Supplemental Document

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Modeling nonlinear microscopy near index-mismatched interfaces: supplement

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DATA AVAILABILITY

The data (experimental data and code) used to generate the figures is archived on Zenodo[1], and will be updated on GitHub (https://github.com/NOLab/FDTD_for_NL_microscopy)

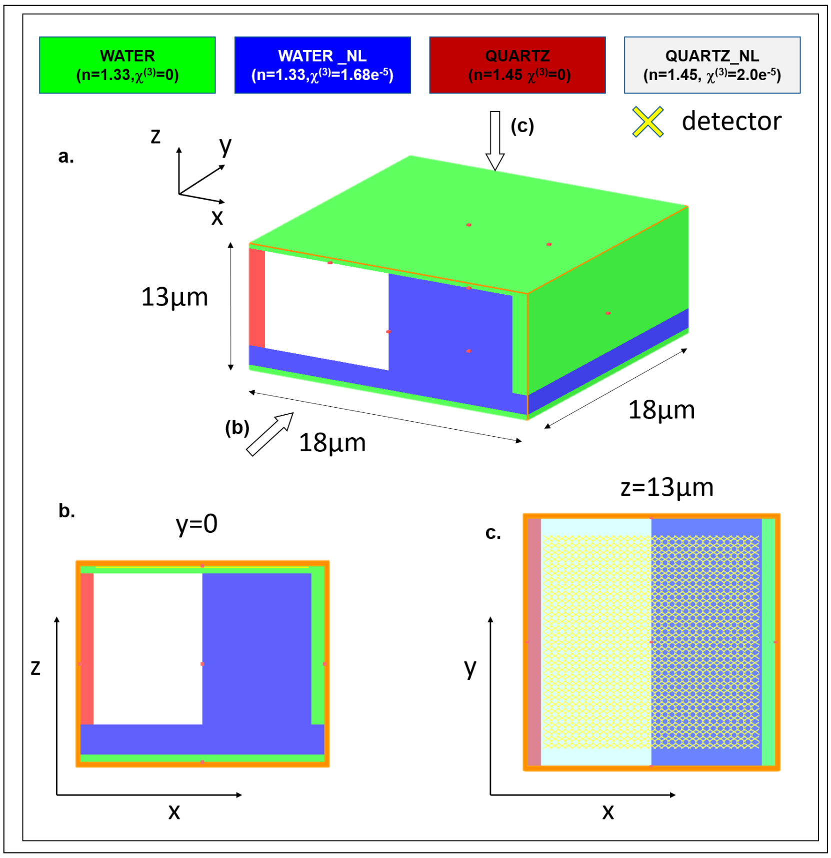

Fig. S1. Schematics of the THG FDTD simulation domain used for Figures 2-5. (a) 3D rendering of the simulation domain with materials represented by different colors. (b) side view (y=0) (c) top view showing the detector array at the end of the simulation domain. Note that light propagates from bottom to top in this figure (and top to bottom in the main text).

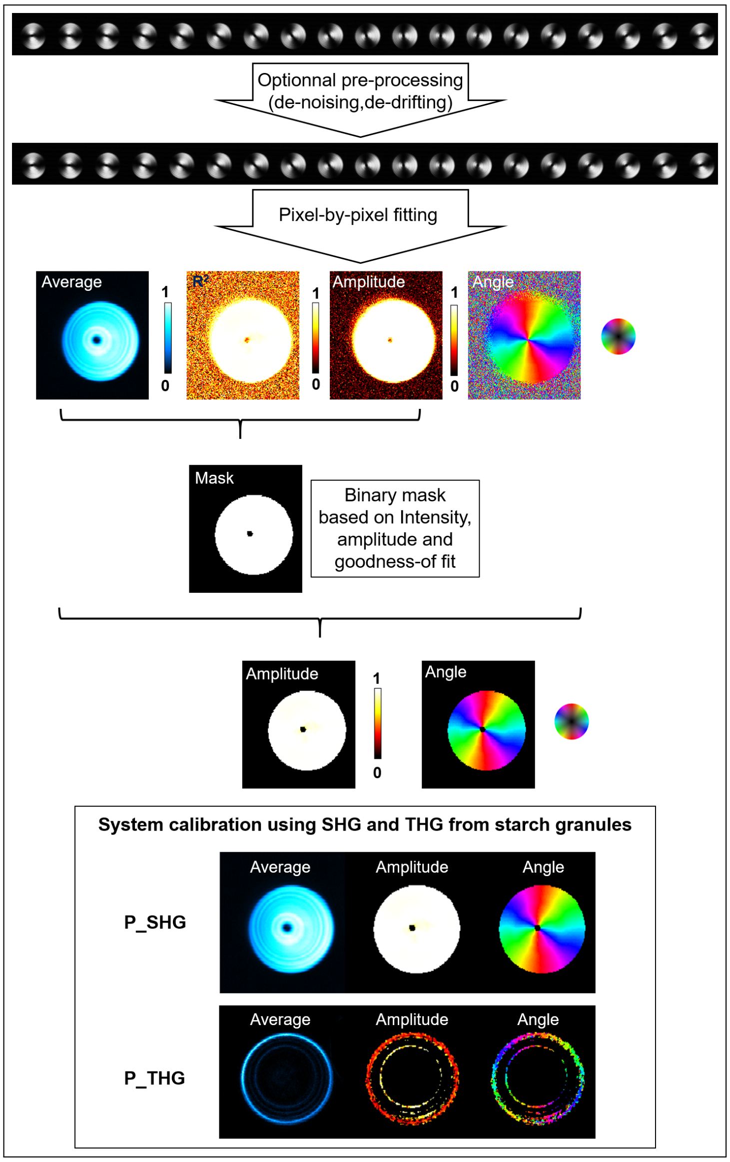

Fig. S2. PTHG image analysis workflow and example of calibration data on a starch granule. THG images taken with different polarizations were converted into a XY-P stack, with an optional de-noising / de-drifting step. Then each pixel was fitted with a function ($y = a + b\cos^2(x - c)$) to extract the average intensity, the amplitude and the phase (polarization angle yielding maximum signal) of the modulation. The goodness-of-fit parameter was also saved, and together with the average intensity and amplitude was used to generate a binary mask, which was then applied to the phase and amplitude maps for cleaner visualization. We show an example of the end result of the analysis pipeline on SHG/THG imaging of starch granules, which we performed before all experiments to correct for polarization offsets.

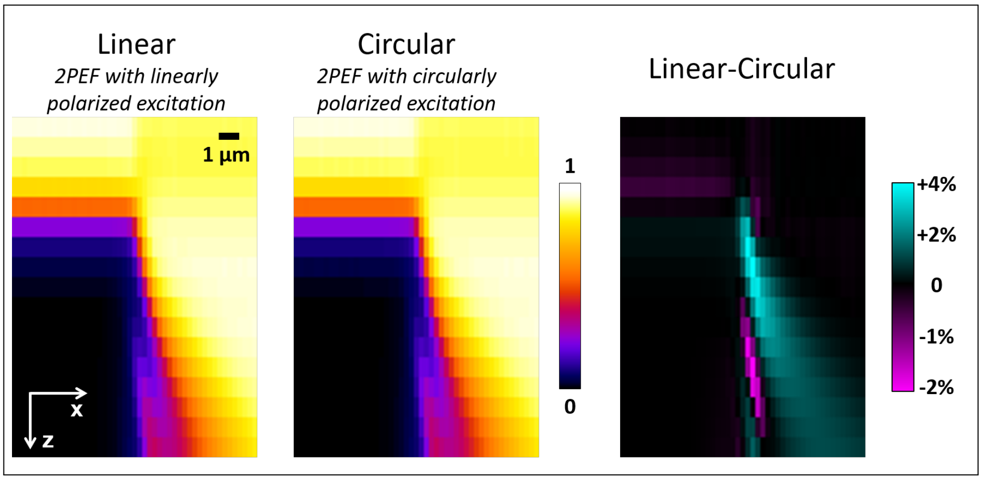

Fig. S3. FDTD simulation of 2PEF imaging near index-mismatched interfaces using linear and circular incident polarization. Simulation parameters were as in Figure 2. We simulated a region of $x \in [-6\mu m; 6\mu m]$ on both sides of the vertical interface and $z \in [-4\mu m; 12\mu m]$ around the horizontal interface. Sampling was $0.25\mu m$ along x and $1\mu m$ along z.

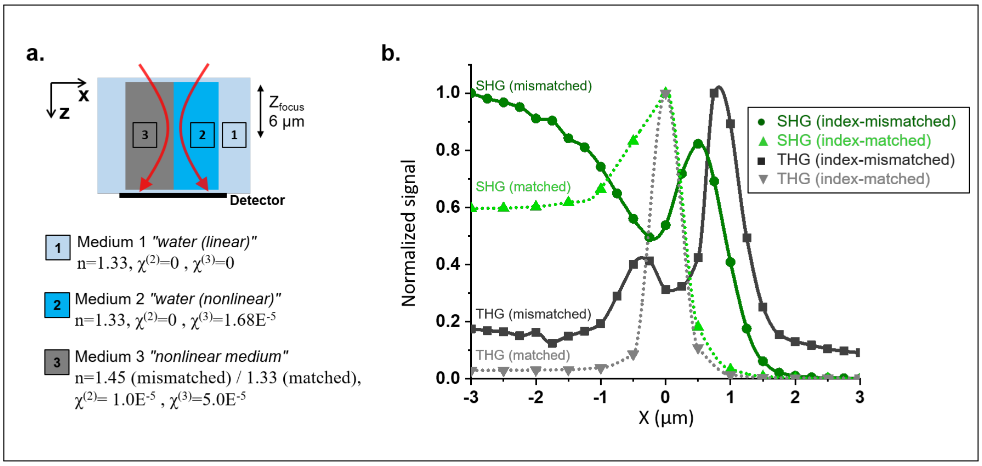

Fig. S4. FDTD simulation of SHG and THG near a vertical interface. In order to investigate the capability of FDTD methods to model SHG imaging in index-mismatched media, we considered the simple case of a vertical interface between water and a material with a non-zero diagonal second-order susceptibility. We simulated both SHG and THG profiles for comparison. (a) Simulation geometry and properties of the nonlinear materials considered. (b) FDTD calculations of SHG and THG when the excitation beam is scanned horizontally across a vertical interface at a depth of $6\mu m$. The results are shown in the case of an index-matched (n = 1.33/1.33) and for an index-mismatched (n = 1.33/1.45) interface. The dotted green curve shows the SHG profile for index-matched materials, and the plain dark green curve shows the SHG profile for index-mismatched materials. As seen in this simple model, index mismatch results in significant profile distortions, including a lateral shift of the peak signals both in the case of SHG and THG. These calculations suggest that taking these distortions into account is necessary for quantitatively interpreting SHG (and THG) images recorded in heterogeneous media. *As a word of caution, we note that in order to speed up calculations, the integration domains used for this simulation were reduced compared to the ones used in Figure 2, and the nonlinear susceptibility was chosen differently. Consequently the predicted index-mismatched THG profile slightly differ from the one shown in Figure 2. The general shape of the profiles remains however valid. Full quantitative analysis of SHG in index-mismatched samples will require additional studies beyond this simple illustration.*

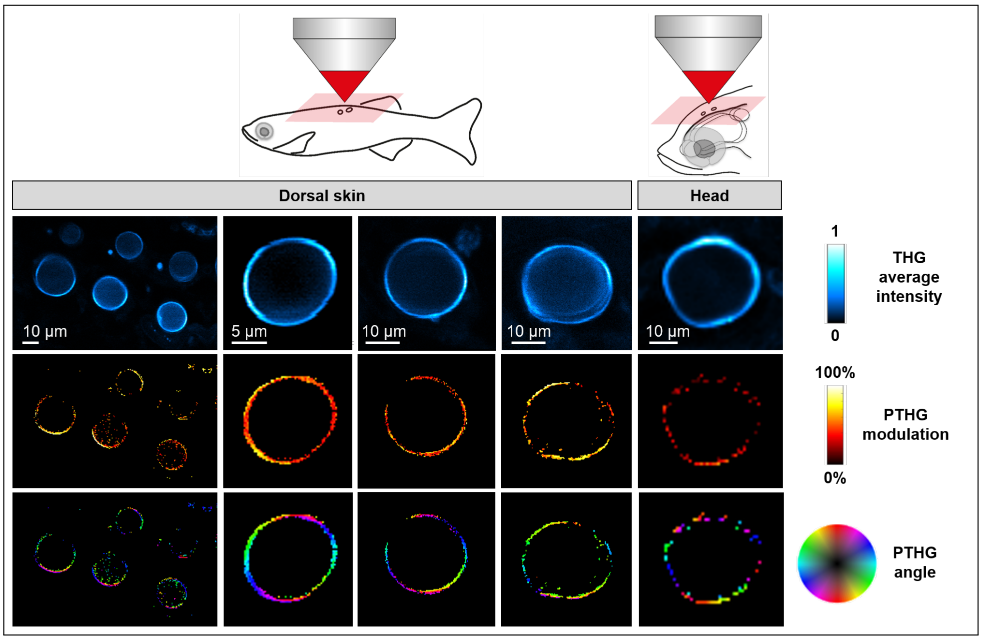

Fig. S5. PTHG imaging of lipid droplets in an adult Zebrafish. The first four columns present images recorded in the same dorsal region as in Figure 5 at a depth of 40-50 μm. The last column shows data recorded in another fish between skin and skull at a depth of 80-100 μm. Overall, these lipidic structures display consistent PTHG modulation values (30-60%) and angles, despite their differences in size and shape.

REFERENCES

1. “Fdtd for nl microscopy,” Zenodo, http://doi.org/10.5281/zenodo.4722857.