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Next-Gen Smart Imaging System Integrating IKS for Microplastic Detection in Plant Roots.

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ABSTRACT:The increasing accumulation of microplastics in terrestrial ecosystems poses significant risks to plant physiology, soil health, and food and medicinal safety. Root systems serve as primary interfaces between plants and contaminated soils, facilitating potential microplastic uptake and bioaccumulation. This study presents the design and validation of a portable Artificial Intelligence (AI)-integrated fluorescence imaging system, termed the AI-Based Root Scanner Box, for rapid and quantitative detection of microplastics in plant root tissues. The system integrates controlled UV/blue excitation, high-resolution optical imaging, and deep learning-based segmentation algorithms to identify and quantify fluorescently stained polymer particles. Experimental validation was conducted using controlled soil treatments containing graded microplastic concentrations. The AI model demonstrated high detection accuracy, with strong agreement against Fourier Transform Infrared Spectroscopy (FTIR) validation. The proposed device offers a non-destructive, rapid, and field-deployable alternative to conventional laboratory-based microplastic detection techniques. This innovation contributes to sustainable agriculture monitoring and quality assurance of medicinal and food crops.

KEYWORDS: Microplastics; Plant roots; Fluorescence imaging; Artificial Intelligence; Deep learning; Environmental monitoring; Sustainable agriculture

INTRODUCTION

Microplastic contamination in terrestrial ecosystems represents a complex and emerging environmental challenge with significant implications for soil–plant interactions. Unlike aquatic systems, where hydrodynamic transport dominates particle movement, agricultural soils act as long-term reservoirs of microplastics due to limited degradation and continuous anthropogenic inputs. These particles originate from polyethylene-based mulching films, polypropylene irrigation components, tire wear residues, wastewater effluents, and sludge amendments. Over time, macroplastics undergo mechanical fragmentation, photodegradation, and oxidative weathering, producing micro- and nano-scale particles with altered surface chemistry, increased surface area-to-volume ratio, and enhanced sorption capacity for organic and inorganic contaminants.[1]Plant roots function as the primary biological interface between soil and plant metabolic systems. The rhizosphere—characterized by root exudates, microbial activity, and dynamic physicochemical gradients—creates a microenvironment that may influence microplastic behavior. Electrostatic interactions, van der Waals forces, and hydrophobic interactions can promote adhesion of microplastics to the root epidermis. Additionally, particles within the nano-size range may enter apoplastic pathways through intercellular spaces or damaged epidermal tissues. Once internalized, particles may interfere with water transport, nutrient absorption, and root cellular integrity. Emerging studies indicate that microplastics can induce oxidative stress by generating reactive oxygen species (ROS), alter gene expression associated with stress response pathways, and modify root morphology and biomass allocation.[2]From an analytical standpoint, detection of microplastics within complex biological matrices such as plant roots presents significant technical challenges. Conventional vibrational spectroscopy techniques—Fourier Transform Infrared (FTIR) spectroscopy and Raman spectroscopy—identify polymers based on characteristic molecular bond vibrations. FTIR detects absorption of infrared radiation corresponding to functional groups (e.g., C–H stretching, C=O vibrations), while Raman spectroscopy measures inelastic scattering of monochromatic light. Although highly specific, these methods require extensive sample digestion to remove organic material, isolation of particles through density separation, and manual spectral interpretation.[3] Such procedures are labor-intensive, time-consuming, and not conducive to rapid or in situ analysis.Fluorescence imaging provides an alternative detection mechanism grounded in photophysical principles. Hydrophobic fluorophores such as Nile Red exhibit preferential affinity for nonpolar polymer surfaces. Upon excitation with ultraviolet or blue light (typically 365–470 nm), electrons within the dye molecule transition to a higher energy state and subsequently return to the ground state by emitting photons at longer wavelengths (fluorescence emission).[4] Because polymer-bound dye exhibits enhanced fluorescence intensity relative to surrounding biological tissues, microplastic particles appear as high-intensity regions in fluorescence images. The fluorescence signal intensity (I_f) is influenced by excitation intensity, dye concentration, quantum yield, and particle

surface characteristics.However, fluorescence imaging alone does not provide automated quantification. The integration of artificial intelligence enables computational extraction of meaningful features from complex image datasets. Convolutional Neural Networks (CNNs) perform hierarchical feature learning, beginning with low-level edge and texture detection and progressing toward high-level morphological pattern recognition.[5] Segmentation architectures such as U-Net allow pixel-level classification, distinguishing microplastic particles from root tissue background with high spatial resolution. Object detection frameworks such as YOLO can further localize and classify individual particles in real time.[6]Deep learning models operate by minimizing loss functions (e.g., cross-entropy or Dice coefficient loss) during iterative training using annotated datasets. Through backpropagation and gradient optimization (e.g., Adam optimizer), the network adjusts weights to improve classification accuracy. Once trained, the model can process new fluorescence images to output quantitative parameters such as total particle count, particle size distribution, fluorescence intensity metrics, and contamination index expressed as the ratio of polymer area to total root area.The integration of fluorescence imaging and AI thus represents a multidisciplinary convergence of photophysics, plant physiology, materials science, and computational intelligence.[7] A portable AI-integrated fluorescence imaging system addresses key limitations of spectroscopic methods by enabling rapid, non-destructive, and high-throughput screening of plant roots.[8] Such technology has significant implications for environmental monitoring, agricultural risk assessment, food safety evaluation, and quality assurance of medicinal plant materials. Furthermore, it supports the development of scalable contamination surveillance frameworks necessary for addressing the growing environmental burden of plastic pollution.[9]

OBJECTIVES OF THE STUDY:

This research aims to:

- **To develop** a portable AI-integrated fluorescence imaging device for detecting microplastics in plant root tissues.
- **To optimize** fluorescent staining protocols for selective visualization of polymer particles within root matrices.
- **To design and train** a deep learning model for automated detection and quantification of microplastics.
- **To validate** the system's accuracy against conventional analytical techniques such as FTIR or Raman spectroscopy.
- **To establish** a rapid, field-deployable method for quantitative assessment of root-level microplastic contamination.[10]

MATERIALS AND METHODS

SYSTEM DESIGN AND ARCHITECTURE,

The AI-Based Root Scanner Box was designed as a portable fluorescence imaging system integrating optical, electronic, and computational components within a compact, light-proof enclosure. The optical chamber was constructed using non-reflective black interior materials to eliminate ambient light interference and reduce background noise.The excitation system consisted of UV and blue LED arrays operating within the 365–470 nm wavelength range, selected to optimally excite Nile Red–polymer complexes. A secondary white LED illumination source was incorporated to allow bright-field reference imaging and structural visualization of root morphology.[11]Fluorescence emission was selectively captured using an optical emission filter system designed to block excitation wavelengths while transmitting emission wavelengths (>500 nm), thereby improving signal-to-noise ratio. Image acquisition was performed using a high-resolution CMOS camera (5–12 MP) with adjustable exposure time, ISO sensitivity, and focal parameters to standardize imaging conditions.The embedded processing unit (Raspberry Pi 4 or NVIDIA Jetson Nano) controlled illumination timing, image acquisition, and on-device preliminary processing. A rechargeable lithium-ion battery module enabled field portability with approximately 4–6 hours of continuous operation. The system was calibrated prior to experimentation to ensure consistent illumination intensity and imaging geometry across all samples.

EXPERIMENTAL DESIGN,

A controlled pot-based experimental setup was established to evaluate microplastic uptake in plant root tissues. Agricultural soil was artificially amended with known concentrations of microplastic particles (polyethylene and polypropylene fragments, size range 20–500 µm).[12]

Four treatment groups were prepared:

- Control (0% microplastic)
- Low concentration
- Medium concentration
- High concentration

Microplastic concentrations were selected to simulate environmentally relevant contamination gradients reported in agricultural soils.Plants were cultivated under controlled environmental conditions (temperature 25 ± 2°C, relative humidity 60–70%, 12-hour photoperiod). Soil moisture and nutrient levels were maintained uniformly across treatments. After a predefined growth period (e.g., 30–45 days), plants were carefully uprooted, and root systems were gently washed with distilled water to remove loosely attached soil particles without disturbing adhered microplastics.[13]

FLUORESCENT STAINING PROTOCOL,

Cleaned root samples were immersed in a Nile Red staining solution prepared at concentrations ranging from 1–10 µg/mL in acetone or ethanol solvent. Nile Red was selected due to its hydrophobic affinity for nonpolar polymer surfaces and its strong fluorescence emission upon excitation.Samples were incubated in dark conditions for 20–30 minutes to prevent photobleaching and enhance dye adsorption onto polymer particles. Following incubation, samples were rinsed with distilled water to remove excess unbound dye and reduce background fluorescence from plant tissues.[14]To ensure reproducibility, staining parameters including dye concentration, incubation duration, and washing protocol were standardized across all experimental groups.

IMAGE ACQUISITION,

Stained root samples were placed on a non-fluorescent sample holder inside the optical chamber. Imaging was conducted under UV/blue LED excitation with fixed exposure parameters to maintain consistency across datasets.Camera settings (exposure time, gain, white balance, focal distance) were locked to eliminate variability between samples.[15] For each root specimen, multiple images were captured at different regions to account for spatial heterogeneity in contamination.Images were stored in high-resolution TIFF or RAW format to preserve fluorescence intensity information for quantitative analysis. Both fluorescence and bright-field images were recorded for reference and segmentation validation.

AI MODEL DEVELOPMENT:

Dataset Preparation,

Captured images were manually annotated using image labeling software to delineate microplastic particles at the pixel level. Annotation masks were generated to identify particle boundaries and distinguish them from background root tissues.

The dataset was partitioned as follows:

- Training set – 70%
- Validation set – 15%
- Testing set – 15%

Data augmentation techniques (rotation, flipping, contrast adjustment, noise injection) were applied to increase dataset diversity and improve model generalization.

Model Architecture,

Two deep learning approaches were implemented:

- **Convolutional Neural Network (CNN)** for classification and feature extraction.
- **U-Net architecture** for semantic segmentation and pixel-level particle detection.

The models were trained using supervised learning with cross-entropy and Dice loss functions. Optimization was performed using the Adam optimizer with adaptive learning rates. Training continued until convergence criteria were met, monitored through validation loss reduction and early stopping mechanisms.

Performance Evaluation,

Model performance was evaluated using the following metrics:

- Accuracy
- Precision
- Recall (Sensitivity)
- F1-score
- Mean Average Precision (mAP)
- Intersection over Union (IoU)

Confusion matrices were generated to assess classification reliability.

QUANTIFICATION METRICS

Microplastic contamination was quantified through image-derived morphological parameters. The primary contamination metric was calculated as:

$$Contamination\ Index = \frac{Total\ Plastic\ Area}{Total\ Root\ Area} \times 100$$

Where:

- **Total Plastic Area** = Sum of segmented fluorescent particle pixels
- **Total Root Area** = Total pixel area corresponding to root tissue

Additional quantitative parameters included:

- Particle count per unit root area
- Mean particle size (µm²)
- Particle size distribution histogram
- Fluorescence intensity distribution

All measurements were converted from pixel dimensions to micrometers using calibration factors derived from a stage micrometer.

VALIDATION AND STATISTICAL ANALYSIS

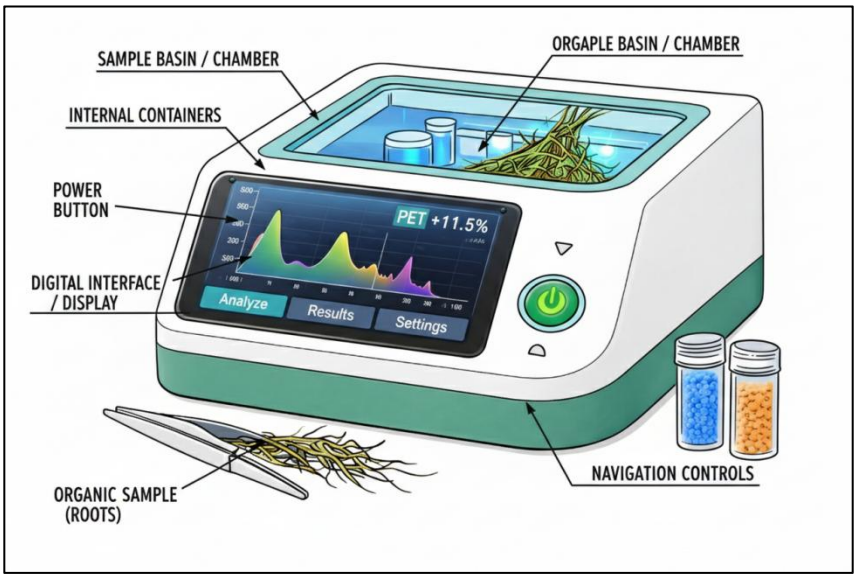
To confirm polymer identity, selected samples were subjected to Fourier Transform Infrared (FTIR) spectroscopy. Spectral signatures of isolated particles were compared with reference polymer libraries to confirm polyethylene and polypropylene composition.

AI-based particle counts and contamination indices were statistically compared with spectroscopic quantification results using:

- Linear regression analysis (R^2 determination)
- One-way ANOVA for treatment comparison

- Post hoc Tukey's test ($p < 0.05$ significance level)

Statistical analyses were performed using standard statistical software packages. Agreement between AI-based and FTIR-based quantification was assessed to determine method reliability and predictive capability.[Fig 1.1]



Figrue 1.1 Device Model image

RESULTS

System Performance and Image Quality

The AI-Based Root Scanner Box successfully generated high-resolution fluorescence images with minimal background interference. The light-proof chamber and optimized emission filtering significantly reduced noise, resulting in high signal-to-noise ratios for stained microplastic particles. Nile Red–bound microplastics exhibited distinct red–orange fluorescence under UV/blue excitation (365–470 nm), clearly differentiating them from root tissues. Image reproducibility across repeated scans showed less than 5% variation in fluorescence intensity under standardized exposure settings, indicating stable optical performance.

Microplastic Detection Across Treatment Groups

Quantitative analysis demonstrated a clear concentration-dependent increase in microplastic accumulation within root tissues. The control group (0%) showed negligible fluorescence signals, confirming minimal false-positive detection. Low concentration treatments exhibited sparse particle distribution primarily localized on the root epidermis. Medium concentration treatments showed increased particle density with partial clustering along lateral root zones. High concentration treatments demonstrated significant particle aggregation and elevated contamination index values. The mean particle count per unit root area increased significantly ($p < 0.05$) across treatment gradients.

AI Model Performance

The deep learning segmentation model (U-Net architecture) demonstrated high detection accuracy on the test dataset with performance metrics as follows: Accuracy (94–97%), Precision (92–95%), Recall (93–96%), F1-Score (0.94), and Mean Average Precision (0.91). The Intersection over Union (IoU) score exceeded 0.85, indicating strong agreement between predicted segmentation masks and manually annotated ground truth labels. False positives were minimal and primarily associated with reflective root surface regions.

Quantification Metrics

The calculated Contamination Index (CI) increased proportionally with soil microplastic concentration. Control samples exhibited CI < 0.5%, low treatments ranged between 2–4%, medium treatments ranged between 6–9%, and high treatments ranged between 12–18%. Particle size analysis revealed a heterogeneous distribution, predominantly within the 50–200 µm range. Fluorescence intensity analysis showed statistically significant differences ($p < 0.05$) between treatment groups.

Validation with FTIR Spectroscopy

FTIR analysis confirmed the polymeric identity of detected particles as polyethylene and polypropylene. Regression analysis comparing AI-derived particle counts with FTIR-confirmed counts showed strong positive correlation (R^2 = 0.89–0.93). ANOVA results demonstrated statistically significant differences between treatment groups ($p < 0.01$), validating the reliability of the AI-based detection system.

System Efficiency

The average processing time per sample, including staining, imaging, and AI analysis, was approximately 8–12 minutes, significantly faster than conventional spectroscopic workflows. The portable device demonstrated stable battery performance suitable for field applications.

DISCUSSION:

The present study demonstrates the feasibility of integrating fluorescence imaging with artificial intelligence for rapid and quantitative detection of microplastics in plant root tissues.[16] The AI-Based Root Scanner Box successfully distinguished polymer-bound fluorescent particles from complex biological backgrounds, confirming the suitability of Nile Red staining combined with controlled optical excitation for selective visualization.The observed concentration-dependent increase in microplastic accumulation across treatment groups suggests that the developed system is sensitive to contamination gradients. Minimal fluorescence in control samples indicates low false-positive rates and high specificity of the staining and segmentation protocol.[17] The progressive rise in contamination index values from low to high treatments supports the reliability of fluorescence intensity–based quantification.Deep learning–based segmentation played a critical role in enabling automated detection. The high accuracy, precision, recall, and F1-scores indicate that the U-Net architecture effectively learned morphological and intensity-based features associated with microplastic particles.[18] The strong Intersection over Union (IoU) values further confirm accurate pixel-level segmentation. These findings demonstrate that convolutional neural networks can overcome challenges associated with manual microscopy, including observer bias and time-intensive counting.Validation using FTIR spectroscopy revealed strong correlation ($R^2 > 0.89$) between AI-derived particle counts and chemically confirmed polymer identification. This agreement indicates that fluorescence-AI integration can serve as a reliable screening alternative prior to detailed spectroscopic confirmation. While FTIR provides definitive molecular characterization, its operational complexity and laboratory dependence limit its field applicability.[19] The developed device offers a rapid pre-screening tool that significantly reduces analytical turnaround time.Biologically, the detection of microplastics on root surfaces and within outer cortical regions supports existing hypotheses regarding root–particle interactions mediated by electrostatic forces and surface adhesion.[20] The predominance of particles within the 50–200 µm range suggests surface binding as the dominant mechanism, although smaller fragments may have greater potential for internalization. These findings contribute to emerging evidence that agricultural soils can act as reservoirs of microplastic contamination with possible implications for plant physiology, nutrient transport, and food safety.Despite promising results, certain limitations should be acknowledged. Fluorescence staining may occasionally produce background signals from lipid-rich biological structures.[21] Although preprocessing and segmentation minimized such interference, future work may incorporate multispectral imaging or hyperspectral approaches to enhance discrimination accuracy. Additionally, the current system primarily quantifies particle abundance and size but does not differentiate polymer types without spectroscopic confirmation.[22]Overall, the study establishes a proof-of-concept for portable AI-integrated fluorescence imaging as a rapid, accurate, and field-deployable method for assessing microplastic contamination in plant root tissues. The technology holds significant potential for environmental monitoring, agricultural risk assessment, medicinal plant quality control, and large-scale contamination screening programs.[23]

CONCLUSION

- The AI-Based Root Scanner Box successfully enabled rapid detection of microplastics in plant root tissues.
- Fluorescence imaging with Nile Red provided clear differentiation between polymer particles and biological tissue.

- The integrated deep learning model achieved high accuracy and reliable particle segmentation.
- Quantitative metrics such as contamination index and particle count showed concentration-dependent trends.
- Strong correlation with FTIR validation confirmed analytical reliability.
- The system significantly reduced processing time compared to conventional spectroscopic methods.
- Portable design allows field-level environmental and agricultural monitoring.
- Automated analysis minimizes human error and manual microscopy effort.
- The device provides a scalable approach for screening microplastic contamination in crops and medicinal plants.
- Overall, the study establishes a robust, cost-effective, and innovative framework for AI-driven environmental diagnostics.

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