amplification; if the threshold is not crossed, the curve is determined to represent non-amplification.

SUMMARY

[0004] Automated determination of amplification is important for increasing throughput of sample analysis, which in turn can both advance scientific research and improve provision of time-sensitive, clinically important information. Existing methods of automatically determining amplification have relied on combinations of techniques and parameters to improve accuracy. Machine learning techniques, including deep learning networks such as artificial neural networks, can help improve accuracy. Improved machine learning techniques are needed to further improve accuracy and reduce the instances in which human review is required. At the same time, it is important to effectively and optimally determine when human review is needed, and the criteria for triggering human review of amplification curves that have been evaluated by a computer system can vary by application context. Improving the overall call-rate accuracy of an amplification-calling machine learning system does not necessarily fully address the problem of knowing *which* individual amplification curve automated call results are at most risk of being wrong and therefore require human review.

[0004a] According to an aspect of the present disclosure, there is provided a method of computerized processing of output of a quantitative polymerase chain reaction (qPCR) instrument, the qPCR instrument being configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample, the method implemented by executing, using

one or more computer processors, processing comprising: pre-processing a plurality of amplification curves generated by the qPCR instrument to obtain, for an amplification curve of the plurality of amplification curves, engineered features and a pre-processed amplification curve; processing, by one or more amplification-calling deep learning networks, the pre-processed amplification curve and one or more of the engineered features to generate amplification call data; processing, by one or more curve-quality calling deep learning networks, the pre-processed amplification curve to generate curve-quality call data; and using the amplification call data and the curve quality call data to provide an interactive electronic graphical user interface (GUI) configured to facilitate user evaluation of the output of the qPCR instrument.

[0004b] According to another aspect of the present disclosure, there is provided a method of computerized processing of output of a quantitative polymerase chain reaction (qPCR) instrument, the qPCR instrument being configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample, the method implemented by executing, using one or more computer processors, processing comprising: pre-processing a plurality of amplification curves generated by the qPCR instrument to obtain, for an amplification curve of the plurality of amplification curves, engineered features and a pre-processed amplification curve; processing, by one or more amplification-calling deep learning networks, the pre-processed amplification curve and one or more of the engineered features to generate amplification call data; processing, by one or more curve-quality calling deep learning networks,

the pre-processed amplification curve to generate curve-quality call data; and using the curve-quality call data to determine whether to invalidate any of the amplification call data.

[0004c] According to another aspect of the present disclosure, there is provided a computer program product comprising executable code stored in a non-transitory computer readable medium, the executable code being executable on one or more computer processors to implement one or more amplification-calling deep learning networks for use in processing output of a quantitative polymerase chain reaction (qPCR) instrument, the qPCR instrument being configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample, an amplification calling deep learning network of the one or more amplification-calling deep learning networks comprising: a first portion configured to receive and process one or more first engineered features obtained from pre-processing the amplification curve; a second portion configured for extracting learned features by receiving and processing a pre-processed amplification curve corresponding to the amplification curve, or, optionally, by processing the pre-processed amplification curve together with one or more second engineered features obtained from pre-processing the amplification curve and/or the pre-processed amplification curve; and a third portion configured for processing output of the first portion and the second portion together to generate amplification call data.

[0004d] According to another aspect of the present disclosure, there is provided a computer program product comprising executable code stored in a non-transitory computer readable medium, the executable code being executable on one or more computer processors to implement

one or more curve-quality calling deep learning networks for use in processing output of a quantitative polymerase chain reaction (qPCR) instrument, the qPCR instrument being configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample, a curve-quality calling deep learning network of the one or more curve-quality calling deep learning networks comprising: a first portion configured for extracting learned features by receiving and processing a pre-processed amplification curve corresponding to the amplification curve, or, optionally, by processing the pre-processed amplification curve along with one or more engineered features obtained from the amplification curve; and a second portion configured for processing output of the first portion to generate curve quality call data.

[0004e] According to an aspect of the present disclosure, there is provided a computer program product comprising executable code stored in a non-transitory computer readable medium, the executable code being executable on one or more computer processors to implement one or more amplification-calling deep learning networks for use in processing output of a quantitative polymerase chain reaction (qPCR) instrument, the qPCR instrument being configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample, an amplification calling deep learning network of the one or more amplification-calling deep learning networks comprising: a first portion configured to receive and process one or more first engineered features obtained from pre-processing the amplification curve; a second portion

configured for extracting learned features by receiving and processing a pre-processed amplification curve corresponding to the amplification curve, or, optionally, by processing the pre-processed amplification curve together with one or more second engineered features obtained from pre-processing the amplification curve and/or the pre-processed amplification curve; and a third portion configured for processing output of the first portion and the second portion together to generate amplification call data; wherein at least one of the first portion and third portion comprises one or more dropout layers.

[0004f] According to another aspect of the present disclosure, there is provided a computer program product comprising executable code stored in a non-transitory computer readable medium, the executable code being executable on one or more computer processors to implement one or more curve-quality calling deep learning networks for use in processing output of a quantitative polymerase chain reaction (qPCR) instrument, the qPCR instrument being configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample, a curve-quality calling deep learning network of the one or more curve-quality calling deep learning networks comprising: a first portion configured for extracting learned features by receiving and processing a pre-processed amplification curve corresponding to the amplification curve, or, optionally, by processing the pre-processed amplification curve along with one or more engineered features obtained from the amplification curve; and a second portion configured for processing output of the first portion to generate curve quality call data, wherein the second portion comprises one or more dropout layers.

[0004g] According to another aspect of the present disclosure, there is provided a method of computerized processing of output of a quantitative polymerase chain reaction (qPCR) instrument, the qPCR instrument being configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample, the method implemented by executing, using one or more computer processors, processing comprising: pre-processing a plurality of amplification curves generated by the qPCR instrument to obtain, for an amplification curve of the plurality of amplification curves, a pre-processed amplification curve; processing, by an ensemble of amplification calling deep learning networks, the pre-processed amplification curve to generate amplification call data; processing the amplification call data to generate an ensemble amplification prediction and first confidence data corresponding to the ensemble amplification prediction; and using the ensemble amplification prediction and the first confidence data to provide an interactive electronic graphical user interface (GUI) configured to facilitate user evaluation of the output of the qPCR instrument.

[0004h] According to another aspect of the present disclosure, there is provided a biological analysis system comprising: a quantitative polymerase chain reaction (qPCR) instrument configured to generate amplification data, referred to herein as amplification curves, an amplification curve comprising data corresponding to fluorescence measurements obtained for a plurality of thermal cycles of a qPCR instrument used to conduct a qPCR assay on a biological sample; and one or more computer processors configured to execute processing in accordance with the executable code as described above.

[0005] Embodiments of the invention address aspects of this “which curve” problem by using an improved amplification calling machine learning system (in some embodiments, an improved artificial neural network) in parallel with a machine learning system (e.g., a deep learning network such as an artificial neural network) for evaluating the quality of an amplification curve. This can help identify potential problem curves. In some embodiments, alternative amplification calling algorithms are used to help determine which curves called by a machine-learning (e.g.,