Hyperspectral Fluorescence Imaging and Machine Learning for the Detection and Classification of Microplastics in Tissues

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Problem Definition

- Microplastics are emerging environmental and biological contaminants
- Current identification methods can be time-consuming and require extensive sample preparation
- Rapid fluorescence-based methods could improve screening capabilities
- Long-term applications include the detection of microplastics in complex biological tissue

Research Question

Can hyperspectral fluorescence imaging microscopy be combined with machine learning (ML) to rapidly detect and classify dye-stained microplastics in biological tissues?

Methodology

Microplastics were stained using selective UV-Fluorescent dyes: 4-Dimethylamino-4'-nitrostilbene (DANS) and Nile Red. The structures of these dyes are shown below in Figures 1 and 2 respectively.

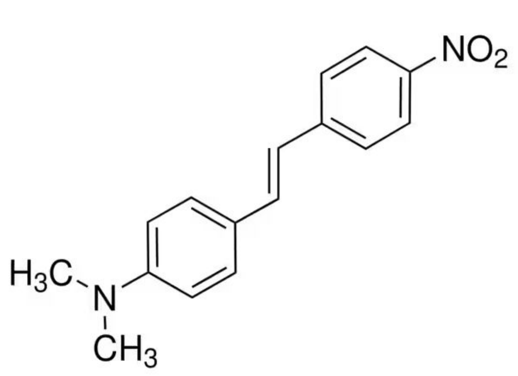

Figure 1: DANS

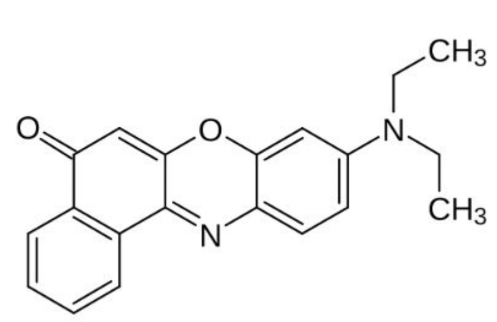

Figure 2: Nile Red (NR)

When stained with either of these dyes, different polymers exhibit different fluorescence emission spectra as shown in Figure 3.

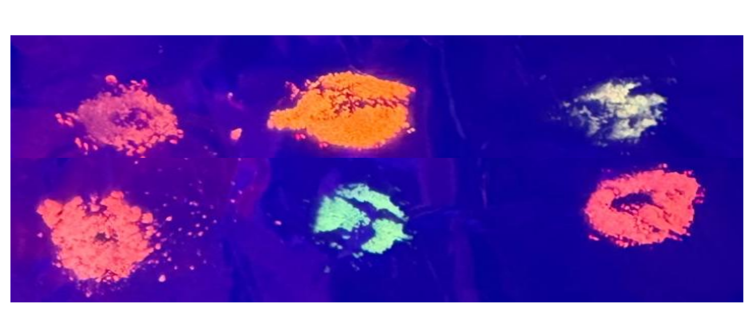

Figure 3: Solvatochromic fluorescence of Nile Red-stained microplastics

Identification of polymer types through emission spectra allows for material identification in substrates, such as tissues, where traditional microplastic identification techniques such as μFTIR struggle against low signal and high background.

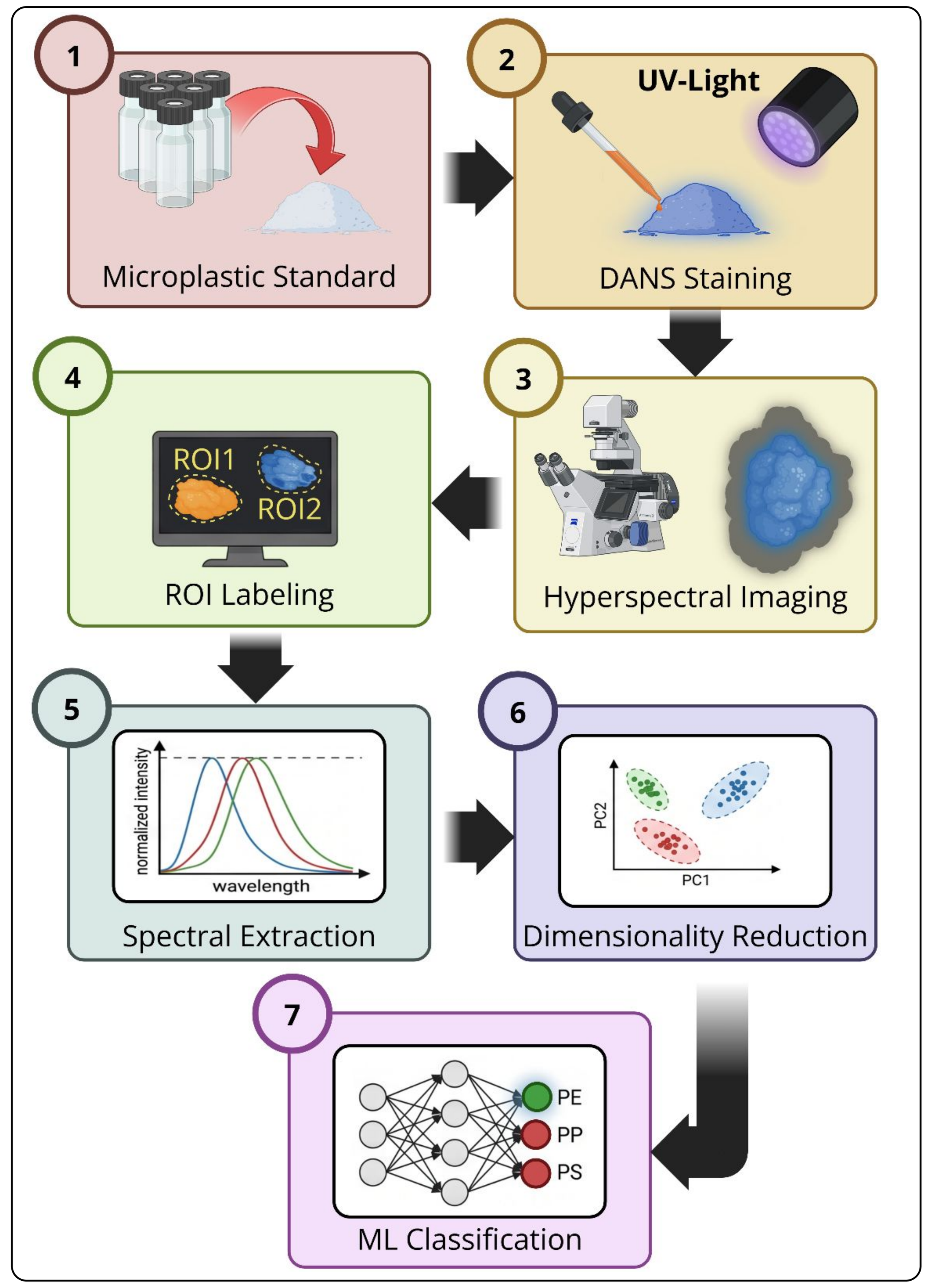

Figure 4: Process 1, Workflow for acquiring hyperspectral imaging microscopy data from microplastic standards

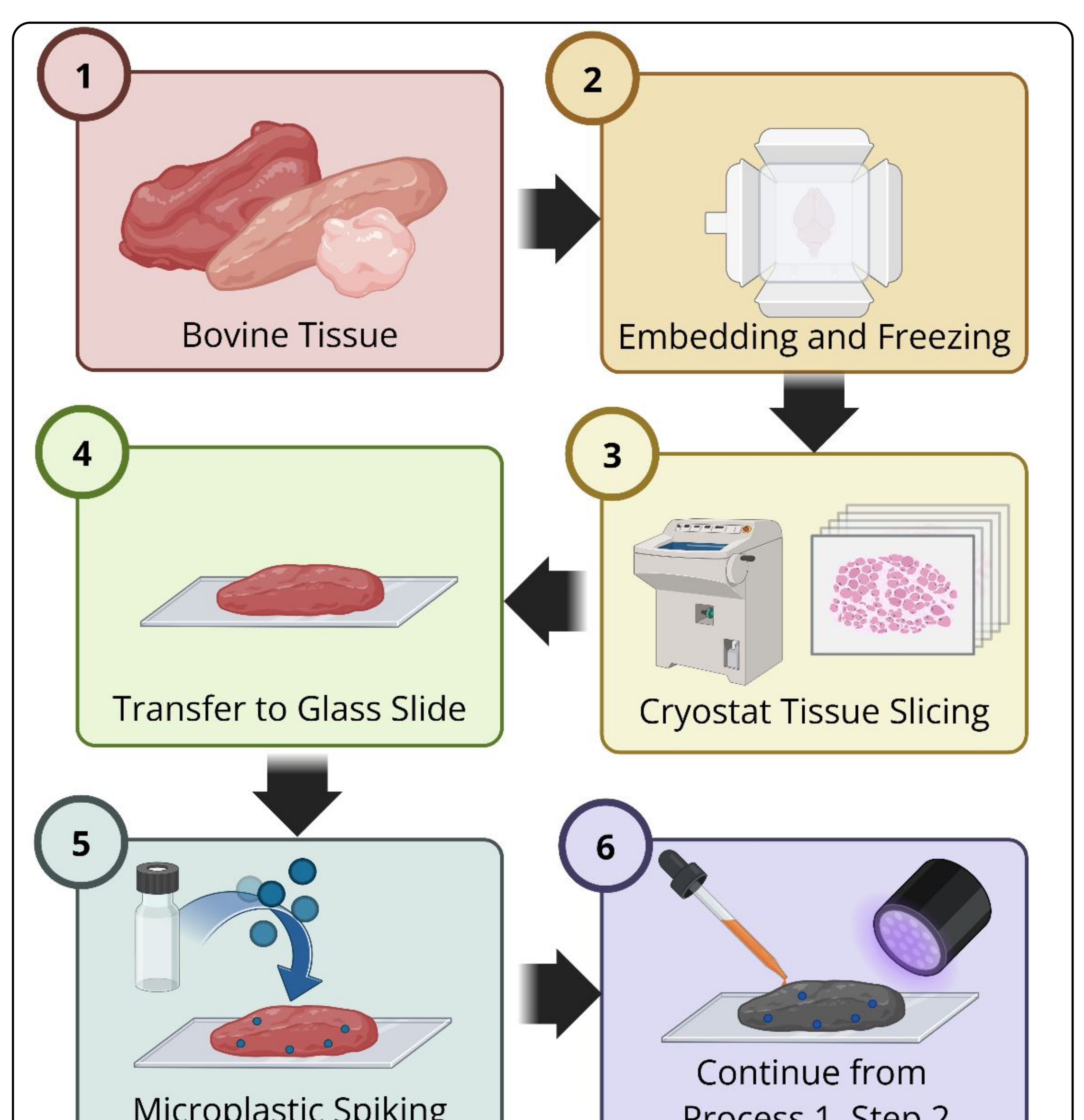

Figure 5: Process 2, Workflow for acquiring microplastic-spiked tissue hyperspectral imaging microscopy data

Results

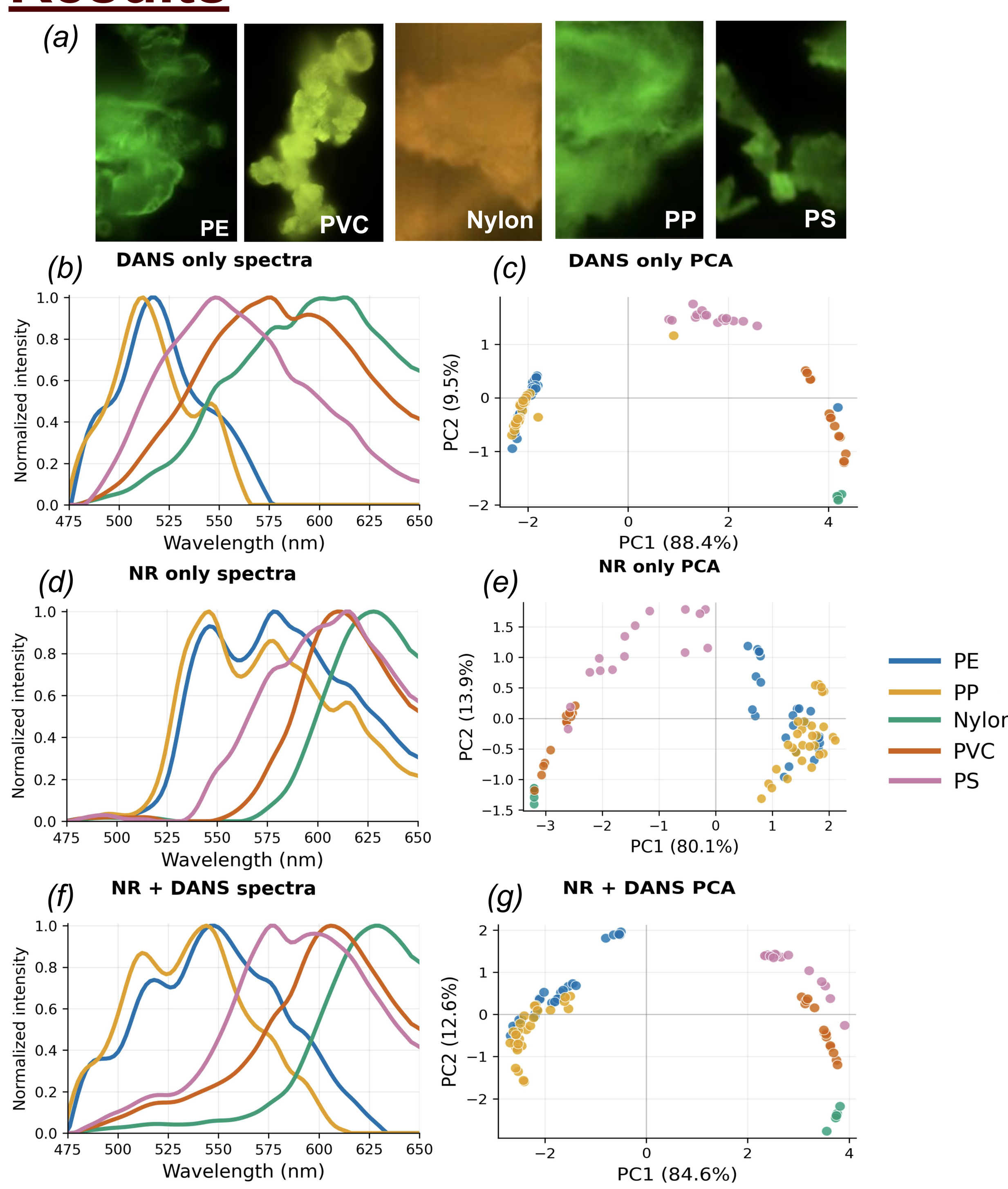

Figure 6: Example colorized hyperspectral images (a), emission spectra, and PCA of microplastics stained with DANS (b,c), Nile Red (d,e), and DANS + Nile Red combination (f,g)

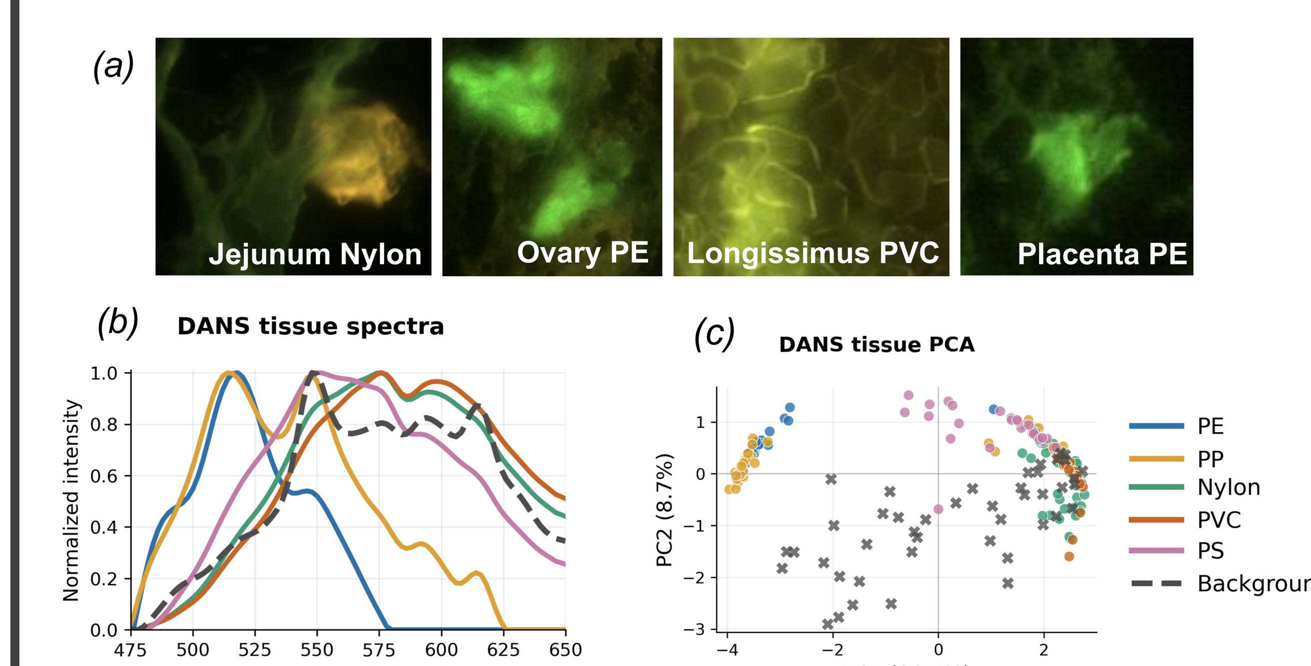

Figure 7: Example colorized hyperspectral images (a), emission spectra (b), and PCA plot (c) of microplastics stained with DANS against tissue background

Table 1: Training-test split and bovine tissue types used for background analysis

Plastic	Bovine Tissue	Train	Test
PE	Ovary, Placenta, Uterus	22	7
PP	Ovary, Placenta, Jejunum, Spiral colon	29	10
Nylon	Jejunum, Spiral colon, Ventral Reticulum	27	9
PVC	Longissimus, Uterus	18	6
PS	Spiral colon, Ventral Reticulum	19	7
Total	All Tissues	115	39

Confusion matrices for machine-learning models trained to classify polymer types under varying staining and background conditions are presented in Figure 8.

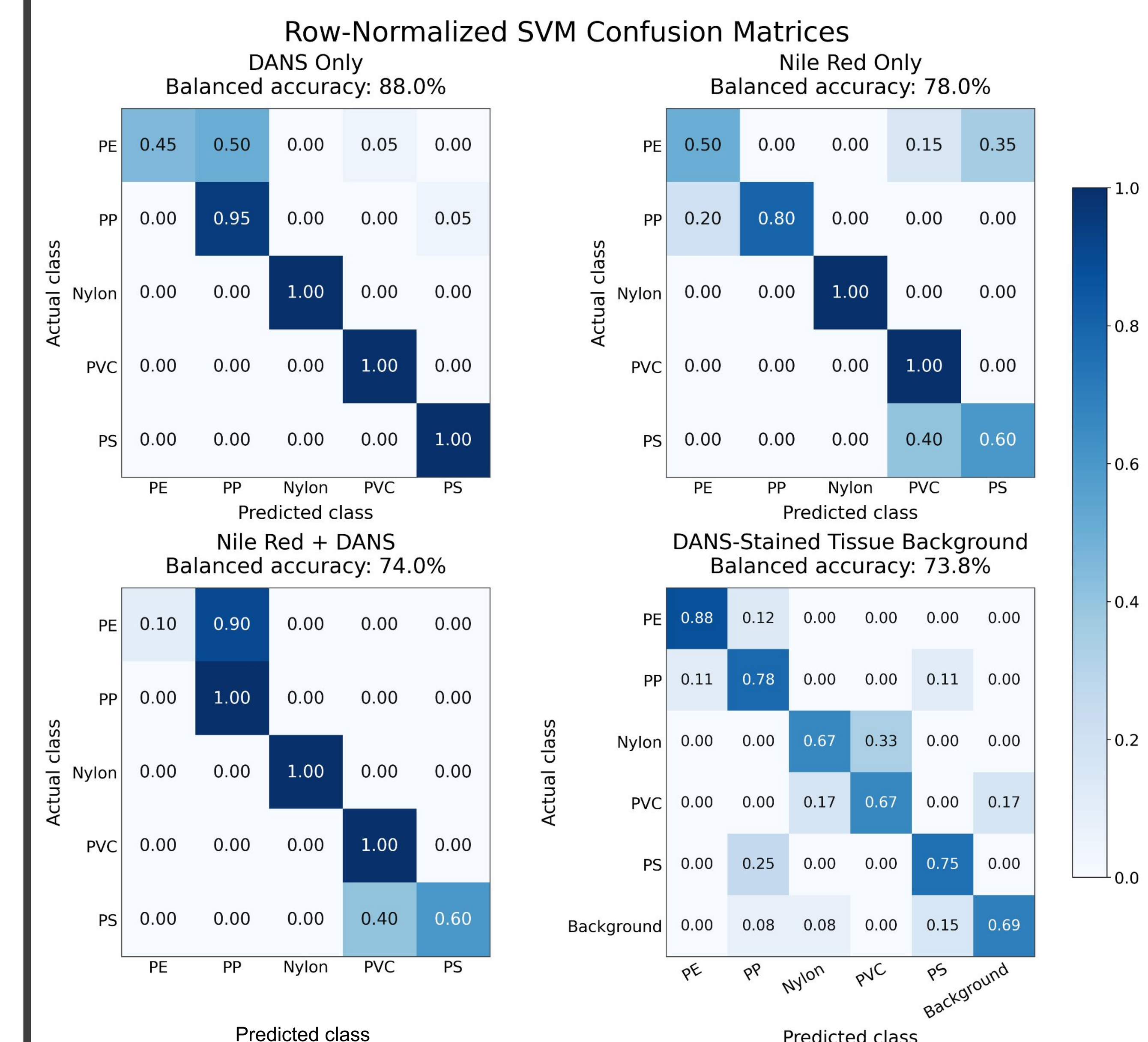

Figure 8: Row-normalized confusion matrices showing polymer classification performance using (a) DANS only, (b) Nile Red only, (c) combined DANS and Nile Red, and (d) DANS with a tissue background.

Conclusions & Future Work

DANS-only staining provided the best polymer separability under controlled conditions, while Nile Red was best at differentiating PE versus PP. No significant benefits from the combined DANS and Nile Red stain were observed. Polymer classification remained feasible against tissue backgrounds, achieving 73.8% balanced accuracy.

These data indicate that hyperspectral fluorescence imaging combined with machine learning provides a promising approach for microplastic identification and lays the foundation of microplastic identification in biological tissue.

Future efforts should focus on expanding the number of samples stained with DANS and Nile Red to improve predictive reliability and identify more complex patterns. Additionally, embedding microplastics within tissue can better replicate their in vivo distribution, improving the model's translational performance to real tissue samples.

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