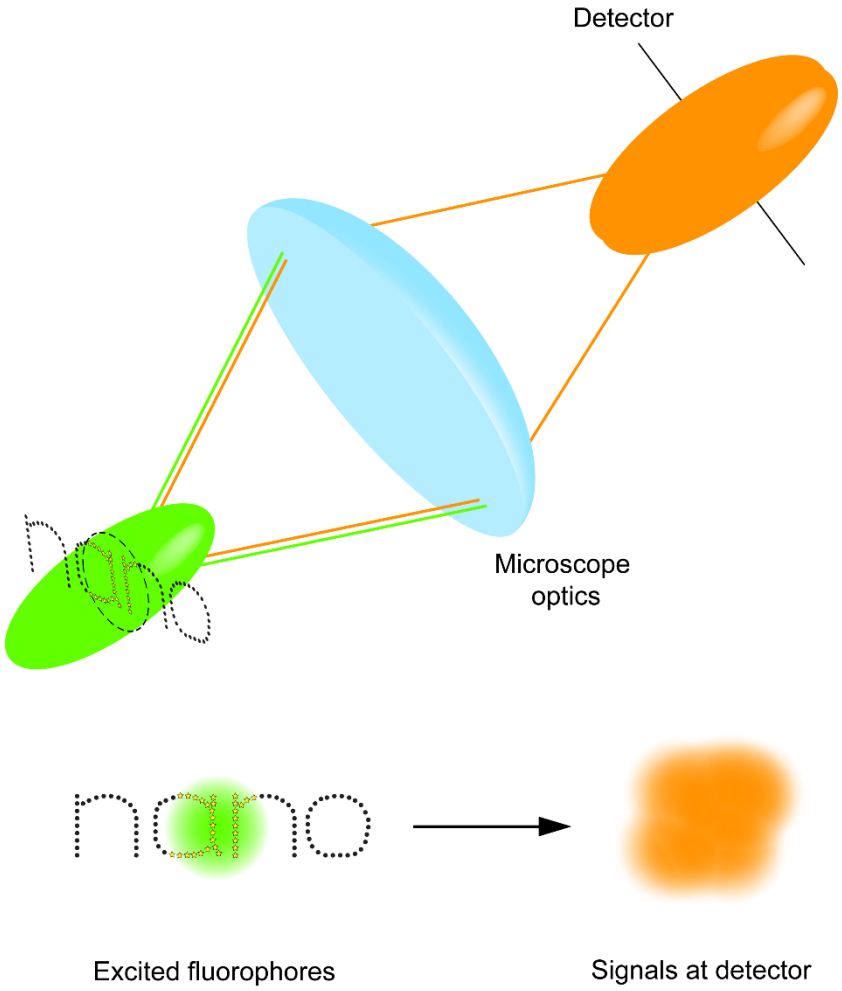
In format provided by Sahl, S. J. *et al.* (doi:10.1038/nrm.2017.71)



Supplementary Box 1 | The diffraction limit of optical microscopy (schematic). Focusing of excitation light (green) by the microscope objective lens cannot occur more tightly than the diffraction limit (Abbe's limit). As a result, all molecules within this diffraction-limited region are illuminated together, emit virtually together (orange), and cannot be told apart upon refocusing at the detector. This is because at the detector – be this a camera, a photodiode, or even the human eye – the fluorescent signals overlap and cannot be separated. The diffraction limit has lateral (x,y) dimensions of $\Delta x, \Delta y \approx \lambda/(2n \sin\alpha)$ and is $\Delta z \approx \lambda/(n \sin^2\alpha)$ along the axial dimension (optic axis z), with λ, α, and n denoting the wavelength, the semiaperture angle of the lens, and the refractive index of the medium, respectively. The optical system is only schematic, not showing the actual arrangement of lenses (objective, tube lens), filters and other components in a real microscope. For an in-depth discussion of resolution and contrast in optical microscopy, see[1].

Reference

1. J. B. Pawley, Ed., Handbook of Biological Confocal Microscopy (Springer, New York, ed. 2, 2006).