MIR Spectroscopy: The Medical Diagnosis Swiss Knife?

Accessing global information, useful for fast detection of various diseases

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For more than two decades, mid-infrared (MIR) spectroscopy appeared a promising method for fast medical diagnostics. However, some material limitations slowed down its development until the past five years. Now, the first CE-marked diagnosis solutions are coming onto the market and the future of this technology as a near-patient fast screening test looks bright.

Vibrational spectroscopy is the study of the interaction between molecular bonds and electromagnetic radiation. Mid-infrared (MIR) refers to the light spectrum of wavelengths between 2 and 25 µm. MIR is the area where bodies at room temperature radiate.

This is the reason why MIR cameras are also called 'thermal imagers', as they show warm bodies' light emission, or 'night vision cameras' as by using these bodies' emitted light they do not need ambient light to visualize objects.

The other interesting feature of MIR is that the fundamental (strongest) molecular absorptions of biological elements fall within this mid-infrared area.

As shown in Fig. 2 on the right, atomic bonds exhibit periodic oscillations within the molecule. Most carbon to oxygen, nitrogen and hydrogen bonds vibrate within the MIR area, so they generate strong absorption bands that can be observed and assigned by MIR spectroscopy. Hence, a MIR absorption spectrum reflects most of the organic elements present within a biologic sample. Any chemical or structural change within the said samples, caused for instance by a disease, affects the spectrum. These changes can then be detected and used as biomarkers of the disease that generated them.

MIR spectroscopy's major advantage is that a spectrum collects global information instead of distinct molecular targets, allowing access to a rich pool of information. It also requires minimal if no sample preparation for a measurement close to a patient. Last but not least, results are quickly provided, in an average of 15 minutes. It has however some limitations, such as a lack of sensitivity. An atomic bond may have several absorption bands, and common atomic bonds like C-H or C=0 are present in many organic molecules. Hence, MIR

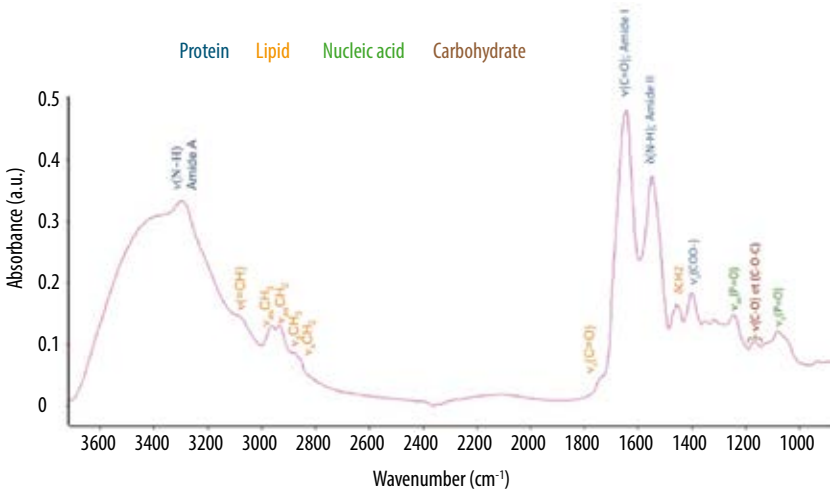

Fig. 1 Example of an MIR absorption spectrum from 3700 to 900 cm^{-1} (or 2.7 to 11 µm wavelength)

spectroscopy cannot identify a specific metabolite within complex biofluids but rather categories (cf. Fig. 1). Water also has strong absorbance around 3400 and 1700 cm^{-1} that may screen some information, so sample water content has to be minimal.

MIR spectra collect a comprehensive molecular signature from a biological sample. This global information is often seen as a metabolic fingerprint of the analyzed biofluid. It can provide a powerful biomarker of a specific disease or patient condition that alters the global metabolic status. Its simplicity and speed make it an ideal screening tool to select patients that may require further examination.

Tumors, bacteria and obviously metabolic diseases have an impact on metabolic status, and therefore significantly affect some biomarkers. Hence, potential applications are limitless and proof of concept systems are available for most cancers, chronic diseases and infections, diagnosis and prognosis. For instance, MIR spectroscopy has demonstrated its capability to quickly detect infection in most body fluids, such as synovial fluid (joints), cerebrospinal fluid (brain, spine), and ascites (abdomen). More than two hundred papers rely on MIR spectroscopy capabilities for cancer detection. However, MIR spectroscopy-based diagnosis is still very rare within hospitals. The first reason is the above-mentioned limitation of a lack of selectivity, so diagnosis development requires a significant number of patients for calibration, then again for validation (>300 patients). Although this may appear a small number compared to the thousands needed for drugs development, these are significant numbers for in vitro diagnosis, where development budgets are smaller and a smaller number of patients are used, if any.

Material

The second restraint to clinical development of MIR spectroscopy application has been tools' availability. MIR spectroscopy equipment has some internal limitation that prevented its use in routine clinical practice, especially for biofluids analysis. Initial dispersive spectrometers had limited power, which implied long measurement times and the use of thin samples to limit overall absorption. Fourier transform IR spectrometers were a first answer as they allowed more powerful signal capture from the samples. However, measurement implementation was still cumbersome as it had to use transmission measurement in order to get a proper signal. This meant preparing thin and thickness-controlled samples between two MIR transmitting materials. The alternative was the ATR (attenuated total reflection) sampling technique, where a sample was deposited onto an IR crystal waveguide. Part of the signal was absorbed by the sample, allowing the spectrum to be recorded. This method lacked sensitivity, and moreover, ATR plates are not single-use, implying a thorough cleaning process between two measurements to avoid cross contamination. Fiber evanescent wave spectroscopy (FEWS, Fig. 4) brought some interesting improvements, however, here again simple and sensitive tools were missing for biological analysis as the main fiber material on the market are silver halogenides that deteriorate in the presence of water, the main biolog-

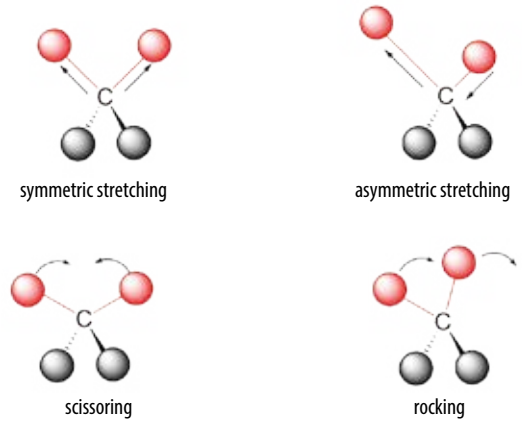

Fig. 2 Bond vibration types

Company

DIAFIR

DIAFIR was established in 2011 following twenty years of research by Rennes University laboratories. Using this unique knowledge in MIR optical material, it has developed the SPID™ platform, using patented single-use sensors, for fast near patient diagnosis. Its first applications are quick articular joint infection and NASH metabolic disease diagnoses for obese patients. Some applications are also appearing for the veterinary and agro-food industries.

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Fig. 3 Spid compact platform, wasy to use near patient

ical compound element. Chalcogenide glass fiber was the next step; it made it possible to make thinner fibers, and thereby more sensitive sensors. And as a hydro-resistant and hydrophobic material, the chemical inertia of such fibers towards biologic samples is ensured.

Part of the light going through an optical fiber actually escapes from the fiber as it is reflected at the surface; it is named the evanescent wave. This part of the signal can be absorbed by a sample in contact with the fiber.

At the fiber exit, we get the initial signal minus the quanta absorbed by the medium; this yields the fingerprint.

Here again, the chalcogenide brittleness prevented their development. Encapsulated chalcogenide glass fiber sensors, coupled to an uncooled detector FTIR spectroscope solve this issue by providing a simple and affordable sampling method. They are disposable, and avoid any cleaning and cross contamination issues. Their sensitivity and reproducibility have been demonstrated over more than two thousand measurements.

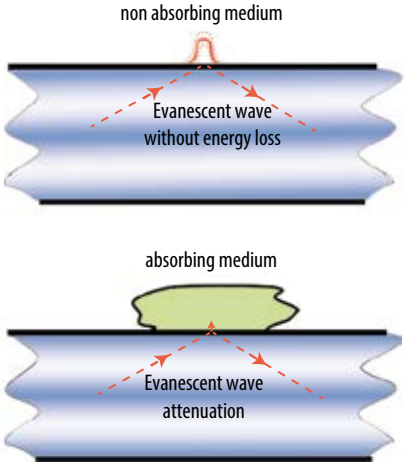

Fig. 4 Fiber evanescent wave spectroscopy (FEWS) principle

It is then possible to obtain a spectrum by simply placing a 7-µl drop onto the sensor. A dedicated, compact, touchscreen operated spectroscope has been developed to operate these sensors. It records an infrared spectrum within ten minutes after just a single one-hour training period, a process adapted to clinical practice.

To get diagnostic information out of the MIR spectrum, machine learning techniques will allow the detection of the metabolic fingerprint from a calibration subject. This requires a patient group with MIR measurements, associated to a diagnosis given by an approved method, usually the one considered as the gold standard. Algorithms such as partial least square regression, random forest, genetic ones, will then identify the metabolic fingerprint from correlated discriminant wavelengths.

To overcome the lack of sensitivity of the method, a robust group of several hundred patients is necessary. The model formula is as follows:

$$D = e^{\Sigma(\beta_i \lambda_i)} / (1 + e^{\Sigma(\beta_i \lambda_i)}) \times 100,$$

where:
D is the diagnostic score, when D is above a set threshold, the patient has a significant probability to exhibit the disease
λ_i are the significantly discriminant wavelengths selected during the learning phase
β_i are weights of the predictive model

The model gives a score from 0 (healthy) to 100 (disease). Several thresholds can be determined to help the practitioners to segregate different conditions (severely ill, probably ill, probably healthy, healthy …). The wavelengths used by the model are checked against known effects of the disease to confirm their biologic relevance. Then the model is validated against a second independent group of patients. This validation group has to be a minimum of half the size of the calibration group.

Fast detection of joint infection

Synovial fluid infection, or septic arthritis, is due to bacterial presence within the joint. It is a therapeutic emergency as it can evolve into septicemia leading to death, or at least create irreversible joint damage and motion loss. Unfortunately, current diagnosis by cell culture only delivers a result within about 48 hours. As a consequence, most patients with acute arthritis and fever, and hence at risk of septic arthritis, are treated with preventive antibiotic doses, delivered by an intravenous procedure. This has numerous drawbacks: for the patient, an annoying treatment, with a hospital stay, that prove useless when the cell culture result is negative. It also favors antibiotic resistance development, a long-term concern for public care. And for the community it generates unnecessary hospital and treatment costs. Here, MIR spectroscopy is a precious tool to exclude the non-septic patients within fifteen minutes with 98 % certainty, avoiding the burdens above. This demonstrates the full capability of the technique. The test is affordable, and possibly near patient thanks to the compact FTIR device, for an immediate decision. Avoiding unnecessary antibiotic treatment brings a three thousand euro saving per patient for the community and makes several days of hospital stay

Fig. 5 Single-use mid-infrared medical sensor

for the patient unnecessary. With more than 100,000 patients at risk yearly, the gain is obvious.

A promising future

MIR spectroscopy development will benefit from current technological evolution. Development of simpler IR spectrometers will increase the prevalence of the technology, from large hospitals to local care units. Furthermore, development of new diagnoses will allow wider use of each piece of equipment and further reduce individual test costs. And the improvement of machine learning techniques will help the search for biomarkers within metabolic profiles global information, without a priori, to develop new diagnoses.

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