

001  
10/02  
2042  
3096 PCT

**CORRECTED  
VERSION\***

5-5361



WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

**PCT**

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : <b>C12Q 1/66, 1/00, C12M 1/00, G01N 33/53, 21/76, 15/06</b>		<b>A1</b>	(11) International Publication Number: <b>WO 97/20947</b>
(21) International Application Number: <b>PCT/US96/19472</b>		(43) International Publication Date: <b>12 June 1997 (12.06.97)</b>	
(22) International Filing Date: <b>5 December 1996 (05.12.96)</b>		(81) Designated States: CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: <b>007,982 5 December 1995 (05.12.95) US</b>		Published <i>With international search report.</i>	
(71) Applicant (for all designated States except US): <b>UNIVERSITY OF UTAH RESEARCH FOUNDATION (US/US); 210 Park Building, Salt Lake City, UT 84112 (US).</b>			
(72) Inventors; and (75) Inventors/Applicants (for US only): <b>ANDRADE, Joseph, D. [US/US]; 6009 Highland Drive, Salt Lake City, UT 84121 (US). WANG, Chung-Yih [-]; 7327 South Spring Crest Court, Midvale, UT 84047 (US). HLADY, Vladimir [HR/HR]; 3091 South 3340 East, Salt Lake City, UT 84109 (US). TRIOLO, Philip, M. [US/US]; 148 South 1200 East, Salt Lake City, UT 84102 (US). SCHEER, Robert, J. [US/US]; 963 East Wilson Avenue, Salt Lake City, UT 84105 (US).</b>			
(74) Agents: <b>BOND, Laurence, B; et al.; Trask, Britt &amp; Rossa, P.O. Box 2550, Salt Lake City, UT 84110 (US).</b>			

(54) Title: **METHOD OF MEASURING CHEMICAL CONCENTRATION BASED ON SPATIAL SEPARATION AND RESOLUTION OF LUMINESCENCE**

(57) Abstract

A method and associated apparatus for measuring chemical concentration in a liquid sample based on spatial separation and resolution of light is disclosed. The method is preferably applied to sensitive, quantitative, luminescence-based biosensors which reads the analyte concentration via spatial distribution of the emitted light. The detection of light is used to assess the spatial position, rather than the intensity or wavelength, of emitted light. A bioluminescent or chemiluminescent reaction requiring, for example, ATP, NADPH or NADH as a specific, and sensitive co-factor is used. ATP or NADH concentration is modulated, "tuned" and/or regulated via, for example, an enzyme which consumes (consumase) ATP, NADPH, or NADH, thereby producing a spatial distribution of ATP or NADH and a spatial distribution in the emitted light. By appropriate control of the consumase or "synthase" activity and kinetics, a sensitive, specific, and easy readable luminescent pattern is produced, permitting detection. The method is applicable to a wide range of analytes, biochemicals and substrates by use of additional substrate-specific enzymes dependent on ATP concentration for their activity. The figure graphically depicts a single channel ATP sensor, where a specific ATP sample (22) is contacted with the ATP consumase immobilized in or on a hydrophilic gel matrix (24) causing a lower concentration of ATP to enter the transduction region (26) and react with, for example luciferase and luciferin, photons emitted and detected by operator (28).

