

towards the use of a high-wavenumber (HW) regime (e.g., 2800-3600 cm^{-1}) since this spectral range exhibits stronger tissue Raman signals as well as less interferences from the silica background from fiber-optic Raman probes. At present, however, there are no techniques which enable identification of cancerous cells *in vivo* that have sufficient accuracy to make this type of technique a viable option. A need exists for Raman spectroscopic techniques that enable higher accuracy identification of cancerous cells *in vivo* and *ex vivo*.

SUMMARY

Embodiments in accordance with the present disclosure seek to overcome at least some of the problems associated with the prior art and current endoscopic techniques for characterizing, identifying, and/or diagnosing the type and/or nature of tissue(s) such as abnormal growths, for instance, as cancers and the like in essentially any part of the body. Embodiments in accordance with the present disclosure also seek to provide an enhanced accuracy system and method based on Raman spectroscopy for rapidly characterizing, identifying, and/or diagnosing the type or nature of abnormal tissues, such as polyps and pre-cancer, *in vivo* during endoscopic investigations (e.g., gastrointestinal endoscopic investigations such as colonoscopy).

Various embodiments in accordance with the present disclosure describe a system and method for Fiber-optic Raman spectroscopy, which provides a label-free vibrational spectroscopic technique enabling enhanced accuracy optical biopsy at the bio-molecular level *in vivo*. Multiple embodiments enable a combination of simultaneous measurements from both FP and HW spectral ranges during ongoing endoscopy. The rationale for combining the FP and HW spectral ranges for *in vivo* and *ex vivo* Raman measurements are diverse:

- (i) For tissues that could exhibit intense auto-fluorescence (e.g., gastric, lung, colon, liver) which overwhelm the tissue Raman signals in the FP range, the HW range can still contain intense tissue Raman peaks with diagnostic information.
- (ii) The FP and HW ranges contain complementary bio-molecular

information (e.g., of proteins, lipids, DNA and water) and can therefore improve tissue characterization and diagnosis.

- (iii) Different bonds vibrate in different spectral ranges, so using the two different spectral ranges (FP and HW) increases the bio-molecular information obtained in a single scan.

In accordance with an embodiment of the present disclosure, a combined FP and HW fiber-optic confocal Raman spectroscopy technique (e.g., involving the simultaneous acquisition of FP and HW spectra) can improve the real-time diagnosis of cancer, pre-cancer, and/or other abnormal growths *in vivo* during examinations of the body. The combined FP and HW technique can also be used *ex vivo* on tissue samples, to more accurately identify the types of abnormal growth present in the samples from any part of the body.

In accordance with an aspect of the present disclosure, a Raman spectroscopy apparatus includes: a first illumination source configured for directing illumination into a tissue; an additional illumination source configured for outputting additional illumination into the tissue; a hot mirror filter configured for compensating for illumination interference between the illumination output by the first illumination source and the additional illumination output by the additional illumination source; a Raman spectrograph configured for simultaneously detecting FP and HW Raman spectra from illumination scattered by the tissue; and a computerized control and analysis module comprising at least one processing unit and a memory storing program instructions executable by the at least one processing unit for analyzing discrete spectral sub-intervals of the detected Raman spectra in FP and HW wavelength ranges to identify a match with one or more reference markers in one or both wavelength ranges.

In multiple embodiments, the Raman spectrograph has a single broadband diffraction grating. The first illumination source includes a source of collimated illumination for generating an excitation energy to apply to the tissue, and the apparatus further includes a probe for transmitting the collimated illumination to the tissue and returning the detected Raman spectra from the tissue to the Raman spectrograph.

The one or more reference markers can include or be specific peaks in the detected Raman spectra. The computerized control and analysis module can

include program instructions executable by the at least one processing unit for diagnosing an abnormal growth based upon the match.

The probe can include or be a confocal fiber-optic probe. The apparatus can further include an endoscope having an elongate shaft having an instrument channel within which the probe is carried.

The computerized control and analysis module can include program instructions executable by the at least one processing unit for dynamically adjusting a power of the collimated illumination, and/or dynamically adjusting a time to which the tissue is exposed to the collimated illumination.

The apparatus can include a calibration apparatus configured for standardizing the probe or the entire Raman apparatus with respect to at least one calibration reference.

In accordance with an aspect of the present disclosure, a method performed by a Raman spectroscopy apparatus includes: directing illumination output by a first illumination source into a tissue; directing additional illumination into the tissue using an additional illumination source while directing the illumination output by first illumination source into the tissue; compensating for illumination interference between the illumination output by the first illumination source and the additional illumination output by the additional illumination source using a hot mirror filter; simultaneously detecting by way of a probe FP and HW Raman spectra from illumination scattered by the tissue; and analyzing discrete spectral sub-intervals in the detected Raman spectra in both FP and HW wavelength ranges to identify a match with one or more reference markers in one or both wavelength ranges.

Simultaneously detecting FP and HW Raman spectra can include diffracting illumination in both FP and HW wavelength ranges using a single broadband diffraction grating.

The method can further include dynamically adjusting the power of the illumination, and/or dynamically adjusting a time to which the tissue is exposed to the illumination.

The method can include performing a calibration or standardization procedure to standardize the probe or the entire Raman apparatus with respect to at least one calibration reference prior to illuminating the tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

Reference will now be made, by way of example, to the accompanying drawings to provide a better understanding of embodiments in accordance with the present disclosure. The drawings should not be interpreted to be limitative and dimensions may not be to scale.

Figure 1 is a schematic diagram of a confocal Raman spectroscopy system for *in vivo* tissue diagnosis and characterization, in accordance with an embodiment of the disclosure;

Figure 2 is a block diagram of a broadband fiber-optic confocal Raman spectroscopy system developed for improved or enhanced accuracy tissue diagnosis and characterization during endoscopy, in accordance with an embodiment of the disclosure;

CLAIMS

1. A Raman spectroscopy apparatus comprising:
 - a first illumination source configured for directing illumination into a tissue;
 - an additional illumination source configured for outputting additional illumination into the tissue;
 - a hot mirror filter configured for compensating for illumination interference between the illumination output by the first illumination source and the additional illumination output by the additional illumination source;
 - a Raman spectrograph configured for simultaneously detecting fingerprint (FP) and high wavenumber (HW) Raman spectra from illumination scattered by the tissue; and
 - a computerized control and analysis module comprising at least one processing unit and a memory storing program instructions executable by the at least one processing unit for analyzing discrete spectral sub-intervals of the detected Raman spectra in FP and HW wavelength ranges to identify a match with one or more reference markers in one or both wavelength ranges.
2. The apparatus of claim 1, wherein the Raman spectrograph has a single broadband diffraction grating.
3. The apparatus of claim 2, wherein the first illumination source comprises a source of collimated illumination for generating an excitation energy to apply to the tissue, and wherein the apparatus further comprises a probe for transmitting the collimated illumination to the tissue and returning the detected Raman spectra from the tissue to the spectrograph.
4. The apparatus of claim 3, wherein the one or more reference markers comprise specific peaks in the detected Raman spectra.
5. The apparatus of claim 3, wherein the computerized control and analysis module includes program instructions executable by the at least one processing unit for diagnosing an abnormal growth based upon the match.
6. The apparatus of claim 3, wherein the probe comprises a confocal fiber-optic probe.

7. The apparatus of claim 6, further comprising an endoscope having an elongate shaft having an instrument channel within which the probe is carried.
8. The apparatus of claim 3, wherein the computerized control and analysis module includes program instructions executable by the at least one processing unit for dynamically adjusting a power of the collimated illumination.
9. The apparatus of claim 3, wherein the computerized control and analysis module includes program instructions executable by the at least one processing unit for dynamically adjusting a time to which the tissue is exposed to the collimated illumination.
10. The apparatus of claim 3, further comprising a calibration apparatus configured for standardizing the probe or the entire Raman apparatus with respect to at least one calibration reference.
11. A Raman spectroscopy method performed by a Raman spectroscopy apparatus, the method comprising:
 - directing illumination output by a first illumination source into a tissue;

directing additional illumination into the tissue using an additional illumination source while directing the illumination output by first illumination source into the tissue;

compensating for illumination interference between the illumination output by the first illumination source and the additional illumination output by the additional illumination source using a hot mirror filter;

simultaneously detecting by way of a probe fingerprint (FP) and high wavenumber (HW) Raman spectra from illumination scattered by the tissue; and

analyzing discrete spectral sub-intervals in the detected Raman spectra in both FP and HW wavelength ranges to identify a match with one or more reference markers in one or both wavelength ranges.

12. The method of claim 11, wherein simultaneously detecting FP and HW Raman spectra comprises diffracting illumination in both FP and HW wavelength ranges using a single broadband diffraction grating.

13. The method of claim 11, wherein the one or more reference markers are specific peaks in the detected Raman spectra.

14. The method of claim 11, further comprising dynamically adjusting the power of the illumination.

15. The method of claim 11, further comprising dynamically adjusting a time to which the tissue is exposed to the illumination.

16. The method of claim 11, further comprising performing a calibration or standardization procedure to standardize the probe or the entire Raman apparatus with respect to at least one calibration reference prior to illuminating the tissue.