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Coherent backscattering of light by an anisotropic biological network

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The scattering strength of a random medium relies on the geometry and spatial distribution of its components as well as on their refractive index. Anisotropy can therefore play a major role in the optimisation of the scattering efficiency both in biological and synthetic materials. In this work, we show that by exploiting the coherent backscattering phenomenon it is possible to characterise the optical anisotropy in the *Cyphochilus* beetle scales without the need of changing their orientation or their thickness. For this reason, such a static and easily accessible experimental approach is particularly suitable for the study of biological specimens. Moreover, the estimation of the anisotropy in the *Cyphochilus* beetle scales might provide inspiration for improving the scattering strength of artificial white materials.

1. Introduction

An object is opaque white when the light incident on it undergoes multiple scattering events before exiting the medium, *i.e.* when the object is optically thick. [1] The optical thickness is defined as the ratio between the physical thickness of an object and the transport mean free path (ℓ_t), namely the distance that light travels before losing information about its starting propagation direction [2, 3]. Commonly, ℓ_t is of the order of tens of microns in low-refractive index white materials. [4, 5] Therefore, opacity is only achieved for relatively large thicknesses (of the order of hundreds of microns) to allow a sufficient number of scattering events.

Nature, however, provides a different approach that can serve as an inspiration for the manufacturing of polymeric, thin but still opaque white materials. [6, 7, 8] In particular, the brilliant whiteness shown by the *Cyphochilus* beetle is known to be generated by multiple scattering of light inside the extremely thin scales ($\simeq 7\,\mu\mathrm{m}$ thick) that cover its exoskeleton (Figure 1a-b). [4, 9, 10, 11, 12] The beetle intra-scale structure is composed of a nanostructured network of chitin filaments with a filling fraction of around 45%. [13] The chitin fibers inside the beetle scales are organised anisotropically, *i.e.* mainly oriented parallel to the surface of the scales (Figure 1c). This structural anisotropy increases the scattering strength in the orthogonal direction to the scale surface, at expense of the in-plane scattering, which is not as relevant for the total reflectance of the insect. [12] With such morphology and geometry, the beetle achieves a high total reflectance (about 70% over the whole visible range) with a thin, lightweight, and anisotropic network made of low refractive index material. [4, 12, 13]

In the recent years a number of different techniques have been used to characterise anisotropic media, for example, spatially resolved reflectance, [14, 15] imaging diffuse transmission, [12, 16, 17] spatio-temporal visualisation of transmitted light, [18] and coherent backscattering. [19, 20, 21, 22] As of yet, the accuracy in the determination of the in-plane and out-of-plane components of the light transport mean free math in the *Cyphochilus* beetle scales has been limited by the strong thickness dependency of the experimental techniques used. [4, 12]

In this work, we show that the coherent backscattering technique is well suited for studying the scattering properties of biological samples, allowing to estimate the anisotropy of a system without the need of changing its orientation and its thickness. Moreover, the CBS provides a precise evaluation of the in-plane transport mean free path without requiring samples with different thicknesses, in contrast with other static and easily accesible techniques. Our experimental results contribute to the understanding of scattering optimisation in the *Cyphochilus* beetle scales, providing a valuable guide for the development of novel sustainable materials by

showing how to obtain a strong optical response whilst using a low-refractive index biopolymer as building block.

2. Results and Discussion

To characterise light propagation in the *Cyphochilus* scales, we performed a coherent backscattering (CBS) experiment. We measured the angular resolved light scattered by the sample around the backscattering direction, which shows a characteristic peak profile. [23] The CBS is the Fourier transform of the spatial distribution of light exiting the sample in the backscattering direction. [3] This phenomenon is often understood as an interference effect that originates from the superposition of a large number of two-wave interference patterns from reciprocal waves. [23, 24, 25, 26, 27] These waves have traveled the same optical path inside the medium but in opposite directions (Figure 2a) and are therefore phase-related. The resulting CBS intensity distribution has a conical shape whose width provides a direct measurement of the light transport mean free path of a material. [3]

The experimental setup is shown in Figure 2b. A collimated laser diode (peak wavelength at 635 nm, spot-size of 2.5 mm, and output power of 1.2 mW) was used as light source. The scattered signal was focused, using a parabolic mirror, on a $100\,\mu\mathrm{m}$ core fiber connected to a spectrometer. The angular resolution of our setup was $\simeq 0.25$ mrad. To acquire the CBS line shape, the speckle pattern, which occurs due to the high spatial coherence of the light source, needs to be averaged out. [26] This was done by placing the sample on a motorised rotation mount whose axis was the same of the propagation direction of the incoming laser beam. This averaging procedure precludes the possibility to investigate a potential in-plane, xy, anisotropy. However, it has been recently shown that the *Cyphochilus* scales are characterised by an isotropic spectral density in the xy plane. [13] For this reason, our work focused only on the study of an out-plane anisotropy.

The enhancement factor of the coherent signal, *i.e.* the ratio between the intensity at exact backscattering angle and the incoherent background, strongly depends on the polarisation. This is maximised when the single-scattered photons, which do not have a reciprocal counterpart and therefore contribute only to the incoherent background, are filtered out. This can be done by acquiring the CBS in the *helicity conserving channel* (HC) (Figure 2). The HC signal was then normalised to the one acquired in a *linear non conserving* (LNC) configuration, as discussed in Ref. [28].

The measured CBS signal is reported in Figure 3. The experimental data show a maximum lower than the theoretical value for semi-infinite media of 1 and a rounded top. This deviation is a consequence of the small thickness of the *Cyphochilus* scales and can be described by the isotropic theory for finite media: [29]

$$\begin{aligned}\gamma_c = 3e^{-b\nu}\Bigg[&\cos(bu)\left[\left(\frac{(\nu-(\nu-\alpha)\cosh(2z_e\alpha)}{(\nu-\alpha)^2+u^2}\right)+\left(\frac{(\nu-(\nu+\alpha)\cosh(2z_e\alpha)}{(\alpha+\nu)^2+u^2}\right)\right]\\&+\sin(bu)\left(\frac{u}{(\alpha+\nu)^2+u^2}-\frac{u}{(\nu-\alpha)^2+u^2}\right)\sinh(2z_e\alpha)\\&+\frac{(\nu-\alpha)\cosh((\nu-\alpha)b-2z_e\alpha)-\nu\cosh((\nu-\alpha)b)}{(\nu-\alpha)^2+u^2}\\&+\frac{(\nu+\alpha)\cosh((\nu+\alpha)b-2z_e\alpha)-\nu\cosh((\nu+\alpha)b)}{(\alpha+\nu)^2+u^2}\Bigg]\end{aligned} \tag{2.1}$$

where $\mu=\cos(\theta)$, $\nu=\frac{1}{2}(1+\frac{1}{\mu})$, $u=k\ell_t(1-\mu)$, $\alpha=k\ell_t\sin(\theta)$, $B=b+2z_e$. The parameters k, ℓ_t, and b are the wave vector, the isotropic transport mean free path, and the optical thickness, respectively. For semi-infinite media, *i.e.* $b\to\infty$, Equation 2.1 reduces to: [29]

$$\gamma_{c,semi}=\frac{3\left[\alpha+\nu\left(1-e^{-2z\alpha}\right)\right]}{2\alpha\mu\nu\left[(\alpha+\nu)^2+u^2\right]} \tag{2.2}$$

The reduction of the theoretical maximum in finite media is caused by the suppression of long light paths, which are responsible for the formation of the cusp of the CBS profile for semi-infinite media. [2] To accurately determine the transport mean free path, the effect of the internal reflections at the scale interface on the light path distribution inside the chitin network was accounted in the extrapolation length $z_e=(2/3)(1+R)/(1-R)$. [30, 31, 32] R is the angle- and polarisation-averaged reflection coefficient at the slab interface and can be obtained by an angular integration of the Fresnel's coefficients. [31, 33] These coefficients depend on the effective refractive index (n_e) of the chitin network, which was estimated by the Maxwell-Garnett's theory. [34] The expression of z_e used in this work, and in the related literature regarding the study of the *Cyphochilus* beetle, is the one derived for isotropic systems. This approximation is justified by the absence of an analytical formula of the extrapolation length in anisotropic media. [35]

Using a filling fraction of $(45\pm6)\%$, [13] we calculated that $n_e=(1.22\pm0.03)$, $R=(0.32\pm0.04)$, and $z_e=(1.29\pm0.11)$. Finally, using the extrapolation length found from the expression above, we obtained $\ell_t=(1.40\pm0.09)$ µm from the fit shown in Figure 3.

In the literature, the isotropic theory has been used to obtain information about media where the anisotropy is in the plane perpendicular (xy) to the incoming beam (z direction). [19, 36] This type of anisotropy gives rise to a CBS cone whose line shape differs when acquired along the x and y directions. [36] It has been recently shown that the isotropic theory cannot be used to obtain quantitatively reliable information about the anistropic light transport along perpendicular directions. [35] However, the anisotropy can be qualitatively estimated as the ratio between the widths of the CBS line shapes acquired along the x and y directions, which can be individually described by the isotropic theory. [36] Similarly, in the case of birefringent media as nematic liquid

crystals, the isotropic theory can be used to describe the CBS line shapes originating from different polarisation configurations. [19]

In the case of the *Cyphochilus* beetle, the anisotropy is in the xz- and yz-plane (defining z as perpendicular to the surface of the scales) [12, 13] and therefore the resulting CBS profile is isotropic (for light incoming along the z direction). Due to the particularly small thickness of the scales ($\simeq 7\ \mu\mathrm{m}$), the probing direction of the incoming beam cannot be changed.

As the anisotropy cannot be investigated directly, to gain insight on the light transport inside the scales we performed anisotropic Monte Carlo simulations for scalar waves. A Monte Carlo technique is well suited for describing light propagation in disordered media, where the photons paths can be mathematically mapped into random walks. [2, 3, 37] Monte Carlo simulations have been extensively used both to investigate theoretical aspects of anisotropic diffusion, [14, 35, 38, 39, 40, 41] and to accurately describe experimental results regarding light propagation in anisotropic media. [15, 19, 22]

Here, the photons paths in the white beetle scales were modeled by a series of random steps. [42] The anisotropy of the system was introduced *via* a direction-dependent step size, *i.e.* the components of the step vector were sampled from two different negative exponential distributions with mean ℓ_{xy} and ℓ_z for the in-plane and out-of-plane components, respectively. The angular component of each of the random steps was sampled from a distribution of points uniformly distributed on the surface of a unit sphere. [43, 44] The collection of the initial and final positions of the walkers that escaped the material from the same face they entered it, that corresponds to reflected photons, was then used to reconstruct the CBS line shape. [3, 29] The effect of residual absorption on the CBS line shape is not considered, due to the negligible absorption of chitin in the visible. [45] The only parameters required for our Monte Carlo simulations are the random steps distribution, the scales thickness and the reflection coefficient at the scale interface (R). A schematic of the simulations parameters is illustrated in Figure 4a.

Figure 4b-c show how the CBS line shape is affected both by changing the in-plane (ℓ_{xy}) and out-of-plane (ℓ_z) components of the transport mean free path. In particular, ℓ_{xy} determines the width of the CBS profile, while the optical thickness, defined as $OT = L/\ell_z$ (where L is the thickness of the medium), specifies the enhancement of the coherent signal. These results can be qualitatively explained by the fact that the CBS depends only on the distance between the positions of the first scattering event (when the photon enters the medium) and the last (when the photon exits the medium). When a large number of photons is considered, these two positions have on average the same z coordinate (which is of the order of ℓ_z) and therefore their distance can be considered to be z-independent. However, as discussed previously for the isotropic theory, when the optical thickness of the medium is small (*i.e.* when long light paths are not allowed

by the finite thickness of the medium) the top of the CBS is rounded. The limited influence of the optical thickness on the width of the CBS line shape allows to obtain a precise value of ℓ_{xy} without requiring samples with different thicknesses. By fitting the experimental data with the Monte Carlo simulations, it is possible to disentangle the contribution of ℓ_{xy} and ℓ_z to the CBS line shape. In particular, we obtained a value of $\ell_{xy} = (1.40 \pm 0.09)$ µm and an optical thickness $OT = (6.89 \pm 0.13)$. The data were fitted by minimising the χ^2. The errors in ℓ_{xy} and OT were estimated by performing simulations were the two parameters were gradually changed. This procedure was then repeated for taking into account the uncertainty in the determination of reflection coefficient (R).

The measured optical thickness is in good agreement with the total transmission data reported in the literature. [4, 12] The total transmission (T) for slab geometry media in the diffusion approximation is:

$$T = \frac{2z_e}{L + 2z_e}, \tag{2.3}$$

where L is the slab thickness. Equation 2.3 is the limit for negligible absorption of the expression derived in Ref. [46]. Using $T = (0.29 \pm 0.02)$, as reported in Ref. [4, 12], and the extrapolation length previously calculated we obtained $OT_{lit} = (6.3 \pm 1.2)$, consistent with what we measured.

From the measured OT and assuming $L = (7 \pm 1)$ µm, where L and its error represent the mean and *1* σ of the distribution reported in Ref. [12], we obtained $\ell_z = (1.02 \pm 0.15)$ µm. The error in ℓ_z, which is mainly determined by the uncertainty in the thickness of the sample, can be affected by systematic errors given by surface roughness and curvature. [3, 47, 48]

Comparing ℓ_z with ℓ_{xy}, the measured optical anisotropy (OA) is:

$$OA = \frac{\ell_{xy} - \ell_z}{\ell_z} = (0.37 \pm 0.24). \tag{2.4}$$

Our experimental result is in agreement with the 3D reconstruction of the chitin network reported in Ref. [13], which predicts $OA \simeq 0.5$.

3. Conclusions

In conclusion, we demonstrated that the transport mean free path and optical anisotropy of light in the *Cyphochilus* beetle scales can be determined by measuring the CBS and that the results are in agreement with the one predicted in Ref. [13]. Exploiting the CBS effect provides a measurement of the optical anisotropy which describes more accurately the scattering properties of the *Cyphochilus* beetle compared to the results reported in the literature. In addition, the experimental technique reported here allows to estimate the anisotropy of a system without the need of changing its orientation and its thickness, making the CBS a technique particularly suitable for the study of biological specimens.

Authors Contribution.

G.J., O.D.O., A.D.L., R.S., and S.V. designed the experiments. G.J., J.B., R.S., and S.V. conceived and planned the simulations. G.J. carried out the experiments and the simulations. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Data Accessibility.

The raw data and the Monte Carlo code regarding the publication can be found on the online figshare repository.

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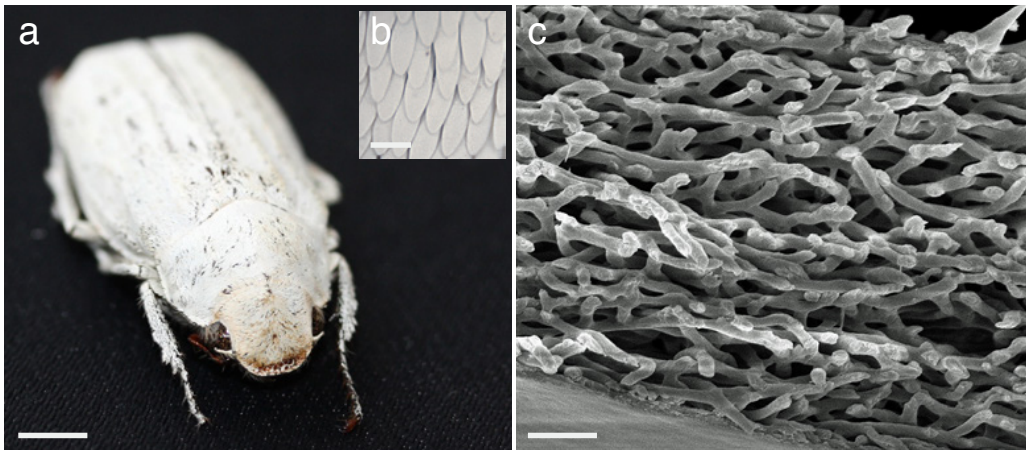

Figure 1. Images of the white beetle at different magnifications: a) photograph of the *Cyphochilus* beetle; b) micrograph of the scales organisation; c) SEM image of the cross-section of a *Cyphochilus* scale showing the interconnected network of chitin filaments which is responsible for the white appearance of the insect. Scale bar: 1 cm for a, $200\,\mu\mathrm{m}$ for b, $1\,\mu\mathrm{m}$ for c.

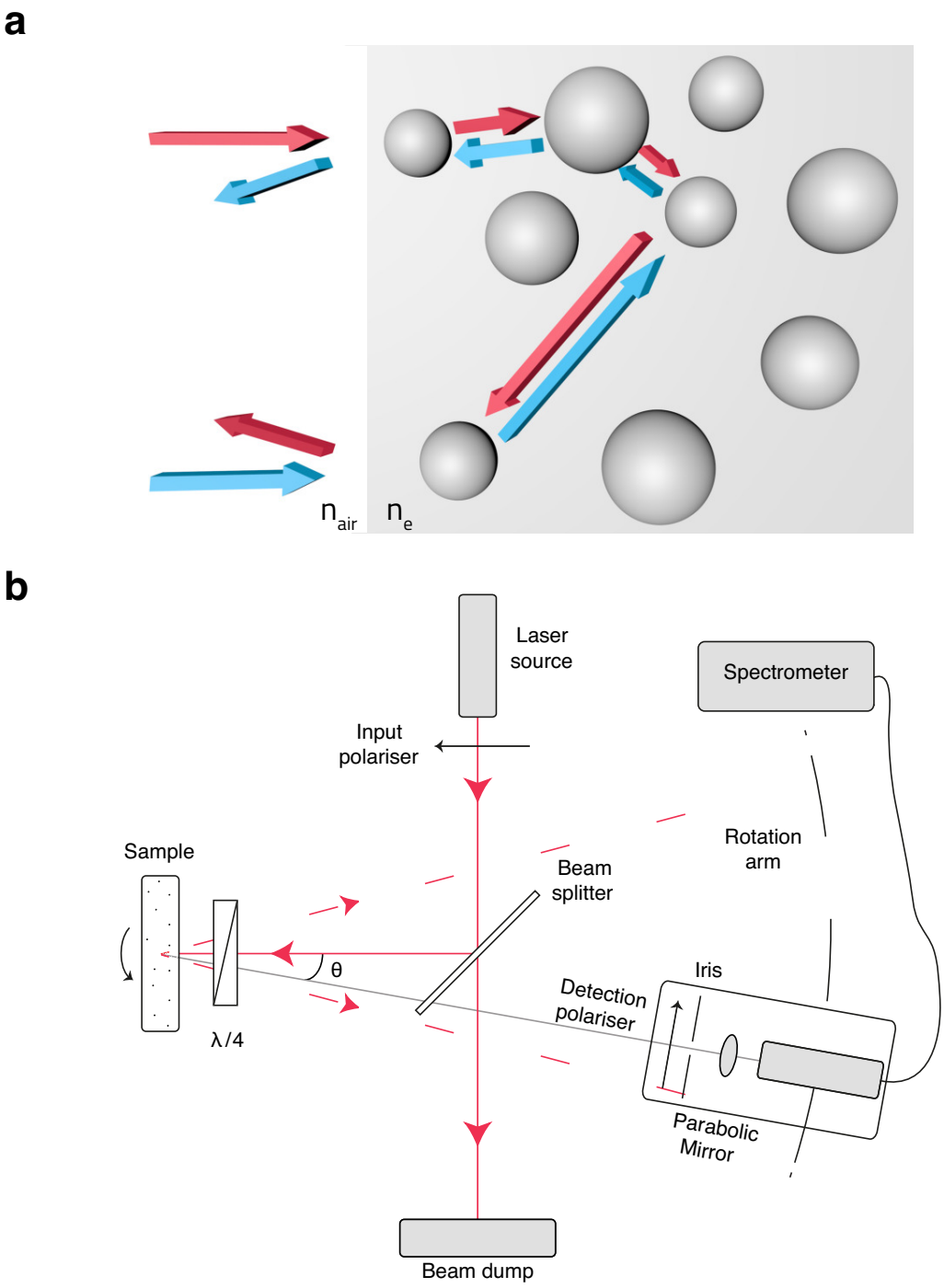

Figure 2. a) Illustration of two counter-propagating photons, red and light blue arrows. For simplicity, the scattering centers are represented as spheres. b) Schematic representation of the CBS setup in a HC configuration: the red dashed lines define the cone of backscattered intensity, while the gray line represents the detection rotation. The sample was mounted on a rotation mount, whose axis was perpendicular to the propagation direction of the laser beam, to average over different disorder realisations.

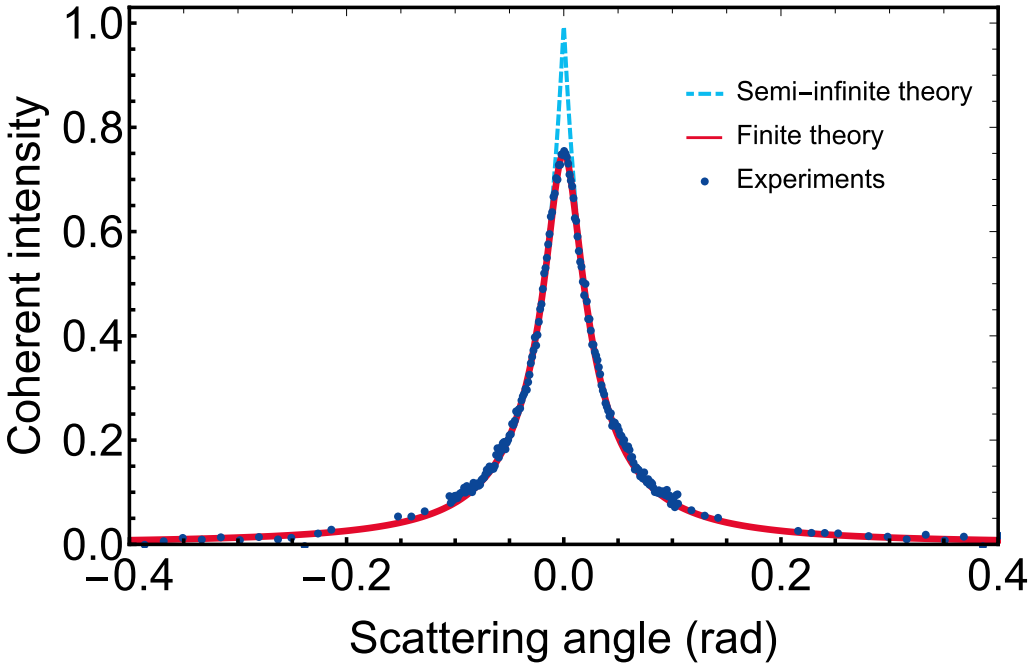

Figure 3. Theoretical fit of the experimental data (blue dots) using the isotropic theory for semi-infinite (light blue dashed line) and finite media (red solid line). Both curves were normalised to the maximum value of the semi-infinite theory. The experimental points were obtained normalising the signal measured in HC configuration to that acquired in the LNC setup.

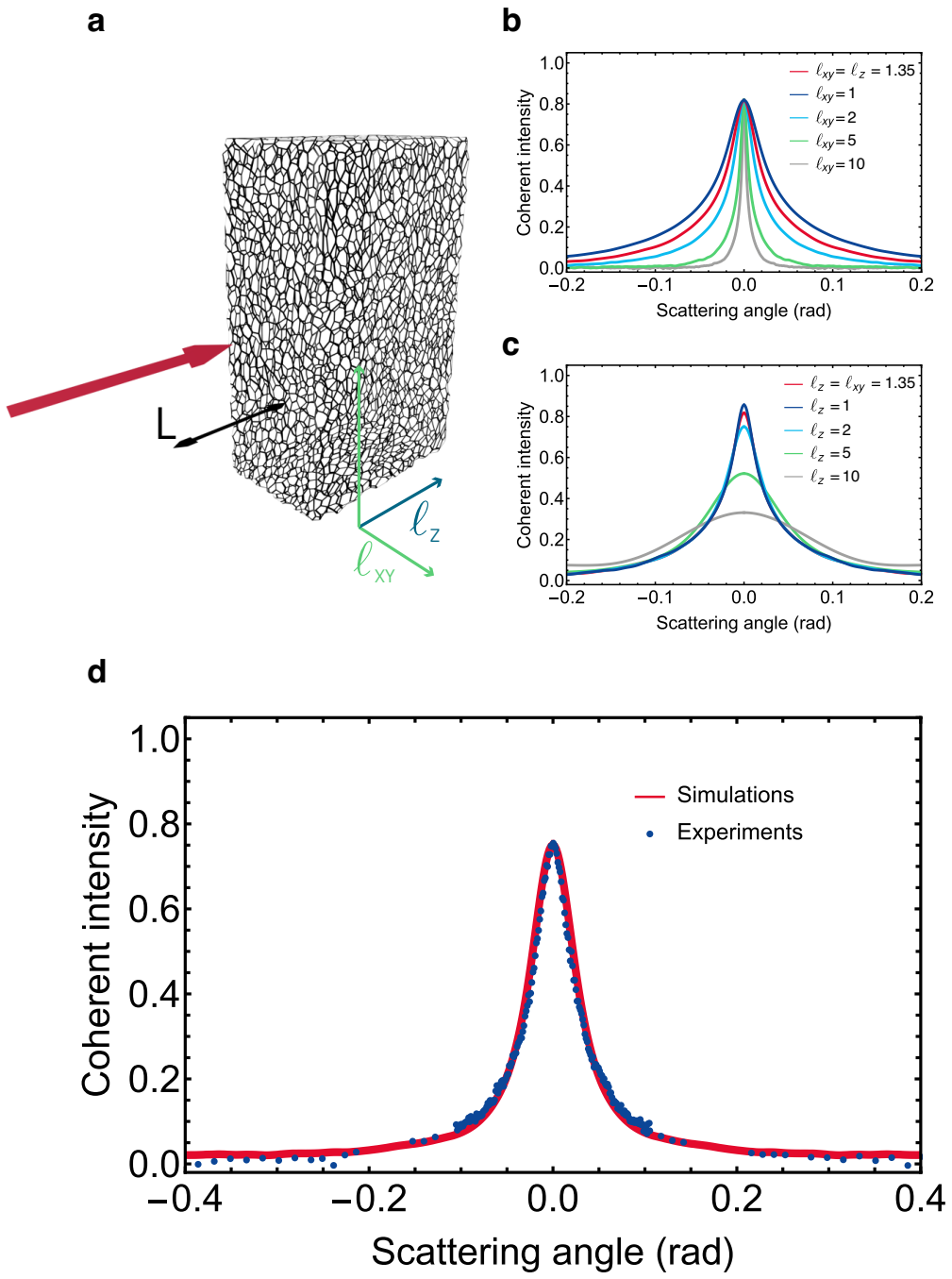

Figure 4. Monte Carlo simulation of the CBS line shape by an anisotropic medium: a) illustration of the simulation parameters, b-c) varying the in-plane components and the out-of-plane one of the mean free path. For both simulations the thickness of the slab (L) was fixed at 15 μm. d) Fit of the experimental data with the anisotropic simulation. All the simulation were performed using 1 million photons. The simulated curves were normalised to the maximum value of a simulation with OT=1000.