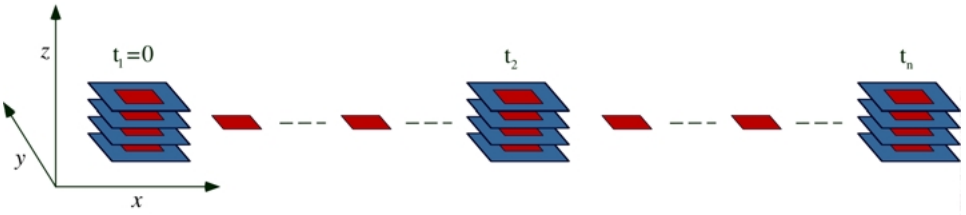

FIGURE 18-3 Paradigm of image acquisition during the time-lapse experiments. A series of optical sections in depth (shown in blue) was acquired at the beginning of the experiment ($t_1 = 0$) to provide the baseline location and morphology of one or a few axons within the region of interest. A maximum-projection image of such a data set is shown in Figure 6. The procedure was repeated at regular intervals ranging between 30 and 60 minutes ($t_2, \ldots, t_n$). In between, high-power (shown in red) single images were acquired at short intervals (60–120 s) to document the changes in morphology and behavior of one or two growth cones within the region of interest. Such data are shown in Figure 7. The difference in size of the blue and red images is meant to reflect the different magnifications (selected by the zoom factor of the confocal).

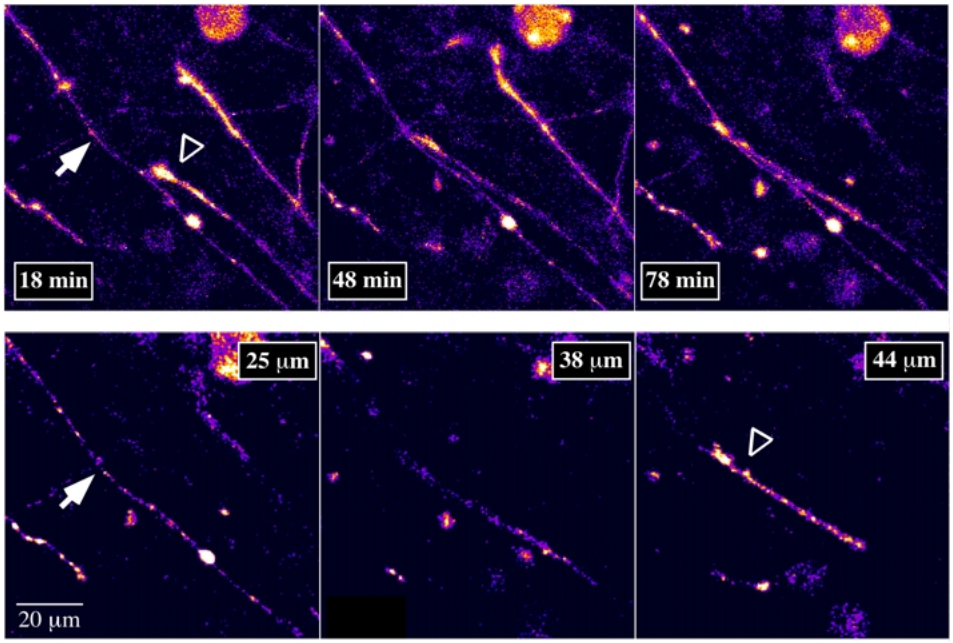

FIGURE 18-4 The panels in the top row are maximum-projection images, generated from a series of confocal optical sections spanning 30 µm in depth and separated by time intervals of 30 minutes. It can be seen that the axon towards the centre (open arrowhead) is extending forward and, in the third top panel, appears to be growing on top of another axon (closed arrow). However, analysis of the single optical sections (bottom row) reveals that the two axons are in fact separated by about 20 µm. Maximum-projection images were created by a "brightest-point algorithm" that performs a pixel-by-pixel comparison of a series of optical sections and uses the brightest point from each one in order to create the projection image. Projection algorithms (using either the brightest point or the average) are an integral part of all 3D image-analysis software packages presently available, as well as some of the image-acquisition software packages that accompany confocal systems.