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SUPPLEMENTARY INFORMATION

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Harmonic optical tomography of nonlinear structures

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Supplemental Information for

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1. Propagation of second-harmonic fields in inhomogenous media

The propagation of the optical field in a static inhomogeneous medium, *U*, satisfies the nonlinear wave equation (see Chapter 2 in Ref. [1])

$$\nabla^2 U(\mathbf{r},t) - \frac{n^2(\mathbf{r})}{c^2}\frac{\partial^2 U(\mathbf{r},t)}{\partial t^2} = \mu_0 \frac{\partial^2 P_{NL}(\mathbf{r},t)}{\partial t^2}, \qquad [S1]$$

where n is the refractive index (RI) of the inhomogeneous medium at temporal frequency ω; c is the speed of light in vacuum; μ_0 is the magnetic permeability of free space. In Eq. S1, *U* consists of the incident, second-harmonic, as well as the scattered fields produced by the RI and second-order susceptibility inhomogeneities. Here, $n(\mathbf{r};\omega)$ is considered slowly varying at both fundamental and harmonic frequencies. For second-harmonic generation (SHG), the nonlinear induced polarization can be written as $P_{NL} = \varepsilon_0 \chi^{(2)}(\mathbf{r}) U^2(\mathbf{r},t)$, where ε_0 is the permittivity of free space, $\chi^{(2)}$ is the medium's second-order susceptibility, assumed to be instantaneous, as the frequencies are away from electronic resonances.

Taking the Fourier transform with respect to *t*, Eq. S1 can be expressed as

$$\nabla^2 U(\mathbf{r},\omega) + n^2(\mathbf{r},\omega)\frac{\omega^2}{c^2}U(\mathbf{r},\omega) = -\frac{\omega^2}{c^2}\chi^{(2)}(\mathbf{r})[U(\mathbf{r},\omega) \circledcirc_{\omega} U(\mathbf{r},\omega)], \qquad [S2]$$

where $\circledv_{\omega}$ is a convolution with respect to ω. For simplicity, a function shares the same symbol as its Fourier transform, but with different arguments (*i.e.*, $f\left(t\right) \leftrightarrow f\left(\omega\right)$, where $\leftrightarrow$ denotes the Fourier transform operation). Invoking the undepleted pump approximation[1], we consider that the generation of second-harmonic is only due to the contribution of the incident light at the fundamental frequency, U_i . For an incident field at frequency ω_0 , $U_i(\mathbf{r},\omega) = U_i(\mathbf{r})\delta(\omega-\omega_0)$, the right side of Eq. S2 can be simplified to

$$\nabla^2 U(\mathbf{r},\omega) + n^2(\mathbf{r},\omega)\frac{\omega^2}{c^2}U(\mathbf{r},\omega) = -\beta_2^2\chi^{(2)}(\mathbf{r})U_i^2\left(\mathbf{r}\right)\delta(\omega-2\omega_0), \qquad \text{[S3]}$$

where $\beta_2 = 2\omega_0 / c$, is the wavenumber of the second-harmonic light. In Eq. S3, we used the convolution property of the δ -function, namely,[2]

$\frac{\omega^2}{c^2}\left[\delta(\omega-\omega_0)\circledv_{\omega}\delta(\omega-\omega_0)\right] = \frac{\left(2\omega_0\right)^2}{c^2}\delta(\omega-2\omega_0)$. Separating Eq. S3 into two equations, one for the fundamental at ω_0 and one for SHG at $2\omega_0$, we obtain

$$\nabla^2 U^{\omega_0}(\mathbf{r}) + n^2(\mathbf{r},\omega_0)\beta_1^2 U^{\omega_0}(\mathbf{r}) = 0 \qquad \text{[S4a]}$$

$$\nabla^2 U^{2\omega_0}(\mathbf{r}) + n^2(\mathbf{r},2\omega_0)\beta_2^2 U^{2\omega_0}(\mathbf{r}) = -\beta_2^2\chi^{(2)}(\mathbf{r})\left[U_i^{\omega_0}\left(\mathbf{r}\right)\right]^2. \qquad \text{[S4b]}$$

The superscript ω_0 or $2\omega_0$ denotes a field's frequency, and $\beta_1 = \omega_0 / c$ is the wavenumber for the fundamental field. Eq. S4a describes the linear scattering process, where the total field (U^{ω_0}) is the sum of the incident and linear scattering components. The nonlinear scattering process described in Eq. S4b can be further split into two equations, describing the unscattered SHG field, $U_{\mathrm{SHG}}^{2\omega_0}$, and its scattered components, $U_s^{2\omega_0}$, such that $U^{2\omega_0} = U_{\mathrm{SHG}}^{2\omega_0} + U_s^{2\omega_0}$,

$$\nabla^2 U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{r}) + \left(\bar{n}_2\beta_2\right)^2 U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{r}) = -\beta_2^2 \chi^{(2)}(\mathbf{r}) \left[U_i^{\omega_0}(\mathbf{r})\right]^2 \quad \text{[S5a]}$$

$$\nabla^2 U_s^{2\omega_0}(\mathbf{r}) + \left(\bar{n}_2\beta_2\right)^2 U_s^{2\omega_0}(\mathbf{r}) = -\beta_2^2 \chi(\mathbf{r}) U^{2\omega_0}(\mathbf{r}), \quad \text{[S5b]}$$

where $\bar{n}_2$ is the spatially averaged refractive index, $\bar{n}_2 = \sqrt{\left\langle n^2\left(\mathbf{r}, 2\omega_0\right)\right\rangle_{\mathbf{r}}}$; $\chi(\mathbf{r})$ is the scattering potential, $\chi(\mathbf{r}) = n^2(\mathbf{r}, 2\omega_0) - \bar{n}_2{}^2$. Similarly to the linear scattering problem[3-5], Eqs. S5a and S5b can be solved in the spatial frequency domain $(\mathbf{k}_\perp, z)$, by applying the first-order Born approximation and selecting the forward-scattered field. Under this approximation, the analytic expression of $U_{\mathrm{SHG}}^{2\omega_0}$ and $U_s^{2\omega_0}$ can be written as

$$U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{k}_\perp, z) \approx \frac{\beta_2^2}{2\gamma_2} e^{i\gamma_2 z} \left[\chi^{(2)}(\mathbf{k}) \circledcirc_k U_i^{\omega_0}(\mathbf{k}) \circledcirc_k U_i^{\omega_0}(\mathbf{k})\right]\Big|_{k_z=\gamma_2}, \quad \text{[S6a]}$$

$$U_s^{2\omega_0}(\mathbf{k}_\perp, z) \approx \frac{\beta_2}{2\bar{n}_2} e^{i\gamma_2 z} \left[\chi(\mathbf{k}) \circledcirc_k U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{k})\right]\Big|_{k_z=\gamma_2}, \quad \text{[S6b]}$$

where $\mathbf{k}_\perp$ is the transverse spatial frequency, z indicates the axial translation, and $\gamma_2 = \sqrt{\left(\bar{n}_2\beta_2\right)^2 - \left|\mathbf{k}_\perp\right|^2}$. Under the first-order Born approximation, the refractive index has weak spatial fluctuations relative to the mean at the second harmonic frequency, $\chi(\mathbf{r}) \approx 2\bar{n}_2\left[n(\mathbf{r}, 2\omega_0) - \bar{n}_2\right]$, meaning that $n(\mathbf{r}, 2\omega_0) - \bar{n}_2$ is very small for biological specimens (typically ≤ 0.05 [6]). Thus, in the spatial domain, the convolution in Eq. S6b takes the form

$$\begin{aligned} \left|\chi(\mathbf{r}) U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{r})\right| &\approx \left|2\bar{n}_2\left[n(\mathbf{r}, 2\omega_0) - \bar{n}_2\right] U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{r})\right| \\ &\ll \left|U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{r})\right| \end{aligned}. \quad \text{[S7]}$$

Equation S7 shows that the magnitude of $U_s^{2\omega_0}$ is small compared to that of $U_{\mathrm{SHG}}^{2\omega_0}$, which suggests the effect of scattering of the SHG signal can be neglected for single scattering samples, *i.e.*, when the thickness of the specimen is below the transport mean free path. In addition, for an imaging system, the direction of the propagating field is limited by the system's pupil function. Including this factor, the SHG field reaching the image plane becomes

$$U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{k}_\perp, z) = \frac{\beta_2^2}{2\gamma_2} e^{i\gamma_2 z} A_o\left(\mathbf{k}_\perp\right)\left[\chi^{(2)}(\mathbf{k}) \circledvee_k U_i^{\omega_0}(\mathbf{k}) \circledvee_k U_i^{\omega_0}(\mathbf{k})\right]\Big|_{k_z=\gamma_2}, \quad \text{[S8]}$$

where $A_o\left(\mathbf{k}_\perp\right)$ is the aperture stop of the imaging optics, *i.e.*, the objective.

For an incident monochromatic plane wave, the fundamental field can be expressed as

$$U_i^{\omega_0}(\mathbf{k}_\perp, k_z) = A_i\left(\mathbf{k}_{i\perp}\right)\delta\left(\mathbf{k}_\perp - \mathbf{k}_{i\perp}\right)\delta\left(k_z - \gamma_1\right), \quad \text{[S9]}$$

where $A_i\left(\mathbf{k}_{i\perp}\right)$ is the illumination amplitude at transverse spatial frequency $\mathbf{k}_{i\perp}$, governed by the condenser aperture, $\gamma_1 = \sqrt{\left(\bar{n}_1\beta_1\right)^2 - \left|\mathbf{k}_\perp\right|^2}$, and $\bar{n}_1 = \sqrt{\left\langle n^2\left(\mathbf{r}, \omega_0\right)\right\rangle_{\mathbf{r}}}$. Plugging the incident field (Eq. S9) into Eq. S6a, the resulting SHG field collected by the objective can be expressed as

$$U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{k}_\perp, z) = \frac{\beta_2^2}{2\gamma_2} e^{i\gamma_2 z} A_o\left(\mathbf{k}_\perp\right) A_i\left(\mathbf{k}'_{i\perp}\right) A_i\left(\mathbf{k}''_{i\perp}\right)\chi^{(2)}(\mathbf{k}_\perp - \mathbf{k}'_{i\perp} - \mathbf{k}''_{i\perp}, \gamma_2 - \gamma'_1 - \gamma''_1). \quad \text{[S10]}$$

Equation S10 implicitly reveals the phase-matching condition in inhomogeneous objects under non-collinear excitation. It is insightful to write $\chi^{(2)}$ in Eq. S10 as

$$\chi^{(2)}(\Delta\mathbf{k}_\perp, \Delta\gamma) = \int_0^L \chi^{(2)}(\Delta\mathbf{k}_\perp, z) e^{i\Delta\gamma z} dz\ , \quad \text{[S11]}$$

where $\Delta\mathbf{k}_\perp = \mathbf{k}_\perp - \mathbf{k}'_{i\perp} - \mathbf{k}''_{i\perp}$ and $\Delta\gamma = \gamma_2 - \gamma'_1 - \gamma''_1$ indicate the transverse and longitudinal momentum mismatch, respectively. The result of the integral in Eq. S11 dictates the output of the SHG signal, $U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{k}_\perp, z)$. For a fixed pair of $\mathbf{k}'_{i\perp}$ and $\mathbf{k}''_{i\perp}$, if $\Delta\gamma \simeq 0$ (perfect phase matching), we have

$$\chi^{(2)}(\Delta\mathbf{k}_\perp, \Delta\gamma = 0) = \int_0^L \chi^{(2)}(\Delta\mathbf{k}_\perp, z)dz \tag{S12}$$

Eq. S12 indicates that the fields emerging at each axial position *z* add up in phase (the complex exponential vanished), favoring SHG generation. At the same time, the particular $\Delta\mathbf{k}_\perp$ at which $\chi^{(2)}$ is evaluated also affects the result: we can imagine particular media for which $\chi^{(2)}(\Delta\mathbf{k}_\perp)$ is very small or even vanishes at some particular values of $\Delta\mathbf{k}_\perp$, even though axially the waves are matched, $\Delta\gamma \simeq 0$. The $\mathbf{k}_\perp$ dependence of our case brings in some complexity and richness to the problem: axial-phase matching does not guarantee maximum SHG signal for an object with spatial variation in $\chi^{(2)}$. On the other hand, the diversity of $\mathbf{k}'_{i\perp}, \mathbf{k}''_{i\perp}$ from the open condenser aperture, broadens the availability of wavevectors to create phase matching. Integrating over the entire condenser aperture is likely to always find phase matched waves. This is the reason why SHG with focused beams tends to always yield a signal. Finally, note that, for crystals assumed to be homogeneous, the integrand in Eq. S12 becomes constant and the phase matching integral yields a *sinc* function in *z* direction (Fourier transform of a rectangular function), which is typically found in nonlinear optics textbooks (see, *e.g.*, Section 2.3 in Ref [7]).

In the next section, we derive a relationship between the nonlinear susceptibility and image.

2. Effective transfer function

In HOT, by recording an interferogram created by the SHG and a reference field, we extract the complex (phase and amplitude) SHG signal. The SHG reference field is expressed as $U_r(\mathbf{k}_\perp, z) = \delta(\mathbf{k}_\perp - \mathbf{k}_{r\perp})e^{i\gamma_r z}$ with unit amplitude, and thus, the cross spatial-frequency spectral density can be calculated

$$\begin{aligned} W(\mathbf{k}_\perp, z) &= U_{\mathrm{SHG}}^{2\omega_0}(\mathbf{k}_\perp, z) \circledvee_{\mathbf{k}_\perp} U_r^*(-\mathbf{k}_\perp, z) \\ &= \frac{\beta_2^2}{2\gamma_2} e^{i(\gamma_2-\gamma_r)z} A_o(\mathbf{k}_\perp + \mathbf{k}_{r\perp}) A_i(\mathbf{k}'_{i\perp}) A_i(\mathbf{k}''_{i\perp}) \chi^{(2)}(\mathbf{k}_\perp - \mathbf{k}'_{i\perp} - \mathbf{k}''_{i\perp} + \mathbf{k}_{r\perp}, \gamma_2 - \gamma'_1 - \gamma''_1). \end{aligned}$$

[S13]

Since the reference is just the SHG of the incident field, $\mathbf{k}_{r\perp} \approx \mathbf{k}'_{i\perp} + \mathbf{k}''_{i\perp}$, and $\gamma_r \approx \gamma'_1 + \gamma''_1$. Therefore, the cross-spectral density in Eq. S10 can be simplified to

$$W(\mathbf{k}_\perp, z) \approx \frac{\beta_2^2}{2\gamma_2} e^{i(\gamma_2-\gamma'_1-\gamma''_1)z} A_o(\mathbf{k}_\perp + \mathbf{k}'_{i\perp} + \mathbf{k}''_{i\perp}) A_i(\mathbf{k}'_{i\perp}) A_i(\mathbf{k}''_{i\perp}) \chi^{(2)}(\mathbf{k}_\perp, \gamma_2 - \gamma'_1 - \gamma''_1) . \text{[S15]}$$

Next, performing a Fourier transform with respect to z, $W(\mathbf{k}_\perp, z)$ can be presented in $(\mathbf{k}_\perp, k_z)$ space as

$$W(\mathbf{k}_\perp, k_z) \approx \frac{\beta_2^2}{2\gamma_2} A_o(\mathbf{k}_\perp + \mathbf{k}'_{i\perp} + \mathbf{k}''_{i\perp}) \delta\left[k_z - (\gamma_2 - \gamma'_1 - \gamma''_1)\right] A_i(\mathbf{k}'_{i\perp}) A_i(\mathbf{k}''_{i\perp}) \chi^{(2)}(\mathbf{k}_\perp, k_z) . \text{[S16]}$$

It is easy to see that Eq. S16 takes the form of a linear system response. By integrating over all the possible angles of illumination, the effective transfer function can be derived as

$$\mathrm{H}(\mathbf{k}_{\perp},k_z;\beta_2)=\beta_2^2\iint\frac{1}{2\gamma_2}A_o\left(\mathbf{k}_{\perp}+\mathbf{k}'_{\perp}+\mathbf{k}''_{\perp}\right)\delta\left(k_z-\gamma_2+\gamma'_1+\gamma''_1\right)A_i\left(\mathbf{k}'_{\perp}\right)A_i\left(\mathbf{k}''_{\perp}\right)d^3\mathbf{k}'d^3\mathbf{k}''$$
$$=\beta_2^2P\left(\mathbf{k}_{\perp},k_z\right)\otimes\left[U_i\left(\mathbf{k}_{\perp},k_z\right)ⓥU_i\left(\mathbf{k}_{\perp},k_z\right)\right]$$

,[S17]

where $\otimes$ represents the correlation operator; P is the 3-dimensional pupil function, $P\left(\mathbf{k}_{\perp},k_z\right)=\frac{1}{2\gamma_2}A_o\left(\mathbf{k}_{\perp}\right)\delta\left(k_z-\gamma_2\right)$; U_i is a collection of plane waves originating from the condenser plane, $U_i\left(\mathbf{k}_{\perp},k_z\right)=A_i\left(\mathbf{k}_{\perp}\right)\delta\left(k_z-\gamma_1\right)$. In Eq. S17, the convolution takes into account the second-harmonic scattering, while the correlation takes into account the phase mismatch of SHG in the forward scattering process. When a broadband incident light source is used, we obtain the general description of the system transfer function.

$$\mathrm{H}(\mathbf{k}_{\perp},k_z)=\int\beta_2^2W\left(\omega_0\right)P\left(\mathbf{k}_{\perp},k_z\right)\otimes\left[U_i\left(\mathbf{k}_{\perp},k_z\right)ⓥU_i\left(\mathbf{k}_{\perp},k_z\right)\right]d\omega_0, \quad \text{[S18]}$$

where $W\left(\omega_0\right)$ is the cross spectral density of the second harmonic reference and SHG scattered fields.

3. Complex SHG field retrieval

The procedure for SHG field retrieval is illustrated in Fig. 1 in the main text. Each SHG image recorded at a distinct z_0 is loaded into Matlab where a two-dimensional fast Fourier transform (2D-FFT) is applied to transform the image to the transverse spatial frequency ($\mathbf{k}_{\perp}$) domain. The product of the SHG field and the complex conjugate of the reference beam appears at a distinct sideband centered at the incident transverse **k**-vector of the reference field. The relevant sideband is isolated by applying a band-pass filter. The isolated sideband is re-centered on the origin of the

2D transverse spatial frequency plane and brought back into the spatial domain through a 2D-IFFT (two-dimensional inverse fast Fourier transform).

4. Tomographic imaging with low condenser NA

To demonstrate the impact of illumination aperture on tomographic reconstruction, 4 biological specimens were prepared and measured by HOT with a 0.15 NA condenser lens, while the rest of the hardware settings were identical. The reconstructed results are shown in Fig. S1. Featured by elongated structures in the axial direction, these renderings suffered from poor sectioning capabilities while maintaining a similar resolution in the transverse plane.

5. Third-harmonic generation

The theoretical framework described in section 1 can be applied to study higher-order nonlinear processes. Take third-harmonic generation as an example, where the three fundamental photons are converted into a single photon with triple the frequency of the fundamental. In this situation, the nonlinear induced polarization density takes the form $P_{NL} = \varepsilon_0 \chi^{(3)}(\mathbf{r}) U^3(\mathbf{r}, t)$. Following the same derivation procedure presented in Section 1, the analytical expression of the scattered third-harmonic field can be written as

$$U_{\mathrm{SHG}}^{3\omega_0}(\mathbf{k}_\perp, z) \approx \frac{\beta_3^2}{2\gamma_3} e^{i\gamma_3 z} \left[\chi^{(3)}(\mathbf{k}) ⓥ_k U_i^{\omega_0}(\mathbf{k}) ⓥ_k U_i^{\omega_0}(\mathbf{k}) ⓥ_k U_i^{\omega_0}(\mathbf{k}) \right]\Big|_{k_z=\gamma_3}, \qquad \text{[S19]}$$

where β_3 is the wavenumber of the second-harmonic field, $\beta_3 = 3\omega_0 / c$, $\bar{n}_3$ the spatially averaged refractive index at the second-harmonic frequency $\bar{n}_3 = \sqrt{\left\langle n^2\left(\mathbf{r}, 3\omega_0\right)\right\rangle_{\mathbf{r}}}$, γ_3 is the longitudinal wavenumber ($\gamma_3 = \sqrt{\bar{n}_3^{\,2}\beta_3^{\,2} - \left|\mathbf{k}_\perp\right|^2}$).

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Figure S1. **HOT imaging of various biological samples using a low NA condenser lens. a.** Pumpkin Stems; **b.** Ox motor neuron; **c. Mammalian tongue tissue; d.** Rabbit tendon. The samples are measured by 0.9 NA/100× objective and 0.15 NA condenser. Each 3D volume is approximately 30× 30×30 μm^3