METHODS AND SYSTEMS FOR BIOLOGICAL INSTRUMENT CALIBRATION

BACKGROUND

[0001] Generally, there is an increasing need to simplify the installation and setup of biological analysis systems so that operators can more quickly and efficiently use biological analysis systems for their intended purpose.

[0002] Installation and calibration of laboratory instrumentation can be a time consuming and expensive process. In many cases, engineers from the instrument supplier must be on site to perform these processes. This cost is generally passed on to the user. In some cases, experienced users can successfully calibrate properly manufactured instruments using multi-step procedures. During such calibration, physical standards and well plates may be used in combination with manual procedures. Manual calibration processing and data inspection is error prone and may rely on ad hoc or subjective measures. While a final system verification step may provide resilience against accepting suboptimal calibrations, automation offers improved objectivity and uniformity during such activities.

SUMMARY

[0003] In one exemplary embodiment, a method for calibrating an instrument is provided. The instrument includes an optical system capable of imaging florescence emission from a plurality of reaction sites. The method includes performing a region-of-interest (ROI) calibration to determine reaction site positions in an image. The ROI calibration minimizes at least one of the following groups of reaction site determination errors: dye saturation within the plurality of reaction sites, grid rotation, variation of magnification factors, and optical radial distortion. The method further includes performing a pure dye calibration to determine the contribution of a fluorescent dye used in each reaction site by comparing a raw spectrum of the fluorescent dye to a pure spectrum calibration data of the fluorescent dye. The method further includes performing an instrument normalization calibration to determine a filter normalization factor. The method includes performing a validation assay to validate the instrument is capable of distinguishing between two different quantities of sample.

[0004] In another exemplary embodiment, a non-transitory computer readable storage medium encoded with processor-executable instructions for calibrating an instrument is provided. The instrument includes an optical system capable of imaging florescence

emission from a plurality of reaction sites. The instructions include instructions for performing a region-of-interest (ROI) calibration to determine reaction site positions in an image. The ROI calibration minimizes at least one of the following groups of reaction site determination errors: dye saturation within the plurality of reaction sites, grid rotation, variation of magnification factors, and optical radial distortion. The instructions include instructions for performing a pure dye calibration to determine the contribution of a fluorescent dye used in each reaction site by comparing a raw spectrum of the fluorescent dye to a pure spectrum calibration data of the fluorescent dye. The instructions further include instructions for performing an instrument normalization calibration to determine a filter normalization factor. The instructions further include instructions for performing a validation assay to validate the instrument is capable of distinguishing between two different quantities of sample.

[0005]

[0006] In another exemplary embodiment, a system for calibrating an instrument is provided. The instrument includes an optical system capable of imaging florescence emission from a plurality of reaction sites. The system includes an ROI calibrator configured to determine reaction site positions in an image. The ROI calibration minimizes at least one of the following groups of reaction site determination errors: dye saturation within the plurality of reaction sites, grid rotation, variation of magnification factors, and optical radial distortion. The system includes a pure dye calibrator configured to determine the contribution of a fluorescent dye used in each reaction site by comparing a raw spectrum of the fluorescent dye to a pure spectrum calibration data of the fluorescent dye. The system further includes an instrument normalization calibrator configured to determine a filter normalization factor. The instrument includes a validator using a known assay configured to validate the instrument is capable of distinguishing between two different quantities of sample. The system further includes a display engine configured to display calibration results.

CLAIMS

1. A method for calibrating an instrument, wherein the instrument includes an optical system capable of imaging florescence emission from a plurality of reaction sites, the method comprising:

performing a region-of-interest (ROI) calibration to determine reaction site positions in an image, wherein the ROI calibration minimizes at least one of the following groups of reaction site determination errors: dye saturation within the plurality of reaction sites, grid rotation, variation of magnification factors, and optical radial distortion;

performing a pure dye calibration to determine the contribution of a fluorescent dye used in each reaction site by comparing a raw spectrum of the fluorescent dye to a pure spectrum calibration data of the fluorescent dye;

performing an instrument normalization calibration to determine a filter normalization factor; and

performing a validation assay to validate that the instrument is capable of distinguishing between two different quantities of sample.

2. The method of claim 1, wherein the ROI calibration comprises:

estimating initial region of interest (ROI) from fluorescence thresholds from each sample well;

estimating the center locations of each ROI;

estimating the size of each ROI;

determining the average size of the ROIs from the plurality of reaction sites; deriving global gridding models;

applying the global gridding models to the ROIs, wherein the application of the global gridding models improve the precision of the ROI center locations;

recovering missing ROIs; and

adjusting the radius of the ROIs, wherein the adjustment improves the signal-to-noise ratio of the optical system.

3. The method of claim 1, wherein the validation assay comprises an assay of the RNase P gene.

4. The method of claim 1, wherein the pure dye calibration comprises:

imaging a sample holder, loaded into the instrument, at more than one channel, the sample holder comprising a plurality of reaction sites and more than one dye type, each dye occupying more than one reaction site;

identifying a peak channel for each dye on the sample holder;

normalizing each channel to the peak channel for each dye; and

producing a dye matrix comprising a set of dye reference values.

5. The method of claim 1, wherein the optical system comprises a plurality of excitation filters and a plurality of emission filters, and wherein the instrument normalization calibration comprises:

determining a first correction factor for each of the excitation filters and emission filters; calculating

a second correction factor for a pair of filters, wherein each pair of filters comprises one excitation filter and one emission filter; and

applying the second correction factors to filter data.

6. The method of claim 1, wherein the validation assay comprises:

receiving amplification data from a validation plate to generate a plurality of amplification curves, wherein the validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region;

determining a set of fluorescence thresholds based on the exponential regions of the plurality of amplification curves;

determining, for each fluorescence threshold of the set, a first set of cycle threshold (Ct) values of amplification curves generated from the samples of the first quantity and a second set of Ct values of amplification curves generated from the samples of the second quantity; and

calculating if the first and second quantities are sufficiently distinguishable based on Ct values at each of the plurality of fluorescence thresholds.

7. The method of claim 1, further comprising:

performing an auto-dye correction for real-time spectral calibration of the multi-component data;

performing a plate detection to determine whether there is a plate loading error;

performing an auto-background calibration to compensate for background changes; and

performing instrument normalization using a reflective material to detect any changes or variability in fluorescent emissions.

8. A non-transitory computer readable storage medium encoded with processor-executable instructions for calibrating an instrument, wherein the instrument includes an optical system capable of imaging florescence emission from a plurality of reaction sites, the instructions comprising instructions for:

performing a region-of-interest (ROI) calibration to determine reaction site positions in an image, wherein the ROI calibration minimizes at least one of the following groups of reaction site determination errors: dye saturation within the plurality of reaction sites, grid rotation, variation of magnification factors, and optical radial distortion;

performing a pure dye calibration to determine the contribution of a fluorescent dye used in each reaction site by comparing a raw spectrum of the fluorescent dye to a pure spectrum calibration data of the fluorescent dye;

performing an instrument normalization calibration to determine a filter normalization factor; and

performing a validation assay to validate the instrument is capable of distinguishing between two different quantities of sample.

9. The non-transitory computer readable storage medium of claim 8, wherein the instructions for ROI calibration comprise instructions for:

estimating initial region of interest (ROI) from fluorescence thresholds from each sample well;

estimating the center locations of each ROI;

estimating the size of each ROI;

determining the average size of the ROIs from the plurality of reaction sites;

deriving global gridding models;

applying the global gridding models to the ROIs, wherein the application of the global gridding models improve the precision of the ROI center locations;

recovering missing ROIs; and

adjusting the radius of the ROIs, wherein the adjustment improves the signal-to-noise ratio of the optical system.

10. The non-transitory computer readable storage medium of claim 8, wherein the validation assay comprises an assay of the RNase P gene.

11. The non-transitory computer readable storage medium of claim 8, wherein the instructions for pure dye calibration comprise instructions for:

imaging a sample holder, loaded into the instrument, at more than one channel, the sample holder comprising a plurality of reaction sites and more than one dye type, each dye occupying more than one reaction site;

identifying a peak channel for each dye on the sample holder;

normalizing each channel to the peak channel for each dye; and

producing a dye matrix comprising a set of dye reference values.

12. The non-transitory computer readable storage medium of claim 8, wherein the optical system comprises a plurality of excitation filters and a plurality of emission filters, and wherein the instructions for instrument normalization calibration comprise instructions for:

determining a first correction factor for each of the excitation filters and emission filters;

calculating a second correction factor for a pair of filters, wherein each pair of filters comprises one excitation filter and one emission filter; and

applying the second correction factors to filter data.

13. The non-transitory computer readable storage medium of claim 8, wherein the instructions for the validation assay comprise instructions for:

receiving amplification data from a validation plate to generate a plurality of amplification curves, wherein the validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region;

determining a set of fluorescence thresholds based on the exponential regions of the plurality of amplification curves;

determining, for each fluorescence threshold of the set, a first set of cycle threshold (Ct) values of amplification curves generated from the samples of the first quantity and a second set of Ct values of amplification curves generated from the samples of the second quantity; and

calculating if the first and second quantities are sufficiently distinguishable based on Ct values at each of the plurality of fluorescence thresholds.

14. The non-transitory computer readable storage medium of claim 8, further comprising instructions for:

performing an auto-dye correction for real-time spectral calibration of the multi-component data;

performing a plate detection to determine whether there is a plate loading error;

performing an auto-background calibration to compensate for background changes; and

performing instrument normalization using a reflective material to detect any changes or variability in fluorescent emissions.

15. A system for calibrating an instrument, wherein the instrument includes an optical system capable of imaging florescence emission from a plurality of reaction sites, the system comprising:

a region-of-interest (ROI) calibrator configured to determine reaction site positions in an image, wherein the ROI calibration minimizes at least one of the following groups of reaction site determination errors: dye saturation within the plurality of reaction sites, grid rotation, variation of magnification factors, and optical radial distortion;

a pure dye calibrator configured to determine the contribution of a fluorescent dye used in each reaction site by comparing a raw spectrum of the fluorescent dye to a pure spectrum calibration data of the fluorescent dye;

an instrument normalization calibrator configured to determine a filter normalization factor;

a validator utilizing a known assay configured to validate the instrument is capable of distinguishing between two different quantities of sample; and

a display engine configured to display calibration results.

16. The system of claim 15, wherein the ROI calibrator is configured to:

estimate initial region of interest (ROI) from fluorescence thresholds from each sample well;

estimate the center locations of each ROI; estimate the size of each ROI;

determine the average size of the ROIs from the plurality of reaction sites;

derive global gridding models;

apply the global gridding models to the ROIs, wherein the application of the global gridding models improve the precision of the ROI center locations;

recover missing ROIs; and

adjust the radius of the ROIs, wherein the adjustment improves the signal-to-noise ratio of the optical system.

17. The system of claim 15, wherein the pure dye calibrator is configured to:

image a sample holder, loaded into the instrument, at more than one channel, the sample holder comprising a plurality of reaction sites and more than one dye type, each dye occupying more than one reaction site;

identify a peak channel for each dye on the sample holder;

normalize each channel to the peak channel for each dye; and

produce a dye matrix comprising a set of dye reference values.

18. The system of claim 15, wherein the optical system comprises a plurality of excitation filters and a plurality of emission filters, and wherein the instrument normalization calibrator is configured to:

determine a first correction factor for each of the excitation filters and emission filters;

calculate a second correction factor for a pair of filters, wherein each pair of filters comprises one excitation filter and one emission filter; and

apply the second correction factors to filter data.

19. The system of claim 15, wherein the validator is configured to:

receive amplification data from a validation plate to generate a plurality of amplification curves, wherein the validation plate includes a sample of a first quantity and a second quantity, and each amplification curve includes an exponential region;

determine a set of fluorescence thresholds based on the exponential regions of the plurality of amplification curves;

determine, for each fluorescence threshold of the set, a first set of cycle threshold (Ct) values of amplification curves generated from the samples of the first quantity and a second set of Ct values of amplification curves generated from the samples of the second quantity; and

calculate if the first and second quantities are sufficiently distinguishable based on Ct values at each of the plurality of fluorescence thresholds.

20. The system of claim 15, further comprising:

an auto-dye corrector configured to perform real-time spectral calibration of the multi-component data;

a plate detector configured to determine whether there is a plate loading error;

an auto-background calibrator configured to compensate for background changes; and

an instrument normalizer configured to use a reflective material to detect any changes or variability in fluorescent emissions.