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(54) Title: SYSTEMS AND METHODS FOR HIGH THROUGHPUT DETECTION AND IMAGING OF SAMPLE ARRAYS USING SURFACE PLASMON RESONANCE

(57) Abstract: A detection system for detecting an array of samples is provided. The system comprises an electromagnetic radiation source, a reference arm, and a sample arm comprising a sensing surface having a plurality of sample fields configured to receive the array of samples. The detection system further comprises a spatial phase difference generator configured to introduce differences in pathlengths of one or more samples a path length difference along a first direction in the array of samples, a carrier signal generator configured to introduce a carrier signal along a second direction in the array of samples, and an imaging spectrometer configured to image one or more samples in the array of samples.

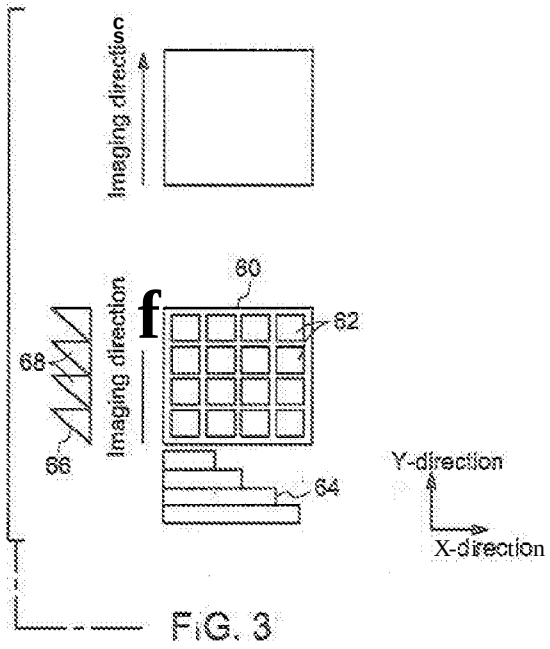

FIG. 3

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Systems and methods for high throughput detection and imaging of sample arrays using surface plasmon resonance

BACKGROUND

[0001] The invention relates to detection and imaging, and more particularly to systems and methods for optical detection and imaging of sample arrays.

[0002] Surface plasmon resonance (SPR) detection is an optical detection technique that is used to detect molecular adsorptions and interactions. The SPR detection is used in a wide variety of chemical systems, including biosensors. Typically, SPR sensors comprise an arrangement where a prism supports a thin metal layer. A ligand molecule is immobilized on one side of the thin metal layer to form a modified metal surface. A sample is disposed on the modified metal surface. A light beam incident on the sample excites surface plasmons in the thin metal layer in a resonant manner. The surface plasmons propagate in a direction parallel to the interface formed between the thin metal layer and the prism (metal/prism interface). Since the surface plasmons are present at the boundary of the thin metal layer and an external medium (e.g., air or water), the oscillations of the surface plasmons are responsive to any changes in the boundary of the metal and the external medium, such as the adsorption of molecules on the metal surface. SPR phenomenon is typically detected by sensing refractive index changes near the surface of a thin metal layer. A reflection spectrum of the modified metal surface may be determined by measuring the intensity of a reflected light as a function of an angle of incidence or a wavelength of the incident light. The sensitivity of the SPR phenomenon towards refractive index changes at the boundary is useful in observing and quantifying chemical reactions at a thin metal film/sample solution interface.

[0003] Typically, there are two paths in an interferometer, namely a reference arm and sample arm. The incident light from the radiation source is split into two portions, a first portion travels through the reference arm, and the second portion travels through the sample arm and is incident on the samples. A path length difference is introduced for the

samples on the sample arm with respect to the reference arm. The reflected beams from the two arms are interfered, and spectrally detected by a detector. The path length difference of the sample beam with respect to the reference beam results in fringes which may be inverse Fourier transformed to localize the samples.

[0004] However, because the Fourier transformation of a real valued function is Hermitian symmetric, the Fourier transformation of fringes representative of spectral characteristics of the samples, produces a complex conjugate artifact. Disadvantageously, the complex conjugate artifact mirrors with the reconstruction about a zero-path length difference in the complex space. This mirroring of the complex conjugate artifact results in unresolved positive and negative side of the peaks representative of the spectral characteristics of the samples. To overcome the mirroring effect and to obtain unambiguous reconstruction, it is desirable to eliminate any overlap between the reconstructed sample characteristics and their conjugate counterparts. The overlap may be eliminated by selecting the path length difference, such that the sample beam and the reference beam comprises either positive or negative path length difference with respect to each other. However, using this approach, only half of the complex space is utilized in reconstruction. Utilizing lesser space reduces a throughput of the system. Also, selecting one of the positive or negative path length difference increases system complexity due to increased constraint with regard to the device used for introducing the phase difference. In addition to the increased system complexity, the finite spectral resolution of the system adds a further disadvantage to the maximum path length difference that may be realized due to sensitivity fall-off with increase in path length difference. As the sensitivity of the system is highest around the zero path length difference, it is useful that the reconstruction of the sample characteristics is performed by placing the zero-phase delay inside the sample.

[0005] Therefore, it is desirable to provide improved systems and methods which can eradicate complex ambiguity in the reconstruction, and utilize full complex space to accommodate more samples, thereby increasing the throughput of imaging arrays of the samples.

BRIEF DESCRIPTION

[0006] In one embodiment, a detection system for detecting an array of samples is provided. The detection system comprises an electromagnetic radiation source, a reference arm, and a sample arm comprising a sensing surface having a plurality of sample fields configured to receive the array of samples. The detection system further comprises a spatial phase difference generator configured to introduce differences in pathlengths of one or more samples a path length difference along a first direction in the array of samples, a carrier signal generator configured to introduce a carrier signal along a second direction in the array of samples, and an imaging spectrometer configured to image one or more samples in the array of samples.

[0007] In another embodiment, a surface plasmon resonance (SPR) detection and imaging system for detecting and imaging an array of samples is provided. The SPR detection and imaging system comprises a broadband light source configured to illuminate the array of samples, and an optical engine. The optical engine comprises a SPR sensing surface having sample fields and at least one reference field, a spatial phase difference generator configured to introduce pathlength differences in one or more samples along a first direction in the array of samples, a carrier signal generator configured to introduce a carrier signal along a second direction in the array of samples, an image acquisition unit configured to acquire image data, and a signal processing unit for processing the acquired image data.

[0008] In one example, a method for imaging spectral characteristics of samples in an array of samples is provided. The method comprises illuminating a reference arm and sample arm using a reference beam and sample beam, respectively, to produce a resultant reference beam and sample beam, respectively, introducing a path length difference in at least one of the reference beam, sample beam, resultant reference beam, resultant sample beam, or a combination thereof, and introducing a carrier signal in at least one of the reference beam, sample beam, resultant reference beam, resultant sample beam, or a combination thereof. The method further comprises interfering the resultant reference beam with the resultant sample beam to form interference spectra, acquiring the interference spectra, and imaging the spectral characteristics of the samples based on the acquired interference spectra.

DRAWINGS

[0009] These and other features, aspects, and advantages of the invention will become better understood when the following detailed description is read with reference to the accompanying drawings in which like characters represent like parts throughout the drawings, wherein:

[0010] FIG. 1 is a block diagram of an example high throughput detection and imaging system for simultaneous detection of an array of samples;

[0011] FIG. 2 is a schematic drawing of an example optical engine of FIG. 1;

[0012] FIGS. 3-5 are cross-sectional views of reference and sample arms using spectral and imaging phase difference generators;

[0013] FIG. 6 is a cross-sectional view of an example high throughput surface plasmon resonance (SPR) detection and imaging system for simultaneous detection of an array of samples;

[0014] FIG. **7** is a cross-sectional view of an example sensing surface configured for free-solution SPR;

[0015] FIG. 8 is a cross-sectional view of an example sensing surface configured for localized SPR;

[0016] FIG. 9 is a cross-sectional view of an example sensing surface configured for nano-grating SPR;

[0017] FIG. 10 is a cross-sectional view of an example sensing surface configured for reflectometric interference spectroscopy;

[0018] FIG. 11 is a flow chart of an example method for high throughput detection and imaging of an array of samples;

[0019] FIG. 12 is an example of an interferometric signal comprising a carrier signal;

[0020] FIG. 13 is an example interferometric signal representative of a single row of samples in an array of samples;

[0021] FIG. 14 is an example graph of a carrier signal added to each column in an imaging direction;

[0022] FIG. 15 is an example of an inverse Fourier transform of a complex signal derived from the interferometric signal of FIG. 13;

[0023] FIG. 16 is an example of a filtering technique used to separate individual peaks in the inverse Fourier transform of FIG. 15; and

[0024] FIG. 17 is an example of Fourier transform of peaks identified in FIG. 16.

DETAILED DESCRIPTION

[0025] Systems and methods for high throughput detection and imaging of samples are provided. The systems and methods may be configured for simultaneous detection of a plurality of samples. The samples may be disposed in a one-dimensional (ID) or a two dimensional (2D) array. In certain embodiments, the simultaneous detection may comprise detecting the samples in a single shot or multiple frames. The systems and methods may be suitable for a high throughput detection and imaging of samples. Individual images of spectral characteristics of the various samples in the sample array may be reconstructed by introducing a phase difference in the array of samples in a first direction (e.g., x-direction), and a carrier signal in a direction other than the first direction. In one embodiment, the phase difference may be introduced in a spatial direction, and the carrier signal may be introduced in a second direction (e.g., y-direction). In this embodiment, the imaging may be performed in the second direction, that is, the second direction may be an imaging direction. In one example, the spatial direction may be a direction about perpendicular to the imaging direction. In an array of samples, if the direction of traversing a row is considered a spatial direction, the direction of traversing a column may be considered an imaging direction.

[0026] In certain embodiments, a detection system for detecting an array of samples is provided. The detection system may comprise an electromagnetic radiation source, a reference arm, and a sample arm comprising a sensing surface having a plurality of

sample fields configured to receive the array of samples. The detection system may further comprise a spatial phase difference generator configured to introduce differences in pathlengths of one or more samples a path length difference along a first direction in the array of samples, and a carrier signal generator configured to introduce a carrier signal along a second direction in the array of samples. An imaging spectrometer configured to image one or more samples in the array of samples may be used in the detection system.

[0027] In certain embodiments, the path length difference or phase difference may be introduced between the sample beam and reference beam in the first direction to spectrally detect the samples in the direction of the phase difference introduced. The phase difference may be introduced for incident radiation or resultant reflected radiation (reflected by the samples or reference). In one example where the phase difference is introduced in the incident sample radiation, the resultant reflected beams from the sample fields in the first direction may be phase separated. The phase difference may be introduced using a phase difference generator as described in detail in the commonly assigned U.S. Patent Application No. 12/964,602 titled "Systems and methods for detection and imaging of two-dimensional sample arrays", which is hereby incorporated by reference. Such a phase difference generator is referred to as a "spatial phase difference generator" throughout the present application.

[0028] In certain embodiments, the carrier signal may be introduced in the imaging direction of the reference arm or sample arm, or both. The carrier signal may be a continuous function or a discontinuous function in the imaging direction. In embodiments where the carrier signal is a discontinuous function, the carrier signal may be continuous within sample, and may comprise discontinuities between the samples. The carrier signal may be a linear ramp function, step function, non-linear function, or a combination thereof. The carrier signal may be generated using a carrier signal generator. The carrier signal generator may be a mechanical or a non-mechanical device. In one embodiment, the carrier signal generator may comprise one or more imaging phase difference generators in the reference arm, sample arm, or both. The path length introduced by the imaging phase difference generators in the imaging direction may be a constant value, or a value that varies as a function of distance traversed in the imaging direction. For example, the path length may be a linear ramp function, step function, non-linear function, or a combination thereof. In one embodiment, a similar amount of phase

difference may be introduced in each of the sample rows in the imaging direction. In another embodiment, a different amount of phase difference may be introduced in one or more sample rows in the imaging direction. In one embodiment, the carrier signal may be a sinusoidal wave.

[0029] In one embodiment, the carrier signal generator may comprise one or more imaging phase difference generators. In one example, the carrier signal may comprise a first imaging phase difference generator operatively coupled to the sensing surface, and a second imaging phase difference generator operatively coupled to a reference arm.

[0030] In certain embodiments, the carrier signal generator may comprise one or more imaging phase difference generators that may comprise any geometrical or non-geometrical shape. The carrier signal generator may comprise a shape and design that provides a continuous carrier signal to one or more samples in the imaging direction.

[0031] In certain embodiments, the imaging phase difference generators may comprise a plurality of portions. The plurality of portions may be such that a shape, range of refractive index values, thickness values, or a combinations thereof, of a portion of the carrier signal generator may be the same or different than that of other portions.

[0032] In one embodiment, the carrier signal generator may comprise a stepped or planar structure having a plurality of portions, wherein each of the portions has a refractive index that is different from the other portions. In one embodiment, the imaging phase difference generator may comprise portions having varying thicknesses. In one example, the portions having varying thicknesses comprise planar surfaces, curved surfaces, stepped-surfaces, or combinations thereof.

[0033] In instances where the carrier signal is introduced in the reference arm, the carrier signal may be introduced by tilting the reference arm, or by disposing an imaging phase difference generator in an optical path of the incident or resultant reflected radiation. In embodiments where the carrier signal is introduced in the sample arm, the carrier signal may be introduced in the sample arm by tilting the sample arm in the imaging direction or by disposing a phase difference generator in an optical path of the incident or resultant reflected radiation. In instances where the carrier signal is introduced in both reference and sample arms, the resultant carrier signal in the imaging direction may be a combination of the carrier signals of the reference and sample arms.

[0034] In certain embodiments, the carrier signal generator may be made of a material that is transparent to the incident radiation reference beam, or the resultant sample beam. For example, the imaging phase difference generator may be made of the material that is transparent to the incident radiation reference beam, or the resultant sample beam. Non-limiting examples of the materials for the imaging phase difference generator may include glass, polymer, dielectric material, or a combination thereof. In one embodiment, the imaging phase difference generator may be made of a multilayer dielectric materials, a stack of glass plates, a liquid crystal display, a sandwich structure of different refractive index materials, computer generated holographic data, or a combination thereof.

[0035] Zero phase delay is the delay corresponding to the common distance travelled by both the reference and sample beams. An imaging signal, such as a surface plasmon resonance (SPR) signal, is a complex signal that comprises a real part and an imaginary part. Signal processing of the complex signal yields true image and complex conjugate image. The phase difference introduced in the spatial direction may be used to spectrally resolve the samples in the spatial direction. The carrier signal in the imaging direction may introduce a phase difference in the imaging direction. The carrier signal introduces periodic phase shift in imaging direction such that an analytic signal corresponding to the signal in the spatial direction may be obtained. The phase difference in the imaging direction may be provided to utilize the complex space about the zero delay line.

[0036] Introducing a carrier signal in the imaging direction may provide a negative complex conjugate of the image. This negative complex when added with the real value and its complex conjugate may be canceled, thereby providing only the real value in the image.

[0037] Signal processing may be used to obtain true peaks by cancelling out the complex conjugate image. In one example, a Fourier transform of the analytic signal may be performed to obtain true peaks. Hence, true peaks appear on one side of the zero delay line, while the space on the other side of the zero delay line is made available due to cancellation of the complex image. The available space on the other side of the zero delay line may be used to accommodate additional peaks in the spatial direction. Hence, the number of samples that may be imaged may be increased by up to two times, thereby increasing the throughput of the system.

[0038] In one example, for a sample array having two samples A and B disposed in the spatial direction. Let SA(λ), and *S*B(λ) be the absorption spectra of spots A and B, respectively. Let ZA and ZB be the path length introduced by a spatial phase difference generator for spots A and B, respectively.

[0039] Light intensity in the interference spectra of the two points is represented as I(λ) as shown by Eq. 1.

$$I(X) = [R + O_A + O_B][R + O_A + O_B]^H \quad \text{Eq. (l)}$$

[0040] where, **R** is the reflected wave from the reference arm, $\mathbf{O_A}$ and $\mathbf{0_B}$ are the reflected waves from the spots A and B, on the sample arm respectively. If **R** is represented as a propagating wave Y(λ), $\mathbf{O_A}$ and $\mathbf{0_B}$ is defined as below:

$$0_A = Y(\lambda)¾\ (\lambda) exp\ (-j\frac{2\pi Z_A}{\lambda}) \quad \text{Eq.(2)}$$

$$0_B = Y(X)S_B(\lambda)exp\left(-i\frac{2\pi Z_B}{\lambda}\right) \quad \text{Eq.(3)}$$

[0041] Assuming that a carrier signal with frequency f_c is added to the reference arm. In one example, the carrier signal may be introduced by tilting the reference mirror. Assuming that the samples are irradiated with broadband source of spectrum U (λ), where U (λ) = Y(λ)Y*(λ). Further assuming that the absorption spectra of the two samples A and B satisfy the Eqs. (4) to (7).

$$S_A(X)exp(j\omega_A) + S_B(\lambda)exp(j\omega_B) = Pexp(i<p_p) \quad \text{Eq. (4)}$$

$$S_A(\lambda)^H exp(-j\omega_A) + S_B(\lambda)^H exp(-j\omega_B) = Qexp(i\varphi_q) \quad \text{Eq. (5)}$$

$$S_A(\lambda)S_B(\lambda)^H exp(j\omega_A - j\omega_B) = Rexp(i\varphi_{pq}) \quad \text{Eq. (6)}$$

$$S_A(\lambda)^H S_B(\lambda)exp(j\omega_B - j\omega_A) = Texp(i\varphi_{qp}) \quad \text{Eq. (7)}$$

[0042] By substituting and rearranging the terms in Eq. (1) with the addition of carrier signal results in Eq. (8) as follows:

$$-\hat{\wedge}-\hat{\ } = [1 + \boldsymbol{exp(j f_c x) Pexp(i\varphi_p) + exp(-j f_c x) Qexp(i\varphi_q) + Rexp(i\varphi_{pq}) + Texp(-i\varphi_{qp})]} \quad \text{Eq. (8)}$$

[0043] Eq. (8) represents a sum of complex sinusoids in the spatial direction (x-direction) with peaks at DC and some distribution around DC due to the cross talk component, $Rexp(i\varphi_{pq})$, and its conjugate $\boldsymbol{Texp(i\varphi_{qp})}$, and two peaks centered around $\boldsymbol{f_c}$ and $\boldsymbol{-f_c}$. In one example, the analytic signal, $\boldsymbol{Pexp(i\varphi_p)}$ may be obtained by performing Fourier filtering around the frequency, $\boldsymbol{f_c}$. The amplitude and phase of the $\boldsymbol{Pexp(i\varphi_p)}$ may be determined to obtain an imaging signal represented by Eq. (9) as follows:

[0044] $7/(2) = \boldsymbol{S_A(\lambda) exp\left(j\frac{2\pi Z_A}{\lambda}\right) + S_B(X) exp\left(j\frac{2\pi Z_B}{\lambda}\right)}$ Eq. (9)

[0045] Thus, by adding a carrier signal, the complex conjugate component and cross talk component in the real valued intensity signal may be removed. Hence, full complex space may be utilized to accommodate more samples in the spatial direction.

[0046] Eq. (9) may be extended in the second direction for reconstructing the spectral absorption for each sample row. For example, in the case of a 8 X 8 sample array that comprises 8 sample rows and 8 sample columns. Eq. (9) may be applied for each sample row. The processing may be performed row by row to obtain imaging spectral characteristics of the samples in the array of samples.

[0047] FIG. 1 illustrates a high throughput detecting and imaging system 10 for simultaneously detecting two or more samples in an array of samples. System 10 comprises an electromagnetic radiation source 12 for irradiating the array of samples with electromagnetic radiation 14. The electromagnetic radiation source 12 may produce visible light, or near infrared light depending on the types of samples to be detected. Non-limiting examples of the radiation source 12 may comprise a light emitting diode, super luminescent light emitting diode, broadband light source, or a combination thereof. The broadband light source may emit a continuous spectrum output over a range of wavelengths at any given point in time. The broadband light source may include sources such as, but not limited to, a tungsten lamp, white light source, xenon lamp, metal halide lamp, phosphor source, or a combination thereof.

[0048] The radiation 14 from the radiation source 12 may be directed to an optical engine 16. The optical engine 16 comprises an optical arrangement for directing the radiation 14 to a sensing surface (not shown) and a reference (not shown). The sensing surface may form a part of the optical engine 16. The optical engine 16 may be in a Michelson or Mach Zehnder interferometer configuration. The system 10 may comprise two optical paths, namely a reference arm and a sample arm. The sample arm may comprise an optical path from a radiation source 12 to the samples and from the samples to an imaging spectrometer. Similarly, the reference arm may comprise an optical path from the radiation source 12 to the reference and from the reference to the imaging spectrometer.

[0049] The sensing surface may comprise a plurality of sample fields for disposing the samples. The sample fields may be disposed on the sensing surface as a ID or 2D array. The samples may be disposed in some or all the sample fields. The sample fields to dispose the samples may be chosen in a determined geometrical pattern. Alternatively, the sample fields may be chosen in an irregular fashion in which to dispose the samples.

[0050] In certain embodiments, one or more sample fields configured to receive samples may be functionalized. In these embodiments, the sample fields may be immobilized with functionalizing material, such as ligand molecules.

[0051] A microfluidic device 18, such as a microfluidic chip, may be operatively coupled to the sensing surface to provide samples to the sensing surface. The microfluidic device 18 may be configured to provide the samples to the corresponding sample fields on the sensing surface. A microfluidic controller 20 may be provided to control the microfluidic operations of the microfluidic device 18. Optionally, a sample handling unit 21 may be operatively coupled to the microfluidic device 18. The sample handling unit 21 may be coupled to fluid ports of the microfluidic device 18 for transporting samples to and from the microfluidic device 18, or for carrying off waste flows from the microfluidic device 18. The sample handling unit 21 may comprise chambers or reagent reservoirs for storing sample solution, flow through port for transporting samples, a pumping device, and a sample flow controller. The sample handling unit 21 may be configured to modify the transport of samples based on the detection of samples by the system 10. The sample handling unit 21 may be configured to accommodate a variety of samples including liquid and gaseous samples. The sample

handling unit 21 may comprise provisions for sample preparation and processing, such as but not limited, metering, mixing and diluting. The sample handling unit 21 may comprise a thermal element for heating or cooling the samples.

[0052] A phase difference may be introduced in the incident radiation 14 or a resultant reflected radiation in the first direction. The phase difference may be introduced in the reference arm or the sample arm using a spatial phase difference generator 22.

[0053] In certain embodiments, the carrier signal may be introduced in the imaging direction of the reference arm or sample arm, or both. The carrier signal may be introduced in the second direction using a carrier signal generator. The first direction may be the spatial direction and the second direction may be the imaging direction. As will be appreciated, a sample field expands over a certain number of pixels in the imaging direction. The carrier signal may be added such that the phase difference between the pixels in each row is less than about 180 degrees. The carrier signal may be introduced in the reference arm, sample arm, or both. In instances where the carrier signal is introduced in both reference and sample arms, the resultant carrier signal in the imaging direction may be a combination of the carrier signals of the reference and sample arms.

[0054] In certain embodiments, the carrier signal generator may comprise microelectromechanical systems (MEMS) based structure, liquid crystal phase modulator, wave plate, computer generated holographic data, a dielectric material structure to introduce a path difference in the imaging direction of the sample arm, reference arm, or both. In one embodiment, the carrier signal generator may be an imaging phase difference generator that may comprise plurality of portions having different refractive indices or thicknesses, such that at least one of the portions comprises a refractive index or a thickness that is different from the other portions. The imaging phase difference generator may comprise shapes, such as but not limited to, a wedge shape, a cube shape, a cuboid shape, a stepped structure, or a combination thereof. A surface of the imaging phase difference generator disposed parallel to the imaging direction may be a planar surface, non-planar surface, or a combination thereof.

[0055] The optical engine 16 comprises an optical arrangement for directing the radiation to the sample arm and the reference arm. The optical engine 16 comprises a

phase difference generator for inducing the first phase difference in the radiation illuminating the sample with respect to the reference beam.

[0056] The system 10 comprises an arrangement for simultaneous acquisition of images. The acquisition may be either a time-domain or Fourier-domain signal. The system 10 may be configured for sweep-source or spectrometer-based Fourier domain analysis.

[0057] The samples are detected by analyzing interference spectra formed by interfering the sample radiation with reference radiation. The interference spectra from the optical engine 16 are received by the image acquisition subsystem 22. The image acquisition subsystem 22 acquires image data that includes interference in spectral domain. The image acquisition subsystem 22 may include a combination of a detector and a grating. In one embodiment, the grating comprises 4200 lines per mm, however, other values of grating lines per mm may also be selected. The grating may be oriented such that ruling of the gratings are parallel to the orientation of the rows (x-direction) on the sensing surface

[0058] The image acquisition subsystem 22 may include additional optical elements such as lens for collimating or focusing the radiation. The acquired image may be processed using a signal processing unit 24. A user interface 26, such as but not limited to a graphical user interface, may be used to provide a user interface to allow the user to interact with the detection system 10.

[0059] The signal processing unit 24 may comprise a microprocessor, microcontroller and a digital signal processor (DSP). The system 10 may also comprise a storage device (not shown) for at least temporarily storing one or more images. The storage device may comprise, but is not limited to, any suitable hard drive memory associated with the processor such as the ROM (read only memory), RAM (random access memory) or DRAM (dynamic random access memory) of a CPU (central processing unit), or any suitable disk drive memory device such as a DVD or CD, or a zip drive or memory card. The storage device may be remotely located from the signal processing unit 24 or the imaging device, and yet still be accessed through any suitable connection device or communications network including but not limited to local area networks, cable networks, satellite networks, and the Internet, regardless whether hard wired or wireless.

[0060] FIG. 2 illustrates an example of the optical arrangement within the optical engine 16. The optical engine 16 comprises an optical arrangement for directing a portion of the radiation 14 to a splitter/coupler 28. The splitter/coupler 28 splits the light into two portions, a first portion or a reference beam 30 is directed towards a reference sample 32. The second portion or a sample beam 34 is directed towards an array 36 of samples 38. The samples 38 may be chemical or biological samples. In one embodiment, the samples 38 may be chemically or biologically samples. The chemically or biologically active samples 38 may produce a determined response when they come in contact with a chemical or a biological entity, respectively. In one example, the samples 38 may have a time constant optical property. The samples 38 may comprise optically active materials. In one example, the samples 38 may absorb, transmit, or reflect the incident radiation.

[0061] In certain embodiments, the reference sample 32 may comprise conventionally used reference solutions such as, but not limited to, high index solutions, low index solutions wherein the high and low refractive indices are the refractive index values that fall outside a resonance range of a device disposed in the optical engine 16, such as a waveguide. In certain other embodiments, the reference sample 32 may comprise a material with determined or known spectral absorption values. The known spectral absorption values may comprise a known constant value or a known time varying value. In one embodiment, the reference sample may be a non-absorptive sample. The reference sample 32 may comprise a material that reflects major portion of the incident radiation. In certain other embodiment, the reference sample 32 may be an optical element, such as, but not limited to, a mirror, total internal reflection surface.

[0062] The sample array 36 may be disposed on a sensing surface 40. The sensing surface 40 may comprise a plurality of sample fields 42 that contain the samples 38. The array 36 may be of varying sizes such as, but not limited to, a 4x4 array, a 6x6 array, or an 8x8 array of samples 38. Due to the ability of the system to remove complex conjugate peaks, in one embodiment, the system enables detection of columns that is two times more than the number of columns typically detectable in conventional detection systems.

[0063] The sensing surface 40 may be a spectrally modifying surface that may reflect, absorb, or transmit at least a portion of the incident sample beam 34. The sensing

surface 40 may be selected based on the detection techniques that are used. Non-limiting examples of the detection techniques may include surface plasmon resonance (SPR) such as but not limited to, a localized SPR (LSPR), nano-grating SPR, label-free SPR, or other techniques such as but not limited to reflectometric interference spectroscopy (RIfS). In the case of LSPR, the sensing surface 40 may include a glass substrate having metal structures. In the case of RifS the sensing surface 40 may include a glass surface. In the case of nano-grating SPR, the sensing surface 40, may include a glass surface having nano gratings.

[0064] The sample fields 42 may be formed on the sensing surface 40 by processing corresponding portions of the sensing surface 40. The processing may comprise fabrication techniques such as but not limited to, etching, patterning, or functionalizing at least portions of the sensing surface 40 corresponding to the sample fields 42. In one embodiment, portions of a top index layer of the sensing surface 40 may be etched to form trenches to define the sample fields. In the case of SPR detection, the sample fields 42 may be coated with a thin metal film, such as but not limited to, gold or silver, to enable SPR when the sample fields are irradiated with excitation radiation. In one example, the thin metal film may have a thickness in a range from about 0.001 microns to about 1 micron to provide for the SPR. In one example, the dimensions of the sample fields may be in a range from about 10 microns to about 112 microns. The volume of the sample fields may be sufficient for the detection of volumes of chemical or biological agents as low as micro-liters or pico-liters.

[0065] The sample fields 42 on the sensing surface 40 may be functionalized with one or more functional materials. The functional materials may comprise a coating of specific antibodies, proteins, DNA sequences, ligand molecules or amino acid sequences that are sensitive and specific to chemical or biological agents of interest. The functional material may be present in the form of a layer or a coating, also referred to as a functionalized coating. By varying the thickness of the metal film or functional layer the systems and methods may be used for linear detection or for threshold detection of predetermined agents. In one embodiment, the detection may be based on the competitive binding of the sample to the binding sites of the ligand. Same or different ligands may be disposed in the different sample fields 42 of the sample array 36. The functional materials may be disposed in discrete areas of the sensing surface 40. These

discrete areas may correspond to the sample fields 42. Thus, the functional materials may be present in the form of an array of discrete sample-binding regions. The different sample fields 42 may comprise same or different functional materials. For example, one or more of the sample fields 42 may comprise a ligand molecule different than the other sample fields. In one embodiment, all the different sample fields 42 may comprise different ligand molecules. The ligands may comprise one or more of a biopolymer, an antigen, antibody, nucleic acids and hormone ligands or a combination thereof. In one example, for antibody binding measurements, an antigen may be immobilized on the sample fields 42 and the sensing surface may be exposed to a solution containing the antibody of interest, after which binding proceeds.

[0066] The functionalizing material may saturate due to high concentrations of the samples in the array 36, or due to exposure of the sensing surface 40 to the sample solution for a long period time. In a case of a saturation of the functionalizing material, the corresponding sample field 42 or the sensing surface 40 needs to be regenerated to continue the detection. In one embodiment, the sensing surface 40 may be regenerated to allow the detection system to be used over and over again, thereby reducing the working material required, with a consequent significant cost reduction. In one example, the regeneration of the sensing surface 40 may be achieved by applying a different solution than previously used. In one example, the sensing surface 40 may be exposed to a base solution, such as sodium hydroxide, or to an acidic solution, such as, glycine hydrogen chloride buffer having pH 2.0, to regenerate the sensing surface. The regeneration of the ligands considerably reduces the cost of the sensor assembly. In one embodiment, regeneration of the ligands enables detection of different sample solutions. In this embodiment, the ligands are regenerated after detecting existing sample solution in a sample field and before providing the next sample solution in the sample field.

[0067] In one embodiment, a plurality of flow cells of a microfluidic device (not shown) may be operatively coupled to the sensing surface 40 to provide the samples 38 to the one or more sample fields 42 on the sensing surface 40. Each flow cell may correspond to one or more sample fields 42 on the sensing surface 40. For example, each of the fluidic channels may be aligned to a particular sample field 42. Each flow cell may comprise at least one fluidic channel. In embodiments where the different sample fields

42 may comprise different ligand molecules, the different sample fields 42 may be aligned with a corresponding fluidic channel having a corresponding ligand molecule.

[0068] A definer component 48 may be provided to define the geometry and the number of sample fields 42. Also, the contrast between the sample fields 42 and their intermediate regions may be determined by the definer component 48. In certain embodiments, a definer component 48 may be disposed in selected regions of the sensing surface 40. For example, the definer component 48 may be disposed in regions around the sample fields 42. The definer component 48 may be a patterned film of a suitable material.

[0069] In certain embodiments, a reference beam 30 may be directed towards the reference sample 32. The resultant reference radiation, generally referred to by the reference numeral 44, may be a reflective radiation produced by interaction of the reference beam 30 with the reference sample 32.

[0070] The radiation source 12 provides a sample beam 34 that is directed towards the sample array 36 and interacts with the samples 38. The size of the sample beam may be large enough to irradiate the sample array 36. Alternatively, the beam 34 may be directed to multi-spot generator optics to produce two or more spatially-spread discrete spots. In one example, the spatially-spread discrete spots are incident on a 2D array of samples. In one example, each of the spatially-spread discrete spots corresponds to a sample from the array 36 of samples 38.

[0071] The optical engine 16 may also include other optical elements such as lenses, filters, and collimators. For example, a lens each may be disposed in the reference arm and the sample arm to direct the radiation to the detector.

[0072] The resultant sample radiation, generally referred to by the reference numeral 46, may be a reflective radiation or a transmissive radiation. Interference spectra are produced by interaction of the resultant sample radiation from the reference sample with the resultant sample radiation from each of the samples.

[0073] In certain embodiments, a phase difference may be introduced in the spatial direction in the incident radiation 14 or resultant radiation in the sample or reference

using a spatial phase difference generator 50. The path length difference may be translated to phase difference in interference spectra.

[0074] In certain embodiments, the spatial phase difference generator 50 may introduce the phase difference between the incident sample beams 34 and reference beams 30. In certain other embodiments, the spatial phase difference generator 50 may introduce a phase difference between the resultant radiation 44 and 46. In these embodiments, the resultant radiation may be passed through a spatial phase difference generator 50 before reaching the detector 56. In the illustrated embodiment, a path length difference may be introduced in the incident reference beam 30.

[0075] In certain embodiments, a carrier signal may be introduced in the imaging direction (y-direction). The carrier signal may be introduced in the one or more samples in the imaging direction. The carrier signal introduced in the one or more samples in the imaging direction may be same or different. The carrier signal may be a continuous function or a discontinuous function. The carrier signal may be a linear or a non-linear function. The carrier signal may be configured to introduce a phase difference in the one or more samples. In one embodiment, a carrier signal generator 52 may be disposed in an imaging direction to provide path length difference to the one or more samples in the imaging direction. The carrier signal generator 52 may be disposed in the sample arm or the reference arm, or both. The phase difference, that is a path length difference, may be introduced in the incident or resultant radiation in the imaging direction.

[0076] The phase difference in the first direction obtained between the reference 32 and the samples 38 may be used to spatially separate various sample locations with respect to the spectral characteristics of the samples 38 corresponding to those sample locations. In one example, the spectral characteristics for various sample locations may be reconstructed using Fourier transform. The carrier signal facilitates removal of the conjugate complex peaks. The carrier signal may be used to provide a phase difference to the one or more samples in the imaging direction in the samples 38 or reference 32. The phase difference introduced in the imaging direction may be same or different from the phase difference introduced in the spatial direction. The carrier signal resolves the samples 38 in both x- and y-directions.

[0077] Resultant radiation 46 from the various samples 38 interfere with the resultant radiation 44 from the reference sample 32 and produces interference spectra. Introducing the phase difference in the first and a carrier signal in the second direction provides a condition under which interference between the resultant beams from the reference and detectable samples may occur giving rise to intensity variations of the beam emerging from the sensing surface 40. The phase difference introduced in the incident radiation 14 or the resultant radiation 44 and/or 46 may be present in the interference spectra. The intensity of the beam received at an imaging spectrometer 54 may depend on the difference in the path length of the beams in the detectable and reference samples.

[0078] In addition to the phase shift caused by the spatial phase difference generator 50 and carrier signal generator 52, the samples 38 disposed in the sample fields 42 may also contribute to the phase shift in the resultant sample radiation. The phase shift produced by the samples 38, may be a fraction of the phase shift produced by the generators 50 and 52. The small phase shift components contributed by the sample 38 may shift the corresponding fringes in the interference pattern. The shift of the fringes corresponds to the properties of the sample 38 at that sample field 42. The additional shift in the resultant radiation caused by the sample 38 may be useful in determining the chemical or optical properties of the sample 38.

[0079] The imaging of the locations of the samples 38, for example a 2D array of samples, may be obtained by reconstructing absorption spectra of the samples 38 using signal processing algorithms, such as but not limited to Fourier transform. Information regarding positions of the reference samples may be provided to the spectrometer 54. The resultant radiation 44 and 46 may be separately identified by the spectrometer 54. In certain embodiments, the samples 38 may be imaged in a single shot. The Fourier transform may be used to determine the spatially separated points (samples 38) from the acquired spectra without movement of any mechanical part or the reference beam, thereby improving the imaging speed.

[0080] The interference spectra between the resultant reference and sample radiation 44 and 46, respectively, may be analyzed and imaged using the imaging spectrometer 54. The imaging spectrometer 54 may include a spectrally separated detector 56 and a grating 58. The spectrally separated detector 56 may be a 2D detector. The spectral frequencies in the interference spectrum are separated using the detector 56 and the grating 58. The

detector 56 detects a change in the optical properties of the reflected light from the 2D array of samples 38. The detector 56 may detect the analytes concentration, or the chemical or biological composition in the sample.

[0081] The imaging spectrometer 54 may be operatively coupled to a signal processing unit 24 that measures interference spectra acquired by the detector 56. In the case of SPR detection and imaging, the wavelength sensitivity of the resonance may be used by maintaining the angle of incidence constant, and measuring the SPR effect as a function of wavelength. The reflectance spectrum exhibits a pronounced minimum due to the SPR effect in the visible to infrared wavelengths. In one embodiment, the position of the reflectance minimum shifts in wavelength upon the adsorption of sample molecules onto the metal surface due to the change of index of refraction at the sample-metal film interface. The detection system may be used to study the adsorption on a chemically modified metallic surface from the gas phase as well as from liquid solutions. In particular, the adsorption of biological molecules such as DNA, proteins, antibodies, and enzymes from aqueous solutions can be monitored in situ with the detection system. Advantageously, the detection system of the invention provides wavelength stability and measurement reproducibility, fast data acquisition rates and high signal-to-noise outputs, and broadened spectral range.

[0082] The imaging spectrometer 54 may be coupled to detection circuitry that may form part of the signal processing unit 24. In one example, the detection circuitry may convert current signal to voltage signal. Also, the detection circuitry may amplify the signal received from the imaging spectrometer 54. The detection circuitry may include components, such as but not limited to, data processor, for receiving measurements of interference pattern from the detector 56, such as a spectrometer, and for conducting analysis thereon, wherein the analysis comprises determining a parameter of an interference spectrum. Non-limiting examples of such parameters may include frequency, phase, and intensity of the interference fringes.

[0083] The detector 56 may be a photo-detector, a spectrometer, or a charge-coupled device (CCD), complementary metal oxide semiconductor (CMOS), a photodiode (such as an avalanche photodiode), solid state photomultiplier tube (PMT), image receptor, or a camera for measuring reflected light from the sample over a selected range of wavelengths. In embodiments where the detector 56 is a CCD or a camera, the detector

56 may record the spectrum of the reflected light from the sample. For each of the samples 38 on the sensing surface 40 there is a corresponding column or row in the 2D spectrometer to measure the interference spectrum of the corresponding sample on the sensing surface 40. If the imaging is done in a y-direction on the sensing surface 40 (which is e.g., a direction of columns), the different samples in a column are individually identified. However, for the samples 38 disposed in x-direction (which is e.g., a direction of rows) the different samples in a row are separately identified by introducing a phase difference using the spatial phase difference generator 50. After imaging using the Fourier transform, the samples 38 in the 2D array of samples are individually identified by the detector.

[0084] A computer may be used to process and display the signals and may form part of the signal processing unit 24. The computer may be used to generate a variety of quantitative and qualitative measures. For example, in quantitative measurements, the abscissa may represent time and the ordinate may represent percentage of concentration of an analyte. In addition, the computer may have a spectrum library, which stores the information regarding the spectral characteristics of various elements or chemical compounds. This spectrum library may be used to identify unknown samples by comparing the spectral information received from an unknown sample with spectral patterns retained in the library, and identification of the unknown substance may be made by comparison.

[0085] The detection and imaging system may be used in different detection techniques to obtain a one-shot/simultaneous detection for ID or 2D array of samples. The sensing surface may be modified depending on the different applications. Also, other arrangement, such as relative position of the camera and the detector may be changed based on the application.

[0086] FIG. 3 illustrates an arrangement for a high throughput detection and imaging of an array 60 of samples 62. A spatial phase difference generator 64 may be disposed in the sample arm. The phase difference generator 64 introduces a phase difference in the spatial direction in the spatial direction. The spatial phase difference generator 64 may be disposed in the optical path of the incident or resultant reflection of the sample arm. An imaging phase difference generator 66 may be disposed along an imaging direction. The imaging phase difference generator 66 may be used to introduce phase differences along

the imaging direction in the samples 62. Same amount of phase differences may be introduced in the samples 62 along the imaging direction. The values of phase differences introduced in the various samples may be same or different. A phase difference introduced in a sample 62 may vary from one location to another in the sample. In the illustrated embodiment, the phase difference may comprise a ramp value. The increasing phase difference may be such that the aggregate phase difference for each sample 62 may provide a negative complex conjugate of the image. This negative complex when added with the real value and its complex conjugate may be canceled, thereby providing only the real value in the image.

[0087] The imaging phase difference generator 66 may comprise any geometrical or non-geometrical shape that provides a continuous carrier signal to one or more samples in the imaging direction. The phase difference generator 66 comprises saw tooth structure, where the portions 68 may be same or different. In the illustrated embodiment, a shape or a range of thickness values of a portion (tooth) may be the same or different than the shape or range of thickness values for other portions.

[0088] FIG. 4 illustrates an arrangement for determining a high throughput detection and imaging of an array 60 of samples 62. In the illustrated embodiment, the imaging phase difference generator 70 may be disposed in the reference arm. The imaging phase difference generator 70 may be disposed in the optical path of the incident or resultant reflection of the reference arm. The imaging phase difference generator 90 may comprise any geometrical or non-geometrical shape that provides a continuous carrier signal to one or more samples in the imaging direction. The carrier signal may be continuous or discontinuous between the samples. The path length introduced by the use of the imaging phase difference generator 70 may be same or different for the different samples.

[0089] Alternatively, in FIGS. 3 and 4, the spatial phase difference generator 64 may be disposed in the reference arm. The surfaces 68 (see FIG. 3) and 72 (see FIG. 4) of the phase difference generators 66 and 70 may be planar, curved, or a combination thereof. In one example, the surface 68 may comprise a single curve, or a combination of difference curves.

[0090] FIG. 5 illustrates another arrangement for determining a high throughput detection and imaging of an array 60 of samples 62. The phase difference generator 64 is

disposed in the sample arm. The phase difference generator 64 may be disposed in the optical path of the incident or resultant reflection of the sample arm. A first imaging phase difference generator 74 may be disposed in the sample arm, and a second imaging phase difference generator 76 may be disposed in the reference arm. The first and second imaging phase difference generators 74 and 76 may be disposed along the imaging direction. The position of the phase difference generators 74 and 76 may be interchanged. For example, the phase difference generator 74 may be disposed in the reference arm, and vice versa. The imaging phase difference generators 74 and 76 may be disposed in the optical path of the incident or resultant reflection of the sample and reference arms, respectively.

[0091] The sample regions disposed in the direction parallel to steps 78 are spectrally separated by the spatial phase difference generator 64 Whereas, the sample regions disposed in the direction perpendicular to the steps 78 are imaged on to the imaging spectrometer. The number of steps in the x-direction may depend on the number of samples disposed in that direction such that each of the steps 78 corresponds to a sample.

[0092] The carrier signal is introduced using the imaging phase difference generator 74 in the incident radiation for the sample regions 62 disposed in the direction parallel to the steps 80. Another carrier signal is introduced in the reference arm using the imaging phase difference generator 76. The resultant carrier signal in the imaging direction is a combination of the carrier signals introduced by the imaging phase difference generators 74 and 76. The design of the imaging phase difference generator 74 and 76 may be such that the resultant carrier signal in the imaging direction may provide a complex conjugate in the imaging signal. In the illustrated embodiment, the imaging phase difference generators 74 and 76 are illustrated as wedge and stepped structures, however, the phase difference generators 74 and 76 may be of any other geometrical or non-geometrical shape that provides a continuous function of the carrier signal. The continuous function of the carrier signal may be determined and the same may be deducted during processing of the imaging signal.

[0093] FIG. 6 illustrates an example of a SPR imaging system 82 for SPR detection and imaging of an array of samples (e.g., 2D array of samples). The system comprises a broadband light source 84 for emitting broadband radiation. The radiation source 84 is optically coupled to the collimator 86 using an optical fiber 88. In one embodiment, the

SPR sensing surface may be made of a prism 90 coated with one or more thin metal films. The sensing surface may comprise an array of sample fields. A beam splitter 92 divides the collimated radiation 94 into two portions a reference beam 96 and a sample beam 106. The reference beam 96 is incident on the reference 100 and is passed through a spatial phase difference generator 102. The phase difference generator 102 introduces relative path length difference in a first direction. A carrier signal may be introduced in the imaging direction for the reference or sample array using an imaging phase difference generator 104. The sample beam 106 is incident on the sensing surface after passing through the imaging phase difference generator 104. The carrier signal may introduce a relative path length difference in samples along a second direction. The second direction may be an imaging direction. The sample beam 106 when incident on the sensing surface undergoes internal reflection in the prism 90 and is reflected by the thin metal films and out of the sample fields on the sensing surface. The thin films may comprise metals, such as but not limited to, gold, silver, copper, or a combination thereof. The prism 90 may be made of glass, although various other materials having suitable optical properties for internally reflecting the sample beam 106 and transmitting the resultant reflected SPR beam 108 may also be used. In one embodiment, a material of the definer component and the metal required for SPR phenomenon may be selectively disposed or patterned onto the sensing surface. That is, certain portions of the sensing surface that are configured to receive the sample may include gold, while the other portions may not include gold, but may include the material of the definer component.

[0094] The reference beam 96 is directed to an imaging spectrometer 110 using a right angle prism and a beam splitter 112. At the beam splitter 112, a portion of the reference beam 96 having the phase difference induced by the spatial phase difference generator 102 and a portion of the resultant sample beam 106 interfere and produce interference spectra 114. At the beam splitter 112 a condition is thus created under which interference between the reference beams and the reflected sample beams can occur giving rise to intensity variations of the beam emerging from the SPR surface. The interference spectra 114 are acquired by the imaging spectrometer 110. The spectrometer 110 comprises a detector 116, a grating 118 and one or more optical elements, such as a cylindrical lens 120. The interference spectra 114 pass through the cylindrical lens 120 and are received by a monochromator 122. In the illustrated embodiment, the

monochromator 122 comprises cylindrical mirrors 124 and the grating 118. The reflected light from the monochromator 122 is received by the detector 116.

[0095] The interference spectra 114 pass through the grating 118, which may split and diffract the interference spectrum into different wavelengths of light. The different wavelengths of light are then incident on the detector 116. The SPR imaging system may include a generally closed housing having an exit beam port therein to direct the beam at the imaging spectrometer 110. The broadband light source may be disposed within the housing and the detector may be disposed outside the housing.

[0096] As illustrated in FIG. **7** the sensing surface 130 in the SPR may be configured for free-solution (label free) SPR. The sample fields 132 may be enclosed volumes (e.g., channels, cavities) that comprise one or more functionalizing agents, such as but not limited to, ligand molecules. One of the sample fields 132 may be configured to receive the sample solution or act as a reference sample.

[0097] As illustrated in FIG. 8, the sensing surface 134 in the SPR may be configured for localized SPR (LSPR). The sample fields 136 may comprise electrically conductive structures 138 disposed at least in portions of the sample fields. One of the sample fields 136 may be configured to receive the sample solution, or act as a reference sample. Alternatively, the thin metal film, such as but not limited to, gold or silver, that is present to enable SPR phenomenon, may be patterned/textured to form the electrically conductive structures. In one embodiment, a patterned film may be used.

[0098] Resonance conditions of the LSPR may depend on the refractive index and dielectric constant of the environment surrounding the electrically conductive structures 138. The incident radiation interacts with the localized plasmons on surfaces of the electrically conductive structures 138. A change in the resonance conditions may be detected by measuring a change in the interference spectrum of the resultant projected to and transmitted through the electrically conductive structure of the sample fields. In one example, a biological reaction may cause a change in the dielectric constant of the electrically conductive structures 138 this change may be utilized for detection. In another example, an occurrence of an antigen-antibody reaction around the electrically conductive structure may be detected using the LSPR. In another embodiment, isolated particles may be disposed on the thin metal film. Non-limiting examples, of electrically

conductive structures may include silver particles. The particles may be nanoparticles or microparticles.

[0099] FIG. 9 illustrates a nano-grating SPR arrangement comprising a transmitting substrate 140 (such as a glass substrate). The arrangement comprises a grating structure 142 disposed on the transmitting substrate 140. The grating structure 142 may be present in the form of a patterned film. Sample solutions may be disposed in sample fields 144 present on the sensing surface formed by the substrate 140 and the grating structure 142 to generate SPR phenomenon. Non-limiting examples of the patterned film may comprise a gold film, silver film, copper film, or combinations thereof. In one example, the grating structure may comprise a gold film disposed on a silver film. The grating structure 142 may include, but is not limited to, gold, silver, copper, or combinations thereof. The grating structure 142 may be a periodic metallic grating structure. In one embodiment, the grating structure 142 may comprise a spacing of between 50 and 500 nm between the gratings. The grating structure 142 may be fabricated using fabricating techniques, such as but not limited to, nano-imprinting technology, E-beam lithography, ultraviolet lithography, interference lithography, or other nanometric technologies, which are configured to achieve a nano-metric structures.

[0100] FIG. 10 illustrates an example of a RIfS device 150. The device 150 comprises a sensing surface 154 having sample fields 155. The device 150 comprises a sensing surface 154 having sample fields 155. The sensing surface 154 may comprise a multilayer structure 156 disposed on a transmitting substrate 158. In one example, the multilayer structure 156 comprises a plurality of layers. The various layers of the plurality of layers may comprise silica layers, high refractive index layers (such as but not limited to tantalum oxide layer). Beams incident on samples may be at least partially reflected and transmitted at phase boundaries formed between two adjacent layers of the multilayer structure 156. The reflected beams from the various samples may superimpose resulting in an interference spectrum. One or more samples may be configured to act as reference samples. In one embodiment, one or more layers of the multilayer structure 156 may be functionalized using functional agent 160 to facilitate interaction of a portion of the layer with target molecules. Interaction of the functionalized layers with the target molecules may provide a change in a thickness and the refractive index of the functionalized layers. Optical thickness is a product of physical thickness and refractive

index, the optical thickness (pathlength) may be changed by changing the physical thickness and the refractive index of the layer. A change in the optical thickness of one or more layers of the plurality of layers may result in a modulation of the interference spectrum. Monitoring the modulation of the interference spectrum over time may be used to observe the binding behavior of the target molecules.

[0101] The arrangements illustrated in FIGS. 7-10 may be used in the optical engine 16 of FIGS. 1 and 2 to provide high throughput detection and imaging system. In these embodiments, a phase difference may be introduced in a spatial direction, and a carrier signal may be introduced in the imaging direction. The phase difference may be introduced in a first direction that is different from an imaging direction to spectrally separate the samples in the first direction.

[0102] FIG. 11 illustrates an example of a method for high throughput detection of an array of samples. At step 170, an incident radiation is provided. The incident radiation may be provided by a single source, such as a broadband light source, or multiple sources, such as a plurality of light emitting diodes. At step 172, the reference and samples in the sample array are illuminated with the incident radiation. The resultant sample beam from the samples may be a reflective or transmissive beam. At step 174, a path length difference may be introduced in a determined direction in the array of samples. The path length difference may be introduced in a spatial direction. In one example, the spatial direction may be perpendicular to a ruling direction of the grating of the spectrometer. Assuming that the direction of traversing the samples in a row is x-direction, which is also a direction perpendicular to the ruling direction of the grating. The phase difference generator may be disposed such that the portions of the phase difference generator having different paths are parallel to the x-direction. In this way, the samples disposed in a particular row will have a path length added in their corresponding incident beams or resultant reflected beams. At step 176, a carrier signal may be introduced in an imaging direction in the incident or resultant radiation of the sample arm or reference arm. The carrier signal may be introduced by adding a path length in incident beams or resultant reflected beams for sample or reference arm in the imaging direction.

[0103] Interference spectra may be formed by interference of the resultant sample beams with the reference beam. At step 188, the interference spectra may be acquired.

The interference spectra may be received by the detector. Spectral differences may be produced in the interference spectra by passing the interference spectra through a grating before receiving the spectra by the detector. The spectral difference may be produced by passing the interference spectra through a grating before receiving the spectra by a 2D detector. The samples of a particular column may be spectrally resolved using a 2D detector. At step 180, the acquired interference spectra may be processing using signal processing algorithms and Eqs. (l)-(9) described above to remove the complex conjugate from the analytic signal along with the cross-talk. At step 182, sample locations of the sample array may be reconstructed from the interference spectra by using signal processing algorithms. In one example, a column (imaging direction) of the 2D data corresponding to a particular wavelength is selected. Fourier transform is performed on the data to obtain peaks centered around carrier frequencies fc and -fc. The peaks around fc or -fc may be filtered to remove the complex conjugate and cross-talk. The resultant signal is then inverse Fourier transformed to obtain the analytic signal corresponding to the signal in the spatial direction.

[0104] At step 184, filtering may be performed on the reconstructed image to separate the individual sample locations. The individual sample locations may be filtered depending on frequencies used by the individual samples. In one embodiment, a windowing technique may be used to separate the individual sample points. In another embodiment, the data may be analyzed using time frequency analysis to determine spectra and/or content of the different sample points. At step 186, the absorption spectrum of each sample may be retrieved. In one embodiment, a Fourier Transform may be applied to retrieve the frequencies corresponding to the different spatial locations of the samples.

[0105] FIG. 12 illustrates a 2D image of a fringe map (interferometric signal) to which a carrier signal is added. In the illustrated embodiment, four spots or sample fields on a sensing surface are simulated. The addition of carrier signal in the imaging direction results in tilted fringes in the interferometric signal. The interferometric signal is representative of four sample locations disposed on a sensing surface.

[0106] FIG. 13 is an example interferometric signal for a single row. FIG. 14 is an example carrier signal added to each column in the imaging direction. Typical signal on a single row for the image shown in FIG. 13. The interferometric signal illustrated in

FIG. 13 may be first converted to a complex signal using the carrier signal shown in Fig 14.

[0107] FIG. 15 shows the inverse Fourier transform of the signal of FIG. 13. This step is followed by individually separating the spots by filtering and computing Fourier transform of each filtered signal to identify the absorption spectrum of the spots. FIG. 16 shows a filtering of a signal shown in FIG. 15. In the illustrated example of FIG. 16, a windowing technique may be used to spectrally separate the individual sample points. FIG. 17 is a Fourier transform of peaks identified in FIG. 16 and shows the spectral absorption at each sample on the sensor chip.

[0108] A shift of the wavelength for surface plasmon resonance may be used to detect biochemical molecules, and whereby a low-cost, compact and portable planar SPR detector is achieved. Advantageously, the nano-grating SPR may facilitate a low-cost, compact and portable planar surface plasmon resonance detector.

[0109] The systems and methods may be used in a variety of applications, for example, in molecular biology and medical diagnostics where specific binding of bioactive molecules to their corresponding binding partners, for example, DNA, proteins, may need to be determined. Based on the electro-optical detection of specific molecular binding events, the affinity sensor may be used to monitor, for example, molecules, viruses, bacteria, and cells in the most diverse samples, such as clinical samples, food samples, and environment samples such as, plants, whereby such monitoring is performed in a time efficient manner. The systems and methods may be used in the fields of molecular detection and concentration analysis of biomolecules, kinetic and equilibrium analysis of biochemical reactions, control of fermentation processes, evaluation of ligand-cell-interactions, clinical analysis, and cell demotion. The systems and methods may be used in determination of active concentration, screening and characterization in terms of both affinity and kinetics. Unlike fluorescence and chemiluminescence methods, no dye-marked samples are needed in SPR for the protein to be tested.

[0110] The systems and methods do not require repeating the methods steps for each sample of the plurality of samples and are configured to simultaneously detect (and image) a plurality of samples in the sample array. The methods do not require mechanical movement of parts of the systems for simultaneous detection of the plurality

of samples. The lack of mechanical movement facilitates longer lifetime of the instruments and provides relative immunity to the system from mechanical vibrations. In addition, self-referencing facilitates the use of a single interferometer path instead of two (sample and reference). Self-referencing the samples using one or more of the samples disposed on the sensing surface makes the system less prone to vibrations that may otherwise affect systems that employ separate sample and reference paths. Having one interferometer path instead of two, provides for a less complex and more robust system design while minimizing beam misalignment.

[0111] A large number of SPR curves for a sample array may be imaged in a single shot without mechanical angle-scanners or translation stages. The Fourier transform approach to 2D SPR provides high signal-to-noise outputs, high wavelength precision, and reproducibility, and high ordinate precision. Single shot imaging allows higher frame rates in data gathering improving SNR. Advantageously, the monitoring or detection may be performed in real-time. For example, binding reactions may be monitored in real time, thereby reducing cost. The systems and methods may be used to analyze any binding reaction, including, but not limited to, those involving biological molecules. For antibody binding affinity measurements, an antigen typically is immobilized on the sensing surface. The sensing surface is exposed to a solution containing the antibody of interest, and binding proceeds. Once binding has occurred, the sensing surface is exposed to buffer solution (e.g. one that initially has no free antibody) and the dissociation rate is continuously monitored in real time.

[0112] While only certain features of the invention have been illustrated and described herein, many modifications and changes will occur to those skilled in the art. It is, therefore, to be understood that the appended claims are intended to cover all such modifications and changes as fall within the scope of the invention.

CLAIMS:

1. A detection system for detecting an array of samples, comprising:

an electromagnetic radiation source;

a reference arm;

a sample arm comprising a sensing surface having a plurality of sample fields configured to receive the array of samples;

a spatial phase difference generator configured to introduce differences in pathlengths of one or more samples along a first direction in the array of samples;

a carrier signal generator configured to introduce a carrier signal along a second direction in the array of samples; and

an imaging spectrometer configured to image one or more samples in the array of samples.

2. The detection system of claim 1, wherein the carrier signal generator is operatively coupled to the sensing surface, a reference, or both.

3. The detection system of claim 1 or 2, wherein the carrier signal generator comprises one or more imaging phase difference generators.

4. The detection system of claim 3, wherein the imaging phase difference generators are disposed along an imaging direction of the sensing surface, a reference, or both.

5. The detection system of claim 3, wherein the carrier signal generator comprises a first imaging phase difference generator operatively coupled to the sensing surface, and a second imaging phase difference generator operatively coupled to a reference arm.

6. The detection system of claim 3, wherein the spatial phase difference generator, imaging phase difference generators, or both comprise a plurality of portions

having different refractive indices or thicknesses, wherein at least one of the portions comprises a refractive index or a thickness that is different from the other portions.

7. The detection system of claim 3, wherein the spatial phase difference generator, imaging phase difference generators, or both comprise a stepped structure having a plurality of portions, wherein each of the portions have a refractive index that is different from the other portions.

8. The detection system of claim 3, wherein one or more imaging phase difference generators comprise portions having varying thicknesses.

9. The detection system of claim 8, wherein the portions having varying thicknesses comprise planar surfaces, curved surfaces, stepped-surfaces, or combinations thereof.

10. The detection system of claim 3, wherein the imaging phase difference generators comprise a dielectric material, a stack of glass plates, a liquid crystal, computer generated holographic data, or a combination thereof.

11. The detection system according to anyone of the preceding claims, wherein the imaging spectrometer comprises a detector operatively coupled to a grating.

12. The detection system of claim 11, wherein the grating is configured to rotate.

13. The detection system according to anyone of the preceding claims, wherein the sensing surface comprises a thin metal film disposed on a prism, electrically conductive structures disposed on a transmitting substrate, gratings disposed on a transmitting substrate, or combinations thereof.

14. A surface plasmon resonance (SPR) detection and imaging system for detecting and imaging an array of samples, comprising:

a broadband light source configured to illuminate the array of samples;

an optical engine, comprising:

a SPR sensing surface having sample fields and at least one reference field;

a spatial phase difference generator configured to introduce pathlength differences in one or more samples along a first direction in the array of samples;

a carrier signal generator configured to introduce a carrier signal along a second direction in the array of samples;

an image acquisition unit configured to acquire image data; and

a signal processing unit for processing the acquired image data.

15. A method for imaging spectral characteristics of samples in an array of samples, comprising:

illuminating a reference arm and sample arm using a reference beam and sample beam, respectively, to produce a resultant reference beam and sample beam, respectively;

introducing a path length difference in at least one of the reference beam, sample beam, resultant reference beam, resultant sample beam, or a combination thereof;

introducing a carrier signal in at least one of the reference beam, sample beam, resultant reference beam, resultant sample beam, or a combination thereof;

interfering the resultant reference beam with the resultant sample beam to form interference spectra;

acquiring the interference spectra; and

imaging the spectral characteristics of the samples based on the acquired interference spectra.

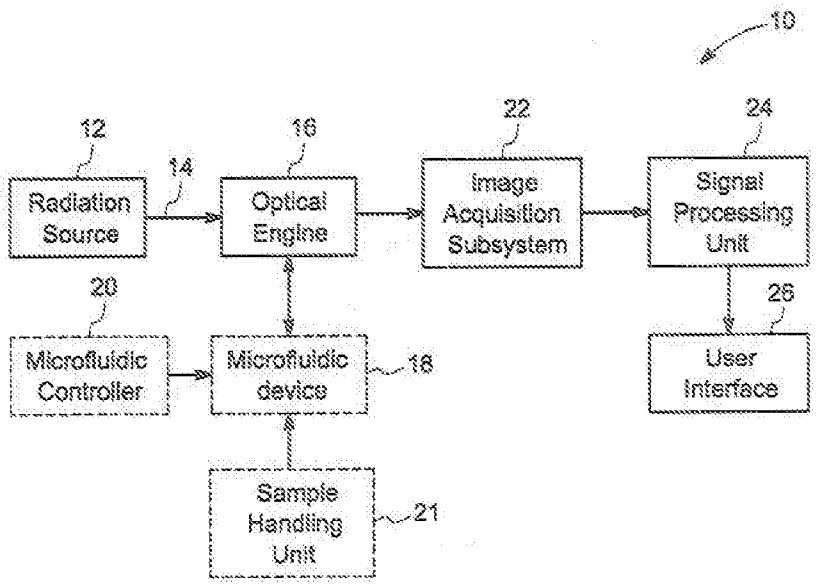

FIG. 1

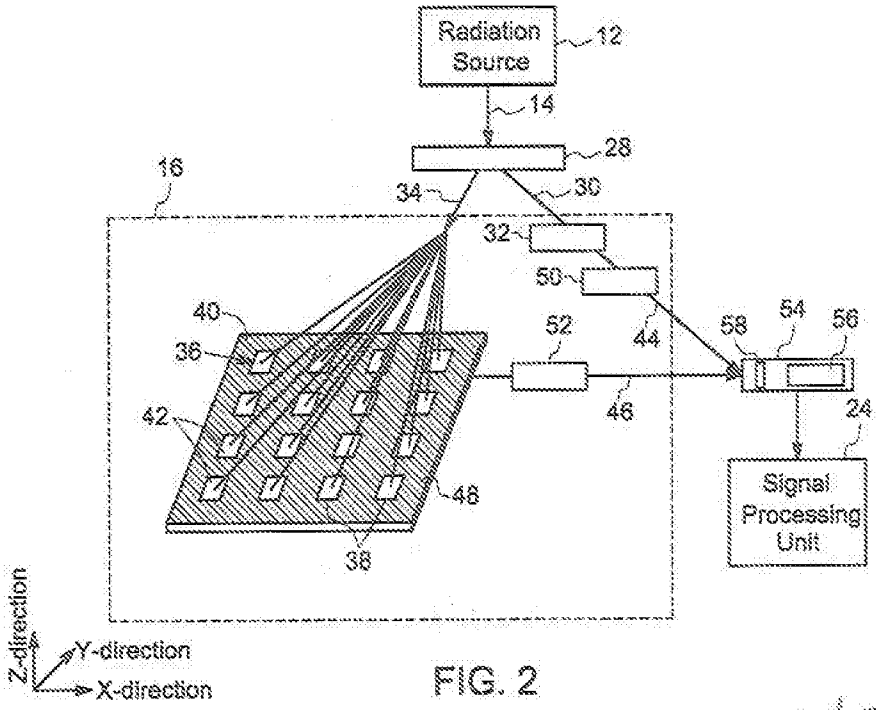

FIG. 2

Patent Agent Name: SHWETA SARASWAT
Reg. No.: IN/PA-1468

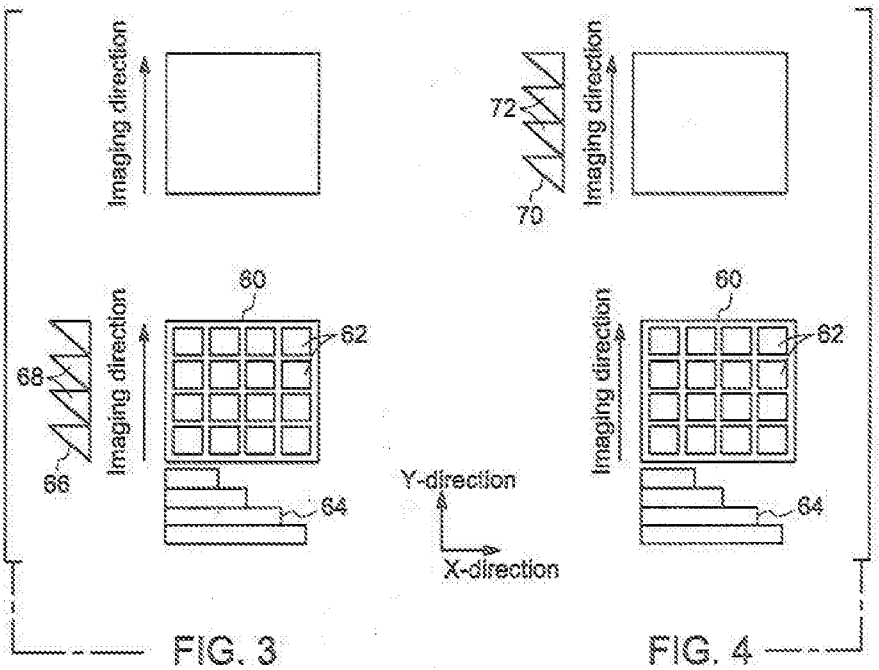

FIG. 3

FIG. 4

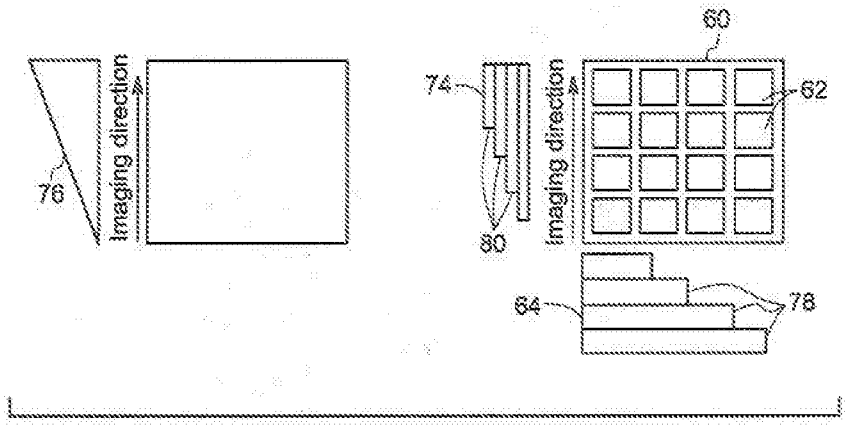

FIG. 5

Patent Agent Name: SHWETA SARASWAT
Reg. No.: IN/PA-1458

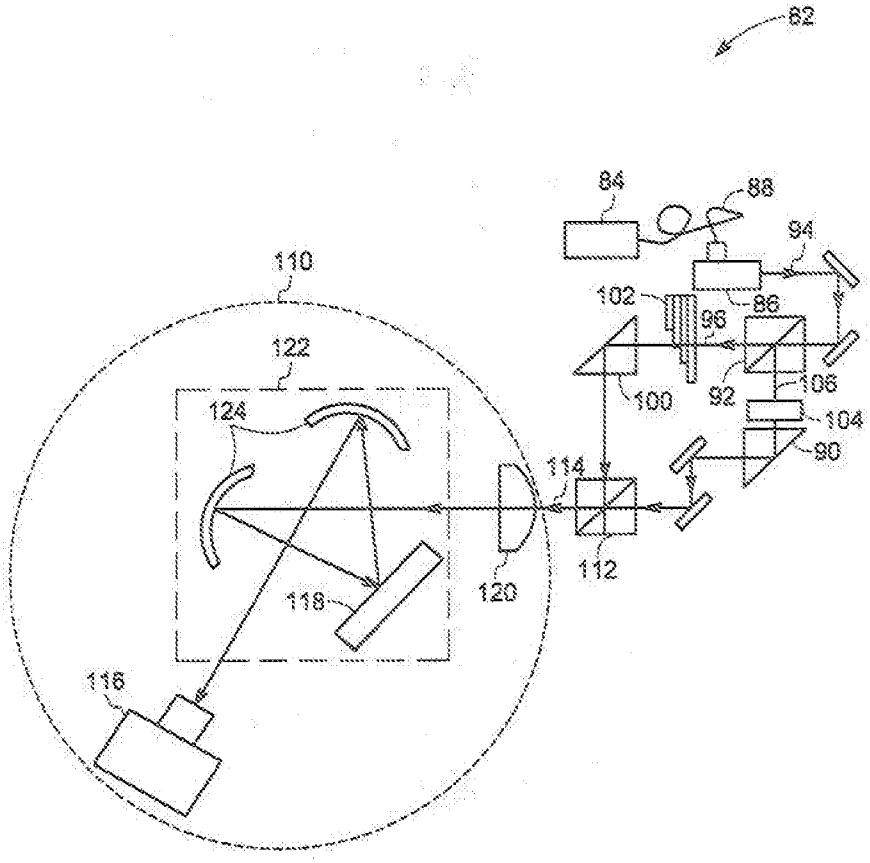

FIG. 6

Patent Agent Name: SHWETA SHRASWAT
Reg. No.: IN/PA-1468

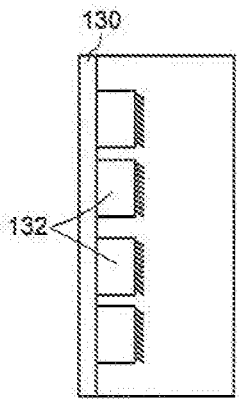

FIG. 7

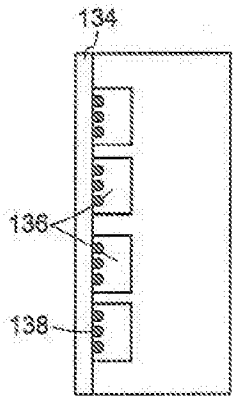

FIG. 8

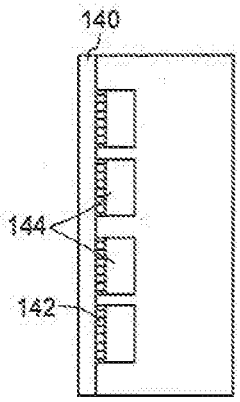

FIG. 9

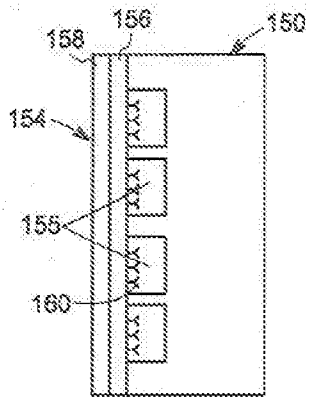

FIG. 10

Patent Agent Name: SHWETA SARASWAT
Reg. No.: IN/PA-1458

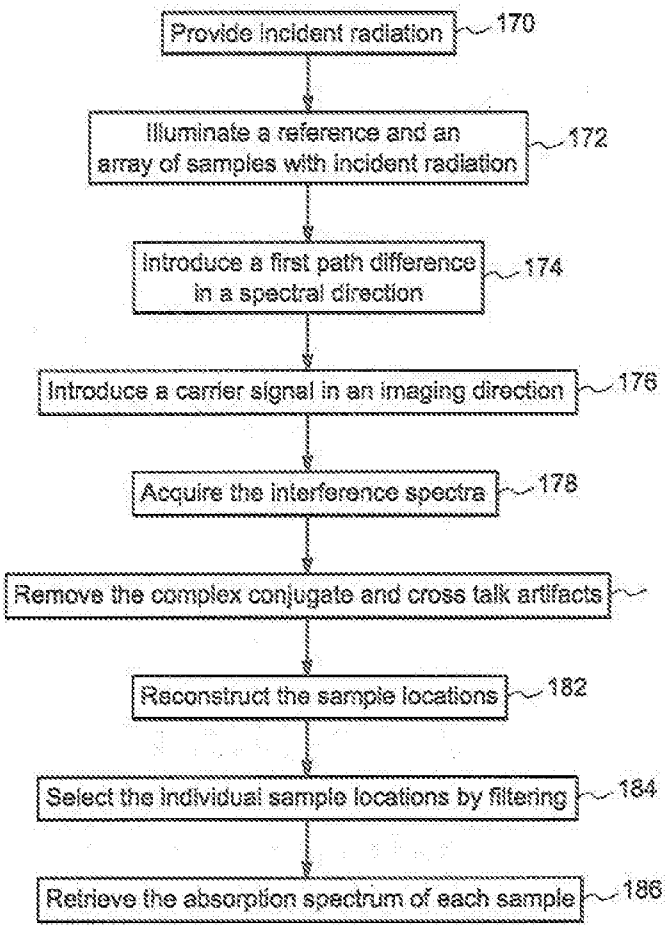

FIG. 11

Patent Agent Name: SHWETA SARASWA
Reg. No.: IN/PA-1950

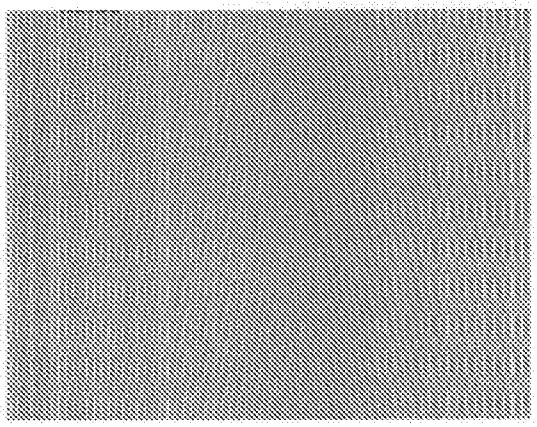

FIG. 12

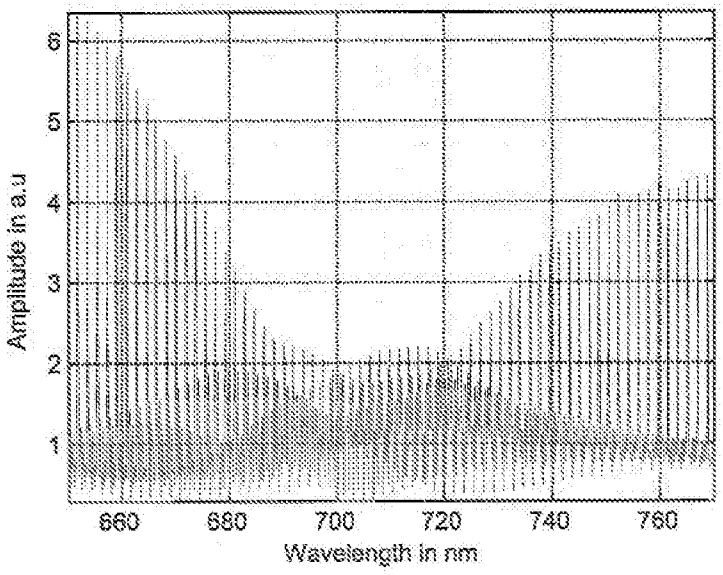

FIG. 13

Patent Agent Name: Sangeeta Saraswat
Reg. No.: IN/PA-1458

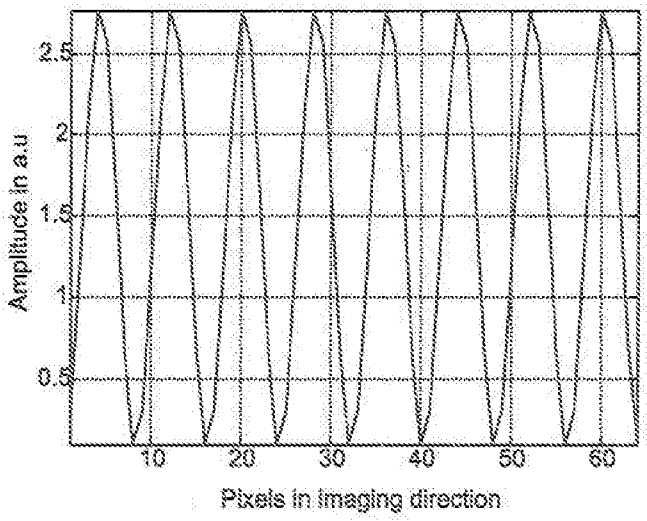

FIG. 14

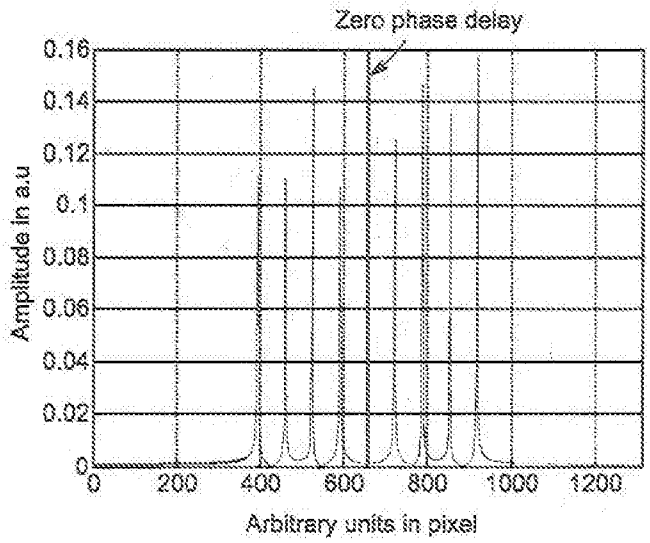

FIG. 15

Patent Agent Name: SHWETA SARASWA
Reg. No.: IN/PA-1458

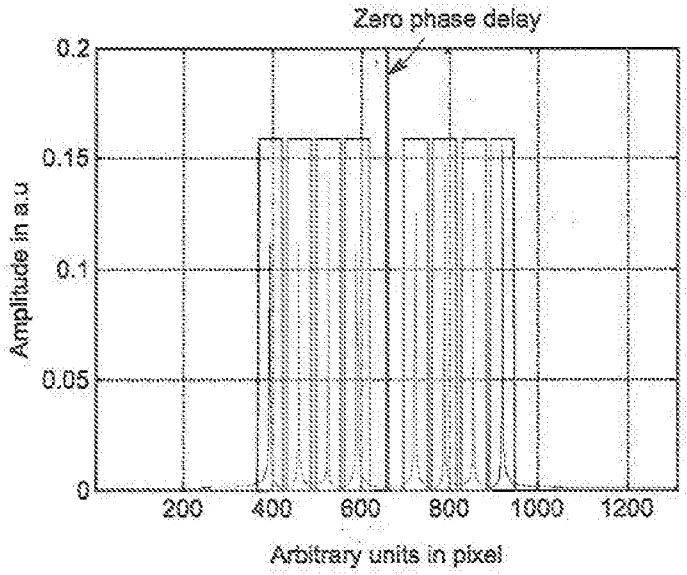

FIG. 16

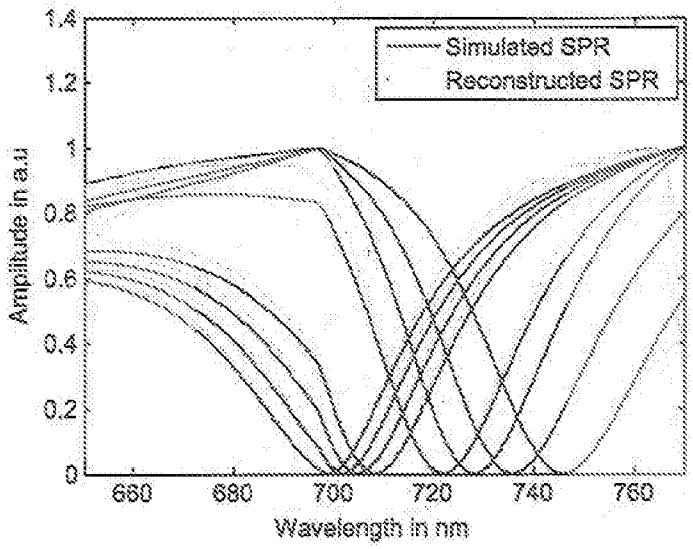

FIG. 17

Patent Agent Name: SHWETA SHARMA
Reg. No.: IN/PA - 1458

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE201 2/051 373

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet

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)

IPC: G01 N

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

SE, DK, FI, NO classes as above

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

EPO-Internal, PAJ, WPI data, BIOSIS, COMPENDEX, INSPEC, MEDLINE, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003021 9809 A 1 (CHEN SHEAN-JEN ET AL), 27 November 2003 (2003-1 1-27); abstract --	1-15
A	US 2008021 8860 A 1 (ROBERTSON WILLIAM M), 11 September 2008 (2008-09-1 1); abstract --	1-15
A	EP 1574832 A2 (AGILENT TECHNOLOGIES INC), 14 September 2005 (2005-09-1 4); abstract --	1-15
A	US 200701 66763 A 1 (HO HO P ET AL), 19 July 2007 (2007-07-1 9); abstract; page 1, paragraph [001 0] - page 2, paragraph [001 8] --	1-15

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

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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 fding 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
24-04-201 3	25-04-201 3

Name and mailing address of the ISA/SE	Authorized officer
Patent- och registreringsverket Box 5055 S-1 02 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Lars Jakobsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE201 2/051 373

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004020985 A1 (MEDICAL BIOSYSTEMS LTD ET AL), 11 March 2004 (2004-03-1 1); abstract -- --------	1-15

International application No.
PCT/SE201 2/051 373

Continuation of: second sheet

International Patent Classification (IPC)

G01N 21/55 (2006.01)
G01N 21/45 (2006.01)
G01N 33/53 (2006.01)

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				EP	1977220	A 1	08/1 0/2008
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				KR	200801 00343	A	17/1 1/2008
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				US	2009008621 0	A 1	02/04/2009
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