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Development of high throughput Dual Laser system incorporating CCD-based multichannel Czerny – Turner spectrometer for real-time qPCR fluorescence intensity measurements

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ABSTRACT

In this article, the development of a commercial high throughput system incorporating a multichannel CCD-based Czerny – Turner spectrometer for real-time fluorescence quantitative polymerase chain reaction (qPCR) measurements is described. The system employs two 20 mW lasers (532 ± 1 nm and 637 ± 3 nm) 2.5× Galilean beam expander-focuser, 5×4-200 μm core diameter fiber-optic bundle and a two-axis galvanometer for exciting fluorescent dyes. Further, the optical measurement system can detect twenty reaction vessels in asynchronous manner and up to five different fluorescent dyes (5-plex) at 1 nM dye concentration in each of the reaction vessels simultaneously. Moreover, PCR curves obtained using the optical measurement system for a genomic deoxyribonucleic acid (DNA) template containing three fluorescent generic binding dyes (FAM, Q670 and CRF610) is discussed. The measurement system repeatability is <1% CV (coefficient of variance). Finally, a performance comparison between the reported qPCR laser-based measurement system and an LED-based qPCR measurement system described by the author in ref. 14 is provided.

1. Introduction

PCR (Polymerase Chain Reaction) refers to a method used to amplify DNA or RNA through repeated cycles of enzymatic replication followed by denaturation of the DNA double strand and the formation of new DNA double strand. The denaturation and naturation of the DNA double strand is performed by altering the temperature of the DNA amplification reaction mixture. Further, quantitative real-time PCR (qPCR) refers to a PCR process in which a signal that is correlated to the total amount of amplified DNA in the reaction is monitored during the amplification process without opening the reaction vessel. The monitored signal is often fluorescence radiation; however, other detection methods are possible [1]. qPCR technology has gained wide applications in fields related to diagnosing infectious diseases, genetic fingerprinting, drug screening, and tissue typing [2–7]. *Chalmydia trachomatis*, *Neisseria gonorrhoeae*, methicillin-resistant Staphylococcus aureus (MRSA), human immunodeficiency virus (HIV) and herpesvirus-5 (HHV-5) are examples for the diseases that can be detected using qPCR amplification.

In this work, a description for an optical system used for detecting fluorescence radiation related to the amount of DNA or RNA product formed during the process of PCR amplification is provided. The optical detection system is part of a newly patented PCR processing station described in detail in reference [8]. The station can perform DNA or RNA extraction, purification, amplification, detection and information management. The fully-automated and random access PCR station is capable of detecting DNA or RNA products from twenty reaction vessels and up to five different fluorescent dyes (5-plex) at approximately 1 nM dye concentration from each of the twenty reaction vessels. Additionally, PCR amplification curves (fluorescence signal amplitude versus thermal cycle number) obtained using the optical measurement system from a genomic DNA template containing three fluorescent generic binding dyes (FAM, Q670 and CRF610) are presented. The reported optical system is part of a platform which combines DNA/RAN extraction, purification and amplification in one platform (only system in the market place).

2. Measurement system description

The DNA amplification fluorescence measurement system consists of seven major parts: two CW lasers (532 ± 1 nm and 637 ± 3 nm), refractive laser beam expander focuser, two-axis

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galvanometer, 22-channel excitation fiber optic bundle, 20 thermocycling modules [9], 22-channel fiber-optic collection bundle and a multi-channel Czerny – Turner spectrometer [10].

Laser beams emitted from the 532 ± 1 nm (20 mW) and the 637 ± 3 nm (20 mW) lasers are passed through two laser line optical filters and expanded using a 2.5X refractive Galilean telescopes [11]. Both filters are tilted by 2° to prevent back reflections into the laser cavities. The emerging 637 nm laser beam is directed towards a plano-convex focusing lens (FL = 60.00 mm, N-BK-7) using aluminum front surface coated mirror and the 532 nm laser beam is directed towards the focusing lens using a beam splitter (>95% Reflectance (average) at 532 nm; >95% Transmittance (average) at 637 nm). Both the mirror and the beam splitter are mounted at 45° relative to the two incident laser beams. The two laser beams are aligned slightly non-collinearly to avoid crosstalk between the two excitation laser channels. The two laser beams are slightly collinear and strike the focusing lens at approximately 1.0° from the optical axis. The laser beams are coupled into the selected excitation fiber or qPCR channel sequentially. A two-axis galvanometer incorporating x and y rotatable mirrors is used to steer either the 532 nm or the 637 nm laser beams into one of the 22 fused silica fibers forming the excitation fiber optic bundle (CeramOptec, fiber p/n Optran WF, Length = 1.5 m). At the same time, the galvanometer x and y rotatable mirrors direct the other laser beam outside the 22 fibers array to prevent crosstalk between the two 532 nm and 637 nm laser excitation channels. The excitation fiber bundle consists of twenty two fibers (two unused spare fiber channels) arranged in 5×4 array with 0.425 mm spacing between the cores of the multimode optical fibers. The core diameter and the numerical aperture of each fiber are 200 μm and 0.12, respectively. Light emerging from each excitation fiber is focused into a reaction vessel by the means of a short focal length lens (FL = 4.50 mm, N-BK-7). The alignment of the two-axis galvanometer with the position of each of the twenty two excitation fibers is carried out by scanning separately the 532 nm and 637 nm laser beams across the excitation fibers and by recording the x and y galvanometer positions at which the maximum power is produced from the output end of each excitation fiber.

Two identical 2.5X refractive Galilean telescopes and the focusing lens (FL = 60 mm, N-BK-7) are designed to focus the 532 nm and 640 nm laser beams to a spot <90 μm in diameter at the excitation fiber optic bundle input surface. The <90 μm focused beam diameter limit ensures that the focused laser beams are always coupled into the 200 μm core diameter of the excitation fibers for the distribution of beam waist sizes and beam waist locations provided by the laser manufacturer for both lasers. Also, the <90 μm focused beam diameter limit ensures that the focused laser beams are always coupled into the excitation fibers for the worst galvanometer drift, galvanometer repeatability, system operating temperature and laser beam pointing stability error conditions [11].

Fluorescence radiation emitted from the reaction vessel in each cell is coupled into 800 μm core diameter fused silica fiber using a molded glass aspheric lens (FL = 6.24 mm, ECO-550). Two slits are placed after the excitation lens and before the collection lenses to control the amount of radiation and to clean the excitation and emission spatial beam profiles. The twenty two (two spare fibers) 800 μm core diameter fibers are arranged in a 2×11 array and coupled into the entrance of Czerny – Turner multi-channel spectrometer which is equipped with uncooled CCD imaging sensor. As shown in Fig. 1, a specially designed optical filter is placed inside the spectrometer after the output end of the excitation fiber optic bundle to prevent the excitation wavelengths and the background radiation from entering the spectrometer. The spectral bandwidth of each spectrometer channel was set to detect fluorescence radiation between 500 nm and 750 nm.

The spectrometer can read only one cell or reaction vessel at a time. The spectrometer starts the integration (integration time = 50 ms; 1×5 binning) of the fluorescence signals received from a selected channel when a trigger pulse is received. The spectrometer trigger out signal commands the start of the galvanometer excitation for a selected fiber or channel. The galvanometer couples the excitation laser beam (i.e., 532 nm or 637 nm) into an excitation fiber only when the read event is underway.

The PCR reaction is formed in twenty capped reaction vessels (typical volume 40 – 50 μL) [8] which were inserted inside

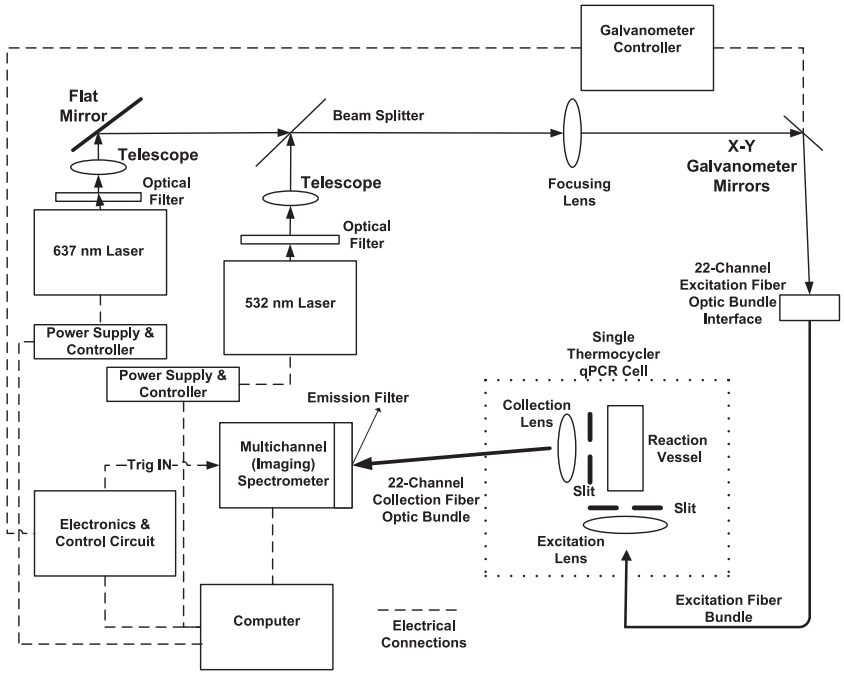

Fig. 1. Schematic showing the optical layout for the DNA or RNA amplification detection system.