ABSTRACT

Intraoperative tumor resection relies on the ability of the surgeon to discriminate tumor from healthy tissue, either visually or by palpation. Small tumor nodules can be missed or tumor margins may be inadequately removed, resulting in the need for secondary treatment. Intraoperative fluorescence imaging can help improve the initial resection, both improving outcomes and reducing cost. First-in-human studies have examined this technique using untargeted fluorescent dyes.[1] However, dyes conjugated to a targeting moiety have superior specificity for the tumor itself and provide enhanced guidance for the surgeon to localize the tumor and determine the margin of resection.[2]

The new Solaris system was recently introduced specifically for intraoperative imaging in research environments.[3] The system supports 4 different fluorescence channels to image common dyes (e.g. indocyanine green (ICG) and Fluorescein sodium (FD)) and more unique functionalized targeted or activated near-infrared (NIR) fluorescent agents in an ambient light environment. Recent data illustrate sensitivities of 10 nM for static snapshots and 10-100 nM for real-time acquisition at video frame rates, all under ambient light conditions. To achieve increased sensitivity focused fluorescence images are required, therefore an algorithmic autofocus approach has been implemented to find the ideal focal plane. Additionally, snapshots acquired at different wavelengths can be overlaid to enable multiplexing and improve tumor identification; previously published studies have shown this to be useful for sentinel lymph node mapping.[4] For FITC, where the fluorescent agents is feasible using the new Solaris imaging system. Intraperitoneal resection of tumors identified with both targeted and activatable agents. Subcutaneous tumors have been resected with the aid of intraoperative imaging in animals injected with IntegriSense 680. Tumor cell lines have also been implanted intraperitoneally in rats; the subsequent deep tissue tumors were identified intraoperatively and removed after injection with either BombesinRSense 680 or ProSense 750. Lymphatic draining, a common problem in tumor metastasis, was also investigated in mice using AngioSense 680. These results suggest that intraoperative resection of tumors identified with both targeted and activatable fluorescent agents is feasible using the new Solaris imaging system.

Instrument Setup

**Instrument Features Include [5]:**

- Tablet based control
- Adjustable imaging arm & head
- Fixed focal length optics to position the system outside of the sterile field
- Movable cart and lockable foot pedal
- Surgical grade white light LED based illumination
- Video rate or long exposure acquisitions
- Four channels (470, 660, 750, 800) to support visible and near infrared (NIR) dyes; <50nM sensitivity for video, <10 nM sensitivity for snapshot
- Custom liquid crystal tunable filter (LCTF) for autofluorescence reduction
- Two sCMOS cameras for simultaneous fluorescence and color image capture

Ambient Light Imaging

Room Lights ON
Room Lights OFF

Spectral gating and a real time background subtraction approach are used in the Solaris imaging system.[5] This facilitates imaging in an ambient light environment while using the surgical grade illumination (left). When a mouse was injected with IntegriSense 750, on the flank, the fluorescence image was unaffected by the ambient lighting (right).

3 Subcutaneous Tumors

### A. Quantification of subcutaneous tumors

<table>
<thead>
<tr>
<th>Pre-Op</th>
<th>Prepped</th>
<th>Incision</th>
<th>Resected</th>
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HCT116 cells were implanted subcutaneously in rats. IntegriSense 680 was injected 24 hours prior to fluorescence guided tumor resection. ROIs around the tumor (green box) or background (yellow box) indicate SNR of 1.7-1.8.

### B. Incomplete tumor resection

Eight rats were implanted with subcutaneous tumors and injected with IntegriSense 680 (24 nmol/kg) 24 hours prior to surgery. Tumors were palpable pre-operatively and were detectable by Solaris imaging (left). This signal was used to guide tumor resection (2nd from left). Residual signal was visible in the surgical bed (2nd from right) and after reclassing the surgical site (right). White arrows indicate residual tumor mass.

Intraperitoneal Tumors

### A. Dual Agent Injection, Surgical Necropsy

One rat with intraperitoneal tumors was injected with both BombesinRSense 680 and ProSense 750 FAST (24 nmol/kg) 24 hours prior to surgery. Post-operative images show a large tumor mass with both agents (white arrows). BombesinRSense had a much higher non-specific signal in the GI tract than ProSense 750 FAST (yellow arrows).

### B. Orthotopic Colon Cancer

HT-29-luc2 cells were implanted subcutaneously in donor mice. After 10-14 days, IntegriSense 680 was injected. The tumors were resected and surgically sutured to the cecum of host mice. Bioluminescent (BLI) and fluorescent images acquired 9 days post-implantation show that the tumor had taken in the host. Twenty-four hours prior to necropsy, mice were injected with IntegriSense 680. The transplanted tumor (green arrows) was palpable and detectable. Exposing the intraperitoneal cavity revealed small metastatic nodules (white arrows) that would otherwise be missed. The second node was a lumbar node (LU).

Lymphatic Draining

### A. Tail Base Injection

AngioSense 680EX was injected into the tail base and a video stream was recorded to monitor dye trafficking. Drainage was visible as early as 1 minute, with clear visualization of an inguinal node (solid circle) after 2 minutes. Drainage continued toward an axillary node (dashed circle) for the duration of the 7 minute long video.

### B. Foot Pad Injection

AngioSense 680EX was injected into the foot pad and a video stream was recorded to monitor dye location. Drainage to the first node (solid circle) was visible as early as 1 minute and continued to a second node for the duration of the 5 minute long video. To confirm that these were nodes, after completion of the imaging study the animals were euthanized and the signal sites were exposed. This confirmed that the first node nearest the tail was the popliteal node (PO) and that the second node was a lumbar node (LU).

Summary

The Solaris imaging system has been designed for intraoperative use and can be used to image fluorescent signals in an ambient light environment. The use of NIR fluorescent guided resection can improve outcomes by ensuring that no residual tumor is left in the surgical bed. Multiple imaging channels can be used to look at different probes and, furthermore, small nodules can be detected that would otherwise be missed. These nodules represent possible metastatic pathways so, to investigate them further, the imaging can also be used to track draining via the lymphatic system.

References

[3] JA Meganck et al., WMIC 2014