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Multi-spectral reflectance confocal microscopy on skin

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Multi-Spectral Reflectance Confocal Microscopy on Skin

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Abstract

Reflectance confocal microscopy is a powerful *in-vivo* modality for imaging superficial layers of biological tissue, especially for human skin. Three dimensional imaging capability enables confocal microscopy to resolve structures of upper skin layer cells. However, sub-celluar structure and corresponding functional organelles play more important parts in skin diseases diagnosis and monitoring. We present a new multi-spectral reflectance confocal microscopy to achieve sub-celluar functional imaging in skin by utilizing our unique *Keck* multi-modality microscope. Spectral information and a modified *Mie* scattering model are incorporated to identify distribution of melanin and mitochondria in cells. *Ex-vivo* and phantom experimental results are presented. Further development of this new modality may lead to future clinical applications.

Introduction

Significance and Challenges

- Multi-spectral confocal microscopy incorporates 4-D information to achieve sub-celluar functional imaging.
- Direct spectral analysis of conventional reflectance confocal images may avoid invasive stain procedure of fluorescent dyes.
- A proper spectral un-mixing algorithm may be needed to separate the measured mixture spectra into independent physically meaningful spectra for further identifying small organelle within cells.
- A scattering model for spherical scatter with laser beam is needed to develop a physical-based classification procedure.

Technical Approaches

- Utilize ability of tunable wavelength of our *Keck* multi-modality microscope to obtain spectral confocal images.
- Apply a non-negative un-mixing algorithm of alternating least square and multivariate curve resolution (ALS-MCR) procedure for spectra separation. [2]
- Adopt a localization approximation (LA) to model light scattering of spherical particles with incident gaussian laser beam. [3,4]

State of the Art

- Spectrally encoded confocal microscopy is developed to increase resolving ability, but spectral information is not utilized. [1]

- Spectral analysis with confocal microscopy is mainly focused on fluorescence imaging, but no work on reflectance spectral confocal microscopy has been reported in the literature yet.

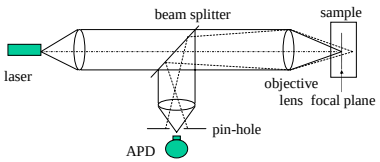


Fig.1 Confocal Microscope Working Diagram

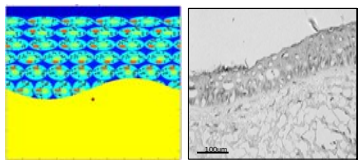
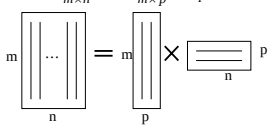


Fig.2 Skin Model [5], and DIC image of a skin histology

1. Un-mixing Spectrum by ALS-MCR:

- A non-negative matrix decomposition is applied to decomposes multi-component data matrix ***D*** as product of component concentration matrix ***C*** and spectra matrix ***S***, which both has physical meaning and are non-negative.

$$D_{m\times n} = C_{m\times p} S^{T}_{p\times n}$$



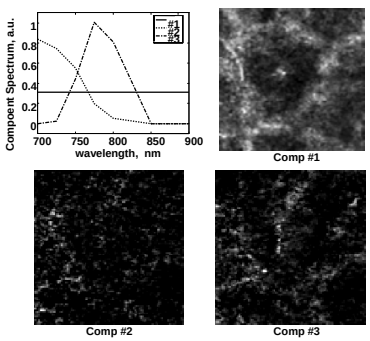


Fig.3 ALS-MCR decomposition results, three pure components and spectra

2. Photon Scattering Model

- Local Approximation (LA), a modified *Mie* scattering theory for gaussian laser beam.
  - In geometric optics, wavefront can be thought of as being made up of separate independent localized rays, LA extended this principle by introducing a beam shape parameters.
  - LA is validated by original *Mie* theory and FP resonator at small and large particle limit.

3. Components identification by spectra matching

- Compare un-mixed spectra with theoretical back-scattering spectra by LA
- Identify components as organelles (*i.e.* melanin and mitochondria)

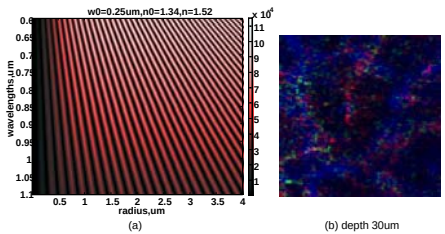


Fig.4, (a) Back-scattering intensity map *v.s.* wavelength and particle sizes; (b)pseudo RGB composition map. (Blue: cell/Nuclei, Red: melanin, Green: mitochondria)

4. Validation with mixed bead phantom

- Two kinds of beads with different size (0.5 and 1.0um in diameter) and refraction index (1.52 and 1.38) are used.
- One kind of bead is fluorescence tagged and can be imaged by two-photon fluorescence microscope to easily identify one bead from the other, as ground truth.

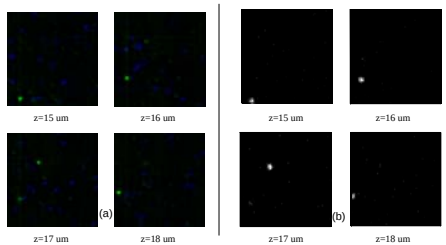


Fig.5 (a) pseudo RGB composition map of bead phantom at different depths. (Green: fluorescence tagged bead, Blue: non-tagged bead) (b) Two-photon fluorescence images at the corresponding depths

Opportunities for Technology Transfer

- Plan to transfer the technology to clinical instrument for spectral skin diagnosis and monitoring in four years.

- Contact with parties might be interested: Memorial Sloan Kettering Cancer Center, a manufacturer of commercial confocal microscopes, and CRI(Cambridge Research & Instrumentation).

Results

- Multi-spectral confocal reflectance images and classification results for real skin sample are demonstrated in Figure 3. and 4.

- Validation of the proposed method by using phantom, images and analysis are shown in Figure 5.

Conclusions/Future Work

Conclusion

- We have developed multi-spectral reflectance confocal modality for sub-celluar functional imaging in skin.
- Built phantoms with mixed beads and took 4-D spectral confocal images for validation;
- Achieved reasonable sub-celluar imaging ability with skin samples.

Future Plans

- Validate the feasibility of the imaging modality with more *ex-vivo* skin samples.
- Collaborate with UPRM on automation for registration and classification procedures.
- Write NIH proposal (R01) and as part of NSF proposal.

References

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