Abstract:

Title: MICROSPECTROSCOPY APPARATUS AND METHOD

FIG 1

Scanned probe near-field infra-red micro-spectroscopy apparatus comprising: a probe operable to be scanned over a surface of a sample; and at least one semiconductor light source arranged to illuminate the substrate thereby to heat the sample by absorption of infra-red radiation, the apparatus being arranged to be responsive by means of the probe to thermal expansion of the sample due to a temperature rise of the sample induced by illumination of the sample by the light source thereby to obtain micro-spectroscopy data in respect of the sample.
MICROSPECTROSCOPY APPARATUS AND METHOD

FIELD OF THE INVENTION

This invention is concerned with high spatial resolution photothermal spectroscopy and imaging using a scanning probe microscope (SCM) and a source of radiation focused onto the point where the probe contacts the surface of the sample (the subjects of IR microspectroscopy and scanning probe microscopy are well established and will not be described here).

BACKGROUND

There are already in the literature methods for this type of measurement [1-11]. They all comprise a method of measuring a spectrum of infrared absorption of a sample (sometimes on a sub-wavelength scale [4-7, 9-11]) comprising the steps of optically coupling, in most cases, a benchtop source [1-8, 11] of sometimes coherent [4-11] and sometimes non-coherent [1-3] infrared radiation to a region of a sample, modulating the intensity of the radiation incident on the sample (in some cases at a frequency 1000Hz or more [9-11]), interacting a cantilever probe with a tip (sometimes with a sub-micron end radius [3-11]) with the sample such that the probe responds to IR radiation absorbed by the sample [1-11]. The wavelength of the infrared radiation supplied by the benchtop source can be swept, in some cases [8-11], over a region of the mid-IR range while detecting a probe response to the modulated infrared radiation absorbed by the sample as a function of wavelength. An alternative to sweeping the wavelength is to use an interferometer with a broadband source or sources that together provide many wavelengths at once [1-3].

Acquiring the probe response as a function of wavelength over the desired wavelength range can, in some cases, be done in a second or less, for example an interferogram can be acquired in a fraction of a second and this can provide a spectrum over the entire mid infrared region. Moving the probe over the surface of the sample to acquire a spatial map of infrared map of a sample with submicron spatial resolution is also possible at relatively high speeds so a map of 100×100 pixels or more can be acquired in minutes [9-11] even when using a benchtop infrared source [7-9].

Of particular interest are methods that use one or more of the following sources of infra
red (IR) radiation; a globar [1-3], lasers [4-7] (particularly but not exclusively quantum
cascade lasers or QCLs because of their ability to work in the 'fingerprint' region of IR
spectroscopy) and optical parametric oscillators [8-11] or OPOs (the term optical
parametric generators, or OPG is also used, this is explained in detail below). A type of
source new to this application but which considerable potential is a light emitting diode
(LED). All of these devices are commercially available and/or described in the literature
and all can cover all or a significant part of the IR spectral region which is generally
taken to be 2-20 micrometers in wavelength. The inventors have been involved in all of
these methods in all of the types of combinations that have been realised to date.

Where the source is broadband, such as the globar the LED and some kinds of QCL
(such as Fabry-Perot QCL's) or combinations of these to create a multiplicity of or
sources each with a different wavelength or range of wavelengths then, as noted above,
an interferometer can be used.

There are two fundamental methods that have been demonstrated for detecting when an
object has absorbed the incident radiation. Both of these are based on the fact that an
object that absorbs IR radiation increases in temperature. The temperature change can
be detected directly by a temperature sensitive probe that has a tip that is capable of
measuring temperature fluctuations [1-3, 8] (this method is referred to herein as
photothermal microspectroscopy or PTMS, as discussed in further detail below.

Other methods detect absorption by reference to thermal expansion of the sample. In
one case the expansion is measured directly [3-7], in another the occurrence of resonant
motion in the cantilever of the probe induced by rapid expansion of the object can be
detected [9-11]. This method is referred to herein as acousto-optic microspectroscopy
or AOMS, as discussed below.

A probe that is always in contact with the surface can be used [1-3, 8-11] (known as a
'contact mode' of operation) or a dynamic technique that moves the tip up and down [4-7]
(known as a 'tapping mode' or 'intermittent contact' mode), also a heterodyne
approach can be employed [4-7].

Top down illumination has been successfully demonstrated [1-8, 11] as well as the use
of attenuated total internal reflection (ATR) [9-10] where a thin sample is placed on an IR
transparent material under conditions where the beam is internally reflected thus the sample is illuminated from below.

It is to be understood that the inventors have recognised that all of the different IR sources could be used with all of the different methods of detection using both methods of illumination although some combinations would be more difficult to realise in practice than others. It is also to be understood that it would be possible to use combinations of different sources and different methods of detection either sequentially or simultaneously. Thus, for example, a probe capable of measuring temperature fluctuations might also measure thermal expansion including induced resonant motion.

An advantage of all of these methods is that they can perform localised spectroscopy and imaging with a spatial resolution that is not diffraction limited, this means they make possible higher spatial resolution than is possible with conventional far-field optics.

STATEMENTS OF THE INVENTION

In a first aspect of the invention there is provided scanned probe near-field infra-red micro-spectroscopy apparatus comprising: a probe operable to be scanned over a surface of a sample; and at least one semiconductor light source arranged to illuminate the substrate thereby to heat the sample by absorption of infra-red radiation, the apparatus being arranged to be responsive by means of the probe to thermal expansion of the sample due to a temperature rise of the sample induced by illumination of the sample by the light source thereby to obtain micro-spectroscopy data in respect of the sample.

In a second aspect of the invention there is provided a method of performing scanned probe near-field infra-red micro-spectroscopy comprising: scanning a probe over a surface of a sample; illuminating the sample with light from at least one semiconductor light source thereby to heat the sample by absorption of infra-red radiation; and sensing by means of the probe a thermal expansion of the sample due to a temperature rise of the sample induced by illumination of the sample by the light source thereby to obtain micro-spectroscopy data in respect of the sample.

Embodiments of the invention may be understood by reference to the appended claims.
In a further aspect of the invention there is provided a scanned probe near-field infra-red micro-spectroscopy apparatus comprising: a probe operable to be scanned over a surface of a substrate; and at least one semiconductor light source arranged to illuminate the substrate thereby to heat the sample by absorption of infra-red radiation, the apparatus being arranged to detect by means of the probe a temperature rise of the sample due to illumination of the sample by the light source thereby to obtain micro-spectroscopy data in respect of the substrate.

Preferably the at least one light source may be arranged to provide a source of broadband illumination.

The at least one light source may be arranged to generate mid-infra-red radiation having a substantially continuous spread of frequencies over at least a portion of the range from around 5 microns to around 10 microns.

The at least one light source may be operable in a continuous wave mode.

Alternatively the at least one light source may be operable in a pulsed mode.

The at least one light source may comprise a light emitting diode (LED).

The LED may comprise $\text{Al}_{x}\text{In}_{1-x}\text{Sb}$.

Alternatively or in addition the at least one light source may comprise a quantum cascade laser (QCL) device.

The QCL device may be a tunable QCL device.

The apparatus may comprise a plurality of light sources.

The plurality of light sources may be arranged to emit light having different respective frequencies.

The light sources may be arranged to emit light having different respective ranges of frequency.
The probe may be a temperature sensing probe.

The apparatus may be arranged to perform photothermal micro-spectroscopy.

The probe may be an atomic force microscopy probe.

The apparatus may be arranged to perform acousto-optic micro-spectroscopy.

Preferably the apparatus is arranged whereby variations between any two spectra obtained from a sample are significant to within a confidence of ninety percent.

Preferably the at least one light source is arranged to generate mid-infra-red radiation having a substantially continuous spread of wavelengths over at least a portion of the range from around 5 microns to around 10 microns.

The variations between any two spectra obtained from a sample are significant to within a confidence of ninety percent.

In a still further aspect of the invention there is provided a method of performing scanned probe micro-spectroscopy comprising: providing a probe operable to be scanned over a surface of a substrate; illuminating the substrate with at least one semiconductor light source thereby to heat the sample by absorption of infra-red radiation; detecting by means of the probe a temperature rise of the sample due to illumination of the sample by the light source thereby to obtain micro-spectroscopy data in respect of the substrate.

The method may comprise the step of operating the at least one light source in a continuous wave mode.

Alternatively or in addition the method may comprise the step of operating the at least one light source in a pulsed mode.

The at least one light source may comprise a light emitting diode (LED).

The LED may comprise Al_{x}In_{1-x}Sb.

The at least one light source may comprise a quantum cascade laser (QCL) device.
The QCL device may be a tunable QCL device.

The method may comprise the step of illuminating the substrate with a plurality of light sources.

The method may comprise the step of illuminating the substrate with a plurality of light sources arranged to emit light having different respective frequencies.

The light sources may be arranged to emit light having different respective ranges of frequency.

The probe may be a temperature sensing probe.

The method may comprise the step of operating the apparatus thereby to perform photothermal micro-spectroscopy.

The probe may be an atomic force microscopy probe.

The method may comprise the step of operating the apparatus to perform acousto-optic micro-spectroscopy.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention will now be described with reference to the accompanying figures in which:

FIGURE 1 shows a prior art arrangement of an apparatus for performing photothermal micro-spectroscopy (PTMS) in Fourier-Transform infra-red (FTIR) spectroscopy mode;

FIGURE 2 shows a prior art arrangement of an apparatus for performing acousto-optical micro-spectroscopy (AOMS);

FIGURE 3 shows emission spectra for five $\text{Al}_x\text{lni}_{1-x}\text{Sb}$ light emitting diodes (LEDs) having different respective Al compositions;
FIGURE 4 shows apparatus according to an embodiment of the invention in which a metal coated sample is scanned by an AFM probe;

FIGURE 5 shows emission spectra obtained from a commercially available quantum cascade laser as a function of operating power level in continuous wave mode;

FIGURE 6 shows a probe for use in some embodiments of the invention having an offset tip design;

FIGURE 7 shows (a) a plot of waveform as a function of time used to modulate a continuous wave source in one embodiment of the invention; (b) an isolated small object O before (left-hand cartoon) and after (right-hand cartoon) irradiation with IR radiation; and (c) waveform intensity as a function of time for illumination of wavelength A and wavelength B showing a continuous sum of intensities A and B;

FIGURE 8 shows an embodiment of the invention in which a conventional interferometer design is employed;

FIGURE 9 shows an embodiment of the invention in which a new radial interferometer design is employed;

FIGURE 10 shows a Fast Fourier Transform (FFT) of the resonant oscillations induced in a probe where these are used as the detector signal and the source is a pulsed optical parametric oscillator;

FIGURE 11 shows a plot of FTIR absorption and of PTIR absorption as a function of wavenumber;

FIGURE 12 shows data from a fuzzy analysis, where the FFT frequencies providing the best discrimination between on-sample and off-sample locations are indicated;

FIGURE 13 shows data from a fuzzy analysis in which the feature weights are used as a measure of each wavenumber enabling a spectrum to be constructed that is in good agreement with data from [ ] ;
FIGURE 14 is a plot of mean misfit as a function of postulated break point showing the measure of fit to a two-phase model allowing different diffusion lengths at different wavenumbers; and

FIGURE 15 shows spectral data obtained using a globar source and a temperature sensitive probe as detector from (a) a sample of Aerogel; (b) a 2 micrometre thick sample of PET on Aerogel; and (c) a 2 micrometre thick sample of Araldite on Aerogel.

DESCRIPTION OF THE INVENTION

The present invention comprises new approaches to overcoming the limitations suffered by existing methods. First we will consider the two types of scanning probe infrared (IR) microspectroscopy known as PTMS and AOMS. These two techniques already enable spectroscopic analysis to be performed at spatial resolutions high enough to circumvent the diffraction limit of far-field infrared microscopy [9-1, 141], but the spatial resolution, signal-to-noise ratio and clarity of data interpretation may be greatly enhanced in some embodiments of the present invention.

Moreover, embodiments of the invention also make possible the recording of IR images at sub-micron resolution to be performed. In some embodiments the source may be one two recently-described particular types of high-intensity IR light source.

The resulting temperature rise of an individual region of the sample depends upon the particular molecular species present as well as the wavelength of the infrared beam.

Differences in temperature rise between regions are detected either by a miniature temperature-sensing probe, or with an ordinary atomic force microscope probe, or with a specially modified atomic force microscopy probe used to probe the local transient deformation induced when the laser is pulsed.

Either type of tip may be mounted in a scanning probe microscope which will thereby be used to give multiple images of the sample, such that the image contrast will correspond to variations in either surface topography or chemical composition.

Techniques for the photothermal characterisation of solids and thin films are widely used [12]. Recently the value of adding spatial resolution to these techniques has become of technical interest in the general area of electronic and optical devices. Methods originally
employed suffered from the limitations imposed by the finite optical wavelengths of the
detection systems used.

Most conventional methods of thermal imaging employ an energy beam that emerges
from a small source and spreads out according to the rules of diffraction. The extent of
this spreading is normally governed by the wavelength associated with
the energy flux.

However, if the sample is within the "near-field" region, i.e. significantly less than one
wavelength away from the source, then a greatly reduced beam diameter can be
achieved. In this case, the beam diameter is not much larger than the size of the source
itself.

This principle is applied in scanning probe microscopy whereby a sharp probe is brought
in close proximity to the surface of a sample. Some probe/sample interaction takes
place. This interaction is monitored as the probe is scanned over the surface. An image
contrast is then computer generated. The image contrast represents variations of some
property (e.g., physical, mechanical, chemical) of the sample across the scanned area.
One such probe microscope is the atomic force microscope (AFM). In conventional AFM,
the height of a probe above the surface being scanned is controlled by a feedback
system. The feedback system keeps the force between the probe and the surface of the
sample constant. The probe height is monitored, and provides the data that is used to
create image contrast which represents the topography of the scanned area.

The use of miniature temperature-sensing probes, as part of a scanning probe
microscopy system, allowed the limitations imposed by diffraction to be overcome, so
that near-field scanning photothermal spectroscopy PTMS is recognised as an
established technique ([2] and numerous other publications).

The method is described in U.S. Patent No. 6,260,997 (hereinafter referred to as the
"997 patent"), to Hammiche et al., which is incorporated herein by reference, and which
discloses the use of a probe in the form of a tiny platinum resistance thermometer for
use with a scanning probe microscope. The sample is exposed to IR radiation, and
heats up. IR absorption causes photon-induced vibrations of specific molecular bonds,
such as stretching and bending. As these molecular vibrations relax, evanescent
thermal waves are emitted via a non-radiative process (Williams and Fleming 1997).
If the IR beam is intensity-modulated, as in a normal Fourier transform infrared spectrometer (FTIR), the measured temperature, and hence the output of the thermal probe, fluctuate accordingly. Moreover, variation of the modulation frequency may be used to control the depth below the surface that is being sampled.

In the FTIR version of PTMS, this time-varying output signal is amplified and fed into the external input of the same spectrometer, thus providing an interferogram which replaces the interferogram normally obtained by means of direct detection of the IR radiation transmitted by the sample. The Fourier-transform algorithm is performed on this interferogram after digitisation, in order to convert it to a photothermal spectrum by transformation from the time domain to the frequency domain to give a spectrum. So that this may be performed as a function of location on the sample surface, the probe is mounted on a simple positioning system, or, if images are required, in an AFM. In either case, an appropriate optical interface is required, to direct the spectrometer's external IR beam onto the sample and to increase the flux at the sample surface in order to increase the signal-to-noise ratio (SNR).

The IR beam is focused to a spot, which is ca. 2 mm in diameter. Probe and sample surface are brought into contact at the focal point, see FIG 1.

FIG. 1 shows an arrangement in which a surface of a sample 1 is scanned by means of a thermal probe having a thermal probe tip 2 attached to a cantilever 3. The thermal probe is manipulated by an actuator 4.

Radiation from an IR source 5 passes through an interferometer 6 and is subsequently directed towards the thermal probe tip 2 by means of a mirror 8.

As an alternative to the FTIR version of the technique, the 997 patent also describes dispersive infrared PTMS, using radiation whose wavelength has been restricted to a chosen band within the infrared region of the electromagnetic spectrum: the relevant exact wording in that patent was as follows -

"Another object of the present invention is to perform dispersive infrared microscopy at a high spatial resolution that is not diffraction-limited, using radiation whose wavelength
has been restricted to a chosen band within the infrared region of the electromagnetic
spectrum.

In one embodiment of the present invention shown in FIG. 1, the wavelength of the
incident electromagnetic radiation is restricted to a chosen band within the infrared
region of the electromagnetic spectrum using a monochromator or a tunable filter. The
data is not Fourier transformed to obtain a spectrum, but otherwise the operation of the
instrument is similar to that described above for the calculations based upon well-
established principles of photoacoustic spectroscopy.

In some embodiments, a broadband source may be provided arranged to provide a
source of illumination providing light of different wavelengths. An array of broadband
sources may be provided. Alternatively or in addition an array of two or more
narrowband sources operating at different respective wavelengths or ranges of
wavelength may be provided. The array may be arranged to simulate a broadband
source.

Thus, illumination having more than one wavelength of radiation may be supplied, in
which case an embodiment of the present invention may be worked in which an
interferometer is employed and a Fourier transform approach as described above.

Thus, both these options are open when measuring temperature and/or thermal
expansion.

PTMS: existing limitations and disadvantages

One disadvantage of the temperature-sensing types of probe is that they tend to require
a larger tip than that of a normal AFM probe, and to have a much higher cost.
Accordingly, the possibility of using the sharper and cheaper normal type of probe to
obtain an IR spectrum is attractive. Indeed, AFM-type micro-cantilevers have been used
as highly-sensitive and selective chemical sensors. One approach is to coat the surface
of the lever with a material that selectively adsorbs or binds a given target substance
e.g., cellular material. When the cantilever comes in contact with the target substance,
the interaction may cause bending of the cantilever and this will typically cause a shift in
the resonant frequency of the cantilever. For a variety of reasons, so far the use of
cantilevers as chemical sensors has involved physical contact with an analyte, thus
severely limiting the possibility of an automated, controlled and localised analysis of tissue samples or cellular material.

The possibility of using standard AFM cantilevers to obtain IR spectra has been explored by a number of research teams. For example a photo-thermo-mechanical recording method has been used [14] employing a standard AFM cantilever probe of low spring constant. The operation is relatively simple: the probe is placed a few micrometres (µm) above the surface of the sample which is illuminated by IR radiation. As the sample absorbs radiation it heats up, and a heavily-damped heat wave is generated in the layer of air in contact with the sample surface.

The cantilever, being located within this layer, vibrates. The mechanical vibrations of the cantilever are sensed as in a standard AFM, and the recorded signal is fed to the external input of an FTIR spectrometer as in a PTMS experiment. Fourier transformation then generates a true absorption spectrum.

In reference [3] a conventional AFM probe was used with the tip in contact with the sample and the sample's thermal expansion was measured thereby, instead of through a layer of air.

To summarise, we conclude that the PTMS technique as described in the 997 patent and in publications to date suffers from two serious limitations:

(i) when used to obtain spectra over a wide range of wavenumbers using a broadband light source, the only such sources considered so far are either the "globar" type commonly used in classical FTIR spectroscopy, or a synchrotron radiation source. The weakness of the globar source means that in order to obtain a sufficient signal-to-noise ratio in PTMS, co-addition of several hundreds or thousands of successively acquired spectra is sometimes required. A powerful synchrotron source has been used for PTMS [14] but access to such facilities is difficult and extremely costly;

(ii) the apparent need to use a costly and fragile temperature sensing probe (of either the thermocouple type or the resistive type), rather than the cheaper, more robust standard type of AFM probe that has also the advantage of being sharper and therefore better suited to the acquisition of data at the highest spatial resolution. The 997 patent does not include the possibility of using a normal AFM probe.
The first of these limitations may be overcome by use of one or more semiconductor light sources, such as one or more low-cost mid-infrared light-emitting diodes (LEDs). For example, one or more LEDs of composition Al$_x$In$_{1-x}$Sb or similar may be used, operating in continuous waveform mode as a broadband light source, as claimed and described below ("Apparatus"). The 997 patent does not include the possibility of using an LED source.

For many areas of research or development (one example being biomedical studies), typically one would like a laser that is tuneable over the wavelength range 5 µm to 10 µm, costing no more than a few thousand dollars and with modest space requirements.

Dye lasers are highly tuneable but their spectral range covers only part of the required spectrum, and for the infrared in particular, depend on costly difference frequency mixing techniques. Also, a dye laser tends to have a relatively short lifetime, lasting for only a limited number of pulses. A serious disadvantage of all laser sources considered so far is the fact that even if to some extent tuneable, they operate at discrete wavelengths. Consequently, unlike broadband sources used in FTIR spectroscopy, they are not suitable in situations where spectra over a wide range of wavenumbers are required.

With semiconductor laser light-emitting diodes (LEDs), the lead salt type suffers from very low power and very limited tunability. There have been very few reports of LEDs emitting beyond 5 µm, but recently LEDs having a total emitted power of 27 mW/cm$^2$ and peak emission at 5.3 µm, with a useful upper limit to the wavelength greater than 7 µm have been reported, see FIG. 3. Because these LED can be made to have a very large emitting area, the effective flux of the light onto the sample may be made to greatly exceed that obtained with a globar source, and even to a level comparable with that achieved in practice by researchers using a synchrotron IR source.

In practice the maximum flux will be that tolerable by the particular sample. By varying the aluminium content of the device it is possible to tune the peak emission wavelength (see FIG. 3).

The LED is operable in either continuous waveform mode or pulsed mode. An array of one or more mid-infrared Al$_x$In$_{1-x}$Sb light-emitting diodes in continuous waveform mode will make an excellent broadband light source for PTMS FTIR spectromicroscopy, in
order to obtain spectra over a wide range of wavenumbers at high signal-to-noise ratio. No refrigeration or vacuum technology is required for the operation, and the fabrication cost is low enough that an array of such diodes could in practice cover the whole spectrum of interest for many applications. It is to be understood that LEDs made of other materials and other devices are also useful.

The actual setup can profit from the fact that each Al$_x$In$_{1-x}$Sb LED may be arranged to emit light from a relatively small area (such as a 1mm-square facet), so that several of these facets may be combined into an array of any suitable shape. Such a shape may be designed with such a geometry as to maximise the intensity of the light received by the sample.

Such an array of one or more of the same type of diode, used in either pulsed or continuous waveform mode, will also serve as an excellent light source for AOMS-based spectromicroscopy.

We also include another embodiment of the present invention involving the use of an array of quantum cascade laser light sources. To overcome the second limitation, we include the possibility of measuring the thermoelectric signal resulting from contact between a normal metalized AFM probe and a metal-coated sample, see FIG. 4. This would have the very significant cost and resolution advantages mentioned above.

FIG. 4 shows an embodiment in which a sample 11 has been coated with a coating 11c. A metallised AFM probe comprising a cantilever 13 having a tip 12 provided at a free end thereof is arranged to be positioned at a location of the coating 11c. The coating 11c is pierced by the tip 12 to form a hole h, the tip 12 then being positioned in contact with the edge of the hole h. The tip 12 is ideally positioned gently at the edge of the hole h so as not to cause further perforation.

A beam of IR illumination is arranged to strike the sample surface through the hole h.

It is to be understood that the hole h need not be a through-hole in that it passes through the entire thickness of the coating 11c.

Quantum cascade laser light sources (QC devices) use transitions between sub-bands created by quantum confinement in a semiconductor heterostructure. In the so-called
distributed feedback-type, a grating is etched in the active region, to force the operation of
the laser at a very specific wavelength determined by the grating periodicity. As a consequence,
the laser operates at a single frequency that may be adjusted slightly by changing the temperature of the active region.

QC devices have been shown to be capable of operating at wavelengths ranging from below 4 μm up to tens of μm. One commercial supplier offers a QC device that achieves 4-12 microns. The required instrumentation includes pulse generator, laser diode driver, laboratory laser housing, and power supply of internal cooling elements via temperature controller. Tuning is performed by varying the temperature of the active region, but each laser has a limited tuneability, the tuning range being a few percent (from 1% to 8%) of peak emission wavelength. However, use of a combination of two tuning methods, coarse (achieved by rotating the grating) and fine (changing the cavity length and the laser chip temperature), to obtain a wavenumber range of 175 cm⁻¹ around a centre frequency of 1850 cm⁻¹.

Despite the limited tuneability, in principle a dozen such lasers could be custom-manufactured, each delivering a different wavelength. In some arrangements a broadband source is simulated by changing the emitted wavelengths and this would enable the use of an interferometer with the benefits for signal to noise and speed of data acquisition that such an approach provides.

Another recently-described approach to infrared micro-spectroscopy (the acousto-optic microspectroscopical AOMS method [9, 10]) is an extension of the thermal expansion measurement demonstrated by Hammiche et al [3]. It also uses a normal AFM probe, thereby avoiding the need for a temperature-sensing probe. Chemical mapping of various objects with sub-wavelength lateral resolution is achieved by using an infrared oscillatory signature characterizing different molecular species. The AFM tip is used to probe the local transient thermal expansion induced by an infrared-pulsed laser tuned to the absorbing wavelength of the sample. Pulse heating of the sample is used to generate a thermal expansion-time profile, whose shape contains information on the local thermal expansion coefficient, and also the thermal diffusivity (from the rate of decay of the expansion).

The tip oscillates at resonant frequencies whose amplitude can be correlated with local absorption. Under favourable conditions the system acts as an amplifier of extremely
small motions induced by optical absorption and the different frequencies provide different information, leading to a full description of the sample deformation.

FIG. 2 shows known apparatus for performing AOMS. The apparatus has a probe provided by a cantilever 23 having a tip 22 arranged to be scanned across the surface of a sample 21 by means of an actuator 28. The acoustic wave 21a generated by thermal expansion of the sample causes oscillation of the probe 22 at a resonant frequency of the probe.

This photoacoustic method has to date used pulsed laser irradiation [8-11]. Typically, when using a free electron laser, each macro-pulse contains about 500 micro-pulses of ~2 ps long [9-10]. When using an optical parametric oscillator (OPO), single pulses of around 10 ns are typical [11].

As outlined above, in one version the sample is deposited on the upper surface of a ZnSe prism and the pulsed IR laser illuminates it from below at the total internal reflection angle (30°) [8-9], in another the sample is illuminated from above [1-7, 10-11]. The pulse produces a very rapid transient local absorption-induced heating of the sample, giving an acoustic wave that propagates up the AFM cantilever. Detection of the transient resonances of the cantilever vibration can be a much more sensitive way of quantifying the surface thermal expansion of the sample than measuring this directly.

Depending on the Q of the cantilever, the duration of the signal is a few hundred µsec. Because the time response of the AFM feedback loop is too slow to compensate for the transient deformation induced by the laser burst, separate images representative of the topography and of local IR absorption can be generated.

Moreover, the effect of thermal diffusion, which could limit spatial resolution, may be reduced. The wavelength spectra are obtained by recording the FFT power density spectra of the cantilever deflection as a function of wavelength.

**AOMS: existing limitations and disadvantages**

The AOMS method described in the literature to date has disadvantages and limitations. The main problem is the availability of sources that cover the required mid-IR range of the electromagnetic spectrum. The IR sources considered so far are either a free-
electron laser source (FEL) [9-10], or a single-wavelength tuneable laser such as an optical parametric amplifier (OPA) [8, 11]. Unfortunately, free electron laser facilities are few and far between. All the published work by Dazzi's group using this method has been done with a free-electron laser. Clearly such a source cannot be part of a commercial product. As an alternative, the OPA type of bright tuneable source employs an optical parametric oscillator (OPO) [15], i.e., an oscillator operating at optical frequencies whose resonance frequency and damping parameters vary in time in a defined way. Nonlinear optical interaction is used to convert a near-\(\lambda\) monochromatic input ("pump") laser wave into two output waves of lower frequency, via a three-wave mixing process. This process is known as optical parametric generation (OPG).

The heart of an OPO is a nonlinear-optical crystal, consisting, for example, of epitaxially-grown GaAs, AgGaS\(_2\), lithium niobate, or ZnGeP\(_2\). In this crystal, the pump photon decays into two less energetic photons (signal and idler). Tuneability is achieved by using a crystal of periodically-modulated orientation, which thereby behaves as an artificially-created grating of optical nonlinearity: the period of this grating determines the output wavelengths of the OPO.

The resultant OPA source of laser radiation can thus emit light of variable wavelengths. Tuning either the pump wavelength or moving the nonlinear-optical crystal or changing the temperature of the crystal allows the mid-IR output to be tuned over a wide range, e.g., from 0.5 \(\mu\)m to as much as 10 \(\mu\)m if provision is made to allow a choice of OPO crystal. Typical specifications include a pulse of typical length in the tens of ns range, delivered at a repetition rate of 1 kHz.

Submicrometer-scale infrared surface imaging has been achieved using such a source [11]. But again, all such commercially available lasers cost six-figure amounts, and have difficulty supplying sufficient power at the longer wavelengths where most of the interesting spectral features are usually to be found. Consequently, the widespread use of these methods is severely restricted.

The two Dazzi papers quoted above were published before any details of that method were patented, the first over 1 year before the first patent application was filed. Since those papers appeared, a U.S patent application (U.S. 2008/0283755) and international application WO 2008143817 have been published that describe various aspects of the
experimental setup, including the use of an OPG source needed to create a commercial version.

It should be noted that so far, all sources mentioned in connection with the AOMS method have been used some type of laser, in pulsed mode: in contrast, the use of a source in continuous waveform mode would have several advantages as regards the elimination of spurious signal components etc.

Again, the source may consist of the previously-mentioned type of array of one or more low-cost mid-infrared diodes of composition AlₓIn₁₋ₓSb (see FIG. 3) or similar, although other devices are also useful. The need to use an FEL or an OPA, or indeed a laser of any kind may therefore be avoided.

Moreover these devices can be used in pulse mode (as described in AOMS publications to date) and a continuous wave mode, which constitutes a further very substantial advantage. Another of our inventions included here, involving the use of an array of quantum cascade laser light sources some of which are broadband such as Fabry-Perot and others of which are narrow band and can be tuned, see FIG 5) will also provide another promising alternative to an FEL or an OPA and can be used both in pulsed and continuous wave modes.

Continuous wave modulation has already been demonstrated for PTMS [1-2] and when directly measuring thermal expansion [3]. There are also a number of advantages that can be derived from the use of continuous waves when practising AOMS.

Pulsed sources have been used to date but the choice of a pulse has been dictated by the limitations of the hardware. The OPO has to be pulsed as continuous wave mode is not available, furthermore each pulse is of the same duration. However, there are advantages when the intensity of the incident radiation is modulated in a way that ensures a broad range of frequencies.

This condition may be fulfilled by having pulses (preferably of variable duration) that constitute an approximation to a Dirac delta function, a sequence of sine waves of varying frequencies, a complex waveform composed of many simultaneous sine waves, saw-tooth modulation or stochastic (or 'white noise') modulations. Other waveforms are also useful.
One advantage is that this enables a more complete characterisation of how the measuring system itself functions (see below). Another is that it can substantially increase the quantity and quality of information that can be obtained for thermal expansion measurement whether by the direct measurement of thermal expansion and contraction [3] or the AOMS method [9, 10].

Furthermore, not only is it possible to have a continuous wave that defines the changes in intensity of the light as a function of time, i.e. amplitude modulation or AM, but frequency modulation or FM can be employed in addition or instead.

Having an array of sources enables the total light intensity to be increased by having more than one of the same type of source. Because each semiconductor light source covers only a limited part of the mid IR range, having more than one source can also enable the total wavelength range to be extended.

In addition to these advantages the use of 2 or more sources simultaneously confers a significant advantage.

How the advantages can overcome limitations and disadvantages of the existing technique will be explored below.

Pulsed lasers typically have a duty cycle of the order of a few percent. This reduces the signal that can be measured because there is a substantial amount of dead time. In the case that a continuous wave is employed this problem is eliminated. The signal to noise ratio can therefore be improved because there will be more events to average in a given period of time.

For AOMS to work, rapid thermal expansion of the sample must be induced in order to create an acoustic wave. A pulse might therefore be considered to be the optimum waveform.

However, very rapid expansion might mean that inertia of the probe cannot be overcome. As a consequence the sample may flow around the tip thereby eliminating or reducing the amplitude of the acoustic wave and thereby the apparent absorption. The sample may also suffer damage.
One solution would be to diminish the intensity of the beam, however this also reduces the amplitude of the acoustic wave. It follows that there is an optimum rate of increase of intensity and this can be achieved with a continuous wave source.

A typical waveform might be a linear increase in intensity with time where the rate of rise can be varied to determine the optimum value for a given material. This is illustrated in FIG. 7 (a) where the rate of increase and decrease of intensity of successive periods of the waveform can be seen to change from pulse to pulse. It is to be understood that such an approach is not possible with a pulsed source.

In the example shown in FIG. 7(a) both the rate of increase and the rate of decrease of intensity change from period to period; in this example the rates and the length time at maximum intensity are arranged such that the total amount of power in each successive period of the waveform remains substantially constant from period to period. (It is to be understood that reference to period does not necessarily imply that the periods are of equal length, although in many embodiments this will be the case).

Other arrangements are also useful. For example, the rate of increase or decrease of intensity may be changed from one period to another period but the rate of decrease or increase held constant from one period to the other.

Continuous waves enable waveforms that have relatively long periods at high intensity. Why this is an advantage is illustrated in FIG 7(b).

A problem when using AOMS is that of the isolated small object surrounded by material. When the material surrounding the isolated small object expands, as the result of absorbing a pulse, even when the isolated object does not absorb it appears to do so because the motion of the surrounding material affects it. In the example shown in FIG. 7(b), following irradiation and expansion of the surrounding medium, it can be seen (right hand figure) that the object is compressed such that it expands in a direction normal to the surface of the sample.

We now consider a waveform that consist of continuous illumination until steady state or quasi steady state is achieved. An example of such a waveform is shown in FIG. 7(c).
If, instead of the rapid increase in intensity from zero quickly followed by a return to zero that characterizes a pulse, we have a rapid decrease in intensity from that at which a steady state or quasi steady state has been achieved, followed by a rapid return to the previous (steady state/quasi steady state level), then this may produce an acoustic wave because of a rapid contraction followed by a rapid expansion or it might not.

If it is the isolated small object that is absorbing while the surrounding material is not then the small object can rapidly cool and contract when the incident radiation decreases because of its high surface area to volume ratio. It then rapidly expands again when the illumination resumes its previous intensity resulting in the generation of an acoustic wave.

However, if it is the surrounding material that absorbs while the object does not, then since the small object has a much lower surface area to volume ratio it cannot rapidly cool. As a consequence when the intensity increases substantially no acoustic wave is generated. In some examples a smaller acoustic wave is generated compared with that generated when applying a rapid increase in temperature when the entire sample is at ambient temperature.

The rate at which the intensity decreases can be changed and as it becomes slower larger objects may produce an acoustic wave.

In this way the downward spike in intensity becomes a powerful tool for probing the surface area to volume ratio of an absorbing component and thereby its size.

Now, returning to the problem of the isolated small object, whether it is the object absorbing the incident radiation or the surrounding material can be determined by the cooling spike and with this information a spectrum for the object can be determined that is not contaminated with that of the surrounding material. This is something that cannot be achieved with a pulse. Combining cooling spikes with heating spikes enables a comparison between the two to be made. This can be achieved by the complex continuous waveforms illustrated in FIG. 7(c). It is to be understood that it is only with a continuous wave that this can be achieved.

There are numerous advantages associated with using top down illumination rather than the ATR configuration. The latter requires that the sample be thin, typically 1 micrometer
or less, this usually requires sample preparation such as microtoming including cryo-
microtoming. Many samples are too friable to be microtomed.

The performance of this configuration crucially depends on the thin film being in good
contact with the ATR crystal. Microtomed sections often are curved and/or contain kinks
and therefore large areas of the sample may not be in good contact with the crystal. This
suggests that top down illumination would usually be preferred, however, an observation
that was made in [11] was that, with this configuration, even when the sample was not
absorbing resonant motion was induced in the cantilever.

This then constitutes an impediment to quantifying the sample absorption, which is an
objective of the experiment. A method based on simple inspection of the data was
proposed in [11]. A much more sophisticated and novel mathematical approach is
described below.

Here we describe a hardware solution to this problem that could, if necessary, be used
together with the sophisticated mathematical approach.

The observation made in [11] means that a pulse of substantially any wavelength
produces a resonant motion in the cantilever of significant amplitude. For the reasons
given above, a pulse is not the optimum waveform, however, this motion is a response to
a fast deformation and so even rapid non-pulse waveforms create the same or a similar
effect.

A solution to this problem is to have two sources at two different wavelengths A, B
operating simultaneously. In the simplest case one of the wavelengths would be one at
which the sample beneath the tip does not absorb while the second would be at a
wavelength at which the sample beneath the tip does absorb.

If the intensity of the first laser (wavelength A) is increased in concert with the decrease
in intensity of the second (wavelength B) then, in terms of total power, there is no
change, rapid or otherwise. Thus, where the probe response is the same at any
wavelength (or at least at wavelengths A and B) or where any differences have been
calibrated and accounted for, if the sample does not absorb there will be no response
from the cantilever thus greatly simplifying that task of distinguishing between cantilever
motions that derive from sample absorption and those that are unwanted.
If the sample does absorb at one of wavelengths A or B but not the other, then cantilever motion may be observed.

As an alternative to the synchronous changes described above, alternating waveforms can be used where differences between the absorbing and non-absorbing behaviour may be arranged to be due to sample absorption. Such differences can be assessed using the mathematical methods described below, see FIG. 7.

It should be noted that all of the above benefits of using a source operated to follow a continuous wave can be achieved using a continuous source of invariant intensity together with a separate device to control the intensity such as an optical chopper or a light valve such as Micralyne's spatial light valve. Such valves can achieve modulation frequencies in the MHz regime and significant increases in intensity on time scales of the order of tens of nanoseconds.

A combination of an invariant source and these types of devices is functionally equivalent to directly controlling the intensity of the source by, for example, controlling the supplied current. This approach can also be used with a wideband source such as a globar.

Above we have considered how broadband semiconductor sources, such as LED's and some kinds of QCL, can be modulated in both AM and FM modes and how they can be subject to complex continuous wave forms at high speeds to facilitate the measurement of thermal expansion through the AOMS approach and/or direct measurement of thermal expansion.

It should be noted that the present inventors have recognised that substantially all types of sources could be used in combination.

Where a broadband source is used or an array of broadband sources there are advantages to using an interferometer when measuring temperature and/or thermal expansion and/or resonant motion of the cantilever as a function of mirror position. These advantages include that all wavelengths are measured simultaneously so the wavelengths are not swept as is the case of a dispersive measurement. This means
that the time required to acquire a full spectrum is considerably reduced compared to the
case where a single source is used.

Another advantage is that the application of multiple sources means greater incident
power can be achieved and this increases signal to noise.

In [11], where a single OPO source was used, we see a method of measuring a
spectrum of infrared absorption of a sample on a sub-wavelength scale comprising the
steps of: optically coupling a benchtop source of coherent infrared radiation to a region
of a sample, modulating the intensity of the radiation incident on the sample at a
frequency of 100 1kHz, interacting a cantilever probe with a tip with a sub-micron end
radius with the sample such that the probe responds to IR radiation absorbed by the
sample, sweeping the wavelength of the infrared radiation supplied by the benchtop
source over the range of the non-linear crystal in the OPO, a range of roughly
250cm\(^{-1}\), and detecting a probe response to modulated infrared radiation absorbed by
the sample as a function of wavelength.

Although no information is given in [11] on the rate at which the wavelengths were swept
one of ordinary skill in the art would know that the nonlinear-optical crystal can be
translated through its entire range in less than a second. At a pulse rate of 1kHz this
would be adequate to take sufficient points for the spectrum.

Daylightsolutions, a manufacturer of tuneable QCL in a press release on November 2
2009 said

"Daylight Solutions, Inc., the leading manufacturer of advanced molecular detection and
imaging solutions in the Mid IR, today announced that it has extended its own previous
world record for extreme tuneability in a solid state, mid-infrared laser source. By
integrating the laser into the company's Swept Sensor\textsuperscript{TM} OEM module, customers can
now collect over 300 wave numbers of spectra in a single sweep."

It can be seen that a single laser sweeps through its available wavelength (wave
number) range, the probe response to the absorbed radiation is detected as the
wavelength is swept and thus the response is detected as a function of wavelength.
In contrast, when using multiple sources simultaneously within an interferometer, the response is not detected as a function of wavelength, indeed it is not clear what this means when more than one wavelength is simultaneously incident on the sample. Instead, the probe response is detected as a function of mirror position. Wikipedia provides the following description:

"Fourier transform infrared (FTIR) spectroscopy is a measurement technique that allows one to record infrared spectra. Infrared light is guided through an interferometer and then through the sample (or vice versa). A moving mirror inside the apparatus alters the distribution of infrared light that passes through the interferometer. The signal directly recorded, called an "interferogram", represents light output as a function of mirror position."

By using an interferometer and an array of broadband sources there is no need to sweep the wavelengths of the sources and so the time taken to acquire a spectrum can be much smaller for a given intensity of radiation.

Furthermore, when an array of sources includes tuned QCL's then these are equivalent to a special type of broadband source, many narrowband sources all emitting a different wavelength simulate a broadband source, and so the same process applies. Once the data has been acquired the spectrum is calculated by applying the Fourier transform method.

In USPTO patent application number US2009/0249521, claim 16 says:

"16. A method of obtaining a spatial map of infrared absorption of a sample with sub micron resolution using a cantilever probe with sub-micron radius, comprising the steps of: bringing the probe tip into interaction with a surface of the sample; generating relative lateral motion between the probe and the sample; directing infrared radiation from a benchtop source towards an area of the sample; detecting a probe response to absorption of infrared radiation by the sample as a function of relative probe-sample position; and, creating a spatial map from the detected probe response indicative of infrared absorption of a region of the sample, wherein the map has a pixel density of at least 100 x 100 pixels and the map is completed in a time of less than thirty minutes."
In [4-7] we see how a benchtop source was modulated at greater than 4kHz and images with submicron resolution of more than 100x100 pixels were obtained. No mention is made of the speed at which the image was acquired but one of ordinary skill in the art could conclude that, given the rapidity of the photothermal measurement then each image would take no more than a few minutes at most. When an interferometer is used then its ability to measure a spectrum rapidly opens up the possibility of acquiring an image with an entire spectrum for each pixel. Alternatively, when a plurality of sources is available then these can be used in rapid succession more rapidly than a single source can retune. By having a response at more than one wavelength or wavelength range better discrimination can be achieved between different materials.

A challenge that must be met when using multiple sources with an interferometer is how to get all of the sources focused on the region around the tip.

If a conventional interferometer is used then each source can be directed onto a separate mirror such that each source is focused onto the same location; instead of individual mirrors one mirror with many facets could be used. Such an embodiment is shown in FIG 8.

In the arrangement of FIG. 8 three light sources 105A, 105B, 105C are shown arranged to direct respective beams A, B, C of radiation to a beam splitter device 120 in the form of a half-silvered mirror oriented so as to reflect a portion of each beam towards an adjustable reflector member 140. In the embodiment shown the splitter device 120 is arranged to reflect the portion of each beam through an angle of substantially 90°.

Other arrangements are also useful.

A remaining portion of the beam passes through the splitter device 120 and follows a path of substantially fixed length to a fixed reflector member 130 which reflects the beam back towards the splitter member 120 along a substantially reciprocal path. A portion of the beam is reflected by the splitter device towards a focussing mirror 150.

The adjustable reflector member 140 is also arranged to reflect radiation incident thereon from the sources 105A, 105B, 105C reflected by the splitter member 120 towards the focussing mirror 150 through the splitter member 120. Thus, portions of the beams A, B, C incident on the focussing mirror 150 have a path difference determined by the distance of the adjustable reflector member 140 from the splitter member 120.
The focussing mirror 150 is arranged to focus the beams A, B, C onto the sample 110 which is typically mounted on a support 115. A probe 160 is arranged to be scanned over a surface of the sample 110 as described above.

In the embodiment shown top-down illumination is employed. Other arrangements are also useful including ATR illumination.

Alternatively a radial interferometer may be used. Such a device is illustrated in FIG. 9. This has the advantage that more sources can be used without overcrowding since a relatively compact arrangement of sources and other components may be achieved.

In the embodiment shown the apparatus is configured for operation in ATR mode. Either ATR or top down illumination can be achieved in this and the non-radial device of FIG. 8.

In the embodiment of FIG. 9 the ATR prism 290 has five sides. Other numbers are also useful. An arbitrarily large number of sides may be employed in order to accommodate an arbitrarily large number of light sources.

In the embodiment shown five light sources 205A-E are arranged circumferentially about the ATR prism 290 in the manner of a rim of wheel about a hub as shown in the plan view illustration of FIG. 8(a). The sources 205A-E are arranged to direct respective beams of illumination towards the ATR prism. A sample 210 may be mounted on a surface of the prism 290 such that the sample is illuminated by radiation passing through the prism. The illumination is arranged at an angle such that it experiences attenuated total reflection.

Light from each source is split by a respective splitter device 220 and directed towards either a fixed reflector member 230 or an adjustable reflector member in the form of a radial adjustable reflector member 240.

Adjustment of the radial adjustable reflector member 240 allows a distance of the adjustable reflector member 240 from the splitter device 220 to be varied thereby to vary a path length of the respective beams split by the splitter device 220.
The adjustable reflector member 240 is provided with a plurality of facets, the number of facets corresponding to the number of illumination sources for which independent adjustment of path difference between divided beams of each source is required.

In the arrangement of FIG. 9 it is to be understood that adjustment of the path difference may be effected by movement of the adjustable reflector member 240 towards or away from the ATR prism 290.

Further limitations in the AOMS technique need to be addressed. False image contrast can arise through non-IR absorption-related effects, such as variations in thermal expansion coefficient or mechanical properties. Still more important is the fact that not all the detected transient resonances of the cantilever vibration will arise through the local absorption-induced heating of the exact region of the sample that is being probed by the tip itself at any given time. Accordingly, the signal obtained at any instant is spectrally "contaminated" by signals generated by sample regions lying beneath the cantilever (and not the tip of the probe) but not intentionally being probed at that time.

We include three additional inventions that will further improve the quality of the highly localised spectral data obtained by the AOMS method, as explained below ("Apparatus"). One of these is to coat the sample with a film of gold to reflect the incident IR light (this would also be effective for PTMS). The other is to employ an AFM probe of offset-tip design instead of the normal type of probe (see FIG. 6).

We have seen that in the AOMS technique, not all the detected transient resonances of the cantilever vibration will arise through the local absorption-induced heating of the exact region of the sample that is being probed by the tip itself at any given time. Accordingly, the signal obtained at any instant is spectrally "contaminated" by signals generated by sample regions lying beneath the cantilever but not intentionally being probed at that time. Dazzi's AFM uses V-shaped cantilevers, which have very low torsional compliance. The fundamental mode (mode 0) corresponds to a frequency of 55 kHz and the two arms of the V-shape oscillate in phase. However, due to the V-shape, the deformation is not perfectly sinusoidal.

The mode number 1 has a frequency of 90.2 kHz and the oscillations of the two arms are in opposite phase. Higher modes exhibit the same behaviour: even modes are symmetrical (arms in phase) and odd modes are anti-symmetrical (arms in opposite
phase). Even modes are expected to be excited by a normal displacement of the sample, but the odd modes are likely to be sensitive to lateral deformation.

One object of this invention is to use a type of probe that has the opposite characteristic: by exploiting the offset-tip design, which has recently become readily available commercially (see Fig. 6), high torsional compliance may be achieved.

Detecting the resulting torsional resonance, instead of the flexural resonances used by Dazzi, has advantages for the purposes of quantifying the thermal expansion of the sample.

Firstly, the fact that only the tip, and not the body of the cantilever, is offset, will greatly reduce the proportional effect of heat transmitted from parts of the sample that are not in contact with the tip. Secondly, the torsional motion of the cantilever typically has much higher bandwidth than its flexural motion, so that many harmonics could be observed simultaneously at lower attenuation and higher signal-to-noise ratio, once the tip has been positioned laterally at an off-axis position on the cantilever.

A further approach involves using sophisticated mathematical techniques for signal processing.

Before considering what can be achieved with data processing we must first consider the problems we are seeking to address. Existing methods have provided positive results but there are four fundamental problems that they often encounter:

1. The first is that the detection of sufficient signal to enable good quality images and spectra to be obtained in a reasonable time can be a difficult.
2. The second is that there are many important cases where some responses that are detected do not relate to the sample.
3. The third is that material underneath the cantilever can induce detected responses thus mixing the spectral information from the material beneath the cantilever with that of the material of interest where the material of interest is defined as that is in contact with the tip of the probe or very close to the area of contact, ideally within 50nm or less.
4. The fourth is that absorption by materials adjacent to the material in contact with the tip of the probe can cause a measured response and in this way spectral
information from objects near the material in contact with the tip of the probe can become mixed with the spectral information of interest i.e. as defined above.

These problems decrease the value of potentially commercialisable instruments based on the methods of illumination and detection outlined above. Some solutions to some of these problems have already been identified, and examples are given above. The present invention addresses how these issues can be addressed by mathematical techniques combined with appropriate calibration procedures.

We can consider that the measured response from the probe to any arbitrary function of incident radiation will be the convolution product of a function that describes the behaviour of the measuring system and the response of the material of interest (that is in contact with the tip of the probe or very close to the area of contact) plus any response from adjacent material that is mixed with the response of the material of interest. Ideally the material of interest should be within 50 nm of the contact area or less. Thus, transforming the signal from the time-domain \( t \) to the frequency domain \( \omega \) via a Fourier transform, we have:

\[
\Psi_{\lambda m}(\omega) = g^*(\omega) \cdot \Theta_{\lambda}(\omega)
\]

Where:

\[
\Psi_{\lambda m}(\omega) = \text{the (Fourier transform of the) measured response at wavelength } \lambda
\]

\[
g^*(\omega) = \text{the Fourier transformed function } G(t)
\]

\[
G(t) = \text{the response function ('point-spread function') of the detection system-sample combination}
\]

\[
\Theta_{\lambda}(\omega) = \text{the (Fourier transform of the) amount of energy absorbed by the material giving rise to a measured response at wavelength } \lambda
\]

\[
\Theta_{\lambda}(\omega) = (\Theta_{\lambda,1}(\omega) + \Theta_{\lambda,2}(\omega))
\]

Where \( \Theta_{\lambda,1}(\omega) = \text{the energy absorbed at the wavelength } \lambda \text{ by the material of interest plus any energy transmitted to the material of interest by adjacent material that is closely coupled to the material of interest} \)

\( \Theta_{\lambda,2}(\omega) = \text{the energy detected by the probe that comes from sources other than} \)

\( \Theta_{\lambda,1}(\omega) \)
\[ \Theta_{\lambda u}(\omega) = (\Theta_{\lambda d}(\omega) + \Theta_{\lambda r}(\omega)) \]

\[ \Theta_{\lambda d}(\omega) = \text{the energy at wavelength } \lambda \text{ transferred to the probe in the absence of a sample that absorbs at wavelength } \lambda \]

\[ \Theta_{\lambda r}(\omega) = \text{the energy at wavelength } \lambda \text{ absorbed by sample material remote from where the area where probe tip contacts the sample surface i.e. not the material of interest or material adjacent to the material of interest and closely coupled to it} \]

The relationships implied by equations 2 and 3 can be determined using techniques that determine the correlations between the observed response of the probe when the probe is in contact with a material that does not absorb radiation and the observed response of the probe when in contact with samples with known absorption spectra i.e. model and/or calibration samples. This enables us to address problems 1-3 described above.

This is an improvement over prior art because it is all based on detecting a single signal and assuming it is related to the amount absorbed by the material of interest, this is typically the height or area of a single peak (however selected) or the amplitude and phase of a single sinusoidal signal. In our approach we take the entirety of all the information the detection system can provide and measure to what degree each part of the response correlates with the behaviour of the material of interest. In this way we maximise the signal to noise ratio while minimising or eliminating unwanted information.

Addressing problem 4 is a different type of problem that requires a different approach. This can be expressed as:

\[ \Theta_{\lambda u}(\omega) = (\Theta_{\lambda ct}(\omega) + \Theta_{\lambda cm}(\omega)) \]

Where \( \Theta_{\lambda ct}(\omega) = \) the energy absorbed at the wavelength \( \lambda \) by the material of interest, i.e. material that is in contact with the tip or is very close, for example 50nm distant

\( \Theta_{\lambda cm}(\omega) = \) the energy detected by the probe that comes from material adjacent to the material of interest, for example a distance greater than 50nm

Clearly the definition of 'very close' poses problems. A realistic minimum distance might be of the order of 10nm, the approximate diameter of a typical AFM probe tip, and this might be achievable in some cases. In other cases the material adjacent to the material of interest might absorb strongly and be strongly coupled to the material of interest in
which case the signals detected by the probe will be strongly influenced by the adjacent material and determining the magnitude of the energy derived solely from the material of interest would be problematic. In other words the spatial resolution is degraded and the area contributing to the measured signal might extend hundreds of nm distant from the probe tip. To address this problem a different mathematical technique is required thus we will divide our approach into two distinct processes:

Process 1) Apply procedures that minimise the influence of signals not derived from the material of interest or material adjacent to the material of interest. These might arise from absorption by the probe itself or material beneath the cantilever many tens of microns away from the area of contact of the tip with the sample. These procedures are essentially methods of determining the degree of correlation between the measured signal and the known behaviour of model samples. In this way the effects of noise and responses that are artefacts are minimised or even completely eliminated.

Process 2) Having applied process 1), where necessary, a model is fitted to the data that takes account of the physical coupling between adjacent materials in a way that enables the contributions from material closer to the area of contact of the tip to be disentangled from the response of material that is farther away; the objective being to determine the energy absorbed purely by material as close as possible to the area of contact.

In some cases process 1 might not be necessary; for example a probe that directly measures temperature fluctuations might only be significantly sensitive to material in contact with the tip or very close to the area of contact and it might also not absorb the IR radiation. In other words there are no strong signals derived from the probe itself or from beneath the cantilever. In other cases process 1 might be essential if images and/or spectra of good quality are to be obtained. A good example of this is when using induced resonant motion (AOMS) when illuminating top-down with an OPO [11]. It has been shown that in this experiment it can be the case that the majority of the response of the probe derives from material other than that close to the area of contact of the tip.

To illustrate our invention we have chosen this exemplar for process 1.

In some cases process 2 might not be needed or not be possible but it generally will be desirable where it can be achieved. To illustrate process 2 we have chosen the example of using a temperature sensitive probe with a globar source as this represents the most challenging example because every wavelength has a different modulation frequency
and thus a different degree of coupling to adjacent material. This is an improvement over the prior art because it has not sought to address this problem at all.

Process 1

To illustrate this concept we will take the example of a bench-top OPO used with the induced resonance method of detection (AOMS) with top down illumination analysed using a fuzzy logic method. Figure 10 shows the fast Fourier transform (FFT) of resonant oscillations induced in a probe where these are used as the detector signal and the source is a pulsed OPO.

Five main peaks can be identified in the plot. Peak 4 was judged to be most responsive to IR absorption.

The sample was a polystyrene (PS) particle on mica. A total of ten positions were measured: five positions on the sample (positions 1 to 5) and 5 positions off sample (positions 6 to 10). For each position, a total of 20 measurements were taken at different wavelengths.

Similar results have been published in the literature [11] and the scheme that was adopted was to identify a peak by inspection that was shown to be characteristic of the absorption of incident radiation and the magnitude of this peak (this might be peak height or peak area) was measured as a function of wavelength of incident radiation to obtain a spectrum and as a function of position to obtain an image.

The data of FIG 11 shows how this approach is not always successful. Whilst there is a peak at about 400 Hz that is present when the tip is in contact with an absorbing sample but not present when it is not, the position and magnitude of the peak does not show sufficient stability and quality in terms of signal to noise to allow for good spectra to be obtained. When we attempt to construct a spectrum from the height of peak 4 the result is poor as illustrated in FIG. 11.

It can be seen from the comparison of a spectrum obtained from a bulk sample of polystyrene, which does show the absorption peaks due to CH stretch with that obtained from our polystyrene particle, the agreement is poor. In contrast, when we construct a fuzzy logic model we can obtain much better agreement. FIG.s 12 and 13 show that, by
using the fuzzy logic model a much better match to the known spectrum is obtained. Thus, constructing a fuzzy logic model based on all of the information increases signal to noise and therefore the quality of spectra and images for a given acquisition time. Of course, the signal/noise ratio can always be improved by taking more time and averaging results for a given wavelength and/or position but our objective is to minimise the acquisition time but still obtain good signal to noise.

This approach addresses the first 3 problems described above;

- It increases (and in some cases maximises) the signal to noise and quality of spectra (and thus mapping also) by exploiting all of the data that is acquired rather than confining attention to only one frequency.
- At the same time we can see that there are responses that are measured but are not related to the absorption properties of the sample, such as peak 1 in the above example, thus these data are effectively ignored when we analyse the data for an unknown.
- By acquiring data for a given material when the cantilever is above another material that absorbs radiation in the spectral region of interest and above a material that does not, the fuzzy logic model will identify those modes that are best correlated with the spectral information of the material in contact with or very close to the area of contact of the probe tip with the sample.

The approach we have described is perfectly general. It can be applied to substantially all types of source and methods of detection and directions of illumination.

It is well known that the mechanical properties of the portion of the sample in contact with the probe tip will affect the resonant behaviour of the probe; model samples with different mechanical properties can be used to incorporate this variable into the model.

It can also be applied to methods that simply leave the probe always in contact with the sample and dynamic methods that move the probe periodically in the direction normal to the surface, provided that unknown samples are analysed with the same configuration and methodology used to construct the model.

Although we have used fuzzy logic, a variety of other techniques known to those skilled in the art could also be used such as principal components regression, ridge regression and continuum regression. Further, iterative approaches with more than one step can be
used, and are likely to be particularly appropriate. These methods can also be applied when using the offset tip illustrated in FIG. 6 where the cross-talk between the cantilever normal and torsional modes can be characterised.

5 The reason why such algorithms are likely to work well here is that a large component of the measured signal is likely to represent systematic - but uninteresting - effects, rather than purely 'noise'. Therefore, it is important to track it in the model (so that it can be removed). An algorithm designed to model first the one and then the other will fit best with the reality of the situation. Hybrid algorithms such as a combination of fuzzy logic with principal components regression would be fruitful.

As with all such techniques, pre-processing the data can be advantageous such as normalisation, baseline subtraction etc.

15 Process 2

The general problem is most easily considered as a boundary problem between two chemically different phases such as shown in FIG. 8. Conventional chemometric analysis, in this case hierarchical cluster analysis (HCA), enables the boundary between the two materials to be delineated with a spatial resolution of circa 500nm. However, the well know property of thermal waves, that they travel and attenuate in an exponential manner, usually characterised as the thermal diffusion length, means that spectral mixing will occur some distance from the boundary. Clearly this issue will apply when the detection method being used is temperature but the same general behaviour can be ascribed to the case where thermal expansion is being measured either directly or by AOMS.

Here we will consider the problem with a temperature sensor as the detector and a broadband IR source hence an interferometer was used. Again top-down illumination is being used.

We take the set of data and ask the question, can it be said to be compatible with a situation where there are two materials with a sharp boundary and a thermal diffusion length (L) that, in this case, changes with the changing wavelength as follows:

\[ L = V(2D/w) \]
where \( w \) is the angular frequency of the excitation and \( D \) is the heat-diffusion coefficient for the material in question, with

\[
D = \frac{\text{thermal conductivity}}{\text{(density) } \times \text{ (specific heat capacity)}}
\]

From the simple geometry of this case, we can easily find (by integration) the signal received at a point \((x=0, \text{ say})\) from a homogeneous region \([0,r]\) as follows:

\[
\frac{L}{V^2} \left( \cos\left(\frac{\tau}{4} - wt\right) - \exp(-d/r)L \cos\left(d/rL + \frac{\tau}{4} - wt\right) \right)
\]

where \( d \) is the pixel-width (here, 0.5 micron)

From the above, one can directly also find the signal received at \( x=0 \) from the interval \([r, \infty)\), across the region \([0,r]\), and hence fit the model to the data for each postulated break-point. Then, the choice of break-point that minimises the misfit is the best estimate.

FIG. 14 is a plot of mean misfit as a function of postulated break point. The best-fit for the break-point is around column 14. This is consistent with what is known from other (physical) analysis of this sample.

The direct model-fitting approach described above requires that we have separate estimates of the 'pure' spectra of each of the two phases under consideration (collected a long way away from the break-point), which is a limitation. However, by incorporating an additional iterative loop into the algorithm, we have implemented an approach that reconstructs estimates of the pure spectra from the 'mixed' data alone. We do this by first assuming a fixed position for the break point, then, separately for each 'point' in the spectrum of the excitation, estimating the values for each phase from a fit of the model to the data (the model is non-linear in this case, because of the phase differences). Cycling this entire process through all possible values of the break-point and comparing overall fits produces the optimum solution.

Further, collecting data at two (or more) mirror-speeds (of the interferometer that sits in front of the globar IR source in this example) or different modulation frequencies of the incident radiation where an interferometer is not being used, will enable better model-
fitting for this step, as such data will contain more information; allowing for discrimination between a boundary that is genuinely diffuse (because of physical mixing) and the spectral mixing due to the effects of thermal diffusion.

5 The influence of the measuring system

In the examples we have given we have not explicitly taken account of the influence on the measured signal of the measuring method itself. To some extent step 1 implicitly corrects for this. However an explicit correction for the measuring system is desirable when it can be achieved; this means determining G(t) to some reasonable approximation. To do this we must consider the response of a given detection system with a given source of radiation and a given direction of illumination. It can be determined by characterising the behaviour of the system-sample combination with a sample of known thermal properties and spectrum. In order to obtain a good characterisation of the function that defines the response function, the manner in which the intensity of the incident radiation is modulated must be in some sense 'rich', in other words, as mentioned above, it must contain a broad range of frequencies. This condition could be fulfilled by having pulses (preferably of variable duration) that constitute an approximation to a Dirac delta function, a sequence of sine waves of varying frequencies, a complex waveform composed of many simultaneous sine waves, saw-tooth modulation or stochastic (or 'white noise') modulations. Another possibility is to construct a waveform that is dependent on the iteration of some suitable function, with starting value selected from the Julia set of that function. The modulations of such a waveform are completely deterministic, and yet act in a chaotic manner, which is a suitable way of investigating non-linear behaviour.

Such a strategy enables us to investigate how the behaviour of the detector-sample combination changes as a function of frequency. This performs the dual function of improving the quantitative accuracy of our measurements and also enabling us to characterise the higher frequency behaviour and it is this behaviour at higher frequencies that will give the highest spatial resolution.

Increasing signal by physical means

It has been observed that decreasing sample thickness can lead to a x5 improvement in signal because the sample does not act as its own heat sink provided it is suspended
above a good thermal insulator such as air [3]. However this is impractical, it would not
generally be possible to stretch thin sections on a frame so that a significant area is
suspended above air because such sections are small and fragile and the probe itself
applies a force to the sample thus potentially deforming it or damaging it. Clearly this
implies that placing a thin sample on a thermal insulator could improve signal to noise
but the type of samples often of interest, such as polymers, are generally considered to
be thermal insulators. To find a better thermal insulator is problematic and even if a
candidate is identified then if it is a strong IR absorber then it could still pose problems
because it could contaminate the IR spectrum of the sample. This is because it may
create thermal waves that will travel through the thin sample, thereby complicating
interpretation and making more difficult identification by searching a database of
standard spectra.

A known thermal insulator is Aerogel but it can be shown to have a strong photothermal
spectrum.

FIG. 15(a) is a plot of signal as a function of wavenumber for a sample of aerogel using
a globar light source and a temperature sensitive probe.

We have made a surprising discovery. Although a strong photothermal spectrum is
obtained from Aerogel when using a globar source and a temperature sensitive probe as
detector placed on the surface of the Aerogel, when a thin sample, say 2 micrometers
thick, is placed on the Aerogel a good spectrum of the sample can be obtained without
contamination from the Aerogel. This is illustrated in FIG. 15(b) which shows a spectrum
obtained from a 2 micrometre thick sample of PET on aerogel. FIG. 15(c) shows a
spectrum obtained from a 2 micrometre thick sample of araldite (RTM) on aerogel.

It can be seen that the spectra of PET and araldite are not significantly contaminated by
the aerogel. Thus, the sample can be supported so that it can be imaged while
providing high signal to noise reported in [3] thus providing a practical solution to
supporting the sample.

It is surprising that thermal waves that can induce a strong signal in the thermal probe
cannot travel through a thin section and so become mixed with the sample spectrum.
While it is possible to speculate on the mechanism that makes this possible it sufficient
for the purposes of this document to note that it works and can be exploited to improve the strength of the signal from any photothermal measurement.

To overcome the practical limitation of lack of chemical functionality with the AFM would be of huge benefit to industries concerned with polymers, pharmaceuticals and biomedical applications. Accordingly these two new types of mid-IR sources have recently been described, both highly suitable for use in improved versions of IR spectromicroscopy, with adequate power and the ability to operate in a continuous wave mode as well as pulsed mode. This is one of the subjects to which the present inventions relate.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", means "including but not limited to", and is not intended to (and does not) exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

References


9) A Dazzi, R Praveres, F Glotin, J M Ortega, Optics Letters, 30(18), 2005, 2388


15) Vodopyanov et al. (2000; 2004)
CLAIMS:

1. Scanned probe near-field infra-red micro-spectroscopy apparatus comprising:
   a probe operable to be scanned over a surface of a sample; and
   at least one semiconductor light source arranged to illuminate the substrate
   thereby to heat the sample by absorption of infra-red radiation,
   the apparatus being arranged to be responsive by means of the probe to thermal
   expansion of the sample due to a temperature rise of the sample induced by illumination
   of the sample by the light source thereby to obtain micro-spectroscopy data in respect of
   the sample.

2. Apparatus as claimed in claim 1 arranged to irradiate the sample with radiation
   having a plurality of different respective wavelengths.

3. Apparatus as claimed in claim 1 or claims 2 wherein the at least one
   semiconductor light source is arranged to provide a source of broadband illumination.

4. Apparatus as claimed in any preceding claim comprising a plurality of different
   semiconductor light sources each source being arranged to illuminate the sample with
   radiation of a different respective wavelength or different respective range of
   wavelengths.

5. Apparatus as claimed in any preceding claim comprising a plurality of different
   semiconductor light sources each source being arranged to illuminate the sample with
   radiation of a different respective wavelength or different respective range of
   wavelengths substantially simultaneously.

6. Apparatus as claimed in any preceding claim comprising a plurality of different
   semiconductor light sources each source being arranged to illuminate the sample with
   radiation of a different respective wavelength or different respective range of
   wavelengths substantially sequentially.

7. Apparatus as claimed in any preceding claim wherein the at least one
   semiconductor light source is operable in a substantially continuous wave mode, the
   apparatus being operable to modulate an intensity of illumination from the at least one
   semiconductor light source that is directed towards the sample as a function of time.
8. Apparatus as claimed in claim 7 operable to modulate the intensity of illumination from the at least one semiconductor light source such that the rate of change of intensity of illumination as the illumination intensity increases during a modulation cycle is different from one modulation cycle to another.

9. Apparatus as claimed in claim 7 or claim 8 operable to modulate the intensity of illumination such that the rate of change of intensity of illumination as the illumination intensity is decreased during a modulation cycle is different from one modulation cycle to another.

10. Apparatus as claimed in claim 8 or claim 9 wherein the intensity is modulated such that the total amount of power of the illumination of the sample in a given modulation cycle is substantially constant from cycle to another.

11. Apparatus as claimed in claim 5 or any one of claims 6 to 10 depending through claim 5 wherein a plurality of different semiconductor light sources are arranged to illuminate the sample at a given moment in time, the intensity of illumination of each source being arranged to vary in synchrony with one another.

12. Apparatus as claimed in claim 11 wherein the intensity of illumination of each source is arranged to vary in synchrony with one another whereby the total power of the illumination of the sample is substantially constant as a function of time.

13. Apparatus as claimed in claim 7 or any one of claims 8 to 12 depending through claim 7 operable to modulate the intensity of illumination of the sample thereby to achieve relatively slow increase in intensity followed by a relative rapid decrease and then increase in intensity thereby to detect an isolated small object.

14. Apparatus as claimed in claim 7 or any one of claims 8 to 13 depending through claim 7 operable to modulate the intensity of illumination of the sample thereby to achieve relatively slow decrease in intensity followed by a relative rapid increase in intensity.

15. Apparatus as claimed in claim 7 or any one of claims 8 to 12 depending through claim 7 arranged to detect an isolated small object by modulate the intensity of
illumination of the sample thereby to achieve a relatively slow increase in intensity thereby to cause absorbing material to heat up followed by a relative rapid decrease and then increase in intensity thereby to induce cantilever vibration if the isolated small object is an absorbing material and the surrounding material is not, subsequently to achieve a relatively slow decrease in intensity followed by a relative rapid increase in intensity.

16. Apparatus as claimed in any preceding claim comprising an interferometer, the apparatus being configured whereby radiation from the at least one semiconductor light source passes through the interferometer as it travels from the source to a detector of the apparatus.

17. Apparatus as claimed in claim 16 wherein the interferometer comprises a beam path adjuster member being a member operable to adjust an amount of a path difference between respective first and second portions of the beam.

It is to be understood that the apparatus is operable to detect sample response as a function of mirror position.

18. Apparatus as claimed in claim 16 or 17 wherein the interferometer is a radial interferometer.

19. Apparatus as claimed in any one of claims 16 to 18 as depending through claim 4 comprising a plurality of interferometers, each interferometer being arranged to receive a beam of light from a different respective semiconductor light source.

20. Apparatus as claimed in claim 19 as depending through claim 17 wherein the plurality of interferometers are arranged to share a common beam path adjuster member, the adjuster member being operable to adjust a path difference between first and second portions of a beam of radiation from each of the plurality of semiconductor light sources substantially simultaneously.

21. Apparatus as claimed in claim 19 or 20 wherein the plurality of interferometers are arranged circumferentially about the common adjuster member.
22. Apparatus as claimed in claim 17 or any one of claims 18 to 21 depending through claim 17 wherein the adjuster member is operably movable towards and away from the sample thereby to effect beam path difference adjustment.

23. Apparatus as claimed in any preceding claim operable to perform a Fourier transform of data obtained by the apparatus.

24. Apparatus as claimed in any preceding claim operable to position the probe with respect to a surface of the sample thereby to detect movement of the probe due to thermal expansion of the sample when the sample is irradiated with illumination.

25. Apparatus as claimed in claim 24 operable to excite resonance of the probe due to the thermal expansion of the sample.

26. Apparatus as claimed in any preceding claim arranged to position the probe in contact with the surface thereby to detect thermal expansion of the sample.

27. Apparatus as claimed in any preceding claim arranged to position the probe in a spaced apart relationship with the surface thereby to detect thermal expansion of the sample.

28. Apparatus as claimed in any preceding claim operable to perform acousto-optical micro-spectroscopy (AOMS).

29. Apparatus as claimed in claim 28 operable to perform Fourier transform infra-red (FTIR) AOMS.

30. Apparatus as claimed in any preceding claim wherein the at least one semiconductor light source comprises a light emitting diode (LED).

31. Apparatus as claimed in claim 30 wherein the LED comprises $\text{In}_{x}\text{Ga}_{1-x}\text{Sb}$.

32. Apparatus as claimed in any preceding claim wherein the at least one light semiconductor source comprises a quantum cascade laser (QCL) device.
33. Apparatus as claimed in claim 32 wherein the QCL device is a tunable QCL device.

34. Apparatus as claimed in any preceding claim wherein the probe comprises an atomic force microscopy probe.

35. Apparatus as claimed in any preceding claim wherein the probe comprises a temperature sensing probe.

36. Apparatus as claimed in any preceding claim arranged to perform photo-thermal micro-spectroscopy (PTMS).

37. Apparatus as claimed in any preceding claim arranged to perform measurements of an amount of thermal expansion of a sample due to irradiation of the sample with radiation.

38. Apparatus as claimed in any preceding claim wherein the at least one semiconductor light source is arranged to generate mid-infra-red radiation having a substantially continuous spread of wavelengths over at least a portion of the range of wavelengths from around 5 microns to around 10 microns.

39. Apparatus as claimed in any preceding claim arranged wherein variations between any two spectra obtained from a sample are significant to within a confidence of ninety percent.

40. Apparatus as claimed in any preceding claim comprising an optical intensity modulator provided between the source and the sample, the intensity modulator being operable to vary the fraction of the illumination emitted by the source that reaches the sample.

41. Apparatus as claimed in claim 40 wherein the intensity modulator comprises an optical chopper.

42. Apparatus as claimed in claim 40 or 41 wherein the intensity modulator comprises a MEMs-based assembly having a reflective surface.
43. Apparatus as claimed in any preceding claim wherein a source of illumination is provided by a globar.

44. A method of performing scanned probe near-field infra-red micro-spectroscopy comprising:
   - scanning a probe over a surface of a sample;
   - illuminating the sample with light from at least one semiconductor light source thereby to heat the sample by absorption of infra-red radiation; and
   - sensing by means of the probe a thermal expansion of the sample due to a temperature rise of the sample induced by illumination of the sample by the light source thereby to obtain micro-spectroscopy data in respect of the sample.

45. A method as claimed in claim 44 comprising the step of irradiating the sample with radiation having a plurality of different respective wavelengths.

46. A method as claimed in claim 44 or 45 comprising the step of generating by means of the at least one semiconductor light source a beam of broadband illumination.

47. A method as claimed in any one of claims 44 to 46 comprising illuminating the sample by means of a plurality of sources by radiation of different respective wavelengths or different respective ranges of wavelengths.

48. A method as claimed in any one of claims 44 to 47 comprising the step of illuminating the sample by means of the plurality of sources by radiation of different respective wavelengths or different respective ranges of wavelengths substantially simultaneously.

49. A method as claimed in any one of claims 44 to 47 comprising the step of illuminating the sample by means of the plurality of sources by radiation of different respective wavelengths or different respective ranges of wavelengths substantially sequentially.

50. A method as claimed in any one of claims 44 to 49 comprising the step of irradiating the sample by means of the at least one semiconductor light source with radiation in a substantially continuous wave mode and modulating an intensity of
illumination from the at least one semiconductor light source that is directed towards the sample as a function of time.

51. A method as claimed in claim 50 comprising the step of modulating the intensity of illumination from the at least one semiconductor light source such that the rate of change of intensity of illumination as the illumination intensity increases during a modulation cycle is different from one modulation cycle to another.

52. A method as claimed in claim 50 or claim 51 comprising the step of modulating the intensity of illumination from the at least one semiconductor light source such that the rate of change of intensity of illumination as the illumination intensity is decreased during a modulation cycle is different from one modulation cycle to another.

53. A method as claimed in claim 51 or claim 52 comprising the step of modulating the intensity such that the total amount of power of the illumination of the sample in a given modulation cycle is substantially constant from one cycle to another.

54. A method as claimed in claim 48 or any one of claims 49 to 53 depending through claim 48 comprising the step of illuminating the sample by means of a plurality of different semiconductor light sources at a given moment in time and modulating the intensity of illumination of each source in synchrony with one another.

55. A method as claimed in claim 54 comprising varying the intensity of illumination of the sources in synchrony with one another whereby the total power of the illumination of the sample is substantially constant as a function of time.

56. A method as claimed in claim 50 or any one of claims 51 to 55 depending through claim 50 comprising the step of modulating the intensity of illumination of the sample thereby to achieve a relatively slow increase in intensity followed by a relative rapid decrease and then rapid increase in intensity thereby to detect an isolated small object.

57. A method as claimed in claim 50 or any one of claims 51 to 56 depending through claim 50 operable to modulate the intensity of illumination of the sample thereby to achieve relatively slow decrease in intensity followed by a relative rapid increase in intensity.
58. A method as claimed in claim 50 or any one of claims 51 to 55 depending through claim 50 arranged to detect an isolated small object by modulating the intensity of illumination of the sample thereby to achieve a relatively slow increase in intensity thereby to cause absorbing material to heat up followed by a relative rapid decrease and then increase in intensity thereby to induce cantilever vibration if the isolated small object is an absorbing material and the surrounding material is not, and modulating the intensity to achieve a relatively slow decrease in intensity followed by a relative rapid increase in intensity.

59. A method as claimed in any one of claims 44 to 58 comprising the step of passing radiation from the at least one semiconductor light source through an interferometer as it travels from the source to a detector of the apparatus.

60. A method as claimed in claim 59 comprising the step of adjusting an amount of a path difference between respective first and second portions of the beam that are produced by the interferometer.

61. A method as claimed in claim 59 or 60 comprising the step of passing the radiation through a radial interferometer

62. A method as claimed in any one of claims 59 to 61 as depending through claim 47 comprising the step of passing light beams from different respective semiconductor light sources through a respective different one of a plurality of interferometers.

63. A method as claimed in claim 62 as depending through claim 60 wherein the plurality of interferometers are arranged to share a common beam path adjuster member, the method comprising the step of adjusting the adjuster member thereby to adjust a path difference between first and second portions of a beam of radiation from each of the plurality of semiconductor light sources substantially simultaneously.

64. A method as claimed in claim 62 or 63 wherein the plurality of interferometers are arranged circumferentially about the common adjuster member.
65. A method as claimed in claim 60 or any one of claims 61 to 64 depending through claim 60 comprising the step of moving the adjuster member towards or away from the sample thereby to effect beam path difference adjustment.

66. A method as claimed in any one of claims 44 to 65 comprising the step of performing a Fourier transform of data obtained by the apparatus.

67. A method as claimed in any one of claims 44 to 66 comprising the step of positioning the probe with respect to a surface of the sample thereby to detect movement of the probe due to thermal expansion of the sample when the sample is irradiated with illumination.

68. A method as claimed in claim 67 comprising the step of exciting resonance of the probe due to the thermal expansion of the sample.

69. A method as claimed in any one of claims 44 to 68 comprising the step of positioning the probe in contact with the surface thereby to detect thermal expansion of the sample.

70. A method as claimed in any one of claims 44 to 69 comprising the step of positioning the probe in a spaced apart relationship with the surface thereby to detect thermal expansion of the sample.

71. A method as claimed in any one of claims 44 to 70 comprising the step of performing acousto-optical micro-spectroscopy (AOMS).

72. A method as claimed in claim 71 comprising the step of performing Fourier transform infra-red (FTIR) AOMS.

73. A method as claimed in any one of claims 44 to 72 wherein the at least one semiconductor light source comprises a light emitting diode (LED).

74. A method as claimed in claim 73 wherein the LED comprises Al_xIn_{1-x}Sb.

75. A method as claimed in any one of claims 44 to 74 wherein the at least one semiconductor light source comprises a quantum cascade laser (QCL) device.
76. A method as claimed in claim 75 wherein the QCL device is a tunable QCL device.

77. A method as claimed in any one of claims 44 to 76 wherein the probe comprises an atomic force microscopy probe.

78. A method as claimed in any one of claims 44 to 77 wherein the probe comprises a temperature sensing probe.

79. A method as claimed in any one of claims 44 to 78 comprising the step of performing photo-thermal micro-spectroscopy (PTMS).

80. A method as claimed in any one of claims 44 to 79 comprising the step of performing measurements of an amount of thermal expansion of a sample due to irradiation of the sample with radiation.

81. A method as claimed in any one of claim wherein the at least one semiconductor light source is arranged to generate mid-infra-red radiation having a substantially continuous spread of wavelengths over at least a portion of the range of wavelengths from around 5 microns to around 10 microns.

82. A method as claimed in any one of claims 44 to 81 arranged wherein variations between any two spectra obtained from a sample are significant to within a confidence of ninety percent.

83. A method as claimed in any one of claims 44 to 82 comprising modulating the amount of illumination emitted by the source that reaches the sample by means of an optical intensity modulator provided between the source and the sample.

84. A method as claimed in claim 83 comprising modulating the amount of illumination emitted by the source that reaches the sample by means of an optical chopper provided between the source and the sample.

85. A method as claimed in claim 83 or 84 wherein the intensity modulator comprises a MEMs-based assembly having a reflective surface.
86. A method as claimed in any one of claims 44 to 85 wherein a source of illumination is provided by a globar.

87. Apparatus substantially as hereinbefore described with reference to the accompanying drawings.

88. A method substantially as hereinbefore described with reference to the accompanying drawings.
FIG. 2 (Prior Art)
Sample FFT spectrum for on sample

FIG. 10
FIG. 11
Fuzzy analysis - 2 classes

FIG. 12
FIG. 13
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Aerogel spectra

Small amount of surfactant

Wavenumber (cm⁻¹)

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FIG. 15a

Bulk reference

PET sections - 2 μm

Wavenumber (cm⁻¹)

PT signal (a.u.)

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FIG. 15b

Araldite sections - 2 μm

Wavenumber (cm⁻¹)

PT signal (a.u.)

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FIG. 15c

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