Supporting information for

Evolution of Membrane Fouling Revealed by Label-Free Vibrational Spectroscopic Imaging

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This supporting information contains 10-page document, including 2 tables with references, 6 figures and this cover page.

Table S1. Averages of SRS Intensity at 2969 cm⁻¹ before and after Membrane Fouling

membrane	pure PVDF	BSA-fouled	dextran-fouled
normalized SRS intensity	1.633±0.062	2.038±0.333	1.762±0.057

method	chemical information	3D distribution	label-free	reference
IR, ATR	\checkmark	poor	\checkmark	1-4
SEM	×	×	\checkmark	5
OCT	×	\checkmark	\checkmark	6,7
fluorescence microscope	\checkmark		×	8,9
SERS SRS imaging		$\stackrel{\times}{\checkmark}$		10,11

Table S2. Comparison Between our SRS Imaging Approach with Previous Methodsfor Characterizing Memebrane Fouling Process

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Figure S1. Schematic illustration of the membrane filtration system



Figure S2. Calibration of Raman shifts in the spectral focusing system. (a) Normalized SRS spectra of dimethyl sulfoxide (DMSO), methanol (MeOH), and two-photon absorption spectrum of rhodamine 6G (R6G); and (b) Correlation of the Raman shift with respect to the pump-Stokes delay.



Figure S3. Spatial resolution estimation of imaging system using a 1951 USAF resolution test grid. The horizontal and vertical intensity profiles are plotted at the bottom and on the right side of the image. The measured FWHMs of Gaussion fitting are 0.50 and 0.57 μ m, respectively.



Figure S4. 3D reconstructed SRS imaging of PVDF membrane substrate at 2969 cm⁻¹ after 100 min filtration of dextran solution.



Figure S5. Cross-section view of foulants on the membrane during the BSA/dextran mixture (1:1) filtration (a); and the corresponding 3D reconstructed SRS images (b). Size: $120 \ \mu m \times 120 \ \mu m \times 20 \ \mu m$



Figure S6. Cross-section view of the protein (a) and polysaccharide (b) foulants on the membrane along *xz* plane using fluorescence confocal microscopy, and the overlay image (c) along *xy* plane after the filtration of 200 mg/L 1:1 phycocyanin and dextran-FITC mixture for 45 min. *xyz* size: $250 \ \mu\text{m} \times 250 \ \mu\text{m} \times 50 \ \mu\text{m}$.