[0005] In a first aspect, the present invention refers to a method for measuring coagulation time of a blood sample of a subject, comprising (a) initiating a coagulation reaction of the blood sample of the subject; (b) obtaining a set of more than one of image of said coagulation reaction; and (c) analyzing said set of images to measure the coagulation time of said blood sample. In a second aspect, the present invention refers to a method for measuring coagulation time of a blood sample of a subject, comprising: (a) obtaining a blood sample from the subject of 1 ml or less; (b) initiating a coagulation reaction of said blood sample; (c) obtaining a set of more than one image of said coagulation reaction; and (d) analyzing said set of images to measure the coagulation time of said blood sample, wherein the amount of time for carrying out steps (a) to (d) is less than or about 1 hour. In a third aspect, the present invention refers to a method for measuring a plurality of coagulation parameters of a blood sample of a subject, the method comprising: (a) obtaining the blood sample of the subject, wherein said blood sample is less than or about 1 ml; and (b) performing a plurality of assays using said sample to measure said plurality of coagulation parameters. In a fourth aspect, the present invention refers to a device for measuring coagulation time of a blood sample of a subject, comprising: (a) a component configured to add a coagulation initiation reagent to the blood sample from the subject under a condition suitable for clot formation, thereby initiating the coagulation reaction; (b) a component configured to obtain set of more than one image of said coagulation reaction; and (c) a component that analyzes said set of images to measure the coagulation time of said blood sample. In a fifth aspect, the present invention refers to a system for measuring coagulation time of a blood sample of a subject, comprising: (a) a device configured to add a coagulation initiation reagent to the blood sample from the subject under a condition suitable for clot formation, thereby initiating the coagulation reaction; (b) a camera configured to obtain a set of more than one image of said coagulation reaction; and (c) a computer configured to analyze said set of images to measure the coagulation time of said blood sample. In some ~~embodiments~~examples, methods for assaying coagulation include: A) monitoring coagulation in a very small volume of blood sample (e.g. 20 □l or less), and/or B) diluting all or part of a blood sample and using the diluted blood sample for an assay, thus reducing the total amount of sample used for the assay.

[0006] In one ~~aspect~~example, various techniques are provided for performing coagulation assays with diluted or undiluted samples. In one ~~embodiment~~example, a coagulation assay is performed in a small container which has a high surface to volume ratio which aids in the adhesion of an incipient clot to the surface of the container. In another ~~embodiment~~example, an exogenous material (for example, fibrinogen) which increases clot strength and/or the turbidity (due to light scattering) generated during the clotting process is added to a sample. In another ~~embodiment~~example, small beads are added to a blood sample, and video imaging is used to track the movement of the beads as they settle by gravity and then reduce or cease movement upon clot formation. In another ~~embodiment~~example, small fluorescent beads

are added to a blood sample, and fluorescence microscopy is used to track the movement of beads as they are moved by Brownian motion, convention and/or airflow and reduce or cease movement upon clot formation. In another ~~embodiment~~example, a blood sample is propelled through a container by a force, and video imaging is used to track the movement of the sample and the reduction or cessation in movement of the sample in the container upon clot formation.

[0007] In one ~~aspect~~example, ~~provided~~described herein is a method for measuring coagulation time of a blood sample of a subject. The method comprises (a) initiating a coagulation reaction of the blood sample of the subject, (b) obtaining a set of images of the coagulation reaction, and (c) analyzing the set of images to measure the coagulation time of the blood sample.

[0008] In one ~~aspect~~example, ~~provided~~described herein is a method for measuring coagulation time of a blood sample of a subject. The method comprises (a) obtaining a blood sample from the subject from blood obtained from the subject via a non-venous route, (b) initiating a coagulation reaction of the blood sample, (c) obtaining a set of images of the coagulation reaction, and (d) analyzing the set of images to measure the coagulation time of the blood sample wherein the amount of time for carrying out steps (a) to (d) is less than or about 1 hour. In some ~~embodiments~~examples, the amount of time for carrying out steps (a) to (d) is less than or about 30 minutes. In some ~~embodiments~~examples, the amount of time for carrying out steps (a) to (d) is less than or about 10 minutes.

[0009] In some ~~embodiments~~examples, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 μl or less of blood sample is used for the coagulation assay.

[0010] In some ~~embodiments~~examples, the volume of the coagulation reaction is less than or about 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 μl.

[0011] In some ~~embodiments~~examples, an individual image of the set of images is pixilated and comprises at least 1, 10, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10,000 pixels.

[0012] In some ~~embodiments~~examples, the method further comprises diluting the blood sample such that the coagulation time of the sample after dilution is between about 30 seconds and about 10 minutes.

[0013] In some ~~embodiments~~examples, the method further comprises adding fibrinogen to the coagulation reaction.

[0014] In some ~~embodiments~~examples, the initiating step comprises adding a coagulation initiation reagent to the blood sample.

[0015] In some ~~embodiments~~examples, the blood sample is a plasma sample.

[0016] In some ~~embodiments~~examples, the blood sample is obtained using a finger prick.

[0017] In some ~~embodiments~~examples, the images are light scattering images of the coagulation reaction.

[0018] In some ~~embodiments~~examples, the coagulation time is measured based on a transition of the intensity of the scattered light.

[0019] In some ~~embodiments~~examples, the method further comprises adding a plurality of beads to the blood sample prior to obtaining the set of images. For example, at least 2, 3, 4, 5, 10, 100, or 1000 beads may be added to the blood sample.

[0020] In some ~~embodiments~~examples, the plurality of beads include beads of at least two different sizes.

[0021] In some ~~embodiments~~examples, the plurality of beads have sizes from about 1 μm to 5 μm.

[0022] In some ~~embodiments~~examples, the plurality of beads have sizes from about 5 μm to 50 μm.

[0023] In some ~~embodiments~~examples, the plurality of beads include one or more of polystyrene, latex, acrylic, or glass. In certain ~~aspects~~examples, the beads may have a different refractive index from the reaction medium (e.g. higher or lower by about 1, 2, 3, 4, 5, 8, 10, 15, 16, 20, 25, 30, 35, 40, or 50%), or may be opaque. In addition, the beads may have a density different from the reaction medium (e.g. higher or lower by about 1, 2, 3, 4, 5, 8, 10, 15, 16, 20, 25, 30, 35, 40, or 50%). In some assays, the reaction medium has a density of about 1.01 g/cc.

[0024] In some ~~embodiments~~examples, the step of analyzing the set of images comprises locating a time point when the beads become substantially motionless.

[0025] In some ~~embodiments~~examples, the step of analyzing the set of images comprises locating the time point when a transition of the mobility of the beads occurs.

[0026] In some ~~embodiments~~examples, the transition of the mobility of the beads is evidenced by deceleration of the settling of the beads in the coagulation reaction.

[0027] In some ~~embodiments~~examples, the step of analyzing the set of images comprises comparing two images of the set of images to measure the motion of the beads.

[0028] In some ~~embodiments~~examples, the beads are substantially motionless if the two images are substantially the same.

[0029] In some ~~embodiments~~examples, the beads are labeled.

[0030] In some ~~embodiments~~examples, the beads are labeled with a fluorescent label.

[0031] In another ~~aspect~~example, a method is provided for measuring a plurality of coagulation parameters of a blood sample of a subject. The method comprises (a) obtaining the blood sample of the subject, wherein the blood sample is less than or about 50 μl, (b) preparing a plasma sample from the blood sample, and (c) performing a plurality of assays using the plasma sample to measure the plurality of coagulation parameters, wherein at least one of the plurality of parameters is coagulation time.

[0032] In some ~~embodiments~~examples, one of the coagulation parameters is selected from the group consisting of: Activated Partial Thromboplastin Time (aPTT), prothrombin time (PT), International Normalized Ratio (INR), bleeding time, coagulation factor, anti-phospholipid antibody, dilute Russell's viper venom time (dRVVT), and platelet function, thromboelastography (TEG or Sonoclot), and euglobulin lysis time (ELT).

[0033] In some ~~embodiments~~examples, less than about 10 μl of the plasma sample is utilized for an individual assay of the plurality of assays. In some ~~embodiments~~examples, less than about 2 μl of the plasma sample is utilized for an individual assay of the plurality of assays.

[0034] In some ~~embodiments~~examples, the reaction volume of some or each of the plurality of assays is about or less than 6 μl. In some ~~embodiments~~examples, the reaction volume of some or each of the plurality of assays is about or less than 10 μl.

[0035] In some ~~embodiments~~examples, the amount of time for carrying out an assay to measure coagulation time is less than or about 1 hour, 30 minutes, 10 minutes, 5 minutes, or 1 minute.

[0036] In some ~~embodiments~~examples, obtaining a set of images of a coagulation reaction includes (i) obtaining a set of images of at least one of the plurality of assays and (ii) analyzing the set of images to measure the coagulation time.

[0037] In one ~~aspect~~example, a device for measuring coagulation time of a blood sample of a subject is provided. The device comprises (a) a component configured to add a coagulation initiation reagent to the blood sample from the subject under a condition suitable for clot formation, thereby initiating the coagulation reaction, (b) a component configured to obtain a set of images of the coagulation reaction, and (c) a component that analyzes the set of images to measure the coagulation time of the blood sample.

[0038] In one ~~aspect~~example, a system for measuring coagulation time of a blood sample of a subject is provided. The system comprises (a) a device configured to add a coagulation initiation reagent to the blood sample from the subject under a condition suitable for clot formation, thereby initiating the coagulation reaction (b) a camera that obtains a set of images of the coagulation reaction, and (c) a computer that analyzes the set of images to measure the coagulation time of the blood sample.

[0039] In some ~~aspects~~examples, a blood sample that is anticoagulated may be used with any of the assays provided herein. To prepare an anticoagulated blood sample for use with a coagulation assay, a reagent which reverses the effect of an anticoagulant is added in excess over the anticoagulant. For the anticoagulants ethylenediaminetetraacetic acid (EDTA), citrate, and oxalate, a suitable reagent is calcium (Ca^{2+}); for the anticoagulant heparin, a suitable reagent is polybrene.

[0040] In some ~~embodiments~~examples, ~~provided~~described herein is a method of assaying a mixture for coagulation, comprising: initiating a coagulation reaction in a mixture comprising a quantity of an original biological sample, wherein the mixture: i) has a total volume of no greater than 500 microliters, ii) contains no greater than 50 microliters of the original biological sample, and iii) contains no greater than 95% by volume original biological sample; illuminating the mixture and detecting light from the mixture; and analyzing the mixture for coagulation based on an analysis of light from the mixture.

[0041] In ~~embodiments~~examples, in method ~~provided~~described herein involving a mixture comprising a quantity of an original biological sample, the mixture may have a total volume of no greater than 500, 400, 300, 200, 100 75, 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, or 0.1 microliters.

[0042] In ~~embodiments~~examples, in method ~~provided~~described herein involving a mixture comprising a quantity of an original biological sample, the mixture may contain no greater than 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, or 0.1 microliters of the original biological sample.

[0043] In ~~embodiments~~examples, in method ~~provided~~described herein involving a mixture comprising a quantity of an original biological sample, the mixture may contain no greater than 95%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% by volume original biological sample.

[0044] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light from a mixture, detecting light from the mixture comprises obtaining an image of the mixture.

[0045] In ~~embodiments~~examples, in methods ~~provided~~described herein involving obtaining an image of the mixture, the method further comprises analyzing the image to identify a region of interest (ROI) of the image, wherein the region of interest of the image contains imaged information of the mixture.

[0046] In ~~embodiments~~examples, in methods ~~provided~~described herein involving identifying a ROI in an image, the method further comprises analyzing the ROI of the image to determine average light intensity in the ROI.

[0047] In ~~embodiments~~examples, in methods ~~provided~~described herein involving obtaining an image of the mixture, the method comprises obtaining at least a first image and a second image of the mixture over a period of time, wherein a coagulation time of the mixture is determined based on an analysis comprising determining the difference in light intensity in a ROI of the first image and a ROI in the second image.

[0048] In ~~embodiments~~examples, in methods ~~provided~~described herein involving obtaining an image of a mixture, the image of the mixture is obtained with a CCD or CMOS sensor.

[0049] In ~~embodiments~~examples, in methods ~~provided~~described herein involving obtaining an image of a mixture, obtaining an image of the mixture comprises obtaining a video of the mixture.

[0050] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light from a mixture, detecting light from the mixture comprises detecting light transmitted through the mixture.

[0051] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light transmitted through a mixture, the light transmitted through the mixture is detected over a period of time.

[0052] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light transmitted through a mixture, a coagulation time of the mixture is identified based on an analysis comprising determining a time when the intensity of light transmitted through the mixture is reduced by a selected amount as compared to a baseline intensity of light transmitted through the mixture.

[0053] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light transmitted through a mixture, a coagulation time of the mixture is identified based on an analysis comprising determining a time when the intensity of light transmitted through the mixture is reduced by 20% or more as compared to a baseline intensity of light transmitted through the mixture.

[0054] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light from a mixture, detecting light from the mixture comprises detecting light scattered by the mixture.

[0055] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light scattered by the mixture, the light scattered by the mixture is detected over a period of time.

[0056] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light scattered by the mixture, a coagulation time of the mixture is identified based on an analysis comprising determining a time when the intensity of light scattered by the mixture is increased by a selected amount as compared to a baseline intensity of light scattered by the mixture.

[0057] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light scattered by the mixture, a coagulation time of the mixture is identified based on an analysis comprising determining a time when the intensity of light scattered by the mixture is reduced by 20% or more as compared to a baseline intensity of light scattered by the mixture.

[0058] In ~~embodiments~~examples, in methods ~~provided~~described herein involving detecting light transmitted through or scattered by the mixture, light transmitted through or scattered by the mixture is detected with a CCD sensor, CMOS sensor, photodiode, or photomultiplier tube (PMT).

[0059] In ~~embodiments~~examples, in methods ~~provided~~described herein involving a coagulation reaction in a mixture comprising a quantity of an original biological sample, the mixture comprises exogenous fibrinogen. In ~~embodiments~~examples, the exogenous fibrinogen may be in the mixture at a concentration of at least 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or 150 mg/ml. In ~~embodiments~~examples, the exogenous fibrinogen may be in the mixture at a concentration of no more than 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150 or 200 mg/ml. In ~~embodiments~~examples, the exogenous fibrinogen may be in the mixture at a concentration of at least 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or 150 mg/ml and no more than 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, or 200 mg/ml.

[0060] In ~~embodiments~~examples, in methods ~~provided~~described herein involving a coagulation reaction in a mixture comprising a quantity of an original biological sample, the original biological sample is whole blood. In ~~embodiments~~examples, the original biological sample may be whole blood from a subject which has not been diluted by the addition of any liquid to the whole blood after its extraction from the subject.

[0061] In ~~embodiments~~examples, in methods ~~provided~~described herein involving a coagulation reaction in a mixture comprising a quantity of an original biological sample, the original biological sample is plasma. In ~~embodiments~~examples, the original biological sample may be plasma from a subject which has not been diluted by the addition of any liquid to the plasma after its extraction from the subject.

[0062] In ~~embodiments~~examples, in methods ~~provided~~described herein involving a coagulation reaction in a mixture comprising a quantity of an original biological sample, the mixture is illuminated with a white light source.

[0063] In ~~embodiments~~examples, in methods ~~provided~~described herein involving a coagulation reaction in a mixture comprising a quantity of an original biological sample, the mixture is illuminated with light of one or more selected wavelengths.

[0064] In ~~embodiments~~examples, in methods ~~provided~~described herein involving a coagulation reaction in a mixture comprising a quantity of an original biological sample, the mixture is illuminated with light from a light-emitting diode (LED).

[0065] In ~~embodiments~~examples, an optical signal disclosed herein may be light. In ~~embodiments~~examples, an optical signal disclosed herein may be electromagnetic radiation outside of the visual spectrum.

[0066] This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter.

INCORPORATION BY REFERENCE

[0067] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. Incorporated publications include U.S. Patent Publication Number 2010/0081144 A1; U.S. Patent Number 7,888,125; U.S. Patent Publication Number 2011/0093249 A1; U.S. Patent Publication Number 2009/0318775 A1; U.S. Patent Number 7,291,497; U.S. Patent Number 8,012,744; U.S. Patent Publication Number 2006/0264783 A1; U.S. Patent Publication Number 2007/0224084 A1; and U.S. Application Number 13/244,947.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] In the drawings,

[0069] Figure 1 shows an increase in light scattering from before coagulation (left vessel) to after coagulation (right vessel) of a plasma sample.

[0070] Figure 2 shows a plot of mean light scattering signal versus time during coagulation of a plasma sample.

[0071] Figure 3 shows a plot of mean light scattering signal versus time fit to a four-parameter log-logistic function progress curve.

[0072] Figure 4 shows a representative reaction time course analyzed by the PSNR method.

[0073] Figure 5 shows a schematic of a bead settling embodiment.

[0074] Figure 6 shows a schematic of a fluorescent microscopy embodiment.

[0075] Figure 7 shows a schematic of a cuvette suitable for microscopy embodiments.

[0076] Figure 8 shows a plot of correlation factor as a function of time for measurement of PT activation factor of a plasma sample.

[0077] Figure 9 shows a method for excluding background from video images.

[0078] Figure 10 shows exemplary results of measuring PT by light scattering.

[0079] Figure 11 shows exemplary results of measuring aPTT by light scattering.

[0080] Figure 12 shows exemplary results of measuring PT by bead settling.

[0081] Figure 13 shows exemplary results of measuring aPTT by bead settling.

49. The method of any of claims 1-42, wherein the set of more than one image comprise a video image.

50. A method for measuring coagulation time of a blood sample of a subject, comprising:

(a) initiating a coagulation reaction of the blood sample of the subject;

(b) obtaining a video of said coagulation reaction; and

(c) analyzing said video to measure the coagulation time of said blood sample.

51. The method of any of claims 1-42 or 50, wherein the method is performed at a point of service location.

52. The device or system of any of claims 43-46, wherein at least a portion of the device or system is located at a point of service location.

53. The device or system of any of claims 43-46 or 52, wherein the device or system may be dynamically controlled through a cloud computing infrastructure.

54. A method of assaying a mixture for coagulation, comprising:

A) initiating a coagulation reaction in a mixture comprising a quantity of an original biological sample, wherein the mixture: i) has a total volume of no greater than 200 microliters, ii) contains no greater than 20 microliters of the original biological sample, and iii) contains no greater than 80% by volume original biological sample;

B) illuminating the mixture and detecting an optical signal from the mixture; and

C) analyzing the mixture for coagulation based on an analysis of the optical signal from the mixture.

55. The method of claim 54, wherein the step of detecting the optical signal from the mixture comprises obtaining an image of the mixture.

56. The method of claim 55, further comprising analyzing the image to identify a region of interest (ROI) of the image, wherein the region of interest of the image contains imaged information of the mixture.

57. The method of claim 55, further comprising analyzing the ROI of the image to determine average optical signal intensity in the ROI.

58. The method of claim 55, further comprising obtaining at least a first image and a second image of the mixture over a period of time, wherein a coagulation time of the mixture is determined based on an analysis comprising determining the difference in optical signal intensity in the ROI of the first image and the ROI in the second image.

59. The method of any of claims 55-58, wherein an image of the mixture is obtained with a CCD or CMOS sensor.

60. The method of any of claims 55-59, wherein the step of obtaining an image of the mixture comprises obtaining a video of the mixture.

61. The method of claim 54, wherein the step of detecting the optical signal from the mixture comprises detecting an optical signal transmitted through the mixture.

62. The method of claim 61, wherein the optical signal transmitted through the mixture is detected over a period of time.

63. The method of claim 62, further comprising identifying a coagulation time of the mixture based on an analysis comprising determining a time when the intensity of the optical signal transmitted through the mixture is reduced by a selected amount as compared to a baseline intensity of the optical signal transmitted through the mixture.

64. The method of claim 62, further comprising identifying a coagulation time of the mixture based on an analysis comprising determining a time when the intensity of the optical signal transmitted through the mixture is reduced by 20% or more as compared to a baseline intensity of the optical signal transmitted through the mixture.

65. The method of claim 54, wherein the step of detecting the optical signal from the mixture comprises detecting the optical signal scattered by the mixture.

66. The method of claim 65, wherein the optical signal scattered by the mixture is detected over a period of time.

67. The method of claim 66, further comprising identifying a coagulation time of the mixture based on an analysis comprising determining a time when the intensity of the optical signal scattered by the mixture is increased by a selected amount as compared to a baseline intensity of the optical signal scattered by the mixture.

68. The method of claim 66, further comprising identifying a coagulation time of the mixture based on an analysis comprising determining a time when the intensity of the optical signal scattered by the mixture is reduced by 20% or more as compared to a baseline intensity of the optical signal scattered by the mixture.

69. The method of any of claims 61-68, wherein the optical signal transmitted through or scattered by the mixture is detected with a CCD sensor, CMOS sensor, photodiode, or photomultiplier tube (PMT).

70. The method of any of claims 54-69, wherein the mixture comprises exogenous fibrinogen.

71. The method of claim 70, wherein the concentration of the exogenous fibrinogen in the mixture is at least 1 mg/ml.

72. The method of claim 70, wherein the concentration of the exogenous fibrinogen in the mixture is between 0.5 and 20 mg/ml.

73. The method of any of claims 54-72, wherein the original biological sample is whole blood.

74. The method of any of claims 54-73, wherein the original biological sample is plasma.

75. The method of any of claims 54-74, wherein the mixture has a total volume of no greater than 50 microliters.

76. The method of any of claims 54-75, wherein the mixture contains no greater than 5 microliters of the original biological sample.

77. The method of any of claims 54-76, wherein the mixture contains no greater than 50% by volume original biological sample.

78. The method of any of claims 54-77, wherein the mixture is illuminated with a white light source

79. The method of any of claims 54-77, wherein the mixture is illuminated with light of one or more selected wavelengths.

80. The method of any of claims 54-77, wherein the mixture is illuminated with light from a light-emitting diode (LED).