(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 July 2008 (31.07.2008)

PCT

(10) International Publication Number
WO 2008/090330 A1

(51) International Patent Classification:
G01N 15/14 (2006.01)

(21) International Application Number:
PCT/GB2008/000216

(22) International Filing Date: 22 January 2008 (22.01.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0701201.6 22 January 2007 (22.01.2007) GB

(71) Applicant *(for all designated States except US)*: **CANCER RESEARCH TECHNOLOGY LTD** [GB/GB]; Sardinia House, Sardinia Street, London, Greater London WC2A 3NL (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **VOJNOVIC, Borivoj** [GB/GB]; University of Oxford, Gray Cancer Institute, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR (GB). **BARBER, Paul R** [GB/GB]; University of Oxford, Gray Cancer Institute, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR (GB). **POPE, Iestyn** [GB/GB]; University of Oxford, Gray Cancer Institute, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR (GB). **SMITH, Paul James** [GB/GB]; Cardiff University, Heath Park, Cardiff, South Wales CF14 4XN (GB). **ERRINGTON, Rachel Jane** [GB/GB]; Cardiff University, Heath Park, Cardiff, South Wales CF14 4XN (GB).

(74) Agents: **WALKER, Edmund** et al.; Mewburn Ellis LLP, York House, 23 Kingsway, London, Greater London WC2B 6HP (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV,

[Continued on next page]

(54) Title: DETECTING OBJECTS

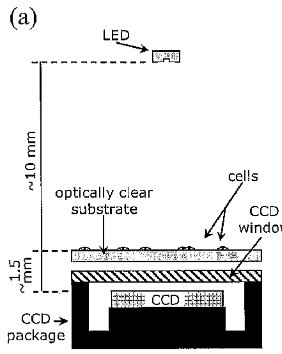

Figure 1a

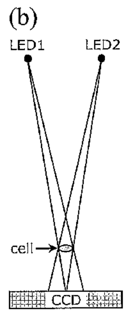

Figure 1b

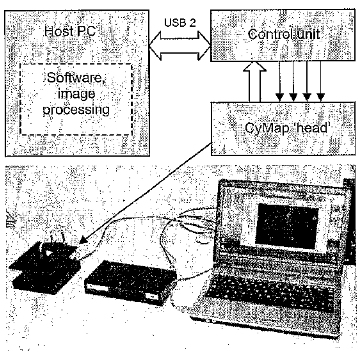

Figure 1c

(57) Abstract: The invention provides apparatus and methods for detecting objects in samples. The sample is held in the transmission path of light from a light source to a detector, whereby light from the light source interacts with objects in the sample. The patterns of light incident on the detector subsequent to its interaction with the objects are directly used to determine the presence of objects in the sample.

WO 2008/090330 A1

SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with amended claims*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

Date of publication of the amended claims: 2 October 2008

AMENDED CLAIMS
received by the International Bureau on
08 AUG 2008 (08.08.08)

1. Apparatus for detecting biological cells or bacteria in a sample, the apparatus comprising:

an incoherent light source;

a detector positioned to detect light from the light source;

a sample holding area spaced from the detector at which the sample can be held in the transmission path of light from the light source to the detector, whereby light from the light source interacts with cells or bacteria in the sample and the light is incident on the detector in the form of diffraction or interference light patterns subsequent to its interaction with the cells or bacteria; and

a processor for receiving an input from the detector, the input being indicative of the patterns of light incident on the detector.

2. Apparatus according to claim 1, comprising a plurality of light sources spaced from one another, light from each of the light sources being incident on the detector subsequent to passing through the sample holding area, and wherein the apparatus further comprises a light source controller for controlling the light sources such that not all of the light sources are emitting light simultaneously.

3. Apparatus according to claim 2, wherein the light source controller controls the light sources to emit light sequentially.

4. Apparatus according any one of the preceding claims, wherein the or each light source is a point-like or near point-like light source.

5. Apparatus according to claim 4, wherein the or each light source is apertured to a diameter or no more than 100μm.

6. Apparatus according to any one of the preceding claims, wherein the or each light source is a light emitting diode.

7. Apparatus according to any one of claims 1 to 5, wherein the or each light source is an optical fibre.

8. Apparatus according to any one of the preceding claims, wherein the detector is a pixelated detector.

9. Apparatus according to claim 8, wherein the detector is a CCD or CMOS detector.

10. Apparatus according to any one of the preceding claims, wherein the processor is arranged to process the input incident light patterns to determine the presence of a cell or bacterium within the sample.

11. Apparatus according to claim 10, wherein the processor is arranged to determine the presence of the cell or bacterium directly from the input incident light patterns.

12. Apparatus according to any one of the preceding claims, wherein the processor is arranged to determine the spatial location within the sample holding area of a detected cell or bacterium from the input incident light patterns.

13. Apparatus according to claim 12, wherein the spatial location is determined in two dimensions in a plane parallel to the detection plane of the detector.

14. Apparatus according to claim 12, wherein the spatial location is determined in three dimensions.

15. Apparatus according to claim 3, wherein the processor is arranged to determine the spatial location in three dimensions within the sample holding area of a detected cell or bacterium from the input incident light patterns.

16. Apparatus according to any one of the preceding claims, wherein the detector captures a series of two or more sequential light patterns over a period of time and the processor is arranged to analyse the series of light patterns to determine whether the location of a detected cell or bacterium within the sample holding area has changed.

17. Apparatus according to claim 16, wherein the processor is arranged to record a sequence of locations of a cell or bacterium within the sample holding space to determine the trajectory of the cell or bacterium.

18. Apparatus according to any one of the preceding claims, wherein the detector captures a series of two or more sequential light patterns over a period of time and the processor is arranged to analyse the series of light patterns to detect a change in the light pattern associated with a specific detected cell or bacterium, indicating a change in relation to a detected cell or bacterium other than a change in its location.

19. Apparatus according to claim 18, wherein the change is cell attachment or cell mitosis or cell death.

20. Apparatus according to any one of the preceding claims, wherein the processor is arranged to analyse the detected light patterns to count the number of detectable cells or bacteria in the sample.

21. Apparatus according to any one of the preceding claims, wherein the detected light is in the form of diffraction patterns.

22. Apparatus according to any one of the preceding claims, wherein the detected light is in the form of interference patterns.

23. A microfluidic platform comprising apparatus for detecting biological cells or bacteria according to any one of the preceding claims.

24. Apparatus for detecting biological cells or bacteria in a plurality of samples, the apparatus comprising a plurality of sample holders and a detecting apparatus according to any one of claims 1 to 21.

25. Apparatus according to claim 24, wherein the plurality of sample holders are provided by a multi-well plate.

26. Apparatus according to claim 24 or claim 25, comprising mean for moving the detecting apparatus relative to said plurality of sample holders to sequentially bring said sample holders into the sample holding area of the detecting apparatus.

27. Apparatus according to claim 24 or claim 25, comprising a plurality of detecting apparatus according top any one of claims 1 to 22, whereby a plurality of said sample holders can be located in respective sample holding areas of the detecting apparatus simultaneously.

28. A method for detecting biological cells or bacteria in a sample, the sample being held in a sample holding location in the transmission path of light from an incoherent light source to a detector, the sample holding location being spaced from the detector, whereby light from the light source interacts with cells or bacteria in the sample and the light is incident on the detector subsequent to its interaction with the cells or bacteria; the method comprising:

exposing the sample to light from the light source;

detecting at the detector incident diffraction or interference light patterns from the light source; and

determining the presence of cells or bacteria in the sample from the detected light patterns.

29. A method according to claim 28, wherein the sample is exposed sequentially to light from a plurality of light sources, the light from each of the light sources being incident on the detector subsequent to its interaction with cells or bacteria in the sample, the detected diffraction or interference light patterns being used to determine the spatial location of the or each detected cell or bacteria within the sample.

30. A method according to claim 28 or 29, wherein the or each light source is a point-like or near point-like light source.

31. A method according to claim 30, wherein the or each light source is apertured to a diameter of no more than 100µm.

32. A method according to any one of claims 28 to 31, wherein the or each light source is a light emitting diode.

33. A method according to any one of claims 28 to 31, wherein the or each light source is an optical fibre.

34. A method according to any one of claims 28 to 33, wherein the detector is a pixelated detector.

35. A method according to claim 34, wherein the detector is a CCD or CMOS detector.

36. A method according to any one of claims 28 to 35, wherein the presence of cells or bacteria within the sample is determined directly from the detected light patterns.

37. A method according to any one of claims 28 to 36, wherein a series of two or more sequential light patterns detected over a period of time are used to determine whether the location of a detected cell or bacterium within the sample has changed.

38. A method according to claim 37, comprising using a sequence of detected locations of a cell or bacterium within the sample to determine the trajectory of the cell or bacterium.

39. A method according to any one of claims 28 to 38, comprising capturing a series of two or more sequential diffraction or interference light patterns over a period of time and analysing the series of light patterns to detect a change in the light pattern associated with a specific detected cell or bacterium, indicating a change in relation to a detected cell or bacterium other than a change in its location.

40. A method according to claim 39, wherein the change is cell attachment or cell mitosis or cell death.

41. A method according to any one of claims 28 to 40, comprising analysing the detected light patterns to count the number of detectable cells or bacteria in the sample.