## (19) World Intellectual Property **Organization**

International Bureau





(43) International Publication Date 24 July 2003 (24.07.2003)

PCT

(10) International Publication Number WO 2003/060477 A3

(51) International Patent Classification<sup>7</sup>:

G01N 33/53

English

(21) International Application Number:

PCT/US2003/000777

(22) International Filing Date: 10 January 2003 (10.01.2003)

(25) Filing Language:

English (26) Publication Language:

(30) Priority Data:

60/347,625 10 January 2002 (10.01.2002) US

(71) Applicant (for all designated States except US): THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, 12th floor, Oakland, CA 94607-5200 (US).

(71) Applicants and

- (72) Inventors: KLEINFELD, David [US/US]; 6190 Inspiration Way, La Jolla, CA 92037 (US). SQUIER, Jeffrey [US/US]; 10584 Wincheck Road, San Diego, CA 92131 (US). TSAI, Philbert [US/US]; 3735 Miramar Street, Apartment F, La Jolla, CA 92037 (US).
- (74) Agents: STEFFEY, Charles, E. et al.; Schwegman, Lundberg, Woessner & Kluth, P.O. Box 2938, Minniapolis, MN 55402 (US).

- (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, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, 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, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, 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: 3 June 2004

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: ITERATIVE OPTICAL BASED HISTOLOGY

(57) Abstract: Femtosecond laser pulses are used to iteratively cut and image fixed as well as exsanguinated fresh tissue. Such images help to automate three-dimensional histological analysis of biological tissue. Cuts are accomplished with approximately 0.3 to 100 microJoule pulses to ablate tissue with one-micrometer precision. Permeability, immunoreactivity, and optical clarity of the remaining tissue is retained after pulsed laser cutting. Samples from transgenic mice that express fluorescent proteins retained their fluorescence to within micrometers of the cut surface.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/00777

		101/0503/0	
A. CLASSIFICATION OF SUBJECT MATTER  IPC(7) : G01N 33/53  US CL : 435/7.1  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) U.S.: 435/7.1			
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			
C. DOCUMENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim No.
X	US 6,166,385 A (WEBB et al) 26 December 2000 (26.12.2000), see entire document		1-12, 19, 21
 Y			20, 22, 23, 24, 28-57
X, P	X, P US 6,344,653 B1 (WEBB et al) 05 February 2002 (05.02.2002), see entire documents		1-12, 19, 21
			13-18, 20, 22, 23, 24, 28-57
Y 	-		nt 1-4, 11-20, 23, 24, 28, 48
A			5-10, 21,22, 25-27, 29- 47, 49-57
Y US 5,720,894 A (NEEV et al) 24 February 1998 (24.		.02.1998), see entire document	23-24, 28-57
Y	US 6,665,556 B1 (ALFANO et al) 16 December 2003 (16.12.2003), see entire document		23-24, 28-57
Further documents are listed in the continuation of Box C. See patent family annex.			
* Special categories of cited documents: "T" later document published after the international filing date or price			the international filing date or priority
	defining the general state of the art which is not considered to be lar relevance	date and not in conflict with the principle or theory underlying t	application but cited to understand the
"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)  "O" document referring to an oral disclosure, use, exhibition or other means		"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	
Date of the actual completion of the international search 04 March 2004 (04.03.2004)		Date of mailing of the international search resert  22 APR 2004	
NY 1 11 Annual Col. TOA (TTO			
Mail Stop PCT, Attn: ISA/US Commissioner for Patents		Nelson Fange Bell Harrefu	
P.O. Box 1450 Alexandria, Virginia 22313-1450 Telephone No. (571) 272-1600			
Facsimile No. (703) 305-3230			