

CLAIMS

1. A device for investigating a nucleic acid analyte characterised by comprising:

- a first zone comprising an attachment site to which the analyte is attached; a first pathway for causing a first fluid medium to flow over the attachment site thereby allowing the nucleic acid to be progressively pyrophosphorolysed into its constituent nucleoside triphosphates; a second pathway for the nucleoside triphosphates from around the attachment site and a means for creating in the second pathway a second medium comprised of aqueous microdroplets in a water-immiscible carrier;

- a microdroplet manipulation zone for manipulating the microdroplets using optically-mediated electrowetting comprised of:

- a first composite wall comprised of

- a first transparent substrate
- a first transparent conductor layer on the substrate having a thickness in the range 70 to 250nm;
- a photoactive layer activated by electromagnetic radiation in the wavelength range 400-1000nm on the conductor layer having a thickness in the range 300-1000nm and
- a first dielectric layer on the conductor layer having a thickness in the range 120 to 160nm;

- a second composite wall comprised of

- a second substrate;
- a second conductor layer on the substrate having a thickness in the range 70 to 250nm and
- a second dielectric layer on the conductor layer having a thickness in the range 120 to 160nm

in which the exposed surfaces of the first and second dielectric layers are disposed less than 10µm apart to define a microfluidic space adapted to contain microdroplets;

- an A/C source to provide a voltage across the first and second composite walls connecting the first and second conductor layers;
 - at least one source of first electromagnetic radiation having an energy higher than the bandgap of the photoactive layer adapted to impinge on the photoactive layer to induce corresponding ephemeral first electrowetting locations on the surface of the first dielectric layer;
 - means for manipulating the points of impingement of the electromagnetic radiation on the photoactive layer so as to vary the disposition of the ephemeral electrowetting locations thereby creating at least one first electrowetting pathway along which the microdroplets may be caused to move;
 - an detection zone disposed downstream of the microdroplet manipulation zone or integral therewith and
 - a fluorescence or Raman-scattering detection system comprising a source of second electromagnetic radiation adapted to impinge on the microdroplets in the detection zone and a detector for detecting fluorescence or Raman-scattering emitted therefrom.
2. A device according to Claim 1 characterised in that the detection zone comprises an array of electrowetting locations.
 3. A device according to Claim 1 characterised in that the detection zone comprises a block of capillary tubes through which the microdroplets pass the point(s) of impingement of the second electromagnetic radiation being the outlet(s) of the microfluidic channel(s).
 4. A device according to Claim 1 in which a bead is immobilised in a region over which discrete microdroplets are driven sequentially such that each droplet wets the bead for a period of time after which it de-wets or partially de-wets the bead.
 5. A device according to Claim 4 in which a molecule of DNA is attached to the bead and the pyrophosphorolytic degradation of the DNA on the bead takes place when the bead is in temporary contact with the aqueous droplet which is driven over it.
 6. A device according to any preceding claim characterised in the attachment site is adapted to receive a bead to which the analyte is attached.

7. A device according to any of Claims 4 to 6 characterised by further comprising upstream of the first zone a preparation zone for preparing the bead and in which the bead is moved from the preparation zone to the first zone by means of a second electrowetting pathway.
- 5 8. A device according to Claim 7 characterised in that the second electrowetting pathway is comprised of second electrowetting locations corresponding to dielectric-coated electrodes and/or dielectric-coated photoactive layers.
9. A device according to any preceding claim characterised in that the first and second composite walls further comprise first and second antifouling layers on respectively the first and second dielectric layers.
- 10 10. A device according to any preceding claim characterised in that the microfluidic space is further defined by a spacer attached to the first and second composite walls.
11. A device according to any preceding claim characterised in that the microfluidic space has depth from 2 to 8µm.
- 15 12. A device according to any preceding claim characterised by further comprising a plurality of first electrowetting pathway running concomitantly to each other.
13. A device according to Claim 12 characterised by further comprising a plurality of third electrowetting pathways adapted to intersect with the first electrowetting pathways to create at least one microdroplet-coalescing location.
- 20 14. A device according to any preceding claim in which a series of feeder channels of continuous fluid are interfaced to the microdroplet manipulation zone and are used to supply the input reagents which are subsequently dropletised and manipulated.
15. A device according to any preceding claim characterised in that the means for manipulating the points of impingement of the electromagnetic radiation on the photoactive layer comprises a microprocessor for creating some or all of the first, second and third
- 25 15. A device according to any preceding claim characterised in that the means for manipulating the points of impingement of the electromagnetic radiation on the photoactive layer comprises a microprocessor for creating some or all of the first, second and third electrowetting pathways and synchronising the movement of each microdroplet in the pathway(s) relative to all the others.
16. Use of a device according to any preceding claim to sequence DNA or RNA of natural or synthetic origin.