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Visualizing Fluorescence: Using a Homemade Fluorescence "Microscope" to View Latent Fingerprints on Paper

Christopher N. LaFratta, **Sun Phill Huh**, **Allistair C. Mallillin**, **Peter J. Riviello**, and **David R. Walt***
Department of Chemistry, Tufts University, Medford, Massachusetts 02155

Abstract

We describe an inexpensive handheld fluorescence imager (low-magnification microscope), constructed from poly(vinyl chloride) pipe and other inexpensive components for use as a teaching tool to understand the principles of fluorescence detection. Optical filters are used to select the excitation and emission wavelengths and can be easily interchanged to accommodate different fluorescent samples. As a demonstration, we used the fluorescence imager to view lawsone-dyed fingerprints on paper, which fluoresce red when illuminated with green light. This emission can be seen by viewing the sample through the instrument by eye, or the fluorescence can be captured by a camera. The entire imager can be built for less than $300.

Keywords

Second-Year Undergraduate; Hands-On Learning/Manipulatives; Fluorescence Spectroscopy; Instrumental Methods; Upper-Division Undergraduate; Analytical Chemistry; Laboratory Instruction; Forensic Chemistry; Laboratory Equipment/Apparatus

Every student has seen something fluoresce, but few students appreciate how important the process of fluorescence is in modern chemical analysis. Many biochemical analyses (1), such as imaging an electrophoretic gel or DNA microarray, use fluorescence. Also, fluorescence microscopy is one of the most important forms of analytical microscopy used by chemists, material scientists, and biologists. From an educational perspective, fluorescence is important because it relates the concepts of energy levels, electronic transitions, and the interaction of light with matter. These ideas are central to the quantum mechanical view of the atom and explain a range of phenomena from the shape of absorption spectra to how lasers operate (2).

Fluorescence is typically introduced to laboratory students in conjunction with a spectrofluorimeter. This first experience can be overwhelming because of the confusion that accompanies concepts such as excitation spectra, emission spectra, and Stoke's shift. This confusion is made even worse because the student cannot see the sample during the data-collection process and probably has no idea what the sample looks like when it is excited at a particular wavelength. We encountered similar confusion when training undergraduate researchers to use a fluorescence microscope. Most students did not understand how the microscope was capturing the fluorescent images. We decided to make a simple fluorescence microscope to teach the students how it works. This approach effectively teaches the

*david.walt@tufts.edu.

Supporting Information Available

A student handout; instructor notes including a parts list with costs, detailed assembly instructions, as well as tips concerning taking pictures. This material is available via the Internet at http://pubs.acs.org.

fundamental concepts of fluorescence because of the simplicity of the instrument's design compared to a spectrofluorimeter and because the images are more understandable than spectra for introductory students.

In this article, we describe a simple fluorescence imager (a low-magnification microscope) built from poly(vinyl chloride) (PVC) pipe that uses a light-emitting diode (LED), a pair of lenses, filters, and a digital camera. The imager produces fluorescence images upon illumination of a fluorescent sample. To demonstrate the utility of the device, we observed lawsone-dyed (2-hydroxy-1,4-naphthoquinone) fluorescent fingerprints on paper. A fingerprint is a convenient sample to observe and offers a connection to forensic science, a field with great appeal to students. Other reports of low-cost fluorescence devices are in the literature (3–8), including a spectrofluorimeter made from a shoebox (8). This article shares the same underlying philosophy as these other articles: when students assemble or dissemble the components, the mystery of what is inside a fluorescence microscope disappears, and they can see and understand the small number of functional parts. Unlike these previous articles, our instrument produces images rather than graphs, providing a clear illustration of fluorescence in action.

Experimental

Constructing the Fluorescence Imager

A schematic and a cross-sectional view of the fluorescent imager are shown in Figure 1. Three pieces of 1 in. i.d. PVC pipe were cut to 30, 40, and 20 mm in length for the excitation, sample, and emission sections, respectively. The LED was connected to a 9 V battery with a current-limiting resistor and a pushbutton switch in series. The LED was placed in a hole drilled in the end cap and secured using tape. The 25 mm focal-length (FL) lens and excitation filter were wrapped together in polytetrafluoroethylene (PTFE) tape and inserted at the end of the 30 mm pipe. PTFE tape was used to tighten the fit of the optics in PVC. The 30 mm pipe was inserted into the end cap with the lens near the LED and the depth of insertion was adjusted until the LED emission was collimated. The PVC T-junction was cut diagonally such that one of the three arms of the T was detached. The dichroic mirror was taped between these two pieces of the T-junction as shown in Figure 1. The excitation arm was inserted into the central opening of the T-junction. The 50 mm FL lens was placed in the 40 mm length piece of pipe and this pipe was fit into the T-junction. The emission filter was inserted into the 20 mm section of pipe and placed in the detection arm of the T-junction. The optics used account for ~95% of the cost, which, not including the digital camera, were less than $300.

The transmission spectra of the filters are shown in Figure 2 along with the LED emission spectrum. Two of the filters were found on the clearance section at Chroma Technology Corp (9) for ~20% of their retail cost. Alternative filters can be substituted; however, the emission and excitation filters should have an optical density greater than or equal to 3 outside their band pass to thoroughly block the excitation light from the detector.

Using the Fluorescence Imager

The device can be held by hand, placed up to a fluorescent sample, and simply viewed by eye. Alternatively, a digital camera can be used to capture the image. When using a camera, it is easiest to use a ring stand with a clamp to secure the imager and camera to avoid blurred pictures due to camera movement. Using a setup similar to the one shown in Figure 3, sharp pictures can be obtained for exposure times of several seconds and it is possible to capture fluorescence that is invisible by eye.

Developing Latent Fingerprints on Paper

Fingerprints were made on copy-machine paper by touching a piece of paper with the pad of the finger. The fingers were not treated in any way before touching the paper, other than that hands were washed about 30 min before. The fingerprints were stained using lawsone, which reacts with amino acids left behind on the paper. To prepare the lawsone solution, 50 mg of lawsone, 2-hydroxy-1,4-naphthoquinone, was dissolved in 10 mL of ethyl acetate and then 40 mL of petroleum ether was added as a co-solvent. The paper containing the print was then dipped in the solution of lawsone and allowed to air dry for 1 min. After 1 min, the paper was placed on a hot plate at 150 °C for 45 min to develop. Following the heat treatment, the portion of the paper exposed to the lawsone solution was light brown and the fingerprint was usually either invisible or very faint brown. The fingerprint could be fluorescently imaged as shown in Figure 3 with a camera or by eye.

Hazards

Lawsone, 2-hydroxy-1,4-naphthoquinone, is a skin, eye, and respiratory irritant and is incompatible with strong oxidizing agents. The lawsone solution uses extremely volatile and flammable solvents. Keep the hot plate away from the solvents and avoid electrostatic discharges that could ignite the vapors. The lawsone powder and solution will stain the skin so always use gloves. Ethyl acetate and petroleum ether are flammable liquids and vapors; they are harmful if swallowed or inhaled and cause irritation to skin, eyes, and respiratory tract. The excitation LED is quite bright and students should be instructed not to look directly at it while it is on.

Results

A fluorescence microscope performs two tasks: (i) it illuminates a sample at a particular excitation wavelength and (ii) it forms an image of the sample at a defined emission wavelength. The fluorescence-imaging device shown in Figure 1 is composed of three arms: an excitation arm, a sample arm, and a detection arm. We used a green LED as the excitation source. A 25 mm FL lens collimates the LED light and a green excitation filter selects a narrow wavelength band of the LED output. After the green light is collimated and filtered, it is reflected off a dichroic mirror and sent through a 50 mm FL lens onto the sample. At this point, the 50 mm FL lens focuses the light slightly to increase the intensity at the sample. The fluorescence emitted from the sample is then collected by the 50 mm FL lens and sent through the dichroic mirror. The emitted light is then filtered by an emission filter to remove any excitation light, and the red fluorescence is viewed by eye or with a camera. The 50 mm FL lens, in addition to focusing the excitation light to increase intensity, also acts as a magnifying lens to enlarge the image of the sample approximately twofold.

A typical fluorescent fingerprint as viewed through the fluorescence imager is shown in Figure 4. The lawsone reacts with primary amines from amino acids residues in the proteins left on the paper by the finger (10). The hypothesized reaction mechanism is given in detail by Almog et al. (10). Briefly, the intermediate formed by the reaction of lawsone with a primary amine reacts with another molecule of lawsone to produce a molecule with extended conjugation that fluoresces. The reaction is simple to perform, requiring only inexpensive reagents and a hot plate. Fluorescent fingerprints were visible on paper in 80% (20/25) of the trials. Failed trials are believed to be caused by a lack of residue on the finger due to touching objects before the fingerprint sample was collected. Since the paper also fluoresces slightly, fluorescent images of fingerprints can be made clearer by subtracting a background fluorescence image of the paper without a fingerprint. We performed the background subtraction using the free image processing software ImageJ (11). The developed fingerprints could be viewed repeatedly with

little photobleaching and remained fluorescent for several months when stored under ambient light.

Discussion

This fluorescence imager can be implemented in an introductory curriculum to achieve several learning objectives: (i) The usefulness of fluorescence as a technique to make invisible objects visible can be demonstrated. The simple fingerprint visualization is an approachable, hands-on example of how sophisticated fluorescence microscopes are able to produce data-rich images of samples such as fluorescently stained cells and gene expression microarrays. (ii) The green excitation and red emission illustrates the mechanism of fluorescence by the absorption of short wavelength light followed by emission at a longer wavelength. (iii) The use of visible excitation demonstrates that fluorescence is not isolated to the special case of UV excitation. (iv) Visible excitation also shows that emitted fluorescence is much less intense than the excitation light, which is not obvious with invisible UV excitation. The ratio of excitation/emission intensity is clear in this experiment as the green light overwhelms the resulting red fluorescence if the emission filter is removed.

These aspects about fluorescence are taught using an instrument whose components are simple, inexpensive, and versatile. Students can readily assemble and disassemble the imager to aid their understanding. The fluorescence imager is amenable both to a classroom demonstration where it could be passed around the room or to a laboratory where students could build and use their own devices. Although fingerprints are convenient and can be connected to topics in forensics, other samples could be viewed instead. For example, the imager could be useful to view bands obtained from thin-layer chromatography if a component of the mixture was fluorescent. Finally, the wavelengths for excitation and emission are readily tunable using a different set of filters or LED, which can easily be exchanged within the device.

Conclusion

In this article we have demonstrated the fabrication of a simple and inexpensive fluorescence-imaging device. Using readily available materials, including PVC pipe and a digital camera, combined with simple optical filters and lenses, we were able to capture fluorescent images of latent fingerprints on paper. Given the importance of fluorescence in modern chemical and biochemical analysis and the confusion that often accompanies a student's first experience with a spectrofluorimeter, we believe this device will clarify the concepts of fluorescence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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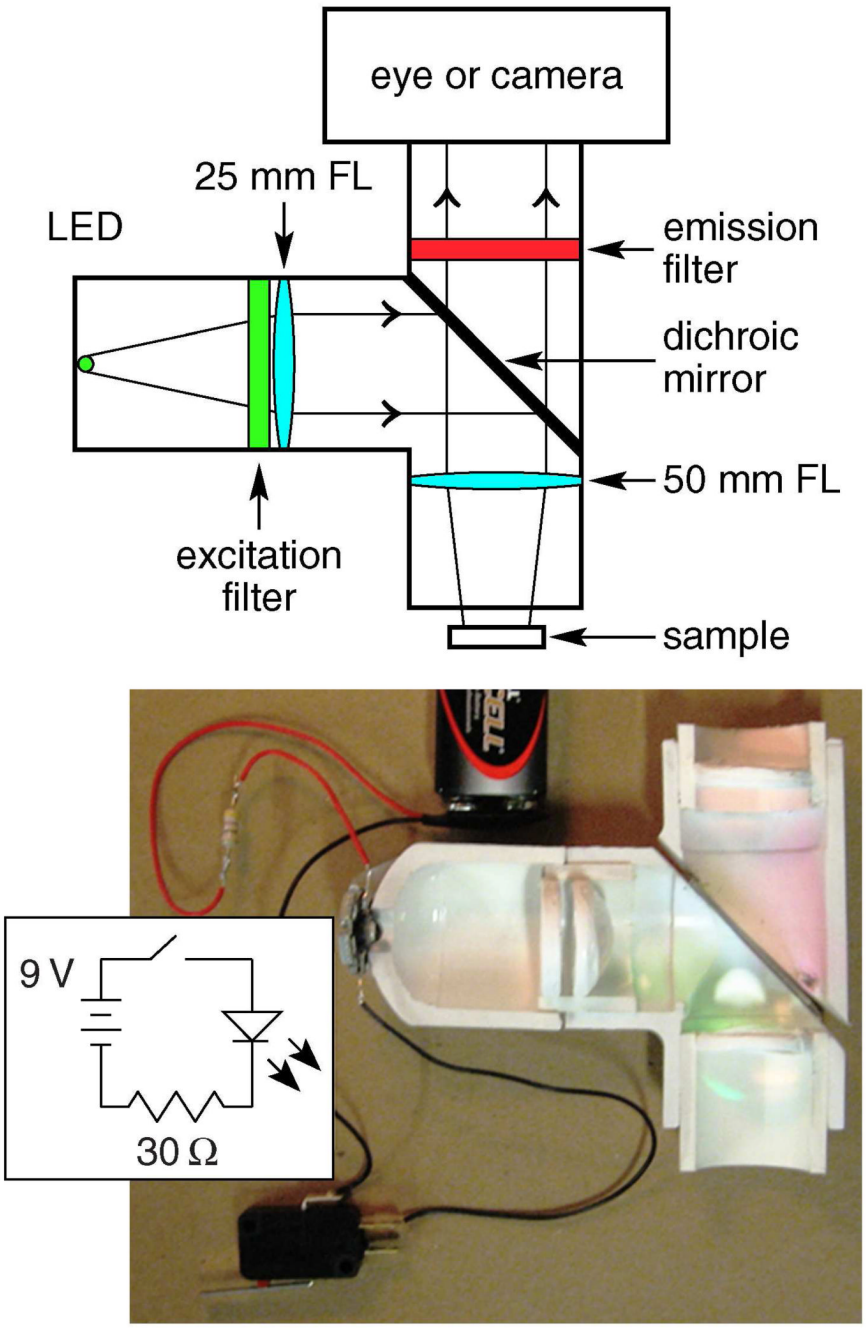

Figure 1.

(Top) Schematic of the fluorescence imager. The green LED excitation source is collimated and filtered before reflecting off the dichroic mirror toward the sample. The excitation is focused to ~7 mm wide spot at the sample by the 50 mm FL lens. The fluorescent emission is collected by the same 50 mm FL lens. After going through the emission filter, the emission is imaged by eye or with a camera. (Bottom) Picture showing a cross-sectional cut-away view of the fluorescence imager. The filters and lenses are wrapped in PTFE tape to make them fit tightly. Note the green reflection and red transmission of room light through the dichroic mirror. The inset shows the circuit diagram to power the LED.

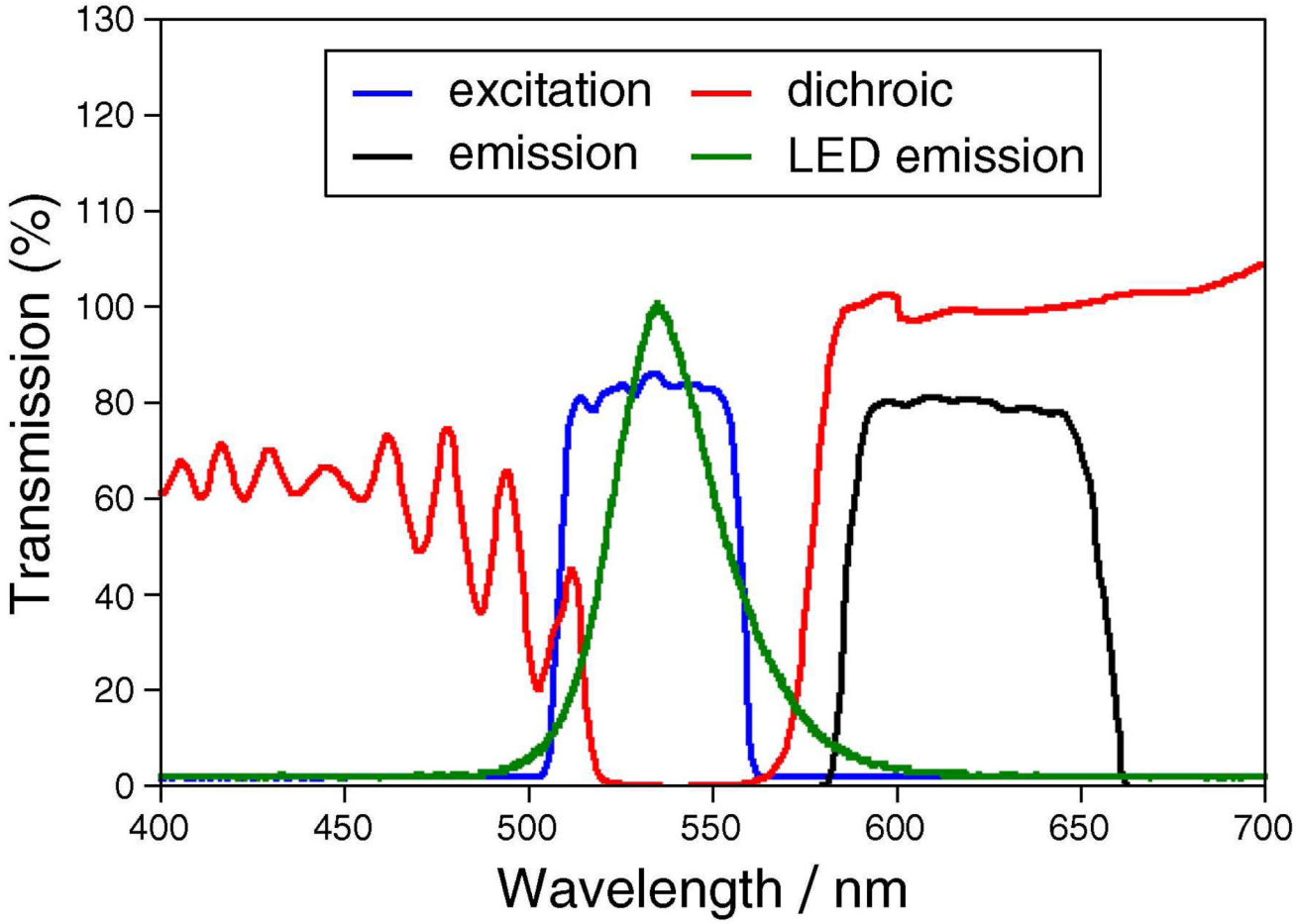

Figure 2.
Transmission spectra of the filters and dichroic mirror along with the emission curve of the LED.

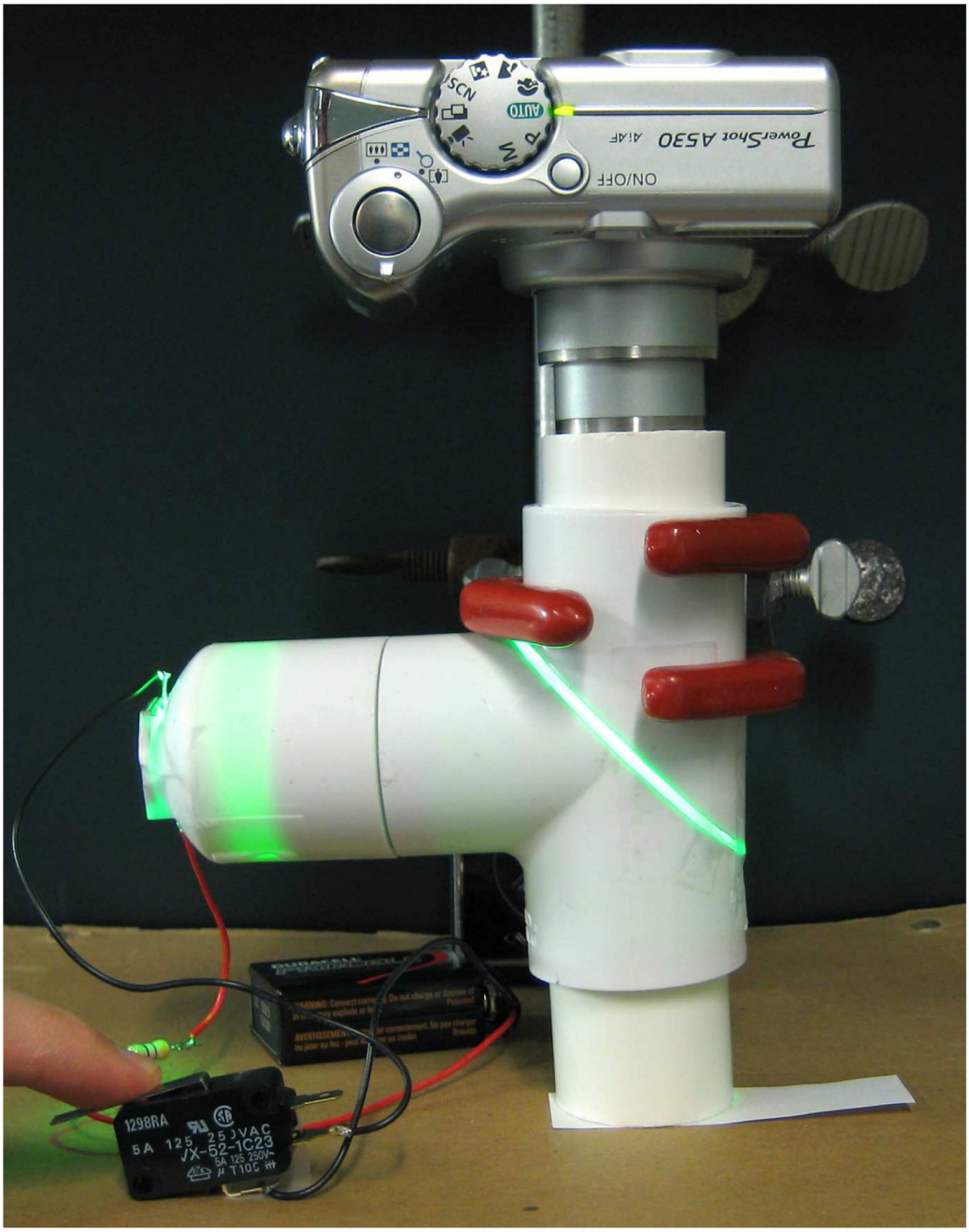

Figure 3.
Arrangement for fluorescence imaging of paper samples.

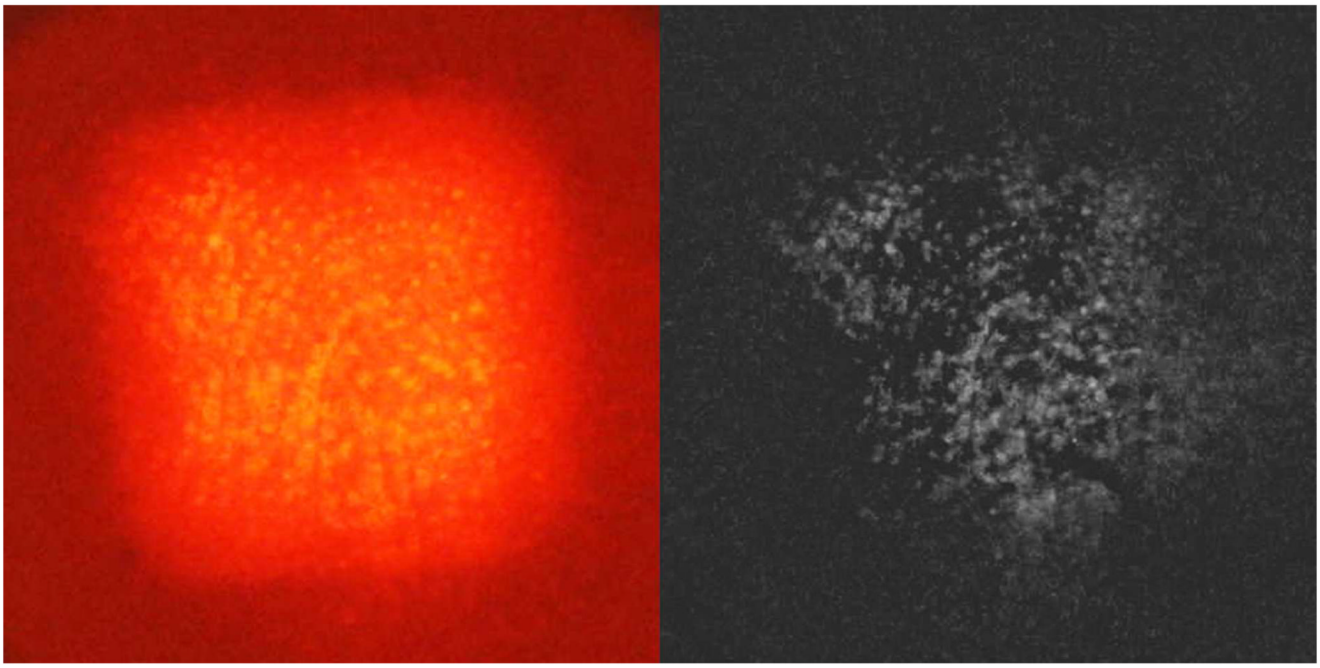

Figure 4.
(Left) Fluorescent fingerprint on paper viewed through the fluorescence imager. (Right) Background-subtracted black and white image of the same fingerprint. The detailed print is much more visible after background subtraction.