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Optimizing Microfluidic Flow Cell Geometry for In-Situ Resonant Soft X-ray Characterization of Molecular Nanostructures

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Liquid-phase resonant soft X-ray scattering (LP-RSoXS) is an emerging label-free technique to probe chemically resolved nanostructures of molecular or hybrid materials in liquid environments. Still, quantitative analysis is hindered by the pressure-induced deformation of thin silicon nitride (SiN) membranes used as windows in microfluidic flow cells, which attenuates the signal in nonlinear ways, making experimental optimization difficult. Here, we directly characterize this deformation under experimental conditions for a variety of cell configurations. We use this to develop a predictive model that combines transmission effects of SiN bowing, incident X-ray beam profiles, and material-dependent resonant scattering cross sections to simulate the effective scattering intensity at the detector across the carbon K-edge. Maps of the total signal across the flow cell window reveal that increasing the window width and polymer concentration shifts the anisotropic intensity distributions from the center toward the edges of the window. It was determined that an optimal SiN thickness of 50 nm, with a window aperture of 104 μm , maximizes the total signal for typical solute concentrations and energies across the carbon K-edge. Our results overturn the assumption that corner regions dominate the scattering signal, offering explicit design guidelines for maximizing LP-RSoXS signals and significantly advancing the quantitative application of this technique to the characterization of molecular and hybrid nanostructured materials in liquids.

Introduction

Liquid in-situ resonant soft X-ray scattering (LP-RSoXS) is particularly valuable for probing molecular-scale structures in liquids due to its ability to distinguish molecular components using chemical contrast without the need for labels.^{1–6} This capability has enabled significant advances in characterizing nanostructured and polymeric materials, including micellar systems and organic nanocarriers in aqueous solutions.^{7–14} In contrast to conventional methods such as electron microscopy or neutron scattering, which require disruptive labeling, LP-RSoXS allows label-free material characterization.^{15–22} However, extending LP-RSoXS to quantitative analysis remains challenging due to the low penetration depth of soft X-rays and sensitivity to scattering geometry.²³

Conventional small-angle X-ray scattering (SAXS) has historically relied on capillary tubes and static hydrated pocket cells for studying nanoparticles in liquid environments. Capillary tubes, typically made of quartz or glass, offer chemical

resistance and optical transparency but present significant limitations, including opacity to soft X-rays, fixed sample environments, restricted fluid control, and susceptibility to beam damage due to static liquid conditions. Similarly, static hydrated pocket cells allow for smaller sample volumes but are prone to sealing failures, background noise, and limited environmental adjustments,^{10,14} restricting their use in dynamic experiments.²⁴

Researchers have adapted microfluidic flow cells initially developed for in-situ transmission electron microscopy (TEM) to LP-RSoXS experiments to overcome these constraints.⁸ These devices provide precise control of fluid flow, temperature, and chemical gradients, enabling dynamic manipulation during measurements. Their unique ability to support in-situ characterization of structural transformations and chemical processes in real-time has made them essential for nanoparticle studies.²⁵ The predictable flow profiles and tunable reaction conditions offered by microfluidic platforms also facilitate high-resolution LP-RSoXS measurements of structural dynamics, advancing nanoscale material characterization.^{26–28}

One critical barrier to quantitative LP-RSoXS analysis is the ability to properly account for the effects of the mechanical deformation, or “bowing,” of the thin silicon nitride (SiN) membranes used as X-ray transparent windows in microfluidic flow cells.²⁹ This bowing arises from differential pressure between the liquid-filled sample chamber and the surrounding environment, altering the optical path length and complicating the interpretation of scattering data.³⁰ Mechanical properties of

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Supplementary Information available: Includes a model vs. experimental % difference plots, window thickness versus intensity for 50nm and 100nm thick SiN windows, optical constants and contrast function, deformation model solution for both windows, and full total transmitted intensity formula.

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SiN, including Young's modulus, Poisson's ratio, and membrane thickness, strongly influence the bowing behavior. Moreover, window bowing affects beam attenuation, making absolute scattering intensity measurements difficult, which are essential for quantitative analysis.³¹

Previous studies modeled window bowing in TEM experiments, but often oversimplified pressure dynamics and assumed parabolic deformation profiles.³² Additionally, fluid-induced pressure variations caused by flow rate and microfluidic channel dimensions were not systematically considered. These limitations have restricted the applicability of previous models to dynamic experimental conditions.

To address these challenges, we directly characterized membrane deformation under experimental conditions and varying cell geometries to develop a predictive model that accounts for both mechanical and fluidic parameters, enabling accurate simulations of SiN window bowing and ultimately optimizing resonant scattering signals through flow cell design. Our approach integrates pressure-dependent mechanical deformation, incident X-ray beam profile, Beer's Law attenuation, and resonant scattering cross sections. By incorporating these factors, we predict the effective scattering intensity at the detector as a function of window dimensions, cell pressure, and X-ray resonant energies of polyethylene oxide (PEO), which can easily be extended to any material and concentration. Our findings challenge the conventional assumption that scattering predominantly arises from window corners, highlighting a more complex interplay of mechanical deformation and beam-sample interaction. This integrated modeling framework advances the quantitative application of LP-RSoXS, enabling precise and reproducible structural and chemical characterization of soft and hybrid nanostructured materials in liquid environments.

Results and Discussion

Figure 1 depicts microfluidic flow cells (available commercially) assembled from a sandwich of two Si chips with SiN films grown on one side. The Si is etched away in a slit pattern such that only the SiN films form the barrier between the liquid sample and high vacuum of electron microscope and X-ray instruments, allowing electrons or soft X-rays to penetrate through the cell where the slits overlap in a square aperture as shown. Spacers deposited on the corners of the SiN-side of the chips define the general thickness of the liquid channel, typically $\geq 0.5 \mu\text{m}$. However, the pressure differential across the exposed SiN membrane causes the mechanical deformation depicted schematically (not to scale) in **Figure 1**. This window 'bowing' causes the liquid sample to bulge in the center of the aperture, resulting in the central region being opaque to electrons in electron microscopes. The extent of this bowing depends on the internal pressure, window size, and membrane thickness. In scattering, this bowing causes both an increased signal (due to more sample) and decreases signal (due to lower transmission).

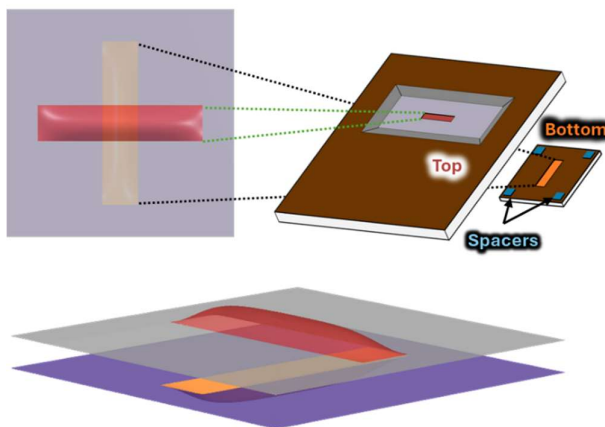


Figure 1 - Illustration of the location of the Silicon Nitride (SiN) membrane window (thickness 20-200 nm) in the larger Silicon chip (thickness $\sim 675 \mu\text{m}$) and the relative orientation of the top and bottom chip windows. The depicted bowing (not to scale) of the membranes is an example of the shape SiN takes during an experiment. The grey region of the top right chip is partially etched to allow for increased scattering angle but does not influence the effects depicted in this study. The "spacers" control the thickness (0.5-1.0 μm) of the sheet of liquid that flows between the two chips and represent the narrowest part of the flow cell.

The bowing of custom-etched SiN membranes of various thicknesses and window sizes was characterized in a scanning transmission X-ray microscope (STXM) under experimental flow conditions. Flow of pure deionized water allowed simple Beer's Law characterization of the total liquid thickness translating directly into bowing of each membrane. The 30 nm resolution of the STXM enabled experimental high-resolution maps of bowing in the aperture, one of which is depicted in **Figure 2a**. All maps were similar in that the apertured cell thickness is minimum at the corners with increased thickness along the edges due to the crossed slit geometry of the two chips that

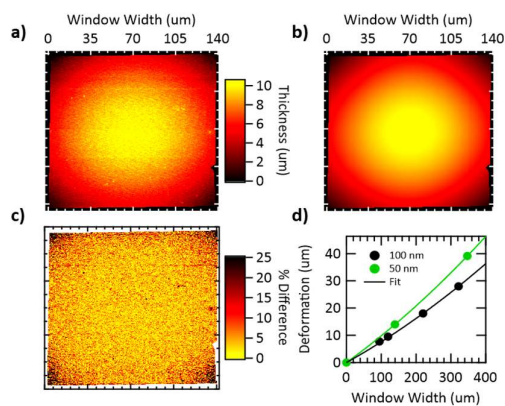


Figure 2 - Analysis of the square aperture created by overlapping two SiN windows to create a flow cell. **a)** Experimental thickness map of the interior-to-interior distance between SiN windows with 0.5 μm spacers and water flowing between as measured under experimental water flow conditions. **b)** Deformation fits results using Kirchhoff-Love Plate Theory as described in the main text **c)** Spatial maps of the percent difference between experimental and modeled deformation. **d)** Modeled deformation at the center of all window sizes measured for both 50 nm (Green) and 100 nm (Black) SiN membrane thicknesses as a function of experimentally determined aperture size. The data is fitted with a quadratic function including a data point at the origin.

form the cell. By far the maximum thickness/bowing occurs at the center of the aperture, where, in the example shown, the cell thickness becomes approximately 10 μm . Given the 0.5 μm spacers used in this study, the bowing here is 20x the undeformed thickness of the fluid sheet between the chips.

Because the thickness of the SiN windows have a thickness-to-width ratio of less than 0.1, Kirchhoff-Love Plate Theory (KLPT) was used to describe the overall shape of the bowing.³³ Under our assumption that the pressure in the cell induces a uniform load, KLPT takes the form of the following differential equation.

$$\nabla^2 \nabla^2 \omega(x, y) = P \frac{12(1 - \nu^2)}{H^3 Y} \quad (1)$$

where ω is the position-dependent distance of deflection of the plate (window) in the z-direction, P is the uniform applied load (pressure), H is the thickness of the window, Y is Young's modulus, and ν is the Poisson's ratio. We use Navier's solution to **Equation 1** for a simply supported rectangular plate with a uniformly distributed load³³ as shown in **Equation S1**. This solution was applied to both the top and bottom SiN membranes separated by a spacer of thickness d with the total thickness of the liquid layer at any given point calculated as

$$\mathcal{B}(x, y) = \omega_1(x, y) + \omega_2(x, y) + d \quad (2)$$

where ω_1 and ω_2 represent bowing of the two crossed windows in opposite directions, as seen in **Figure 1**. Their individual forms can be found in **Equation S2**.

Figure 2b shows the model, $\mathcal{B}(x, y)$, used to fit the raw data in **Figure 2a**. There is a total of four fit parameters that are part of calculating **Equation 2**: Uniform pressure, P , two window dimensions a and b , and spacer thickness, d . The percent difference between the experimental data and the model in **Figure 2c** shows excellent agreement except directly at the corners, where the model predicts the sample thickness to be up to 25% thinner than measured. This is due to the model not accounting well for the deformation effects as the SiN window comes into contact with the silicon wafer. We measured multiple window sizes and SiN thicknesses, applying the same analysis to each as shown in **Figure S1**. Our use of KLPT is important, since previous published window deformation models using crossed parabolas are insufficient to forward model the scattering and attenuation effects in the critical region away from the direct center of the window.^{30,34} **Figure S2** shows the same percent difference maps as **Figure S1**, using crossed parabolas instead of KLPT for comparison, highlighting the benefits of using KLPT.

The deformation at the center of each window is shown in **Figure 2d**, where the green and black data correspond to 50 nm and 100 nm SiN membrane thicknesses. Quadratic fits to these thicknesses (lines) fall within an average of 2% of each datapoint and were used to calculate the center thickness of any other window sizes, under the same experimental conditions. Fit coefficients are in **Table S1**. Both the measurements and predicted trends show that the total window bowing is

approximately 10% of the window width for both membrane thicknesses measured, which is more than an order of magnitude larger than the original spacer-defined gap from which new fluid emerges from the microfluidic lines. Though the difference between deformation at the center of the two membrane thicknesses follows a constant ratio of 0.78, our measurement of only two thicknesses precludes the need to model any others because the decrease in bowing from the 100 nm thick membrane is not substantial enough to overcome the decrease in transmission ($\sim 2\times$), **Figure S3**. Due to the transmission loss, we find that the 50 nm thick membrane is the better of the two options.

A quantitatively accurate window bowing model is key in predicting the calculated intensity that reaches the detector for LP-RSoXS analyses, $I_{det}(x, y)$. In particular I_{det} involves three key effects: the intrinsic scattering intensity $I_{sc}(x, y)$ from the sample in the cell, X-ray transmission $T(x, y)$ through the cell, and the incident beam profile $I_0(x, y)$ which is typically not uniform across the window aperture:

$$I_{det} = I_{sc} \cdot T \cdot I_0 \quad (3)$$

The first two terms are dependent on the specific window bowing behavior, while the last term is specific to the scattering instrument or beamline being used.

The details of the nanostructure will be encoded in the first term I_{sc} . For generality, we calculate the Porod Invariant which is the total scattering power of a structure and is independent of the details of that structure. Most structures can be approximated as a two phase system yielding $I_{sc}(x, y) = 4\pi^2 dV(x, y) \Delta n^2 \phi(1 - \phi)/\lambda^4$, where $dV = \mathcal{B}(x, y) dx dy$ is the differential sample volume that varies across the aperture due to the bowing, ϕ is the volume fraction (concentration) of nanoparticles in solvent or polymer in a hydrogel, λ is the photon wavelength, and Δn^2 is the scattering contrast between the solute and solvent, defined by the complex index of refraction $n = 1 - \delta + i\beta$. This model also works for continuous (phase separated or porous) solids where $\phi \sim 1$. Since we are focused on resonant scattering of carbon molecular systems, we simulate a plausible polyethylene oxide (PEO) polymer in water across the carbon absorption edge with dilute $\phi \ll 1$ for nanoparticles in water and $\phi \sim 1$ for a hydrogel. At the absorption edge, Δn varies dramatically with photon energy, giving RSoXS exquisite sensitivity to specific chemical moieties within the molecule¹ and must be calculated from absorption spectroscopy measurements of the energy-dependent mass absorbance $\mu = 2\pi\beta/\lambda$. The components of the index (δ and β) are presented in Figure S4a for PEO, water, and SiN across the carbon K-edge.

The transmission through the cell is calculated using Beer's Law through the two SiN membranes and the solution between them as $T(x, y) = \exp[-2\mu_{SiN} t_{SiN} - \mathcal{B}(x, y) \sum_i c_i \mu_i]$. While

the SiN windows are of a singular material, the fluid between can be a mixture based on the mass concentration (c_i) of each component. Due to the bowing of the SiN membrane windows, the photon path through the sample will be longer in the middle of the aperture, decreasing transmission through attenuation while simultaneously increasing the scattering signal through an increase in scattering volume. The nonlinear trade-off between these two effects governs the optimum flow cell geometry.

We note that the relatively long photon path lengths in liquid flow cells compared to solid thin films, raise the question of multiple scattering events. Following the common ‘rule of thumb’ from neutron and x-ray scattering, multiple scattering becomes important when the product of scattering cross-section (σ), particle concentration (c), and sample path length (L) approaches unity, $\sigma c L \gtrsim 1$.³⁵ However, this ignores absorption, which is a critical piece when photon energies are near the absorption edge. In practice, absorption reduces the chance that a photon survives to be scattered a second time, effectively raising the threshold for multiple scattering events. Absorption can then be included in our estimated threshold by including the attenuation factor, $\sigma c L e^{-\mu L} \gtrsim 1$, effectively making multiple scattering irrelevant and single scattering remains the dominant process.

For the final term in **Equation 3**, we approximate the incident beam profile, $I_0(x, y)$, as a uniform two dimensional Gaussian shown in **Figure 3**. This is an idealized representation matching the FWHM of the beam at the sample at the Advanced Light Source RSoXS Beamline 11.0.1.2, but will be different for other instruments. Superimposed on this profile are the apertures for cell window widths of 200 μm , 100 μm , and 50 μm

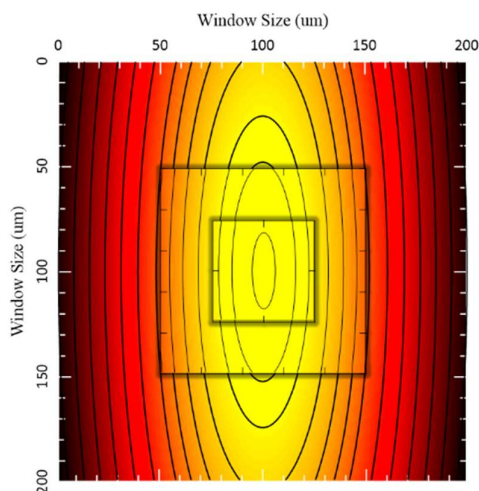


Figure 3 - 200 μm , 100 μm , and 50 μm apertured beam profiles, which are idealized representations of that at the ALS Beamline 11.0.1.2. These profiles are the 2D incident intensity maps $I_0(x, y)$ used in the window-bowing models.

μm . These apertures show that the right and left edges of the window are exposed to less intensity than the top and bottom. Additionally, the smallest aperture, 50 μm , exhibits a more uniform intensity distribution, whereas the 200 μm aperture receives very little to no intensity at the right and left edges. As such, the effect of I_0 on I_{det} becomes more impactful as the window aperture increases.

The full analytical model that quantitatively combines all discussed effects on I_{det} is shown in **Equation S3** while example results of this model are shown in **Figure 4a** for various window

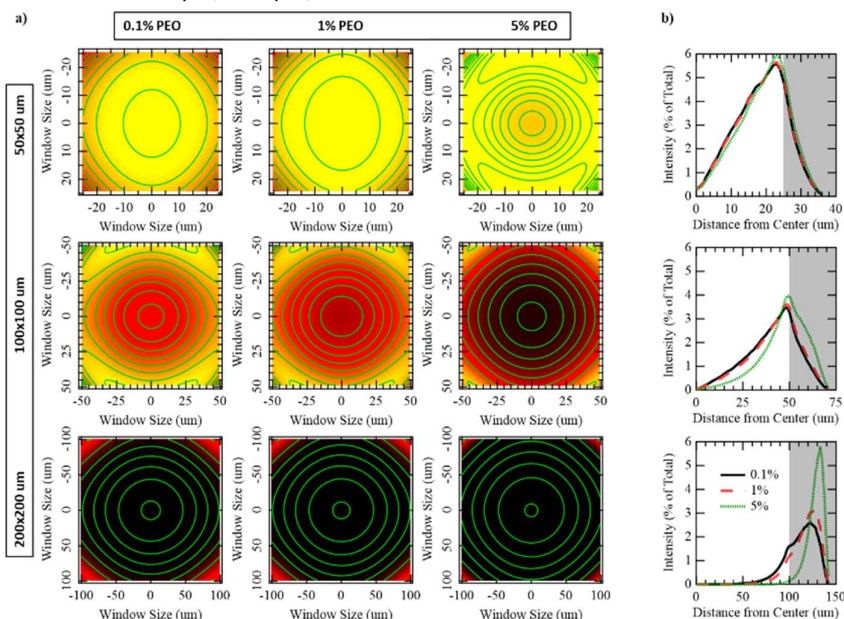


Figure 4 - **a)** 2D Images of $I_{det}(x, y)$ at 289 eV for varying mass fraction concentrations (0.1%, 1%, and 5%) of polyethylene oxide (PEO) in water for different window sizes. Constant intensity contour lines are overlaid. All three window sizes at a given concentration are displayed on the same yellow-red-black color scale. Each image has eight contour lines, log-distributed between 95% of the maximum and 1% of the minimum pixel intensity. **b)** Normalized azimuthal integration of each window from the center $I_m(r)$. Black – 0.1% PEO, Red – 1% PEO, and Green – 5% PEO. The shaded region represents radii extending past the window’s edge, i.e., radii that only represent the corners of the window.

sizes and PEO solute concentrations – all at the same color scale and with a 50 nm thick SiN membrane. The photon energy used (289 eV) maximizes the contrast with water, as shown in **Figure S4b**. For the smallest window (50 μm , **Figure 4a top row**), the bowing increases the scattering signal in the center due to increased sample volume that does not appreciably attenuate. At the highest PEO concentration, however, the lower transmission in the center slightly attenuates the signal due to the increased absorption by the PEO chains. This results in an annulus around the center as the most intense region for I_{det} . Notably, the vertical beam profile also affects the intensity map, causing increased intensity from the top and bottom of the aperture compared with the sides (best revealed by the contour lines). The attenuation in the center worsens for the 100 μm windows (**Figure 4a middle row**), where now the brightest region is a wide annulus that touches the sides of the windows, and the transmission function more severely attenuates the center region, which again gets worse with higher PEO concentration. For the 200 μm windows (**Figure 4a bottom row**), the transmission function completely dominates the other terms, attenuating all the signal except for the corners where deformation is held to a minimum.

The traces in **Figure 4b** represent the azimuthal integration $I_m(r)$ of each window's I_{det} from the center, normalized to the total integrated intensity across the entire window I_{tot} . The white region represents radii where all angles of the azimuth contribute (from the center to the sides of the window), whereas the gray area indicates the window corners. This representation tells a similar story as the I_{det} heat maps where a peak in $I_m(r)$ within the white region for the smallest window (top panel) indicates that most of the signal originates from the

center. A decline toward $r = 0$ is due to less area (fewer pixels) at that radius rather than a decline in intensity. Importantly, out of the total area, very little signal originates from the window corners, as can be estimated by comparing the areas under the curve in the two regions. The middle graph shows that at 100 μm window sizes, the intensity is indeed very concentrated in an annulus directly at the window edges. Finally, the bottom graph in **Figure 4b** demonstrates how nearly all of the intensity comes from the window corners, with increasing PEO concentration causing the intensity to be pushed further into the corner.

$I_{det}(x, y)$ and $I_m(r)$ in **Figure 4** show the distribution of where the signal comes from in the window, but each window size has a different area, affecting the total signal, and both signal and attenuation depend on the material's optical constants in a complex manner across the carbon absorption edge. To capture this, we show the total signal I_{tot} to the detector as a heat map in **Figure 5** as a continuous function of window size and PEO concentration for three representative energies across the carbon K-edge: pre-edge (270 eV, **Figure 5a**), at resonance (289 eV, **Figure 5b**), and post-edge (320 eV, **Figure 5c**). Each heat map can then be used to find the optimal window width and PEO concentration to maximize the scattering signal. Alternatively, vertical line profiles can be extracted to determine the best window width for a specific targeted PEO concentration, green lines in **Figure 5**. The model consistently shows a localized maximum signal, which is bounded in window size by the aperture at the small end and attenuation from bowing at the high end. In the PEO concentration dimension, intensity is bounded by scattering signal at the low end and absorption at the high end.

At non-resonant or pre-edge energies (**Figure 5a**), the maximum signal primarily occurs at a window size of 110 μm but over a large range of PEO concentrations centered on that of a hydrogel rather than a dilute sample. This is because the PEO chains do not absorb at this energy, such that the 57.6% concentration will produce the most scattering. Conversely, at X-ray energies that the PEO chains absorb, the region of maximum scattering signal is compressed to significantly lower concentrations and smaller window sizes, with the most compression occurring at the energy of highest absorbance (289 eV), resulting in maximum signal at PEO concentrations of 4.4% for window sizes of 78 μm . Notably, at hydrogel concentrations (>40%), the ideal window size is less than 40 μm , since any bowing significantly attenuates the signal at these concentrations.

Although the results in **Figure 5** can help one choose sample concentrations if that flexibility exists, it is generally impractical to choose a different window size for different X-ray energies. Given the difficulty of manufacturing microfluidic flow cells, we turn to evaluating an optimal window size in the case of typical solute concentrations such as 0.1%, 1%, and 5% PEO. This analysis is plotted as a continuous function of energy across the carbon K-edge in **Figure 6a**. The matching color-shaded regions depict the window sizes associated with I_{tot} reduced by less

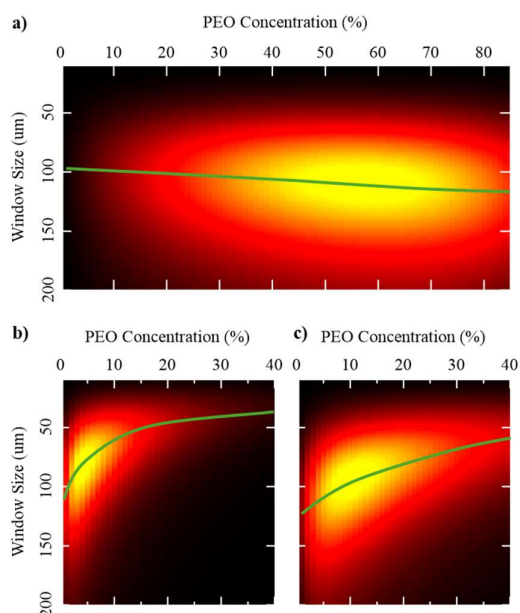


Figure 5 - Yellow-red-black heat map of total intensity at the detector I_{tot} versus window size and PEO concentration at an incident photon energy of **a)** 270 eV, **b)** 289 eV, **c)** 320 eV. The green line on each heat map follows the window size with the maximal intensity for each PEO concentration.

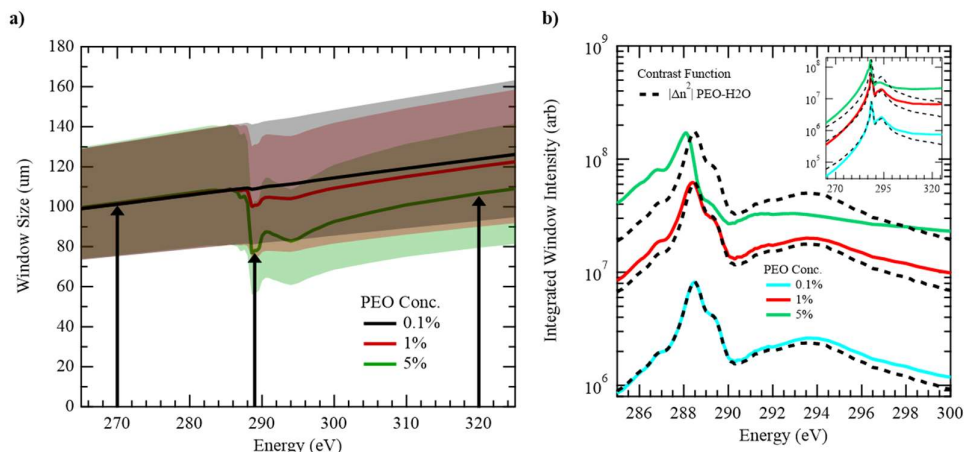


Figure 6 - a) Optimal window size versus incident photon energy at three concentrations of PEO in water: Black – 0.1%, Red – 1%, Green – 5%. The corresponding shaded region for each concentration represents the distribution of window sizes where I_{tot} is reduced by less than 20% from the maximum value. The three black arrows indicate the three energies displayed in Figure 5. **b)** Expected total calculated signal, I_{tot} , across the carbon K-edge for the common optimal window width of 104 μm for PEO in water: Blue – 0.1%, Red – 1%, Green – 5%. All three PEO concentrations have the same overlaid standard contrast function between PEO and H₂O (Black) that have been arbitrarily scaled so that the intensity of the maximum peaks are the same, to highlight any differences in features. The primary graph shows an energy range between 285 eV and 300 eV, where the most dramatic difference in features is located, whereas the inset at the top right shows an increased energy range of 265 eV to 325 eV.

than 20% from the maximum, representing a range of window sizes that produce scattering signals similar to the maximum. Across the absorption edge, there are common window sizes between the chosen concentrations and across the energy range. By taking the weighted mean of all energies for all three concentrations, the resulting common optimal window width is 104 μm . Notably, this width falls within the optimal range for all energies and PEO concentrations simulated.

Choosing this optimal window size, we plot the expected total signal, I_{tot} , for each of the simulated PEO concentrations across the carbon K-edge in **Figure 6b**, providing an attenuation and scattering intensity corrected contrast function. The overlaid standard PEO:H₂O contrast function, Δn^2 , is arbitrarily scaled so that the intensity of the maximum peaks are equal for qualitative comparison, highlighting some stark differences in features. Notably, at low concentrations, 0.1% PEO, the standard contrast function matches quite well; however, as we move to 5% PEO, the energies of maximum intensity change significantly by 0.4 eV, and other features are either substantially suppressed or enhanced. This apparent difference indicates that the contrast functions being used for in-situ nanoparticle scattering are incorrect when the solutions under study begin to exceed the dilute limit. However, this should not be a deterrent, as the features of **Figure 6b** clearly show that the 5% PEO solution exhibits a total signal enhancement of approximately 1.5 orders of magnitude over that of the 0.1% PEO solution across even an extended energy range (see **Figure 6b** inset).

Put together, these results challenge the assumption that the primary scattering intensity originates from the corners of the windows. Instead, we demonstrate that the primary signal can originate from the center, an annulus, or the corners,

depending in a complex manner on the geometry, bowing, sample concentration, and X-ray energy used. Although outside the scope of this study, KLPT refined by our measurements can also be used to predict ideal cell geometries and conditions for use in electron microscopes, for which these platforms are primarily used.

These results also challenge the simple model of smooth laminar flow of liquid through the aperture. All cell geometries measured, including the ideal one, are expected to have bowing >10x that of the flow inlet defined by the cell spacers. Thus, new fluid entering the aperture will not continue to flow with the previous velocity in a sheet across the aperture but begin to mix in the relatively large three-dimensional droplet inside the cell. Consequently, considerations of turbulence and dilution effects must be included in any dynamics measured in such a system, the timescales of which are relatively unknown.

Although our results appear to point to one optimal window geometry, it should be noted that hydrogels will require different geometries for the best signal due to the additional attenuation from their higher carbon concentration. The results also depend on the pressure applied across the membrane. In this study, we used a constant flow microfluidic system, though future studies would benefit from implementing constant pressure control, which would enable a more direct and reproducible correlation between applied pressure and membrane deformation, providing a more systematic route to optimizing cell geometry and potentially enhancing the ultimate signal beyond the parameters investigated here. Though the observed deformation suggests that SiN membranes remain highly elastic, we did not directly measure the applied pressures or quantify fracture strain limits. Determining the maximum sustainable deformation before failure will require future

experiments with controlled pressure loading. In practice, however, we have repeatedly cycled SiN flow cells in LP-RSoXS experiments without failure, suggesting robust operation under typical conditions, though systematic studies of long-term or fatigue effects have not been performed.

Additional considerations are also important for future work. Finite element simulations, for example, could complement the experimentally validated KLPT framework presented here. However, such simulations require well defined mechanical parameters for ultrathin SiN membranes, which often deviate from bulk values and therefore must be measured or carefully calibrated. The use of other, more rigid membrane materials has also recently been demonstrated with aluminium oxide membranes,³⁶ which are likely to reduce bowing and may prove superior to SiN, while also enabling studies at the Nitrogen K-edge. Alternatively, patterned support structures may reduce bowing but will also introduce parasitic scattering features that can obscure weak LP-RSoXS signals.

Experimental Section

Flow Cell SiN Chip Fabrication: Custom SiN microfluidic flow cells were fabricated from silicon wafers coated with SiN films (50 nm or 100 nm thickness). Windows were etched via photolithography with aluminum spacer pads deposited by physical vapor deposition to maintain a uniform spacing of 500 nm. Photomasks defined window sizes of 800 μm in length and widths of 70, 100, 200, or 300 μm . Wafer dicing ensured precise chip separation and fit for tight sealing into a custom microfluidic platform fabricated by Protochips.

Scanning Transmission X-ray Microscopy (STXM) STXM measurements were performed under vacuum at Beamline 5.3.2.2 of the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory (LBNL), using a Protochips custom liquid flow cell.⁸ A photon energy of 530 eV (just below the oxygen edge) was used to measure the transmission through the flow cell across all SiN window configurations. During the experiment, a constant flow of DI water was passed through the cell to mimic similar experimental parameters used during a scattering experiment.

Optical Constants: PEO was dissolved in H₂O prior to being spun-cast as a dry film. Total electron yield (TEY) NEXAFS spectra of PEO were collected at BL 6.3.2³⁷ of the ALS at LBNL. The imaginary component of the index of refraction, Beta, was scaled to bare atom mass absorption calculated from the CXRO database²³. Beta for H₂O and SiN were taken directly from the CXRO database. The real component of the index of refraction, Delta, was calculated via Kramer-Kronig transformation methods.³⁸

Data Analysis and Modeling: All data analysis and modeling presented in this manuscript were conducted using Igor Pro 9 (Wavemetrics) with custom-written code. The code and associated scripts are available upon request.

Conclusions

We have directly characterized SiN membrane deformation in experimental conditions of a flow cell, refining a physical model to predict window bowing in a microfluidic experiment under vacuum. We found that window bowing increases the total fluid sample thickness by more than 10x the original spacer thickness up to about 10% of the window width. Further, the membrane thickness does not have as significant an influence on the total amount of bowing as hypothesized. We apply these measurements to a comprehensive model including scattering volume, resonant cross-section, sample concentration, beam attenuation, and beam profile to determine from where within the cell scattering signal originates and what the optimal cell geometry is. We find that, contrary to previous assumptions, the signal can originate from any part of the cell depending on the conditions, but at the optimum window width of 104 μm , the majority of the signal originates from an annulus region that touches the sides of the cell. This model can be used to predict and optimize resonant scattering experiments as well as provide a basis for increasingly quantitative spatiochemical analyses of organic and hybrid nanostructures in liquid environments.

Author contributions

B.A.C. conceived and B.A.C. and C.W. oversaw the project with significant input from D.G. and T. M. D.G. and T. M. carried out all experimental measurements with assistance in setting up beamlines from M.A.M. Analysis and model development were primarily done by D.G. with some input from T.M. The manuscript was written by D.G. with contributions from B.A.C.

Conflicts of interest

There are no conflicts to declare.

Data availability

The raw data, as collected from LBNL ALS beamline 5.3.2, and the code used to support this article has been included as part of the Supplementary Information. The raw data is also available on LBNL ALS serves upon request to the current beamline scientist.

yield (TEY) NEXAFS

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References

- 1 B. A. Collins and E. Gann, *J. Polym. Sci.*, 2021, **60**, 1199–1243.
- 2 J. H. Carpenter, A. Hunt and H. Ade, *J. Electron Spectrosc. Relat. Phenom.*, 2015, **200**, 2–14.
- 3 F. Liu, M. A. Brady and C. Wang, *Eur. Polym. J.*, 2016, **81**, 555–568.
- 4 S. Rongpipi, J. T. Del Mundo, E. D. Gomez and E. W. Gomez, *MRS Commun.*, 2021, **11**, 1–17.
- 5 W. Zhong, F. Liu and C. Wang, *J. Phys. Condens. Matter*, 2021, **33**, 313001.
- 6 D. Grabner, H. Heilman, A. Patterson, T. M. Melody and B. A. Collins, *Annu. Rev. Mater. Res.*, DOI:10.1146/annurev-matsci-080323-040123.
- 7 D. Grabner, P. D. Pickett, T. McAfee and B. A. Collins, *Langmuir*, 2024, **40**, 7444–7455.
- 8 T. McAfee, T. Ferron, I. A. Cordova, P. D. Pickett, C. L. McCormick, C. Wang and B. A. Collins, *Nat. Commun.*, 2021, **12**, 3123.
- 9 E. B. Gowd, T. Koga, M. K. Endoh, K. Kumar and M. Stamm, *Soft Matter*, 2014, **10**, 7753–7761.
- 10 B. Ingham, G. D. Erlangga, A. Smialowska, N. M. Kirby, C. Wang, L. Matia-Merino, R. G. Haverkamp and A. J. Carr, *Soft Matter*, 2015, **11**, 2723–2725.
- 11 J. Korpanty, C. Wang and N. C. Gianneschi, *Nat. Commun.*, 2023, **14**, 3441.
- 12 Y. Yang, I. Roh, S. Louisia, C. Chen, J. Jin, S. Yu, M. B. Salmeron, C. Wang and P. Yang, *J. Am. Chem. Soc.*, 2022, **144**, 8927–8931.
- 13 Y. Yang, S. Louisia, S. Yu, J. Jin, I. Roh, C. Chen, M. V. Fonseca Guzman, J. Feijóo, P.-C. Chen, H. Wang, C. J. Pollock, X. Huang, Y.-T. Shao, C. Wang, D. A. Muller, H. D. Abruña and P. Yang, *Nature*, 2023, **614**, 262–269.
- 14 D. Ye, T. P. Le, B. Kuei, C. Zhu, P. H. Zwart, C. Wang, E. D. Gomez and E. W. Gomez, *Structure*, 2018, **26**, 1513–1521.e3.
- 15 K. P. Mineart, J. J. Ryan, M.-S. Appavou, B. Lee, M. Gradzielski and R. J. Spontak, *Langmuir*, 2019, **35**, 1032–1039.
- 16 L. Willner, A. Poppe, J. Allgaier, M. Monkenbusch and D. Richter, *Europhys. Lett. EPL*, 2001, **55**, 667–673.
- 17 S.-H. Choi, T. P. Lodge and F. S. Bates, *Phys. Rev. Lett.*, 2010, **104**, 047802.
- 18 J. Lu, F. S. Bates and T. P. Lodge, *Macromolecules*, 2015, **48**, 2667–2676.
- 19 A. J. Peters and T. P. Lodge, *Macromolecules*, 2017, **50**, 6303–6313.
- 20 J. T. Early and T. P. Lodge, *Macromolecules*, 2019, **52**, 7089–7101.
- 21 E. Mahieu and F. Gabel, *Acta Crystallogr. Sect. Struct. Biol.*, 2018, **74**, 715–726.
- 22 T. P. Russell, *Macromolecules*, 1993, **26**, 5819.
- 23 CXRO X-Ray Interactions With Matter, https://henke.lbl.gov/optical_constants/, (accessed October 13, 2024).
- 24 S. S. Welborn and E. Detsi, *Nanoscale Horiz.*, 2020, **5**, 12–24.
- 25 B. F. B. Silva, *Phys. Chem. Chem. Phys.*, 2017, **19**, 23690–23703.
- 26 J. Feng, M. Kriechbaum and L. (Emily) Liu, *Nanotechnol. Rev.*, 2019, **8**, 352–369.
- 27 A. Yaghmur and I. Hamad, *Molecules*, 2022, **27**, 4602.
- 28 T. Li, A. J. Senesi and B. Lee, Small Angle X-ray Scattering for Nanoparticle Research, <https://pubs.acs.org/doi/abs/10.1021/acs.chemrev.5b00690>, (accessed December 13, 2024).
- 29 S. Labov, S. Bowyer and G. Steele, *Appl. Opt.*, 1985, **24**, 576.
- 30 ProtoChips, 2017.
- 31 T. Ferron, D. Grabner, T. McAfee and B. Collins, *J. Synchrotron Radiat.*, 2020, **27**, 1601–1608.
- 32 M. E. Holtz, Y. Yu, J. Gao, H. D. Abruña and D. A. Muller, *Microsc. Microanal.*, 2013, **19**, 1027–1035.
- 33 S. P. Timošenko and S. Woinowsky-Krieger, *Theory of plates and shells*, McGraw-Hill, New York, 2. ed., [Nachdr.], 1996.
- 34 N. J. Zaluzec, M. G. Burke, S. J. Haigh and M. A. Kulzick, *Microsc. Microanal.*, 2014, **20**, 323–329.
- 35 G. V. Jensen and J. G. Barker, *J. Appl. Crystallogr.*, 2018, **51**, 1455–1466.
- 36 D. Ye, S. Rongpipi, J. H. Litofsky, Y. Lee, T. E. Culp, S. H. Yoo, T. N. Jackson, C. Wang, E. W. Gomez and E. D. Gomez, *MRS Commun.*, 2019, **9**, 224–228.
- 37 J. H. Underwood and E. M. Gullikson, *J. Electron Spectrosc. Relat. Phenom.*, 1998, **92**, 265–272.
- 38 H. Yan, C. Wang, A. R. McCarn and H. Ade, *Phys. Rev. Lett.*, 2013, **110**, 177401.