



# Development of a commercial LEDs-based fluorescence polarization microplate reader for molecular biology measurements: System performance evaluation using calibration standards



Sami D. Alaruri\*

Independent Scholar, PO Box 13123, Mill Creek, WA 98082, USA

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## ABSTRACT

In this work, the design of a commercial LEDs-based fluorescence polarization microplate reader which can be used for molecular bioanalytical measurements is described. The obtained validation measurements for the fluorescence polarization microplate reader indicate that the detection limit is 5 mP at 10 nM fluorescein (1 s integration time) measured with a black 96-well plate and the instrument dynamic range is >5 decades. Furthermore, the microplate reader fluorescence measurement mode can detect 1.5 fmol/200  $\mu$ L fluorescein at 1 s integration time setting using a 96-well black microplate.

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## 1. Introduction

Fluorescence polarization is a powerful tool for studying in solution molecular binding events of a fluorescently-labeled ligand or a tracer in order to understand the impact of pH, temperature or salt concentration on the molecular binding interactions. Also, fluorescence polarization measurement techniques are routinely used in dissociation and association molecular reaction studies [1–5].

Fluorescence polarization measurements are based on exciting molecules in solution with linearly-polarized radiation and by measuring the emitted fluorescence radiation intensity in two orthogonal polarization orientations. The calculated ratio of fluorescence intensity measurements in the two mutually orthogonal polarization orientations provide information related to the changes in the molecule size and its rotational relaxation rate [1–5].

Some of the applications of fluorescence polarization measurements include high-throughput drug screening, receptor–ligand binding, immunoassays, DNA protein binding and DNA hybridization [5].

In this work, a description for the operation of a commercial fluorescence polarization microplate reader that incorporates multiple LEDs as light excitation sources and a channel photomultiplier detector operated in the photon counting mode is provided. In addition to the fluorescence polarization measurement

mode the multimode microplate reader is capable of performing five additional measurement modes, namely, fluorescence intensity top and fluorescence intensity bottom, time-resolved fluorescence, absorbance and luminescence. The focus of this article will be on the operation of the fluorescence polarization mode of the microplate reader. The described microplate reader [6–10] is the first commercial microplate reader in the market place that incorporates LEDs as excitation sources instead of flash lamps or lasers.

The described fluorescence polarization microplate reader optical performance is comparable to that of well-established readers that incorporate flash lamps such as Tecan [17] and PerkinElmer [18,19].

The use of LEDs as excitation sources in analytical instruments offer several advantages when compared with flash lamps (typical lifetime ~2000 h) or lasers (typical lifetime 15,000 h) [11–16]. Some of the major advantages of using LEDs are long lifetime (>50,000 h), low current consumption, low cost, low maintenance, small footprint and robustness.

Finally, measurements collected with the microplate reader using commercial fluorescence polarization calibration microplates are presented.

## 2. System description

The fluorescence polarization detection mode described in this article is part of a microplate multimode reader which can perform in addition to the fluorescence polarization fluorescence intensity

\* Corresponding author.

E-mail address: [sami.laser@yahoo.com](mailto:sami.laser@yahoo.com)

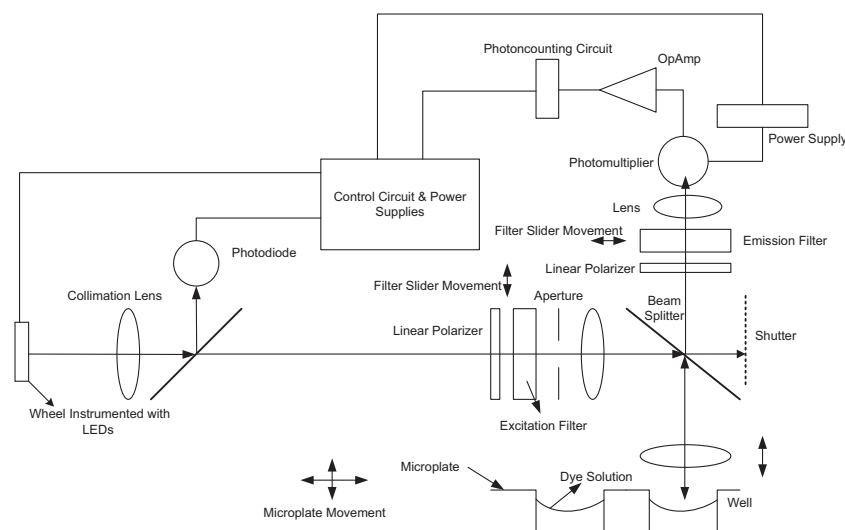


Fig. 1. Schematic showing the optical layout of the fluorescence polarization detection mode of the multimode reader [6–10].

top, fluorescence intensity bottom, absorbance, time-resolved fluorescence, and glow luminescence measurements. A full description for the optical layout of the microplate reader different detection modes is provided in references [6–10]. The focus of this paper will be on the operation of the fluorescence polarization mode of the microplate reader.

The fluorescence polarization detection mode consists of seven major parts: LED light sources, collimation and focusing optics, excitation filters slider, linear polarizers, emission filters slider, a channel photomultiplier, and a microplate mechanical transport system which can accommodate 6, 12, 24, 48, 96 and 384-well microplate formats is schematically shown in Fig. 1. The microplate is placed in the transport system either in the landscape or portrait orientation.

As shown in Fig. 1, the LEDs are mounted on a motorized wheel. Light emitted from a selected LED is collimated and passed through a narrow band-pass emission optical filter which is mounted on a motorized emission filter slider. The microplate reader LEDs cover a wide excitation spectral range and operator selected (excitation wavelengths: white LED (450–750 nm) and LEDs centered at 365, 375, 405, 470, 540 and 590 nm). The LEDs output, which are operated in the continuous mode at constant current settings, ranges between 2 mW and <1 W at  $I_F = 20$  mA. The microplate LEDs rotating wheel can accommodate up to fifteen different LEDs. Then the radiation is directed toward a short focal length (FL = 13.5 mm) quartz lens utilizing a 50:50 dichroic beam splitter (230 nm to 700 nm transmission). Thereafter, a quartz lens focuses the excitation radiation into a well in the microplate the height of which is optimized relative to the liquid meniscus using a z-height motorized stage and by measuring the collected fluorescence radiation from the solution contained in a well. The generated fluorescence radiation passes through a beam splitter and an emission optical filter and then is focused using a quartz lens (FL = 16 mm) into a channel photomultiplier detector (spectral range 390–750 nm) operated in the photon counting mode [20,21]. The emission interference optical filter is mounted on a motorized filter slider which can accommodate up to six filters. As depicted in Fig. 1, the stability of the excitation beams emitted from a selected LED is monitored by using a silicon photodiode. The instrument integration time per well can be set between 10 and 10,000 ms.

All the excitation and emission filter slide positions are designed to accommodate linear polarizers. The pairing of the excitation and emission filters is selected by the operator through the instrument user interface software. Furthermore, measurement attributes such

as the excitation wavelength and the photomultiplier integration time are selected by the operator.

Finally, it is worth noting that the microplate reader chamber is equipped with a heater which can incubate a microplate up to 45 °C.

### 3. Results and discussion

#### 3.1. Background

Fluorescence polarization was first described by Jean Perrin in 1926 using the following mathematical expression

$$\frac{r_0}{r} = 1 + \frac{\tau}{\theta} \quad (1)$$

where  $r_0$  is the fundamental anisotropy,  $r$  is the measured anisotropy,  $\tau$  is the fluorophore fluorescence lifetime and  $\theta$  is the fluorophore rotational correlation time. The fluorophore rotational correlation time,  $\theta$ , or the time it takes to rotate through an angle of 68.5° is proportional to the solution viscosity ( $\eta$ ), molecular volume ( $V$ ), the gas constant ( $R$ ) and the absolute temperature ( $T$ ) is given by

$$\frac{\theta \alpha \eta V}{RT} \quad (2)$$

Experimentally the fluorescence polarization ratio value ( $P$ ) and the emission anisotropy ( $r$ ) can be measured by illuminating the fluorophores in solution with linearly polarized light and then by measuring the emitted fluorescence radiation in two orthogonal polarization states (i.e.  $I_{\text{par}} - I_{\text{per}}$ ) with respect to the polarized light used to excite the solution in the microplate wells.

The fluorescence polarization ratio value ( $P$ ) can be expressed in units of mP (milliP) as

$$P = \frac{I_{\text{par}} - I_{\text{per}}}{I_{\text{par}} + I_{\text{per}}} \times 1000 \quad (3)$$

where  $I_{\text{par}}$  and  $I_{\text{per}}$  are the polarized emitted fluorescence intensity radiation measured in the two orthogonal polarization states. Then, Eq. (3) can be written as

$$P = \frac{1 - (I_{\text{par}} - I_{\text{per}})}{1 - (I_{\text{par}} + I_{\text{per}})} \quad (4)$$

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