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论 文 题 目：Visualizing Nanoplastics: New Fluorophore and its Applications in Plastic Particle Imaging

Visualizing Nanoplastics: New Fluorophore and its Applications in Plastic Particle Imaging

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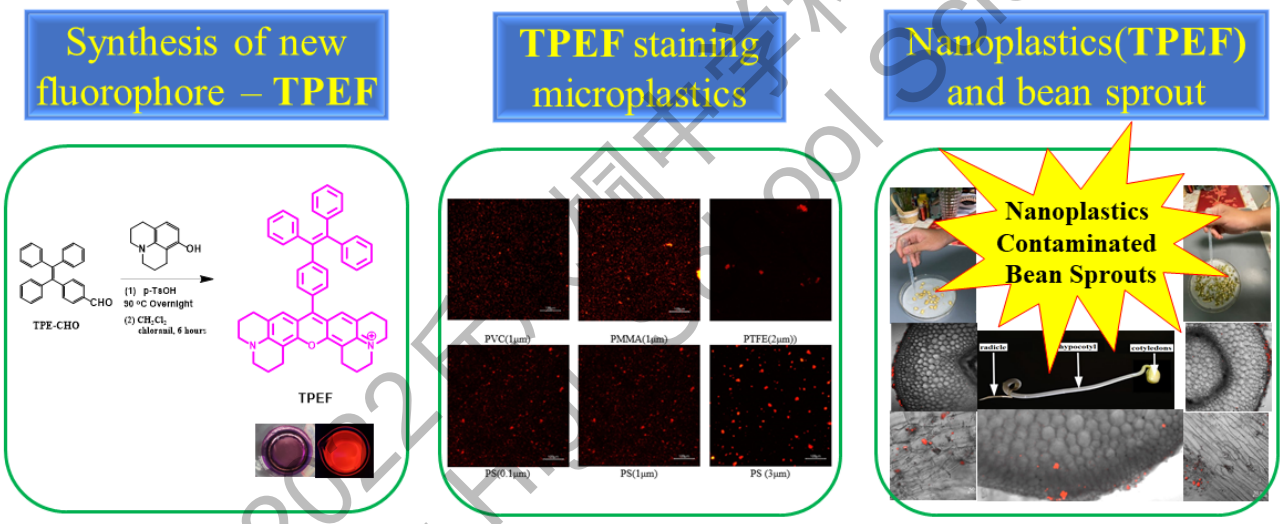
1. Graphical Abstract



2. Abstract

Microplastics are global, persistent contaminants. Among microplastics, research shows that nanoplastics (<1 μm) can be absorbed by plants, drawing attention for its potential harms to organisms. Microplastics are in water and food chains, and the potential associated effects threaten human health. Understanding the spread of microplastics in the environment and in living organisms becomes extremely important. However, because of their small size, it is difficult to detect and observe microplastic. Fluorescent dyes, also known as fluorophores, is a vital tool in detecting microplastic.

In this study, a new fluorophore -**TPEF**- was designed and synthesized. We, for the first time, dyed various microplastics (PS, PMMA, PVC, PET, etc.) with **TPEF**. Also for the first time, we cultured bean sprouts with water containing fluorescently stained PS microplastics. The results show that microplastic particles could accumulate on sprout epidermis, and even enter the sprouts. This study shows that the new fluorophore **TPEF** is a good microplastic dye, especially good for nanoplastics. This study also reveals that bean sprouts can be contaminated by water containing microplastics during growth, which directly threatens human health.

This work provides a new choice for future studies regarding nanoplastics in organisms. **TPEF** has potential to be a widely used fluorophore for visualizing nanoplastics.

Keywords: Fluorophore; Synthesis; Microplastics; Nanoplastics; Visualizing; Bean Sprouts.

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3. Introduction

3.1. Microplastics

As a man-made polymer, plastic is used extensively in building materials, agriculture, engineering, construction, and everyday life due to its light weight, anti-corrosive properties, and low cost. (Figure 1) Globally, 368 million tons of plastic are produced each year, and it is expected that the output may double in 20 years [1]. However, less than 10% of plastic products are recycled or incinerated, most of the rest either goes to landfills or enters the environment [2].

In the environment, plastic degrades into minuscule particles under physical and chemical forces (such as UV radiation, mechanical forces, bio degradation etc). These particles, specifically particles with a diameter under 5mm, are microplastics. [3]

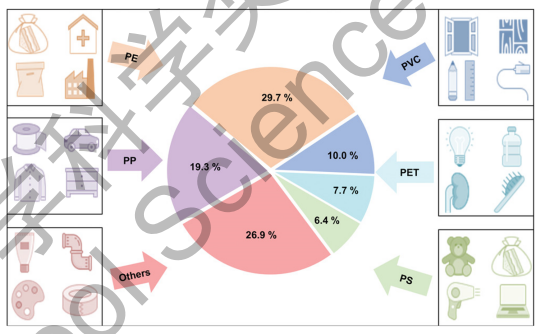


Figure 1: Chemical composition of plastics.

Microplastics are increasingly widespread in the environment. Microplastics degrade slowly in the environment. They exist for a long time in water, sediments and other environmental media, and accumulate in organisms. Because of their extensiveness and longevity, microplastics have become a concerning environmental hazard.

Research show that microplastics are prevalent in bodies of freshwater around the world. [6] Many studies and surveys have shown that microplastics with different concentrations can be found in surface rivers, lakes, reservoirs and water bodies near residential areas. For example, in China's fresh water bodies, microplastics have been detected in the Yellow Sea, Taihu Lake, Yangtze River, and Poyang Lake [7].

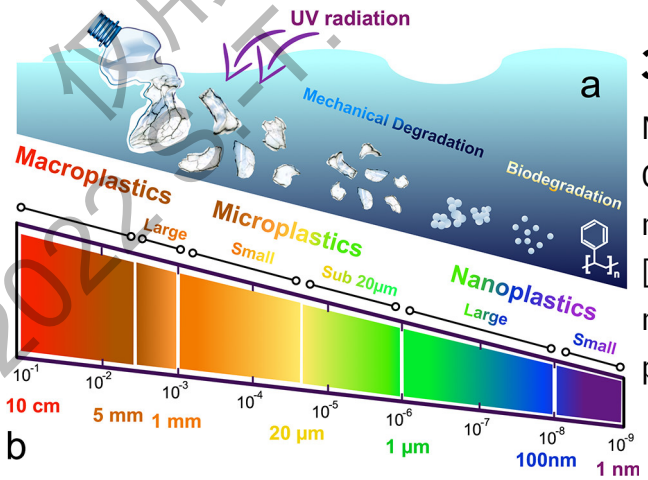


Figure 2: Degradation flow and size-based definition of plastics.

3.2. Microplastic Varieties

Microplastics are categorized by size. (Figure 2) Currently, nanoplastics were defined as microplastics with diameters less than 1000 nm [8, 9]). The distinguishing feature of microplastic particles that differ from ordinary plastic pollutants is their small size.

Microplastics may invade blood vessels to deposit, adhere to cell membranes, crossing biological barriers, and potentially harm to cells.

Microplastics are also categorized by composition. They can be made of many plastics, such as: polyethylene (PE), polystyrene (PS), Polyphenylene Sulfide (PPS), Polymethyl Methacrylate (PMMA) Polyamide (PA), polypropylene (PP), polychlorinated Ethylene (PVC), polytetrafluoroethylene (PTFE) and polyethylene terephthalate (PET). (Figure 3)

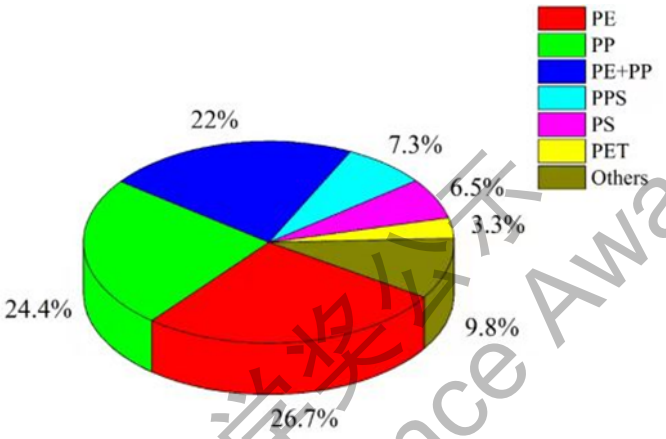


Figure 3: Microplastic types in freshwater samples.

3.3. Microplastic Harms

Microplastics are widespread in the terrestrial environment as well as in marine and freshwater ecosystems because of human activities. [10] Because of their small size, wide source and complex chemical composition, microplastics have strong diffusivity. They contain plasticizers, and because of their large specific surface area, they are carriers of environmental pollutants (pesticides, antibiotics, cyclic aromatic hydrocarbons, heavy metals, etc.), making them toxic and harmful chemical substances with the characteristics of biotoxicity, environmental persistence, and bio-accumulation. [11] Moreover, microplastics have numerous interactions with biotic and abiotic factors in the ecosystem, and therefore have multiple impacts on the food chain. (Figure 4)

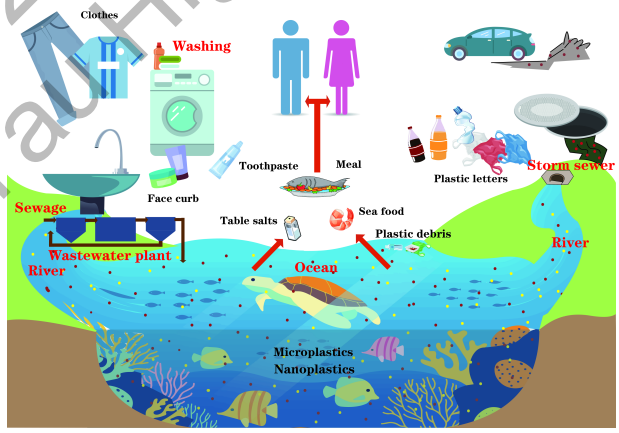


Figure 4: Microplastics and the food chain.

Since plastics will not decompose for many years, the entire cycle and movement of microplastics in the entire environment is unclear. Its widespread presence in the

environment raises concerns to various established and uncertain hazards to living organisms.

Microplastics are ubiquitous in the global biosphere, raising concerns about their impact on humans. At present, human beings are constantly exposed to microplastics environment leading to long-term inhalation or ingestion of microplastics. The latest research shows that the microplastic particles detected in human blood are ≥700nm, and the total quantified concentration of plastic particles in blood is 1.6 μg / ml. The study shows that microplastics can enter the bloodstream through the digestive tract or respiratory tract, and may already be widespread in the human body. [12]

However, microplastics' specific effects on the health of humans is still not clear. Critical data on exposure and hazards are severely lacking and need to be filled to better understand the impact of microplastics on human health. [13]

3.4. Microplastic Visualization

There are, at present, several ways of seeing environmental microplastics.

For those large-sized plastic particles (larger than microplastics), the most common (and economic) detection method is visual inspection. Because the larger particles are easy to identify, the results of this method are relatively accurate.

For microplastics, the microscope is an important tool. [14] With the development of technology, and people's attention increased to microplastics, the focus of microscopes has transitioned from the ordinary optical microscope to the fluorescent microscope, and even the current con-focal microscope, the size of the detected microplastics is getting increasingly small. [15] However, most plastic itself are colorless and easily lend into background noise. Because of this, fluorescent dyes, or fluorophores, are used to help people distinguish and identify microplastics in various samples. [16]

Fluorescence microscopy is commonly used to study the migration and translocation of microplastic particles between tissues. The method of fluorescent staining has become a crucial method to effectively inspect microplastics in environmental samples and biological samples. The methods could help people understanding the effects of microplastic, understanding microplastic size, distribution, chemical composition, as well as tracking temporal and distributional change.

3.5. Research Content

Earlier this year, I entered Prof. Xing's group and studied the design and synthesis of new fluorescent probes, with a particular focus on developing novel fluorescent probes with the tetraphenyl ethylene functional group. This research is focused on the synthesis a fluorescent probe through joining the tetraphenyl ethylene to a fluorescent core and studying its applications in cell imaging. Incidentally, the new molecule exhibits an excellent affinity to plastic materials, the plastic tube and the gasket on the column were dyed and exhibit pink fluorescence under the UV irradiation (Figure 5).

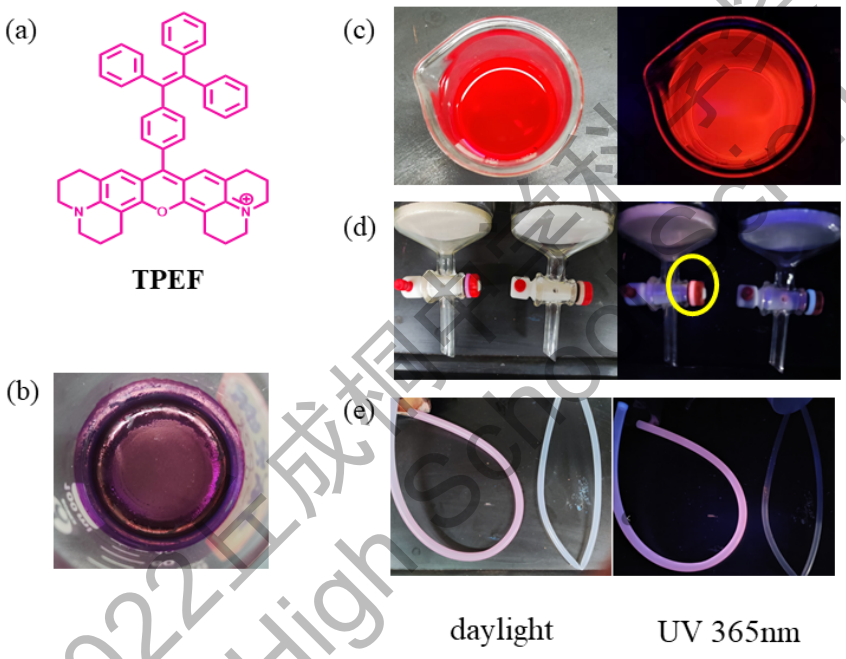


*Figure 5: (a) Structure of compound **TPEF**; (b) Sample of compound **TPEF** (purple solid); (c) **TPEF** methanol solution ; (d) Plastic Gasket on the glass column (left: after the contact of **TPEF** methanol solution of ; right: without the contact of **TPEF** methanol solution); (e) Plastic tube (left: after the contact of methanol solution of **TPEF**; right: without the contact of **TPEF** methanol solution).*

When I first discovered this phenomenon, I tried to use solvents to clean these fluorescent dye-contaminated rubber tubes and column plastic gaskets, but it took a lot of effort to realize that **TPEF** has a very strong dyeing ability, and these dyed Plastic products simply cannot be restored to their original state. Because I have seen reports about microplastics

when I usually read, an idea suddenly popped into my mind, that is, can **TPEF** dye those microplastics to make them easier to identify?

Bean sprouts are the sprouted products of legume seeds after soaking in water, and are used to produce vegetables for human consumption. Bean sprouts can provide essential proteins, antioxidants, and minerals worldwide [17]. Bean sprouts are one of the most common and economic vegetables and extensively cultivated and consumed in Asian countries, including Japan, Korea, and China. The most commonly used legumes are mung beans, soybeans, etc., which are called mung bean sprouts and soybean sprouts after finished products. It was estimated that more than 42,000 tons of soybean sprouts were consumed in Korea in 2017. [17] To the best of our knowledge, while there have been many reports on the accumulation of microplastics in plant organisms, the absorption of microplastics by bean sprouts is not reported. According to our aforementioned discovery, we attempted to use compound **TPEF** as a fluorescent dye to tracking the microplastic and nanoplastic in soybean sprouts and the mung bean sprouts.

4. Experiments

4.1. General Information on Material and Instruments

All reagents were purchased from commercial sources and used as is without further purification. Nile Red (CAS #: 7385-67-3) was purchased from Picasso Reagent Co., Ltd. **TPE-CHO** were prepared according to reference method. [18] The microparticles were purchased from purchased from Dong Guan Ming Yu Xing Plastic starting materials Ltd (Table 1). Seeds of plant species mung bean (Vigna radiata) and soybean (Glycine max) were purchased locally. and used as is. Wahaha (Hangzhou, China) deionized water was used.

The microplastic particles used are shown in Table 1.

Table 1: Microplastics used in this project.

Microplastic	PVC	PMMA	PTFE	PS	PS	PS	PE	PPS	PET
Size (μm)	1	1	2	0.1	1	3	5	48	5

4.2. Synthesis

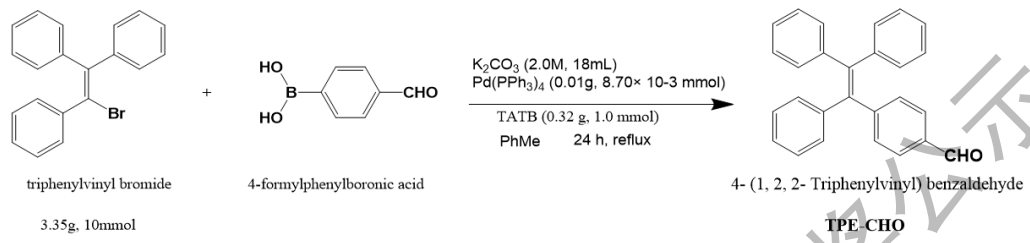


*Figure 6: Synthesis of **TPE-CHO***

TPE-CHO (4- (1, 2, 2- Triphenylvinyl) benzaldehyde) was synthesized according at the condition in the reference. Yellowish powder was got. ^{1}H NMR (400 MHz, Chloroform-d6) δ 9.88 (s, 1H), 7.60 (d, 2H), 7.19 (d, 2H), 7.13-7.06 (m, 9H), 7.04-6.99 (m, 6H). ^{13}C NMR (75 MHz, Chloroform-d6, 298 K): δ 191.8, 150.5, 143.0, 142.9, 142.8, 139.7 134.2, 131.9, 131.23, 131.22, 131.17, 129.1, 127.88, 127.70, 127.0, 126.84, 126.81. The data are in consistent with reported results. [18]

The synthesis process of **TPE-CHO** is shown in Figure 6.

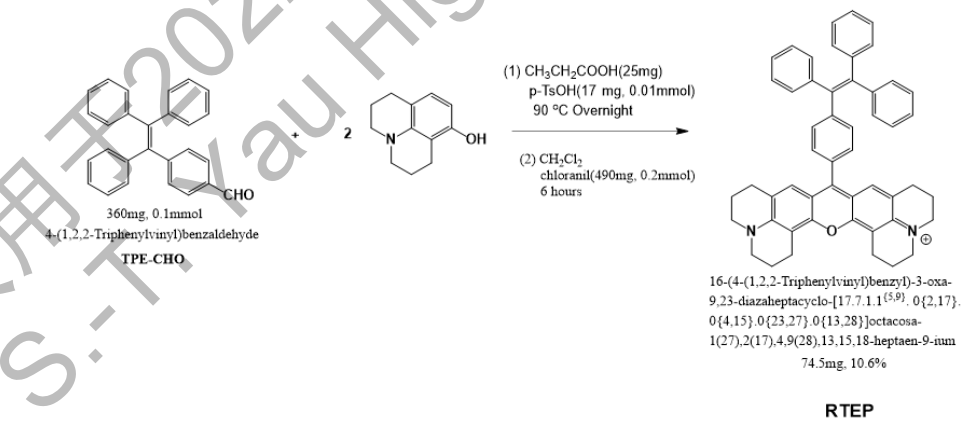


*Figure 7: Synthesis of **TPEF***

TPEF was synthesized according at the condition in Figure 7, and purified with Al2O3 chromatograph (eluted with dichloromethane:methol 20:1) and obtained a purple powder.

[19] ^{1}H NMR (400 MHz, Chloroform-d6) δ 7.98 (s, 1H), 7.44 (s, 2H), 6.96 (s, 2H), 4.55 – 4.15 (m, 2H), 3.84 – 3.33 (m, 10H), 2.67 – 2.49 (m, 1H), 2.01 (s, 1H), 1.94 (s, 3H), 1.58 – 1.42 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-d6) δ 175.61, 174.08, 147.25, 144.16, 132.59, 128.53, 125.67, 100.99, 73.26, 72.25, 68.82, 68.69, 62.96, 61.74, 52.68, 48.41, 40.93, 30.29, 22.69. HRMS(ESI+): (M)+ Calcd for C51H45N2O, 701.3526; found 701.3525.

Pictures of the synthesis process are shown in Figure 8.

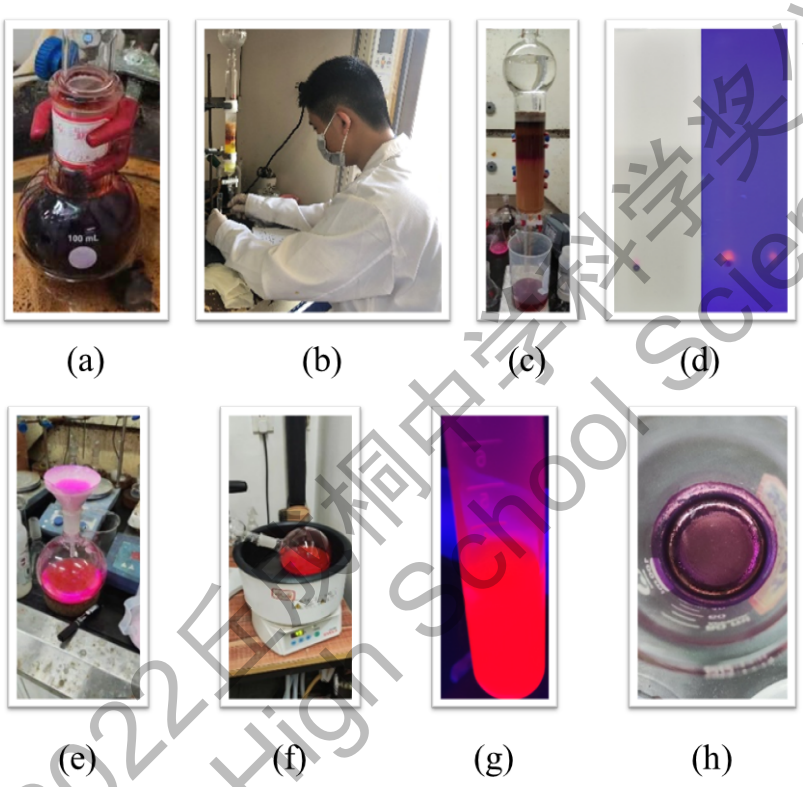


*Figure 8: Some photos in the process of synthesis of **TPEF**: (a) Synthesis of **TPEF**; (b) Purify the **TPEF** through Silica Gel Column; (c) Silica Gel Column; (d) Thin Layer Chromatograph; (e) Filtrate the **TPEF** solution; (f) Rotary evaporation to remove solvent; (g) **TPEF** in methanol under 365nm UV light; (h) **TPEF** product (purple solid).*

4.3. Characterization

^{1}H and ^{13}C NMR(Bruker AVANCE III-400), Mass spectrum (Thermo scientific Q Exactive HF Orbitrap-FTMS mass spectromete) Fluorescence spectra(Shimadzu RF-6000 fluorophotometer). Ultraviolet visible (UV-Vis) absorption spectra (Agilent Cary 300

spectrophotometer) were recorded to characterize the **TPE-CHO** and **TPEF**. Gaussian 16 suite of program[20] was used to calculate **TPEF**'s HOMO and LUMO orbitals with the density functional theory (DFT) at the level of B3LYP/6-311+G (d, p). The energy gap (Eg) of the HOMO and LUMO was calculated.

4.4. Imaging

Imaging was performed using a light microscope (AOSVI L208F-3M830F) with a light-emitting diode (LED) providing the fluorescence source. Using green light at 495~555 nm for excitation of the stained microplastic particles. Confocal Laser Scanning Microscopy (Germany LSM800(Zeiss)) was used to observe the microplastic in the hypocotyl epidermis and sliced hypocotyl of mung bean sprouts or soybean sprouts. Images were corrected for brightness and contrast. The sliced bean sprout was embedded on glass microscopy slides.

4.4.1. Preparation of Fluorescent Microplastic Particles

In this study, stock solutions of 0.03mmol/mL fluorescent dye **TPEF** in absolute ethanol were prepared. Add 100mg plastic particles respectively into 15ml fluorescent dyes stock solutions and treated with ultrasound irritation in a water bath (preheated to 60°C) for 30 minutes (the photo of the ultrasonic instrument was taken here). The solutions are cooled with ice-water bath for 15 minutes. The mixtures were filtered with microporous membrane (0.2μm pore size) under the reduced pressure, For the Nano plastic PS(0.1μm) mixture, the centrifugation (13000 rpm) was used to collect the PS particles. After the centrifugation, the excess EtOH was decanted. All the particles were further rinsed with EtOH until the effluent or decant was colorless. And the dyed PS particles was air dried. After drying, the stained microplastics were transferred to glass slides and observed under a fluorescence microscope (L208F-3M830F assisted 8.3 million-pixel electronic eyepiece, the fluorescence microscope parameters also need to be given here) at a magnification of 4-10× staining results.

4.4.2. Imaging of Microplastic and Nanoplastic in Bean Sprouts

The mung bean sprouts and soybean sprouts were grown in Petri dishes. Rinse the seeds four times a day for 5 days. All of mung bean sprouts or soybean sprouts were divided into ten groups respectively (Table 2). The groups containing four control groups (the bean sprouts were rinsed with water(M1), 100μg/mL 0.1μm PS in water(M2), 100μg/mL 1μm PS suspended in water(M3), and 100μg/mL 3μm PS in water(M4) respectively) [22], those control groups were used to figure out that the possibility of detection of microplastics

particles in mung bean and soybean sprouts. And in the 6 test groups (test group M1 - M6), the bean sprouts were rinsed with aqueous suspension of dyed PS particles (100μg/mL), the PS particles were dyed with **TPEF** or Nile Red)

At the same time, prior to the preparation of thin sections, an extra bean sprouts group samples were selected bean sprouts were collected and they were rinsed with running distilled water for several minutes to dislodge foreign particles (incl. microplastic) from the hypocotyl epidermis to determine the microparticles, the purpose of it was to figure out whether washing could remove the microplastic particles on the hypocotyl epidermis of the bean sprouts.

Pictures recording the process is shown in Figure 9.

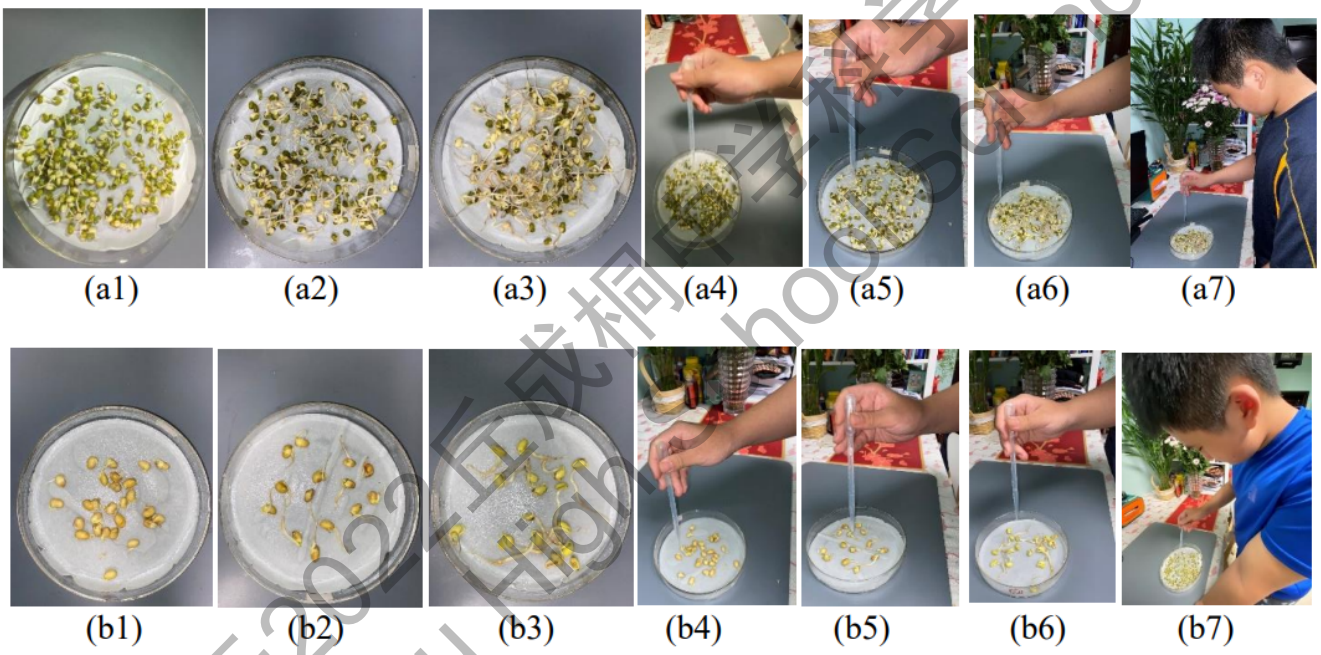


Figure 9: Rinsing the bean sprouts. Mung bean Sprouts: (a1) day 1; (a2) day 3; (a3) day 5; (a4) day 1; (a5) day 3; (a6) day 5; (a7) experiment operator; Mung bean Sprouts: (b1) day 1; (b2) day 3; (b3) day 5; (b4) day 1; (b5) day 3; (b6) day 5; (b7) experiment operator;

The concentration of 100μg/mL of aqueous suspension of microplastic (1μm and 3μm PS) and nanoplastic (0.1μm PS) were prepared. All grouped bean sprouts were sampled at one day, three days and five days of growth. The sampled bean sprouts were sliced in the middle of the hypocotyl with a scalpel and placed on a glass slide and covered with a cover glass. The hypocotyl epidermis was peeled off with tweezers. All the samples were observed with confocal microscope. All bean sprouts for each of the three replicates had the same experimental results, therefore we only present selected images in the following sections.

Table 2: Groups of mung and soy bean sprouts used in this project

No.	Bean	Groups	PS Size (μm)	PS Content (mg/L)	Fluorescent Dye
1	mung bean	Control group M-1	None	0	None
2	mung bean	Control group M-2	0.1	100	None
3	mung bean	Control group M-3	1	100	None
4	mung bean	Control group M-4	3	100	None
5	mung bean	Test group M-1	0.1	100	**TPEF**
6	mung bean	Test group M-2	1	100	**TPEF**
7	mung bean	Test group M-3	3	100	**TPEF**
8	mung bean	Test group M-4	0.1	100	Nile Red
9	mung bean	Test group M-5	1	100	Nile Red
10	mung bean	Test group M-6	3	100	Nile Red
11	soybean	Control group S-1	None	0	None
12	soybean	Control group S-2	0.1	100	None
13	soybean	Control group S-3	1	100	None
14	soybean	Control group S-4	3	100	None
15	soybean	Test group S-1	0.1	100	**TPEF**
16	soybean	Test group S-2	1	100	**TPEF**
17	soybean	Test group S-3	3	100	**TPEF**
18	soybean	Test group S-4	0.1	100	Nile Red
19	soybean	Test group S-5	1	100	Nile Red
20	soybean	Test group S-6	3	100	Nile Red

5. Results and Discussion

5.1. Characterization

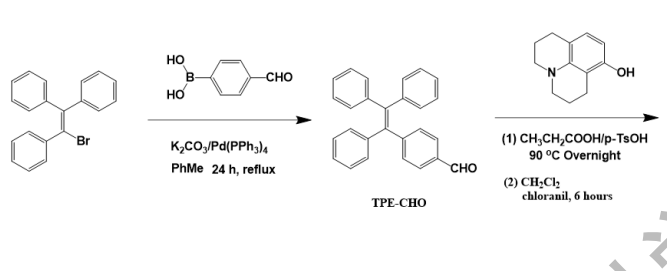


*Figure 10: Synthesis of **TPE-CHO** and **TPEF***

As shown in Figure 10. **TPEF** is composed of a tetraphenyl ethylene unit and a rigid fluorophore linked by the C-C bond. **TPE-CHO** was synthesized via Suzuki reaction (Figure 10). Condensation and oxidative cyclization **TPE-CHO** and 8-Hydroxyjulolidine provided **TPEF** as a purple solid. The **TPEF** were characterized by NMR (^1H and ^{13}C) coupled with high-resolution mass spectrometry (HRMS) (Figure 12).

The NMR results of **TPE-CHO** is shown in Figure 11.

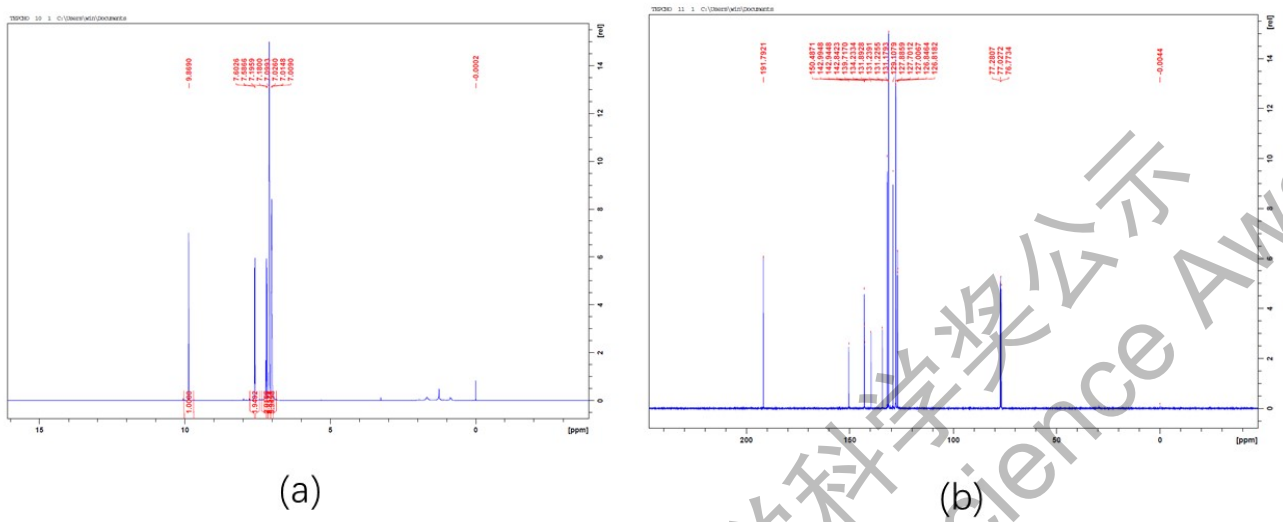


*Figure 11: NMR of **TPE-CHO**: (a) ¹H NMR; (b) ¹³C NMR*

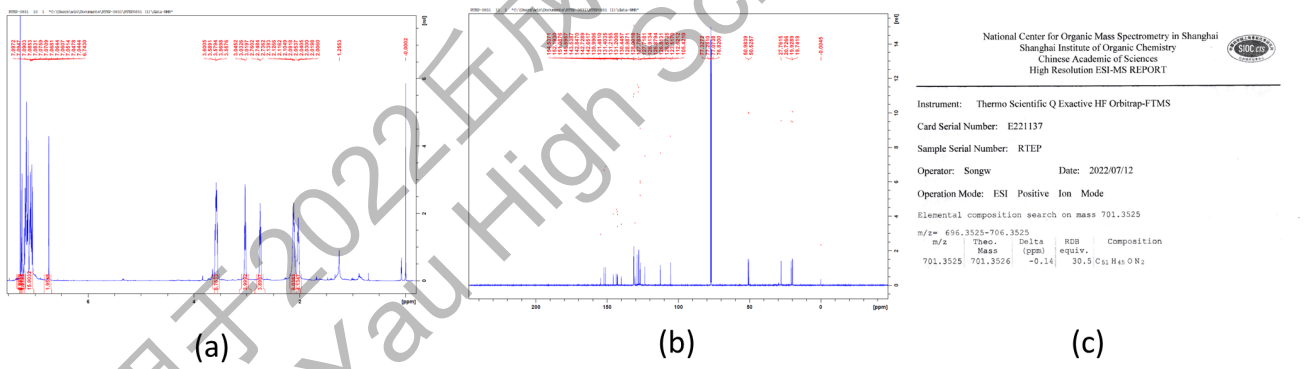


*Figure 12: NMR of **TPEF**: (a) ¹H NMR; (b) ¹³C NMR; (c) HR-MS result.*

The UV–vis absorption of **TPEF** in several common solvents and Excitation/Emission Fluorescence spectra of fluorophore **TPEF** is shown in Figure 13. As depicted in Figure 13a, in different solvents (peak), the absorption bands of **TPEF** all show one main absorption peak. There was a negligible shift at the peak in a long wavelength region with increasing solvent polarity.

The maximum emission wavelength of TPEF(602nm) reaches the far-infrared region (>600 nm), which can effectively avoid the background fluorescence interference of biomolecules in vivo, and is more suitable forfluorescence imaging in biological samples.

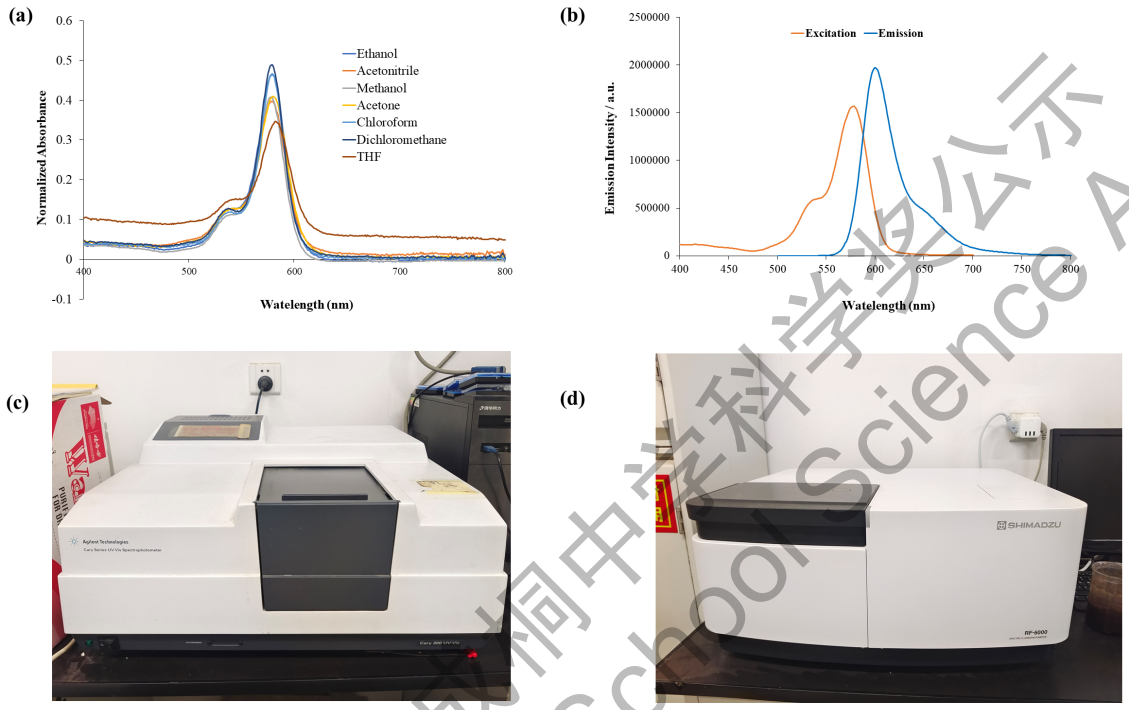


*Figure 13: (a) Normalized UV–vis absorption of **TPEF** in different solvents (0.1 mmol/L); (b) Excitation/Emission Fluorescence spectra of **TPEF** in MeOH (0.1 mmol/L); (c) Agilent Cary 300 spectrophotometer; (d) Shimadzu RF-6000 fluorophotometer.*

For the electron distribution and energy levels of **TPEF**, the optimized ground-state geometry was obtained through density functional theory (DFT) calculation at the level of B3LYP/6-311+G (d, p), then electron distributions of HOMOs and LUMOs of **TPEF** were gained from the optimized ground state structure at the same level. As shown in Figure 13, the lowest unoccupied molecular orbital (LUMO) occupied the rigid ring units, whereas the highest occupied molecular orbital (HOMO) was mainly localized on the tetraphenylethylene moieties. The calculated HOMO and LUMO energy levels of **TPEF** were -0.349 and -0.0718 eV, respectively. The corresponding energy gap (Eg) between the HOMO and LUMO energy levels was calculated to be 0.277 eV.

The HOMO-LUMO results are shown in Figure 14.

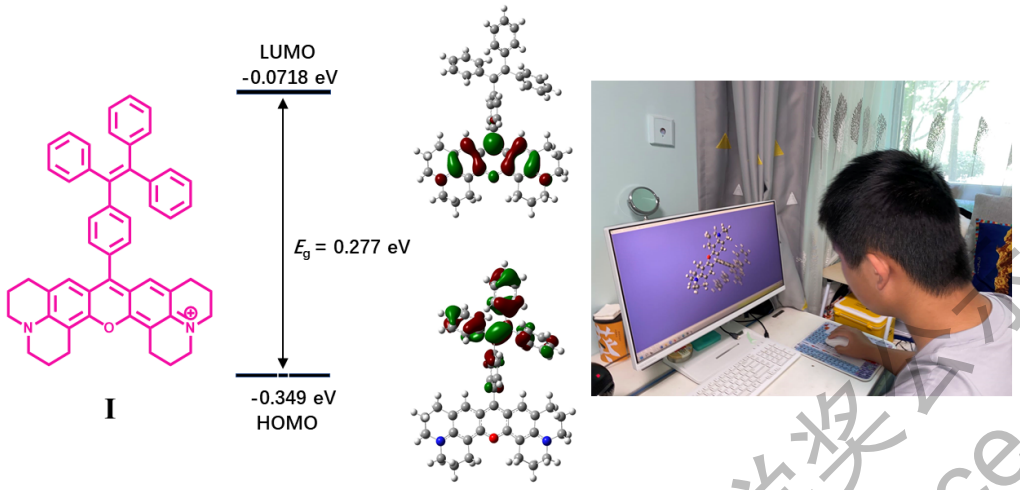


*Figure 14: Calculated HOMO/LUMO distributions of **TPEF**.*

5.2. Imaging

5.2.1. Microplastic Particles Dyeing

Plastics including PS (0.1 μm, 1 μm and 3 μm), PVC (1 μm), PMMA (1 μm), PE (5 μm), PPS (48 μm), PTFE (2 μm) and PET (5 μm) were dyed using **TPEF**, through fluorescence microscope observation, it is found that the compound has strong fluorescent dyeing effect on PS with three different particle sizes, as well as PVC and PMMA. **TPEF** dyed PTFE, PPS and PET show moderate fluorescence intensity, and the PE exhibit weak fluorescence intensity, as shown below in Figure 15.

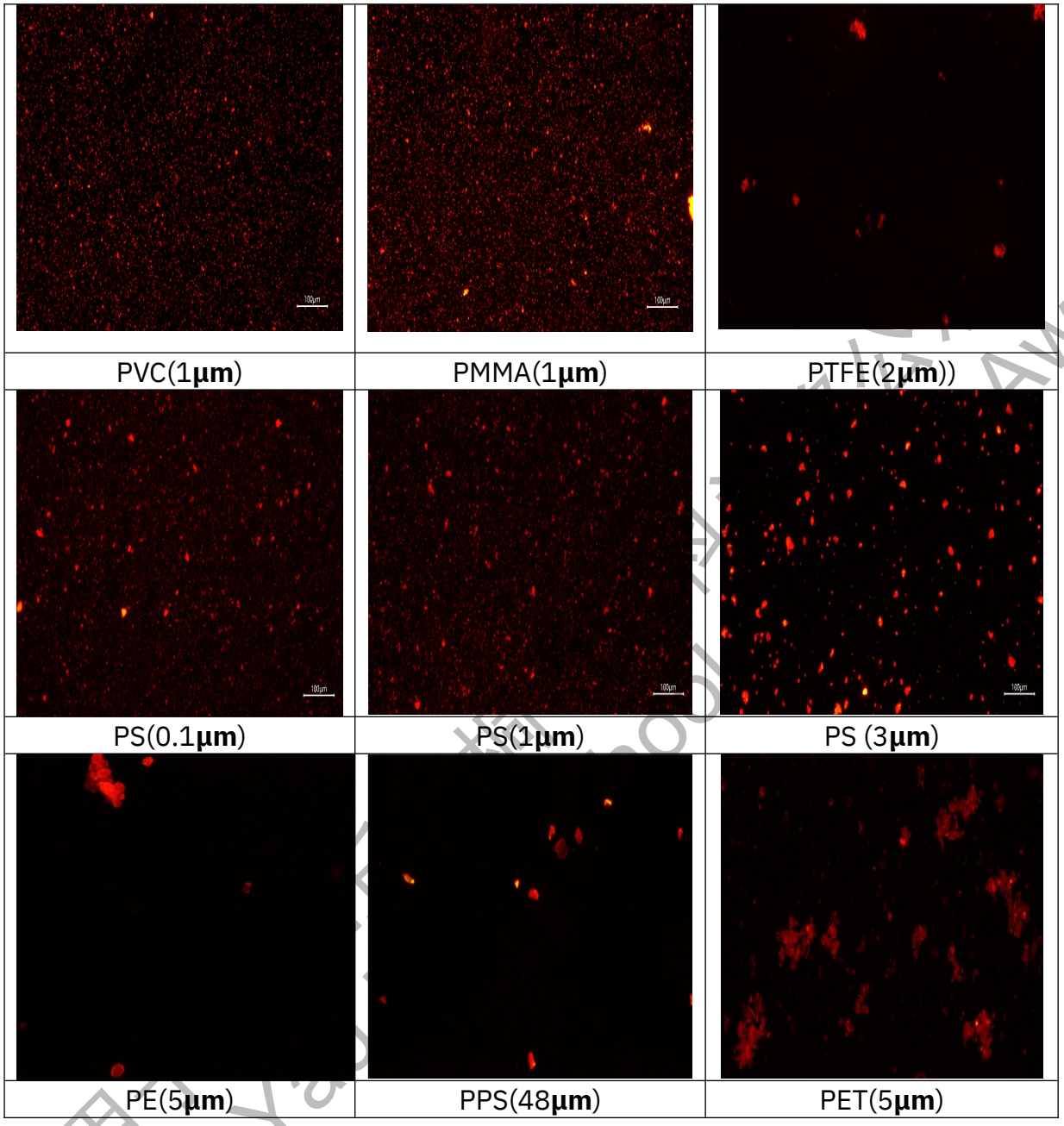


*Figure 15: **TPEF** dyed microplastic particles*

According to the dyeing effect, and considering that in most of our microplastic studies, PS is a representative microplastic, in the following experiments, we used **TPEF** to dye PS particles with three different particle sizes (0.1 μm, 1 μm and 3 μm). The dyed or undyed PS was added to the water for culturing mung bean sprouts and soybean sprouts, and they were rinsed with these waters every 6 hours. The samples of bean sprouts were collected on the first, third and fifth days of bean sprouts growth. In the middle section, the deposition of PS

particles in the hypocotyl epidermis and hypocotyl section was observed by confocal microscope, and compared with the control group.

For fifth days' samples, prior to the preparation of thin sections, an extra bean sprouts group samples were selected bean sprouts were collected and they were rinsed with running distilled water for several minutes to compare with the unwashed group, in order to figure out whether washing could remove the microplastic particles on the hypocotyl epidermis of the bean sprouts.

5.2.2. Association of Microplastic and Nanoplastic with Mung Bean and Soybean Sprouts

The main structure of soybean sprouts is cotyledon, hypocotyl and radicle, among which the cotyledon and hypocotyl are the edible parts of bean sprouts, mung bean sprout has the similar structure of soybean sprout. The middle of the hypocotyl of the bean sprouts were selected as the observation site (as shown below in Figure 16). Mung bean sprouts have a comparable structure.

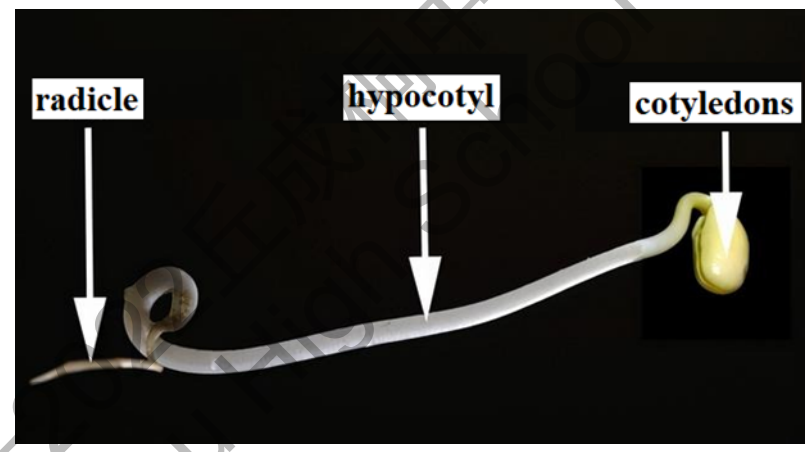
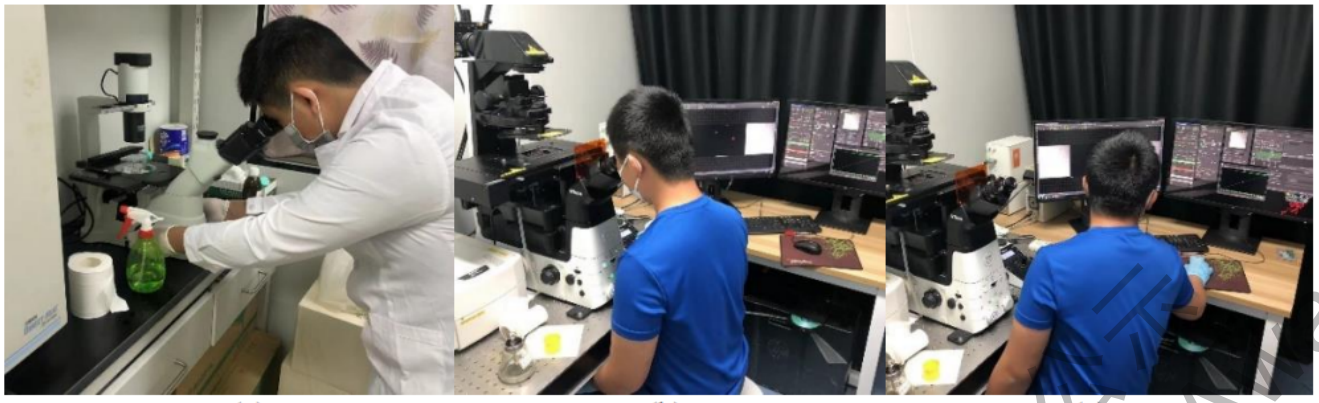


Figure 16: Soybean sprout structure

Our hypothesis is that the microplastics in the water may be absorbed by the bean sprouts during growth, and thus stay on the surface or inside of the bean sprouts. In this experiment, we hope to study whether the microplastics will be adsorbed or intake by the bean sprouts by culturing bean sprouts in water containing microplastic or nanoplastic particles.



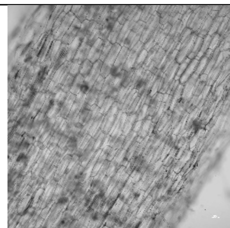
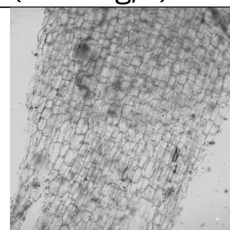
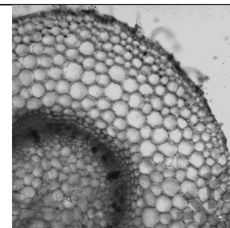
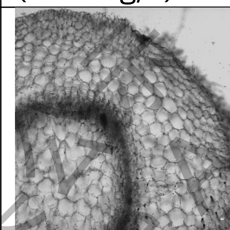
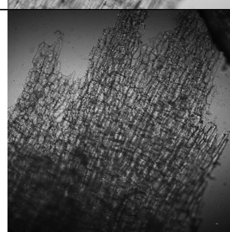
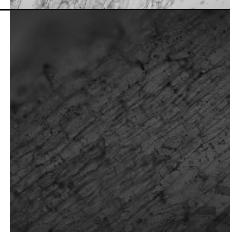
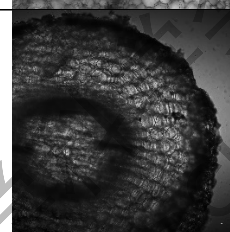
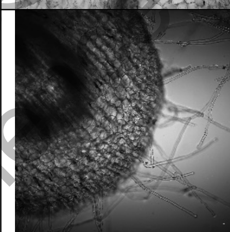
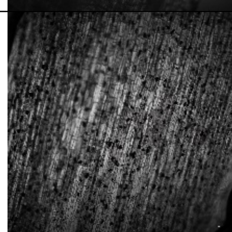
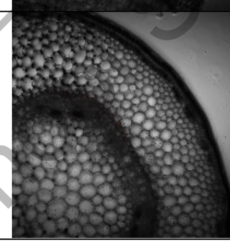
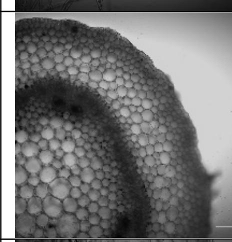
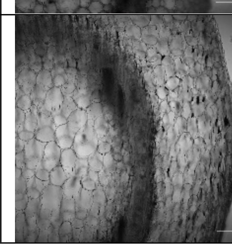
(a) (b) (c)

Figure 17: Observe the microplastics with: (a)fluorescent microscope; (b,c)confocal microscope.

In the control group, because mung bean sprouts and soy bean sprouts were incubated with water alone or with the addition of unstained PS particles (0.1 μm in diameter). Confocal microscopy of the hypocotyl of the bean sprouts in water showed there is no evidence for intake or accumulation of microplastics on the surface of or in the hypocotyl of the mung bean sprouts (Table 3) and soybean sprouts (Table 4).

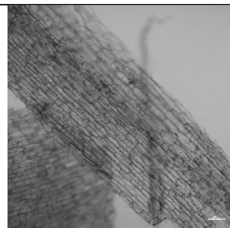
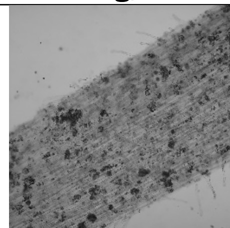
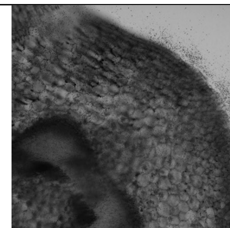
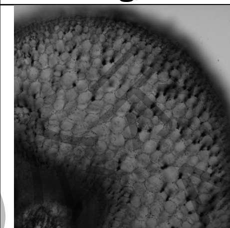
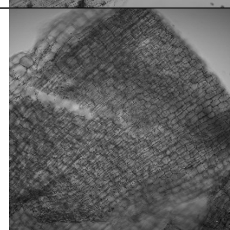
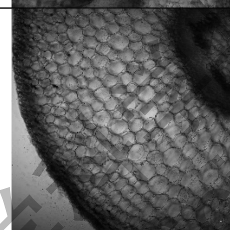
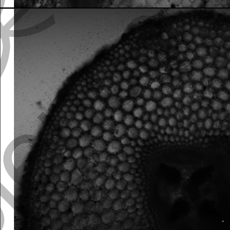
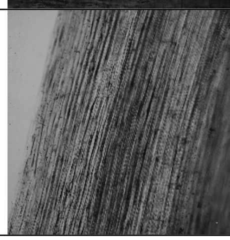
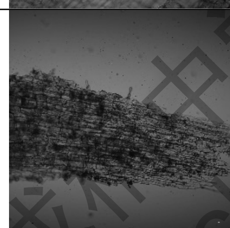
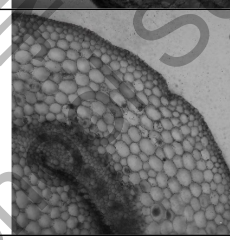
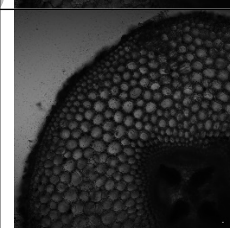
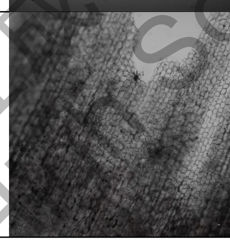
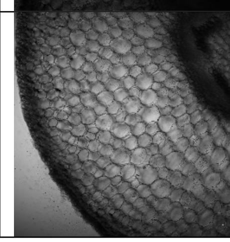
Pictures depicting the process is shown in Figure 17.

Table 3: Confocal microscopy views of mung bean sprouts control samples

Particles Time (Day)	hypocotyl epidermis		cross-sections of hypocotyls	
	Water	0.1μm PS in water (100 mg/L)	Water	0.1μm PS in water (100 mg/L)
1				
3				
5				
5 (washed with water)	None		None	

From the tables 3 and 4, the mung bean sprouts and soybean sprouts rinsed with pure water or water containing unstained PS particles were observed by confocal microscope for the whole testing process (1 day old, 3 days old and 5 days old), No PS microplastics were observed under confocal microscopy in all samples. Similar results were found for the 1μm and 3 μm PS particles in water were used to rinse the bean sprouts.

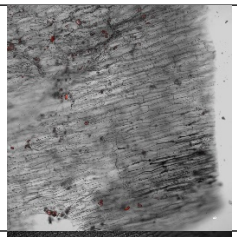
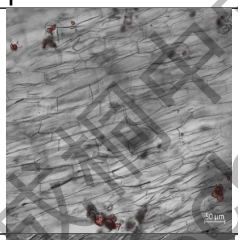
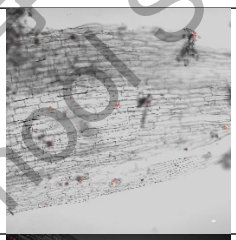
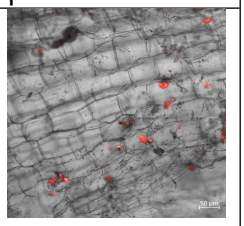
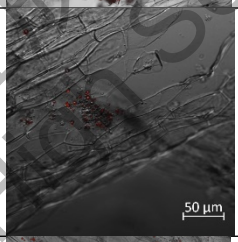
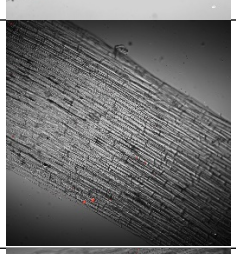
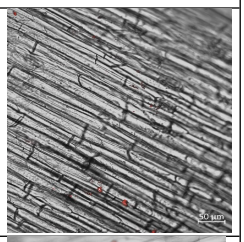
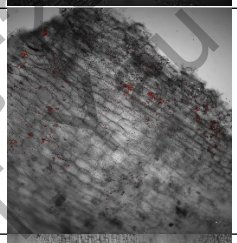
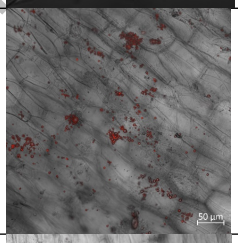
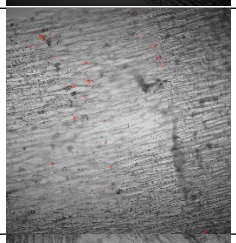
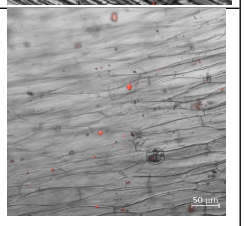
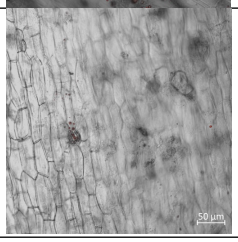
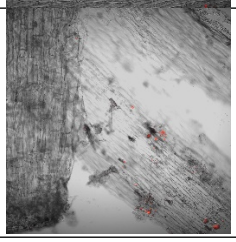
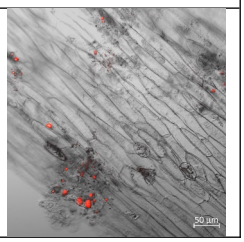
Table 4: Confocal microscopy views soybean sprouts control samples

Particles Time (Day)	hypocotyl epidermis		cross-sections of hypocotyls	
	Water	0.1μm PS in water (100 mg/L)	Water	0.1μm PS in water (100 mg/L)
1				
3				
5				
5 (washed with water)	None		None	

In the test groups of mung bean sprouts rinsed with the suspensions of 0.1μm PS particles in water, those particles were dyed with Nile red or **TPEF**, the confocal microscopy views of hypocotyl epidermis showed fluorescent particles accumulated on the hypocotyl epidermis (Table 5). In order to observe the difference in the adsorption of microplastics with different particle sizes during the growth of mung bean sprouts, three different particle sizes of PS (0.1 μm, 1 μm and 3 μm) were selected in the experiment and added to the water for rinsing mung bean sprouts. Mung bean sprouts were sampled at 1 day, 3 days and 5 days and the adsorption of these PS microplastics on mung bean sprouts hypocotyl epidermis and cross section of hypocotyl was observed by confocal microscopy. Aggregation of fluorescent PS microparticles on the hypocotyl epidermis of the bean sprouts were easily imaged. From the enlarged photos, **TPEF** stained PS particles exhibit brighter light that from Nile Red stained PS particles.

For the 5 days old mung bean sprouts, in order to figure out whether the washing of the bean sprouts (to mimic everyday washes before bean sprouts are ingested) could remove the PS particles on the hypocotyl epidermis, both washed and unwashed 5 days old mung bean sprouts were observed, results shows that although it seems that the number of PS particles on hypocotyl epidermis has decreased a little, there are still residual particles, which means that if the bean sprouts product is infected with microplastics, washing with water cannot completely remove such contamination, and the microplastics may follow.

Table 5: Confocal microscopy views of hypocotyl epidermis in mung bean sprouts test samples.

Particles Time (Days)	Nile Red stained 0.1μm PS in water (100 mg/L)		**TPEF** stained 0.1μm PS in water (100 mg/L)	
		Enlarged photoes		Enlarged photoes
1				
3				
5				
5 (washed with water)				

In Table 6, the con-focal microscopy views show cross sections of hypocotyl of the mung bean sprouts. There is some evidence for the presence of the 0.1 μm (Yellow arrow in Table

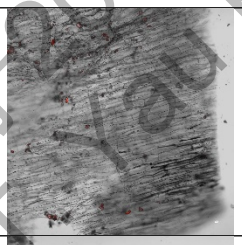
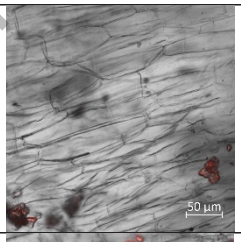
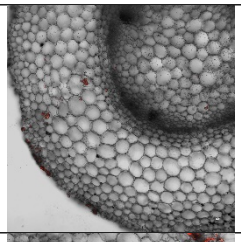
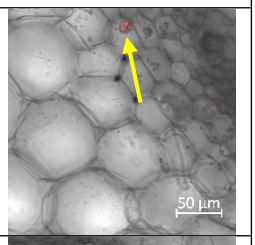
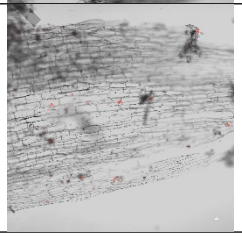
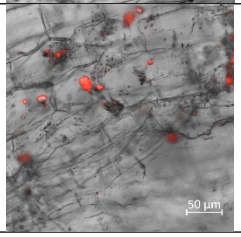
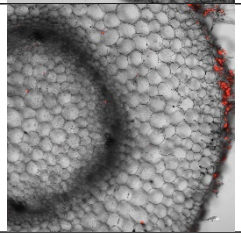
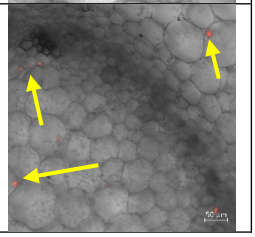
6) were noticed to extend into the internal hypocotyl structure. Light yellow arrows show particles inside sprouts and on the inside the hypocotyl. As can be seen from Table 6, since the particle size of PS microplastic particles of 0.1 μm is smaller, the particles entering the mung bean hypocotyl are more than those of PS microplastics with a particle size of 1 μm or 3 μm. These particles stay in gaps between cells. However, no particles are visible internal to the active bean sprouts cells.

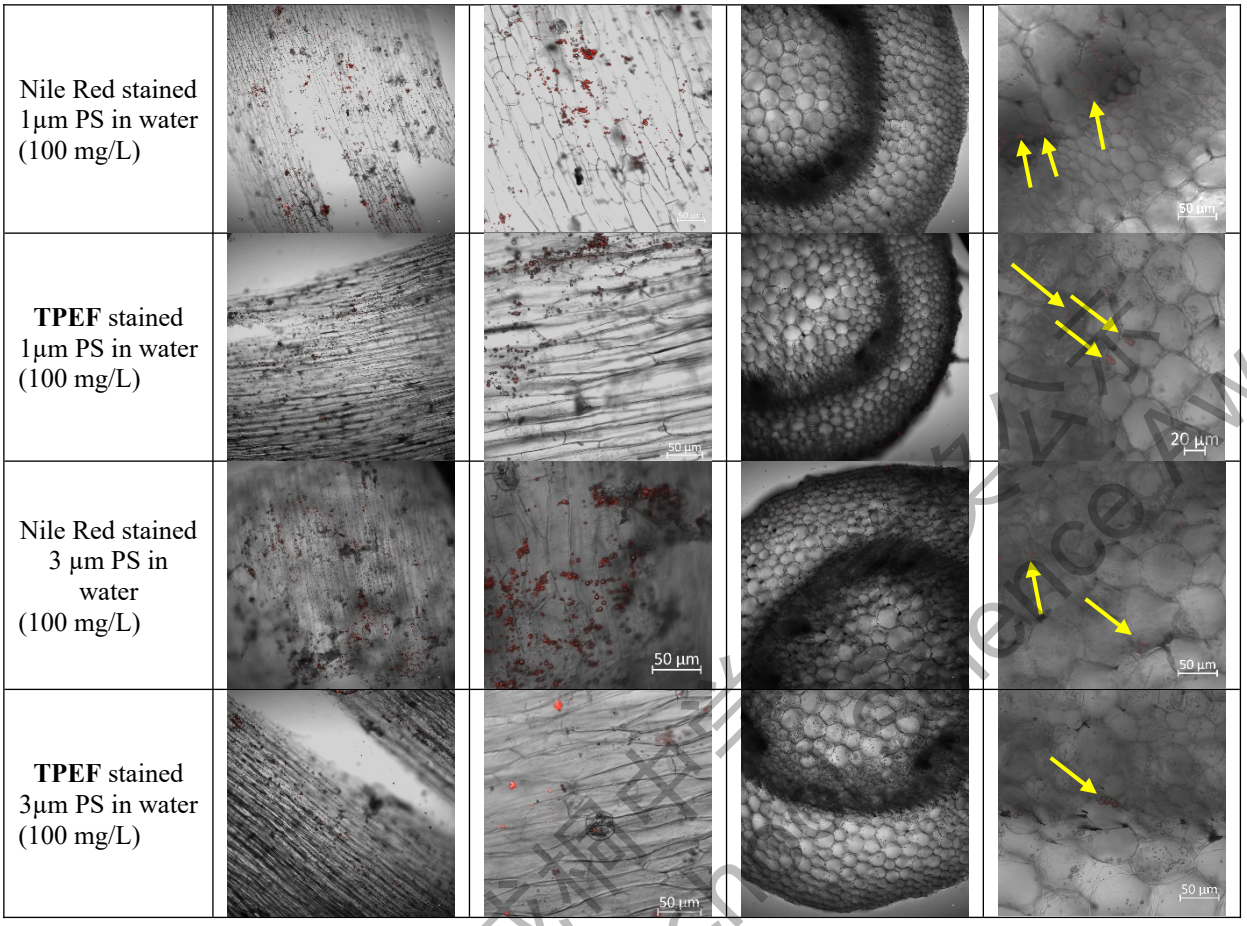
Since the Nile red-stained PS particles exhibited weaker fluorescence, the TEPF-stained microplastic particles were more helpful to observe the microplastic particles inside the hypocotyl than the Nile red-stained particles.

Table 6: Confocal microscopy views of cross section of hypocotyl of mung bean sprouts test samples.

Table 7 lists PS particles of different sizes (0.1 μm, 1 μm and 3 μm) stained with Nile Red and **TPEF**, respectively, adsorbed on hypocotyl epidermis and into hypocotyl on the fifth day of mung bean sprouts growth. It can be seen from the table that PS particles exist in both hypocotyl epidermis and hypocotyl. Considering that PS particles entering hypocotyl cannot be washed away, these particles are likely to enter the human body with bean sprouts as food. In addition, it can also be seen from the figure that **TPEF** staining works best for PS particles with a size of 0.1 μm.

Table 7: Confocal microscopy views of test samples on 5 days old mung bean sprouts.

Test samples / MPs	hypocotyl epidermis	Enlarged photo	cross section of hypocotyl	Enlarged photo
Nile Red stained 0.1μm PS in water (100 mg/L)				
TPEF stained 0.1μm PS in water (100 mg/L)				



For soybean sprouts, the experiment was similar to that of mung bean sprouts mentioned above. First, we cultured soybean sprouts in water containing Nile red and **TPEF**-stained PS particles (0.1 μm), respectively, and took samples on the 1st, 3rd, and 5th days of soybean sprouts growth, and observed hypocotyls by confocal microscopy to figure out the distribution of PS particles on the hypocotyl epidermis and in the hypocotyl.

In the test groups of soybean sprouts rinsed with the suspensions of 0.1μm PS particles in water, those particles were dyed with Nile red or **TPEF**, the confocal microscopy views of hypocotyl epidermis showed fluorescent particles accumulated on the hypocotyl epidermis (Table 8). In Table 9, the confocal microscopy views show cross sections of hypocotyl of the soybean sprouts. Similarly, from the enlarged photos, **TPEF** stained PS particles exhibit brighter light that from Nile Red stained PS particles. And PS particles were found that not only PS particles accumulated on the hypocotyl epidermis, but also, they entered in the hypocotyl. These particles stay in between cells. However, no particles are visible internal to the active bean sprouts cells.

Similar to that of mung bean sprouts, washing with water can not remove the PS nanoplastics from the soybean sprouts. And because the particle size of PS microplastic

particles of 0.1 µm is smaller, the particles entering the soy bean hypocotyl are more than those of PS microplastics with a particle size of 1 µm or 3 µm.

Table 8: Confocal microscopy views of hypocotyl epidermis of soybean sprouts test samples.

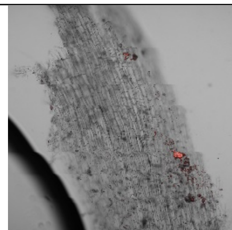
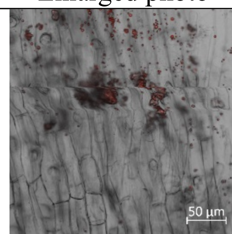
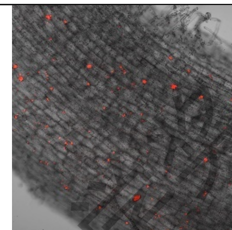
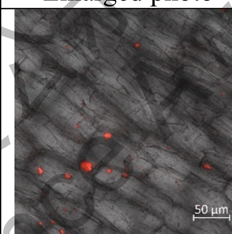
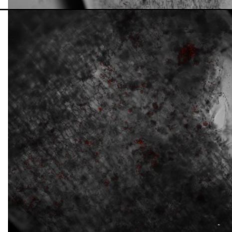
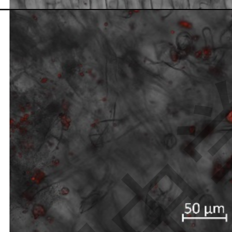
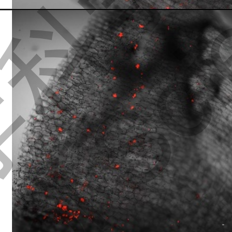
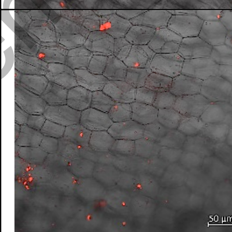
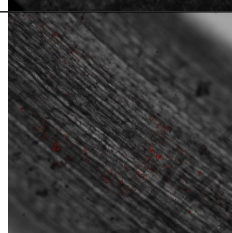
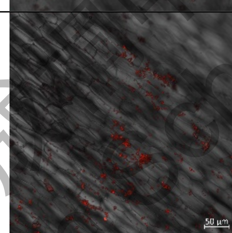
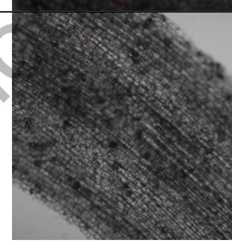
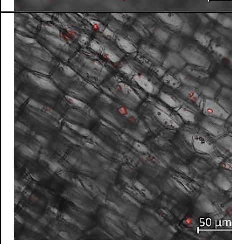
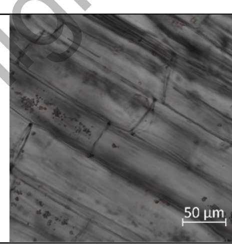
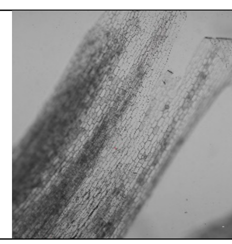
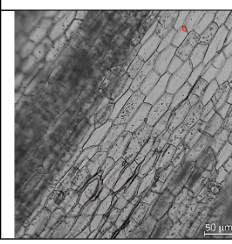
Particles / Time (days)	Nile Red stained 0.1µm PS in water (100 mg/L)		**TPEF** stained 0.1µm PS in water (100 mg/L)	
		Enlarged photo		Enlarged photo
1				
3				
5				
5 (washed with water)				

Table 9: Confocal microscopy views of cross section of hypocotyl of mung bean sprouts test samples.

Particles / Time (days)	Nile Red stained 0.1µm PS in water (100 mg/L)	**TPEF** stained 0.1µm PS in water (100 mg/L)

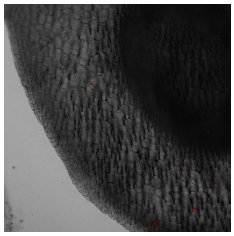
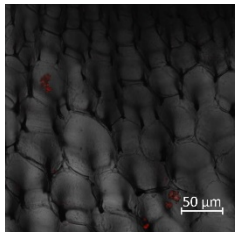
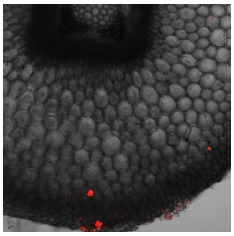
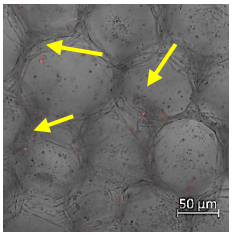
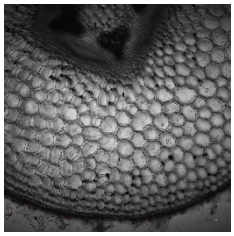
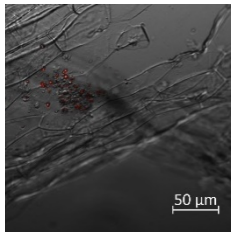
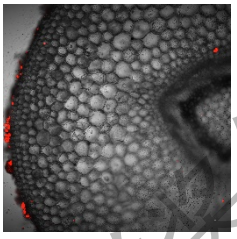
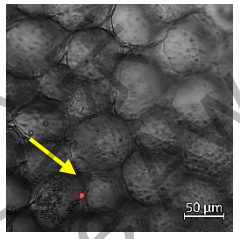
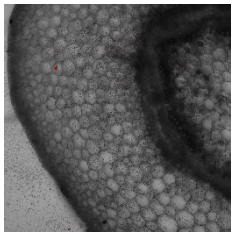
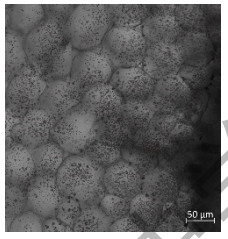
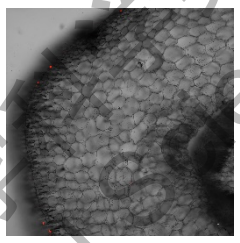
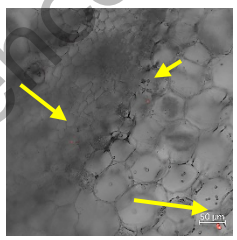
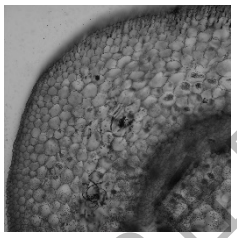
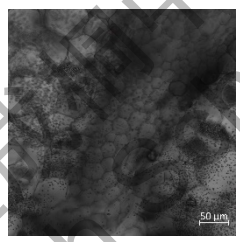
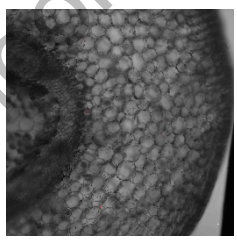
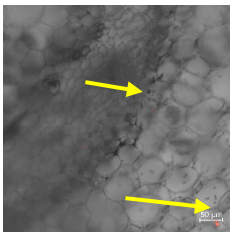
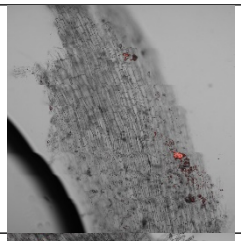
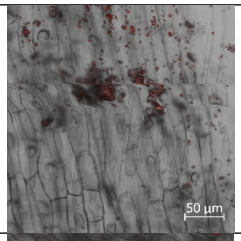
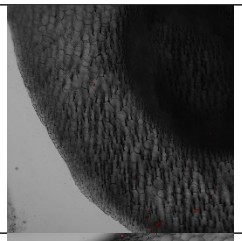
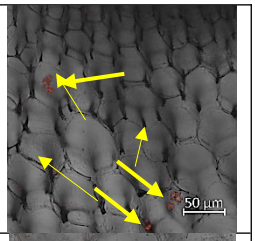
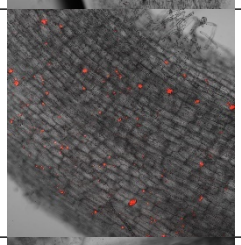
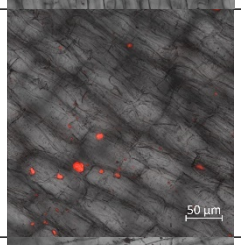
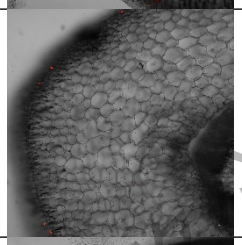
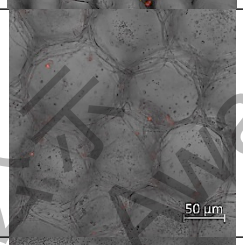
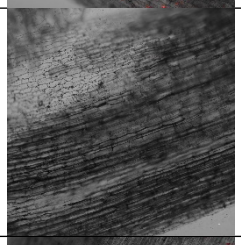
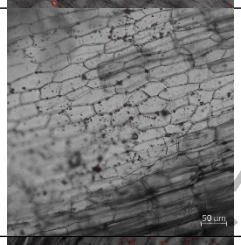
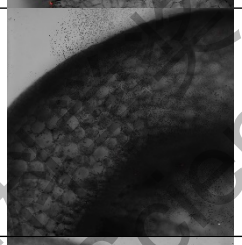
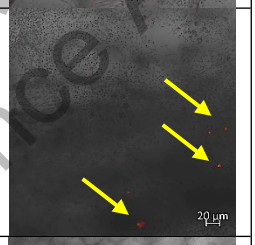
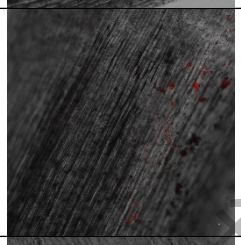
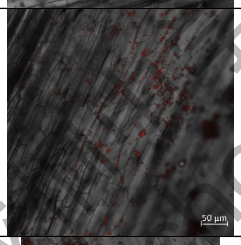
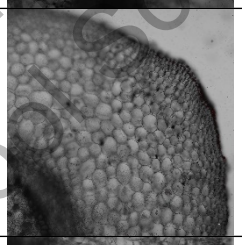
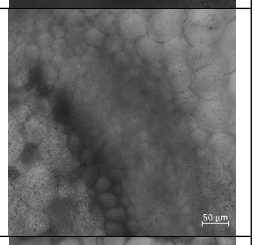
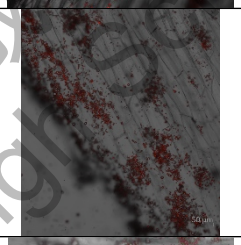
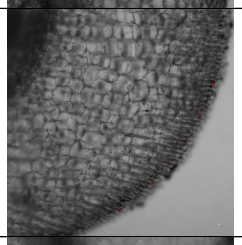
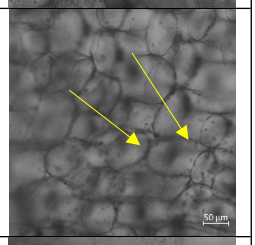
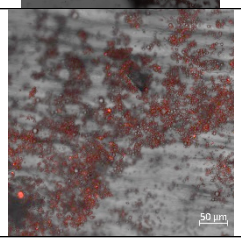
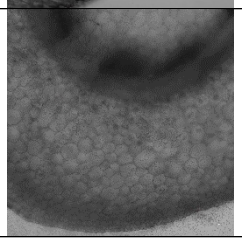
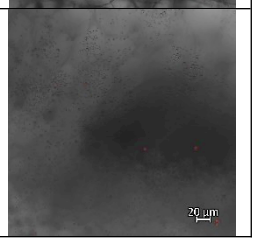
1				
3				
5				
5 (washed with water)				

Table 10 lists PS particles of different sizes (0.1 μm, 1 μm and 3 μm) stained with Nile Red and **TPEF**, respectively, adsorbed on hypocotyl epidermis and into hypocotyl on the fifth day of soybean sprouts growth. It can be seen from the table that PS particles exist in both hypocotyl epidermis and hypocotyl. And washing the soybean sprouts could not remove the PS particles in both hypocotyl epidermis and hypocotyl, which is a potential risk for humans.

The nanoplastic particles were more extensive in its contamination in the sprouts.

Table 10: Confocal microscopy views of control samples on 5 days old mung bean sprouts.

position / Particles	hypocotyl epidermis	cross section of hypocotyl

Nile Red stained 0.1µm PS in water (100 mg/L)				
TPEF stained 0.1µm PS in water (100 mg/L)				
Nile Red stained 1µm PS in water (100 mg/L)				
TPEF stained 1µm PS in water (100 mg/L)				
Nile Red stained 3 µm PS in water (100 mg/L)				
TPEF stained 3µm PS in water (100 mg/L)				

6. Conclusions

6.1. Key Findings

1. The research synthesized, characterized, and applied a new fluorophore-**TPEF**. This molecule requires mild synthesis conditions, is easy to synthesize and purify, and has desirable fluorescent characteristics.

2. During purification, it was accidentally discovered the molecule's outstanding affinity to plastic. After testing on several plastic particles, it was discovered that the molecule can be used in plastic imaging. In particular, using **TPEF** to fluoresce nanoplastic, filling an area that current conventional Fluorophore cannot achieve. This compound can be easily used to dye a variety of microplastic and nanoplastic particles, providing a new choice for studies that track microplastic and nanoplastic particles.

3. Bean sprouts is a staple in numerous east-Asian cultures. With the world's waters already contam-inated with microplastic, it is crucial to determine if microplastic and nanoplastic particles' presence in bean sprouts.

   After growing mung sprouts and soy sprouts in water contaminated in various dyed microplastic and nanoplastic particles, we studied their biological behavior. We found that microplastic and nanoplastic particles were able to strongly bind on the hypocotyl epidermis. With enough time, it was found that microplastic andnanoplastic particles were able to penetrate the epidermis and enter the sprout. This means microplastic and nanoplastic particles almost certainly can enter humans via bean sprouts, leading to potential health hazards, reminding us the importance of plastic waste management, as well as further, more extensive research regarding microplastics and nanoplastics.

6.2. Prospects

Based on current results, combined with the development of the field, prospects are as follows:

1. Expand the types of microplastic particles used. This includes both the size and chemical composition of microplastics.
2. Study their biological behavior in more hydroponic plants, and expand the research to the fluorescence of the interaction between plastic particles and in-virto cells imaging research.
3. Expand the research to human cells, such as HeLa cells.

7. References

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