Supplementary Material: A multi-mode digital holographic microscope

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A. 3D reconstruction with inline holography

Figure S1: Full frame of the bacterial data acquired using inline holographic microscopy and shown in figure 3a−c.
Figure S2: Localizing bacteria in 3D using inline holographic microscopy.
Figure S3: Video scrolling through the reconstructed 3D volume of *E. coli* data, acquired by inline holography.

B. 3D reconstruction with dark field off-axis holography

Figure S4: Procedure for recovering the object term in dark field off-axis holography.
Figure S5: Localizing gold nanoparticles in 3D using dark field off-axis holography.

C. Phase unwrapping with bright field off-axis holography

Figure S6: Procedure for unwrapping phase to generate a phase map of samples imaged using bright field off-axis holography.

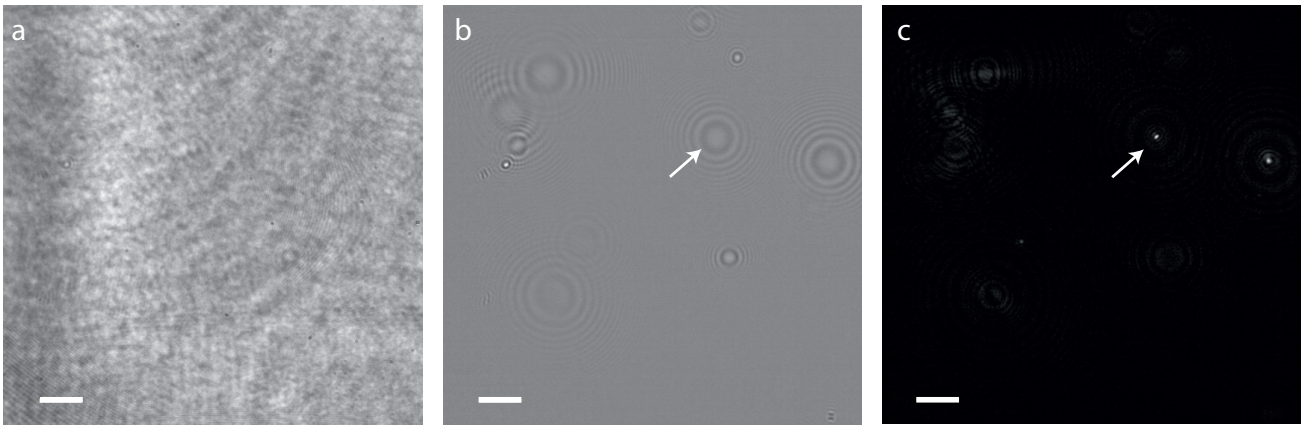

FIG. S1. Full frame images of the bacterial data shown in figure 3a−c. The sample is a dilute suspension of wild type *E. coli* in a tape tunnel slide recorded at 45× magnification. (a) is the raw inline hologram; (b) is the normalized hologram; (c) is a reconstructed plane at 60.8μm above the hologram plane. The arrows indicate the bacterium shown in figure 3a−c. Scale bars are 10μm.

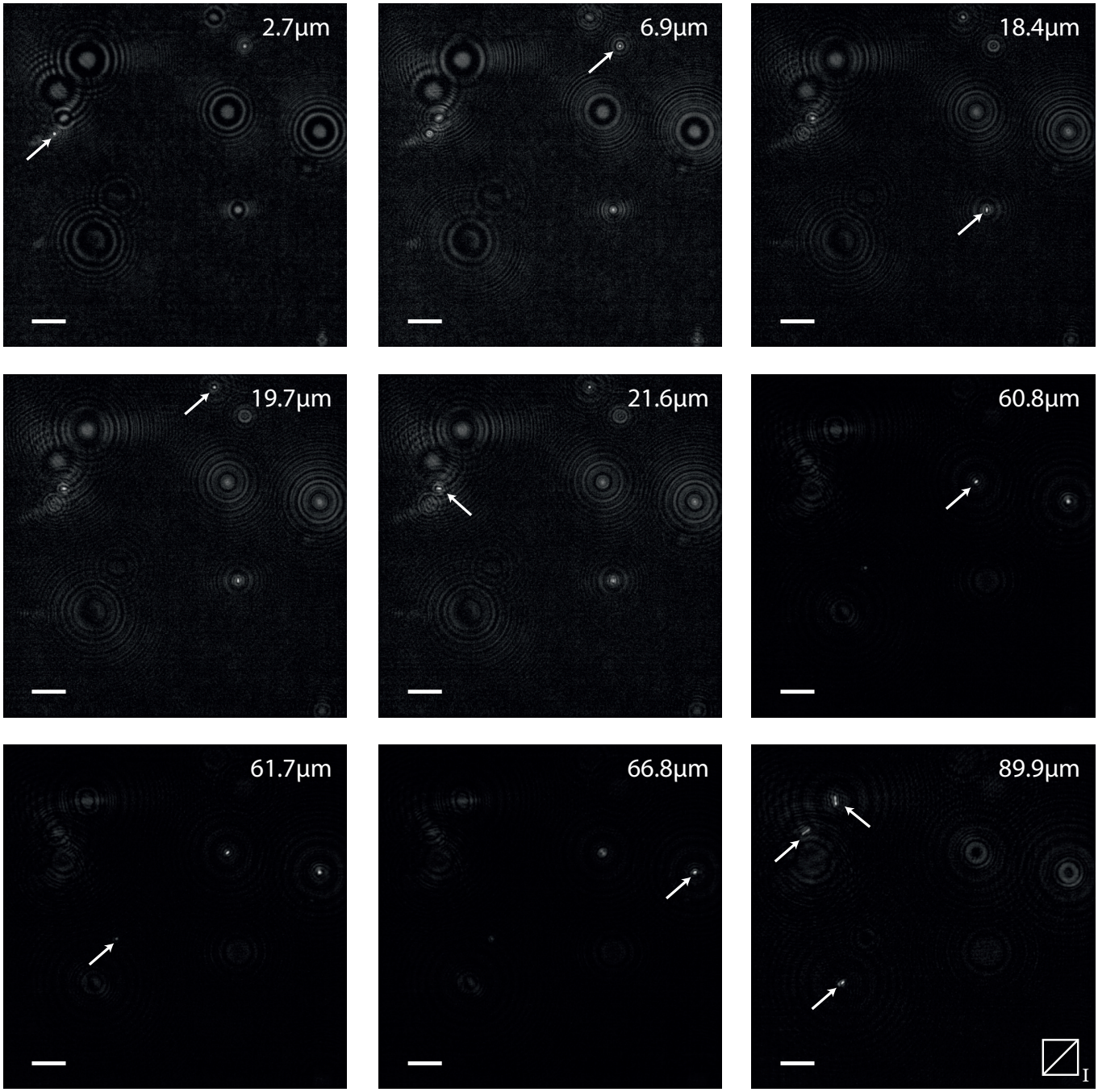

FIG. S2. Reconstructed planes of a single frame of an inline holography time series of a sample of motile *E. coli*. The numbers indicate the location of the reconstruction from the sample plane along the optical axis and are chosen to highlight bacteria that are in focus in that particular plane. Eleven bacteria are identified in this particular frame. The characteristic rod shape of E. coli is visible in several planes (notably at 21.6μm and 89.9μm) where the cell is parallel to the reconstruction plane. The axes in the lower right image show the scale used to display the images: intensity (I) is along the horizontal axis and gray level (0−255) is along the vertical axis. Scale bars are 10μm.

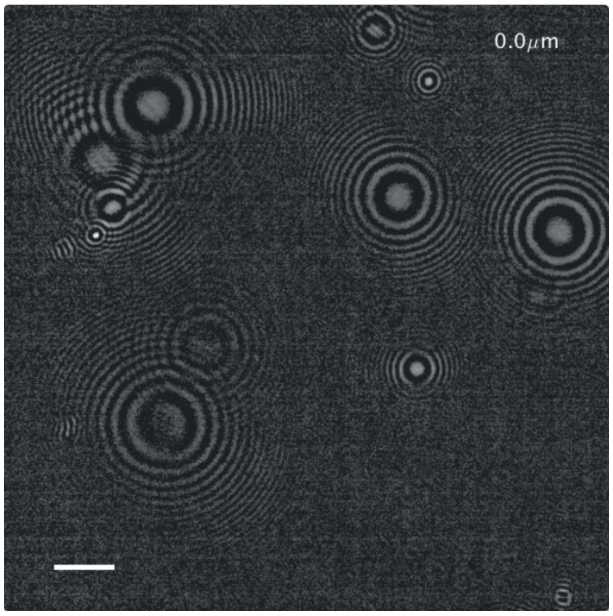

FIG. S3. Video demonstrating a scroll-through a reconstructed volume of a single frame of an inline holography time series of a sample of motile *E. coli*, as per figure S2. The video scrolls from the hologram plane to 100μm above the hologram plane in 0.5μm steps. The scale bar is 10μm.

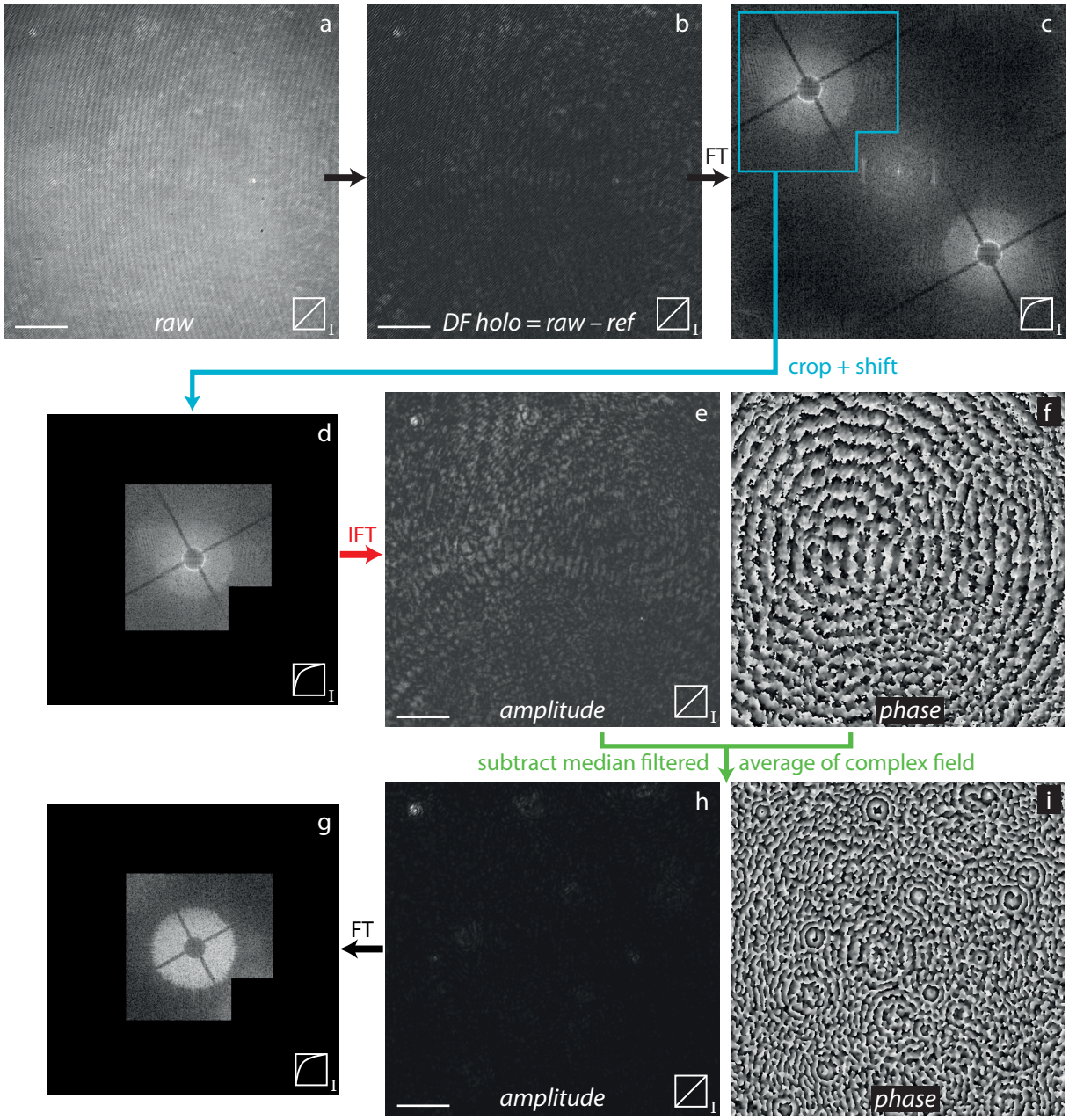

FIG. S4. Procedure for recovering the object term in dark field off-axis holography. (a) shows a raw off-axis hologram of a field of 100nm gold nanoparticles illuminated under dark field conditions. A separate recording of the reference beam is subtracted, resulting in the dark field hologram (b), whose Fourier transform (FT) is shown in (c). The spatial frequencies of the desired object term are cropped and shifted to center of the Fourier plane (d). An inverse Fourier (IFT) transform recovers the amplitude (e) and phase (f) of the object field. In this case, the light scattered from the ball bearing mask occludes the majority of the signal from the nanoparticles. The spatial frequencies of this scattered light can be seen as a bright ring around the ball bearing mask in (d) and the amplitude and phase of this light shows as noise in (e) and (f) respectively. The effect of this noise is eliminated by subtracting a complex field reconstructed from a median filtered average of all frames in the video series, which removes the bright ring around the ball bearing in the Fourier transform (g) and most of the noise in the reconstructed amplitude (h); the reconstructed phase (i) now clearly shows the characteristic ring patterns of the nanoparticles. The axes in the images show the scale used to display the images: intensity (I) is along the horizontal axis and gray level (0−255) is along the vertical axis. Scale bars are 5μm.

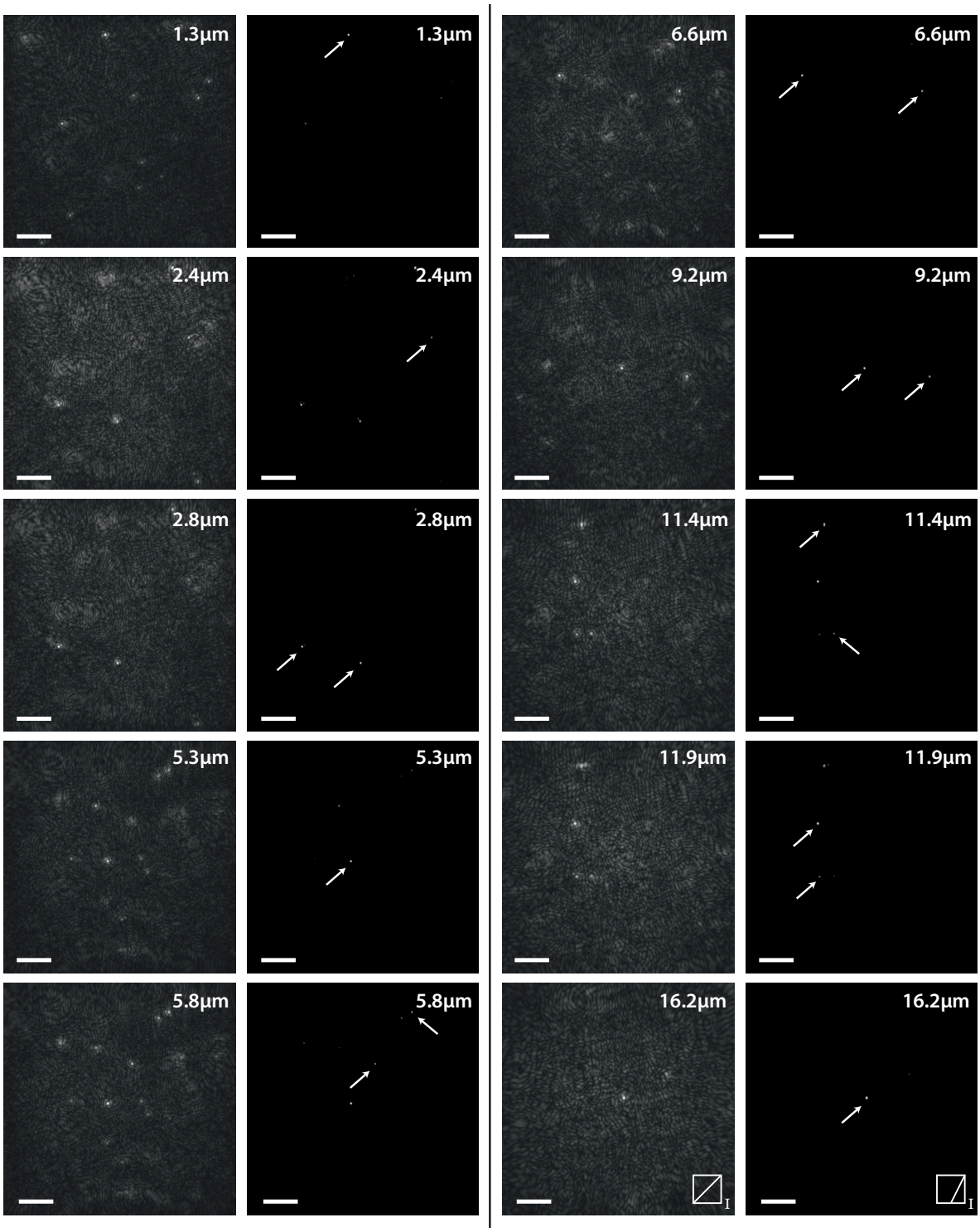

FIG. S5. Intensity reconstructions of a single frame from a time series dark field off-axis holography recording of 100nm gold nanoparticles. The left hand image in each pair shows the intensity of the reconstruction at the indicated distance from the focal plane along the optical axis. The right hand image is the same reconstruction with a threshold applied. This is used to determine the 3D positions of the nanoparticles. Sixteen distinct nanoparticles are indicated by the white arrows. The axes in the lower right pair of images show the scale used to display the images: intensity (I) is along the horizontal axis and gray level (0−255) is along the vertical axis. Scale bars are 5μm.

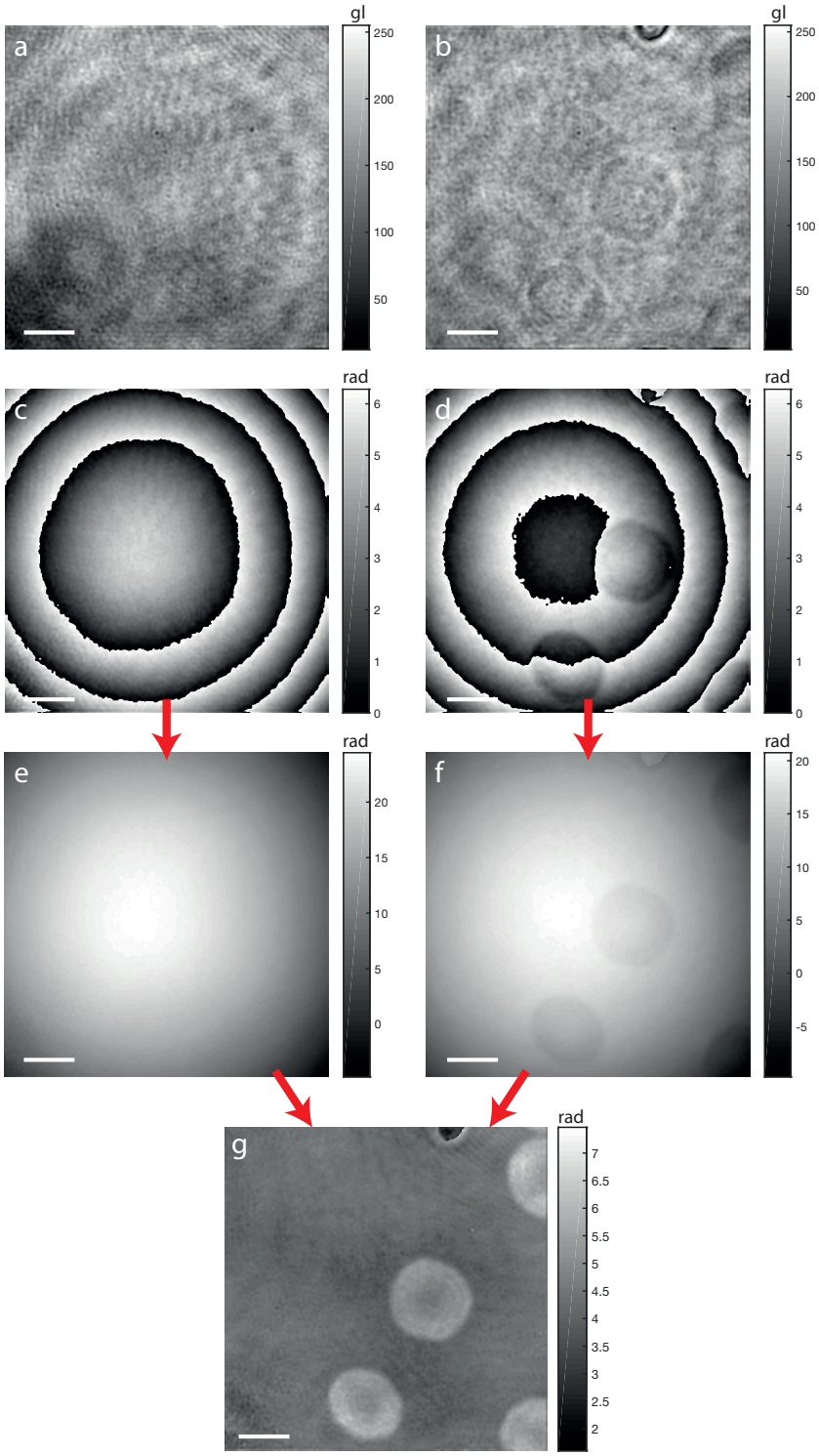

FIG. S6. Procedure for phase unwrapping using bright field off-axis holography. The sample shown is a suspension of human erthyrocytes in saline solution. A quarter of the CMOS chip is used to acquire bright field off-axis holograms. (a) and (b) show the reconstruction of the amplitude of images of the background (no blood cells in the field of view) and sample of interest, respectively. The color bar indicates gray level (gl) of the 8-bit image. (c) and (d) show the phase reconstruction of the respective views. Our microscope is sensitive to the absolute phase difference between object and reference beams, as shown by the circular wave pattern most clearly in the background phase. Color bars indicate phase in radians. These phase maps are unwrapped, shown in (e) and (f) and the difference map (g) is arrived at by subtracting the unwrapped background phase from that of the sample. Scale bars are 5μm.