

[0006] The (m x n) format in most quantitative polymerase chain reaction (qPCR) platforms is designed for sample-by-assay experiments, in which PCR results need to be addressable for post-run analysis. For dPCR, however, the specific position or well of each PCR result may be immaterial and only the number of positive and negative replicates per sample may be analyzed.

[0007] The read-out of dPCR, that is, the number of positive reactions and the number of negative reactions, is linearly proportional to the template concentration, while the read-out of qPCR (signal vs. cycle) is proportional to the log of the template concentration. For this reason, dPCR typically is constrained to a narrow dynamic range of template input. As a result of the log versus linear, the dPCR analysis offers better resolution for close fold changes than qPCR. Furthermore, the resolution remains approximately linear across the entire dynamic range that is supported.

[0008] However, to determine positive and negative counts for a dPCR result after thermal cycling, also known as endpoint reads, requires determining or setting a fluorescence threshold. Thus, it is a challenge to determine where a threshold should be set to accurately distinguish between negatives and positives because there may be no or low amplifications for each sample. Furthermore, it is also a challenge to determine whether a sample volume contains one copy or multiple copies, for example.

SUMMARY

[0009] In one exemplary embodiment, a computer-implemented method of generating a digital polymerase chain reaction (dPCR) result is provided. The method includes detecting a first set of emission data from a plurality of samples, each included in a sample region of a plurality of sample regions, at a first time during an amplification period; detecting a second set of emission data from the plurality of samples at a second time during the amplification period, wherein the first and second set of emission data is included in amplification curve data for each of the plurality of samples; ~~The method further includes~~ determining a positive or negative amplification determination for each sample of the plurality of samples based in part on the ~~first set of emission data~~ corresponding amplification curve data; ~~A;~~ generating a dPCR result is generated based on the positive or negative amplification determinations for the plurality of samples; and displaying to a user the amplification curve data and the positive or negative determinations.

[0009a] According to another exemplary embodiment, there is provided a computer-readable medium encoded with instructions, executable by a processor, the instructions comprising instructions for: detecting a first set of emission data from a plurality of samples, each included in a sample region of a plurality of sample regions, at a first time during an amplification period; detecting a second set of emission data from the plurality of samples at a second time during ~~an~~the amplification period, wherein the first and second set of emission data is included in amplification curve data for each of the plurality of samples; determining a positive or negative amplification determination for each sample of the plurality of samples based in part on the corresponding amplification curve data; ~~and~~ generating a dPCR result based on the positive or negative amplification determinations for the plurality of samples; and displaying to a user the amplification curve data and the positive or negative determinations.

[0009b] According to another exemplary embodiment, there is provided system for generating a digital polymerase chain reaction result (dPCR), the system comprising: a thermal cycler for performing amplification on a plurality of samples; a processor; and a memory encoded with instructions for: detecting a first set of emission data from a plurality of samples, each included in a sample region of a plurality of sample regions, at a first time during an amplification period; detecting a second set of emission data from the plurality of samples at a second time during ~~an~~the amplification period, wherein the first and second set of emission data is included in amplification curve data for each of the plurality of samples; determining a positive or negative amplification determination for each sample of the plurality of samples based in part on the corresponding amplification curve data; ~~and~~ generating a dPCR result based on the positive or negative amplification determinations for the plurality of samples; and displaying to a user the amplification curve data and the positive or negative determinations.

DESCRIPTION OF THE FIGURES

[0010] FIG. 1 is a block diagram that illustrates a computer system, upon which embodiments of the present teachings may be implemented;

CLAIMS

What is claimed is:

1. A computer-implemented method of generating a digital polymerase chain reaction (dPCR) result, the method comprising:
 - detecting a first set of emission data from a plurality of samples, each included in a sample region of a plurality of sample regions, at a first time during an amplification period;
 - detecting a second set of emission data from the plurality of samples at a second time during ~~an~~ the amplification period, wherein the first and second set of emission data is included in amplification curve data for each of the plurality of samples;
 - determining a positive or negative amplification determination for each sample of the plurality of samples based in part on the corresponding amplification curve data; ~~and~~
 - generating a dPCR result based on the positive or negative amplification determinations for the plurality of samples; and
 - displaying to a user the amplification curve data and the positive or negative determinations.
2. The computer-implemented method of claim 1, further comprising detecting emission data from the plurality of samples after each stage of thermal cycling in the amplification period to generate the amplification curve data.
3. The computer-implemented method of claim 1, further comprising detecting real-time emission data during the amplification period to generate the amplification curve data.
4. The computer-implemented method of claim 1, wherein the determining the positive or negative amplification determination for each sample of the plurality of samples comprises:
 - determining a quantification cycle for each of the plurality of samples;
 - binning the plurality of determined quantification cycles; and
 - displaying a histogram of the binned quantification cycles for the plurality of samples.
5. The computer-implemented method of claim 1 further comprising:
 - determining a valid testing of a sample in the plurality of samples based on the amplification curve data of the sample.

~~6.~~ The computer-implemented method of claim 1, further comprising displaying to the user the amplification curve data and the positive or negative determinations.

~~76.~~ The computer-implemented method of claim 4, wherein the amplification curve data is displayed along with the histogram to the user.

~~87.~~ The computer-implemented method of claim 4, wherein a quantification cycle lower than a predetermined threshold is determined to be an invalid testing.

~~98.~~ The computer-implemented method of claim 4, wherein a quantification cycle higher than a predetermined threshold is determined to be a valid testing.

~~109.~~ The computer-implemented method of claim 4, wherein a quantification cycle within a predetermined range is determined to be a valid testing.

~~110.~~ A computer-readable medium encoded with instructions, executable by a processor, the instructions comprising instructions for:

detecting a first set of emission data from a plurality of samples, each included in a sample region of a plurality of sample regions, at a first time during an amplification period;

detecting a second set of emission data from the plurality of samples at a second time during ~~an~~ the amplification period, wherein the first and second set of emission data is included in amplification curve data for each of the plurality of samples;

determining a positive or negative amplification determination for each sample of the plurality of samples based in part on the corresponding amplification curve data; ~~and~~

generating a dPCR result based on the positive or negative amplification determinations for the plurality of samples; ~~and~~

displaying to a user the amplification curve data and the positive or negative determinations.

~~1211.~~ The computer-readable medium of claim ~~110~~, wherein the instructions for determining the positive or negative amplification determination for each sample of the plurality of samples comprises instructions for:

determining a quantification cycle for each of the plurality of samples;

binning the plurality of determined quantification cycles; and

displaying a histogram of the binned quantification cycles for the plurality of samples.

~~1312.~~ The computer-readable medium of claim ~~110~~ further comprising instructions for:

determining a valid testing of a sample in the plurality of samples based on the amplification curve data of the sample.

~~14. The computer-readable medium of claim 11, wherein the instructions further comprise instructions for displaying to the user the amplification curve data and the positive or negative determinations.~~

~~15~~13. The computer-readable medium of claim ~~12~~11, wherein the amplification curve data is displayed along with the histogram to the user.

~~16~~14. The computer-readable medium of claim ~~12~~11, wherein a quantification cycle lower than a predetermined threshold is determined to be an invalid testing.

~~17~~15. The computer-readable medium of claim ~~12~~11, wherein a quantification cycle higher than a predetermined threshold is determined to be a valid testing.

~~18~~16. The computer-readable medium of claim ~~12~~11, wherein a quantification cycle within a predetermined range is determined to be a valid testing.

~~19~~17. A system for generating a digital polymerase chain reaction result (dPCR), the system comprising:

a thermal cycler for performing amplification on a plurality of samples;

a processor; and

a memory encoded with instructions for:

detecting a first set of emission data from a plurality of samples, each included in a sample region of a plurality of sample regions, at a first time during an amplification period;

detecting a second set of emission data from the plurality of samples at a second time during ~~an~~ the amplification period, wherein the first and second set of emission data is included in amplification curve data for each of the plurality of samples;

determining a positive or negative amplification determination for each sample of the plurality of samples based in part on the corresponding amplification curve data; ~~and~~

generating a dPCR result based on the positive or negative amplification determinations for the plurality of samples; and

displaying to a user the amplification curve data and the positive or negative determinations.

~~2018.~~ The system of claim ~~1917~~, further comprising detecting emission data from the plurality of samples after each stage of thermal cycling in the amplification period to generate the amplification curve data.

~~2119.~~ The system of claim ~~1917~~, further comprising detecting real-time emission data during the amplification period to generate the amplification curve data.

~~2220.~~ The system of claim ~~1917~~, wherein the determining the positive or negative amplification determination for each sample of the plurality of samples comprises:

determining a quantification cycle for each of the plurality of samples;

binning the plurality of determined quantification cycles; and

displaying a histogram of the binned quantification cycles for the plurality of samples.

~~2321.~~ The system of claim ~~1917~~, further comprising:

determining a valid testing of a sample in the plurality of samples based on the amplification curve data of the sample.

~~24. The system of claim 19, further comprising displaying to the user the amplification curve data and the positive or negative determinations.~~

~~2522.~~ The system of claim ~~2220~~, wherein the amplification curve data is displayed along with the histogram to the user.

~~2623.~~ The system of claim ~~2220~~, wherein a quantification cycle lower than a predetermined threshold is determined to be an invalid testing.

~~2724.~~ The system of claim ~~2220~~, wherein a quantification cycle higher than a predetermined threshold is determined to be a valid testing.

~~2825.~~ The system of claim ~~2220~~, wherein a quantification cycle within a predetermined range is determined to be a valid testing.