A Cost-Effective Fluorescence-Based Framework for Microplastic Detection in Water Using Nile Red Dye

[1]Dr. M. Raju., [2]Sanjana. S. S*., [2]Sanjai. S. M

[1]Assistant Professor, Department of Software Systems & AIML, Sri Krishna Arts and Science College, Coimbatore, India

[2]Department of Software Systems & AIML, Sri Krishna Arts and Science College, Coimbatore, India

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ABSTRACT

Microplastic pollution has created a growing need for simple and affordable methods that can support preliminary screening of water samples. This paper presents a fluorescence-based framework that combines Nile Red staining, camera-based imaging, and basic image-processing techniques to identify suspected microplastic particles. The proposed workflow includes sample preparation, staining, controlled fluorescence imaging, image enhancement, segmentation, and particle analysis.

The performance of Nile Red-based screening can be affected by several factors, including dye concentration, staining conditions, excitation wavelength, optical filters, and sample composition. For this reason, the framework also considers contamination control, positive and negative controls, and confirmatory polymer identification.

Independent experiments were not carried out as part of this study; therefore, quantitative performance values are not reported. Future work should include controlled laboratory validation, replicate testing, and confirmation using established techniques such as FTIR or Raman spectroscopy. The proposed framework is therefore intended for preliminary screening rather than as a replacement for established analytical methods.

Keywords: Microplastics, Nile Red Dye, Fluorescence-Based Detection, Camera-Based Imaging, Image Processing, Water Quality Analysis, Cost-Effective Monitoring.

INTRODUCTION

Microplastic pollution, caused by the breakdown of larger plastic materials, synthetic fibers, industrial waste, and other sources, has become a growing environmental concern. The presence of microplastics in water can affect aquatic ecosystems and water quality, creating a need for reliable methods to detect and monitor these particles. Identifying microplastics in water samples requires careful analysis, particularly when the particles are small, transparent, or difficult to distinguish from other materials. However, commonly used laboratory-based detection methods have several practical limitations.

[1] High Cost: FTIR and Raman spectroscopy require specialized instruments and trained personnel, which can make routine analysis expensive.

[2] Lack of Portability: These techniques are mainly used under controlled laboratory conditions, which may limit their use for rapid field-level screening.

[3] High Equipment and Processing Requirements: Conventional methods often depend on specialized equipment and analytical procedures, making large-scale or frequent monitoring more difficult. To address these limitations, this study proposes a fluorescence-based approach that combines Nile Red staining, camera-based image acquisition, and lightweight image-processing techniques for preliminary microplastic screening in water samples.

The objective of this study is to develop a structured and accessible framework for Nile Red fluorescence-based microplastic screening and to identify the methodological requirements needed for its future experimental implementation. Particular attention is given to staining conditions, controlled fluorescence imaging, image-processing procedures, contamination control, positive and negative controls, quantitative performance measures, and confirmatory polymer identification. Since independent experiments were not conducted as part of the present study, experimental validation is considered an important direction for future work.

Table 1: Comparison of FTIR/RAMAN and Proposed Framework

Feature	FTIR / Raman	Proposed Framework
Detection Cost	High	Low
Equipment Requirement	Lab-grade instruments	Standard instruments
Portability	Limited	High
Processing Complexity	High	Moderate
Field Deployability	Not Suitable	Suitable
Energy Consumption	High	Low
Polymer Confirmation	Direct chemical identification	Requires FTIR/Raman

The proposed fluorescence-based framework is designed to use comparatively accessible hardware and lightweight image-processing techniques. These characteristics may provide potential advantages for portable and field-level preliminary screening. However, its cost, energy requirements, detection performance, and field applicability must be confirmed through controlled experimental evaluation.

Related Work

Several methods have been used for microplastic detection in water, including spectroscopic techniques, microscopic observation, fluorescence staining, and image-based analysis. Each of these methods offers useful detection and identification capabilities, but their practical use may be limited by factors such as equipment cost, sample preparation, analysis time, and dependence on laboratory facilities.

1. Spectroscopy-Based Detection: FTIR and Raman spectroscopy are widely used for microplastic analysis because they can provide reliable information about polymer composition. However, these techniques generally require specialized instruments, trained personnel, and controlled laboratory conditions, which may limit their use for rapid or field-level screening.

2. Manual Visual and Microscopic Analysis: Optical and stereo microscopes are commonly used to examine suspected microplastic particles. However, manual identification can be time-consuming and may depend on the experience of the person carrying out the analysis. Small, transparent, or irregularly shaped particles can also be difficult to distinguish from non-plastic materials.

3. Fluorescence-Based Detection: Nile Red fluorescence has been studied as a rapid screening method because the dye interacts with hydrophobic polymer surfaces and can improve particle visibility under suitable excitation conditions. However, the fluorescence response may vary depending on polymer type, dye concentration, solvent, staining conditions, and illumination parameters.

4. Image-Based and Automated Detection Techniques: Digital image-processing techniques such as contrast enhancement, noise reduction, thresholding, segmentation, and morphological analysis can support the detection and counting of suspected particles. These methods may reduce manual analysis effort, but fluorescence and image processing alone cannot confirm polymer composition. Some non-plastic organic materials may also produce fluorescence and lead to false-positive detections.

The proposed framework combines Nile Red fluorescence, camera-based imaging, and lightweight image-processing techniques for preliminary microplastic screening. Since the framework has not yet been independently validated through experiments, confirmatory methods such as FTIR or Raman spectroscopy are still required for reliable polymer identification.

PROPOSED METHODOLOGY

A. System Architecture: The proposed framework uses fluorescence-assisted imaging to support the preliminary screening of suspected microplastic particles in water samples. It consists of four main stages: sample preparation, fluorescence image acquisition, image preprocessing, and particle detection and analysis.

1. Water Sample Preparation

What they do: The water sample is prepared to remove large debris and other unwanted materials so that the analysis can focus on smaller suspended particles, including possible microplastics.

How they work: Nile Red dye is added to enhance the fluorescence of hydrophobic polymer particles.

For example: Gentle mixing and sufficient incubation time can help the dye interact more evenly with the particles and improve fluorescence consistency.

2. Fluorescence Image Acquisition

What they do: Fluorescence images of the stained particles are captured for further analysis.

How they work: The samples are illuminated under controlled light conditions, and images are recorded using a standard digital camera.

For example: Camera-based imaging may provide a simpler option for preliminary screening and reduce dependence on specialized microscopic equipment.

3. Image Pre-processing

What they do: The captured images are improved so that suspected particles can be distinguished more clearly from the background.

How they work: Image-processing techniques such as contrast enhancement, noise reduction, and intensity-based thresholding are applied.

For example: Segmentation can help separate fluorescence-positive regions from the surrounding background for further analysis.

4. Microplastic Detection and Analysis

What it does: The processed images are examined to identify and highlight suspected microplastic particles.

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How it works: Size- and shape-based filtering can be used to reduce likely artifacts and non-target regions in the image.

For example: The remaining candidate particles can be marked or counted for further evaluation.

B. Methodological Parameters for Nile Red-Based Detection

The performance of Nile Red fluorescence-based screening depends on several experimental conditions. Important factors include Nile Red concentration, staining solvent, incubation time and temperature, sample volume, filtration conditions, excitation wavelength, optical filtering, camera settings, and image-acquisition conditions.

Previous studies have reported different staining conditions depending on the polymer type and the characteristics of the sample. Therefore, a single Nile Red concentration or staining condition may not produce the same fluorescence response for every type of microplastic. During future experimental validation, different Nile Red concentrations should be tested using known reference polymers to determine suitable conditions that produce clear fluorescence while reducing background signals.

The staining solvent, incubation time, and temperature should also be kept consistent during testing.

Fluorescence image acquisition should also be carried out under controlled conditions. Factors such as excitation wavelength, optical-filter properties, camera settings, and ambient light should remain consistent throughout image capture.

Representative parameters reported in previous research are summarized in **Table 2**.

Table 2. Literature-Reported Nile Red Staining and Imaging Parameters

Study	NR Staining Conditions	Imaging Conditions
Maes et al. [1]	10 µg/mL; acetone; 30 min	Blue LED 450–510 nm; orange filter 529 nm
Shim et al. [2]	5 mg/L; n-hexane	Green fluorescence detection
Kang et al. [3]	10/100/1000 mg/L; chloroform; plate dried 24 h	UV 365 nm; camera under dark conditions

Fluorescence-Based Visualization of Microplastics:

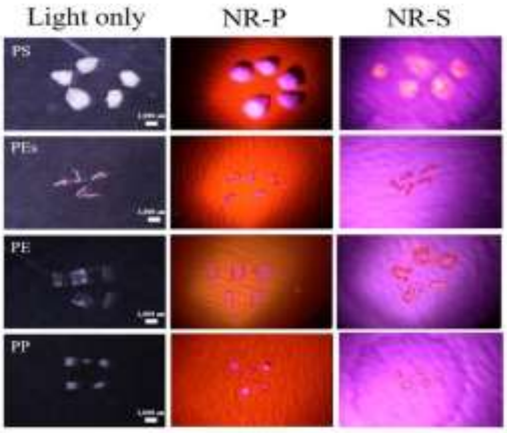

Fig. 1. Comparison of microplastic shapes under visible light, Nile Red plate (NR-P), and Nile Red staining (NR-S) methods. Reproduced from Kang et al. [3], under CC BY 4.0.

A. Potential Advantages of Fluorescence-Based Microplastic Screening

1. Visibility Enhancement:

Small or transparent particles can be difficult to observe under normal lighting because they may have very little contrast with the background. Nile Red staining can improve the fluorescence of several hydrophobic polymer particles under suitable excitation conditions, making suspected microplastic particles easier to identify during preliminary screening.

2. Shape Visualization:

Fluorescence imaging can make the edges and shapes of particles easier to observe compared with normal visible-light examination. This can help in studying particle morphology, although the shape of a particle alone cannot confirm that it is plastic.

3. Potential Improvement in Screening:

Manual visual inspection may miss particles that are very small, transparent, or have low contrast. Nile Red fluorescence may improve the visibility of these particles and support preliminary screening. However, suitable positive and negative controls are still required to evaluate false-positive and false-negative detections.

4. Cost and Accessibility:

Techniques such as FTIR and Raman spectroscopy require specialized analytical instruments. In comparison, the proposed framework uses more accessible components such as a conventional camera, controlled illumination source, and optical filtering. This may reduce hardware requirements for preliminary screening, although the actual cost-effectiveness of the framework still needs to be evaluated experimentally.

5. Compatibility with Image Processing:

Fluorescence images can be processed using techniques such as contrast enhancement, noise reduction, thresholding, segmentation, and morphological operations. These methods may help with particle counting and further image analysis. Machine-learning-based classification could also be explored in future studies once suitable experimentally validated datasets are available.

6. Potential for Field-Level Application:

The relatively simple imaging setup may support future portable use in field or resource-constrained environments. However, field-level performance has not been experimentally validated in the present study. Future evaluation should therefore consider factors such as ambient light, sample composition, contamination, and other environmental conditions that may affect detection performance.

Potential Application Scenarios:

The following scenarios show how the proposed framework could be applied in different water-monitoring situations. These are conceptual examples based on previously reported microplastic-screening approaches and do not represent experiments carried out in the present study.

A. Potential Application 1: Surface-Water Screening

Scenario: Surface-water samples may contain sediment, fibres, biological material, and other suspended particles along with suspected microplastics. These materials can interfere with fluorescence-based screening, so proper sample preparation is important.

Proposed Process:

1. Sample Preparation: Surface-water samples may be collected and filtered to separate suspended particles from the water.

2. Nile Red Staining: The filtered particles may be treated with Nile Red under standardized staining conditions.

3. Fluorescence Imaging: Images may be captured under controlled illumination using a suitable optical filter and a digital camera.

4. Image Processing: Techniques such as contrast enhancement, thresholding, segmentation, and morphological processing may be used to identify fluorescence-positive regions.

5. Interpretation: Fluorescence-positive particles should be treated as suspected microplastics until their polymer composition is confirmed using FTIR or Raman spectroscopy.

B. Potential Application 2: Drinking-Water Screening

Scenario: Drinking-water samples may also require preliminary screening for suspected microplastic contamination. Because particle concentrations may be low, suitable sample volumes and careful contamination control are important.

Proposed Process: Drinking-water samples may be filtered, stained with Nile Red, imaged under controlled fluorescence conditions, and analysed using image-processing techniques. Fluorescence-positive particles may then be recorded as candidate particles for further investigation.

Interpretation: The absence of visible fluorescence should not be taken as proof that microplastics are completely absent, since very small particles or polymers with weak fluorescence may remain undetected.

C. Potential Application 3: Field-Level Environmental Screening

Scenario: Portable fluorescence imaging may have potential for preliminary microplastic screening in field or resource-constrained environments.

Proposed Process: A conventional camera, controlled excitation source, optical filter, and lightweight image-processing system may be combined to support portable screening.

Limitation: Field-level performance has not been experimentally validated in the present study. Factors such as ambient light, sample composition, contamination, temperature, and imaging conditions may influence the screening results.

Proposed Performance Evaluation

A. Proposed Experimental Validation Strategy

The proposed framework has not yet been independently tested through experiments. Therefore, quantitative performance values are not reported in the present study.

Future validation should include known polymer positive controls, non-plastic negative controls, procedural blanks, and multiple independent replicates. Reference polymers such as polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) may be tested under standardized staining and imaging conditions.

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Known numbers of reference particles may be added to controlled water samples and processed through the complete workflow, including filtration, Nile Red staining, fluorescence imaging, and image processing. This would make it possible to evaluate the performance of the proposed screening framework quantitatively.

B. Proposed Quantitative Evaluation Metrics

Future experimental studies should evaluate the framework using recovery rate, sensitivity, specificity, precision, accuracy, and F1-score

Table 3: Performance Evaluation Metrics for Microplastic Detection

Metric	**Formula**
Recovery (%)	**(Number of particles detected / Number of particles added) × 100**
Sensitivity	**TP / (TP + FN)**
Specificity	**TN / (TN + FP)**
Precision	**TP / (TP + FP)**
Accuracy	**(TP + TN) / (TP + TN + FP + FN)**
F1-Score	**2TP / (2TP + FP + FN)**

TP represents plastic particles that are correctly detected, while TN represents non-plastic particles that are correctly rejected. FP refers to non-plastic particles that are incorrectly identified as plastic, and FN refers to plastic particles that are not detected.

Results from replicate measurements should be reported using mean ± standard deviation to assess consistency and repeatability. Particle-size-dependent testing should also be carried out to determine the smallest particle size that can be reliably detected using the proposed imaging setup.

Future Machine-Learning Integration

A. Scope of Future Machine-Learning Integration

Machine learning was not implemented or experimentally tested in the present study. It is therefore considered a possible future extension of the proposed fluorescence-based screening framework rather than a validated part of the current work.

Future studies may develop a labelled dataset containing fluorescence images of confirmed plastic particles together with representative non-plastic materials. Machine-learning models could then be explored for the automated analysis and classification of candidate particles based on their visual and fluorescence characteristics.

Possible input features may include particle size, shape, texture, colour information, fluorescence intensity, and other morphological characteristics. These features may help distinguish plastic from non-plastic particles once a sufficiently large and experimentally validated dataset is available.

B. Proposed Machine-Learning Development Strategy

1. Dataset Generation

Develop a labelled dataset using fluorescence images of known plastic and non-plastic particles. Wherever possible, polymer identity should be confirmed using FTIR or Raman spectroscopy before the images are used for model training.

2. Feature Extraction

Extract useful characteristics such as particle size, shape, texture, colour information, and fluorescence intensity from the segmented candidate particles.

3. Model Selection

Evaluate suitable lightweight machine-learning algorithms or convolutional neural networks for image-classification tasks.

4. Model Training and Validation

Divide the dataset into separate training, validation, and testing sets. Model performance should be evaluated using previously unseen test data.

5. Performance Evaluation

Future models should be evaluated using measures such as accuracy, sensitivity, specificity, precision, recall, F1-score, and confusion matrices.

6. System Integration

The trained model should only be integrated into the image-processing pipeline after satisfactory experimental validation has been achieved.

Limitations of The Proposed Framework

The present study proposes a fluorescence-based screening framework and does not include independent experimental validation. The fluorescence response of Nile Red may vary depending on factors such as polymer type, particle size, staining conditions, imaging setup, and sample composition.

Some non-plastic materials may also fluoresce and could lead to false-positive results. For this reason, fluorescence-positive particles should be considered suspected microplastics rather than confirmed plastic particles.

Further confirmation using established analytical techniques such as FTIR or Raman spectroscopy is still necessary to verify polymer identity.

CONCLUSION AND FUTURE DEPLOYMENT

A. Conclusion

This study presents a fluorescence-based framework for preliminary microplastic screening in water using Nile Red staining, camera-based image acquisition, and lightweight image-processing techniques. The framework brings together sample preparation, fluorescence visualization, image enhancement, segmentation, and candidate-particle analysis in a structured workflow.

Independent experimental validation was not carried out in the present study. Therefore, no quantitative claims are made regarding detection accuracy, sensitivity, specificity, recovery rate, repeatability, or detection limits. Particles identified through fluorescence imaging should be treated as suspected microplastics until their polymer composition is confirmed using established methods such as FTIR or Raman spectroscopy.

The proposed framework provides a basis for future development of accessible and portable microplastic-screening systems. Controlled experimental validation, optimization of staining and imaging conditions, suitable controls, replicate testing, and polymer confirmation will be necessary before the framework can be considered suitable for routine monitoring or field-level use.

B. Future Deployment

Future work should focus on experimentally validating and optimizing the proposed framework. Important parameters such as Nile Red concentration, staining solvent, incubation time, excitation wavelength, optical-filter characteristics, camera settings, sample volume, filtration conditions, and particle-size range should be systematically evaluated.

Validation should include known polymer standards, non-plastic negative controls, procedural blanks, and multiple independent replicates. Quantitative measures such as recovery rate, sensitivity, specificity, precision, accuracy, F1-score, and operational detection limits should also be determined.

Future studies may also explore machine-learning techniques using experimentally validated and labelled fluorescence images. After successful validation, the framework may be adapted for portable or mobile platforms to support preliminary on-site water-quality screening.

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