

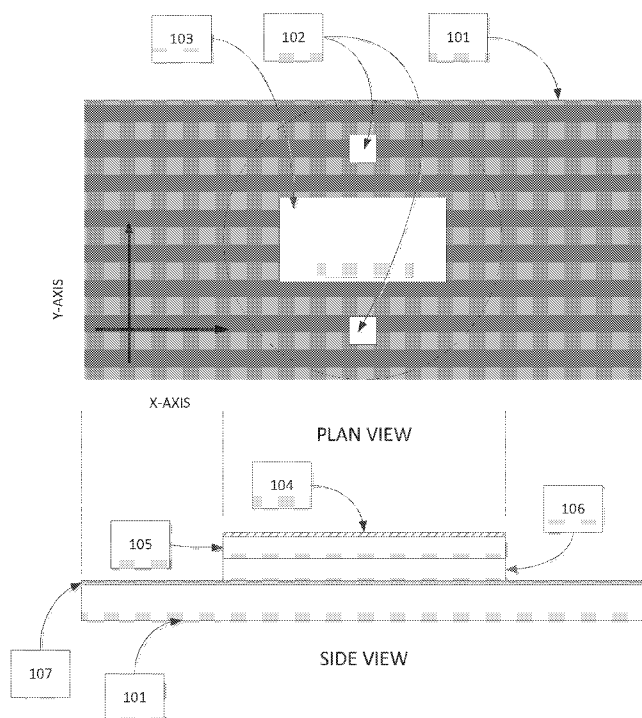


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(54) Title: FLUORESCENT STANDARD DEVICE AND METHOD OF USE

FIG. 1 – PLAN AND SIDE VIEW OF THE FLUORESCENT STANDARD
DEVICE



(57) Abstract: A fluorescent standard device that includes a rectangular cover glass, a cylindrical piece of a glass that produces a fluorescent signal when exposed to a excitation beam and a cylindrical piece of background glass. The fluorescent standard device is configured to provide a long-term, stable, standardized fluorescent response to an excitation source for use in diagnostic clinical analyzers employing Cy-5 dyes. Preferred methods of use are also disclosed.



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FLUORESCENT STANDARD DEVICE AND METHOD OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This patent application claims priority of United States Provisional Application Number 62/052,142, filed September 18, 2014, and entitled: FLUORESCENT STANDARD DEVICE FOR AUTOMATIC RECALIBRATION, the disclosures of which are incorporated by reference in their entireties.

10

FIELD OF THE INVENTION

The invention relates generally to a fluorescent standards device and methods used to normalize and calibrate clinical diagnostic analyzers or instruments that use fluorometry as a measurement mechanism.

15

BACKGROUND OF THE INVENTION

- The present invention pertains to photometric measurement systems which require a reference standard in order to normalize a population of subordinate clinical diagnostic analyzers to a master clinical diagnostic analyzer, and monitor and
- 20 correct for instrumentation drift with respect to time. Various components of the photometric measurement system will change, for example, illumination sources will dim, optical components may haze or become coated with contaminants, detection systems may drift, etc. In order to monitor and correct for such phenomena, a reference standard must be used. However, in the case of fluorescence
- 25 measurement, a stable reference material is not readily available. Solid materials currently available photo bleach thereby producing a reduced fluorescent output over time for a specific excitation input. Likewise, liquid fluorescence standards can be benchmarked and used as a reference, but require a high degree of technical skill to assess. Also liquid fluorescence standards are not only prone to photo

bleaching, but are affected by unintended sources of fluorescence such as inappropriate diluents and contaminants. Furthermore, both solid and liquid standards are temperature sensitive. A simple, robust and stable reference source is required to ensure that measurements remain accurate over time.

5

SUMMARY OF THE INVENTION

One object of the present invention is to provide a device which has a significant resistance to photo bleaching and will provide a stable reference over time. Such a device has been designed utilizing a phosphate matrix glass giving the glass fluorescent properties such as a NIST (National Institute of Standards and Technology) developed material consisting of a phosphate matrix glass doped with bismuth ion, giving the glass fluorescent properties. Its excitation and emission spectra fall in the same region as many of the Cy5-like family of dyes, including Alexa Fluor® 647. This characteristic makes it an ideal solid fluorescent reference material for instruments that process Cy-5 like dyes.

Another object of the invention is to provide a device which is self-indicating with respect to photo bleaching such that measurements on the device itself can indicate when replacement is required.

The foregoing and further objects of the invention are accomplished according to one aspect of the invention that provides a fluorescent standard device comprising a cover glass, a glass that produces a fluorescent signal when exposed to an excitation beam, and a background glass.

Still another aspect of the invention provides a method of using said fluorescent standard device article of manufacture to provide a standardized fluorescent signal comprising, determining the y-axis position of an excitation beam, determining the x-axis position of said excitation beam, determining the difference between a determined position of said excitation beam and a desired read position, moving the

position of said excitation beam from said determined position to said desired read position, and effecting a standardized read.

Further objects, features and advantages of the present invention will be apparent to those skilled in the art from detailed consideration of the preferred embodiments that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a plan and side view of the fluorescent standard device.

FIG. 2 is a view of the three major components of the fluorescent standard device. These components are the rectangular cover glass **101**, a cylindrical piece of NIST SRM 2944 glass **106**, and a cylindrical piece of background glass **105**, usually B270 borosilicate crown glass.

FIG. 3 is a diagram of the scanning method used to make sure that the final fluorescent read is in the proper position. Diagram **301** shows the scanning setup for determining the y-axis position and diagram **302** shows the scanning setup for determining the x-axis position.

FIG. 4 is a view of the two major scanning sites for the final fluorescent read. Diagram **401** shows the read site "C" centered in the rectangular window and diagram **402** shows the read site "C" offset to the left in the rectangular window along with alternative read site "D" offset to the right in the rectangular window.

FIG. 5A shows the starting position of a one-dimensional scan across (bottom to top) a square window when the scan is initiated near the bottom edge of the window. FIG. 5B shows the starting position of a one-dimensional scan a (bottom to top) cross a square window when the scan is initiated a moderate distance away

from the bottom edge of the window. FIG. 5C shows the starting position of a one-dimensional scan across (bottom to top) a square window when the scan is initiated quite far away from the bottom edge of the window. In each case the active scan positions are indicated by an ordinate value of 100% transparency.

- 5 FIG. 6 shows the relative scan intensity of a one-dimensional scan across the excitation beam starting at some distance below the bottom of the beam and continuing across (bottom to top) some distance above the beam.

FIG. 7A shows the response to the excitation light beam as a function of position as the beam scans across (bottom to top) the square window where the excitation beam is initially positioned near the bottom edge of the square window. FIG. 7B shows the response to the excitation light beam as a function of position as the beam scans across (bottom to top) the square window where the excitation beam is initially positioned some distance away from the bottom edge of the square window. FIG. 7C shows the response to the excitation light beam as a function of position as the beam scans across (bottom to top) the square window where the excitation beam is initially positioned quite far away from the bottom edge of the square window.

FIG.8 is a diagram of the methodology used to locate the center of the square window for the case where the scan starts near the bottom of the square window. Regression lines are constructed using the ascending and descending data points associated with the scan response. Where there two regression lines cross provides an estimate of the center of the square window.

FIG. 9A presents the details of the analysis used to produce the regression lines for the ascending and descending data of FIG. 7A which is the case where the scan response has the square window near the bottom of the scan. FIG. 9B presents the details of the analysis used to produce the regression lines for the ascending and descending data of FIG. 7B which is the case where the scan response has the square window near the center of the scan. FIG. 9C presents the

details of the analysis used to produce the regression lines for the ascending and descending data of FIG. 7C which is the case where the scan response has the square window near the top of the scan.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

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While the present invention is described with respect to preferred embodiments as detailed below and shown in the figures, the present invention is limited only by the metes and bounds of the claims that follow.

10 A preferred embodiment of the present invention consists of a manufactured device housing NIST SRM 2944 glass which consists of a phosphate matrix glass doped with bismuth ion to cause fluorescence when exposed to an excitation light source. While SRM 2944 is a preferred glass, for different wavelength fluorometry a different material other than NIST SRM 2944 would be used, such as other
15 phosphate doped glasses also available from NIST, including SRM 2943, copper doped glass, spectral correction standard for blue fluorescence. Optical access to the NIST SRM 2944 glass **106** is provided via several optically transparent windows fabricated into the rectangular cover glass **101**. One surface of the NIST SRM 2944 glass **106** is attached to the back of the rectangular cover glass **101**
20 and is further enclosed with a B270 borosilicate crown background glass **105** adhered to the other surface of the NIST SRM 2944 glass **106**. It should be noted that there exists many other NIST and otherwise certified glass targets that could be used in this application instead of NIST SRM 2944 glass.

25 One of the benefits of this manufactured device is that it provides a highly stable fluorescent source when exposed to an excitation light source that is resistant to photo bleaching. Another benefit is that the excitation light source exposure can be tightly controlled as to location such that excitation exposures in other locations can be used to detect any longer term reduction in fluorescent response. Yet another
30 other benefit is that the inventive device is a key component in a method to nor-

malize a population of subordinate clinical diagnostic analyzers to a master clinical diagnostic analyzer which is the subject of co-pending US patent application “Normalizing the Response of a Fluorescent Instrument using Spectral Response” by David Heavner (Attorney Docket No. CDS5169WOPCT), incorporated by reference in its entirety.

For a general understanding of the disclosed methods, reference is made to the drawings. In the drawings, like reference numerals have been used to designate identical elements. In describing the disclosed methods, the following terms have been used in the description.

The terms “clinical diagnostic analyzer,” “diagnostic analyzer,” and “instrument” refer to devices that accept a patient sample or specimen, analyze the sample or specimen for a specific analyte, and report the result of that analysis. These terms are meant to encompass clinical chemistry analyzers, immunohematology analyzers, lateral flow device readers, and the like.

The term “normalize” refers to the a method applied to two clinical diagnostic analyzers or instruments, a master instrument “A” and a subordinate instrument “B”, such that the response of “B” to a specific sample or specimen containing a certain concentration of analyte can be converted to the response of “A” to the same sample or specimen by using a multiplicative factor.

The terms “square window,” “square alignment window,” “alignment target,” and “alignment window” are used interchangeably and refer to the either of the upper or lower optically transparent sections in the rectangular cover glass.

In FIG. 1, there is a plan view and a side view of the instant inventive device. The plan view shows the front of the rectangular cover glass **101** in gray. It has three transparent windows, namely, a center rectangular window **103**, and upper and lower square windows **102**. The section of the rectangular cover glass **101** shown

in gray is opaque to any excitation light source. The side view in FIG. 1 shows the stacked glass components of the inventive device. The rectangular cover glass **101** shows an opaque coating **107** on the rear surface. The rear surface of the rectangular cover glass **101** is attached to the front surface of the NIST SRM 2944 glass **106**. The rear surface of the NIST SRM 2944 glass **106** is attached to the front surface of the background glass **105**. The rear surface of the background glass **105** is frosted **104**.

The inventive device is manufactured from three components: a rectangular cover glass **101**, a cylindrical piece NIST SRM 2944 glass **106**, and a cylindrical piece of background glass **105** as shown in FIG. 2. Each is an integral component of the device. The cover glass **101** is made of a non-fluorescing material, for example, B270 borosilicate crown glass, and is rectangular for device installation alignment. The rear side of the glass is coated with an opaque and non-reflective finish relative to the wavelength of excitation light. Three clear windows are provided in the coating; the largest is preferably rectangular in shape and is centered on the cover glass. This rectangular window **103** has been designed to be significantly larger than the excitation source illumination beam. In this case, the rectangular window **103** width was set twice the required width as dictated by the long axis of the excitation illumination beam in order to facilitate two read regions if desired. It is important that sufficient clear space is created for the reads. If the excitation light were to shine upon the coated region it would be perceived as a reduction in signal due to a fluorescence change. The two other square windows **102** are alignment targets. Only one of the two may be used, but in order to make the assembly mistake proof, a second target is created, making the rectangular cover glass **101** symmetrical. In this instance the alignment targets **102** are square; the height has been set to approximately the short axis of the excitation beam. Square targets will yield the highest transitions from the non-reflective mask to the fluorescence material for scanning, but are not required to be square to work. The alignment targets **102** have been positioned sufficiently distant from the read area so that when scanned the illumination spot will not strike the rectangular window **103**

read area and cause photo bleaching. All three features must reside within a suitable space defined by the short axis of the rectangular cover glass **101**. Within this space will be the area of the NIST SRM 2944 glass **106** and background glass **105** bonded to the coated side of the rectangular cover glass **101**.

5

The fabrication of the inventive device proceeds in the following manner. First the rectangular cover glass **101** is cut and opaque coating **107** is applied to the rear surface such that the previously identified windows, specifically the center rectangular window **103**, and the two upper and lower square alignment windows **102**,
10 are allowed to remain transparent. Then the NIST SRM 2944 glass **106** is then cut and polished into a suitable shape, preferably cylindrical, and is then glued between the rectangular cover glass **101** and background glass **105** with an optical adhesive. The resulting stack of components has both optical and protective functions. The rectangular cover glass **101** includes a coating to establish a coordinate
15 frame for consistent measurement while providing a barrier against moisture. The background glass **105** provides a non-reflective opaque background for the fluorescence standard while also providing a barrier against moisture. The rear surface of the background glass **105** is frosted to aid in the assembly of the inventive device. This configuration is important in providing a stable reference material.
20 The NIST SRM 2944 glass **106** will slightly photo bleach as it is exposed to excitation light. Therefore, reference measurements must be structured to minimize the exposure of the NIST SRM 2944 glass **106** to the fluorescence excitation source. To accomplish this, the alignment target (a square alignment window **102**) is positioned such that scanning of the target will not expose the center rectangular win-
25 dow **103** to light. Once the alignment target has been scanned, the measurement system is located over a defined region of the NIST SRM 2944 glass **105** relative to the measured alignment target location. When in position, a preferred method consists of a brief single read (area "C" in FIG. 4) taken as the reference measurement. Measuring the same relative location mitigates variability in the measurement. Measuring the same relative location mitigates variability in the meas-
30 urement due to NIST SRM 2944 glass **106** non-uniformity. By taking the measurement in the same location and making that measurement as brief as possible a

very accurate reference reading can be obtained with minimal photo bleaching over the life of the product. The fluorescence readings made by the instrument will be adjusted inversely to changes observed in the signal read from the inventive device as dictated by well-understood, routine, and conventional predefined limits and rules. Specifically see, James O. Westgard, Basic QC Practices, 3rd edition, 2010, published by Westgard QC, Inc. which is hereby incorporated in its entirety by reference. For instance, if the signal read from the inventive device has dropped by “1%” from its initial factory values, the raw fluorescence values from the clinical diagnostic analyzer would be adjusted up by “1%” to compensate.

10

To further extend the life of the reference material, an alternative preferred method is to use two non-overlapping reference locations relative to the alignment target. One location (area “D” in in diagram **402** in FIG. 4) would be used as a “gold standard” to be measured infrequently in order to determine degradation of the other measurement location (area “C” in diagrams **402** in FIG. 4) that would be used for each system measurement. This would facilitate monitoring and correction of drift in the frequently used reference by comparing it against the infrequently used “gold standard”.

The NIST SRM 2994 material **106** is a glass doped with molecules that will fluoresce when excited with light. The molecules selected are to fluoresce in approximately the same region as the assays that will be processed on the instrument. The NIST SRM 2994 glass **106** is cut in a circle (cored) as is common practice by optical component manufacturers. The thickness of the glass can be used to set the intensity of fluorescence when excited; thinner glass emits less light. While common processing produces a circularly shaped glass, the present invention is not limited to such a shape.

The background glass **105** is preferably the same material and coating as the cover glass **101**. However, it has no clear features as the cover glass and is preferably the same dimensions as the NIST SRM 2994 glass **106**. Like the cover glass

101, the coated surface of the background glass **105** is bonded to the NIST SRM 2994 glass **106** using a non-fluorescing optical adhesive. In order to mistake proof the assembly of the background glass, the side opposite the coated surface of the background glass **105** is frosted **104**; otherwise it is difficult to determine which
5 side should be bonded to the NIST SRM 2994 glass **106**. In a preferred embodiment, in order to reduce cost, simplify assembly and provide perfect alignment between the NIST SRM 2994 glass **106** and background glass **105**, an uncut background glass is created to match the size of the polished but not cored NIST glass wafers. The NIST / background glass stack is then cored to create the individual
10 subassemblies. This subassembly is then bonded to the coated side of the cover glass **101** to complete the assembly.

The size of the assembly is dictated by the illumination spot size. In a preferred embodiment, the spot size is substantially 1.2(width) x 0.7(height) mm. The reference standard device scales from that to be an overall size of substantially
15 10(width) x 5(height) x 3(depth) mm. The read window dimensions are substantially 3(width) x 1.2(height) mm in order to accommodate two side by side read areas if desired. The alignment targets are substantially 0.5(width) x 0.5(height) mm and are spaced substantially 0.9 mm from the read window to provide sufficient
20 space between the alignment target (square window) **102** and read window **103** so that the read area does not photo bleach during a scan of the alignment target **102**.

An alternative preferred embodiment for cost reduction would be to remove the
25 alignment target **102** and read window **103** features from the cover glass **101** and machine them into the instrument. Rather than mounting the device into the instrument with a relatively large opening to reveal the cover glass features, the cover glass **101** would have no coating. The NIST SRM 2994 glass **106** would be visible through the cover glass over its entire diameter. The location in which the
30 device would be mounted would have a machined hole to act as the alignment target and a machined window to reveal the read area. A second alignment target

would be unnecessary since the orientation during assembly is no longer an issue. With the features removed from the cover glass there would be no need for it to be rectangular. The cover glass **101** could, like the background glass **105**, be bonded to the polished NIST SRM 2994 glass **106** wafer prior to coring. This would significantly reduce the cost of the device. The round device would be mounted behind the alignment target **102** and read window **103** in the device then securely mounted to prevent any rotation or translation.

Reference standard measurements are made in three steps.

Step A: In FIG. 3, the read head digitally scans across the alignment target **102** normal to the long axis of the excitation beam (designated by the "A" arrow in diagram **301** in FIG. 3) to determine the Y location of the alignment target **102**. At the start of the scanning operation, the position of the read head may not be accurately known. In FIG. 5A the read head's start position is just below the alignment target **102**, in FIG. 5B the read head's start position is some distance below the alignment target **102**, and in FIG. 5C the read head's start position is a considerable distance below the alignment target **102**. Fig. 6 shows a one-dimensional representation of the excitation beam such that the total beam spans 61 scan positions and the illumination portion of the excitation beam spans 33 scan positions in the center of the total beam. As this beam is digitally stepped across the alignment target **102** for the positioning of FIG. 5A, the output response of FIG. 7A is produced. Two sets of data are then extracted from the FIG. 7A data, namely an ascending set composed of the data points included in the left ramp feature **701** and a descending set composed of the data points included in the right ramp feature **702**. A linear regression line is then fitted to each of the two data sets; the ascending set regression results displayed in the left panel of FIG. 9A and the descending set regression results displayed in the right panel of FIG. 9A. The ascending regression equation was found to be:

$$C1 = -14.00 + 1.00 \cdot C2$$

[eqn. 1]

where C1 is the ordinate and C2 is the abscissa values. The descending regression equation was found to be:

5 $C1 = 58.00 - 1.00 \cdot C2$ [eqn. 2]

where C1 is the ordinate and C2 is the abscissa values. FIG. 8 indicates the general method used to find the center of the window along the y-axis. The ascending regression line **801** is extended upward as is the descending regression line **802**
10 until the two lines intersect at a point **803**. This point on the y-axis is the center of the window. Mathematically, this is equivalent to solving the above two equation set such that the value of C2 is determined when C1 is the same in both equations, that is,

15 $-14.0 + 1.00 \cdot C2 = 58.0 - 1.00 \cdot C2$

Rearranging results in,

20 $2.00 \cdot C2 = 58.0 + 14.0$

Or,

$$2 \cdot C2 = 72.0 \quad \text{and} \quad C2 = 36.$$

25 This value is then adjusted by subtracting 14 (the leading number of zeros in the scan function in FIG. 6) and then by subtracting 16 (the half width of the illumination portion of the excitation beam in FIG. 6) to arrive at a Y position estimate of $36 - 14 - 16 = 6$ which is the correct placement (as per the center point of the square window position as shown in FIG. 5A). In a similar manner, for the alignment target **102** position in FIG. 5B reference to the scan result for the center square window in FIG. 7B and the associated ascending and descending regressions in FIG.

9B show that the correct Y position estimate of 27 is obtained. Additionally, for the alignment target **102** position in FIG. 5C reference to the scan result for the top square window in FIG. 7C and the associated ascending and descending regressions in FIG. 9C show that the correct Y position estimate of 49 is obtained.

5

Step B: Using the Y location from "Step A", the read head digitally scans across the alignment target **102** normal to the short axis of the excitation beam (designated by the "B" arrow in diagram **302** in FIG. 3) to determine the X location of the alignment target in the same manner as shown in "Step A."

10

Step C: Using the X and Y location from Step A and Step B alignment procedure above and a predefined offset, a read location is determined near the middle of the rectangular window **103**. The read head is moved with the excitation source OFF to the read location which is position "C" as shown in diagram **401** in FIG. 4. A single read is taken as the reference reading. The read head is moved from the rectangular window **103** with the excitation beam OFF. This value is compared against the original reading taken at the factory and used to adjust sample readings.

15

20 Step D (alternative read procedure): Periodically position "D", as shown in diagram **402** in FIG. 4, is read as a reference standard to correct the reads taken at position "C". In this method, as indicated above, position "C" is not centered but is located as shown in diagram **402** in FIG. 4. Both position "C" and position "D" have been measured at the factory as references. The use of these two read positions requires a different offset adjustment when compared to the offset of "Step C."

25

It will be apparent to those skilled in the art that various modifications and variations can be made to the article of manufacture disclosed herein. Thus, it is intended that the present invention cover such modifications and variations, provided they come within the scope of the appended claims and their equivalents.

30

The disclosure of all publications cited above is expressly incorporated herein by reference in their entireties to the same extent as if each were incorporated by reference individually.

We claim:

1. A fluorescent standard device comprising
 - 5 a cover glass,

a glass that produces a fluorescent signal when exposed to an excitation beam,

10 and a background glass.
2. A fluorescent standard device as recited in Claim 1, where said cover glass is substantially rectangular in shape.
- 15 3. A fluorescent standard device as recited in Claim 1, where said glass that produces a fluorescent signal is substantially cylindrical in shape.
4. A fluorescent standard device as recited in Claim 1, where said background glass is substantially cylindrical in shape.
- 20 5. A fluorescent standard device as recited in Claim 2, where at least one surface of said cover glass is coated with an opaque coating containing at least one transparent window.
- 25 6. A fluorescent standard device as recited in claim 1, wherein the glass is a phosphate matrix glass.
7. A fluorescent standard device as recited in claim 6, wherein the phosphate matrix glass is a bismuth-doped phosphate glass.

30

8. A fluorescent standard device as recited in Claim 7, where said glass that produces a fluorescent signal is NIST SRM 2944 glass.
9. A fluorescent standard device as recited in Claim 6, where said NIST SRM 2944 glass is mounted to said cover glass such that the said NIST SRM 2944 glass is in optical communication with an excitation beam directed through said at least one transparent window in said cover glass and a remaining surface is exposed.
10. A fluorescent standard device as recited in Claim 7, where said background glass is mounted to said remaining exposed surface of said NIST SRM 2944 glass thereby creating an exposed surface of said background glass.
11. A fluorescent standard device as recited in Claim 8, where said exposed surface of said background glass is frosted.
12. A method of using said fluorescent standard device to provide a standardized fluorescent signal comprising,
- determining the y-axis position of an excitation beam,
- determining the x-axis position of said excitation beam,
- determining the difference between a determined position of said excitation beam and a desired read position,
- moving the position of said excitation beam from said determined position to said desired read position,
- and affecting a standardized read.

13. The method of claim 12, wherein the desired read position is configured into two separate, non-overlapping regions such that a first region is used for said standardized read and that a second region is infrequently read to determine degradation of the first region.

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FIG. 1 – PLAN AND SIDE VIEW OF THE FLUORESCENT STANDARD DEVICE

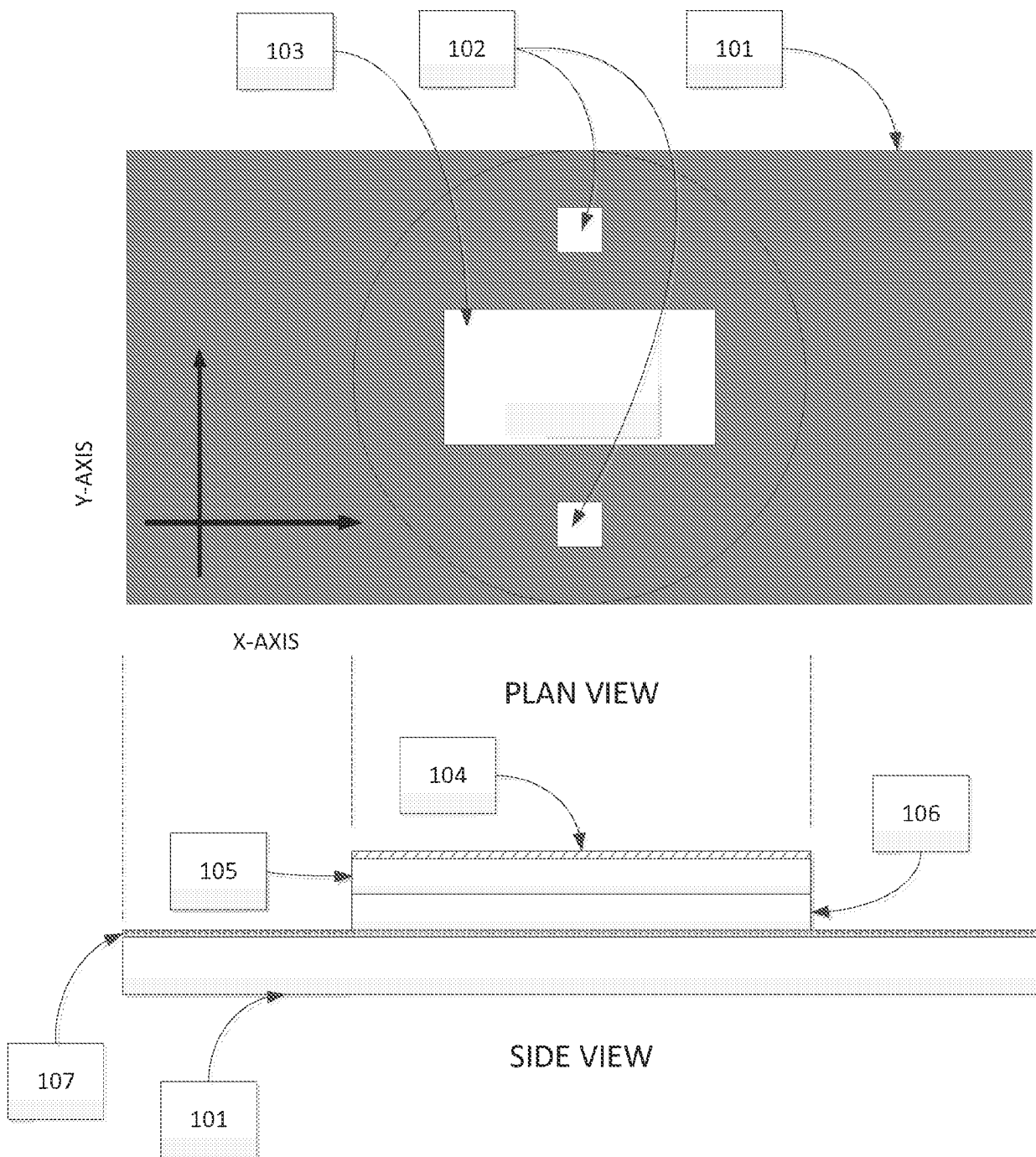


FIG. 2 – MAJOR COMPONENTS OF THE FLUORESCENT STANDARD
DEVICE

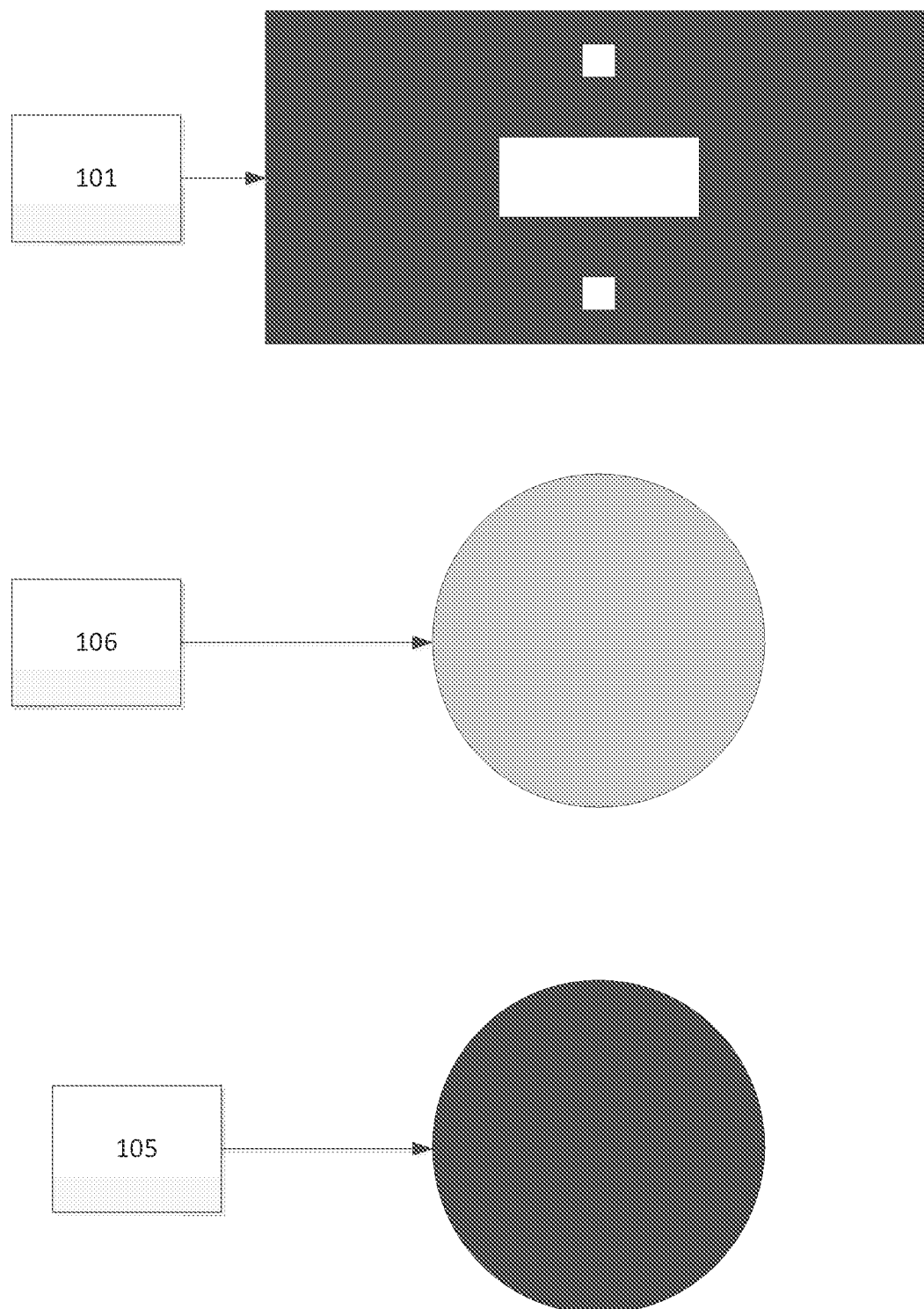
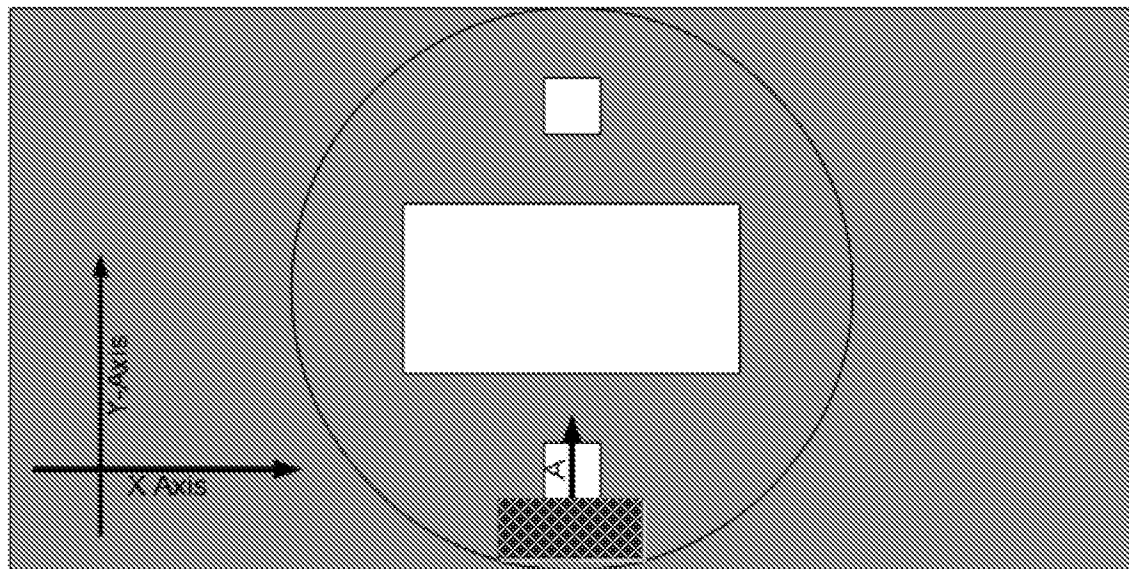
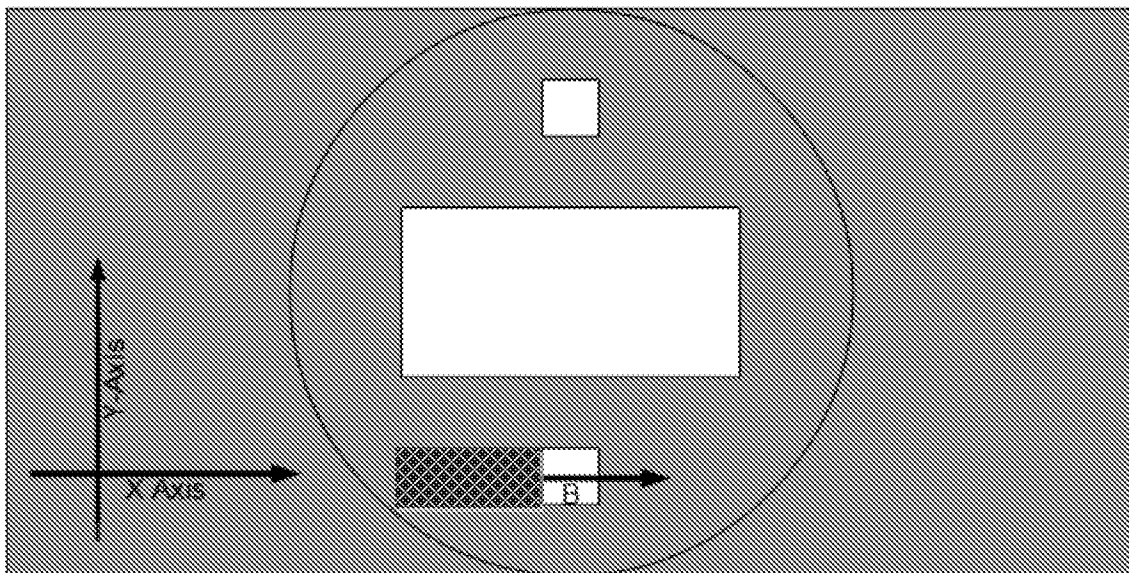


FIG. 3 – SCANNING CONFIGURATIONS FOR DETERMINING THE POSITION OF THE SQUARE WINDOW

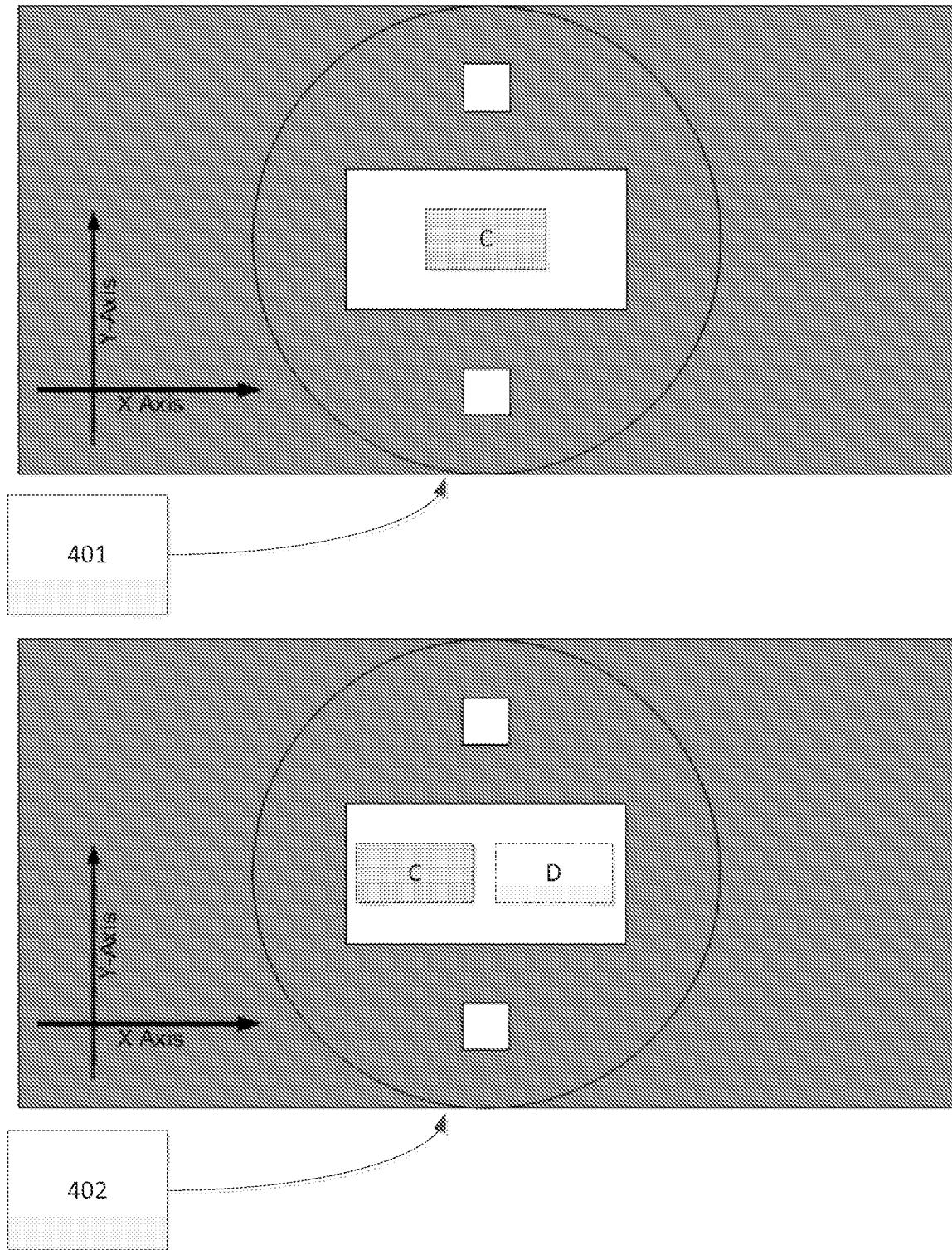


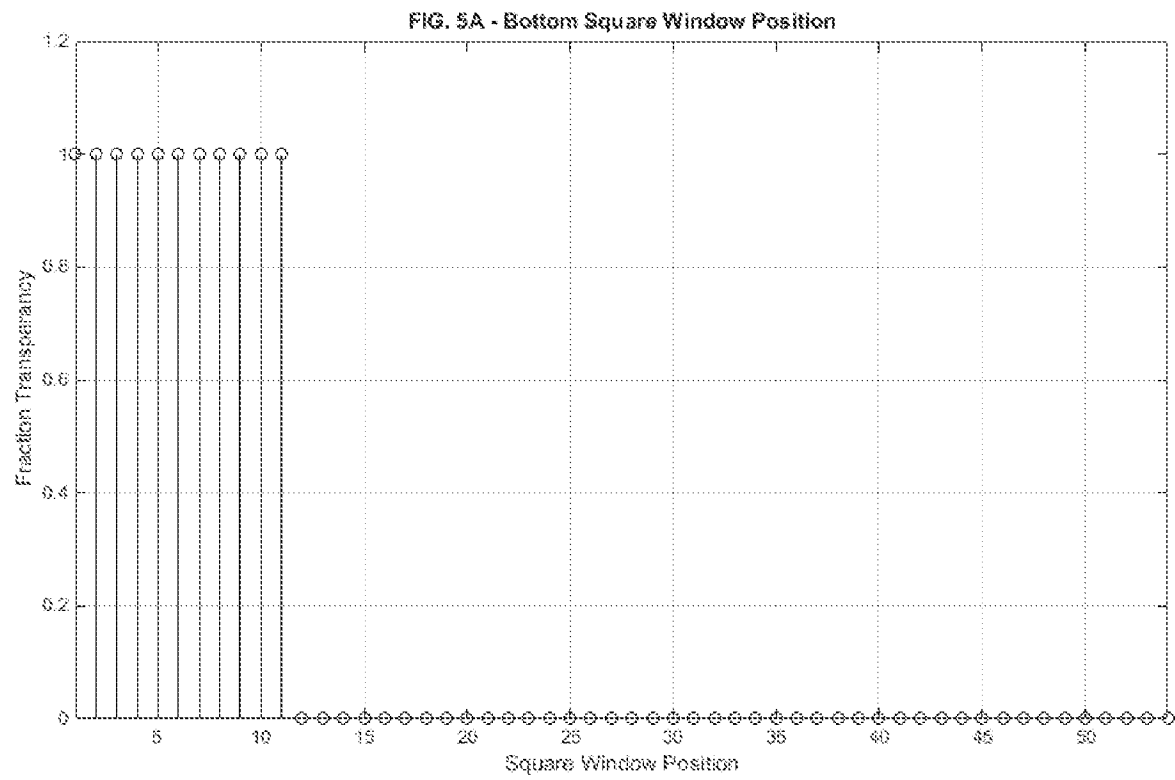
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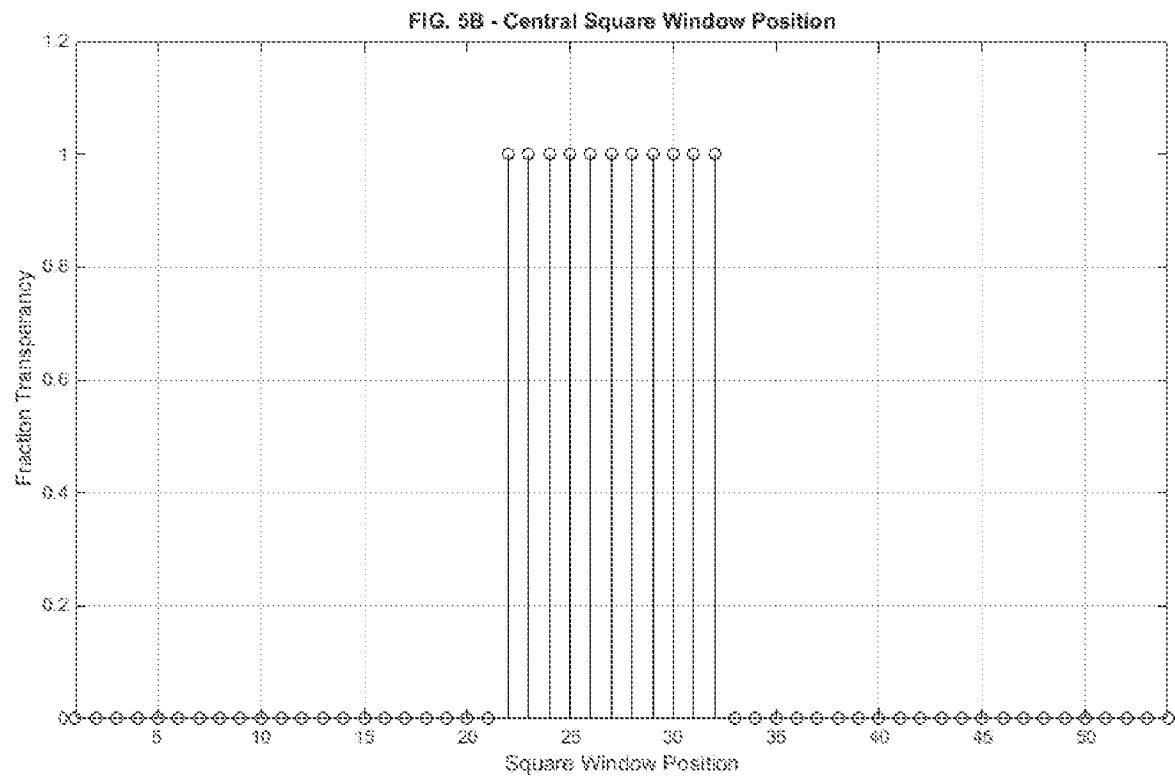


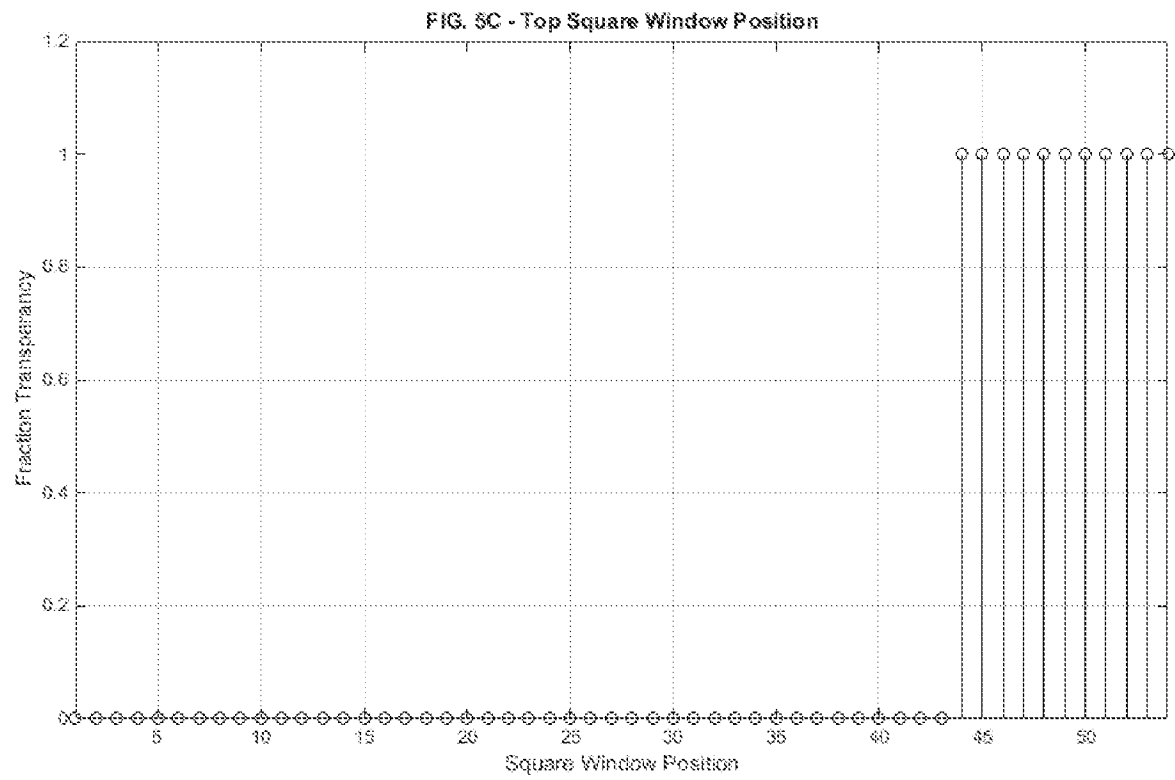
302

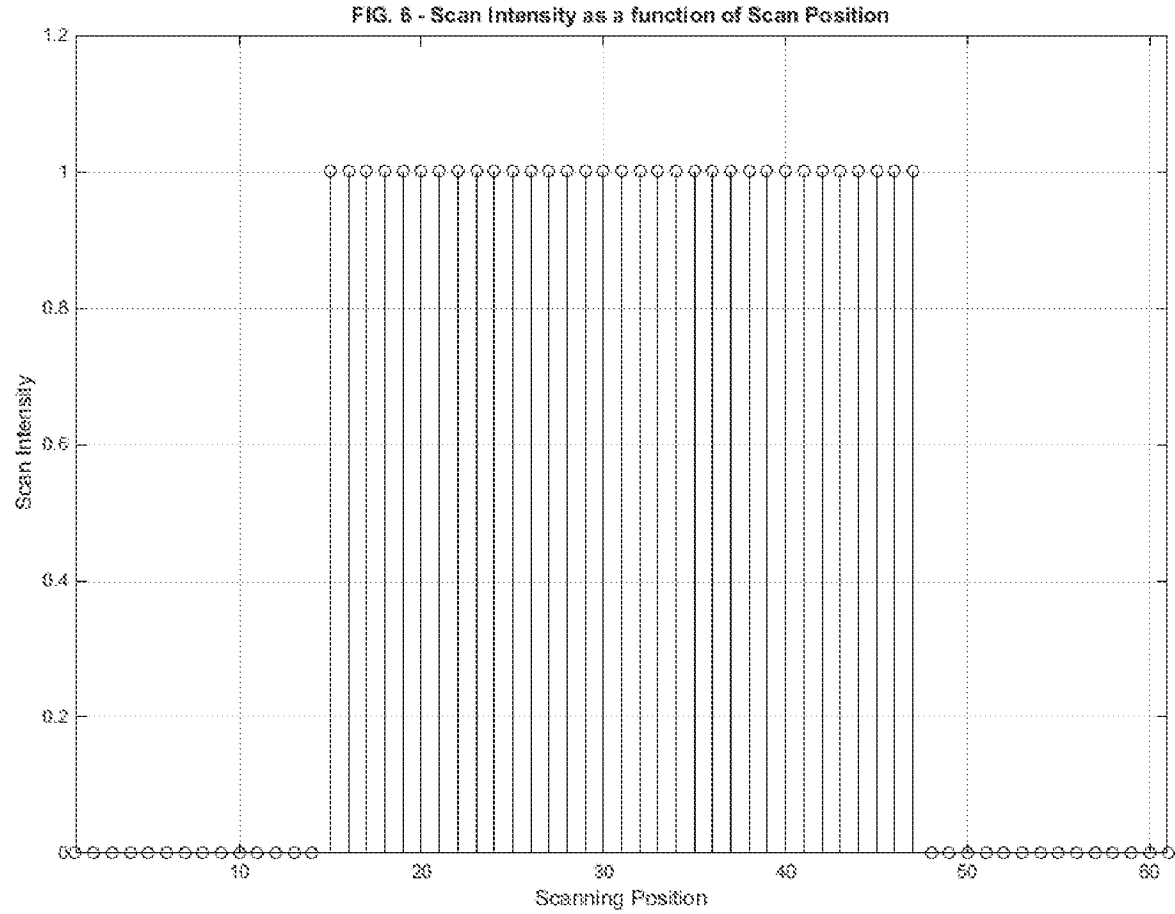
FIG. 4 – PRIMARY AND ALTERNATIVE SCANNING SITES

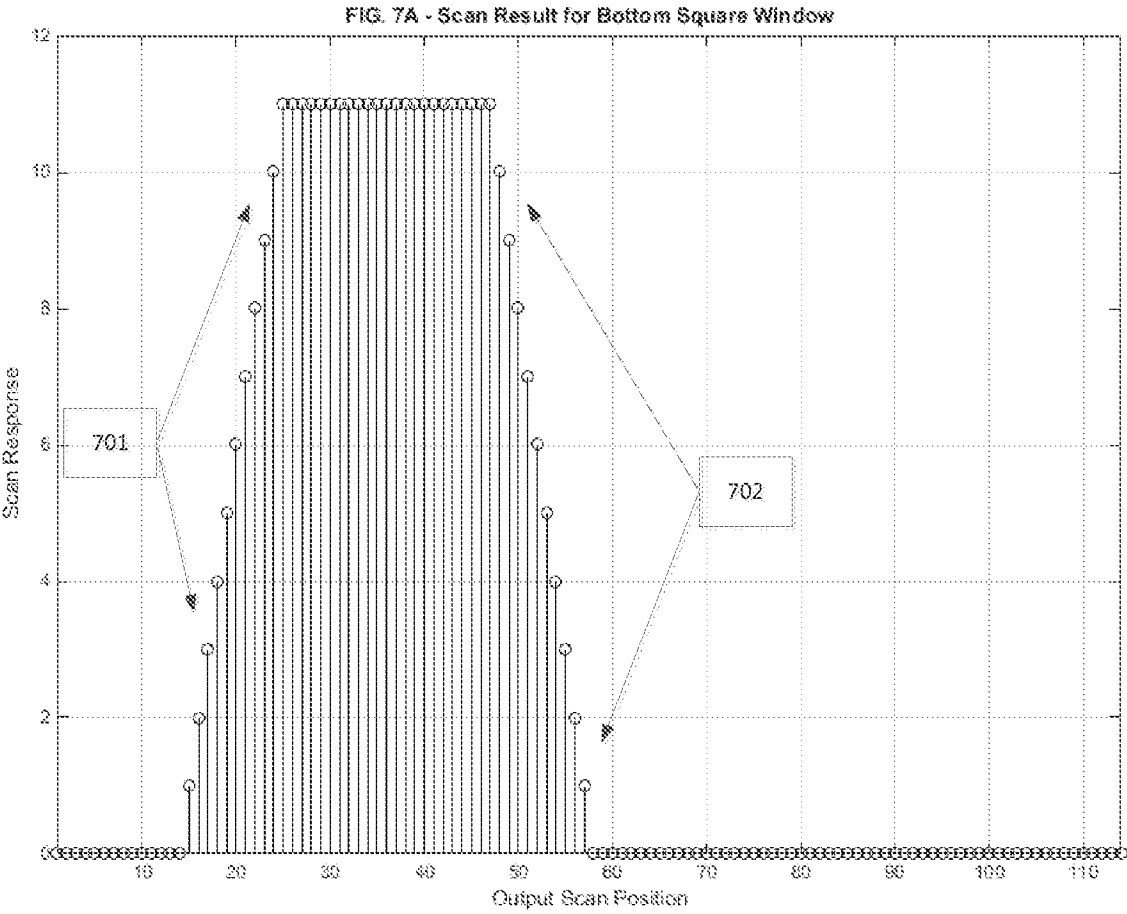


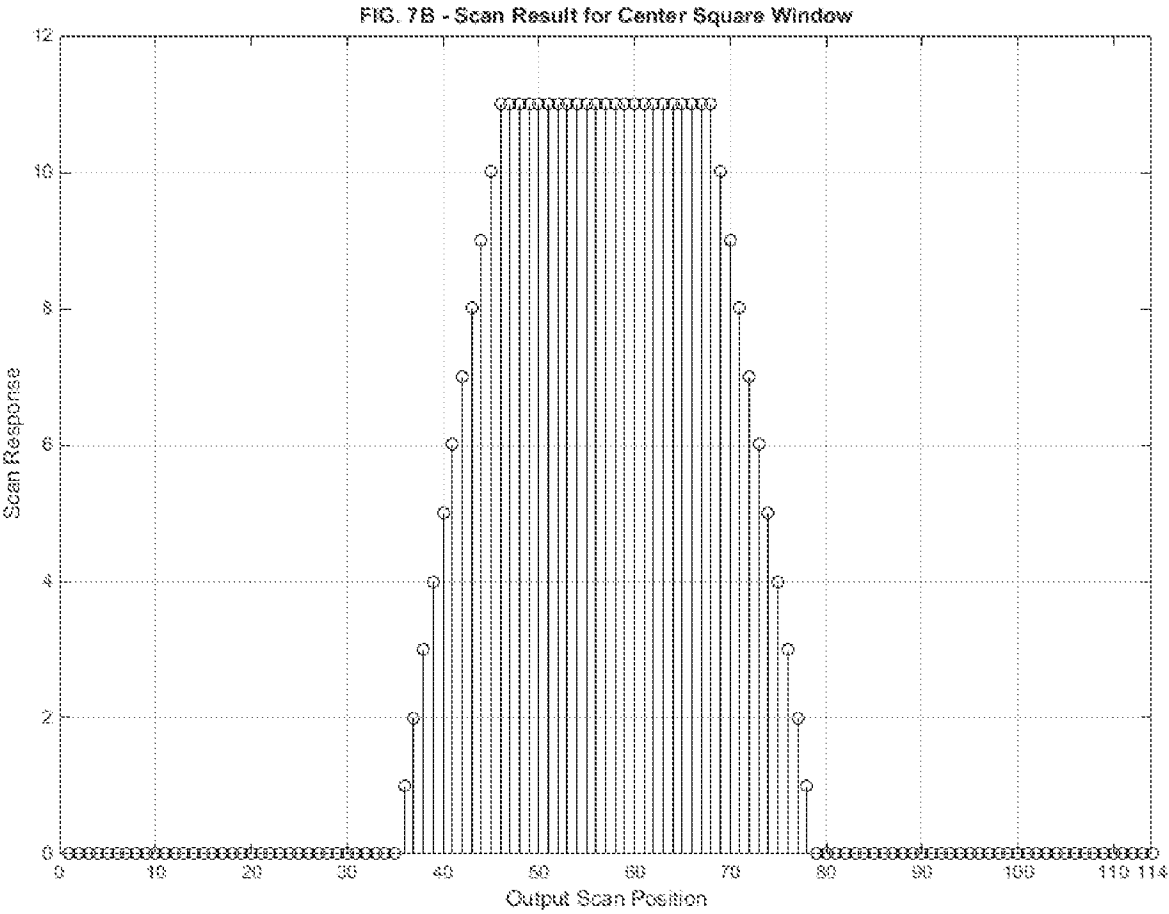


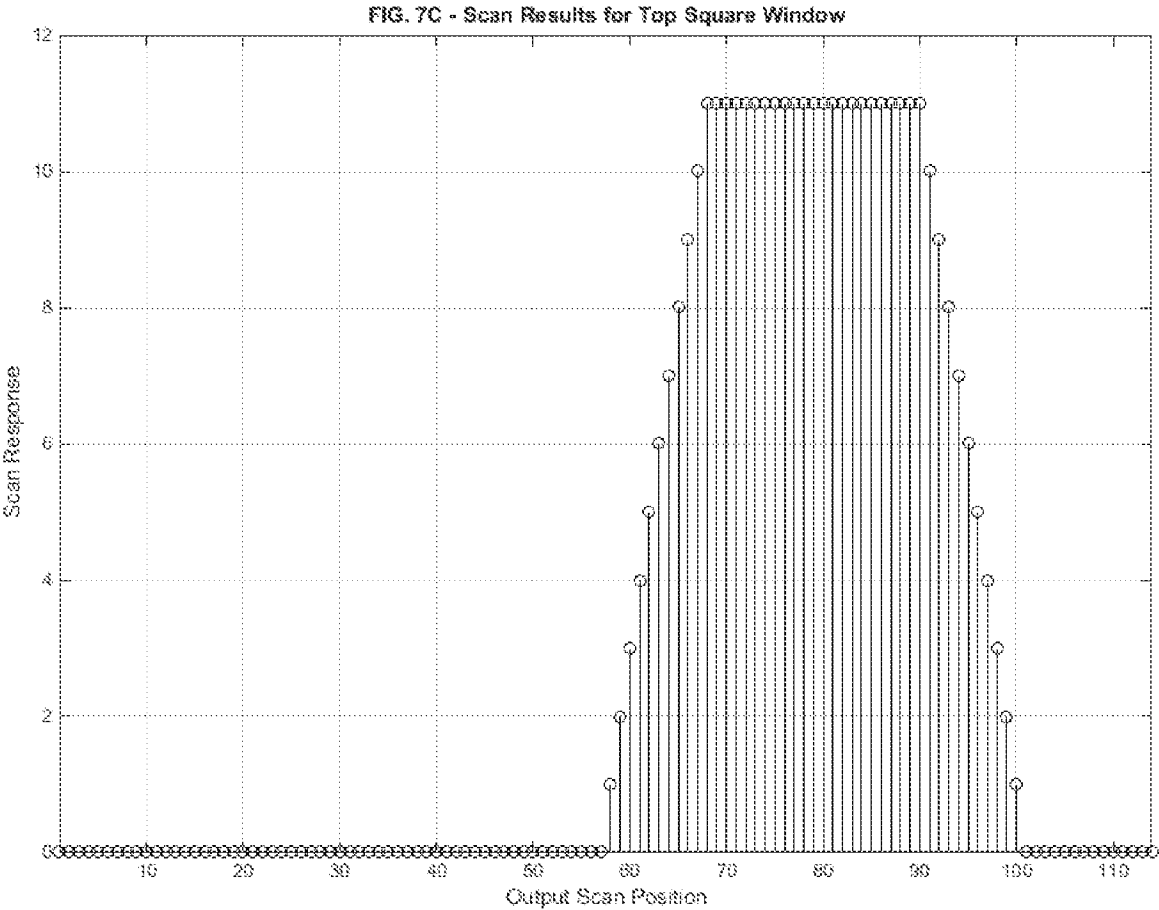












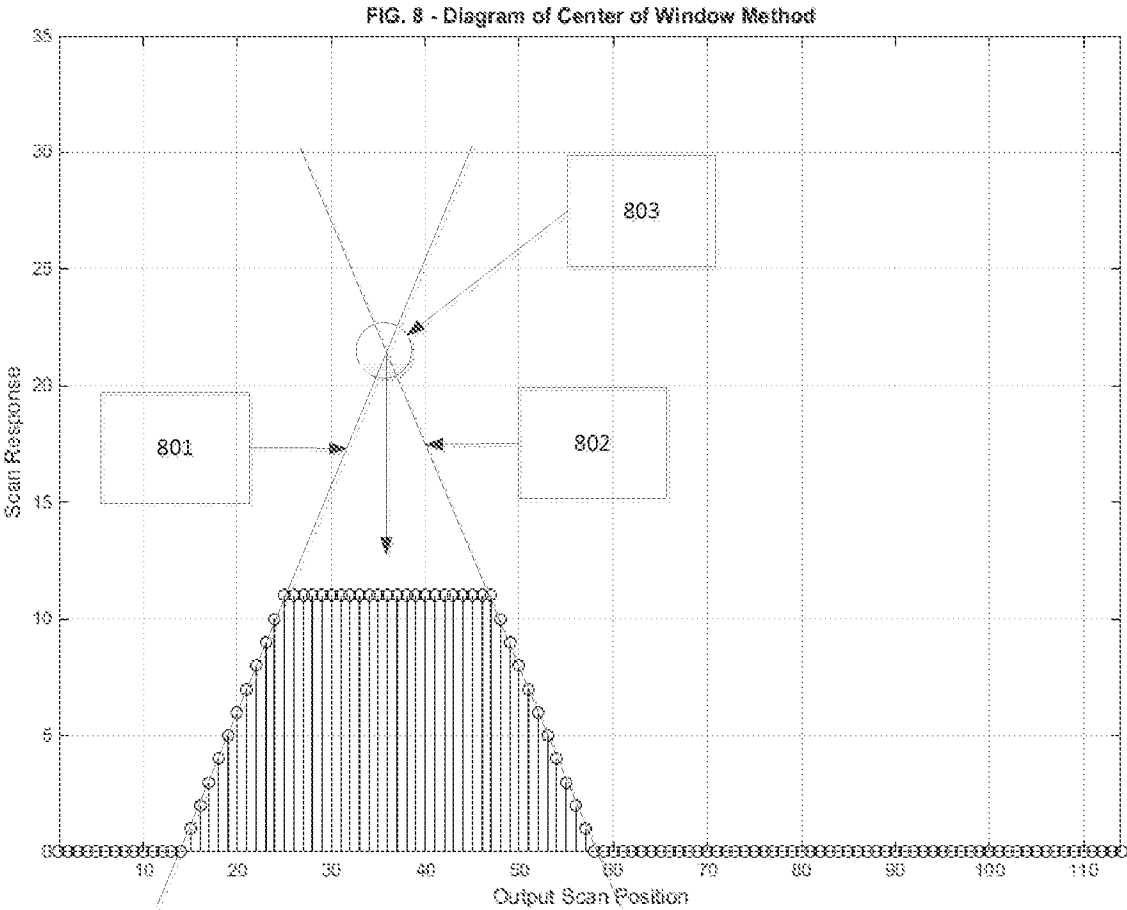


FIG. 9A – Minitab Outputs for Data of FIG. 7A

Session

Minitab 15 - Regression Analysis: C1 versus C2

Analysis of Variance (ascending data for FIG. 7A)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	143.000	143.000	*	*
C2	1	143.000	143.000	*	*
Error	10	0.000	0.000		
Total	11	143.000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-14.00	0.00	*	*	
C2	1.000	0.000	*	*	1.00

Regression Equation

$$C1 = -14.00 + 1.000 C2$$

#	C1	C2	C3	C4	C5
1	0	14			
2	1	15			
3	2	16			
4	3	17			
5	4	18			
6	5	19			
7	6	20			
8	7	21			
9	8	22			
10	9	23			
11	10	24			
12	11	25			

Session

Minitab 15 - Regression Analysis: C1 versus C2

Analysis of Variance (Descending data for FIG. 7A)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	143.000	143.000	*	*
C2	1	143.000	143.000	*	*
Error	10	0.000	0.000		
Total	11	143.000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	58.00	0.00	*	*	
C2	-1.000	0.000	*	*	1.00

Regression Equation

C1 = 58.00 - 1.000 C2

#	C1	C2	C3	C4	C5
1	11	47			
2	10	48			
3	9	49			
4	8	50			
5	7	51			
6	6	52			
7	5	53			
8	4	54			
9	3	55			
10	2	56			
11	1	57			
12	0	58			

FIG. 9B – Minitab Outputs for Data of FIG. 7B

Session

Minitab 15 - Regression Analysis: C1 versus C2

Analysis of Variance (Ascending data for FIG. 7B)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	143.000	143.000	*	*
C2	1	143.000	143.000	*	*
Error	10	0.000	0.000		
Total	11	143.000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-35.00	0.00	*	*	
C2	1.000	0.000	*	*	1.00

Regression Equation

$$C1 = -35.00 + 1.000 C2$$

Worksheet1 ***

#	C1	C2	C3	C4	C5
1	0	35			
2	1	36			
3	2	37			
4	3	38			
5	4	39			
6	5	40			
7	6	41			
8	7	42			
9	8	43			
10	9	44			
11	10	45			
12	11	46			

Session

Minitab 15 - Regression Analysis: C1 versus C2

Analysis of Variance (Descending data for FIG. 7B)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	143.000	143.000	*	*
C2	1	143.000	143.000	*	*
Error	10	0.000	0.000		
Total	11	143.000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	79.00	0.00	*	*	
C2	-1.000	0.000	*	*	1.00

Regression Equation

$$C1 = 79.00 - 1.000 C2$$

Worksheet1 ***

#	C1	C2	C3	C4	C5
1	11	68			
2	10	69			
3	9	70			
4	8	71			
5	7	72			
6	6	73			
7	5	74			
8	4	75			
9	3	76			
10	2	77			
11	1	78			
12	0	79			

FIG. 9C – Minitab Outputs for Data of FIG. 7C

Session

Minitab 15 - Regression Analysis: C1 versus C2

Analysis of Variance (Ascending data for FIG. 7C)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	143.000	143.000	*	*
C2	1	143.000	143.000	*	*
Error	10	0.000	0.000		
Total	11	143.000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-87.00	0.00	*	*	
C2	1.000	0.000	*	*	1.00

Regression Equation

C1 = -87.00 + 1.000 C2

Worksheet1

	C1	C2	C3	C4	C5
1	0	57			
2	1	58			
3	2	59			
4	3	60			
5	4	61			
6	5	62			
7	6	63			
8	7	64			
9	8	65			
10	9	66			
11	10	67			
12	11	68			

Session

Minitab 15 - Regression Analysis: C1 versus C2

Analysis of Variance (Decending data for FIG. 7C)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	143.000	143.000	*	*
C2	1	143.000	143.000	*	*
Error	10	0.000	0.000		
Total	11	143.000			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	101.0	0.0	*	*	
C2	-1.000	0.000	*	*	1.00

Regression Equation

$$C1 = 101.0 - 1.000 C2$$

Worksheet1

	C1	C2	C3	C4	C5
1	11	90			
2	10	91			
3	9	92			
4	8	93			
5	7	94			
6	6	95			
7	5	96			
8	4	97			
9	3	98			
10	2	99			
11	1	100			
12	0	101			

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/050608

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 21/01 (2015.01)

CPC - G01N 21/278 (2015.10)

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(8) - G01J 1/10; G01N 21/01, 33/36 (2015.01)

USPC - 356/243.1, 243.2, 243.4, 243.6, 243.8, 246

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

CPC - G01N 21/274, 21/278, 21/4785 (2015.10) (keyword delimited)

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

Orbit, Google Patents, ProQuest

Search terms used: fluorescent, cover glass, excitation, photometric, borosilicate crown glass, moisture barrier, calibration, normalizing, fluorescence standard

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0159624 A1 (RESCH-GENGER et al) 12 July 2007 (12.07.2007) entire document	1-4, 6, 12
Y		5, 7-11, 13
Y	US 2008/0056951 A1 (ANGROS) 06 March 2008 (06.03.2008) entire document	5
Y	US 2009/0116809 A1 (DIANOV et al) 07 May 2009 (07.05.2009) entire document	7-11
Y	US 2005/0287040 A1 (GIEBELER et al) 29 December 2005 (29.12.2005) entire document	11
Y	US 2003/0173525 A1 (SEVILLE) 18 September 2003 (18.09.2003) entire document	13
A	US 3,901,588 A (LONGHENRY) 26 August 1975 (26.08.1975) entire document	1-13
A	US 2007/0212793 A1 (DEJNEKA et al) 13 September 2007 (13.09.2007) entire document	1-13

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

☐ See patent family annex.

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"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

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Date of the actual completion of the international search

02 November 2015

Date of mailing of the international search report

20 JAN 2016

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