

capacitance of C45 and resistance of R25 are chosen so as to impact the time constant τ_c (equal to the product of R25 and C45) of the circuit as well as gain of the detection circuit. The higher the time constant, the more sluggish is the response of the system to incident light. It typically takes the duration of a few time constants for the photodetector to completely charge to its maximum current or to discharge to zero from its saturation value. It is important for the photo current to decay to zero between measurements, however. As the PCR systems described herein are intended to afford rapid detection measurements, the product $R_{25}C_{45}$ should therefore be made as low as possible. However, the gain of the pre-amplifier whose circuitry is shown is directly proportional to the (fluorescent-activated) current generated in the photodetector and the resistance R_{25} . As the fluorescence signal from the microfluidic channel device is very faint (due to low liquid volume as well as small path lengths of excitation), it is thus important to maximize the value of R_{25} . In some embodiments, R_{25} is as high as 100 Giga-Ohms (for example, in the range 10-100 G Ω), effectively behaving as an open-circuit. With such values, the time-constant can take on a value of approximately 50-100 ms by using a low-value capacitor for C45. For example, the lowest possible available standard off-the-shelf capacitor has a value of 1 pF (1 picoFarad). A time-constant in the range 50-100 ms ensures that the photocurrent decays to zero in approximately 0.5 s (approx. 6 cycles) and therefore that approximately 2 samplings can be made per second. Other time constants are consistent with effective use of the technology herein, such as in the range 10 ms-1 s, or in the range 50 ms-500 ms, or in the range 100-200 ms. The actual time constant suitable for a given application will vary according to circumstance and choice of capacitor and resistor values. Additionally, the gain achieved by the pre-amplifier circuit herein may be in the range of 10^7 - 5×10^9 , for example may be 1×10^9 .

[0240] As the resistance value for R25 is very high (~100 G Ω), the manner of assembly of this resistor on the optics board is important for the overall efficiency of the circuit. Effective cleaning of the circuit during assembly and before use is important to achieve an optimal time-constant and gain for the optics circuit.

[0241] It is also important to test each photo-diode that is used, because many do not perform according to specification.

Sensitivity and Aperturing

[0242] The LED light passes through a filter before passing through the sample in the micro-fluidic channel (as described herein, typically 300 μ deep). This is a very small optical path-length for the light in the sample. The generated fluorescence then also goes through a second filter, and into a photo-detector. Ultimately, then, the detector must be capable of detecting very little fluorescence. Various aspects of the detector configuration can improve sensitivity, however.

[0243] The illumination optics can be designed so that the excitation light falling on the PCR reactor is incident along an area that is similar to the shape of the reactor. As the reactor is typically long and narrow, the illumination spot should be long and narrow, i.e., extended, as well. The length of the spot can be adjusted by altering a number of factors, including: the diameter of the bore where the LED is placed (the tube that holds the filter and lens has an aperturing effect); the distance of the LED from the PCR reactor; and the use of proper lens at the right distance in between. As the width of the beam

incident on the reactor is determined by the bore of the optical element (approximately 6 mm in diameter), it is typical to use an aperture (a slit having a width approximately equal to the width of the reactor, and a length equal to the length of the detection volume) to make an optimal illumination spot. A typical spot, then, is commensurate with the dimensions of a PCR reaction chamber, for example 1.5 mm wide by 7 mm long. FIG. 35A shows the illumination spot across 12 PCR reactors for the two different colors used. A typical aperture is made of anodized aluminum and has very low autofluorescence in the wavelengths of interest. FIG. 35B illustrates a cross-section of a detector, showing an exemplary location for an aperture 802.

[0244] The optimal spot size and intensity is importantly dependent on the ability to maintain the correct position of the LED's with respect to the center of the optical axis. Special alignment procedures and checks can be utilized to optimize this. The different illuminations can also be normalized with respect to each other by adjusting the power current through each of the LED's or adjusting the fluorescence collection time (the duration for which the photodetector is on before measuring the voltage) for each detection spot. It is also important to align the detectors with the axis of the micro-channels.

[0245] The aperturing is also important for successful fluorescence detection because as the cross-sectional area of the incident beam increases in size, so the background fluorescence increases, and the fluorescence attributable only to the molecules of interest (PCR probes) gets masked. Thus, as the beam area increases, the use of an aperture increases the proportion of collected fluorescence that originates only from the PCR reactor. Note that the aperture used in the detector herein not only helps collect fluorescence only from the reaction volume but it correspondingly adjusts the excitation light to mostly excite the reaction volume. The excitation and emission aperture is, of course, the same.

[0246] Based on a typical geometry of the optical excitation and emission system and aperturing, show spot sizes as small as 0.5 mm by 0.5 mm and as long as 8 mm \times 1.5 mm have been found to be effective. By using a long detector (having an active area 6 mm by 1 mm) and proper lensing, the aperture design can extend the detection spot to as long as 15-20 mm, while maintaining a width of 1-2 mm using an aperture. Correspondingly, the background fluorescence decreases as the spot size is decreased, thereby increasing the detection sensitivity.

Use of Detection System to Measure/Detect Fluid in PCR Chamber

[0247] The fluorescence detector is sensitive enough to be able to collect fluorescence light from a PCR chamber of a microfluidic substrate. The detector can also be used to detect the presence of liquid in the chamber, a measurement that provides a determination of whether or not to carry out a PCR cycle for that chamber. For example, in a multi-sample cartridge, not all chambers will have been loaded with sample; for those that are not, it would be unnecessary to apply a heating protocol thereto. One way to determine presence or absence of a liquid is as follows. A background reading is taken prior to filling the chamber with liquid. Another reading is taken after microfluidic operations have been performed that should result in filling the PCR chamber with liquid. The presence of liquid alters the fluorescence reading from the chamber. A programmable threshold value can be used to