

Supplementary Video 1 (SV1)

Aligned tilt series of a 150 nm thick section of freeze-substituted and resin-embedded wild type *S. cerevisiae* sample. The tilt series illustrate a “grazing” section of a dividing bud, close to the cell membrane. Filaments running perpendicular to the mother-bud axis are clearly visualized, even in the tilt series. Data was collected from angles -60 to 60 with a pixel size of 6.9 Å.

Supplementary Video 2 (SV2)

Second tilt series from the sample shown in movie SV1 visualized after it was rotated 90 degrees in the dual axis holder. The data was collected from angles -60 to 60 with a pixel size of 6.9 Å.

Supplementary Video 3

Three-dimensional tomographic reconstruction obtained by combining the tilt series displayed in SV1 and SV2. The movie shows successive slices in the z direction in the tomogram. One of these slices, near the plasma membrane is displayed in Figure 2A.

Supplementary Video 4

Small region within the three dimensional reconstruction of a medial 150 nm thick section of freeze-substituted and resin-embedded wild type budding yeast. The movie displays the successive slices of the tomogram where axial filaments are particularly well resolved. One of these slices is shown in Figure 2B.

Supplementary video 5

Tomographic reconstruction and rendering of membranes and filaments at the bud neck of a dividing budding yeast cell that had been freeze-substituted and embedded. Circumferential filaments are seen most clearly on the grazing slices close to the top of the section, while long axial filaments are more clearly seen in deeper slices. The tomogram includes a region of a dividing nucleus with four spindle microtubules, as well as endomembrane (likely ER) adjacent to the nucleus and the bud filaments.

Supplementary Video 6

Three dimensional reconstruction of a tomogram from a 50 nm thick cryo-section of wild type *S. cerevisiae*. This sample is fully hydrated and was not subjected to chemical embedding or staining. One of the slices in this tomogram is shown in Figure 4.