AIP | Review of Scientific Instruments

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Citation: Review of Scientific Instruments **85**, 103108 (2014); doi: 10.1063/1.4897247
View online: http://dx.doi.org/10.1063/1.4897247
View Table of Contents: http://scitation.aip.org/content/aip/journal/rsi/85/10?ver=pdfcov
Published by the AIP Publishing

REVIEW OF SCIENTIFIC INSTRUMENTS 85, 103108 (2014)

Grazing incidence angle based sensing approach integrated with fiber-optic Fourier transform infrared (FO-FTIR) spectroscopy for remote and label-free detection of medical device contaminations

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(Received 11 July 2014; accepted 23 September 2014; published online 9 October 2014)

Contamination of medical devices has become a critical and prevalent public health safety concern since medical devices are being increasingly used in clinical practices for diagnostics, therapeutics and medical implants. The development of effective sensing methods for real-time detection of pathogenic contamination is needed to prevent and reduce the spread of infections to patients and the healthcare community. In this study, a hollow-core fiber-optic Fourier transform infrared spectroscopy methodology employing a grazing incidence angle based sensing approach (FO-FTIR-GIA) was developed for detection of various biochemical contaminants on medical device surfaces. We demonstrated the sensitivity of FO-FTIR-GIA sensing approach for non-contact and label-free detection of contaminants such as lipopolysaccharide from various surface materials relevant to medical device. The proposed sensing system can detect at a minimum loading concentration of approximately 0.7 μg/cm^2. The FO-FTIR-GIA has the potential for the detection of unwanted pathogen in real time. [http://dx.doi.org/10.1063/1.4897247]

I. INTRODUCTION

Healthcare associated infections (HAIs) in clinics and hospitals are a major concern for public safety and impose significant medical, social, and economic consequences. Approximately, 1 in every 20 inpatients has an infection associated with hospital care.[1] In 2002, 1.7×10^6 HAI occurred in U.S. hospitals and approximately 99 000 deaths were associated with it.[2] Recently published CDC report showed that 2×10^6 people in the United States become infected and at least 23 000 people die due to antibiotic resistant bacteria.[3] Department of Health and Human service (DHHS) and associated organization including the U.S. Food and Drug Administration (FDA) have already setup their action plan to identify the reduction of HAI caused by any infectious agent, including bacteria, fungi, viruses, etc.[1,4]

Although there are many factors related to HAI, medical devices in clinical setting are one of the major risk factor as the devices are being extensively used in clinical practices for diagnostics, therapeutics, and indwelling devices such as medical implants. There are several techniques available in healthcare facilities to validate cleaning process for preventing the spread of infection to patients and healthcare community caused by medical device contamination. These techniques are based on *ex situ* approaches such as swap/wipe sampling, which are complex, time consuming, and not adequate to monitor and detect pathogen contamination in real time.

In order to reduce HAI for protecting public health, alternative methods for quantitative, accurate, easy-to-use and real-time detection, and identification of microorganism contaminations on medical devices surface in clinical setting are needed. We have recently presented a novel proof-of-concept platform for label-free, remote, and rapid detection of medical device surface contamination employing a fiber-optic Fourier Transform Infrared (FO-FTIR) spectroscopy methodology.[5] FTIR has a potential for providing qualitative and quantitative spectral signature information about the targeted samples.[6–9] Furthermore, the developed reflection based FO-FTIR method ensures some unique benefits such as intrinsic biochemical specificity, non-destructive, non-contact, and sensitive contamination detection with potential for miniaturization for *in situ* on site applications. We demonstrated the feasibility and sensitivity of the FO-FTIR technology for detecting and analyzing some reference low-concentration protein (such as $\leq$ 0.0025% or $\leq 4 \times 10^{11}$ molecules of BSA) and bacterial endotoxins (such as 0.5% or 0.5 EU/ml endotoxin).[5] However, since the FO-FTIR design uses a reflection sensor mode with a relatively small angle of incidence of about 20°, it is more effective for testing samples with highly reflected surfaces. In practice, medical device surfaces are made of different types of materials from metals to dielectric (such as vinyl, glass, etc.) with various surface finish quality from smooth to rough, which provides lower surface reflection modes. Therefore, to enhance the FO-FTIR sensitivity for measurement of thin layer of samples on non-reflective or semi-reflective surface, a significantly increased sensor path-length through the tested sample is required, which can be achieved using a grazing incidence angle (GIA) sensing approach integrated to the FO-FTIR methodology.

Reflection spectroscopic measurement at GIA is a broadly employed sensing method for various applications.[10–14] Currently, some commercially available

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GIA spectroscopic probes have been used in food and drug manufacturing plants for cleaning validation.[11–15] However, a major challenge of this technique is its applicability to any target area on medical device surfaces due to large and flat designs employed. As a continuation of the study, we have developed a novel simple sensing approach (FO-FTIR-GIA) using a flexible IR hollow-core fiber probe operating in a GIA mode that is integrated in a FO-FTIR spectroscopy platform. FO-FTIR-GIA sensing method can provide a non-contact, label-free tool for detection and identification of contaminants on various remote target areas including tough-to-reach areas of different medical device surfaces such as metal and dielectric materials. In this study, we investigated the sensitivity of the proposed FO-FTIR-GIA sensing method utilizing different types of target surfaces (substrates) relevant to medical device surfaces with biological contaminant such as lipopolysaccharide as an example. The results suggest that the FO-FTIR-GIA method provides reasonable sensitivity for *in situ* identification of pathogenic contaminant on medical device surface.

II. MATERIAL AND METHODS

A. Reagents and chemicals

Lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* was commercially supplied by Sigma-Aldrich (St. Louis, MO) in powder form and endotoxin-free water was purchased from Fisher Scientific (Fair Lawn, NJ).

B. Sample preparation

All chemicals were used without further purification. A thin plastic film (0.1 mm thick) was used as a homogeneous sample to validate the sensitivity of the experimental setup with different types of sample substrates. LPS was used as a contaminant sample relevant to microorganism commination on medical device surface. 100% stock solution of LPS was prepared by adding 1mg of LPS to 1 ml of endotoxin free water at room temperature. The solution was stirred for 10 min or until the LPS dissolved completely. Using the stock solution, different concentration samples (such as 50%, 25%, 10%, 5%, etc.) were prepared and stored at 4°C until the measurements were completed, for a maximum of 1 day.

C. Substrate

Different types of substrates were selected relevant to medical device surfaces including metals (rough and smooth stainless steel, aluminum, etc.) and dielectric (vinyl and glass). The highly reflected surface (99.9%) of a 25 mm diameter gold mirror (ThorLabs Inc., Newton, NJ) was used as a standard sample substrate.

D. Fiber-optic sensor system

A schematic diagram of the measurement setup is shown in Fig. 1. We have designed and developed a prototype of GIA sensing probe that is integrated with the fiber-optic FTIR spectroscopy platform for remote and *in situ* detection of microorganism. As shown in Fig. 2, the GIA probe includes two flexible hollow-core fiber arms to set incidence and detection angles, respectively. The incidence angle can vary from 70° to 85° as compared to the normal incidence angle (0°), which allows the sensitivity of IR reflectance measurements to be maximized for thin layers of biochemical contaminants on any types of metallic (such as steel, aluminum, etc.) and dielectric (such as glass, polymer, etc.) materials surfaces. In this study, we used a fixed incidence angle of 85° to the surface for all measurements of contaminations on different types of reflecting surfaces. The FO-FTIR-GIA sensor head is connected to the external ports of the FTIR spectrometer (Vertex 70, Bruker Optiks, Ettlingen, Germany) by two mid-IR hollow-core optical fibers (Hollow Waveguide with Acrylate Buffer, HWEA7501200, Polymicro Technologies, Phoenix, AZ) with diameters of 750 μm and a numerical aperture of 0.05. One of the sensor fibers is employed for light delivery from a light source (Halogen) to the sample, and the other fiber for transmitting the signal light to the detector (liquid Nitrogen cooled MCT) after absorption by the sample. By using an adjustable stage, the sensor head was placed above the sample at a distance where the signal intensity is maximal. Each spectrum was averaged over 256 scans in the range of 850–5000 cm^{-1} at a 4 cm^{-1} resolution. Preceding sample measurement, the background signal was collected from the corresponding surface (substrate) without the sample.

Prior to FTIR experiments, the substrate of different types were cleaned with 70% isopropyl alcohol wipes and dried with scientific grade wipes. Surface finish of different types was characterized using a digital microscope (VH-Z500, Keyence Corp., Itasca, MA) with 2000× magnification. As representative model of a homogenous sample, a plastic thin film is placed on different types of substrates and measured. LPS solution of different concentrations were placed on each plate of different material composition in 2 μl drops of equal size (∼4 mm diameter) and allowed to dry for ∼30 min under a covered area to decrease the dust landing on

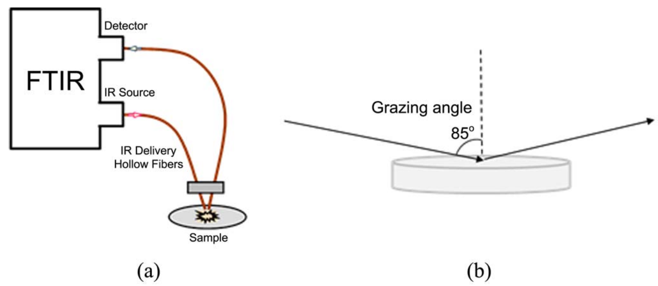

FIG. 1. (a) Schematic diagram of the measurement system and (b) grazing incidence angle.

FIG. 2. Proposed grazing incidence angle (GIA) sensing head.

the plates. Before each measurement, background spectrum of the clean surface was collected prior to sample deposition. Three trials were recorded and averaged at different positions on the sample.

III. RESULTS AND DISCUSSION

In our previous study,[5] we demonstrated the feasibility to use a fiber-optic FTIR (FO-FTIR) reflection sensing head for real time detection and analysis of surface contaminations. The method has a potential for detecting surface contamination in order to control transmission of HAI in health care facility. However, the sensor head was limited to use for highly reflected surface with an incidence angle of 22°.

In this study, FO-FTIR-GIA sensing head using a flexible IR hollow-core fiber probe operating in a GIA mode with an incidence angle from 70° to 85° was developed for FTIR spectroscopic measurement to improve the detection sensitivity of various types of sample material surfaces (high-, semi- or non-reflective) relevant to medical device surface contamination detection. The angle of incidence of the FO-FTIR-GIA sensing head was optimized from any kind of distortion of the spectrum by comparing the spectrum of a plastic film (0.1 mm homogeneous thickness) on a mirror surface to the spectra obtained from reflection sensing head. The spectrum distortion factors could include peak shift, band shape changes, band splitting, etc., caused by various refractive index of surface materials, wavelength, incidence angle, etc.[16] After fixing the incidence angle of FO-FTIR-GIA sensing head at 85°, the comparative absorption spectra of the reference plastic film on a mirror surface are obtained using the FO-FTIR-GIA and FO-FTIR reflection sensing head. Figure 3 illustrates typical absorption spectra which are identical and reproducible. Moreover, we did not observe any incidence angle dependent distortion in these spectra.

The effectiveness and sensitivity of the FO-FTIR-GIA sensing head at the 85° incidence angle was further investigated by measuring the absorption spectra of the plastic film on different types of metallic (steel and aluminum) and dielectric (clear vinyl and glass) surfaces as well as a gold mirror surface. As shown in Fig. 4, the measured spectra of the plastic film on different types of substrates are intense and the peaks are readily identifiable of the material. The absorption peaks from plastic film on metal surfaces were found to be highly reproducible as compare to mirror surfaces. In addition, we did not observe any significant changes due to surface roughness for stainless steel substrates.

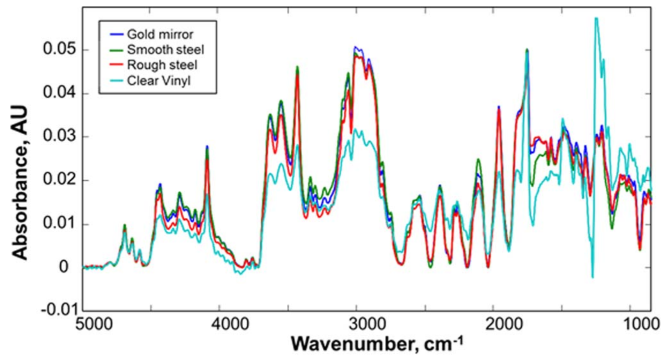

FIG. 4. Absorbance spectra of plastic film on different types of metallic and dielectric surfaces.

Furthermore, in case of dielectric material surfaces, although these surfaces are generally not reflective enough to allow beam to successfully reflect off the surface, the proposed novel FO-FTIR-GIA sensing approach provides adequate sensitivity which enable the IR light to pass through the contaminant on a non-reflective surface and to be detected. In Fig. 4, the absorbance spectra of plastic film on vinyl surface is lower compared to the metallic surface, but the quality of spectra is good enough for the identification of peaks. However, in the spectral range lower than 1500 cm^{-1} wavenumber, the spectrum of plastic film on vinyl surface is distorted and high-intensity fluctuation effects are observed in comparison with metallic or mirror surfaces. Similar but more intense effects are also observed for clear glass substrate in the same region as shown in Fig. 5. These effects on glass surfaces have been observed and reported in the literature.[11] However, in the region above 1500 cm^{-1} in the spectra for the plastic film on glass substrate are identical to the metallic surface as shown in Fig. 5 (inset). At wavenumber greater than 1500 cm^{-1}, glass or vinyl materials are transparent to mid-IR radiation, but there is a strong absorption band at longer wavelengths

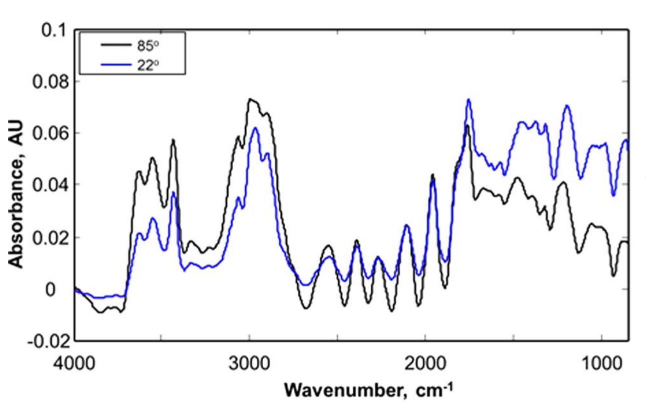

FIG. 3. Comparative absorbance spectra of plastic film (0.1 mm thick) on mirror surface obtained by GIA sensing head at incidence angle 85° and reflection sensing head at incidence angle 22° to the surface normal.

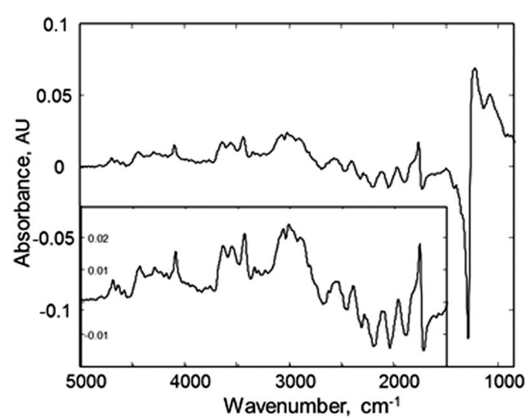

FIG. 5. Absorbance spectra of plastic film on glass surface. (Inset) Enlargement of the region containing the peak used for comparison.

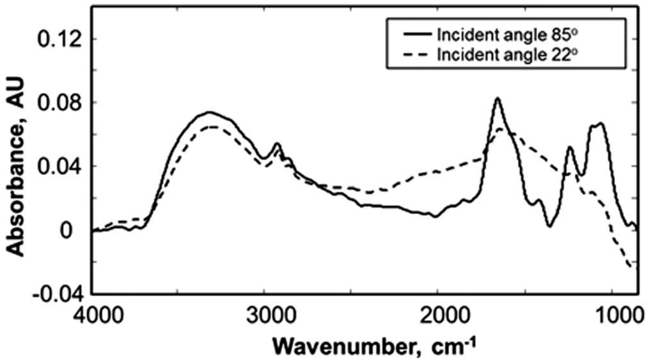

FIG. 6. Absorbance spectra obtained from lipopolysaccharide (LPS) on aluminum surface using incidence angle 22° (reflection sensing head) and 85° (GIA sensing head).

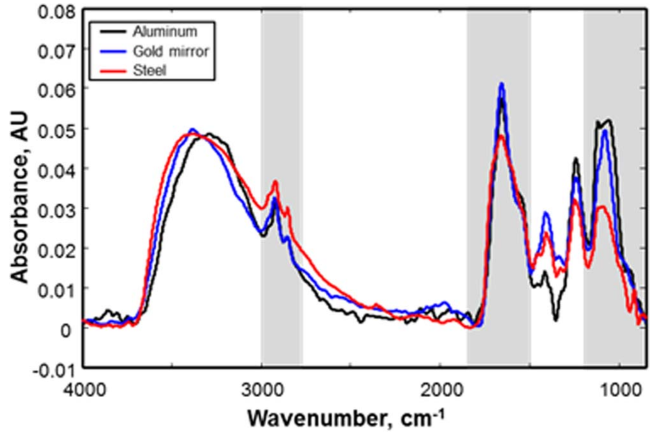

FIG. 8. Absorbance spectrum of lipopolysaccharide (LPS) on various types of surface.

that is associated with refractive index of the materials of the surface.

Medical device contaminations in clinical environment are mostly pathogen growing on the surface or harmful residue left on the device surface. As a representative sample relevant to medical device microorganism contaminations, standard lipopolysaccharide (LPS) was used in this study.[17,18] LPS is the major component of the outer membrane of Gram-negative bacteria and consists of lipid and polysaccharide. LPS acts as endotoxins and an excessive amount of LPS (1 μg/kg) in blood may induce shock in human.[19] We tested the feasibility and sensitivity of the proposed FO-FTIR-GIA sensing head as compared to the reflection head (an incident angle of 22°) for detecting LPS on different type of surfaces. The comparative spectra of LPS on aluminum surface are shown in Fig. 6. As compared to the proposed FO-FTIR-GIA sensing head, the reflection sensing head is not sensitive enough to record spectra of LPS from aluminum surface (semi-reflective), but there is no significant difference between the spectra of LPS for a higher reflecting surface such as smooth stainless steel or mirror surface. Typical GIA absorption spectra of LPS in dry condition are shown in Fig. 7. The LPS spectra can be sub-divided in accordance to the constituents of biological cells, for example, fatty acid region or lipid (3000–2800 cm^{-1}), amide region (1800–1500 cm^{-1}), polysaccharide region (1200–900 cm^{-1}), etc.[8] Each region of the recorded spectra was found to be intense and identifiable thus enabling identification of the LPS. The LPS spectral signatures observed in this study are similar to those published earlier.[20] Repeated measurements of LPS on smooth stainless steel demonstrate a high reproducibility as shown in Fig. 7(a)., whereas in the case of aluminum surface excellent reproducibility in the LPS spectra (position and peaks) is accompanied slight variations in the absorbance magnitudes (Fig. 7(b)). This may be attributed to the higher degree of LPS homogeneity over the smooth stainless steel surface relative to rough aluminum surface. Similar characteristics in the reproducibility were also observed from other surface types such as rough stainless steel, vinyl, glass, etc. The acquisitions of LPS spectra from dielectric surfaces (vinyl, glass, etc.) are limited to observations above 1500 cm^{-1} wavenumber due to high absorption of mid-IR light as mentioned in previous paragraph. The LPS signature spectra obtained from various metals and mirror surfaces are shown in Fig. 8. The spectra of LPS from various substrates

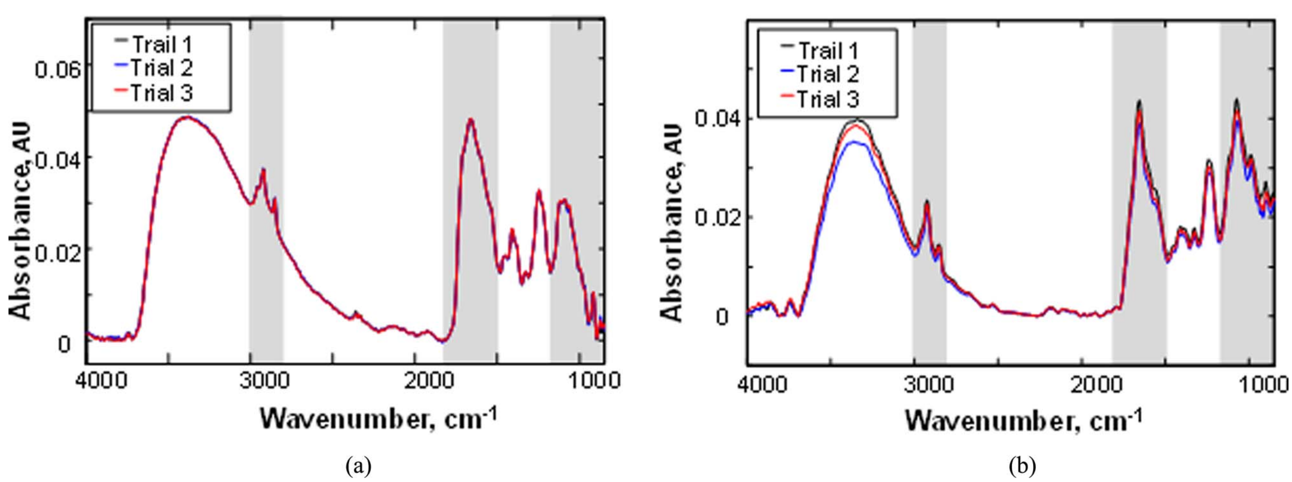

FIG. 7. Typical absorbance spectrum of lipopolysaccrade (LPS) using three consecutive trials (a) on smooth stainless steel surface and (b) on aluminum surface. The region can be defined according to the components of the cell: lipid (3000–2800 cm^{-1}), amide region (1800–1500 cm^{-1}), and polysaccharide region (1200–900 cm^{-1}).

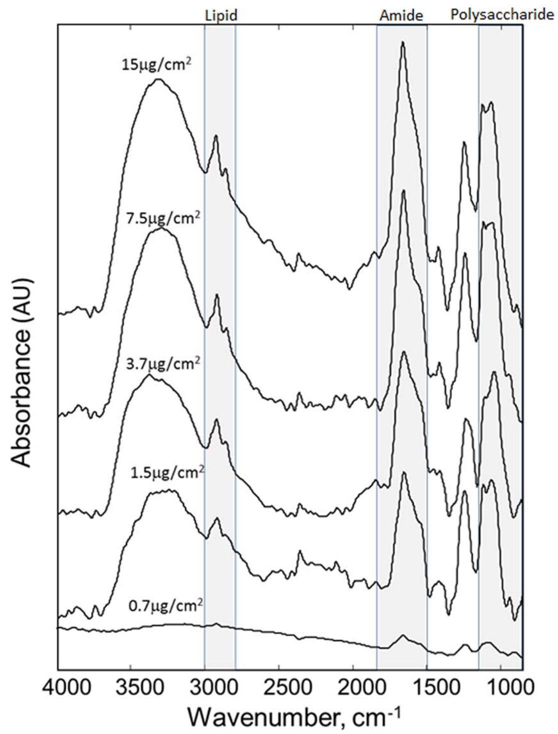

FIG. 9. Typical absorbance spectra of lipopolysaccharide (LPS) of different concentrations on aluminum with surface loading 15 μg/cm^2–0.7 μg/cm^2.

are identical to each other. We also investigated the sensitivity of the FO-FTIR-GIA sensing head for LPS of different concentrations (from 1 μg/ml to 0.025 μg/ml) after depositing on each plate in 2 μl drops, which provide average surface concentrations of LPS ranged from 0.7 $\mu g\,cm^{-2}$ to 15 $\mu g\,cm^{-2}$. Measured absorption spectra of LPS at different loading concentrations on aluminum surface are shown in Fig. 9. In this case, we did not observe a linear dependence in the absorbance intensity due to the inhomogeneous LPS distributions after depositing on the surface in the dry condition. We identified a minimum detection limit of $\sim$0.7 $\mu g\,cm^2$ using the GIA sensing probe for detecting LPS surface residues on aluminum when the specific spectral signals are above the system noise level. As compared to other commercially available devices, the proposed FO-FTIR-GIA sensing probe provides a detecting sample area much smaller due to the single detecting hollow-core fiber design with a numerical aperture of 0.05 used for the system (spot size approximately 0.5 mm). The minimal detection threshold is also in agreement with other surface materials such as metals and dielectric materials used in this study. In addition, depending on the specific quantitative applications or area of interest, the minimum threshold level could be improved by adjusting the spot size of fiber-optic sensor system.

IV. CONCLUSIONS

Employing the mid-infrared FTIR sensing methodology, we have developed FO-FTIR-GIA sensing approach for non-contact, label-free identification of pathogen from various types of surface materials relevant to medical device surfaces in real time. Due to the fiber-optic advanced features, the FO-FTIR-GIA sensing head is flexible to fit at any target area on medical device surface. However, further work is required to determine the limits for *in situ* detection and identification of pathogen contamination from medical device in clinical environment. The proposed sensing system has the potential for real time identification of pathogen in conjunction with mathematical algorithm and possible to control transmission of infection in healthcare industry as well as to address infectious disease threats for the nation determined by public health needs and Emergency Medical Countermeasures Enterprise.[21] In addition, the proposed technique could be useful for regulatory agencies such as U.S. FDA as an alternative test method to implement regulatory guidelines.

ACKNOWLEDGMENTS

This study is supported by the intramural research program of Medical Counter Measure initiative (MCMi) of Center for Devices and Radiological Health (CDRH), U.S. Food and Drug Administration (FDA). We like to thank Dr. Darrell Tata for his useful discussions.

The authors have no conflicts of interest or financial ties to disclose.

The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the U.S. Food and Drug Administration (FDA), Department of Health and Human Services.

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