Image-Guided Surgery and Pathology using Invisible Near-Infrared Fluorescent Light

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Disclosures

• This talk describes the off-label use of indocyanine green and methylene blue, two NIR fluorophores that are FDA-approved for other indications.

• The FLARE™ imaging system is investigational only and not approved for the indications shown.

Harvard Medical School Conflict List

Employment: No conflict of interest to disclose
Research support: No conflict of interest to disclose
Scientific advisory board: No conflict of interest to disclose
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Speakers bureau: No conflict of interest to disclose
Major stockholder: Curadel, Curadel ResVet Imaging, Curadel Surgical Innovations
Patents: FLARE™ Imaging Systems and Contrast Agents
Honoraria: No conflict of interest to disclose
Travel support: No conflict of interest to disclose
Other: No conflict of interest to disclose
Outline

I. Why NIR Fluorescence?

II. Imaging System Design

III. Contrast Agent Chemistry

IV. Clinical Trials in Surgery

V. Clinical Trials in Digital Pathology

VI. Unlocking the Potential of the Technology
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Why NIR Fluorescence for Image-Guided Surgery?

Because of 4 physical principles:
1) Photon absorption is minimal
2) Photon scatter is minimal
3) Autofluorescence is minimal
4) NIR light is invisible to the human eye

Benefits are:
• High contrast: “Bright stars on a black sky”
• No change to the look of the surgical field
• Any object can be potentially be highlighted
• Real-time intraoperative imaging
• Millimeter, instead of micron, depth detection
1) Absorption is Minimal in the NIR

2) Scatter is Minimal in the NIR

**Skin** (Rayleigh-Type, i.e. Wavelength-Dependent Scatter)

**Breast** (Mie-Type, i.e., Non-Wavelength-Dependent Scatter)
3) Autofluorescence is Minimal in the NIR


4) NIR Light is Invisible to the Human Eye

The Visible Spectrum

NIR Window

FLARE™ Channel 1

FLARE™ Channel 2

Sensitivity ($\log_{10}$)

Wavelength (nm)
NIR Contrast in Thick, Scattering Tissue: “Bright Stars on a Black Sky” **

** Provided that optimal imaging systems and contrast agents are available.
So, Why all the Excitement?

• Sensitive, real-time, high-resolution tumor margin detection (tumor deposits as small as 10 cells wide)

• Avoidance of blood vessels, nerves, ureters, bile ducts, and other vital structures during tumor resection

• Rapid identification of sentinel lymph nodes and other sub-surface structures without the need for ionizing radiation

• Reduced anesthesia time

• Lower patient morbidity from damage to vital structures

• Increase surgeon and OR throughput (raise income)

• Improved surgical outcomes lead to lower healthcare costs

• Proof of principle in 1000 patients worldwide (500 w/ FLARE™)
Fundamentals of NIR Fluorescence Imaging

1) Medical Device: Imaging System

2) Pharmaceutical (Drug): NIR Fluorescent Contrast Agent
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Imaging System Components and Form Factors

**Form Factors**
- Open surgery
- Fiberoscopy

**Key Parameters**
- Fluence rate adjusted for laser Class 3R
- Fluence rate adjusted below photobleaching
- NIR-compatible optics
- Real-time acquisition and display

An Optical Imaging Platform for Image-Guided Surgery
Fluorescence-Assisted Resection and Exploration (FLARE™)
FLARE™ Imaging System Filter Design

(Chroma Sputtered Filters, 4-5 logs at boundaries, ≥ 98% transmission)
Second-Generation Mini-FLARE™ Imaging System

First Commercial Production System
Curadel ResVet Imaging’s Lab-FLARE™ Model R1

Applications

- Stem cell tracking
- Tissue perfusion
- Development of novel NIR fluorescent contrast agents
- Development of novel image-guided surgery procedures
- Tissue engineering
- Tumor detection

Specifications*

Cart: 2’ x 2’ x 3’, medical grade locking casters, 250 lbs.
Worldwide custom shipping crate

Arm: Custom retracting with 6-DOF positioning
48” lateral and 60” vertical reach

Electrical: 100-240 V, 50/60 Hz, 8 A

Optical: Custom lens with 12”-18” working distance
15X zoom providing FOV from 1.5 to 20 cm
Resolution as high as 10 cells wide

Laser: Custom. 4W and 10W sub-systems

White Light: Custom. 40,000 lx at 15” working distance

Software: 2 Independent NIR channels (700 nm & 800 nm) with simultaneous acquisition of color video

* Specifications are subject to change

For Research Use Only. Not for Veterinary or Human Use.
The FLARE Product Portfolio – Generation 1

Open Surgery

Minimally-Invasive Surgery

Surgical Microscopes

NIR Pathology Microscopes

Eyepiece adapter replaces custom lens

Microscope Body Replaces Custom Lens

US and European Patents 8,229,548 & 8,473,035
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Novel Class of Zwitterionic NIR Fluorophores
(i.e., geometrically-balanced, net-neutral, polyionic polymethines)

- Lowest non-specific background than any other agent described to date
- ZW800-1, CA: CAS #1239619-02-3
- ZW800-1, NHS: CAS #1360738-78-8
- One-step conjugation
- $1M tox package paid for by NCI
- Difficult cGMP synthesis, formulation, and lyophilization completed
- Pharma partner program – develop bespoke companion imaging agents based on therapeutic drugs

ZW800-1, Succinimidyl Ester
Chemical Formula: C_{55}H_{70}N_{5}O_{11}S_{2}^{+}
Exact Mass: 1040.45
Molecular Weight: 1041.30
Modular Chemistry*: One of Many Efficiencies Built into Our Platform

Homing to Cancer or Normal

Targeting Ligand (Small Molecule, Peptide or Antibody) → NH₂ → NHS Ester → Linker → NIR Fluorophore

Targeting Ligand (Small Molecule, Peptide or Antibody) → Linker → NIR Fluorophore

* Humblet et al., Contrast Agents & Molecular Imaging. 2006; 1: 196-211.
**Novel “Structure-Inherent Targeting”**

- Superb pipeline (315 novel NIR fluorophores) whose chemical structures have been engineered for both targeting and NIR fluorescence.
- All 315 compounds have been validated in rodent and pig
- 40 have been selected for their unique targeting ability
- Highly efficient cGMP synthesis and aseptic fill-finish already developed. Routine 100 g scale to date.
- Single intravenous injection and short clearance time
- Development of first clinical products and overall priority based on market size and clinical need
- Pipeline and pharma partnering program (conjugate)
Examples of Proprietary Contrast Agents Validated in Large Animals

- Cartilage
- Pan-Lymph Nodes
- Adrenal Glands
- Thyroid
- Parathyroid

NIR Antibodies (Strategic Alliances): Herceptin, Erbitux, Etc.

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Clinically-Available NIR Fluorophores
(1000 patients worldwide reported in the literature to date; 500 of these with FLARE™)

700 nm Fluorescence

**Methylene Blue**
- Ureter imaging
- Bile duct imaging
- Cardiac perfusion imaging
- Certain Solid Tumor Imaging

800 nm Fluorescence

**Indocyanine Green**
- Sentinel lymph node mapping
- NIR angiography
- Perforator flap mapping
- Certain Solid Tumor Imaging
- Intraluminal imaging

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M.W. 320

M.W. 776
Tumors

Parathyroid Tumor Resection in the Human Neck

Where’s the tumor?

Where are the recurrent laryngeal nerves? Damage to them leads to voice loss.

Where are the major blood vessels? Damage to them can be catastrophic.
The New Gold Standard from Curadel

Parathyroid Tumor Resection in the Human Neck using Methylene Blue

* Van der Vorst et al., Head and Neck. 2014; In Press
Breast Cancer Sentinel Lymph Node Mapping

100-Patient Trial in Breast Cancer SLN Mapping

- Radioactivity necessary for identification of initial SLNs
- Identification of SLN only by NIR fluorescence
- Identification of macrometastases only by NIR fluorescence

Verbeek et al., Breast Cancer Res Treat, 2014; In Press.
100-Patient Trial in Breast Cancer SLN Mapping (Cont.)

Verbeek et al., *Breast Cancer Res Treat*, 2014; *In Press*.
ICG: Vulvar Cancer

ICG: Cervical Cancer

- No difference ± albumin
- Sensitivity 78%-100 for SLN identification


Identification/ Resection of Colon Cancer Metastases in Liver

Van der Vorst et al., Cancer, 2014: in press.
Methylene Blue: Breast Tumor Imaging

Negative Margin Example

Tummers et al., Eur J Surg Onc. 2014; In Press

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Methylene Blue: Breast Tumor Imaging
Positive Margin Example

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NIR Fluorescence Digital Pathology

Updated Version of H&E Staining from 1876

Gibbs et al., Manuscript in Review.
NIR Fluorescence Digital Pathology

Prostate Cancer Disease Detection

<table>
<thead>
<tr>
<th></th>
<th>H&amp;E</th>
<th>AMACR (700 nm)</th>
<th>CK903 (800 nm)</th>
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Gibbs et al., Manuscript in Review.
NIR Fluorescence Digital Pathology

Automated Disease Detection at Whole Mount or Single Cell Level

Gibbs et al., Manuscript in Review.
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The Field is Presently like the Tip of an Iceberg
Unlocking the Potential of the Technology

• The next generation of FDA/EMEA-approved contrast agents won’t peak for approximately 10 years.

• Expand clinical trials with MB and ICG.

• Quantification of OUTCOMES using “imperfect” agents MB and ICG:

  Can surgery be performed faster, better, and/or cheaper using NIR fluorescence compared to the standard of care?

  If any of these are true then benefits to patients and the healthcare system are profound.
FLARE™ Imaging Network

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