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# **A cost-effective fluorescence detection system for pulsed laser analysis**

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## **Abstract**

A cost-effective fluorescence detection system has been developed using a National Instruments PCI-6251 data acquisition (DAQ) board that is driven by LabVIEW Signal Express. The signal is collected using a collimator and transported to the DAQ board using a fiber-coupled detector. The same detection system has been incorporated into an inverted microscope fitted with internal dichroic mirrors to provide a cost-effective alternative to commercial fluorescence microscopes. The detection system has been used to measure fluorescence intensity and generate a laser power curve that can be used for pulsed lasers. Different data analysis approaches have been compared and standard deviation and signal-to-noise ratios have been determined.

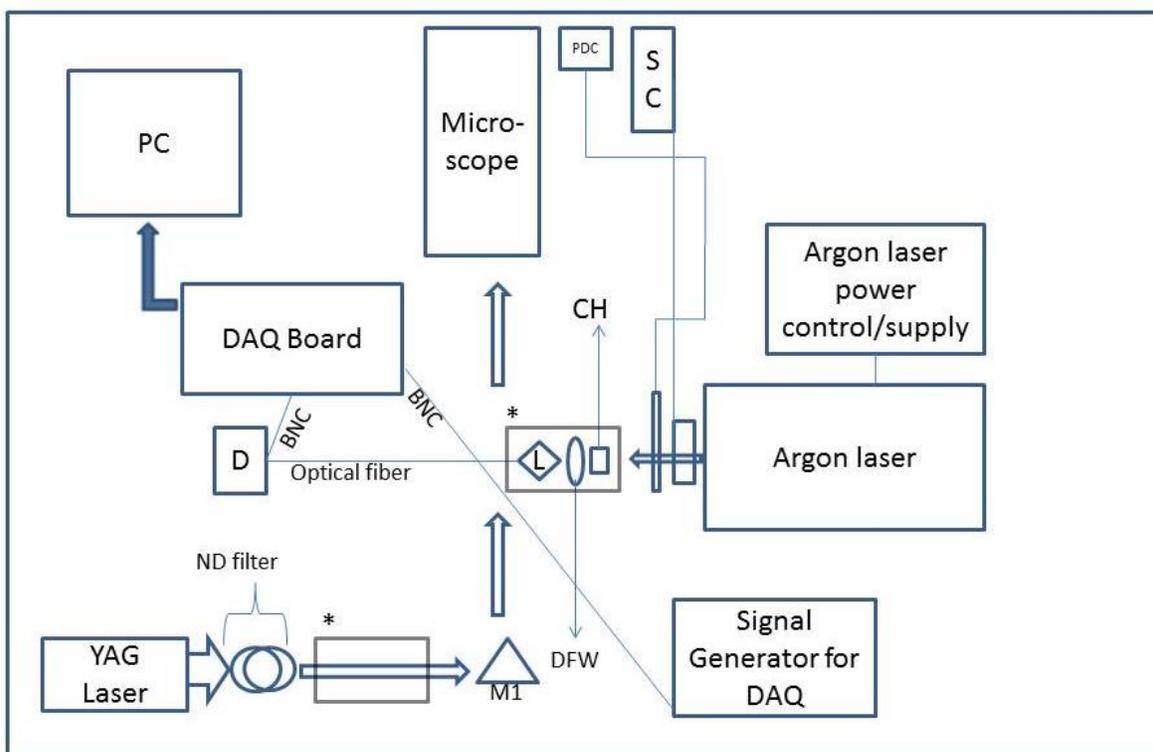
**Keywords:** Fluorescence microscopy, LabVIEW, data acquisition

## 1. Introduction

Fluorescence microscopy is a widely used and very versatile tool for analyzing molecules and intermolecular interactions at low concentrations [1]. Many publications focus on fluorescence imaging but not the detection of intensities from samples that cannot be studied using conventional fluorescence spectrometers [1, 2]. The detection of fluorescence intensity or subtle changes in intensity due to interactions can be difficult at times due to sensitivity and resolution limitations as well as the short excited-state lifetime of many fluorophores. Typical fluorescence microscopes come fitted with detection optics and software that are often cost-prohibitive for many researchers. Also, the implementation of fluorescence detection optics through the side port of a microscope has its challenges, with the biggest challenge being the long working distance required for a C-mount setup that leads to greatly diminished signal. An inverted microscope, like the one used in this work, does not have the capability of allowing detection using a mounted photomultiplier tube that upright microscopes have [3, 4]. In this communication, we present the design of a low-cost fluorescence setup, with flexible signal acquisition and analysis that can work with a standard cuvette arrangement or with a microscope. Using a fiber optic detector connected to a data acquisition board via a detector, fluorescence signal has been collected and analyzed using LabVIEW Signal Express. Given that many laser power meters do not have a high enough damage threshold to measure the power that is being emitted from pulsed lasers, this setup has also been used to measure laser power and provide a non-damaging alternative to direct pulsed laser power measurements. Upper and lower limits of detection, and signal-to-noise ratios have been measured. Various data analysis approaches have been implemented and compared. This is an upgrade to a microscope that is already used for imaging (white-light) and photopolymerization [5, 6]. This will enable us to carry out experiments on low-volume (<100 $\mu$ L) samples and track subtle fluorescence changes in processes like FRAP (fluorescence recovery after photobleaching) and FRET (fluorescence resonance energy transfer). Previously, a detector had to be mounted above the slide to capture the signal [6].

## 2. Experimental

The experimental setup is shown in figure 1 and the prices and part numbers of the individual components of the detection system have been provided. The microscope and lasers have been used for other applications in the past. The primary excitation source for the fluorescence experiments was the 514 nm output of an Ar<sup>+</sup>-ion laser (Melles-Griot) which was directed using a single mirror into the laser port of an inverted microscope (Zeiss Axiovert). The laser is modulated using a shutter which is programmed to open for 10 ms and remain closed for one second. The laser beam is then steered upward through the objective (20x, 0.5NA), which focuses the light onto the sample. The concentration and exposure dosage (time of exposure and laser frequency) were varied to determine optimum parameters. The concentration of Rose Bengal was varied from 10mM down to 1nM.



**Figure 1.** The schematic of the experimental setup. M1 represents the horizontal turning mirror which directs the beam into the inverted microscope where it arrives at the sample and can be moved to line up with the Ar<sup>+</sup>-ion laser as needed. SC is the shutter control (Thorlabs SH05 and SC10, \$1070), CH is the

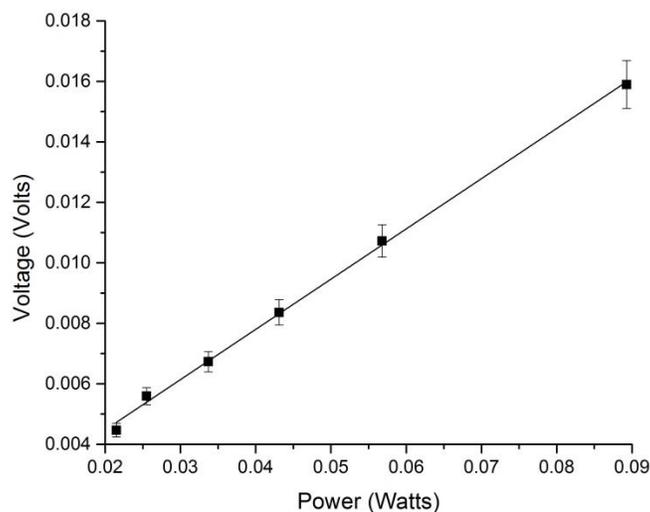
cuvette holder, DFW is the dichroic filter wheel, ND represents the neutral density filters, D is the detector (Thorlabs DET02AFC, \$269) and L is the collimator (Cable: Thorlabs M65L01, \$105; Collimator: Thorlabs F810SMA-543, \$196).

For microscopy experiments, the fluorescence signal passes through a dichroic filter which filters out the excitation light, and the emission is collected through the side port by a collimator which is attached to the optical fiber. Experiments involving bulk (2-3 mL) solutions of dyes were carried out with a dichroic filter wheel placed between the collimator and cuvette.

The optical fiber transfers the signal to a detector that is connected to a BNC block (National Instruments 777643-01 and 192061-02, \$508) which relays it to a data acquisition (DAQ) board (NI PCI-6251, \$1154). The signal is collected and processed using LabVIEW Signal Express. This data is then exported to an Excel file for further analysis using Origin 8.6.

### **3. Results and Discussion**

The signal to noise ratio for this setup an experiment was determined with a 1.0  $\mu\text{M}$  aqueous Rose Bengal solution which was placed in a cuvette and excited at 514 nm using the  $\text{Ar}^+$ -ion laser. Rose Bengal is a xanthene photoactivator that has been used to cross-link proteins on the micron and sub-micron scale [5, 6, 7]. Rose Bengal has a high absorption cross-section and extinction coefficient [8]. The power of the laser was adjusted and measured using a ThorLabs power meter before each sampling. A relationship between the fluorescence intensity and power was determined (figure 2). This relationship has been used to calculate the power of the pulsed laser, which has a high peak power and can damage sensitive optics and detectors. By attenuating the pulsed laser with neutral density filters, the voltage has been measured and the best fit equation used to calculate the power.



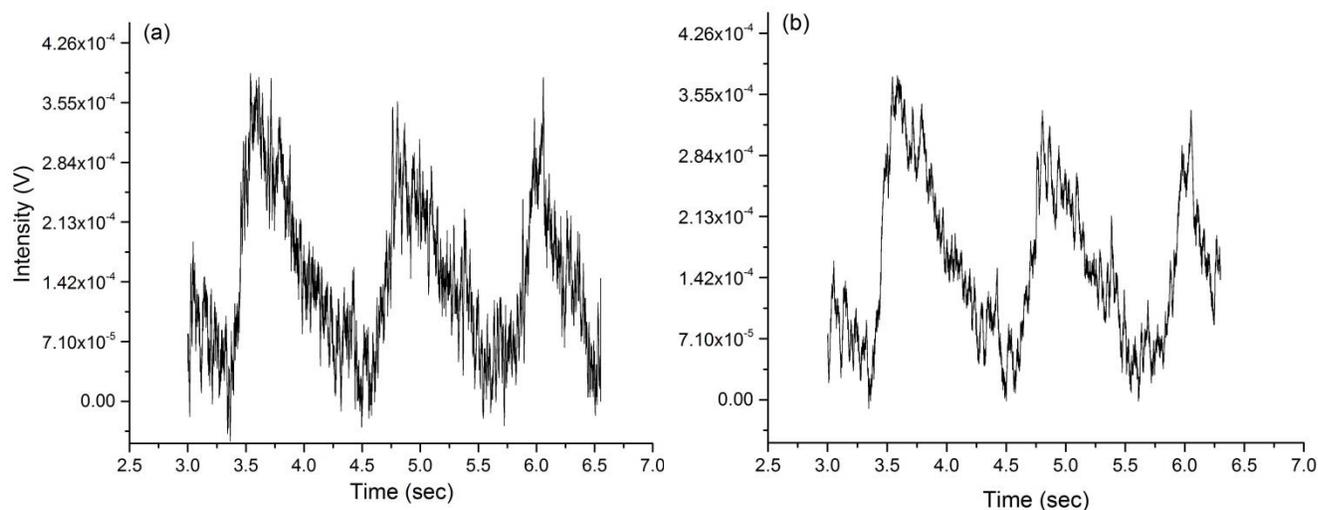
**Figure 2.** Plot of measured voltage as a function of laser power. Best fit equation:  $y = 0.16612x + 0.00116$ .  $R^2 = 0.99804$ .

Table 1 summarizes the S/N for power measurements using this setup and the percent error in power measurements when averaged across five measurements.

**Table 1.** Signal-to-noise and percent error measurements at different laser powers.

<b>Power (mW)</b>	89.3	56.8	43.1	33.7	25.5	21.5
<b>S/N</b>	4.7	3.7	2.6	2.3	2.0	1.8
<b>Percent Error</b>	0.8	1.5	1.3	1.5	2.8	3.7

When the fluorescence emission is collected from the side port of the inverted microscope the signal is weak. In order to collect reliable data, a high sampling rate (6000 points per second) was used. Due to the large number of data points (18000 for three seconds), the peaks are not discernible on the screen or when plotted in Excel or Origin. Data analysis was carried out using two different approaches. In the first approach, 100 and 200 point adjacent averaging was carried out on the entire data set (figure 3).



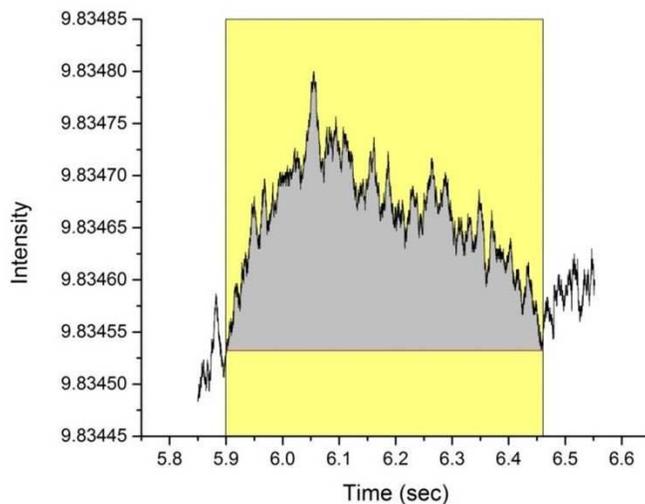
**Figure 3.** Fluorescence measurements as a function of time for a 10 $\mu$ M solution of Rose Bengal. (a) 100-point adjacent averaging, (b) 200-point adjacent averaging.

Each fluorescence event is represented by the peaks. The area under the curve was calculated and used as a measure of the fluorescence intensity of the sample. While the S/N for the 100-point averaging method was worse than the 200-point averaging, there was little difference in the calculated intensities. The results are summarized in table 2.

**Table 2.** Fluorescence intensities for five concentrations of Rose Bengal. Standard deviations for 3-event averages are on the order of  $10^{-7}$ .

Concentration	Fluorescence Intensity (a.u.)		
	100 point	200 point	3-event average
<b>0.1 mM</b>	$1.58 \times 10^{-4}$	$1.61 \times 10^{-4}$	$1.60 \times 10^{-4}$
<b>10<math>\mu</math>M</b>	$7.10 \times 10^{-5}$	$7.60 \times 10^{-5}$	$7.40 \times 10^{-5}$
<b>1 <math>\mu</math>M</b>	$4.37 \times 10^{-5}$	$4.42 \times 10^{-5}$	$4.40 \times 10^{-5}$
<b>0.1 <math>\mu</math>M</b>	$6.08 \times 10^{-6}$	$6.22 \times 10^{-6}$	$6.19 \times 10^{-6}$
<b>10 nM</b>	$2.75 \times 10^{-6}$	$2.78 \times 10^{-6}$	$2.75 \times 10^{-6}$

In the second method, the raw data for three individual fluorescence events were averaged without any adjacent averaging (figure 6). The area under the curve was calculated and there was a good agreement between the results from each of the methods.



**Figure 4.** Average of three successive fluorescence measurements. The area under the curve (shaded) is proportional to the fluorescence intensity.

The concentration detection limit of the instrument was determined. As the concentration of the dye was increased in 0.1 M increments, starting at 1 nM, the measured intensity began to level off (Supplementary Information, figure 1a), indicating that signal saturation is occurring at high concentrations, which is consistent with Beer's law and basic fluorescence spectroscopy principles. The relationship is linear in the 10 nM – 1  $\mu$ M range (Supplementary Information, figure 1b). This shows that the setup is very sensitive with a low detection limit and expected saturation at high concentrations.

#### 4. Conclusion

A low-cost fluorescence detection system has been developed. The total cost of the detection system for the microscope is \$3300, not including software. The system is flexible as it can be used with a cuvette as well as in a microscopy setup. The system offers good resolution and S/N, as well as detection capabilities

in the nanomolar range. Data acquisition is carried out by LabVIEW Signal Express and different averaging approaches show good agreement with each other. Finally, fluorescence intensities measured using this setup are in good agreement with those obtained using a standard benchtop fluorimeter.

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