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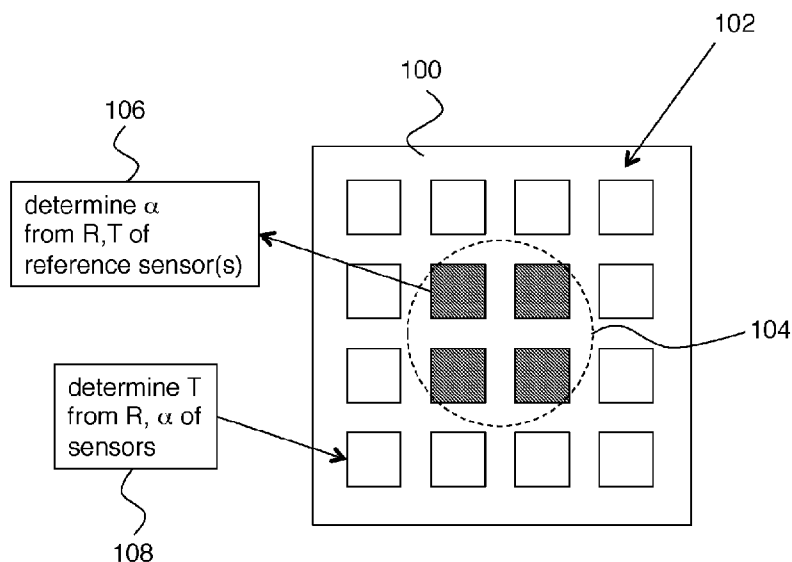


FIG. 1

(57) Abstract: Improved temperature measurement and correction is provided for magnetoresistive sensor arrays. A linear coefficient of resistance vs. temperature is determined from one or more reference sensors in the array by measuring resistance of the reference sensors at known temperatures, which enables resistance measurements to be used to determine unknown temperatures in all sensors of the array. Double modulation leads to MR sensor outputs having center tones which can be used to correct temperature dependence in side tone MR signals. This correction can be according to a linear fit or a polynomial fit. Applications include biological assays requiring accurate temperature data, such as accurate determination of DNA melting curves.



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Method for accurate temperature measurement on GMR
biosensor arrays

5

FIELD OF THE INVENTION

This invention relates to improved methods for
magnetoresistive (MR) biosensing applications.

10 BACKGROUND

To accurately measure temperature dependent properties
of biological interaction (e.g. : DNA melting temperature,
affinity, avidity, adsorption and desorption rates etc.),
accurate knowledge of the reaction conditions is required.
15 In the particular case of GMR (giant magnetoresistance)
biosensor arrays, the working assumption of uniform
temperature across the chip area holds true only with the
system at thermal equilibrium at room temperature. In every
other situation, the inherent shape of the chip and details
20 of the temperature controlling method cause a temperature
gradient across the chip surface. Accordingly, it would be
an advance in the art to provide improved methods for
dealing with temperature measurement and/or calibration in
magnetoresistive sensor arrays.

25

SUMMARY

In an exemplary embodiment, the GMR biosensor chip
includes more than 80 GMR resistors distributed in a square
array, with a pitch of about 400 μm . The resistivity of the

GMR material is linearly dependent on the temperature in the working range 0-100°C. By measuring the resistance of each sensor on the array, it is possible to characterize the temperature gradient across the chip and correct for it
5 in subsequent data analysis.

We provide a method to measure temperature of each sensor by means of a double modulation sensing protocol that allows for synchronous measurement of magnetic nanoparticle binding events (for biosensing) as well as
10 resistivity variation due to temperature. This method can be applied to each sensor individually, thus ensuring two-dimensional quantification of the temperature across the chip. Furthermore, we describe a method to correct for
15 temperature dependency in the sensor sensitivity to magnetic field that would affect the magnetic nanoparticle detection.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an embodiment of the invention.

20 FIG. 2 is a diagram of the idealized output signal in a double modulation sensing system.

FIG. 3 is a table of measured temperatures from an exemplary magnetoresistive sensor array.

25 FIG. 4 shows $\Delta ST/ST_0$ vs. $\Delta CT/CT_0$ curves measured for several MR sensors on the chip.

FIG. 5A shows DNA signal vs. time during a temperature ramp at 0.05C/s for several MR sensors on the chip.

30 FIG. 5B is a table of melt delay in minutes for each sensor in the array, relative to the first sensor on the chip to melt (at row 5, column 6).

FIG. 6A shows melting curves from sensors in rows 2-4 after temperature calibration.

FIG. 6B is a table of melting temperature measured on each sensor after temperature calibration.

5 FIG. 7 is a flow diagram of an assay suitable for practice in connection with embodiments of the invention.

FIG. 8 shows an exemplary apparatus for carrying out the assay of FIG. 7.

10 FIGs. 9A-B show further details relating to the assay of FIG. 7.

DETAILED DESCRIPTION

A) Exemplary hardware configuration

15 FIG. 1 shows an exemplary magnetoresistive sensor array operating in an embodiment of the invention. In this example, an array of magnetoresistive sensors 102 is disposed on a substrate 100, and substrate 100 is disposed on a temperature control member 104. In the top view of FIG. 1, temperature control member 104 is shown with dotted lines because it is in contact with the back side of
20 substrate 100 and therefore is not visible in a top view. As shown on FIG. 1, it is usually the case that some of the magnetoresistive sensors in array 102 are disposed such that they have better thermal contact to temperature
25 control member 104 than the other magnetoresistive sensors. It is convenient to refer to these one or more MR sensors as reference MR sensors. The reference MR sensors on FIG. 1 are shaded. Briefly, the main idea is to use one or more reference sensors to determine the linear temperature
30 coefficient of zero-field resistance, α , by measuring resistance R at two or more known temperatures T (106 on

FIG. 1). Once α is known, temperature can be determined from resistance for any sensor in the array (108 on FIG. 1). It is convenient to first consider the implications of double modulation sensing before providing
 5 further details of this temperature measurement approach.

B) Double modulation sensing

GMR sensor are biased with a voltage V at frequency f_v . An external magnetic field H is applied at frequency f_h . The
 10 output signal (i.e. current in the sensor) has ideally 4 main components, as shown on FIG. 2. A center tone (CT) component at frequency f_v , two identical side tone (ST) components at $f_v \pm f_h$ and a magnetic pickup noise component induced by the field at f_h . Working with $f_v \gg f_h$ it is
 15 possible to isolate the signal from the noise at f_h as well as from the $1/f$ noise. The CT and ST components are detected by spectral analysis. These are found to be:

$$CT = \frac{V}{R_0}, \quad (1)$$

$$ST = -\frac{V\Delta R_0}{4R_0^2}, \quad (2)$$

20 where V is the bias voltage, R_0 is the zero-field resistance of the sensor and ΔR_0 is the change in resistance when a field H is applied. The figure of merit for magnetoresistive sensor is the magnetoresistive ratio, defined as $MR = \Delta R_0/R_0$. In double modulation sensing the
 25 appropriate figure of merit becomes $MR = 4ST/CT$. The magnetoresistive ratio is affected by the presence of magnetic nanoparticles at or near the sensor surface, allowing for biosensing.

C) Temperature measurement

The resistivity of a GMR sensor depends on temperature, thus, in a double modulation scheme:

$$R(T) = R_0(1 + \alpha\Delta T), \quad (3)$$

$$5 \quad CT \sim \frac{V}{R_0}(1 - \alpha\Delta T), \quad (4)$$

where α is a thermal coefficient that depends on the GMR material but can be assumed constant over the chip. The coefficient α can be obtained by measuring resistances at two known temperatures for one or several reference sensors that are in better thermal contact with the temperature regulation system. If more than one sensor is used, the individual α values can be averaged.

Once the thermal coefficient α is known, we can measure temperature for each sensor on the chip. More explicitly, a method for temperature measurement in an array of magnetoresistive sensors includes the following steps:

1) providing an array of magnetoresistive sensors, where the array of magnetoresistive sensors includes at least one reference magnetoresistive sensor;

2) R_0 is a zero field resistivity at a reference temperature T_0 , T is temperature, α is a material parameter of the magnetoresistive sensors, and the magnetoresistive sensors have a zero field resistivity R given by

$$25 \quad R = R_0(1 + \alpha(T - T_0));$$

3) measuring zero field resistivity of the reference magnetoresistive sensor(s) at two or more known temperatures to determine α ; and

4) measuring zero field resistivity of one or more
magnetoresistive sensors of the array of magnetoresistive
sensors to determine corresponding temperatures T of the
one or more magnetoresistive sensors from measured R and
5 known α .

In cases where more than one reference
magnetoresistive sensor is employed, determining α can be
done by averaging α values of the two or more reference
magnetoresistive sensors. Once α is known, temperature can
10 be determined from zero-field resistance for any sensor in
the array, whether or not it was used as a reference sensor
for determining α .

A temperature controlled cartridge used in one
embodiment of this invention had a similar configuration to
15 that of FIG. 1. The cartridge was assembled such that the
back side of the GMR chip die (i.e., substrate 100) was in
thermal contact with the temperature controlled cartridge
(i.e., temperature control member 104). The contact area in
this case was circular, with a diameter of 1-2 mm. The
20 limited contact area causes a temperature gradient on the
chip surface. FIG. 3 shows a temperature distribution
across the chip for this example as determined by the
above-described method. The temperature varies about 1.5 C
from the center to the edge of the chip. The procedure here
25 described allows for accurate temperature measurement of
each sensor and for correction for temperature gradients
across the GMR chip

D) Temperature correction for double modulation

30 Side tone (ST) also depend on temperature, since both
the resistivity and the magnetoresistivity of the GMR

sensor are temperature dependent. This is normally assumed to be linear for small temperature variation. Thus

$$ST \sim -\frac{V\Delta R_0}{4R_0^2} (1 + (\beta - \alpha)\Delta T) \quad (5)$$

$$CT \sim \frac{V}{R_0} (1 - \alpha\Delta T) \quad (6)$$

5 Normalizing CT and ST by their initial values

$$\frac{\Delta CT}{CT_0} \sim -\alpha\Delta T \quad (7)$$

$$\frac{\Delta ST}{ST_0} \sim (\beta - \alpha)\Delta T \quad (8)$$

From which we obtain

$$\frac{\Delta ST}{ST_0} \propto K \frac{\Delta CT}{CT_0} \quad (9)$$

10 Thus, CT can be used as an internal temperature measurement to characterize and correct the temperature dependency of ST.

$$1 + (\beta - \alpha)\Delta T = 1 + K \frac{\Delta CT}{CT_0} \quad (10)$$

$$ST_{corr} = \frac{ST}{1 + K \frac{\Delta CT}{CT_0}} \quad (11)$$

15 The coefficient K varies across the chip as shown in FIG 4. More than 10% variation of K was found. The calibration of ST to CT should therefore be performed on each individual sensor.

For small temperature variation of ± 10 °C, the method
 20 presented above is sufficient to correct ST since $\Delta ST/ST_0$ vs. $\Delta CT/CT_0$ can be considered linear. For a larger temperature range, the relation between $\Delta ST/ST_0$ and $\Delta CT/CT_0$ was found to be non-linear. A higher order polynomial (P_n)

should be fitted to the curve to correct completely the temperature dependency of ST.

$$ST_{corr} = \frac{ST}{1 + P_n \left(\frac{\Delta CT}{CT_0} \right)} \quad (12)$$

A polynomial of order 3 or higher should be used in those applications in which precise correction of the temperature driven variation is necessary. In one specific case, a polynomial of 5th order was identified as the optimal model.

According to these principles, the above described temperature correction methods can further include double modulation according to the following steps:

- 1) disposing the array of magnetoresistive sensors in a tickling magnetic field having a modulation frequency of f_h ;
- 2) providing an electrical excitation to the array of magnetoresistive sensors having a modulation frequency of f_v , wherein f_h and f_v are distinct, and where an output spectrum of each magnetoresistive sensor in the array of magnetoresistive sensors includes a carrier tone (CT) at frequency f_v and side tones (STs) at frequencies $f_v \pm f_h$, where the carrier tone has a CT amplitude and the side tones have an ST amplitude;
- 3) for each magnetoresistive sensor in the array of magnetoresistive sensors, individually determining a baseline relation between the CT amplitude and the ST amplitude in the output spectrum;
- 4) for each magnetoresistive sensor in the array of magnetoresistive sensors, individually measuring corresponding CT and ST amplitudes when a sample is present to provide an input CT measurement and an input ST measurement;

5) for each magnetoresistive sensor in the array of magnetoresistive sensors, individually correcting the input ST measurement using the corresponding baseline relation and the corresponding input CT measurement to provide a
5 corresponding corrected ST measurement (e.g., according to Eq. 11 for a linear correction and according to equation 12 for a polynomial fit correction); and

6) for each magnetoresistive sensor in the array of magnetoresistive sensors, individually providing the
10 corrected ST measurement as an output.

The baseline relation between CT amplitude and ST amplitude can be determined individually for each sensor in the array by collecting ST vs. CT data for each sensor and determining a corresponding curve fit to that ST vs. CT
15 data. The result is curve fits individually corresponding to each sensor in the array. As indicated above, these curve fits can be linear fits and/or higher order polynomial curve fits.

20 E) Application to biological assays

Biological assays are an important application of the present methods. Here we consider DNA melting curve measurements as an example of temperature sensitive biological measurement. In this example, a magnetic labeled
25 single stranded DNA target is hybridized to surface tethered single stranded DNA probes. After binding, the temperature is raised and a melting curve is measured while the temperature is increased.

FIG. 5A shows the time series for such a melting. A
30 sigmoid fit was applied to the curves to calculate the time of melt for each sensor. The relative time delay results are shown in the table of FIG. 5B. The central sensors in

FIG. 5B are at a higher temperature and thus melt the DNA hybrids earlier. A melting time difference of 0.7 min corresponds to a temperature difference of 2.1 C that must be accounted for.

5 Using the temperature measured for each sensor as described above, we can provide this correction, as shown on FIGs. 6A-B. In FIG. 6A, the measured melting curves are similar to each other after this temperature correction and calibration. FIG. 6B shows the melting temperature
10 obtained by sigmoid fitting. The measured melting temperature for rows 1-5 vary less than 1 C. The other rows are affected by the wiring and epoxy potting in this particular case.

 According to these principles, the above described
15 temperature correction and double modulation methods can further include biological assays where the sample includes analytes labeled with magnetic nanoparticles and where one or more of the magnetoresistive sensors has capture reagents disposed thereon corresponding to the analytes,
20 such that binding and immobilization of analytes with corresponding capture reagents provides a magnetoresistive assay.

 More specifically, an exemplary assay according to this approach has the analytes and capture species being
25 complementary DNA strands, and further includes the following steps:

- 1) performing bisulphite conversion of DNA strands containing methylated and unmethylated sites to create converted DNA strands with mismatched base pairs;
- 30 2) performing polymerase chain reaction (PCR) amplification of the converted DNA strands;

3) magnetically labeling single strand target DNA strands among the PCR amplified converted DNA strands;

4) hybridizing the magnetically labeled single strand target DNA strands with complementary DNA strands
5 immobilized onto the magnetoresistive sensor array;

5) increasing a stringency condition to cause the magnetically labeled single strand target DNA strands to be denatured from the complementary DNA strands immobilized onto the magnetoresistive sensor array;

10 6) reading out in real time during the increasing of the stringency condition a denaturation signal resulting from the denatured magnetically labeled single strand target DNA strands; and

7) determining stringency conditions of methylated and
15 unmethylated DNA strands from the denaturation signal.

In this approach, the denaturation signal can be combined with the corresponding magnetoresistive sensor temperature T to individually provide accurate DNA melting curves for each magnetoresistive sensor, as in the examples
20 of FIGs. 6A-6B above.

E1) Further details on biological assay applications

FIG. 7 is a flow chart providing an overview of a method of methylation detection providing a quantitative
25 description of the methylation density in DNA strands. In step 700, bisulphite conversion of the DNA strands containing methylated and unmethylated sites is performed to create converted DNA strands with mismatch base pairs. In step 702, PCR amplification of the converted DNA strands
30 is performed. In step 704, single strand target DNA strands among the PCR amplified converted DNA strands are

magnetically labeled. In step 706, the magnetically labeled single strand target DNA strands are hybridized with complementary DNA strands immobilized onto a magneto-resistive (MR) sensor array. In some
5 implementations, during hybridizing a binding signal is read out in real time.

In step 708, a stringency condition is increased to cause the magnetically labeled single strand target DNA strands to be denatured from the complementary DNA strands
10 immobilized onto a magneto-resistive (MR) sensor array. In step 710, during the increasing of the stringency condition a denaturation signal resulting from the denatured magnetically labeled single strand target DNA strands is read out in real time. In step 712, stringency conditions
15 of methylated and unmethylated DNA strands are determined from the denaturation signal.

The stringency condition may be temperature, in which case increasing the stringency condition comprises increasing the temperature while salt concentration is held
20 constant. Determining the stringency conditions of the methylated and unmethylated DNA strands in this case comprises determining melting temperatures of the methylated and unmethylated DNA strands. Alternatively, the stringency condition may be salt concentration, in which
25 case increasing the stringency condition comprises decreasing the salt concentration while temperature is held constant. Determining the stringency conditions of the methylated and unmethylated DNA strands in this case comprises determining melting salt concentrations of the
30 methylated and unmethylated DNA strands.

In some implementations, the method may be used to determine mutation sites simultaneously with methylation sites. In this case, performing bisulphite conversion of

the DNA strands containing methylated and unmethylated sites includes performing bisulphite conversion of the DNA strands containing methylated and unmethylated sites and wild type genes and mutated genes. In addition, determining stringency conditions of methylated and unmethylated DNA strands from the denaturation signal in this case includes determining stringency conditions of methylated and unmethylated DNA strands and wild type genes and mutated type genes from the denaturation signal.

FIG. 8 provides a schematic overview of a protocol for the detection of magnetically labeled DNA using a GMR biosensor device 800. After denaturation of the reverse strand and labeling, PCR products are injected into the reaction well over the chip 804. In a hybridization step 812, DNA labeled with MNPs (magnetic nanoparticles) hybridizes to complementary surface-tethered probes for 1 hour at 37 °C, resulting in hybridized DNA labeled with MNPs 808. Unbound sample is removed by washing. In melting step 814, the temperature is swept from 20 °C to 65 °C, causing denaturation of the DNA from the probes 810 to measure the melting temperature, T_m .

To detect DNA mutations, we PCR amplified the genomic regions of interest using non-discriminatory primers. The PCR products were then magnetically labeled using biotinylated primers and streptavidin-coated magnetic nanoparticles (MNPs). After magnetic column separation and denaturation of the double-stranded PCR products, ssDNA conjugated to MNPs (MNP-ssDNA) was introduced to the GMR biosensor array where multiple DNA probes were separately tethered to the surface of each sensor. Upon hybridization of the injected MNP-ssDNA to surface-tethered complementary probes, GMR biosensors produced changes in sensor magneto-resistive ratio (ΔMR) proportional to the bound

MNPs. To genotype a mutation, we employed a set of two probes complementary to the wild type (WT) and mutant type (MT) sequences of the sample. During hybridization at low stringency, amplicons hybridized to both WT and MT probes
5 with similar affinity. To obtain single base specificity, stringent washing is typically used after hybridization in DNA microarray. To achieve a more flexible system for detection of single-base mutations, we challenged the hybrids by increasing the temperature and continuously
10 measuring DNA melting simultaneously for all probes on the GMR biosensor array.

FIGs. 9A-B are schematic illustrations of the bisulphite conversion process. Upon bisulphite treatment 902, 912, unmethylated cytosines in DNA 910 are converted
15 to uracil in DNA 914 (FIG. 9B) whereas 5-methylcytosines in DNA 900 are retained in DNA 904 (FIG. 9A). In the subsequent PCR 906, 916, which produces products 908, 918, uracil in 914 is substituted by thymine. Thus, the methylated cytosines are mapped to single base alterations
20 (C>T) of the amplicons.

CLAIMS

1. A method for temperature measurement in an array of magnetoresistive sensors, the method comprising:

5 providing an array of magnetoresistive sensors, wherein the array of magnetoresistive sensors includes at least one reference magnetoresistive sensor;

wherein R_0 is a zero field resistivity at a reference temperature T_0 , wherein T is temperature, wherein α is a material parameter of the magnetoresistive sensors, and
10 wherein the magnetoresistive sensors have a zero field resistivity R given by $R = R_0(1 + \alpha(T-T_0))$;

measuring zero field resistivity of the at least one reference magnetoresistive sensor at two or more known
15 temperatures to determine α ;

measuring zero field resistivity of one or more magnetoresistive sensors of the array of magnetoresistive sensors to determine corresponding temperatures T of the one or more magnetoresistive sensors from measured R and
20 known α .

2. The method of claim 1, wherein there are two or more reference magnetoresistive sensors, and wherein determining α comprises averaging α values of the two or more reference
25 magnetoresistive sensors.

3. The method of claim 1, further comprising

disposing the array of magnetoresistive sensors in a tickling magnetic field having a modulation frequency of f_h ;

providing an electrical excitation to the array of magnetoresistive sensors having a modulation frequency of f_v , wherein f_h and f_v are distinct, and wherein an output spectrum of each magnetoresistive sensor in the array of magnetoresistive sensors includes a carrier tone (CT) at frequency f_v and side tones (STs) at frequencies $f_v \pm f_h$, wherein the carrier tone has a CT amplitude and the side tones have an ST amplitude;

for each magnetoresistive sensor in the array of magnetoresistive sensors, individually determining a baseline relation between the CT amplitude and the ST amplitude in the output spectrum;

for each magnetoresistive sensor in the array of magnetoresistive sensors, individually measuring corresponding CT and ST amplitudes when a sample is present to provide an input CT measurement and an input ST measurement;

for each magnetoresistive sensor in the array of magnetoresistive sensors, individually correcting the input ST measurement using the corresponding baseline relation and the corresponding input CT measurement to provide a corresponding corrected ST measurement; and

for each magnetoresistive sensor in the array of magnetoresistive sensors, individually providing the corrected ST measurement as an output.

4. The method of claim 3, wherein the individually determining a baseline relation between CT amplitude and ST amplitude comprises collecting ST vs. CT data for each magnetoresistive sensor in the array of magnetoresistive sensors followed by determining a curve fit relating baseline ST amplitude to baseline CT amplitude for each

magnetoresistive sensor in the array of magnetoresistive sensors.

5 5. The method of claim 4, wherein for one or more of the magnetoresistive sensors in the array of magnetoresistive sensors, the curve fit is a linear fit.

6. The method of claim 4, wherein for one or more of the magnetoresistive sensors in the array of magnetoresistive
10 sensors, the curve fit is a polynomial fit.

7. The method of claim 6,

wherein the sample includes analytes labeled with magnetic nanoparticles, and

15 wherein one or more of the magnetoresistive sensors has capture reagents disposed thereon corresponding to the analytes, whereby binding and immobilization of analytes with corresponding capture reagents provides a magnetoresistive assay.

20

8. The method of claim 7, further comprising combining an assay signal of the magnetoresistive assay with the corresponding magnetoresistive sensor temperature T to individually provide accurate assay curves for each
25 magnetoresistive sensor.

9. The method of claim 8 wherein the assay signal is a DNA denaturation signal and wherein the assay curve is a DNA melting curve.

30

10. The method of claim 3,

wherein the sample includes analytes labeled with magnetic nanoparticles, and

wherein one or more of the magnetoresistive sensors
5 has capture reagents disposed thereon corresponding to the analytes, whereby binding and immobilization of analytes with corresponding capture reagents provides a magnetoresistive assay.

10 11. The method of claim 10, further comprising combining an assay signal of the magnetoresistive assay with the corresponding magnetoresistive sensor temperature T to individually provide accurate assay curves for each magnetoresistive sensor.

15

12. The method of claim 11 wherein the assay signal is a DNA denaturation signal and wherein the assay curve is a DNA melting curve.

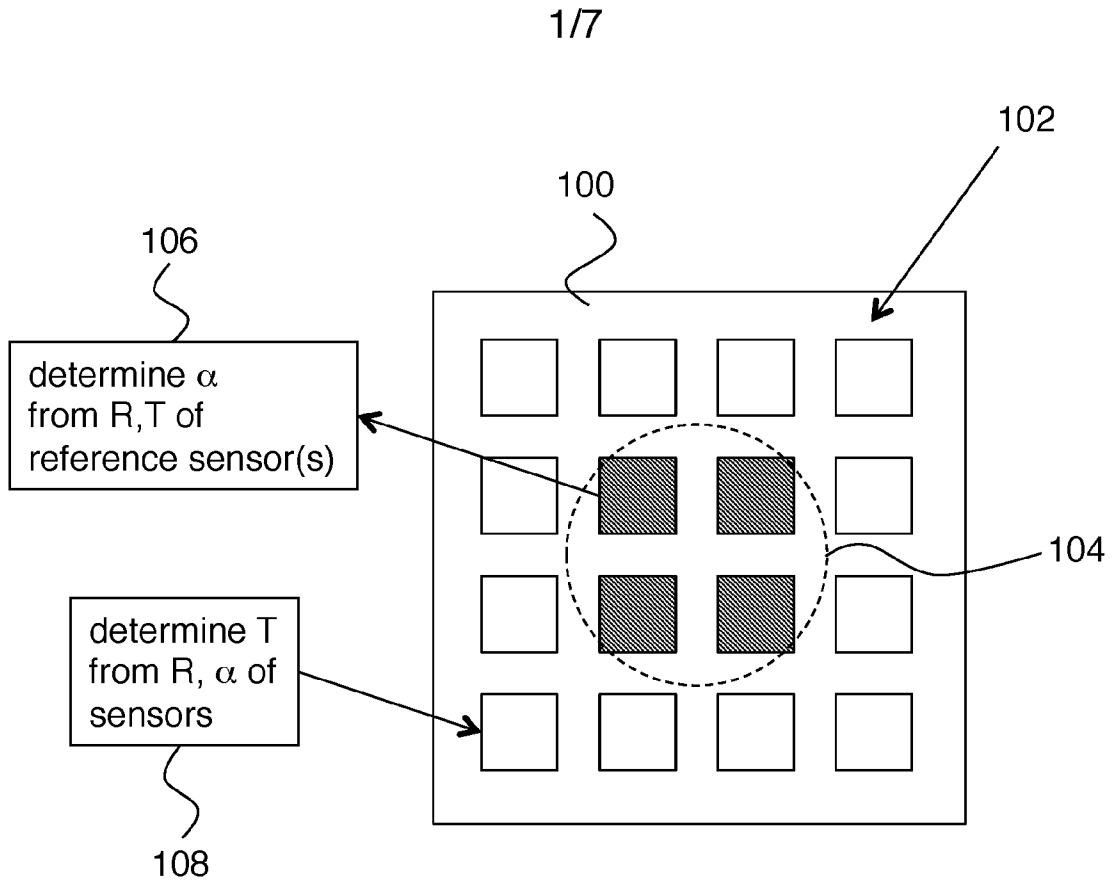


FIG. 1

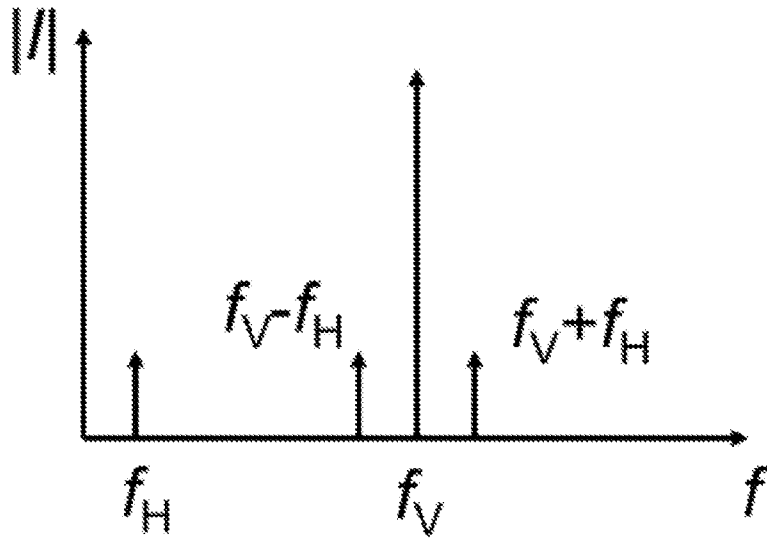


FIG. 2

NA	47.9	NA	48.8	48.5	NA	48.8	NA	NA	NA
48.6	48.5	48.7	48.2	48.8	48.9	48.8	48.3	48.5	48.3
48.0	48.2	48.5	48.9	48.4	48.8	48.3	48.4	48.4	48.2
48.6	48.2	48.4	NA	48.8	48.3	NA	49.0	48.5	48.2
49.0	48.6	48.7	48.6	48.5	48.4	48.1	48.3	48.4	47.6
49.3	49.0	49.0	48.4	48.7	48.9	48.7	48.2	NA	48.4
50.2	49.2	49.6	49.3	49.7	49.3	49.0	49.4	49.0	49.2
51.4	52.6	52.4	51.7	51.6	51.3	51.7	51.0	50.2	49.5

FIG. 3

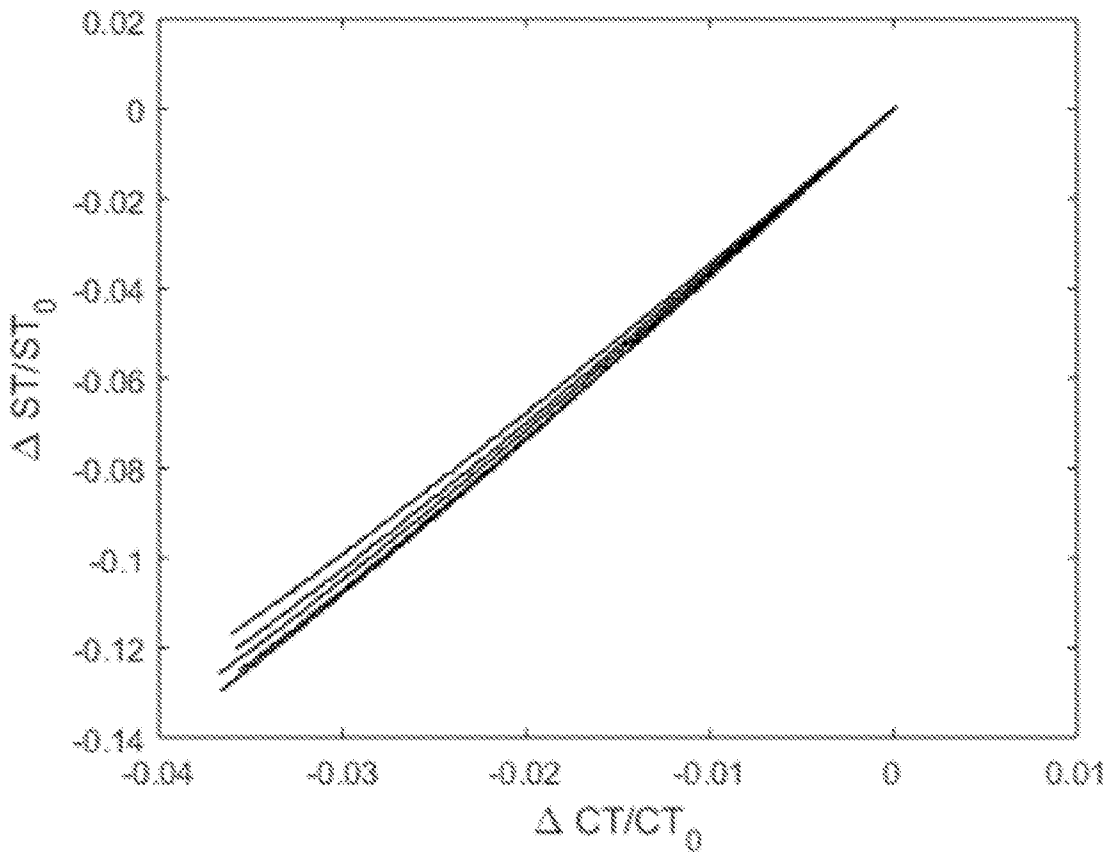


FIG. 4

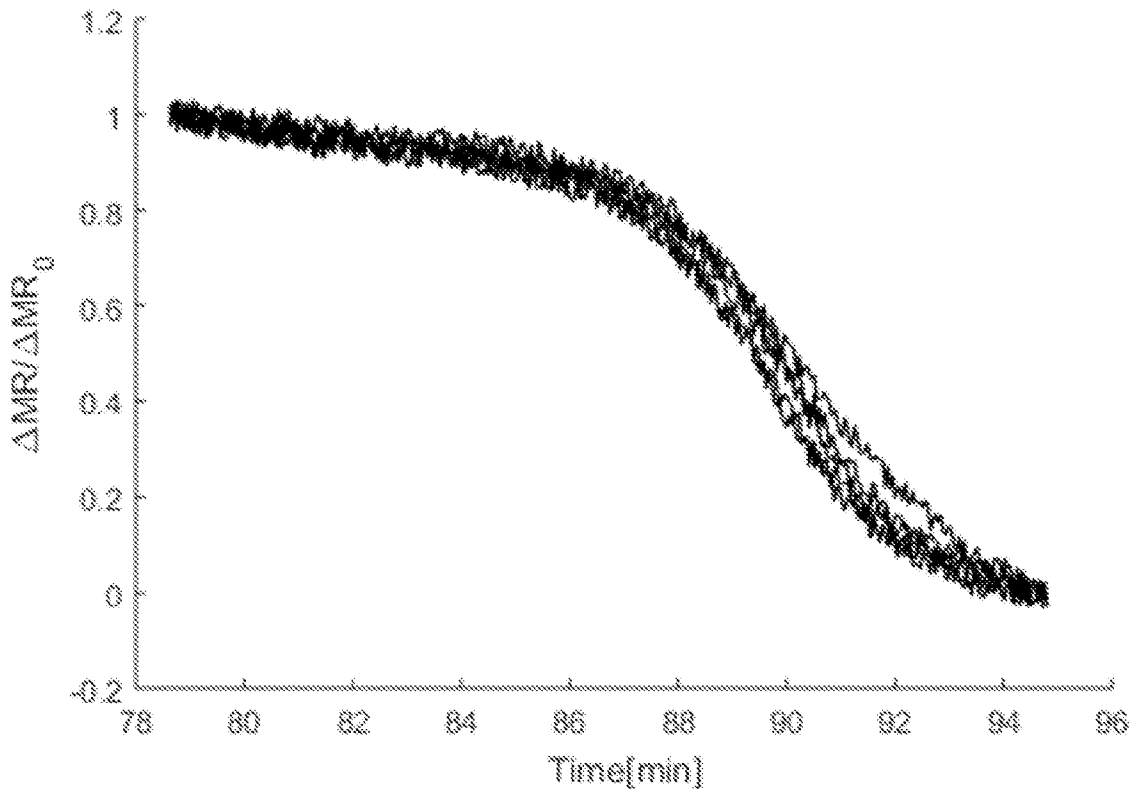


FIG. 5A

NA	0.6	NA	0.6	0.7	NA	0.7	NA	NA	NA
0.7	0.5	0.5	0.4	0.4	0.5	0.4	0.4	0.5	0.5
0.5	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.5
0.5	0.5	0.3	NA	0.2	0.2	NA	0.2	0.4	0.5
0.7	0.5	0.3	0.2	0.1	0.0	0.1	0.2	0.4	0.5
0.7	0.5	0.4	0.3	0.1	0.1	0.2	0.3	NA	0.5
1.0	0.7	0.6	0.4	0.3	0.3	0.3	0.6	0.5	0.8
1.0	1.7	1.5	1.3	1.1	1.0	1.2	1.1	0.9	0.9

FIG. 5B

4/7

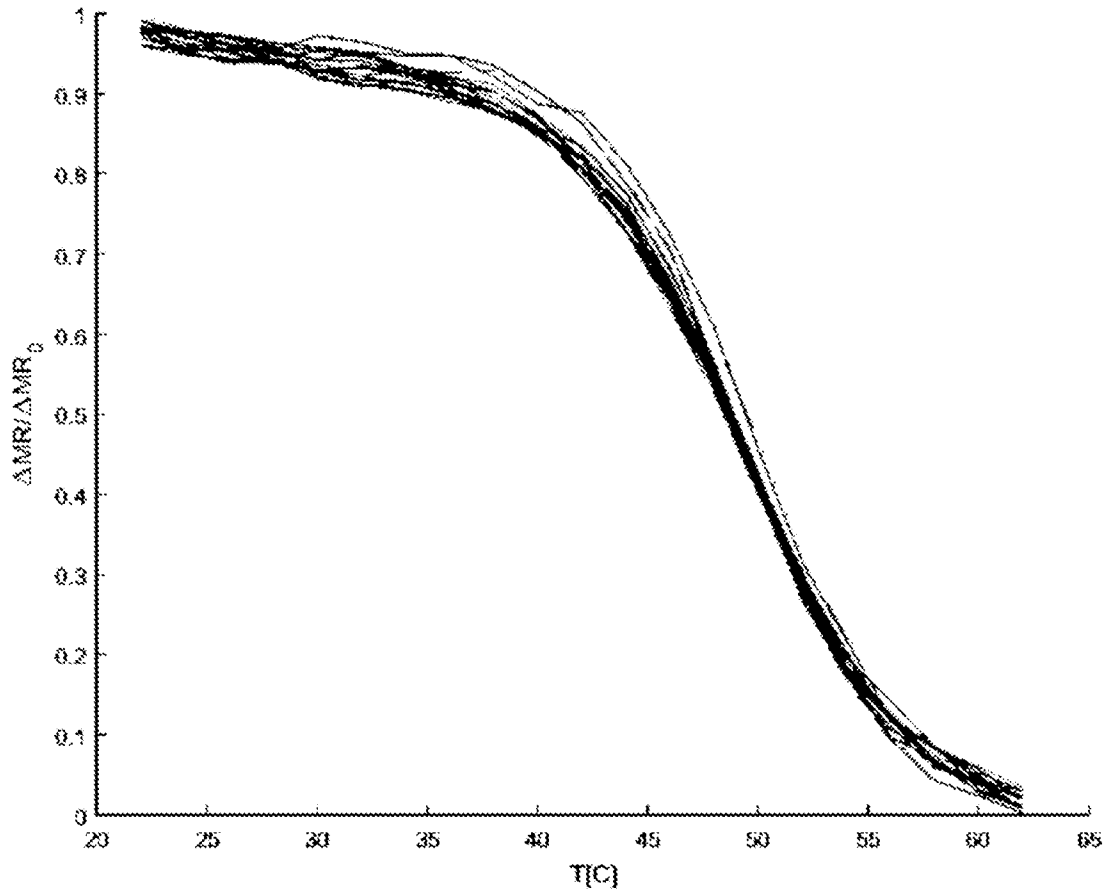


FIG. 6A

57.6	58.0	58.3	58.5	58.6	58.7	58.5	58.3	58.1	57.8
57.8	58.1	58.4	58.6	58.8	58.7	58.4	58.4	58.1	57.7
57.9	58.2	58.4	58.5	58.7	58.8	58.7	58.5	58.3	58.0
58.0	58.4	58.6	58.9	59.0	58.9	58.5	58.3	58.2	57.9
57.6	58.0	58.3	58.9	59.3	59.3	59.1	58.7	58.3	57.9
57.8	58.1	58.5	59.0	59.3	59.3	59.0	58.4	58.0	57.6
57.6	58.0	58.4	58.8	59.1	59.2	59.0	58.6	58.2	57.9
57.7	58.0	58.4	58.7	59.0	59.0	58.6	58.2	57.9	57.6
57.4	57.7	58.0	58.3	58.5	58.6	58.5	58.2	58.0	57.7

FIG. 6B

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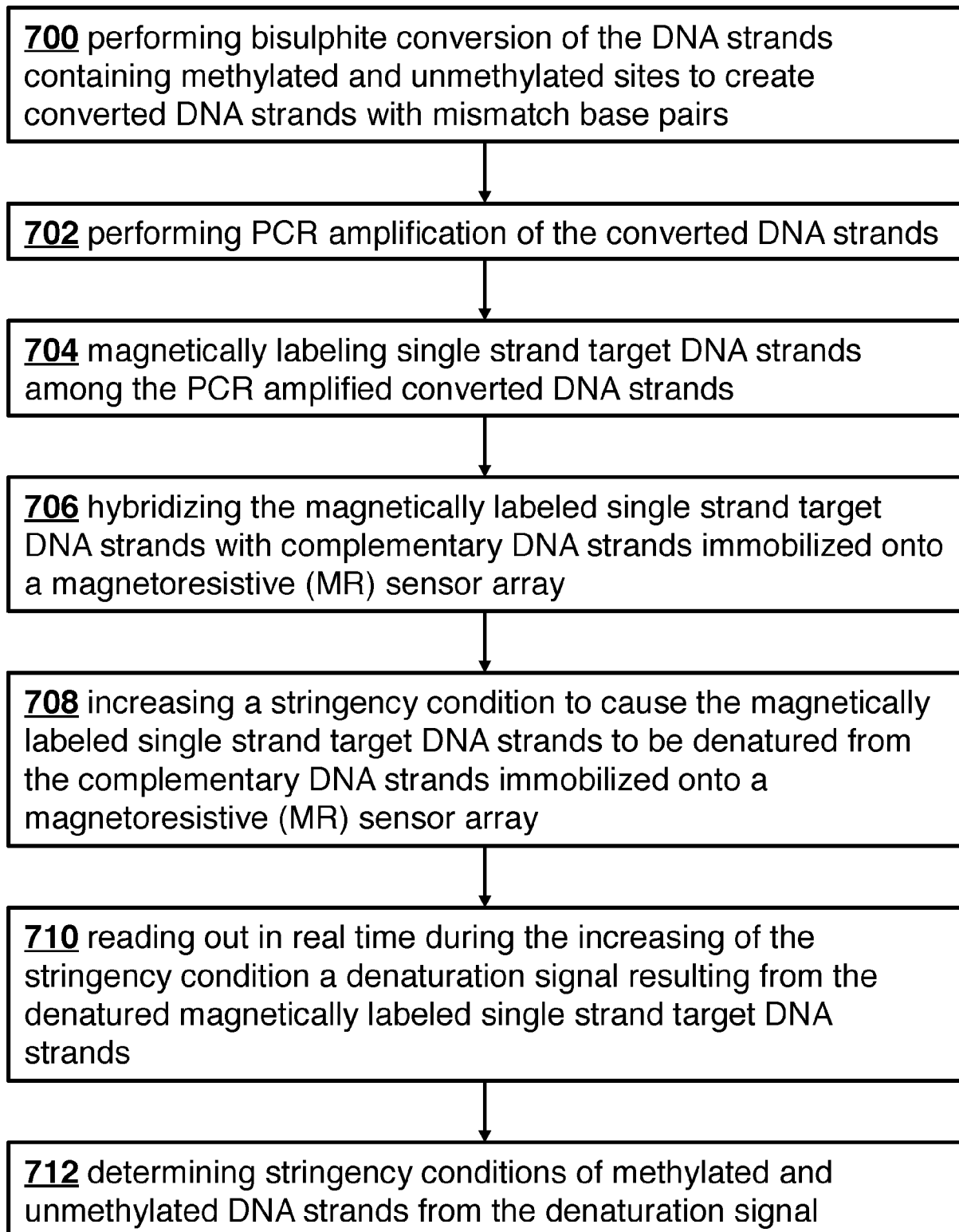


FIG. 7

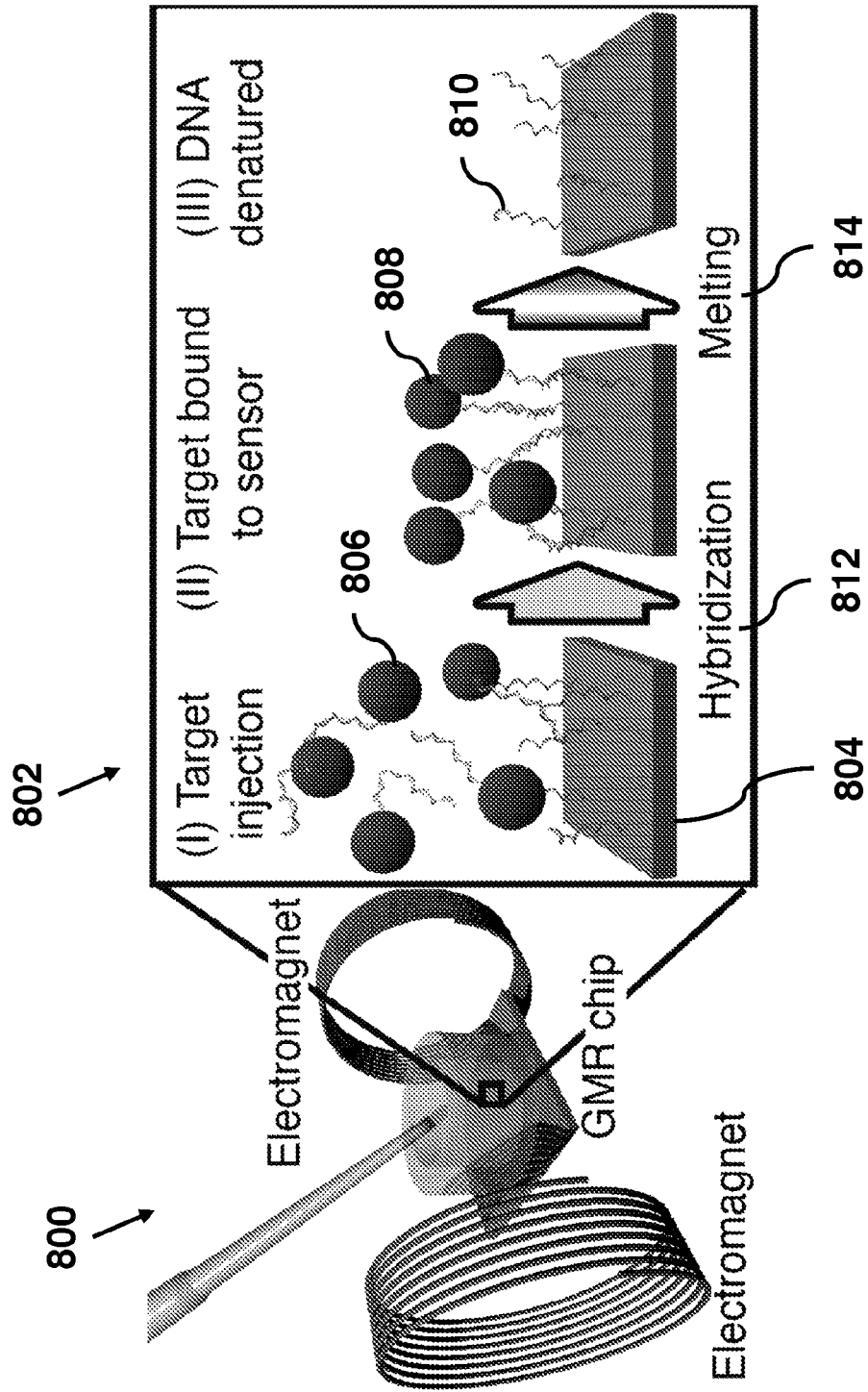


FIG. 8

FIG. 9A

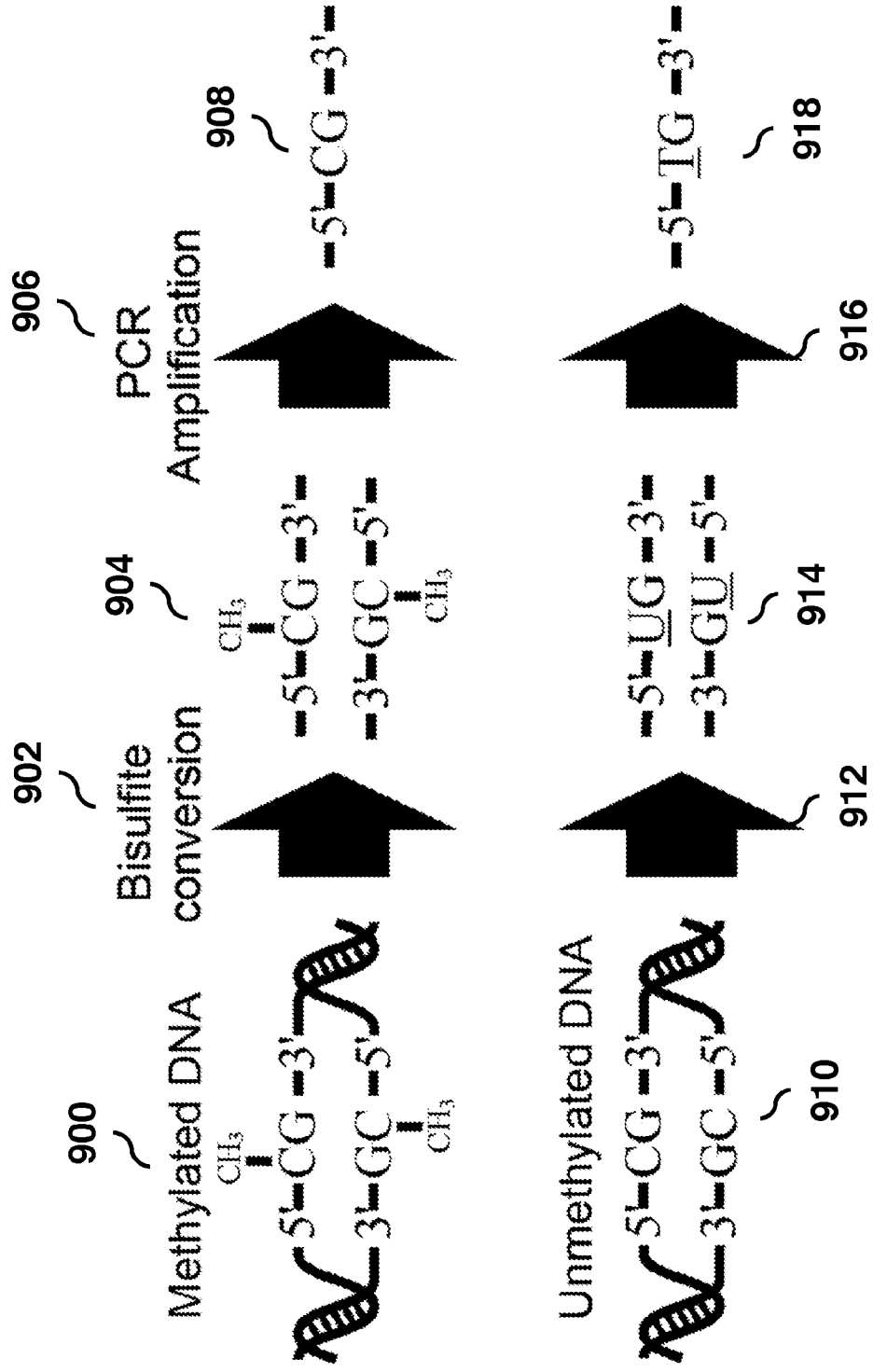


FIG. 9B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/30445

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01R 33/06; G01R 33/09 (2018.01)

CPC - B82Y 25/00; G01R 33/007; G01R 33/0082; G01R 33/0094; G01R 33/06; G01R 33/09

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)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	US 2010/0231213 A1 (NIEUWENHUIS et al.) 16 September 2010 (16.09.2010), Fig 1-3, abstract, para [0044], [0079], [0080]	1, 3-12 ----- 2
Y --- A	US 2016/0161343 A1 (ADVANCED LIQUID LOGIC, INC.) 09 June 2016 (09.06.2016), abstract, para [0091]-[0096]	1, 3-12 ----- 2
Y	US 8,405,385 B2 (HALL et al.) 26 March 2013 (26.03.2013), Fig 1, 4, abstract, col 2, ln 38-61, col 3, ln 28-46, col 4, ln 42-57, col 5, ln 10-38	3-12
Y	US 6,106,777 A (FUJITA et al.) 22 August 2000 (22.08.2000), abstract, col 2, ln 60-65	9, 12
A	US 7,508,203 B2 (TAKENAGA et al.) 24 March 2009 (24.03.2009), abstract	2

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

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"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

27 June 2018

Date of mailing of the international search report

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