

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 November 2002 (28.11.2002)

PCT

(10) International Publication Number
WO 02/095070 A3

- (51) International Patent Classification⁷: **C12Q 1/68**
- (21) International Application Number: PCT/GB02/02345
- (22) International Filing Date: 20 May 2002 (20.05.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0112238.1 18 May 2001 (18.05.2001) GB
- (71) Applicant (for all designated States except US): **MEDICAL BIOSYSTEMS LITD.** [GB/GB]; The Old Mill, Beaston Cross, Broadhempston, Nr- Totnes, Devon TQ9 6BX (GB).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **DENSHAM, Daniel, Henry** [GB/GB]; The Old Mill, Beaston Cross, Broadhempston, Nr- Totnes, Devon TQ9 6BX (GB).
- (74) Agent: **JAPPY, John, William, Graham**; Gill Jennings & Every, Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report:
18 September 2003
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/095070 A3

(54) Title: POLYNUCLEOTIDE SEQUENCING METHOD

(57) Abstract: A method for determining the sequence of a polynucleotide comprising the steps of (i) contacting a polynucleotide processive enzyme immobilised in a fixed position, with a target polynucleotide under conditions sufficient to induce enzyme activity; (ii) detecting an effect consequent on the interaction of the enzyme and the polynucleotide, wherein the effect is detected by measurement of a non-linear optical signal or a linear signal coupled to a non-linear signal.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/02345

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 53805 A (ARMES NIAL ANTONY ;STEMPLE DEREK LYLE (GB); ASM SCIENT INC (US)) 14 September 2000 (2000-09-14)	1-7,9, 13-17, 19,21, 23,25
Y	abstract page 3, paragraph 3 -page 4, paragraph 3 page 14, paragraph 1 -page 19, paragraph 2 claims 1-23 --- -/--	8,10-12, 18,20, 22,24

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

31 July 2003

Date of mailing of the international search report

12/08/2003

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Madlener, M

INTERNATIONAL SEARCH REPORT

Inte sional Application No

PCT/GB 02/02345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	<p>WO 01 16375 A (RUBENS DENISE ;US HEALTH (US); SCHNEIDER THOMAS D (US)) 8 March 2001 (2001-03-08)</p> <p>abstract page 3, line 36 -page 6, line 39 examples 1,2,4 figures 1,2 claims 1-45</p> <p>---</p>	<p>1,2,5-7, 9,16,17, 20,23,25 3,4,8, 10-15, 18,19, 21,22,24</p>
X Y	<p>WO 00 70073 A (CORNELL RES FOUNDATION INC) 23 November 2000 (2000-11-23)</p> <p>abstract page 5, line 11 -page 7, line 23 page 18, line 7 -page 18, line 32 page 29, line 22 -page 32, line 24 claims 1-61 figure 1C</p> <p>---</p>	<p>1-4,9, 13,14, 16,17, 19,20, 23,25 5-8, 10-12, 15,18, 21,22,24</p>
X Y	<p>WO 01 25480 A (DENSHAM DANIEL HENRY ;MEDICAL BIOSYSTEMS LTD (GB)) 12 April 2001 (2001-04-12)</p> <p>abstract page 3, line 10 -page 5, line 19 page 8, line 33 -page 9, line 33 claims 1-21</p> <p>---</p>	<p>1,2,5-7, 9,16,17, 23,25 3,4,8, 10-15, 18-22,24</p>
X Y	<p>WO 99 05315 A (DENSHAM DANIEL HENRY ;MEDICAL BIOSYSTEMS LTD (GB)) 4 February 1999 (1999-02-04) cited in the application</p> <p>abstract page 2, line 26 -page 3, line 24 page 4, line 10 -page 6, line 2 claims 1-29</p> <p>---</p> <p style="text-align: center;">-/--</p>	<p>1,2,9, 16,17</p> <p>3-8, 10-15, 18-25</p>

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/02345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 95 06138 A (UNIV CALIFORNIA) 2 March 1995 (1995-03-02) abstract page 3, last paragraph -page 6 page 10, last paragraph -page 14 claims 12-19 ---	1,2,9, 16,17 3-8, 10-15, 18-25
X Y	WO 00 60114 A (DENSHAM DANIEL HENRY ;MEDICAL BIOSYSTEMS LTD (GB)) 12 October 2000 (2000-10-12) abstract page 1, line 9 -page 1, line 33 claims 1-6 ---	1,2,10, 16,17 3-9, 11-15, 18-25
X Y	LEMON KATHERINE P ET AL: "Localization of bacterial DNA polymerase: Evidence for a factory model of replication." SCIENCE (WASHINGTON D C), vol. 282, no. 5393, 20 November 1998 (1998-11-20), pages 1516-1519, XP002248280 ISSN: 0036-8075 cited in the application the whole document - in particular Fig. 1 in combination with the summary of ref. 6 (p. 1519, left col.) ---	24 22
Y	EP 0 740 156 A (POLYTECH TRANSFER LTD) 30 October 1996 (1996-10-30) claims 1-7 column 5, line 41 -column 6, line 2 column 4, line 23 -column 4, line 49 column 2, line 27 -column 3, line 55 column 1, line 5 -column 1, line 16 abstract ---	1-25
Y	WO 01 23459 A (ARRAY BIOSCIENCE CORP) 5 April 2001 (2001-04-05) abstract page 9, line 24 -page 12, line 7 page 54, line 13 -page 54, line 18 claims 1-64 ---	1-25
Y	WO 00 36152 A (WILLIAMS JOHN G K ;LI COR INC (US)) 22 June 2000 (2000-06-22) abstract page 2, line 28 -page 4, line 12 claims 1-30 ---	1-25
	---	-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/02345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 958 696 A (CRUTE JAMES J) 28 September 1999 (1999-09-28) abstract column 2, line 3 - line 35 claims 1-10 ---	10-12
Y	JENKINS R H ET AL: "A novel Raman spectrophotometric method for quantitative measurement of nucleoside triphosphate hydrolysis." BIOSPECTROSCOPY, vol. 5, no. 1, 1999, pages 3-8, XP009014146 ISSN: 1075-4261 the whole document ---	1-25
Y	CAMPAGNOLA PAUL J ET AL: "High-resolution nonlinear optical imaging of live cells by second harmonic generation." BIOPHYSICAL JOURNAL, vol. 77, no. 6, December 1999 (1999-12), pages 3341-3349, XP002248209 ISSN: 0006-3495 the whole document ---	1-25
Y	PELEG G ET AL. : "Gigantic optical non-linearities from nanoparticle-enhanced molecular probes with potential for selectively imaging the structure and physiology of nanometric regions in cellular systems" BIOIMAGING, vol. 4, 1996, pages 215-224, XP001118469 cited in the application the whole document ---	1-25
A	WO 99 44045 A (MASSACHUSETTS INST TECHNOLOGY) 2 September 1999 (1999-09-02) page 3, line 19 -page 5, line 26 abstract ---	1-25
A	PELEG G ET AL: "Nonlinear optical measurement of membrane potential around single molecules at selected cellular sites." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA. UNITED STATES 8 JUN 1999, vol. 96, no. 12, 8 June 1999 (1999-06-08), pages 6700-6704, XP002248210 ISSN: 0027-8424 the whole document ---	1-25
	--- -/--	

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/GB 02/02345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LEWIS A ET AL. : "Second-harmonic generation of biological interfaces: probing the membrane protein bacteriorhodopsin and imaging membrane potential around GFP molecules at specific sites in neuronal cells of C. elegans" CHEMICAL PHYSICS, vol. 245, 1999, pages 133-144, XP002248211 cited in the application abstract	1-25
A	--- SCHULTZ S ET AL: "Single-target molecule detection with nonbleaching multicolor optical immunolabels" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 97, no. 3, 1 February 2000 (2000-02-01), pages 996-1001, XP002227348 ISSN: 0027-8424 the whole document	1-25
A	--- PUPPELS G J ET AL: "CONFOCAL RAMAN MICROSCOPY IN BIOLOGY: APPLICATIONS AND FUTURE DEVELOPMENTS" TRAC, TRENDS IN ANALYTICAL CHEMISTRY, ANALYTICAL CHEMISTRY. CAMBRIDGE, GB, vol. 10, no. 8, 1 September 1991 (1991-09-01), pages 249-253, XP000219626 ISSN: 0165-9936 the whole document	1-25
A	--- NIE S ET AL. : "Probing Single Molecules and Single Nanoparticles by Surface-Enhanced Raman Scattering" SCIENCE, vol. 275, 21 February 1997 (1997-02-21), pages 1102-1106, XP002248212 the whole document	1-25
A	--- HA T ET AL: "Probing the interaction between two single molecules: Fluorescence resonance energy transfer between a single donor and a single acceptor" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 13, 1996, pages 6264-6268, XP002171249 ISSN: 0027-8424 the whole document	1-25
	--- -/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/02345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MCGILP J F: "A REVIEW OF OPTICAL SECOND-HARMONIC AND SUM-FREQUENCY GENERATION AT SURFACES AND INTERFACES" JOURNAL OF PHYSICS D. APPLIED PHYSICS, IOP PUBLISHING, BRISTOL, GB, vol. 29, no. 7, 14 July 1996 (1996-07-14), pages 1812-1821, XP000621099 ISSN: 0022-3727 the whole document -----	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/02345

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0053805	A	14-09-2000	AU 3174600	A 28-09-2000
			CA 2366112	A1 14-09-2000
			EP 1159453	A1 05-12-2001
			WO 0053805	A1 14-09-2000
			JP 2002537858	A 12-11-2002
WO 0116375	A	08-03-2001	AU 7086800	A 26-03-2001
			WO 0116375	A2 08-03-2001
WO 0070073	A	23-11-2000	AU 5027600	A 05-12-2000
			CA 2373385	A1 23-11-2000
			EP 1179085	A1 13-02-2002
			JP 2002543847	T 24-12-2002
			WO 0070073	A1 23-11-2000
			US 2003044781	A1 06-03-2003
			US 2003092005	A1 15-05-2003
WO 0125480	A	12-04-2001	AU 7546700	A 10-05-2001
			BR 0014468	A 20-08-2002
			CA 2386115	A1 12-04-2001
			CN 1391615	T 15-01-2003
			EP 1226271	A2 31-07-2002
			WO 0125480	A2 12-04-2001
			JP 2003511043	T 25-03-2003
WO 9905315	A	04-02-1999	AT 225858	T 15-10-2002
			AU 735898	B2 19-07-2001
			AU 8455998	A 16-02-1999
			BR 9812270	A 18-07-2000
			CN 1265158	T 30-08-2000
			DE 69808661	D1 14-11-2002
			DE 69808661	T2 31-07-2003
			DK 1017848	T3 10-02-2003
			EP 1229133	A2 07-08-2002
			EP 1017848	A2 12-07-2000
			ES 2183394	T3 16-03-2003
			WO 9905315	A2 04-02-1999
			JP 2001511358	T 14-08-2001
			NZ 502557	A 01-02-2002
			NZ 515315	A 30-06-2003
			PT 1017848	T 28-02-2003
			RU 2198221	C2 10-02-2003
WO 9506138	A	02-03-1995	WO 9506138	A1 02-03-1995
			US 5620854	A 15-04-1997
WO 0060114	A	12-10-2000	AU 3978100	A 23-10-2000
			BR 0009529	A 29-01-2002
			CA 2367277	A1 12-10-2000
			CN 1345379	T 17-04-2002
			EP 1198590	A2 24-04-2002
			WO 0060114	A2 12-10-2000
			JP 2002540800	T 03-12-2002
EP 0740156	A	30-10-1996	CA 2174360	A1 28-10-1996
			DE 69615254	D1 25-10-2001
			EP 0740156	A1 30-10-1996
			JP 8320322	A 03-12-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/02345

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0123459	A	05-04-2001	AU 7616100 A	30-04-2001
			AU 7714800 A	30-04-2001
			AU 7716500 A	30-04-2001
			EP 1221047 A1	10-07-2002
			EP 1222432 A1	17-07-2002
			EP 1242510 A1	25-09-2002
			JP 2003510065 T	18-03-2003
			JP 2003510552 T	18-03-2003
			JP 2003510607 T	18-03-2003
			TW 445362 B	11-07-2001
			WO 0123822 A1	05-04-2001
			WO 0123459 A1	05-04-2001
			WO 0123888 A1	05-04-2001
			US 2001053521 A1	20-12-2001
			US 2002132371 A1	19-09-2002
US 2001054495 A1	27-12-2001			
WO 0036152	A	22-06-2000	AU 2180200 A	03-07-2000
			AU 2360200 A	03-07-2000
			CA 2355816 A1	22-06-2000
			EP 1141409 A1	10-10-2001
			JP 2002532104 T	02-10-2002
			WO 0036151 A1	22-06-2000
			WO 0036152 A1	22-06-2000
			US 6255083 B1	03-07-2001
			US 6232075 B1	15-05-2001
			US 2001018184 A1	30-08-2001
US 2002115076 A1	22-08-2002			
US 5958696	A	28-09-1999	NONE	
WO 9944045	A	02-09-1999	US 2002150938 A1	17-10-2002
			WO 9944045 A1	02-09-1999