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2016

Lim, H.-T. & Murukeshan, V. M. (2016). Spatial-scanning hyperspectral imaging probe for bio-imaging applications. Review of Scientific Instruments, 87, 033707-.

<https://hdl.handle.net/10356/83660>

<https://doi.org/10.1063/1.4943968>

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Spatial-scanning hyperspectral imaging probe for bio-imaging applications

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(Received 21 October 2015; accepted 1 March 2016; published online 18 March 2016)

The three common methods to perform hyperspectral imaging are the spatial-scanning, spectral-scanning, and snapshot methods. However, only the spectral-scanning and snapshot methods have been configured to a hyperspectral imaging probe as of today. This paper presents a spatial-scanning (pushbroom) hyperspectral imaging probe, which is realized by integrating a pushbroom hyperspectral imager with an imaging probe. The proposed hyperspectral imaging probe can also function as an endoscopic probe by integrating a custom fabricated image fiber bundle unit. The imaging probe is configured by incorporating a gradient-index lens at the end face of an image fiber bundle that consists of about 50 000 individual fiberlets. The necessary simulations, methodology, and detailed instrumentation aspects that are carried out are explained followed by assessing the developed probe's performance. Resolution test targets such as United States Air Force chart as well as bio-samples such as chicken breast tissue with blood clot are used as test samples for resolution analysis and for performance validation. This system is built on a pushbroom hyperspectral imaging system with a video camera and has the advantage of acquiring information from a large number of spectral bands with selectable region of interest. The advantages of this spatial-scanning hyperspectral imaging probe can be extended to test samples or tissues residing in regions that are difficult to access with potential diagnostic bio-imaging applications. © 2016 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4943968>]

I. INTRODUCTION

Hyperspectral imaging (HSI) has been used in airborne and spaceborne remote sensing as early as 1989 using electronic recording devices^{1,2} to record intensity-related information of adjacent spectral bands. HSI yields a datacube, which is a three-dimensional (3-D) set of information in a spatial-spatial-spectral domain. Multiple two-dimensional (2-D) spatial-spatial images each corresponding to a particular spectral band can be extracted from the datacube. Each voxel in the datacube holds the intensity-related information of a particular spectral band from one spatial point in the 2-D sample.³ A spectrum is acquired by extracting the information from a spatial point along the spectral domain. Since the spectrum is made up of information from adjacent spectral bands, rich spectral information can be acquired. The spectrum can then be used for classification and quantification by comparing it with a set of data library using algorithms. Besides remote sensing,⁴ HSI has now been used in a wide variety of applications such as quality assessment of agriculture and farm products,^{5,6} biomedical applications,⁷⁻⁹ and counterfeiting of fraudulent documents.¹⁰

HSI does not have a definition which is universally adopted. Puschell had defined HSI as having ~30–300 spectral bands, spaced less than 10 nm apart.¹¹ While Fresse *et al.* had defined HSI as having 100–200 spectral bands with a spectral precision of 0.01.¹² Taking into account that the definition used

by Fresse *et al.* is stricter and more recent as compared to that of Puschell, this paper will adopt the definition of Fresse *et al.* of HSI. There are three commonly used methods to perform HSI, namely, spatial-scanning, spectral-scanning, and snapshot. The advantages and limitations of each method should be considered before applying it for certain applications. These three methods have been reported to build the hyperspectral (HS) datacube which contains multiple 2-D spatial-spatial images at different spectral bands.^{8,13,14}

A spatial-scanning imager usually uses a spectrograph as a spectral dispersive element to split the light into its constituent spectral bands before detection by the sensor array. Spatial-scanning imagers can be further divided into two types, namely, the whiskbroom and pushbroom imagers. A whiskbroom imager acquires the intensity spectrum of only a spatial position in each scan¹¹ to give one-dimensional spectral information. Multiple scans in two orthogonal spatial directions are required before gathering enough information to build the datacube. Each scan by a pushbroom imager acquires the intensity spectrum of multiple spatial positions in a straight line, giving 2-D spatial-spectral information. This is accomplished by having a narrow slit before the spectrograph.¹⁵ Multiple scans are required in only one direction (transverse to the slit) to form the 3-D datacube. Since a pushbroom imager acquires more information at once, it can be a time-saving alternative compared to the whiskbroom imager. Spatial-scanning imager requires a relative displacement between itself and the object. The movement of such airborne or spaceborne imager can provide the displacement, while mechanical stages are used in many other ground-based HSI systems.

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Electronically tunable filters, such as liquid crystal¹⁶ and acousto-optical tunable filters,¹³ are used in spectral-scanning HS imager. The tunable filter has spectral transmission that can be controlled electronically³ to select the spectral band permitted to pass through and be detected by the sensor array. Each scan by the sensor is the image of the object at a particular spectral band, giving 2-D spatial-spatial information. By controlling the tunable filter to transmit different spectral bands, multiple spatial-spatial images are acquired to form the 3-D datacube. Unlike the spatial-scanning imager, relative displacement between the object and the system is not required in the spectral-scanning imager. A snapshot imager is able to acquire all the information to build the 3-D datacube in a single scan. Therefore both sequential scanning and relative displacement between the object and system are not required. Many methods have been adopted to produce a snapshot imager, such as compressive sensing¹⁷ and image mapping spectroscopy.¹⁴

HSI has been configured for use as an optical probe and in some cases as an endoscope to image the body cavity. They have been used to image the vasculature of the lower lip for oxy-hemoglobin studies,¹⁴ as well as the skin^{18,19} and in otolaryngoscopic investigations²⁰ for cancer studies. Although there are three commonly used methods to perform HSI, only the spectral-scanning and snapshot methods have been demonstrated in a HS imaging probe. Both the liquid crystal¹⁸ and acousto-optical tunable filters¹⁹ have been used in spectral-scanning HS imaging probe, and image mapping spectroscopy has been used in snapshot HS imaging probe.¹⁴ These HS imaging probes usually use a fiber bundle to deliver light from the sample (distal end) to the proximal end where HS measurement is performed. One reason why the spatial-scanning method has been left out for such an application is that the mechanical scanning required during HS measurement makes it much slower compared to the other two methods. However, existing HS imaging probes that use spectral-scanning or snapshot method has so far only provided up to ~50 spectral bands.^{14,20}

In this context, this paper reports the instrumentation, performance, and capability of a pushbroom HS imaging probe which has not been reported in the literature. This fills the gap in translating the spatial-scanning pushbroom HSI

system to a pushbroom HS imaging probe. Also, the advantage of using such a configuration is that information from a large number of spectral bands, of the order of several hundreds, can be acquired. The feasibility and efficiency of the probe system are illustrated by performing HS measurements on a United States Air Force (USAF) resolution chart and chicken breast tissue with blood clot as test samples.

II. PUSHBROOM HYPERSPECTRAL IMAGING PROBE

A. Instrumentation

The pushbroom HS imaging probe is shown in Fig. 1 and is divided into two main parts, the imaging probe and the pushbroom HS imager. The imaging probe is an assembly of an imaging fiber optic bundle and a gradient-index (GRIN) lens. The fiber bundle (Sumitomo Electric, IGN 11/50) has an active and an outer diameter of ~1 mm and 1.2 mm, respectively. Attached to the distal end of the fiber bundle is a GRIN lens (Grintech, GT-IFRL-100-cus-50-NC) with a diameter and length of ~1 mm and 4.2 mm, respectively. The GRIN lens was custom fabricated for 1× magnification with a working distance of 0.3 mm at 532 nm in air at both sides. The fiber bundle and the GRIN lens are attached to each other using stainless steel tubing with an outer diameter and length of 1.6 mm and 25 mm, respectively. The maximum diameter on the distal end of the imaging probe is 1.6 mm, due to the stainless steel tubing, making it suitable to be used as an optical probe in confined places.

The pushbroom HS imager has been previously reported.⁸ A broadband fiber-optic pigtailed source (Edmund Optics MI-150) is used to provide sample illumination. The GRIN lens produces an image of the sample on the distal end-face of the fiber bundle. This image is transferred to the proximal end of the fiber and directed towards the forelens (Navitar 2-50145 doublet lens). Before HS scanning, the sliding mirror is initially pushed into the quadocular adapter and directs light towards the video camera, as shown by Path 1 in Fig. 1. The video camera (iDS UI-1550LE-C-HQ) allows direct video imaging of the proximal end-face of the fiber bundle where the sample image is formed. This allows easy positioning of the proximal end of the fiber bundle by the 3-axis motorized

FIG. 1. Schematic diagram of the developed pushbroom HS imaging probe.

stage (Physik Instrumente, x and y -axes: M-112.2DG, z -axis: M-110.1DG). When the sliding mirror is pulled out of the quadrocular adapter, the image travels towards the spectrograph (Specim ImSpector V10E, spectral resolution 2.8 nm) and the detector camera (Andor EMCCD LucaEM DL-604M-OEM) (Path 2 in Fig. 1). The spectrograph has a slit allowing only one line of the image to reach the other optical elements in the spectrograph for spectral dispersion. The detector camera records the resulting 2-D spatial-spectral information. HS scanning is done by the y -axis motorized stage to carry out sequential line-scanning of the proximal end-face of the fiber bundle. The pushbroom HS imager was reported to have a spectral range covering 400–1000 nm with 756 spectral bands.⁸

B. HyperSpec

HyperSpec⁸ is an in-house developed software using LabView® allowing the user to choose a particular region within the video camera's field-of-view as the region of interest (ROI). The position of the proximal end-face of the fiber bundle may vary, but as long as it is within the field-of-view of the video camera, user can select the proximal fiber end-face as the ROI. HyperSpec is then able to automatically control the y -axis stage to perform sequential pushbroom HS scanning. Such flexibility allows easy positioning of the proximal fiber end-face and makes the system alignment convenient. HyperSpec takes into account the calibrations of the system, user-defined parameters, and the subsequent hardware synchronization during HS line-scanning measurement.

III. GRIN LENS

A GRIN lens is used as a miniature objective lens so that the image of the sample falls onto the distal end-face of the image fiber. The GRIN lens was initially designed for use with 532 nm light source with a working distance of 0.3 mm, considering only the on-axis optical performance. Using ZEMAX simulation, the length required for the GRIN lens was determined to be ~ 4.2 mm. The GRIN lens is attached to the end face of the fiber bundle by Grintech to form the imaging probe.

In order to integrate this fiber probe with the HS imaging probe, more factors have to be considered. The most important factor includes the spectral range of interest from 400 to 1000 nm along with the on and off axes optical performances. Five equally weighted wavelengths (400 nm, 550 nm, 700 nm, 850 nm, and 1000 nm) are used in the simulation to represent the spectral range of interest from 400 to 1000 nm. Three field positions are considered, on-axis, 0.32 mm (0.707 full-field) and 0.45 mm (full-field). Using ZEMAX's default merit function (type: RMS, criteria: wavefront, reference: centroid, pupil integration: Gaussian quadrature with three rings and six arms), the optimized object-lens distance was found to be ~ 0.316 mm (Fig. 2).

At the optimized object-lens distance of ~ 0.316 mm, it can be observed from Fig. 2 that each wavelength behaves differently as it moves through the GRIN lens. This causes wavelength-dependent optical characteristics on the distal

FIG. 2. Optimized layout of distal end of HS imaging probe at five representative wavelengths.

end-face of the fiber bundle. It is evident from Fig. 2 that the focus positions shift away from the object as wavelength increases, causing the images formed at the distal end-face of the fiber bundle to be wavelength-dependent. The on-axis root-mean-square radii with centroid references for 400 nm, 550 nm, 700 nm, 850 nm, and 1000 nm are 41.440 μm , 2.511 μm , 14.872 μm , 24.018 μm , and 29.415 μm , respectively. It can also be seen that the amount of light collected by the GRIN lens decreases as the object is positioned away from the center of the lens. This may cause the features collected from the peripheral of the fiber bundle to be suppressed by the high intensity of light collected from the center. Although GRIN lens is useful as a miniature objective lens, a detailed study on the use of such a GRIN lens in a HS imaging probe is important as this can affect the spatial resolutions of the spectral images captured by the system.

IV. METHODS

Each scan during HS measurement had an integration time of 0.1 s. The results presented in this paper are not in terms of intensity but were referenced to take into account the non-uniform collection of light by the GRIN lens and any uneven illumination of sample. Each sample was placed at ~ 0.3 mm away from the GRIN lens using the 3-axis mechanical stage. The data were processed using MATLAB®.

HS measurement of the USAF resolution chart shows the system's performance and was done in transmission mode. In order to get the *transmittance* data, the *sample* data were corrected by dark reference (*Dark*) and white reference (*White*) using Eq. (1). *Sample* data were acquired when the bars on the USAF resolution chart were imaged. *Dark* data were acquired when the light source was turned off and the forelens covered. It represents the image with dark current noise where the transmittance was 0%. *White* data were acquired by imaging a clear region of the USAF resolution chart where the transmittance was taken to be 100%. x and y refer to the orthogonal spatial dimensions and λ refers to the spectral band. *Smooth* is the 11-point moving average in the spectral direction for spectrum

smoothing,

$$\begin{aligned} \text{Transmittance}(x, y, \lambda) \\ = \text{Smooth} \left[\frac{\text{Sample}(x, y, \lambda) - \text{Dark}(x, y, \lambda)}{\text{White}(x, y, \lambda) - \text{Dark}(x, y, \lambda)} \right]. \end{aligned} \quad (1)$$

HS measurement of the chicken breast tissue with blood clot shows the system's capability to acquire biological images in reflection mode with access to only one side of the sample. In order to get the *reflectance* data, the *sample* data were corrected by dark reference (*Dark*) and white reference (*White*) using Eq. (2). *Sample* data were acquired by imaging the chicken breast tissue. *Dark* data were acquired when the light source was turned off and the forelens covered. It represents the image with dark current noise where the reflectance was 0%. *White* data were acquired by imaging a 99% reflectance standard (Labsphere, SRS-99-010) where the reflectance was taken to be 99%,

$$\begin{aligned} \text{Reflectance}(x, y, \lambda) \\ = \text{Smooth} \left[\frac{\text{Sample}(x, y, \lambda) - \text{Dark}(x, y, \lambda)}{\text{White}(x, y, \lambda) - \text{Dark}(x, y, \lambda)} \right] \times 0.99. \end{aligned} \quad (2)$$

V. RESULTS AND DISCUSSION

A. Scale and orientation

Figure 3 shows the image of the selected ROI using a USAF chart [vertical bars of Group 2 Element 4 (G2E4)] and two intensity mappings of 660 nm from the datacube, placed beside and below the ROI. The circular dashed lines indicate the position of the imaged proximal end-face of the fiber bundle within the ROI. Data outside the circular dashed lines are the background and thus are ignored. The four straight dashed lines match features in the ROI to the same features in both the intensity mappings. The longer horizontal line also shows that the ROI and the HS data have the same orientation. These show that both the horizontal and vertical scales between the ROI and HS are the same and that the system is properly aligned and calibrated.

FIG. 3. Comparison of ROI and intensity mappings of USAF chart G2E4 at 660 nm.

FIG. 4. Images of horizontal bars of USAF chart G1E6. (a) ROI and (b) 660 nm intensity mapping.

B. Effective field-of-view of HS data

The horizontal bars of G1E6 of the USAF chart are imaged, as shown in Fig. 4. They are specifically chosen as they fit nicely within the image of the fiber bundle as captured by the video camera. The results in Fig. 4(b) show that the entire end-face of the fiber bundle can be used to acquire HS data, making it the effective field-of-view of HS data.

C. Lateral resolution

Group 3 of the USAF chart has been imaged in three separate measurements, as seen in Fig. 5. Using the 505 nm intensity mapping, the vertical and horizontal bars of G3E5 (Fig. 5(f)) can still be distinguished. Therefore the vertical and horizontal lateral resolution of this system at 505 nm is evaluated to be $\sim 40 \mu\text{m}$.

As a representation, nine out of 756 intensity mappings of G3E5 and G3E6 are selected from the entire spectral range (400–1000 nm) and shown in Fig. 6. It can be seen that some features, more evidently the vertical bars of G3E5, do not appear to be the same in all spectral bands. They appear to be sharper at 505 and 570 nm, but became worse as wavelength increases. The HS data in Fig. 6 show that the system's lateral resolution is a function of wavelength. This result coincides with the ZEMAX simulation results of the GRIN lens where the path of each wavelength varies, resulting in different optical performance of the wavelengths on the distal end-face of the fiber bundle.

D. Biological images

A chicken breast tissue with blood clot is used as the test sample to demonstrate the imaging capability of the integrated HS imaging probe. The sample used and the ROI of the measurement are shown in Fig. 7. The datacube of this measurement has a size of $100 \times 293 \times 756 (x, y, \lambda)$, and a cut datacube is shown in Fig. 8 to reveal the features within the datacube. The intensity mappings of four selected spectral bands are also shown in Fig. 9. The spectra of the blood clot and the chicken breast tissue were acquired from three regions each, indicated by the red and black boxes, respectively, in Figs. 7(b) and 9. Each region is $\sim 0.1 \times 0.1 \text{ mm}^2$ and corresponds to $30 y\text{-pixel} \times 10 x\text{-pixel}$ in the datacube. The 900 spectra in the three regions of blood clot and chicken breast tissue were averaged to give the representative spectra. The center white lines in the plots shown in Fig. 10 are the representative spectra,

FIG. 5. Images of USAF chart Group 3. ROIs of (a) G3E1 and G3E2, (b) G3E3 and G3E4, (c) G3E5 and G3E6, 505 nm intensity mappings of (d) G3E1 and G3E2, (e) G3E3 and G3E4, and (f) G3E5 and G3E6.

while the black areas surrounding the white lines represent the standard deviations. The spectral results show that the integrated HS imaging probe is able to acquire the distinct spectra from different parts of the sample reliably. Such spectral data can be stored in a data library and used for the classification and quantification of other samples.

The proposed and illustrated HSI system has given a new dimension of performing endoscopic imaging possibility with the addition of the imaging probe. The proposed concept of spatial-scanning pushbroom HSI probe offered many advantages compared to other existing pushbroom HSI systems which can only perform table-top imaging.^{21–24} Using a video

FIG. 6. Nine selected intensity mappings of USAF chart G3E5 and G3E6.

FIG. 7. Sample of chicken breast tissue with blood clot. (a) Sample on glass slide and (b) ROI.

FIG. 8. Cut datacube of chicken breast tissue with blood clot.

FIG. 9. Four selected intensity mappings of chicken breast tissue with blood clot.

camera in the proposed system⁸ offers flexibility in positioning the proximal end-face of the fiber bundle and allows convenient system alignment. It also gives a color image of the ROI so that a visual comparison can be made between the color image and the HS data. The system detects 756 spectral bands within the spectral range from 400 to 1000 nm. With this arrangement, existing table-top pushbroom HS imager can be made to perform both table-top and endoscopic imaging, making it suitable for more diagnostic bio-imaging appli-

FIG. 10. Mean reflectance spectra (white lines) and standard deviation (black areas) of chicken breast tissue and blood clot.

cations such as endoscopy in the gastrointestinal tract and pharynx.

VI. CONCLUSION

We have proposed, optically configured and demonstrated a pushbroom HS imaging probe based on spatial-scanning method, for the first time. The imaging probe is an assembly of a GRIN lens and imaging fiber optic bundle and is used to deliver the sample image to the proximal fiber end-face of the fiber bundle for HS measurement. The system offers 756 spectral bands for detection within the full spectrum range of the system. It is also noted that lateral resolutions of the system are wavelength-dependent. This result is in agreement with the ZEMAX simulation results of the GRIN lens. The horizontal and vertical lateral resolutions at 505 nm are $\sim 40 \mu\text{m}$. A chicken breast tissue with blood clot is used as test sample for demonstrating bio-imaging capability and the reflectance spectra of the chicken breast tissue and blood clot were acquired. The spectra were found to be easily distinguishable from one another.

The pushbroom HS imaging probe can be used on samples that are difficult to reach and close to being stationary. The main advantage is that it can provide hundreds of spectral images. It is envisaged that the hundreds of spectral images that are available for an efficient analysis can contribute to potential diagnostic bio-imaging applications in the near future. We have also extended the scope of application of the developed table-top pushbroom HS imager by enabling it to perform endoscopic imaging using a flexible imaging probe. This configuration can then be used for endoscopic bio-imaging applications.

ACKNOWLEDGMENTS

The authors acknowledge the financial support received through Ministry of Education (MOE-AcRF Tier 1) and COLE-EDB.

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