Influence of Lightpipes on Readers for Microtubes Fluorescence Detection

Eduardo de Araujo Rocha[1*], Clara Mariah Loureiro da Silva[1], Valmara Silveira Ponte[1], Valéria Loureiro da Silva[1], Vinicius Pinto Rocha[2], Milena Botelho Pereira Soares[2,3]

[1]Optic and Photonic Engineering Laboratory, SENAI CIMATEC; [2]Institute of Innovation in Advanced Health Systems – ISI SAS; [3]Gonçalo Moniz Institute, Oswaldo Cruz Foundation - FIOCRUZ; Salvador, Bahia, Brazil

Lightpipes are light guides based on total internal reflection used for LED illumination systems with many applications. This work investigates using lightpipes in a fluorescent reader for microtubes. It is part of a project to develop an RT-RPA multiplex test and fluorescence reader for SARS-COV 2 and H1N1. We investigated the lightpipes interaction with an excitation filter. The results do not show a benefit in using them on the emission side due to the capture of LED scattered light.

Keywords: Lightpipes. Lightguides. Fluorescence Readers.

Introduction

Lightpipes are used when it is necessary to direct light from the source to a particular area that requires illumination. They are made up of a transparent material, usually glass or plastic, and tiny filaments capable of transmitting light signals through internal reflections [1]. In essence, the light pipe reflects the rays of light through total internal reflection until the light reaches the pipe's end and can exit. They are often used in applications that require a homogeneous light beam and can be commonly found in stage fixtures and automotive applications [1].

The total internal reflection occurs at angles of incidence more significant than the critical angle when the light beam goes from a medium with a higher index of refraction to a medium with a lower index of refraction, as illustrated in Figure 1. The incident light ray is refracted at a critical angle tangentially to the refractive index interface. When the angle of incidence is greater than the critical angle, the incident ray is reflected [2].

In the automotive industry, there has been exponential growth in the use of lightpipes in lighting systems, both for external and internal vehicle lighting [3]. Furthermore, the discovery of the broad applicability of light guides, combined with the advent of powerful LED light sources, enables innovative designs and concept styles using total internal reflection [3]. This work is part of a project to develop an RT-RPA multiplex test and fluorescence reader for SARS-COV 2 and H1N1 [4]. This article investigated the use of a lightpipe for capturing the fluorescence emitted by the fluorophores. A fluorophore is a molecular functional group that absorbs the energy of a specific wavelength and reacts by emitting light at a wavelength longer than that absorbed [5].

Materials and Methods

The influence of different lightpipes from three manufacturers was investigated, whose characteristics we presented in Table 1. The reader uses a BPX61 photodiode, a PCI board for amplification, and an STM32 microcontroller to control the excitation LEDs and filter the emission signal [4]. For the tests, the photodiode was replaced by a 400 μm optical fiber (Ocean Insight QP400-2-SR) and an Avantes spectrometer (AvaSpec-3648) to enable spectral analyses of the captured light. As fluorescent markers, the developed RT-RPA tests use three fluorophores, FAM, ROX, and Cy5. However, for characterizing the lightpipes, an aqueous solution of Rhodamine 6G with a

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Address for correspondence: Eduardo de Araujo Rocha. Rua Santa Isabela, 100, Cond. Elegance Garibaldi, Ap 2202 C, Engenho Velho da Federação, Salvador, Bahia, Brazil. Zipcode: 40221-225. DOI 10.34178/jbth.v5i4.240.

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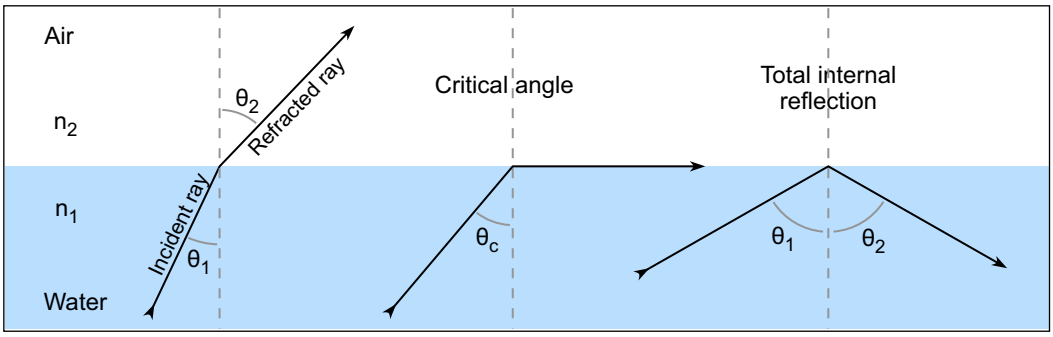
Figure 1. Illustrative image demonstrating the process of total internal reflection.



Source: Jaycon Systems.

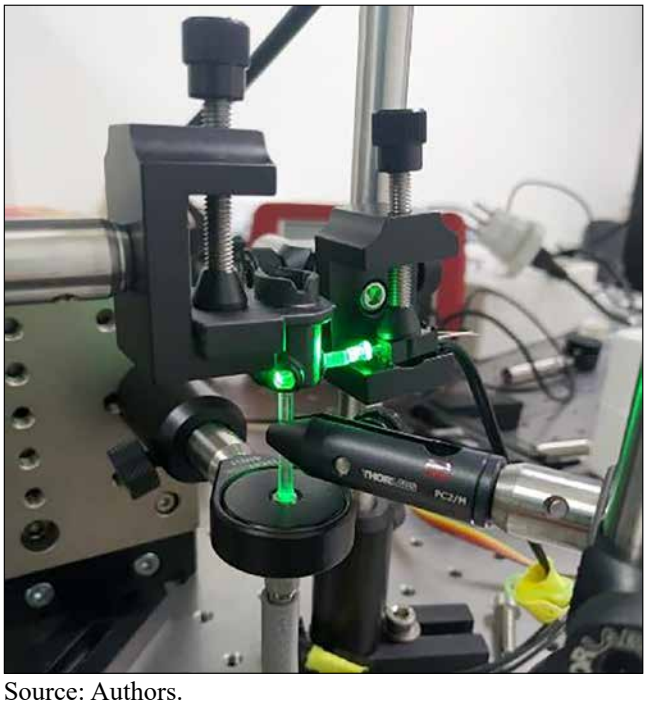
Table 1. Physical characteristics of the analyzed lightpipes.

Model (Manufacturer)	Length	Internal Diameter	External Diameter
LFB100CTP (VCC)	25.4mm	2.84mm	3.30mm
515-1302-0900F (Dialight)	22.86mm	2.36mm	3.30mm
PLP2-500 (BIVAR)	12.7mm	2.80mm	3.30mm
PLP2-750 (BIVAR)	19.0mm	2.80mm	3.30mm

concentration of 0.01 mg/mL was used due to its high fluorescence and convenience. For the tests discussed in the article, twenty microliters were placed into a microtube, which a 3D-printed holder supported. We investigated the use of lightpipes in two locations (Figure 2). The first was to guide the light from the excitation LED to the microtube. In this position, a PLP2-750 lightpipe was used. The second lightpipe location was to collect the emitted fluorescent light and direct it to the spectrometer via the optical fiber (Figure 3).

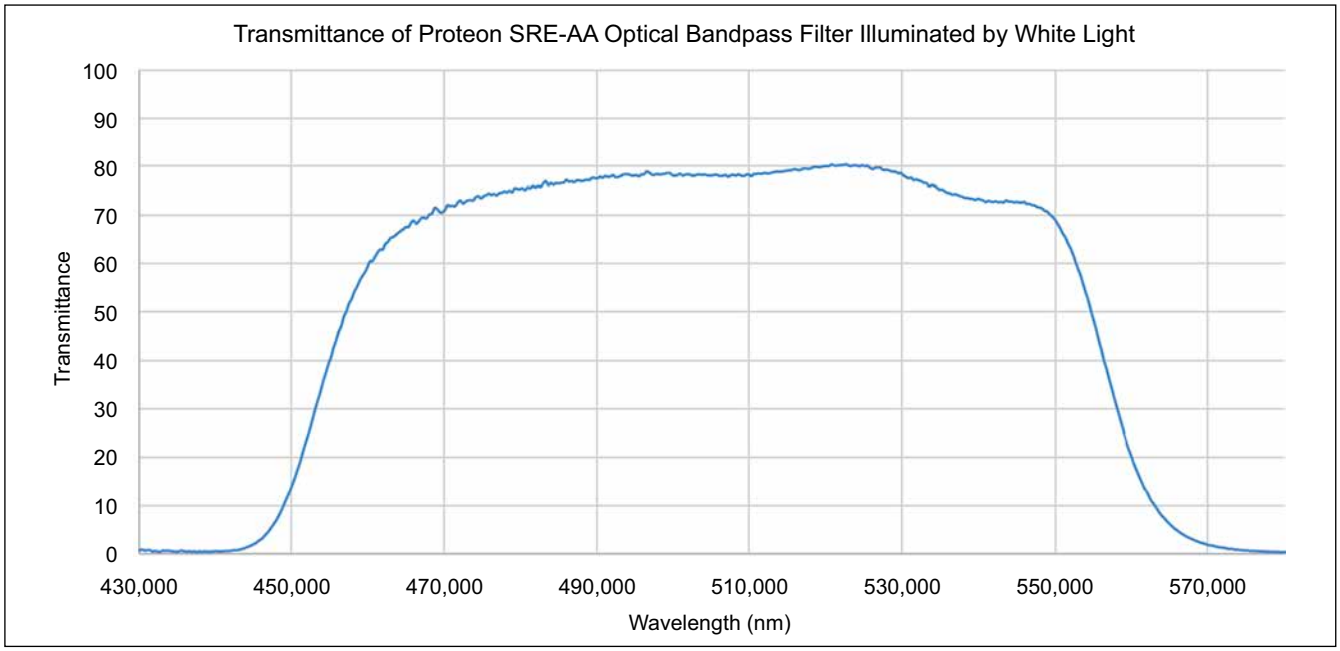
Also, the impact of inserting an optical filter to restrict the excitation of LED spectra was analyzed. A bandpass optical filter (Proteon SRE-AA) was positioned between the LED and the excitation lightpipe to limit the amount of scattered light from the LED that overlaps with the fluorescence spectra. In the measurements, the Avasoft spectrometer software recorded the spectral data, and the data were exported to Microsoft Excel for further analysis.

Figure 2. Picture of the test bench used in the experiments.



Source: Authors.

Figure 3. Measured transmission spectra of the excitation filter.



Source: Authors.

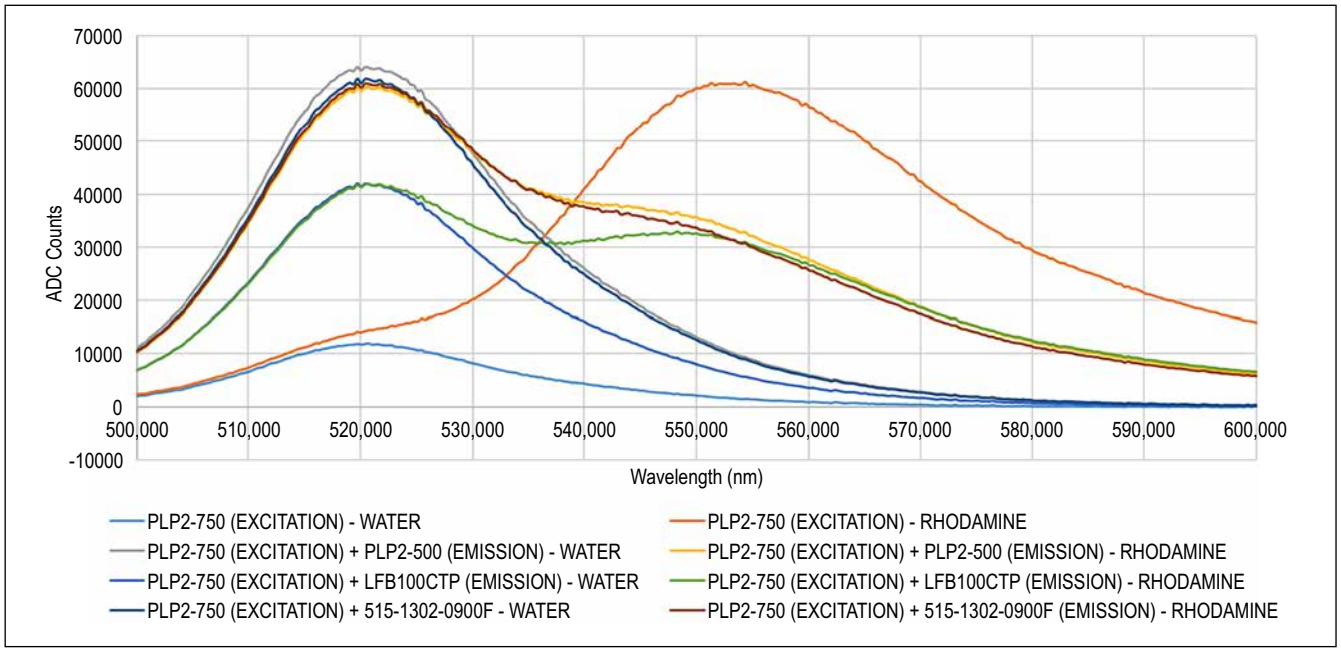
Results and Discussion

Figure 4 shows the spectra obtained without the excitation filter. The blue and grey curves correspond to the spectra obtained with the microtubes filled with deionized water. Therefore, as there is no fluorescence, the peaks around 520 nm correspond to the scattered light from the excitation LED in this case. The other curves show two peaks: the 520 nm scattered light and the fluorescence emission peak around 550 nm. Interestingly, the amount of scattered light at 520 nm in the samples with deionized water and Rhodamine 6G are the same, indicating that the deionized water is a good reference for the undesired, scattered light. The measured spectrum for the case where no lightpipe was used for capturing the fluorescence emission showed a better result, with higher fluorescence at 550 nm than the scattered light at 520 nm. The spectra for the cases where lightpipes are used catch more scattered light (520 nm) than fluorescence (550 nm). Of the lightpipes tested, the LFB100CTP captured the least scattered light.

The addition of an excitation filter (Proteon SRE-AA) changes the relationship between the scattered and emitted fluorescence (Figure 5). The captured scattered light is proportionally smaller than the Rhodamine 6G fluorescence peak. However, the overall intensity of the captured fluorescent light was reduced by five times because of the large thickness of the excitation filter used (6mm). In addition, since the LED has a high divergence, less light is captured and guided by the excitation lightpipe. These measured spectral curves also show a better result for the case where no lightpipe was used for capturing the fluorescence emission.

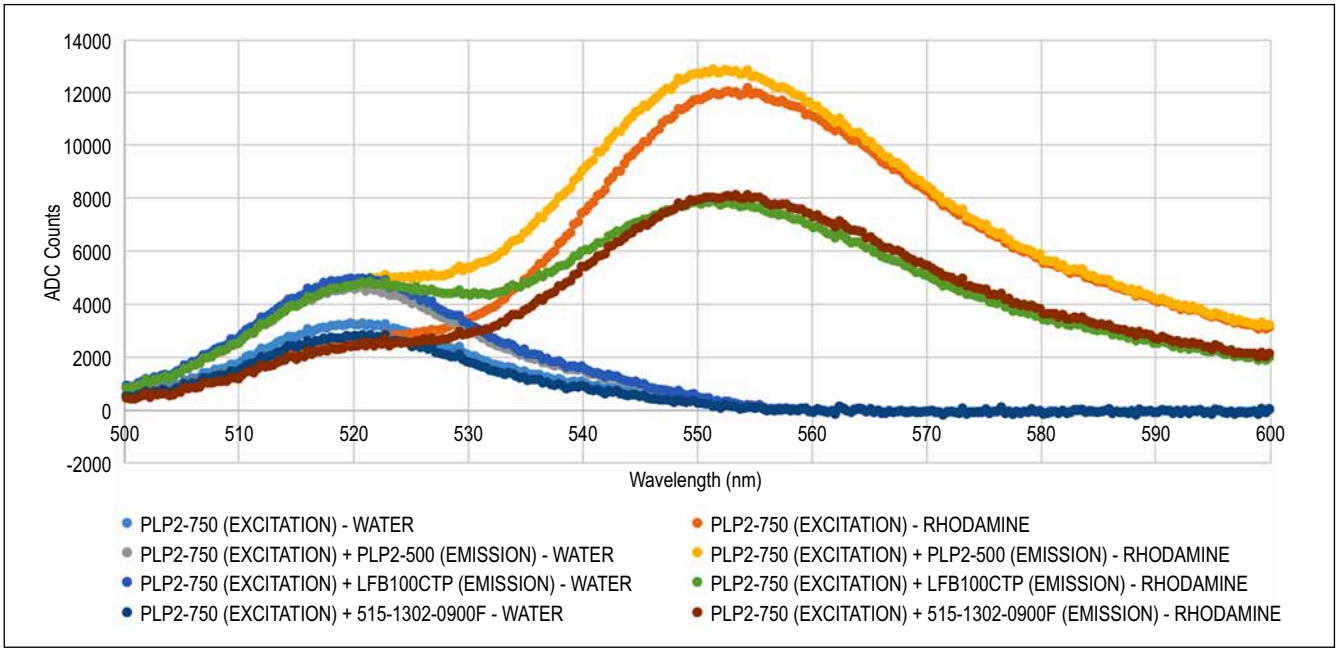
For clarity, Table 2 summarizes the relative intensity for the measured spectral peaks at 520 nm and 550 nm for the deionized water and Rhodamine 6G solution. The addition of the filter improves the relation between the captured fluorescence and scattered lights when a lightpipe at the emission side is used. However, when there is no lightpipe, the improvement is not so clear. The relation between the peak fluorescence and peak scattered light is similar, but the filter helps reduce the scattered light for wavelengths greater than 560 nm. However, the

Figure 4. Samples obtained without the excitation filter.



Source: Authors.

Figure 5. Samples obtained with the excitation filter.



Source: Authors.

Table 2. The leading information contained in Figures 3-5.

CONFIGURATION		SCATTERED LIGHT PEAK INTENSITY AT 520 nm FOR DEIONIZED WATER (ADC COUNTS)	SCATTERED LIGHT PEAK INTENSITY AT 520 nm FOR RHODAMINE 6G (ADC COUNTS)	FLUORESCENCE INTENSITY AT 550 nm PEAK FOR RHODAMINE (ADC COUNTS)	FLUORESCENCE AT 550 nm / SCATTERED LIGHT AT 520 nm FOR RHODAMINE 6G SOLUTION
FILTER	EMISSION LIGHTPIPE				
WITHOUT EXCITATION FILTER	No lightpipe	12000	14000	62000	4,429
	PLP2-500	65000	60000	36000	0,600
	LFB100CTP	42000	41000	32000	0,780
	515-1302-0900F	60000	60000	34000	0,567
WITH EXCITATION FILTER	No lightpipe	3500	3000	12000	4,000
	PLP2-500	4500	5000	13000	2,600
	LFB100CTP	5000	5000	8000	1,600
	515-1302-0900F	3000	2500	8000	3,200

results are always better without the lightpipe on the emission side, indicating the need to restrict the captured scattered light with an adequate emission filter.

Conclusion

Lightpipes are great solutions when it is necessary to guide light from a source to a specific point, and they have been used in various illumination applications. In this paper, we investigated the use of lightpipes in a fluorescence reader for multiplex RT-RPA tests. The results indicate that using lightpipes to capture the fluorescence emission is not advantageous since it captures too much-scattered light. When combined with excitation filters, the use of lightpipes in the excitation is compromised due to reduced light capture. However, spectral filtering of the excitation light can reduce the overall scattered light if combined with an appropriate emission filter.

Acknowledgments

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