

System and Methods for calibrating binding dyes

FIELD OF THE DISCLOSURE

[0001] The present disclosure is directed to biological analysis such as, for example, a polymerase chain reaction (PCR), and biological analysis devices such as, for example, instruments for PCR, especially to dye calibration plates and computer systems and computer software relating to methods for calibration dyes using dye calibration plates.

BACKGROUND

[0002] 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. For example, advances in the calibration of biological analysis instruments advantageously allow for reduced operator error, reduced operator input, and reduced time necessary to calibrate a biological analysis instrument, and its various components, for proper and efficient installation.

SUMMARY OF THE DISCLOSURE

[0003] The present disclosure relates, in some embodiments, to a computer-implemented method for calibrating a fluorescent dye. The method can comprise imaging a sample holder, loaded into an instrument, at each of a plurality of optical channels. The sample holder can comprise a plurality of reaction sites and a plurality of dyes comprising more than one dye type, with each dye occupying more than one reaction site. The method can further comprise identifying a peak channel of a plurality of optical channels for each dye on the sample holder, wherein the peak channel at a reaction site is related to a greatest fluorescence for the dye at that reaction site and wherein each optical channel of the plurality of optical channels corresponds to a filter combination of excitation and emission filters, normalizing, for each dye, each optical channel to the identified peak channel for that dye, and producing a dye matrix serving as a profile of a fluorescence signature of each calibrated dye for each reaction site.

[0004] In an embodiment, a non-transitory computer-readable storage medium encoded with instructions, executable by a processor, can be provided. The instructions can comprise instructions for imaging a sample holder, loaded into an instrument, at each of a plurality of optical channels. The sample holder can comprise a plurality of reaction sites and a plurality of dyes comprising more than one dye type, with

each dye occupying more than one reaction site. The instructions can further comprise instructions for identifying a peak channel of a plurality of optical channels for each dye on the sample holder, wherein the peak channel at a reaction site is related to a greatest fluorescence for the dye at that reaction site and wherein each optical channel of the plurality of optical channels corresponds to a filter combination of excitation and emission filters, normalizing, for each dye, each optical channel of the plurality of optical channels to the identified peak channel for that dye, and producing a dye matrix serving as a profile of a fluorescence signature of each calibrated dye for each reaction site.

[0005] In another embodiment, a system for calibrating a fluorescent dye is provided. The system can comprise a processor and a memory encoded with instructions, executable by the processor. The instructions can comprise instructions for imaging a sample holder, loaded into an instrument, at each of a plurality of optical channels. The sample holder can comprise a plurality of reaction sites and a plurality of dyes comprising more than one dye type, with each dye occupying more than one reaction site. The instructions can further comprise instructions for identifying a peak channel of a plurality of optical channels for each dye on the sample holder, wherein the peak channel at a reaction site is related to a greatest fluorescence for the dye at that reaction site and wherein each optical channel of the plurality of optical channels corresponds to a filter combination of excitation and emission filters, normalizing, for each dye, each optical channel of the plurality of optical channels to the identified peak channel for that dye, and producing a dye matrix serving as a profile of a fluorescence signature of each calibrated dye for each reaction site.

[0006] A sample holder for calibrating fluorescent dyes is also disclosed. The sample holder can comprise a plurality of reaction sites. Each of the plurality of reaction sites can be configured to accept a single dye. The sample holder can be configured to accept more than one dye. Alternatively, the sample holder can be a plate. Also, the plurality of reactions sites can be a plurality of wells.

BRIEF DESCRIPTION OF THE FIGURES

[0007] FIG. 1 is an image of a calibration plate with FAM dye occupying each well of a 96-well calibration plate.

[0008] FIG. 2 is a block diagram that illustrates a computer system 200 that may be employed to carry out processing functionality, according to some exemplary embodiments of the disclosure.

[0009] FIG. 3 is a block diagram that illustrates a PCR instrument 300 upon which embodiments of the present teachings may be implemented.

Claims:

1. A computer-implemented method for calibrating a fluorescent dye, the method comprising:
 - imaging a sample holder, loaded into an instrument, at each of a plurality of optical channels, the sample holder comprising a plurality of reaction sites and a plurality of dyes comprising more than one dye type, each dye occupying more than one reaction site;
 - identifying a peak channel of a plurality of optical channels for each dye on the sample holder, wherein the peak channel at a reaction site is related to a greatest fluorescence for the dye at that reaction site and wherein each optical channel of the plurality of optical channels corresponds to a filter combination of excitation and emission filters;
 - normalizing, for each dye, each optical channel of the plurality of optical channels to the identified peak channel for that dye; and
 - producing a dye matrix serving as a profile of a fluorescence signature of each calibrated dye for each reaction site.
2. The computer-implemented method of Claim 1, wherein the dyes are distributed in a pattern along the reaction sites such that each dye occupies substantially the same number of reaction sites.
3. The computer-implemented method of Claim 1, wherein the dyes are distributed in a pattern along the reaction sites such that dyes occupying adjacent reaction sites are different.
4. The computer-implemented method of Claim 1, wherein the dyes are distributed in a repeating alternating pattern along the reaction sites.
5. The computer-implemented method of Claim 1, further comprising:
 - performing, for a given dye, a global modeling for all reaction sites by using data from reaction sites occupied by the given dye.
6. The computer-implemented method of Claim 5, further comprising:
 - performing a goodness of fit check on results of the global modeling.
7. The computer-implemented method of Claim 5, further comprising:
 - performing, for a given dye, a local modeling for reaction sites not occupied by the given dye by using data from reaction sites occupied by the given dye.

8. A non-transitory computer-readable storage medium encoded with instructions, executable by a processor, for calibrating a fluorescent dye, the instructions comprising instructions for:
 - imaging a sample holder, loaded into an instrument, at each of a plurality of optical channels, the sample holder comprising a plurality of reaction sites and a plurality of dyes comprising more than one dye type, each dye occupying more than one reaction site;
 - identifying a peak channel of a plurality of optical channels for each dye on the sample holder, wherein the peak channel at a reaction site is related to a greatest fluorescence for the dye at that reaction site and wherein each optical channel of the plurality of optical channels corresponds to a filter combination of excitation and emission filters;
 - normalizing, for each dye, each optical channel of the plurality of optical channels to the identified peak channel for that dye; and
 - producing a dye matrix serving as a profile of a fluorescence signature of each calibrated dye for each reaction site.
9. The non-transitory computer-readable storage medium of Claim 8, wherein the dyes are distributed in a pattern along the reaction sites such that each dye occupies substantially the same number of reaction sites.
10. The non-transitory computer-readable storage medium of Claim 8, wherein the dyes are distributed in a pattern along the reaction sites such that dyes occupying adjacent reaction sites are different.
11. The non-transitory computer-readable storage medium of Claim 8, wherein the dyes are distributed in a repeating alternating pattern along the reaction sites.
12. The non-transitory computer-readable storage medium of Claim 8, further comprising:
 - performing, for a given dye, a global modeling for all reaction sites by using data from reaction sites occupied by the given dye.
13. The non-transitory computer-readable storage medium of Claim 12, further comprising:
 - performing, for a given dye, a local modeling for reaction sites not occupied by the given dye by using data from reaction sites occupied by the given dye.
14. A system for calibrating a fluorescent dye, the system comprising:
 - a processor; and

a memory encoded with instructions, executable by the processor, the instructions for:

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

identifying a peak channel of a plurality of optical channels for each dye on the sample holder, wherein the peak channel at a reaction site is related to a greatest fluorescence for the dye at that reaction site and wherein each optical channel of the plurality of optical channels corresponds to a filter combination of excitation and emission filters;

normalizing, for each dye, each optical channel of the plurality of optical channels to the identified peak channel for that dye; and

producing a dye matrix serving as a profile of a fluorescence signature of each calibrated dye for each reaction site.

15. The system of Claim 14, wherein the dyes are distributed in a pattern along the reaction sites such that each dye occupies substantially the same number of reaction sites.
16. The system of Claim 14, wherein the dyes are distributed in a pattern along the reaction sites such that dyes occupying adjacent reaction sites are different.
17. The system of Claim 14, wherein the dyes are distributed in a repeating alternating pattern along the reaction sites.
18. The system of Claim 14, further comprising:

performing, for a given dye, a global modeling for all reaction sites by using data from reaction sites occupied by the given dye.
19. The system of Claim 18, further comprising:

performing a goodness of fit check on results of the global modeling.
20. The system of Claims 18, further comprising:

performing, for a given dye, a local modeling for reaction sites not occupied by the given dye by using data from reaction sites occupied by the given dye.