This document provides supplementary information to "In-vivo volumetric imaging by crosstalk-free full-field OCT," https://doi.org/10.1364/OPTICA.6.000608. It encompasses a detailed description of the crosstalk free Fd-FF-OCT apparatus, a phantom sample, and animations of fly-through 3-D reconstructions of human forearm skin in vivo in the XY, XZ, and YZ planes.

1. EXPERIMENTAL SETUP

The imaging apparatus is a modified Fourier domain, Full-Field Optical Coherence Tomography (Fd-FF-OCT) system with a Deformable Membrane (DM) and a pair of galvo scanners placed in front of a Linnik interferometer (Fig. S1). Acquisition is performed with a fast CMOS camera (Fastcam SA-Z, pixel well depth 16 000 e, dark noise 29 e –) which allows 12-bit images to be collected at a rate of up to 21 GS/s. Light from a tunable laser light source (Broadsweeper) is delivered to the optical setup by a single mode fiber. The surface of the DM is imaged by lenses L1 and L2 (both achromatic doublets of f = 50 mm) forming a 4-f system on one of the mirrors in the galvanometric scanners (G) (2-axis galvo mirrors from Thorlabs GVS 202), which serves as an element enabling angular compounding. Another 4-f system composed of the achromatic doublet lenses L3 and L4 (both f = 75 mm) is used to transfer images from the DM to the non-polarizing beam splitter (BS). Images of the DM surface are formed on the reference mirror and the object by a set of identical optical elements – achromatic doublet L5 or L6 (f = 50 mm) and the 10X microscope objective M01 or M02 (NA = 0.3) in the 4-f systems. Thus, the membrane is placed before the interferometer, at planes conjugated to the sample. An image of the sample and reference radiation are recombined and superimposed on the camera with another 4-f system formed by achromatic doublet lenses L7 and L8 (f = 50 mm and f = 200 mm, respectively), ensuring appropriate magnification. In order to maximize the sensitivity of the setup we matched optical and digital resolutions by setting the magnification of the system to image 1.8x1.8 µm² on a single camera pixel (twice oversampling).

Fig. S1. Cross-talk free Fd-FF-OCT instrument: detailed scheme of the optical set-up also presented in Figure 2. L1-L8 achromatic doublet lenses; GM and M – pair of galvo scanners arranged in classical 2-axis configuration and introducing additional angular compounding; M01 and M02 - 10X Olympus objectives with 0.3NA; RM – reference mirror; BS – beam splitter; NDF – neutral density filter; TS – translational stage; Ob – object; DM – deformable membrane; DET – CMOS camera. Red beam corresponds to the unmodulated beam showing the formation of the illuminating beam on the object. Grey color corresponds to the reference beam. Light back-scattered from the object fills the objective pupil plane. Inset shows formation of the image of the DM surface on the object- the size of active DM elements and magnification of the system both determine the effective size of the Spatial Coherence Area.
2. TRANSVERSE RESOLUTION
In order to characterize the transverse resolution, we performed a classical measurement of the Optical Transfer Function (OTF) using Ronchi Rulers (Thorlabs Combined Resolution and Distortion Test Target). First, we characterized the transverse resolution of the basic microscopic set-up with a blocked reference arm by acquiring camera screen shots for Ronchi Rulers varying from 20 to 200 lines per mm, shown in Fig S2(c). For this experiment the Broadsweeper was set to emit quasi-monochromatic light and the DM was turned on. Normalized OTF is shown in Fig S2(a). In this particular case the phase modulation introduced by DM is small enough to not affect the Gaussian profile of the illuminating beam. Therefore, we could still use an approximation of Gaussian optics to calculate PSF, shown in Fig S2(b), and determine the transverse resolution as Full Width Half Maximum of the Gaussian profile to be 3.5 µm. In order to determine the transverse resolution of cross-talk free Fd-FF-OCT we performed an interferometric measurement of the USAF Resolving Power Test Target (Edmunds Scientific) with swept optical frequencies and DM turned on. We identified the 4th element of the 7th group to be the smallest resolvable with full contrast and clearly visible bars. The width of 1 line of this group is 2.76 µm indicating that we improved transversal resolution to 2.5 µm by applying both the Spatial and Temporal Coherence Gating (SCG) giving the final value of the transverse resolving power of the cross-talk free Fd-FF-OCT.

Fig. S2. Characterization of transverse resolving power of cross-talk free Fd-FF-OCT: a. Normalized Optical Transfer Function. Red curve – Gaussian fit; green curve – Gaussian function plotted based on the fitted parameters. b. Point Spread Function calculated as FFT of the Gaussian reconstruction in Figure a. c. Representative image of the Ronchi Ruler acquired without interference; d. Fd-FF-OCT reconstruction of the surface of the USAF Resolving Power Test Target; e. Zoomed detail of the enface cross-sectional image from panel d.

3. MEASUREMENT OF BIOLOGICAL SAMPLE IN VITRO
To demonstrate an improvement of crosstalk free imaging we acquired two sets of data measured with- and without DM and compared it to OCT reconstructions acquired by scanning OCT set-up. As a sample we used a portion of thermally processed pork skin. The results are shown in Figure S3. The contrast is clearly improved in both XZ and XY cross-sectional image with DM On. It is hard to identify biological structures after thermal processing of in vitro skin but still the details visible in XY projection obtained by crosstalk free technique differ significantly from those imaged by the scanning OCT set-up. For this comparison we used the scanning OCT system described in the section 5 of the main text.

Fig. S3. Comparison of crosstalk free Fd-FF-OCT, Fd-FF-OCT without spatial phase modulation and scanning OCT imaging performed on pork skin in vitro.

4. OPTIMIZATION OF THE SENSITIVITY AND THE DYNAMIC RANGE
Limited optical power density of light emitted by the Broadsweeper and illuminating the sample (20 nW per voxel) results in relatively low values of the Sensitivity (around 70dB) and the DR (around 60dB) for single shot Fd-FF-OCT measurements. Such a Sensitivity level is below requirements for in vivo OCT imaging. Therefore, we decided to perform multiple averages over measurements to improve both parameters. Figure S4 shows Sensitivity and DR as a function of the number of averaged volumes. Saturation of both parameters at 30-40 averages is caused by the presence of residual structured noise corresponding to parasitic reflections from optical elements of the system (coherence noise).

Fig. S4. Measured Sensitivity and Dynamic Range in Fd-FF-OCT as a function of the number of averaged volumes.

These parameters may be improved in the future if specular reflections from optical components and their mounts are carefully removed. Based on the measurements presented in Fig. S4 we decided to perform 36 averages corresponding to the plateau of the Sensitivity curve at 93dB and DR curve at 75dB, which results in a total acquisition time of 0.7 s. Possible bulk motion of the sample was corrected in the post processing by careful registration of volumes based on three dimensional cross-correlations. In the case of sensitivity measurement, optical power coming from the sample arm was attenuated 3x10^4 times that enabled suppression of some residual interferences coming from random reflections in the system, which dominate in DR measurements.
5. Efficiency of Angular Compounding

In order to take advantage of multiple measurements and averaging process we additionally used angular compounding to reduce the contrast of the speckle field and increase SNR. To analyze the efficiency of angular compounding we applied a method described earlier by Desjardins et al. [1, 2]. The extent to which the speckle contrast is decreased by the angular compounding depends on the level of decorrelation between volumes acquired with different illumination angles. The maximum number of independent illumination angles defines the number of decorrelated volumes and it depends on the total span of accessible angles and the angle range within which correlation remains. In order to quantify the number of decorrelated volumes acquired within the accessible range of illumination angles (limited by the NA) we calculated the normalized autocorrelation function of amplitude variations as a function of consecutive angles of illumination. Normalized autocorrelations were calculated for 600 arbitrarily chosen points of Fd-Ff-OCT reconstruction and averaged out to reduce the influence of static scatterers with dimensions similar or larger to the size-scale of the resolution. FWHM of the autocorrelation peak was used to estimate the effective number of different angles that contribute to the speckle contrast reduction. In order to test the efficiency of the speckle reduction with defocus the analysis was repeated for 22 axial positions of the object (corresponding to 440 µm of defocus). To have fully controlled experimental conditions we imaged a silver mirror with a rough surface made by deposition of silver nanoparticles on a silicon base. The surface roughness did not exceed 500 nm – hence we observed only a single layer in OCT reconstructions and we precisely adjusted the position of the mirror surface with respect to the objective lens. Results are shown in Figure S5. The number of decorrelated images increases with the introduced defocus – effectively causing more scatterers to contribute to the speckle formation for defocused images. The width of the autocorrelation function decreases with the introduction of larger defocus, meaning that a smaller angular shift is required to achieve decorrelation with introduced defocus. Hence, there is larger number of independent illumination angles available as the range of illumination angles is fixed and depends on the optical setup (NA). The number of decorrelated volumes in focus is only 3.5times giving 2x reduction in speckle contrast (6 deg out of 20 deg of the full angular span – Fig S5b). It increases to 12 uncorrelated volumes for the introduced defocus of ~150 µm, resulting in 3.5x reduction of speckle contrast (1 deg out of 20 deg of the full angular span – Fig S5b). Defocusing also results in worsening transverse resolution to the value of 12 µm. We expect that the speckle contrast reduction is slightly better in real in vivo imaging situations, but it is nearly impossible to perform such measurements since it is hard to find a perfectly optically homogeneous object to measure the true number of decorated images.

Fig. S5. Speckle reduction introduced by angular compounding: a. Number of decorrelated volumes as a function of defocus in the object arm is shown in red squares; angle is changed by 7x10⁻³ rad between the measurements; for comparison purposes the transverse resolution is shown as a function of defocus (blue circles); b. Averaged autocorrelation as a function of angle for focused (bottom) and defocused (top) cases.

6. Fabrication Process of the 3-D Scattering Phantom

Scattering phantoms were prepared using reusable epoxy stamps, which were fabricated by means of a soft-lithography based technique (Fig.S6). First, a multilevel pattern was defined on a silicon wafer using two-step photolithography (SU-8 2100, MicroChem Corp.). The first layer of the pattern contained an array of IPC logotypes ("IChF") with letters of width 100 µm, height 700 µm and thickness about 110 µm. The second layer consisted of four supporting corners 1 mm wide and about 220 µm high. The pattern was designed using Autocad 2016 software (Autodesk) and printed on low-resolution 3000 dpi photomasks (Prepress Service S.C.). The finished relief pattern on the silicon wafer was then transferred to a negative PDMS mould (Sylgard 184). In order to reduce its susceptibility to deformation in high temperatures, the negative mould was permanently bonded to a thick glass plate using corona plasma treater (BD-20ACV, Electro-Technic Products). In the next step, an epoxy stamp was cast against the negative PDMS mould. For this purpose, Conapoxy® FR-1080 was used according to the attached manual. After removing the cured stamp from the PDMS mould, it was levelled with a grinder and washed.

Fig. S6. Epoxy stamp - top and front view (left) and expanded scheme of the 3-D scattering phantom (right).

The phantom was composed of two scattering layers immersed in a transparent material. Preparation of both layers started with production of thin membranes with microstructures made of transparent PDMS. First, PDMS was poured onto the epoxy stamp and degassed for a better penetration of microcavities. Next, a microscope glass slide was pressed against the stamp squeezing out excess PDMS. The “sandwich” was secured using “bulldog” paper clips on opposite sides and put into an oven for curing (75°C, ~30 minutes). The stamp was carefully removed from the cured membrane, leaving it attached to the glass slide. PDMS mixed with TiO2 (10 mg/mL, Kremer Pigments) was poured onto the membrane and degassed. A bottom, thicker layer was then placed directly into the oven. The top layer was first squeezed with a second glass slide using a paper clip and left for curing with the bottom layer (75°C, ~30 minutes). Finally, two layers were aligned and bonded with corona treater.
7. VISUALIZATION OF MEMBRANE DYNAMICS

In order to understand the dynamic performance of the deformable membrane we performed two additional measurements by using a camera with sub microsecond integration time $T_{exp}$. We placed the membrane in the sample arm of the on-axis interferometer and used a monochromatic laser to record holograms, shown in Fig. 7.a. Thanks to the short integration time we were effectively able to "freeze" the phase modulation introduced by the membrane and analyze the phase masks introduced by the membrane. Multiple rings around "active" elements suggest that the phase shift ('stroke') should be larger than the wavelength. Another panel (Fig. 7.b) shows a time-lapse of the bright-field illumination (recorded without the reference arm) field incident on the sample in out-of-focus case. The DM introduces phase modulation to produce a "wrinkled" illumination away from the focal plane. Once in the focal plane the illumination is homogenous (see Fig. 3 in the main text.)

![Fig. 7.](image)

**Fig. 7.** a. Interference image of DM recorded with the shutter speed of 6.3 Mfps (integration time of ~0.15 μs, image size 1 x 1mm). b. Time series of out-of-focus patterns of DM recorded every 50 μs with the shutter speed of 2.3 Mfps (integration time of ~0.4 μs).

**LIST OF VISUALIZATIONS**

- Visualization S1
  - presents the fly through 3-D cross-noise free Fd-FF-OCT reconstruction of human forearm skin in vivo in the XY plane

- Visualization S2
  - presents the fly through 3-D cross-noise free Fd-FF-OCT reconstruction of human forearm skin in vivo in the XZ plane

- Visualization S3
  - presents the fly through 3-D cross-noise free Fd-FF-OCT reconstruction of human forearm skin in vivo in the YZ plane

**References**

1. A. E. Desjardins, B. J. Vakoc, G. J. Tearney, and B. E. Bouma, "Speckle reduction in OCT using massively-parallel detection and frequency-domain ranging," Opt. Express **14**, 4736-4745 (2006).

2. A. E. Desjardins, B. J. Vakoc, W. Y. Oh, S. M. Motaghiannezam, G. J. Tearney, and B. E. Bouma, "Angle-resolved optical coherence tomography with sequential angular selectivity for speckle reduction," Opt Express **15**, 6200-6209 (2007).