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(54) **Title**: MULTI-WAVELENGTH REFERENCE MICROPLATE FOR LABEL-INDEPENDENT OPTICAL READER

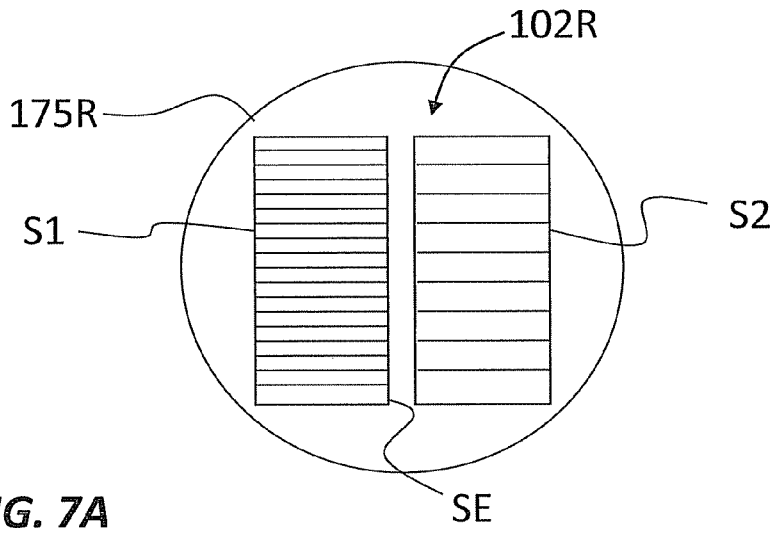

FIG. 7A

(57) **Abstract**: A multi-wavelength reference microplate for a label-independent optical reader is disclosed. The microplate includes a support plate that supports a plurality of reference wells. At least one of the reference wells is configured as a multi-wavelength reference well having disposed therein two or more resonant waveguide grating sections that respectively reflect two or more different reference resonant wavelengths within the light source wavelength band. Methods for making and using the microplates are also disclosed.

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MULTI-WAVELENGTH REFERENCE MICROPLATE FOR LABEL-INDEPENDENT OPTICAL READER

CLAIMING BENEFIT OF PRIOR FILED U.S. APPLICATION

[0001] This application is a non-provisional application and claims the benefit of U.S. Provisional Application Serial No. 61/257,061, filed on November 2, 2009.

CROSS-REFERENCE TO RELATED COPENDING APPLICATION

[0002] Commonly owned and assigned co-pending application USSN 61/257058 (filed concurrently herewith) entitled "MULTI-GRATING BIOSENSOR FOR LABEL-INDEPENDENT OPTICAL READERS".

FIELD

[0003] The present disclosure relates to label-independent optical readers, and in particular relates to multi-wavelength microplates for such readers.

BACKGROUND

[0004] Label-independent (LID) optical readers are used, for example, to detect a drug binding to a target molecule such as a protein. Certain types of LID optical readers measure changes in refractive index on the surface of a resonant waveguide grating (RWG) biosensor for an array of RWG biosensors. The individual RWG biosensors are located in respective wells of a microplate. Broadband light from a broadband light source is directed to each RWG biosensor. Only light whose wavelength is resonant with the RWG biosensor is strongly reflected. This reflected light is collected and spectrally analyzed to determine the resonant wavelength, which is representative of a refractive index change and thus biomolecular binding to the RWG biosensor.

[0005] Spurious changes to the refractive index of the RWG biosensor and other system effects can reduce the accuracy of the resonant wavelength measurement.

Consequently, a reference microplate can be used with standardized RWGs that produce a resonant wavelength within the optical reader's operating spectral bandwidth λ_{FWHM}, which is typically approximately 824 nm to 844 nm. However, broadband light sources can have variations (noise) that are not detected by present-day reference microplates.

SUMMARY

[0006] An aspect of the disclosure is a multi-wavelength reference microplate for a LID optical reader having a light source with a wavelength band. The microplate includes a support plate that supports a plurality of reference wells. At least one of the reference wells is configured as a multi-wavelength reference well having disposed therein two or more RWG sections that respectively reflect two or more different reference resonant wavelengths within the light source wavelength band.

[0007] Another aspect of the disclosure is a multi-wavelength reference microplate for a LID optical reader having a light source with a wavelength band. The microplate includes a support plate that operably supports a plurality of multi-wavelength reference wells each having a RWG biosensor disposed therein that includes two or more RWG sections that respectively have two or more reference resonant wavelengths. The microplate also includes a fill material that at least partially fills each multi-wavelength reference well, with the fill material having a refractive index similar to that of water, such as of about 1.3, within the light source wavelength band.

[0008] Another aspect of the disclosure is a method of using a reference microplate with reference wells to measure multiple reference resonant wavelengths in a LID optical reader system. The method includes providing in at least one reference well two or more RWG sections each having a different reference resonant wavelength. The method also includes irradiating each of the two or more RWG sections to generate respective reflected light therefrom. The method further includes spectrally analyzing the respective reflected light to measure the two or more reference resonant wavelengths.

[0009] These and other aspects of the disclosure will be described by reference to the following written specification, claims and appended drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] A more complete understanding of the present disclosure may be had by reference to the following detailed description when taken in conjunction with the accompanying drawings, wherein:

[0011] **FIG. 1** is a generalized schematic diagram of an example optical reader system suitable for use with the multi-wavelength reference microplate of the disclosure;

[0012] **FIG. 2** shows an exemplary RWG biosensor array operably supported in regions or "wells" of a microplate, which in turn is held by a microplate holder;

[0013] **FIG. 3** is an example plot of the resonant wavelength λ_R (nm) vs. position (mm) across the RWG biosensor;

[0014] **FIG. 4** is a plot of the peak amplitude (photon counts) versus spectrometer pixel location, which corresponds to wavelength and illustrates how the resonant wavelength shifts;

[0015] **FIG. 5** is a plot of the intensity (dB) versus wavelength (nm) for a typical superluminous diode (SLD) measured through a linear polarizer and illustrates the typical SLD spectral bandwidth λ_{FWHM}, which has fringes (ripples) when polarized;

[0016] **FIG. 6** is a plot of the resonant wavelength λ_R as a function of time (minutes) as calculated using numerical modeling based on shifting fringes in the SLD spectrum plot of **FIG. 5**;

[0017] **FIGS. 7A** through **7C** are close-up plan views of respective multi-wavelength reference wells of an example multi-wavelength reference microplate, wherein each multi-wavelength reference well has a reference RWG biosensor that includes multiple RWG sections with different resonant-wavelength reflectivities;

[0018] **FIGS. 8A** and **8B** illustrate an example method of forming a multi-wavelength reference RWG biosensor by disposing multiple RWGs on a common substrate;

[0019] **FIGS. 9A** through **9C** are similar to **FIGS. 7A** through **7C** and illustrate example embodiments of multi-wavelength reference wells wherein the reference RWG biosensors have contiguous RWG sections;

[0020] **FIGS. 10A** through **10C** illustrate a first method of forming RWG sections using a mask-based approach;

[0021] **FIGS. 11A** through **11D** include perspective and cross-sectional views that illustrate a second method of forming contiguous RWG sections using multiple coatings to form different waveguide thicknesses that define the different RWG sections;

[0022] **FIGS. 12A** and **12B** are cross-sectional views of an example multi-wavelength reference well of a multi-wavelength reference microplate, wherein **FIG. 12B** shows a multi-wavelength reference well filled with a water mimic;

[0023] **FIG. 13** is similar to **FIG. 3** and shows an example multi-wavelength reference microplate having *m* sets of multi-wavelength reference wells, with the insets A through C showing different example configurations for the multi-wavelength reference wells in the different sets;

[0024] **FIG. 14** is a plot similar to **FIG. 6** and shows a schematic wavelength spectrum for an SLD light source, along with the different reference resonant wavelengths λ_{RR} (dashed lines) associated with three different sets of multi-wavelength reference wells for an example multi-reference microplate such as shown in **FIG. 13**;

[0025] **FIG. 15A** is a plot of the change in wavelength noise (picometers) versus reference well number as measured on an example optical reader system having a stable broadband light source, for both a prior art reference microplate (dashed line) and the multi-wavelength reference microplate (solid line) of the present disclosure; and

[0026] **FIG. 15B** is the same plot as **FIG. 15A**, except that the optical readers system uses an unstable broadband light source, and illustrates how the multi-wavelength reference microplate (solid line) detects the light source variations while the prior art reference microplate (dashed line) does not.

DETAILED DESCRIPTION

[0027] Reference is now made to embodiments of the disclosure, exemplary embodiments of which are illustrated in the accompanying drawings.

[0028] **FIG. 1** is a generalized schematic diagram of an example optical reader system ("system") **100** used to interrogate one or more RWG biosensors **102** each having a surface **103** to determine if, for example, a biological substance **104** is present on the RWG biosensor. Inset **A** shows a close-up of an exemplary RWG biosensor **102**. System **100** is suitable for use in combination with the multi-wavelength reference microplate **170R** of the disclosure as introduced and discussed in greater detail below.

[0029] **FIG. 2** is a plan view of an example microplate **170** that comprises a support plate **171** with a surface **173** having a plurality of wells **175** formed therein. An example support plate **171** has a two-part construction of an upper plate and a lower plate (not shown), as described, for example, in U.S. Patent Application Publication No. 2007/0211245, which is incorporated by reference herein.

[0030] Microplate **170** of **FIG. 2** illustrates an exemplary configuration where RWG biosensors **102** are arranged in an array **102A** and operably supported in wells **175**. For a "sample" microplate **170S** that includes actual biological samples, each well **175** contains a "sample region" **175S** and a "reference region" **175R**. Each well region has a resonant wavelength, generally referred to as λ_R. The sample or "signal" resonant wavelength of sample RWG biosensor **102S** is denoted as λ_{RS} and the reference resonant wavelength of a reference RWG biosensor **102R** is denoted as λ_{RR}. It is important that biological samples do not attach to the reference region **175R**. Therefore, reference regions within wells **175** are biologically altered, or adjacent wells ("reference" wells **175R**) will be used as shown in **FIG. 2**. On a reference microplate **170R**, the RWG biosensors **102** are referred to as "reference RWG biosensors **102R**".

[0031] An exemplary RWG biosensor array **102A** has a 4.5 mm pitch for RWG biosensors **102** that are 2 mm square, and includes 16 RWG biosensors per column and 24 RWG biosensors in each row. In embodiments, fiducials **428** can be used to position, align, or both, the microplate **170** in system **100**. A microplate holder **174** is also shown holding microplate **170**. Many different types of plate holders can be used as microplate holder **174**. Here again, microplate **170** can be the actual sample microplate **170S** or a reference microplate **170R** used to calibrate or troubleshoot system **100**.

[0032] With reference again to **FIG. 1**, system **100** includes a light source **106** that generates light **120**. Light source **106** may include one or more of a lamp, laser, diode, filters, attenuators, and like devices or combinations thereof. An example light source **106** includes a broad-band light source such as a super luminescent diode (SLD), discussed in greater detail below. Light **120** from light source **106** is directed by a coupling device **126** (e.g., a circulator, optical switch, fiber splitter or like device) to an optical system **130** that has an associated optical axis **A1** and that transforms light **120** into an incident optical beam **134I**, which forms a light spot **135** at RWG biosensor **102** (see inset **B**). Incident optical beam **134I** (and thus light spot **135**) is scanned over the RWG biosensor **102** by either a scanning operation of scanning optical system **130** or by the movement of microplate **170** via microplate holder **174**.

[0033] Incident optical beam **134I** reflects from RWG biosensor **102,** thereby forming a reflected optical beam **134R**. Reflected optical beam **134R** is received by optical system **130** and light **136** therefrom (hereinafter, "guided light signal") is directed by coupling device **126** to a spectrometer unit **140,** which generates an electrical signal **S140** representative of the spectra of the reflected optical beam. In embodiments, a controller **150** having a processor unit ("processor") **152** and a memory unit ("memory") **154** then receives electrical signal **S140** and stores in the memory the raw spectral data, which is a function of a position (and possibly time) on RWG biosensor **102.** Thereafter, processor **152** analyzes the raw spectral data based on instructions stored therein or in memory **152.**

[0034] The result is a spatial map of resonant wavelength (λ_R) data such as shown in **FIG. 3**, which shows the calculated resonance wavelength centroid as a function of the position of the scanning spot across the sensor for a number of different scans. The variation of the resonance wavelength λ_R indicates if a chemical or biological reaction happened for a specific RWG biosensor **102**. In embodiments, controller **150** includes or is operably connected to a display unit **156** that displays measurement information such as spectra plots, resonant wavelength plots, and other measurement results, and system status and performance parameters. In embodiments, spectra can be processed immediately so that only the resonant wavelengths (as calculated, for example, as the respective centroids of measured spectra) are stored in memory **154**.

[0035] Example RWG biosensors **102** make use of changes in the refractive index at sensor surface **103** that affect the waveguide coupling properties of incident optical

beam **134I** and reflected optical beam **134R** to enable label-free detection of biological substance **104** (e.g., cell, molecule, protein, drug, chemical compound, nucleic acid, peptide, carbohydrate) on the RWG biosensor. Biological substance **104** may be located within a bulk fluid deposited on RWG biosensor surface **103,** and the presence of this biological substance alters the index of refraction at the RWG biosensor surface.

[0036] To detect biological substance **104**, RWG biosensor **102** is probed with incident optical beam **134I**, and reflected optical beam **134R** is received at spectrometer unit **140**. Controller **150** is configured (e.g., processor **152** is programmed) to determine if there are any changes (e.g., 1 part per million) in the RWG biosensor refractive index caused by the presence of biological substance **104**. In embodiments, RWG biosensor surface **103** can be coated with, for example, biochemical compounds (not shown) that only allow surface attachment of specific complementary biological substances **104**, thereby enabling RWG biosensor **102** to be both highly sensitive and highly specific. In this way, system **100** and RWG biosensor **102** can be used to detect a wide variety of biological substances **104**. Likewise, RWG biosensor **102** can be used to detect the movements or changes in cells immobilized to RWG biosensor surface **103**, for example, when the cells move relative to the RWG biosensor or when they incorporate or eject material, a refractive index change occurs.

[0037] If multiple RWG biosensors **102** are operably supported as an array **102A**, then they can be used to enable high-throughput drug or chemical screening studies. For a more detailed discussion about the detection of a biological substance **104** (or a biomolecular binding event) using scanning optical reader systems, reference is made to U.S. Patent Application Ser. No. 11/027,547. Other optical reader systems are described in U.S. Patent No. 7,424,187 and U.S. Patent Application Publications No. 2006/0205058 and 2007/0202543.

[0038] The most commonly used technique for measuring biochemical or cell assay events occurring on RWG biosensors **102** is spectral interrogation. Spectral interrogation entails illuminating RWG biosensor **102** with a multi-wavelength or broadband beam of light (incident optical beam **134I**), collecting the reflected light (reflected optical beam **134R**), and analyzing the reflected spectrum with spectrometer unit **140**. An exemplary reflection spectrum from an example spectrometer unit **140** is shown in **FIG. 4**, where the "peak amplitude" is the number of photon counts as

determined by an analog-to-digital (A/D) converter in the spectrometer. The resonance is covered by about 10 pixels and the wavelength range is from about 824 nm to 840 nm. When chemical binding occurs at RWG biosensor surface **103**, the resonance shifts slightly in wavelength, as indicated by the arrow, and such shift is detected by spectrometer unit **140**.

Light source noise

[0039] As discussed above, in an example of system **100**, light source **106** employs a broadband light source such as an SLD. **FIG. 5** is a plot of the intensity (dB) versus wavelength (nm) for a typical SLD as measured through a linear polarizer and illustrates the typical SLD waveform (spectrum), which has fringes (ripples) **202** when polarized. Fringes **202** have a period of about 1.27 nm. The actual fringe period of a light source will vary based on its design and the polarizer used. Example locations of the signal resonant wavelength λ_{RS} and reference resonant wavelength λ_{RR} are indicated in **FIG. 5**, and are typically a few nm apart. Light source **106** has a full-width half-maximum (FWHM) spectral bandwidth λ_{FWHM} .

[0040] If fringes **202** shift over time, the power level of the waveguide resonant wavelength is altered and the resulting signal resonant wavelength λ_{RS} reported by the LID detection system shifts. **FIG. 6** is a plot of the signal resonant wavelength λ_{RS} as a function of time (minutes) as calculated using numerical modeling based on shifting fringes **202**. The plot of **FIG. 6** shows how the shifting fringes cause a shift in the signal resonant wavelength λ_{RS} over time. The signal and reference resonant wavelengths λ_{RS} and λ_{RR} are affected differently, causing additional noise to the resulting measurement, and possibly masking any biomolecular binding signal.

[0041] Consequently, reference microplate **170R** of the present disclosure is a "multi-wavelength reference microplate" configured to verify the stability of an SLD-based light source **106**. Multi-wavelength reference microplate **170R** has at least one and preferably multiple multi-wavelength reference wells **175R** that each provide multiple reflected wavelengths in a manner that approximates the sample microplate **170S,** that matches one half the fringe period of the SLD light source, or both. Multi-wavelength reference microplate **170R** provides the capability to sample multiple wavelengths within the operating wavelength spectral bandwidth λ_{FWHM} of light source **106** to more accurately measure or otherwise characterize the optical reader system's

noise performance. In embodiments, all of the reference wells **175R** of multi-wavelength microplate **170R** are multi-wavelength reference wells, while in other embodiments, the multi-wavelength microplate includes one or more but not all multi-wavelength reference wells.

[0042] **FIGS. 7A** through **7C** are close-up plan views of respective multi-wavelength reference wells **175R** of an example multi-wavelength reference microplate **170R**. Each multi-wavelength reference well **175R** has a reference RWG biosensor **102R** that includes multiple ($n \geq 2$) RWG sections $\mathbf{S}_n$ having different reference resonant-wavelengths λ_{RRn}. **FIGS. 7A** through **7C** illustrate respective multi-wavelength reference wells **175R** having two, three, and four RWG sections $\mathbf{S}_n$ (labeled as $\mathbf{S}_1$, $\mathbf{S}_2$, $\mathbf{S}_3$ and $\mathbf{S}_4$) having different respective reference resonance wavelengths λ_{RR1}, λ_{RR2}, λ_{RR3} and λ_{RR4} . The number n of RWG sections $\mathbf{S}_n$ employed in a given reference RWG biosensor **102R** depends on how many reference resonance wavelengths λ_{RRn} are needed to adequately sample the wavelength spectral bandwidth λ_{FWHM} of light source **106**. RWG sections $\mathbf{S}_n$ are shown by way of an example as being spaced apart, thereby providing edges **SE** that can be used to identify which RWG grating section is being interrogated by incident beam **134I**. In embodiments, the RWG sections $\mathbf{S}_n$ are spaced apart a distance equal to or greater than the size (diameter) of light spot **135**. For example, if light spot **135** has a diameter of 100 μm, an example spacing between adjacent RWG sections $\mathbf{S}_n$ is 200 μm.

[0043] In embodiments, RWG sections $\mathbf{S}_n$ are formed separately to have different grating periods and thus different reference resonant wavelengths λ_{RRn}. The separate RWG sections $\mathbf{S}_n$ are then disposed on an upper surface **212** of a support substrate **210**, as illustrated in **FIGS. 8A** and **8B**, thereby forming a multi-segment reference RWG biosensor **102R**. The multi-segment reference RWG biosensor **102R** is then disposed in a well **175** to form a multi-wavelength reference well **175R**.

[0044] **FIGS. 9A** through **9C** are similar to **FIGS. 7A** through **7C** and illustrate example embodiments of multi-wavelength reference wells **175R** wherein reference RWG biosensors **102R** have contiguous RWG sections $\mathbf{S}_n$. In one case, the contiguous grating sections $\mathbf{S}_n$ are formed at the same time using, for example, standard photolithographic techniques. Here, with reference to **FIGS. 10A** through **10C**, a single mask **230** having multiple regions **232** with different grating periodicities is irradiated with light **240** to form contiguous sections $\mathbf{S}_n$ of gratings **242** on a

photosensitive surface **233** of substrate **234**. Contiguous grating sections $\mathbf{S}_n$ are shown slightly separated for the sake of illustration. Photosensitive surface **233** may include, for example, a layer of photoresist. This mask exposure is followed by applying a single coating **246** over RWG sections $\mathbf{S}_n$ on substrate surface **233** to form reference RWG biosensor **102R** shown in **FIG. 10C** in cross-sectional view, with three contiguous RWG sections $\mathbf{S}_1$, $\mathbf{S}_2$ and $\mathbf{S}_3$ demarcated by dashed lines. Note that coating **246** is substantially conformal to the underlying gratings **242**.

[0045] **FIGS. 11A** through **11D** illustrate another example method of forming contiguous RWG sections $\mathbf{S}_n$ that employs multiple coatings that changes the grating thickness. With reference first to **FIG. 11A**, an initial RWG biosensor **102R** having a substrate **234** with a grating **242** of period $\mathbf{P}_0$ and a reference resonant wavelength λ_{RR0} is provided. Then, with reference to **FIG. 11B**, the reference RWG biosensor **102R** of **FIG. 11A** is covered with a first coating **261** designed to increase the waveguide thickness without substantially altering the waveguide period $\mathbf{P}_0$, thus shifting the reference resonant wavelength from λ_{RR0} to wavelength λ_{RR1} within the light source spectral bandwidth λ_{FWHM}. In embodiments, period $\mathbf{P}_0$ can be chosen so that the reference resonant wavelength λ_{RR0} is already within the light source spectral bandwidth λ_{FWHM}.

[0046] Then, with reference to **FIG. 11C**, a portion of first coating **261** can be coated with a second coating **262** designed to locally alter the grating thickness and thus to reflect a reference resonant wavelength λ_{RR2} within the light source spectral bandwidth λ_{FWHM}.

[0047] Then, with reference to **FIG. 11D**, a portion of second coating **262** can be coated with a third coating **263** designed to locally alter the grating thickness and thus to reflect a reference resonant wavelength λ_{RR3} within the light source spectral bandwidth λ_{FWHM}. This process results in the formation of a multi-segment reference RWG biosensor **102R** having three RWG sections $\mathbf{S}_1$, $\mathbf{S}_2$ and $\mathbf{S}_3$ that respectively have reference resonant wavelengths λ_{RR1}, λ_{RR2} and λ_{RR3} all within light source spectral bandwidth λ_{FWHM}. Thus, two or more RWG sections $\mathbf{S}_n$ can be formed in this manner. The cross-sectional views of FIG. **11C** through **11D** are taken through the multiply coated sections.

[0048] Note that the grating period $\mathbf{P_0}$ is on the order of hundreds of nanometers while the thickness increases due to the coatings are on the order of 5 nm to 10 nm. The period $\mathbf{P_0}$ does not change due to addition of the coatings, thought there is a slight changed in the duty cycle, which has a negligible effect on the performance of the multi-segment reference RWG biosensor **102R**.

[0049] In embodiments, layers **261**, **262,** and **263** can be applied using known selective mask-based deposition techniques. In embodiments, coatings **261**, **262,** and **263** can comprise niobia.

[0050] **FIGS. 12A** and **12B** are cross-sectional views of an example multi-wavelength reference well **175R** of a multi-wavelength reference microplate **170R**. Multi-wavelength reference well **175R** includes a bottom **302**, an interior **304** and an open top **306**. **FIG. 12A** shows a reference RWG biosensor **102R** disposed at well bottom **302**, with interior **304** filled with air.

[0051] Since the sample microplate **170S** will have its sample wells **175S** filled with water, multi-wavelength reference wells **175R** must also be filled with either water or a material that mimics water by having substantially the same refractive index (e.g., of about 1.3) within light source spectral bandwidth λ_{FWHM}. The use of distilled water to fill multi-wavelength reference wells **175R** is an option, though it is generally not preferred because water may cause RWG biosensors to degrade (e.g., delaminate) over time. Distilled water also evaporates, and can spill out of the reference wells **175R** if reference microplate **170R** is not carefully handled or the wells not sealed.

[0052] With reference to **FIG. 12B**, in an example embodiment, multi-wavelength reference wells **175R** are at least partially filled (and in an example embodiment, completely filled) with a fill material **310** that has a refractive index and thermal properties similar to that of water. An exemplary fill material **310** is solid at room temperature and is not easily perturbed by the environment. In an example embodiment, fill material **310** comprises an elastomer, an optical epoxy, or a combination thereof. Use of elastomers in reference microplates is discussed in the aforementioned U.S. Patent Application Publication No. 2007/0211245.

[0053] An example elastomer fill material **310** suitable for use in filling multi-wavelength reference wells **175R** is sold under the brand name of Sylgard-184® elastomer, available from the Dow Corning Corporation, Midland, Michigan. The

Sylgard-184® elastomer has the following properties/characteristics as provided in Table 1:

Table 1: Sylgard-184® elastomer properties

Physical Form	**Liquid**
Color:	Colorless
Odor:	Some odor
Specific Gravity @ 25°C:	1.05
Viscosity:	5000 cSt or 3900 cpsi
Boiling Point:	> 35°C/95°F
One or two parts:	2
Durometer:	50A
Working Time RT:	> 2 hours
Room Temp Cure Time:	48 hours
Heat Cure Time:	45 min @ 100C
Thermal Conductivity	0.18 (watts/meter- K)
Refractive Index:	about 1.41 to 1.42
dn/dT:	about 450 ppm/degree C

[0054] It is noted here that any fill material **310** that is known or is subsequently developed that has properties and characteristics substantially the same as that of the Sylgard-184™ elastomer is or will be suitable for use in the present disclosure.

[0055] Fill material **310** is added to interior **306** of multi-wavelength reference wells **175R** either manually using a positive displacement pipette or by an automated filling process. Multi-wavelength reference microplate **170R** is then allowed to cure for approximately two days at room temperature, after which time the elastomer fill material **310** within the multi-wavelength reference wells **175R** has fully cured and is ready for use.

[0056] **FIG. 13** is similar to **FIG. 3** and shows an example multi-wavelength reference microplate **170R** having *m* sets **350** (e.g., **350-1**, **350-2**, …**350-*m***) of multi-wavelength reference wells **175R**. The different sets **350** of multi-wavelength reference wells **175R** respectively contain multi-segment reference RWG biosensors **102R** having different reference resonant wavelengths λ_{RR}, namely for set **350-1**: λ_{RR1A}, λ_{RR1B}, …; for set **350-2**: λ_{RR2A}, λ_{RR2B}, …; and for set **350-3**: λ_{RRmA}, λ_{RRmB}, … .

[0057] Consider by way of example a multi-wavelength reference microplate **170R** having three different multi-wavelength reference well sets **350-1**, **350-2** and **350-3**. The first set **350-1** includes multi-wavelength reference wells **175R1** having multi-segment reference RWG biosensors **102R** with two sections $\mathbf{S_1}$ and $\mathbf{S_2}$

configured to reflect reference wavelengths λ_{RR1A} = 825 nm and λ_{RR2A} = 830 nm (see inset A). The second set **350-2** includes multi-wavelength reference wells **175R2** having multi-segment reference RWG gratings **102R** again with two sections S_1 and S_2 configured to reflect reference wavelengths λ_{RR2A} = 834.5 nm and λ_{RR2B} = 837 nm (see inset B). The third set **350-3** includes multi-wavelength reference wells **175R3** having multi-segment reference RWG gratings **102R** with three sections S_1, S_2 and S_3 configured to reflect reference wavelengths λ_{RR3A} = 840 nm, λ_{RR3B} = 842 nm and λ_{RR3C} = 844 nm (see inset C).

[0058] The result is a multi-wavelength reference microplate **170R** that provides wavelength information at multiple wavelengths within the broadband light source spectral bandwidth λ_{FWHM}. **FIG. 14** is a plot similar to **FIG. 6** and shows a schematic example wavelength spectrum for an SLD light source **106**. The plot of **FIG. 14** shows the seven different reference wavelengths λ_{RR} reflected by the different reference wells **175R** of the example reference microplate **170R** of **FIG. 13**. The number *m* of reference well sets **350** can be selected to provide as complete a wavelength coverage as needed or desired.

[0059] **FIG. 15A** is a plot of the change in wavelength noise (picometers) versus reference well number as measured on an example optical reader system **100** having a stable broadband light source **106**. The "noise" is the difference between the measured signals resonant wavelength λ_{RS} versus the measured reference resonant wavelength λ_{RR}. The standard (prior art) reference microplate was used (dashed line) and the multi-wavelength reference microplate **170R** of the present disclosure was also used (solid line). For a stable light source **106**, the two types of microplates give essentially the same constant reading for the change in wavelength noise

[0060] **FIG. 15B** is a plot similar to **FIG. 15A**, except that an unstable broadband light source **106** was used in the optical reader system. The plot of **FIG. 15B** reveals that the prior art reference microplate shows substantially no change in the wavelength noise, while the multi-wavelength reference microplate **170R** of the present disclosure shows a significant change in the wavelength noise due, as one would expect, to variations in the (polarized) light source output spectrum. Thus, the prior art reference microplate is unable to detect spectral variations in light source **106** that cause measurement noise in the optical reader system.

[0061] Multi-wavelength reference microplates **170R** can be employed by end-users to ensure system performance prior to running an assay, or use them as a reader control during an assay. The multi-wavelength reference microplates **170R** provide a more realistic simulation of the customer assay then the prior art reference microplates. It is also noted that multi-wavelength reference plates **170R** simplify field support efforts by providing multi-wavelength (and up to full spectrum) verification of the optical reader system in a single reference microplate. Currently, field support personnel must carry multiple microplates and additional metrology equipment (notch filters, etc) if they need to fully evaluate the optical reader's optical spectrum.

[0062] It will be apparent to those skilled in the art that various modifications to the preferred embodiment of the disclosure as described herein can be made without departing from the scope of the disclosure as defined in the appended claims. Thus, the disclosure covers the modifications and variations provided they come within the scope of the appended claims and the equivalents thereto.

What is claimed is:

1. A multi-wavelength reference microplate for a label-independent optical reader, the reader having a light source with a wavelength band, comprising:

a support plate that supports a plurality of reference wells, at least one reference well being configured as a multi-wavelength reference well having disposed therein two or more resonant waveguide grating (RWG) sections that respectively reflect two or more different reference resonant wavelengths within the light source wavelength band.

2. The microplate of claim 1, wherein all the reference wells are configured as multi-wavelength reference wells.

3. The microplate of claim 1, wherein the two or more RWG sections are contiguous.

4. The microplate of claim 1, wherein the two or more RWG sections are spaced apart from each other.

5. The microplate of claim 4, wherein the two or more RWG sections are disposed on a common substrate.

6. The microplate of claim 1, wherein the two or more RWG sections have respective two or more coatings of different thicknesses that respectively define two or more different grating periods that in turn respectively define the two or more different reference resonant wavelengths.

7. The microplate of claim 1, wherein the microplate includes two or more sets of multi-wavelength reference wells, with the multi-wavelength reference wells within each set having the same two or more reference resonant wavelengths and the different sets having different two or more reference resonant wavelengths.

8. The microplate of claim 1, wherein each multi-wavelength reference well is filled with a fill material having a refractive index of about 1.3 within the light source wavelength band.

9. The microplate of claim 8, wherein the fill material comprises an elastomer, an optical epoxy, or a combination thereof.

10. A multi-wavelength reference microplate for a label-independent optical reader, the reader having a light source with a wavelength band, comprising:

a support plate that supports a plurality of multi-wavelength reference wells each having a reference resonant waveguide grating (RWG) biosensor disposed therein that includes two or more RWG sections that respectively have two or more reference resonant wavelengths; and

a fill material that at least partially fills each multi-wavelength reference well, wherein the fill material has a refractive index of about 1.3 within the light source wavelength band.

11. The multi-wavelength reference microplate of claim 10, wherein the fill material comprises an elastomer, an optical epoxy, or a combination thereof.

12. The multi-wavelength reference microplate of claim 10, wherein the two or more RWG sections are contiguous.

13. The multi-wavelength reference microplate of claim 10, wherein the two or more RWG sections are spaced apart from each other.

14. The multi-wavelength reference microplate of claim 10, wherein the two or more RWG sections include respective coatings having different thicknesses.

15. A method of using a reference microplate with reference wells to measure multiple reference resonant wavelengths in a label-independent optical reader system, comprising:

providing in at least one reference well two or more resonant waveguide grating (RWG) sections each having a different reference resonant wavelength;

irradiating each of the two or more RWG sections to generate respective reflected light therefrom; and

spectrally analyzing the respective reflected light to measure the two or more reference resonant wavelengths.

16. The method of claim 15, wherein the two or more RWG sections are contiguous.

17. The method of claim 15, wherein the two or more RWG sections are spaced apart.

18. The method of claim 15, wherein the two or more RWG sections are separate sections on a common substrate.

19. The method of claim 15, further comprising filling the at least one reference well with a fill material having a refractive index of about 1.3 within the light source wavelength band, wherein the fill material comprises an elastomer, an optical epoxy, or a combination thereof.

20. The method of claim 15, further comprising defining the two or more RWG sections by:

providing a single grating with a single grating period; and

providing different coating thicknesses in different sections to form two or more grating periods in the two or more RWG sections.

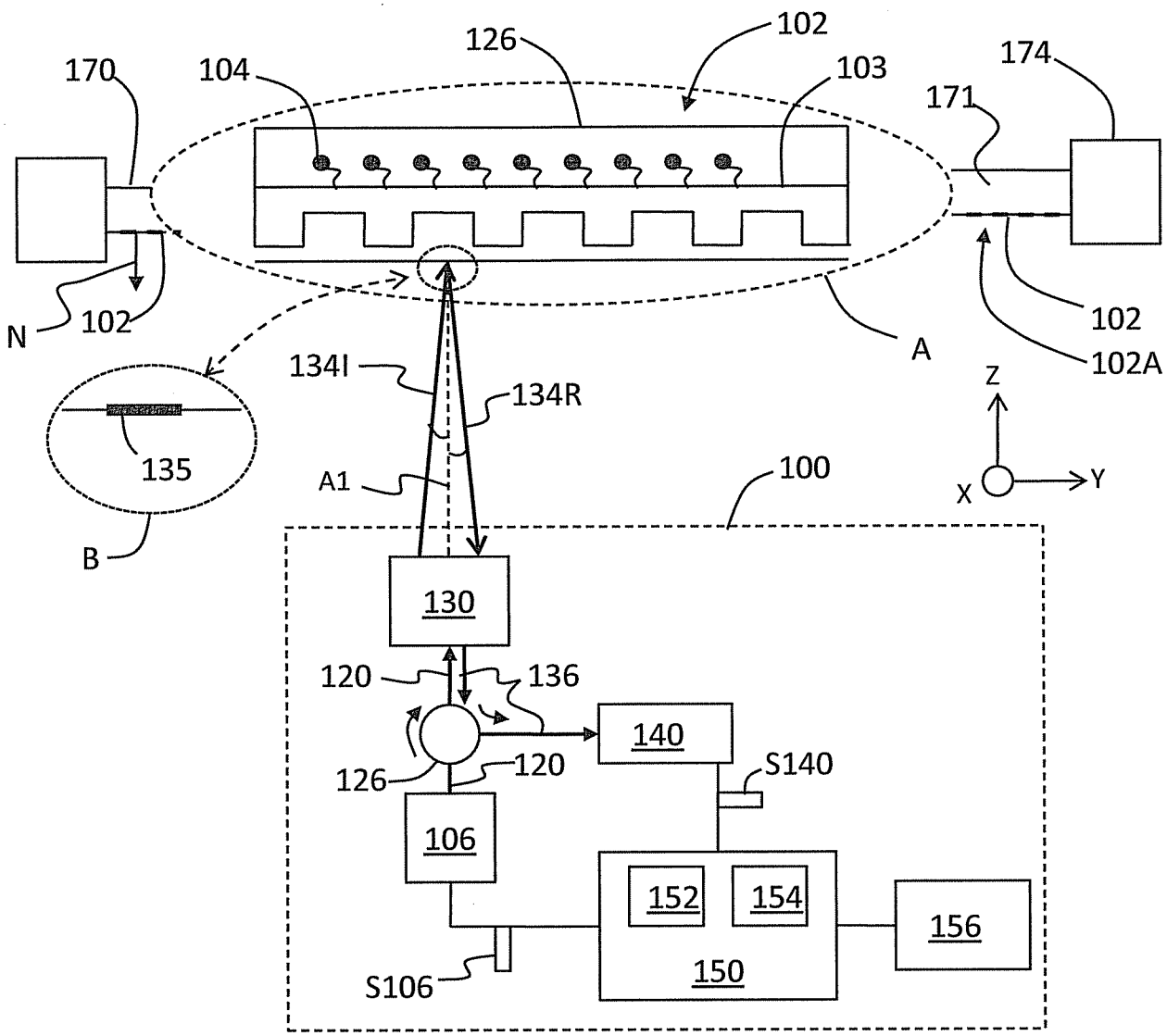
170
104
126
102
103
174
171
N
102
A
102
102A
134I
134R
135
A1
100
Z
X
Y
B
130
120
136
140
S140
126
120
106
152
154
156
150
S106

FIG. 1

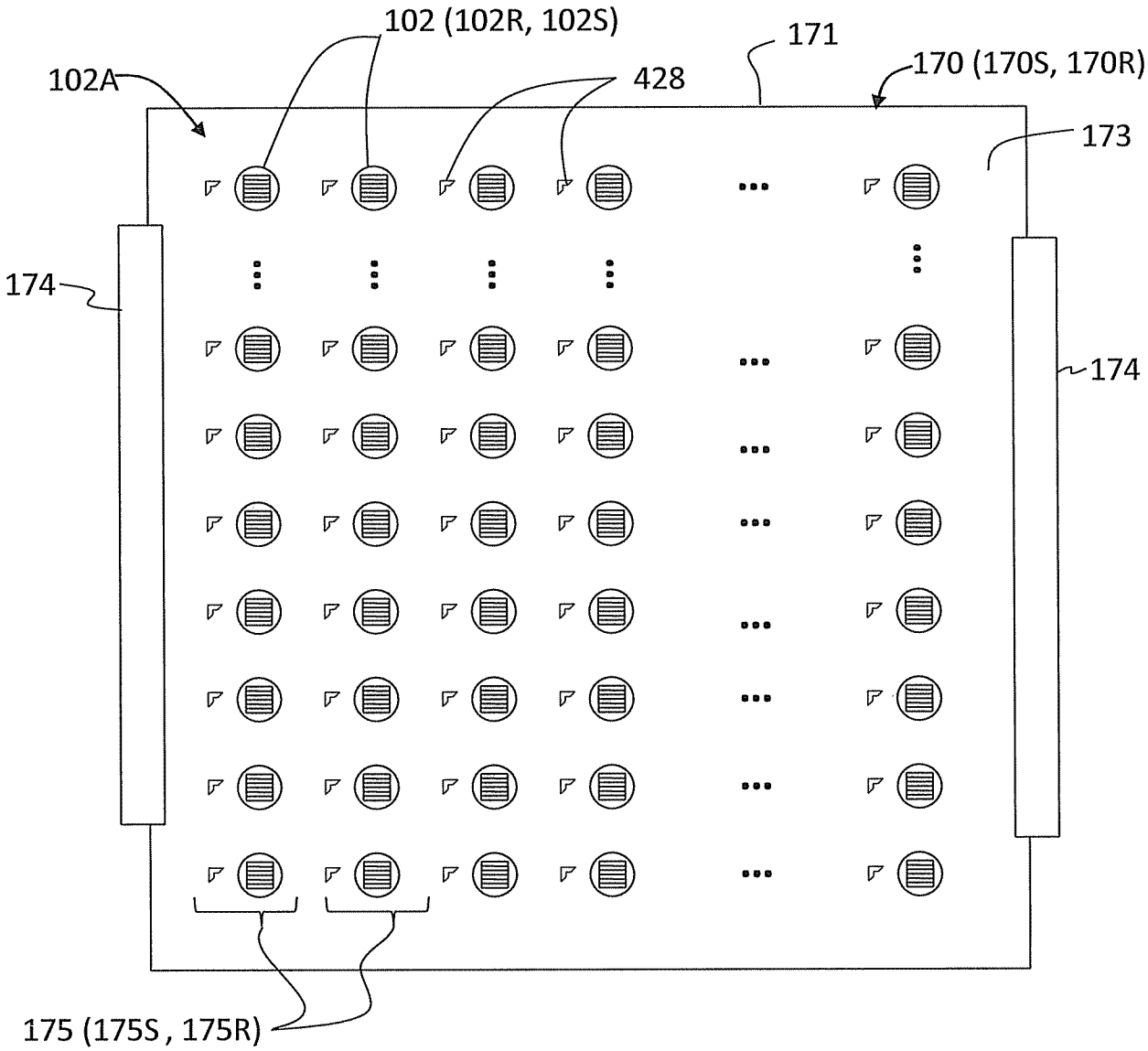
102 (102R, 102S)
171
102A
428
170 (170S, 170R)
173
174
174
175 (175S , 175R)

FIG. 2

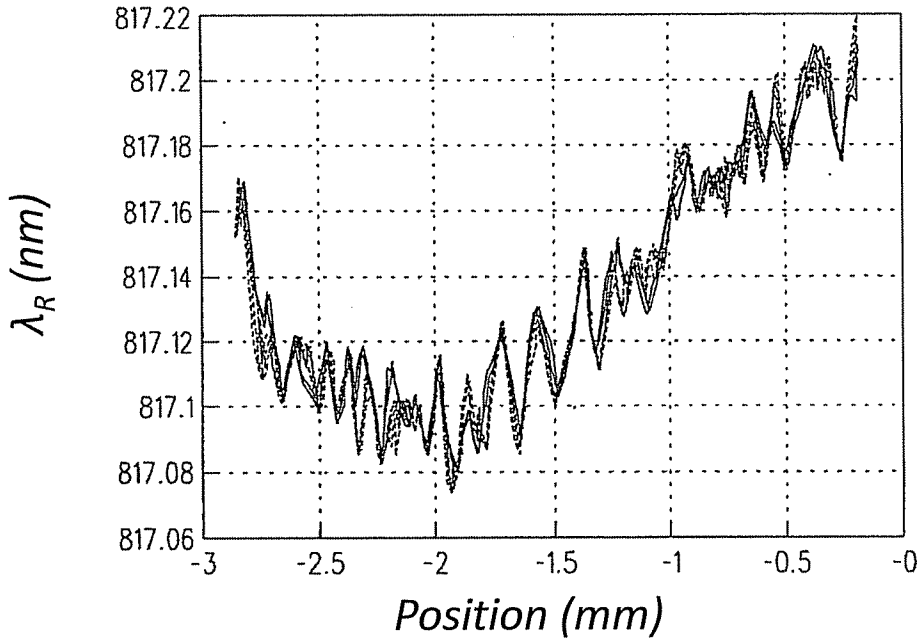
817.22
817.2
817.18
817.16
817.14
817.12
817.1
817.08
817.06
λ_R (nm)
-3
-2.5
-2
-1.5
-1
-0.5
-0
Position (mm)

FIG. 3

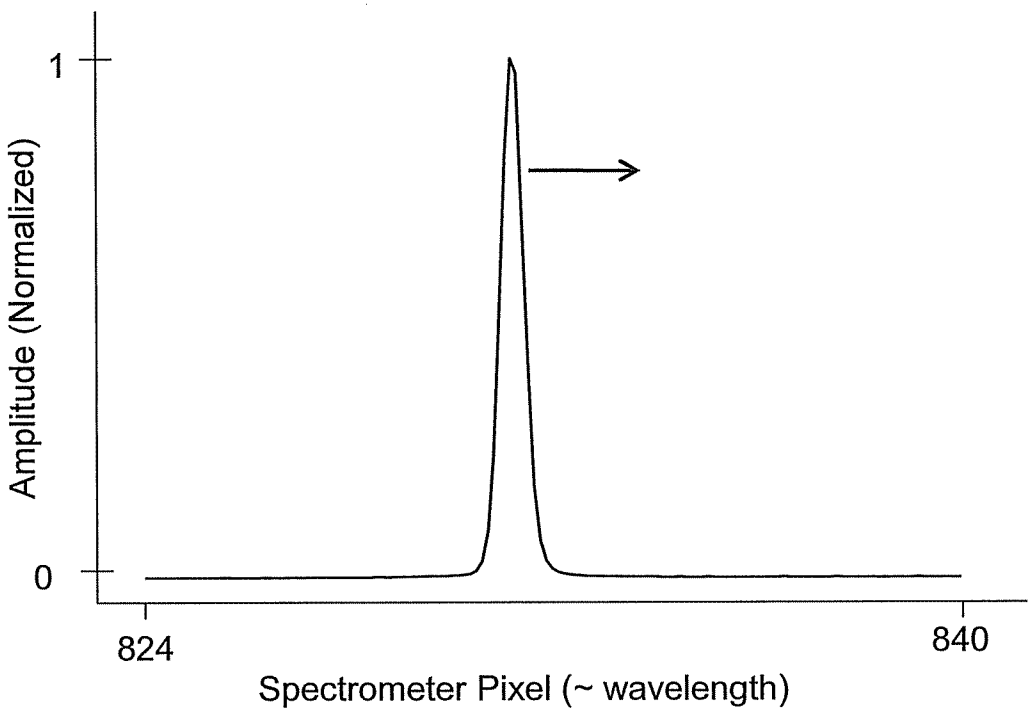
1
0
Amplitude (Normalized)
824
840
Spectrometer Pixel (~ wavelength)

FIG. 4

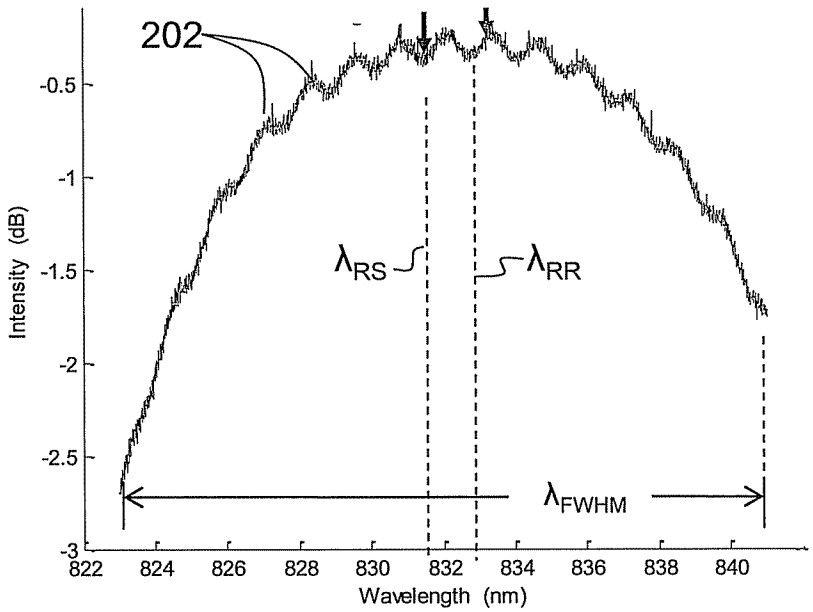
202
-0.5
-1
-1.5
-2
-2.5
-3
Intensity (dB)
λ_{RS}
λ_{RR}
λ_{FWHM}
822
824
826
828
830
832
834
836
838
840
Wavelength (nm)

FIG. 5

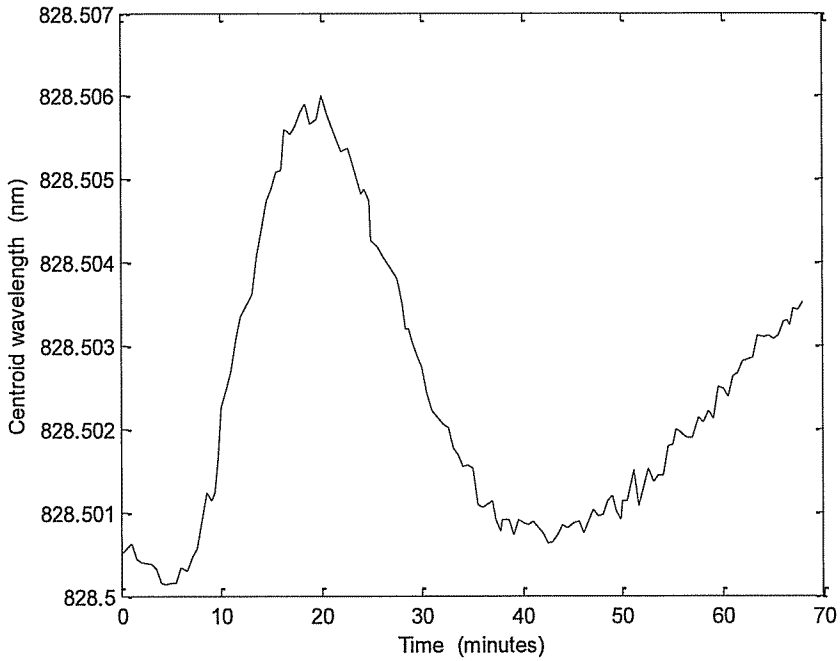
828.507
828.506
828.505
828.504
828.503
828.502
828.501
828.5
Centroid wavelength (nm)
0
10
20
30
40
50
60
70
Time (minutes)

FIG. 6

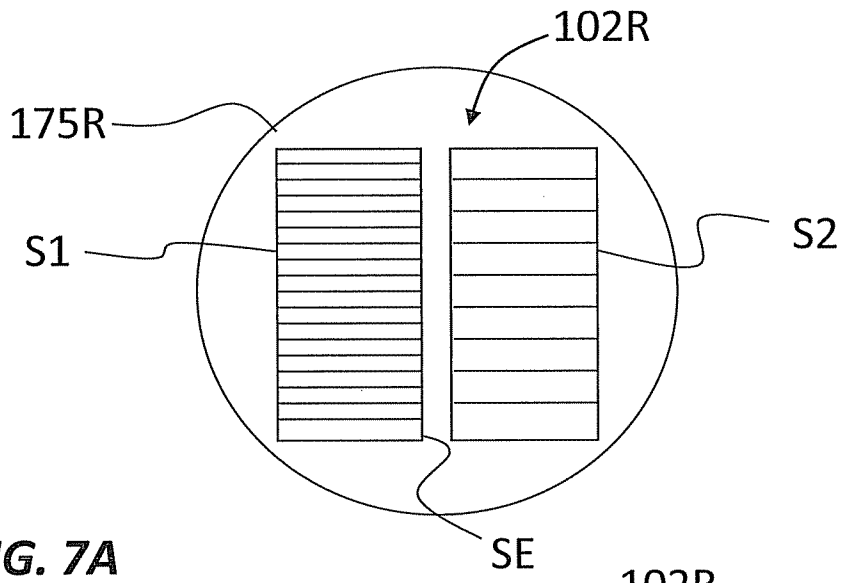

FIG. 7A

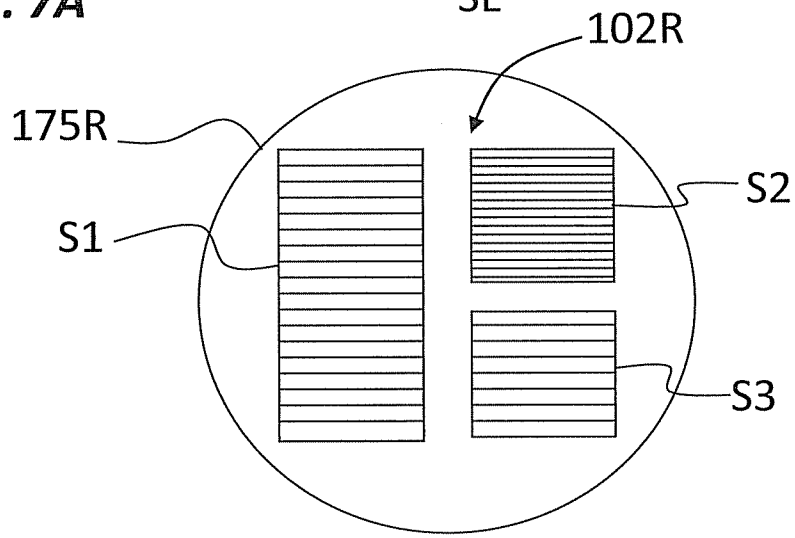

FIG. 7B

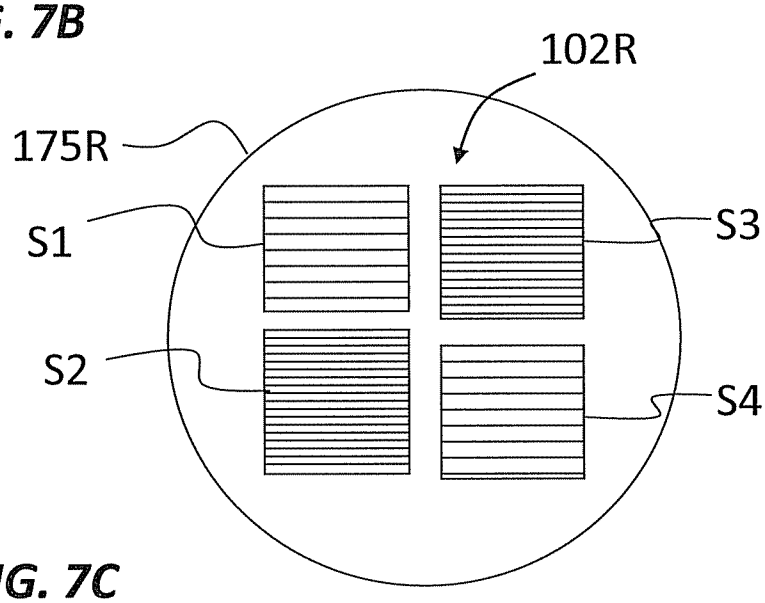

FIG. 7C

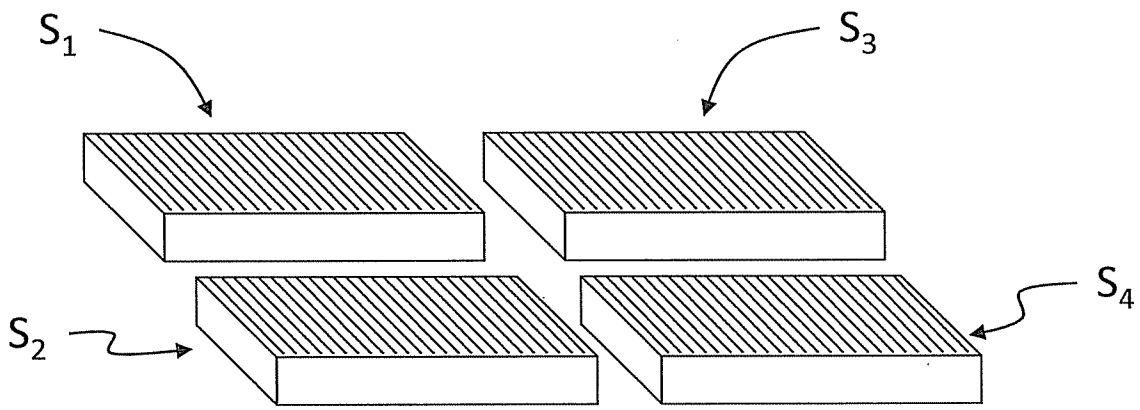

FIG. 8A

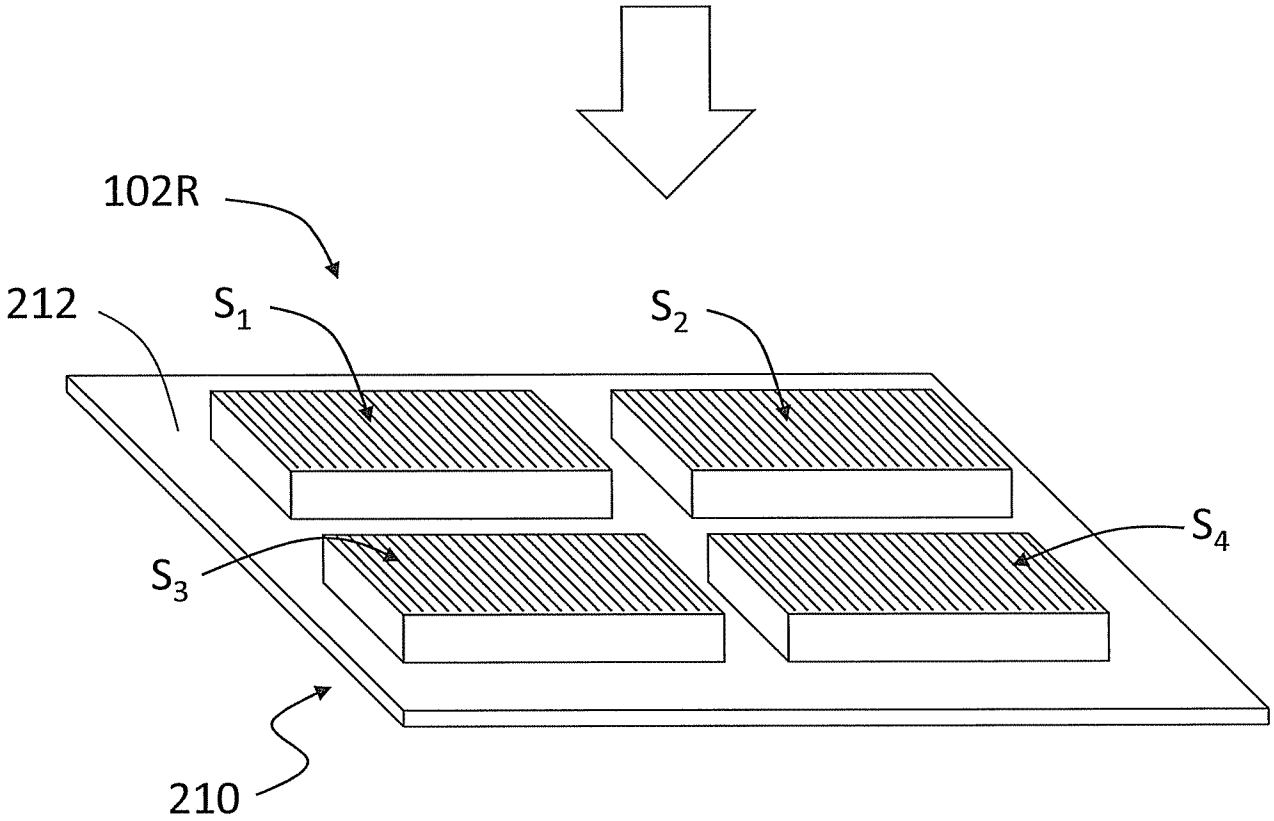

FIG. 8B

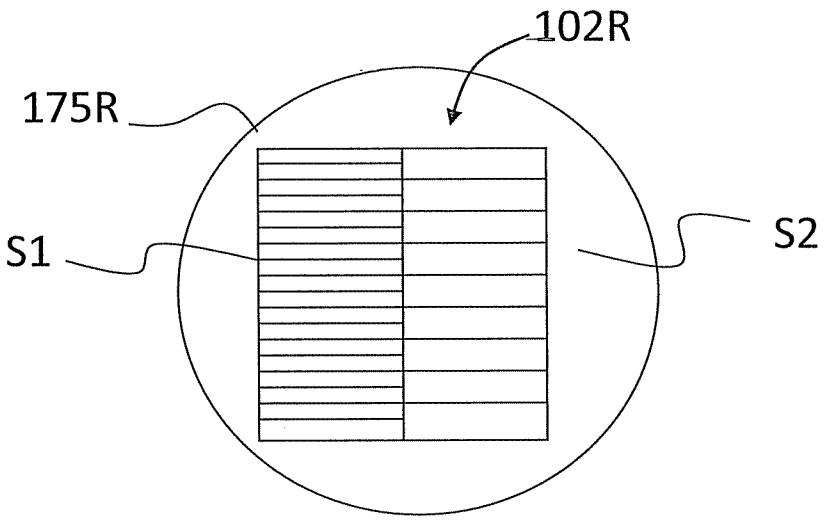

FIG. 9A

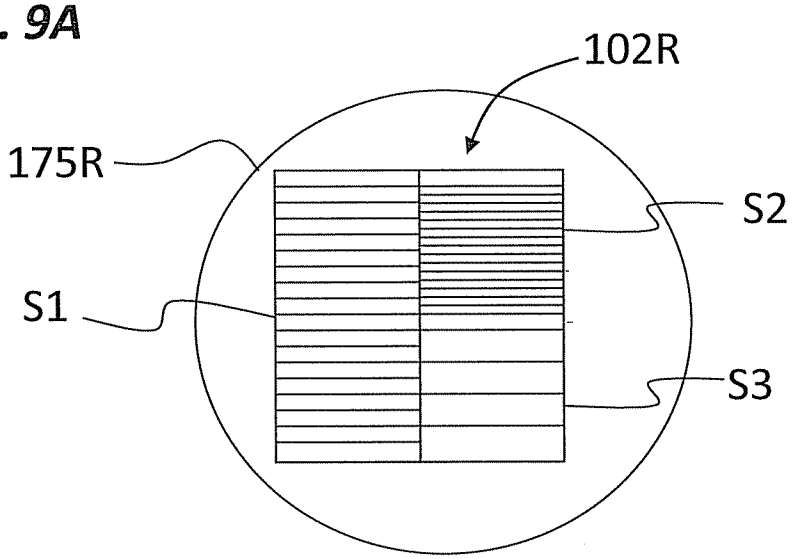

FIG. 9B

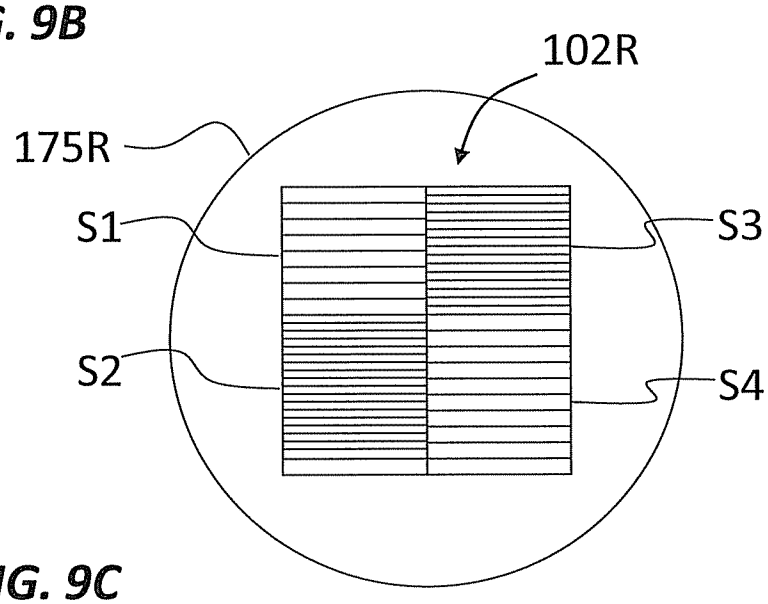

FIG. 9C

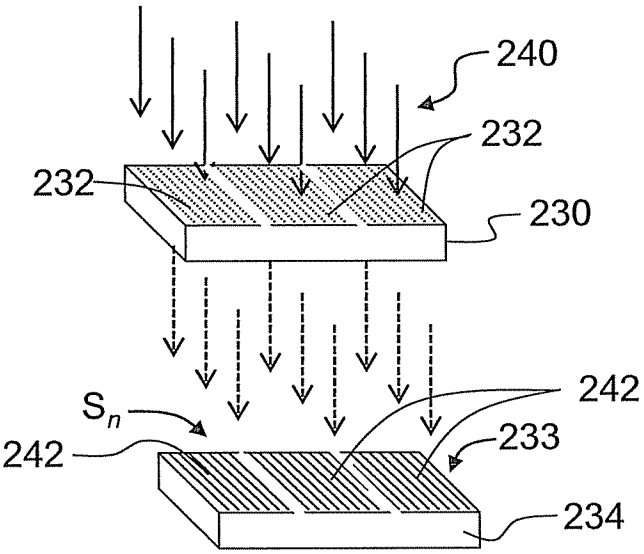

FIG. 10A

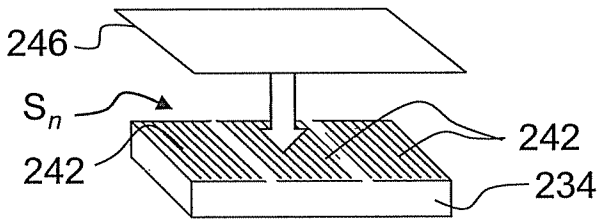

FIG. 10B

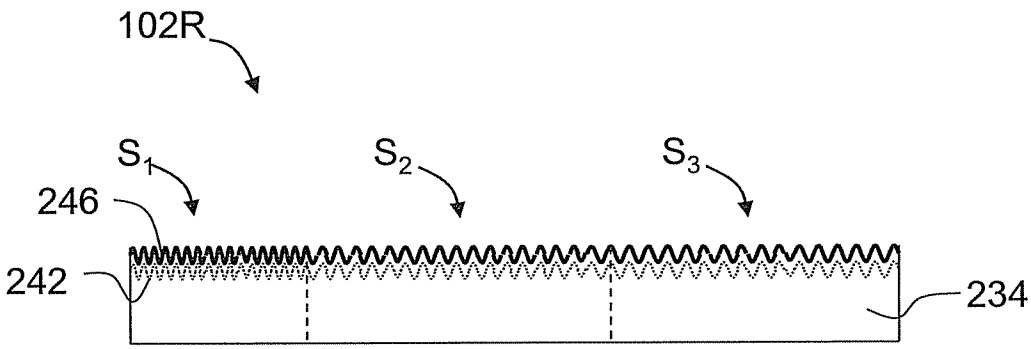

FIG. 10C

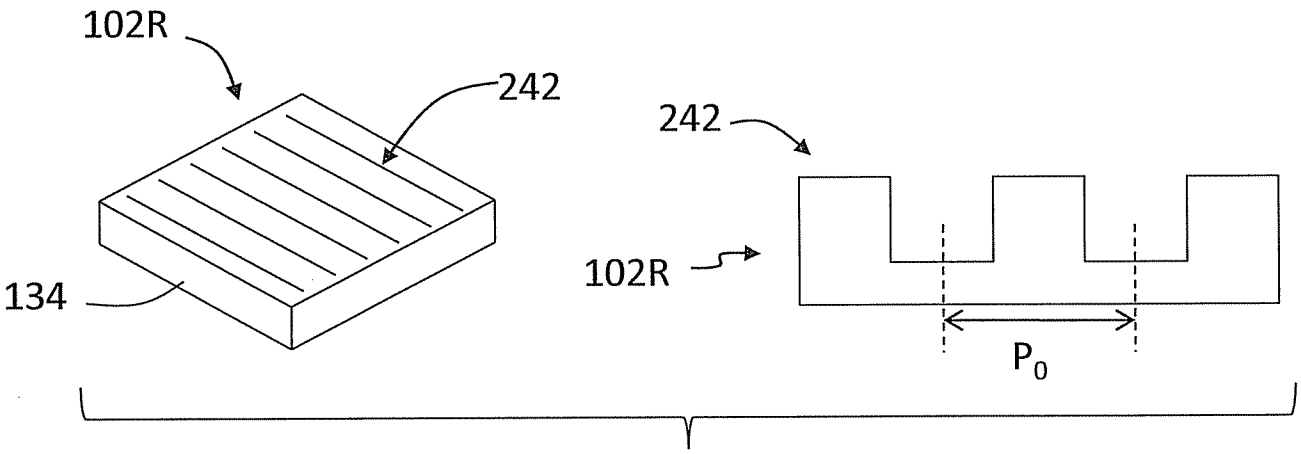

FIG. 11A

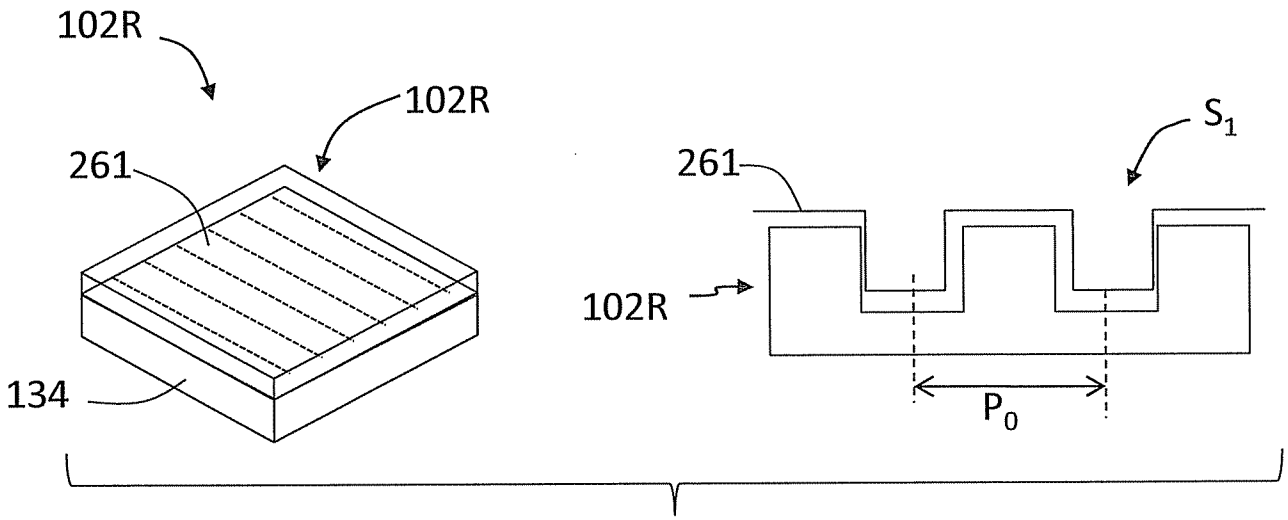

FIG. 11B

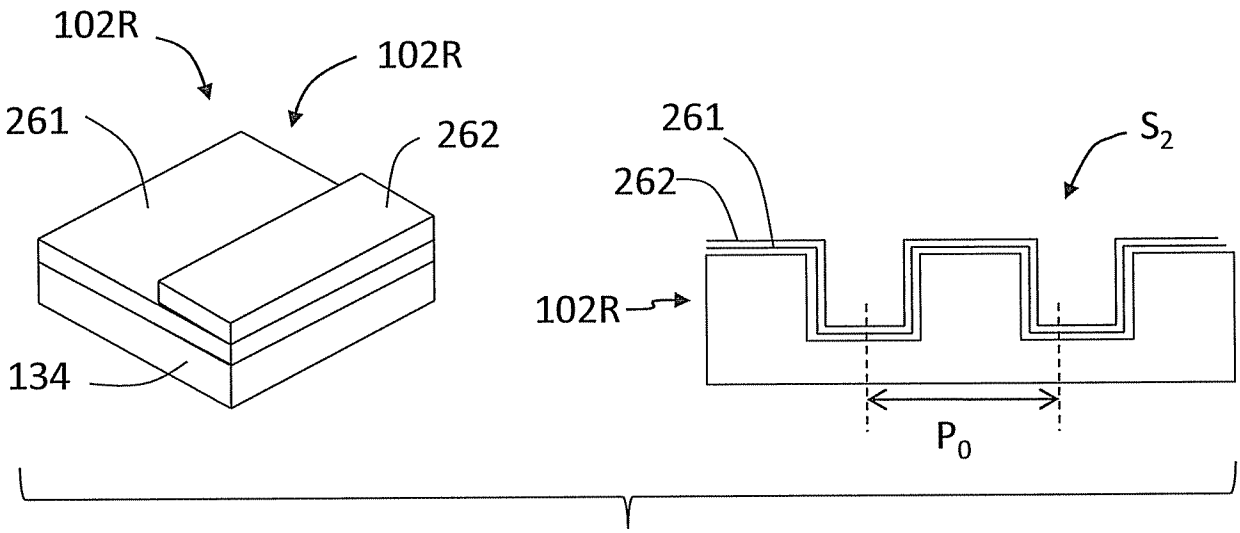

FIG. 11C

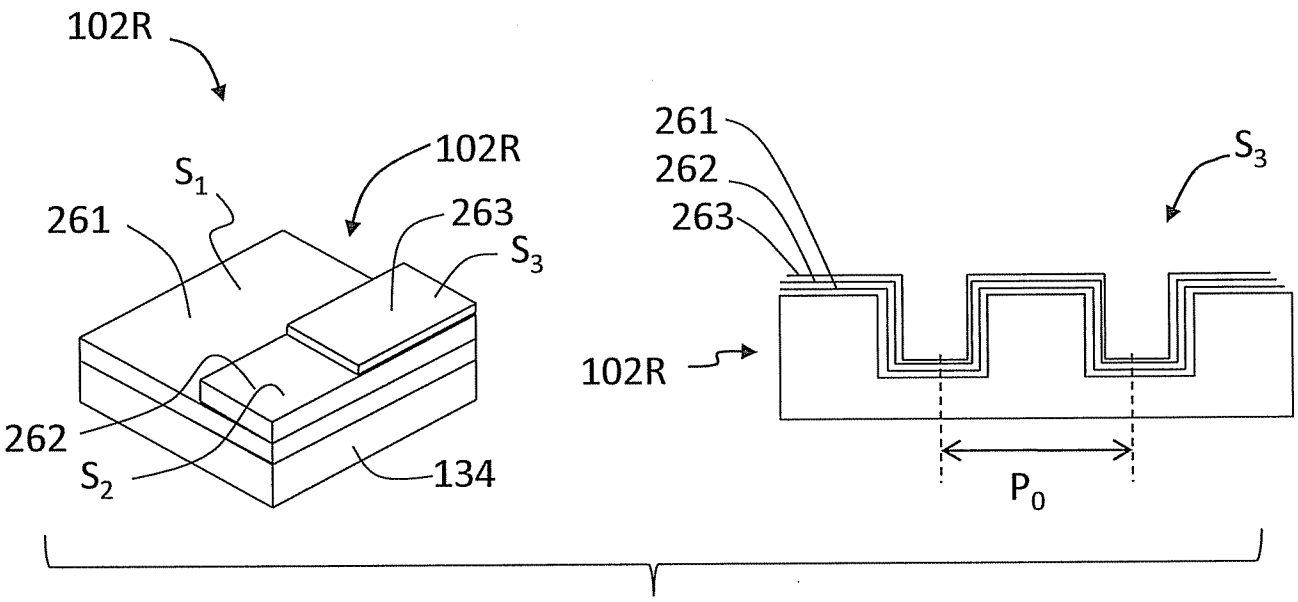

FIG. 11D

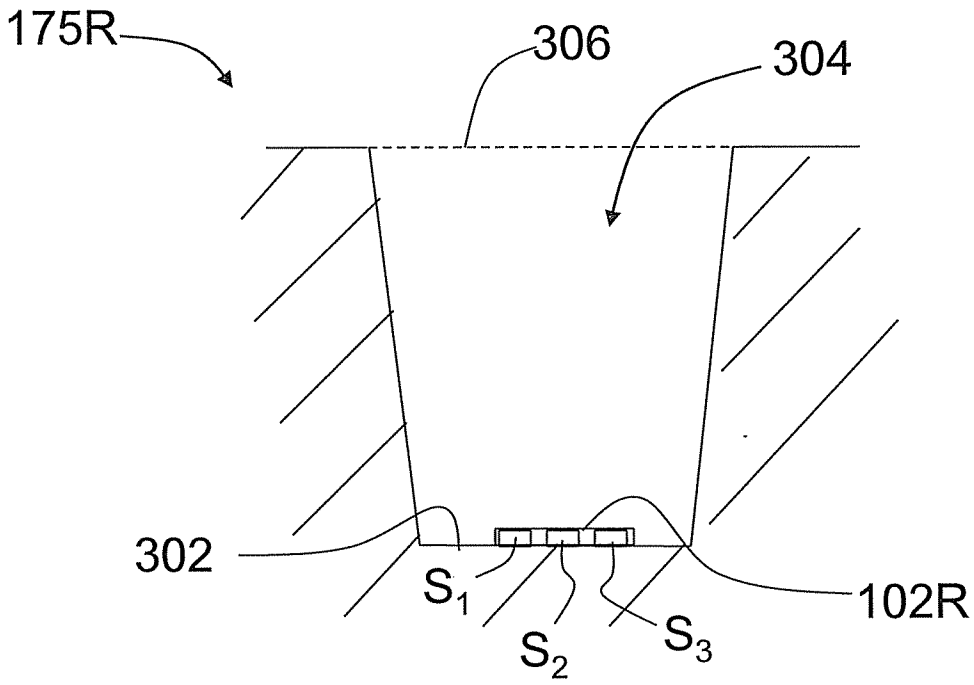

FIG. 12A

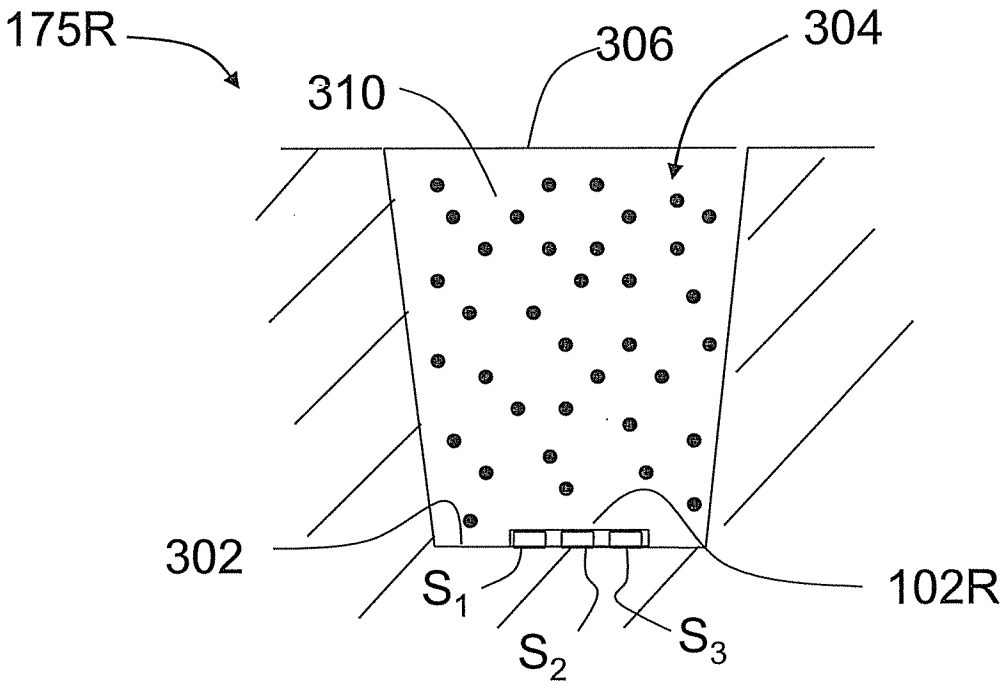

FIG. 12B

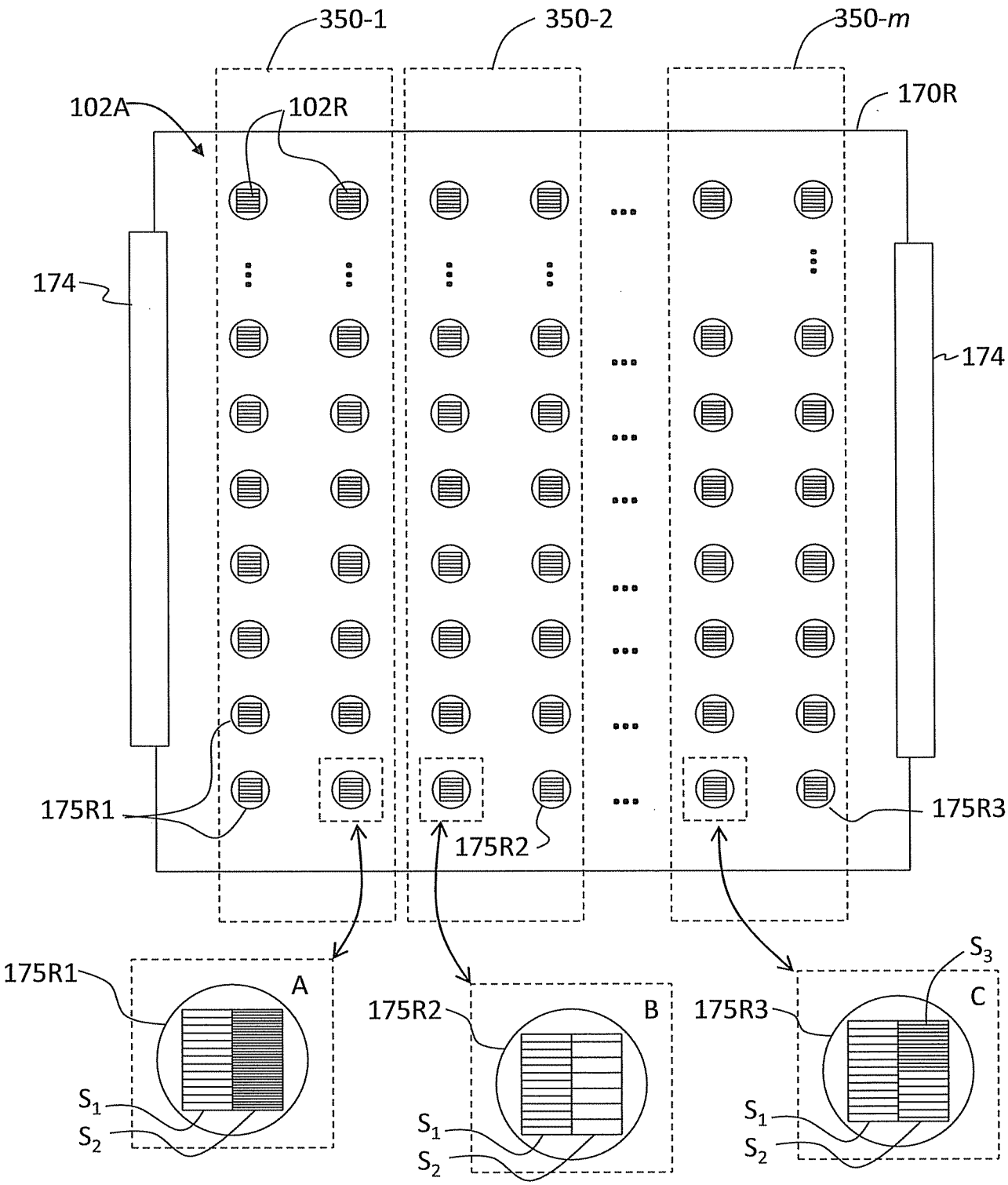
350-1
350-2
350-m
102A
102R
170R
174
174
175R1
175R2
175R3
175R1
A
S1
S2
175R2
B
S1
S2
S3
175R3
C
S1
S2

FIG. 13

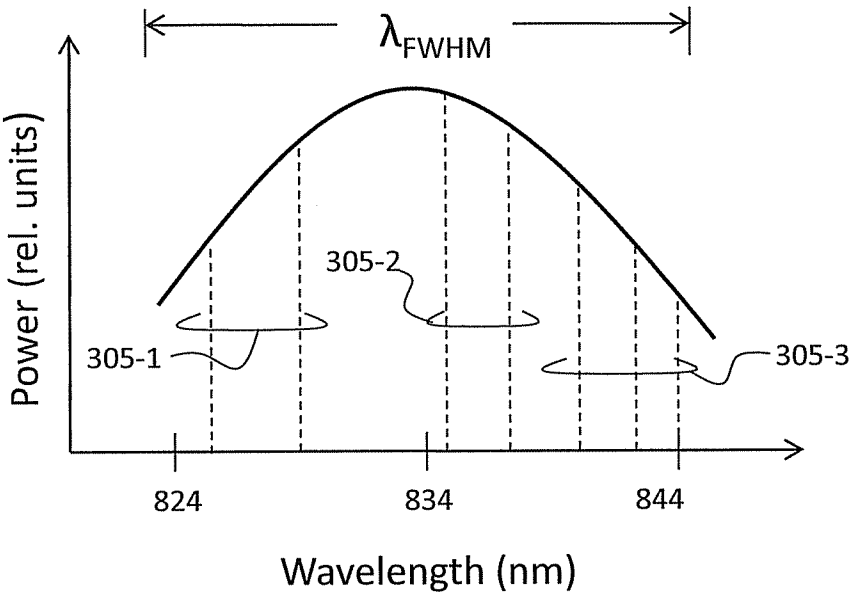
λFWHM
Power (rel. units)
305-1
305-2
305-3
824
834
844
Wavelength (nm)

FIG. 14

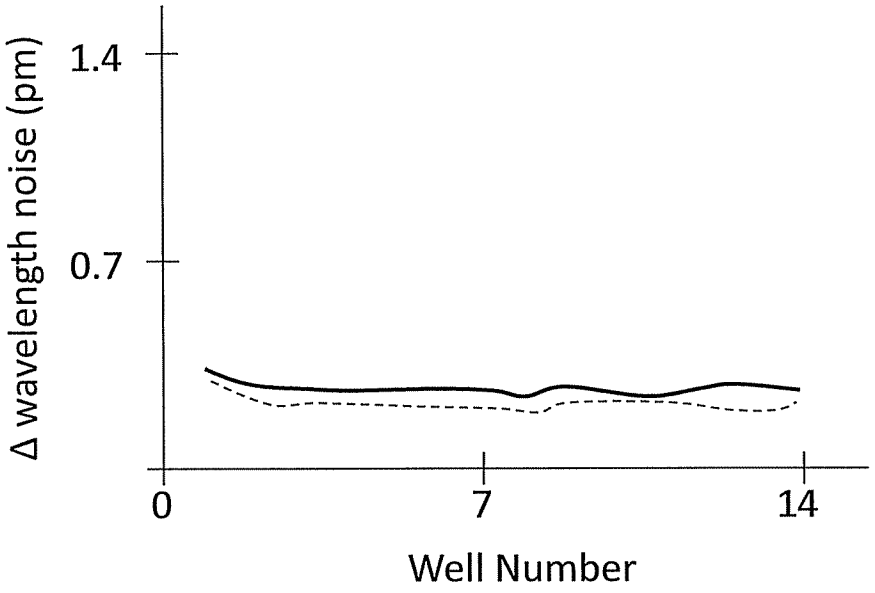

FIG. 15A

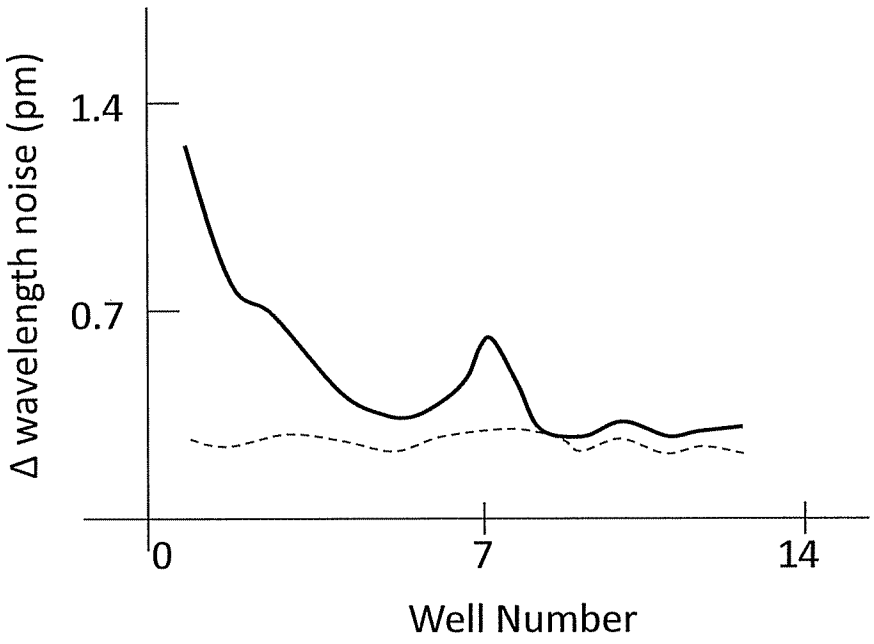

FIG. 15B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/054949

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/27 G01N21/77 B01L3/00
ADD.

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)
G01N B01L

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/202543 A1 (GOLLIER JACQUES [US] ET AL) 30 August 2007 (2007-08-30) cited in the application	1,2,4-8, 10, 13-15, 17,18,20
Y	paragraphs [0016], [0020], [0026], [0027], [0029] figures 1,7A	9,11,19

X	US 2006/062509 A1 (KROL MARK F [US] ET AL) 23 March 2006 (2006-03-23)	1-3,5,8, 10,12, 15,16
	* abstract paragraphs [0014], [0015], [0055] figure 1A	

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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
10 February 2011	21/02/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	D'Alessandro, Davide

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/054949

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2007/211245 A1 (PASTEL DAVID A [US] ET AL) 13 September 2007 (2007-09-13) cited in the application paragraphs [0016], [0018], [0044], [0065] - [0076] -----	9,11,19
Y	US 2003/081875 A1 (KOCHERGIN VLADIMIR [US] ET AL) 1 May 2003 (2003-05-01) paragraphs [0003], [0007], [0036], [0056] figure 2A -----	9,11,19
Y	EP 0 255 302 A2 (ARES SERONO RES & DEV LTD [US] ARS HOLDING 89 NV [AN]) 3 February 1988 (1988-02-03) page 2, line 3 - line 16 page 2, line 34 - line 47 page 6, line 11 - line 14 -----	9,11,19
A	US 2005/025421 A1 (CARACCI STEPHEN J [US] ET AL) 3 February 2005 (2005-02-03) * abstract paragraphs [0054], [0056] -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2010/054949

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US 2007202543 A1	30-08-2007	US 2006139641 A1	29-06-2006
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		AU 7600687 A	28-01-1988
		CA 1301470 C	26-05-1992
		DE 3789923 D1	07-07-1994
		DE 3789923 T2	08-09-1994
		ES 2053545 T3	01-08-1994
		IL 83280 A	05-11-1990
		JP 2511057 B2	26-06-1996
		JP 63081226 A	12-04-1988
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		JP 2007501432 T	25-01-2007
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