

# Selection of Hyperspectral Endmember Extraction Algorithm for Tumor Delineation in Animal Models

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**Abstract:** Advanced algorithms used in geospatial imaging were adopted for biomedical application to analyze hyperspectral datasets. To demonstrate the effectiveness, endmember extractions method was applied for delineating tumors in animal models of cancer. © 2021 The Author(s)

## Introduction

Hyperspectral imaging (HSI) generates three dimensional datasets (datacubes), with spatial and spectral dimensions. Each pixel carries the entire high-resolution spectrum. HSI offers an exciting opportunity to explore optical properties of objects and discover hidden features not accessible by other techniques, all based on the small changes in the spectra without the use of contrast agents. Combined with the appropriate algorithms, HSI leads to a substantially improved ability to classify, differentiate, and quantify the objects based on their spectral signatures, enabling even small, otherwise unnoticeable, features to be amplified.

The concept of hyperspectral imaging (HSI) began more than forty years ago for geospatial applications and has been used most widely by geologists and environmentalists for the mapping of minerals and monitoring the processes on Earth. Geospatial HSI significantly expanded from just a few systems even 20 years ago to hundreds and thousands in 2020. Rapidly growing number of HSI systems installed on small satellites, planes and drones decrease the cost of remote imaging and turned hyperspectral approaches into affordable tools that are widely used in different aspects of geospatial monitoring including environmental screening, mining, agriculture, forestry, and construction.

Relatively recently, HSI has moved to the area of biomedical applications [1]. As spectral imaging hardware (full body scanners, handheld HSI cameras, HSI microscopes) become available, the needs for imaging algorithms to handle the multidimensional datasets followed. Many of these algorithms can be adapted from geospatial imaging [2, 3]. Here, we applied a set of well-known geospatial algorithms based on hyperspectral endmember extraction to identify tumors in the animal models of cancer without the use of any contrast agents.

## Experimental section

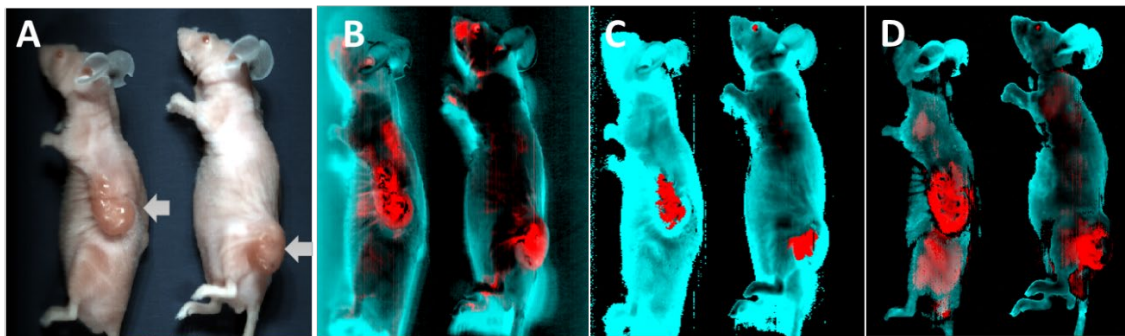
Animal studies were performed under the guidance of Washington University IUCAC. Athymic male mice 10-12 weeks old were implanted with HT1080 human fibrosarcoma cells subcutaneously in their left flanks. The tumors were allowed to grow until about 10 mm maximum diameter. Prior to the imaging, the mice were anesthetized with an intraperitoneal injection of ketamine and xylazine cocktail. (90 mg/kg and 10 mg/kg, respectively).

The mice were imaged using a benchtop hyperspectral imaging described in [4]. The imaging HSI pushbroom data system featured a SWIR sensitive 2D InGaAs thermoelectrically cooled CCD camera (Ninox, Raptor), a 25 mm focal length SWIR lens (StingRay Optics), an imaging spectrograph Inspector N17E (Specim), equipped with a 30  $\mu\text{m}$  slit which provides 5 nm spectral resolution, and a linear, PC-controlled movable stage (Middleton Inc.). These components were integrated by Middleton Inc. into a stand-alone image acquisition system. To minimize specular reflection from the skin, mice were illuminated with quartz halogen lamps at 45°. Data analysis was performed on a hyperspectral imaging analysis software, IDCube (HSpEQ, USA).

## Results

The goal of this work was to investigate hyperspectral imaging algorithms to identify and delineate tumors with high contrast using endmember extraction techniques. In geospatial imaging, an endmember corresponds to a pure mineral with a specific individual spectrum. Each pixel corresponds to the mixture of different components. Identification of the individual mineral from the image requires spectral unmixing analysis known as endmember extraction. We used three different endmember extraction methods: N-finder (N-FINDR) [5], FIPPI [6] and PPI [7]. Identification and delineation of tumors presents a similar task, since the spectra signal from the tumor are mixed with the skin and other tissue components.

The hyperspectral images were made in a shortwave infrared spectral range to increase the depth penetration of photons through the skin. Two mice, each with a tumor, were imaged. A pseudo-RGB image of mice composed of three wavelengths in shortwave infrared is shown in **Fig. 1A**. The number of the endmembers present in a hyperspectral datacube was performed by using the noise-whitened Harsanyi–Farrand–Chang (NWHFC) method. Overall, 27 unique endmembers were identified. After the extraction procedures, all endmembers were presented through the corresponding abundance maps. A map for each endmember was visually evaluated and the best combinations of two endmembers corresponding to the tumors and the other healthy tissue were overlapped as shown in **Figure 1B–D**. The computational method was considered to be successful if both tumors are identified with a minimum overlap from the surrounding tissues. The results demonstrate that FIPPI based algorithm provides the best contrast. Other endmember extraction methods suffer substantial overlap with the normal tissue.



**Figure 1.** HSI imaging of athymic male mice 10-12 weeks old implanted with 4t1 mouse mammary carcinoma tumors. Arrows point to the tumors. A: Pseudo RGB: Red: 1094 nm; Green: 1144; Blue: 1296 nm. Total 27 endmembers were identified. Endmember extraction. B: N-FINDR, C: FIPPI, D: PPI.

## Conflict of Interest

Berezin is the founder and CEO of HSpEQ LLC.

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