

NileScope: Software-Assisted Detection of Microplastics Using Nile Red Fluorescence Image

Selvarani D¹, T Siva Dhanush Reddy², Pooja K² & Chaithra S²

¹Assistant Professor, Department of Information Science and Engineering
Sri Sairam College of Engineering, Bengaluru

²Final Year B.E. (Information Science and Engineering), Sri Sairam College of Engineering, Bengaluru

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Abstract: Manual microscopic inspection of microplastics is slow, subjective and difficult to scale. This paper presents NileScope, a browser-based pipeline that detects and quantifies microplastics in Nile Red fluorescence microscopy images. The pipeline performs pre-processing, adaptive-threshold segmentation, watershed particle separation, multi-feature extraction (shape, intensity, colour and texture) and three-class machine-learning classification, labelling each particle as plastic, uncertain or non-plastic according to classifier confidence. It then estimates particle counts, concentration and contamination level, and generates a shareable report. All core modules are implemented and integrated, and an illustrative run on a fluorescence image demonstrates the complete upload-to-report workflow. Quantitative validation against ground-truth samples is identified as the next phase of work.

Keywords: Microplastics, Nile Red Staining, Fluorescence Microscopy, Machine Learning

INTRODUCTION

Plastic debris that has fragmented, or been manufactured, to a size below five millimetres is classified as microplastic. Because of its small size, low density and chemical variability, microplastic is now found in marine, freshwater, soil and airborne environments, and its presence in environmental, food and biological samples has become a widely studied indicator of pollution. Microplastic particles have also been reported in human tissue: Ragusa et al. (2021) reported the first evidence of microplastic fragments in human placenta, and Leslie et al. (2022) detected and quantified plastic particles in human blood. These findings have intensified interest in detection methods that are sensitive, reliable and practical enough for routine use rather than only in specialised analytical laboratories.

Detecting and counting microplastics from microscope images remains largely manual. An analyst scans a slide, separates candidate plastic fragments from sand, organic debris and fibres, and counts them by eye, a process that is slow, does not scale and varies between analysts. Laboratory-grade identification by Fourier-transform infrared (FTIR) or Raman spectroscopy offers high chemical specificity but needs expensive instrumentation and trained personnel, which limits routine use in resource-constrained teaching and research laboratories. Nile Red, a lipophilic fluorescent dye, selectively stains plastic surfaces so that particles fluoresce brightly while most non-plastic material stays comparatively dark, making it a popular low-cost front end for screening. Converting the

resulting image into a reliable count and classification, however, still depends on manual scoring or on offline, single-purpose scripts and desktop tools with limited accessibility.

Nile Red staining was established as a practical basis for microplastic identification by Shim et al. (2016), who showed that solvent choice and dye concentration strongly influence staining consistency. Meyers et al. (2022) combined staining with RGB colour features and supervised machine learning to semi-automate plastic versus non-plastic classification, showing that simple colour statistics can carry enough signal for automated screening. Ho et al. (2024) reviewed Nile Red fluorescence imaging and identified the lack of standardised staining, imaging and processing protocols as a barrier to reproducible automated analysis, while Ho et al. (2025) introduced a multispectral platform combining K-means segmentation with nearest-neighbour classification, noting that very small particles and separation from natural organic matter remain open challenges. Hidalgo-Ruz et al. (2012) reviewed identification methods more broadly and indicated that fluorescence screening is best used as a fast first-pass filter ahead of confirmatory spectroscopy. Table 1 summarises these works.

A recurring gap across this literature is the absence of an accessible, browser-based pipeline that takes a single fluorescence image through pre-processing, segmentation, feature extraction, classification and concentration estimation in one workflow, without specialised desktop software or spectroscopic instrumentation. This paper presents NileScope to fill that gap. Its contributions are: (i) an end-to-end, browser-accessible pipeline integrating image pre-processing, segmentation, feature extraction and machine-learning classification behind a single upload-to-report workflow; (ii) a confidence-banded three-class labelling scheme that separates confidently plastic and confidently non-plastic particles from ambiguous ones rather than forcing a binary decision; and (iii) a reporting layer that converts raw counts into an estimated concentration and a qualitative contamination level that non-specialists can interpret.

LITERATURE REVIEW

Nile Red fluorescence staining was established as a practical basis for microplastic identification by Shim et al. [1], who determined optimal staining conditions for reliable fluorescence-based detection and showed that solvent choice and dye concentration strongly influence staining consistency. Building on staining protocols, Meyers et al. [2] combined Nile Red staining with RGB color feature extraction and supervised machine-learning models to semi-automate plastic versus non-plastic classification and polymer-type identification, reporting high classification accuracy on spiked environmental samples and demonstrating that simple color statistics, rather than full spectroscopic data, can carry enough signal for automated screening. Ho et al. [3] reviewed the chemistry and instrumentation of Nile Red fluorescence imaging and identified a lack of standardized staining, imaging and processing protocols as a barrier to reproducible automated analysis,

arguing that comparability across studies requires agreement on excitation wavelength, filter choice and image-processing conventions. More recently, Ho et al. [4] introduced a multispectral fluorescence imaging platform combining K-means segmentation with nearest-neighbor machine-learning classification, achieving high classification precision across multiple polymer types while noting that reliable detection of very small particles and separation from natural organic matter remain open challenges. Table 1 summarizes these works.

Collectively, this body of work shows a progression from manual visual scoring, to semi-automated RGB/ML classification, to multispectral and segmentation-based pipelines. Two broader threads of literature further motivate this work. First, human-exposure studies such as Ragusa et al. [6] and Leslie et al. [7] establish that microplastic contamination is not only an environmental concern but a direct human-health one, which strengthens the case for detection tools that are cheap and easy enough to deploy widely rather than confined to a handful of specialized laboratories. Second, general reviews of microplastic identification methods, such as Hidalgo-Ruz et al. [5], show that the field has historically relied on a mix of visual sorting, density separation and spectroscopic confirmation, each with its own cost and throughput trade-offs, and that image-based fluorescence screening is best positioned as a fast first-pass filter ahead of any confirmatory spectroscopy rather than as a full replacement for it.

A recurring gap across this literature is the absence of an accessible, browser-based pipeline that takes a single fluorescence image through pre-processing, segmentation, feature extraction, classification and concentration estimation in one integrated workflow, without requiring specialized desktop software, command-line tooling or spectroscopic instrumentation. NileScope is designed to fill this gap by packaging the stages surveyed above into a single web application with a guided upload-to-report interface.

CORE OF RESEARCH INNOVATION

A. Problem Formulation

Microplastic particles are very small and hard to identify reliably in environmental samples, and visually separating genuine plastic from organic or mineral particles is difficult. Four sub-problems motivate NileScope. The first is throughput: a single filter sample can contain hundreds of candidate particles, and reviewing each under magnification does not scale to routine monitoring. The second is subjectivity: analysts can disagree on borderline particles because brightness and colour judgements are not perfectly repeatable. The third is accessibility: FTIR and Raman spectroscopy are typically confined to well-funded laboratories, excluding many teaching institutions, small monitoring programmes and citizen-science efforts. The fourth is reporting: converting a raw count into an estimated concentration or contamination level is a separate, often manual step that is easy to skip or perform inconsistently.

B. Proposed Architecture

Figure 1 shows the system architecture, organised into four elements. In the sample-to-image stage, an environmental sample is stained with Nile Red and imaged under a fluorescence microscope to produce a digital image. In the software pipeline stage, the image passes through pre-processing (resizing, noise reduction and contrast enhancement), segmentation (adaptive thresholding and morphological operations), particle detection and separation (connected-component analysis and watershed separation of touching particles), feature extraction and a three-class classifier. In the output stage, the system reports total particles detected, the plastic, uncertain and non-plastic counts, the estimated concentration in particles per litre, a qualitative contamination level (low, medium or high) and a stored analysis report and history. The fourth element, the project workflow overview, ties these stages back to the physical laboratory process. The layered design lets stages, such as the classifier or segmentation method, be revised independently.

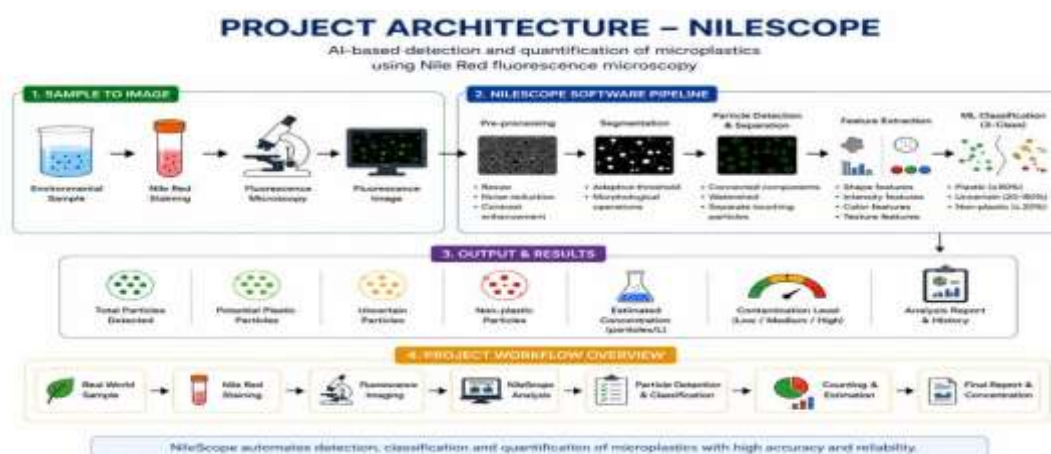


Figure 1 Project Architecture of NileScope: Sample-to-Image Acquisition, Software Analysis Pipeline and Outputs

C. Methodology

The NileScope workflow proceeds through eleven stages, shown in Figure 2: sample collection and Nile Red staining, fluorescence imaging and upload, pre-processing, segmentation, particle separation, feature extraction, classification, particle counting, concentration estimation, report generation and performance evaluation. The final stage allows results to be validated against ground truth using accuracy, precision, recall and F1-score.

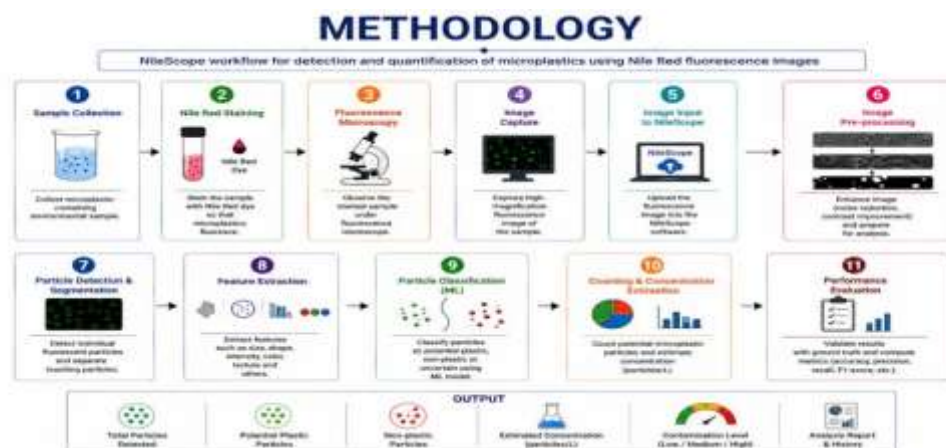


Figure 2 NileScope Workflow for Detection and Quantification of Microplastics Using Nile Red Fluorescence Images

Sample preparation and imaging. The collected sample is filtered, stained with Nile Red dissolved in a suitable solvent, and incubated so the dye adsorbs onto plastic surfaces. The stained filter or slide is examined under a fluorescence microscope with excitation and emission filters matched to the Nile Red spectrum, and a digital image is captured through a microscope camera at a magnification sufficient to resolve individual particles.

Pre-processing and segmentation. The image is resized to a standard working resolution, denoised and contrast-enhanced so fluorescent particles stand out from the dark background. Adaptive thresholding then converts the enhanced grayscale image into a binary particle mask. Because it sets a local threshold for each image region instead of a single global cutoff, it tolerates uneven illumination across the field of view. Morphological opening and closing remove small noise artefacts and smooth particle boundaries.

Particle detection and feature extraction. Connected-component analysis identifies candidate particles, and a watershed step separates touching or overlapping particles so that each physical particle is a single object. For every particle, four feature groups are computed: shape (area, perimeter, circularity, aspect ratio), intensity (mean and peak brightness), colour (RGB and HSV channel statistics, following the RGB-based approach of Meyers et al., 2022) and texture (local variation within the particle boundary). Together these form the input vector for classification.

Classification and quantification. A supervised machine-learning classifier, trained to distinguish plastic from sand grains, biological debris and fibres, receives each feature vector. Rather than a binary label, NileScope reports a confidence score and assigns one of three classes: plastic, non-plastic or uncertain (the intermediate band). This banding makes the system's uncertainty visible instead of silently forcing a decision on borderline particles, and responds to the misclassification risk noted in earlier Nile Red work (Ho et al., 2025; Meyers et al., 2022). The plastic-labelled count and the known sample volume then give a

concentration in particles per litre, which is mapped to a low, medium or high contamination level.

D. Mathematical Formulation

For a particle i with classifier confidence p_i (expressed as a percentage), the three-class labelling rule and the concentration estimate are:

$$\text{Label}(i) = \text{Plastic, if } p_i \geq 80 \quad (1)$$

$$\text{Label}(i) = \text{Non-plastic, if } p_i \leq 20 \quad (2)$$

$$\text{Label}(i) = \text{Uncertain, otherwise} \quad (3)$$

$$\text{Concentration} = N_{\text{plastic}} / V \quad (4)$$

where N_{plastic} is the number of particles labelled plastic and V is the known sample volume in litres. When ground-truth annotations are available, classification performance is evaluated with the standard measures:

$$\text{Accuracy} = (\text{TP} + \text{TN}) / (\text{TP} + \text{TN} + \text{FP} + \text{FN}) \quad (5)$$

$$\text{Precision} = \text{TP} / (\text{TP} + \text{FP}) \quad (6)$$

$$\text{Recall} = \text{TP} / (\text{TP} + \text{FN}) \quad (7)$$

$$\text{F1} = 2 \times \text{Precision} \times \text{Recall} / (\text{Precision} + \text{Recall}) \quad (8)$$

where TP, TN, FP and FN denote true positives, true negatives, false positives and false negatives respectively.

E. Implementation

NileScope is a browser-based application built with React, Vite and HTML5 Canvas, so it runs on any modern browser without installation. Table 2 lists the hardware needed to prepare and image a stained sample and the software stack used to build and run the pipeline. The interface guides the user through four steps: uploading the fluorescence image, selecting the sample, running the analysis and viewing the generated report (Figure 3).

The application is organised into cooperating modules. The upload module validates the format and size of the fluorescence image. The pre-processing and segmentation module implements resizing, denoising, contrast enhancement, adaptive thresholding and morphological clean-up on the Canvas to produce a binary mask. The detection and feature-extraction module runs connected-component analysis and watershed separation and computes the four feature groups for each particle. The classification module applies the trained three-class model and returns a label and confidence score, and the reporting module aggregates per-particle results into counts, an estimated concentration, a contamination level and a stored, shareable report that the user can revisit as history.

A browser front end was chosen over a native desktop application to keep NileScope installation-free and cross-platform. HTML5 Canvas was chosen because it gives the direct pixel-level access needed for thresholding and morphological operations while remaining entirely client-side, which keeps the workflow simple for a single-machine laboratory. The confidence-banded scheme was preferred over a plain binary classifier so that uncertain particles are surfaced to the user rather than hidden behind an overconfident label.

RESULTS AND DISCUSSION

A. Current Implementation Status

The image acquisition, pre-processing, segmentation and classification modules have been implemented and integrated into a single end-to-end pipeline. The system has been exercised on a sample fluorescence image to confirm that data flows correctly between stages, from upload through segmentation and feature extraction to classification and report generation. The interface is functional for the upload, sample-selection, analysis and report-viewing steps.

B. Illustrative Analysis Run

Figure 3 shows the interface and outputs for one analysis run. Panel (a) is the guided workflow screen, panel (b) overlays the detected particles as labelled bounding boxes, panel (c) lists per-particle results (identifier, classification, confidence, area, equivalent diameter and fluorescence intensity), and panel (d) gives the summary report. Table 3 reproduces the summary values. The pipeline detected 647 candidate particles in 0.25 s. None reached the potential-microplastic class: 325 were labelled uncertain and 322 non-plastic candidates, with an average screening confidence of 45.2%, so the estimated concentration was 0.0 particles/L.



Figure 3 NileScope User Interface Showing the Upload, Select Sample, Analyse and Report Workflow

Table 3 Summary of the Illustrative Analysis Run Shown in Figure 3(d)

Metric	Value
Image quality score	66/100
Total particles detected	647
Potential microplastics	0
Non-plastic candidates	322
Uncertain	325
Average screening confidence	45.2%
Estimated concentration	0.0 particles/L
Processing time	0.25 s

C. Discussion

The run illustrates the intended behaviour of the confidence-banded scheme. Every one of the 647 particles received a confidence score in the intermediate range, and the system reported this explicitly by flagging 325 particles as uncertain, where a binary classifier would have forced a plastic or non-plastic decision on each. The report also states that the detection threshold is derived from the smallest detected particle and the entered optical calibration and is not a validated analytical detection limit.

These values demonstrate that the pipeline works end to end; they are not evidence of detection accuracy. The zero concentration should not be read as a plastic-free sample, because no ground truth exists for this image. The dense, overlapping particle labels in the upper region of Figure 3(b) also suggest that segmentation parameters may need tuning for crowded fields. Quantitative evaluation using accuracy, precision, recall and F1-score (Equations 5 to 8) on ground-truth annotated Nile Red images is therefore the immediate next step, together with testing on a larger set of laboratory-stained environmental samples that vary in particle size, staining intensity and imaging conditions. Following Ho et al. (2024), imaging conditions will be standardised, and, in line with Hidalgo-Ruz et al. (2012), a subset of samples should be confirmed by FTIR or Raman spectroscopy. Until this benchmark exists, no quantitative accuracy claims are made.

CONCLUSION

This paper presented NileScope, a software-assisted pipeline that pairs Nile Red fluorescence staining with automated image analysis to detect, classify and quantify microplastic particles. By combining pre-processing, segmentation, multi-feature extraction and a three-class machine-learning classifier in one browser-based tool, NileScope aims to reduce the manual effort and subjectivity of conventional screening while remaining usable without spectroscopic instrumentation. The core modules are implemented and integrated, and an illustrative run demonstrates the full upload-to-report workflow. Future work will validate detection performance against ground-truth annotated and laboratory-stained samples before any accuracy claims are made.

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