

increase the rate of the binding of the detection reagents to immobilized assay components in the chambers, and iii) expelled from the detection chambers to the waste chambers 2540 A and B (FIG. 26, panels 2620-2622). Optionally, residual detection reagent solution is washed from the detection chambers 2550A and B by aspirating at the waste chamber vents with the reagent chamber B vent valve 2432B open (and, preferably, alternating opening reagent chamber B vent valve 2432B and air vent valve 2422 so as to segment the fluid stream) and then with air vent valve 2422 continuously open to draw the excess assay diluent into the waste chambers (FIG. 26, panels 2623-2625). Alternatively, washing can be accomplished using the wash reagent by repeating the steps in panels 2613-2616.

[0332] To provide an appropriate environment for the ECL measurement, detection chambers 2550A and 2550B are filled with the wash reagent (which preferably, is an ECL read buffer comprising an ECL coreactant). Accordingly, wash reagent is introduced into the detection chambers by operating the pump with reagent A chamber vent valve 2432A and waste chamber vent valves 2442A and/or 2442B open so as to aspirate wash reagent into sample conduit branches 2515A and 2515B. Operating the pump with air vent valve 2422 and waste chamber valves 2442A and/or 2442B open introduces slugs wash fluid into the detection chambers (FIG. 16, panels 2628-2631). The above assay is described for a two-step assay that employs two binding steps. An analogous protocol may be used for a one step protocol with one binding step, preferably, by omitting the steps in FIG. 26, panels 2617-2625. In the one step format, all the detection reagents used in the assay are, preferably, stored as dry reagents in sample conduit branches 2515A and 2515B so that they are reconstituted during passage of the sample through the branches. Optionally, reagent chamber B 2530B may be omitted.

[0333] Preferably, an ECL measurement is conducted by stimulating/firing working electrodes in the detection chamber. Preferably, the immobilized binding reagents of the detection chambers are immobilized on one or more working electrodes, more preferably on an array of electrodes, most preferably an array of electrodes configured to be fired in a pair-wise fashion (as described above). Electrical potential is applied to the working electrodes to stimulate ECL, preferably in the pair-wise fashion discussed above. The light so generated is detected using an optical detector, e.g., using a photodiode or the like. The cartridge and/or light detector may be moved during the pair-wise firing process so as to align the active electrode with the light detector. Optionally, an array of light detectors or a sufficiently large light detector is used so that movement of the cartridge and/or light detector is not required. Predefined assay-specific conversion parameters may be used to derive concentrations/results from the measured ECL counts; e.g., empirically derived from test data or computed from theoretical predictions/models. In particularly preferred embodiments different types of cartridges may have different electrode patterns but would preferably employ a common cartridge electrode contact pattern/area. Some of the electrode contacts may not be used for lower density cartridge formats.

[0334] A preferred sequence of operations that one embodiment of the cartridge reader may employ for firing each read location will now be described. The discussion will reference a photodiode as the optical detector but it should be understood that any suitable optical detector known in the art may be employed. The photodiode assembly (or alternatively,

the cartridge) is moved into position; e.g., to the appropriate side of the cartridge's electrode array. The cartridge is then positioned such that the first read location to be processed is brought into a predetermined alignment position with the photodiode (e.g., positioned in registered alignment) and electrical contact is made to the electrode contacts. Once the contact has been made, the reader preferably performs a diagnostic measurement to detect potential anomalies that may interfere with proper operation of the electrode array and/or its components (leads, contacts, electrodes, etc.). Anomalies that are preferably detected include manufacturing defects, surface bubbles, or the like. This diagnostic measurement may be accomplished by preferably applying either a 500 Hz AC voltage or a very low voltage (e.g., less than 100 mV), low current (e.g., less than 1 μ A) DC signal to the electrodes and measuring the surface capacitance. An appropriate predetermined algorithm could then be utilized to determine the presence and/or effect of any such anomalies; e.g., compare measured signal to fixed thresholds, or the like. Preferably, if anomalies are detected, the cartridge reader would record the error and proceed accordingly; e.g., if the anomaly is isolated to a particular electrode/electrode pair, the cartridge reader would skip reading this location and proceed to the next pair and/or next operation. Upon confirming operational status, ECL from the first pair of electrodes is initiated by application of a voltage waveform; data acquisition from the light detector is also begun. After completion of the ECL measurement, the cartridge/light detector are realigned to measure ECL from the second electrode pair and the ECL induction/measurement process is repeated. The cycle is repeated for each electrode pair to be analyzed.

[0335] In certain preferred embodiments, once a full set of data points has been acquired, the cartridge reader can either store the acquired data later retrieval/inspection, preferably on machine readable storage medium, and conclude the read cycle by performing the necessary finalization steps (detailed below) or can post-process, preferably performed in real-time, the acquired data and store either the post-processed data alone or in combination with the raw acquired data. Since it is often times important to inspect raw data (e.g., troubleshooting, diagnostics, data cleansing/filtering, and the like), where data is stored only in post-processed format, the corresponding parameters utilized in converting the data may be stored as well so that the raw acquired data can be computed/determined as needed. Alternatively, both the raw acquired data as well as the post-processed data may be stored. Still further, the raw acquired data may only be subjected to a subset of predetermined data conversion/analysis operations in real-time and stored for further post-processing offline, i.e., not in real time; post-processing can be performed by the cartridge reader itself or another device, e.g., a general purpose programmable computer.

[0336] In certain preferred embodiments employing ECL detection technology, data conversion/analysis operations may include one or more of: background subtraction; conversion to ECL counts; conversion of ECL counts to concentrations; and/or performance of quality checks on the acquired data. Since it is preferable that the resulting data set represents only the light generated by ECL background subtraction is employed to adjust the measured light to correct for the influence of ambient light or "background" signal. Background subtraction consists of subtracting the background signal from the photodiode signal.