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- (71) **Applicant:** LI-COR, INC. [US/US]; 4647 Superior Street, Lincoln, NE 68504 (US).
- (72) **Inventors:** DRANEY, Daniel, R.; c/o Li-Cor, Inc., 4647 Superior Street, Lincoln, NE 68504 (US). VOLCHECK, William, M.; c/o Li-Cor, Inc., 4647 Superior Street, Lincoln, NE 68504 (US). SCHAEPE, Katie; c/o Li-Cor, Inc., 4647 Superior Street, Lincoln, NE 68504 (US). ELITZIN, Vassil; c/o Li-Cor, Inc., 4647 Superior Street, Lincoln, NE 68504 (US). KOVAR, Joy; c/o Li-Cor, Inc., 4647 Superior Street, Lincoln, NE 68504 (US).
- (74) **Agents:** SNYDER, Joseph, R. et al.; Kilpatrick Townsend and Stockton LLP, Two Embarcadero Center, Eighth Floor, San Francisco, CA 94111 (US).
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[Continued on next page]

(54) Title: IR DYES FOR FLUORESCENCE IMAGING

FIG. 2A

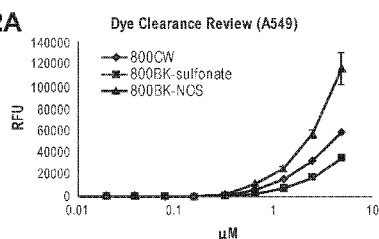


FIG. 2B

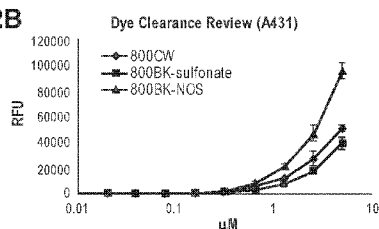
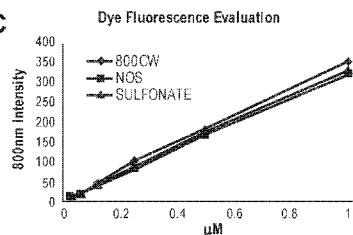


FIG. 2C



(57) **Abstract:** A method for organ imaging, comprising: administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy) cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate or 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-(4-sulfonatophenoxy) cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonato-butyl)-3H-indol-1-ium-5-sulfonate. In one embodiment, the organ includes one or more of kidney, bladder, liver, gall bladder, spleen, intestine, heart, lungs and muscle.



DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT,
LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE,
SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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IR DYES FOR FLUORESCENCE IMAGING

5 CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/084,971, filed November 26, 2014, the teachings of which are hereby incorporated by reference in their entities for all purposes.

10 FIELD OF THE INVENTION

[0002] The present disclosure provides for methods of fluorescence imaging.

BACKGROUND OF THE INVENTION

[0003] Conventional laparoscopic and robotic laparoscopic techniques are both rapidly
15 growing practices in the fields of colorectal and gynecologic surgery. Recent studies as described in Simorov *et al.*, "Laparoscopic colon resection trends in utilization and rate of conversion to open procedure: a national database review of academic medical centers" *Ann Surg.* 2012 256(3), 462-468; Wright *et al.*, "Robotically assisted vs laparoscopic hysterectomy among women with benign gynecologic disease" *JAMA* 2013 309(7), 689-698;
20 and, Park *et al.*, "Ureteral injury in gynecologic surgery: a 5-year review in a community hospital" *Korean J. Urol.* 2012 53(2), 120-125, report 42% of all colorectal resections and greater than 30% of gynecologic procedures are attempted in this manner.

[0004] A laparoscopic approach offers several advantages over traditional open abdominal surgery, including decreased postoperative pain, shorter hospital length of stay, fewer
25 surgical site infections, and decreased overall hospital cost, as described in Kiran *et al.*, "Laparoscopic approach significantly reduces surgical site infections after colorectal surgery: data from national surgical quality improvement program" *J Am Coll Surg.* 2010 211(2) 232-238; Bilimoria *et al.*, "Laparoscopic-assisted vs. open colectomy for cancer: comparison of short-term outcomes from 121 hospitals" *J Gastrointest Surg.* 2008 12(11) 2001-2009; Juo *et al.*, "Is Minimally Invasive Colon Resection Better Than Traditional Approaches?: First
30 Comprehensive National Examination With Propensity Score Matching" *JAMA Surg.* 2014 149(2) 177-184; Wilson *et al.*, "Laparoscopic colectomy is associated with a lower incidence

of postoperative complications compared with open colectomy: a propensity score-matched cohort analysis” *Colorectal Dis.* 2014 16(5) 382-389; Kobayashi *et al.*, “Total laparoscopic hysterectomy in 1253 patients using an early ureteral identification technique” *J Obstet Gynaecol Res.* 2012 38(9) 1194-1200; Makinen *et al.*, “Ten years of progress--improved hysterectomy outcomes in Finland 1996-2006: a longitudinal observation study” *BMJ Open* 2013 3(10) e003169.

[0005] However, limited tactile sensation and two-dimensional images can lead to iatrogenic ureter injury. Although infrequent, laparoscopic ureter injury remains a serious complication with significant associated morbidity. Reports indicate an incidence between 0.1 – 7.6% for colorectal and gynecologic surgery with more than 80% of these failing to be recognized intraoperatively, as described in Park *et al.*; Palaniappa *et al.*, “Incidence of iatrogenic ureteral injury after laparoscopic colectomy” *Arch Surg.* 2012 147(3), 267-271; and, da Silva *et al.*, “Role of prophylactic ureteric stents in colorectal surgery” *Asian J Endosc Surg.* 2012 5(3), 105-110. Current techniques for intraoperative ureter identification include ureteral stent placement, x-ray fluoroscopy, and visible dyes; however, both stents and fluoroscopy carry additional risk to the patient, and visible dyes are often not sensitive, as described in Tanaka *et al.*, “Real-time intraoperative ureteral guidance using invisible near-infrared fluorescence” *J Urol.* 2007 178(5), 2197-2202; Bothwell *et al.*, “Prophylactic ureteral catheterization in colon surgery. A five-year review” *Dis Colon Rectum.* 1994 37(4), 330-334; Wood *et al.*, “Routine use of ureteric catheters at laparoscopic hysterectomy may cause unnecessary complications” *J Am Assoc Gynecol Laparosc.* 1996 3(3), 393-397; and, Brandes *et al.*, “Diagnosis and management of ureteric injury: an evidence-based analysis” *BJU Int.* 2004 94(3), 277-289.

[0006] Fluorescence imaging using cyanine dyes is a rapidly emerging field to support surgical navigation and provide real-time illumination of anatomic structures. Emissions in the 700 – 900 nm range may avoid interference from tissue auto-fluorescence and can penetrate approximately 1 cm of tissue, as described in Adams *et al.*, “Comparison of visible and near-infrared wavelength-excitable fluorescent dyes for molecular imaging of cancer” *J Biomed Opt.* 2007 12(2), 024017; and, Keereweer *et al.*, “Optical Image-Guided Cancer Surgery: Challenges and Limitations” *Clin Cancer Res.* 2013 19(14), 3745-3754.

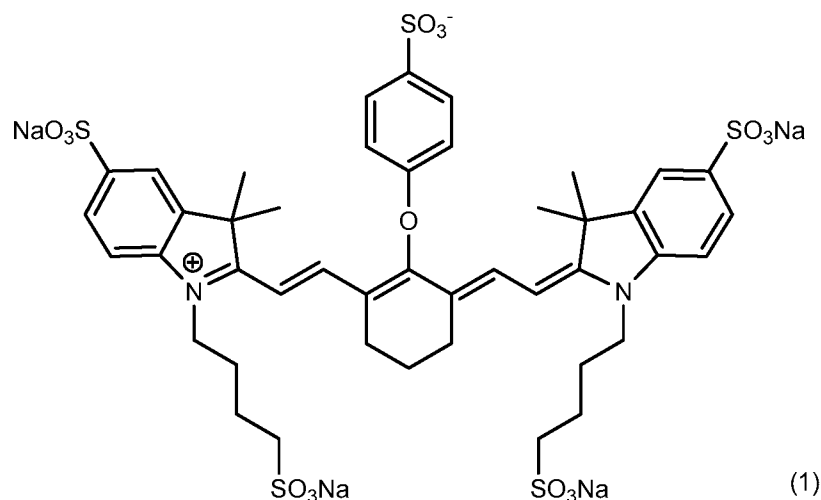
[0007] Another application of fluorescence imaging is for the real-time intra-operative imaging of the biliary anatomy, including the biliary duct and cystic duct. Current methods

often use indocyanine green (ICG) dye by either intra-biliary injection or intravenous injection before surgery. However, studies have shown clear problems in using ICG dye. These include poor efficiency and kinetics of excretion into bile (Tanaka *et al.*, “Real-time intraoperative assessment of the extrahepatic bile ducts in rats and pigs using invisible near-infrared fluorescent light” *Surgery* 2008 144(1) 39-48) and adverse reaction with the patient (Benya *et al.*, “Adverse reactions to indocyanine green: a case report and a review of the literature” *Cathet Cardiovasc Diagn.* 1989 17(4) 231-233).

[0008] There exists a need for sensitive compositions and methods to detect and measure an internal target non-invasively. Specifically, there exists a need for improved, stable cyanine dyes to detect injuries to various organs that may occur during laparoscopic or robotic surgery. The present invention satisfies these and other needs.

BRIEF SUMMARY OF THE INVENTION

[0009] In an embodiment, the present invention includes a method for kidney ureter imaging, comprising: administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-(4-sulfonatophenoxy)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate compound of Formula 1:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation, wherein the administering is performed at one or more times selected from the group consisting of before a procedure, during a procedure, after a procedure and combinations thereof, exposing tissue of the subject's renal system to electromagnetic radiation; and detecting fluorescence radiation from the compound.

[0010] In one embodiment, the method includes administering the compound of Formula 1 intravenously.

[0011] In one embodiment, the method includes administering the compound of Formula 1 wherein the pharmaceutically acceptable cation is selected from the group consisting of potassium or sodium.

[0012] In one embodiment, the method includes administering the compound of Formula 1 in combination with a pharmaceutically acceptable carrier selected from the group consisting of physiological sterile saline solution, sterile water solution, pyrogen-free water solution, isotonic saline solution, and phosphate buffer solution.

[0013] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 3000.0 $\mu\text{g/kg}$ and approximately 1500.0 $\mu\text{g/kg}$.

[0014] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 1500.0 $\mu\text{g/kg}$ and approximately 1000.0 $\mu\text{g/kg}$.

[0015] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 1000.0 $\mu\text{g/kg}$ and approximately 500.0 $\mu\text{g/kg}$.

[0016] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 500.0 $\mu\text{g/kg}$ and approximately 170.0 $\mu\text{g/kg}$.

[0017] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 170.0 $\mu\text{g/kg}$ and approximately 120.0 $\mu\text{g/kg}$.

[0018] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 120.0 $\mu\text{g/kg}$ and approximately 60.0 $\mu\text{g/kg}$.

[0019] In one embodiment, the method includes measuring a fluorescence intensity of the administered compound of Formula 1 remaining at the tissue of the subject's renal system at

a time period after administering. In certain aspects, the methods provide visualizing the compound of Formula 1 in urine or bile.

[0020] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 1 is background fluorescence approximately 24 hours after administering.

[0021] In one embodiment, the method includes the procedure selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic laparoscopic procedure, and an open procedure.

[0022] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 1 is higher in the kidney as compared to a measured fluorescence intensity of the administered compound of Formula 1 in one or more of the spleen, intestine, heart, lungs, muscle, or combinations thereof approximately up to six hours after administering.

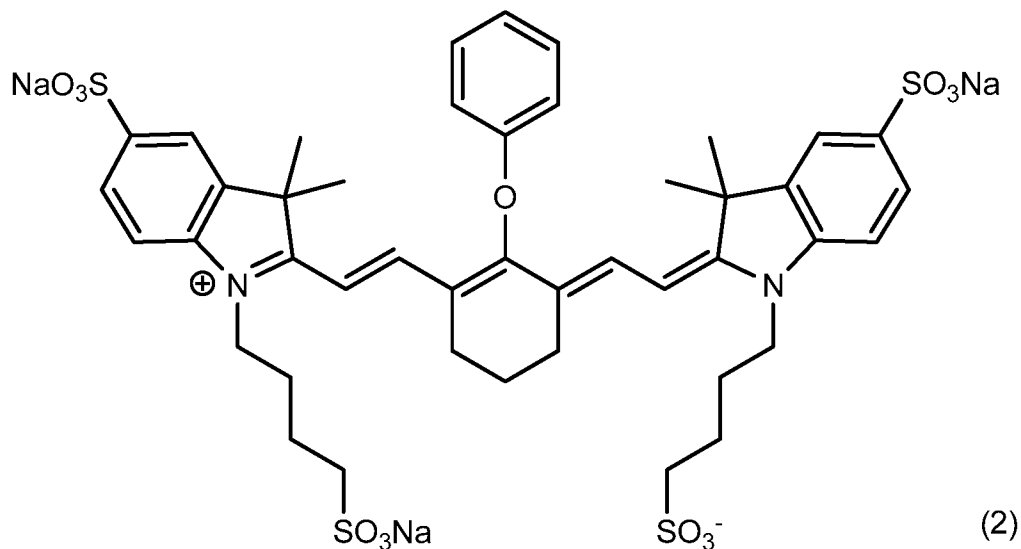
[0023] In one embodiment, the present invention provides a solid form (a polymorph), which is Form A of Formula 1. Form A of Formula 1 has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 4.3°. Form A has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 4.3°, about 9.6°, about 12.9°, about 18.3°, and about 20.8°. Form A has an X-ray powder diffraction pattern substantially as shown in FIG. 1E.

[0024] In one embodiment, the present invention provides a method for biliary duct imaging, comprising administering a compound of Formula 1 to a subject; and detecting fluorescence radiation from the compound.

[0025] In another embodiment, the invention provides a polymorph or solid form (Form A) of the compound of Formula 1 in that it provides an XRPD pattern comprising peaks substantially as set out in Table 2.

[0026] In one embodiment, the present invention provides a solid form (a polymorph), which is Form B of Formula 1. Form B of Formula 1 has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 21.2°. Form B has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 5.3°, about 14.2°, about 14.3°, about 20.7°, and about 21.2°. Form B has an X-ray powder diffraction pattern substantially as shown in FIG. 1H.

[0027] In an embodiment, the invention includes a method for liver biliary imaging, comprising: administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy-cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate of Formula 2:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation, wherein the administering is performed at one or more times selected from the group consisting of before a procedure, during a procedure, after a procedure and combinations thereof, exposing tissue of the subject's liver system to electromagnetic radiation; and detecting fluorescence radiation emission from the compound.

[0028] In one embodiment, the method includes administering the compound of Formula 2 intravenously.

[0029] In one embodiment, the method includes administering the compound of Formula 2 wherein the pharmaceutically acceptable cation is selected from the group consisting of potassium or sodium.

[0030] In one embodiment, the method includes administering the compound of Formula 2 in combination with a pharmaceutically acceptable carrier selected from the group consisting of physiological sterile saline solution, sterile water solution, pyrogen-free water solution, isotonic saline solution, and phosphate buffer solution.

[0031] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 3000.0 µg/kg and approximately 1500.0 µg/kg.

[0032] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 1500.0 µg/kg and approximately 1000.0 µg/kg.

[0033] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 1000.0 µg/kg and approximately 500.0 µg/kg.

[0034] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 500.0 µg/kg and approximately 170.0 µg/kg.

[0035] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 170.0 µg/kg and approximately 120.0 µg/kg.

[0036] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 120.0 µg/kg and approximately 60.0 µg/kg.

[0037] In one embodiment, the method includes measuring a fluorescence intensity of the administered compound of Formula 2 remaining at the tissue of the subject's liver system at a time period after administering.

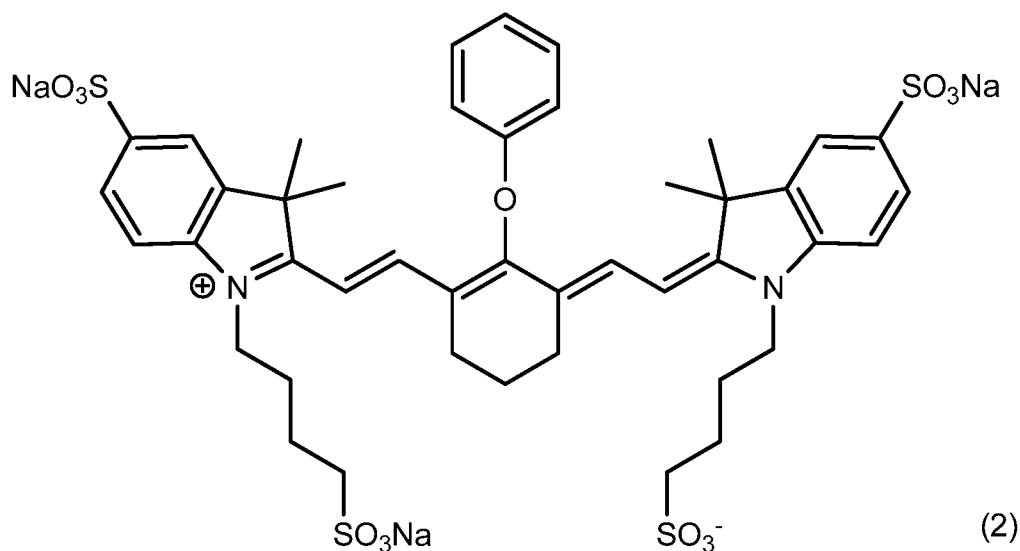
[0038] In certain aspects, the methods provide visualizing the compound of Formula 2 in urine or bile.

[0039] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 2 is background fluorescence approximately 24 hours after administering.

[0040] In one embodiment, the method includes the procedure selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic laparoscopic procedure, and an open procedure.

[0041] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 2 is higher in the liver as compared to a measured fluorescence intensity of the administered compound of Formula 2 in one or more of the spleen, intestine, heart, lungs, muscle, or combinations thereof approximately up to six hours after administering.

[0042] In an embodiment, the invention includes a method for liver cystic duct imaging, comprising: administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate of Formula 2:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation, wherein the administering is performed at one or more times selected from the group consisting of before a procedure, during a procedure, after a procedure and combinations thereof, exposing tissue of the subject's liver system to electromagnetic radiation; and detecting fluorescence radiation from the compound.

[0043] In one embodiment, the method includes administering the compound of Formula 2 intravenously.

[0044] In one embodiment, the method includes administering the compound of Formula 2 wherein the pharmaceutically acceptable cation is selected from the group consisting of potassium or sodium.

[0045] In one embodiment, the method includes administering the compound of Formula 2 in combination with a pharmaceutically acceptable carrier selected from the group consisting of physiological sterile saline solution, sterile water solution, pyrogen-free water solution, isotonic saline solution, and phosphate buffer solution.

5 [0046] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 3000.0 µg/kg and approximately 1500.0 µg/kg.

[0047] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 1500.0 µg/kg and approximately 1000.0 µg/kg.

10 [0048] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 1000.0 µg/kg and approximately 500.0 µg/kg.

[0049] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 500.0 µg/kg and approximately 170.0 µg/kg.

[0050] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 170.0 µg/kg and approximately 120.0 µg/kg.

20 [0051] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 120.0 µg/kg and approximately 60.0 µg/kg.

[0052] In one embodiment, the method includes measuring a fluorescence intensity of the administered compound of Formula 2 remaining at the tissue of the subject's liver system at a time period after administering.

25 [0053] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 2 is background fluorescence approximately 24 hours after administering.

[0054] In one embodiment, the method includes the procedure selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic laparoscopic procedure, and an open procedure.

[0055] In one embodiment, the present invention provides a method for kidney ureter imaging, comprising administering a compound of Formula 2 to a subject; and detecting fluorescence radiation from the compound.

[0056] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 2 is higher in the liver as compared to a measured fluorescence intensity of the administered compound of Formula 2 in one or more of the spleen, intestine, heart, lungs, muscle, or combinations thereof approximately up to six hours after administering.

[0057] In one embodiment, a pharmaceutical composition comprises a diagnostic imaging amount of a compound of Formula 1, a pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier.

[0058] In one embodiment, the pharmaceutical composition comprising a diagnostic imaging amount of a compound of Formula 1, a pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier is contained in an intravenous (IV) bag.

[0059] In one embodiment, a pharmaceutical composition comprises a diagnostic imaging amount of a compound of Formula 2, a pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier.

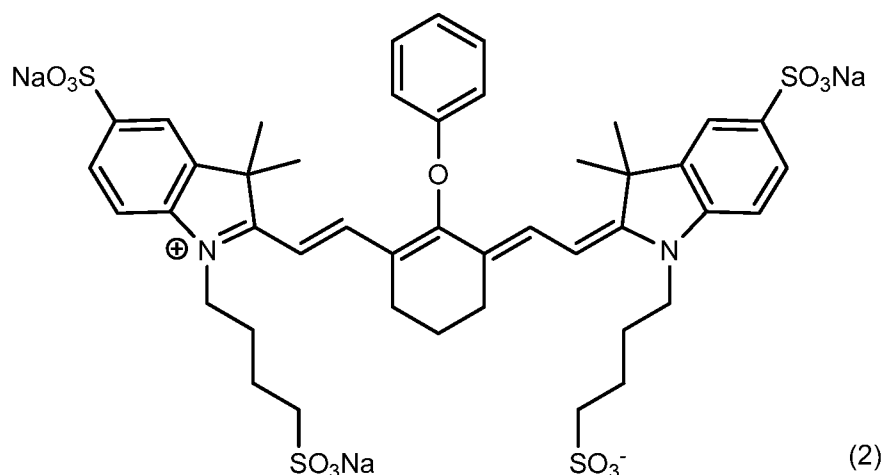
[0060] In one embodiment, the pharmaceutical composition comprising a diagnostic imaging amount of a compound of Formula 2, a pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier is contained in an intravenous (IV) bag.

[0061] In one embodiment, the invention includes a kit containing the pharmaceutical composition comprising a diagnostic imaging amount of a compound of Formula 1, a pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier, and an instruction manual or instructions for use. The pharmaceutical composition can be contained in an intravenous (IV) bag.

[0062] In other embodiments, the invention includes a kit containing the pharmaceutical composition comprising a diagnostic imaging amount of a compound of Formula 2, a

pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier, and an instruction manual or instructions for use. The pharmaceutical composition can be contained in an intravenous (IV) bag.

[0063] In an embodiment, the invention includes a composition of matter comprising: 2-
 5 ((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy-cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate of Formula 2:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation.

[0064] Other embodiments, aspects, and objects will become better understood when read with the detailed description and drawings which follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0065] In each of the drawings 800CW corresponds to the compound set forth in

Comparative example 1; 800BK-sulfonate refers to compound of Formula 1; 800BK-NOS refers to compound of Formula 2.

[0066] FIGS. 1A-1I show X-ray powder diffraction (XRPD) patterns for various samples. Samples shown in FIGS. 1A and 1C represent Form A. FIG. 1B is Form A, but less crystalline. FIG. 1D and 1E show the XRPD pattern of crystalline Form A. FIG. 1F shows the XRPD of the amorphous form. FIG. 1H and FIG. 1I show the XRPD pattern of crystalline Form B. For comparison, FIG. 1G is identical to FIG. 1D.

[0067] FIGS. 2A, and 2B illustrate in vitro cell-based analyses for A549 cells (A) and A431 cells (B). FIG. 2C shows fluorescence as a function of concentration of each dye prepared for in vivo imaging (0.03-1 μ M).

[0068] FIGS. 3A-C illustrates dorsal view fluorescence images of mice for the compound of comparative example 1 (FIG. 3A), the compound of Formula 1 (FIG. 3B), and the compound of Formula 2 (FIG. 3C) probes. Images are presented for the approximate time points post probe administration: 30 min, 2 hrs, 5 hrs, and 24 hrs. A Pearl[®] Impulse small animal imaging system was used for all animal and organ image acquisitions

[0069] FIGS. 4A-C illustrate ventral view fluorescence images of the same mice for the compound of comparative example 1 (FIG. 4A); the compound of Formula 1 (FIG. 4B), and the compound of Formula 2 probes (FIG. 4C). Images are presented for the approximate time points post probe administration: 30 min, 2 hrs, 5 hrs, and 24 hrs. Arrows pinpoint bladder regional bladder signal. White arrows point to gall bladder signal.

[0070] FIG. 5 illustrates whole body fluorescence intensity numbers for the images of FIG. 4 for the compound of comparative example 1, the compound of Formula 1, and the compound of Formula 2 probes.

[0071] FIGS. 6A-D show fluorescence data for mouse organs for the compound of comparative example 1 (FIG. 6A), the compound of Formula 2 (FIG. 6B), the compound of Formula 1 (FIG. 6C): liver, spleen, intestine, brain, heart/lung, kidney, brain, and muscle (FIG. 6D). Fluorescence signal (800nm) adjusted to area (pixel) for all probes.

[0072] FIG. 7 illustrates image captured of ureters, kidneys and bladder in mouse after administration of the compound of Formula 1 probe.

[0073] FIGS. 8A-B illustrate fluorescence detection of probes in a histogram of stained electrophoresis gels of selected proteins showing non-specific or affinity of the named dyes to human (H), bovine (B), ovalbumin (O), or 5% FBS (fetal bovine serum) (FIG. 8A). In the fluorescence images of stained electrophoresis gels dark grey represents the signal from the compound being examined, light grey represents a Coomassie Blue assessment of protein loading and white represents where both colors reside (FIG. 8B).

[0074] FIG. 9 illustrates fluorescent signal (800 nm) analysis of mouse urine for a control sample (no probe), the compound of Formula 1, the compound of Formula 2, and indocyanine green (ICG) dye.

[0075] FIGS. 10A, 10B, 10C, 10D, and 10E illustrate biliary tract imaging for: 50 nmole of ICG in phosphate buffer saline (PBS) solution (FIG. 10A), 1 nmole of the compound of Formula 2 in PBS solution (FIG. 10B), 0.5 nmole of the compound of Formula 2 in PBS solution (FIG. 10C), and 0.1 nmole of the compound of Formula 2 in PBS solution (FIGS. 10D and 10E). The imaging areas displayed for each condition were recorded at 1 min, 30 min, 2 hrs, 5 hrs, and 24 hrs., respectively (left to right).

[0076] FIGS. 11A-11H show images of various mouse organs after administration. Fluorescence images of various mouse organs at 24 and 72 hrs post-administration for the comparative control (FIG. 11A), compound of Formula 1 (FIGS. 11C and 11E) and compound of Formula 2 (FIG. 11B and 11D). Organs examined included: heart (Ht), lungs (Ln), kidney (Kd), liver (Lv), spleen (Spl), intestine (Int), brain (Br), and muscle (Ms). At 24 hrs, there remains some signal in the liver and kidney but by 72 hrs the signal is diminished substantially. FIGS. 11F-11H show intraoperative fluorescence (800 nm, compound of Formula 2) (FIG. 11G), white light (FIG. 11F), and composite images of the liver, gall bladder, and bile duct for compound of Formula 2 (0.1 nmole) (FIG. 11H).

[0077] FIG. 12 shows a spot test of the injected dye solutions-800CW, 800BK-sulfonate (compound of Formula 1; 800BK or BK) and 800BK-NOS (compound of Formula 2; 800NOS or NOS).

[0078] FIGS. 13A-13C show whole animal imaging of dorsal and ventral view of a representative mouse injected with one of the dyes tested. Each panel of figures shows a dorsal and ventral view of the animal at 15 minutes, 1 hour, 2 hours, 4 hours 6 hours and 24 hours after injection. An animal injected with 800CW dye, 800NOS dye, and 800BK dye are shown in FIG. 13A, FIG. 13B and FIG. 13C, respectively.

[0079] FIG. 14 shows the whole animal LUT scale, the organ $1/10^{\text{th}}$ LUT scale, and the organ $1/100^{\text{th}}$ LUT scale.

[0080] FIGS. 15A-15D show the signal intensity of the kidney (lower left corner), liver (lower right corner), lungs (upper left corner) and muscle (upper right corner) on the organ $1/10^{\text{th}}$ LUT scale. Organs from an animal injected with no probe (control), 800CW dye, 800NOS dye, and 800BK dye are shown in FIG. 15A, FIG. 15B, FIG. 15C, and FIG. 15D, respectively.

[0081] FIGS. 16A-16D show the signal intensity of the kidney (lower left corner), liver (lower right corner), lungs (upper left corner) and muscle (upper right corner) on the organ 1/100th LUT scale. Organs from an animal injected with no probe (control), 800CW dye, 800NOS dye, and 800BK dye are shown in FIG. 16A, FIG. 16B, FIG. 16C, and FIG. 16D, respectively.

DETAILED DESCRIPTION OF THE INVENTION

I. DEFINITIONS

[0082] The terms “a,” “an,” or “the” as used herein not only include aspects with one member, but also include aspects with more than one member. For example, an embodiment of a method of imaging that comprises using a compound set forth herein would include an aspect in which the method comprises using two or more compounds set forth herein.

[0083] The term “approximately” or “about” as used herein to modify a numerical value indicates a defined range around that value. If “X” were the value, “approximately X” or “about X” would indicate a value from 0.9X to 1.1X, and more preferably, a value from 0.95X to 1.05X. Any reference to “approximately X” or “about X” specifically indicates at least the values X, 0.95X, 0.96X, 0.97X, 0.98X, 0.99X, 1.01X, 1.02X, 1.03X, 1.04X, and 1.05X. For example, “approximately X” or “about X” is intended to teach and provide written description support for a claim limitation of, *e.g.*, “0.98X.”

[0084] When the quantity “X” only allows whole-integer values (*e.g.*, “X carbons”) and X is at most 15, “about X” indicates from (X-1) to (X+1). In this case, “about X” as used herein specifically indicates at least the values X, X-1, and X+1. If X is at least 16, the values of 0.90X and 1.10X are rounded to the nearest whole-integer values to define the boundaries of the range.

[0085] When the modifier “approximately” or “about” is applied to describe the beginning of a numerical range, it applies to both ends of the range. Thus, “from approximately 700 to 850 nm” is equivalent to “from approximately 700 nm to approximately 850 nm.” Thus, “from about 700 to 850 nm” is equivalent to “from about 700 nm to about 850 nm.” When “approximately” or “about” is applied to describe the first value of a set of values, it applies to all values in that set. Thus, “about 680, 700, or 750 nm” is equivalent to “about 680 nm, about 700 nm, or about 750 nm.”

[0086] “Balanced charge” as used herein includes the condition that the net charge for a compound and its associated counterions be zero under standard physiological conditions. In order to achieve a balanced charge, a skilled person will understand that after the first additional sulfonato group that balances the +1 charge of the indolinium ring, a cationic counterion (*e.g.*, the cation of a Group I metal such as sodium) must be added to balance the negative charge from additional sulfonato groups. Similarly, anionic counterions must be added to balance any additional cationic groups (*e.g.*, most basic amino groups under physiological conditions).

II. EMBODIMENTS

[0087] While preferred embodiments of the invention are shown and described herein, such embodiments are provided by way of example only and are not intended to otherwise limit the scope of the invention. Various alternatives to the described embodiments of the invention may be employed in practicing the invention.

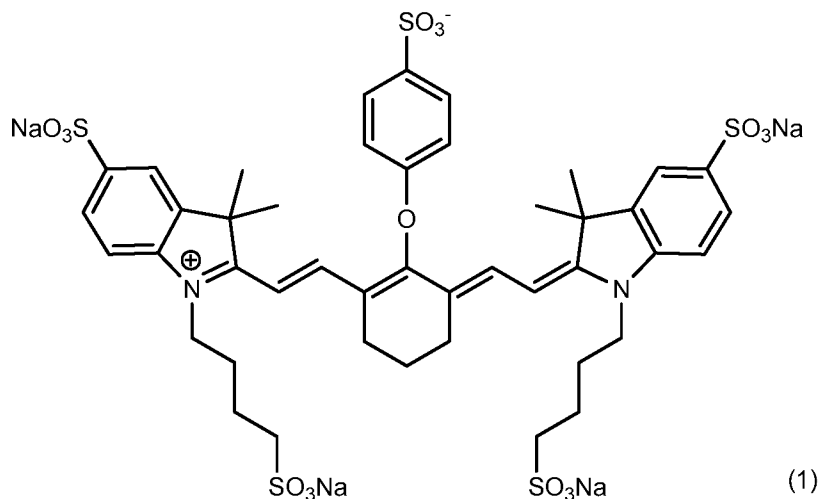
[0088] Some advantages of cyanine dyes include: (1) cyanine dyes strongly absorb and fluoresce light; (2) many cyanine dyes do not rapidly photo-bleach under the fluorescence microscope; (3) many structures and synthetic procedures are available and the class of dyes is versatile; and (4) cyanine dyes are relatively small (a typical molecular weight is about 1,000 daltons) so they do not cause appreciable steric interference.

[0089] Generally, cyanine dyes are prepared according to the procedures taught in Hamer, F. M., *Cyanine Dyes and Related Compounds*, Weissberger, Mass., ed. Wiley Interscience, N.Y. 1964. For example, U.S. Pat. Nos. 6,663,847; 6,887,854; 6,995,274; 7,504,089; 7,547,721; 7,597,878 and 8,303,936, incorporated herein by reference, describe synthesis mechanisms for a variety of cyanine dyes.

[0090] Other cyanine dyes are known which contain reactive functional groups. For example, U.S. Pat. Nos. 4,337,063; 4,404,289 and 4,405,711, incorporated herein by reference, describe a synthesis for a variety of cyanine dyes having N-hydroxysuccinimide active ester groups. U.S. Pat. No. 4,981,977, incorporated herein by reference, describes a synthesis for cyanine dyes having carboxylic acid groups. U.S. Pat. No. 5,268,486, incorporated herein by reference, discloses a method for making arylsulfonate cyanine dyes. U.S. Pat. No. 6,027,709, incorporated herein by reference, discloses methods for making cyanine dyes having phosphoramidite groups. U.S. Pat. No. 6,048,982, incorporated herein by reference, discloses methods for making cyanine dyes having a reactive group selected

from the group consisting of isothiocyanate, isocyanate, phosphoramidite, monochlorotriazine, dichlorotriazine, mono- or di-halogen substituted pyridine, mono- or di-halogen substituted diazine, aziridine, sulfonyl halide, acid halide, hydroxysuccinimide ester, hydroxy sulfosuccinimide ester, imido ester, glyoxal and aldehyde.

- 5 [0091] As described herein, the present invention provides for the use of a cyanine dye of the compound having Formula 1:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation.

- [0092] In one embodiment, the present invention provides a solid form (a polymorph), which is Form A of Formula 1. Form A of Formula 1 has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 4.3°. Form A has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 4.3°, about 9.6°, about 12.9°, about 18.3°, and about 20.8°. Form A has an X-ray powder diffraction pattern substantially as shown in FIG. 1E.
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- [0093] In one embodiment, the present invention provides a solid form (a polymorph), which is Form B of Formula 1. Form B of Formula 1 has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 21.2°. Form B has an X-ray powder diffraction pattern comprising a peak, in terms of 2-theta, at about 5.3°, about 14.2°, about 14.3°, about 20.7° and about 21.2°. Form B has an X-ray powder diffraction pattern substantially as shown in FIG. 1H.
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[0094] In certain aspects, the polymorphs of the invention are formulated into a composition prior to administration to a subject.

[0095] The compounds of Formula 1 can exist in crystalline or noncrystalline form, or as a mixture thereof. For salts of the invention that are in crystalline form, the skilled artisan will appreciate that pharmaceutically acceptable solvates may be formed wherein solvent molecules are incorporated into the crystalline lattice during crystallization. Solvates may involve nonaqueous solvents such as ethanol, isopropanol, DMSO, acetic acid, ethanolamine, and ethylacetate. In one embodiment, the invention provides the sodium salt of the compound of Formula 1 incorporated into the crystalline lattice.

[0096] In one aspect, the present invention provides a polymorph of the compound of Formula 1 in isolated or pure form. "Isolated" or "pure" or "substantially pure" form refers to a sample in which the polymorph is present in an amount of >75%, particularly >90%, >91%, >92%, >93%, >94%, >95%, >96%, >97%, >98%, or >99%, relative to other materials which may be present in the sample.

[0097] The polymorphs made according to the methods of the invention can be characterized by any methodology according to the art. For example, the polymorphs made according to the methods of the invention may be characterized by X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), hot-stage microscopy, and spectroscopy (e.g., Raman, solid state nuclear magnetic resonance (ssNMR), and infrared (IR)).

[0098] XRPD

[0099] Polymorphs according to the invention may be characterized by X-ray powder diffraction patterns (XRPD). The relative intensities of XRPD peaks can vary, depending upon the sample preparation technique, the sample mounting procedure and the particular instrument employed. Moreover, instrument variation and other factors can affect the 2θ values. Therefore, the XRPD peak assignments can vary by plus or minus about 0.2 degrees.

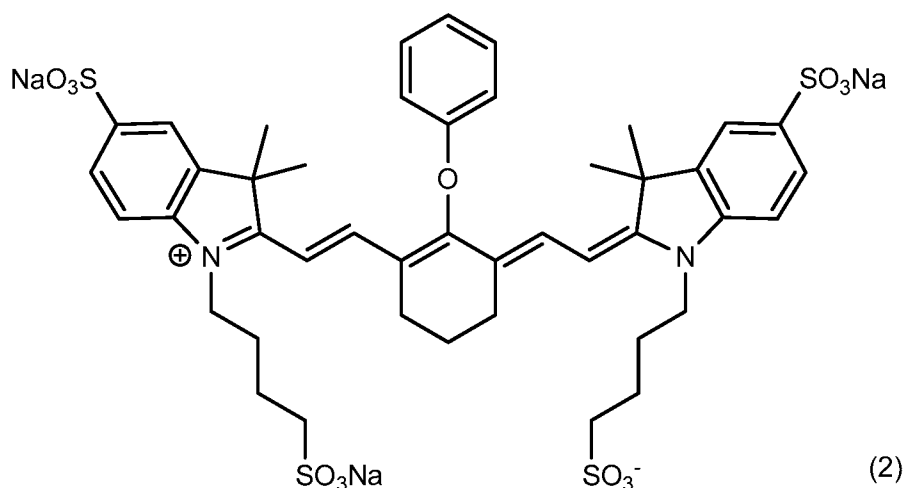
[0100] The polymorph forms of the invention are useful in the production of imaging agents and can be obtained by means of a crystallization process to produce crystalline and semi-crystalline forms. In various embodiments, the crystallization is carried out by either generating the compound of Formula 1 in a reaction mixture and isolating the desired polymorph from the reaction mixture, or by dissolving raw compound in a solvent, optionally with heat, followed by crystallizing/solidifying the product by cooling (including active cooling) and/or by the addition of an antisolvent for a period of time. The crystallization can

be followed by drying carried out under controlled conditions until the desired water content is reached in the end polymorphic form.

[0101] In another embodiment, the present invention provides a method of making a polymorph of a compound of Formula 1 (Form A or Form B). In various embodiments, the invention is directed to methods of making a polymorph of the compound of Formula 1, wherein the method involves converting an amorphous form into a desired polymorph. In certain embodiments, the methods comprise exposing a composition comprising an amorphous form to conditions sufficient to convert at least about 50% of the total amount of the amorphous form into at least about 50% of the desired polymorph, and isolating the desired polymorph as needed.

[0102] In certain instances, a crystalline solid will be more amenable to purification than an amorphous solid and the crystalline form is able to be made in higher purity. This is because, under proper conditions, the formation of the crystals tends to exclude impurities from the solid, in contrast to amorphous solids formed in a less controlled manner. Similarly, a crystalline solid will often have better stability than an amorphous solid, as the crystal packing gives a protective effect. For materials which are polymorphic, some crystal forms will normally be more effective than others at excluding impurities and enhancing stability.

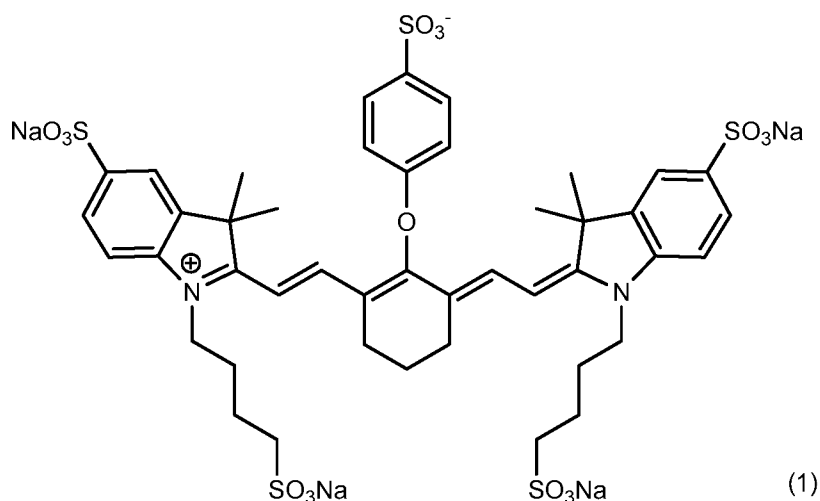
[0103] In one embodiment, the present invention provides for a composition according to Formula 2:



or a hydrate or polymorph thereof and having a pharmaceutically acceptable cation.

[0104] One embodiment of the invention includes a method for organ imaging, comprising: administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-

dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-(4-sulfonatophenoxy)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate compound of Formula 1:



- 5 or a solvate or polymorph thereof and having a pharmaceutically acceptable cation, wherein the administering is performed at one or more times selected from the group consisting of before a procedure, during a procedure, after a procedure and combinations thereof, exposing tissue of the subject's organ system to electromagnetic radiation; and detecting fluorescence radiation from the compound. In one embodiment, the organ includes one or more of kidney, bladder, liver, spleen, intestine, heart, lungs and muscle. In one embodiment, the organ is kidney, bladder or combinations of. In another embodiment, the organ is the ureter of a kidney.

[0105] In one embodiment, the method includes administering the compound of Formula 1 intravenously. The compound of Formula 1 can be administered as a bolus injection, *e.g.*, an intravenous bolus injection. In some embodiments, about 5 mL to about 10 mL of a composition comprising the compound of Formula 1 is administered in a bolus injection.

[0106] In one embodiment, the method includes administering the compound of Formula 1 wherein the pharmaceutically acceptable cation is selected from the group consisting of potassium or sodium.

20 [0107] In one embodiment, the method includes administering the compound of Formula 1 in combination with a pharmaceutically acceptable carrier selected from the group consisting of physiological sterile saline solution, sterile water solution, pyrogen-free water solution, isotonic saline solution, and phosphate buffer solution.

[0108] The compound of Formula 1 can be highly soluble in water. In some embodiments, the compound of Formula 1 is re-suspended in water to a concentration of at least 200 mg/mL, or about 300 mg/mL to about 320 mg/mL.

[0109] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 3000.0 µg/kg and approximately 1500.0 µg/kg.

[0110] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 1500.0 µg/kg and approximately 1000.0 µg/kg.

[0111] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 1000.0 µg/kg and approximately 500.0 µg/kg.

[0112] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 500.0 µg/kg and approximately 170.0 µg/kg.

[0113] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 170.0 µg/kg and approximately 120.0 µg/kg.

[0114] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 120.0 µg/kg and approximately 60.0 µg/kg.

[0115] In one embodiment, the method includes administering the compound of Formula 1 at a diagnostic effective amount of the compound ranging between approximately 30.0 µg/kg and approximately 500.0 µg/kg.

[0116] The compounds of Formula 1 can be non-toxic. They can absorb and fluoresce light, and do not rapidly photo-bleach under fluorescence imaging. Upon administration, the compounds of Formula 1 can be transported to tissues and organs of the subject via the natural flow of bodily fluids in the subject. As such, compounds of Formula 1 can be carried or transferred from the site of administration to the desired sites, tissues and organs for, *e.g.*, visualization.

[0117] In certain embodiments, the compounds and methods herein can image the biliary tract which includes any part of the liver, gall bladder, spleen, small intestine, and associated ducts. In certain instances, the biliary tract includes the intrahepatic bile ducts, cystic duct—gallbladder to common bile duct—and common bile duct—liver and gallbladder to small intestine. In some instances, the compounds herein are found in a subject's bile or urine at a time period after administering. The present invention provides compositions of the compounds of Formula 1 or 2 in urine or bile.

[0118] In certain embodiments, the methods herein provide visualizing the compounds of Formula 1 or 2 in urine or bile.

[0119] In one embodiment, the method includes measuring a fluorescence intensity of the administered compound of Formula 1 remaining at the tissue of the subject's organ at a time period after administering. In one embodiment, the organ includes one or more of kidney, bladder, liver, gall bladder, spleen, intestine, heart, lungs and muscle. In one embodiment, the organ is kidney, bladder or combinations. In another embodiment, the organ is the ureter of a kidney. In some embodiments, the method includes measuring a fluorescence intensity of the administered compound of Formula 1 remaining in the subject's urine or bile at a time period after administering.

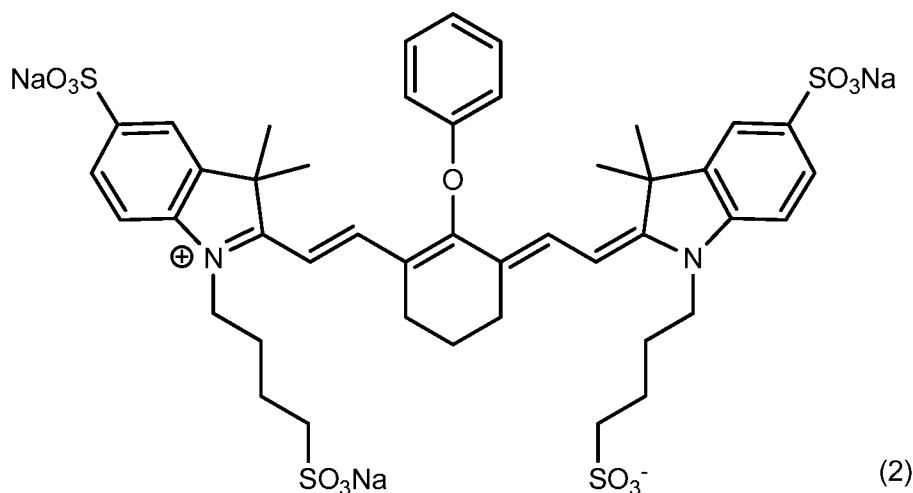
[0120] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 1 is background fluorescence approximately 24 hours after administering.

[0121] In one embodiment, the method includes the procedure selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic laparoscopic procedure, an endoscopic procedure, and an open procedure.

[0122] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 1 is higher in the kidney as compared to a measured fluorescence intensity of the administered compound of Formula 1 in one or more of the spleen, intestine, heart, lungs, muscle, or combinations thereof approximately up to six hours after administering.

[0123] In one embodiment, a pharmaceutical composition comprises a diagnostic imaging amount of a compound of Formula 1, a pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier.

[0124] In another embodiment, a method for organ imaging, comprising: administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy-cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate of Formula 2:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation, wherein the administering is performed at one or more times selected from the group consisting of before a procedure, during a procedure, after a procedure and combinations thereof, exposing tissue of the subject's organ system to electromagnetic radiation; and detecting fluorescence radiation from the compound. In one embodiment, the organ includes one or more of kidney, bladder, liver, spleen, intestine, heart, lungs and muscle. In one embodiment, the organ is a liver. In one embodiment, the organ is the biliary duct of a liver. In one embodiment, the organ is the cystic duct of a liver.

[0125] In one embodiment, the method includes administering the compound of Formula 2 intravenously. The compound of Formula 2 can be administered as a bolus injection, *e.g.*, an intravenous bolus injection. In some embodiments, about 5 mL to about 10 mL of a composition comprising the compound of Formula 2 is administered in a bolus injection.

[0126] In one embodiment, the method includes administering the compound of Formula 2 wherein the pharmaceutically acceptable cation is selected from the group consisting of potassium or sodium.

[0127] In one embodiment, the method includes administering the compound of Formula 2 in combination with a pharmaceutically acceptable carrier selected from the group consisting

of physiological sterile saline solution, sterile water solution, pyrogen-free water solution, isotonic saline solution, and phosphate buffer solution.

[0128] The compound of Formula 2 can be highly soluble in water. In some embodiments, the compound of Formula 2 is resuspended in water to a concentration of at least 200 mg/mL, about 300 mg/mL or about 320 mg/mL.

[0129] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 3000.0 $\mu\text{g/kg}$ and approximately 1500.0 $\mu\text{g/kg}$.

[0130] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 1500.0 $\mu\text{g/kg}$ and approximately 1000.0 $\mu\text{g/kg}$.

[0131] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 1000.0 $\mu\text{g/kg}$ and approximately 500.0 $\mu\text{g/kg}$.

[0132] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 500.0 $\mu\text{g/kg}$ and approximately 170.0 $\mu\text{g/kg}$.

[0133] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 170.0 $\mu\text{g/kg}$ and approximately 120.0 $\mu\text{g/kg}$.

[0134] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 120.0 $\mu\text{g/kg}$ and approximately 60.0 $\mu\text{g/kg}$.

[0135] In one embodiment, the method includes administering the compound of Formula 2 at a diagnostic effective amount of the compound ranging between approximately 30.0 $\mu\text{g/kg}$ and approximately 500.0 $\mu\text{g/kg}$.

[0136] The compounds of Formula 2 can be non-toxic. They can absorb and fluoresce light, and do not rapidly photo-bleach under fluorescence imaging. Upon administration, the compounds of Formula 2 can be transported to tissues and organs of the subject via the natural flow of bodily fluids in the subject. As such, compounds of Formula 2 can be carried

or transferred from the site of administration to the desired sites, tissues and organs for, *e.g.*, visualization.

[0137] In one embodiment, the method includes measuring a fluorescence intensity of the administered compound of Formula 2 remaining at the tissue of the subject's organ system at a time period after administering. In one embodiment, the organ includes one or more of kidney, bladder, liver, gall bladder, spleen, intestine, heart, lungs and muscle. In one embodiment, the organ is the liver. In another embodiment, the organ is the biliary duct of a liver. In another embodiment, the organ is the cystic duct of a liver. In some embodiments, the method includes measuring a fluorescence intensity of the administered compound of Formula 2 remaining in the subject's urine or bile at a time period after administering.

[0138] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 2 is background fluorescence approximately 24 hours after administering.

[0139] In one embodiment, the method includes the procedure selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic laparoscopic procedure, an endoscopic procedure, and an open procedure.

[0140] In one embodiment, the method includes the measured fluorescence intensity of the administered compound of Formula 2 is higher in the liver as compared to a measured fluorescence intensity of the administered compound of Formula 2 in one or more of the spleen, intestine, heart, lungs, muscle, or combinations thereof approximately up to six hours after administering.

[0141] In one embodiment, a pharmaceutical composition comprises a diagnostic imaging amount of a compound of Formula 2, a pharmaceutically acceptable cation, and a pharmaceutically acceptable carrier.

[0142] In some embodiments, a pharmaceutical composition of compound of Formula 1 or compound of Formula 2 are in combination with a pharmaceutically acceptable cation and a pharmaceutically acceptable carrier in a diagnostic effective amount. In such embodiments, the pharmaceutical composition is contained in an intravenous drip bag.

[0143] In selected embodiments, the diagnostic effective amount of each of the compound of Formula 1 and the compound of Formula 2, is independently less than, for example, 3000.0, 2800.0, 2600.0, 2400.0, 2200.0, 2000.0, 1800.0, 1600.0, 1400.0, 1200.0, 1000.0,

950.0, 900.0, 850.0, 800.0, 750.0, 700.0, 650.0, 600.0, 550.0, 500.0, 490.0, 480.0, 470.0, 460.0, 450.0, 440.0, 430.0, 420.0, 410.0, 400.0, 390.0, 380.0, 370.0, 360.0, 350.0, 340.0, 330.0, 320.0, 310.0, 300.0, 290.0, 280.0, 270.0, 260.0, 250.0, 240.0, 230.0, 220.0, 210.0, 200.0, 190.0, 180.0, 170.0, 160.0, 150.0, 140.0, 130.0, 120.0, 110.0, 100.0, 95.0, 90.0, 85.0, 80.0, 75.0, 70.0, 65.0, 60.0, 55.0, 50.0, 49.0, 48.0, 47.0, 46.0, 45.0, 44.0, 43.0, 42.0, 41.0, 40.0, 39.5, 39.0, 38.5, 38.0, 37.5, 37.0, 36.5, 36.0, 35.5, 35.0, 34.5, 34.0, 33.5, 33.0, 32.5, 32.0, 31.5, 31.0, 30.9, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 30.2, 30.1 µg/kg.

[0144] In selected embodiments, the diagnostic effective amount of each of the compound of Formula 1 and the compound of Formula 2, is independently greater than, for example,

2800.0, 2600.0, 2400.0, 2200.0, 2000.0, 1800.0, 1600.0, 1400.0, 1200.0, 1000.0, 950.0, 900.0, 850.0, 800.0, 750.0, 700.0, 650.0, 600.0, 550.0, 500.0, 490.0, 480.0, 470.0, 460.0, 450.0, 440.0, 430.0, 420.0, 410.0, 400.0, 390.0, 380.0, 370.0, 360.0, 350.0, 340.0, 330.0, 320.0, 310.0, 300.0, 290.0, 280.0, 270.0, 260.0, 250.0, 240.0, 230.0, 220.0, 210.0, 200.0, 190.0, 180.0, 170.0, 160.0, 150.0, 140.0, 130.0, 120.0, 110.0, 100.0, 95.0, 90.0, 85.0, 80.0, 75.0, 70.0, 65.0, 60.0, 55.0, 50.0, 49.0, 48.0, 47.0, 46.0, 45.0, 44.0, 43.0, 42.0, 41.0, 40.0, 39.5, 39.0, 38.5, 38.0, 37.5, 37.0, 36.5, 36.0, 35.5, 35.0, 34.5, 34.0, 33.5, 33.0, 32.5, 32.0, 31.5, 31.0, 30.9, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 30.2, 30.1, 30.0 µg/kg.

[0145] In selected embodiments, the diagnostic effective amount of each of the compound of Formula 1 and the compound of Formula 2, is independently in the range from

approximately 30.0 µg/kg to approximately 3000.0 µg/kg, approximately 30.1 µg/kg to approximately 2800.0 µg/kg, approximately 30.2 µg/kg to approximately 2600.0 µg/kg, approximately 30.3 µg/kg to approximately 2400.0 µg/kg, approximately 30.4 µg/kg to approximately 2200.0 µg/kg, approximately 30.5 µg/kg to approximately 2000.0 µg/kg, approximately 30.6 µg/kg to approximately 1800.0 µg/kg, approximately 30.7 µg/kg to approximately 1600.0 µg/kg, approximately 30.8 µg/kg to approximately 1400.0 µg/kg, approximately 30.9 µg/kg to approximately 1200.0 µg/kg, approximately 31.0 µg/kg to approximately 1000.0 µg/kg, approximately 31.5 µg/kg to approximately 950.0 µg/kg, approximately 32.0 µg/kg to approximately 900.0 µg/kg, approximately 32.5 µg/kg to approximately 850.0 µg/kg, approximately 33.0 µg/kg to approximately 800.0 µg/kg, approximately 33.5 µg/kg to approximately 750.0 µg/kg, approximately 34.0 µg/kg to approximately 700.0 µg/kg, approximately 34.5 µg/kg to approximately 650.0 µg/kg, approximately 35.0 µg/kg to approximately 600.0 µg/kg, approximately 35.5 µg/kg to approximately 550.0 µg/kg, approximately 36.0 µg/kg to approximately 500.0 µg/kg,

approximately 36.5 µg/kg to approximately 490.0 µg/kg, approximately 37.0 µg/kg to approximately 480.0 µg/kg, approximately 37.5 µg/kg to approximately 470.0 µg/kg, approximately 38.0 µg/kg to approximately 460.0 µg/kg, approximately 38.5 µg/kg to approximately 450.0 µg/kg, approximately 39.0 µg/kg to approximately 440.0 µg/kg, approximately 39.5 µg/kg to approximately 430.0 µg/kg, approximately 40.0 µg/kg to approximately 420.0 µg/kg, approximately 41.0 µg/kg to approximately 410.0 µg/kg, approximately 42.0 µg/kg to approximately 400.0 µg/kg, approximately 43.0 µg/kg to approximately 390.0 µg/kg, approximately 44.0 µg/kg to approximately 380.0 µg/kg, approximately 45.0 µg/kg to approximately 370.0 µg/kg, approximately 46.0 µg/kg to approximately 360.0 µg/kg, approximately 47.0 µg/kg to approximately 350.0 µg/kg, approximately 48.0 µg/kg to approximately 340.0 µg/kg, approximately 49.0 µg/kg to approximately 330.0 µg/kg, approximately 50.0 µg/kg to approximately 320.0 µg/kg, approximately 55.0 µg/kg to approximately 310.0 µg/kg, approximately 60.0 µg/kg to approximately 300.0 µg/kg, approximately 65.0 µg/kg to approximately 290.0 µg/kg, approximately 70.0 µg/kg to approximately 280.0 µg/kg, approximately 75.0 µg/kg to approximately 270.0 µg/kg, approximately 80.0 µg/kg to approximately 260.0 µg/kg, approximately 85.0 µg/kg to approximately 250.0 µg/kg, approximately 90.0 µg/kg to approximately 240.0 µg/kg, approximately 95.0 µg/kg to approximately 230.0 µg/kg, approximately 100.0 µg/kg to approximately 220.0 µg/kg, approximately 110.0 µg/kg to approximately 210.0 µg/kg, approximately 120.0 µg/kg to approximately 200.0 µg/kg, approximately 130.0 µg/kg to approximately 190.0 µg/kg, approximately 140.0 µg/kg to approximately 180.0 µg/kg, and approximately 150.0 µg/kg to approximately 170.0 µg/kg.

[0146] In one embodiment, the diagnostic effective amount of the compound of Formula 1 is in the range from approximately 3000.0 µg/kg to approximately 1500.0 µg/kg.

[0147] In one embodiment, the diagnostic effective amount of the compound of Formula 1 is in the range from approximately 1500.0 µg/kg to approximately 1000.0 µg/kg.

[0148] In one embodiment, the diagnostic effective amount of the compound of Formula 1 is in the range from approximately 1000.0 µg/kg to approximately 500.0 µg/kg.

[0149] In one embodiment, the diagnostic effective amount of the compound of Formula 1 is in the range from approximately 500.0 µg/kg to approximately 170.0 µg/kg.

[0150] In one embodiment, the diagnostic effective amount of the compound of Formula 1 is in the range from approximately 170.0 µg/kg to approximately 120.0 µg/kg.

[0151] In one embodiment, the diagnostic effective amount of the compound of Formula 1 is in the range from approximately 120.0 µg/kg to approximately 60.0 µg/kg.

[0152] In one embodiment, the diagnostic effective amount of the compound of Formula 1 is in the range from approximately 30.0 µg/kg to approximately 500.0 µg/kg.

5 [0153] In one embodiment, the diagnostic effective amount of the compound of Formula 2 is in the range from approximately 3000.0 µg/kg to approximately 1500.0 µg/kg.

[0154] In one embodiment, the diagnostic effective amount of the compound of Formula 2 is in the range from approximately 1500.0 µg/kg to approximately 1000.0 µg/kg.

10 [0155] In one embodiment, the diagnostic effective amount of the compound of Formula 2 is in the range from approximately 1000.0 µg/kg to approximately 500.0 µg/kg.

[0156] In one embodiment, the diagnostic effective amount of the compound of Formula 2 is in the range from approximately 500.0 µg/kg to approximately 170.0 µg/kg.

[0157] In one embodiment, the diagnostic effective amount of the compound of Formula 2 is in the range from approximately 170.0 µg/kg to approximately 120.0 µg/kg.

15 [0158] In one embodiment, the diagnostic effective amount of the compound of Formula 2 is in the range from approximately 120.0 µg/kg to approximately 60.0 µg/kg.

[0159] In one embodiment, the diagnostic effective amount of the compound of Formula 2 is in the range from approximately 30.0 µg/kg to approximately 500.0 µg/kg.

20 [0160] The diagnostic effective amount of each of the compound of Formula 1 and the compound of Formula 2 according to the invention is effective over a wide dosage range. The exact dosage will depend upon the route of administration, the form in which each compound is administered, the gender, age, and body weight of the subject to be treated, and the preference and experience of the administrator.

25 [0161] In selected embodiments, the fluorescence intensity of each of the administered compound of Formula 1 and the administered compound of Formula 2 may be independently measured at a time period of, for example, less than 24 hrs, 23 hrs, 22 hrs, 21 hrs, 20 hrs, 19 hrs, 18 hrs, 17 hrs, 16 hrs, 15 hrs, 14 hrs, 13 hrs, 12 hrs, 11 hrs, 10 hrs, 9.5 hrs, 9.0 hrs, 8.5 hrs, 8.0 hrs, 7.5 hrs, 7.0 hrs, 6.5 hrs, 6.0 hrs, 5.5 hrs, 5.0 hrs, 4.75 hrs, 4.50 hrs, 4.25 hrs, 4.00 hrs, 3.75 hrs, 3.50 hrs, 3.25 hrs, 3.00 hrs, 2.75 hrs, 2.50 hrs, 2.25 hrs, 2.00 hrs, 1.75 hrs, 1.50

hrs, 1.25 hrs, 1.00 hrs, 0.90 hrs, 0.80 hrs, 0.70 hrs, 0.60 hrs, 0.50 hrs, 0.40 hrs, 0.30 hrs, 0.20 hrs after administering.

[0162] In selected embodiments, the fluorescence intensity of each of the administered compound of Formula 1 and the administered compound of Formula 2 may be independently measured at a time period of, for example, greater than 23 hrs, 22 hrs, 21 hrs, 20 hrs, 19 hrs, 18 hrs, 17 hrs, 16 hrs, 15 hrs, 14 hrs, 13 hrs, 12 hrs, 11 hrs, 10 hrs, 9.5 hrs, 9.0 hrs, 8.5 hrs, 8.0 hrs, 7.5 hrs, 7.0 hrs, 6.5 hrs, 6.0 hrs, 5.5 hrs, 5.0 hrs, 4.75 hrs, 4.50 hrs, 4.25 hrs, 4.00 hrs, 3.75 hrs, 3.50 hrs, 3.25 hrs, 3.00 hrs, 2.75 hrs, 2.50 hrs, 2.25 hrs, 2.00 hrs, 1.75 hrs, 1.50 hrs, 1.25 hrs, 1.00 hrs, 0.90 hrs, 0.80 hrs, 0.70 hrs, 0.60 hrs, 0.50 hrs, 0.40 hrs, 0.30 hrs, 0.20 hrs, 0.10 hrs after administering.

[0163] In selected embodiments, the fluorescence intensity of each of the administered compound of Formula 1 and the administered compound of Formula 2 may be independently measured at a time period in the range from approximately 0.10 hrs to approximately 24 hrs, approximately 0.20 hrs to approximately 23 hrs, approximately 0.30 hrs to approximately 22 hrs, approximately 0.40 hrs to approximately 21 hrs, approximately 0.50 hrs to approximately 20 hrs, approximately 0.60 hrs to approximately 19 hrs, approximately 0.70 hrs to approximately 18 hrs, approximately 0.80 hrs to approximately 17 hrs, approximately 0.90 hrs to approximately 16 hrs, approximately 1.00 hr to approximately 15 hrs, approximately 1.25 hrs to approximately 14 hrs, approximately 1.50 hrs to approximately 13 hrs, approximately 1.75 hrs to approximately 12 hrs, approximately 2.00 hrs to approximately 11 hrs, approximately 2.25 hrs to approximately 10 hrs, approximately 2.50 hrs to approximately 9.5 hrs, approximately 2.75 hrs to approximately 9.0 hrs, approximately 3.00 hrs to approximately 8.5 hrs, approximately 3.25 hrs to approximately 8.0 hrs, approximately 3.50 hrs to approximately 7.5 hrs, approximately 3.75 hrs to approximately 7.0 hrs, approximately 4.00 hrs to approximately 6.5 hrs, approximately 4.25 hrs to approximately 6.0 hrs, approximately 4.50 hrs to approximately 5.5 hrs, approximately 4.75 hrs to approximately 5.0 hrs after administering.

[0164] In the embodiments of the methods described herein, the sample is illuminated with a wavelength of light selected to give a detectable optical response, and observed with a means for detecting the optical response. Equipment that is useful for illuminating the dye compounds of the invention includes, but is not limited to, tungsten lamps, hand-held ultraviolet lamps, mercury arc lamps, xenon lamps, light emitting diodes (LED), lasers and

laser diodes. These illumination sources are optionally integrated into surgical cameras, laparoscopes and microscopes. Preferred embodiments of the invention are dyes that are excitable at or near the wavelengths 633-636 nm, 647 nm, 660 nm, 680 nm and beyond 700 nm, such as 780 nm, 810 nm and 850 nm as these regions closely match the output of
5 relatively inexpensive excitation sources. The optical response is optionally detected by visual inspection, or by use of any of the following devices: CCD cameras, video cameras, photographic film.

[0165] The NIR imaging probe used was the compound of Formula 1; excitation 773 nm and emission 790 nm or independently, Formula 2, excitation 772 nm and emission 787 nm.

10 [0166] The compound of Formula 1 and the compound of Formula 2 as described herein can be administered in a manner compatible with the dosage formulation, and in such amount as will be effective or suitable for *in vivo* imaging. The quantity to be administered depends on a variety of factors including, *e.g.*, the age, body weight, physical activity, and diet of the individual, the tissue or organ to be imaged, and type of procedure or surgery to be
15 performed. In certain embodiments, the size of the dose may also be determined by the existence, nature, and extent of any adverse side effects that accompany the administration of the compound in a particular individual.

[0167] It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including
20 the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, hereditary characteristics, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

[0168] In certain embodiments, the dose may take the form of solid, semi-solid, or
25 lyophilized powder forms, preferably in unit dosage forms suitable for simple administration of precise dosages. In some embodiments, the dose is provided in a container, vial or syringe at a particular dosage for one or more administrations.

[0169] As used herein, the term "unit dosage form" refers to physically discrete units suitable as unitary dosages for humans and other mammals, each unit containing a
30 predetermined quantity of an imaging agent calculated to produce the desired onset, tolerability, and/or fluorescent effects, in association with a suitable pharmaceutical excipient (*e.g.*, an ampoule). In addition, more concentrated dosage forms may be prepared, from

which the more dilute unit dosage forms may then be produced. The more concentrated dosage forms thus will contain substantially more than, *e.g.*, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times the amount of the imaging agent.

[0170] Methods for preparing such dosage forms are known to those skilled in the art (*see, e.g., REMINGTON'S PHARMACEUTICAL SCIENCES*, 18th ED., Mack Publishing Co., Easton, PA (1990)). The dosage forms typically include a conventional pharmaceutical carrier or excipient and may additionally include other medicinal agents, carriers, adjuvants, diluents, tissue permeation enhancers, solubilizers, and the like. Appropriate excipients can be tailored to the particular dosage form and route of administration by methods well known in the art (*see, e.g., REMINGTON'S PHARMACEUTICAL SCIENCES*, 18th ED., Mack Publishing Co., Easton, PA (1990)).

[0171] In certain embodiments, the dosage forms contain a stabilizing agent for the storage, isolation, purification, and/or lyophilization of the compounds. Agents for lyophilization include, but are not limited to, a saccharide such as a monosaccharide, a disaccharide or dextran. Other saccharides include glucose, galactose, xylose, glucuronic acid, trehalose, dextran, hydroxyethyl starch, mannitol, or 5% dextrose.

[0172] For parenteral administration, *e.g.*, intravenous injection, intra-arterial injection, subcutaneous injection, intramuscular injection and the like, the effective dose can be in the form of sterile injectable solutions and sterile packaged powders. Preferably, injectable solutions are formulated at a pH of from about 4.5 to about 7.5, or physiological pH.

[0173] In some embodiments, the effective dose for imaging contains a lyophilized compound described herein in a high-quality, easily dissolved form which is stable for months at room temperature. The lyophilized compound of Formula 1 or Formula 2 can be stored in any suitable type of sealed container, such as a sealed vial or syringe that contains an amount of the compound for a single dosage for a subject, such as a human adult. The term "vial" is used broadly herein, to refer to any drug-packaging device that is designed and suitable for sealed and sterile storage, shipping, and handling of small (*e.g.*, single-dosage) quantities of drugs. Single-chamber vials (which would contain only the lyophilized compound, with no water) are well known; a typical single-chamber vial may be designed for use with an intravenous infusion bag. Alternatively, two-chamber vials can be used that contain both the lyophilized compound and a sterile aqueous solution, to enable immediate

reconstitution and injection of an aqueous liquid containing the compound of Formula 1 or Formula 2.

[0174] Provided herein are kits containing a compound of Formula 1 or a compound of Formula 2. In some embodiments, a kit comprises one or more vials or syringes containing compound of Formula 1 in, for example, a lyophilized form. In other embodiments, a kit comprises one or more vials or syringes containing compound of Formula 2 in, for example, a lyophilized form. Such kits can also include a pharmaceutically acceptable carrier or a sterile aqueous solution, *e.g.*, sterile water for reconstituting the compound prior to administration. In some cases, the kit also includes a sterile syringe for parenteral administration of the compound or for use with an intravenous infusion bag. The kit can also include an instruction manual for use.

[0175] When ranges are used herein, all combinations and subcombinations of ranges and specific embodiments therein are intended to be included. Use of the term “approximately” when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary from, for example, between 1% and 15% of the stated number or numerical range. The term “comprising” (and related terms such as “comprise” or “comprises” or “having” or “including”) includes those embodiments that “consist of” or “consist essentially of” the described features.

III. EXAMPLES

EXAMPLE 1

[0176] The compound of Formula 1 may be synthesized by dissolving 3,3-Dimethyl-2-[2-[2-chloro-3-[2-[1,3-dihydro-3,3-dimethyl-5-sulfo-1-(4-sulfobutyl)-2H-indol-2-ylidene]-ethylidene]-1-cyclohexen-1-yl]-ethenyl]-5-sulfo-1-(4-sulfobutyl)-3H-indolium hydroxide, innersalt, trisodium salt (1 g, 1.05 mmol) in 25 mL of water and sparging with nitrogen for 15 minutes. Sodium 4-hydroxybenzenesulfonate dihydrate (875 mg, 3.77 mmol) was dissolved in 3.6 mL 1N NaOH (3.6 mmol) and added to the reaction mixture. The reaction mixture was placed in an oil bath at 40°C and stirred for 16 hours. The solution was dried by rotary evaporation and the product was then recrystallized from 80:20 ethanol:water. The compound was filtered then washed with ethanol and dried under vacuum at 60°C for 18 hours.

[0177] The polymorph of the compound of Formula 1 (Form A) can be prepared as follows:

[0178] A mixture of 3,3-Dimethyl-2-[2-[2-chloro-3-[2-[1,3-dihydro-3,3-dimethyl-5-sulfo-1-(4-sulfobutyl)-2H-indol-2-ylidene]-ethylidene]-1-cyclohexen-1-yl]-ethenyl]-5-sulfo-1-(4-sulfobutyl)-3H-indolium hydroxide, innersalt, trisodium salt (20 g, 21 mmol) and Sodium 4-hydroxybenzenesulfonate dihydrate (5.84 g, 25.2 mmol) is suspended in water (120 ml). The suspension is heated to 85 °C where complete dissolution is observed. Aqueous sodium hydroxide (10N, 2.5 ml, 25 mmol) is added dropwise and the reaction stirred for 45 min. Isopropanol (360 ml) is added slowly to maintain the reaction temperature above 60 °C. The mixture is then slowly cooled to ambient temperature and the resulting slurry filtered. The filter cake is rinsed with 40 ml isopropanol:water (3:1) and twice with 40 ml isopropanol and dried at 50-60 °C under vacuum to obtain 18.4 g of the compound of Formula 1 as a dark green solid. Ten grams (9 mmol) of this material is then recrystallized by dissolving in water (50 ml) and isopropanol (100 ml) at approximately 70 °C, and slowly cooling the mixture to ambient temperature. The solids are collected by filtration and rinsed with 20 ml isopropanol:water (2:1) and twice with 20 ml isopropanol and dried at 50-60 °C under vacuum to obtain 6.7 g of the compound Formula 1 as a crystalline dark green solid.

[0179] X-Ray Powder Diffraction (XRPD) for Form A

[0180] XRPD analysis was carried out on a PANalytical X'pert pro, scanning the samples between 3 and 35° 2θ. The material was gently ground and loaded onto a multiwell plate with Kapton or mylar polymer film to support the sample. The multiwell plate was then loaded into a Panalytical diffractometer running in transmission mode and analyzed.

[0181] The XRPD data are shown in FIG. 1A-1F. As illustrated, FIG. 1A and 1C are identical and are both moderately crystalline and represent Form A. FIG. 1B is similar in crystallinity and is Form A.

[0182] FIG. 1D is crystalline. FIG. 1E is an enlargement of FIG. 1D. FIG. 1D and FIG. 1E represent Form A.

[0183] FIG. 1F is the amorphous form.

[0184] Characteristic XRPD angles and d-spacings for the solid state form are summarized in Table 1 for Form A. Peak positions were measured and tabulated.

Table 1: Peak Data of IRDye 800BK Lot: BV736185 (FIG. 1A) XPRD Analysis

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
4.2384	362.12	0.1279	20.84818	29.2
5.2771	550.29	0.0895	16.74659	44.37
5.4888	295.59	0.0768	16.10118	23.83
5.9607	293.22	0.0512	14.82759	23.64
9.5414	221.36	0.1535	9.26957	17.85
10.5728	314.69	0.0768	8.36752	25.37
10.9522	224.35	0.1023	8.07854	18.09
11.3914	371.25	0.0768	7.76803	29.93
11.9362	322.74	0.0768	7.41466	26.02
12.8323	579.36	0.0895	6.89882	46.71
14.1854	785.5	0.1279	6.24366	63.33
15.0743	305.87	0.1279	5.87743	24.66
15.3653	384.4	0.1279	5.76676	30.99
15.5532	262.25	0.1023	5.69752	21.15
15.8747	444.26	0.0512	5.58284	35.82
16.3152	405.54	0.1535	5.43308	32.7
16.7216	378.51	0.0768	5.30195	30.52
17.3776	514.77	0.064	5.10327	41.51
18.2698	226.8	0.1023	4.856	18.29
19.1518	465.59	0.1535	4.63431	37.54
19.5492	393.65	0.1279	4.54099	31.74
20.1805	277.83	0.1023	4.40035	22.4
20.7531	1146.9	0.1151	4.2802	92.47
21.2128	1240.24	0.1151	4.18848	100
21.8521	229.87	0.2047	4.06738	18.53
22.6123	239.17	0.1535	3.93232	19.28
23.389	331.71	0.064	3.80346	26.75
23.8748	148.63	0.1535	3.72716	11.98
24.3664	124.78	0.1535	3.65307	10.06
24.9407	221.2	0.1279	3.57024	17.84
26.4158	297.98	0.1279	3.37412	24.03
28.658	136.22	0.1791	3.11503	10.98

[0185] Characteristic XRPD angles and d-spacings for the solid state form are summarized in Table 2 for Form A. Peak positions were measured and tabulated.

Table 2: Peak data of IRDye 800BK Lot: VE-759-24-1 (FIG. 1E) XRPD Analysis

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
4.272	1845.05	0.064	20.68408	100
9.6191	863.9	0.0895	9.19491	46.82

10.5611	267.84	0.1023	8.37677	14.52
12.4823	217.24	0.0768	7.09145	11.77
12.7274	748.04	0.0895	6.95545	40.54
12.93	1108.7	0.0768	6.84692	60.09
13.4567	353.68	0.1279	6.58007	19.17
14.197	188.01	0.1023	6.23861	10.19
15.4372	537.33	0.064	5.74009	29.12
15.8734	703.35	0.0384	5.58329	38.12
16.1055	637.51	0.064	5.50336	34.55
16.6046	222	0.1279	5.33906	12.03
17.1929	293.61	0.1023	5.15766	15.91
17.559	419.11	0.1023	5.05094	22.72
18.0101	705.32	0.1023	4.92543	38.23
18.2864	1035.04	0.1663	4.85163	56.1
18.7831	635.82	0.0512	4.72445	34.46
19.1781	617.24	0.0895	4.62801	33.45
19.4323	420.97	0.4093	4.56805	22.82
19.7836	680.51	0.064	4.48772	36.88
20.2392	744.22	0.0512	4.38771	40.34
20.5039	801.47	0.1151	4.33166	43.44
20.8076	960.22	0.1407	4.26912	52.04
21.1497	546.41	0.1791	4.20083	29.61
21.7763	541.2	0.064	4.08136	29.33
22.5703	573.98	0.0512	3.93954	31.11
23.1948	292.69	0.1023	3.83487	15.86
23.6245	311.01	0.1279	3.76608	16.86
24.6218	243.08	0.1535	3.61575	13.17
25.2623	343.69	0.1791	3.52551	18.63
26.3234	274.93	0.1791	3.38575	14.9
28.0536	225.6	0.1535	3.18075	12.23
28.6694	301.22	0.2047	3.11382	16.33

[0186] In one embodiment, it is possible to make Form B from Form A. Form A is crystalline and exists as small birefringent particles with no defined morphology. The material is hygroscopic (27 wt% uptake up-to 90 %RH) and can change form under stress conditions. A form change does occur when Form A is subjected to 40 °C/75% RH. This form corresponds with the form found with the post-GVS sample. HPLC purity showed no change.

[0187] FIG. 1H and FIG. 1I represent Form B. For comparison, FIG. 1G is identical to FIG. 1D.

[0188] Characteristic XRPD angles and d-spacings for the solid state form are summarized in Table 3 for Form B. Peak positions were measured and tabulated.

Table 3: Peak data of IRDye 800BK Lot: VE-759-24-1 Form B XRPD Analysis

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
5.2989	1193.55	0.064	16.67783	52.81
5.9816	649.44	0.0512	14.77594	28.74
9.535	387.23	0.064	9.27578	17.13
10.6007	690.31	0.1023	8.3456	30.55
10.9387	376.49	0.0895	8.08848	16.66
11.3924	413.39	0.064	7.76734	18.29
11.9715	817.54	0.0768	7.39285	36.18
12.8677	587.96	0.1279	6.8799	26.02
14.2153	1273.22	0.0936	6.22544	56.34
14.2827	1000.8	0.078	6.21161	44.28
15.9468	606.88	0.2496	5.55317	26.85
16.3781	600.12	0.1092	5.4079	26.55
16.7597	562.34	0.1092	5.28561	24.88
17.3714	797.24	0.078	5.10083	35.28
17.7698	277.37	0.1872	4.98736	12.27
18.4312	246.4	0.156	4.80986	10.9
18.6816	475.06	0.1872	4.74595	21.02
19.1392	766.24	0.078	4.63351	33.91
19.5987	600.29	0.0936	4.52589	26.56
20.1626	509.11	0.0624	4.40057	22.53
20.7073	1255.16	0.1404	4.28603	55.54
21.2464	2259.95	0.1092	4.17848	100
21.8382	456.8	0.156	4.06656	20.21
22.6653	385.85	0.1872	3.92	17.07
23.4295	644.04	0.2808	3.79383	28.5
23.9381	351.56	0.1872	3.71438	15.56
24.9551	451.86	0.2184	3.56525	19.99
26.4745	574.6	0.0468	3.36399	25.43

5 EXAMPLE 2

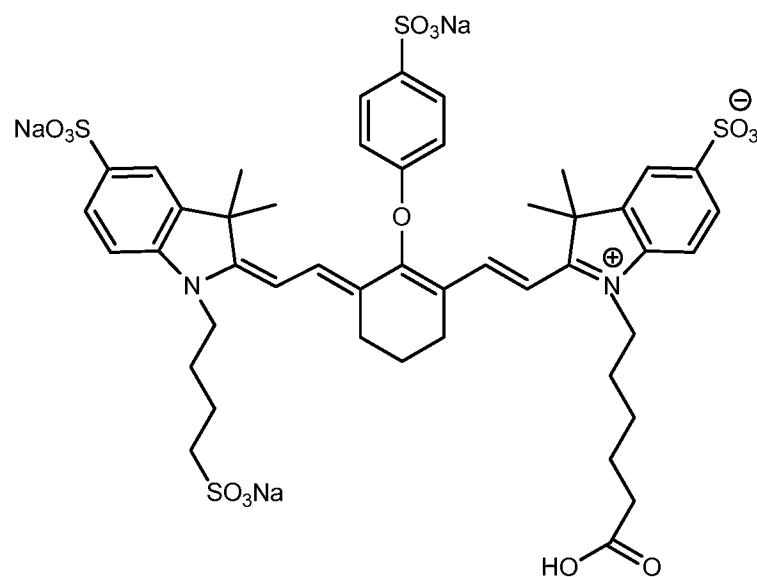
[0189] The compound of Formula 2 may be synthesized by dissolving 3,3-Dimethyl-2-[2-[2-chloro-3-[2-[1,3-dihydro-3,3-dimethyl-5-sulfo-1-(4-sulfobutyl)-2H-indol-2-ylidene]-ethylidene]-1-cyclohexen-1-yl]-ethenyl]-5-sulfo-1-(4-sulfobutyl)-3H-indolium hydroxide, innersalt, trisodium salt (1 g, 1.05mmol) in 25 mL of water and sparging with nitrogen for 15 minutes. Sodium phenoxide trihydrate (390 mg, 2.29mmol) was dissolved in 2 mL ultrapure water and added to the reaction mixture. The reaction mixture was placed in an oil bath at

10

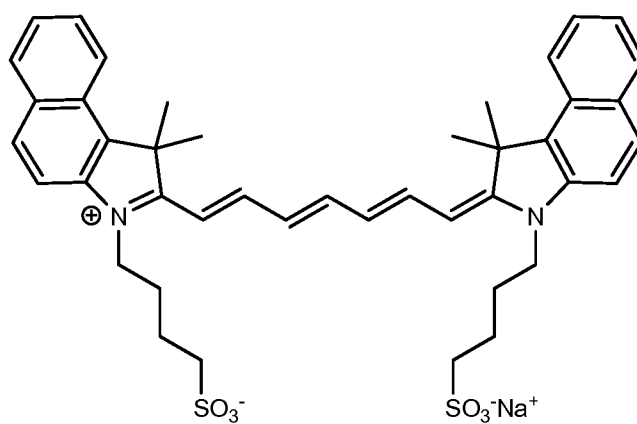
40°C and stirred for 4 hours. The solution was dried by rotary evaporation and the product was then recrystallized from 90:10 ethanol:water. The compound was filtered then washed with ethanol and dried under vacuum at 60°C for 18 hours.

COMPARATIVE EXAMPLE 1

- 5 [0190] The structure of the compound of comparative example 1, also referred to as 800CW, is shown below:



- [0191] The structure of indocyanine green (ICG) dye is shown below:



10

EXAMPLE 3: In vitro

- [0192] In vitro cell-based assays with two carcinoma cell lines (A431 and A549) were done to assess non-specific binding of the compound of Formula 1 and the compound of Formula 2. The dyes were compared to 800CW. This is shown in **FIGS. 2A, 2B, and 2C**.

[0193] The compound of Formula 2 demonstrated a significant increase in non-specific binding compared to either the compound of Formula 1 and 800CW by two-fold. The compound of Formula 1 was more comparable to 800CW.

[0194] To confirm all three compounds have comparable fluorescence, a dilution series was made and imaged on an Odyssey SA fluorescence imaging system (LI-COR Biosciences). The fluorescence in each well was quantified with the instrument software.

FIG. 2C shows that all three compounds in this test were very close in fluorescence, thus confirming that the increased fluorescence at higher concentrations noted in plate-based assay is likely due to a tendency of the compound to bind non-specifically to cells.

[0195] In general, low non-specific binding is an advantage. Both cell lines show low non-specific binding for all compounds at concentrations below 1 μ M. However all begin to show some binding at higher concentrations.

EXAMPLE 4: In vivo

[0196] Animals injected with the probes were imaged over time using the 800 fluorescence channel of the Pearl Impulse Imaging System (LI-COR Biosciences). Clearance profiles for 800CW, the compound of Formula 1, and the compound of Formula 2 were carried out in nude mice (dose of 1 nmole for all compounds). Serial images and excised organs were evaluated over a 24 hr period. Dorsal image series are presented in **FIG. 3**. A Pearl Impulse small animal imaging system was used for all animal and organ image acquisitions.

[0197] The compound of comparative example 1 is used as the reference. From the Dorsal view there is no kidney signal for any probe. At 2 hrs post injection, the compound of Formula 2 exhibits high signal, emanating in the liver region visible from the back. The compound of Formula 2 never fully disseminates because it is collected rapidly in the liver and by 5 hr post injection, the whole body signal is low compared to 800CW and the compound of Formula 1.

EXAMPLE 5: Ventral images

[0198] Ventral images are presented in **FIG. 4**. From visual examination, the compound of Formula 1 and 800CW are similar in their clearance pattern. These two probes appear to clear rapidly via the kidney. This feature is very useful for imaging the urinary system.

[0199] The compound of Formula 2, on the other hand, clears through the liver. This feature is a very useful attribute for a biliary duct monitoring dye which may “mimic” ICG that is currently used for this clinical application. From the in vitro plate-based assays it appeared both the compound of Formula 1 and the compound of Formula 2 have similar fluorescence intensities to 800CW. However, from the in vivo data, it appeared that the compound of Formula 1 moves rapidly through the tissues but is retained for an extended period in organs.

[0200] Bladder signal is the strongest signal noted for 800CW and the compound of Formula 1 probes (purple arrows).

[0201] Whole body fluorescence numbers for the three probes (ventral view) are shown in FIG. 5. Non-invasive imaging will not capture signals for the full depth of the animal but the intensity values are useful to show a relative trend.

[0202] There is a slower clearance for 800CW and the compound of Formula 1 as compared to the compound of Formula 2. The signal for the compound of Formula 2 appears to be lower overall than the signal for 800CW and the compound of Formula 1, however, it appears to rapidly collect in the liver. At 24 hrs post administration, the remaining fluorescence is diminished for all three probes to roughly the same extent.

[0203] Another way of examining fluorescence signals is by measuring the % of the compound remaining at a particular time point. The 30 min time point is the baseline to which other time points are normalized. This data are presented below in Table 4.

	30 min	120 min	300 min	1440 min
Compound of comparative example 1 (800CW)	100	85	23	0.8
Compound of Formula 1 (800BK-sulfonate)	100	81	19	1.4
Compound of Formula 2 (800BK-NOS)	100	70	14	1.4

[0204] The percent signal remaining at each time is shown with the 30 min time point post-injection, which is used as the baseline.

[0205] The greatest reduction occurs for the compound of Formula 2 at 2 hrs. The compound of Formula 1 is very similar to 800CW and by 24 hrs post injection, all compounds are very similar in remaining signals at less than 2% of baseline.

EXAMPLE 5: Organs

5 [0206] Organs were excised at 24 hrs and imaged. Data is presented in **FIG. 6**.

[0207] The compound of Formula 1 is retained at higher levels in the liver and kidney compared to the compound of Formula 2 at 24 hrs post injection. Surprisingly, the compound of Formula 2, which clears predominantly from the liver, has lower liver signals at 24 hrs when compared to 800CW and the compound of Formula 1. This may be explained
10 by a slower overall clearance from tissue for the compound of Formula 1 as compared to 800CW and the compound of Formula 2.

[0208] None of 800CW or the compound of Formula 1 or the compound of Formula 2 is detected in the brain and is likely due to their inability to cross the blood brain barrier.

[0209] **FIG. 7** illustrates the ureters along with kidney and bladder. When the compound
15 of Formula 1 was administered to screen the ureter of the kidney, an image of the region was captured post-administration.

[0210] **FIG. 8** illustrates fluorescence images of stained electrophoresis gels of selected proteins showing non-specific or affinity of the named compounds to human (H), bovine (B), ovalbumin (O), or 5% FBS (fetal bovine serum). It is clear that the compound of Formula 2
20 has potential to be useful for biliary duct examinations, similar to ICG.

EXAMPLE 7: Mouse Urine

[0211] With the primary elimination route being renal for the compound of Formula 1, the urine output in mice was analyzed. Urine was obtained from mice that received: neither of the compound of Formula 1 nor the compound of Formula 2 (control), the compound of
25 Formula 1, ICG, and the compound of Formula 2. The urine was extracted with an acetonitrile:methanol mix and imaged on an Odyssey CLx imager (LI-COR Biosciences) as shown in **FIG. 9**.

[0212] No fluorescent signal was seen for the control and ICG. ICG is eliminated by the liver so no signal is expected. There was a slight fluorescence for the compound of Formula
30 2 that suggests liver excretion is not quite exclusive.

EXAMPLE 8: Comparison with ICG

[0213] A comparison between the compound of Formula 2 and ICG was conducted for their common application in imaging the biliary duct (**FIGS. 10A** and **10B**). A Pearl Impulse small animal imaging system was used for all animal and organ image acquisitions. ICG is not very soluble in aqueous media and gives a much weaker fluorescent signal than the compound of Formula 2 in vivo. A 50 nmole solution of ICG in phosphate buffer saline solution was prepared, injected intravenously, and compared for localization with a 1 nmole solution of the compound of Formula 2 at time periods of 1 min, 30 min, 2 hrs, 5 hrs, and 24 hrs.

[0214] A comparison of the dose responses for the compound of Formula 2 are shown in **FIG. 10B** (1 nmole), **FIG. 10C** (0.5 nmole), and **FIGS. 10D** and **10E** (0.1 nmole) at time periods of 1 min, 30 min, 2 hrs, 5 hrs, and 24 hrs. All images are presented using the same LUT table except **FIG. 10E** where the LUT for **FIG. 10D** has been reduced to brighten the 800nm signal so they are visible.

[0215] The gall bladder is visible by 30 min post-intravenous administration for the 1 nmole (**FIG. 10B**) and 0.5 nmole (**FIG. 10C**) doses and continues to be visible in the 2 hrs and 5 hrs images. The images for the 0.1 nmole dose in **FIG. 10D** appear to show very low detection signal using the same scale as the 1 nmole and 0.5 nmole dose series. However, if the scale is adjusted as in **FIG. 10E**, the gall bladder target region is visible on a very low background. A wide range of dosing is possible to accommodate a particular imaging system or application.

EXAMPLE 9: Excised organ evaluation

[0216] Fluorescent signal intensities of various organs were examined at 24 hrs and 72 hrs for the compound of Formula 1 and the compound of Formula 2 (**FIG. 11A-11E**). Organs examined included: heart (Ht), lungs (Ln), kidney (Kd), liver (Lv), spleen (Spl), intestine (Int), brain (Br), and muscle (Ms). At 24 hrs, there remains some signal in the liver and kidney but by 72 hrs the signal is diminished substantially.

[0217] **FIG. 11A** shows the results using the control (800CW), 24 hours post administration.

[0218] FIG. 11B shows the results using a compound of Formula 2, 24 hours post administration. FIG. 11C shows the results using a compound of Formula 1, 24 hours post administration.

[0219] FIG. 11D-11E show the results 72 hours post administration of Formula 2 and Formula 1, respectively.

[0220] FIG. 11F shows a surgically opened animal for determining whether biliary structures are visible. The animal's liver was positioned to expose the critical structures. The image series of FIG. 11F-11H present the white light image of the liver with lobes raised to expose the gall bladder and biliary duct associated with Calot's triangle; the 800 nm image as detected by fluorescence studies of a compound of Formula 2; and a composite image overlaying the 800 nm image atop the white light image.

EXAMPLE 10: Elimination routes in mice

[0221] This example describes an experiment to evaluate the excretion of the dyes described herein from animals injected with such. The dyes tested were stable cyanine dyes (IRDyes): 800CW, 800BK-sulfonate (compound of Formula 1; 800BK or BK) and 800BK-NOS (compound of Formula 2; 800NOS or NOS). In this study 12 nude mice (3 per treatment group) were injected with either (1) no probe (control group); (2) 800CW; (3) 800NOS; or (4) 800BK. The three probes (800CW-1166 g/mole (should have been 1091.1 g/mole), 800NOS-1011.09 g/mole, and 800BK-1113.14 g/mole) were dissolved in PBS. A spot test of the three probes was performed to detect the fluorescent signal when diluted to the injection dose of 1 nmole/100 μ l (FIG. 12). The fluorescence of 800CW was slightly higher than that of 800NOS and 800BK.

[0222] The mice received 1 nmole of dye by tail vein injection. The mice were imaged serially over 24 hours after IV injection: 5 minutes, 1 hour, 2 hours, 4 hours, 6 hours and 24 hours after injection. A representative series of images are presented in FIGS. 13A-13C of one animal per dye. All the data was normalized to the same LUT. A control mouse (no probe) was used as a reference (no signal control). Imaging was performed using the Pearl[®] Trilog Imaging System (LI-COR[®]).

[0223] The mice were then sacrificed and their organs and tissue (i.e., liver, kidney, lungs, spleen and muscle) were harvested. The organs were imaged to detect fluorescence. When the same LUT was used for the animal and organ images, fluorescence in the organs was not

detectable. As such, the low end of the animal scale was expanded such that the images of the organs could be evaluated (**FIG. 14**). For example, the “Organs 1/10th scale” was used for the images of **FIGS. 15A-15D** and the “Organs 1/100th scale” was used for the images of **FIGS. 16A-16D**.

5 **[0224]** **FIG. 15A** shows no fluorescence in the organs of the control animal. Kidneys (lower left corner) and liver (lower right corner) had a detectable signal in the animals treated with 800CW (**FIG. 15B**), 800NOS (**FIG. 15C**), and 800BK (**FIG. 15D**). No muscle (top right corner) or lung tissue (top left corner) was visible in the treatments except for the 800CW treatment. At the 1/10th LUT scale, the high level is 1/10th that of the whole animal LUT scale. The data shows that there is only residual dye remaining in these organs when
10 imaging at the whole animal LUT scale.

[0225] **FIGS. 16A-D** show some signal in the organs if the whole animal LUT scale is reduced to 100x such that the adjusted scale is 1/100th the whole animal scale. **FIG. 16A** shows the signal from the control animal. **FIGS. 16B, 16C and 16D** shows the signal from
15 organs of the 800CW treated animal, the 800NOS treated animal and the 800BK treated animal, respectively.

[0226] 800CW and 800BK was excreted renally. The higher overall signal in the 800CW animals is mainly due to the accidentally higher concentration of the probe injected into the animals.

20 **[0227]** 800NOS was eliminated from the body via the biliary tract (liver, gall bladder and bile ducts) and intestines. In the 800NOS treated animals, the liver was visible at 15 minutes and then the gall bladder, as the dye rapidly left the liver. The data also showed that the dye was moved into the intestines for excretion. The predominant signal between 2-6 hours was in the intestines. At 24 hours post injection the intestinal signal was barely detectable.

25 **[0228]** The organs were imaged under three LUT scales; each being progressively smaller in the signal range covered. The lower levels of each progressive scale were expanded to cover the full red to blue color range (**FIG. 14**). Very little signal remained in any of the target organs such as liver, kidney, lungs and muscle. Similar signal intensities were found in the kidney for 800CW and 800BK. In the liver, 800BK had a higher signal compared to
30 800CW. The results show that 800BK has a longer retention time in the liver compared to 800CW and 800NOS. This may be due to increased (higher) plasma protein binding of 800BK than 800CW.

[0229] This example shows that the clearance of IReDye 800NOS is rapid from the whole body to the biliary system with a very short retention time in the liver and also rapid excretion into the intestines. The rapid clearance by the liver implies that 800NOS has low plasma protein binding activity.

5

EXAMPLE 11: Ureter Visualization during a hysterectomy

[0230] The pharmaceutical formulation of Formula 1 is dissolved in a vial (25 mg) with 5 mL of saline (0.9% sodium chloride) and is administered to a patient via a bolus injection at the concentration of 5mg/mL, 15 minutes prior to the surgery. The medical device for this procedure is the PINPOINT Endoscopic Fluorescence Imaging System (Novadaq, Mississauga, Ontario, Canada). At any point during the procedure, when the surgeon needs to identify the ureter, the device's mode is switched to the near-infrared detection imaging, and the surgeon visualizes the ureter via the monitor or display of the medical device. This identification of the ureter is visualized on the monitor as an overlay image, in which the surgeon can simultaneously locate the ureter with white light imaging.

15

EXAMPLE 12: Biliary Duct Visualization during a cholecystectomy

[0231] The pharmaceutical formulation of Formula 2 is dissolved in a vial (25mg) with 5 mL of saline (0.9% sodium chloride) and is administered to a patient via a bolus injection at the concentration of 5 mg/mL, 15 minutes prior to the surgery starting. The medical device for this procedure is the da Vinci Firefly Surgical System (Intuitive Surgical, Sunnyvale CA). During the procedure, when the surgeon needs to identify the biliary duct, the device's mode is switched to the near-infrared detection imaging, and the surgeon identifies the biliary duct via the monitor or display of the medical device by switching between the white light image and the near-infrared image as needed.

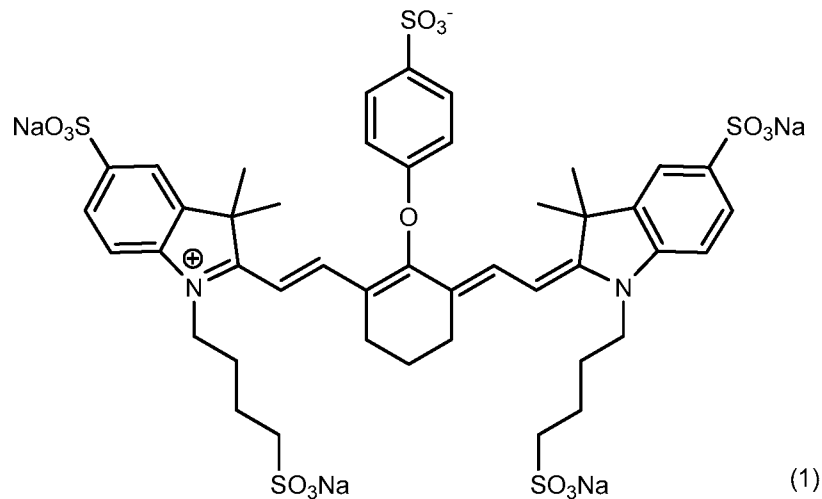
25

[0232] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

30

WHAT IS CLAIMED IS:

1. A method for kidney ureter imaging, the method comprising:
 administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-
 ((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-(4-
 sulfonatophenoxy)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-
 ium-5-sulfonate compound of Formula 1:



or a solvate or polymorph thereof and having a pharmaceutically acceptable
 cation, wherein the administering is performed at one or more times selected from the group
 consisting of before a procedure, during a procedure, after a procedure and combinations
 thereof,
 exposing tissue of the subject's renal system to electromagnetic radiation; and
 detecting fluorescence radiation from the compound.

2. The method of claim 1, wherein the administering is conducted
 intravenously.

3. The method of any one of claims 1-2, wherein the pharmaceutically
 acceptable cation is selected from the group consisting of potassium or sodium.

4. The method of any one of claims 1-3, wherein the compound is
 administered in combination with a pharmaceutically acceptable carrier selected from the
 group consisting of physiological sterile saline solution, sterile water solution, pyrogen-free
 water solution, isotonic saline solution, and phosphate buffer solution.

1 **5.** The method of any one of claims **1-4**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 3000.0 µg/kg and approximately 1500.0 µg/kg.

1 **6.** The method of any one of claims **1-5**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 1500.0 µg/kg and approximately 1000.0 µg/kg.

1 **7.** The method of any one of claims **1-6**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 1000.0 µg/kg and approximately 500.0 µg/kg.

1 **8.** The method of any one of claims **1-7**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 500.0 µg/kg and approximately 170.0 µg/kg.

1 **9.** The method of any one of claims **1-8**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 170.0 µg/kg and approximately 120.0 µg/kg.

1 **10.** The method of any one of claims **1-9**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 120.0 µg/kg and approximately 60.0 µg/kg.

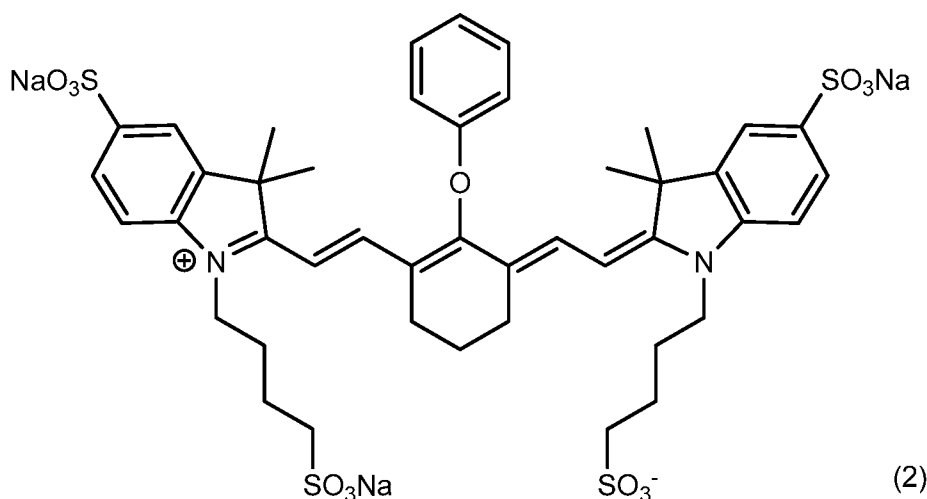
1 **11.** The method of any one of claims **1-10**, further comprising measuring a
2 fluorescence intensity of the administered compound remaining at the tissue of the subject's
3 renal system at a time period after administering.

1 **12.** The method of any one of claims **1-11**, wherein the measured
2 fluorescence intensity of the administered compound is background fluorescence
3 approximately 24 hours after administering.

1 **13.** The method of any one of claims **1-12**, wherein the procedure is
2 selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic
3 laparoscopic procedure, and an open procedure.

14. The method of any one of claims **11-13**, wherein the measured fluorescence intensity of the administered compound is higher in the kidney as compared to a measured fluorescence intensity of the administered compound in one or more of the spleen, intestine, heart, lungs, muscle, or combinations thereof approximately up to six hours after administering.

15. A method for liver biliary imaging, the method comprising:
administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy-cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate of Formula 2:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation, wherein the administering is performed at one or more times selected from the group consisting of before a procedure, during a procedure, after a procedure and combinations thereof,

exposing tissue of the subject's liver system to electromagnetic radiation; and detecting fluorescence radiation from the compound.

16. The method of claim **15**, wherein the administering is conducted intravenously.

17. The method of any one of claims **15-16**, wherein the pharmaceutically acceptable cation is selected from the group consisting of potassium or sodium.

1 **18.** The method of any one of claims **15-17**, wherein the compound is
2 administered in combination with a pharmaceutically acceptable carrier selected from the
3 group consisting of physiological sterile saline solution, sterile water solution, pyrogen-free
4 water solution, isotonic saline solution, and phosphate buffer solution.

1 **19.** The method of any one of claims **15-18**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 3000.0 µg/kg and approximately 1500.0 µg/kg.

1 **20.** The method of any one of claims **15-19**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 1500.0 µg/kg and approximately 1000.0 µg/kg.

1 **21.** The method of any one of claims **15-20**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 1000.0 µg/kg and approximately 500.0 µg/kg.

1 **22.** The method of any one of claims **15-21**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 500.0 µg/kg and approximately 170.0 µg/kg.

1 **23.** The method of any one of claims **15-22**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 170.0 µg/kg and approximately 120.0 µg/kg.

1 **24.** The method of any one of claims **15-23**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 120.0 µg/kg and approximately 60.0 µg/kg.

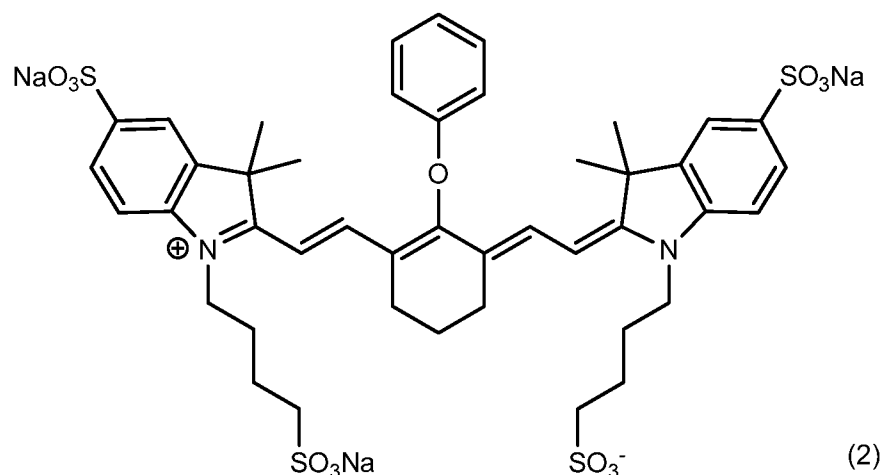
1 **25.** The method of any one of claims **15-24**, further comprising measuring
2 a fluorescence intensity of the administered compound remaining at the tissue of the subject's
3 liver system at a time period after administering.

1 **26.** The method of claim **25**, wherein the measured fluorescence intensity
2 of the administered compound is background fluorescence approximately 24 hours after
3 administering.

27. The method of any one of claims **15-26** wherein the procedure is selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic laparoscopic procedure, and an open procedure.

28. The method of claim **25**, wherein the measured fluorescence intensity of the administered compound is higher in the liver as compared to a measured fluorescence intensity of the administered compound in one or more of the spleen, intestine, heart, lungs, muscle, or combinations thereof approximately up to six hours after administering.

29. A method for liver cystic duct imaging, the method comprising:
administering to a subject a diagnostic effective amount of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy-cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate of Formula 2:



or a solvate or polymorph thereof and having a pharmaceutically acceptable cation, wherein the administering is performed at one or more times selected from the group consisting of before a procedure, during a procedure, after a procedure and combinations thereof,

exposing tissue of the subject's liver system to electromagnetic radiation; and detecting fluorescence radiation from the compound.

30. The method of claim **29**, wherein the administering is conducted intravenously.

31. The method of any one of claims **29-30**, wherein the pharmaceutically acceptable cation is selected from the group consisting of potassium or sodium.

1 **32.** The method of any one of claims **29-31**, wherein the compound is
2 administered in combination with a pharmaceutically acceptable carrier selected from the
3 group consisting of physiological sterile saline solution, sterile water solution, pyrogen-free
4 water solution, isotonic saline solution, and phosphate buffer solution.

1 **33.** The method of any one of claims **29-32**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 3000.0 µg/kg and approximately 1500.0 µg/kg.

1 **34.** The method of any one of claims **29-33**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 1500.0 µg/kg and approximately 1000.0 µg/kg.

1 **35.** The method of any one of claims **29-34**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 1000.0 µg/kg and approximately 500.0 µg/kg.

1 **36.** The method of any one of claims **29-35**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 500.0 µg/kg and approximately 170.0 µg/kg.

1 **37.** The method of any one of claims **29-36**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 170.0 µg/kg and approximately 120.0 µg/kg.

1 **38.** The method of any one of claims **29-37**, wherein the administering is
2 conducted at a diagnostic effective amount of the compound ranging between approximately
3 120.0 µg/kg and approximately 60.0 µg/kg.

1 **39.** The method of any one of claims **29-38**, further comprising measuring
2 a fluorescence intensity of the administered compound remaining at the tissue of the subject's
3 liver system at a time period after administering.

1 **40.** The method of claim **39**, wherein the measured fluorescence intensity
2 of the administered compound is background fluorescence approximately 24 hours after
3 administering.

1 **41.** The method of any one of claims **29-40**, wherein the procedure is
2 selected from the group consisting of a laparoscopic procedure, a robotic procedure, a robotic
3 laparoscopic procedure, and an open procedure.

1 **42.** The method of claim **39**, wherein the measured fluorescence intensity
2 of the administered compound is higher in the liver as compared to a measured fluorescence
3 intensity of the administered compound in one or more of the spleen, intestine, heart, lungs,
4 muscle, or combinations thereof approximately up to six hours after administering.

1 **43.** A pharmaceutical composition comprising a diagnostic imaging
2 amount of a compound of Formula 1, a pharmaceutically acceptable cation, and a
3 pharmaceutically acceptable carrier.

1 **44.** The pharmaceutical composition of claim **43**, wherein the composition
2 is contained in an intravenous (IV) bag.

1 **45.** A pharmaceutical composition comprising a diagnostic imaging
2 amount of a compound of Formula 2, a pharmaceutically acceptable cation, and a
3 pharmaceutically acceptable carrier.

1 **46.** The pharmaceutical composition of claim **45**, wherein the composition
2 is contained in an intravenous (IV) bag.

1 **47.** A solid form, which is Form A of Formula 1.

1 **48.** The solid form of claim **47**, having an X-ray powder diffraction
2 pattern comprising a peak, in terms of 2-theta, at about 4.3°.

1 **49.** The solid form of claim **47**, having an X-ray powder diffraction
2 pattern comprising a peak, in terms of 2-theta, at about 4.3°, about 9.6°, about 12.9°, about
3 18.3°, and about 20.8°.

1 **50.** The solid form of claim **47**, having an X-ray powder diffraction
2 pattern substantially as shown in FIG. 1E.

1 **51.** A solid form, which is Form B of Formula 1.

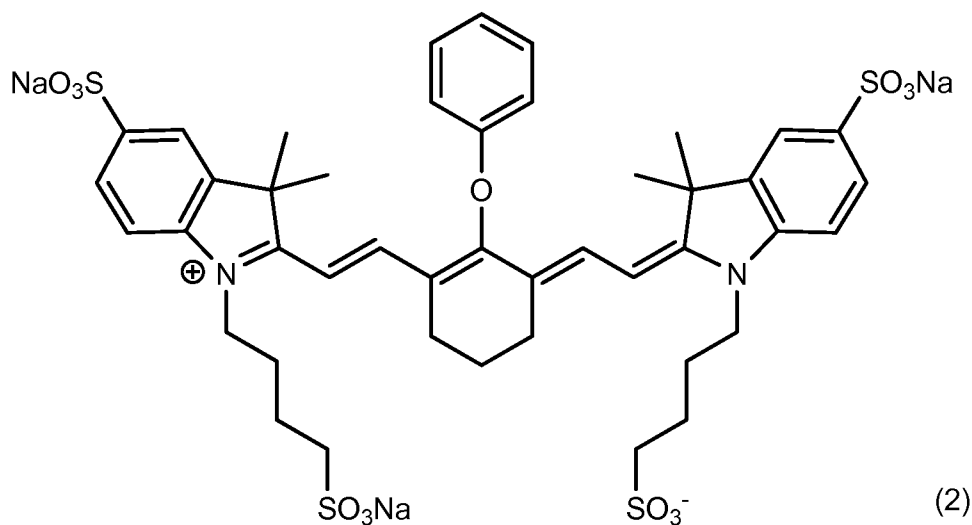
1 **52.** The solid form of claim **51**, having an X-ray powder diffraction pattern
2 comprising a peak, in terms of 2-theta, at about 21.2°.

1 **53.** The solid form of claim **51**, having an X-ray powder diffraction pattern
2 comprising a peak, in terms of 2-theta, at about 5.3°, about 14.2 °, about 14.3 °, about 20.7°
3 and about 21.2°.

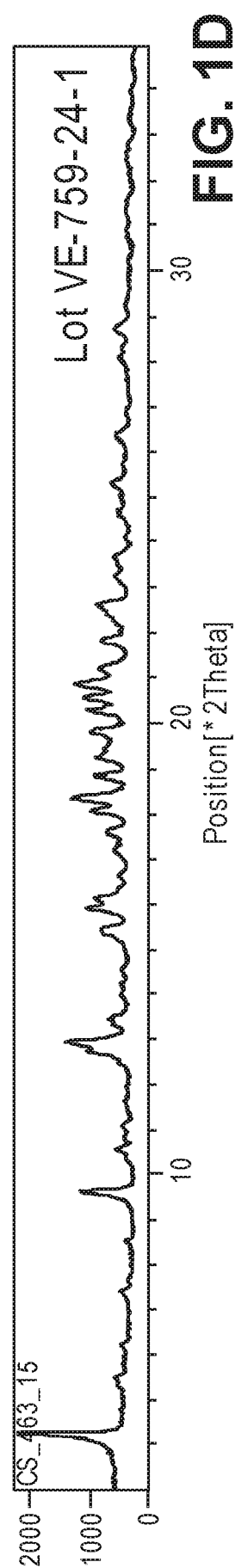
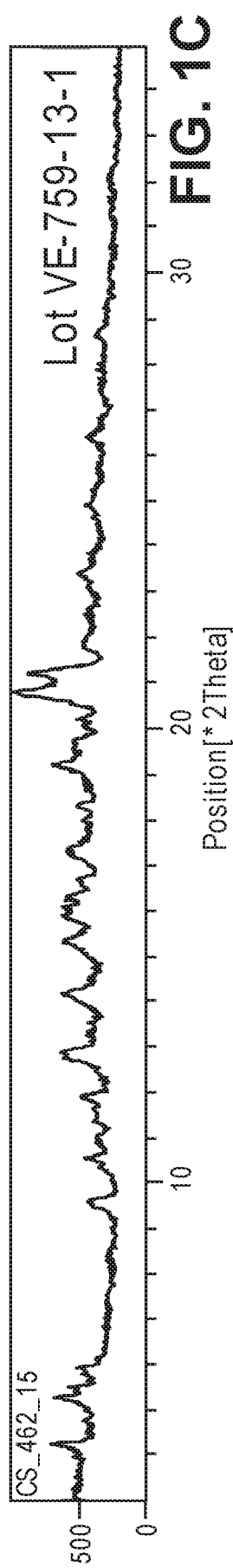
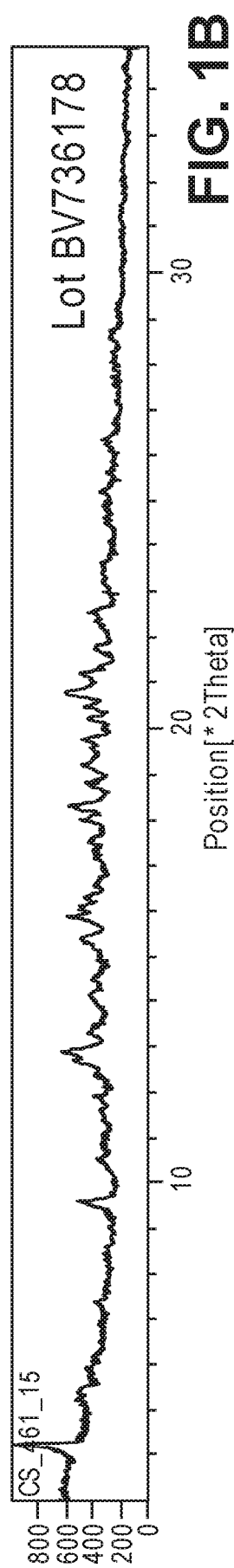
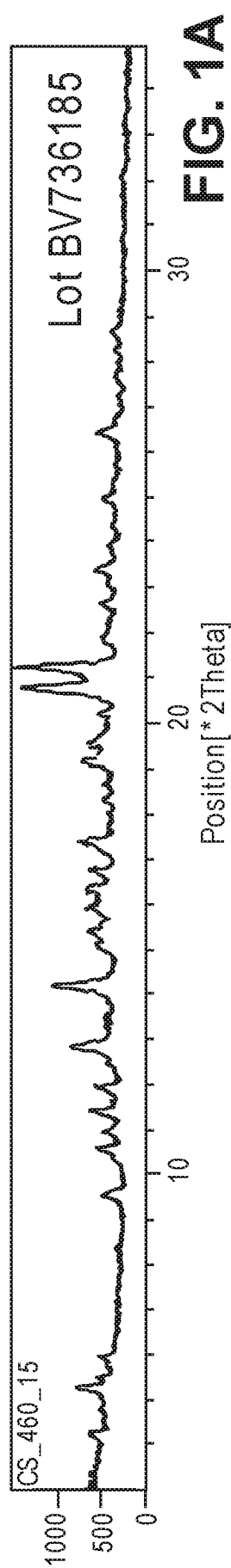
1 **54.** The solid form of claim **51**, having an X-ray powder diffraction
2 pattern substantially as shown in FIG. 1H.

1 **55.** A kit comprising the pharmaceutical composition of any one of claims
2 **43** to **50** and an instruction manual.

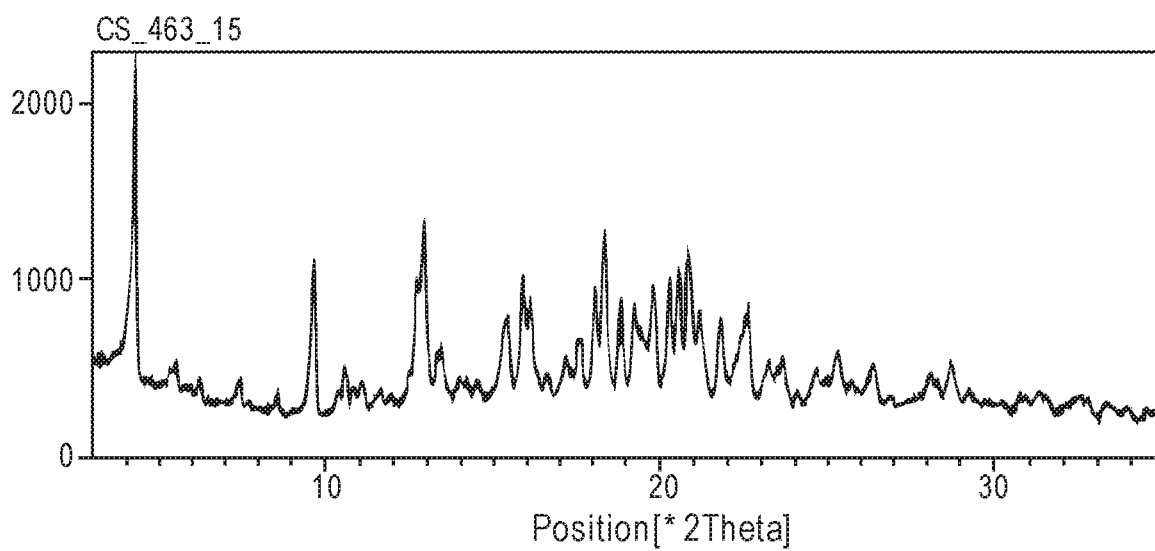
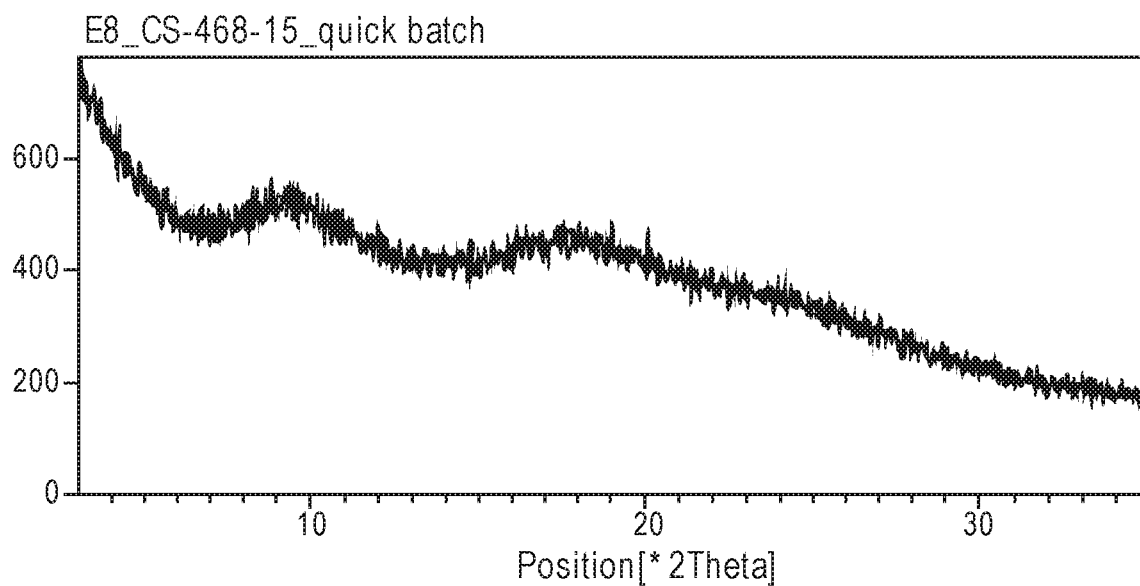
1 **56.** A composition of matter comprising: 2-((E)-2-((E)-3-(2-((E)-3,3-
2 dimethyl-5-sulfonato-1-(4-sulfonatobutyl)indolin-2-ylidene)ethylidene)-2-phenoxy-cyclohex-
3 1-en-1-yl)vinyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3H-indol-1-ium-5-sulfonate of Formula 2:

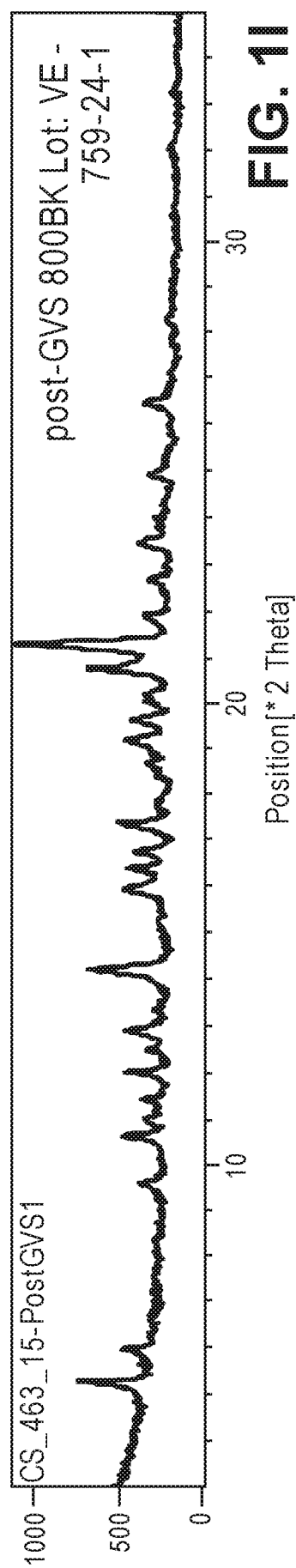
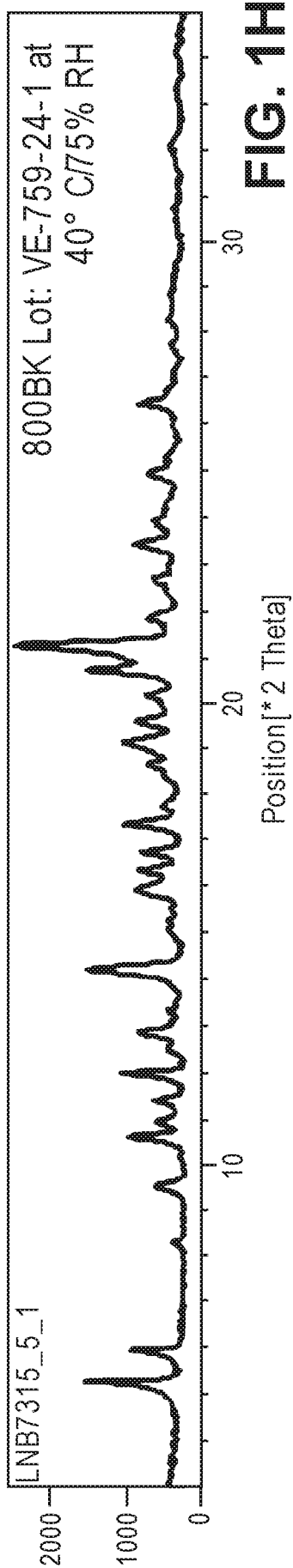
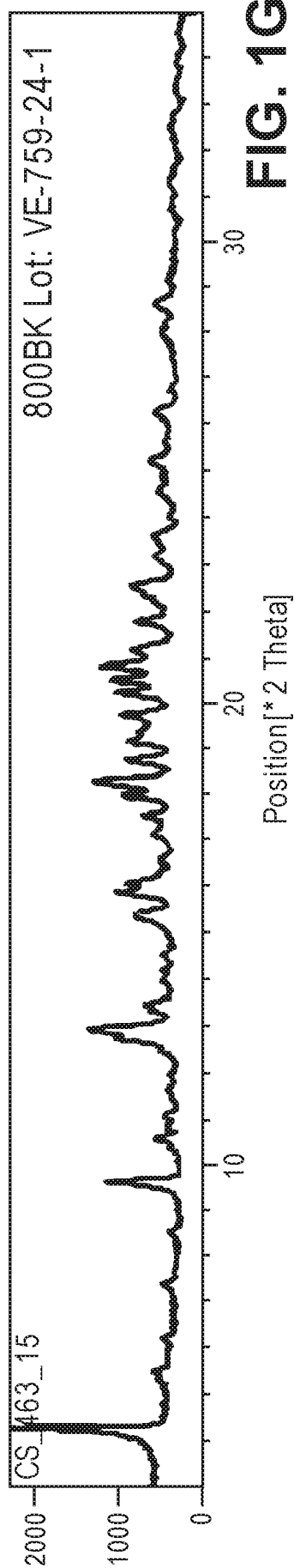


4 or a solvate or polymorph thereof and having a pharmaceutically acceptable
5 cation.
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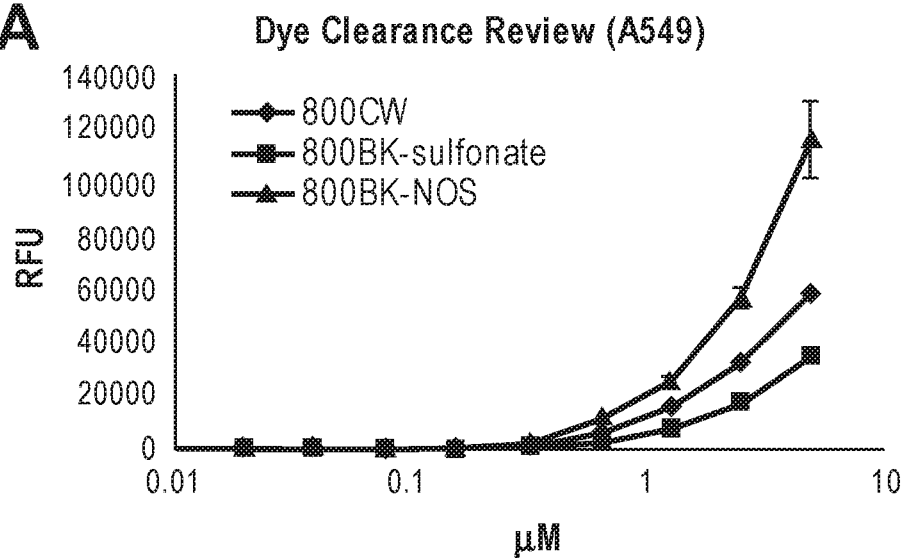
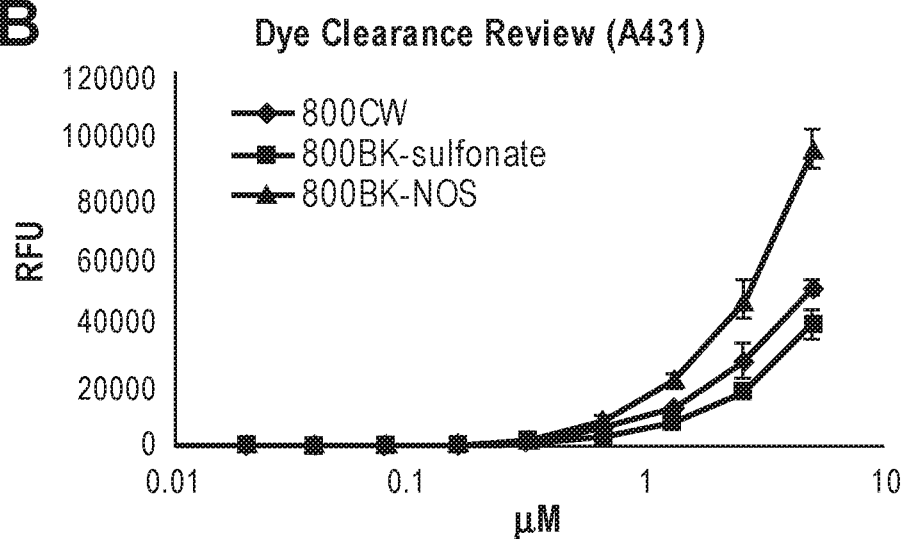
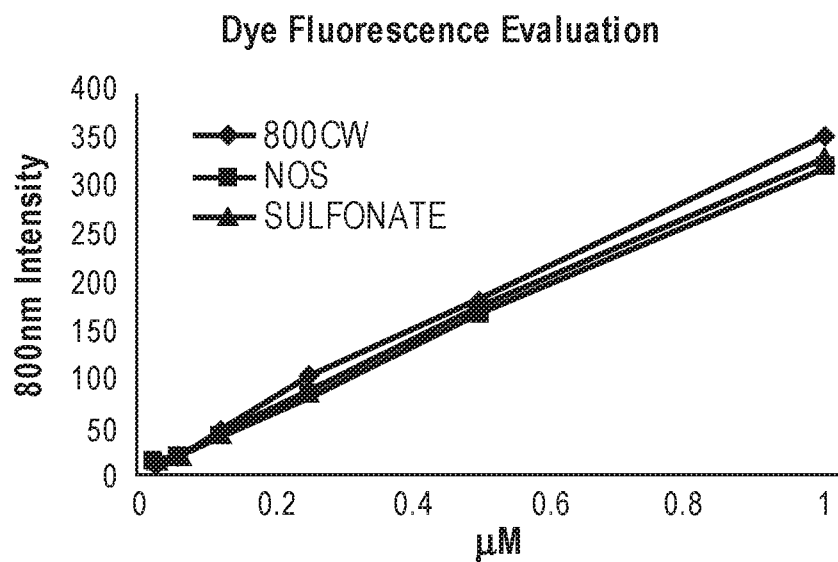


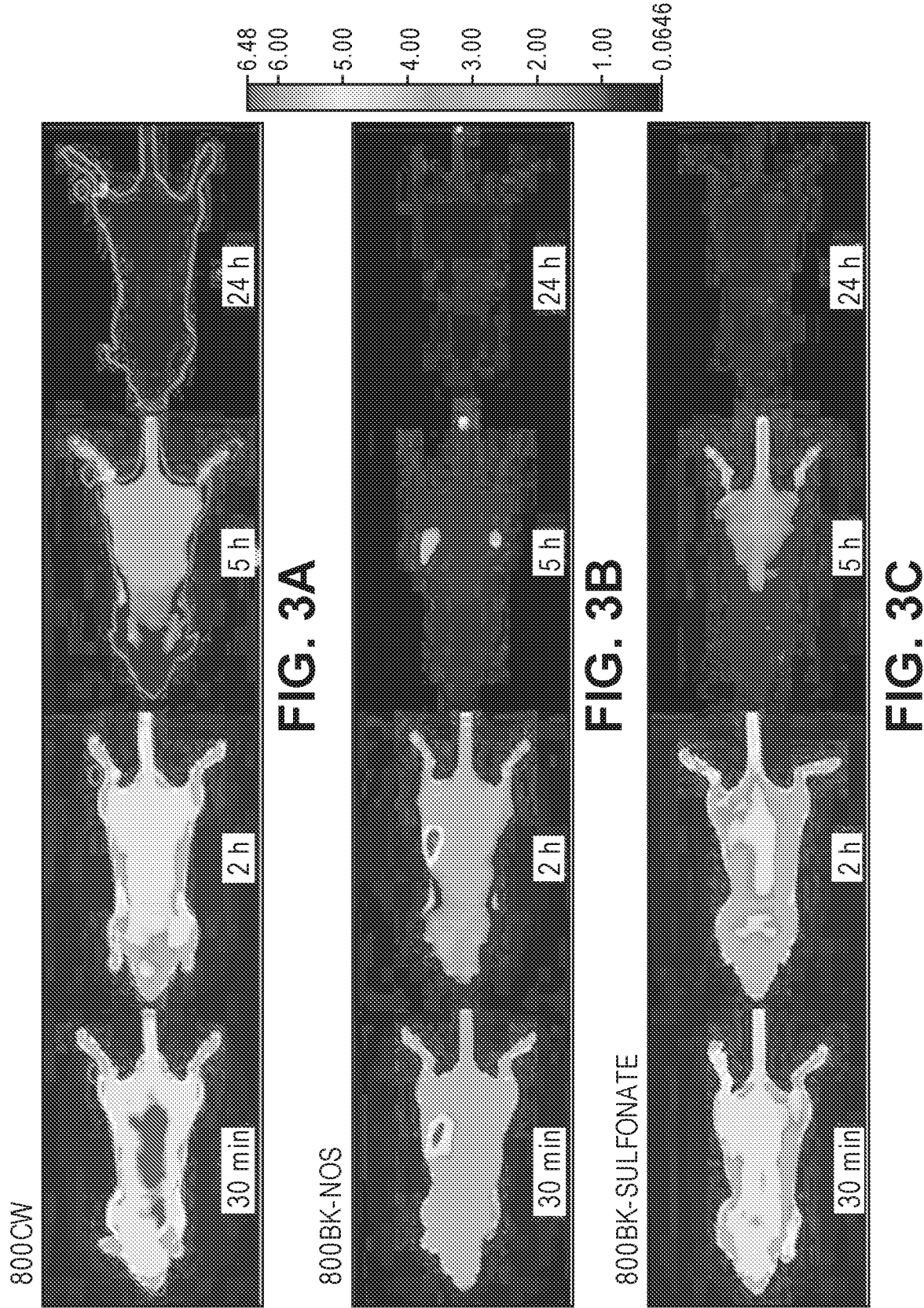
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**FIG. 1E****FIG. 1F**

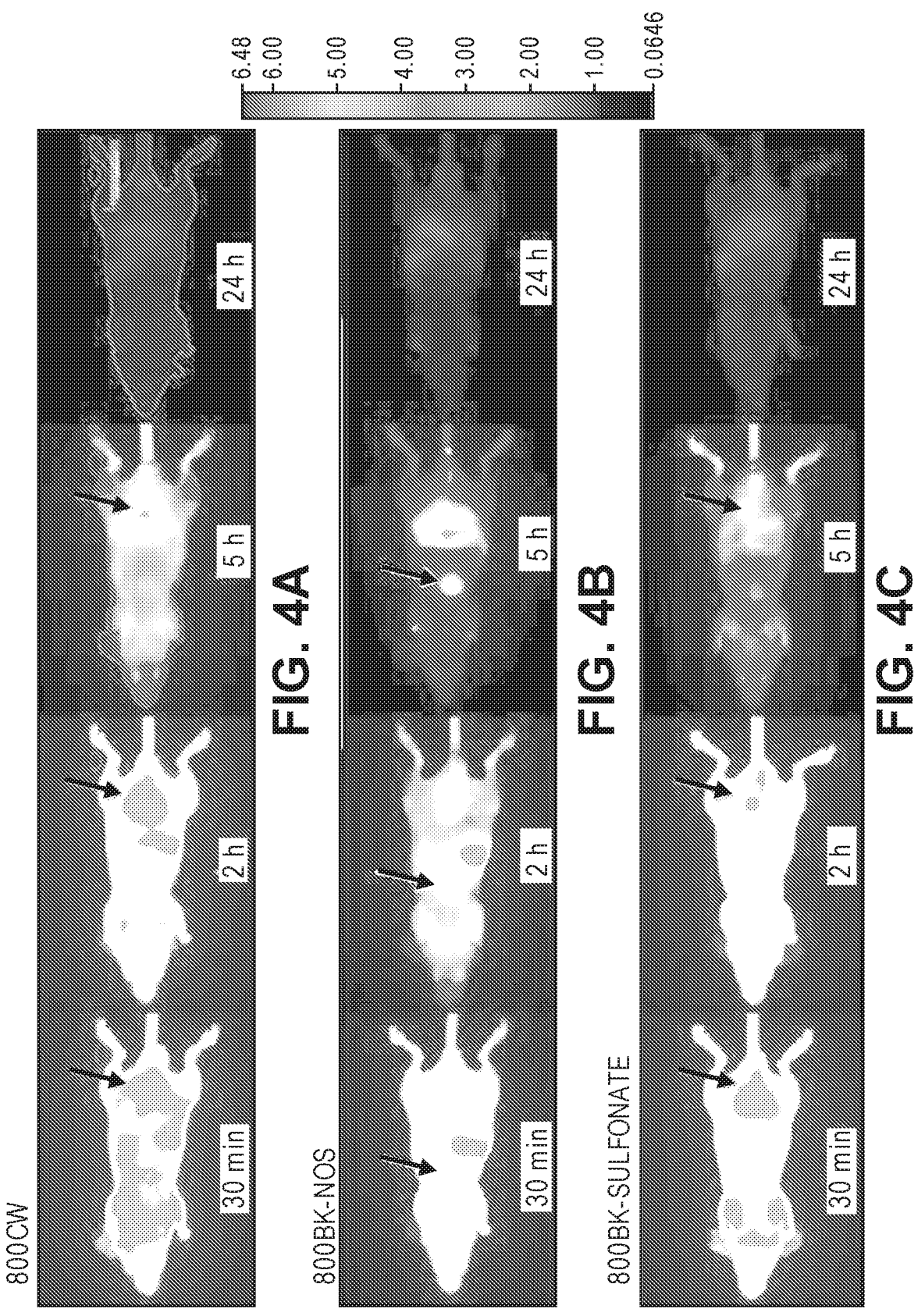


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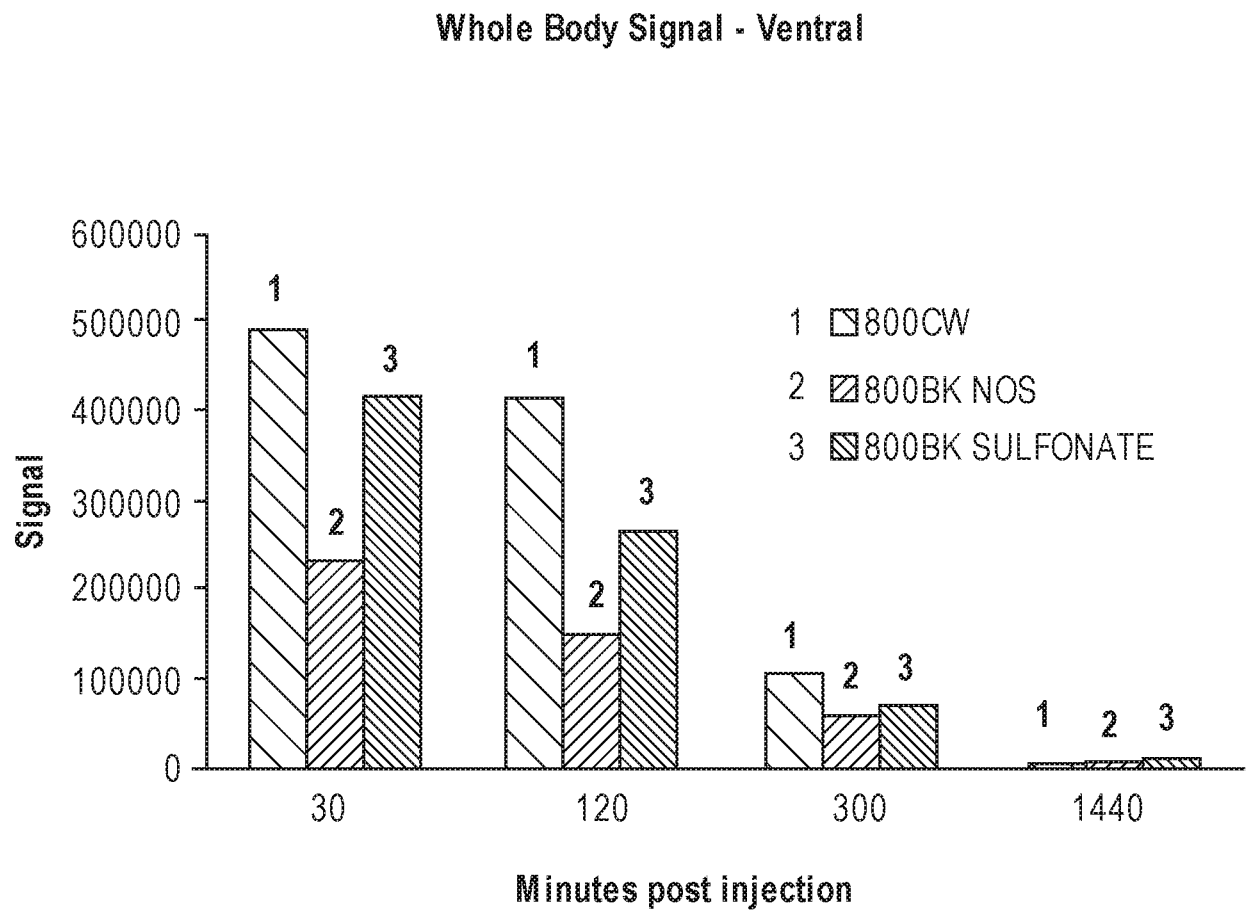
FIG. 2A**FIG. 2B****FIG. 2C**

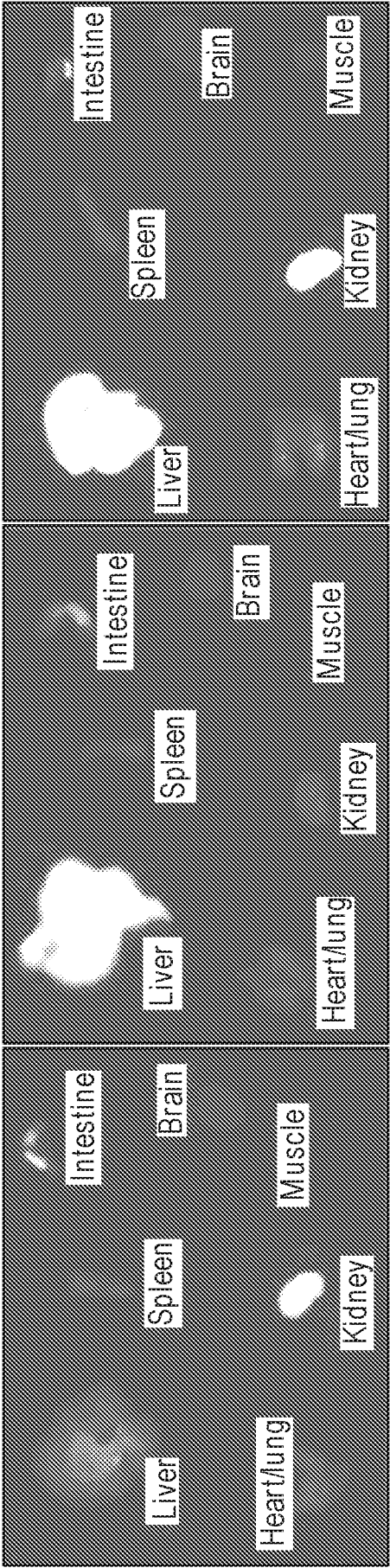
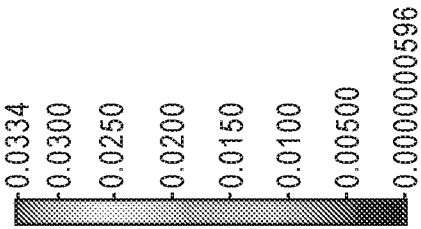


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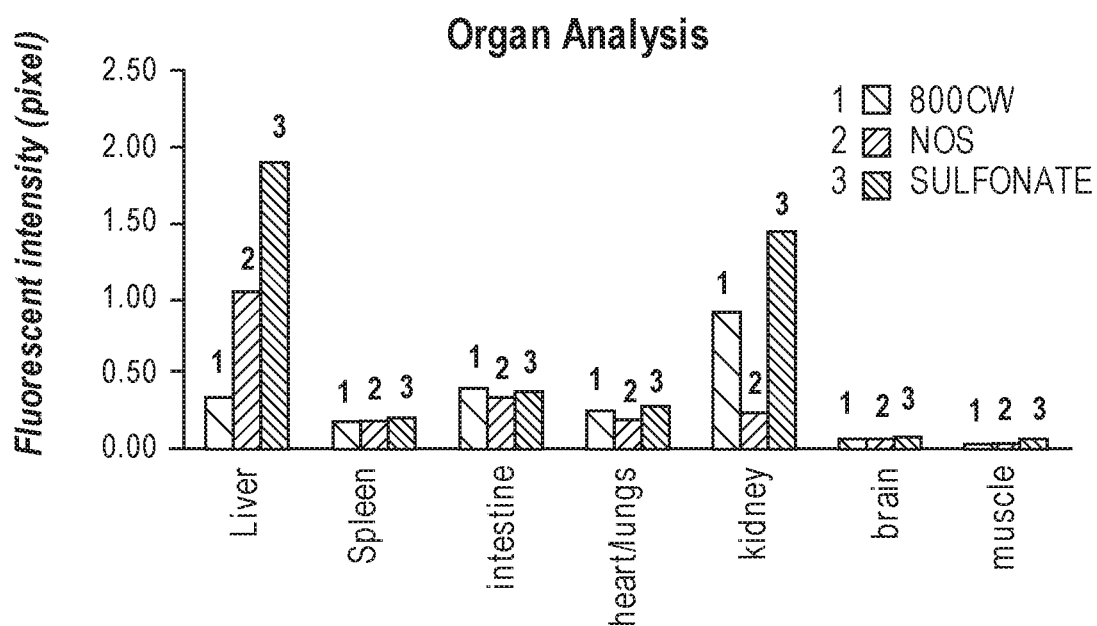
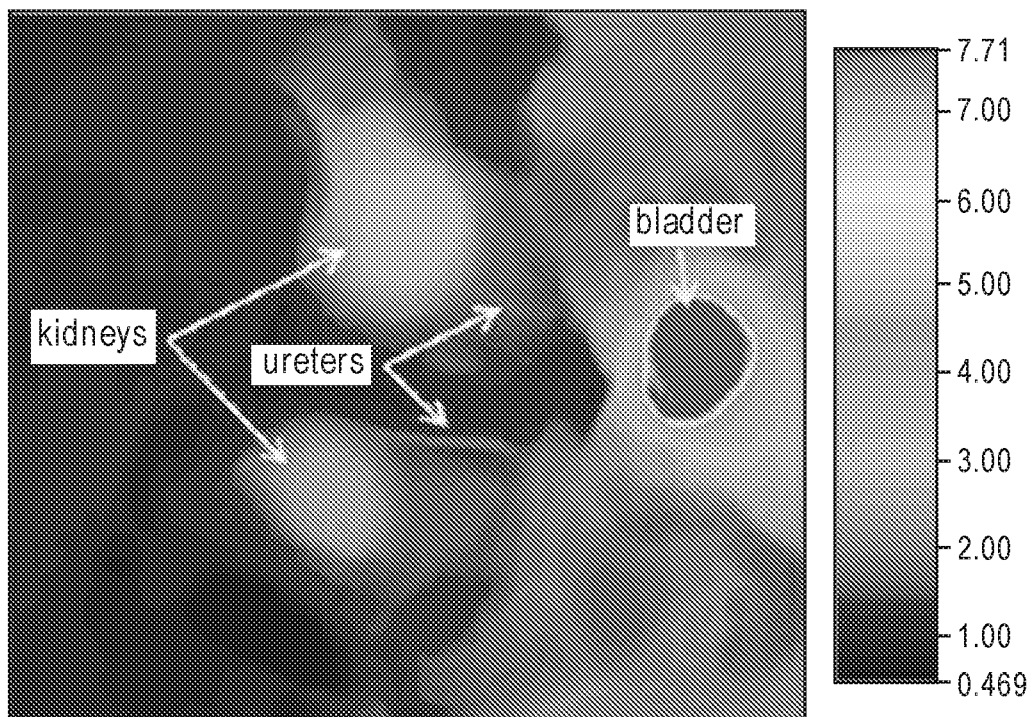


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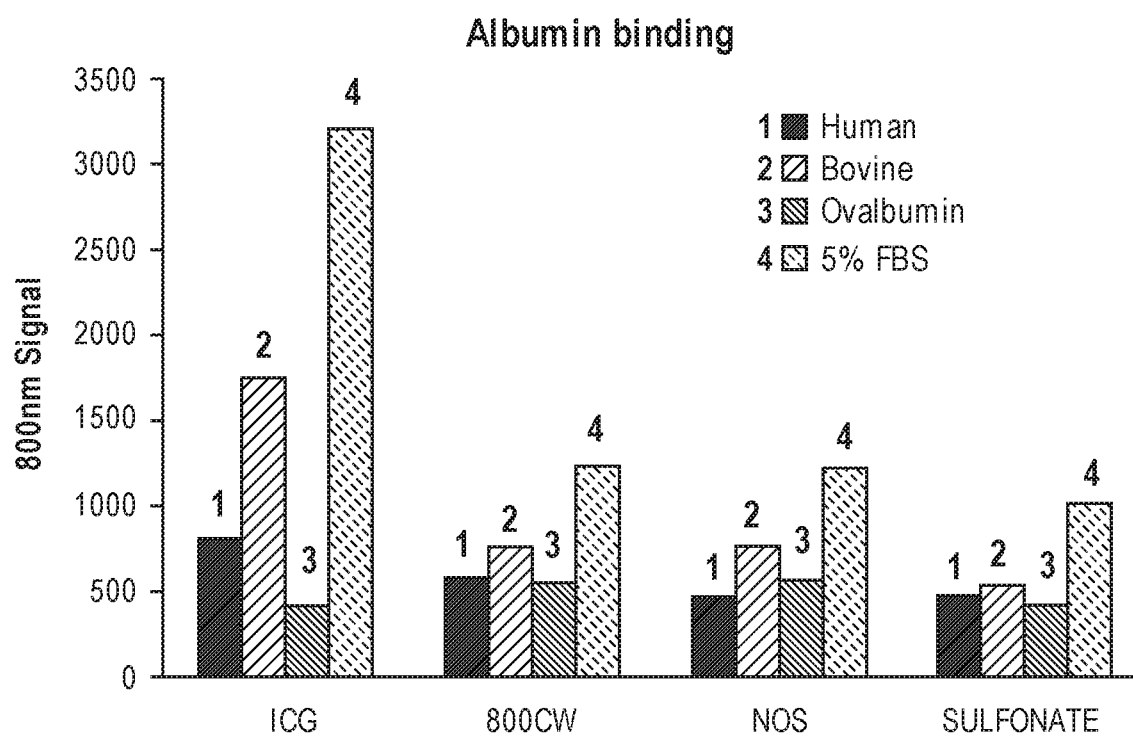
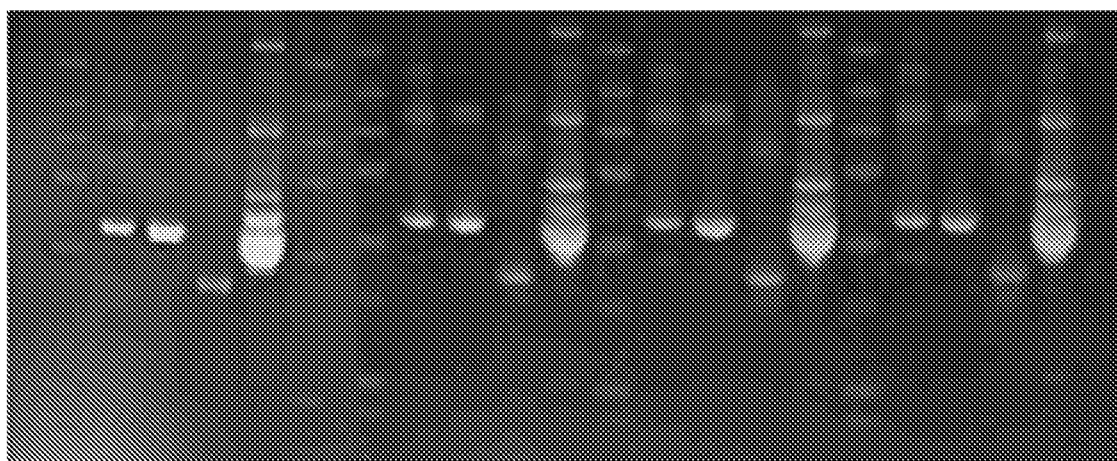
**FIG. 5**



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**FIG. 6D****FIG. 7**

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**FIG. 8A****FIG. 8B**

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Control

800BK-S

ICG

800BK-NOS

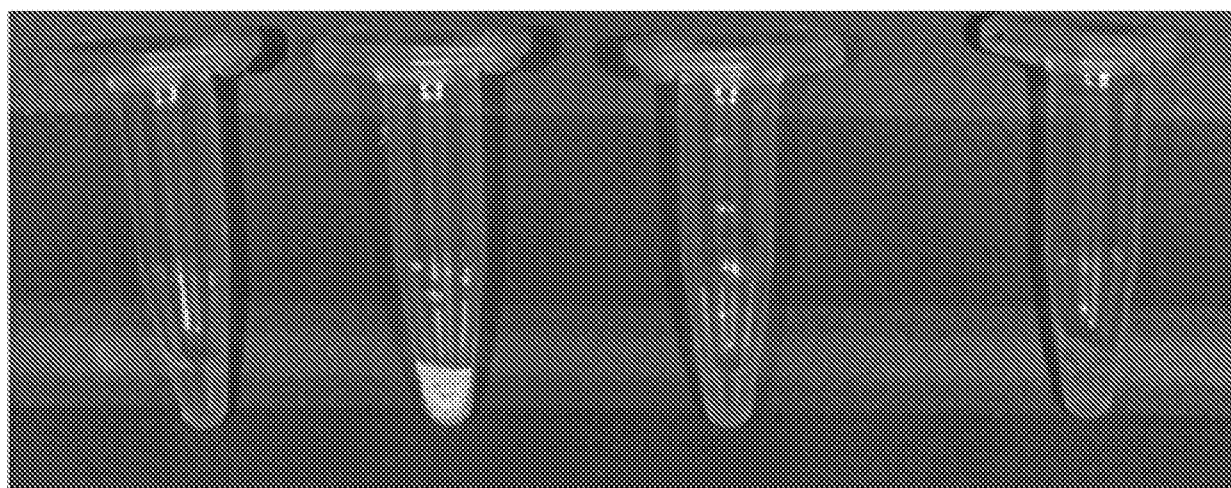
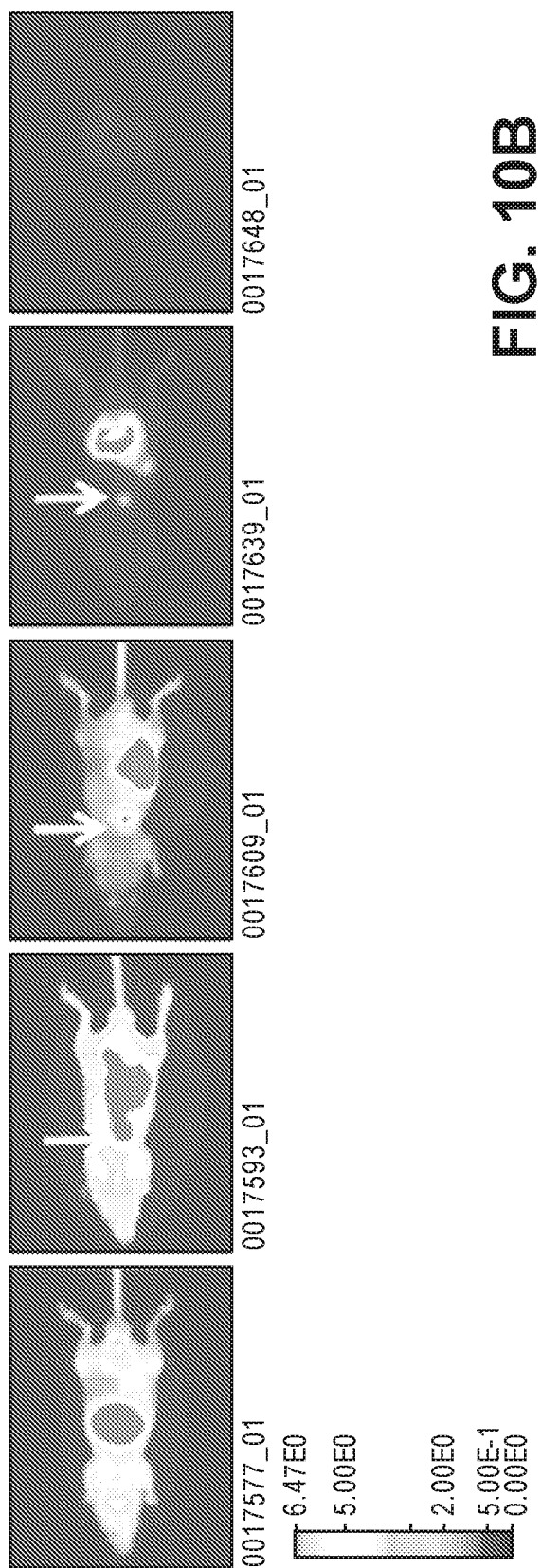
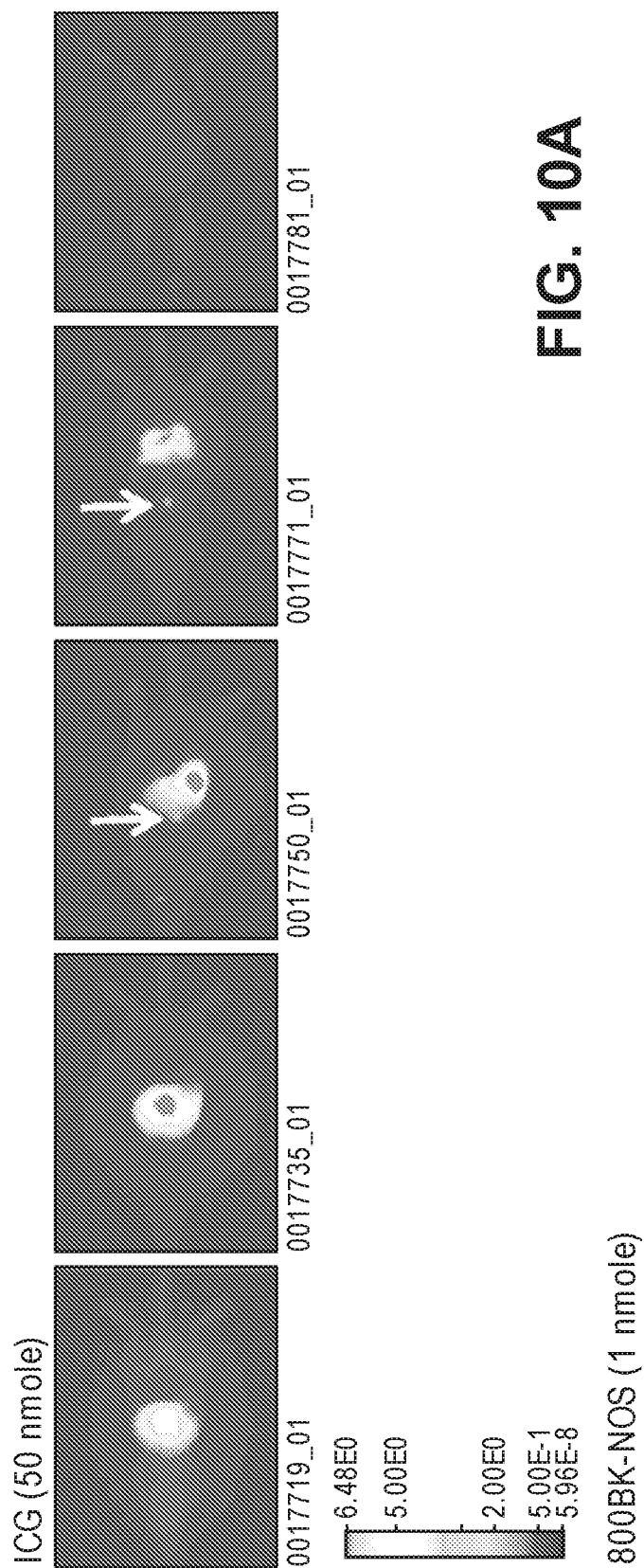
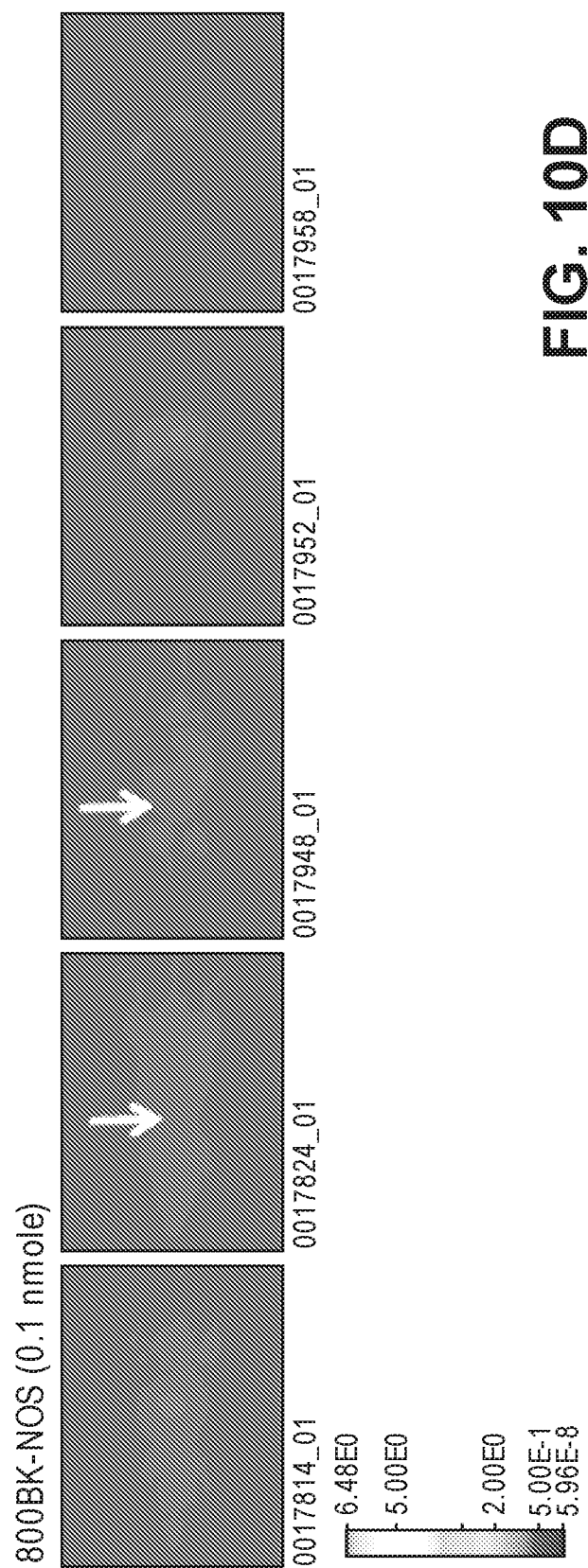
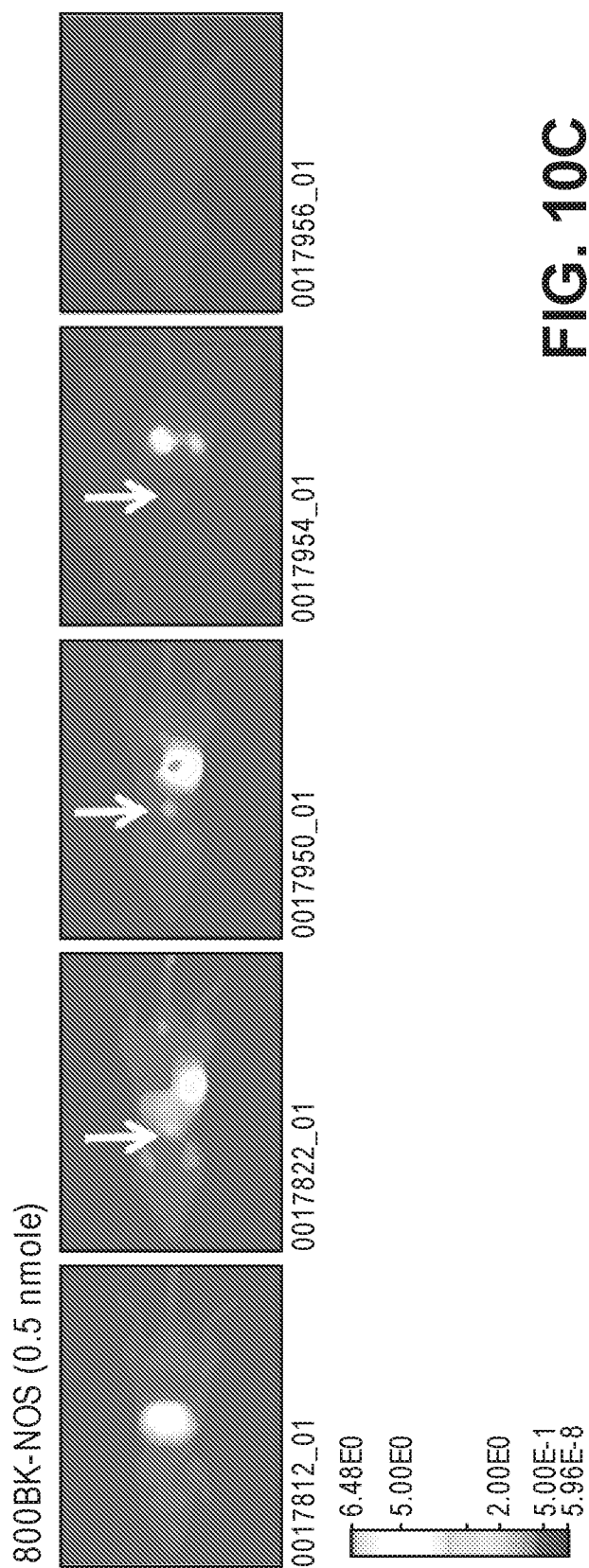


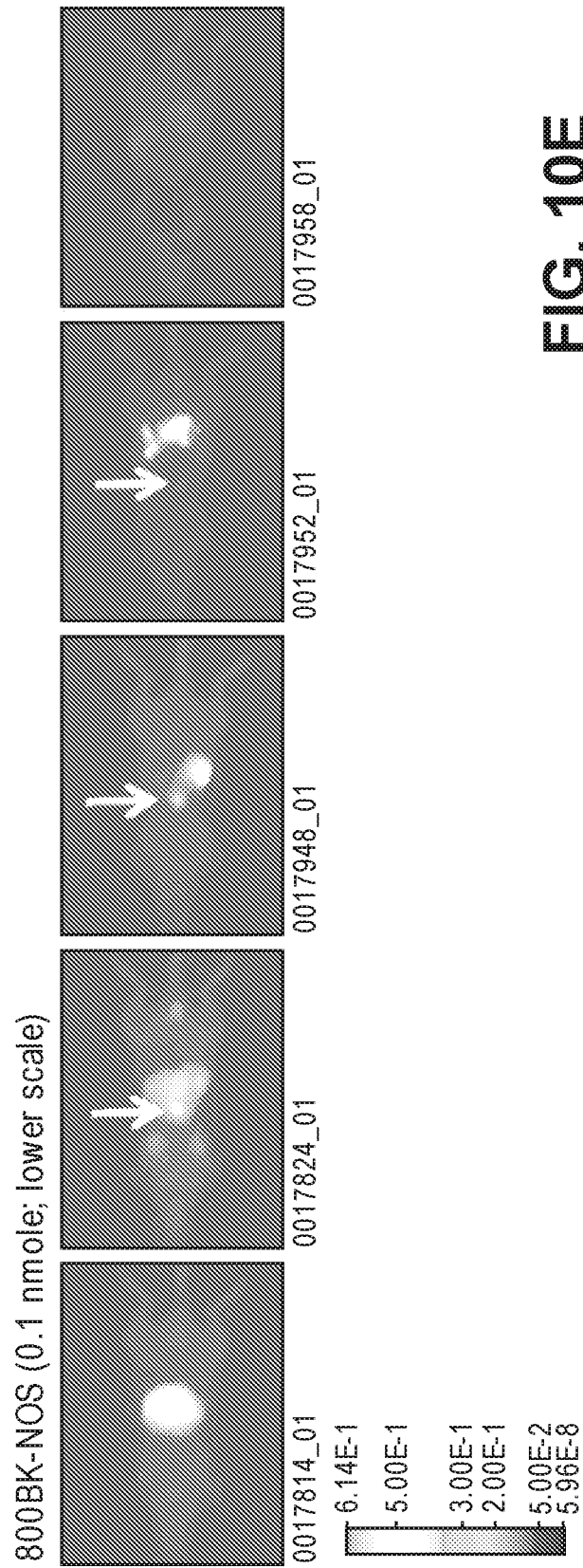
FIG. 9

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FIG. 11A

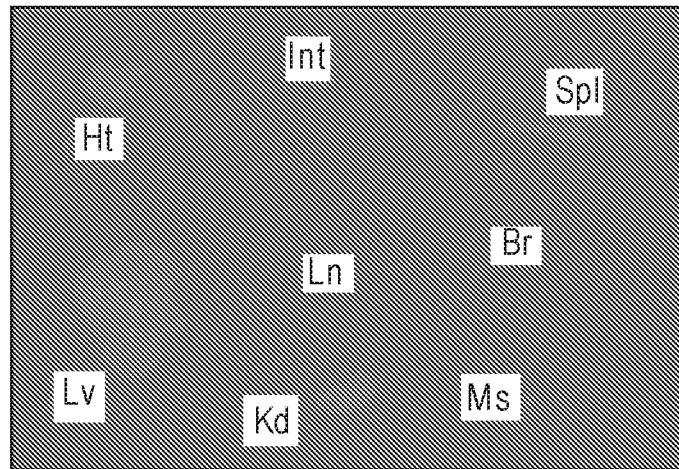


FIG. 11B

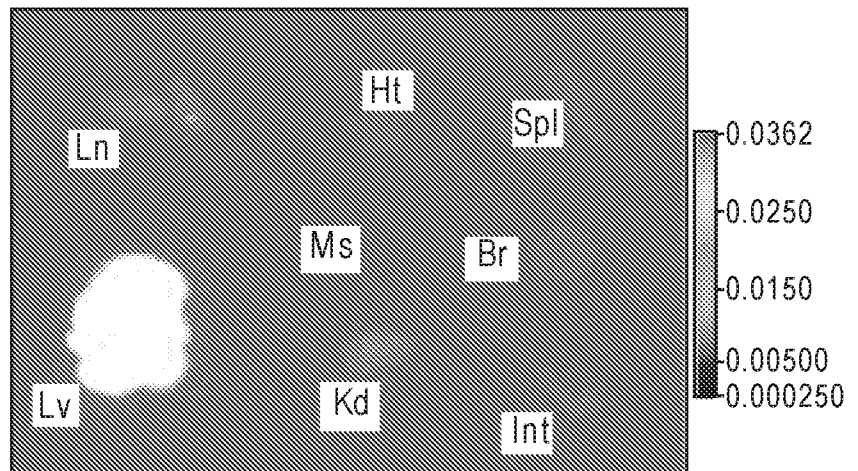
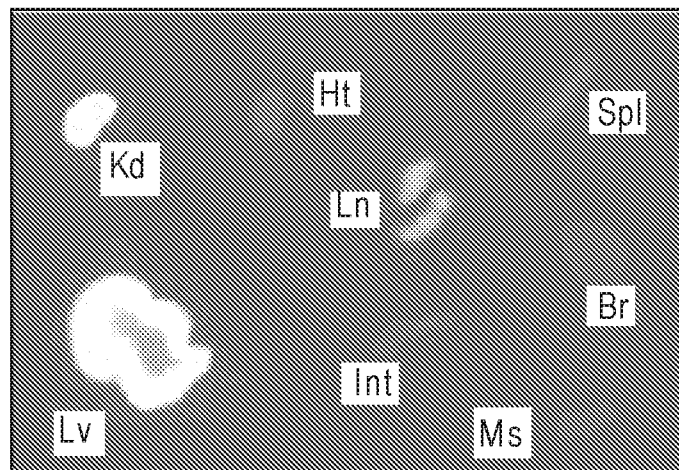
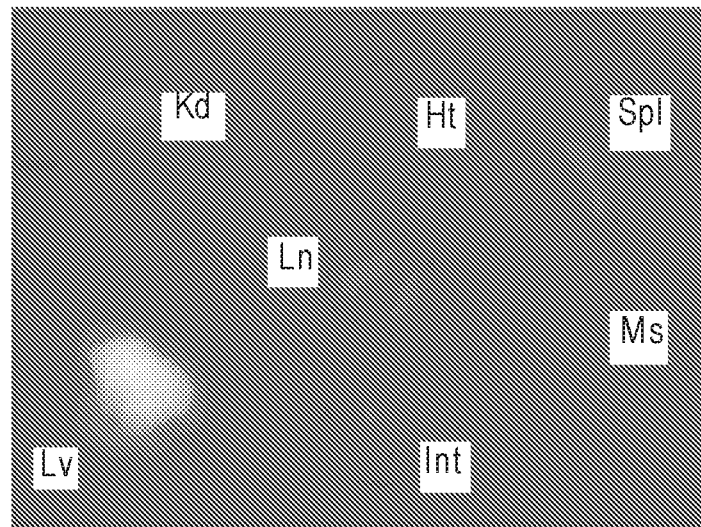
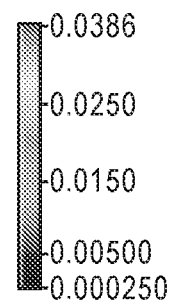
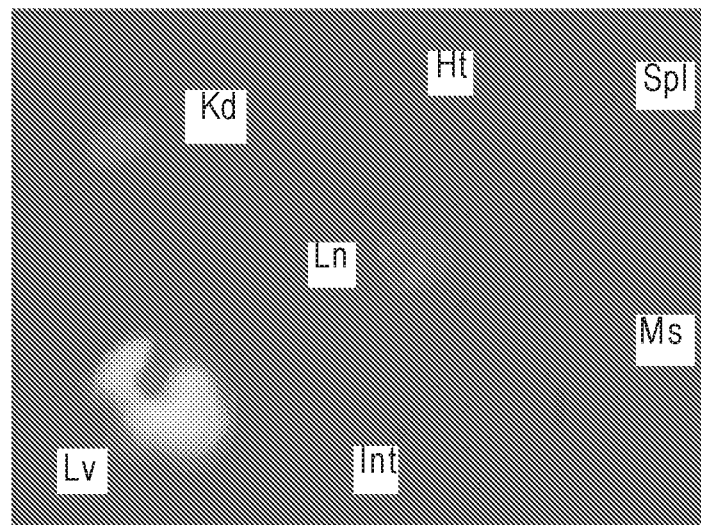


FIG. 11C



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72 h post administration

FIG. 11D**FIG. 11E**

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800BK-NOS (0.1 nmole)

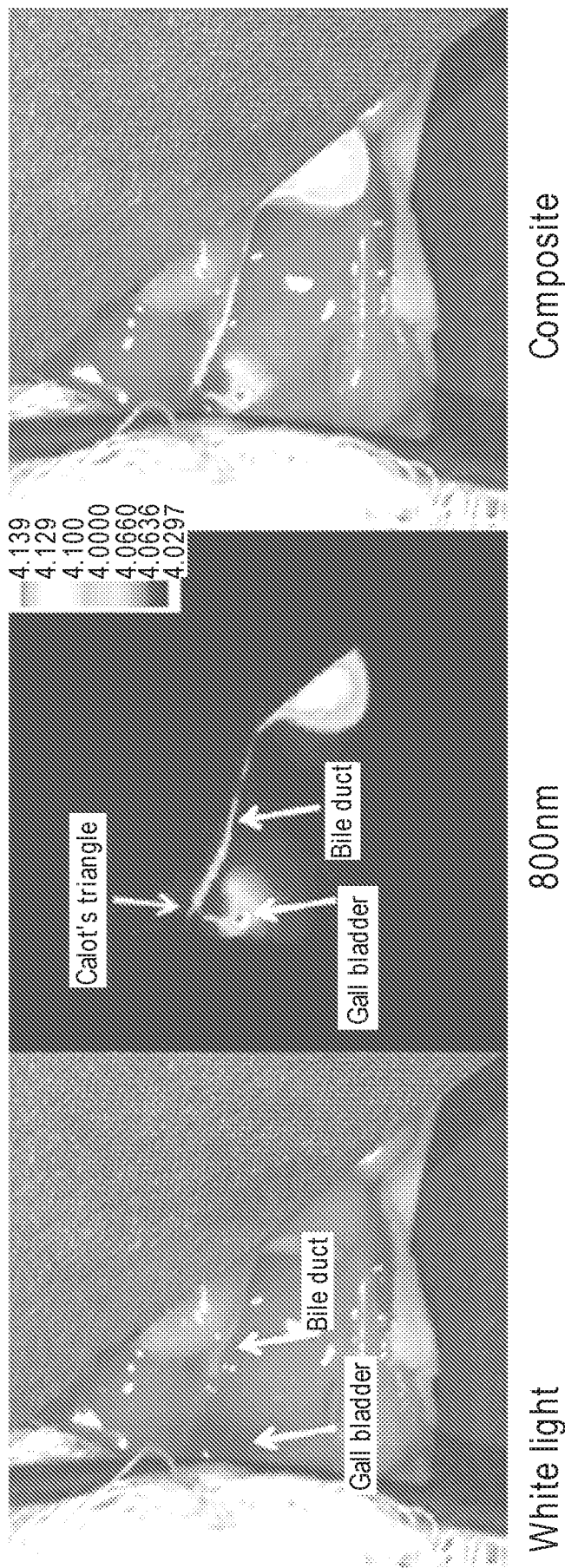


FIG. 11F

FIG. 11G

FIG. 11H

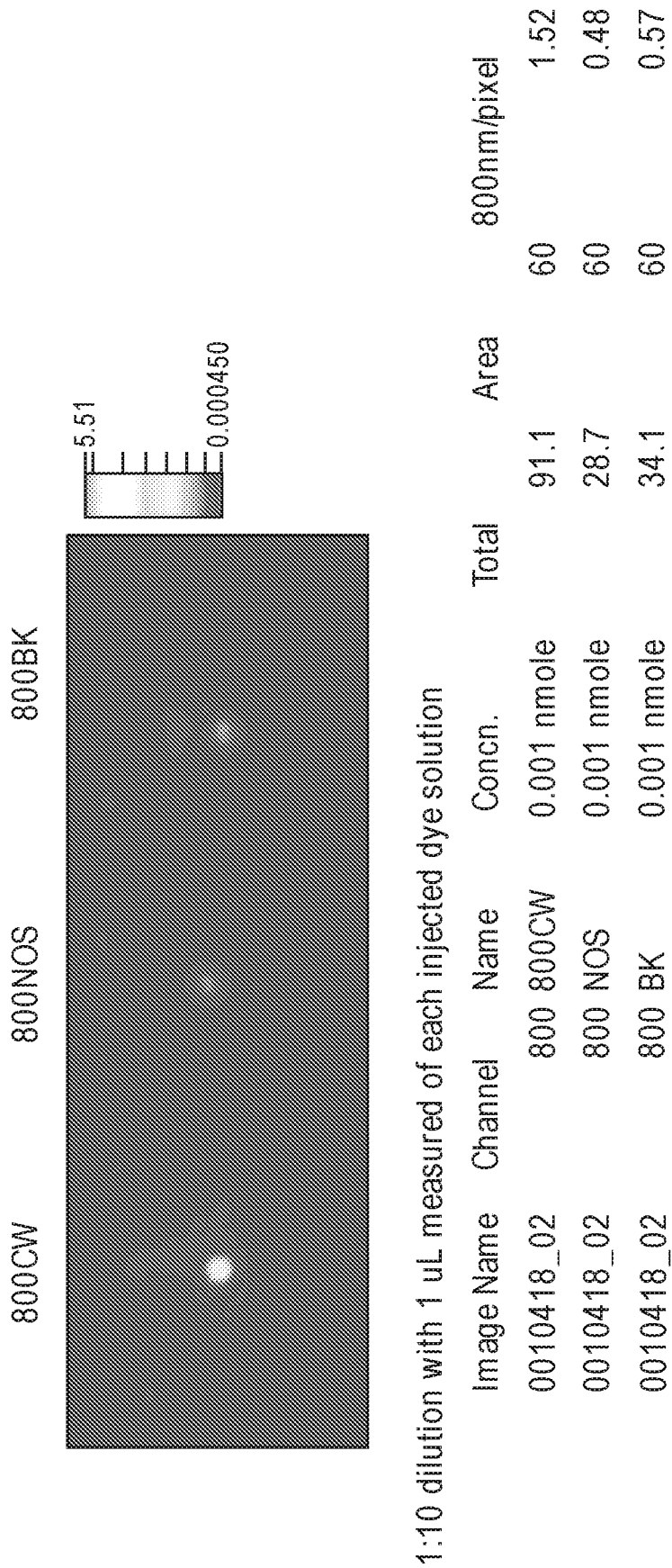
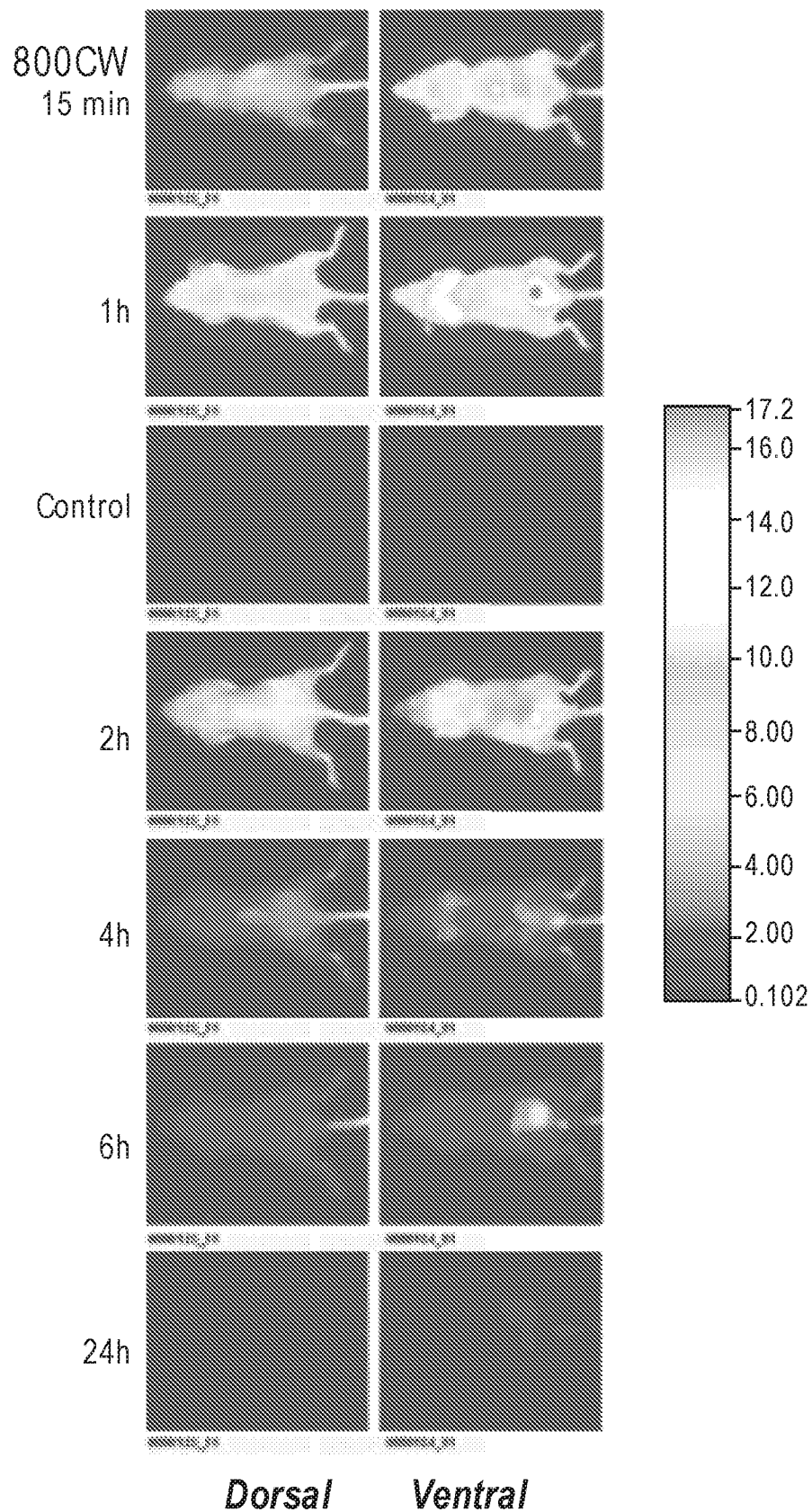


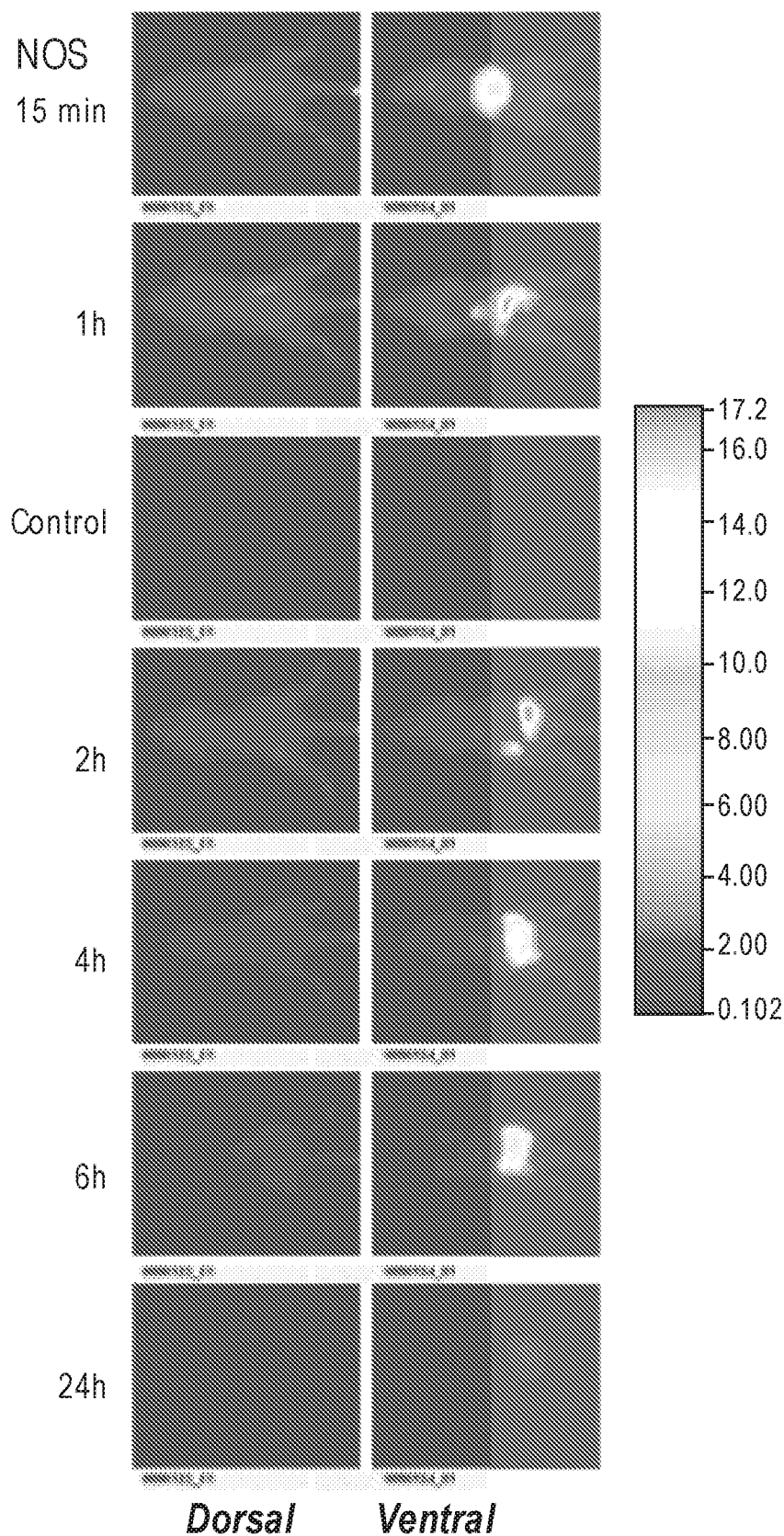
FIG. 12

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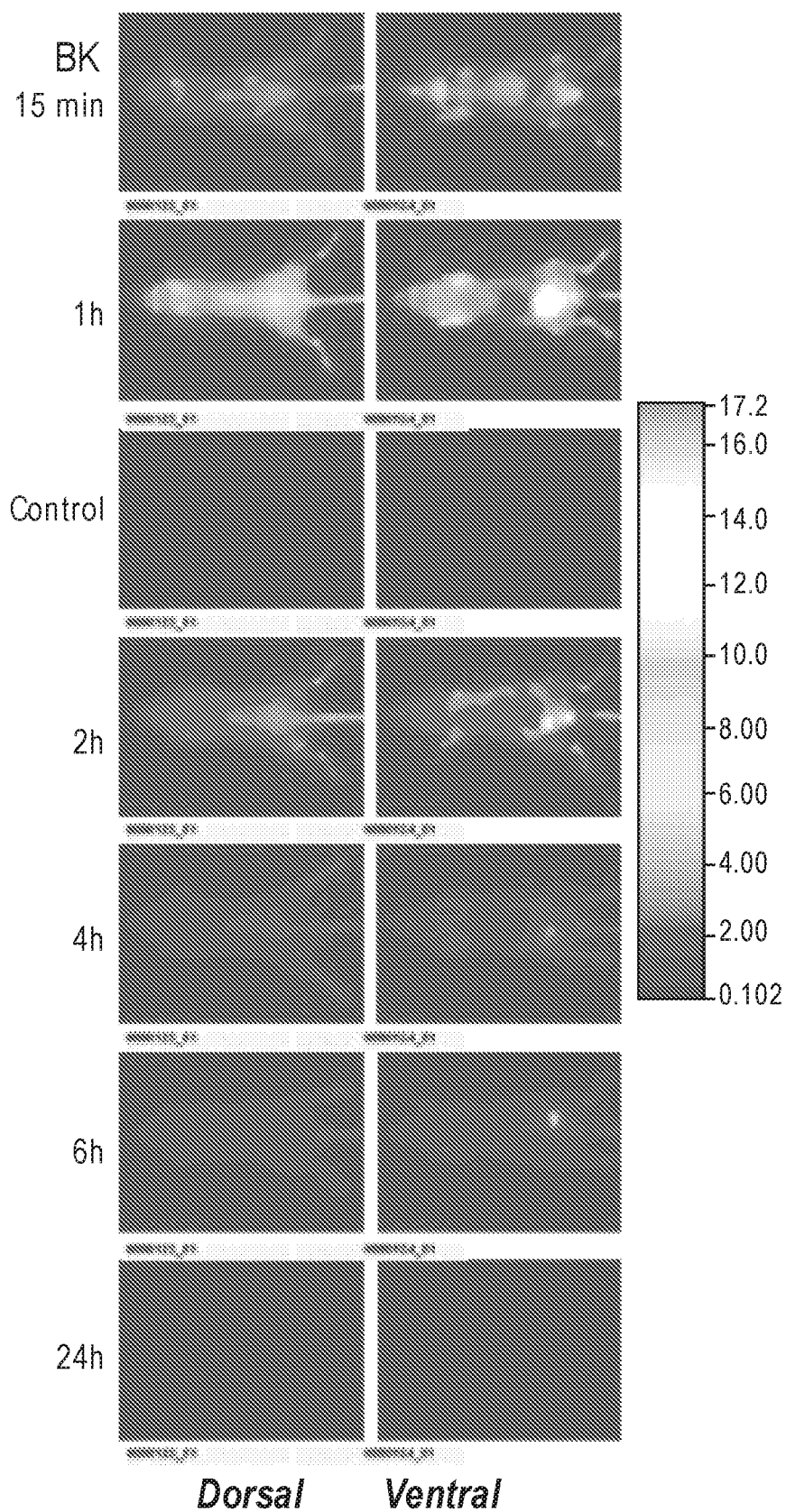
FIG. 13A



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FIG. 13B*Compound of Formula 2*

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FIG. 13C*Compound of Formula 1*

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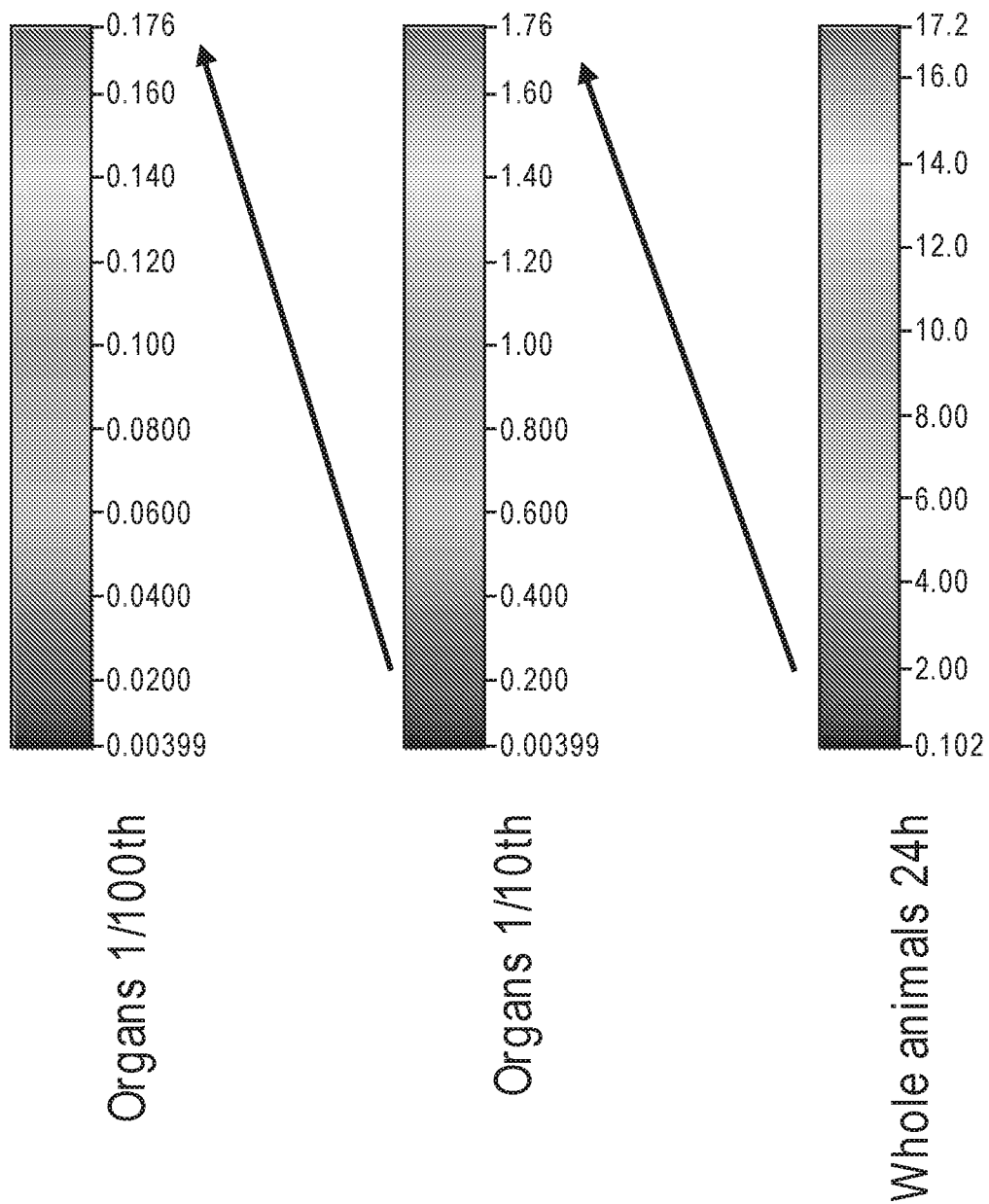
**FIG. 14**

FIG. 15A

FIG. 15B

FIG. 15C

FIG. 15D

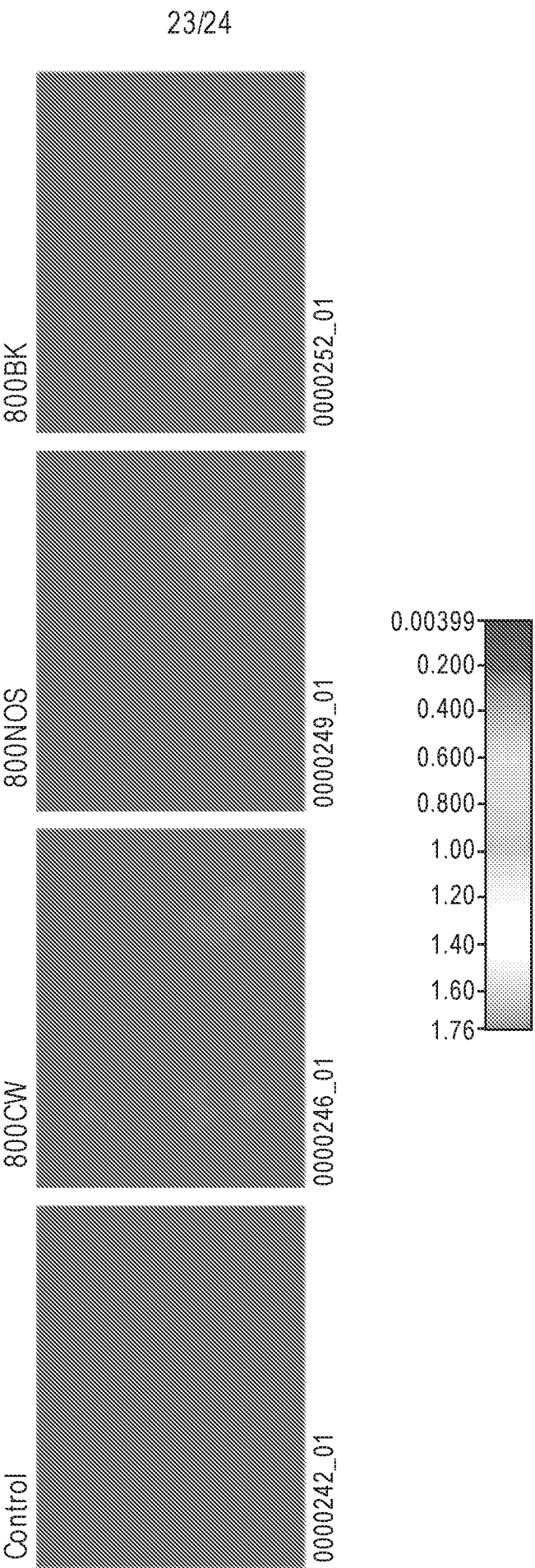


FIG. 16A

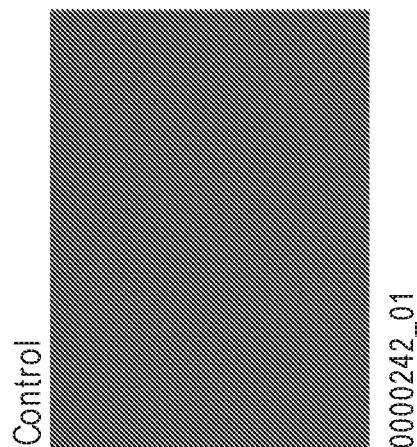


FIG. 16B

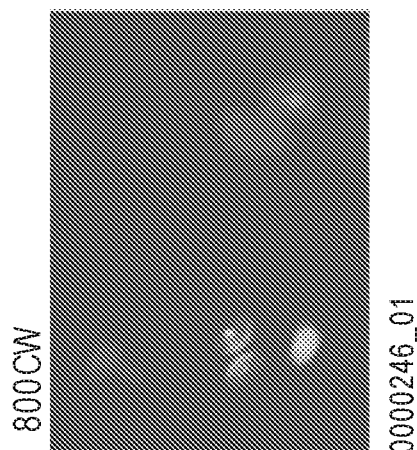


FIG. 16C

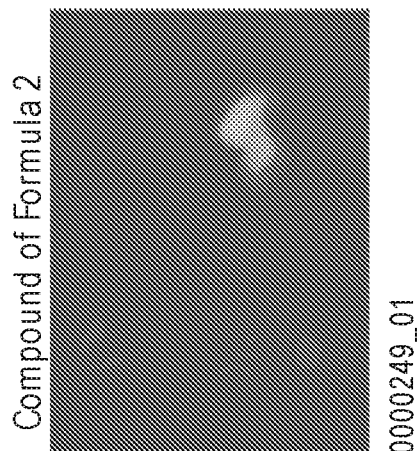
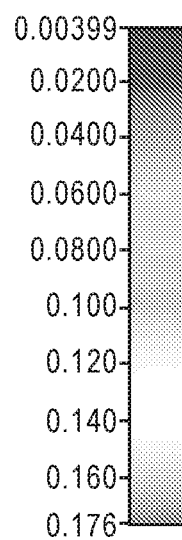
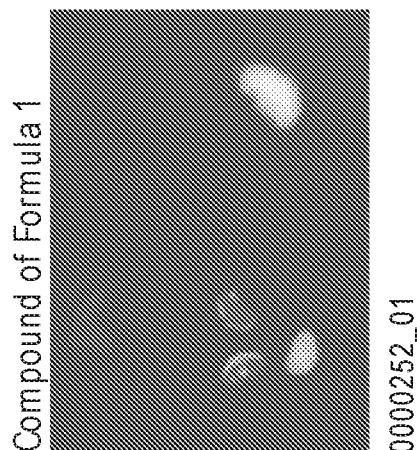


FIG. 16D



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/062212

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K49/00 C09B23/01
ADD.

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)
A61K C09B

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)

EPO-Internal, BIOSIS, EMBASE, DISSERTATION ABS, PASCAL, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZAHEER A ET AL: "IRDYE78 CONJUGATES FOR NEAR-INFRARED FLUORESCENCE IMAGING", MOLECULAR IMAGING, vol. 1, no. 4, 1 October 2002 (2002-10-01), pages 354-364, XP008029547, ISSN: 1535-3508, DOI: 10.1162/153535002321093963 abstract page 357 page 361	1-14,43, 44,47-55
X	EP 0 753 584 A1 (LI COR INC [US]) 15 January 1997 (1997-01-15) examples claim 4	1-14,43, 44,47-55

☒ Further documents are listed in the continuation of Box C.

☒ 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

"E" earlier application or patent 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

9 February 2016

Date of mailing of the international search report

03/05/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Dullaart, Anwyn

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2015/062212

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TANAKA ET AL: "Real-Time Intraoperative Ureteral Guidance Using Invisible Near-Infrared Fluorescence", JOURNAL OF UROLOGY, vol. 178, no. 5, 9 October 2007 (2007-10-09), pages 2197-2202, XP022295257, ISSN: 0022-5347, DOI: 10.1016/J.JURO.2007.06.049 cited in the application abstract page 2198, right-hand column, last paragraph - page 2200, left-hand column, line 4 page 220, right-hand column, last paragraph figures -----	1-14,43, 44,47-55
Y	WO 2008/076467 A2 (BETH ISRAEL HOSPITAL [US]; FRANGIONI JOHN V [US]; EIICHI TANAKA [JP];) 26 June 2008 (2008-06-26) example 5 -----	1-14,43, 44,47-55
Y	WO 2010/091243 A1 (BETH ISRAEL HOSPITAL [US]; FRANGIONI JOHN V [US]) 12 August 2010 (2010-08-12) example 9 figure 4 -----	1-14,43, 44,47-55
Y	AKERS WALTER J ET AL: "Predicting in vivo fluorescence lifetime behavior of near-infrared fluorescent contrast agents using in vitro measurements", JOURNAL OF BIOMEDICAL OPTICS, vol. 13, no. 5, September 2008 (2008-09), pages 054042-1-054042-9, XP002754039, ISSN: 1083-3668, DOI: 10.1117/1.2982535 abstract figure 3 table 3 pages 054042-8 -----	1-14,43, 44,47-55
Y	GOIFFON REECE J ET AL: "Dynamic noninvasive monitoring of renal function in vivo by fluorescence lifetime imaging", JOURNAL OF BIOMEDICAL OPTICS, vol. 14, no. 2, March 2009 (2009-03), pages 020501-1-020501-3, XP002754040, ISSN: 1083-3668, DOI: 10.1117/1.3095800 abstract figures pages 020501-3 -----	1-14,43, 44,47-55

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/062212

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:

see additional 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 an additional fees, this Authority did not invite payment of 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.:

1-14, 43, 44, 47-54(completely); 55(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14, 43, 44, 47-54(completely); 55(partially)

Method for kidney ureter imaging using a compound of formula (1), a pharmaceutical composition containing a compound of formula (1), and a solid form of a compound of formula (1).

2. claims: 15-42, 45, 46, 56(completely); 55(partially)

Method for liver biliary imaging or liver cyst duct imaging, using a compound of formula (2), a pharmaceutical composition containing a compound of formula (2), and a composition of matter comprising a compound of formula (2).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2015/062212

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0753584	A1	15-01-1997	AU 723952 B2 07-09-2000
		AU 5940896 A	23-01-1997
		CA 2180894 A1	12-01-1997
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		US 6086737 A	11-07-2000
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		EP 2393420 A1	14-12-2011
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		WO 2010091243 A1	12-08-2010
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摘要

用于器官成像的方法，该方法包括：给个体施用诊断有效量的 2-((E)-2-((E)-3-(2-((E)-3, 3-二甲基-5-磺酸根合-1-(4-磺酸根合丁基)二氢吡啶-2-亚基)亚乙基)-2-苯氧基环己-1-烯-1-基)乙烯基)-3, 3-二甲基-1-(4-磺酸根合丁基)-3H-吡啶-1-鎓-5-磺酸盐或 2-((E)-2-((E)-3-(2-((E)-3, 3-二甲基-5-磺酸根合-1-(4-磺酸根合丁基)二氢吡啶-2-亚基)亚乙基)-2-(4-磺酸根合苯氧基)环己-1-烯-1-基)乙烯基)-3, 3-二甲基-1-(4-磺酸根合丁基)-3H-吡啶-1-鎓-5-磺酸盐。在一个实施方案中，所述器官包括肾、膀胱、肝、胆囊、脾、肠、心脏、肺和肌肉的一种或多种。