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Perspective

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Is Infrared Spectroscopy Ready for the Clinic?

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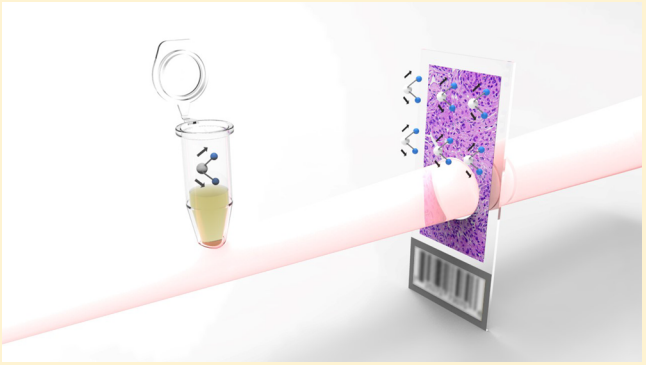
ABSTRACT: Fourier transform-infrared spectroscopy (FT-IR) represents an attractive molecular diagnostic modality for translation to the clinic, where comprehensive chemical profiling of biological samples may revolutionize a myriad of pathways in clinical settings. Principally, FT-IR provides a rapid, cost-effective platform to obtain a molecular fingerprint of clinical samples based on vibrational transitions of chemical bonds upon interaction with infrared light. To date, considerable research activities have demonstrated competitive to superior performance of FT-IR strategies in comparison to conventional techniques, with particular promise for earlier, accessible disease diagnostics, thereby improving patient outcomes. However, amidst the changing healthcare landscape in times of aging populations and increased prevalence of cancer and chronic disease, routine adoption of FT-IR within clinical laboratories has remained elusive. Hence, this perspective shall outline the significant clinical potential of FT-IR diagnostics and subsequently address current barriers to translation from the perspective of all stakeholders, in the context of biofluid, histopathology, cytology, microbiology, and biomarker discovery frameworks. Thereafter, future perspectives of FT-IR for healthcare will be discussed, with consideration of recent technological advances that may facilitate future clinical translation.



The potential of infrared (IR) spectroscopy as a powerful clinical tool is abundantly clear as demonstrated from the myriad proof-of-principle studies boasting high specificity and sensitivity for disease detection and classification.[1−9] There is an ever present search to develop novel, low-cost, and rapid diagnostics platforms to prevent bottlenecks in healthcare workflows and subsequently time delays in patient care, the ramifications for which are poorer patient outcomes and huge economic burdens upon healthcare institutions.[10−12] Additionally, aging populations and increased burden of chronic disease demand greater diagnostic throughput capabilities to diagnose and stratify patients given the number of people aged over 65 is predicted to swell by 71% by year 2050 in developed nations.[13] The future implementation of IR spectroscopic techniques extends to, and may redefine, numerous stages of clinical management from screening all the way through to treatment monitoring.[14] It is versatile in the clinical applications due to the ability to discriminate individual IR active molecular species making both unianalyte and multianalyte observation possible. Samples are interrogated using an infrared beam by stimulating intermolecular bond vibrations which, depending on the particular molecular bonding environment, will selectively absorb specific infrared wavelengths in accordance with Beer−Lambert's law.[15] From this, a multitude of clinically relevant biomolecules can be quickly and accurately identified and quantified for disease diagnosis.[16,17] Furthermore, sophisticated multivariate analysis techniques can be employed to discriminate between patient disease states and provide comprehensive biomolecular "fingerprint" profiles from a variety of patient sample types.[1,7,18−20] All of this has been demonstrated on a wide range of biological sample types, such as biofluids, cells, and tissues.[1,15,21−24]

Infrared spectroscopy is generally posited as being simple to operate, requiring no reagents, inexpensive, noninvasive, and nondestructive[14] and as such is already utilized in an expansive range of applications such as in the pharmaceutical, food, environmental, and forensics industries. It should be obvious, then, that there is a place for IR spectroscopy in the clinical environment which raises the question as to why clinical translation has been so elusive? The aims of this Perspective is to assess the suitability and highlight future directions of infrared spectroscopy within specific clinical situations from the perspective of spectroscopists, clinicians, and financial contributors.

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DEVELOPING THE TRANSLATION

Demonstrating potential as a powerful clinical tool does not necessarily demonstrate clinical suitability. It should be understood that successful translation of a technology into healthcare pathways requires more than simply being able to inform clinical decisions. Clinical translation is, in large part, an entrepreneurial endeavor and not only a humanitarian one. It can therefore appear a daunting and ambitious undertaking requiring proficiency outwith the typical spectroscopists field of expertise, especially when considering the large amount of time such a project can consume.[25] Development of a medical device is a project in communication not only within the research team but with contacts from a broad range of disciplines including intellectual property (IP) law, health economics, manufacturing, assembly, distribution, sales and marketing, and healthcare professionals.

Translation of IR spectroscopy is by and large progressed by "technical push" rather than "clinical pull", whereby existing spectroscopic technologies are being adapted in order to enter the clinical market.[14] This means the technology in its current form may not be suitable for the clinic for a variety of reasons, such as by not being user-friendly, disrupting clinical workflows, cost, lab footprint, and incompatibility with automation. These are absolutely valid reasons to be hesitant about adopting new healthcare technologies as the ramifications for disrupting clinical workflows, for example, should not be underestimated. Continued end user (e.g., clinicians, laboratory technicians, etc.) input and support is incredibly valuable in this regard as in-depth knowledge of the clinical environment is the best way to resolve issues during development stages and achieve the most market ready product as possible before embarking on clinical trials. However, spectrometer instrument manufacturers will not substantially modify their products for a clinical environment because it is not a market they are established in and therefore view expansion into this market as a significant risk.[26] Confirming the existence of a consumer base through thorough examination of an unmet clinical need, proof-of-concept studies and financial projections via health economics analysis is a good way to reassure manufacturers that tailoring their instruments toward clinical applications will help the growth of their own company. An example of this can be seen with Glyconics, a Cambridge based IR diagnostics company, working with Spectrolytics, a German IR spectrometer development and supply company, who together are producing a hand-held point-of-care (POC) FT-IR device specifically for use in a clinical setting. Through this partnership, Glyconics are commencing a major clinical trial in Q1 of 2019 to validate claims that the device can be used to differentiate chronic obstructive pulmonary disease (COPD) from other disease states and also monitor the conditions progression.[27,28] This is a significant step on the road toward clinical translation and would not have been possible without a mutually beneficial relationship between medical diagnostic IR spectroscopy experts and instrument developers. Clinspec Diagnostics Ltd., a spin−out from the University of Strathclyde, present another example of the impending clinical translation of FT-IR technology. They recognized through proof-of-concept studies that attenuated total reflection-Fourier transform-infrared (ATR-FT-IR) could be used to identify and classify brain cancers from blood serum samples.[1] The company then undertook a health economics study where they collaborated with clinical experts and health economists to inform economic models investigating the cost and health benefits an ATR-FT-IR serum based triage tool could have when implemented into brain tumor healthcare pathways. The study predicted that the test had a high probability of being cost-effective and improving the Quality Adjusted Life Years (QALYs) of brain tumor patients within the NHS.[29] This helped ClinSpec Diagnostics Ltd. secure significant funding for further development. This is an emblematic example of why protection of IP and a health economics assessment should be carried out as soon as possible when identifying that a nonclinical technique has potential value for a specific clinical application. In simple terms, a health economics study will help elucidate the answers to two vitally important questions: (1) Will the proposed technique improve patient outcomes? (2) Will it alleviate financial strains imposed on healthcare systems while generating revenue? Confidence in satisfying both conditions is invaluable toward gaining clinical support and informing investment decision. There are other stand out examples of translational research of IR spectroscopic techniques. Biotech Resources, whose founding members have received international acclaim for their work using ATR-FT-IR to screen malaria in low-income areas,[8] were granted large amounts of investment from the Australian government toward development of a rapid FT-IR based diagnostic platform for the detection of blood borne pathogens associated with sepsis. Cireca Theranostics have developed "spectral histopathology" protocols for reagent free objective, machine learning based analyses of tissue sections for use in situations where histopathologists cannot reach an agreement of how to classify a sample.[30,31] These promising enterprises along with a strong mutual desire from both researchers and clinicians to inform healthcare decision making with state-of-the-art analytics indicates imminent arrival of IR spectroscopy to the clinical environment. A versatile array of techniques makes IR spectroscopy relevant to a wide variety of clinical applications and thereby an attractive opportunity to investors provided the appropriate evidence is clearly demonstrated to all concerned parties.

IR SPECTROSCOPY OF BIOFLUIDS

Biofluids are irrefutably a high value diagnostic resource due to their ease of collection with minimal invasiveness and the abundance of biochemical information contained within and are perhaps the most effective means to carry out objective, cost-effective, and rapid diagnosis and triaging of patients.[32] However, the procedures from sample collection to results delivery are hugely diverse depending on the target molecule or molecular signature. Multiple physical operations and chemical reactions may have to be carried out before the target molecule can be quantified. Compound this with the amount of parallel processes required to test for several biomolecules within each sample across several patients and it is clear how labor intensive and susceptible to errors clinical chemistry can become. Modern clinical laboratories are configured to handle this problem, though, through clever automation devices and systems,[33] but infrared spectroscopy offers a different perspective on how to combat this issue. Since infrared spectroscopy captures a biochemical signature of all the bonding environments present within the sample, it follows that the resultant data set is influenced by all the samples' constituent molecules which are therefore detectable and quantifiable. Many infrared spectroscopy proof of concept

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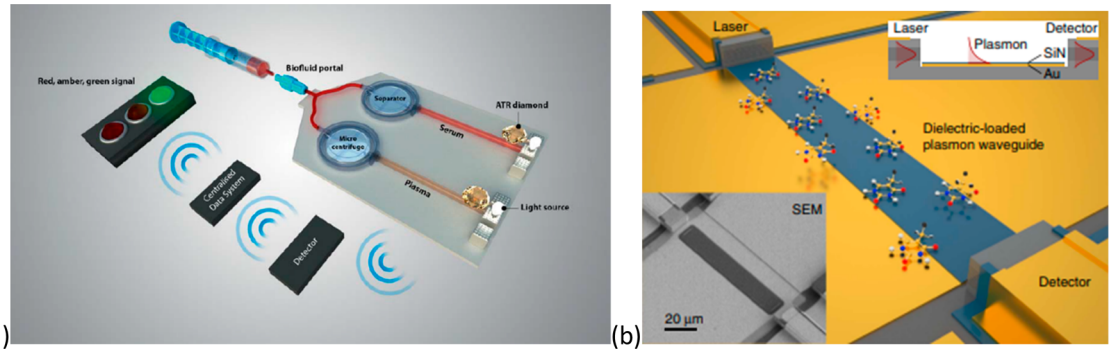

Figure 1. (a) Concept drawing shows blood separated into serum and plasma derivatives for simultaneous ATR spectral analysis, the results from which are wirelessly communicated to centralized data systems for classification. (b) Miniaturized QCL/D with an SPP waveguide provides an ideal sensing stage for mid-IR microfluidic devices.

studies have already established that a wide variety of clinically significant findings can be retrieved from a range of biofluids such as serum,[34] plasma,[35] whole blood,[36] sputum,[37] bile,[2] amniotic fluid,[38] cerebrospinal fluid,[39] urine,[40] saliva,[41] and even tears.[5] The utility of IR spectroscopy in the clinic extends beyond a univariate approach with the implementation of multianalyte pattern recognition algorithms for disease detection and the potential to discover novel biomarkers.[19,42,43] Indeed, spectroscopic biofluid analysis could be used to inform clinical decisions by providing a comprehensive biochemical panel as well as indicating the likelihood of the presence of specific diseases, from cancers to chronic or infectious disease.[3,9,18,44−48] Sepsis diagnosis is one such condition where not only do the causal pathogens need to be identified, but biomarker concentrations, such as lactate and c-reactive protein, are important in stratifying patients and determining clinical actions.[49] Blood cultures are the current gold standard for determining the presence of infection in the blood but take up to several days to return results while the host's condition deteriorates.[50] Researchers at Monash University, Australia, are currently addressing this unmet clinical need by developing methods of detecting blood borne pathogens using ATR-FT-IR on aqueous blood films with the aim of delivering results at the point-of-care.[51,52]

Infrared spectroscopy faces a variety of obstacles to overcome when analyzing biological materials and even more technologies and protocols have been developed to overcome them.[15] The specific biofluid being analyzed will influence the preanalytical treatment protocols, the FT-IR mode selected (transmission, transflection, or ATR), the light source used (globar, QCL, or synchrotron), and the detector used (focal plane array, linear array, or single element). Each of these choices maintain strengths and weaknesses, so careful consideration is required for specific clinical applications. For more information on FT-IR sampling considerations, please refer to an in depth protocol by Baker et al.[15] Biofluids in particular have a high water content that absorbs IR light strongly in the mid-IR region and may contain cellular components that give rise to dispersion effects.[53] Water absorption obscures pertinent information on other biomarkers present in the sample, and so there are almost always efforts made to eradicate the water contribution of biofluids. This is usually done by drying samples directly upon the sampling site, but homogeneous sample distribution is difficult due to the "coffee-ring" effect that concentrates large molecular weight molecules in a concentric ring around the droplet deposition site.[54,55] Nevertheless, plenty of proof-of concept studies show excellent results using this method,[1,9,34,56] although drying times and methods have not been standardized. Hands et al. have shown that ATR-FT-IR can analyze dried blood serum films to discriminate between patients with brain cancer and noncancer controls with sensitivity and specificity of 91.5% and 83%, respectively.[57] An earlier study did, however, highlight the time limiting factor created by the need to individually dry samples upon the sampling site for analysis before another sample can be prepared.[1] This would create an obvious bottleneck in clinical workflows and is a technical issue that needs to be overcome before ATR-FT-IR is suitable for the clinical environment. More recently, an advanced sample spotting technique called piezo-jetting, which deposits discrete arrays of tiny (∼130 μm) sample droplets, has been used in conjunction with QCL on serum samples with comparable reproducibility and diagnostic power as the aforementioned study by Hands et al.[1,58] More importantly, drying and acquisition time was significantly reduced, and the use of QCL light sources may better lend itself to POC diagnostics and is more capable at detecting low concentrations of analyte compared to conventional FT-IR techniques.[59] All are significant factors that will progress toward clinical translation. Interchangeable disposable silicon IREs[60] could be used as the central mechanism around which an automated high-throughput ATR-FT-IR system could be constructed, although issues of signal loss due to significant beam attenuation through the silicon crystal would need to be addressed.[61] Mitchell et al.[32] assert that a vibrational spectroscopy based POC device presents an attractive option for an objective, low-cost biofluid screening platform. They go on to propose hypothetical designs of a hand-held FT-IR POC device (Figure 1a) that employs microfluidic separation of blood into its derivatives (plasma/serum) with remote classification for continuous bedside monitoring. Mitchell even suggests that a user-friendly "traffic-light" system could be used to further speed-up triaging decisions where green would indicate no disease, yellow cause for further screening, and red the presence of disease. This design may be perfectly complemented by lab-on-a-chip technology developed by Schwarz et al.[62] who have miniaturized QCL, waveguide, and detector into a monolithically integrated mid-IR molecular sensing platform (Figure 1b) that can be fully submerged in liquid samples. Two major technical developments permitted this design. The first is a bifunctional quantum cascade laser/detector (QCL/D) that can act as both light source and

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detector depending on the applied bias. The second is a surface plasmon polariton (SPP) waveguide comprised of a narrow dielectric stripe (silicon nitride) printed atop an unpatterned gold layer. This constrains the mid-IR plasmon waves with high-coupling efficiency of the beam from emitter to receiver while also promoting strong interaction with the analyte. An evanescent decay that can range from 2 to 50 μm in length can be achieved depending on the thickness of SiN layer. This rather elegant single-chip system is a low-cost IR spectroscopic platform that may prove ideally suited to biofluid screening applications pending further investigation.

Aside from the multitude of technical milestones that are being surpassed and investigated, there are preanalytical biofluid sample handling and treatment considerations that need to be standardized.[55] Biofluids likely to contain micrometer scale structures such as whole blood, for example, that is usually fractionated so that the derivatives, serum or plasma, may be analyzed. This is because micrometer scale structures, like erythrocytes (~8.2 μm), are of sizes that match the wavelength of infrared light (2.5−25 μm) will result in dispersion artifacts, such as Mie scattering, in the acquired spectra.[63] One minor complication in handling is in storage. Biofluids are either analyzed fresh or stored frozen (−80 °C), incurring an added complication since variance may be introduced through repeat freeze−thaw cycles, which should be avoided where possible.[64] Biofluids may also be fractionated to partition high molecular weight (HMW) molecules from low-molecular weight molecules (LMW) so that each fraction's constituent molecules are less obfuscated by spectral contributions from each other.[40] For instance, Bonnier et al.[16] were able to improve the precision and accuracy of quantitative models for estimating glucose concentrations in spiked serum samples by eliminating the HMW component of the samples prior to spectral analysis. This technique may help overcome the FT-IR limitation of being unreliable at measuring low molecule concentrations by removing competing molecular signatures. It is evident from the literature described here that there are a multitude of projects underway to tailor IR spectroscopic techniques and processes for biofluid analysis and move much needed technologies toward clinical suitability.

INFRARED SPECTROSCOPY FOR HISTOPATHOLOGY

Histopathological evaluation of clinical tissue samples is fundamental to the diagnosis, prognosis, and treatment of medical conditions, including cancers, where morphological features of neoplasms facilitate detection of malignancies and elucidation of tumor grades. Today, clinical histopathological practice remains predominantly unchanged since its inception over 100 years ago and primarily concerns microscopic examinations of formalin-fixed, paraffin-embedded (FFPE) tissue sections, where hematoxylin and eosin (H&E) stains provide necessary contrast for visual inspection of tissue architectures. Consequently, histopathology is intrinsically subjective with significant reported inter- and intraoperative variability,[65,66] especially for disease stratification of tumor subclasses.[67] Additionally, biopsies are often restricted to clinically assigned areas of interest, due to inherent time restrictions of laborious sample preparation and analytical procedures, which may inadvertently lead to mischaracterization of heterogeneous tumors.[68] Recently, concurrent use of immunohistochemistry has enabled specific identification of molecular biomarkers associated with cancer pathogenesis, thereby providing much needed molecular content on samples and improving clinical diagnosis. However, immunohistochemistry is synonymous with antibody reagents that are notoriously expensive, limit the scope of molecular analysis to available antigen−antibody affinity based interactions, and restrict applicability to a single protein biomarker for a given clinical tissue section.[69] In times when worldwide new cancer cases are projected to rise to ~26 million annually by 2030,[70] with pathologists spending vast amounts of time evaluating noncancerous samples,[71] amidst escalating economic strain on health care resources, it is pertinent to explore emerging modalities for clinical translation to assist future histopathology laboratories.

Spectral histopathology describes the coupling of conventional mid-infrared FT-IR/Raman technologies with optics where projections of infrared light at 2−14 μm wavelengths on to clinical tissue sections permit identification of spatially resolved spectroscopic transitions indicative of the chemical composition of sample constituents. Spectral histopathology represents an attractive molecular based imaging technology for translation to clinical histopathology laboratories, where complete clinical sample sections may be chemically profiled in a nondestructive manner without the requirement for stains, antibodies, and secondary labeled reagents.[72] Hence, heterogeneous samples may be classified according to unique biochemical signatures of cell types and molecules arising from nucleic acid, lipid, protein, and carbohydrate contributions,[15] thereby, providing additional diagnostic information on tissue sections. Moreover, spectral histopathology has no additional sample preparation requirements and permits analysis of dewaxed and paraffin embedded tissue[73] and thus represents an ideal candidate for integration in to current histopathological practice.

Spectral histopathology has demonstrated through proof-of-principle studies immense potential for clinical translation and integration with histopathological laboratories, with sensitive and specific diagnosis demonstrated for several neoplastic tissues, from lung[21] to breast cancers.[22] Yet, major barriers to clinical translation persist, both regarding technical spectroscopic and clinically orientated challenges, which must be surmounted if routine adoption of spectral histopathology is to be realized in health care settings.

One of the major perceived barriers to clinical translation is spectral acquisition time for clinical tissue samples. Today, clinical histopathology departments analyze hundreds of samples per day consisting of tissue sections of 1−5 μm thickness mounted to standard glass 75 mm × 25 mm microscope slides. Consequently, current tissue volumes employed for clinical diagnosis present a major obstacle for spectroscopic translation, where single point mode analysis of tissue with single element detectors is intrinsically slow and time prohibitive for clinical translation.[74] Recently, emergence of focal plane array detectors has transformed spectral imaging capabilities for the clinic, where collection of thousands of spectra concurrently enables substantial reduction in spectral acquisition times.[73] Still, spectroscopic interrogation of large tissue sections may take several hours,[75] which restricts clinical sample throughput. Alternatively, emergence of tissue microarrays whereby multiple tissue cores of submillimeter diameter positioned on single microscope slides from different clinical regions of interest may facilitate high-throughput spectral histopathology, as recently demonstrated for prostate cancer-

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ous tissues.[76] However, there remains a trade-off between acquisition time and tissue volume, where reduced representation of samples may promote mischaracterization of heterogeneous tumors.[77] Hence, this poses a critical question as to the optimum method from a time perspective for clinical translation, including whether collecting information over the entire mid-infrared wavenumber region is conducive to translation or entirely necessary for accurate diagnosis? Recently, emergence of quantum cascade lasers (QCLs) has revolutionized the potential for translation of spectral histopathology, which project discrete frequencies of infrared light rather than entire continuous mid-infrared spectra. Hence, QCLs may solely probe molecular biomarkers pertinent to clinical diagnosis as opposed to the complete chemical constituents of tissue samples,[78] which has dramatic implications for analysis time and represents a paradigm shift for clinical implementation of spectral histopathology.

Another perceived technological hurdle for clinical translation of spectral histopathology concerns whether spectral images contain sufficient physiological detail to inform difficult clinical diagnosis. Today, optical microscopy for histopathology is limited by the diffraction limit of visible light, with optimum spatial resolution capabilities of 0.2 μm that can discern small subcellular details pertinent for early cancer diagnosis.[79] Conversely, spectral histopathology is restricted by the diffraction limit of infrared light, with optimum spatial resolutions of 2.5 μm in the clinically important fingerprint region.[73] Yet, conventional FT-IR spectrometers rarely operate at such resolution to achieve adequate signal-to-noise and overcome inherently noisy Globar sources, making inspection of subcellular details difficult.[73] However, new infrared sources, such as QCLs, free electron lasers, and super continuum sources, allow excellent signal-to-noise and diffraction limited resolutions[69] and may facilitate future routine inspection of small subcellular features. Although contact modalities such as AFM-IR may overcome the diffraction limit of infrared light,[80] current techniques are time and cost prohibitive for near future clinical translation, and therefore understanding current spectral imaging capabilities in a clinical context is paramount for successful integration into health care.

A major barrier from a clinical perspective for translation of spectral histopathology is how can such technology be implemented in an economically viable manner without disrupting current histopathology practices. Currently, histopathology is performed on glass microscope slides, which are not transparent over the entire mid-infrared frequency range, limiting the acquisition of biological information from tissue sections.[81] Hence, adoption of spectral histopathology may require implementation of different analytical substrates, either barium or calcium fluoride for transmission or reflective coated slides for transflection experiments, which have their own limitations for clinical translation. Specifically, barium and calcium fluoride substrates incur costs of ~£60 per slide and do not permit automatic handling due to poor mechanical properties, while reflective coated slides suffer from spectral artifacts arising from the standing wave effect, further exacerbated by variation in sample thickness.[73] Therefore, such limitations pose critical questions as to whether it is economically and practically viable to propose shifts from glass microscope slides in histopathology and must be clearly demonstrated before clinical translation can be realized. Similarly, another critical clinical question concerns how medical staff would interpret vast amounts of spectral data? Bhargava's group recently demonstrated the potential of stainless staining where biological signatures obtained from spectroscopic interrogation of normal breast, precancer, nonmalignant, and malignant tissue microarrays were computationally transformed to represent classical staining results for familiar interpretation by clinicians.[72] In particular, Mayerich showed that computational staining from infrared spectra had competitive to improved diagnostic performance in comparison to both classical H&E dyes and specific immunohistochemical stains, including Masson's trichrome stain, high molecular weight cytokeratin, smooth muscle alpha actin, and vimentin (Figure 2). Hence, infrared spectroscopy has the capacity to obtain a vast array of biomarkers from clinical tissue sections presented in an accessible, digital format for clinical observation and is an important example of how spectroscopy must provide integrated platforms to compliment current clinical practices. Lastly, the wide scope of current research activities in spectral histopathology must converge and agree on a consensus for standardization protocols for different tissue specimens and will be crucial to achieve clinical translation of spectral histopathology.

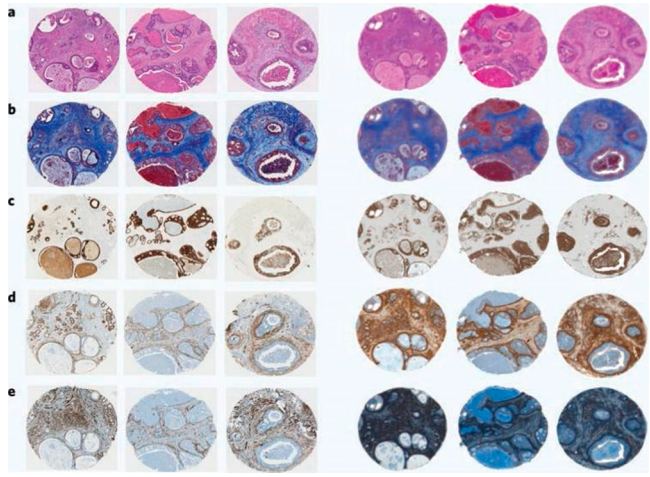

Figure 2. Tissue microarrays (TMAs) representing either normal breast tissue, precancer, nonmalignant, and malignant breast cancer samples. Comparison of three physical (left) and computational (right) stained samples from (a) H&E stains, (b) Masson's trichrome stain, (c) high-molecular weight cytokeratin, (d) smooth muscle alpha actin, and (e) vimentin. Each spot is 1.4 mm diameter.

INFRARED SPECTROSCOPY FOR CYTOPATHOLOGY

Infrared spectroscopy is a proven analytical tool for investigation of eukaryotic cells that has considerable potential for clinical translation to cytopathology to assist diagnosis of precancerous, cancerous, and infectious diseases. Cytopathology concerns the optical evaluation of stained cell morphologies through microscopic assessment of fixed or suspended cells. Cytopathology has revolutionized early detection of certain cancers including cervical cancer in the developed world, where high-volume Papanicolau (Pap) screening of exfoliated cells fixed to glass microscope slides permits inspection of cellular abnormalities indicative of cancer pathogenesis, contributing to 70% reductions in mortality in England.[82] However, the subjective nature of Pap screening produces test sensitivities as low as 55.4%,[83] which may cause

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significant proportions of cervical cancer patients to receive false negative results, and is responsible for 29.3% of clinical errors that lead to development of preventable invasive cervical cancer.[84] Consequently, Pap testing is now routinely performed in conjunction with HPV testing which has been shown to have greater sensitivity; however, the diagnostic procedure is recognized to be costly, be time-consuming, and has lower specificity.[85,86] Furthermore, only 58.6% of Pap test results were anticipated to reach recipients within a 2-week time frame in 2017−2018, despite national policies stating that all women should receive test results within this period.[87] Hence, there is a clear clinical need for low-cost, high-throughput, molecular based tests to assist cancer-screening programs in stretched cytopathology departments.

Infrared spectroscopy has demonstrated promise for detection of cervical neoplasia with observation of distinct spectral biochemical signatures pertinent to clinical diagnosis. Specifically, Cohenford found changes in the shape and intensity of amide I, amide II, and symmetric and asymmetric phosphate and glycogen vibrations were associated with dysplasia and squamous cell carcinoma diagnosis,[24] with spectral features since confirmed by other groups.[88] Similarly, Schubert showed that infrared spectroscopy may detect the presence of HPV infected cells that arise from further changes in DNA, RNA, and ν(CH) vibrational modes.[89] Additionally, Ostrowska demonstrated that spectral changes associated with expression of protein biomarker $p16^{INK4A}$ could be employed to further discriminate between negative HPV-C33A and positive HPV-16/18 copy numbers, thus providing additional information for clinical diagnosis of cervical cancers.[90]

Yet, translation of infrared spectroscopy to clinical cytopathology is largely elusive, which poses critical questions as to why such a promising technology has not so far been integrated into the clinic? First, studies mentioned above have been conducted on small sample populations, and the technology must be further validated with large clinical trials to demonstrate clinical efficacy and establish comparable sensitivities and specificities. Furthermore, some studies mentioned above have been conducted on calcium fluoride substrates, which are cost prohibitive for clinical translation especially in the context of high volume testing. Recently, Wood demonstrated successful spectral distinctions for different stages of squamous cervical epithelium on reflective coated substrates costing $1 per slide,[91] and although cost savings are made from the absence of stains, such substrates are still significantly more expensive than standard glass microscope slides. Therefore, future health economic studies must be conducted to prove cost-effectiveness of such techniques before clinical translation can be realized for cancer screening. Conversely, Neves employed ATR-FT-IR spectroscopy for discrimination between healthy cells and squamous intraepithelial lesions from blood plasma samples with clinically competitive sensitivity and specificity, negating the need for calcium fluoride or reflective coated substrates.[23] However, the study utilizes a diamond internal reflection element, which is inherently expensive and limits analysis to sequential sample measurements, with 30 min drying times for each sample not conducive to high-throughput clinical cytology. Therefore, this highlights the need for critical consideration of cytopathology environments and of the requirement for minimal disruption of new technologies to current cytopathology laboratories.

To this extent, another critical question for clinical translation of infrared spectroscopy to cytopathology is whether the technology can be implemented into clinical laboratory workflows without major disruption? Proposed approaches of integrated cytological and infrared spectroscopic analysis would require cell samples to be examined spectroscopically following inconclusive cytopathology inspections. However, hematoxylin, eosin, and Pap stains currently employed for cytological investigations were found to influence spectral signatures of cells, with an additional band at 1305 cm^{-1} and reduced intensities at 1740, 2850, and 2920 cm^{-1} attributed to exogenous stains and staining procedures, respectively.[92] While it was found that spectral signatures of staining did not compromise diagnostic capabilities for detecting lung cancer cells, the study employed a synchrotron light source with significantly improved spatial resolution over infrared sources conceivable for the clinic.[93] Therefore, large-scale studies must be conducted to assess clinical viability of spectroscopic interrogation of stained cytology samples with conventional FT-IR instrumentation. Furthermore, if there is no clinical appetite or clear demonstration of cost-effectiveness concerning employment of reflective coated slides for spectral cytology, spectroscopists must explore novel strategies to work with standard glass microscope slides. Recently, Bassan demonstrated the possibility of extracting biological information from breast tissue samples fixed to standard glass microscope slides that could similarly be implemented for cytology workflows.[81] In particular, Bassan demonstrated that standard glass microscope slides are infrared transparent at 3 700−2 000 cm^{-1} wavenumber regions, allowing the acquisition of certain vibrations including $\nu_{s/as}(CH_3)$, $\nu_{s/as}(CH_2)$, $\nu_{s/as}(NH)$, and amide A modes pertinent to the identification of biological molecules (Figure 3). Furthermore, Rutter has shown that thin glass coverslips allow spectral acquisition in the amide I and lipid regions that facilitate separation between different cancerous cell types[94] and is another important example of how spectroscopists must adapt to the demands of cytopathology departments if clinical translation to health care is to be achieved.

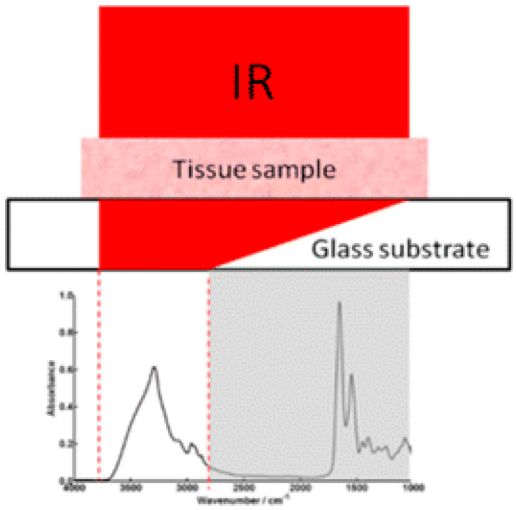

Figure 3. Schematic shows that standard laboratory glass microscope slides are infrared transparent at 3 700−2 000 cm^{-1}, which allows identification of vibrational modes pertinent to the identification of specific biological molecules. Reproduced and adapted with permission from ref 79. Copyright 2014 American Chemical Society.

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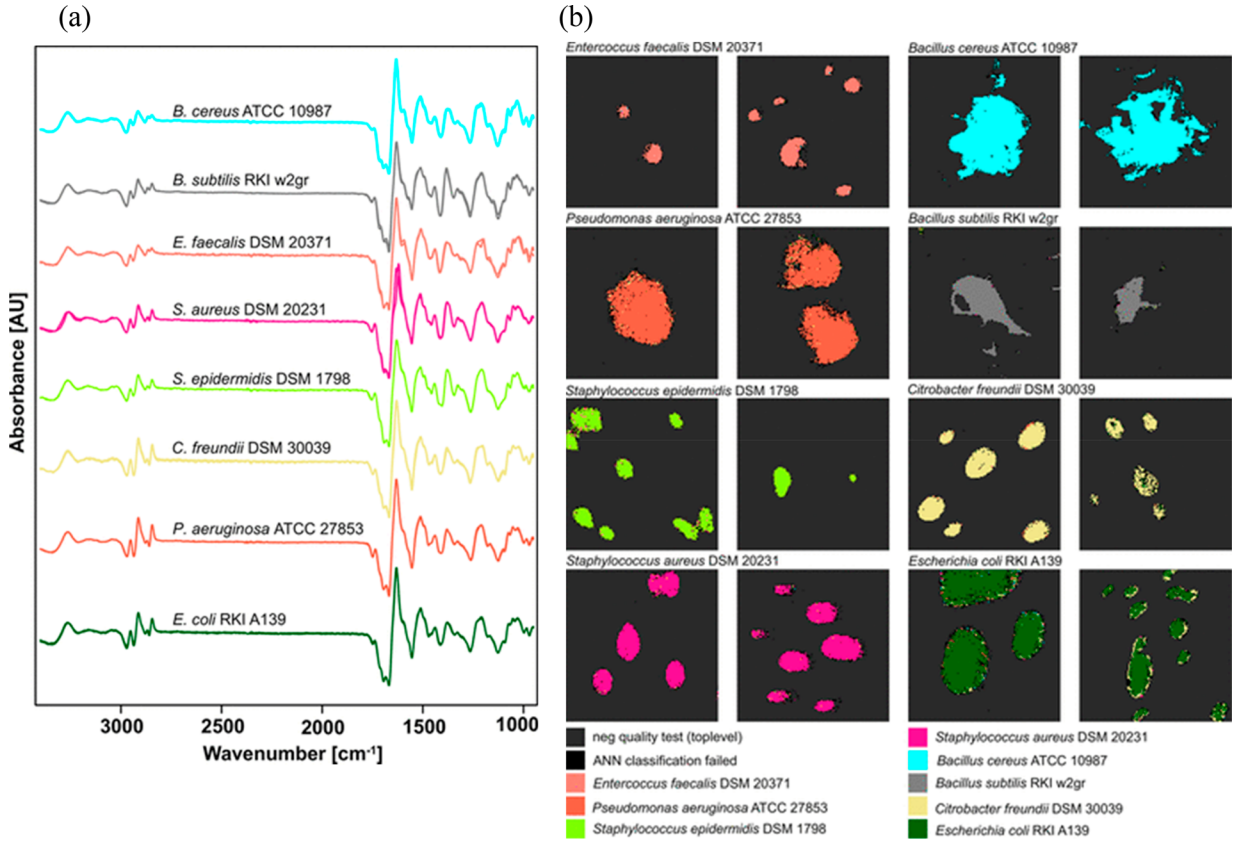

Figure 4. (a) FT-IR mean spectra of the RKI test sample comprising three genera of Gram-positive bacteria (*Bacillus*, *Enterococcus*, and *Staphylococcus*) and three genera of Gram-negative bacteria (*Citrobacter*, *Pseudomonas*, and *Escherichia*). (b) Characterization of microcolony imprints using FT-IR-microscopy for classification of RKI test samples to different taxonomies. Reproduced and adapted with permission from ref 110. Copyright 2018 American Chemical Society.

INFRARED SPECTROSCOPY FOR BACTERIA

Infrared spectroscopy represents an attractive technology for clinical translation to microbiology laboratories where chemical profiling of prokaryotic cells may facilitate classification of clinical pathogenic bacteria. Today, clinical microbiologists employ a wide range of techniques to detect pathogenic bacteria, including microscopy, serology, microbial cultures, and immunological and molecular testing. Preliminary microscopic gram staining of bacteria is subjective, influenced by previous antibiotic administration and cannot identify individual species, thus requiring additional testing.[95] Serology based tests for several infectious diseases suffer from cross-reactivity, poor specificity, and lengthy seroconversion times prevent applicability to acute clinical settings.[96] Similarly, microbial cultures depend on bacterial multiplication and purification strategies to study virulence, antibiotic susceptibility, and genomic and proteomic properties, which is time-consuming, laborious, requires specialized staff, and has limited applicability to <1% of microbes.[97,98] While emergence of immunological and molecular techniques have revolutionized clinical microbiology, both technologies are inherently expensive, with antigen−antibody methods suffering from low sensitivities[96] and nucleic acid testing time-consuming and labor intensive.[99] Hence, there is a clear clinical need for low-cost, high-throughput, and sensitive and specific modalities to detect pathogenic bacteria for early clinical diagnosis and timely therapeutic intervention, to which infrared spectroscopy may be invaluable in the near future.

Infrared spectroscopy has demonstrated significant diagnostic capabilities for microbiology, with early studies showing accurate identification of bacterial pathogens, including the clinically important Staphylococcus, Streptococcus, and Aeromonas species, based on distinctive spectral signatures within defined wavenumber regions.[100,101] Thereafter, several groups reported identification of numerous pathogenic bacteria by analyzing spectra with advanced chemometrics, including Campylobacter,[102] Escherichia,[103] Bacillus,[104] Pseudomonas,[105] and Salmonella,[106] with classification to genus, species, or strain taxonomies. Recently, Sharaha has further shown potential to evaluate the sensitivity of *Escherichia coli* strains to specific antibiotic drugs,[107] which is particularly relevant given current public health challenges concerning antibiotic resistance.[108] Additionally, infrared spectroscopy has demonstrated reduced analysis time compared to current clinical practice typically at 1−5 days for bacteria,[109] with small microcolonies of bacteria cultured and analyzed in 6−8 h.[48]

Yet, despite the significant potential of infrared spectroscopy for microbial detection, the technology has not witnessed translation to clinical laboratories, which poses the complex question: why? One major challenge preventing translation of infrared spectroscopy to the microbiology setting concerns the sensitivity of the technique to different microbial culture environments, such as culture media, temperature, and inoculation time, which alongside different instrumentation and computational methods may lead to variable analytical performance.[110] Hence, standardization protocols and continued development of infrared bacterial databases must be

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realized to validate results and enable analytical confidence across microbiology laboratories. Additionally, the vast majority of studies have employed traditional FT-IR techniques on microbial culture isolates, which does not circumvent current time-consuming and laborious isolation and purification procedures. Interestingly, Ngo-Thi showed FT-IR-microscopy could identify bacterial species from mixed culture with successful classification from diverse microcolonies of 22 Staphylococcus species, although the linear mapping approach is inherently slow for routine clinical testing.[111] Recently, Lasch showed FT-IR-microscopy could accurately classify mixed Gram-positive and Gram-negative genera to different taxonomies by evaluating hyperspectral images and artificial neural networks (ANNs), with spectral collection utilizing FPA detectors enabling significantly faster clinical analysis.[112] In particular, Lasch found distinct spectral differences between pathogenic bacterial taxonomies, with variations in $\nu(CH_2)$ and $\nu(CH_3)$ vibrations at 2800−3000 cm^{-1}, the ester $\nu(C{=}O)$ vibration at 1700−1760 cm^{-1}, $\delta(CH_2)$ and $\delta(CH_3)$ vibrations at 1370−1490 cm^{-1} and polysaccharide contributions at 900−1200 cm^{-1} pertinent to discrimination, with classification highlighted by separate colors in ANN images (Figure 4a,b). However, both FT-IR-microscopy studies described employ reflectance and/or transmission techniques, respectively, with substrates cost prohibitive for clinical translation. Therefore, health economic studies that clearly demonstrate cost effectiveness for specific microbiology applications need to be conducted before translation to microbiology laboratories can be realized. Interestingly, Kirkwood demonstrated microarray printing of microbial cultures enabling high-throughput analysis of multiple microcolonies on a conventional substrate, which reduces analysis cost per slide and possibly could enable future adoption of FT-IR-microscopy for mixed culture applications.[113] Alternatively, future endeavors utilizing traditional FT-IR techniques for mixed culture classification would represent a significant step toward clinical translation due to overall sampling simplicity, although it is recognized to be technically challenging because of the significant overlap of bacterial spectra.[114]

Another barrier for clinical translation of infrared spectroscopy to microbiology laboratories concerns current clinical sample throughput capabilities since present studies primarily utilize transmission or ATR-FT-IR modalities with consecutive transfer and analysis of bacterial cultures. However, Scholz recently demonstrated high throughput capabilities for FT-IR analysis of *Escherichia coli* species, where microliter volumes of microbial cultures were analyzed with transmission on 96-well zinc selenide microtiter plates.[115] Similarly, Kohler showed high-throughput transmission FT-IR of microcultivated yeast samples on infrared transparent 384-well microtiter plates[116] and represents a promising approach for future clinical translation given microtiter plate technology is well established for automated workflows.

Integration of infrared spectroscopy with microfluidics could also be significant for future clinical translation to microbiology cell suspension applications since coupling of both techniques allows precise control of optical path lengths and minimization of spectral contributions from strong infrared absorbing solvents. Recently, Holman demonstrated an integrated infrared spectroscopic and microfluidic platform for real-time monitoring of *Escherichia coli* within biofilm structures.[117] While the study utilized synchrotron radiation which is not conducive to clinical laboratory testing, it is conceivable that QCL sources could in the future facilitate observation of the dynamic biochemical behavior of bacteria at discrete frequencies in their native environments. Similarly, Pousti demonstrated a microfluidic device that utilized ATR-FT-IR for in situ monitoring of Pseudomonas biofilms,[118] and given the portability and miniaturization capabilities of both technologies, it is easy to envisage clinical translation of combined approaches for future detection of pathogenic bacteria in point-of-care settings.

INFRARED SPECTROSCOPY FOR NOVEL BIOMARKER DISCOVERY AND PERSONALIZED MEDICINE

Infrared spectroscopy represents a powerful investigative tool for understanding the molecular landscape and chemical pathologies of various clinical diseases. Specifically, synchrotron infrared sources coupled to FT-IR microscopy has shown immense potential for clinical research where the photon flux density is 100−1000 times greater than traditional Globar sources enabling diffraction limited spatial resolution and high signal-to-noise.[119] Consequently, synchrotron FT-IR microscopy may be employed for nondestructive interrogation of biological matter where small apertures down to 3 μm × 3 μm facilitate subcellular biochemical studies.[120] To date, synchrotron FT-IR microscopy has demonstrated significant clinical applicability to investigate multiple diseases,[121,122] including cancer,[123] with a wide range of studies from cytokinesis of prostate cells[124] to apoptosis of glioma cells.[125] Hence, synchrotron FT-IR is set to play an increasingly vital role for clinical research, and while instrumentation is not conducive to health care settings, it may inform future clinical decisions and diagnosis with conventional medical modalities. To this extent, Dučić utilized synchrotron FT-IR to understand the chemical changes of astrocytes associated with genetic mutations of the metallo-enzyme copper−zinc superoxide dismutase (SOD1) implicated in familial amyotrophic lateral sclerosis, a common type of motor neuron disease (Figure 5).[126] In particular, Dučić found localized enhancement of $\nu_{s/as}(CH_2)$, $\nu_{s/as}(CH_3)$, and $\nu(C{=}O)$ vibrations revealed increased concentrations of lipids in the central regions of ALS astrocytes and significant lipid acyl chain saturation, neither of which were observed in nontransgenic littermate (Ngs) control astrocytes. Furthermore, differences in amide I and II vibrations reflected increased antiparallel β-sheet protein secondary structures, while absence of $\nu_{as}N(CH_3)_3$ vibrations indicate disruption of choline metabolism in ALS astrocytes. Importantly, these spectral signatures have enabled identification of large aggregates of lipid vesicles and SOD1 proteins, lipid peroxidation, and metabolic choline alterations, all of which may allow future biomarker discovery and development of novel therapeutic strategies. Similarly, Pijanka employed synchrotron FT-IR microscopy to interrogate isolated nuclei of CALU-1 lung cancer cells where changes in lipid, protein, and DNA signatures may provide spectral biomarkers toward cancer diagnosis.[92] Furthermore, Nakamura showed synchrotron FT-IR microscopy has the capacity to establish spectral biomarkers for adult stem cells in human corneal epithelium that may help identify their in situ location for clinical applications,[127] with both studies highlighting the potential of synchrotron FT-IR for biomarker discovery that could influence future clinical workflows. Recently, Siddique demonstrated significant potential of synchrotron FT-IR

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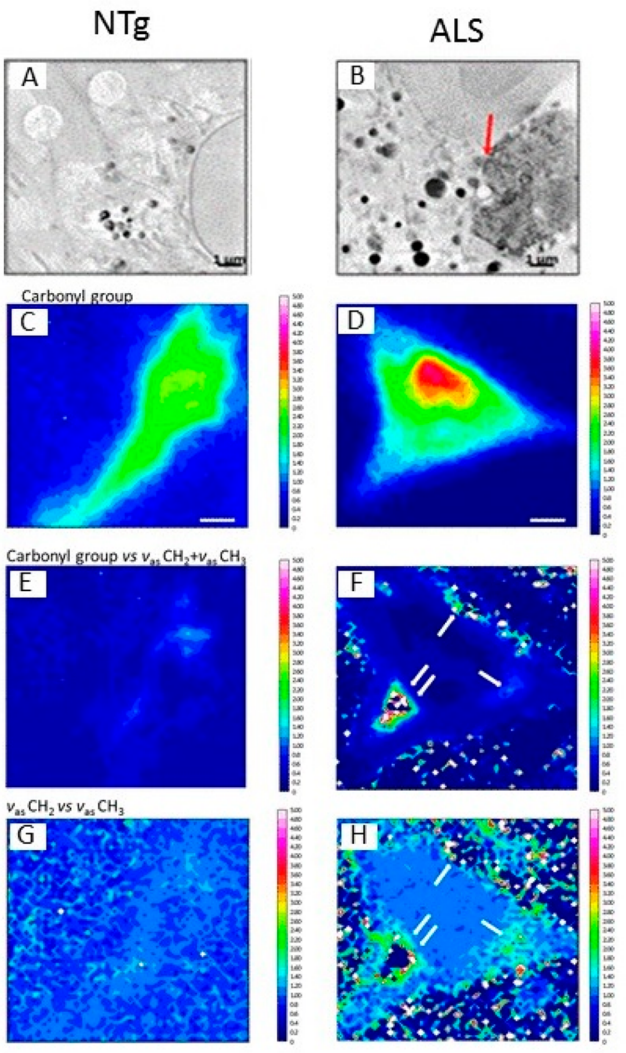

Figure 5. Synchrotron-FT-IR imaging of (A) Ntg and (B) ALS astrocytes where (C,D) ν(C═O) vibrations, (E,F) ratio of ν(C═O)/($\nu_{as}CH_3$ + $\nu_{as}CH_2$) vibrations, and (G,H) ratio of $\nu_{as}CH_2/\nu_{as}CH_3$ vibrations reveal concentrated intracellular lipid compositions and acyl chain unsaturation (white arrows) associated with lipid vesicles and lipid peroxidation. Reproduced and adapted with permission from ref 124. Copyright 2019 American Chemical Society.

microscopy for evaluation of clinical therapeutics, where K562 leukemia cells were analyzed for sensitivity toward Nilotinib, with the view to discovering spectral biomarkers that may in the future enable clinicians to determine patient suitability to specific chemotherapy agents.[128] Similarly, Rutter, expanding on previous FT-IR microscopy studies that analyzed responses of CALU-1 lung cancer cells to gemcitabine,[129] employed synchrotron FT-IR microscopy to identify spectral biomarkers indicative of cell sensitivity or resistance to gemcitabine[130] and underlines the future potential of infrared spectroscopy toward personalized medicine strategies.

It is clear that infrared spectroscopy has a multitude of applications within the clinic and is applicable to all clinical samples types (e.g., biofluids, tissue, and cells) providing high fidelity data that can aid in clinical decision making. Armed with the methodological advances described above the impact within healthcare has the potential to be disruptive to the current approach and can provide reduced mortality and morbidity and enhanced quality of life for patients around the globe. In addition, the new and exciting additions to the technological stability of IR instrumentation with the recent use of QCL, which allows the use of non-liquid nitrogen cooled detectors and supercontinuum sources, the development of optical photothermal infrared (OPTIR) by companies such as Photothermal Spectroscopy Corp. and the methodological varieties for nanoscale based infrared analysis will undoubtedly have a huge impact in this area when the wealth of research using traditional IR can be developed within these techniques. IR has clinical ability and is ready for translation. The particular developments shown above and the move toward an entrepreneurial approach to clinical translation within the spectroscopic community is direly needed, in our opinion, to enable the first product(s) to be released on the market. Following this, the lessons learnt and communicated will enable a wealth of clinically appropriate spectroscopic technology to develop underpinned by close collaboration between clinicians, scientists, entrepreneurs, and investors to change patient processes and management for patient benefit.

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Notes

The authors declare the following competing financial interest(s): Dr. Matthew J. Baker is a Founder, Director, and Chief Scientific Officer of Clinspec Diagnostics Ltd.

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REFERENCES

(1) Hands, J. R.; Dorling, K. M.; Abel, P.; Ashton, K. M.; Brodbelt, A.; Davis, C.; Dawson, T.; Jenkinson, M. D.; Lea, R. W.; Walker, C.; et al. *J. Biophotonics.* **2014**, *7* (3−4), 189−199.

(2) Untereiner, V.; Dhruvananda Sockalingum, G.; Garnotel, R.; Gobinet, C.; Ramaholimihaso, F.; Ehrhard, F.; Diebold, M.-D.; Thiefin, G. *J. Biophotonics.* **2014**, *7* (3−4), 241−253.

(3) Baker, M. J.; Gazi, E.; Brown, M. D.; Shanks, J. H.; Gardner, P.; Clarke, N. W. *Br. J. Cancer* **2008**, *99* (11), 1859−1866.

(4) Wood, B. R.; Quinn, M. A.; Burden, F. R.; Mcnaughton, D. *Biospectroscopy* **1996**, *2*, 143−153.

(5) Travo, A.; Paya, C.; Deleris, G.; Colin, J.; Mortemousque, B.; Forfar, I. *Anal. Bioanal. Chem.* **2014**, *406*, 2367−2376.

(6) Titus, J.; Viennois, E.; Merlin, D.; Unil Perera, A. G. *J. Biophoton.* **2017**, *10*, 465−472.

(7) Gajjar, K.; Trevisan, J.; Owens, G.; Keating, P. J.; Wood, N. J.; Stringfellow, H. F.; Martin-hirsch, P. L.; Martin, F. L. *Analyst* **2013**, *138*, 3917−3926.

(8) Khoshmanesh, A.; Dixon, M. W. A.; Kenny, S.; Tilley, L.; Mcnaughton, D.; Wood, B. R. *Anal. Chem.* **2014**, *86* (9), 4379−4386.

(9) Lechowicz, L.; Chrapek, M.; Gaweda, J.; Urbaniak, M.; Konieczna, I. *Mol. Biol. Rep.* **2016**, *43* (12), 1321−1326.

(10) Weiss, S. L.; Fitzgerald, J. C.; Balamuth, F.; Alpern, E. R.; Lavelle, J.; Chilutti, M.; Grundmeier, R.; Nadkarni, V. M.; Thomas, N. J. *Crit. Care Med.* **2014**, *42* (11), 2409−2417.

DOI: 10.1021/acs.analchem.9b02280
Anal. Chem. XXXX, XXX, XXX−XXX

(11) Gildea, T. R; DaCosta Byfield, S.; Hogarth, D K.; Wilson, D. S; Quinn, C. C *Clin. Outcomes Res.* **2017**, *9*, 261−269.
(12) Mennini, F. S; Viti, R.; Marcellusi, A.; Sciattella, P.; Viapiana, O.; Rossini, M. *Clin. Outcomes Res.* **2018**, *10*, 45−51.
(13) Work. Global Health and Aging. https://www.who.int/ageing/publications/global_health.pdf, 2011, accessed April 25, 2019.
(14) Wilson, B. C.; Jermyn, M.; Leblond, F. *J. Biomed. Opt.* **2018**, *23* (3), 1−13.
(15) Baker, M. J.; Trevisan, J.; Bassan, P.; Bhargava, R.; Butler, H. J.; Dorling, K. M.; Fielden, P. R.; Fogarty, S. W.; Fullwood, N. J.; Heys, K. A.; et al. *Nat. Protoc.* **2014**, *9* (8), 1771−1791.
(16) Bonnier, F.; Blasco, H.; Wasselet, C.; Brachet, G.; Respaud, R.; Carvalho, L. F. C. S.; Bertrand, D.; Baker, M. J.; Byrne, H. J.; Chourpa, I. *Analyst* **2017**, *142*, 1285−1298.
(17) Spalding, K.; Bonnier, F.; Bruno, C.; Blasco, H.; Board, R.; Benz-de Bretagne, I.; Byrne, H. J.; Butler, H. J.; Chourpa, I.; Radhakrishnan, P.; Baker, M. J. *Vib. Spectrosc.* **2018**, *99*, 50−58.
(18) Roy, S.; Perez-Guaita, D.; Andrew, D. W.; Richards, J. S.; McNaughton, D.; Heraud, P.; Wood, B. R. *Anal. Chem.* **2017**, *89* (10), 5238−5245.
(19) Shaw, R. A.; Mantsch, H. H. *Encycl Anal Chem.* **2008**, 1−24.
(20) Li, L.; Bi, X.; Sun, H.; Liu, S.; Yu, M.; Zhang, Y.; Weng, S.; Yang, L.; Bao, Y.; Wu, J.; Xu, Y.; Shen, K. *J. Ovarian Res.* **2018**, *11* (64), 1−10.
(21) Bird, B.; Miljković, M.; Remiszewski, S.; Akalin, A.; Kon, M.; Diem, M. *Lab. Invest.* **2012**, *92* (9), 1358−1373.
(22) Fabian, H.; Lasch, P.; Boese, M.; Haensch, W. *Biopolymers* **2002**, *67* (4−5), 354−357.
(23) Neves, A. C. O.; Silva, P. P.; Morais, C. L. M.; Miranda, C. G.; Crispim, J. C. O.; Lima, K. M. G. *RSC Adv.* **2016**, *6*, 99648−99655.
(24) Cohenford, M. A.; Rigas, B. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95* (26), 15327−15332.
(25) Morris, Z. S.; Wooding, S.; Grant, J. *J. R. Soc. Med.* **2011**, *104*, 510−520.
(26) Baker, M. J.; Byrne, H. J.; Chalmers, J.; Gardner, P.; Goodacre, R.; Henderson, A.; Kazarian, S. G.; Martin, F. L.; Moger, J.; Stone, N.; Sule-Suso, J. *Analyst* **2018**, *143*, 1735−1757.
(27) Whiteman, S. C.; Yang, Y.; Jones, J. M.; Spiteri, M. A. *Ther. Adv. Respir. Dis.* **2008**, *2* (1), 23−31.
(28) Patel, N.; Coleborn, P.; Hampton, A.; Campbell, V.; Allen, M.; Gahkani, A.; Spiteri, M. *Thorax* **2010**, *65*, A124−A125.
(29) Gray, E.; Butler, H. J.; Board, R.; Brennan, P. M.; Chalmers, A. J.; Dawson, T.; Goodden, J.; Hamilton, W.; Hegarty, M. G.; James, A.; et al. *BMJ Open* **2018**, *8*, e017593.
(30) Ergin, A.; Großeruschkamp, F.; Theisen, O.; Gerwert, K.; Remiszewski, S.; Thompson, C. M.; Diem, M. *Analyst* **2015**, *140*, 2465−2472.
(31) Diem, M.; Ergin, A.; Remiszewski, S.; Mu, X. *J. Thorac. Oncol.* **2016**, *11S* (11), S288.
(32) Mitchell, A. L.; Gajjar, K. B.; Theophilou, G.; Martin, F. L.; Martin-hirsch, P. L. *J. Biophotonics.* **2014**, *7* (3−4), 153−165.
(33) Archetti, C.; Montanelli, A.; Finazzi, D.; Caimi, L.; Garrafa, E. *J. Public Health Res.* **2017**, *6*, 881.
(34) Elmi, F.; Movaghar, A. F.; Elmi, M. M.; Alinezhad, H.; Nikbakhsh, N. *Spectrochim. Acta, Part A* **2017**, *187*, 87−91.
(35) Barlev, E.; Zelig, U.; Bar, O.; Segev, C.; Mordechai, S.; Kapelushnik, J.; Nathan, I.; Flomen, F.; Kashtan, H.; Dickman, R.; Madhala-Givon, O.; Wasserberg, N. *J. Gastroenterol.* **2016**, *51* (3), 214−221.
(36) Shen, Y. C., Davies, A. G.; Linfield, E. H.; Taday, P. F.; Arnone, D. D.; Elsey, T. S. Determination of Glucose Concentration in Whole Blood using FTIR Spectroscopy. In *Tera-Hertz Radiation in Biological Research, Investigation on Diagnostics, and Study on Potential Genotoxic Effects*; 2002.
(37) Lewis, P. D; Lewis, K. E; Ghosal, R.; Bayliss, S.; Lloyd, A. J; Wills, J.; Godfrey, R.; Kloer, P.; Mur, L. A. *BMC Cancer* **2010**, *10*, 640.
(38) Liu, K.; Dembinski, T. C.; Mantsch, H. H. *Am. J. Obstet. Gynecol.* **1998**, *178* (2), 234−241.
(39) Yonar, D.; Ocek, L.; Tiftikcioglu, B. I.; Zorlu, Y.; Severcan, F. *Sci. Rep.* **2018**, *8* (1025), 1−13.
(40) Oliver, K. V.; Vilasi, A.; Maréchal, A.; Moochhala, S. H.; Unwin, R. J.; Rich, P. R. *Sci. Rep.* **2016**, *6* (34737), 1−7.
(41) Scott, D. A; Renaud, D. E; Krishnasamy, S.; Meric, P.; Buduneli, N.; Cetinkalp, S.; Liu, K.-Z. *Diabetol. Metab. Syndr.* **2010**, *2*, 48.
(42) Yu, M.-C.; Rich, P.; Foreman, L.; Smith, J.; Yu, M.-S.; Tanna, A.; Dibbur, V.; Unwin, R.; Tam, F. W. K. *Sci. Rep.* **2017**, *7*, 4601.
(43) Siqueira, L. F. S.; Lima, K. M. G. *Analyst* **2016**, *141*, 4833−4847.
(44) Sahu, R. K.; Mordechai, S. *Future Oncol.* **2005**, *1* (5), 635−647.
(45) Ollesch, J.; Heinze, M.; Heise, H. M.; Behrens, T.; Bruning, T.; Gerwert, K. *J. Biophoton.* **2014**, *7* (3−4), 210−221.
(46) Paraskevaidi, M.; Morais, C. L. M.; Freitas, D. L. D.; Lima, K. M. G.; Mann, D. M. A.; Allsop, D.; Martin-Hirsch, P. L.; Martin, F. L. *Analyst* **2018**, *143*, 5959−5964.
(47) Le Corvec, M.; Jezequel, C.; Monbet, V.; Fatih, N.; Charpentier, F.; Tariel, H.; Boussard-Pledel, C.; Bureau, B.; Loreal, O.; Sire, O.; Bardou-Jacquet, E. *PLoS One* **2017**, *12* (10), e0185997−15.
(48) Maquelin, K.; Kirschner, C.; Choo-Smith, L.-P.; Ngo-Thi, N. A.; van Vreeswijk, T.; Stammler, M.; Endtz, H. P.; Bruining, H. A.; Naumann, D.; Puppels, G. J. *J. Clin Microbiol.* **2003**, *41* (1), 324−329.
(49) Carrigan, S. D.; Scott, G.; Tabrizian, M. *Clin. Chem.* **2004**, *50*, 1301−1314.
(50) Biron, B. M.; Ayala, A.; Lomas-neira, J. L. *Biomarker Insights* **2015**, *10s4*, 7−17.
(51) Wood, B. R.; Khoshmanesh, A.; Dixon, M.; Tilley, L.; Mcnaughton, D. *Method and System for Rapid Malaria Detection.* U.S. Patent 9,983,130, May 29, 2018.
(52) Perez-Guaita, D.; Marzec, K. M.; Hudson, A.; Evans, C.; Chernenko, T.; Matthäus, C.; Miljkovic, M.; Diem, M.; Heraud, P.; Richards, J. S.; et al. *Chem. Rev.* **2018**, *118* (11), 5330−5358.
(53) Bassan, P.; Byrne, H. J.; Bonnier, F.; Lee, J.; Dumas, P.; Gardner, P. *Analyst* **2009**, *134* (1), 1586−1593.
(54) Cameron, J. M.; Butler, H. J.; Palmer, D. S.; Baker, M. J. *J. Biophoton.* **2018**, *11* (4), e201700299.
(55) Lovergne, L.; Clemens, G.; Untereiner, V.; Lukaszweski, R. A.; Sockalingum, G. D.; Baker, M. J. *Anal. Methods* **2015**, *7* (17), 7140.
(56) Peters, A. S.; Backhaus, J.; Pfützner, A.; Raster, M.; Burgard, G.; Demirel, S.; Böckler, D.; Hakimi, M. *Vib. Spectrosc.* **2017**, *92*, 20−26.
(57) Hands, J. R.; Clemens, G.; Stables, R.; Ashton, K.; Brodbelt, A.; Davis, C.; Dawson, T. P.; Jenkinson, M. D.; Lea, R. W.; Walker, C.; et al. *J. Neuro-Oncol.* **2016**, *127* (3), 463−472.
(58) Hughes, C.; Clemens, G.; Bird, B.; Dawson, T.; Ashton, K. M.; Jenkinson, M. D.; Brodbelt, A.; Weida, M.; Fotheringham, E.; Barre, M.; et al. *Sci. Rep.* **2016**, *6* (20173), 1−8.
(59) Schwaighofer, A.; Brandstetter, M.; Lendl, B. *Chem. Soc. Rev.* **2017**, *46*, 5903−5924.
(60) Karabudak, E.; Kas, R.; Ogieglo, W.; Rafieian, D.; Schlautmann, S.; Lammertink, R. G. H.; Gardeniers, H. J. G. E.; Mul, G. *Anal. Chem.* **2013**, *85*, 33−38.
(61) Schumacher, H.; Kunzelmann, U.; Vasilev, B.; Eichhorn, K.-J.; Bartha, J. W. *Appl. Spectrosc.* **2010**, *64* (9), 1022−1027.
(62) Detz, H.; Andrews, A. M.; Schwarz, B.; Reininger, P.; Ristanic, D.; Schrenk, W.; Strasser, G. *Nat. Commun.* **2014**, *5* (4085), 1−7.
(63) Bassan, P.; Kohler, A.; Martens, H.; Lee, J.; Byrne, H. J.; Dumas, P.; Gazi, E.; Brown, M.; Clarke, N.; Gardner, P. *Analyst* **2010**, *135*, 268−277.
(64) Lovergne, L.; Bouzy, P.; Untereiner, V.; Garnotel, R.; Baker, M. J.; Thiefin, G.; Sockalingum, G. D. *Faraday Discuss.* **2016**, *187*, 521−537.
(65) Paech, D. C.; Weston, A. R.; Pavlakis, N.; Gill, A.; Rajan, N.; Barraclough, H.; Fitzgerald, B.; Van Kooten, M. *J. Thorac. Oncol.* **2011**, *6* (1), 55−63.
(66) Nakai, Y.; Tanaka, N.; Shimada, K.; Konishi, N.; Miyake, M.; Anai, S.; Fujimoto, K. *BMC Urol.* **2015**, *15* (1), 70.

DOI: 10.1021/acs.analchem.9b02280
Anal. Chem. XXXX, XXX, XXX−XXX

(67) Bueno-de-Mesquita, J. M.; Nuyten, D. S. A.; Wesseling, J.; van Tinteren, H.; Linn, S. C.; van de Vijver, M. J. *Ann. Oncol.* **2010**, *21* (1), 40−47.
(68) Trujillo, K. A.; Jones, A. C.; Griffith, J. K.; Bisoffi, M. *Prostate Cancer* **2012**, *2012*, 1−12.
(69) Hermes, M.; Morrish, R. B.; Huot, L.; Meng, L.; Junaid, S.; Tomko, J.; Lloyd, G. R.; Masselink, W. T.; Tidemand-Lichtenberg, P.; Pedersen, C.; et al. *J. Opt.* **2018**, *20* (2), 023002.
(70) Boyle, P., Levin, B., Eds. *World Cancer Report 2008*; International Agency for Research on Cancer/World Health Organization, 2008; https://publications.iarc.fr/Non-Series-Publications/World-Cancer-Reports/World-Cancer-Report-2008, accessed April 25, 2019.
(71) Weaver, D. L.; Rosenberg, R. D.; Barlow, W. E.; Ichikawa, L.; Carney, P. A.; Kerlikowske, K.; Buist, D. S. M.; Geller, B. M.; Key, C. R.; Maygarden, S. J.; et al. *Cancer* **2006**, *106* (4), 732−742.
(72) Mayerich, D.; Walsh, M. J.; Kadjacsy-Balla, A.; Ray, P. S.; Hewitt, S. M.; Bhargava, R. *Technology* **2015**, *3* (1), 27−31.
(73) Pilling, M.; Gardner, P. *Chem. Soc. Rev.* **2016**, *45* (7), 1935−1957.
(74) Schubert, J. M.; Mazur, A. I.; Bird, B.; Miljković, M.; Diem, M. *J. Biophotonics.* **2010**, *3* (8−9), 588−596.
(75) Bassan, P.; Sachdeva, A.; Shanks, J. H.; Brown, M. D.; Clarke, N. W.; Gardner, P. *Analyst* **2013**, *138* (23), 7066.
(76) Kwak, J. T.; Hewitt, S. M.; Sinha, S.; Bhargava, R. *BMC Cancer* **2011**, *11*, 62.
(77) Wang, H.; Wang, H.; Zhang, W.; Fuller, G. N. *Brain Pathol.* **2002**, *12* (1), 95−107.
(78) Tiwari, S.; Raman, J.; Reddy, V.; Ghetler, A.; Tella, R. P.; Han, Y.; Moon, C. R.; Hoke, C. D.; Bhargava, R. *Anal. Chem.* **2016**, *88*, 10183−10190.
(79) Chen, X.; Zheng, B.; Liu, H. *Anal. Cell. Pathol.* **2011**, *34* (1−2), 5−18.
(80) Centrone, A. *Annu. Rev. Anal. Chem.* **2015**, *8* (1), 101−126.
(81) Bassan, P.; Mellor, J.; Shapiro, J.; Williams, K. J.; Lisanti, M. P.; Gardner, P. *Anal. Chem.* **2014**, *86* (3), 1648−1653.
(82) Mayor, S. *BMJ.* **2016**, *354*, i5026.
(83) Barut, M. U.; Kale, A.; Kuyumcuoğlu, U.; Bozkurt, M.; Ağaçayak, E.; Özekinci, S.; Gül, T. *Med. Sci. Monit.* **2015**, *21*, 3860−3867.
(84) Spence, A. R.; Goggin, P.; Franco, E. L. *Prev. Med.* **2007**, *45* (2−3), 93−106.
(85) Lyng, F. M.; Traynor, D.; Ramos, I. R. M.; Bonnier, F.; Byrne, H. J. *Anal. Bioanal. Chem.* **2015**, *407* (27), 8279−8289.
(86) Cuzick, J.; Arbyn, M.; Sankaranarayanan, R.; Tsu, V.; Ronco, G.; Mayrand, M.-H.; Dillner, J.; Meijer, C. J.L.M. *Vaccine* **2008**, *26*, K29−K41.
(87) NHS Digital. *Cervical Screening Programme*, 2018; https://www.statisticsauthority.gov.uk/code-of-practice/, accessed April 25, 2019.
(88) Walsh, M. J.; German, M. J.; Singh, M.; Pollock, H. M.; Hammiche, A.; Kyrgiou, M.; Stringfellow, H. F.; Paraskevaidis, E.; Martin-Hirsch, P. L.; Martin, F. L. *Cancer Lett.* **2007**, *246* (1−2), 1−11.
(89) Schubert, J. M.; Bird, B.; Papamarkakis, K.; Miljković, M.; Bedrossian, K.; Laver, N.; Diem, M. *Lab. Invest.* **2010**, *90* (7), 1068.
(90) Ostrowska, K. M.; Garcia, A.; Meade, A. D.; Malkin, A.; Okewumi, I.; O'Leary, J. J.; Martin, C.; Byrne, H. J.; Lyng, F. M. *Analyst* **2011**, *136* (7), 1365.
(91) Wood, B. R.; Chiriboga, L.; Yee, H.; Quinn, M. A.; McNaughton, D.; Diem, M. *Gynecol. Oncol.* **2004**, *93* (1), 59−68.
(92) Pijanka, J.; Sockalingum, G. D.; Kohler, A.; Yang, Y.; Draux, F.; Parkes, G.; Lam, K.-P.; Collins, D.; Dumas, P.; Sandt, C.; et al. *Lab. Invest.* **2010**, *90* (5), 797−807.
(93) Miller, L. M.; Dumas, P. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758* (7), 846−857.
(94) Rutter, A. V.; Crees, J.; Wright, H.; van Pittius, D. G.; Yousef, I.; Sule-Suso, J. *Analyst* **2018**, *143* (23), 5711−5717.
(95) Steinbach, W. J.; Shetty, A. K. *Postgrad. Med. J.* **2001**, *77*, 148−156.
(96) Laupland, K. B.; Valiquette, L. *Can. J. Infect Dis Med. Microbiol.* **2013**, *24* (3), 125−128.
(97) Mediannikov, O.; Edouard, S.; Raoult, D.; Lagier, J.-C.; Pagnier, I.; Drancourt, M. *Clin. Microbiol. Rev.* **2015**, *28* (1), 208−236.
(98) Boughner, L. A.; Singh, P. *Postdoc J. a J. Postdr Res. Postdr Aff.* **2016**, *4* (11), 3−17.
(99) Zarnowiec, P.; Lechowicz, Ł; Czerwonka, G.; Kaca, W. *Curr. Med. Chem.* **2015**, *22* (14), 1710−1718.
(100) Helm, D.; Labischinski, H.; Naumann, D. *J. Microbiol. Methods* **1991**, *14* (2), 127−142.
(101) Naumann, D.; Helm, D.; Labischinski, H. *Nature* **1991**, *351* (6321), 81−82.
(102) Muhamadali, H.; Weaver, D.; Subaihi, A.; AlMasoud, N.; Trivedi, D. K.; Ellis, D. I.; Linton, D.; Goodacre, R. *Analyst* **2016**, *141* (1), 111−122.
(103) Gilbert, M. K.; Frick, C.; Wodowski, A.; Vogt, F. *Appl. Spectrosc.* **2009**, *63* (1), 6−13.
(104) Winder, C. L.; Goodacre, R. *Analyst* **2004**, *129* (11), 1118.
(105) Bosch, A.; Minan, A.; Vescina, C.; Degrossi, J.; Gatti, B.; Montanaro, P.; Messina, M.; Franco, M.; Vay, C.; Schmitt, J.; et al. *J. Clin Microbiol.* **2008**, *46* (8), 2535−2546.
(106) Whittaker, P.; Mossoba, M. M.; Al-Khaldi, S.; Fry, F. S.; Dunkel, V. C.; Tall, B. D.; Yurawecz, M. P. *J. Microbiol. Methods* **2003**, *55* (3), 709−716.
(107) Sharaha, U.; Rodriguez-Diaz, E.; Riesenberg, K.; Bigio, I. J.; Huleihel, M.; Salman, A. *Anal. Chem.* **2017**, *89* (17), 8782−8790.
(108) Jindal, A. K.; Pandya, K.; Khan, I. D. *Med. journal, Armed Forces India* **2015**, *71* (2), 178−181.
(109) Erukhimovitch, V.; Pavlov, V.; Talyshinsky, M.; Souprun, Y.; Huleihel, M. *J. Pharm. Biomed. Anal.* **2005**, *37* (5), 1105−1108.
(110) Quintelas, C.; Ferreira, E. C.; Lopes, J. A.; Sousa, C. *Biotechnol. J.* **2018**, *13* (1), 1700449.
(111) Ngo-Thi, N.; Kirschner, C.; Naumann, D. *J. Mol. Struct.* **2003**, *661−662*, 371−380.
(112) Lasch, P.; Stämmler, M.; Zhang, M.; Baranska, M.; Bosch, A.; Majzner, K. *Anal. Chem.* **2018**, *90* (15), 8896−8904.
(113) Kirkwood, J.; Al-Khaldi, S. F.; Mossoba, M. M.; Sedman, J.; Ismail, A. A. *Appl. Spectrosc.* **2004**, *58* (11), 1364−1368.
(114) Davis, R. K.; Mauer, L. J. Fourier Transform Infrared (FT-IR) Spectroscopy: A Rapid Tool for Detection and Analysis of Foodborne Pathogenic Bacteria. In *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*; Formatex Research Center, 2010; Vol. *II*, pp 1582−1594.
(115) Scholz, T.; Lopes, V. V.; Calado, C. R. C. *Biotechnol. Bioeng.* **2012**, *109* (9), 2279−2285.
(116) Kohler, A.; Böcker, U.; Shapaval, V.; Forsmark, A.; Andersson, M.; Warringer, J.; Martens, H.; Omholt, S. W.; Blomberg, A. *PLoS One* **2015**, *10* (2), e0118052.
(117) Holman, H-YN; Miles, R.; Hao, Z.; Wozei, E.; Anderson, L. M.; Yang, H. *Anal. Chem.* **2009**, *81* (20), 8564−8570.
(118) Pousti, M.; Joly, M.; Roberge, P.; Amirdehi, M. A.; Bégin-Drolet, A.; Greener, J. *Anal. Chem.* **2018**, *90* (24), 14475−14483.
(119) Miller, L. M.; Dumas, P. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758* (7), 846−857.
(120) Dumas, P.; Miller, L. *J. Biol. Phys.* **2003**, *29* (2/3), 201−218.
(121) Araki, K.; Yagi, N.; Ikemoto, Y.; Yagi, H.; Choong, C.-J.; Hayakawa, H.; Beck, G.; Sumi, H.; Fujimura, H.; Moriwaki, T.; et al. *Sci. Rep.* **2015**, *5* (1), 17625.
(122) Aboualizadeh, E.; Ranji, M.; Sorenson, C. M.; Sepehr, R.; Sheibani, N.; Hirschmugl, C. J. *Analyst* **2017**, *142* (7), 1061−1072.
(123) Grzelak, M. M.; Wróbel, P. M.; Lankosz, M.; Stęgowski, Z.; Chmura, Ł; Adamek, D.; Hesse, B.; Castillo-Michel, H. *Spectrochim. Acta, Part A* **2018**, *203*, 48−55.
(124) Lyng, F.; Gazi, E.; Gardner, P. Preparation of Tissues and Cells for Infrared and Raman Spectroscopy and Imaging. In *Biomedical Applications of Synchrotron Infrared Microspectroscopy*;

DOI: 10.1021/acs.analchem.9b02280
Anal. Chem. XXXX, XXX, XXX−XXX

RSC Analytical Spectroscopy Series; Royal Society of Chemistry: Cambridge, U.K., 2010; pp 145−191,
(125) Buriankova, L.; Nadova, Z.; Jancura, D.; Refregiers, M.; Yousef, I.; Mikes, J.; Miskovsky, P. *Laser Phys. Lett.* **2010**, *7* (8), 613−620.
(126) Dučić, T.; Stamenković, S.; Lai, B.; Andjus, P.; Lučić, V. *Anal. Chem.* **2019**, *91* (2), 1460−1471.
(127) Nakamura, T.; Kelly, J. G.; Trevisan, J.; Cooper, L. J.; Bentley, A. J.; Carmichael, P. L.; Scott, A. D.; Cotte, M.; Susini, J.; Martin-Hirsch, P. L. *Mol. Vis.* **2010**, *16*, 359−368.
(128) Siddique, M. R.; Rutter, A. V.; Wehbe, K.; Cinque, G.; Bellisola, G.; Sulé-Suso, J. *Analyst* **2017**, *142* (8), 1299−1307.
(129) Draux, F.; Jeannesson, P.; Gobinet, C.; Sule-Suso, J.; Pijanka, J.; Sandt, C.; Dumas, P.; Manfait, M.; Sockalingum, G. D. *Anal. Bioanal. Chem.* **2009**, *395* (7), 2293−2301.
(130) Rutter, A. V.; Siddique, M. R.; Filik, J.; Sandt, C.; Dumas, P.; Cinque, G.; Sockalingum, G. D.; Yang, Y.; Sulé-Suso, J. *Cytometry, Part A* **2014**, *85* (8), 688−697.

DOI: 10.1021/acs.analchem.9b02280
Anal. Chem. XXXX, XXX, XXX−XXX