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(54) **SYSTEMS AND METHODS FOR CALIBRATION USING DYE SIGNAL AMPLIFICATION**

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(57) **ABSTRACT**

The present teachings relate to a method of generating calibration information during a real-time polymerase chain reaction (RT-PCR) or other amplification reaction. A sample well plate or other support can contain one or more dyes or other reference materials that are subjected to the same RT-PCR thermal cycles or other conditions used to conduct amplification or other reactions on a biological sample. A set of maxima values and a set of minimum values, and/or other calibration information useful for adjusting emission data for sample dyes can be recorded, for example, for 10 cycles, 20 cycles, or each cycle of a complete RT-PCR run. Such testing of dye response under realistic operating conditions can enable more accurate characterization of plate, dye, filter, or instrument response and therefore more accurate calibration corrections and other and/or adjustments.

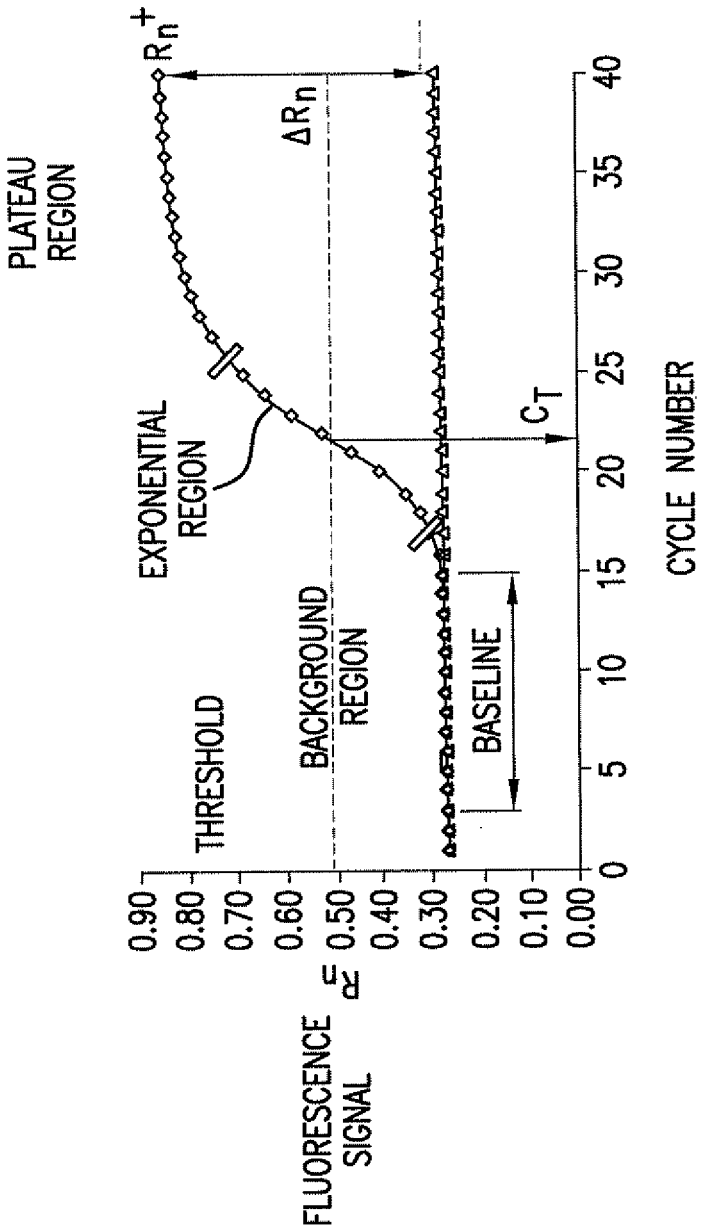
PLATEAU REGION
EXPONENTIAL REGION
THRESHOLD
BACKGROUND REGION
BASELINE
$R_n^+$
$\Delta R_n$
$C_T$
FLUORESCENCE SIGNAL $\Delta R$
CYCLE NUMBER
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
5
10
15
20
25
30
35
40

FIG. 1

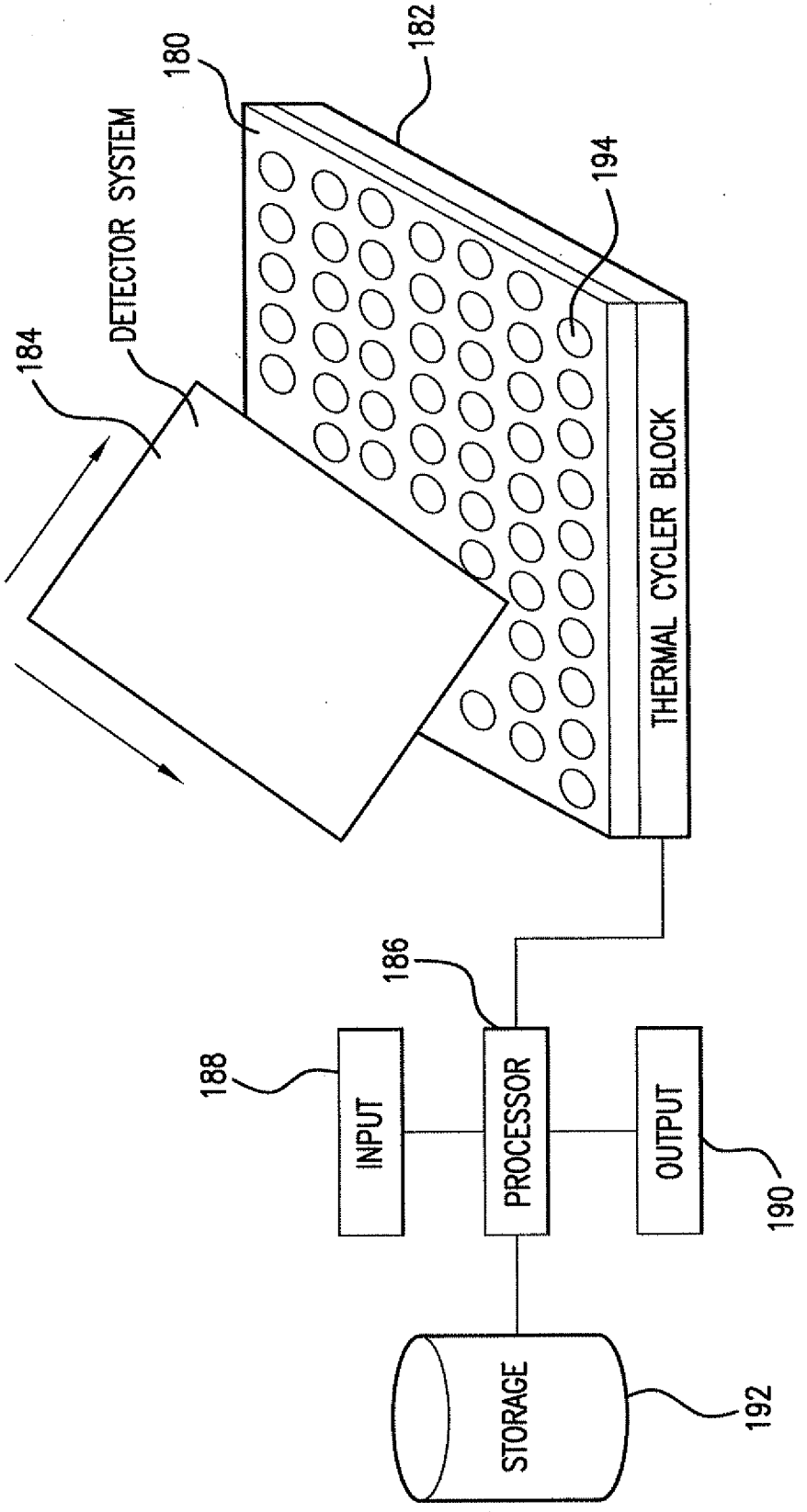
180
182
194
DETECTOR SYSTEM
184
THERMAL CYCLER BLOCK
186
188
INPUT
PROCESSOR
OUTPUT
190
STORAGE
192

FIG. 2

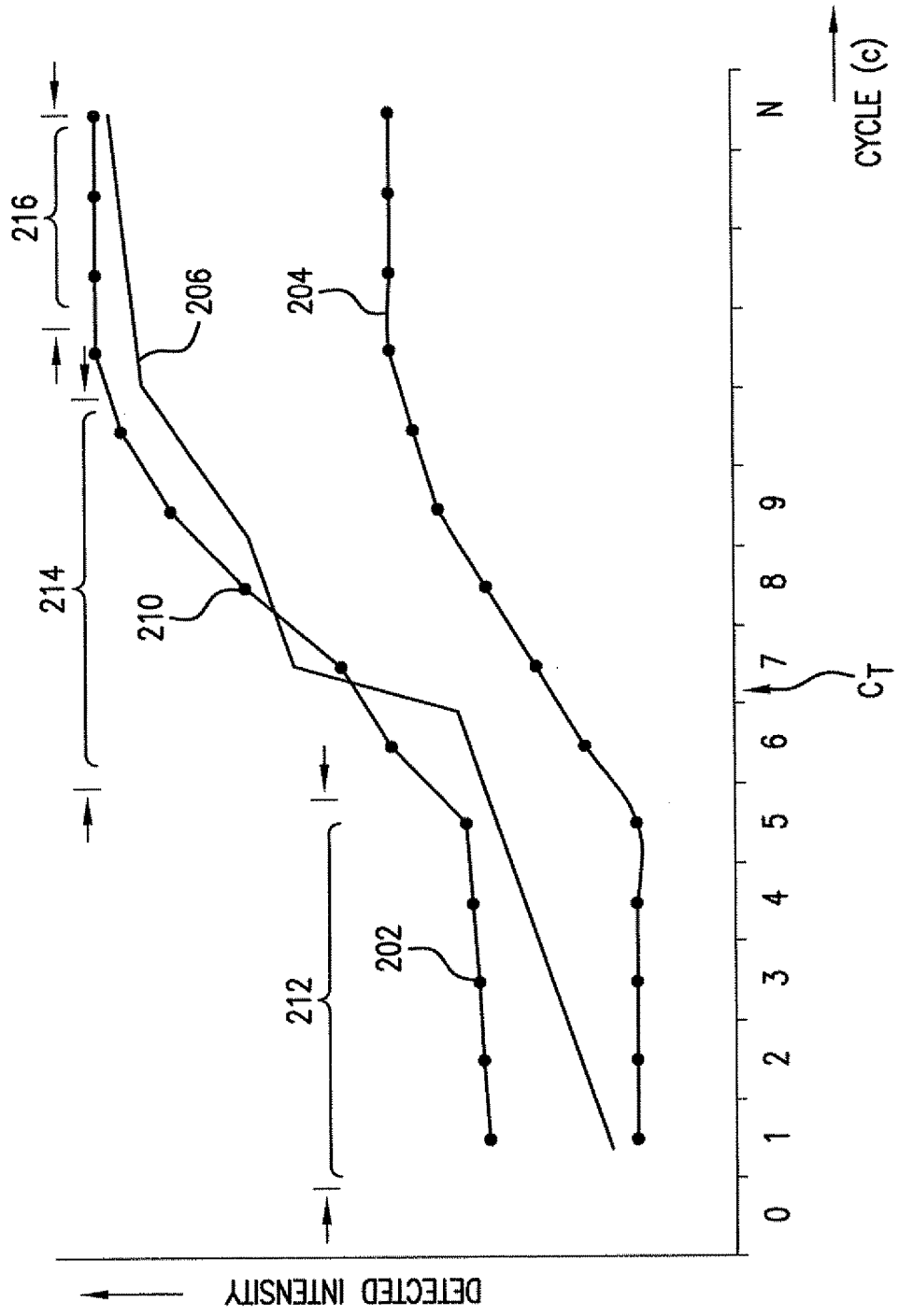
DETECTED INTENSITY
CYCLE (c)
0
1
2
3
4
5
6
7
8
9
N
$C_T$
202
204
206
210
212
214
216

FIG.3

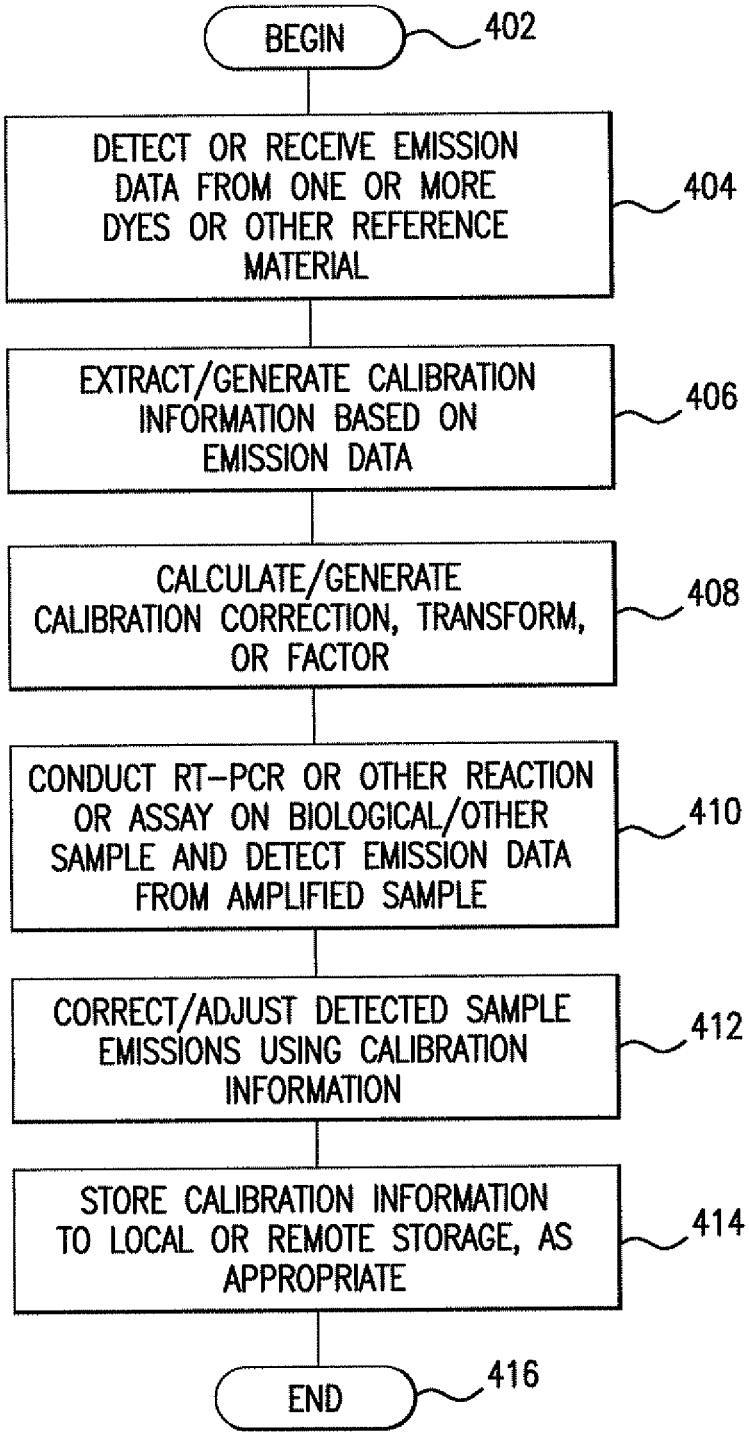
BEGIN
402
DETECT OR RECEIVE EMISSION DATA FROM ONE OR MORE DYES OR OTHER REFERENCE MATERIAL
404
EXTRACT/GENERATE CALIBRATION INFORMATION BASED ON EMISSION DATA
406
CALCULATE/GENERATE CALIBRATION CORRECTION, TRANSFORM, OR FACTOR
408
CONDUCT RT-PCR OR OTHER REACTION OR ASSAY ON BIOLOGICAL/OTHER SAMPLE AND DETECT EMISSION DATA FROM AMPLIFIED SAMPLE
410
CORRECT/ADJUST DETECTED SAMPLE EMISSIONS USING CALIBRATION INFORMATION
412
STORE CALIBRATION INFORMATION TO LOCAL OR REMOTE STORAGE, AS APPROPRIATE
414
END
416

FIG.4

# SYSTEMS AND METHODS FOR CALIBRATION USING DYE SIGNAL AMPLIFICATION

## RELATED APPLICATION

**[0001]** This application is a divisional of application Ser. No. 14/750,463 filed Jun. 25, 2015, which is a continuation of application Ser. No. 13/673,574 filed Nov. 9, 2012, now abandoned, which is a continuation of application Ser. No. 12/022,079 filed Jan. 29, 2008, now abandoned, which claims priority to U.S. Provisional Application No. 60/898,064, filed Jan. 29, 2007, all of which are incorporated herein in its entirety by reference.

## FIELD

**[0002]** The present application relates to biological testing devices, systems that contain such devices, and methods that use such devices and/or systems.

## INTRODUCTION

**[0003]** Real-time polymerase chain reaction (RT-PCR) technology, as presently practiced, relies upon the accurate detection of fluorescent emission signals above an initial baseline. The baseline signal can represent a combination of spurious or unwanted signal contributions such as the residual fluorescence contributed by the plastic or other material of a sample plate, the fluorescence of a buffer or other non-reactant liquid material, noise in the optical detector or detection electronics, or other sources of background signal noise or detection floor that are not a product of the desired PCR amplification. In various known RT-PCR implementations, better accuracy in the detection of an amplification signal, and hence original sample quantity, is frequently sought by characterizing the baseline floor over the first few PCR cycles, and then subtracting the baseline from the detected emissions once an inflection point into the exponential region has been reached.

**[0004]** Known calibration techniques for calibrating or adjusting the detected emission data captured by a RT-PCR or other instrument can involve calibration based on a plate loaded with a pure dye formulation but no sample material. A RT-PCR detector can, for instance, take emission readings from a standard 96-well sample plate or other support, to determine intensity range or spectral behavior, and perform a calibration or normalization based on those values. Such calibration techniques, however, depend on the emission data from a reference dye or other material under static conditions. Moreover, the calibration factors generated by known pure-dye calibration techniques can be limited to fixed scaling factors or other adjustments, whereas the intensity range and spectral behavior of actual emission data can possibly vary with time under the varying cycles and conditions of an RT-PCR or other analytic process. Reference calibration does not match actual performance calibration due to real time running effects, such as thermal cycling, condensation, evaporation, chemistry changes, and the like. A need exists for calibration and related techniques that address these and other issues.

## SUMMARY

**[0005]** Systems and methods according to various embodiments of the present teachings, relate to techniques and platforms to capture, identify, and calibrate emission data of an RT-PCR or other amplification reaction, on a real-time basis in which the reference dye, actual RT-PCR reactions, or other material can itself be subjected to RT-PCR thermal cycling or other dynamic processing. According to various embodiments, the reference dye can be subjected to the same number and type of RT-PCR thermal cycles as a sample being tested is exposed to, and can produce a time-varying emission output. The emission output data can be captured and stored across the entire set of cycles of the RT-PCR or other process. The detected intensity range, spectral response, and other characteristics of the amplified dye or other material can be used to generate a calibration curve, function, or other calibration data set whose ranges, limits, or other parameters can vary with time and be set to correlate with different cycles in an RT-PCR, or other assay or reaction. As a result, better calibration accuracy over the entire RT-PCR process can be achieved. In some embodiments, the calibration occurs during the actual amplification reaction that is sought to be calibrated such that even minor differences due to DNA sequence construction, pH, and other variables that affect the spectrum of a dye can be accounted for.

## FIGURES

**[0006]** FIG. **1** illustrates an exemplary PCR amplification profile or curve, according to various embodiments of the present teachings.

**[0007]** FIG. **2** illustrates a schematic of a PCR detection system, according to various embodiments of the present teachings.

**[0008]** FIG. **3** illustrates a calibration data set for a PCR amplification profile or curve, according to various embodiments of the present teachings.

**[0009]** FIG. **4** illustrates a flowchart of calibration processing, according to various embodiments of the present teachings.

## DESCRIPTION

**[0010]** According to various embodiments, a method of generating calibration information for an amplification reaction is provided. The method can comprise performing a first amplification reaction on at least one reference material in a sample support of an analytical instrument, performing a second amplification reaction on a sample in a sample support of the analytical instrument, receiving first emission data generated by the at least one reference material during the first amplification reaction, receiving second emission data generated by the sample during the second amplification reaction, generating calibration information based on the received first emission data, and adjusting the received second emission data as a function of the calibration information. In some embodiments, the at least one reference material can comprise at least one dye, for example, at least one fluorescent dye. In some embodiments, the first and second amplification reactions can each comprise a polymerase chain reaction. In some embodiments, the first amplification reaction can be performed simultaneously with the second amplification reaction, or before or after the second amplification reaction. Herein, the term "emission" is used to exemplify a signal detected and/or calibrated according to various embodiments of the present teachings. It is to be understood that by "emission" the present teachings are referring to not only electromagnetic radiation but

rather are also referring to any physical or chemical signal or other data that can be read, detected, imaged, or surmised from one or more area of interest, for example, a support region such as a well of a multi-well plate. "Emission" herein is intended to encompass electromagnetic radiation, optical signals, chemiluminescent signals, fluorescent signals, radiation transmission values, and radiation absorption values.

**[0011]** According to various embodiments, the calibration information generated can comprise time-varying information corresponding to multiple cycles of the first amplification reaction. In some embodiments, the calibration information generated comprises at least one of a set of maximum values of the emission data, a set of minimum values of the emission data, and spectral response information derived from the first emission data.

**[0012]** According to various embodiments, a system is provided for generating calibration information. The system can comprise an input unit configured to receive first emission data generated by at least one reference material during an amplification reaction and configured to receive second emission data generated by at least one sample during an amplification reaction. The system can also comprise a processor communicating with the input unit and configured to generate calibration information based on the first emission data and to adjust the second emission data based on the calibration information. In some embodiments, the at least one reference material used in such a system can comprise at least one dye, for example, at least one fluorescent dye. In some embodiments, the system can further comprise at least one sample support and at least one reference material, wherein the at least one reference material comprises a dye and the dye is disposed in the at least one sample support. The input unit can be configured to receive first emission data generated by at least one reference material during a polymerase chain reaction. The processor can be configured to generate calibration information comprising time-varying information corresponding to multiple cycles of an amplification reaction. In some embodiments, the processor can be configured to generate calibration information comprising at least one of a set of maximum values of the first emission data, a set of minimum values of the first emission data, and spectral response information derived from the first emission data.

**[0013]** According to various embodiments, a set of calibration information can be provided that is generated by a method comprising disposing at least one amplifiable reference material into a sample support, performing multiple cycles of an amplification reaction on the at least one reference material in the sample support, receiving emission data generated by the at least one reference material during the multiple cycles of the amplification reaction, and generating a set of calibration information based on the received emission data. In some embodiments, the emission data can comprise fluorescent intensity data generated during at least 10 cycles of polymerase chain reaction. In some embodiments, the emission data can comprise emission data generated during a polymerase chain reaction. In some embodiments, the set of calibration information can comprise time-varying information corresponding to at least 10 cycles of the amplification reaction. In some embodiments, the set of calibration information can comprise at least one of a set of maximum values of the emission data, a set of minimum values of the emission data, and spectral response information derived from the emission data. In various embodiments, the set of calibration information can comprise at least one set of maximum values of time-varying information corresponding to at least 10 cycles of the amplification reaction and at least one set of minimum values of time-varying information corresponding to at least 10 cycles of the amplification reaction.

**[0014]** Various embodiments of the present teachings relate to systems and methods for generating a calibration data set for an RT-PCR or other amplification curve, signature, graph, profile, or data, using the detection of reference dye or other material that is subjected to RT-PCR cycling or other process conditions. According to various embodiments, an amplification curve, signature, graph, profile, or other data can be received from detection of fluorescent emissions of one or more reference dyes, or other reference material, in an RT-PCR instrument or other instrument. The calibration systems and methods can be implemented in or applied to RT-PCR scanning systems or RT-PCR imaging systems, or other systems or platforms. In some embodiments, systems and methods according to the present teachings can be applied to non-real-time PCR instruments.

**[0015]** According to various embodiments, RT-PCR or other processing can take place using a standard sample plate, such as a 96-well or other standard well number and/or well capacity microtiter plate. In some embodiments, each well, container, or other location of a plate or tray can contain samples, for example, samples of DNA fragments or other materials, to which one or more spectrally distinct dye is attached for detection and analysis. A calibration, normalization, or other adjustment can be performed to normalize, adjust, or otherwise increase the consistency and/or accuracy of readings taken from the sample wells. The normalization or calibration can correct or compensate for variations due to or affected by factors which include, for example, differences in overall signal intensity or amplitude, varying dye or sample concentrations, contaminations, spectral or amplitude distortions, deviations in optical path, plate geometry, fluorescent noise floor, sample population or size, or other variations or anomalies that can arise from dye-to-dye, well-to-well, plate-to-plate, or instrument-to-instrument variations.

**[0016]** According to various embodiments, an RT-PCR emission or other amplification graph, chart, or profile typically displays three sections or regions: an initial baseline region, an exponential region, and a plateau region. An example of this is shown in the illustration in FIG. **1**. The baseline region can display a linear, or approximately linear, or other form over the first several cycles, as reaction chemistries have not liberated enough marker dye to rise over the detected background. The next, exponential region represents the rise of amplification product over the noise or background floor, as the PCR reaction kinetics come into force. The plateau region typically exhibits a final flattening or tapering of detected emission intensities, as reagents are exhausted. The combined amplification profile usually resembles a sigmoid or S-shape. In some embodiments, the RT-PCR system can determine a threshold cycle ($C_T$) that represents the cycle point at which the exponential threshold is reached.

**[0017]** According to various embodiments, calibration of an instrument, filter, or channel, sample plate, or other component, equipment, or hardware can be performed in connection with an RT-PCR system, for example, an overall

system as schematically illustrated in FIG. **2**. According to embodiments and as shown, an RT-PCR system can comprise a detector system **184**, such as a scanning or whole-plate imaging optical detection element, that can comprise, for example, a photomultiplier tube, CCD device, or other detection element. Detector system **184** can communicate with a processor **186**. Processor **186** can communicate with an input module **188**, an output module **190**, and/or a storage module **192**, such as a local or networked disk storage. Detector system **184** can also scan or image a sample plate **180**, to detect optical emissions from a set of sample wells **194**, such as wells arranged in a standard 96-well, 384-well, or other multi-well array. According to various embodiments, during operational use, sample wells **194** can contain samples combined with reagents useful to conduct an RT-PCR run. In some embodiments, the RT-PCR processing can comprise operating the system at a series or cycle of RT-PCR temperatures regulated by thermal cycler block **182**. The temperatures can subject the reactants in sample wells **194** to a desired sequence of denaturing, annealing, extension, and other steps.

**[0018]** According to various embodiments, to prepare and perform calibration processing, the sample wells **194** can contain one or more reference dyes or other reference materials, that are subjected to RT-PCR processing. The reference material used for calibration can comprise the same dye or dyes used for PCR processing of biological samples. According to other embodiments, the reference material can comprise one or more different dyes compared to those used for PCR processing of biological samples. The reference material can comprise a fluorescent dye attached to a polynucleotide or it can comprise an intercalating dye affixed to, or that can affix to, a polynucleotide. It will be appreciated that the reference material can comprise a dye or dyes that are not attached or affixed to a polynucleotide. According to various embodiments, the reference material can comprise a liquid dye, a solid dye such as a powder material, or another liquid, solid, or gaseous material. According to various embodiments, the reference material can comprise material that is not a fluorescent dye, or other type of dye.

**[0019]** According to various embodiments, and as shown in FIG. **3**, for example, the output of a calibration run conducted using dye or other reference material by performing part or all of an RT-PCR run, can comprise a set of detected emission data **210**, that can represent detected intensities and/or spectra of fluorescent or other emission profiles produced by the reference material, during an RT-PCR cycle. According to various embodiments, emission data **210** can comprise discrete values. These discrete values can be interpolated, re-sampled, or oversampled, to produce a more dense, or differently-spaced, collection of data points. The emission data **210** can, as illustratively shown, can comprise a set of minimum values **204** and a set of maximum values **202**, for a subject dye or other reference material. The set of minimum values **204** and the set of maximum values **202** can comprise data points taken at discrete cycles in an RT-PCR processing run.

**[0020]** In some embodiments, emission data **210** can comprise a continuous curve or trace. The emission data can extend over a total number of cycles from **1** to N, where N can be the endpoint of an RT-PCR run, such as **30**, **35**, **40**, or another number of cycles. According to various embodiments, the horizontal axis of the illustrative emission signature or profile, as shown in FIG. **3**, can comprise cycle numbers, or it can comprise time units. The vertical axis can comprise fluorescence, absolute or relative amplitude or intensity units, or other measures. In some embodiments, the vertical axis can, for example, reflect detected emission and/or intensity values on a logarithmic scale.

**[0021]** According to various embodiments, the calibration run performed to calibrate the baseline correction can produce information including, but not limited to, a set of minimum values **204** and a set of maximum values **202**. These values can be produced for a subject dye, reference material, or other standard, over a complete set of RT-PCR cycles. Emission data **206** generated by the RT-PCR processing of a biological sample can be adjusted, corrected, or normalized using the set of minimum values **204** and the set of maximum values **202**. For example, according to various embodiments, data points or output curves that deviate outside the set of minimum values **204** and the set of maximum values **202** can be presumed to be invalid. These values can be discarded, corrected, or adjusted to account for the validated intensity range determined by the emission calibration. According to various embodiments, a calibration correction, factor, or other adjustment can comprise a scaling factor or value, or can comprise a function or transform that can apply a correction on a time-varying basis. The calibration based on one or more amplified dyes or other reference materials can be performed before RT-PCR or other processing is conducted on biological samples.

**[0022]** According to various embodiments, the calibration based on one or more amplified dyes or other reference materials can be conducted at the same time as an amplification reaction, or other process, for example, at the same time that an RT-PCR process is conducted on biological samples. In some embodiments, calibration using amplified dye or other reference detection can be conducted in the same RT-PCR processing run. This can be accomplished, for example, by loading reference dye into one or more empty wells, and detecting and processing the amplified dye calibration during the same RT-PCR or other cycles that the samples would be subjected to. According to various embodiments, calibration conducted at the same time or in the same run as sample amplification can eliminate the necessity for preparing a separate preceding dye amplification run, thereby streamlining PCR processing, economizing on technician time, and conserving other materials or resources.

**[0023]** In some embodiments, the set of calibrations, corrections, or adjustments based on dye or other reference material, can be carried out on a set of plates or other platforms, at the time of manufacture. In some embodiments, the calibration correction, or adjustment can be carried out in the field by a technician, for example, before an RT-PCR run. The calibration, correction, or other adjustment can be stored in electronic memory, such as in a read-only memory (ROM) embedded in an instrument, or in a database stored on a local or remote server or stored on another resource. According to various embodiments, the calibration correction, or other adjustment can be stored in portable media, such as in a CD-ROM, or in another type of optical or electronic disc.

**[0024]** According to various embodiments, emission data **210** can be collected for a calibration with respect to multiple dyes or other reference materials, and applied to RT-PCR runs employing one or more of those dyes. It will

be appreciated that the calibration techniques described in embodiments herein, that relate to a calibration using one or more dyes or other reference materials, can be combined with other calibrations or uniformity correction techniques. For example, calibration derived from one or more reference dyes can be combined with baseline normalization to account for spurious fluorescent background. When baselining is employed, techniques such as those described in U.S. Pat. No. 7,228,237 to Woo et al., which is incorporated herein in its entirety by reference, can be used to isolate and identify a baseline region **212** and baseline signal **202** located in baseline region **212** of emission data **210**.

**[0025]** According to various embodiments, and as shown in FIG. **4**, the overall calibration based on dye or other reference amplification can be illustrated in a flowchart. In step **402**, processing can begin. In step **404**, emission data **210** from amplification of one dye or other reference material in an RT-PCR or other amplification process can be detected or received. In step **406**, calibration information such as a set of minimum values **204**, a set of maximum values **202**, spectral response, or other data or information characterizing or related to emission data **210**, can be extracted, calculated, or generated. In step **408**, a calibration constant, or other calibration factor, function, or transform, can be calculated or generated. In step **410**, the emission data from one or more biological samples being tested in an RT-PCR or other reaction or process can be detected and recorded. In step **412**, the emission data produced by the biological or other sample can be corrected, normalized, or otherwise adjusted using the set of normalized values **204**, the set of maximum values **202**, the spectral responses, or other calibration information. For example, emission data for samples taken from sample wells that exceed the maximum values or fall under the minimum values for a given dye or other reference material at a given cycle, can be discarded, adjusted, or flagged for operator review. In step **414**, the calibration information or any part thereof, derived from amplified dyes or other reference materials, can be stored, for example, to a local hard disk, network storage site, or other local or remote location or data store. In step **416**, processing can repeat, return to a prior processing point, proceed to a further processing point, or end.

**[0026]** Various embodiments of the present teachings can be implemented, in whole or part, in digital electronic circuitry, or in computer hardware, firmware, or software, or in a combination thereof. In some embodiments, apparatuses of the present teachings can be implemented in a computer program, software, code, or algorithm embodied in a machine-readable media. This media can comprise electronic memory, a CD-ROM, a DVD disc, a hard drive, or another storage device or media used for execution by a programmable processor. Various method steps can be performed by a programmable processor to generate output data by executing a program of instructions, functions, or processes on input data.

**[0027]** The present teachings can be implemented in one or more computer programs that are executable on a programmable system that can include at least one programmable processor coupled to receive and transmit data and instructions, to and from a data storage system or memory. The system can include at least one input device, such as, a keyboard or mouse, and at least one output device, such as, a display or printer. Each computer program, algorithm, software, or code can be implemented in a high-level procedural or object-oriented programming language, or in assembly, machine, or other low-level language, if desired. The code or language can be a compiled, interpreted, or otherwise processed for execution.

**[0028]** According to various embodiments, processes, methods, techniques, and algorithms can be executed on processors that can include, for example, both general and special purpose microprocessors, such as those manufactured by Intel Corp. or AMD Inc. The processors can also include digital signal processors, programmable controllers, or other processors or devices. According to various embodiments, a processor can receive instructions and data from a read-only memory and/or from a random access memory. A computer implementing one or more aspects of the present teachings can comprise one or more mass storage devices for storing data files, such as a magnetic disk, an internal hard disk, removable disk, magneto-optical disk, a CD-ROM, a DVD, a Blu-Ray disk, or another storage disk or media.

**[0029]** According to various embodiments, memory or storage devices suitable for storing, encoding, or embodying computer program instructions or software and data can comprise, for example, any form of volatile and/or non-volatile memory. This type of memory can comprise, for example, a semiconductor memory device, such as a random access memory, an electronically programmable memory (EPROM), an electronically erasable programmable memory (EEPROM), a flash memory device, and a magnetic disk such as an internal hard disk a removable disk, a magneto-optical disk, and an optical disk. Any of the foregoing can be supplemented by, or incorporated in, ASICs. Processors, workstations, personal computers, storage arrays, servers, and other computer, information, or communication resources used to implement features of the present teachings, can be networked or network-accessible.

**[0030]** It will be appreciated, while various embodiments described above involve the calibration based upon dye amplification or other reference material amplification, using instrument response in the form of intensity ranges or spectral response, more than one type of calibration or correction can be performed, together or in sequence. While the foregoing description has generally described calibration based on amplified dye as the reference material, other materials or types of materials or signals can be used, such as electrical signals, thermal signals or signatures, or other information or output detected from a non-dye reference.

**[0031]** Other embodiments will be apparent to those skilled in the art from consideration of the present specification and practice of the present teachings disclosed herein. It is intended that the present specification and examples be considered as exemplary only.

What is claimed is:

**1**. A method of generating calibration information for an amplification reaction, comprising:

performing a first amplification reaction on at least one reference material in a sample support of an analytical instrument

performing a second amplification reaction on a sample in a sample support of the analytical instrument;

receiving first emission data generated by the at least one reference material during the first amplification reaction;

receiving second emission data generated by the sample during the second amplification reaction;

generating calibration information based on the received first emission data; and

adjusting the received second emission data as a function of the calibration information.

**2**. The method of claim **1**, wherein the at least one reference material comprises at least one dye.

**3**. The method of claim **1**, wherein the at least one reference material comprises at least one fluorescent dye.

**4**. The method of claim **1**, wherein the first and second amplification reactions, each comprise a polymerase chain reaction.

**5**. The method of claim **1**, wherein the calibration information generated comprises time-varying information corresponding to multiple cycles of the first amplification reaction.

**6**. The method of claim **1**, wherein the calibration information generated comprises at least one of a set of maximum values of the emission data, a set of minimum values of the emission data, and spectral response information derived from the first emission data.

**7**. The method of claim **1**, wherein the first amplification reaction is performed simultaneously with the second amplification reaction.

**8**. The method of claim **1**, wherein the first amplification reaction is performed before the second amplification reaction.

**9**. A system for generating calibration information, comprising:

an input unit, configured to receive first emission data generated by at least one reference material during an amplification reaction and configured to receive second emission data generated by at least one sample during an amplification reaction; and

a processor communicating with the input unit and configured to generate calibration information based on the first emission data and to adjust the second emission data based on the calibration information.

**10**. The system of claim **9**, wherein the at least one reference material comprises at least one dye.

**11**. The system of claim **9**, further comprising at least one sample support and at least one reference material, wherein the at least one reference material comprises a dye and the dye is disposed in the sample support.

**12**. The system of claim **9**, wherein the input unit is configured to receive first emission data generated by at least one reference material during a polymerase chain reaction.

**13**. The system of claim **9**, wherein the processor is configured to generate calibration information comprising time-varying information corresponding to multiple cycles of an amplification reaction.

**14**. The system of claim **9**, wherein the processor is configured to generate calibration information comprising at least one of a set of maximum values of the first emission data, a set of minimum values of the first emission data, and spectral response information derived from the first emission data.

**15**. A set of calibration information, the set of calibration information being generated by a method comprising:

disposing at least one amplifiable reference material into a sample support;

performing multiple cycles of an amplification reaction on the at least one reference material in the sample support;

receiving emission data generated by the at least one reference material during the multiple cycles of the amplification reaction; and

generating a set of calibration information based on the received emission data.

**16**. The set of calibration information of claim **15**, wherein the emission data comprises fluorescent intensity data generated during at least 10 cycles of polymerase chain reaction.

**17**. The set of calibration information of claim **15**, wherein the emission data comprises emission data generated during a polymerase chain reaction.

**18**. The set of calibration information of claim **15**, comprising time-varying information corresponding to at least 10 cycles of the amplification reaction.

**19**. The set of calibration information of claim **15**, comprising at least one of a set of maximum values of the emission data, a set of minimum values of the emission data, and spectral response information derived from the emission data.

**20**. The set of calibration information of claim **15**, comprising at least one set of maximum values of time-varying information corresponding to at least 10 cycles of the amplification reaction and at least one set of minimum values of time-varying information corresponding to at least 10 cycles of the amplification reaction.

* * * * *