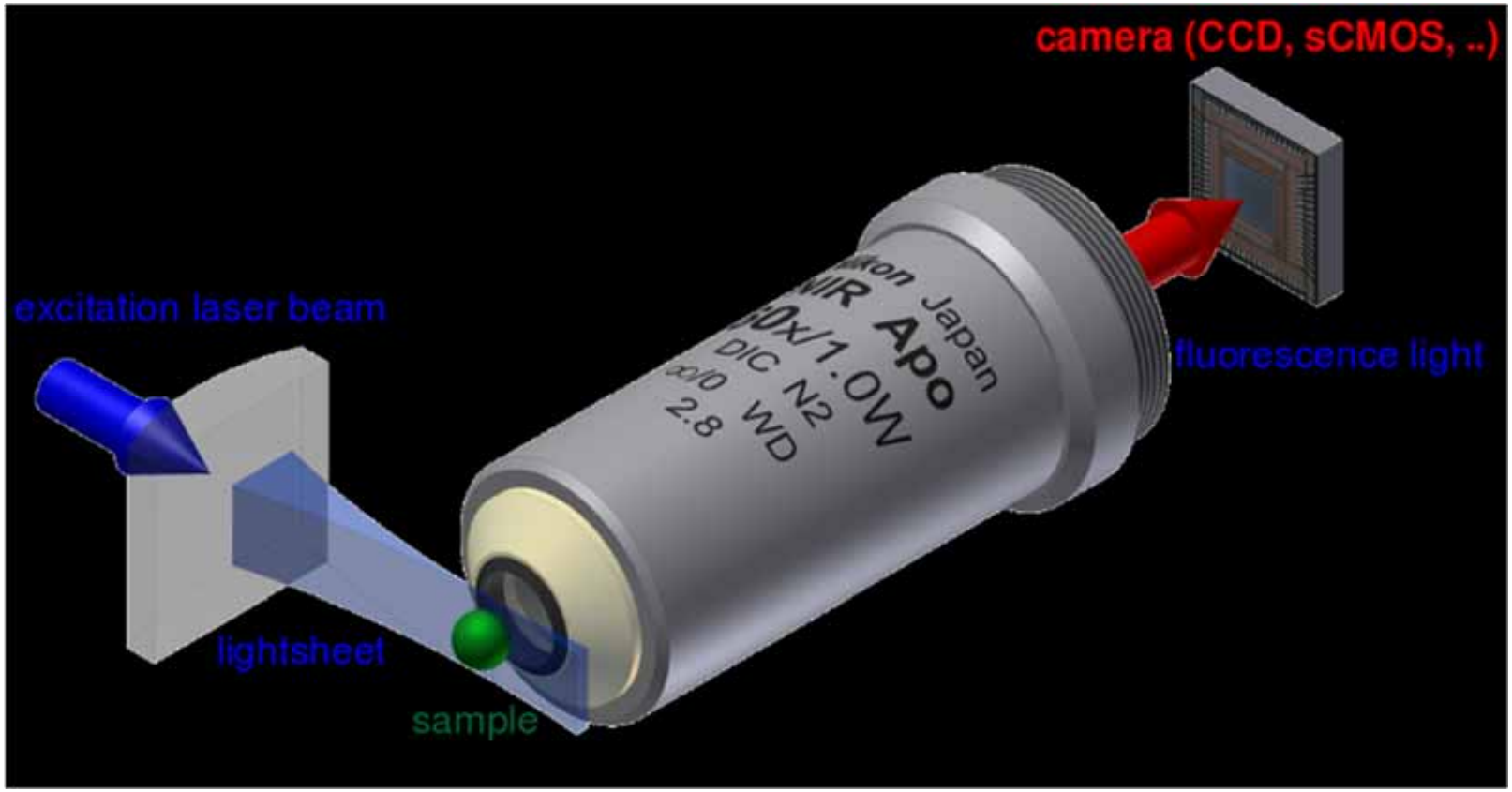
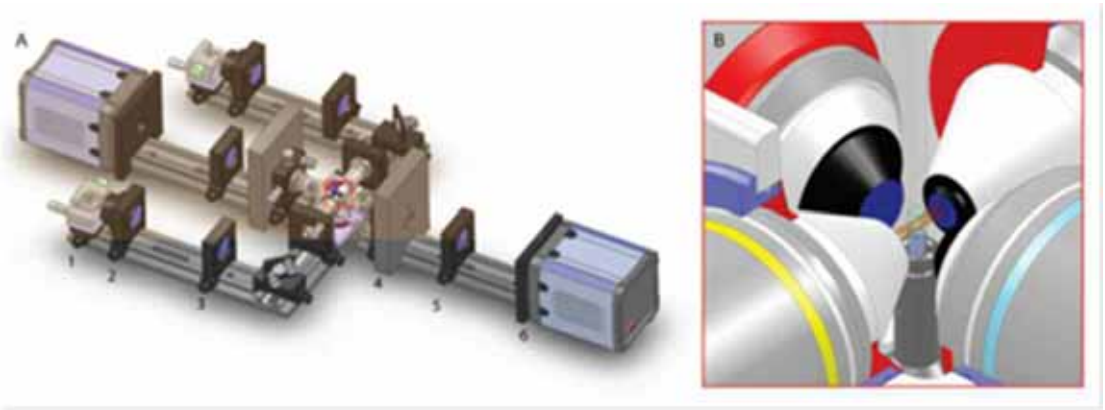
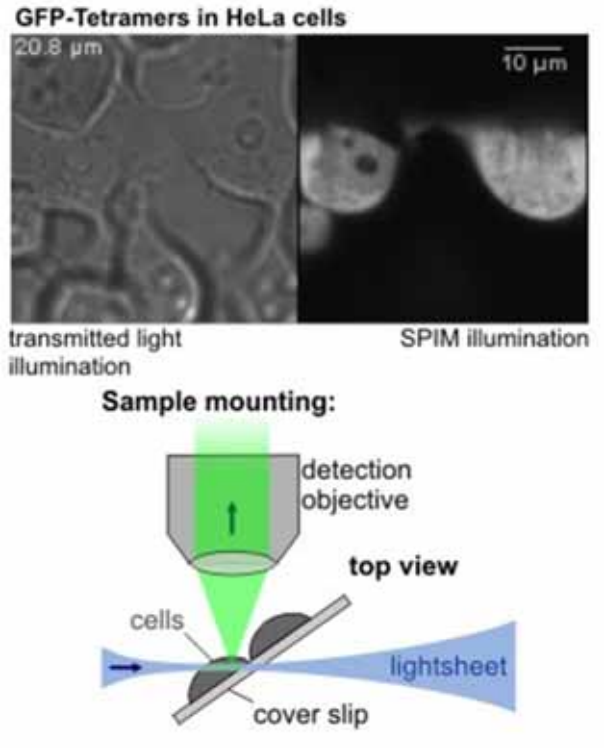
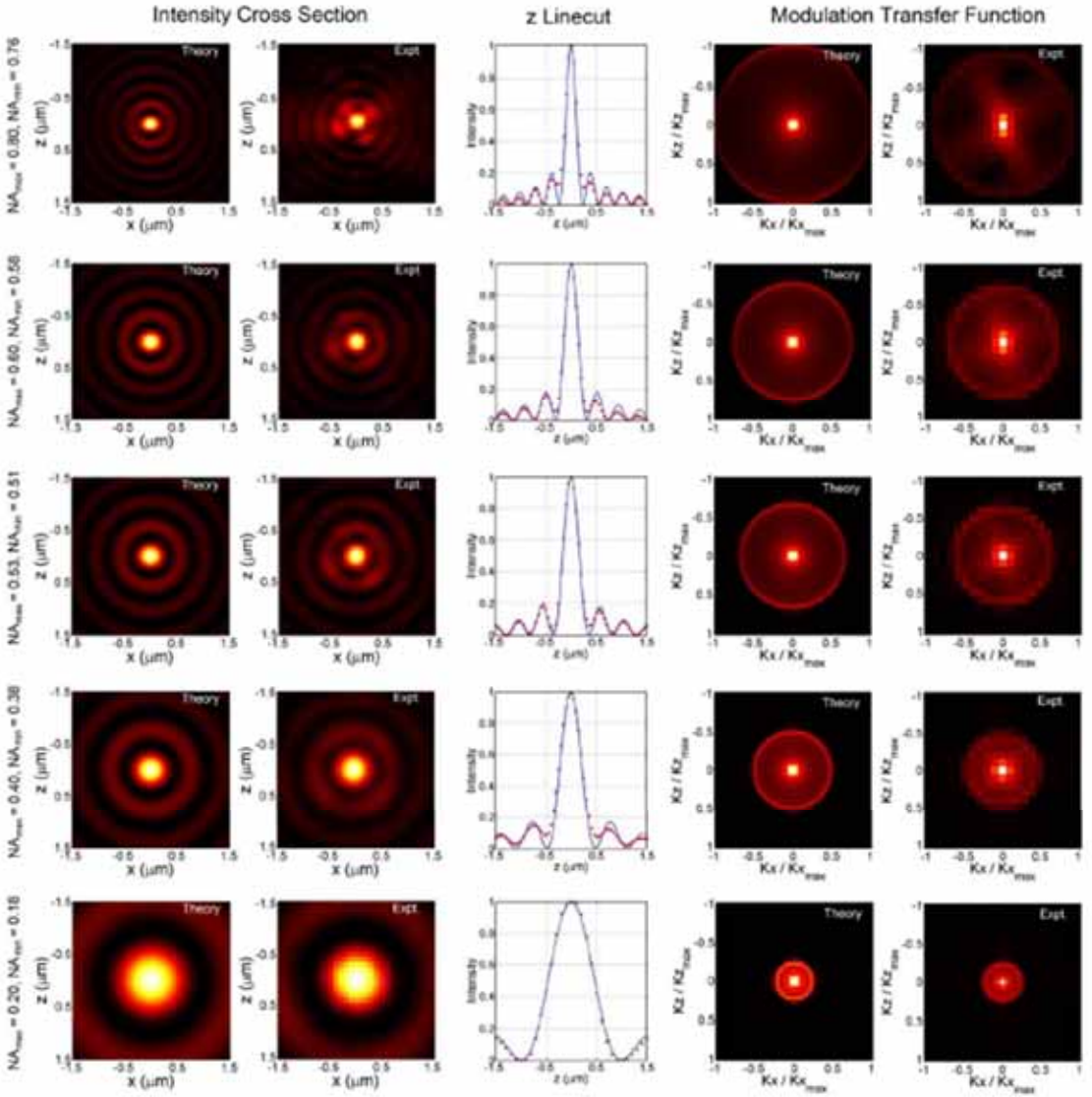
Light sheet fluorescence microscopy




A
B


GFP-Tetramers in HeLa cells
20.8 µm
10 µm
transmitted light illumination
SPIM illumination
Sample mounting:
detection objective
top view
cells
lightsheet
cover slip

Bessel Beam



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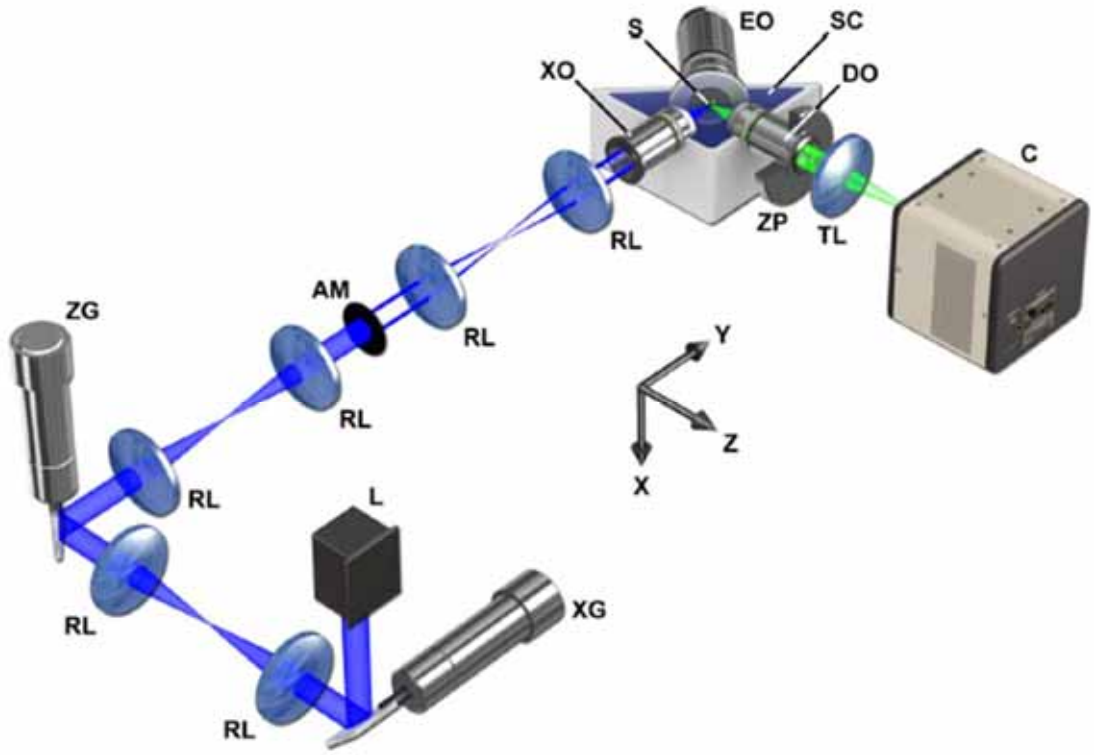


SUPPLEMENTARY FIGURE 3. Simplified schematic of the Bessel beam plane illumination microscope. Light from laser (L) is reflected from x-axis galvanometer (XG) and transmitted in turn by relay lenses (RL) to z-axis galvanometer (ZG) and annular apodization mask (AM). XG, ZG, and AM are all at conjugate planes, so that the Gaussian beam falling on AM does not oscillate as XG and ZG are scanned. Similarly, AM is conjugate to the rear pupil plane of excitation objective (XO) so that the thin annular illumination transmitted through AM produces a Bessel beam within specimen (S) that translates along x and z without tilting. The light sheet created by scanning XG creates fluorescence at the focal plane of detection objective (DO), which is imaged at camera (C) by tube lens (TL). Different planes within S are imaged by translating DO with z-axis piezoelectric collar (ZP) in synchronization with the z axis motion of

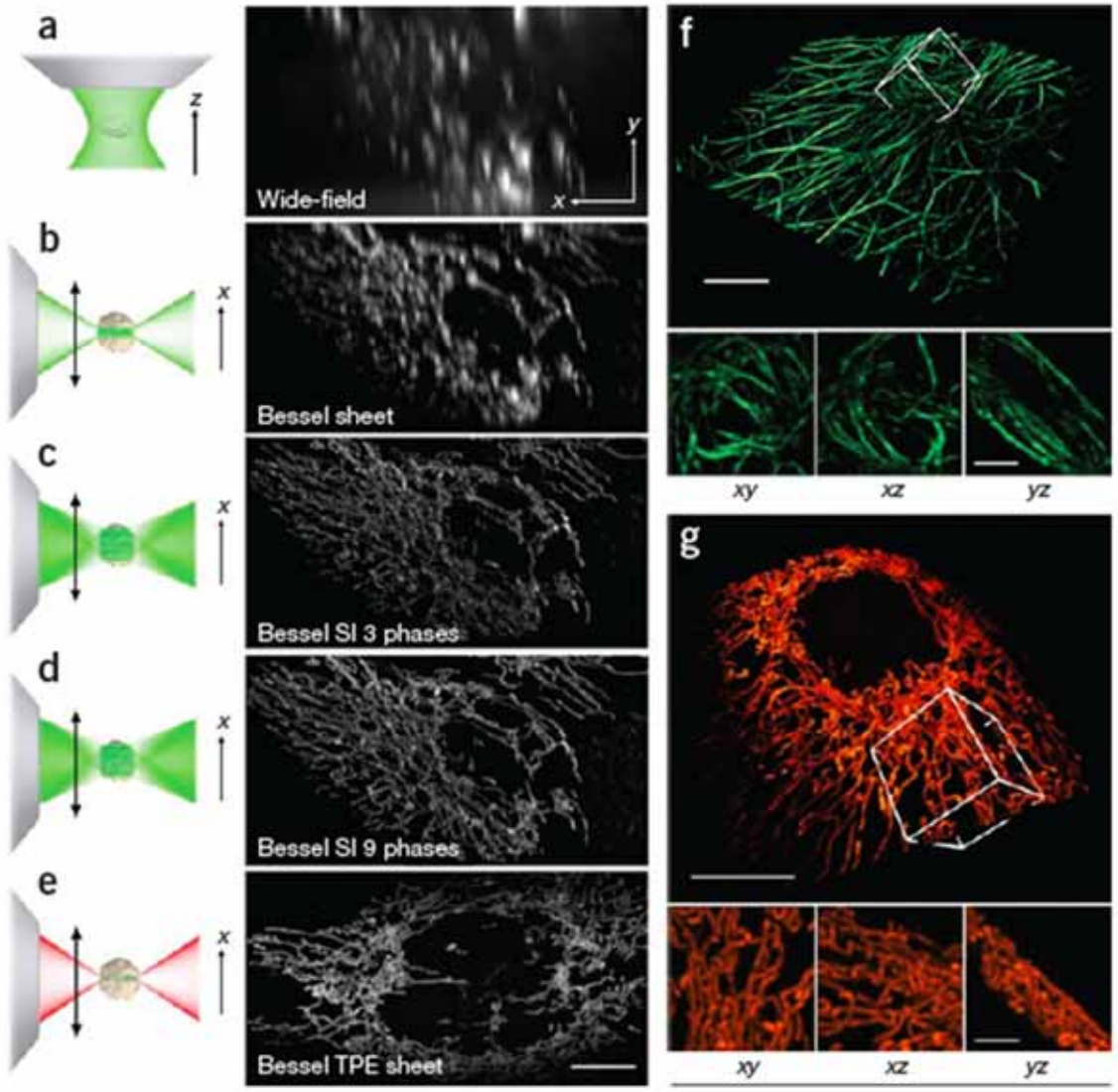
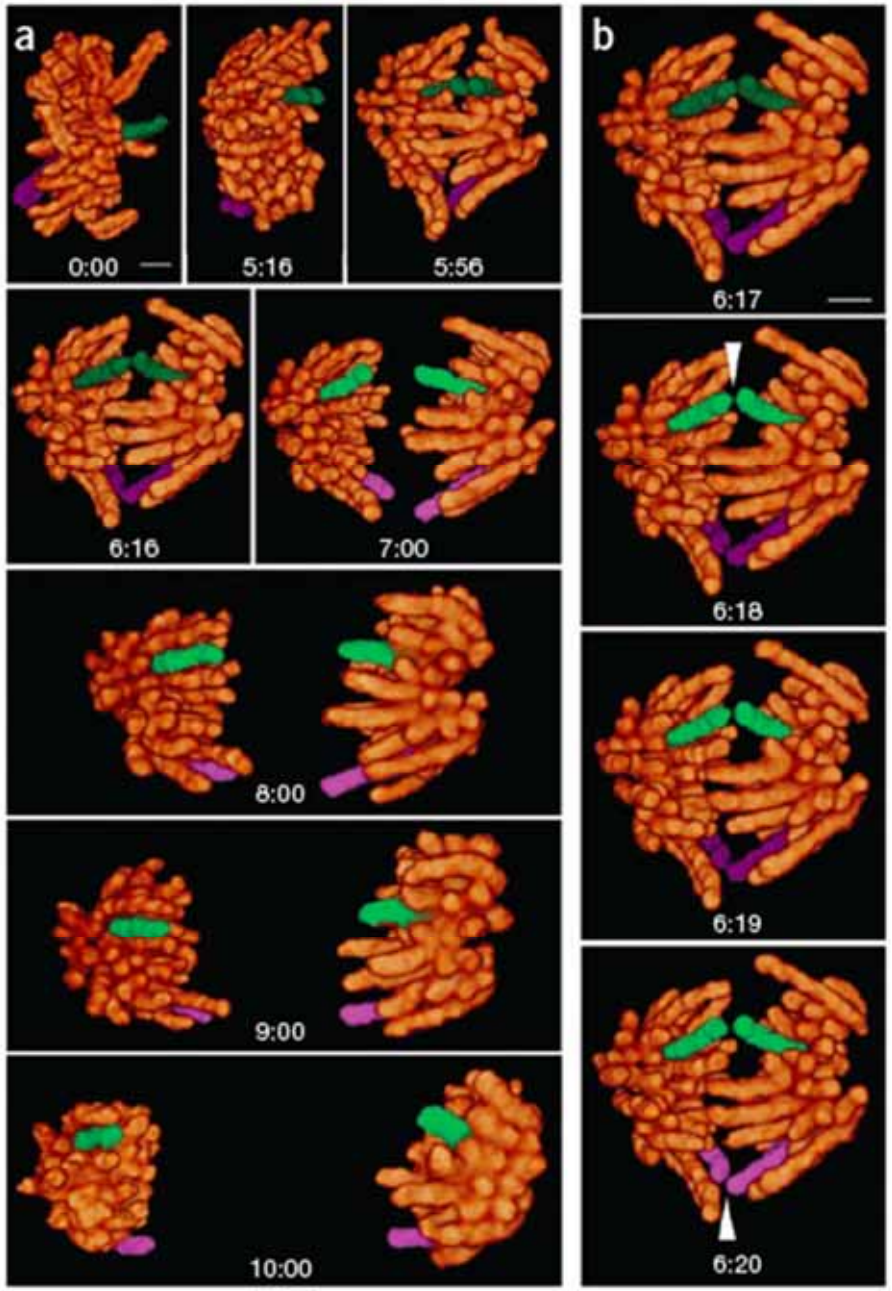
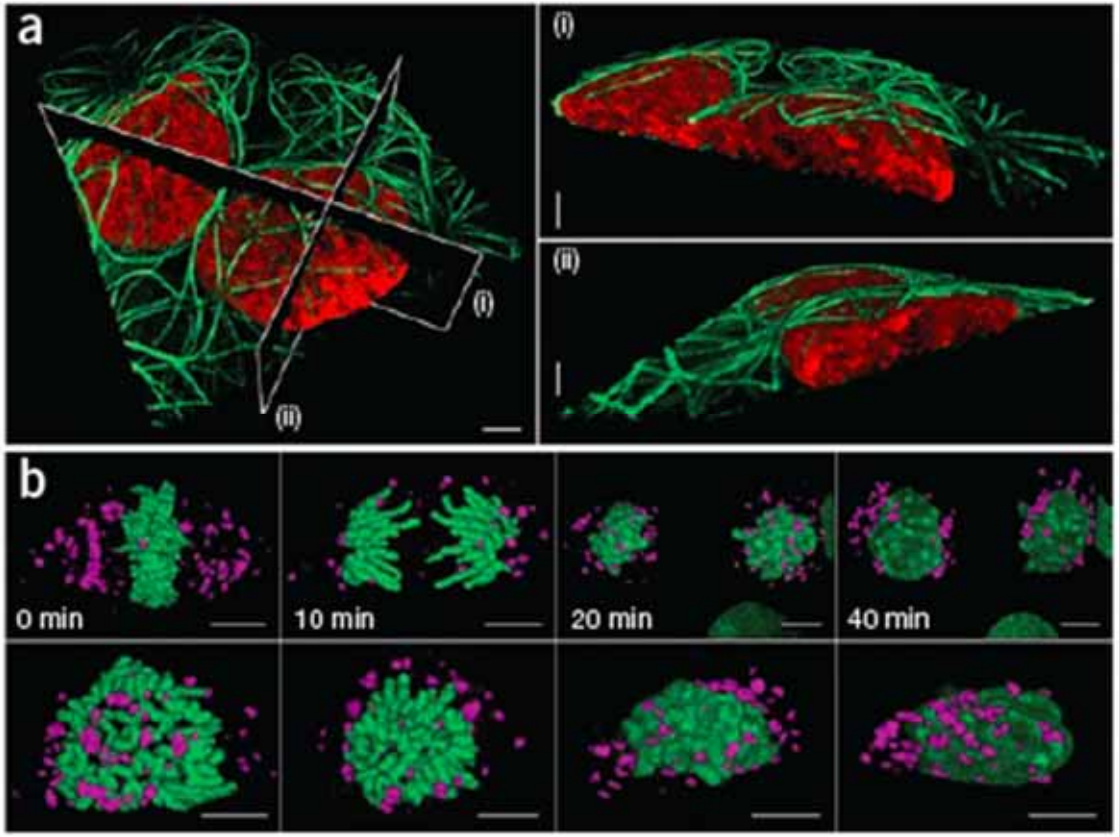


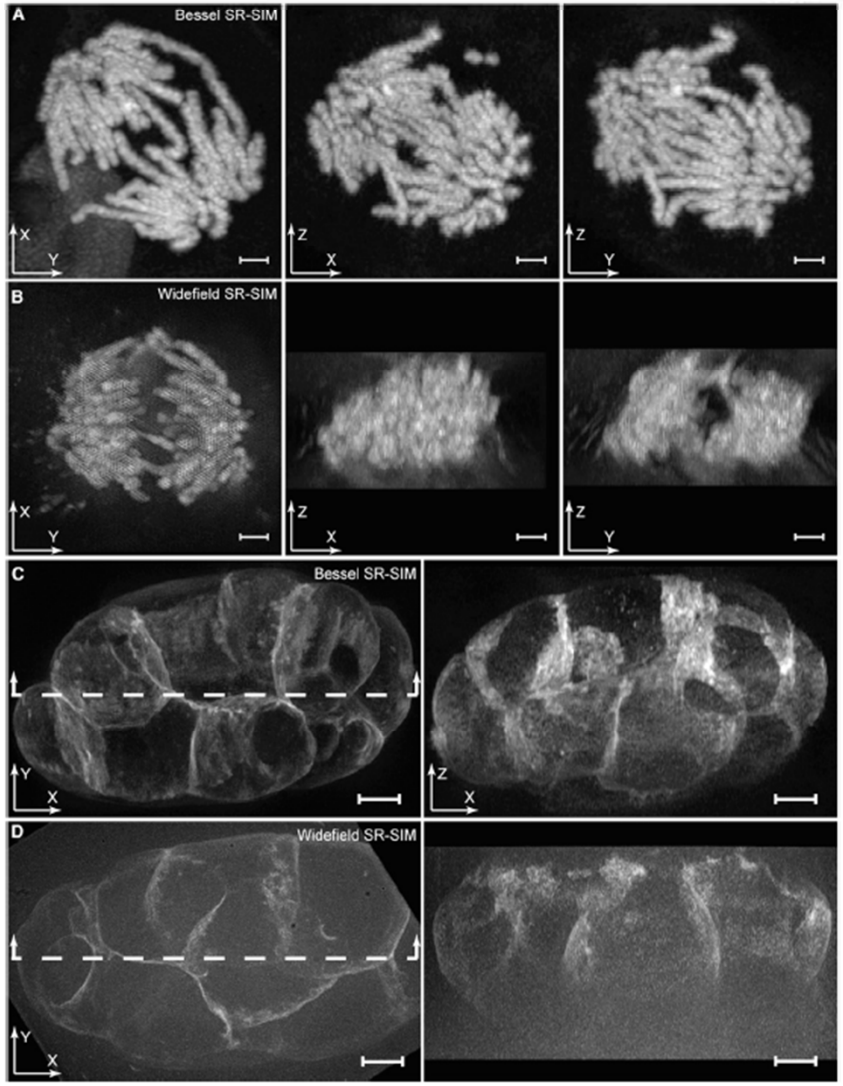
Figure 2 | Modes of Bessel beam plane illumination microscopy. (**a**) Wide-field illumination geometry (left) and maximum-intensity projection (MIP) in the *x*–*z* plane (right; *z*, detection axis) from a 3D image stack of a fixed human osteosarcoma cell (U2OS) transfected with plasmids encoding mEmerald fused to human pyruvate dehydrogenase alpha 1 (PDHA1). (**b**) Bessel sheet mode geometry (left), showing fluorescence excitation from Bessel side lobes (light green) as well as the central peak (dark green), and *x*–*z* plane MIP (right) from same cell as in **a**. (**c**,**d**) Bessel SI mode geometry, showing periodic Bessel beam excitation pattern (left) and *x*–*z* plane MIPs with single-harmonic (**c**) and multiharmonic (**d**) excitation (right). (**e**) Two-photon excitation (TPE) Bessel sheet mode geometry (left), showing infrared excitation (red) of fluorescence in the central peak (green), with negligible fluorescence in side lobes and *x*–*z* plane MIP from a cell (right) similar to those in **a**–**d**. (**f**) Volume rendering in the multiharmonic SI mode (9 phases, 2.4 μm period) of mEmerald-tagged microtubule associated protein 4 (MAP4) in a live U2OS cell. (**g**) Volume rendering in the TPE sheet mode of mEmerald-labeled mitochondria in a live pig kidney epithelial cell (LLC-PK1 cell line). Insets in **f** and **g** show MIPs along orthogonal axes of the cubical volumes shown. Scale bars, 10 μm except 3 μm in insets.


a
0:00
5:16
5:56
6:16
7:00
8:00
9:00
10:00
b
6:17
6:18
6:19
6:20


a
(i)
(ii)
(i)
(ii)
b
0 min
10 min
20 min
40 min

Noninvasive Imaging beyond the Diffraction Limit of 3D Dynamics in Thickly Fluorescent Specimens

1370 Cell *151*, 1370–1385, December 7, 2012 ©2012 Elsevier Inc.



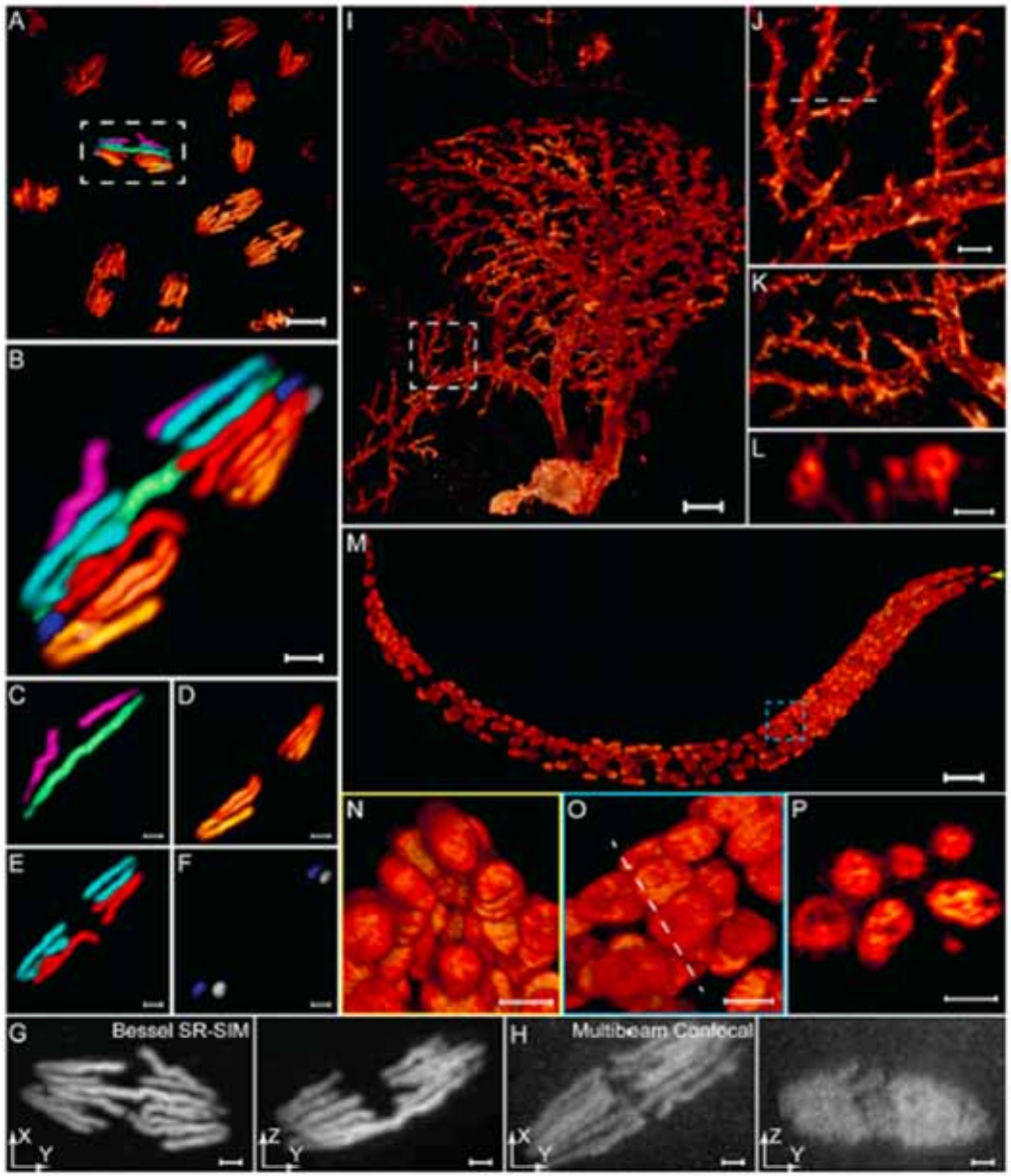


Figure 7. Two-Photon Bessel Beam Plane SR-SIM of Larger Multicellular Specimens

(A) Chromosomes in mitotic nuclei at the surface of a *D. melanogaster* embryo.

(B) Zoom view of the boxed chromosome in (A), with chromatids individually colored.

(C) Sex chromosomes isolated from that shown in (B), identifying the embryo as female.

(D–F) Autosomes 2, 3, and 4 isolated from that shown in (B).