



# Stokes-consistent computational polarimetric reconstruction for label-free imaging of nanoplastic-associated perturbations

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**Abstract:** Polarimetric microscopy offers a label-free readout to probe anisotropy, scattering, and microstructural organization in biological systems, yet quantitative polarization observables become unreliable in low-signal aqueous imaging. In particular, the degree and angle of linear polarization are nonlinear functions of Stokes parameters and are therefore sensitive to noise, undersampling, and channel-dependent distortions in division-of-focal-plane measurements. Here, we introduce a Stokes-consistent computational polarimetric reconstruction framework for recovering reliable linear polarization observables from low-fidelity aqueous measurements. The proposed Nano-PolarSRN (nano-polarimetric super-resolution network) reconstructs four polarization-resolved channels while constraining the solution in both the image and Stokes-derived domains, thereby preserving intensity structure, degree of linear polarization (DoLP), and angle of polarization (AoP). Compared with intensity-supervised reconstruction, the Stokes-consistent framework improves structural fidelity and suppresses physically inconsistent DoLP and AoP artifacts. Applied to live marine rotifers exposed to polystyrene nanoplastics, the method reveals size-dependent polarization perturbations in digestive-vesicle-rich regions. Confocal fluorescence imaging independently confirms nanoplastic uptake, supporting the association between these label-free polarimetric signatures and nanoplastic uptake. This work establishes Stokes-consistent reconstruction as a computational optical strategy for quantitative label-free analysis of nanoplastic-associated perturbations in living aquatic organisms.

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## 1. Introduction

Nanoplastics have emerged as pervasive environmental contaminants that interact with aquatic organisms across multiple biological scales [1,2]. Owing to their small size, large surface-to-volume ratio, and high mobility, nanoplastics can enter feeding pathways, accumulate within digestive compartments [3,4], and perturb biological interfaces [5,6] that are difficult to access using conventional imaging or endpoint assays [7]. However, direct analysis of nanoplastic–organism interactions in living aquatic systems remains challenging [8–11]. Many established approaches, including Raman spectroscopy [12–15], Fourier-transform infrared (FTIR) spectroscopy [16,17], and pyrolysis–gas chromatography–mass spectrometry (py-GC-MS) [18,19], provide important compositional or morphological information but often require sample extraction, drying, labeling, or destructive processing. Fluorescence microscopy can visualize labeled particles in living specimens [20], but the interpretation may be influenced by dye leakage, photobleaching, and labeling efficiency. These limitations motivate a label-free optical strategy capable of probing how nanoplastics disturb living organisms in situ.

Transparent aquatic organisms pose a distinctive optical measurement problem [3]. Their internal structures often exhibit weak absorption contrast, while biologically relevant interfaces

can still modulate the polarization state of transmitted light [21]. Polarimetric microscopy therefore provides a route to access microstructural information that is not available from intensity alone [22–25]. For nanoplastic-exposed organisms, changes in scattering, refractive-index heterogeneity, and local anisotropy can therefore manifest as perturbations in Stokes-derived polarization maps [26,27]. However, quantitative polarimetry in living aqueous specimens is fundamentally limited by measurement fidelity [28,29]. In water, light attenuation, weak scattering contrast, specimen motion, and low photon budget degrade the signal-to-noise ratio (SNR) [21,30]. This degradation is especially problematic for degree of linear polarization (DoLP) and angle of polarization (AoP), because they are nonlinear functions of the measured Stokes parameters. Small channel-dependent errors in the polarization-resolved intensities can be amplified during Stokes reconstruction, and low total intensity can lead to biased DoLP values or spatially unstable AoP estimates. Thus, the central challenge is not simply that aqueous images are blurred or noisy, but that the physical polarization observables themselves become unreliable under low-fidelity acquisition.

Mueller matrix microscopy and holographic polarimetry provide powerful but distinct routes for quantitative polarimetric imaging. Mueller matrix microscopy characterizes the polarization-transfer properties of a specimen under multiple input and output polarization states, enabling analysis of diattenuation, retardance, and depolarization [22]. Holographic polarimetry combines polarization-resolved detection with phase-sensitive or interferometric measurement, providing access to complex-field, phase-related, or scattering-sensitive information [24,26,27,31]. These approaches offer rich physical characterization, but they generally require more complex acquisition schemes, multiple polarization-state measurements, interferometric stability, or more involved calibration. In contrast, this study focuses on compact snapshot division-of-focal-plane (DoFP)-based linear-Stokes imaging, in which  $S_0$ ,  $S_1$ , and  $S_2$ , together with DoLP and AoP, are recovered from low-SNR aqueous measurements.

Within this linear-polarimetry setting, computational super-resolution offers a possible route to recover information from low-quality measurements, but conventional intensity-driven super-resolution is not sufficient for quantitative polarimetric microscopy [32–34]. A reconstruction that appears sharper in the intensity domain may still distort the relative relationships among the four polarization channels. Such distortions can generate visually plausible images while producing physically inconsistent Stokes parameters, DoLP maps, and AoP patterns. This distinction is crucial: in polarimetric imaging, the desired output is not only a high-resolution (HR) image, but a set of polarization-resolved measurements from which meaningful optical observables can be computed. Super-resolution in this setting should therefore be formulated as a constrained inverse problem in the Stokes domain rather than as a purely perceptual image enhancement task. Here, we introduce a Stokes-consistent computational polarimetric reconstruction framework for recovering physically meaningful polarization information from low-fidelity aqueous measurements. The proposed Nano-PolarSRN (nano-polarimetric super-resolution network) reconstructs four polarization-resolved channels from low-resolution (LR) DoFP inputs while enforcing consistency in both the image domain and the Stokes-derived polarization domain. In this work, super-resolution refers to computational reconstruction from degraded LR polarimetric measurements, rather than optical super-resolution microscopy that surpasses the diffraction limit. Accordingly, Nano-PolarSRN aims to improve the effective spatial resolution, SNR, and Stokes-domain consistency of LR polarimetric measurements, rather than to recover spatial frequencies beyond the optical passband of the microscope.

In this work, computational polarimetric super-resolution is treated as a physics-constrained recovery of observables rather than as intensity restoration alone. Nano-PolarSRN combines pixel-wise and perceptual reconstruction losses with polarimetric supervision that constrains Stokes-domain consistency. We show that this design improves structural fidelity while suppressing

polarimetric artifacts introduced by intensity-supervised reconstruction. We apply this Stokes-consistent reconstruction strategy to the label-free analysis of polystyrene (PS) nanoplastic exposure in aquatic organisms. In marine rotifers exposed to 500 nm, 100 nm, and 20 nm particles, the reconstructed polarization maps reveal size-dependent polarimetric signatures associated with nanoplastic exposure in digestive-vesicle-rich regions. Rotifers exposed to larger particles exhibit more localized DoLP attenuation and disrupted AoP coherence, whereas smaller particles show weaker and more diffuse polarization changes. Confocal fluorescence imaging provides an independent validation of nanoplastic uptake, supporting the interpretation that label-free polarization signatures are associated with nanoplastic accumulation. Together, these results establish Stokes-consistent computational polarimetric reconstruction as a computational optical strategy for probing nanoplastic-associated perturbations in living aquatic organisms.

## 2. Materials and methods

### 2.1. DoFP polarimetric microscope and Stokes reconstruction

Polarization-resolved transmission images were acquired with a DoFP polarimetric microscope. Illumination was provided by a halogen lamp (Philips 7724), and the transmitted light was collected through an objective and tube lens imaging path, and recorded by a polarization-sensitive camera (PHX050S1-P/Q, Lucid Vision Labs). The camera contains a  $2 \times 2$  micro-polarizer array that simultaneously samples analyzer orientations of  $0^\circ$ ,  $45^\circ$ ,  $90^\circ$ , and  $135^\circ$  (Fig. 1(a)). The native resolution is  $2448 \times 2048$  pixels, with a subpixel size of  $3.45 \mu\text{m}$ . Interchangeable microscope objectives (S Plan Fluor  $10\times$  and  $40\times$ , Nikon) were used according to the required field of view and sampling density. A linear polarizer, quarter-wave plate, and neutral-density filter were included in the illumination path for polarization-state control and intensity adjustment.

Raw DoFP measurements were separated into four polarization sub-images according to the micro-polarizer pattern [35]. Dark-frame subtraction was applied to remove the camera offset, and flat-field correction was used to compensate for illumination nonuniformity and channel-dependent transmission differences. The four polarization channels were then interpolated onto a common spatial grid and normalized before Stokes reconstruction. The same preprocessing pipeline was applied to all high-fidelity reference images, low-fidelity inputs, and reconstructed outputs to ensure that intensity-domain and polarization-domain comparisons were performed consistently.

Only the linear Stokes components were reconstructed in this work. From the calibrated analyzer-channel intensities,  $I_{0^\circ}$ ,  $I_{45^\circ}$ ,  $I_{90^\circ}$ , and  $I_{135^\circ}$ , the linear Stokes parameters were computed as

$$S_0 = I_{0^\circ} + I_{90^\circ}, \quad S_1 = I_{0^\circ} - I_{90^\circ}, \quad S_2 = I_{45^\circ} - I_{135^\circ}. \quad (1)$$

The DoLP and AoP were then defined as

$$\text{DoLP} = \frac{\sqrt{S_1^2 + S_2^2}}{S_0}, \quad \text{AoP} = \frac{1}{2} \tan^{-1} \left( \frac{S_2}{S_1} \right). \quad (2)$$

The present implementation should be interpreted as snapshot linear Stokes polarimetry. Because the DoFP camera samples only four linear analyzer orientations, Nano-PolarSRN reconstructs  $S_0$ ,  $S_1$ , and  $S_2$ , together with the derived DoLP and AoP maps, but does not recover the circular Stokes component  $S_3$ . Therefore, the method does not characterize circular polarization, ellipticity-related contrast, circular birefringence/dichroism, or Mueller-matrix-derived depolarization behavior. Full-Stokes characterization would require additional polarization modulation or analyzer states, which is beyond the scope of the present DoFP implementation.

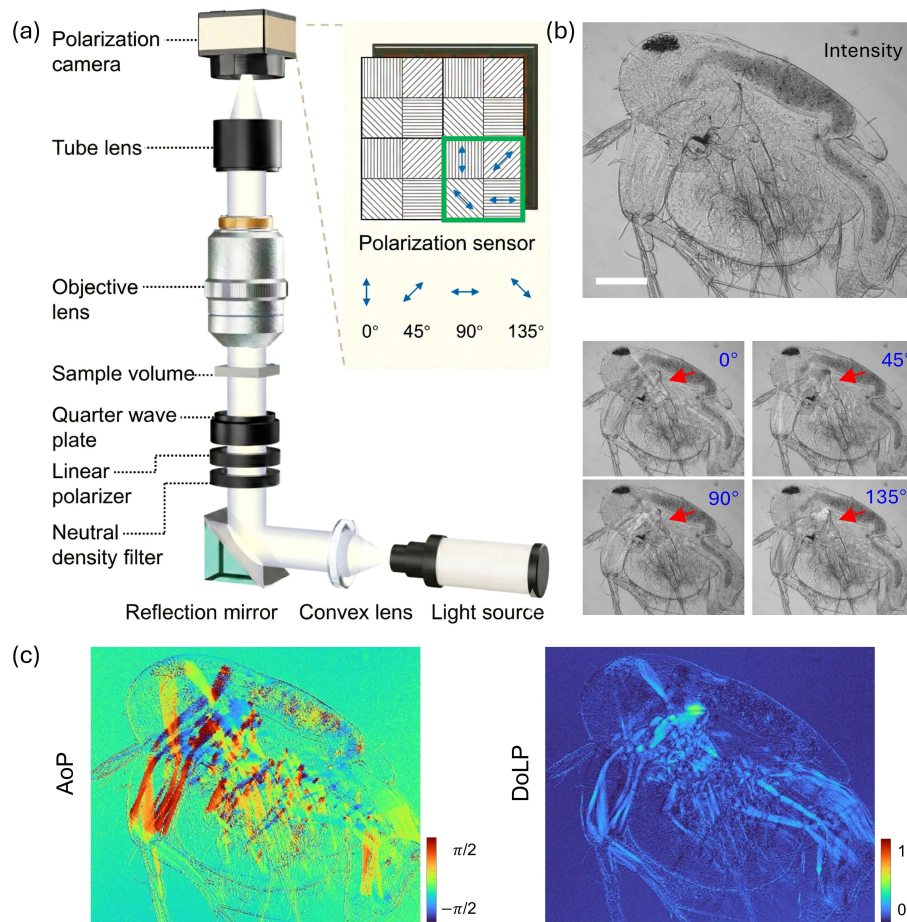


**Fig. 1.** DoFP polarimetric microscopy and label-free polarization contrast in a transparent aquatic organism. (a) Schematic of the DoFP polarimetric microscopy system. The inset shows the  $2 \times 2$  micro-polarizer array, which simultaneously samples four linear analyzer orientations ( $0^\circ$ ,  $45^\circ$ ,  $90^\circ$ , and  $135^\circ$ ). (b) Representative transmitted-intensity image of a *Daphnia* specimen and the corresponding polarization-resolved intensity images. Red arrows indicate a region with orientation-dependent contrast. Scale bar: 500  $\mu\text{m}$ . (c) Reconstructed angle of polarization (AoP, left) and degree of linear polarization (DoLP, right) maps derived from the four polarization channels, revealing spatially heterogeneous polarization signatures associated with internal microstructures.

## 2.2. Aquatic organism preparation and nanoplastic exposure

Marine rotifers (*Brachionus plicatilis*) were used as the primary living organism model. Rotifers were maintained in natural seawater at  $20^\circ$  under a 12 h:12 h light: dark cycle before imaging. For exposure experiments, rotifers were incubated with fluorescent PS nanoplastics with nominal diameters of 20, 100, and 500 nm. Emission spectra confirmed blue fluorescence from PS nanoplastics of all tested sizes. During confocal imaging, this fluorescence was collected using the DAPI/blue detection setting and pseudocolored blue for visualization. Morphology was assessed by scanning electron microscopy (SEM; S-4800, Hitachi) and laser scanning confocal microscopy (AX, Nikon). Polymer identity and surface functionality were validated by optical photothermal infrared spectroscopy (O-PTIR; mIRage, Photothermal Spectroscopy Corp.);

spectra were baseline-corrected and smoothed with PTIR Studio v4.4. A particle-free seawater group was used as the control.

Exposure was performed at a particle concentration of  $5 \text{ mg L}^{-1}$  for 48 h. Each exposure dish contained 2 mL of seawater and approximately 30 rotifers. After exposure, rotifers were gently rinsed three times with filtered seawater to reduce loosely attached external particles and were transferred to glass-bottom dishes for polarimetric and confocal imaging. Quantitative experiments included three biological replicates. Representative *Daphnia* specimens were also imaged to demonstrate label-free polarization contrast in transparent aquatic organisms, while quantitative nanoplastic-exposure analysis was performed on rotifers.

### 2.3. Paired data generation and degradation model

Supervised reconstruction requires paired low-fidelity inputs and high-fidelity references. Because direct acquisition of perfectly registered low- and high-resolution polarimetric image pairs from living aquatic organisms is limited by motion, low photon budget, and polarization-sensitive noise, paired data were generated from experimentally acquired high-SNR references under the same aqueous imaging geometry.

For each field of view, approximately 70 consecutive polarization-resolved frames were acquired under identical illumination, exposure, and camera-gain settings. After preprocessing, motion-free frames were temporally averaged to form the high-SNR HR reference  $\mathbf{X}^{\text{HR}} = \{I_{0^\circ}^{\text{HR}}, I_{45^\circ}^{\text{HR}}, I_{90^\circ}^{\text{HR}}, I_{135^\circ}^{\text{HR}}\}$ .

The corresponding low-fidelity inputs were generated using a physically motivated image degradation model:

$$I_\theta^{\text{LR}} = g_\theta \mathcal{D}_4 \left( G_{\sigma_{\text{add},\theta}} * I_\theta^{\text{HR}} \right) + b_\theta + n_\theta, \quad (3)$$

where  $\theta \in \{0^\circ, 45^\circ, 90^\circ, 135^\circ\}$ ,  $\mathcal{D}_4$  denotes  $4\times$  lateral downsampling,  $G_{\sigma_{\text{add},\theta}}$  is the additional pre-downsampling Gaussian blur for analyzer channel  $\theta$ ,  $*$  represents convolution,  $g_\theta$  and  $b_\theta$  are channel-dependent gain and offset terms, and  $n_\theta$  denotes the noise term. The noise was modeled using an intensity-dependent variance,

$$\text{Var}(n_\theta) = \alpha_\theta I + \beta_\theta, \quad (4)$$

where  $I$  denotes the corresponding clean downsampled intensity, with  $\alpha_\theta$  and  $\beta_\theta$  fitted separately for each analyzer channel.

The effective optical blur was first estimated from USAF resolution-target images acquired under the same optical configuration. Because the high-SNR reference already contains the microscope PSF, the USAF-derived blur was not applied a second time; instead, it was used as a prior to constrain the additional pre-downsampling blur. The final channel-dependent blur values were selected by matching the spatial-frequency spectra of synthetic LR images to real single-frame-derived LR images. Channel-dependent gain and offset were estimated by linear intensity matching, and the noise parameters were fitted from residuals between real single-frame-derived LR images and the corresponding clean downsampled references. The calibrated degradation parameters and supplementary validation analyses are summarized in Table S1 and Figs. S1 and S2 of [Supplement 1](#).

The calibrated synthetic LR images were designed to approximate real single-frame-derived LR measurements in terms of residual noise level and radial spatial-frequency behavior, as shown in Fig. S2. Nevertheless, the degradation model remains an approximation of real live aqueous imaging and may not fully capture specimen motion, out-of-focus scattering, depth-dependent blur, local refractive-index fluctuations, or residual preprocessing artifacts. Therefore, the quantitative evaluation on synthetic LR–reference pairs should be interpreted as a controlled reconstruction benchmark under an experimentally calibrated degradation model, rather than as a complete characterization of all possible real acquisition conditions.

In total, 115 paired LR–HR polarimetric datasets were generated for training and evaluation. When extracting patches, all four channels were cropped and augmented using identical spatial transformations to preserve inter-channel correspondence.

#### 2.4. Nano-PolarSRN architecture

Nano-PolarSRN reconstructs a four-channel super-resolved polarimetric image,  $\hat{\mathbf{X}}^{\text{SR}}$ , from a four-channel low-fidelity input,  $\mathbf{Y}^{\text{LR}}$ . Stokes parameters, DoLP, and AoP are computed only after reconstruction from the four recovered analyzer channels.

The network backbone follows a residual-in-residual dense block (RRDB) design adapted for four-channel polarimetric input [33,36]. A  $3 \times 3$  convolution first embeds the input into a 64-channel feature space. The feature extractor contains 23 RRDBs and a long skip connection adds the shallow feature map to the output of the RRDB cascade. Because the LR input is degraded by a factor of four along each lateral dimension, the upsampling module uses two successive  $2 \times$  upsampling stages, each followed by a  $3 \times 3$  convolution for feature refinement. A final  $3 \times 3$  convolution outputs the four super-resolved analyzer channels.

All models compared in this work used the same four-channel architecture and training split. The intensity-supervised baseline was trained with the polarimetric loss disabled while keeping the pixel and perceptual terms unchanged.

#### 2.5. Stokes-domain supervision

Nano-PolarSRN was optimized using a composite loss that balances image-domain fidelity and Stokes-domain consistency:

$$\mathcal{L}_{\text{total}} = \alpha \mathcal{L}_{\text{pixel}} + \beta \mathcal{L}_{\text{perceptual}} + \gamma \mathcal{L}_{\text{polar}}. \quad (5)$$

The pixel loss was the mean absolute error over the four analyzer channels. The perceptual loss was computed on the total-intensity image  $S_0$  rather than on pseudo-color polarization channels:

$$\begin{aligned} \mathcal{L}_{\text{pixel}} &= \frac{1}{4} \sum_{\theta} \|\hat{I}_{\theta}^{\text{SR}} - I_{\theta}^{\text{HR}}\|_1, \\ \mathcal{L}_{\text{perceptual}} &= \sum_l \left\| \phi_l(\hat{S}_0^{\text{SR}}) - \phi_l(S_0^{\text{HR}}) \right\|_1, \end{aligned} \quad (6)$$

where  $\phi_l(\cdot)$  denotes the feature map of the selected layer  $l$  in a fixed pretrained network.

The polarimetric loss was designed to enforce consistency in the Stokes-derived domain:

$$\mathcal{L}_{\text{polar}} = \lambda_S \mathcal{L}_S + \lambda_D \mathcal{L}_{\text{DoLP}} + \lambda_A \mathcal{L}_{\text{AoP}}. \quad (7)$$

The Stokes loss is

$$\mathcal{L}_S = \sum_{j=0}^2 \left\| \hat{S}_j^{\text{SR}} - S_j^{\text{HR}} \right\|_1. \quad (8)$$

The DoLP loss is

$$\mathcal{L}_{\text{DoLP}} = \left\| \widehat{\text{DoLP}}_{\text{SR}} - \text{DoLP}_{\text{HR}} \right\|_1. \quad (9)$$

Because AoP is an angular observable with  $\pi$ -periodicity, its loss was defined using a circular error:

$$\mathcal{L}_{\text{AoP}} = \left\langle 1 - \cos \left[ 2 \left( \widehat{\text{AoP}}_{\text{SR}} - \text{AoP}_{\text{HR}} \right) \right] \right\rangle. \quad (10)$$

The factor of two accounts for the  $\pi$ -periodicity of linear polarization orientation. All DoLP- and AoP-related losses were evaluated only over valid pixels with sufficient  $S_0$  to avoid low-intensity noise dominating the optimization.

## 2.6. Split-reference validation and effective-resolution analysis

To evaluate effective resolution improvement and assess whether reconstructed fine structures were supported by independent measurements, we performed split-reference validation on held-out temporal image stacks. For each selected field of view, the preprocessed polarization-resolved frames were divided into two non-overlapping subsets while preserving the same frame split across all four analyzer channels. The two subsets were independently averaged to generate split reference A and split reference B. Split reference A was used to synthesize the degraded LR input using the calibrated degradation model, whereas split reference B was reserved only as an independent validation reference.

Before comparison, the LR input was interpolated to the native reference grid. Effective resolution was quantified using Fourier ring correlation (FRC) computed from the reconstructed  $S_0$  intensity images [37,38]. FRC was evaluated between the LR input and split reference B, between the Nano-PolarSRN reconstruction and split reference B, and between split reference A and split reference B. The split-reference comparison served as an empirical upper bound for reproducible spatial information under the same optical configuration. The FRC cutoff frequency was determined using the 1/7 criterion.

Radial Fourier power spectra were also computed to compare the spatial-frequency content of the LR input, Nano-PolarSRN reconstruction, and independent split reference. Line profiles through selected vesicle-associated regions were used to assess whether reconstructed local features were spatially aligned with corresponding structures in the independent split reference. This analysis was used to quantify computational resolution enhancement relative to degraded LR inputs and was not interpreted as evidence of diffraction-unlimited optical resolution.

## 2.7. Confocal validation, quantitative evaluation, and statistics

Laser scanning confocal fluorescence microscopy was used as an independent validation method to confirm the presence and localization of fluorescent PS nanoplastics in exposed rotifers [39]. After polarimetric imaging or after matched exposure preparation, rotifers were transferred to glass-bottom dishes and imaged using the fluorescence channel matched to the particle label. Fluorescence-positive regions were used to verify nanoparticle accumulation, particularly in digestive-vesicle-rich regions. The fluorescence images were not used for Nano-PolarSRN training or loss computation.

Reconstruction performance was evaluated using one intensity-domain metric and two polarization-domain metrics. Structural fidelity was quantified by the structural similarity index measure (SSIM) computed from the reconstructed  $S_0$  image relative to the HR reference. DoLP reconstruction accuracy was quantified by the mean absolute error over valid pixels. AoP accuracy was quantified using the circular angular error

$$E_{\text{AoP}} = \frac{1}{2|\Omega|} \sum_{p \in \Omega} \arccos \left\{ \cos \left[ 2 \left( \widehat{\text{AoP}}^{\text{SR}}(p) - \text{AoP}^{\text{HR}}(p) \right) \right] \right\}. \quad (11)$$

Lower DoLP error and lower AoP circular error indicate improved polarimetric consistency.

For nanoplastic-exposure analysis, vesicle-rich digestive regions were selected from  $S_0$  or brightfield images before viewing the DoLP and AoP maps to reduce selection bias.

Data are reported as mean  $\pm$  standard deviation as specified in the figure captions. Group comparisons were performed using one-way ANOVA with Tukey post hoc test, with  $P < 0.05$  considered statistically significant. The  $1.5 \times$  interquartile range (IQR) rule was used only to define box-plot whiskers and to identify outliers for visualization purposes; no data points were excluded from the statistical analyses.

### 3. Results and discussion

#### 3.1. DoFP polarimetry reveals label-free structural contrast beyond transmitted intensity

We first evaluated whether DoFP polarimetry provides optical contrast beyond conventional transmitted-intensity imaging. A transparent *Daphnia* specimen was used as a representative aquatic organism to illustrate the polarization contrast mechanism. As shown in Fig. 1(a), the DoFP polarimetric microscope simultaneously records four linear polarization-resolved intensity channels through a  $2 \times 2$  micro-polarizer array. This simultaneous acquisition is important for living or weakly immobilized aquatic specimens because it avoids temporal mismatch among polarization channels.

The transmitted-intensity image in Fig. 1(b) reveals the overall morphology of the organism, but many internal structures remain weakly contrasted because of limited absorption contrast. In contrast, the four polarization-resolved channels exhibit orientation-dependent intensity variations. Regions that are only weakly distinguishable in the transmitted-intensity image become more apparent in selected polarization channels, indicating that the transmitted optical field contains polarization-dependent structural information as indicated by the red arrows.

The reconstructed AoP and DoLP maps in Fig. 1(c) further reveal spatially heterogeneous polarization signatures associated with internal microstructures. AoP reports the local orientation of polarization-dependent contrast, whereas DoLP quantifies the degree to which linear polarization is preserved. These Stokes-derived observables therefore provide a physical readout of anisotropy, refractive-index heterogeneity, and interface-associated scattering. This result establishes the optical basis for using polarimetric microscopy to probe weakly absorbing aquatic organisms without exogenous labels.

#### 3.2. Low-fidelity aqueous imaging destabilizes Stokes-derived polarization observables

Although polarimetric microscopy provides a label-free contrast mechanism for probing anisotropy and scattering in transparent aquatic specimens, quantitative linear polarization recovery is strongly limited by measurement fidelity in live aqueous imaging. Figure 2(a) summarizes this measurement bottleneck. During nanoplastic–organism imaging in water, optical blur, scattering, noise, and low SNR degrade the polarization-resolved measurements. These degradations do not merely reduce visual image quality; they can destabilize Stokes-derived observables such as AoP and DoLP, which are the quantities required for interpreting nanoplastic-associated polarization perturbations.

A representative low-fidelity polarimetric measurement of a live rotifer is shown in Fig. 2(b)–(d). The four polarization-resolved intensity images preserve the coarse body morphology of the organism, but the reconstructed AoP and DoLP maps exhibit spatial fluctuations, weak contrast, and fragmented local patterns. The AoP map contains noisy orientation variations across the body region, while the DoLP map shows attenuated and spatially nonuniform polarization contrast. These features indicate that the directly reconstructed polarization observables are unreliable under low-SNR aqueous imaging conditions.

This instability arises from the nonlinear dependence of AoP and DoLP on the reconstructed Stokes parameters. Small channel-dependent errors in the measured polarization intensities propagate into  $S_1$  and  $S_2$ , and are further amplified when  $S_0$  is weak. As a result, low-fidelity measurements can produce biased DoLP values and spatially unstable AoP patterns, even when the overall organism morphology remains visible in the intensity images.

These results define the central reconstruction problem addressed in this work. The objective is not simply to sharpen blurred images, but to recover polarization-resolved measurements that preserve the physical relationships among the four analyzer channels. Reliable analysis of nanoplastic-associated perturbations therefore requires a Stokes-consistent reconstruction

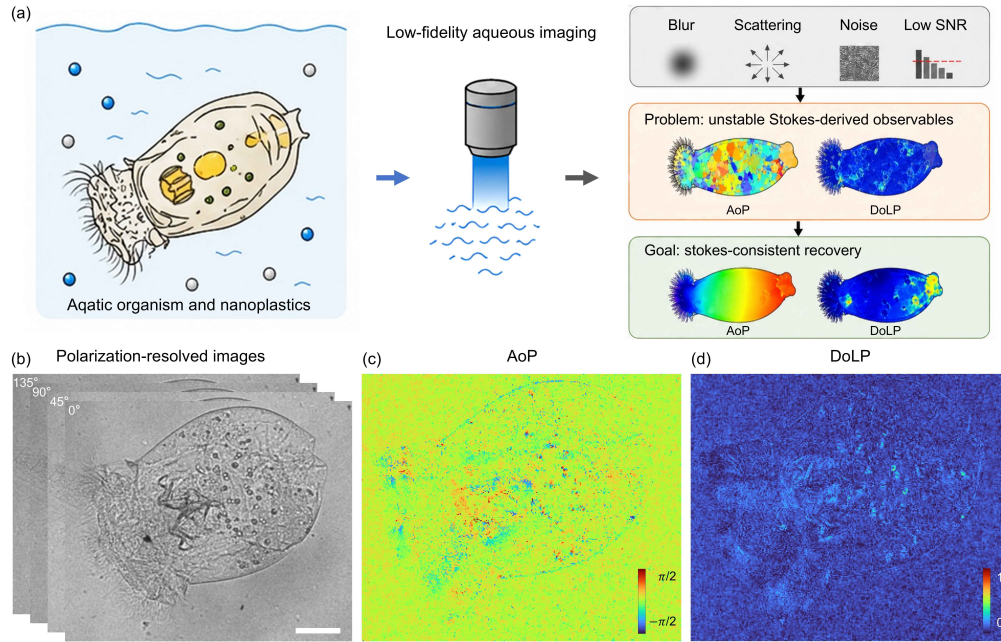


**Fig. 2.** Low-fidelity aqueous polarimetric imaging of a live rotifer. (a) Conceptual illustration of the imaging scenario and motivation. Nanoplastic–organism interactions are observed in an aqueous environment, where blur, scattering, noise, and low SNR degrade polarization measurements and destabilize Stokes-derived observables such as AoP and DoLP. This limitation motivates the development of a Stokes-consistent recovery framework for reliable polarimetric reconstruction. (b) Representative polarization-resolved intensity images acquired simultaneously at four linear analyzer orientations. (c) Reconstructed AoP map derived from the four polarization channels. (d) Reconstructed DoLP map. Weak DoLP contrast and spatially fluctuating AoP patterns illustrate the instability of Stokes-derived polarization observables under low-SNR aqueous imaging conditions. Scale bar: 50  $\mu\text{m}$ .

strategy that restores physically meaningful AoP and DoLP maps from low-fidelity aqueous measurements.

### 3.3. Stokes-consistent Nano-PolarSRN recovers structural and polarimetric fidelity

To address this measurement bottleneck, we developed Nano-PolarSRN as a Stokes-consistent computational polarimetric reconstruction framework. The overall training strategy is summarized in Fig. 3. High-SNR references were obtained by temporally averaging approximately 70 consecutive polarization frames acquired under the same imaging geometry. Corresponding LR inputs were generated using a physically motivated degradation model incorporating calibrated additional blur, 4 $\times$  lateral downsampling, and noise addition. This paired formulation allows the network to learn a fidelity-transfer mapping from low-fidelity aqueous measurements to experimentally grounded high-SNR polarimetric references.

Nano-PolarSRN reconstructs four polarization-resolved channels rather than a single intensity image. This design allows the Stokes parameters, DoLP, and AoP to be computed from the reconstructed measurements. The network is supervised by a composite loss that combines image-domain fidelity, perceptual structure, and polarimetric consistency. The polarimetric loss constrains Stokes-domain reconstruction and penalizes errors in DoLP and AoP, thereby encouraging the network to recover physically interpretable polarization observables.

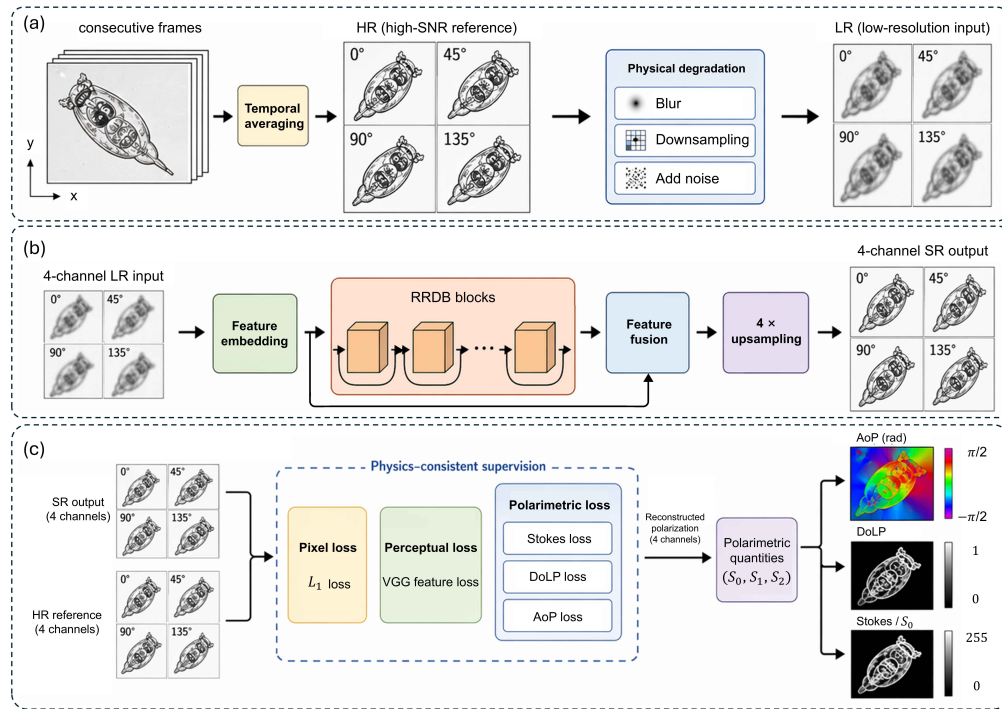


**Fig. 3.** Nano-PolarSRN architecture and training pipeline for Stokes-consistent computational polarimetric reconstruction. (a) Paired dataset generation. Consecutive polarization frames are temporally averaged to obtain a high-fidelity high-SNR reference. The corresponding LR input is synthesized from the reference using the calibrated degradation model. (b) Nano-PolarSRN architecture. A four-channel LR polarimetric input, corresponding to analyzer orientations of 0°, 45°, 90°, and 135°, is embedded into a feature space, processed by residual-in-residual dense blocks (RRDBs), fused, and upsampled by 4× to reconstruct the four high-fidelity analyzer-channel images. (c) Stokes-consistent supervision. The reconstructed SR output and high-SNR reference are compared using a composite loss that combines pixel-wise intensity loss, perceptual loss, and polarimetric loss. The polarimetric loss constrains Stokes-domain consistency and preserves DoLP and AoP information for downstream polarimetric analysis.

Representative reconstruction results are shown in Fig. 4(a). The LR input lacks vesicle-scale detail and exhibits degraded polarization contrast. The SR reconstruction without polarimetric loss improves the apparent sharpness of the intensity image, but its AoP and DoLP maps remain less consistent with the high-SNR reference. In particular, intensity-supervised reconstruction can produce visually plausible structures while introducing distortions in polarization-derived observables. This demonstrates that intensity-domain supervision alone is insufficient for quantitative linear polarimetric recovery.

In contrast, Nano-PolarSRN recovers sharper vesicle-associated structures and produces AoP and DoLP maps that more closely follow the high-SNR reference. Enlarged ROIs show improved recovery of local vesicle-scale intensity features and polarization signatures. The improvement is not simply a visual sharpening effect; it reflects better preservation of relationships among the four reconstructed polarization channels.

Quantitative evaluation in Fig. 4(b) supports this interpretation. SSIM, computed from the reconstructed  $S_0$  image, measures intensity-domain structural fidelity. DoLP MAE and AoP

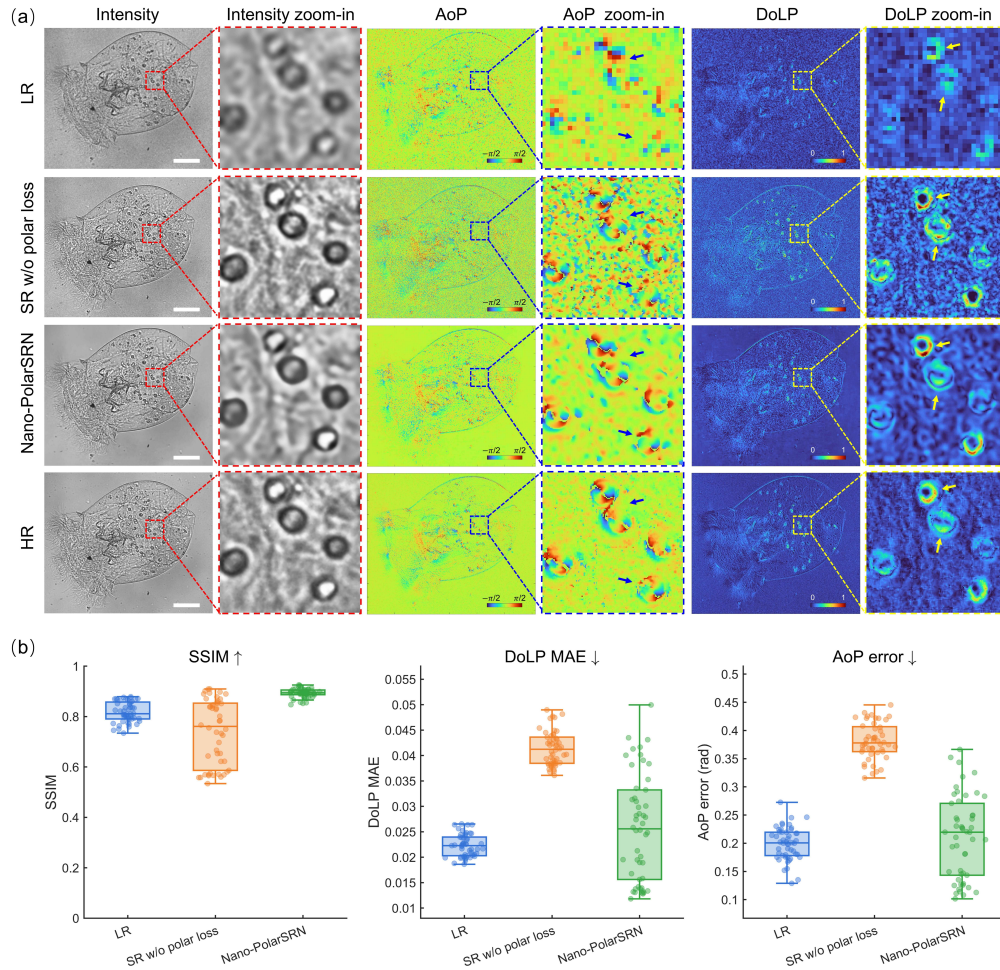


**Fig. 4.** Computational reconstruction performance and linear polarimetric fidelity comparison. (a) Representative reconstruction results of a live rotifer using LR input, SR without polarimetric loss, Nano-PolarSRN, and the high-SNR reference. The SR w/o polar loss baseline uses the same four-channel reconstruction architecture as Nano-PolarSRN but is trained without the polarimetric loss term. Nano-PolarSRN improves vesicle-scale structural recovery and yields AoP and DoLP maps that more closely resemble the high-SNR reference than the intensity-supervised SR baseline. Arrows indicate representative vesicle-associated polarization features. (b) Quantitative comparison using SSIM, DoLP mean absolute error (MAE), and AoP circular error. SSIM was computed from the reconstructed  $S_0$  intensity image relative to the high-SNR reference, while DoLP MAE and AoP error were computed from Stokes-derived polarization observables reconstructed from the four polarization channels. All metrics were calculated relative to the high-SNR reference. Higher SSIM indicates better structural fidelity, whereas lower DoLP MAE and AoP error indicate improved polarimetric consistency. Scale bars: 50  $\mu\text{m}$ .

circular error quantify the recovery of polarization magnitude and orientation, respectively. Nano-PolarSRN achieves the highest structural fidelity and reduces the DoLP and AoP errors introduced by SR without polarimetric loss. The LR input may retain coarse-scale polarization values because of its low-pass nature, but it lacks the vesicle-scale structural information recovered

by the Stokes-consistent reconstruction. These results show that Nano-PolarSRN balances spatial detail recovery with polarimetric consistency, which is essential for downstream analysis of nanoplastic-associated linear polarimetric signatures.

It is important to clarify the meaning of resolution enhancement in this work. The high-SNR images used for supervision were generated by temporal averaging under the same optical configuration and therefore represent high-SNR, diffraction-limited measurements rather than diffraction-unlimited ground truth. Accordingly, Nano-PolarSRN should not be interpreted as recovering spatial frequencies beyond the optical passband of the microscope. Its role is to recover spatial and polarimetric information that is physically present but degraded by low SNR, blur, and undersampling in the low-fidelity input. The vesicle-associated structures emphasized in the reconstructed images are therefore interpreted only when they are supported by the corresponding high-SNR references and polarization-domain consistency, and not as direct evidence of resolving individual sub-wavelength nanoplastic particles.

### 3.4. *Split-reference validation of computational resolution enhancement*

Because the high-SNR references were acquired under the same optical configuration, we further evaluated whether Nano-PolarSRN improves effective resolution relative to degraded LR inputs without claiming diffraction-unlimited optical resolution. For a held-out field of view, the frame stack was divided into two disjoint subsets to generate split reference A and split reference B, as shown in Fig. S1. Split reference A was used to generate the degraded LR input, while split reference B served only as an independent validation reference.

The magnified digestive-vesicle-rich ROI shows that fine structures enhanced by Nano-PolarSRN are also present in the independent split reference (Fig. 5). Line profiles further show that reconstructed local features align with corresponding structures in split reference B, whereas the LR input shows reduced contrast and weaker feature separation.

FRC analysis quantified the effective resolution improvement. The LR input reached a cutoff of approximately 0.155 cycles/pixel relative to split reference B, while Nano-PolarSRN extended the correlated spatial-frequency range to approximately 0.405 cycles/pixel. The split-reference comparison remained above the 1/7 threshold up to near the native Nyquist limit, serving as an empirical upper bound for reproducible spatial information under the same optical configuration. These results indicate that Nano-PolarSRN restores spatial-frequency content present in experimentally acquired high-SNR references but attenuated in LR inputs, without claiming recovery beyond the optical passband.

### 3.5. *Nano-PolarSRN improves visualization of vesicle-associated optical features*

We next examined whether the reconstructed polarimetric images provide improved local visualization in nanoplastic-exposed organisms. Figure 6 shows a representative rotifer exposed to 500 nm PS nanoplastics. Two biologically relevant regions were selected: the ciliary apparatus, which is involved in feeding and particle intake, and a body region containing vesicle-associated internal structures.

In the degraded LR input, local punctate and vesicle-associated features are weak or blurred. After Nano-PolarSRN reconstruction, these structures become more distinguishable in both the ciliary and body regions. The enlarged ROIs reveal enhanced local contrast and sharper feature boundaries. Intensity profiles extracted along the indicated lines further show that Nano-PolarSRN increases the visibility of local feature transitions relative to the LR input.

These observations demonstrate that Stokes-consistent computational reconstruction improves the visualization of local structures in nanoplastic-exposed organisms. Importantly, the enhanced punctate features should be interpreted as vesicle-associated or particle-associated optical signatures supported by the split-reference validation in Fig. 5, rather than as direct evidence of resolving every individual nanoplastic particle or recovering spatial frequencies beyond the optical passband.

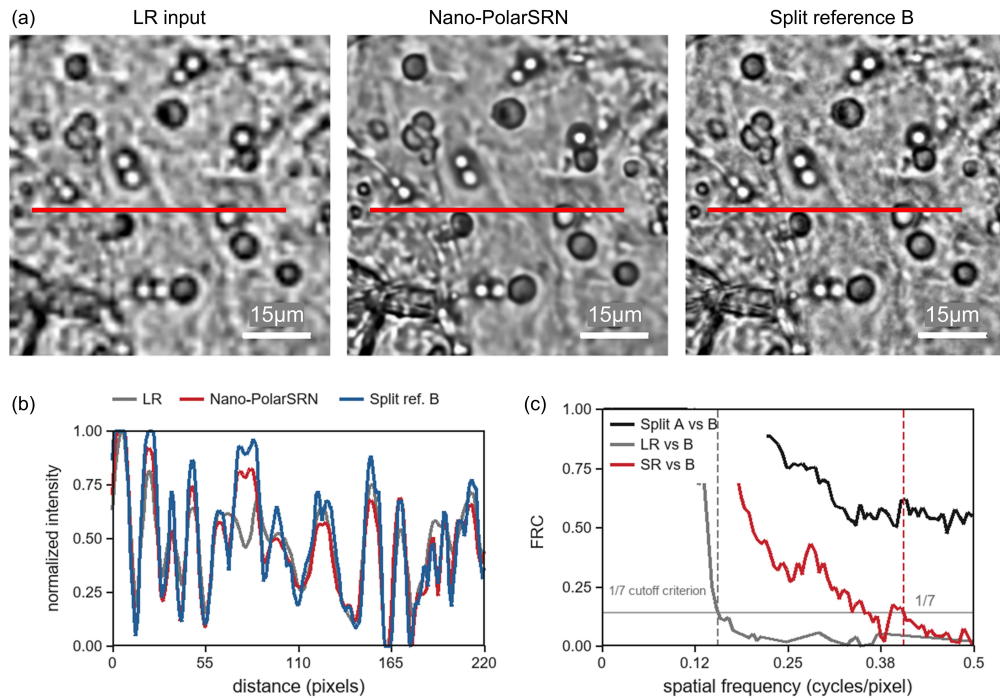


**Fig. 5.** Split-reference validation of computational resolution enhancement by Nano-PolarSRN. (a) Digestive-vesicle-rich regions comparing the LR input, Nano-PolarSRN reconstruction, and split reference B for independent validation. The red lines indicate the line-profile positions. Scale bars: 15 μm. (b) Normalized intensity profiles along the red lines in (a), comparing the LR input, Nano-PolarSRN reconstruction, and split reference B. (c) Fourier ring correlation (FRC) analysis using the 1/7 cutoff criterion. The cutoff increases from approximately 0.155 cycles/pixel for the LR input to 0.405 cycles/pixel for Nano-PolarSRN. The split-reference comparison remains above the 1/7 criterion up to near the native Nyquist frequency.

### 3.6. Nanoplastic exposure is associated with size-dependent polarimetric signatures

We then applied Nano-PolarSRN to rotifers exposed to PS nanoplastics of different sizes. Figure 7 compares representative AoP and DoLP maps from particle-free control rotifers and rotifers exposed to 500 nm, 100 nm, and 20 nm PS nanoplastics. For each condition, the high-SNR reference and the Nano-PolarSRN reconstruction are displayed side by side, allowing direct assessment of whether the reconstructed maps preserve the main polarization features of the high-SNR reference.

In the control group, vesicle-rich digestive regions exhibit coherent AoP patterns and localized DoLP contrast. These signatures are consistent with intrinsic polarization contrast arising from refractive-index gradients and membrane-associated internal structures. Nano-PolarSRN preserves these major AoP and DoLP features, supporting its use for recovering vesicle-scale polarimetric information from low-fidelity measurements.

Rotifers exposed to 500 nm PS nanoplastics exhibit the strongest localized polarization perturbations. The corresponding DoLP maps show regions of attenuated polarization contrast, while the AoP maps exhibit more disrupted orientation patterns in vesicle-rich digestive regions. This behavior is consistent with stronger scattering and depolarization from particles whose size approaches the optical wavelength. The perturbations are spatially localized, consistent

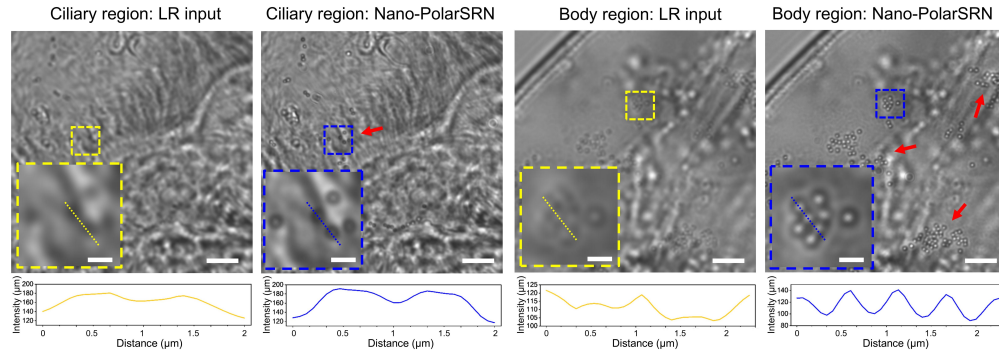


**Fig. 6.** Representative local visualization after Nano-PolarSRN reconstruction in a 500 nm PS-exposed rotifer. Ciliary and body regions are shown as degraded LR inputs and the corresponding Nano-PolarSRN reconstructions. Enlarged ROIs highlight local punctate and vesicle-associated optical features that are weak or blurred in the LR input and become more distinguishable after reconstruction. Red arrows indicate representative particle-associated or vesicle-associated features enhanced by Nano-PolarSRN. Intensity profiles were extracted along the dashed lines in the enlarged ROIs and show improved local contrast after computational reconstruction. Scale bars: 5  $\mu\text{m}$  in the main panels and 1  $\mu\text{m}$  in the enlarged ROIs.

with stronger local optical disturbances in regions containing 500 nm particles or particle-rich vesicular structures.

For 100 nm and 20 nm PS nanoplastics, the polarization changes are weaker and more diffuse. Individual particles at these sizes are not directly resolved by the optical system. Instead, their presence is reflected by more subtle reductions in DoLP contrast and less localized AoP disruption. These observations suggest that sub-wavelength nanoplastic exposure may be detected indirectly through associated changes in Stokes-derived polarization observables rather than through direct particle-resolved imaging. Importantly, these results should be interpreted as nanoplastic-associated linear polarimetric signatures rather than direct single-particle localization of sub-wavelength particles.

To provide an optical rationale for the observed size-dependent trend, we estimated the size parameter  $x = \pi n_m d / \lambda_0$  and scattering efficiency  $Q_{\text{sca}}$  of PS particles in water, using  $n_m \approx 1.33$ ,  $n_{\text{PS}} \approx 1.59$ , and  $\lambda_0 = 550 \text{ nm}$  as a representative wavelength for the broadband visible illumination. As summarized in Table 1, 20 nm particles fall in a weak Rayleigh-like regime, 100 nm particles lie in a Rayleigh-to-Mie transition regime, and 500 nm particles behave as wavelength-scale Mie scatterers. The estimated  $Q_{\text{sca}}$  and scattering cross-section increase by several orders of magnitude from 20 to 500 nm, supporting the optical plausibility of stronger localized DoLP attenuation and AoP disruption in the 500 nm condition and weaker, more diffuse signatures in the 100 nm and 20 nm conditions. This analysis is intended as a semi-quantitative consistency check rather than a complete in vivo forward model. Particle uptake efficiency, aggregation, vesicle morphology, local refractive-index heterogeneity, and the surrounding scattering microenvironment may also contribute to the measured polarimetric signatures.

**Table 1. Semi-quantitative optical scaling of PS particles in water at  $\lambda_0 = 550 \text{ nm}$**

| Diameter | Size parameter $x$ | Optical regime                | Estimated $Q_{\text{sca}}$ |
|----------|--------------------|-------------------------------|----------------------------|
| 20 nm    | 0.15               | Rayleigh-like weak scattering | $2.2 \times 10^{-5}$       |
| 100 nm   | 0.76               | Rayleigh-to-Mie transition    | $1.2 \times 10^{-2}$       |
| 500 nm   | 3.80               | Mie regime                    | order unity                |

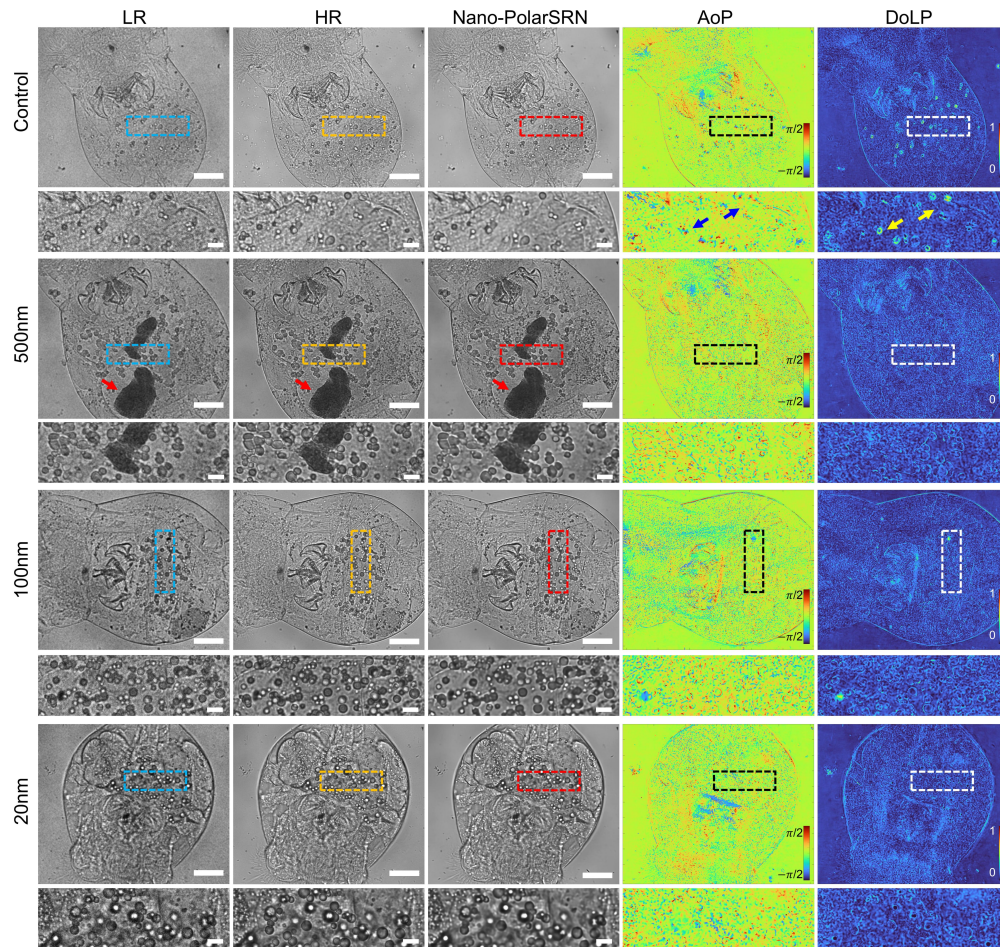


**Fig. 7.** Representative intensity and polarization maps under nanoplastic exposure. Particle-free control rotifers and rotifers exposed to 500 nm, 100 nm, and 20 nm PS nanoplastics are compared. Rows correspond to exposure conditions, and columns show the LR input, high-SNR reference, Nano-PolarSRN reconstruction, AoP map, and DoLP map. Dashed boxes mark vesicle-rich digestive regions, with enlarged ROIs shown below each overview image. Nano-PolarSRN improves the visualization of vesicle-associated structures relative to the LR input while preserving the main features of the high-SNR reference. The reconstructed AoP and DoLP maps show particle-size-dependent polarization variations in digestive regions. Scale bars: 30  $\mu\text{m}$  for main panels, with 5  $\mu\text{m}$  for enlarged insets.

Independent fluorescence validation confirms uptake of fluorescent PS nanoplastics in digestive regions, supporting the interpretation that the observed label-free polarization changes are associated with nanoparticle accumulation (Fig. 8). The fluorescence images were not used for network training; rather, they provide an orthogonal biological reference for interpreting the polarimetric signatures.

The mechanistic interpretation of the observed DoLP and AoP changes should be considered correlative. Confocal fluorescence imaging confirms uptake of fluorescent PS nanoplastics and their accumulation in digestive regions, providing independent support for associating the reconstructed polarimetric signatures with nanoplastic exposure. However, fluorescence imaging does not establish that the measured DoLP attenuation and AoP disruption are caused

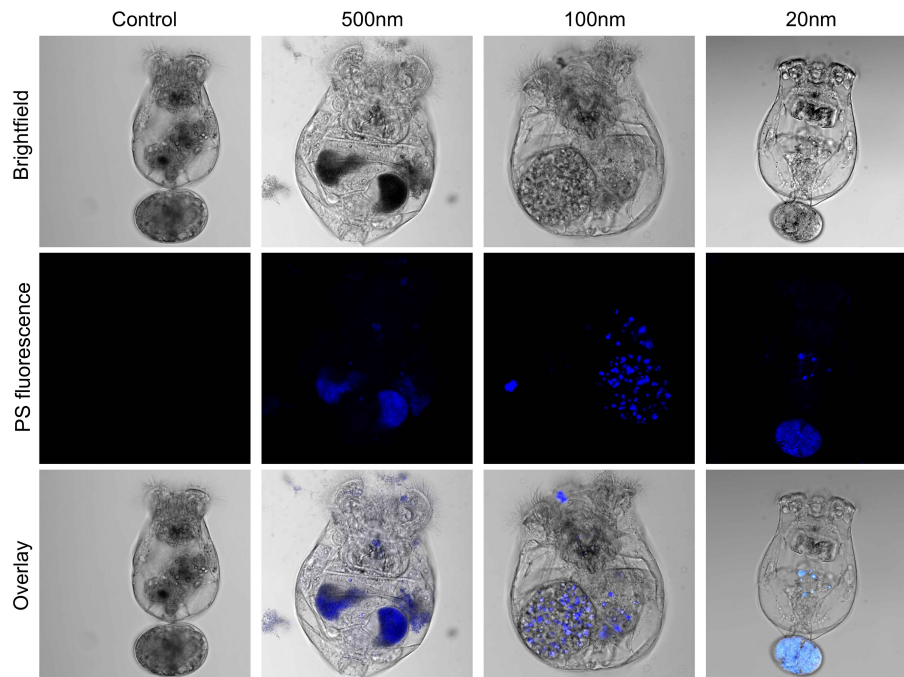


**Fig. 8.** Confocal fluorescence validation of nanoplastic uptake in rotifers. Representative brightfield, fluorescence, and merged confocal images of particle-free control rotifers and rotifers exposed to fluorescent PS nanoplastics with diameters of 500 nm, 100 nm, and 20 nm. Fluorescence signals are absent in the control group, whereas exposed rotifers show particle-associated fluorescence within internal digestive regions.

specifically or solely by direct nanoplastic optical effects. These signatures may arise from a combination of particle-associated scattering, refractive-index contrast, vesicle reorganization, digestive morphology changes, and the surrounding scattering microenvironment. Therefore, we interpret the reconstructed DoLP and AoP variations as nanoplastic-associated linear polarimetric signatures in digestive-vesicle-rich regions, rather than as definitive evidence that individual nanoplastic particles alone directly caused the observed polarimetric changes.

Together, these results indicate that Stokes-consistent polarimetric reconstruction can recover label-free linear polarimetric signatures associated with nanoplastic exposure and digestive-region accumulation in living aquatic organisms.

#### 4. Conclusion

We have presented Stokes-consistent computational polarimetric reconstruction for quantitative label-free imaging of nanoplastic-associated perturbations in living aquatic organisms. The central idea is that computational polarimetric super-resolution should not be treated as image sharpening alone. In DoFP polarimetry, the information of interest is encoded in the physical relationship among four analyzer channels, and nonlinear observables such as DoLP and AoP can become unstable under low-SNR aqueous imaging. By reconstructing four polarization-resolved channels under Stokes-domain supervision, Nano-PolarSRN improves structural recovery while preserving physically meaningful polarization observables.

Applied to nanoplastic-exposed marine rotifers, the method reveals size-dependent polarization perturbations in digestive-vesicle-rich regions. Rotifers exposed to the 500 nm particles exhibit more localized DoLP attenuation and disrupted AoP coherence, whereas 100 nm and 20 nm

particles are associated with weaker and more diffuse polarization changes. Confocal fluorescence imaging further supported nanoparticle uptake, linking the label-free polarimetric signatures to nanoplastic accumulation.

Overall, this work establishes Stokes-consistent reconstruction as a practical computational optical strategy for recovering reliable polarization observables from low-fidelity aqueous measurements. Beyond nanoplastic exposure, the same principle may be extended to other weakly absorbing biological or environmental specimens where relevant information is carried not only by intensity, but also by the physical state of polarization.

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**Data availability.** Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

**Supplemental document.** See [Supplement 1](#) for supporting content.

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