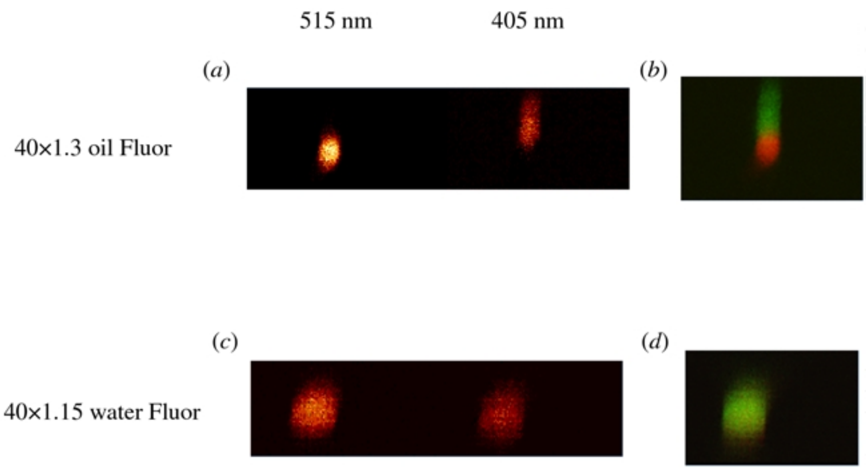

FIGURE 18-2 Illustration of the differences in chromatic correction of the objective lenses indicated. We imaged doubly labeled fluorescent beads, 4 μm in diameter, mounted in water. (*a*) and (*c*) show simultaneously acquired cross-sectional images (viewed along the *x*–*z* plane) of a single bead, at the emission wavelengths indicated. It can be seen in (*a*) that, because of inadequate color correction, the two fluorescence images, coming from the same bead, do not align along the *z* axis. In contrast, when the same bead is imaged with the other objective, the two images are perfectly aligned (*c*). Such a considerable difference in chromatic performance was unexpected, since both objectives had the same chromatic specification (Fluor). This result indicates that significant differences can exist even between objectives of similar specifications, and therefore individual testing is essential when maximum performance is required. Both objectives also have reduced ultraviolet transmission, since the image obtained at 405 nm is significantly dimmer than that obtained at 515 nm, with the oil objective being slightly brighter. For ease of comparison, the images from (*a*) and (*c*) are shown as green–red overlays in (*b*) and (*d*), respectively. Finally, as can be seen in (*a*), the oil-immersion objective lens has significant spherical aberration, because the beads were mounted in water. Thus, the spherical bead appears elongated along the *z* axis. It is possible to compensate for such distortions, by choosing an immersion oil with a different refractive index (Section 4.1.2). Data for the $40 \times /1.15$ objective lens were acquired with higher zoom, and hence the bead appears bigger. All images appear in pseudocolor.