OPTICAL DIFFRACTION TOMOGRAPHY

Volume imaging of anisotropic materials

Revealing the molecular orientations of anisotropic materials is desired in materials science and soft-matter physics. Now, an optical diffraction tomographic approach enables the direct reconstruction of dielectric tensors of anisotropic structures in three dimensions.

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In biological, materials or technological studies, the optical microscope is an essential tool for observing samples at the microscopic scale. It illuminates the sample homogeneously with a lamp and detects the transmitted light through a magnification system. In standard microscopes, the variation of the recorded intensity can be related to the local absorption of the sample. In more sophisticated set-ups, using masks and polarizers on the illumination and detection paths, it reveals optical path differences or changes in the field polarization due to refractive index or birefringence inhomogeneities. However, the information provided by these images cannot be related easily to the three-dimensional (3D) structure of the sample[1]. To address this issue, an approach combining optical microscopy with computed tomography was proposed in the early 2000s[2], and further work in this direction has enabled the 3D imaging and quantitative refractive index mapping of isotropic materials and biological samples[3–7]. However, the recovery of the full dielectric tensor of anisotropic materials has remained inaccessible. Now, writing in *Nature Materials*, Seungwoo Shin and colleagues report a dielectric tensor tomography technique capable of providing quantitative 3D images of local anisotropy[8].

Optical diffraction tomography (ODT) is based on a modified microscope in which the sample is illuminated by a collimated laser beam under various incident angles, and an interferometric configuration records the transmitted (or reflected) light field in phase and amplitude. The 3D image of the sample is reconstructed numerically using a model linking the measured scattered field to the parameter governing the light–matter interaction, namely, the inhomogeneous permittivity tensor. The simplest and most widespread model in ODT reconstructions, valid for weakly scattering samples, states that the scattered electric field is linearly linked to the 3D Fourier transform of the permittivity tensor applied to the polarization vector of the illumination (Fig. 1a).

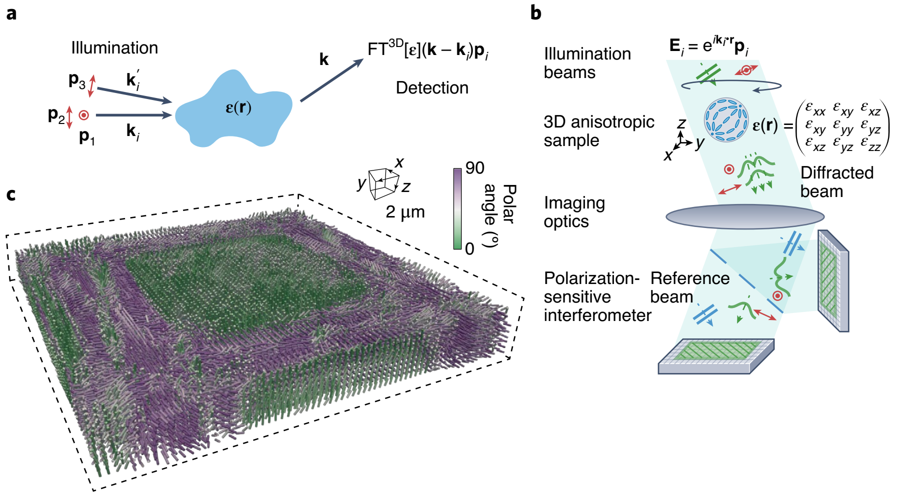

Fig. 1 | Principle of operation of the DTT method. a, The field scattered in the $\mathbf{k}$ direction by a sample illuminated under the $\mathbf{k}_i$ direction is linearly linked to the 3D Fourier transform (FT^{3D}) of the permittivity tensor $\boldsymbol{\varepsilon}$, taken at $\mathbf{k}-\mathbf{k}_i$, applied to the incident polarization vector $\mathbf{p}_i$. To recover the full tensor for a given $\mathbf{k}-\mathbf{k}_i$, three incident polarization states $\mathbf{p}_1$, $\mathbf{p}_2$, $\mathbf{p}_3$ are successively used for illuminating the sample. $\mathbf{p}_1$ and $\mathbf{p}_2$ are normal to $\mathbf{k}_i$ while $\mathbf{p}_3$ is normal to a slightly tilted incident direction, $\mathbf{k}_i'$. This approach assumes that the Fourier transform of $\boldsymbol{\varepsilon}$ does not vary much between $\mathbf{k}-\mathbf{k}_i$ and $\mathbf{k}-\mathbf{k}_i'$. **b**, Schematic of the set-up: the scattered-field vectors are measured using a polarization-sensitive off-axis interferometer for various illumination directions $\mathbf{k}_i$ and polarizations $\mathbf{p}_i$. $\mathbf{E}_i$ is the illumination field and $\mathbf{r}$ is the position vector. The green and blue arrows are the diffracted beam and the reference beam, respectively. **c**, Three-dimensional view of the reconstructed directors of a liquid-crystal polymer network film. Panels **b** and **c** adapted with permission from ref. [8], Springer Nature Ltd.

When the material is isotropic, the permittivity tensor reduces to a complex scalar, corresponding to the square of the refractive index. Then, the measurement of one component of the scattered-field vector for one incident polarization is enough to reconstruct the sample. Optical diffraction tomography has been mainly developed in this particular configuration, with reconstruction models generally neglecting the vectorial nature of the scattered field. It yielded spectacular 3D images of fixed and live biological samples in the form of quantitative refractive index maps with a resolution of about 200 nm in the transverse directions and 300 nm axially[2–7]. More recently, vectorial ODT that measures the scattered-field vector for two incident polarizations and using a rigorous model for the scattered field could improve further the resolution of the reconstructions[9].

When the material is anisotropic, the permittivity tensor is a 3 × 3 tensor. To recover its nine coefficients, one needs to measure the scattered-field vector for three independent incident polarization states. However, because of the transverse nature of propagative waves, only two independent polarization states are available, and recovering the full tensor becomes impossible. This issue was clearly stated in ref. [10], which presented the first ODT experiment for imaging anisotropic samples and limited the reconstructions to the purely transverse coefficients of the permittivity tensor.

Now, Shin and colleagues propose a simple and clever idea to circumvent this limitation. They remark that if the direction of propagation of the incident field is slightly rotated, it is possible to find a new polarization state that forms a 3D basis with the two previous polarization states. Then, assuming that the Fourier transform of the permittivity tensor does not vary much with this slight tilt, they obtain a third additional vectorial equation to complete the extraction of the tensor coefficients.

In practice, the dielectric tensor tomography (DTT) set-up proposed by Shin and colleagues uses a digital micromirror device and a liquid-crystal retarder to vary the illumination angle and control the incident polarization. The transmitted-field vector is detected by off-axis holography using two cameras (Fig. 1b). The field data are recorded in less than 1 second for 43 incident angles and 3 polarizations (the last polarization being obtained with a slight angle tilt of 0.3°). After checking the validity of the DTT method on calibrated targets, the authors were able to reconstruct with submicrometric resolution the inhomogeneous permittivity tensor of liquid-crystal droplets and films. Then, the authors demonstrated the potential of the DTT method for dynamic imaging by studying the rotation of the directors when the droplets were heated or cooled and when an electric field was applied to the films (Fig. 1c). We anticipate, however, that the method could be limited by the signal-to-noise ratio, which should be high enough to extract meaningful information from the third measurement, and by the validity of the approximate model linking the detected field to the permittivity tensor.

The DTT method provides a label-free, non-toxic, non-invasive and relatively quick approach to obtaining quantitative 3D images of the local anisotropy of samples. As the local anisotropy provides insights into the molecular order and orientation, we envision that the DTT method should find many exciting applications in the materials and biological domains; for example, the alignment of collagen fibres in cancer tissues, the local order of DNA microstructures, the growth of microcrystals during biomineralization and the dynamic changes of molecular arrangement in cell membranes are just a few of the many studies that could benefit from this imaging technique. ❐

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https://doi.org/10.1038/s41563-022-01213-5

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Competing interests

The authors declare no competing interests.