

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
6 May 2005 (06.05.2005)

PCT

(10) International Publication Number  
**WO 2005/040755 A3**

(51) International Patent Classification<sup>7</sup>: **G01N 33/00**

(21) International Application Number:  
PCT/US2004/034844

(22) International Filing Date: 20 October 2004 (20.10.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/513,042 20 October 2003 (20.10.2003) US

(71) Applicant (for all designated States except US): **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, 5th Floor, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HELLER, Michael** [US/US]; 1614 Hawkview Drive, Encinitas, CA 92024 (US). **SULLIVAN, Benjamin** [US/US]; 9152 Regents Road, Apt. E, La Jolla, CA 92037 (US). **ZLATANOVIC, Sanja** [YU/US]; 9248 Regents Road, Apt. B, La Jolla, CA 92037 (US). **ESENER, Sadik** [US/US]; 743 San Marino, Solana Beach, CA 92075 (US). **DEHLINGER, Dietrich** [US/US]; 3440 Lebon Drive, Apt. 4203, San Diego, CA 92122 (US).

(74) Agent: **HALL, David, A.**; Heller Ehrman LLP, 4350 La Jolla Village Drive, San Diego, CA 92122-1246 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, 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, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, 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, HU, IE, IT, LU, MC, NL, 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
- 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:  
15 December 2005

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.

(54) Title: NANOSCALE TRANSDUCTION SYSTEMS FOR DETECTING MOLECULAR INTERACTIONS

(57) Abstract: The present invention relates to nanoscale transduction systems that produce reversible signals to facilitate detection. In one respect, the invention relates to the analysis of molecular binding events using higher order signaling nanoscale constructs, or "nanomachines", that allow nanostructures to be individually detectable, even in the midst of high background noise. Such systems are particularly useful for improving the performance of rare target detection methods, as well as being generally useful in any field in which sensitivity, discrimination and confidence in detection are important.



WO 2005/040755 A3

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/34844

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : G01N 33/00 US CL : 435/6, 287.2; 436/94; 536/23.1 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/6, 287.2; 436/94; 536/23.1 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 practicable, search terms used) EAST (USPAT, USPGP, EPO, JPO, Derwent)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/0127574 A1 (Mirkin et al.) 12 September 2002 (12.09.2002), see paragraph 0178.	1, 2, 137, and 138
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"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	
"E" earlier application or patent published on or after the international filing date	"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	
"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)	"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	
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
31 August 2005 (31.08.2005)	21 OCT 2005	
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450	Authorized officer Bradley L. Sisson	
Facsimile No. (703) 305-3230	Telephone No. (571) 272-0500	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/34844

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1,2,137 and 138

- Remark on Protest**
- ☐ The additional search fees were accompanied by the applicant' s protest and, where applicable, the payment of a protest fee.
  - ☐ The additional search fees were accompanied by the applicant' s protest but the applicable protest fee was not paid within the time limit specified in the invitation.
  - ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/34844

### BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-52 and 137-148, drawn to a method.

Group II, claim(s) 53-79, drawn to an apparatus.

Group III, claim(s) 80-100, drawn to a method.

Group IV, claim(s) 116-136, drawn to signaling nanostructure and a related kit.

Group V, claim(s) 149-155, drawn to metallic nanoparticle.

Group VI, claim(s) 156-174, drawn to method for identifying target nucleic acid.

Group VII, claim(s) 175-176, drawn to diagnostic profile.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

The invention of Group I has the following 5,184,000 species:

1. The applied energy is
  - a. Electric field
    - b. DC field
    - c. AC field
    - d. Capacitive field
    - e. Thermal
    - f. Electrical
    - g. Chemical
    - i. ATP
  - ii. NADH
  - h. Photonic
  - i. Magnetic
  - j. Kinetic
  - k. Acoustic

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/34844

- l. Ultrasonic
- m. Microwave
- n. Radiative
2. Reversible alteration is:
  - a. Deformation
    - i. Elastic
    - ii. Inelastic
    - iii. Plastic
  - b. Angular motion
  - c. Separation distance
  - d. A rotation
  - e. A linear displacement
  - f. Helical motion
  - g. In response to shear force
  - h. In response to pressure
3. Interaction is
  - a. Resonant energy
    - i. Dipole coupling
    - ii. Quadrapole coupling
    - iii. Fluorescence resonance energy transfer
  - b. Plasmonic
  - c. Near field coupling
  - d. Photonic
  - e. Capacitive
  - f. Magnetic
  - g. Electrostatic
4. Method further comprises detecting a changed characteristic resulting from the interaction, wherein the characteristic is
  - a. A variation in luminescence
  - b. A variation in fluorescence
  - c. A variation in optical properties
  - d. Color
  - e. A magnetic field
  - f. An electric field
  - g. Raman scattering (SERS)
  - h. Raman spectra
5. The reversibly altering interaction occurs:
  - a. In a solution
  - b. At a fixed location for which there is no a priori knowledge
  - c. In a homogeneous assay
  - d. In a heterogeneous assay
  - e. In an in situ assay
  - f. At a fixed location for which there is a priori knowledge
6. Method is performed on
  - a. A microarray
  - b. A nanoarray
7. Target is
  - a. A nucleic acid
  - b. A protein
  - c. Inorganic surface
  - d. Genomic nucleic acid
  - e. Antigen and wherein the first and second target binding elements are antibodies that bind to the antigen
8. Detecting a target in a biological sample comprising
  - a. Cells on a microscope slide
    - i. Target is nucleic acid
  - b. Tissue on a microscope slide
    - i. Target is nucleic acid
9. The nanostructure further comprises a first target binding region having a target binding element attached thereto that is selective for a predetermined target; and wherein the associated structure further comprises a second target binding region having attached thereto a second target binding element that is selective for the same predetermined target
  - a. The first and second target binding elements are oligonucleotides
10. Method is adapted to be performed in solution, and wherein the method is performed without removing the nanostructure or the associated structure from the solution.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/34844

The apparatus of Group II comprises the following 9,408 species:

1. Nanostructure is a:
  - a. Quantum dot
  - b. Semiconductor nanoparticle
  - c. Photonic crystal
  - d. Metallic nanoparticle
  - e. Ceramic nanoparticle
  - f. Polymeric nanoparticle
  - g. Nanotube
2. Associated structure is:
  - a. Quantum dot
  - b. Semiconductor nanoparticle
  - c. Photonic crystal
  - d. Metallic nanoparticle
  - e. Ceramic nanoparticle
  - f. Polymeric nanoparticle
  - g. Nanotube
3. Apparatus further comprises:
  - a. Fluorophore
  - b. Quencher
  - c. Chromophores
  - d. Phycobillic protein
  - e. Lumiphore
  - f. Fluorescent protein
4. Apparatus further comprises interaction element attached to:
  - a. The nanostructure
  - b. The associated structure
5. Interaction element is
  - a. Pressure responsive element
  - b. Displacement amplifying element
6. Nanostructure attached thereto a fluorescent donor, and wherein the associated structure further comprises a fluorescent quencher
7. The nanostructure and the associated structure have attached thereto individual members of a fluorescent energy transfer (FET) pair
8. The nanostructure and the associated structure are adapted to reversibly and spatially independently interact with each other and a target
  - a. In solution
  - b. On a surface
  - i. Microarray
  - ii. Nanoarray

The invention of Group III comprises the following 192 species:

1. Driving force is
  - a. Photonic
  - b. Electrical
  - c. Thermal
  - d. Magnetic
  - e. Periodic
  - f. A series of impulses
  - g. An impulse
  - h. Constant
2. The information signal is a variation in
  - a. Fluorescence of the nanostructure, associated structure, and target combinations
  - b. Color of the nanostructure, associated structure and target combinations
  - c. Temperature of the nanostructure, associated structure and target combinations
  - d. Electric field strength of the nanostructure, associated structure and target combinations
  - e. Magnetic field strength of the nanostructure, associated structure and target combinations
  - f. Frequency of a characteristic of the nanostructure, associated structure and target combinations
3. Processing the detected information signal
  - a. To classify a molecular binding event
  - b. Utilizing neural networks
  - c. Utilizing Bayesian networks

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/34844

- d. Utilizing MAP detection
- 4. Method further comprising applying a driving force that produces reversibly-altering interaction between a nanostructure, an associated structure, and a target comprising the information signal.

The System of Group IV comprises the following 48 species:

- 1. Imparted energy is
  - a. Photonic
  - b. Electrical
  - c. Thermal
  - d. Magnetic
  - e. Periodic
  - f. A series of impulses
  - g. An impulse
  - h. Constant
- 2. Transduced output is a variation
  - a. Fluorescence of the nanostructure, associated structure, and target combinations
  - b. Color of the nanostructure, associated structure and target combinations
  - c. Temperature of the nanostructure, associated structure and target combinations
  - d. Electric field strength of the nanostructure, associated structure and target combinations
  - e. Magnetic field strength of the nanostructure, associated structure and target combinations
  - f. Frequency of a characteristic of the nanostructure, associated structure and target combinations

The signaling nanostructure and related kit of Group V comprises the following 105 species:

- 1. The signal influencing element is a signal inhibiting element
- 2. The signaling nanostructure is fluorescent and the signal inhibiting element is a fluorescent quencher
- 3. The target binding region and the signal influencing region are asymmetrically patterned on the surface of the signaling nanostructure
- 4. Nanostructure comprises only one target binding region
- 5. The signal influencing element is a metallic nanoparticle
- 6. Target binding element is
  - a. An oligonucleotide
  - b. An antibody
  - c. A polypeptide
- 7. The signaling nanostructure and the signal influencing element are attached via the target binding element
- 8. The signal influencing element further comprises at least one target binding region, and wherein the signaling nanostructure further comprises a second signal influencing element attached thereto
- 9. Tethering group is a
  - a. Synthetic polymer
  - b. Single stranded nucleic acid
  - c. Fatty acid
  - d. Glycosaminoglycan
  - e. Polypeptide

Metallic nanoparticles of Group VI comprise the following 12 species of:

- 1. Signaling element is a
  - a. Quantum dot
  - b. Fluorophore
  - c. FRET donor
  - d. FRET acceptor
- 2. Target binding element is a/an
  - e. Antibody
  - f. Nucleic acid
  - g. Polypeptide

Method of identifying a nucleic acid of Group VII comprises the following 97 species:

- 1. Target nucleic acid molecule is
  - a. DNA
  - b. RNA
- 2. First nucleic acid probe is
  - a. DNA
  - b. RNA
- 3. Second nucleic acid probe is

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US04/34844

- a. DNA
  - b. RNA
  - 4. Pulse electric field is
    - a. Alternating
    - b. Direct current
  - 5. Signaling element is nanoparticle, which comprises:
    - a. Polymer bead
    - b. Quantum dot
    - c. Gold particle
  - 6. Sample associated with solid support
    - a. Array
    - b. Microarray
- Amplifying target nucleic acid

The inventions listed as Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The methods, apparatus, system, and kit are all linked through the presence or use of nanoparticles. US Patent Application Publication US 2002/0127574 A1 teaches the development and use of nanoparticles in forming conjugates with oligonucleotides. Paragraph 0178 teaches of metallic and semiconductor nanoparticles, the presence of fluorescent moieties, and the detection of target molecules, such as nucleic acids. Accordingly, the inventions are not so linked by a special technical feature so as to have unity of invention under PCT Rule 13.1.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The species set forth above are all found within Groups I-VII, which, for reasons set forth above, lack unity of invention.