signal generators. Operation of a PWM generator includes generating a signal with a chosen, programmable, period (the end count) and a particular granularity. For instance, the signal can be 4000 its (micro-seconds) with a granularity of 1 μs, in which case the PWM generator can maintain a counter beginning at zero and advancing in increments of 1 μs until it reaches 4000 μs, when it returns to zero. Thus, the amount of heat produced can be adjusted by adjusting the end count. A high end count corresponds to a greater length of time during which the microfabricated heater receives current and therefore a greater amount of heat produced. It would be understood that the granularity and signal width can take values other than those provided here without departing from the principles described herein.

Fluorescence Detection System, Including Lenses and Filters, and Multiple Parallel Detection for a Multi-Lane Cartridge

[0232] The detection system herein is configured to monitor fluorescence coming from one or more species involved in a biochemical reaction. The system can be, for example, an optical detector having a light source that selectively emits light in an absorption band of a fluorescent dye, and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof, as further described elsewhere herein. Alternatively, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality of different locations of, for example, a microfluidic substrate, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. The detector further has potential for 2, 3 or 4 color detection and is controlled by software, preferably custom software, configured to sample information from the detector.

[0233] The detection system described herein is capable of detecting a fluorescence signal from nanoliter scale PCR reactions. Advantageously, the detector is formed from inexpensive components, having no moving parts. The detector can be configured to couple to a microfluidic cartridge as further described herein, and can also be part of a pressure application system, such as a sliding lid on an apparatus in which the detector is situated, that keeps the cartridge in place.

[0234] FIGS. **29**-**31**B depict an embodiment of a highly sensitive fluorescence detection system that includes light emitting diodes (LED's), photodiodes, and filters/lenses for monitoring, in real-time, one or more fluorescent signals emanating from the microfluidic channel. The embodiment in FIGS. **29**-**31**B displays a two-color detection system having a modular design that couples with a single microfluidic channel found, for example, in a microfluidic cartridge. It would be understood by one skilled in the art that the description herein could also be adapted to create a detector that just detects a single color of light. FIGS. **31**A and **31**B show elements of optical detector elements **1220** including light sources **1232** (for example, light emitting diodes), lenses **1234**, light detectors **1236** (for example, photodiodes) and filters **1238**. The detector comprises two LED's (blue and red, respectively) and two photodiodes. The two LED's are configured to transmit a beam of focused light on to a particular region of the cartridge. The two photodiodes are configured to receive light that is emitted from the region of the cartridge. One photodiode is configured to detect emitted red light, and the other photodiode is configured to detect emitted blue light. Thus, in this embodiment, two colors can be detected simultaneously from a single location. Such a detection system can be configured to receive light from multiple microfluidic channels by being mounted on an assembly that permits it to slide over and across the multiple channels. The filters can be, for example, bandpass filters, the filters at the light sources corresponding to the absorption band of one or more fluorogenic probes and the filters at the detectors corresponding to the emission band of the fluorogenic probes.

[0235] FIGS. **32** and **33** show an exemplary read-head comprising a multiplexed 2 color detection system that is configured to mate with a multi-lane microfluidic cartridge. FIG. **32** shows a view of the exterior of a multiplexed read-head. FIG. **33** is an exploded view that shows how various detectors are configured within an exemplary multiplexed read head, and in communication with an electronic circuit board.

[0236] Each of the detection systems multiplexed in the assembly of FIGS. **32** and **33** is similar in construction to the embodiment of FIGS. **29**-**31**B. The module in FIGS. **32** and **33** is configured to detect fluorescence from each of 12 microfluidic channels, as found in, for example, the respective lanes of a 12-lane microfluidic cartridge. Such a module therefore comprises 24 independently controllable detectors, arranged as 12 pairs of identical detection elements. Each pair of elements is then capable of dual-color detection of a predetermined set of fluorescent probes. It would be understood by one of ordinary skill in the art that other numbers of pairs of detectors are consistent with the apparatus described herein. For example, 4, 6, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 pairs are also consistent and can be configured according to methods and criteria understood by one of ordinary skill in the art.

Detection Sensitivity, Time Constant and Gain

[0237] A typical circuit that includes a detector as described herein includes, in series, a preamplifier, a buffer/inverter, a filter, and a digitizer. Sensitivity is important so that high gain is very desirable. In one embodiment of the preamplifier, a very large, for example 100 GΩ, resistor is placed in parallel with the diode. Other values of a resistor would be consistent with the technology herein: such values typically fall in the range 0.5-100 GΩ, such as 1-50 GΩ, or 2-10 GΩ). An exemplary pre-amplifier circuit configured in this way is shown in FIG. **7**. Symbols in the figure have their standard meanings in electronic circuit diagrams.

[0238] The FIG. **34** shows a current-to-voltage converter/pre-amplifier circuit suitable for use with the detection system. D11 is the photodetector that collects the fluorescent light coming from the microfluidic channel and converts it into an electric current. The accompanying circuitry is used to convert these fluorescent currents into voltages suitable for measurement and output as a final measure of the fluorescence.

[0239] A resistor-capacitor circuit in FIG. **34** contains capacitor C45 and resistor R25. Together, the values of