# Multispectral open-air fluorescence-guided imaging and detection of tumors using a hands-free translational platform with liquid crystal tunable filters (LCTF)

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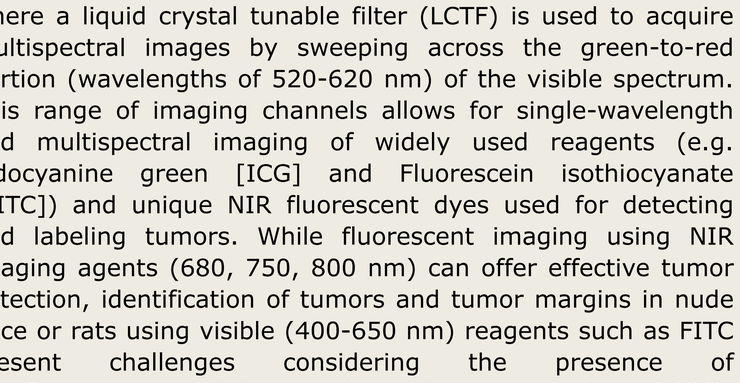
## Abstract

Effective detection and resection of tumors intraoperatively depends upon the surgeon's ability to visually locate and palpate the tumors and tumor margins. Tumor nodules and residual malignant tissue may go undetected or be inadequately removed, with such cases often resulting in the need for secondary treatment or additional surgical intervention. The Solaris™ platform is an open-air fluorescence imaging system designed for translational fluorescence-guided surgery, with the advantage of real-time video-rate acquisition of fluorescence signal under ambient and surgical light conditions.

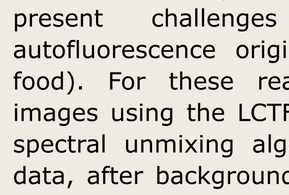
Solaris supports four fixed fluorescent channels ranging from visible to near infrared (NIR), and a multispectral channel where a liquid crystal tunable filter (LCTF) is used to acquire multispectral images by sweeping across the green-to-red portion (wavelengths of 520-620 nm) of the visible spectrum. This range of imaging channels allows for single-wavelength and multispectral imaging of widely used reagents (e.g. indocyanine green [ICG] and Fluorescein isothiocyanate [FITC]) and unique NIR fluorescent dyes used for detecting and labeling tumors. While fluorescent imaging using NIR imaging agents (680, 750, 800 nm) can offer effective tumor detection, identification of tumors and tumor margins in nude mice or rats using visible (400-650 nm) reagents such as FITC present challenges considering the presence of autofluorescence originating from tissue and chow (alfalfa food). For these reagents, Solaris acquires multispectral images using the LCTF under ambient light conditions, and a spectral unmixing algorithm is applied to the multispectral data, after background correction and ambient light removal, to separate tissue and chow autofluorescence from the reagent fluorescent signal.

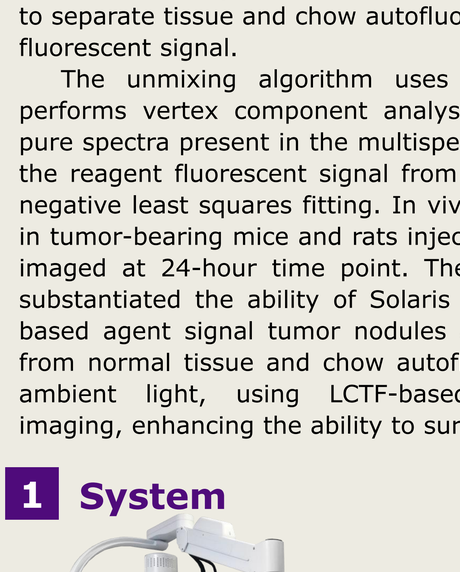
The unmixing algorithm uses library-based spectra or performs vertex component analysis to extract the primary pure spectra present in the multispectral images, and separate the reagent fluorescent signal from autofluorescence by non-negative least squares fitting. In vivo studies were carried out in tumor-bearing mice and rats injected with FITC-dextran and imaged at 24-hour time point. The results of these studies substantiated the ability of Solaris to spectrally unmix FITC-based agent signal tumor nodules and residual tumor tissue from normal tissue and chow autofluorescence imaged under ambient light, using LCTF-based open-air multispectral imaging, enhancing the ability to surgically resect them.

## 1 System



**Liquid Crystal Tunable Filter (LCTF)**

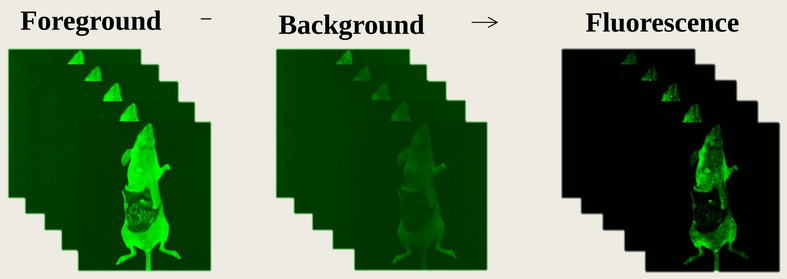


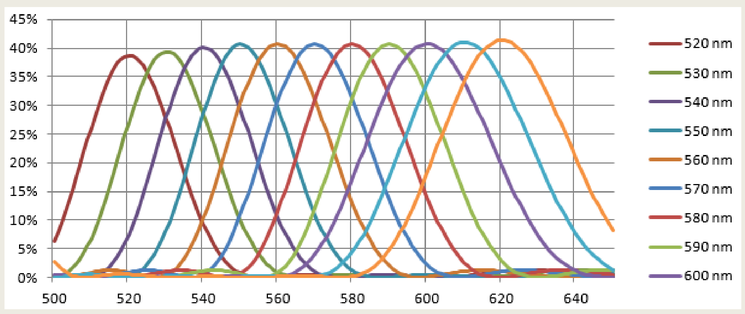
**Instrument Features**

- Surgical grade white light LED based illumination
- sCMOS and CMOS RGB cameras for simultaneous fluorescence and color image capture
- Video rate or long exposure acquisitions
- Temporal and spectral gating of excitation and illumination LEDs to reject ambient light
- 100um spatial resolution at 10cm field of view
- <10-50nM sensitivity in video and snapshot
- Adjustable imaging arm & head
- Auto-focus and range finder
- Fixed focal length optics to position the system outside of the sterile field
- Four channels to support visible and near infrared (NIR) dyes
- Custom liquid crystal tunable filter (LCTF) for spectral unmixing and autofluorescence reduction

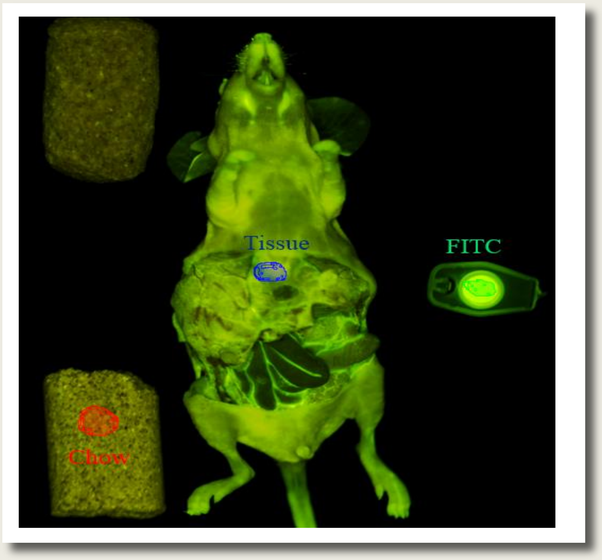
## 2 Open-air Multi-spectral Fluorescence Imaging

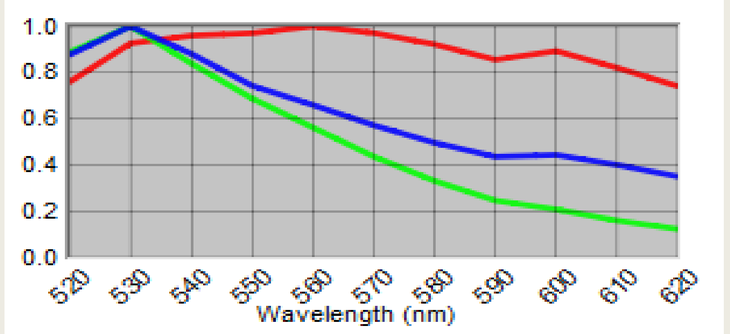


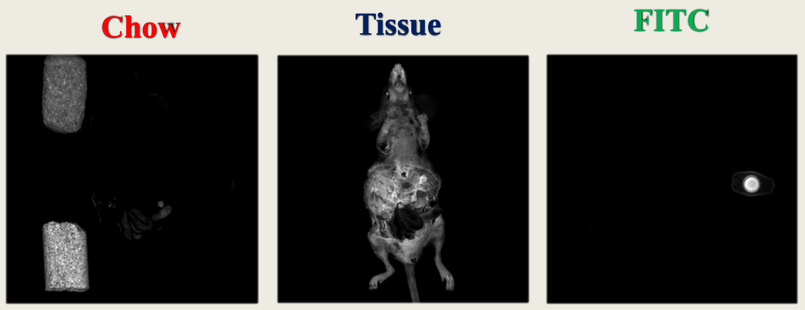

Multispectral image stacks are acquired rapidly using fast tuning of LCTF which provides the spectral bands shown below. Foreground (excitation LEDs on) and background (excitation LEDs off) images are acquired for each band, the fluorescence image is retrieved by performing background subtraction.




## 3 Spectral Unmixing of Fluorescein isothiocyanate (FITC) fluorescence from tissue and chow autofluorescence

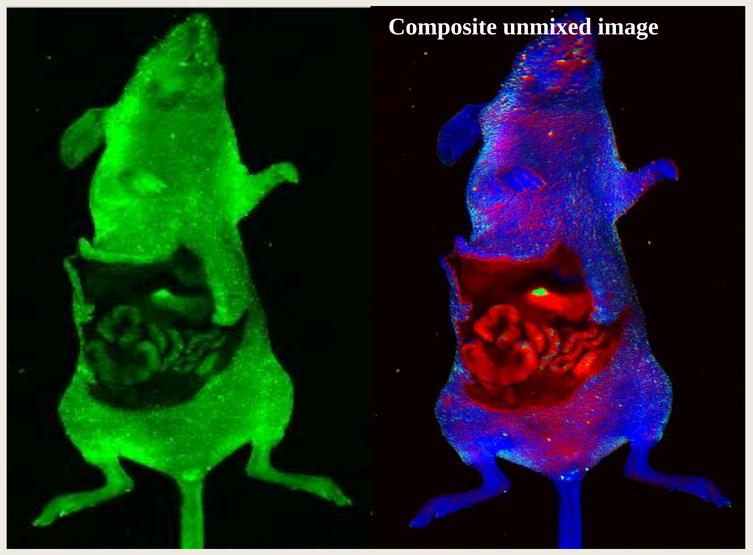


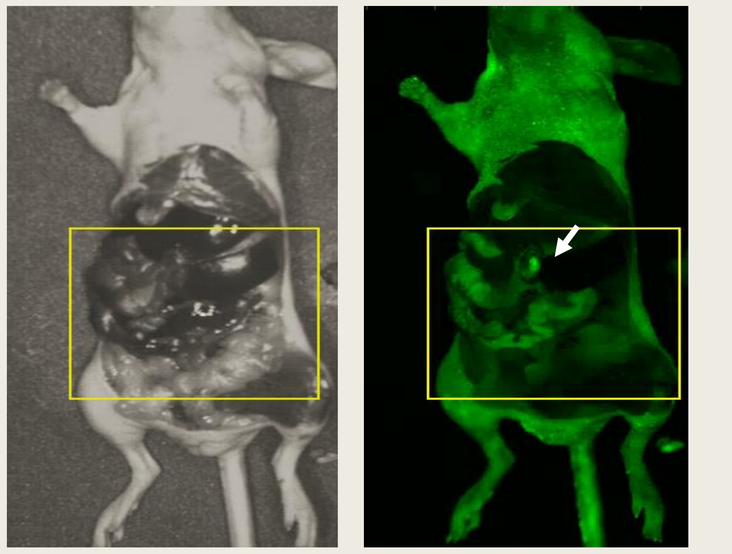



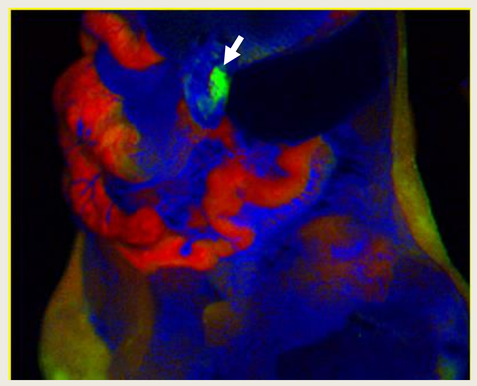

Primary sources of autofluorescence in the visible region are tissue and chow which need to be unmixed from fluorescence signal of FITC-based reagents that tag biological or physiological inclusions such as tumors. The first step of spectral unmixing is to extract the spectral signature of the fluorescent and autofluorescent components. This can be done in a supervised approach as shown above with the user manually selecting the corresponding signal regions. Unsupervised automated methods including vertex component analysis can also be used when manual selection is not available to arrive at the spectra shown above. Once the spectra are characterized, the unmixing can be performed by applying a non-negative least squares to the multispectral images (data) and the spectra (mixing matrix) to yield the three component images corresponding to chow, tissue, and FITC fluorescence as shown above.

## 4 Identification of tumor nodules labeled with FITC-Dextran using multispectral imaging



**Composite unmixed image**



A mouse model of metastatic liver cancer was investigated. Mice were injected with HCT116-Red_Fluc cells intrasplenicly. Mice were splenectomized 10 minutes after injection, and metastatic lesions were detectable by BLI within about a week. Twenty-four hours before intraoperative imaging, mice were injected intravenously with FITC-Dextran. Mice underwent fluorescence-guided open-air intraoperative tumor identification and resection with spectral unmixing in the FITC channel. White arrows indicate small tumor masses with regions of vascular leak (FITC-Dextran).

## Summary

- Intraoperative tumor resection has historically relied solely on a surgeon's ability to palpate and visually distinguish tumors
- Fluorescence-guided surgical imaging in the FITC channel is hampered by intrinsic tissue and chow (food) autofluorescence
- Utilizing spectral unmixing on the Solaris, metastatic liver nodules were spectrally separated from autofluorescent sources, demonstrating the unique feature of the LCTF on the Solaris imaging system
- Spectral unmixing assists in the identification of FITC-tagged residual tumor and metastatic disease in translational preclinical cancer models

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