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(54) Title: METHOD AND SYSTEM FOR ANALYZING A COLORIMETRIC ASSAY

(57) Abstract: Described herein is a method, system and computer program for analyzing a colorimetric assay that includes obtaining an image of the assay, optionally correcting for ambient lighting conditions in the image, converting the intensity data for at least one of the red channel, the green channel, or the blue channel to a first data point, recalling a predetermined standardized curve, comparing the first data point with the standardized curve, and identifying the value for the assay parameter from the standardized curve.

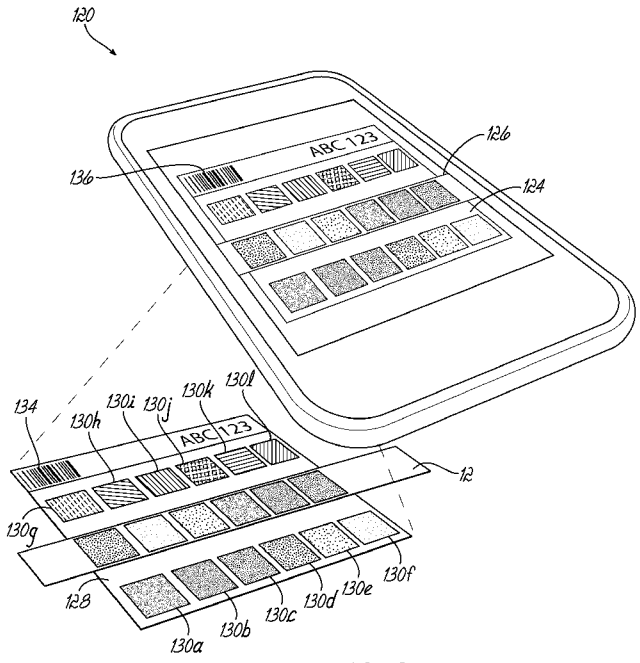


FIG. 2

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METHOD AND SYSTEM FOR ANALYZING A COLORIMETRIC ASSAY

RELATED APPLICATION

The Present application claims priority to U.S. Serial No. 61/594,683 filed February 3, 2012, the disclosure of which is hereby incorporated herein by reference in its entirety.

FIELD

The present invention is directed to colorimetric diagnostic assays and, more particularly, to processes for analyzing colorimetric diagnostic assays.

BACKGROUND

Paper microfluidic analytical devices have emerged in recent years, leading to development of a number of inexpensive and quick point-of-care ("POC") analyses, including HIV chips, paper ELISA, and other low-cost colorimetric diagnostic assays. Such paper microfluidic assays are gaining popularity as a simple and fast way of disease screening in resource limited environments. Although the colorimetric results of these assays can be viewed by naked eye, it is difficult to precisely quantify the analyte amount. Promising colorimetric detection results have been demonstrated using video cameras, digital color analyzers, scanners or custom portable readers. A key drawback of all these methods is the need for specialized instrumentation and for image analysis with a computer.

Mobile devices with wireless connectivity to remote computer systems, such as smart phones and tablets, offer attractive alternatives for imaging, analysis, and communication of results in the field. For example, with 6 billion mobile phone subscriptions worldwide, mobile phones are becoming ubiquitous. Indeed, several investigators have already demonstrated the use of phones in mobile phones for on-site diagnosis in dermatology, ophthalmology, and colorimetric diagnostics. However, mobile phones have yet to gain popularity for colorimetric detection due to three key challenges. First, integrated color balancing functions of a conventional mobile phone are optimized for photography in high ambient light, and are not suited for images when accurate quantitative measurements must be performed. Second, lighting conditions during imaging can be difficult to control, especially outside of a controlled environment like a laboratory. Third, analysis of images can be challenging especially

when small color changes are present, and red, green, blue intensity ("RGB") values alone are not necessarily sufficient. For these reasons, the use of cameras on mobile devices such as mobile phones and tablets has not yet been fully exploited for POC analyses.

SUMMARY

Described herein are methods, systems, and devices for analyzing colorimetric assays, such as those based on paper test strips. The methods, systems, and devices may be utilized with mobile devices such as a cell phone, a tablet, and portable computer. Moreover, the method may also be utilized with less mobile computer systems, such as a desk top system.

An embodiment of the invention is directed to a method of analyzing a colorimetric assay that includes obtaining an image of the assay, optionally correcting for ambient lighting conditions in the image, converting the RGB intensity data to a first data point, recalling a predetermined standardized curve, comparing the first data point with the standardized curve, and identifying the value for the assay parameter from the standardized curve.

The method can also compensate for differences in lighting conditions when taking images under uncontrolled lighting conditions, such as outside of a laboratory settin. The challenges of using a mobile devices as a portable photodetector can all be addressed, as demonstrated herein using two model paper assays – pH paper and urine glucose test strip. Thus, the methods herein allow for the use of mobile devices as a viable tool for quantitative POC analysis.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate various embodiments of the invention and, together with a general description of the invention given above and the detailed description of the embodiments given below, serve to explain the embodiments of the invention.

FIG. 1 is a flow chart illustrating the method of analyzing a colorimetric assay in accordance with embodiments of the invention.

FIG. 2 is a perspective view of a device and system for analyzing a colorimetric assay in accordance with embodiments of the invention.

FIG. 3 is a graph illustrating the insufficiency of RGB data in analyzing a colorimetric assay.

FIG. 4 is a flow chart illustrating the method of compensating for light conditions when analyzing a colorimetric assay in accordance with embodiments of the invention.

FIG. 5 is a block diagram of a processing system in accordance with embodiments of the invention.

FIG. 6A is a grey scale image for an imaging sensor sensitivity test.

FIG. 6B is a three dimensional surface intensity plot of the grey scale image of FIG. 6A.

FIG. 6C is a graph illustrating the grey scale sensitivity of multiple imaging sensors.

FIG. 7A is a CIE-1931 color map with pH values mapped to the color space in accordance with embodiments of the invention.

FIG. 7B is a three dimensional plot of the color map from FIG. 7A in accordance with embodiments of the invention.

FIG. 7C is a graph demonstrating the correlation between the mapped values from FIG. 7A and the actual pH values from the measured samples.

FIG. 8A is a CIE-1931 color map with glucose values from a urine glucose strip mapped to the color space in accordance with embodiments of the invention.

FIG. 8B is a three dimensional plot of the color map from FIG. 8A in accordance with embodiments of the invention.

FIG. 8C is a graph demonstrating the correlation between the mapped values from FIG. 8A and the actual values from the measured samples.

FIG. 9A is a plot demonstrating the linearity between illumination under a standard lighting condition of a 5000k fluorescent light and an ambient condition of a 3500k fluorescent light in accordance with embodiments of the invention.

FIG. 9B is a plot demonstrating the linearity between illumination under a standard lighting condition of a 5000k fluorescent light and an ambient condition of sunshine in accordance with embodiments of the invention.

FIG. 9C is a plot demonstrating the linearity between illumination under a standard lighting condition of a 5000k fluorescent light and an ambient condition of shade in accordance with embodiments of the invention.

FIG. 9D is a plot demonstrating the linearity between illumination under a standard lighting condition of a 5000k fluorescent light and an ambient condition of mobile phone LED in accordance with embodiments of the invention.

FIG. 10 is a color space map demonstrating a shift of measurements taken under an ambient condition when calibrated against the standard light condition in accordance with embodiments of the invention.

DETAILED DESCRIPTION

With reference to FIG. 1, and in accordance with embodiments of the invention, an improved method 10 of analyzing a colorimetric assay 12 (FIG. 2) to identify the value for an assay parameter (referred to herein as a test assay) that includes obtaining an image of the assay (block 100), optionally stabilizing the automated color correcting functions of the device used to obtain the image (block 102), correcting for ambient lighting conditions in the image (block 104), converting the RGB intensity data to a first data point (block 106), recalling a predetermined standardized curve (block 108), comparing the first data point with the standardized curve (block 110), and identifying the value for the assay parameter from the standardized curve (block 112).

The image may be obtained (block 100) using a device capable of generating a digital image of the test assay. The device includes an electronic sensor capable of capturing the blue, green, and red intensities of the image. Exemplary imaging sensors include charge-coupled device ("CCD") sensors, a complementary metal-oxide-semiconductor ("CMOS") sensor, and a contact image sensor ("CIS").

In an exemplary embodiment, the electronic sensor is included in a device such as a mobile phone, a tablet, a portable computer, a computer, and a scanner. Advantageously, the device is a portable device that also includes processors capable of

performing at some of the steps of the methods describes, with preferred portable devices including mobile phone, a tablet, and a portable computer. Other similarly portable devices with similar processing may also be utilized such as devices dedicated to assay analysis. FIG. 2 illustrates an exemplary embodiment wherein the electronic sensor is included in a mobile phone, such as a smart phone 120.

Many mobile phone cameras, such as most smart phone cameras use CMOS arrays, which are low-cost and integrate a range of automated functions, such as *Auto White Balance (AWB),* designed to provide good color reproduction by adjusting the detected RGB signals at different ratios. The resulting images are brighter and more pleasing to the eyes, making the automatic function popular with non-professional photographers. However, changes in RGB values may skew measurements when attempting to use mobile phone cameras for quantitative measurements. A solution to this challenge is to fully control all camera functions to preserve consistency through the tests. However, this approach may not applicable to mobile phones since these functions are not generally accessible in a fully integrated mobile phone camera. Embodiments of the methods described herein allow for the use of a fully integrated imaging sensor, such as a CMOS sensor or CCD sensor, to accurately analyze a colorimetric assay.

An exemplary embodiment of the method includes optionally obtaining an image of a reference chart 124 simultaneous with obtaining an image of the test assay 126 (FIG. 2). An exemplary reference chart 128 includes 12 reference areas 130a-130l with known color intensities to stabilize the automated color correcting functions of a fully integrated imaging sensor. The exemplary reference chart 128 is also useful in compensating ambient light conditions in the subsequent data processing, as disclosed later. The reference chart 128 is similar to color-rendition charts utilized in photography, in which 24 reference areas are included. The present chart contains seven grayscale regions (130a-130g), and five color regions (130h-130l) that range from short wavelength (blue, 130h) to longer wavelengths (green 130i, yellow 130j, orange 130k, and red 130l). The present reference chart 128 nearly eliminates effects of automatic camera functions, making images reproducible and quantifiable. Skewing the reference chart in the direction of warm colors by including yellow, orange, and red regions overcomes the tendency of AWB to decrease gain in the *red* channel of the CMOS imaging array, since silicon exhibits better responsiveness at these longer wavelengths. The reduced complexity of the present reference chart 128 compared to

the more complex color-rendition charts utilized in photography permit simpler and faster analysis, without sacrificing the quality of the image. Moreover, as disclosed later, the 12-region reference chart is sufficient to build a conversion curve to compensate for differences in ambient light.

The reference chart 128 may be a separate sheet or may be printed directly onto the test assay 12. The reference areas 130a-130l may be arranged in any order on the sheet, such as in one or more rows or in a circular arrangement.

The test assay 12, the reference chart 128, or the combination of the test assay 12 and the reference chart 128 may further include a label portion 134 that includes a machine readable code (illustrated as a barcode) and/or an identifier (illustrated as an alpha numeric code), which may be used for identifying an assay, a protocol, a date, a predetermined standard, and so forth. An image 136 of the machine readable code may be used by the processing system 202 (FIG. 5) to identify and access such information, which may be stored locally on the storage device 212, or retrieved from a resource connected to the processing system 202 via a network. For example, the information may be retrieved from a server.

While the reference chart assists with accurate colorimetric imaging, the images of the test assay 126 must still be analyzed. The approach of directly converting RGB intensity-values obtained from the image into the corresponding analyte concentrations does not yield useful data. For example, as demonstrated in FIG. 3, a clear illustration of this can be observed from imaging pH paper, which turns colors from red to dark blue according to pH value of the test solution. The RGB values of images for pH increasing from 1 to 12 plotted in Fig. 3 illustrate that RGB intensities do not exhibit any discernible trend and are difficult to correlate with test solution pH values. Prior methods have attempted to address this inadequacy of the direct RGB measurement by using hue values, taking a ratio between red and green channels, or using subtractive CMYK and hue-based HSL values. However, these methods are not sensitive to dark colors and are application specific. The present method overcomes these problems.

Embodiments of the present method convert the intensity data from at least one of the red channel, the green channel, or the blue channel ("RGB") from at least a portion of the image of the first colorimetric assay to a data point having a first value and a second value that indicate the color of the test colorimetric assay (FIG. 1, block 106). In one

embodiment, the RGB intensity data is converted to a data point using the CIE color space to code the colorimetric image to overcome inadequacies of the simple RGB analysis. The CIE system is the most recognized method in which color is represented, with tristimulus values *X*, *Y*, and *Z* characterizing the emission color of luminescence data across the wavelength range of visible light. Preferably, the CIE 1931 *xyY* system is used, in which the *Y* parameter represents luminance (brightness) of a color and derived parameters *x* and *y* determine the chromaticity of a color. Conversion of the obtained image data into the CIE 1931 *xyY* color space involves three steps, in which the color space terms are derived from the conventional RGB values. In devices that utilize the sRGB color standard, such as digital cameras, cameras in mobile devices such as mobile phones, tablets, and portable computers, the nonlinear sRGB values are converted to linear RGB values using

$$C_{linear} = \left(\frac{C_{srgb}+0.055}{1.055}\right)^{2.4} \quad (1)$$

in which Csrgb stands for Rsrgb, Gsrgb, and Bsrgb, and Clinear indicates Rlinear, Glinear, and Blinear. Then, the linear RGB values can be converted to trisimulus values *X*, *Y*, and *Z* using

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = \begin{bmatrix} 0.4124 & 0.3576 & 0.1805 \\ 0.2126 & 0.7152 & 0.0722 \\ 0.0193 & 0.1192 & 0.9505 \end{bmatrix} \begin{bmatrix} R_{linear} \\ G_{linear} \\ B_{linear} \end{bmatrix} \quad (2)$$

Finally, the chromaticity-values *x* and *y* are obtained by

$$\begin{cases} x = \frac{X}{X+Y+Z} \\ y = \frac{Y}{X+Y+Z} \end{cases} \quad (3)$$

The new color space specified by *x*, *y* and *Y* is represented in a 2-D diagram — the *Horse Shoe shaped* Chromaticity diagram. The pure colors are located on the boundary curve from blue (380nm) to red (700nm), while all the mixed colors, such as yellow and pink, are represented within the area enclosed by the curve. The position of a point in the diagram indicates the chromaticity of the corresponding color. In practice, the first and second values of the data point from the colorimetric assay correspond with the x and y coordinates of the image as plotted on the *xy* chromaticity diagram.

The *xy* chromaticity diagram of the CIE 1931 system can be used to predict the outcome of a mixture of two colors. The mixed color lies along the straight line connecting the two points of the original colors on the *xy* chromaticity diagram. The ratio of the two original colors determines the position of the mixed color. This can be potentially useful in more complicated colorimetric assays. Notably, the hue and saturation of a color, based on which the widely used HSV and HSL models were defined, can be derived from its location on the *xy* diagram. Considering these assets of the CIE 1931 *xyY* color space, the present method to analyzing colorimetric assays to quantify the colors is versatile and works well as demonstrated in the Examples discussed below.

The first data point is compared with a predetermined three-dimensional standardized curve. The standardized curve includes a plurality of data points wherein each data point has an x-value, a y-value, and z-value. The x- and y-values correspond with the x and y-values of the xy chromaticity diagram as disclosed above. The z-value corresponds with a predetermined assay-value, such as, for example, an analyte concentration. An exemplary predetermined standardized curves is generated with analytes over a range of known concentrations by obtaining images of a plurality of colorimetric assays conducted with known analyte concentrations, converting the RGB intensity data for each known analyte concentration to a data point having an x-value and a y-value indicative of the chromaticity of the data point and plotting the x-value and the y-value along with the z-value, indicative of the analyte concentration, on a three dimensional curve. The standardized curve includes not only the data points obtained from the assays conducted with known analyte concentrations but also the data points along the curve connecting the data points from the known analyte concentration assays. An exemplary standardized curve is illustrated in FIGS. 5B and 6B, discussed below with reference to the Examples. The standardized curve may be prepared in advance and stored in a storage medium, such as the device's RAM and

writable media, from which it is recalled for use in analyzing a test assay (FIG. 1, block 108).

The test assay parameter, such as analyte concentration, is identified by comparing the data point from the test assay with the standardized curve (FIG. 1, blocks 110 and 112). In an embodiment, the test assay parameter is the z-value of a data point along the standardized curve having an x-value and a y-value that most closely matches the x-value and y-value from the data point obtained from the test assay. One method of identifying the data point along the standardized curve that most closely matches the data point from the test assay is to calculate the absolute difference between the data point from the test assay and the data points making up the standardized curve. For example, the difference can be calculated between the x-values and the y-values for the test assay and the standardized curve. The absolute values of the x-value difference and the y-value difference can be summed to identify the absolute difference between the data points. The data point along the standardized curve that most closely matches the data point from the test assay will be the standardized curve data point with the smallest absolute difference from the test assay data point. Other methods for identifying the data point along the standardized curve that most closely matches the test assay data point could also be used.

With reference back to FIG. 1, in some embodiments, the method includes a step for compensating for ambient light conditions (block 104). Variations in ambient light conditions are determined by the position of the light source, light temperature, or outdoor lighting environment. An object absorbs light in a specific wavelength range while reflecting the rest. The CMOS pixel used in many portable devices measures intensity of the reflected light based on many factors, such as the ambient light wavelength, the reflection, the color of the object and the RGB responsivity of the CMOS pixels. A practical way is to treat all these factors as a black box and build a mapping algorithm based on the measured RGB intensities of the references.

With reference to FIG. 4, ambient light compensation includes obtaining an image of a reference chart, such as reference chart 128 (FIG. 2), under the same lighting conditions in which the image of the test assay is obtained (FIG. 4, block 140). For example, the image of the reference chart 128 can be obtained simultaneously with the image of the test assay. The reference chart 128 includes at least one reference area 130a-130l (FIG.

2). In an embodiment, reference charts includes a plurality of areas spanning a range of intensity in both grey scale and color. The intensity of the at least one reference area is measured (block 142). The measured intensity of the at least one reference area is compared with a predetermined intensity of the reference area as measured under a predetermined illumination condition to calculate the ambient light correction required for the test assay data point. The measured intensities of the reference areas a reference chart have a linear relationship between different ambient light conditions. In embodiments measuring the intensity of a plurality of reference areas, an ambient lighting correction curve is plotted with one axis being the measured intensity for each reference area and the other axis being the predetermined intensity for each reference area. The formula defining the resulting curve is then utilized to correct the test assay data point. In an embodiment, the RGB intensity values from the image of the test assay are corrected before being converted to an x-value and a y-value. In another embodiment, the x-value and y-value correction is calculated after conversion.

In some circumstances, the obtained image of the test assay may include areas having different ambient lighting conditions, such as a portion of the image may be in a shadow or exposed to brighter lighting conditions. Embodiments of the invention may recognize and account for changes in lighting conditions across the image. For example, if the measure intensity of one or more reference areas does not have a linear relationship with the remainder of the reference areas, then this would indicate that those references areas are exposed to different ambient lighting conditions. As such, the method could prompt the user to reacquire the image under better lighting conditions or if sufficient data from the reference areas is present, the method could correct the data point associated with the different ambient light condition based on a correction from the nearest reference area. In the alternative. the method could exclude areas wherein the ambient light condition is identified as being different from ambient light condition of the remainder of the image of the test assay.

The methods and systems described herein may be useful for analyzing various types of colorimetric assays so long as the assay results in a colorimetric change that can be detected by the methods described herein. Exemplary assays include pH assays, glucose concentration assays, paper ELISA, and assays that detect proteins, nucleic acids, antibodies, or microorganisms, as well as any assay that can detect the exemplary assay parameter disclosed below. Exemplary assay parameters that may be quantified

include an analyte concentration, analyte presence, and analyte activity, such as enzyme activity. Specific exemplary analytes include a hydroxide ion, a hydrogen ion, a carbohydrate, a ketone, an alcohol, a lipid, a peptide, a protein, a nucleic acid, an amino acid, an antibody, a nitrite, a drug, a drug metabolite, a cell, a virus, a metal, such as a nanoparticle of a metal like a gold or silver nanoparticle, a salt, a contaminant, and combinations thereof.

Embodiments of the invention include a program code 200 that includes instructions executable on a processor system 202, such as a mobile phone, a tablet, a portable computer, or computer system, for carrying out the steps of the method. In one embodiment, the program code 200 includes instructions for analyzing a colorimetric assay. Embodiments of the invention, whether implemented as part of an operating system 204, application, component, program code 200, object, module or sequence of instructions executed by one or more processing units 206 are referred to herein as "program code." The program code 200 typically comprises one or more instructions that are resident at various times in various memory 208 and storage devices 212 in the processor system 202 that, when read and executed by one or more processors 206 thereof cause that processor system 202 to perform the steps necessary to execute the instructions embodied in the program code 200 embodying the various aspects of the invention.

While embodiments of the invention are described in the context of fully functioning processing systems 202, those skilled in the art will appreciate that the various embodiments of the invention are capable of being distributed as a program product on a computer readable storage medium. The program product may embody a variety of forms. The invention applies equally regardless of the particular type of computer readable storage medium used to actually carry out the distribution of the program code 200. Examples of appropriate computer readable storage media for the program product include, but are not limited to, non-transitory recordable type media such as volatile and nonvolatile memory devices, floppy and other removable disks, hard disk drives, USB drives, optical disks (e.g. CD-ROM's, DVD's, Blu-Ray discs, etc.), among others.

Any of the individual processes described above or illustrated in the figures may be formed into routines, procedures, methods, modules, objects, and the like, as is well

known in the art. It should be appreciated that embodiments of the invention are not limited to the specific organization and allocation of program functionality described herein.

In addition, the systems for analyzing the test assays may further include a module for generating an image of the test assay (i.e. a image generator) 210 and a module for receiving the image 214. The image generator may include a device having an imaging sensor such as a CCD sensor, a CMOS sensor, and a CIS. Image generators as known in the art may be used in accordance with the invention. The image receiving module includes components and/or program code to receive an image from the image generator module.

EXAMPLE 1

A smart phone was utilized to examine its suitability as a photodetector and to compare its light sensitivity with other more expensive detectors. The smart phone utilized for this example was the Casio G'zone Commando smartphone, which runs the Android operating system and is ruggedized to military standard MIL-STD-810G. Thus, this phone is stronger and more durable than normal consumer electronics, and is able to handle drops, spills and dirt that may accompany activities such as those in harsh work environments or outdoors. Images of ten grey scale standards were obtained (Labsphere) and the intensities in each image were compared against the intensities obtained with other devices (intensity I = 0 is black while the maximum value I = 255 for the Commando's8-bitcolor depth is white). A flatbed desktop scanner (HP Scanjet N6310, 12-bit color depth) and an SLR camera (Canon EOS T3i, 22-bit color depth) were used for sensitivity comparison. FIG. 6A, 6B, and 6C, illustrates the mean intensities of the grey scale regions in each image, with all imaging devices showing a linear response. The differences in intensities are due to the reflectance of the paper as the surface absorbs a portion light while reflecting the rest. The light collected by the smart phone and SLR cameras was the reflected component of ambient light, while the scanner used an internal light source that was substantially brighter, yielding higher intensity-values. Overall, these results demonstrated that sensitivity of a smart phone camera was comparable to a SLR camera or a desktop scanner when sufficient ambient light was present. The Casio smart phone was used in the following examples to

demonstrate the accuracy of the present method in accurately analyzing colorimetric assays.

EXAMPLE 2

The described color quantification method can be applied to commercially available colorimetric test strips. The method was demonstrated using colorimetric pH indicator strips (Micro Essential Laboratory) which were dipped into a range of pH buffer solutions and then imaged with the smart phone camera. The mean RGB intensities of the region of interest (ROI) were calculated and converted to the chromaticity-values x and y. Fig. 7A illustrates that the 2-D diagram not only intuitively reflects the color change of the pH strip, but also the corresponding pH change. The pH value appears as a function of the chromaticity-values x and y in the 3-D space (Fig. 6B). A calibration plane was constructed for pH quantification using chromaticity-values of various standard solutions of known pH. The resulting 2nd order polynomial equation is given by:

$$pH(x,y) = 95.01x^2 - 0.58y^2 + 57.94xy - 98.71x - 21.65y + 29.61 \quad (4)$$

The high coefficient of determination (R^2 = 0.9874) indicated a highly accurate model. By substituting the x and y-values to the calibrated equation, the corresponding pH value was obtained. Fig. 7C illustrates the standard curve for measuring pH in the range from 2 to 10. The observed sensitivity of ~0.5 is limited by the pH strip, and can be improved by using narrow range pH strips for more accurate measurements.

EXAMPLE 3

A calorimetric urine glucose test strip (Science Kit & Boreal Laboratories) was tested with the method. The calorimetric urine glucose test is a quick and inexpensive way to check glucose in urine and is one of the most commonly used colorimetric tests that can be done in the privacy of one's home or in a doctor's office. The normal glucose range in urine is 0-0.8 mM (0-15 mg/dL); when the glucose level exceeds the renal threshold of ~10 mM, glucose can be found in the urine. The linear range covers the 0-60mM urine glucose concentration used in clinical diagnostics, and changes color to blue-green when urine glucose concentration exceeds 5mM. A double sequential enzymatic reaction, involving glucose oxidase and peroxidase in the presence of an indicator is responsible for the color change. To demonstrate test strip operation, urine was spiked

with 0-60mM glucose. FIGS. 8A, 8B, and 8C illustrate the results. After color quantification, the glucose concentration can be expressed as

$$C_{glucose}(x, y) = -3181x^2 + 1803y^2 + 931.8xy + 1005x - 571.1y - 59.36 \quad (5)$$

The high coefficient of determination (R^2 = 0.9874) once again indicates a highly accurate model. The standard curve for measuring urine glucose in the 0-60 mM range is shown in Fig. 8C. The observed limit of detection of 5mM is the limitation of the test strip, but is comparable with results of a colorimetric reader with multiple photodetectors.

It is noted that the calibration curves developed for both pH and urine glucose tests above show dependence on the smart phone CMOS chip. The tests with HTC and BlackBerry phones show slight variations (<5%), indicating necessity of re-calibration for each new smart phone model used if higher precision is desired. Nevertheless, only initial calibration is necessary and the process does not need to be repeated for each measurement. It is also noted that additional measurement errors can be caused by differences in ambient light conditions when test strips are imaged. Thus, the effect of ambient light was examined and an approach for its compensation was developed.

EXAMPLE 4

While the bench top results above demonstrate the capability of using a mobile phone such as a smart phone for quantitative colorimetric analysis, a challenge for everyday use outside of a controlled setting is compensating for ambient light conditions. After measuring the 12 regions on the reference chart, it was found that the measured intensities between different ambient light conditions had a linear relationship. FIGS. 9A, 9B, 9C, and 9D illustrate the red channel intensities of the reference chart regions at 5000K fluorescent light, compared to other light conditions, such as 3500K fluorescence (FIG. 9A), sunshine (FIG. 9B), shade (FIG. 9C), and smart phone LED (FIG. 9D). The high coefficient of determination values (R^2>0.99) indicate excellent linearity of the fits, demonstrating that the reference color chart can be used to compensate the intensity differences caused by ambient light changes. As is illustrated in FIG. 9A, the measured intensities at one light condition I_1 can be mapped to another I_2. The test strips responses and the corresponding 3-D fitting model using a 5000K fluorescent light source were calibrated and the detection tests were run under other

light conditions. By building the compensating equations in the red, green, and blue pixel channels, RGB intensities of any imaged sample can be mapped to the 5000K exposure and the corresponding chromaticity-values calculated. This color mapping approach provides an excellent way to compensate for errors caused by the ambient light changes, making the mobile phone detection approach applicable to any lighting environment from indoors to outdoors, from sunshine to overcast.

As further proof of the light calibration method, the detection algorithm for urine glucose was calibrated at the 5000K ambient light condition. Then the measurement was repeated at the 3500K ambient light condition. Fig. 10 illustrates the measured chromaticity coordinates of the urine test strips' color response to samples with different glucose concentrations. The chromaticity-values measured under 3500K ambient light exhibit an approximately 0.05 shift in x and 0.03 shift in y which causes large error in glucose detection. After the processing, the different intensities caused by the ambient light were compensated, resulting in a new series of chromaticity coordinates which matches the calibration at 5000K very well. The slight deviation is caused by material of the reference color chart and the test strip paper with different reflection. If the reference chart is printed directly on the test strip, the accuracy of this method can be further improved.

The light compensating method creates mapping of signals detected at any light condition to the calibration light condition, enabling improvisatory calibration of the test. To get an accurate measurement, light sources with high color rendering index are still recommended and the image of the color reference and test strip should be taken with care. The method assumes that the ambient is uniformly shining on the sample so that the intensities of the reference colors can be used to build a precise conversion curve for the unknown sample. If the imaging device is too close to the sample, it may block the light and generate a shadow which breaks the uniformity and causes false measurement. Thus, the smartphone should be placed at a proper position and height, depending on the location of the camera, to get accurate readings.

These data demonstrate the following potential advantages and applications. First, any mobile phone, tablet, portable computer or other device with an imaging sensor can be used for imaging colorimetric tests. According to the International Telecommunication Union (ITU), mobile-cellular subscriptions reached 6 billion in 2011, with 75% in

developing countries; a 3-8 megapixel camera is a standard feature for the most of the phones. Using cell phone for imaging colorimetric tests may offer a simple and convenient way to read results (and potentially transmit data to a physician via cellular network). With a smart phone, it may even be possible to process images with the phone for immediate display. Furthermore, using a smart phone for data analysis does not require trained personnel, and can be accomplished in seconds by a novice. Coupled with low-cost paper test strips, smart phones may offer a simple approach to disease screening in developing countries and resource limited settings. It is not expected that smart phone based colorimetric detection will replace traditional microscopic or spectroscopic based diagnostic, but it may offer a low-cost solution for *a priori* screening of a large number of potential patients.

Second, the approach can be easily extended to images taken by other means. Indeed, the method has been successfully applied it to images obtained by a tablet, a microscope and a scanner. Using Matlab, the color conversion process can be automated for processing on a laptop computer or a tablet (e.g., Fujitsu Q550). The CIE 1931 color system projects all the human visible colors onto a 2-D plane regardless of brightness, and is inherently resistant to changes in intensity in ambient light. The present method is simpler and more accurate than the direct RGB measurement using hue values or ratios of red and green channels. Using color space conversion leads to accurate quantification and ambient light compensation. Further, as already mentioned, with a tablet or mobile phone one could take advantage of cellular network transmission and cloud-based data storage, or perform analysis outside laboratory.

Thirdly, the color conversion analysis technique could be extended beyond imaging colorimetric test strips, and could be applied to analysis of any color images including fluorescence data. The recent development in paper microfluidic immunoassays and ELISA chips make these accurate methods directly accessible to end users, without performing complicated sample handling steps. It is envisioned that the present analysis method will provide a user-friendly approach that matches these assays. Ultimately, it is believed that the methods disclosed herein can be broadly applied to POC diagnosis with any type of colorimetric test strip, or to any sensor systems that provide colorimetric response.

While the present invention has been illustrated by the description of specific embodiments thereof, and while the embodiments have been described in considerable detail, it is not intended to restrict or in any way limit the scope of the appended claims to such detail. The various features discussed herein may be used alone or in any combination. For example, the detailed description primarily discloses analyzing a one test assay from an obtained image, however, multiple test assays could be analyzed in a given image, such as with a paper-based ELISA assay in which multiple areas may be analyzed simultaneously. Additional advantages and modifications will readily appear to those skilled in the art. The invention in its broader aspects is therefore not limited to the specific details, representative apparatus and methods and illustrative examples shown and described. Accordingly, departures may be made from such details without departing from the scope or spirit of the general inventive concept.

WHAT IS CLAIMED IS:

1. A method of analyzing a colorimetric assay to identify a value for an assay parameter, the method comprising:

obtaining an image of a first colorimetric assay;

converting intensity data from at least one of the red channel, the green channel, or the blue channels from at least a portion of the image of the first colorimetric assay to a first data point having a first value and a second value wherein the first value and the second value indicate the chromaticity of the test colorimetric assay;

recalling a predetermined standardized curve from a storage medium, the standardized curve including a plurality of data points, each of the plurality of data points having a third value, a fourth value, and a fifth value, the third value and fourth values indicating the chromaticity of the data point along the standardized curve and the fifth value indicating the value of an assay parameter associated with the third value and the fourth value; and

comparing the first data point with the standardized curve to identify the value of the assay parameter for the first colorimetric assay.

2. The method of claim 1 wherein the assay parameter value is identified as the fifth value of the data point along the standardized curve that has a third value and a fourth value that most closely matches the first value and second value of the first data point.

3. The method of claim 2 wherein the most closely matched data point along the standardized curve is identified as the data point having a third value and a fourth value with the smallest absolute difference from the first value and the second value from the first data point.

4. The method of any previous claim wherein the predetermined standardized curve is prepared from a plurality of colorimetric assays having known assay parameter values.

5. The method of any previous claim wherein the predetermined standardized curve is prepared using a device having the same characteristics as the device used to obtain the image of the first colorimetric assay.

6. The method of any previous claim wherein the image is obtained with a device having an imaging sensor selected from the group consisting of a charge-coupled device ("CCD") sensor, a complementary metal-oxide-semiconductor ("CMOS") sensor, and a contact image sensor ("CIS").

7. The method of any previous claim wherein the image is obtained with at least one of a digital camera, a mobile phone, a tablet, a portable computer, a computer, and a scanner.

8. The method of any previous claim wherein the intensity data from the image is converted to a data point using the CIE1931 color space code.

9. The method of any previous claim further comprising:

obtaining an image of a reference chart simultaneously with obtaining the image of the first colorimetric assay, wherein the reference chart includes a plurality of reference areas; and

stabilizing the automated color correcting functions of the device utilized to obtain the image with the reference chart.

10. The method of any previous claim further comprising compensating for the ambient light condition of the image.

11. The method of claim 10 wherein the ambient light compensation step includes:

obtaining an image of a reference chart under the same ambient light conditions as the image of the first colorimetric assay, the reference chart having at least one reference area;

measuring the intensity of the at least one reference area in at least one of the red channel, the green channel, or the blue channel;

comparing the measured intensity of the reference area with a predetermined intensity of the reference area measured under a predetermined illumination condition; and

correcting the first and second values of the first data point based on the comparison of the measured intensity of the reference area with the predetermined intensity of the reference area.

12. The method of claim 11 wherein the first data point is corrected by shifting the RGB intensity data from at least one of the red channel, the green channel, or the blue channel from at least a portion of the image of the first colorimetric assay.

13. The method of claim 11 wherein the first data point is corrected by shifting the first value and the second value of the first data point.

14. The method of one of claims 11 to 13 wherein the measured intensity is the measured intensity of at least one of the red channel, the green channel, or the blue channel; and

the predetermined intensity is the intensity of at least one of the red channel, the green channel, or the blue channel.

15. A method of compensating for ambient light conditions from an image of a colorimetric assay comprising:

obtaining an image of a reference chart under the same ambient light conditions as an image obtained from a colorimetric assay being analyzed, the reference chart having at least one reference area;

measuring the intensity of the at least one reference area in at least one of the red channel, the green channel, or the blue channel;

comparing the measured intensity of the reference area with a predetermined intensity of the reference area measured under a predetermined illumination condition; and

correcting the first and second values of the first data point based on the comparison of the measured intensity of the reference area with the predetermined intensity of the reference area.

16. A computer program product comprising:

a computer readable storage medium; and

program instructions for performing the method of any previous claim,

wherein the program instructions are stored on the computer readable storage medium.

17. A system for analyzing a colorimetric assay to identify a value for an assay parameter, the method comprising:

a data analysis module, the data analysis module being configured to:

a) convert intensity data from at least one of the red channel, the green channel, or the blue channel from at least a portion of an image of a colorimetric assay to a first data point having a first value and a second value wherein the first value and the second value indicate the chromaticity of the test colorimetric assay;

b) recall a predetermined standardized curve from a storage medium, the standardized curve including a plurality of data points, each of the plurality of data points having a third value, a fourth value, and a fifth value, the third value and fourth values indicating the chromaticity of the data point along the standardized curve and the fifth value indicating the value of an assay parameter associated with the third value and the fourth value; and

c) compare the first data point with the plurality of data points from the standardized curve to identify the value of the assay parameter for the first colorimetric assay.

18. The system of claim 17 further comprising an image obtaining module, the image obtaining module configured to receive intensity data points from the colorimetric assay.

19. The system of claim 17 further comprising a reference chart, wherein said reference chart includes a plurality of reference areas, the reference chart being configured to stabilize the automated color correcting functions of the image obtaining module.

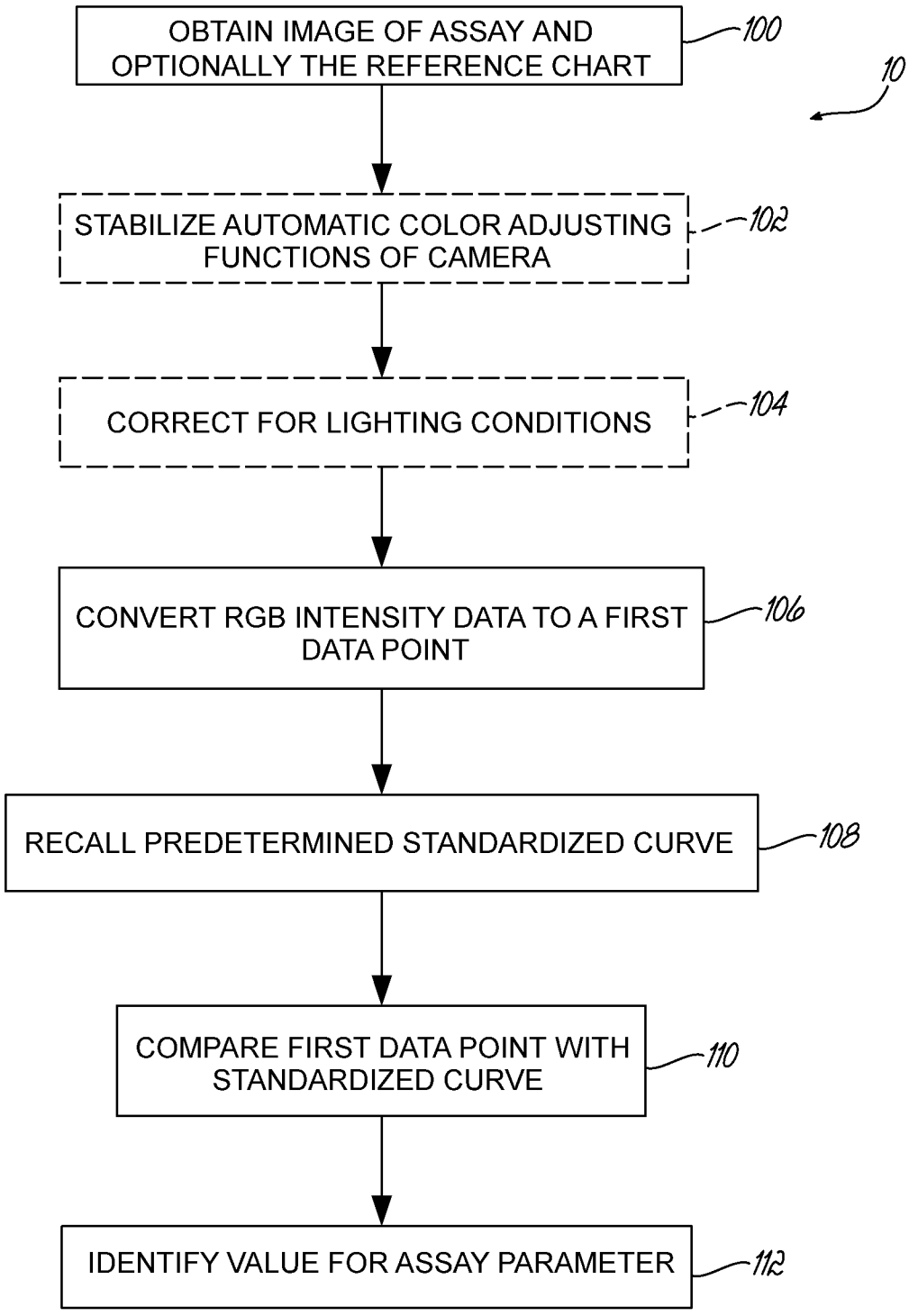

OBTAIN IMAGE OF ASSAY AND OPTIONALLY THE REFERENCE CHART
100
10
STABILIZE AUTOMATIC COLOR ADJUSTING FUNCTIONS OF CAMERA
102
CORRECT FOR LIGHTING CONDITIONS
104
CONVERT RGB INTENSITY DATA TO A FIRST DATA POINT
106
RECALL PREDETERMINED STANDARDIZED CURVE
108
COMPARE FIRST DATA POINT WITH STANDARDIZED CURVE
110
IDENTIFY VALUE FOR ASSAY PARAMETER
112

FIG. 1

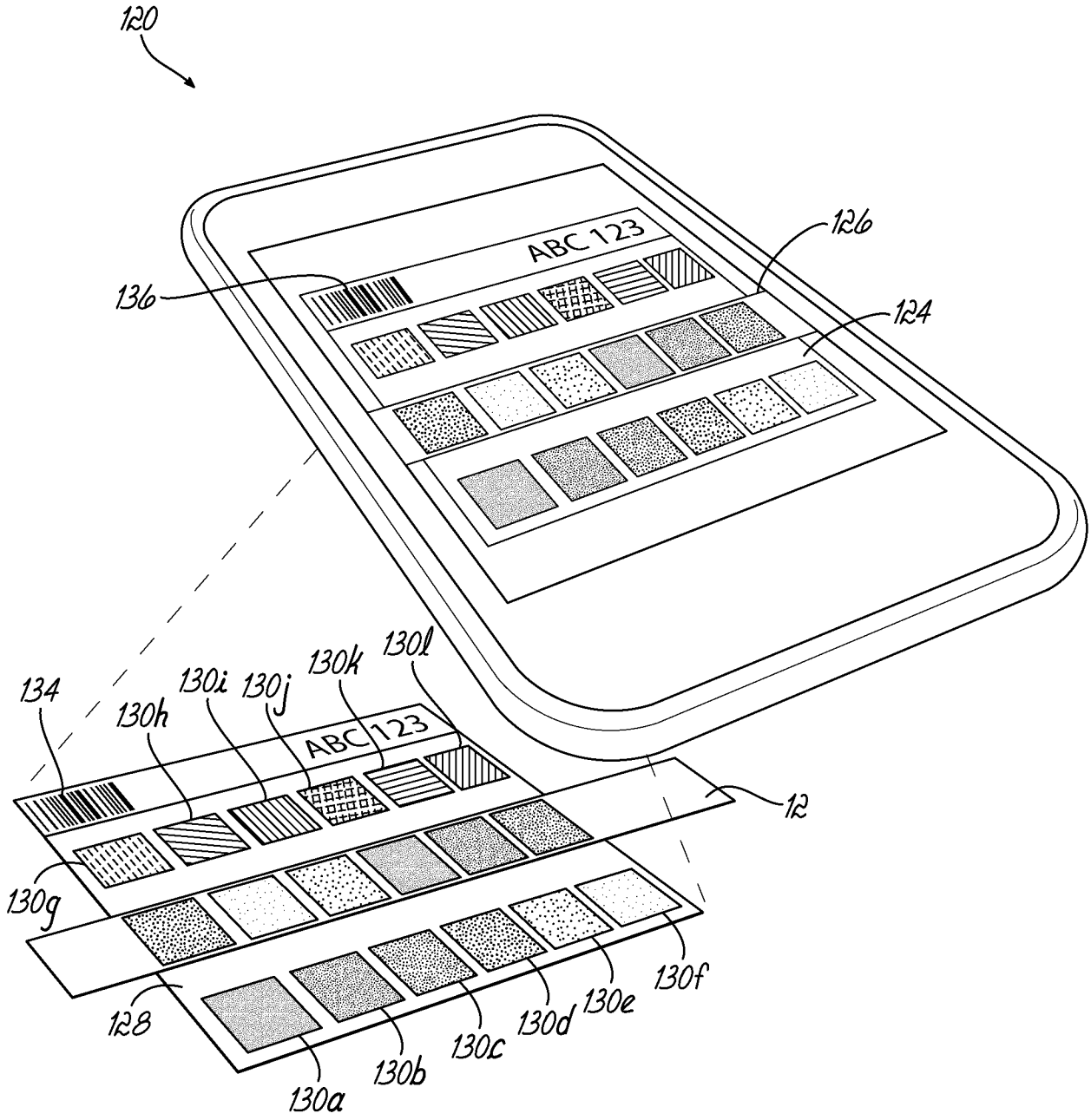

120
ABC 123
126
136
124
134
130h
130i
130j
130k
130l
ABC 123
12
130g
128
130a
130b
130c
130d
130e
130f

FIG. 2

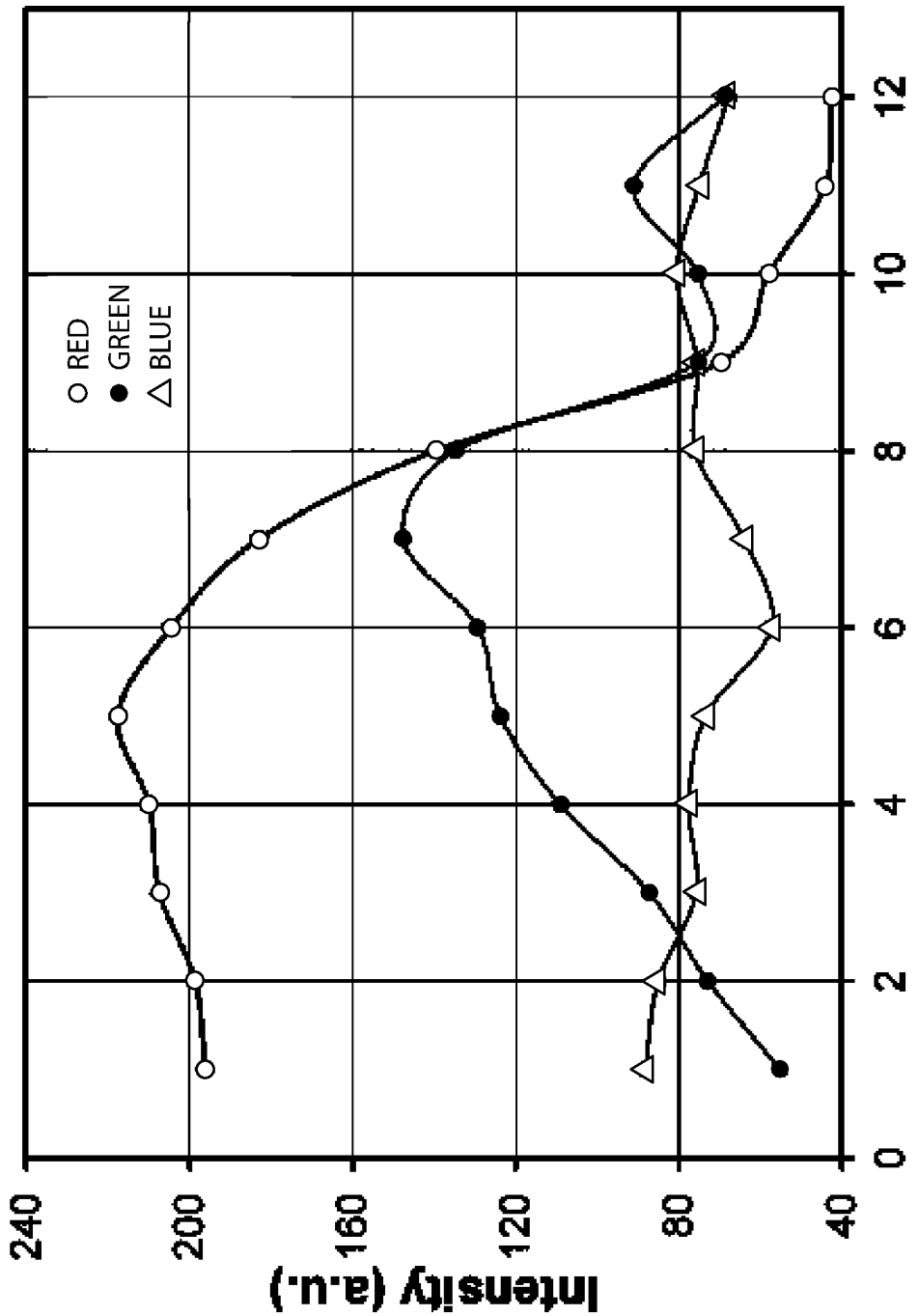

RED
GREEN
BLUE
Intensity (a.u.)
240
200
160
120
80
40
0
2
4
6
8
10
12
pH

FIG. 3

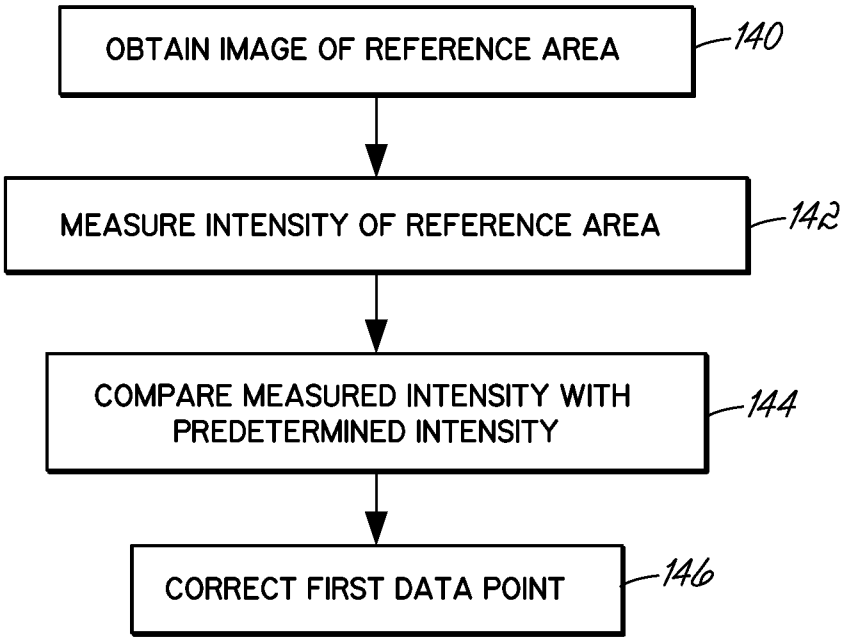

OBTAIN IMAGE OF REFERENCE AREA
140
MEASURE INTENSITY OF REFERENCE AREA
142
COMPARE MEASURED INTENSITY WITH PREDETERMINED INTENSITY
144
CORRECT FIRST DATA POINT
146

FIG. 4

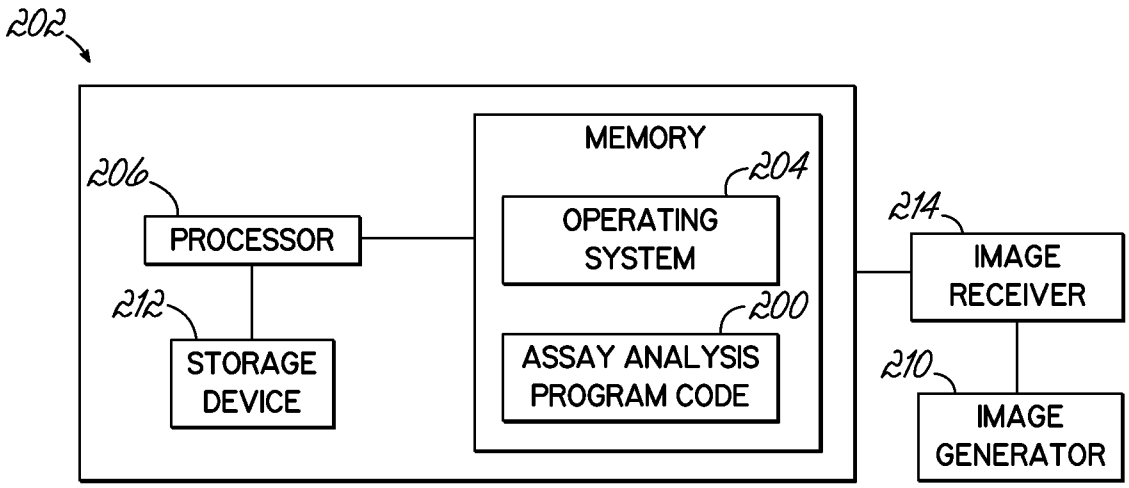

202
MEMORY
204
206
PROCESSOR
OPERATING SYSTEM
214
IMAGE RECEIVER
212
200
STORAGE DEVICE
ASSAY ANALYSIS PROGRAM CODE
210
IMAGE GENERATOR

FIG. 5

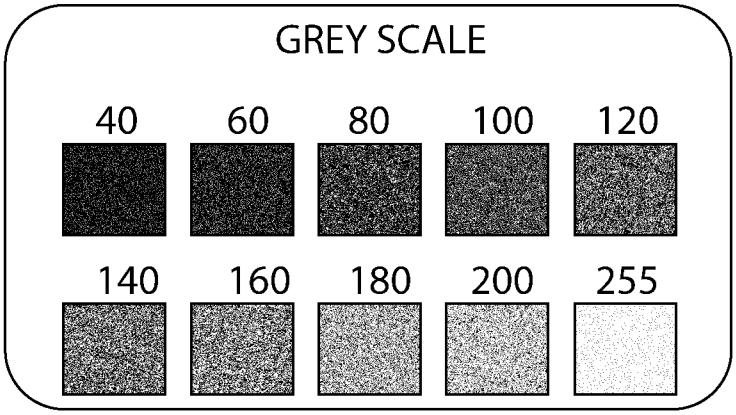

GREY SCALE
40
60
80
100
120
140
160
180
200
255

FIG. 6A

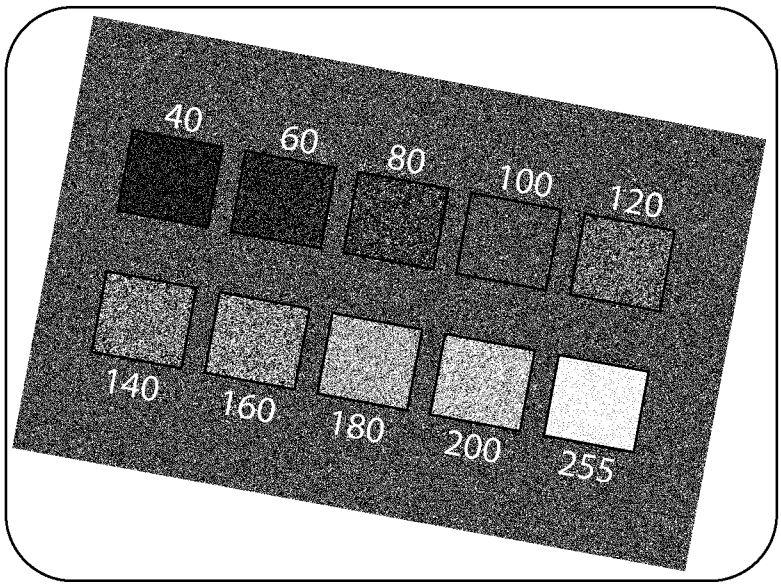

40
60
80
100
120
140
160
180
200
255

FIG. 6B

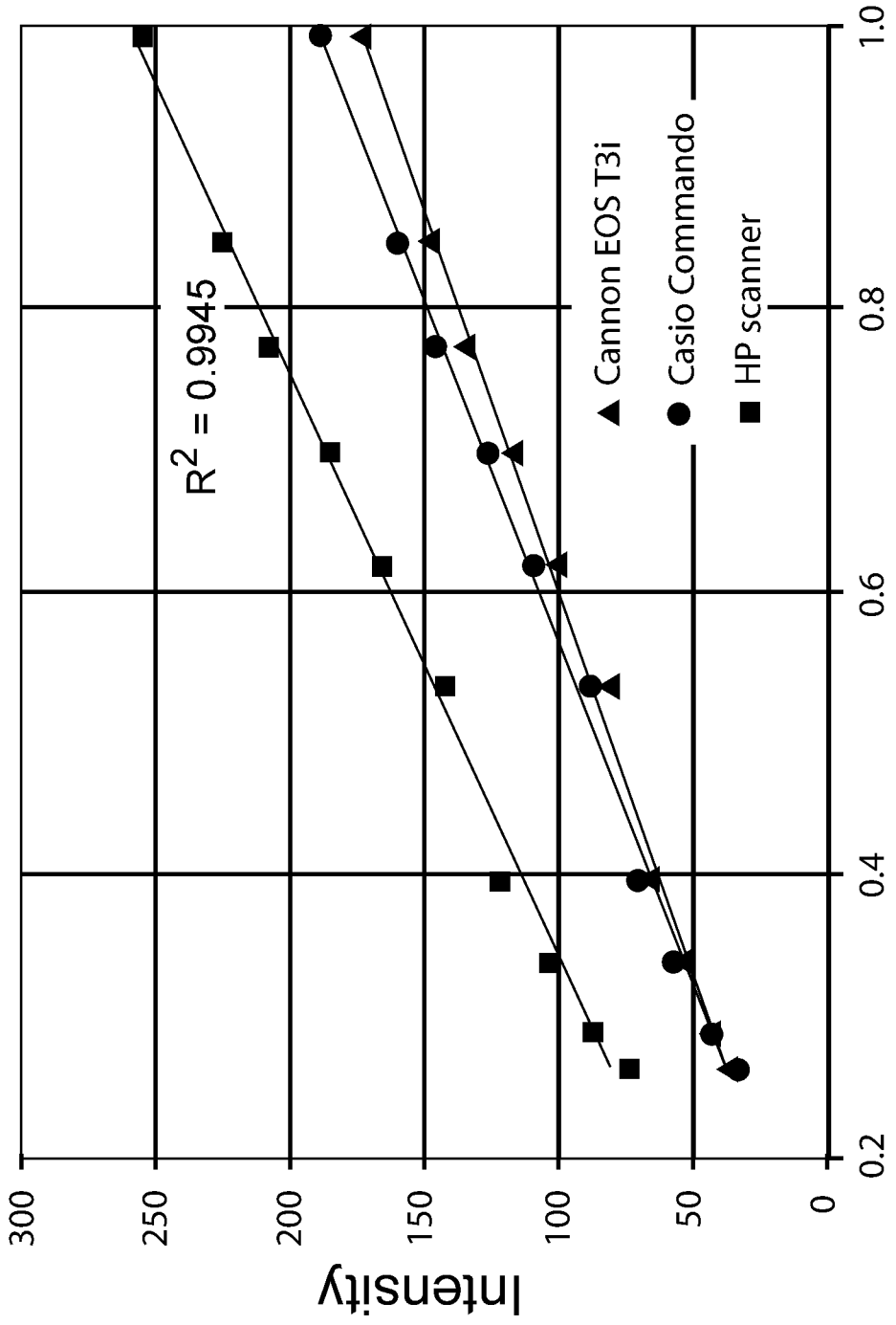

Intensity
300
250
200
150
100
50
0
0.2
0.4
0.6
0.8
1.0
Standard Grey Scale
$R^2 = 0.9945$
Cannon EOS T3i
Casio Commando
HP scanner

FIG. 6C

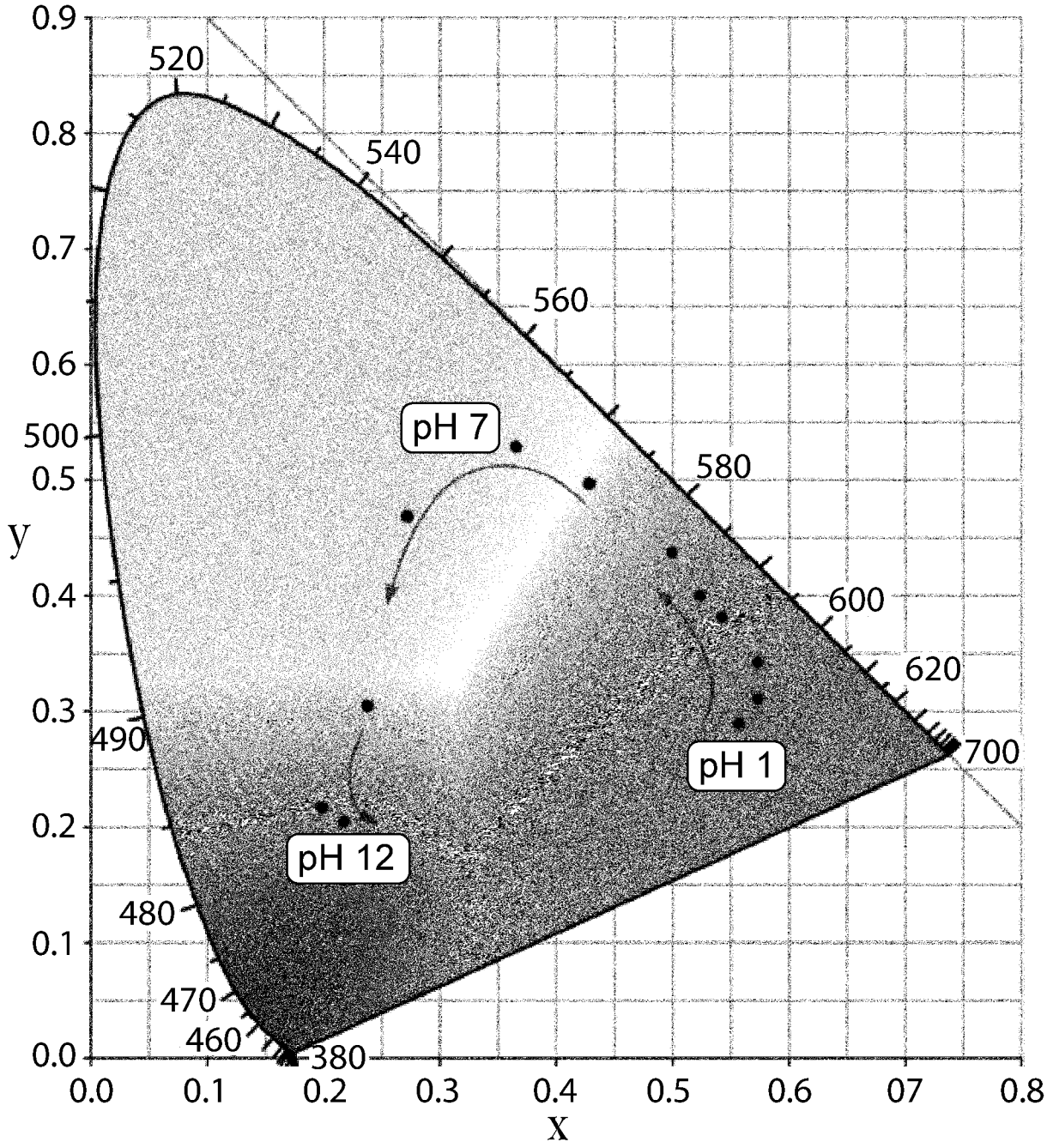

0.9
0.8
0.7
0.6
500
0.5
0.4
0.3
490
0.2
480
0.1
470
460
0.0
520
540
560
580
600
620
700
380
pH 7
pH 1
pH 12
y
0.0
0.1
0.2
0.3
0.4
0.5
0.6
07
0.8
x

FIG. 7A

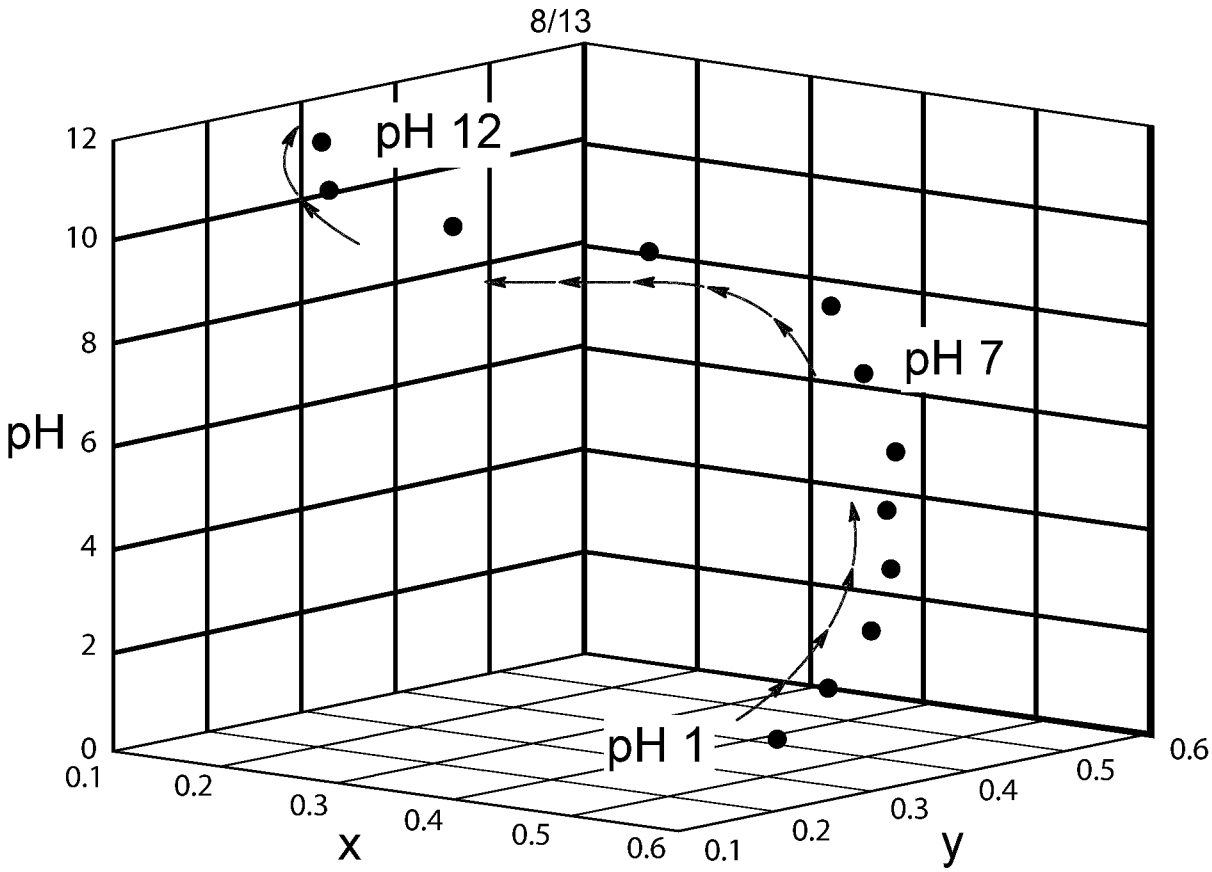

pH 12
pH 7
pH 1
pH
x
y
0
2
4
6
8
10
12
0.1
0.2
0.3
0.4
0.5
0.6

FIG. 7B

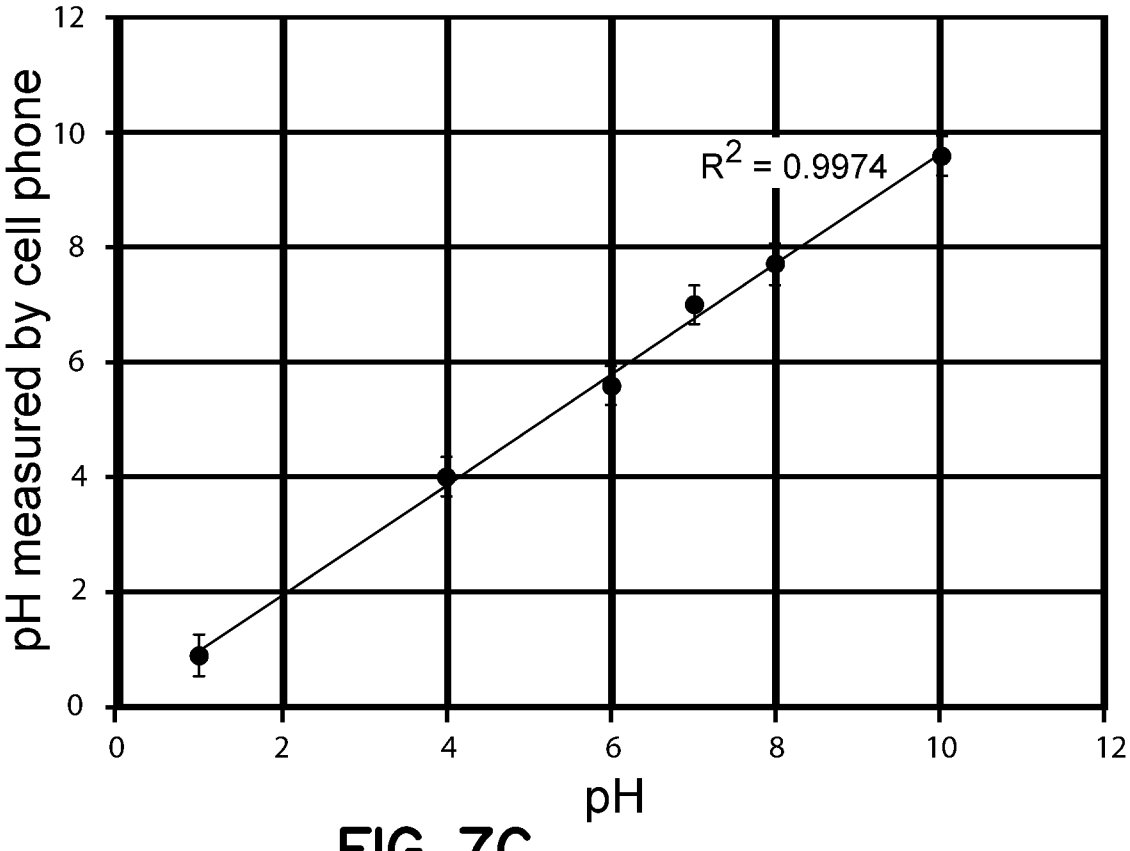

R^2 = 0.9974
pH measured by cell phone
pH
0
2
4
6
8
10
12

FIG. 7C

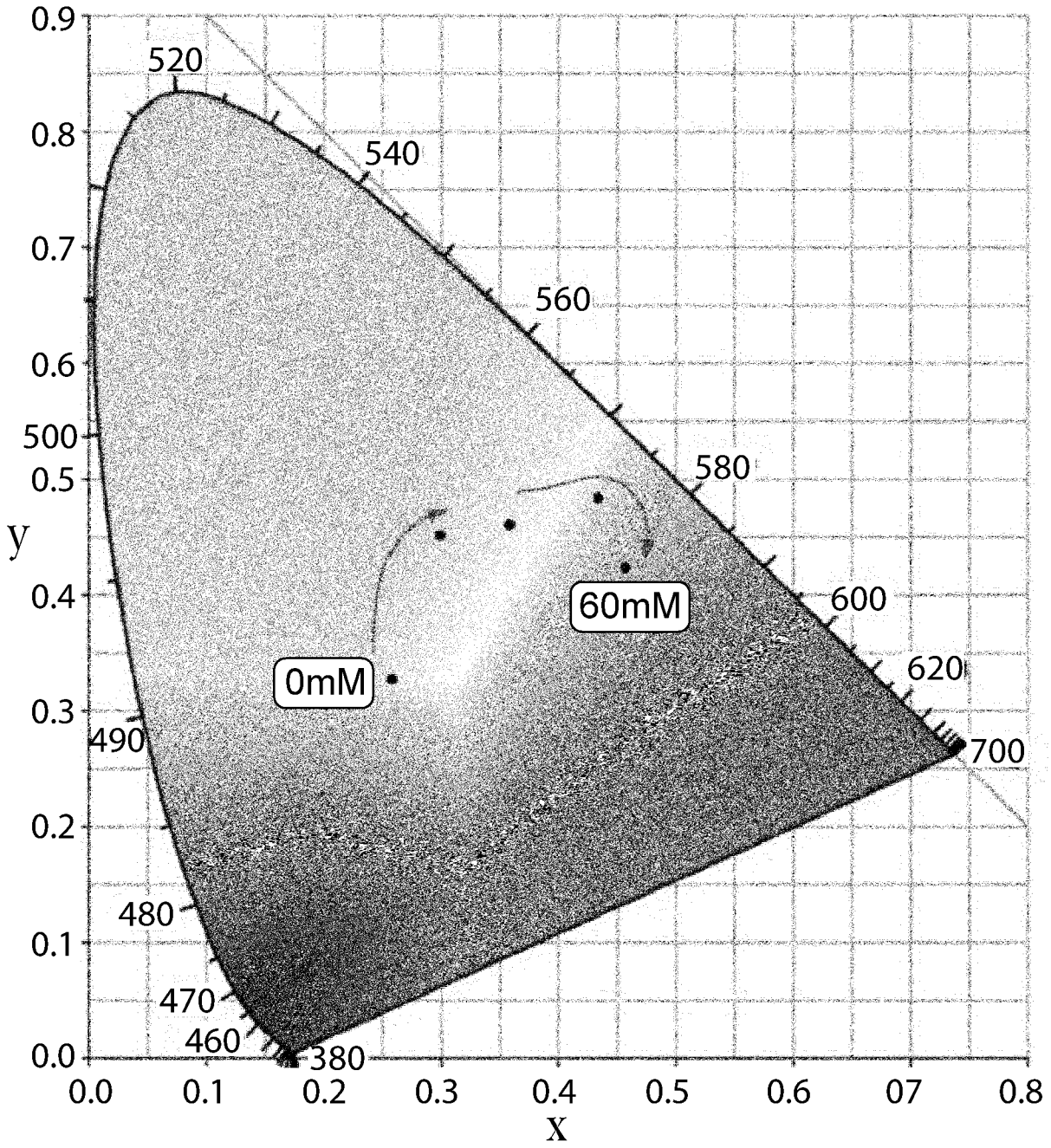

0.9
0.8
0.7
0.6
500
0.5
0.4
0.3
490
0.2
480
0.1
470
460
0.0
520
540
560
580
600
620
700
380
60mM
0mM
y
0.0
0.1
0.2
0.3
0.4
0.5
0.6
07
0.8
x

FIG. 8A

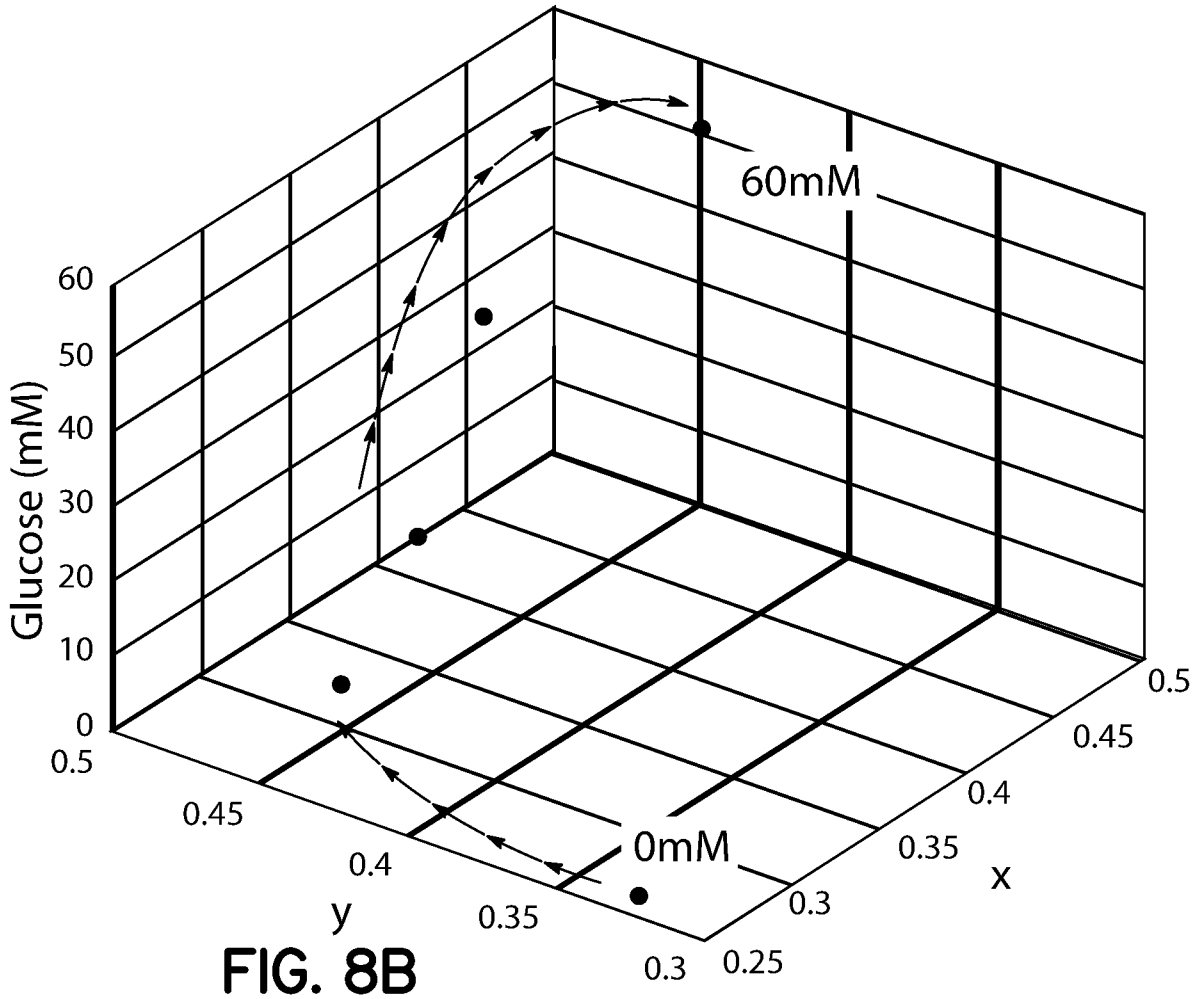

60mM
0mM
Glucose (mM)
60
50
40
30
20
10
0
0.5
0.45
0.4
0.35
0.3
y
0.5
0.45
0.4
0.35
0.3
0.25
x

FIG. 8B

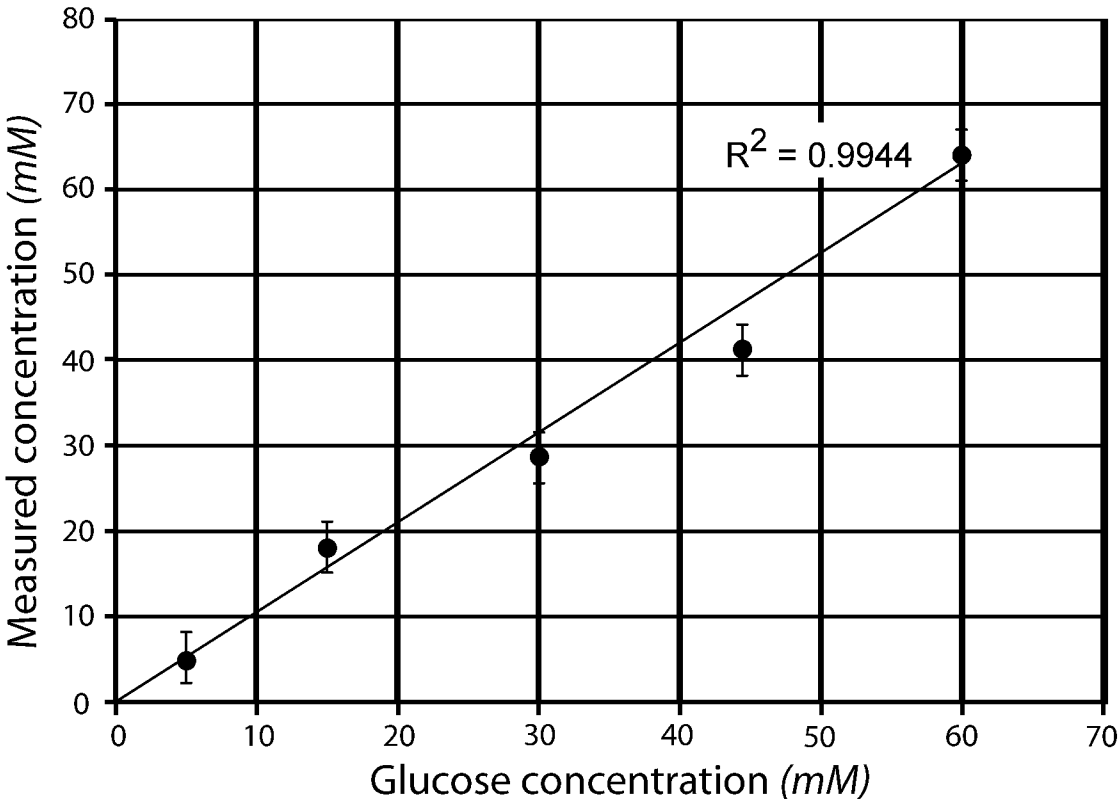

R^2 = 0.9944
Measured concentration (mM)
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Glucose concentration (mM)

FIG. 8C

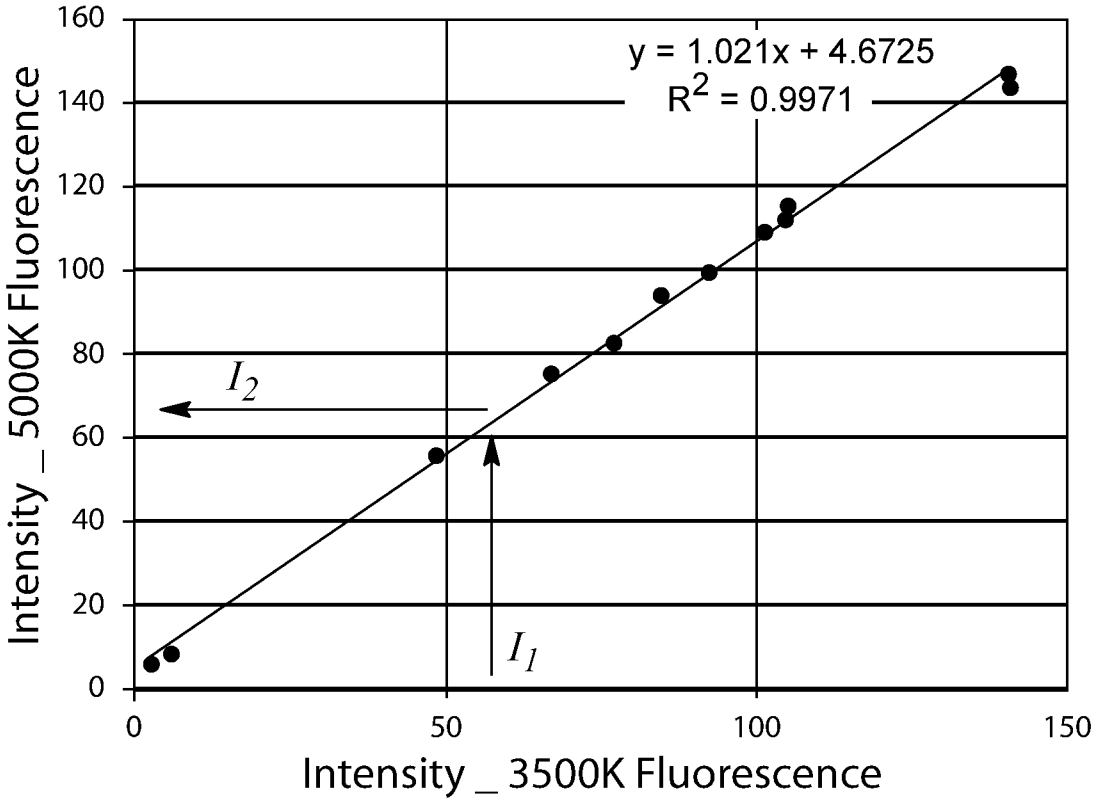

y = 1.021x + 4.6725
R2 = 0.9971
I2
I1
Intensity _ 5000K Fluorescence
Intensity _ 3500K Fluorescence
0
20
40
60
80
100
120
140
160
0
50
100
150

FIG. 9A

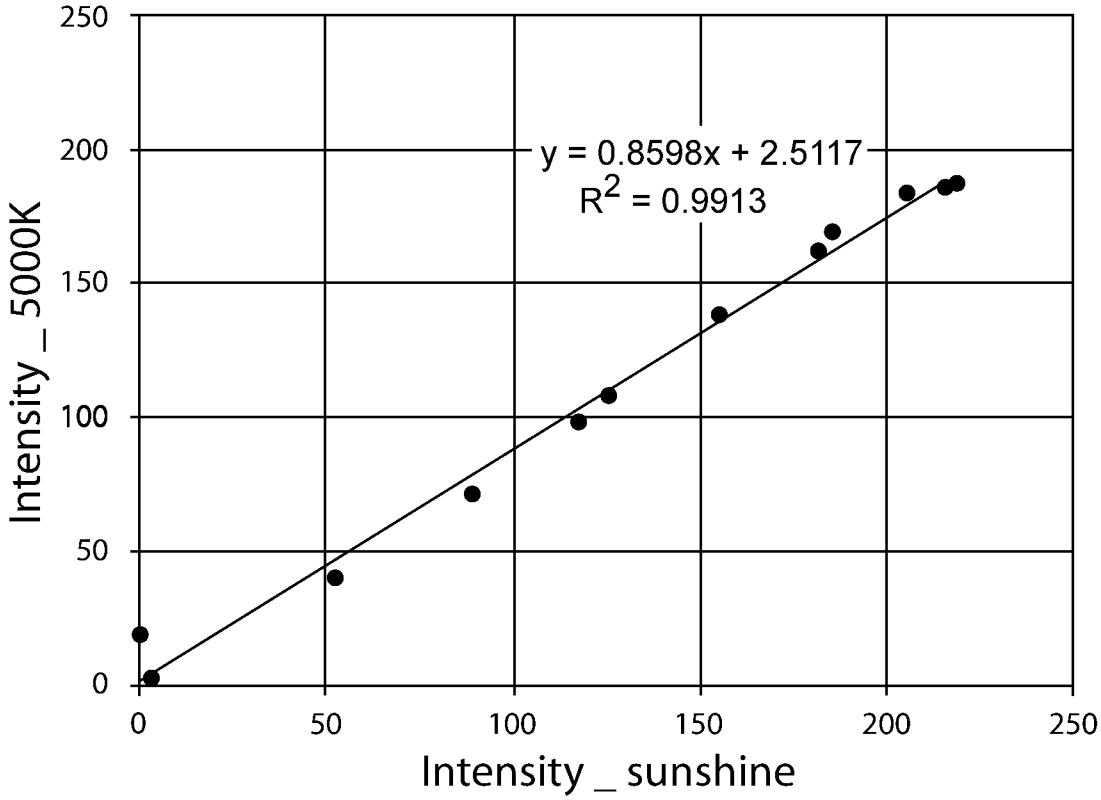

y = 0.8598x + 2.5117
R2 = 0.9913
Intensity _ 5000K
Intensity _ sunshine
0
50
100
150
200
250
0
50
100
150
200
250

FIG. 9B

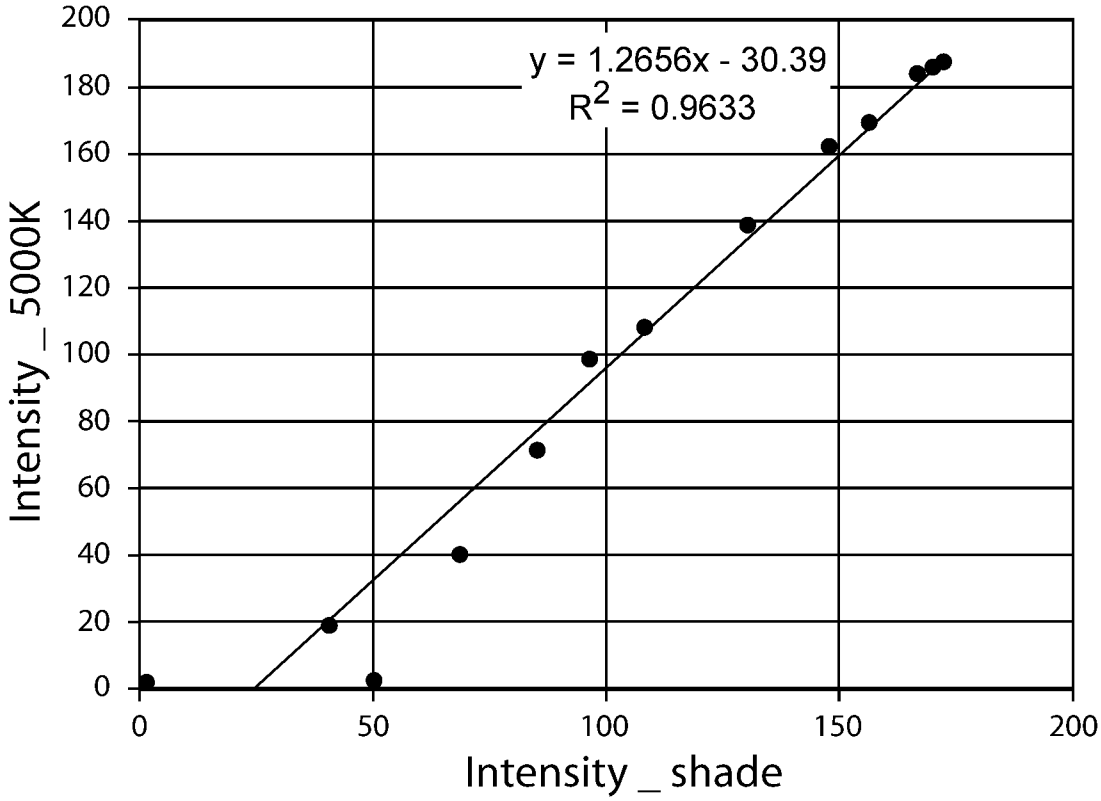

y = 1.2656x - 30.39
R2 = 0.9633
Intensity _ 5000K
Intensity _ shade
0
20
40
60
80
100
120
140
160
180
200
0
50
100
150
200

FIG. 9C

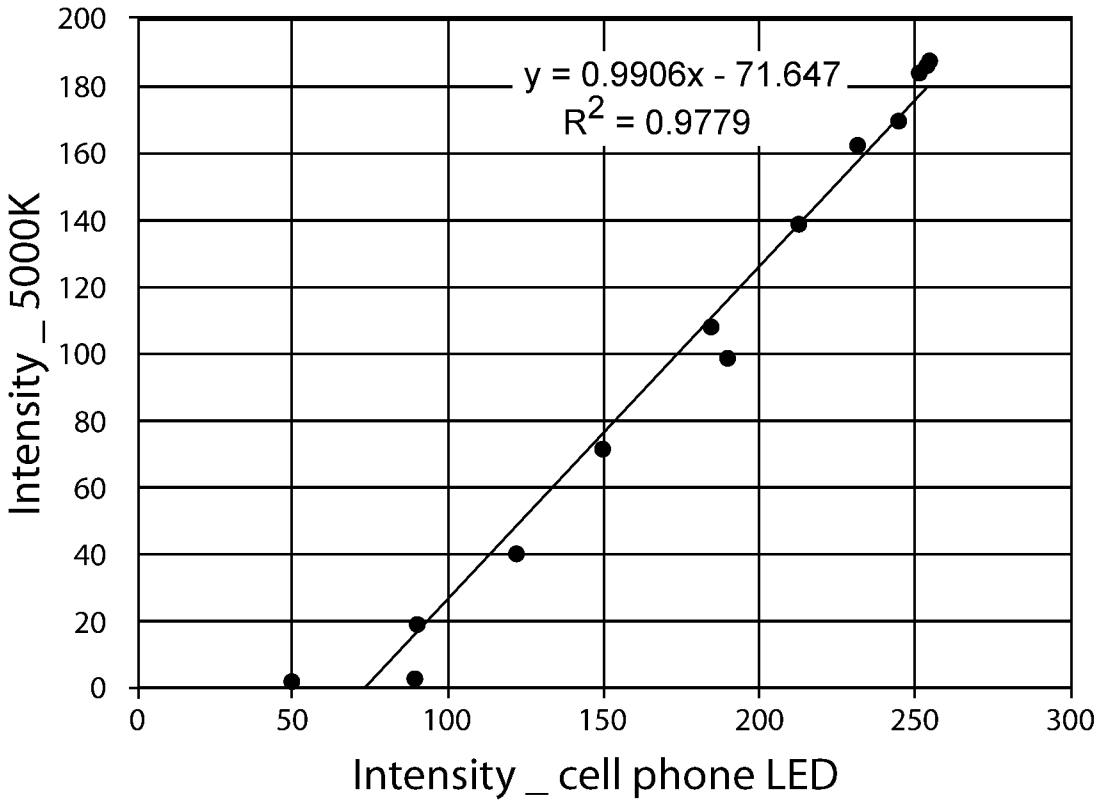

y = 0.9906x - 71.647
R2 = 0.9779
Intensity _ 5000K
Intensity _ cell phone LED
0
20
40
60
80
100
120
140
160
180
200
0
50
100
150
200
250
300

FIG. 9D

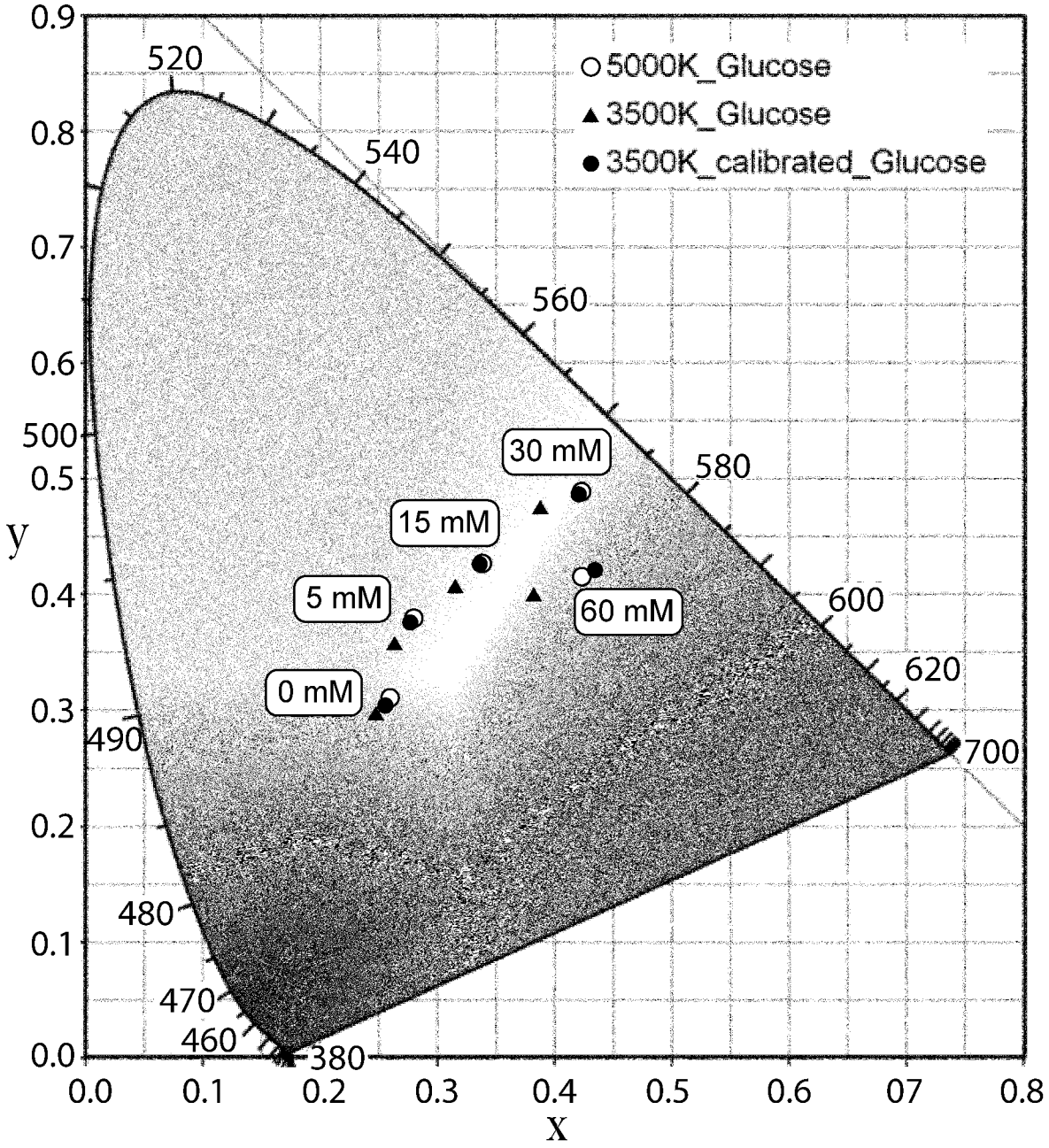

5000K_Glucose
3500K_Glucose
3500K_calibrated_Glucose
30 mM
15 mM
5 mM
0 mM
60 mM
520
540
560
580
600
620
700
500
490
480
470
460
380
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
y
0.0
0.1
0.2
0.3
0.4
0.5
0.6
07
0.8
x

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/024622

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/25
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

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 practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANTONIO GARCA ET AL: "Mobile phone platform as portable chemical analyzer", SENSORS AND ACTUATORS B: CHEMICAL: INTERNATIONAL JOURNAL DEVOTED TO RESEARCH AND DEVELOPMENT OF PHYSICAL AND CHEMICAL TRANSDUCERS, ELSEVIER S.A, SWITZERLAND, vol. 156, no. 1, 17 April 2011 (2011-04-17), pages 350-359, XP028222857, ISSN: 0925-4005, DOI: 10.1016/J.SNB.2011.04.045 [retrieved on 2011-04-27] the whole document ----- -/--	1-19

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

[ ] 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 application or patent but published on or after the international filing date

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2013	11/04/2013

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	Croucher, J

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/024622

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HIRAYAMA E ET AL: "VISUAL AND COLORIMETRIC LITHIUM ION SENSING BASED ON DIGITAL COLOR ANALYSIS", ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 72, no. 3, 1 February 2000 (2000-02-01), pages 465-474, XP000924099, ISSN: 0003-2700, DOI: 10.1021/AC990588W the whole document	1-19
A	ANDRES W MARTINEZ ET AL: "Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis", ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 80, no. 10, 15 August 2008 (2008-08-15), pages 3699-3707, XP008148137, ISSN: 0003-2700, DOI: 10.1021/AC800112R [retrieved on 2008-04-11] the whole document	1-19
A	DAE-SIK LEE ET AL: "A simple and smart telemedicine device for developing regions: a pocket-sized colorimetric reader", LAB ON A CHIP, ROYAL SOCIETY OF CHEMISTRY, vol. 11, no. 1, 7 January 2011 (2011-01-07), pages 120-126, XP007917465, ISSN: 1473-0197, DOI: 10.1039/C0LC00209G the whole document	1-19
A	LAPRESTA-FERNNDEZ A ET AL: "Environmental monitoring using a conventional photographic digital camera for multianalyte disposable optical sensors", ANALYTICA CHIMICA ACTA, ELSEVIER, AMSTERDAM, NL, vol. 706, no. 2, 25 August 2011 (2011-08-25), pages 328-337, XP028326085, ISSN: 0003-2670, DOI: 10.1016/J.ACA.2011.08.042 [retrieved on 2011-09-01] the whole document	1-19