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# Alternative Microplastic Identification Methods using Optical Components to Modify Standard Compound Microscopes for Fluorescence Microscopy

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# Alternative Microplastic Identification Methods using Optical Components to Modify Standard Compound Microscopes for Fluorescence Microscopy

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**Abstract**

This study aimed to use optical components to modify and convert a compound microscope into a functional fluorescence microscope as an alternative identification procedure for microplastics in water samples. The results of this study showed that Nile Red dye was most effective in emitting fluorescence in colorless or clear plastics. Plastics that were colored showed weaker fluorescence. Rit Fabric Dye was also analyzed for fluorescence while bound to microplastics, but no fluorescence was found with this method.

**Introduction**

The presence of plastics in the environment is old news, yet there is a new rendition of this same story that has people concerned. This is the problem of microplastics. Microplastics can be defined as materials made of synthetic polymers that are less than five millimeters in length, meaning any plastic material smaller than a sesame seed. Macroplastics can choke or entangle animals, while microplastics enter the environment through filter feeders. When microplastics end up in primary producers they eventually end up in every animal in the food web, including humans. There are primary microplastics that include plastic directly deposited in the environment like plastic beads. There are also secondary microplastics which result from the degradation of macroplastics. A study conducted by The International Union for Conservation of Nature (Boucher and Friot 2017) identified seven major sources of microplastics. Microplastics come from seemingly surprising and everyday sources. Close to 35% of microplastics come from synthetic fibers that are freed from the normal process of washing clothes. Plastics from tires compose up to 28% of plastics and city dust makes up 24% which includes the soles of shoes or

the normal wear and tear of plastic utensils. The main concern with finding all this plastic in the environment is that as it travels through the food chain, it will end up in our own bodies. A study published in Environment International found the presence of microplastics in human blood (Heather et. Al 2022). Medicine does not yet know the health effects of plastic in the body, and as plastic production continues to grow, the concern grows as well.

Microplastics can be identified using traditional stereo and compound microscopes but it can be quite difficult as the plastics get smaller and are different shapes. In order to get people alerted to the problem of microplastics, it would be beneficial to put them into the curriculum of a typical General Chemistry class. One way to determine whether microplastics are present in a sample is with fluorescence microscopy. Using fluorescence microscopes and fluorescent dye makes it quite easy to identify microplastics in a sample. The downside of this solution is that fluorescence microscopes are very expensive. Buying a set of thirty microscopes for labs to use would not be feasible financially. Recently a paper was published that investigated using optical components to modify a compound microscope for polarization and fluorescence detection (Labbe et. Al. 2020). According to this paper, after modification, students were able to distinguish microplastics from other substances and detritus much easier than with the traditional compound microscopes. This is a unique and cost-effective solution that may allow entry level students to investigate plastics in the environment. Another study investigated the use of common fabric dye as a fluorescent dye as an alternative to much more expensive dyes like Nile Red (Karakolis et al. 2019).

The main objective of this study was to use optical components to convert a standard compound microscope into a working fluorescence microscope to identify microplastics in water samples. Two types of dye were investigated for fluorescence, Nile Red and Rit Fabric Dye.

## Materials and Methods

The procedure for dying and finding fluorescence in this experiment closely followed the procedure outlined by Labbe et al for alternative methods for microplastic identification.

### *Preparation of Nile Red Stock Solution*

Nile Red dye was purchased through the university and used for this procedure. A stock solution of 1 mg/mL was made using 0.005 g of Nile Red and 5 mL of methanol. This stock solution was stored in a glass container and covered with parafilm.

### *Creation of Microplastic Controls*

Several samples were collected to test for fluorescence after being dyed with Nile Red. Samples of lint were collected from the researchers home dryer to investigate microplastics resulting from normal cleaning processes. Used cigarette butts were collected from a local parking lot for analysis of microplastics in the filters of cigarettes. Fibers from a chair and carpet were extracted using metals forceps and scissors. Several plastics were investigated including a clear microscope cover bag, a common grocery bag, and clear stiff plastic from the package of a toothbrush. Distilled water was used to collect samples from the bottom of a pair of shoes and the tire of a car by pouring the water over the object and catching the runoff in a separate container.

Fibrous samples were carefully separated into smaller portions using metal forceps and scissors and placed aside in a small glass beaker. Samples of soft plastic from bags as well as stiff

plastics were cut or torn into appropriate microplastic sizes ranging from the size of a sesame seed or smaller using metal forceps and scissors and placed in glass containers. Samples collected using distilled water were stored in glass bottles and set aside.

Biologically active water samples were also collected to analyze the affects of Nile Red on naturally found organisms like zooplankton. Water was collected in glass bottles from the boat launch in Lake Winona Park and Gilmore Creek in late September. These samples were labeled and stored in the lab refrigerator.

***Sample Preparation using Nile Red Stock Solution***

Following the procedure set out by Labbe et al., for every 6 mL of sample 1 drop of the Nile Red Stock Solution was added. For solid samples not already in water pieces of samples were placed in a small glass container and 6 mL of distilled water was added along with the single drop of Nile Red Solution. Samples were allowed to develop for at least 45 minutes after addition of Nile Red Stock.

The procedure for dying and finding fluorescence in this experiment closely followed the procedure outlined by Karakolis et al. for alternative methods for microplastic identification.

***Preparation of Rit Liquid Dye Stock Solution***

Rit Dye in the color *Super Pink* was purchased from a local store and used for this procedure. The stock solution was prepared of 50% dye and 50% distilled water was made using 40 mL of Rit dye and 40 mL of distilled water. This solution was placed in a glass contained with a glass stopper.

### *Sample Preparation using Rit Dye Stock Solution*

Following the procedure laid out by Karakolis et al., for every 25 mg of sample, 10 mL of Rit Stock Solution was added. These samples were made in small glass vials which were placed in the lab oven set to 70˚C for 2 hours. The lab oven was used to keep the samples in darkness during the duration of the heating. After heating, the microplastic samples were removed from Rit Dye Solution using a filter and rinsed three times with distilled water. The samples were then stored in distilled water in glass containers.

### *Modification Procedure of Traditional Compound Microscope*

The ocular head of the microscope was carefully removed and a precut piece of yellow fluorescence filter was placed over opening where the light shines through. The ocular head was then returned to its original place. Following the procedure described by Labbe et al., a spacer was made from the lid of a yogurt container. A hole was cut in the center to allow light through for Brightfield analysis and the whole lid was painted with black acrylic paint to reduce the amount of reflection. The excitation source for this procedure was a 5 mW 455nm blue laser pointer pen. The laser was secured to a ring stand using clamps and positioned at the highest angle possible without the beam being blocked by the barrel of the objective lens.

### *Procedure to Locate Fluorescence*

Using a glass pipette, 2 or 3 drops of sample was placed on a glass microscope slide. If control plastics were involved, these were also placed on the microscope slide. A glass cover slip was placed on top of each sample as well. Samples were first viewed with normal Brightfield capabilities to identify any microorganisms and suspect microplastics. Standard “lawnmower” technique was used to scan entire sample. To search for fluorescence the optical components of a

yellow emission filter and laser pointer excitation source were put in place. During analysis of fluorescence all microscope lights were off as well as all overhead lights if possible. The same "lawnmower" technique was used to analyze samples thoroughly. Adjustment of laser pen was occasionally necessary to find fluorescence.

**Results and Discussion**

The aim of this study was to develop a procedure to analyze microplastics with more cost effective techniques and tools than traditional fluorescence microscopy. Nile Red was much more effective in emitting fluorescence than the Rit Dye. Using Nile Red as a dye, fluorescence was found in nearly all of the control samples collected. Fluorescence was determined to be a range from greenish-yellow, orange, and bright red, not to be mistaken with reflectance of the blue light from the laser pen. The difference between reflected light and fluorescence can be plainly seen in Figure 9b. which shows the analysis of sweepings from laboratory floor. One single fiber fluoresced bright red while the other fibers in the sample were simply reflecting blue light (Figure 9b.). Originally the beam of the laser pen was focused directly on the sample and field of view. Adjustment of the beam position revealed focusing the beam directly on the sample washed out any possible fluorescence. Having the beam focused on the corner of the cover slip allowed enough excitation, but did not overpower the fluorescence. The procedure from Labbe et al. called for a spacer to be placed on the objective stage to raise the sample and lower background reflectance. A spacer was made for this procedure, but it was discovered that the procedure would work well with or without the spacer. The reason for this difference may originate from the use of petri dishes in the original study compared to the microscope slides that were used in this study (Labbe et al, 2020).

Using Nile Red as a fluorescent dye, clear plastic bags and cigarette filter fibers showed the strongest fluorescence (Figure 2, 3, 4). This may have been because these plastic fibers were mostly colorless, allowing the Nile Red fluorescence to be seen clearly once excited with the laser pen. A white grocery bag was also tested using Nile Red but did not show fluorescence, which may be related to the fact that this plastic was not clear, but dyed white. This also seemed to be the case with black fibers from a white board eraser that were analyzed with Nile Red. Weak fluorescence was found in clothes lint, carpet fibers, and fibers from the cloth covering of a chair. These results show that plastics that are used in cloth products can be identified using this procedure.

The Rit Dye procedure using hue *Super Pink* was not successful in producing fluorescence under the laser pen. After the dying process in Rit Dye, the cigarette filter fibers were noticeably deep pink. This is different from the cigarette fibers that were dyed using Nile Red which were a very pale pink if they took on any color at all. The cigarette filter fibers dyed with Rit did not show fluorescence (Figure 5). The hard clear plastic sample from the package of a toothbrush was also tested and did not show any fluorescence. Similar to the cigarette filter fibers, the clear plastic was noticeably deep red after the Rit dyeing process (Figure 8). The same hard clear plastic was dyed with Nile Red and showed weak fluorescence. The difference between the effectiveness of Nile Red and Rit Dye as a fluorescent dye can be plainly seen in Figures 4 and 5, as well as Figures 7 and 8. This difference could be due to the Rit Dye coating the plastics in a way that prevents fluorescence from being excited.

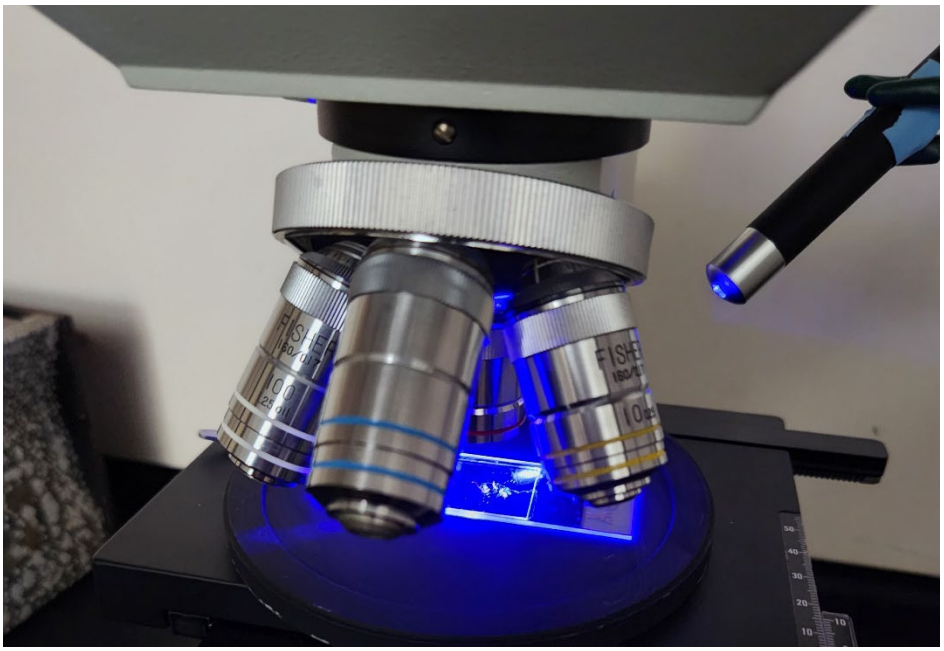

**Figure 1:** Use of a blue laser pointer to excite fluorescence in a sample.

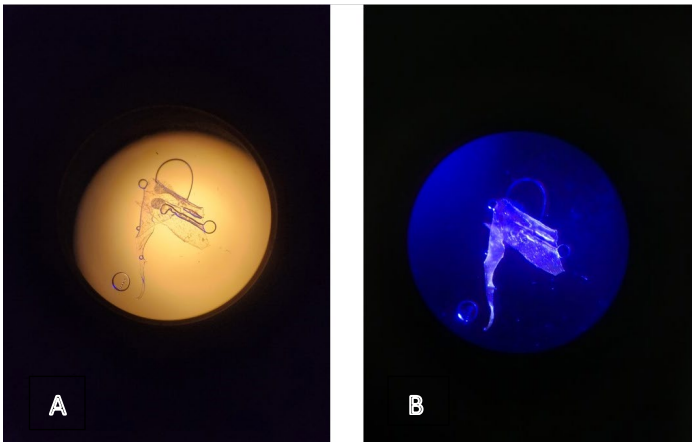


**Figure 2: Nile Red Application:** (a) A piece of clear microscope cover bag as seen through normal Bright Field application. (b) The same piece of bag as seen with fluorescence microscopy.

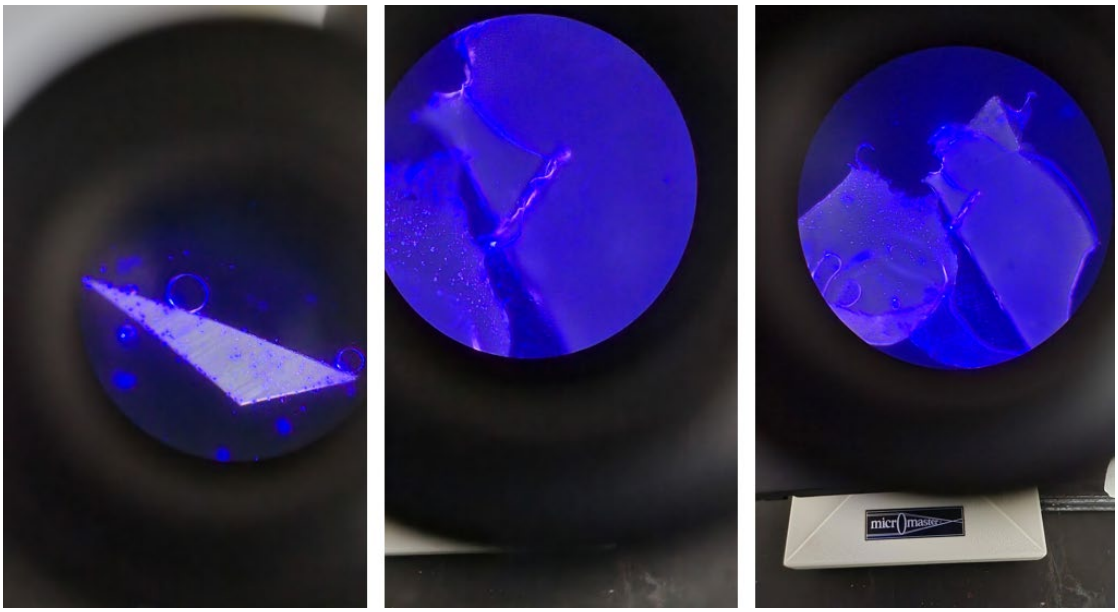

**Figure 3: Nile Red Application:** These are all photos of clear microscope cover bag pieces as seen with fluorescence microscopy.

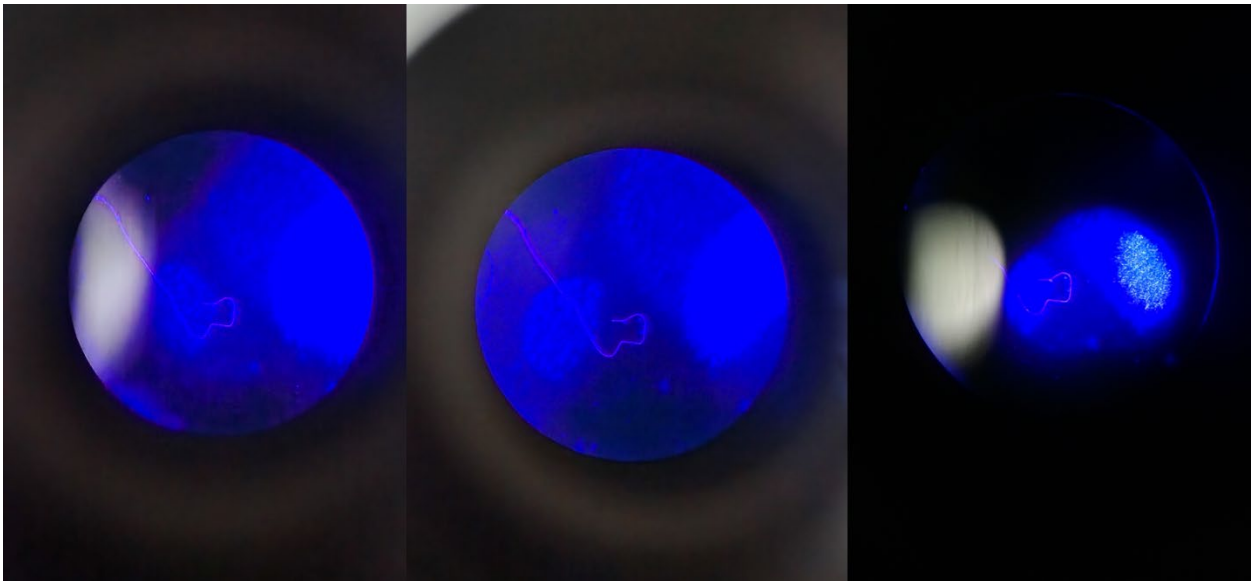

**Figure 4: Nile Red Application:** Photos show fluorescence emitting weakly from a cigarette filter fiber.

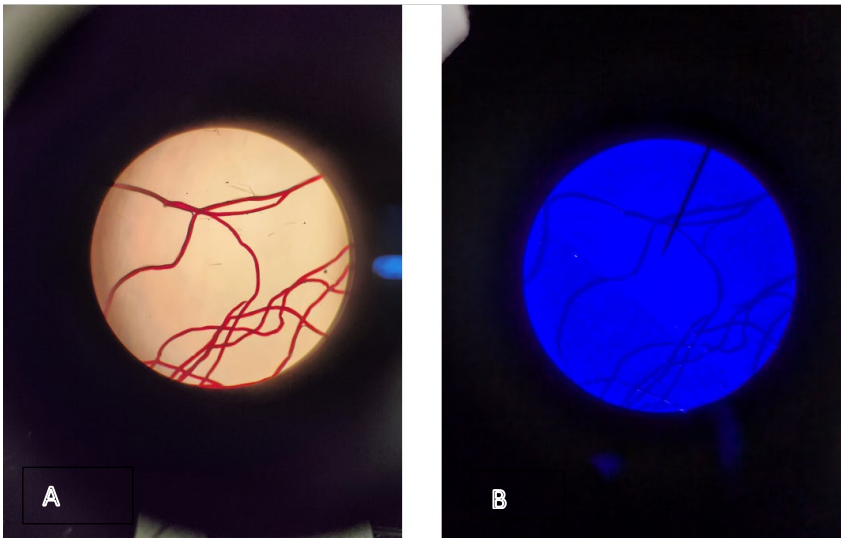


**Figure 5: Rit Dye Application:** (a) Cigarette filter fibers as seen through normal Bright Field application. (b) The same cigarette fibers as seen with optical modifications for fluorescence detection. No fluorescence was found in cigarette fibers after dying with Rit Dye.

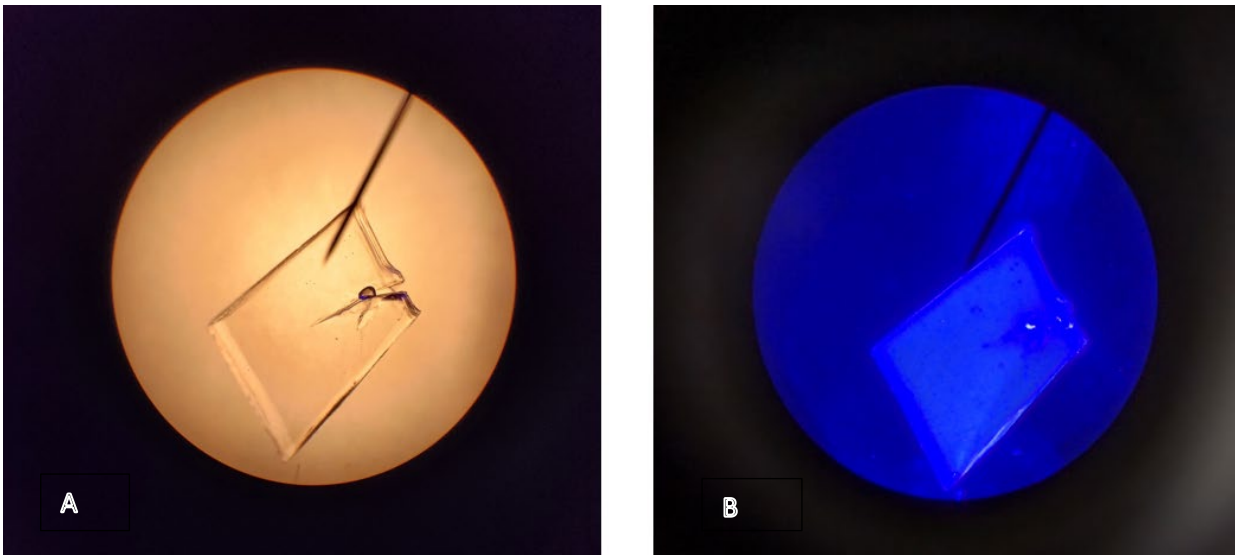


**Figure 7: Nile Red Application:** (a) A piece of clear hard plastic collected from the packaging of a toothbrush as seen through normal Bright Field application. (b) The same piece of plastic as seen with optical modifications for fluorescence detection. This shows weak fluorescence.

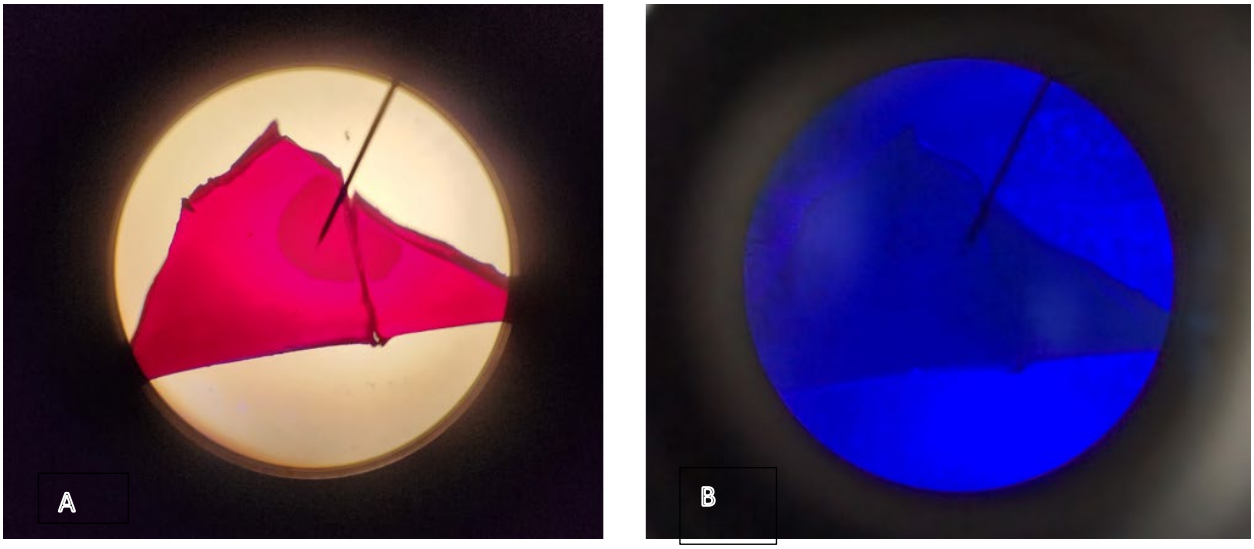


**Figure 8: Rit Dye Application:** (a) A piece of clear hard plastic collected from the packaging of a toothbrush as seen through normal Bright Field application. (b) The same piece of plastic as

seen with optical modifications for fluorescence detection. No fluorescence was observed with this particular plastic after dying with Rit Dye.

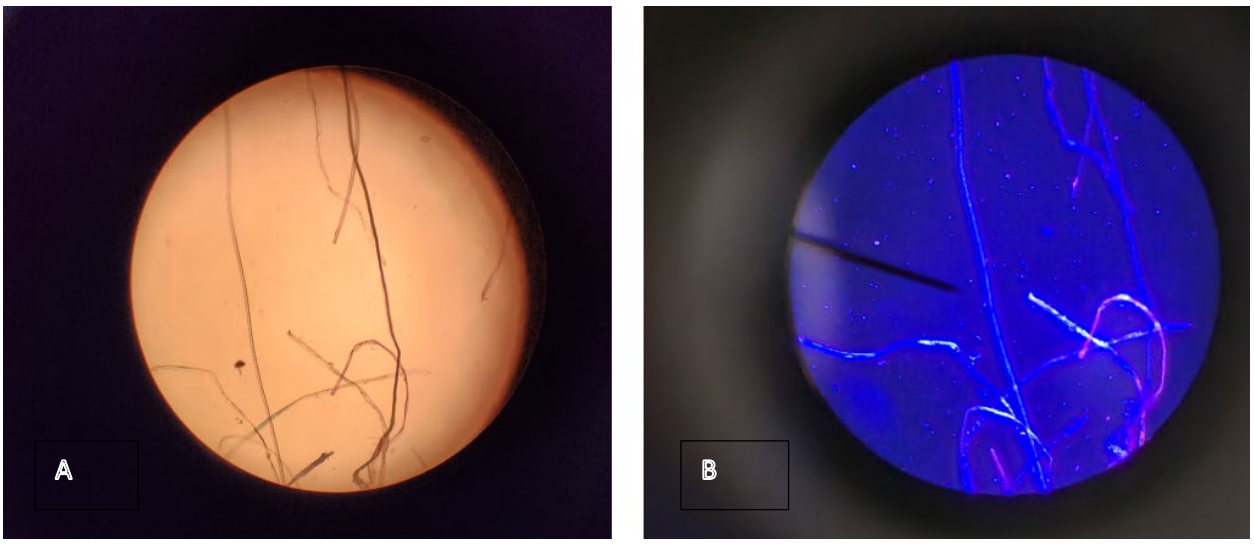


Figure 9: Nile Red Application: (a) Sweepings from lab room floor as seen through normal Bright Field application. (b) Same sweepings as seen with optical modifications for fluorescence detection.

**Conclusion**

The main objective of this study was to use optical components to convert a traditional compound microscope into a functional fluorescence microscope using cost-effective tools and techniques. Two types of dye were tested for fluorescence. Nile Red dye performed very well in adhering to plastics and emitting fluorescence when an excitation source was applied. Rit Fabric dye in the shade *Super Pink* was also analyzed but did not show any fluorescence when bound to plastics. The results of this study show that colorless plastics took on the Nile Red the best and emitted the strongest fluorescence. Fibers that were previously dyed, showed weaker

fluorescence overall. The procedure adapted from this study can be used for entry level chemistry classes to investigate microplastics in water samples.

**References**

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Discovery and quantification of plastic particle pollution in human blood

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Inexpensive Adaptations of Basic Microscopes for the Identification of Microplastic Contamination Using Polarization and Nile Red Fluorescence Detection

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