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Development and Application of A Novel and Compact Long Wavelength Fluorescence Spectrometer

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1. Introduction

Long wavelength (>600nm) fluorescence offers many advantages when applied to analysis, including minimal autofluorescence and scattered light from biological samples and the possibility of compact, robust, yet sensitive instruments. Modern clinical analysis has a number of specific requirements, namely specificity, sensitivity and speed, whilst the ability to monitor samples away from the laboratory is increasingly in demand. Immunoassays possess all these attributes and can be used in the following:

- Environmental monitoring
- Clinical analysis
- Therapeutic drug monitoring

This research work describes a novel portable fluorescence spectrophotometer using long wavelength detection which can be used in all environments.

2. Operation Configuration of Instrument

A fluorescence spectrometer was successfully developed in the house and has employed the these opto-electronics.
Laser diode source: 635nm (2mW), 645nm (2mW) and 670nm (2mW).
Excitation range: 500nm - 900nm, Emission range: 550nm - 1100nm.
High speed, large area and high sensitivity photodiode.
Conventional cut off filters, 645nm, 650nm and 690nm.
A high speed, high precision and low noise amplifier to signal output has been developed for the fluorescence signal amplification. A flow cell or standard cuvette can be either applied in the measurement. The instrument is overall dimension is 34 x 32 x 9.5cm with a digital display, the output for chart recorder and the FlowTEK PC Interface.

Fluorescent dyes used:

Dye	Exmax	Emmax
Naphthofluorescein	600nm	660nm
Cy5	649nm	670nm
Cy5.5	675nm	694nm

Fig. 1 Overview of fluorescence measurement system

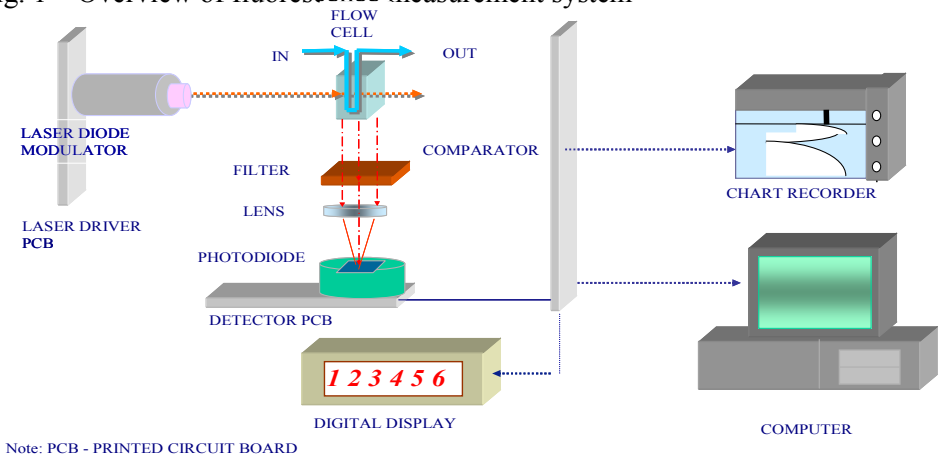
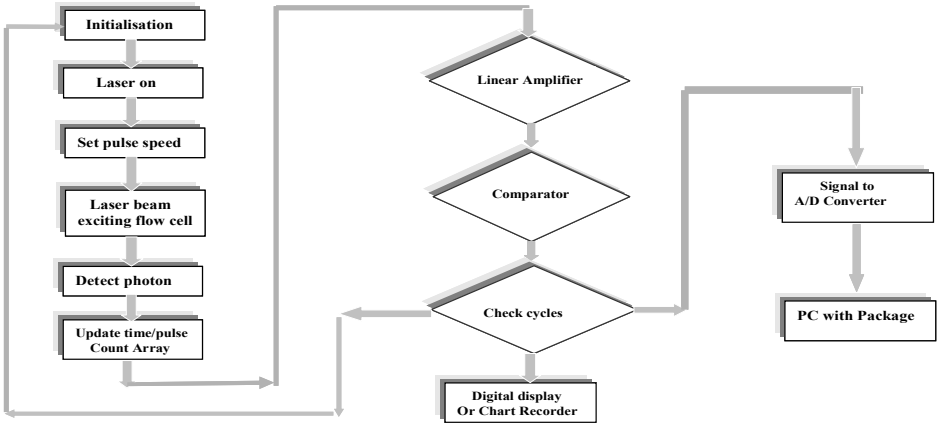


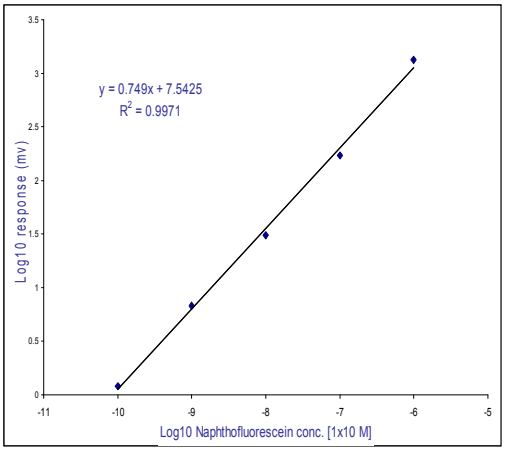
Fig. 2 Flow chart for detection system using laser diode



This instrument makes use of long wavelength fluorescence and solid state sources and detectors which facilitate the development of methods to test samples in non laboratory situations. Such methods provide the analyst with a tool for a number of applications, for example drugs of abuse testing or environmental monitoring of cytotoxins or pesticides. Flow injection immunoassays not only offer the potential for rapid screening and quantitative analysis but they also have the potential to be used in a portable format, supported by a robust, sensitive and reliable detector.

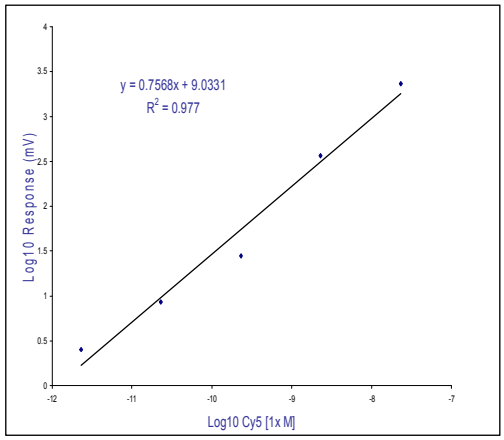
3. Experimentation

Fig. 3 Calibration curve of Naphthofluorescein response



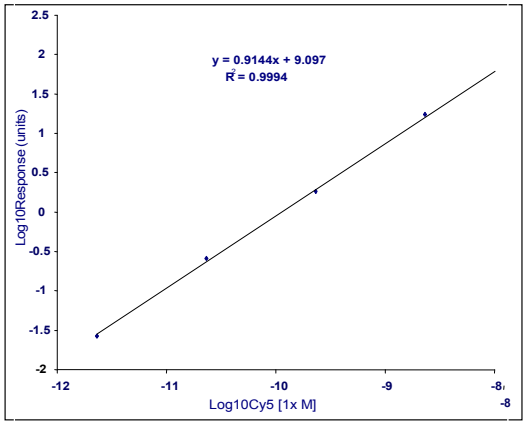
Naphthofluorescein with CHAPS 2.5% w/v, HELLMA 10mm cuvette, Tris buffer pH8.8, RACA-DANA 4005 digital multimeter, 635nm 2mW and Optosigma cutoff filter 645nm

Fig. 4 Calibration curve of Cy5.5 response



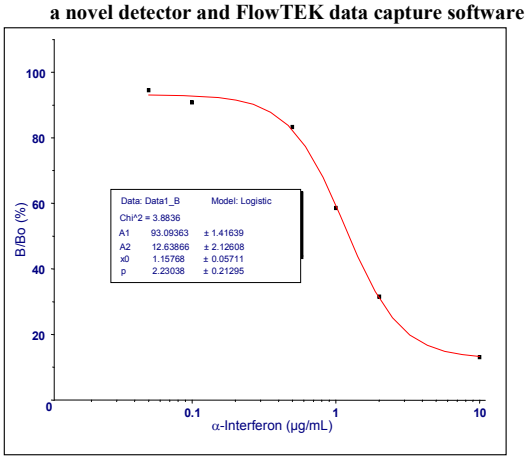
Cy5.5 with Tris buffer pH8.8, HELLMA 10mm cuvette, RACA-DANA 4005 digital multimeter, 670nm, 2mW and Schott cutoff filter 695nm

Fig. 5 Calibration curve of Cy5 response



Cy5 with Citric acid buffer pH2.5, HELLMA 100µl flow cell, FlowTEK data capture software in a PC, 645nm, 2mW and Schott cutoff filter 650nm

Fig.6 Flow injection immunoassay for α-Interferon using a novel detector and FlowTEK data capture software



α-Interferon with Citric acid buffer pH2.5, HELLMA 100µl flow cell, FlowTEK data capture software in a PC, 645nm, 2mW and Schott cutoff filter 650nm

4. Further Development Work

Further development work has been considered to investigate other laser diodes or superluminescent light-emitting diodes (SLEDs) and dye combinations, i.e. Cy2, Cy3, Cy3.5 and Cy7. Further modification work will improve the functionality of the detectoion, i.e. NI DAQ card and Labview5.01 data capture package instead of Global DAQ board and FlowTEKTM data capture package. Environmental monitoring for selected pesticides using flow injection inhibition methods has been discussed to perform with this instrument. The instrument is also being provided to other Ph.D. students for developing long wavelength fluorescence based enzyme immunoassays.

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