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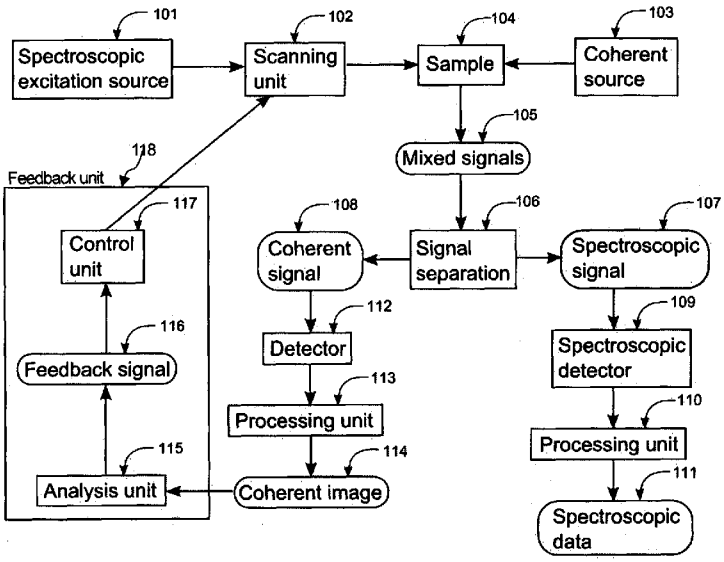
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(54) Title: AN APPARATUS FOR TRACKING IN SPECTROSCOPY THROUGH FAST IMAGING MODALITY

Fig.1



(57) Abstract: An apparatus comprises a coherent source (103) radiating coherent light to a sample, an excitation source (101) radiating excitation light to the sample, a scanning unit (102) controlling at least the radiated location and/or radiated shape of the excitation light on the sample, a signal separation unit (106) dividing a mixed signal from the sample into a coherent signal and spectroscopic signal, at least one detector (109,112) measuring the spectroscopic signal and coherent signal, at least one processing unit (110, 113) converting the spectroscopic signal into spectroscopic data and converting the coherent signal into a coherent image, and a feedback unit (118) controlling the scanning unit (102) based on the coherent image.

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DESCRIPTION

Title of Invention: AN APPARATUS FOR TRACKING IN SPECTROSCOPY THROUGH FAST IMAGING MODALITY

Technical Field

The present invention relates to an apparatus for measuring spectroscopic data of a sample in a spectrally resolved manner and imaging a sample with a narrow-band source simultaneously.

Background Art

Spectroscopy provides, in general, a large amount of information concerning the composition of a sample, through the measurement of the interaction of an electromagnetic wave with the sample, where each measured wavelength is related to a specific chemical composition. Spectroscopy is usually defined by the wavelength range and/or center value of the excitation source, which in turn determines the type of chemical information obtained (atomic composition, molecular composition, chemical bonds characterization, etc.), which also depends on the measured physical property, such as, for example, absorbance, Raman scattering, resonant stimulated emission, etc. Within this wide range of applications, vibrational spectroscopy more specifically measures the chemical bonds in molecules through electronic vibrations, with excitation sources classically in the optical, ultraviolet or infrared range of wavelengths.

In particular in the optical regime, vibrational spectroscopy usually suffers from slow acquisition times, as the signal to be recorded is weak and only long exposure times can overcome the noise of present detectors. Several strategies have been proposed in order to make the measurement faster, such as relying on an amplification of the emitted signal through physical means such as surface-enhanced Raman spectroscopy, or frequency resonance through multiple-source excitation (Patent Literature 1). Other

approaches optimize the detection through parallel schemes with, for instance, 2D detectors (Non-Patent Literature 1), or particular signal integration patterns during scanning (Patent Literature 2).

Moreover, coherent imaging is still widely used nowadays in various ways and covers various imaging possibilities such as absorption imaging, coherent complex-field imaging (Non-Patent Literature 2), and phase imaging through, for instance, phase-retrieval methods (Non-Patent Literature 3). Coherent imaging relates to the use of a relatively narrow-bandwidth emitter as a source, and thus embraces a large class of imaging modalities, as many techniques nowadays employ light sources such as lasers, which are an example of narrow sources. In the present invention, we employ coherent imaging techniques essentially based on wide-field imaging, which do not require scanning during acquisition, and can thus easily provide images at a high temporal rate.

It has been demonstrated recently that spectroscopy and coherent imaging can be employed together to retrieve two measurements (Non-Patent Literature 4), possibly in a simultaneous way (Non-Patent Literature 5).

Citation List

Patent Literature

PTL 1: US 8027032

PTL 2: DE 102009013147

Non Patent Literature

NPL 1: D. K. VEIRS, J. W. AGER III, E. T. LOUCKS AND G. M. ROSENBLATT, *"MAPPING MATERIALS PROPERTIES WITH RAMAN SPECTROSCOPY UTILIZING A 2D DETECTOR"*, APPLIED OPTICS ,1990, **29**, PP.4969-4980.

NPL 2: E. CUCHE, P. MARQUET AND C. DEPEURSINGE, *"SIMULTANEOUS AMPLITUDE-CONTRAST AND QUANTITATIVE PHASE-CONTRAST MICROSCOPY BY NUMERICAL RECONSTRUCTION OF FRESNEL OFF-AXIS HOLOGRAMS"*, APPLIED OPTICS, 1999, **38**(34), PP. 6994-7001.

NPL 3: J. R. FIENUP, *"PHASE RETRIEVAL ALGORITHMS: A COMPARISON"*, APPLIED OPTICS, 1982, **21**(15), PP. 2758-2769.

NPL 4: J. W. KANG, N. LUE, C.-R. KONG, I. BARMAN, N.C. DINGARI, S.J. GOLDFLESS, J. C. NILES, R.R. DASARI AND M. S. FELD, *"COMBINED CONFOCAL RAMAN AND QUANTITATIVE PHASE MICROSCOPY SYSTEM FOR BIOMEDICAL DIAGNOSIS"*, BIOMEDICAL OPTICS EXPRESS, 2011, **2**(9), PP. 2484-2492.

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Summary of Invention

Technical Problem

While spectroscopy can provide information about the molecular or chemical composition of a measured sample, it is very often desired to also be able to make the acquisition specific to a chosen location in the sample, in order to obtain information in a spatially resolved manner. This can even extend to the case where the measurement is performed in a full two-dimensional region, leading to a spectroscopic image. However, spectroscopy is classically limited in its capacity to provide images, as it requires a spatial scanning for measuring information at different locations, so that it is intrinsically limited in its acquisition speed with present detectors. This implies that two-dimensional measurements are inevitably slower than, for instance, standard bright field imaging, so that its capability in measuring two-dimensional signals with a high temporal resolution becomes hindered due to the large amount of information to acquire with the additional spectral dimension.

Accordingly, spectroscopic data provide invaluable information about the molecular content of a measured sample, but classically require a long time for measurement, during which the sample may move or change. This implies that while this modality has a

strong specificity, its temporal sampling is classically low.

On the other hand, coherent imaging can provide rapid acquisitions, but is usually less specific than spectroscopic measurements, as standard coherent imaging provides for example absorption and/or phase data only at the wavelength of the source, and thus carries less information.

In view of the foregoing, the present invention aims at optimizing the acquisition process of a spectroscopic measurement, especially in the temporal and spatial domain, by adding a simultaneous coherent imaging measurement to the spectroscopic one.

Solution to Problem

In accordance with an aspect of the present invention, an apparatus comprises a coherent source radiating coherent light to the sample, an excitation source radiating excitation light to the sample, a scanning unit controlling at least the radiated location and/or radiated shape of the excitation light on the sample, a signal separation device dividing a mixed signal from the sample into a coherent signal and spectroscopic signal, at least one detector measuring the spectroscopic signal and coherent signal, at least one processing unit retrieving spectroscopic data from the spectroscopic signal and a coherent image from the coherent signal, and a feedback unit controlling the scanning unit based on the coherent image.

According to the above arrangement, the apparatus simultaneously measures a spectroscopic signal and rapid coherent signal in an independent way through a signal separation device after interaction with the sample. The rapid coherent image enables tracking the specimen in time and space in the feedback unit, so that the spectroscopic measurement can be precisely targeted towards known locations in the specimen of interest or part of it

by controlling the scanning unit. This specific targeting makes it possible to obtain rapid time- and space-resolved spectroscopic measurements.

Moreover, the present invention increases the amount of information which can be extracted from a sample, by combining two measurement approaches in a simultaneous way, compared to the usual implementations which are restricted to only one modality, or separate modalities employed only sequentially.

Advantageous Effects of Invention

As described above, the apparatus of the present invention makes it possible to optimize the acquisition process of a spectroscopic measurement, especially in the temporal and spatial domain, by adding coherent imaging at the same time at which the spectroscopic measurement is performed. The coherent image enables spatial and/or temporal tracking of specific locations in the sample during spectroscopic measurements.

Furthermore, it can increase the amount of information which can be extracted from a sample, by combining two measurement approaches in a simultaneous way, compared to the usual implementations.

Brief Description of Drawings

Fig.**1** is a block diagram of an apparatus in accordance with one embodiment of the present invention, wherein the apparatus measures the multimodal signals, namely spectroscopic and coherent, and the separation principle in order to obtain two independent and simultaneous signals, so that the coherent image can be used as a feedback signal for rapidly and potentially automatically tracking specific parts of the specimen with the spectroscopic measurement

Fig.**2** is an illustration of the measurement timeline, between the coherent signal acquired in real-time, and the spectroscopic

signal acquired more slowly, where it queries the measurement position based on the processed coherent signal information which provides a feedback signal.

Fig.**3** is an example of the apparatus of the embodiment, where the spectroscopic signal is implemented as a laser-scanning Raman micro-spectroscope, and the coherent signal is implemented as a digital holographic microscope.

Fig.**4** is an illustration of the principle enabling the separation of the two multimodal signals **(a)** for the case of a large band excitation spectroscopic source, and **(b)** for the case of a spectrally narrow spectroscopic source. The coherent signal is situated either far from the large spectroscopic signal band, enabling edge or band filtering (1), or within the spectroscopic band, enabling band filtering (2).

Fig.**5** contains two examples of measurement of both spectroscopic and coherent images measured simultaneously, with **(a)**, **(b)** the coherent images and **(c)**, **(d)** the corresponding spectroscopic images, where each pixel contains spectral information as shown in **(e)**.

Fig.**6** is an example of tracking specific parts of the specimen (here the cell regions without background) through the coherent signal to obtain fast spectroscopic signals. **(a)** Schematic of a standard raster scan, which requires a long time to generate a whole image, and **(b)** Magnitude of the gradient of the coherent image, which highlights edges. **(c)** Refined edges through maxima suppression of the gradient magnitude. **(d)** Segmented cell region, based on the detection of the strong edges in **(c)**, which can be employed as a mask for fast scanning. **(e)** Overlay of the coherent image and segmented region, showing the agreement in detecting the cells. **(f)** Schematic of the resulting fast spectroscopic scanning measurement, where the background is not measured.

Fig.**7** is a block diagram of the feedback unit and the scanning unit. The computing process involved in the analysis unit of the feedback unit for the example of detecting the cell edges, based on the Canny edge detector principle, which can be used to

segment the regions containing cells. The feedback signal is then treated by the control unit to convert the extracted pixel positions into control positions for scanning. The scanning unit, taken here as an example as galvano-mirrors, then transfers the voltages to the mirrors for scanning.

Fig.**8** presents different applications of fast tracking. **(a)** Schematic representation of the scanning based on the mask of Fig.**6(d)**, where only regions containing cells are measured. **(b)** Principle of measurement of chosen points, or **(c)** regions within cells, which minimize the measurement time by their smaller sizes. **(d)** Principle of tracking specific features in the cells in time through trajectories which can be extracted from the coherent image.

Fig.**9** is a variation of the embodiment of an apparatus simultaneously measuring the multimodal signals, and where the spectroscopic and coherent signals are combined together and processed in order to derive additional information about the sample, enabling the use of coherent and/or complementary information for tracking.

Fig.**10** is an example of the principle of additional complementary information, obtained here by subtracting the C-H stretching region of the spectroscopic signal to the coherent image, highlighting the cell nucleus.

Description of Embodiments

Referring to the drawings, an apparatus according to an embodiment of the present invention will be described in detail.

Fig.**1** is a block diagram of the embodiment. A spectroscopic excitation source (101) for spectroscopic measurement provides excitation light, which is modulated by a scanning unit (102), which radiates the excitation light to the sample (104). The scanning unit (102) controls at least the radiated location and/or radiated shape of the excitation light on the sample, and so makes it possible to control and possibly scan the excitation

light within the sample. Coherent source (103) for coherent measurement also independently radiates coherent light to the sample. The coherent source (103) is chosen in order to enable simultaneous acquisition while not limiting the spectroscopic measurement, and is precisely defined in the spectrum to be accurately separated from the spectroscopic measurement by a separation unit (106) described later.

The signals having interacted with the sample are mixed (105), and can be separated through the separation unit (106), which divides the mixed signal into the spectroscopic signal (107) and coherent signal (108) to make them independent for further detection. A spectroscopic detector (109) acquires the spectroscopic signal, which can then be sent to a first processing unit (110), which converts the spectroscopic signal into spectroscopic data (111). Similarly, the coherent signal (108) is acquired by a detector (112), and sent to a second processing unit (113), which can possibly be merged with (110), leading to the coherent image (114).

The coherent image (114) is sent to a feedback unit (118). The feedback unit (118) controls the scanning unit (102) based on the coherent image (114). The feedback unit (118) comprises an analysis unit (115) and a control unit (117). The analysis unit (115) analyzes the coherent image (114) and precisely determines the location and/or shape of the spectroscopic measurement within the sample. Then, the analysis unit (115) outputs a feedback signal (116) to control at least the radiated location and/or radiated shape of the excitation light on the sample. The feedback signal (116) is sent to a control unit (117) which controls the scanning unit (114) based on the feedback signal (116).

As mentioned above, this apparatus makes it possible to obtain

the two signals, spectroscopic and coherent, simultaneously so that the rapid coherent image can be employed as an indicator which can be treated in an automated way by an analysis unit (115), which sends a feedback signal (116) to a control unit (117) which drives the scanning unit (102), so that the spectroscopic data (111) can be acquired rapidly in an automated way on specific known locations.

Furthermore, while the description above presents an automated case, a manual inspection of the coherent signal can also be employed for selecting the spectroscopic measurement location depending on the application, instead of employing an automated analysis unit (115).

Fig.**2** illustrates the principle of the operation of this apparatus, where a typical timeline of the measurement process is presented. The fact of getting a fast coherent signal can be employed to understand the spatial structure of the measured sample, in order to determine the locations of interest to measure the spectroscopic signal. While the coherent signal is measured continuously at high speed (typically fractions of seconds), the spectroscopic signal is measured at a lower rate (typically in the seconds range).

When a spectroscopic signal is about to be measured, the coherent signal is processed by the analysis unit (115), in order to return the measurement position as a feedback signal that the control unit (117) can employ to select the correct location of spectroscopic measurement. Then, a spectroscopic signal is measured. The coherent signal can be continuously measured also during the spectroscopic measurement. After finishing measuring a spectroscopic signal, the coherent signal is processed by the analysis unit (115) again, and the correct location of spectroscopic measurement is selected for continuous tracking.

According to the above arrangement, the apparatus simultaneously measures a spectroscopic signal and rapid coherent signal in an independent way through a signal separation device after interaction with the sample. The rapid coherent image enables tracking the specimen in time and space in the feedback unit, so that the spectroscopic measurement can be precisely targeted towards known locations in the specimen of interest or part of it by controlling the scanning unit. This specific targeting makes it possible to obtain rapid time- and space-resolved spectroscopic measurements.

Fig.**3** is an example of the apparatus of the embodiment through an implementation in microscopy, where the spectroscopic measurement consists in Raman scattering, while the coherent imaging is implemented as a digital holographic microscope, yielding phase images.

The excitation from the spectroscopic excitation source (101) is performed with a continuous wave laser whose light is tightly focused in the object plane with a microscope objective (MO). The back-scattered light, which contains the Raman signal, is separated from the excitation beam with a long-pass dichroic mirror (DM), before being focused on the slit of a spectrometer with a relay optics (RO). The spectroscopic detector (109), composed here of a spectrometer and a low-noise detector, separates the Raman frequencies by employing a grating, and images the spectral line. To perform imaging through scanning microscopy, a set of scanning mirrors (GM1 and GM2) as a part of the scanning unit (102) are employed to control the beam in both directions.

The coherent imaging part of the experimental setup consists in a Mach-Zehnder interferometer, in which the sample is observed in transmission. The light emitted by a laser diode as the coherent source (103), whose wavelength is chosen outside the broad

wavelength range of Raman emission, is split into two beams by a beam splitter (BS), and the object beam illuminates the observed field of view after having been focused by a condenser lens (C). The scattered light emanating from the specimen is collected with the MO and is imaged by the field lens (FL) before being reflected onto the detector (112). The reference beam is recombined with a second BS, and a camera records the interference pattern between the two coherent waves. The complex wave field is retrieved through Fourier filtering methods and demodulated to retrieve the complex field from which the phase can be extracted.

In this example, the separation between the spectroscopic signal and the coherent one is performed through a short-pass dichroic mirror as the separation unit (106) which separates the spectrum below (spectroscopic) and above (coherent).

A processing device such as a computer is connected to the spectroscopic detector (109) and the detector (112), and receives the spectroscopic signal and the coherent signal. The processing device includes a controller, a memory and an interface. The interface is a device which sends or receives signals. The memory is, for example, a flash memory or a non-volatile memory to store the received spectroscopic signal and the received coherent signal, and programs executed by the controller. The controller includes for example a CPU or GPU and a memory for a work area. In the implementation of Fig.**3**, the controller functions as the processing unit (110), the processing unit (113), and the feedback unit (118) by executing programs stored in memory.

By using the controller, the processing unit (110) converts the spectroscopic signal into spectroscopic data (111) and the processing unit (113) converts the coherent signal (108) into the coherent image (114). The processing unit (113) sends the coherent image (114) to the feedback unit (118). The feedback

unit (118) includes the analysis unit (115) and the control unit (117). The analysis unit (115) outputs the feedback signal (116) based on the coherent image (114). The control unit (117) sends the scanning commands, to the scanning unit (102) based on the feedback signal (116). In the case of Fig.**3**, the scanning commands correspond to physical quantities, for example voltages sent to the actuators (102b).

The scanning unit (102) comprises scanning mirrors (GM1 and GM2) (102a) and actuators (102b). The voltage based on the feedback signal (116) drives the actuators (102b) to control the mirrors (102a), so that the excitation light targets toward a known position on the sample. The mirrors (102a) of the scanning unit (102) may comprise one or several scanning mirrors, a diffractive element, a dynamically variable reflective or transmittive device, or an integrated reflective device.

The processing and analysis unit may comprise at least one computer-processing unit, graphics-processing unit, frame grabber, field-programmable gate array, dedicated electronic device, or distributed or network-based computing architecture.

The spectroscopic source (101), whose spectrum may be located in the ultraviolet, visible, infrared or far-infrared region, comprises a wide-band source, a laser source, or a sweeping - dynamically varying wavelength - source. The spectroscopic detector (109) for spectroscopic measurement may comprise a grating, a dispersive element or a dynamic element dispersing wavelengths, and an electronic detector, such as, for example, a photo-detector, a line detector, or an array detector.

The spectroscopic signal (107) may be located in the ultraviolet, visible, infrared or far-infrared region and is, but is not limited to, an absorbance spectrum, a stimulated vibrational spectrum comprising Stokes or anti-Stokes shifts, or a resonance

stimulated spectrum. Furthermore, the spectroscopic signal (107) may be a processed spectrum, modified, for example, through reference calibration, background subtraction, or post-processing procedures.

The coherent source (103) of arbitrary center wavelength and spectral distribution may comprise at least a laser source, such as, for example, a solid-state, gas or diode laser; a filtered white light source, such as a light bulb, a mercury lamp, or a xenon lamp; or an integrated light source, such as a diode, a light-emitting diode, or a superluminescent diode. The detector (112) for the coherent signal may be any analog or electronic detector, such as an array detector. The coherent signal (108) may be composed of an intensity signal, a polarization-dependent signal, or a nonlinear physical effect of at least one impinging electromagnetic wave. The coherent image (114) may consist of an intensity, amplitude, phase, polarization or dispersion optical signal.

The sample may consist of biological samples such as cells, tissue or biological molecules, obtained through *in vitro*, *in vivo*, or *ex vivo* means. Then, the sample may consist of organic compounds, or industrial products. Furthermore, the sample may consist of biological samples relevant to immunology such as cells (macrophage cells, lymphocytes, dendritic cells, etc.), tissue or part of tissues (lymph nodes, spleen, liver, etc.) or biological fluids (blood, interstitial fluid, etc.).

The example given above and demonstrating the feasibility of the approach is not limiting, in the sense that the same concept of invention can be applied to other spectroscopic measurements, such as, for example, optical and/or UV absorbance, infrared spectroscopy, etc. Similarly, other coherent signals could be employed, such as, for example, different phase imaging modalities (phase-shifting, phase-retrieval, etc.), or absorption

imaging as the only limitation is the precise spectral location of the source for the additional modality.

The apparatus described above specifically relies on a way to separate the two mixed signals which are measured. This can be performed by employing the fact that the coherent imaging possesses a precise and defined location in the spectrum, so that it can be separated from the other signal through the signal separation unit (106). The signal separation unit (106) may comprise at least a spectral filtering device such as a dichroic mirror, a notch filter, a dispersive element, a grating, an integrated dispersive element, a dynamically varying dispersive element, or any spectrally capable devices.

The principle of the separation is presented in Fig.**4** for different possible configurations. In Fig.**4(a)**, the case of a large band or sweeping spectroscopic source (gray region) is considered, as typically used for absorbance spectroscopy, with the absorbance spectrum shown within the measurement gray region. The coherent signal (black line) can be placed outside of the spectroscopic source range (coherent signal 1), in which case the separation can be performed with an edge filter (separation 1, dashed line) or a band-pass filter, for example. The same applies with the coherent signal on the left of the spectrum. The coherent signal can also be placed within the spectroscopic source range (coherent signal 2), in which case the separation can be performed through a band-pass filter (separation 2, dashed rectangle), at the cost of losing this band in the spectroscopic measurement.

The same principle can be employed when the spectroscopic source is localized in the spectrum, as shown in Fig.**4(b)**, such as, for example, in the case of Raman spectroscopy, where the spectroscopic signal is shifted compared to the localized source. In this case, the coherent signal can be placed outside the

spectroscopic signal (coherent signal 1), in which case a separation with, for example, an edge filter (separation 1, dashed line) or a band-pass filter is possible. The same applies for a coherent signal on the left of the spectrum. The coherent signal can also be placed within the spectroscopic signal (coherent signal 2), in which case a band-pass filter can be used (separation 2, dashed rectangle).

In the example in Fig.**3**, the separation between the spectroscopic signal and the coherent one is performed through a short-pass dichroic mirror which separates the spectrum below (spectroscopic) and above (coherent), thus corresponding to the case of coherent signal 1 of Fig.**4(b)**.

Fig.**5** presents two examples of the measurement of both spectroscopic and coherent images measured simultaneously, with Fig.**5(a)** and Fig.**5(b)** the coherent images and Fig.**5(c)** and Fig.**5(d)** the corresponding spectroscopic images, where each pixel contains spectral information as shown in Fig.**5(e)**. These two examples are based on measurements on HeLa cells cultured *in vitro* for two different measurements, where we continuously recorded the coherent images during the acquisition of Raman hyper-spectral stacks. The fields of view in phase, shown in Fig.**5(a)** and Fig.**5(b)**, correspond to the HeLa cells at the beginning of the experiment, and can be compared with the Raman acquisitions represented in the C-H stretching band (2890-2960 cm^{-1}) in Fig.**5(c)** and Fig.**5(d)**. As the recording of one Raman stack requires 10 minutes, coherent images in phase were continuously recorded simultaneously during this time, with a limiting frame rate being in this case the speed of the camera at 15 frames per second. To illustrate the more specific information provided by the spectroscopic measurement, Fig.**5(e)** shows the spectrum recorded in the cell cytosol, showing the various spectral bands. As the images in Fig.**5(c)** and Fig.**5(d)** are spectroscopic images, each pixel contains a specific spectroscopic signature as the one

shown in Fig.**5(e)**, which depends on the local molecular content.

An example in Fig.**6** illustrates the principle of tracking specific parts of the specimen (here the cell regions without background) through the coherent signal to obtain fast spectroscopic signals. Fig.**7** is a block diagram of the computing process of tracking specific parts of the specimen, which involves the feedback unit (118) and the scanning unit (102). The example presented in Fig.**6** employs the coherent information as depicted in Fig.**5(b)**.

The analysis unit (115) specifies the shape of the sample, determines the locations of interest on the sample, and outputs a signal corresponding to the locations of interest as a feedback signal. The control unit (117) controls the scanning unit (102) to radiate excitation light to the locations of interest on the sample, based on the feedback signal. In the case corresponding to the implementation of Fig.**3**, the control unit (117) converts the feedback signal into scanning commands corresponding to horizontal and vertical positions, and sends the scanning commands to the scanning unit (102). The scanning unit (102) comprises the actuator (102b) and the mirror (102a), and the actuator (102b) modifies the mirror positions according to the received scanning commands.

As mentioned above, the fact of having a rapid signal such as the coherent image presented above makes it then possible to track specific regions in the sample, in order to specifically and rapidly obtain the spectroscopic information at the desired locations. This tracking can be employed to minimize the measurement time of the spectroscopic signal by measuring for instance only where cells are present, avoiding measuring the background in which no information is contained (dark pixels in Fig.**5(d)**).

It is possible to compare a standard raster scan, as depicted in Fig.**6(a)**, and where each spatial point is sequentially scanned to construct the image, which takes a significant time (10 minutes in the example provided above) with a selective scanning as presented below.

This tracking can be performed by employing, for example, an edge detection technique such as the Canny edge detector, whose process is represented by the analysis unit (115) of the feedback signal (118) in Fig.**7**. The analysis unit (115) is designed in accordance with the type of feedback signal required.

First, in the analysis unit (115), the image is processed with a smoothing and gradients in the horizontal (h_x) and vertical (h_y) directions. The magnitude and phase of the gradient is then computed from horizontal and vertical components, as shown in magnitude in Fig.**6(b)**, where edges are highlighted by the gradient operation. The edges can then be refined through non-maxima suppression, where only the highest values of the gradient magnitude are retained, leading to fine edges as shown in Fig.**6(c)**. The strongest edges can then be detected by hysteresis threshold, where the strongest values in Fig.**6(c)** are used as seeds for following edges along less intense values.

The inside of cells can then be filled, providing a mask showing the cell surface (see Fig.**6(d)**), which corresponds to the region which should be scanned with the spectroscopic measurement. The result of edge detection is compared with the original image in Fig.**6(e)**, where it is possible to see the agreement between the cell region and the segmented lines. The analysis unit (115) specifies the shape of the sample from the result of edge detection, determines the locations of interest on the sample, and outputs the pixel positions corresponding to the locations of interest as the feedback signal. The corresponding spectroscopic measurement, performed only in cells region is schematically

shown in Fig.**6(f)**, where the measurement time has been reduced by not measuring background pixels.

In Fig.**7**, the feedback signal then has to be converted to control the scanning unit (102) in the control unit (117). In the example of implementation shown in Fig.**7**, the feedback signal is treated in the control unit (117) by extracting horizontal and vertical positions of the pixel coordinates, and converting them into voltages as the scanning commands (see the control unit (117) in Fig.**7**). The control unit (117) sends the voltage as scanning commands to the scanning unit (102). The scanning unit (102) corresponds to galvano-mirrors (102a) and the actuators (102b) in Fig.**3**, so that the control corresponds to voltages to apply to the mirror actuators (102b). The actuators (102b) modify the mirror positions according to the voltages.

The conversion from pixel positions to scanning commands given as voltages implies that the scanning unit (102) employed to control the excitation position for the spectroscopic excitation is well adjusted with the image provided in the coherent channel. This can typically require a calibration prior to measurement with a known object in order to map the laser-scanning positions with the coherent image pixels. The automatically extracted voltages are then transferred to the scanning unit (102), where the actuators (102b) modify the mirror positions according to the received voltages.

The principle of tracking based on the cell shape presented in Fig.**6** is only one example of the possible applications of the fast tracking capability of the embodiment. Some other examples of tracking possibilities, which are not exhaustive, are presented in Fig.**8**.

The scanned region based on the cell segmentation described above is shown in Fig.**8(a)**, where the scanning region is smaller than

the full field of view, and can therefore provide a faster measurement with the same information. Other possibilities include the measurement of points as shown in Fig.**8(b)**, where, for example, two specific points can be selected from the coherent image, and monitored with the spectroscopic measurement. Similarly, an arbitrary region can also be selected, as shown in Fig.**8(c)**. Through dynamic tracking, it is also possible to follow specific elements in the specimen by employing the coherent image, in order to follow them in time with the spectroscopic measurement through arbitrary trajectories, as schematically depicted in Fig.**8(d)**.

The principle shown in the feasibility demonstration presented above can easily be generalized in order to track several locations by employing, for example, fast scanning devices which are properly driven to rapidly send the excitation source to the desired location in a successive way. Another possibility is to employ, for example, dynamic diffractive devices which can generate multiple excitation points simultaneously, in order to create a parallel detection onto the desired locations. Finally, this acquisition can then be made temporally resolved by performing successive rapid acquisitions on the chosen locations.

A variation of the embodiment depicted in Fig.**9** further comprises a combining unit (201), where the spectroscopic data (111) and the coherent image (114) are combined by the combining unit (201), which can provide additional complementary information (202) about the sample through the combination of the spectroscopic signal and the coherent signal. This additional information can also be employed for fast tracking, which may not be obtained through the independent measurements alone.
The analysis unit (115) outputs the feedback signal (116) to control at least the radiated location and/or radiated shape of the excitation light by analyzing the complementary information (202).

Furthermore, in another variation, the signals combination is employed for obtaining additional information from the two signals in their complete spatial form, so that the feedback unit (118) becomes optional.

Finally, Fig.**10** is an example of complementary information which can be obtained by combining both spectroscopic and coherent signals, as proposed in the variations presented above and shown in Fig.**9**. In this example of application, an image has been extracted from the spectroscopic data by selecting the C-H stretching region, as shown in Fig.**5(d)**, and subtracted from the coherent image shown in Fig.**5(b)**. Due to the nature of the signals corresponding to the C-H stretching and phase images, the result in this example highlights the cell nuclei.

The information derived by combining the spectroscopic data (111) and the coherent image (114) leads to images which have complementary information (201), which possess specific features which can be used for tracking specific locations in the sample which may be difficult to extract from the coherent image (114) alone. The complementary information (202) shown in Fig.**10**, which results from the subtraction of the C-H stretching region of spectroscopic data and a coherent image by the combining unit (201) can be used for example for tracking the nucleus structure which is highlighted by the signal combination.

The example in Fig.**10** presents one particular example of combination of both signals (subtraction with the C-H stretching region), the principle is not limited to it, and can use other mathematical operators and other combinations. It can also use other spectral regions from the spectroscopic data. Examples of mathematical operation that can be employed to derive additional information are addition, multiplication, division, subtraction, cross-correlation, or any operation employing the two signals to

derive or highlight additional information.

The foregoing description, for purposes of explanation, used specific nomenclature to provide a thorough understanding of the invention. However, it will be apparent to one skilled in the art that the specific details are not required in order to practice the invention. The foregoing descriptions of specific embodiments are presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed. Rather, many modifications and variations are possible in view of the above teachings.

CLAIMS

[Claim 1] An apparatus comprising:

a coherent source radiating coherent light to a sample,

an excitation source radiating excitation light to the sample,

a scanning unit scanning the excitation light while controlling at least a radiated location and/or radiated shape of the excitation light on the sample,

a signal separation unit dividing a mixed signal from the sample into a coherent signal and spectroscopic signal,

at least one detector measuring the spectroscopic signal and coherent signal,

at least one processing unit converting the spectroscopic signal into spectroscopic data and converting the coherent signal into a coherent image , and

a feedback unit controlling the scanning unit based on the coherent image.

[Claim 2] The apparatus according to claim 1, further comprising a combining unit combining the spectroscopic data and the coherent image so as to provide additional complementary information.

[Claim 3] The apparatus according to claim 1 or 2, wherein the feedback unit comprises:

an analysis unit for outputting a feedback signal to control at least the radiated location and/or radiated shape of the excitation light on the sample by analyzing the coherent image, and

a control unit for controlling the scanning unit based on the feedback signal.

[Claim 4] The apparatus according to claim 2, wherein the feedback unit comprises:

an analysis unit for outputting a feedback signal to control at least the radiated location and/or radiated shape of the excitation light by analyzing the complementary information, and

a control unit for controlling the scanning unit based on the

feedback signal.

[Claim 5] The apparatus according to claim 3 or 4, wherein the analysis unit specifies the shape of the sample, determines the locations of interest on the sample, and outputs a signal corresponding to the locations of interest as the feedback signal, and

the control unit controls the scanning unit to radiate excitation light to the locations of interest on the sample based on the feedback signal.

[Claim 6] The apparatus according to one of claims 3 to 5, wherein the control unit converts the feedback signal into scanning commands corresponding to physical quantities that are sent to the scanning unit.

[Claim 7] The apparatus according to claim 6, wherein the scanning unit comprises an actuator and at least one mirror,

wherein the actuator modifies the mirror positions according to the received scanning commands.

Fig.1

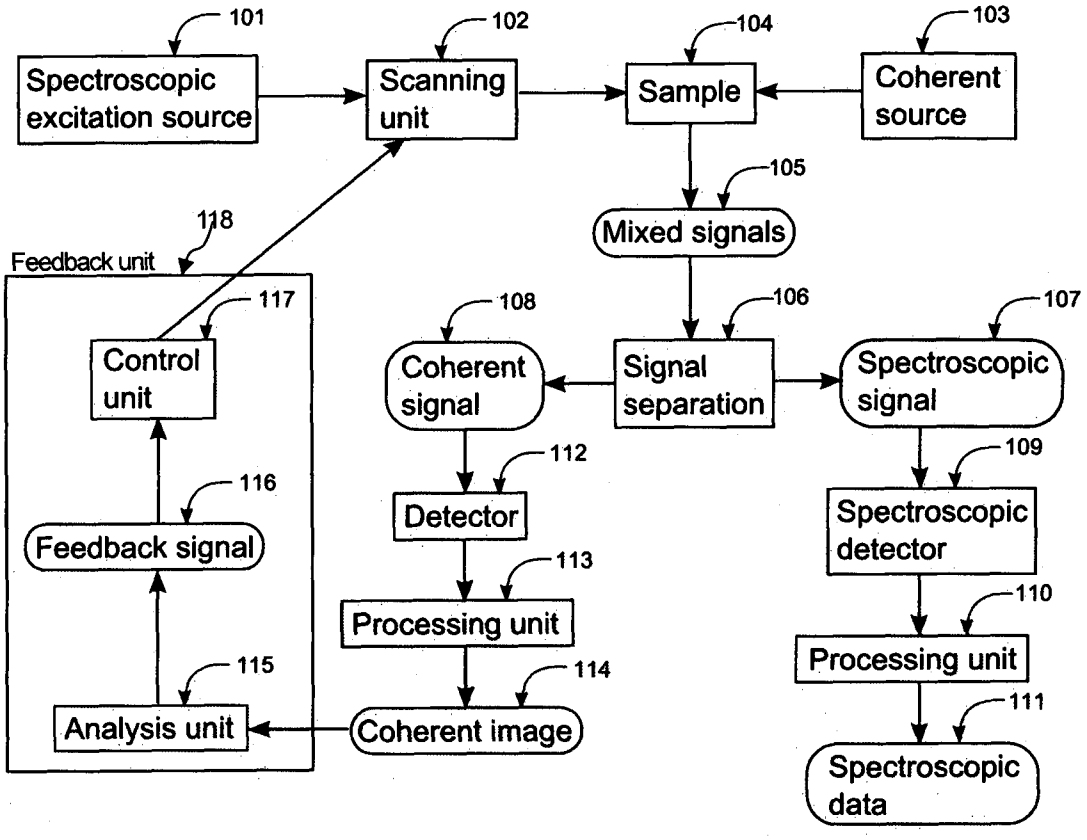

101
Spectroscopic excitation source
102
Scanning unit
104
Sample
103
Coherent source
105
Mixed signals
118
Feedback unit
117
Control unit
116
Feedback signal
115
Analysis unit
108
Coherent signal
106
Signal separation
107
Spectroscopic signal
112
Detector
113
Processing unit
114
Coherent image
109
Spectroscopic detector
110
Processing unit
111
Spectroscopic data

Fig.2

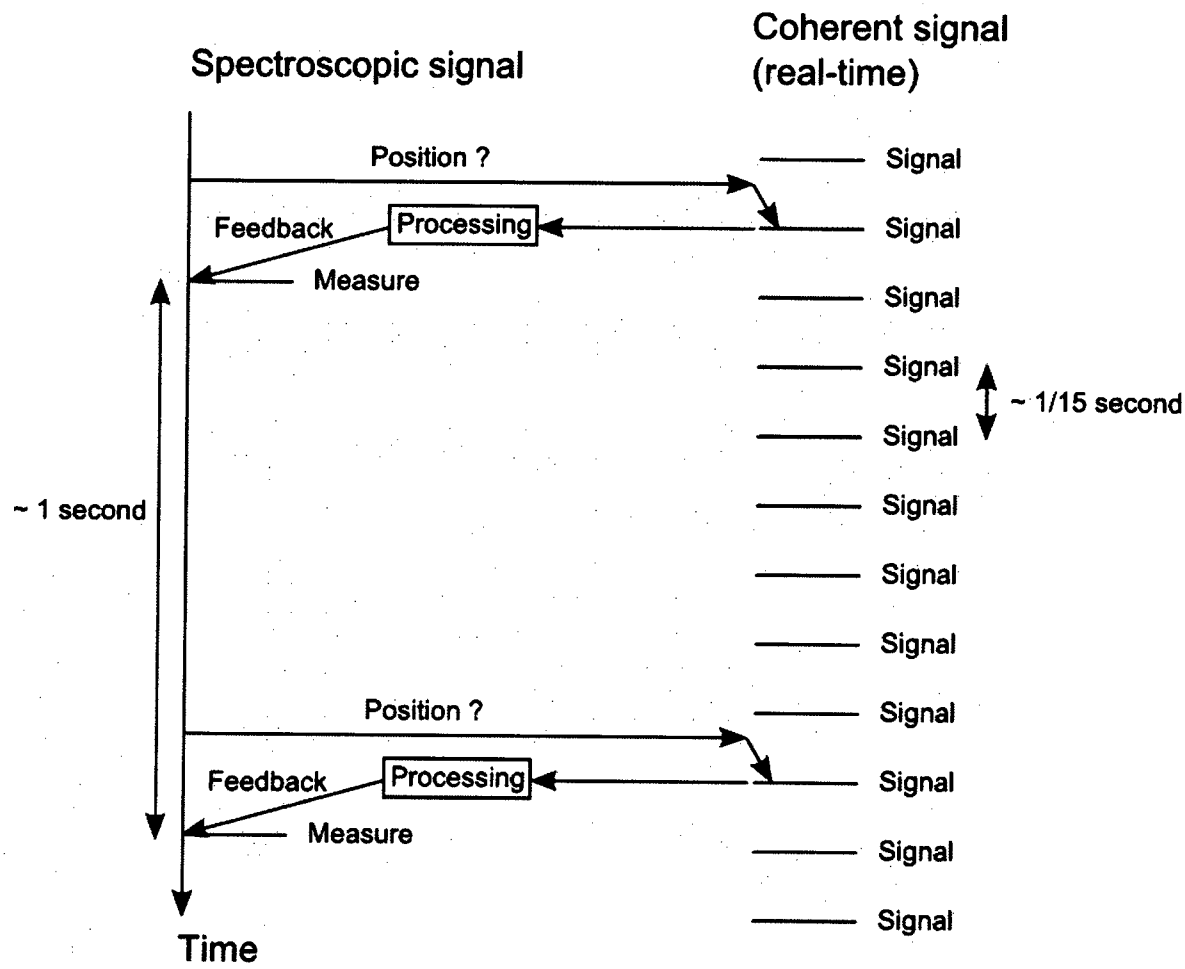

Spectroscopic signal
Coherent signal
(real-time)
Position ?
Feedback
Processing
Measure
~ 1 second
Signal
~ 1/15 second
Time

Fig.3

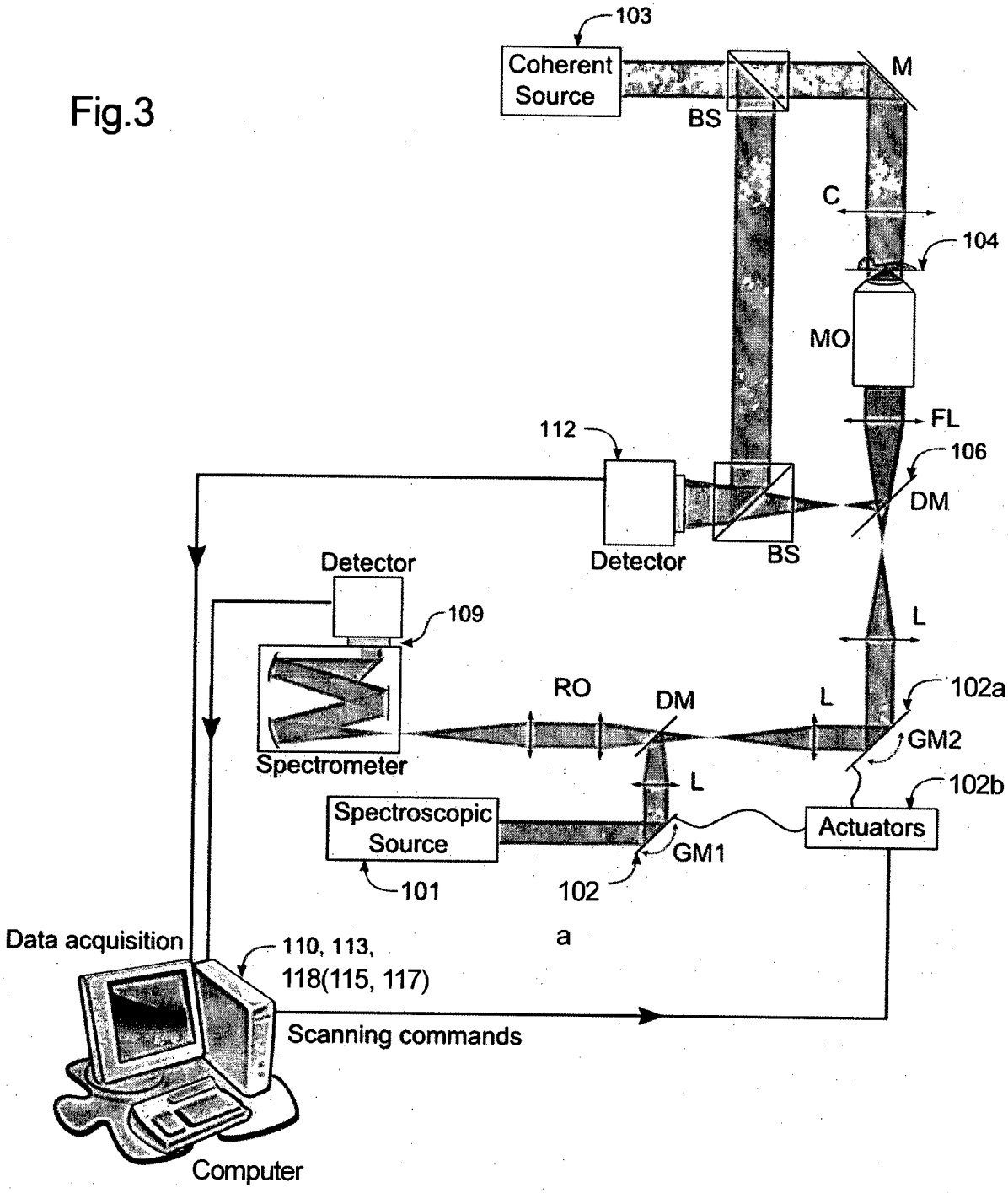

103
Coherent Source
BS
M
C
104
MO
FL
106
DM
112
Detector
Detector
BS
L
109
RO
DM
L
102a
GM2
Spectrometer
L
102b
Spectroscopic Source
Actuators
GM1
101
102
a
Data acquisition
110, 113,
118(115, 117)
Scanning commands
Computer

Fig.4

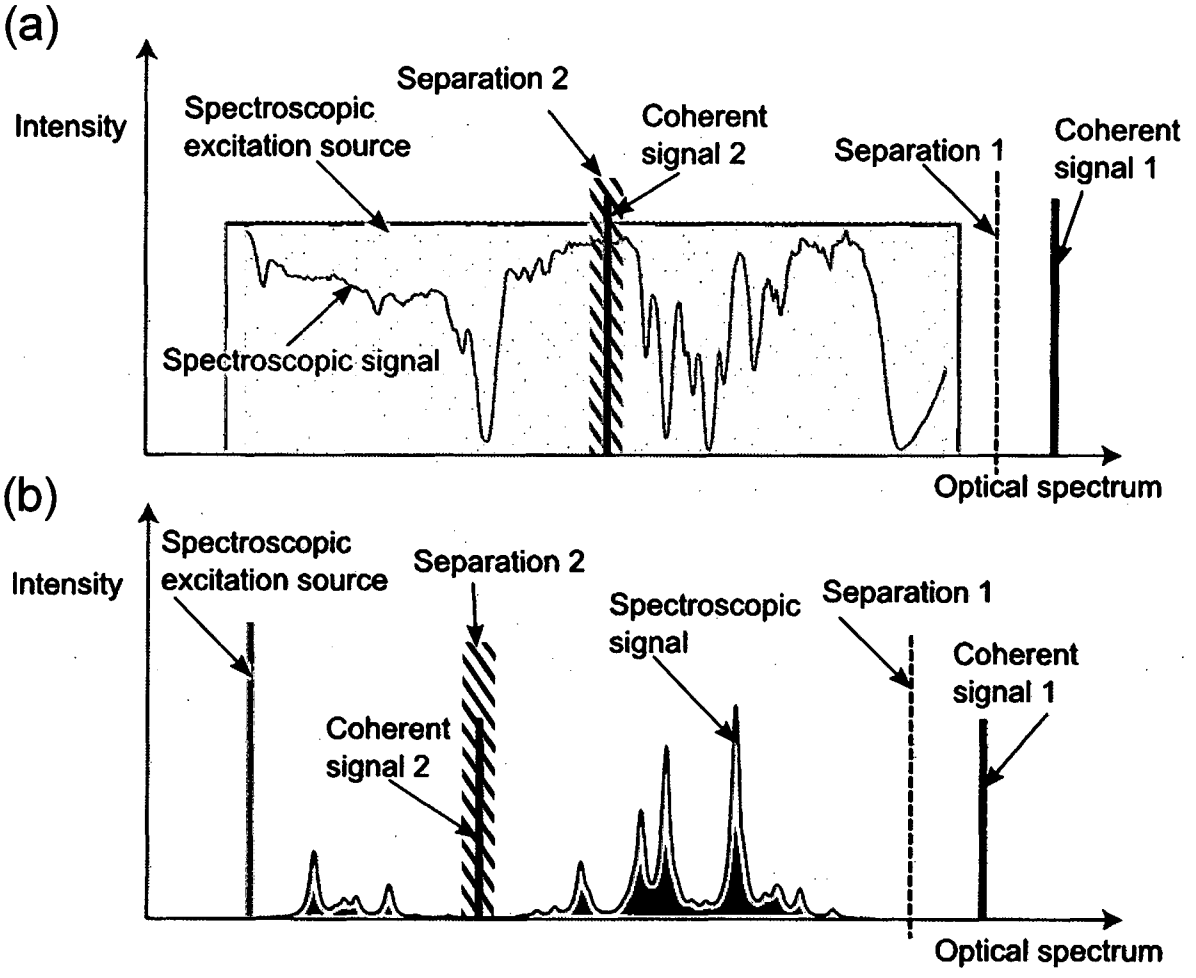

(a)
Intensity
Spectroscopic excitation source
Separation 2
Coherent signal 2
Separation 1
Coherent signal 1
Spectroscopic signal
Optical spectrum
(b)
Intensity
Spectroscopic excitation source
Separation 2
Spectroscopic signal
Separation 1
Coherent signal 1
Coherent signal 2
Optical spectrum

Fig.5

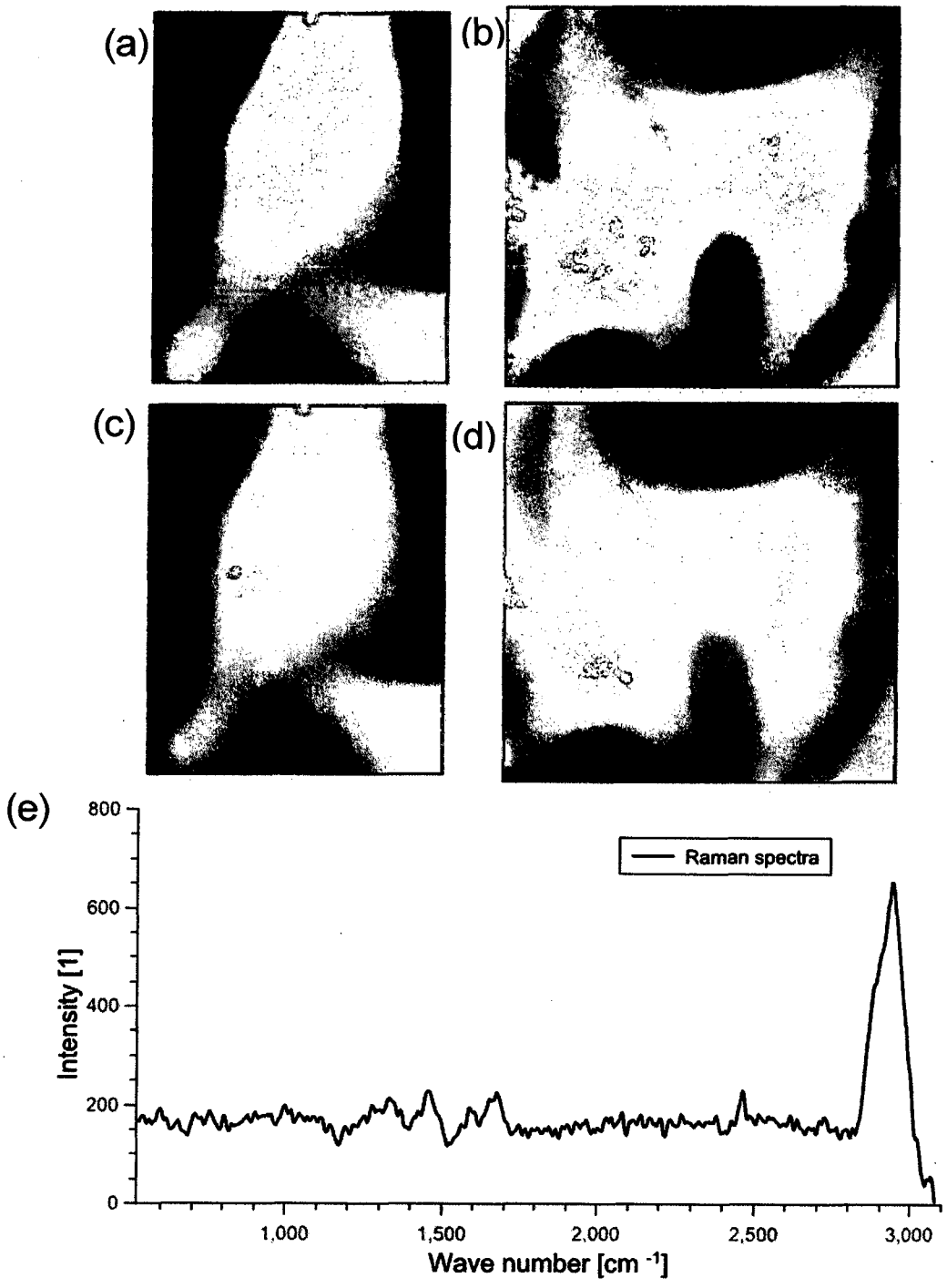

(a)
(b)
(c)
(d)
(e)
Raman spectra
Intensity [1]
800
600
400
200
0
1,000
1,500
2,000
2,500
3,000
Wave number [cm -1]

Fig.6

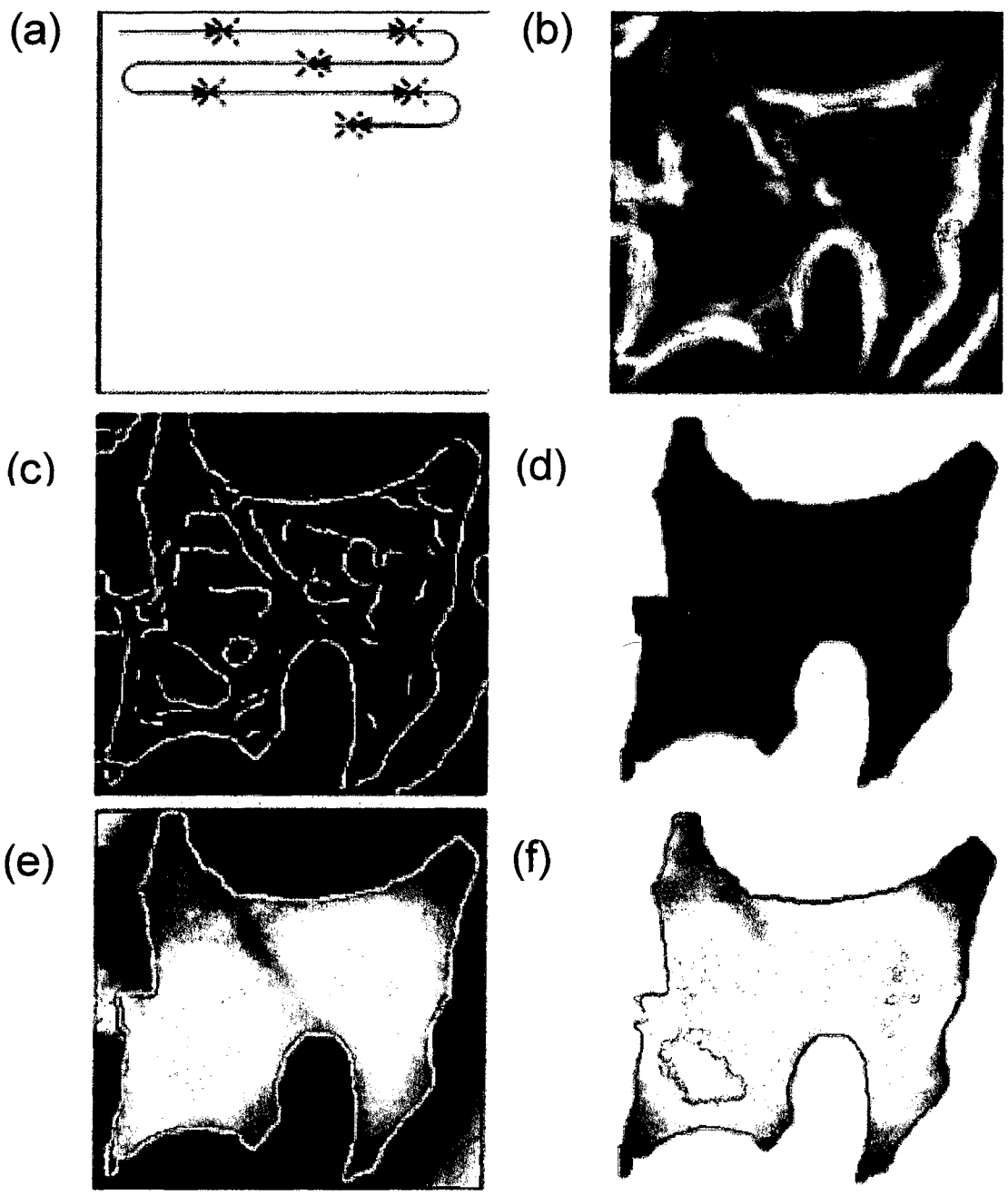


Fig.7

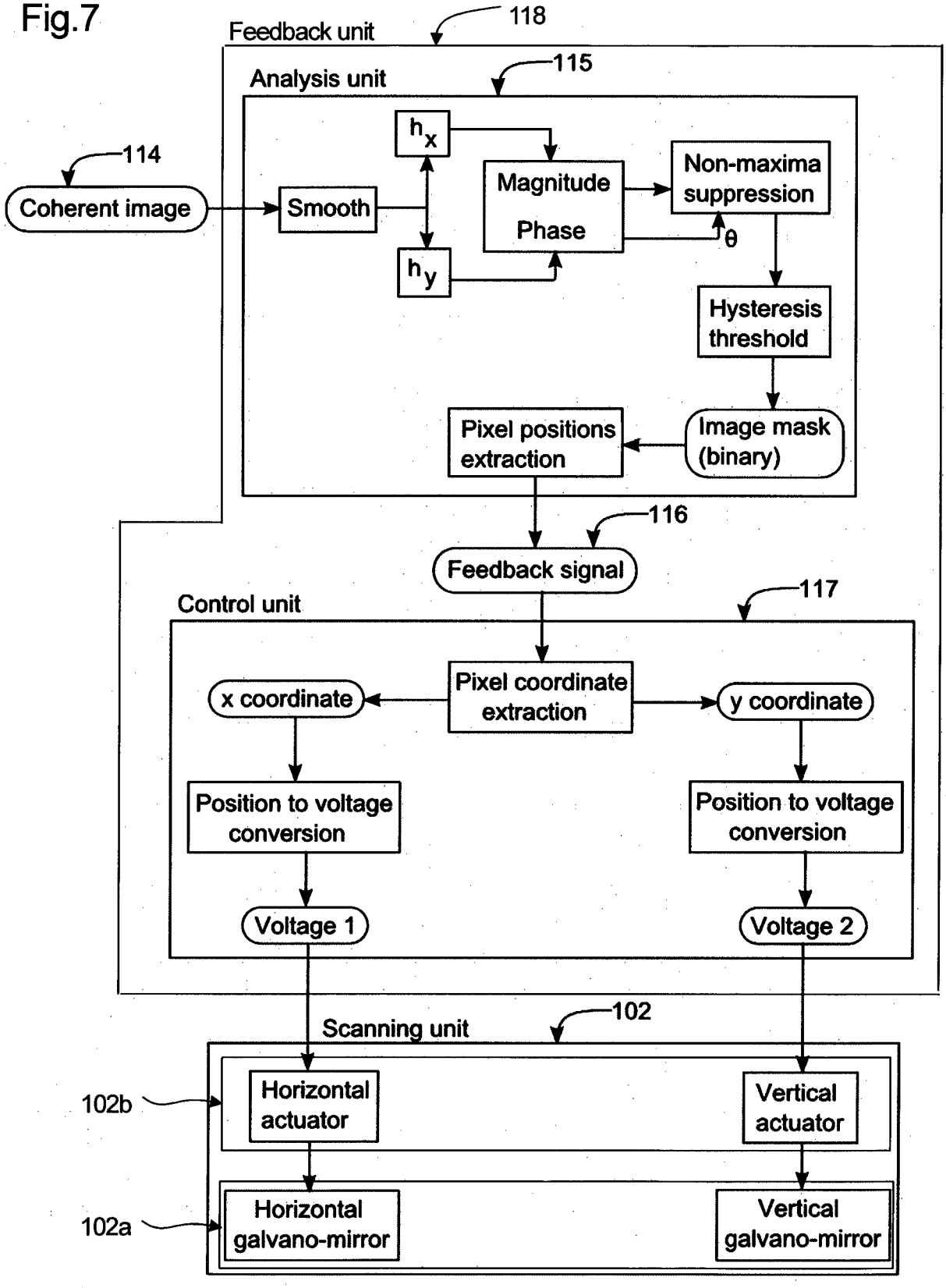

Feedback unit
118
Analysis unit
115
114
Coherent image
Smooth
h_x
h_y
Magnitude
Phase
Non-maxima suppression
θ
Hysteresis threshold
Image mask (binary)
Pixel positions extraction
116
Feedback signal
Control unit
117
Pixel coordinate extraction
x coordinate
y coordinate
Position to voltage conversion
Position to voltage conversion
Voltage 1
Voltage 2
Scanning unit
102
102b
Horizontal actuator
Vertical actuator
102a
Horizontal galvano-mirror
Vertical galvano-mirror

Fig.8

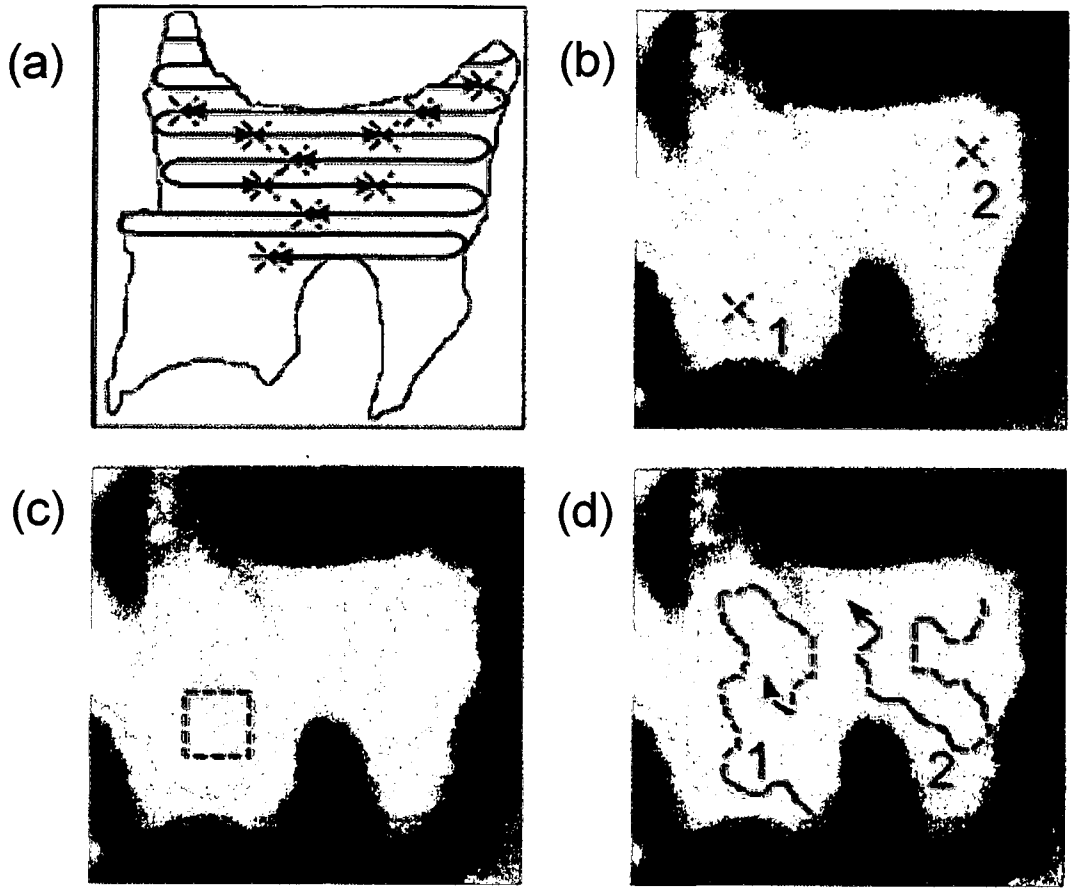


Fig.9

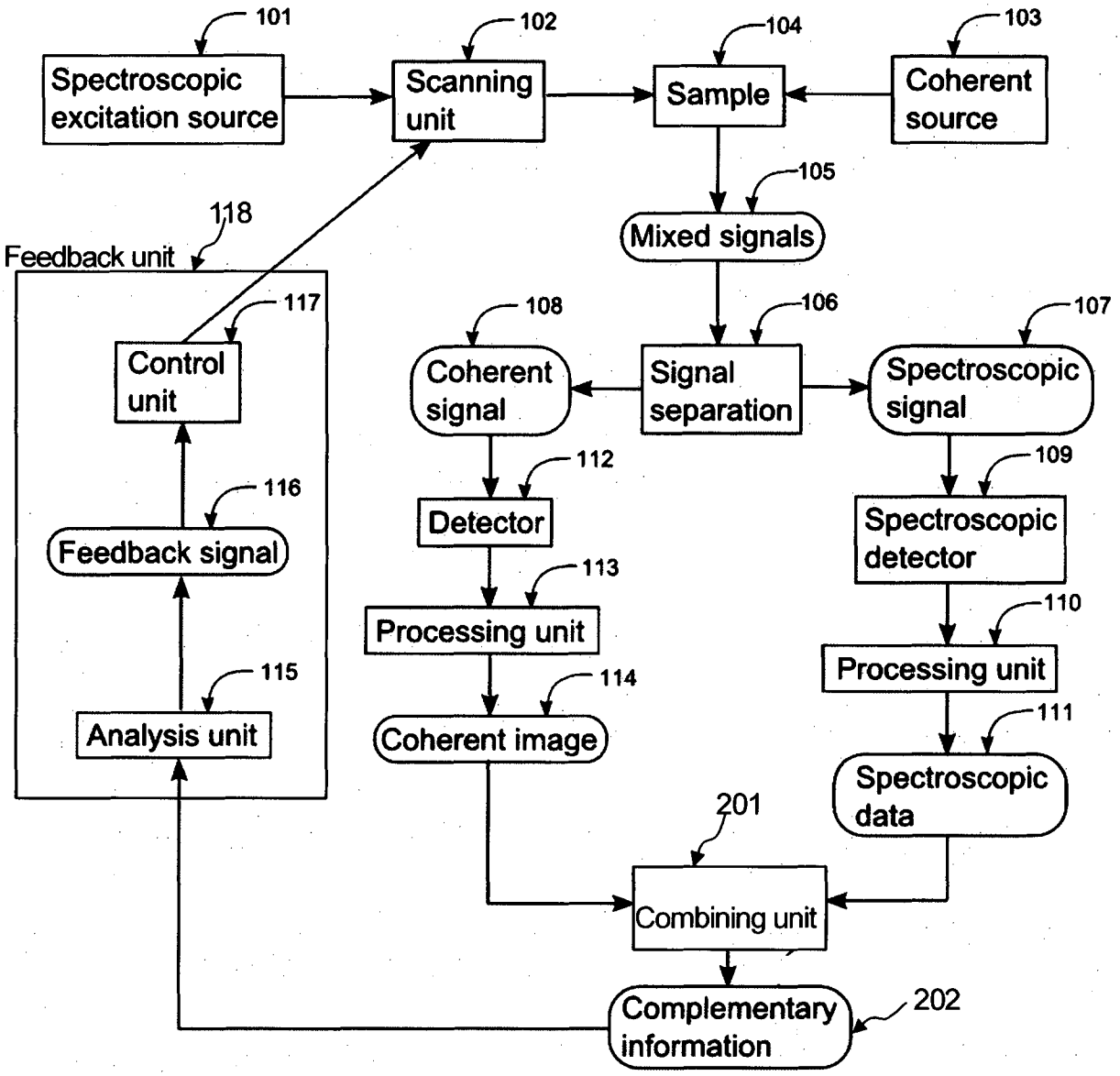
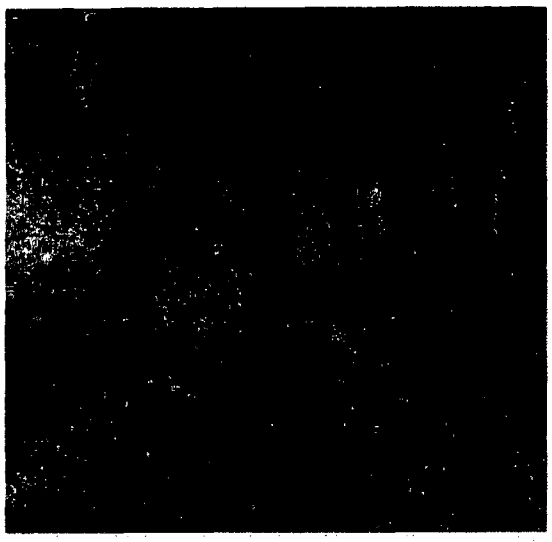

101
Spectroscopic excitation source
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118
Feedback unit
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Control unit
116
Feedback signal
115
Analysis unit
108
Coherent signal
106
Signal separation
107
Spectroscopic signal
112
Detector
113
Processing unit
114
Coherent image
109
Spectroscopic detector
110
Processing unit
111
Spectroscopic data
201
Combining unit
Complementary information
202

Fig.10



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2014/066366

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. G01N21/65(2006.01)i, G01B11/24(2006.01)n

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. G01N 21/00 - G01N 21/74

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2014
Registered utility model specifications of Japan 1996-2014
Published registered utility model applications of Japan 1994-2014

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

JSTPlus(JDreamIII), JMEDPlus(JDreamIII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2011-257691 A (OLYMPUS CORPORATION) 2011.12.22 [0026]-[0056],[0094]-[0100], Figure 1	1-7
A	JP 10-090064 A (FUJITSU CORPORATION) 1998.04.10 [0019]-[0028], Figure 1	1-7
A	WO 2004/081549 A1 (KONINKLIJKE PHILIPS ELECTRONICS N.V.) 2004.09.23 Figure 1	1-7
A	WO 2010/024397 A1 (RIKEN) 2010.03.04 Figures 1, 13	1-7

☐ Further documents are listed in the continuation of Box C.

☑ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08.07.2014	22.07.2014

Name and mailing address of the ISA/JP	Authorized officer
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INTERNATIONAL SEARCH REPORT
Information on patent family members

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PCT/JP2014/066366

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