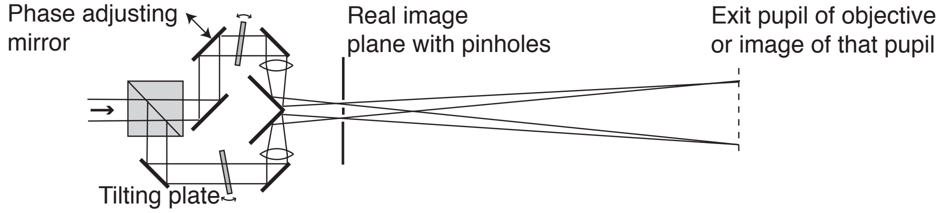
IMPROVING LATERAL RESOLUTION IN THE STED MICROSCOPE

Stephen C. Baer
Photome 10 Poplar Rd. Cambridge, MA baer99@comcast.net

Hell and Wichmann [1, 2] and the present author [3] proposed similar ways to improve resolution in a scanned fluorescence microscope, involving shrinking the scanned spot by optically deexciting fluorescence in its outer parts. In our proposal [3], the deexciting (STED) beam was shaped like a doughnut with a zero intensity central point superimposed on the spot's central point. Fluorescence at this point therefore could not be attenuated, even when points arbitrarily close to the center could be attenuated as much as desired by raising the STED beam power, which therefore became the resolution-limiting factor. Shaping the STED beam by creating an interference minimum at the central point sharpened the distribution around that point, but the distribution was elongated, and remained so even when superimposed with a second interference minimum at right angles to it. We therefore proposed a beam-shaping scheme where two such minima crossed at right angles, but were prevented from interfering with each other [4]. Hell and his coworkers implemented this scheme, where crossed polarizations between the perpendicular interference minima prevented their mutual interference. The authors saw the expected doughnut, but the measured central intensity was1% to 5% of the mean intensity rather than the predicted zero, a discrepancy attributed to wavefront aberration [5]. The optical arrangement described here eliminates several components in that device that could potentially create aberration or spurious reflections.



The pinhole in each arm of the device of [5] is replaced with a pair of pinholes on opposite sides of the optical axis, illuminated 180° out-of-phase. A finite corrected objective avoids the need for a converging lens. Tilting glass plates and a moving mirror together provide achromatic phase shifting, sharpening the minima. The pinhole plane can include the pinholes for the perpendicular minimum and the excitation beam, eliminating two beamsplitters. A 90° phase difference between the minima eliminates their mutual interference [6].

We calculated that for the optimum distance of the pinholes from the axis, the FWHM of the minimum in this device is very similar to that calculated by Engel et al for their device [5]. However because of achromatization of phase delay and because of elimination of some sources of aberration and spurious reflection, we expect that in the present device the actual intensity curve will more closely follow the computed one near the central point. Furthermore the present arrangement appears to allow both STED beams to share the polarization axis of the excitation beam, maximizing quenching effectiveness. Hopefully these factors may help bring to two lateral dimensions the impressive resolution gains recently demonstrated for one [7].

1) Hell, S. and Wichmann, J., *Opt. Lett. 19*:780 (1994).
2) Hell, S. and Wichmann, J., US Patent 5,731,588 (1998).
3) Baer, S., US Patent 5,866,911 (1999).
4) Baer, S., US Patent 5,952,668 (1999).
5) Engel, E., Huse, K., Klar, T.A., and Hell, S., *Appl.Phys.B 77*:111(2003).
6) Baer, S., US Patent 6,259,104 (2001).
7) Westphal, V., Kastrup, L., and Hell, S., *Appl.Phys.B 77*:337 (2003).