Hybrid immersion-polarization method for measuring birefringence applied to spider silks

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Hybrid immersion-polarization method for measuring birefringence applied to spider silks

Douglas J. Little* and Deborah M. Kane

MQ Photonics Research Centre, Department of Physics and Astronomy, Macquarie University, Sydney, NSW 2109, Australia

*Corresponding author: douglas.little@mq.edu.au

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The remarkable mechanical properties of spider silk have been well documented and the function of these properties with regard to prey capture is well understood [1–3]. There is now a growing interest in the optical properties of spider silk, and the optics of transparent spider webs more generally [4]. This interest is fuelled by biologists who seek to use the optical properties of spider silk to explain aspects of spider behavior and evolution [5], and physicists who seek to engineer materials that emulate aspects of the silks themselves.

Study of the optical properties of spider silks has largely been restricted to measurements of reflectivity [5], surface quality [6,7], and refractive index, mainly due to the practical difficulties of handling the silks. Spider silks are very fine (diameter = 1–10 μm) and must be kept under tension. This prohibits cleaving or breaking the silk fibers and rules out the use of most available methods for measuring refractive index. Inverse-scattering-based techniques have been used; these are generally limited to an accuracy of ±0.01 [8–10]. The de Sénarmont method has been applied to measuring birefringence of spider silks [11]. But this method does not give the principal refractive indices of the silk.

A recently developed method for measuring the refractive index of spider silks, dubbed the image contrast immersion method [12], involves immersion of the silk in a series of liquids with calibrated refractive indices and quantifying the visibility of the silk. The refractive index is determined as the minimum in visibility of the silk/liquid interface when the silk is illuminated with light linearly polarized along the axis of the silk and perpendicular to it. This gave measurements of both principal refractive indices and the birefringence is their difference. The technique outlined in this Letter is a further development of the image contrast immersion method. The method involves rotating the orientation of the incident linear polarization on the immersed silk.

There is an orientation where index matching between the silk and liquid is obtained. Once this is repeated for a second liquid, the principal refractive indices of the spider silk are calculated using the method outlined herein. The modified procedure is faster and more accurate compared to that in [12], due to the need to use fewer immersion liquids and the relative simplicity of continuously rotating the orientation of the incident polarization. The drawback of this method is that it can only be used on samples where there are two or more available calibrated immersion liquids that lie between the principal refractive indices. Commercially available sets of immersion liquids typically have intervals of 0.002 [13]. Thus the minimum birefringence that could be measured with these sets is of order 0.003.

This hybrid immersion–polarization method uses the same experimental setup as outlined previously [12]. Light from the metal-halide lamp source was passed through a bandpass filter centered at 589 nm and a linear polarizer that was set in a rotation stage fixed to the microscope assembly.

A number of refractive-index oils (Cargille, series A) with refractive indices in between the principal refractive indices of the spider silk were identified through a trial-and-error approach. The silk was then immersed in a suitable refractive-index oil and the orientation of the polarization (as depicted in Fig. 1) that resulted in index matching between the silk/oil interface was recorded. This was repeated for the second oil, giving two different orientations corresponding to two different oils with a known refractive index. The silk was washed with isopropanol between immersions. Isopropanol did not affect the refractive index of the silk; this was verified.

Fig. 1. Polarizer positions for immersed silk illuminated with light polarized parallel (left) and perpendicular (right) to the silk axis. Minimum visibility occurs at some orientation θ (center).

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by immersing the silk in the same oil before and after an isopropanol wash and checking that the measured values did not change.

Radial silks can be approximated as uniaxial crystals with the birefringent axis parallel to the axis of the silk. For a given orientation of the incident polarization $\theta$ (measured with respect to the silk axis), the refractive index of the silk is given by

$$n_{\text{silk}}(\theta) = \left( \frac{\cos^2 \theta}{n_p^2} + \frac{\sin^2 \theta}{n_s^2} \right)^{-1/2},$$

(1)

where $n_p$ and $n_s$ are the principal refractive indices of the spider silk for the polarizations oriented parallel and perpendicular to the silk length, respectively. Equation (1) was rearranged to give

$$n_s = \left( \frac{n_p^2 n_{\text{silk}}^2 \sin^2 \theta}{n_p^2 - n_{\text{silk}}^2 \cos^2 \theta} \right)^{1/2}.$$

(2)

Refractive-index matching occurred between the silk and oil (where $n_{\text{silk}} = n_{\text{oil}}$) at measured orientations $\theta_1$ and $\theta_2$ for oils with refractive indices $n_{\text{oil}}$ and $n_{\text{oil},2}$, thus,

$$n_s = \left( \frac{n_p^2 n_{\text{oil}}^2 \sin^2 \theta_1}{n_p^2 - n_{\text{oil}}^2 \cos^2 \theta_1} \right)^{1/2};$$

(3)

$$n_s = \left( \frac{n_p^2 n_{\text{oil},2}^2 \sin^2 \theta_2}{n_p^2 - n_{\text{oil},2}^2 \cos^2 \theta_2} \right)^{1/2}.$$

(4)

To obtain the principal refractive indices, Eq. (3) and (4) were solved simultaneously for $n_p$:

$$n_p = n_{\text{oil}} n_{\text{oil},2} \left( \frac{\sin^2 \theta_2 - \sin^2 \theta_1}{n_{\text{oil}}^2 \sin^2 \theta_2 - n_{\text{oil},2}^2 \sin^2 \theta_1} \right)^{-1/2},$$

(5)

Once $n_p$ was evaluated, $n_s$ was calculated by substitution.

A typical $n$ measurement is shown in Fig. 1. The uncertainty of an $n$ measurement consisted of two contributions. The first contribution was due to the uncertainty in $\theta$. For the silk samples studied, $\theta$ could be determined to within an accuracy of $\pm 1^\circ$ by monitoring the intensity profile across the silk image as the polarization was rotated. The second contribution was due to the inhomogeneity of the silk sample and manifested as imperfections in the theoretical fit to experimental data (such as the fit shown in Fig. 2.). At least three oils were needed to assess the uncertainty due to sample inhomogeneity. The more oils that were used, the more accurately the uncertainty could be determined, with up to six used for each $n$ measurement. As the uncertainty due to sample inhomogeneity was larger than the uncertainty due to $\theta$ in the specific case of spider silks, the overall uncertainty in the $n$ measurement was determined by applying Eq. (5) to different pairs of oils and calculating the spread of $n_p$ and $n_s$ values that were obtained as a result. Only pairs of oils where the uncertainty due to $\theta$ was small ($|\theta_1 - \theta_2| > 10^\circ$) were included in this calculation. In general, the procedure for calculating the uncertainty depends on how the contribution due to $\theta$ and sample inhomogeneity compare.

The birefringence of a $P$. $\text{eburnus}$ radial silk was measured as a function of strain by mounting the silk on a Vernier caliper and repeating the procedure outlined above for different strains, applied by adjusting the distance between the teeth of the caliper. The initial length of the radial silk was 15.00 mm and the silks were initially mounted such that the native tension of the web was preserved. The variability of strain within a single web is probably less than 10% [14]. The silk of $P$. $\text{eburnus}$ is not very extensible, though it does contract, keeping the silk fiber taut as the strain is reduced. This is in contrast to most dragline/radial spider silks, which are highly elastic when stretched. The strain was reduced by adjusting the silk to lengths of 14.00, 13.00, and 12.00 mm.

The measured principal refractive indices as a function of strain are shown in Fig. 3. Changes in refractive index were due to the strain-optic effect and changes to the silk structure that occur in response to the applied strain, including density changes and reordering of the nanocrystallite domains [15].

The strain-optic tensor elements are defined as [16]

$$\Delta(1/\kappa_{ij}) = p_{ijkl} f_{kl}.$$

(6)

Fig. 2. Typical set of measurements for measuring the principal refractive indices of a radial silk sample. Three oils were used; data points are shown as black triangles, while the gray curve is Eq. (1) with $n_p = 1.5407$ and $n_s = 1.5329$. Uncertainty bars along both axes are smaller than the data points themselves.

Fig. 3. Measured principal refractive indices of $P$. $\text{eburnus}$ radial silk as a function of strain. Straight lines indicate linear fits to the data.
where $\kappa_{ij}$ is the optical dielectric tensor, $p_{ijkl}$ is the strain-optic tensor, and $\epsilon_{kl}$ is the strain, calculated as $L/L_0 - 1$, where $L$ is the silk length and $L_0$ is the initial silk length. For a uniaxial strain applied along the axis of a silk thread,

$$\Delta(1/n_p^2) = p_{1111}\epsilon,$$

$$\Delta(1/n_s^2) = p_{2211}\epsilon.$$

The measured values for $\Delta(1/n^2)$ as a function of strain are shown in Fig. 4. If we assume that 100% of the thread, for a uniaxial strain applied along the axis of a silk optic tensor, and shown in Table 1. The low values exhibited by the silk silks, and to other micro-optical elements and samples showing birefringence. It represents a new tool for studying strain-optic effects in such birefringent micro-optics.

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### Table 1. Measured $p_{1111}$ and $p_{2211}$ Values for Different Optical Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>$p_{1111}$</th>
<th>$p_{2211}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silk <em>P. eburnus</em></td>
<td>0.040</td>
<td>0.026</td>
</tr>
<tr>
<td>Fused silica [18]</td>
<td>0.126</td>
<td>0.260</td>
</tr>
<tr>
<td>As$_2$S$_3$ [18]</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td>Polymethyl methacrylate [19]</td>
<td>0.300</td>
<td>0.297</td>
</tr>
</tbody>
</table>

Fig. 4. Measured values for $\Delta(1/n^2)$ as a function of strain for a *P. eburnus* radial silk. Dotted and bold lines indicate linear fits for $n_p$ (black points) and $n_s$ (white points), respectively.

### References