

ACCOUNTING FOR ERRORS IN OPTICAL MEASUREMENTS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority from U.S. Provisional Patent Application No. 62/924,229 to Pecker, filed Oct. 22, 2019, entitled "Accounting for errors in optical
5 measurements."

The present application is related to a PCT application filed on even date herewith, entitled "Accounting for errors in optical measurements" to Pecker, claiming priority from U.S. Provisional Patent Application No. 62/924,229 to Pecker, filed Oct. 22, 2019.

The above-referenced applications are incorporated herein by reference.

FIELD OF EMBODIMENTS OF THE INVENTION

Some applications of the presently disclosed subject matter relate generally to analysis of bodily samples, and in particular, to optical density and microscopic measurements that are performed upon blood samples.

BACKGROUND

15 In some optics-based methods (e.g., diagnostic, and/or analytic methods), a property of a biological sample, such as a blood sample, is determined by performing an optical measurement. For example, the density of a component (e.g., a count of the component per unit volume) may be determined by counting the component within a microscopic image. Similarly, the concentration and/or density of a component may be measured by performing optical absorption, transmittance,
20 fluorescence, and/or luminescence measurements upon the sample. Typically, the sample is placed into a sample carrier and the measurements are performed with respect to a portion of the sample that is contained within a chamber of the sample carrier. The measurements that are performed upon the portion of the sample that is contained within the chamber of the sample carrier are analyzed in order to determine a property of the sample.

SUMMARY OF EMBODIMENTS

25 In accordance with some applications of the present invention, a biological sample (e.g., a blood sample) is placed into a sample carrier. While the sample is disposed in the sample carrier, optical measurements are performed upon the sample using one or more optical measurement

devices. For example, the optical measurement devices may include a microscope (e.g., a digital microscope), a spectrophotometer, a photometer, a spectrometer, a camera, a spectral camera, a hyperspectral camera, a fluorometer, a spectrofluorometer, and/or a photodetector (such as a photodiode, a photoresistor, and/or a phototransistor). For some applications, the optical measurement devices include dedicated light sources (such as light emitting diodes, incandescent light sources, etc.) and/or optical elements for manipulating light collection and/or light emission (such as lenses, diffusers, filters, etc.). For some applications, a microscope system is used that is generally similar to the microscope system described in US 2014/0347459 to Greenfield, which is incorporated herein by reference.

10 A computer processor typically receives and processes optical measurements that are performed by the optical measurement device. Further typically, the computer processor controls the acquisition of optical measurements that are performed by the one or more optical measurement devices. For some applications, the optical measurement device is housed inside an optical measurement unit. In order to perform the optical measurements upon the sample, the sample carrier is placed inside the optical measurement unit. Typically, the optical measurement unit includes a microscope system configured to perform microscopic imaging of a portion of the sample. For some applications, the microscope system includes a set of brightfield light sources (e.g. light emitting diodes) that are configured to be used for brightfield imaging of the sample, a set of fluorescent light sources (e.g. light emitting diodes) that are configured to be used for fluorescent imaging of the sample, and a camera (such as a CCD camera and/or a CMOS camera) configured to image the sample. Typically, the optical measurement unit also includes an optical-density-measurement unit configured to perform optical density measurements (e.g., optical absorption measurements) on a second portion of the sample. For some applications, the optical-density-measurement unit includes sets of optical-density-measurement light sources (e.g., light emitting diodes) and light detectors, which are configured for performing optical density measurements on the sample. For some applications, each of the aforementioned sets of light sources (i.e., the set of brightfield light sources, the set of fluorescent light sources, and the set optical-density-measurement light sources) includes a plurality of light sources (e.g. a plurality of light emitting diodes), each of which is configured to emit light at a respective wavelength or at a respective band of wavelengths.

In accordance with some applications of the present invention, various techniques are performed (typically by the computer processor), in order to determine whether various errors have occurred, and optionally to identify the source of the error if an error has occurred. Such errors

may result from the preparation of all of the sample. For example, the sample may have been left in the sample carrier for too long before the measurements were performed (which may result in the sample become degraded, and/or which may result in stains that were mixed with one or both portions of the sample from becoming overly-absorbed by entities within the sample).

5 Alternatively, errors may result from the preparation of a particular portion of the sample. Alternatively or additionally, errors may result from an error with the sample carrier (such as the material of the sample carrier itself being unclean, and/or dirt or spilled blood on the sample carrier), and/or an error with the microscope system itself, such as lighting (e.g., a light emitting diode that used for the brightfield imaging, and/or a light emitting diode that is used during

10 fluorescent imaging of the sample), and/or errors associated with the optical path, and/or motor and stage components or controllers, and/or errors resulting from the environment in which the device is placed (such as, relative humidity, temperature, pressure, particulate concentration, or any other environmental factor). Further alternatively or additionally, there may be an inherent problem with the sample (such as a very low count or a very high count of a certain entity, or too

15 much time having elapsed since the sample collection was performed), which means that the computer processor is unable to perform certain measurements with a sufficient degree of accuracy, and/or which means that the computer processor should flag this to the user.

For some applications, in response to identifying an error, the computer processor outputs a message indicating the error and/or indicating the source of the error. For some applications, the

20 computer processor does not perform certain measurements upon the blood sample in response to identifying an error. For some applications, in response to identifying the error, the computer processor does not perform any measurements on the sample, and/or flags that the sample is invalid to the user, and/or instructs the user to repeat the sample preparation with a new test kit and/or to re-collect the blood sample. Alternatively or additionally, certain parameters of the blood sample

25 are determined by the computer processor by calibrating measurements that are performed upon the blood sample, in order to account for the error.

For some applications, one or more of the following errors is accounted for, e.g., in one or more of the above-described ways:

Errors in the microscopy device, such as:

- 30
- steps-loss in a motor that moves the microscope stage
 - changes in backlash of the movement of the microscope stage
 - changes in timing between the microscope camera and the microscope stage

- alignment between microscope camera and the microscope stage (e.g., due to relative rotation between these elements)
- issues with the optical system (for example, changes in focus quality over time, e.g., caused by samples, or caused by a piece of the microscope stage (e.g., a scratched piece))
- levelling changes in the microscope system
- change in expected focus location along the z-axis (i.e., the optical axis)
- loss of communication between elements
- changes in the camera linear response

Errors caused by environmental factors, such as:

- device outside of allowable temperature, humidity, elevation, etc.
- specific components outside of target values

General errors, caused by factors such as:

- time of scan
- time of device startup
- available working/storage memory

Some examples of the techniques for identifying errors and accounting for such errors are described hereinbelow.

There is therefore provided, in accordance with some applications of the present invention, a method including:

preparing a blood sample for analysis by:

depositing the blood sample within a sample chamber; and

placing the sample chamber, with the blood sample deposited therein, within a microscopy unit;

acquiring one or more microscopic images of the sample chamber with the blood sample deposited therein, using a microscope of the microscopy unit;

based upon the one or more images, determining an amount of one or more cell types within the sample chamber that had already settled within the sample chamber, prior to acquisition of the one or more microscopic images; and

determining a characteristic of the sample, at least partially in response thereto.

In some applications, preparing the blood sample for analysis further includes staining the blood sample with one or more stains.

In some applications:

determining an amount of one or more cell types within the sample chamber that had already settled within the sample chamber, prior to acquisition of the one or more microscopic images includes determining whether more than a threshold amount of red blood cells within the sample chamber had already settled within the sample chamber, prior to acquisition of the one or more microscopic images, and

determining the characteristic of the sample includes, in response to determining that more than the threshold amount of red blood cells within the sample chamber had already settled within the sample chamber prior to acquisition of the one or more microscopic images, invalidating the at least a portion of the sample from being used for performing at least some measurements upon the sample.

In some applications, the method further includes:

subsequent to placing the sample chamber, with the blood sample deposited therein, within the microscopy unit, allowing the one or more cell types within the sample chamber to form a monolayer of cells;

acquiring a set of one or more additional microscopic images of the monolayer of cells; and

performing one or more measurements upon the sample, by analyzing the set of one or more additional microscopic images.

In some applications, the method further includes determining an indication of how long the blood sample has been in the sample chamber prior to acquisition of the one or more microscopic images, based upon the amount of the one or more cell types that had already settled within the sample chamber, prior to acquisition of the one or more microscopic images.

In some applications,

the method further includes performing one or more measurements upon the sample,

determining the amount of the one or more cell types within the sample chamber that had already settled within the sample chamber, prior to acquisition of the one or more microscopic images includes determining whether more than a threshold amount of red blood cells within the sample chamber had already settled within the sample chamber, prior to acquisition of the one or more microscopic images, and

performing one or more measurements upon the sample includes, in response to determining that more than the threshold amount of red blood cells within the sample chamber had

already settled within the sample chamber, prior to acquisition of the one or more microscopic images, calibrating the measurements.

5 In some applications, preparing the blood sample for analysis further includes staining the blood sample with one or more stains, calibrating the measurements includes calibrating the measurements to account for an amount of staining that entities within the blood sample underwent as indicated by more than the threshold amount of red blood cells within the sample chamber having already settled within the sample chamber, prior to acquisition of the one or more microscopic images.

10 There is further provided, in accordance with some applications of the present invention, a method including:

placing a portion of a blood sample within a sample chamber;

acquiring microscopic images of red blood cells within the blood sample, while the red blood cells within the blood sample are settling within the sample chamber;

15 determining a settling-dynamics characteristic of the blood sample by analyzing the images; and

generating an output in response thereto.

20 In some applications, placing the portion of the blood sample within the sample chamber includes placing an undiluted portion of a blood sample within the sample chamber and acquiring microscopic images of red blood cells within the blood sample includes acquiring microscopic images of red blood cells within the undiluted blood sample.

In some applications, determining the settling-dynamics characteristic of the blood sample by analyzing the images includes determining the settling-dynamics characteristic of the blood sample in real-time with respect to the settling of the red blood cells within the blood sample.

25 In some applications, determining the settling-dynamics characteristic of the blood sample by analyzing the images includes determining the settling-dynamics characteristic of the blood sample, while the red blood cells within the blood sample are still settling.

In some applications, determining the settling-dynamics characteristic of the blood sample by analyzing the images includes determining a sedimentation rate of red blood cells in the blood sample by analyzing the images.

30 There is further provided, in accordance with some applications of the present invention, a method including:

placing a first portion of a blood sample within a first sample chamber;
placing a second portion of the blood sample within a second sample chamber;
acquiring microscopic images of the first portion of the blood sample;
performing optical density measurements on the second portion of the blood sample;
5 detecting that a concentration of a given entity within the sample passes a threshold; and
determining a cause for the concentration of the given entity passing the threshold by
comparing a parameter determined from the microscopic images of the first portion of the blood
sample to a parameter determined from the optical density measurements performed on the second
portion of the blood sample.

10 In some applications, determining the cause for the concentration of the given entity
passing the threshold includes determining that the blood sample itself is the cause of the
concentration of the given entity within the sample passing the threshold by determining that a
concentration of the entity as indicated by the microscopic images is similar to the concentration
of the entity as determined from the optical density measurements.

15 In some applications, determining the cause for the concentration of the given entity
passing the threshold includes determining that preparation of one of the portions of the blood
sample is the cause of the concentration of the given entity within the sample passing the threshold
by determining that a concentration of the entity as indicated by the microscopic images is different
from the concentration of the entity as determined from the optical density measurements.

20 There is further provided, in accordance with some applications of the present invention, a
method including:

placing at least a portion of a blood sample within a sample chamber;
acquiring microscopic images of the portion of the blood sample;
identifying, within the microscopic image, at least one type of entity selected from the
25 group consisting of: echinocytes, spherocytes, and crenate red blood cells;
measuring a count of the selected type of entity; and
generating an output in response thereto.

In some applications, identifying the at least one type of entity includes identifying
echinocytes. In some applications, identifying the at least one type of entity includes identifying
30 spherocytes. In some applications, identifying the at least one type of entity includes identifying
crenate red blood cells.

In some applications, generating the output includes invalidating at least the portion of the blood sample from being used to perform at least some measurements upon the blood sample, at least partially based upon the count of the selected type of entity passing a threshold. In some applications, generating the output includes generating an indication of the count to a user. In
5 some applications, generating the output includes generating an indication of an age of the portion of the sample to a user.

There is further provided, in accordance with some applications of the present invention, a method including:

placing at least a portion of a blood sample within a sample chamber;
10 acquiring microscopic images of the portion of the blood sample;
identifying, within the microscopic images, at least one type of entity selected from the group consisting of: echinocytes, spherocytes, and crenate red blood cells;
measuring a count of the selected type of entity; and
determining an indication of an age of the portion of the sample, at least partially based
15 upon the count.

In some applications, the method further includes measuring a parameter of the sample by analyzing the microscopic images, and measuring the parameter of the sample includes performing a measurement upon the microscopic images and calibrating the measurement based upon the determined indication of the age of the portion of the sample.

20 In some applications, the method further includes measuring a parameter of the sample by performing optical density measurements upon a second portion of the blood sample, and measuring the parameter of the sample includes calibrating the optical density measurements based upon the determined indication of the age of the portion of the sample.

In some applications, identifying the at least one type of entity includes identifying
25 echinocytes. In some applications, identifying the at least one type of entity includes identifying spherocytes. In some applications, identifying the at least one type of entity includes identifying crenate red blood cells.

There is further provided, in accordance with some applications of the present invention, a method including:

30 placing at least a portion of a blood sample within a sample chamber that is a cavity that includes a base surface;

allowing the cells in the cell suspension to settle on the base surface of the carrier to form a monolayer of cells on the base surface of the carrier;

acquiring at least one microscope image of at least a portion of the monolayer of cells;

identifying, within the microscopic image, hemolyzed red blood cells;

5 measuring a count of the identified hemolyzed red blood cells; and

generating an output, based upon the count of the identified hemolyzed red blood cells.

In some applications, the method further includes, based upon the count of the identified hemolyzed red blood cells, estimating a total count of hemolyzed red blood cells within the blood sample that is greater than the count of the identified hemolyzed red blood cells, and generating
10 the output includes generating an indication of the estimated total count of hemolyzed red blood cells to a user.

In some applications, generating the output includes invalidating the portion of the sample from being used for performing at least some measurements upon the sample, at least partially based upon the count of the identified hemolyzed red blood cells passing a threshold.

15 In some applications, invalidating the sample from being used for performing at least some measurements upon the sample includes, based upon the count of the identified hemolyzed red blood cells, estimating a total count of hemolyzed red blood cells within the sample that is greater than the count of the identified hemolyzed red blood cells.

In some applications, the method further includes staining the blood sample, and
20 identifying the at least partially hemolyzed red blood cells includes distinguishing the hemolyzed red blood cells from non-hemolyzed red blood cells by identifying red blood cells that are stained by the stain as being hemolyzed.

In some applications, staining the blood sample includes staining the blood sample with a Hoechst reagent.

25 In some applications, acquiring at least one microscope image of at least a portion of the monolayer of cells includes acquiring at least one brightfield microscope image of at least a portion of the monolayer of cells, and identifying red blood cells that are stained by the stain includes identifying red blood cells having an outline that is visible within the brightfield microscope image and having an interior that is similar to a background of the brightfield microscope image.

30 In some applications, acquiring at least one microscope image of at least a portion of the monolayer of cells includes acquiring at least one fluorescent microscope image of at least a

portion of the monolayer of cells, and identifying red blood cells that are stained by the stain includes identifying red blood cells that appear as bright circles within the image.

There is further provided, in accordance with some applications of the present invention, a method including:

- 5 placing a biological sample into a sample chamber that has a plurality of regions, each of which define respective different heights;
- measuring a parameter that is indicative of light transmission through the sample chamber at respective regions; and
- using a computer processor:
- 10 normalizing the parameter as measured at the respective regions with respect to each other;
- detecting that there is a bubble within the sample chamber, at least partially in response thereto; and
- performing an action in response to detecting that there is a bubble within the
- 15 sample chamber.

There is additionally provided, in accordance with some applications of the present invention, a method including:

- placing a blood sample into a sample chamber that has a plurality of regions, each of which define respective different heights;
- 20 measuring a parameter that is indicative of light transmission through the sample chamber at respective regions; and
- using a computer processor:
- based upon an absolute value of the parameter at the selected regions, calculating hemoglobin concentration within the sample;
- 25 normalizing the parameter as measured at the respective regions with respect to each other; and
- validating the calculated hemoglobin concentration based upon the normalized parameter.

There is further provided, in accordance with some applications of the present invention, a method

30 including:

- placing at least a portion of a blood sample within a sample chamber;
- acquiring microscopic images of the portion of the blood sample;

identifying, within the microscopic image, candidates of a given entity within the blood sample;

validating at least some of the candidates as being the given entity, by performing further analysis of the candidates;

5 comparing a count of the candidates of the given entity to a count of the validated candidates of the given entity; and

invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample, at least partially based upon a relationship between the count of candidates and the count of validated candidates.

10 In some applications:

identifying, within the microscopic image, candidates of a given entity within the blood sample includes identifying, within the microscopic image, platelet candidates within the blood sample;

15 validating at least some of the candidates as being the given entity, by performing further analysis of the candidates includes validating at least some of the platelet candidates as being platelets, by performing further analysis of the candidates; and

invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample, includes invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample at least partially based upon a
20 relationship between the count of platelet candidates and the count of validated platelet candidates.

In some applications:

identifying, within the microscopic image, candidates of a given entity within the blood sample includes identifying, within the microscopic image, white blood cell candidates within the blood sample;

25 validating at least some of the candidates as being the given entity, by performing further analysis of the candidates includes validating at least some of the white blood cell candidates as being white blood cells, by performing further analysis of the candidates; and

invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample, includes invalidating at least the portion of the sample from being
30 used for performing at least some measurements upon the sample at least partially based upon a relationship between the count of white blood cell candidates and the count of validated white blood cell candidates.

In some applications, invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample includes invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample based a ratio of the count of validated candidates to the count of candidates exceeding a maximum
5 threshold.

In some applications, invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample includes invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample based a ratio of the count of validated candidates to the count of candidates and being less than minimum
10 threshold.

There is further provided, in accordance with some applications of the present invention, a method including:

placing at least a portion of a blood sample within a sample chamber;
acquiring microscopic images of the portion of the blood sample;
15 identifying, within the microscopic image, white blood cell candidates within the blood sample;
validating at least some of the white blood cell candidates as being given types of white blood cells, by performing further analysis of the white blood cell candidates;
comparing a count of the white blood cell candidates to a count of the white blood cell
20 candidates validated as being the given types of white blood cells; and
invalidating at least the portion of the sample from being used for performing at least some measurements upon the sample, at least partially based upon a relationship between the count of the white blood cell candidates and the count of the white blood cell candidates validated as being given types of white blood cells.

25 There is further provided, in accordance with some applications of the present invention, a method including:

staining a blood sample with one or more stains;
acquiring at least one microscopic image of the blood sample;
identifying contaminating bodies within the sample by identifying stained objects having
30 irregular shapes;
performing a count of one or more entities disposed within the sample, by performing microscopic analysis upon the sample; and

invalidating regions of the sample disposed within given distances from the identified contaminating bodies from being included in the count.

In some applications, staining the blood sample with one or more stains includes staining the blood sample with acridine orange and with a Hoechst reagent, and identifying the debris
5 includes identifying stained objects having irregular shapes and that are stained by both the acridine orange and the Hoechst reagent.

There is further provided, in accordance with some applications of the present invention, a method including:

placing at least a portion of a blood sample within a sample chamber;
10 performing optical measurements upon the sample;
based upon the optical measurements determining that one or more air bubbles are present within the sample chamber;
generating an output at least partially based upon determining that the one or more air bubbles are present within the sample chamber.

15 In some applications, the method further includes determining one or more parameters of the sample based upon the optical measurements, and invalidating at least some of the optical measurements from being used to determine the one or more parameters of the sample, based upon determining that the one or more air bubbles are present within the chamber.

In some applications, generating the output includes invalidating the portion of the blood
20 sample from being used to perform at least some measurements upon the sample, based upon determining that the one or more air bubbles are present within the sample chamber.

In some applications, determining that one or more air bubbles are present within the sample chamber includes analyzing an optical absorption profile of the sample along a given direction along the sample chamber.

25 In some applications, performing optical measurements upon the sample includes performing one or more optical absorption measurements at a wavelength at which hemoglobin does not absorb light, and determining that one or more air bubbles are present within the sample chamber includes analyzing the one or more optical absorption measurements at a wavelength at which hemoglobin does not absorb light.

30 There is further provided, in accordance with some applications of the present invention, a method including:

placing at least a portion of a blood sample within a sample chamber that is a cavity that includes a base surface;

allowing the cells in the cell suspension to settle on the base surface of the carrier to form a monolayer of cells on the base surface of the carrier;

- 5 acquiring at least one microscope image of at least a portion of the monolayer of cells;
identifying, within the microscopic image, a region in which the sample is not present; and
invalidating at least the identified region from being used in microscopic analysis of the portion of the sample.

10 In some applications, identifying the region in which the sample is not present includes identifying an interface between a wet region and a dry region upon the base surface of the sample chamber.

In some applications, identifying the region in which the sample is not present includes distinguishing between the region in which the sample is not present and one or more regions in which the sample is present and the sample has a low cell density.

- 15 There is further provided, in accordance with some applications of the present invention, a method including:

placing at least a portion of a blood sample within a sample chamber that is a cavity that includes a base surface and a top cover;

- 20 allowing the cells in the cell suspension to settle on the base surface of the carrier to form a monolayer of cells on the base surface of the carrier;

acquiring at least one microscope image of at least a portion of the monolayer of cells, while the microscope is focused on a monolayer focal plane, the portion of the monolayer of cells being disposed within the monolayer focal plane;

- 25 identifying that dirt is disposed on the top cover or on an underside of the base surface by identifying a region in which an entity is visible at a focal plane that is different from the monolayer focal plane; and

invalidating at least the identified region from being used in microscopic analysis of the portion of the sample.

- 30 There is further provided, in accordance with some applications of the present invention, a method including:

placing at least a portion of a blood sample within a sample chamber that is a cavity that includes a base surface and a top cover;

allowing the cells in the cell suspension to settle on the base surface of the carrier to form a monolayer of cells on the base surface of the carrier;

acquiring at least one microscope image of at least a portion of the monolayer of cells, while the microscope is focused on a monolayer focal plane, the portion of the monolayer of cells
5 being disposed within the monolayer focal plane;

by identifying a region in which a background intensity of the microscope image is indicative of dirt is disposed on the top cover or on an underside of the base surface; and

invalidating at least the identified region from being used in microscopic analysis of the portion of the sample.

10 There is further provided, in accordance with some applications of the present invention, a method including:

staining at least a portion of a blood sample with a fluorescent stain;

identifying stained cells within the blood sample, by acquiring a plurality of fluorescent microscope images of the portion of the blood sample using a microscopy unit, by illuminating
15 the portion of the sample with a light source that emits light at a given spectral band;

identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source, other than the stained cells; and

determining a characteristic of the light source, based upon the identified fluorescent regions.

20 In some applications, determining the characteristic of the light source, based upon the identified fluorescent regions includes determining whether a spatial distribution of the illumination by the fluorescent light source has changed since a previous measurement.

In some applications, determining the characteristic of the light source, based upon the identified fluorescent regions includes determining whether a spatial uniformity of the illumination
25 by the fluorescent light source has changed since a previous measurement.

In some applications, determining the characteristic of the light source, based upon the identified fluorescent regions includes determining whether a spatial location of the illumination by the fluorescent light source has changed since a previous measurement.

30 In some applications, determining the characteristic of the light source, based upon the identified fluorescent regions includes determining whether vignette effects of the illumination by the fluorescent light source has changed since a previous measurement.

In some applications, determining the characteristic of the light source, based upon the identified fluorescent regions includes determining whether a spectral distribution of the illumination by the fluorescent light source has changed since a previous measurement.

5 In some applications, determining the characteristic of the light source, based upon the identified fluorescent regions includes determining whether an intensity of the illumination by the fluorescent light source has changed since a previous measurement.

In some applications, the method further includes determining a parameter of the blood sample by performing measurements upon the stained cells and normalizing the measurements based upon the determined characteristic of the light source.

10 In some applications, the method further includes invalidating at least some measurements from being performed upon the blood sample, based upon the determined characteristic of the light source.

In some applications, identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying intercellular regions within the blood sample.

15 In some applications, identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying one or more fluorescent regions of the microscopy unit.

In some applications, acquiring the plurality of fluorescent microscope images of the portion of the blood sample includes acquiring the plurality of fluorescent microscope images of the portion of the blood sample while the blood sample is housed in a sample carrier, and identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying one or more fluorescent regions of the sample carrier.

20 In some applications, the sample carrier includes glass and plastic layers that are coupled to each other via a pressure-sensitive adhesive that fluoresces, and identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying the pressure-sensitive adhesive.

25 There is further provided, in accordance with some applications of the present invention, a method including:

30 staining at least a portion of a blood sample with a fluorescent stain;

identifying stained cells within the blood sample, by acquiring a plurality of fluorescent microscope images of the portion of the blood sample, by illuminating the portion of the sample with a light source that emits light at a given spectral band;

- identifying one or more fluorescent regions that are visible within a microscopic image that
5 that is acquired under lighting by the light source, other than the stained cells; and
normalizing fluorescence of the stained cells, based upon the identified fluorescent regions.

In some applications, identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying intercellular regions within the blood sample.

- 10 In some applications, identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying one or more fluorescent regions of the microscopy unit.

- In some applications, acquiring the plurality of fluorescent microscope images of the portion of the blood sample includes acquiring the plurality of fluorescent microscope images of
15 the portion of the blood sample while the blood sample is housed in a sample carrier and identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying one or more fluorescent regions of the sample carrier.

- In some applications, the sample carrier includes glass and plastic layers that are coupled
20 to each other via a pressure-sensitive adhesive that fluoresces, and identifying one or more fluorescent regions that are visible within a microscopic image that that is acquired under lighting by the light source other than the stained cells includes identifying the pressure-sensitive adhesive.

There is further provided, in accordance with some applications of the present invention, a method including:

- 25 identifying entities within a blood sample, by acquiring a plurality of brightfield microscope images of at least a portion of the blood sample, by illuminating the portion of the blood sample with light from a brightfield light source;
analyzing brightfield regions of light emitted by the brightfield light source in an absence of a blood sample; and
30 determining a characteristic of the brightfield light source, based upon analyzing the brightfield regions of light.

In some applications, determining the characteristic of the light source, based upon the identified brightfield regions includes determining whether a spatial distribution of the illumination by the brightfield light source has changed since a previous measurement.

5 In some applications, determining the characteristic of the light source includes determining whether a spatial uniformity of the illumination by the brightfield light source has changed since a previous measurement.

In some applications, determining the characteristic of the light source includes determining whether a spatial location of the illumination by the brightfield light source has changed since a previous measurement.

10 In some applications, determining the characteristic of the light source includes determining whether vignette effects of the illumination by the brightfield light source has changed since a previous measurement.

In some applications, determining the characteristic of the light source includes determining whether a spectral distribution of the illumination by the brightfield light source has
15 changed since a previous measurement.

In some applications, determining the characteristic of the light source includes determining whether an intensity of the illumination by the brightfield light source has changed since a previous measurement.

20 In some applications, analyzing brightfield regions of light emitted by the brightfield light source in an absence of a blood sample includes periodically analyzing brightfield regions of light emitted by the brightfield light source in the absence of the blood sample at fixed intervals of time.

In some applications, analyzing brightfield regions of light emitted by the brightfield light source in an absence of a blood sample includes analyzing brightfield regions of light emitted by the brightfield light source subsequent to a given number of blood sample having been imaged
25 using the brightfield light source.

In some applications, the method further includes determining a parameter of the blood sample by performing measurements upon the entities within the blood sample and normalizing the measurements based upon the determined characteristic of the brightfield light source.

30 In some applications, the method further includes invalidating at least some measurements from being performed upon the blood sample, based upon the determined characteristic of the brightfield light source.

There is further provided, in accordance with some applications of the present invention, a method including:

- staining portions of respective blood samples with at least one type of fluorescent stain;
- using a microscopy unit, acquiring fluorescent microscopic images of the portions of the
- 5 respective blood samples, by illuminating the portion of the sample with a plurality of light sources, each of which emits light at a respective spectral band;
- detecting that there is an error in at least some of the microscopic images; and
- classifying a source of the error, by:
 - in response to detecting that the error was introduced from a given point in time,
 - 10 identifying the fluorescent stain as being the source of the error;
 - in response to detecting that the error gradually increased over time, identifying dirt within the microscopy unit as being the source of the error; and
 - in response to detecting that the error is present only in images acquired under illumination by a given one of the light sources, identifying the given one of the light
 - 15 sources as being the source of the error.

The present invention will be more fully understood from the following detailed description of embodiments thereof, taken together with the drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a block diagram showing components of a biological sample analysis system, in
20 accordance some applications of the present invention;

Figs. 2A, 2B, and 2C are schematic illustrations of respective views of a sample carrier that is used for performing both microscopic measurements and optical density measurements, in accordance with some applications of the present invention;

Figs. 3A, 3B, and 3C are microscopic images of a blood sample that contains hemolyzed
25 red blood cells, acquired in accordance with some applications of the present invention; and

Fig. 4 is a graph showing profiles of normalized light transmission intensity recorded along the length of a sample chamber, in accordance with some applications of the present invention.

DETAILED DESCRIPTION OF EMBODIMENTS

Reference is now made to Fig. 1, which is block diagram showing components of a
30 biological sample analysis system 20, in accordance with some applications of the present

invention. Typically, a biological sample (e.g., a blood sample) is placed into a sample carrier 22. While the sample is disposed in the sample carrier, optical measurements are performed upon the sample using one or more optical measurement devices 24. For example, the optical measurement devices may include a microscope (e.g., a digital microscope), a spectrophotometer, a photometer, a spectrometer, a camera, a spectral camera, a hyperspectral camera, a fluorometer, a spectrofluorometer, and/or a photodetector (such as a photodiode, a photoresistor, and/or a phototransistor). For some applications, the optical measurement devices include dedicated light sources (such as light emitting diodes, incandescent light sources, etc.) and/or optical elements for manipulating light collection and/or light emission (such as lenses, diffusers, filters, etc.). For some applications, a microscope system is used that is generally similar to the microscope system described in US 2014/0347459 to Greenfield, which is incorporated herein by reference.

A computer processor 28 typically receives and processes optical measurements that are performed by the optical measurement device. Further typically, the computer processor controls the acquisition of optical measurements that are performed by the one or more optical measurement devices. The computer processor communicates with a memory 30. A user (e.g., a laboratory technician, or an individual from whom the sample was drawn) sends instructions to the computer processor via a user interface 32. For some applications, the user interface includes a keyboard, a mouse, a joystick, a touchscreen device (such as a smartphone or a tablet computer), a touchpad, a trackball, a voice-command interface, and/or other types of user interfaces that are known in the art. Typically, the computer processor generates an output via an output device 34. Further typically, the output device includes a display, such as a monitor, and the output includes an output that is displayed on the display. For some applications, the processor generates an output on a different type of visual, text, graphics, tactile, audio, and/or video output device, e.g., speakers, headphones, a smartphone, or a tablet computer. For some applications, user interface 32 acts as both an input interface and an output interface, i.e., it acts as an input/output interface. For some applications, the processor generates an output on a computer-readable medium (e.g., a non-transitory computer-readable medium), such as a disk, or a portable USB drive, and/or generates an output on a printer.

For some applications, optical measurement device 24 (and/or computer processor 28 and memory 30) is housed inside an optical measurement unit 31. In order to perform the optical measurements upon the sample, sample carrier 22 is placed inside the optical measurement unit. Typically, the optical measurement unit includes microscope system configured to perform microscopic imaging of a portion of the sample. For some applications, the microscope system

includes a set of brightfield light sources (e.g. light emitting diodes) that are configured to be used for brightfield imaging of the sample, a set of fluorescent light sources (e.g. light emitting diodes) that are configured to be used for fluorescent imaging of the sample, and a camera (such as a CCD camera or a CMOS camera) configured to image the sample. Typically, the optical measurement unit also includes an optical-density-measurement unit configured to perform optical density measurements (e.g., optical absorption measurements) on a second portion of the sample. For some applications, the optical-density-measurement unit includes set of optical-density-measurement light sources (e.g., light emitting diodes) and light detectors, which are configured for performing optical density measurements on the sample. For some applications, each of the aforementioned sets of light sources (i.e., the set of brightfield light sources, the set of fluorescent light sources, and the set optical-density-measurement light sources) includes a plurality of light sources (e.g. a plurality of light emitting diodes), each of which is configured to emit light at a respective wavelength or at a respective band of wavelengths.

Reference is now made to Figs. 2A and 2B, which are schematic illustrations of respective views of sample carrier 22, in accordance with some applications of the present invention. Fig. 2A shows a top view of the sample carrier (the top cover of the sample carrier being shown as being opaque in Fig. 2A, for illustrative purposes), and Fig. 2B shows a bottom view (in which the sample carrier has been rotated around its short edge with respect to the view shown in Fig. 2A). Typically, the sample carrier includes a first set 52 of one or more chambers, which are used for performing microscopic analysis upon the sample, and a second set 54 of one or more chambers, which are used for performing optical density measurements upon the sample. Typically, the chambers of the sample carrier are filled with a bodily sample, such as blood via sample inlet holes 38. For some applications, the chambers define one or more outlet holes 40. The outlet holes are configured to facilitate filling of the chambers with the bodily sample, by allowing air that is present in the chambers to be released from the chambers. Typically, as shown, the outlet holes are located longitudinally opposite the inlet holes (with respect to a sample chamber of the sample carrier). For some applications, the outlet holes thus provide a more efficient mechanism of air escape than if the outlet holes were to be disposed closer to the inlet holes.

Reference is made to Fig. 2C, which shows an exploded view of sample carrier 22, in accordance with some applications of the present invention. For some applications, the sample carrier includes at least three components: a molded component 42, a glass sheet 44, and an adhesive layer 46 configured to adhere the glass sheet to an underside of the molded component. The molded component is typically made of a polymer (e.g., a plastic) that is molded (e.g., via

injection molding) to provide the chambers with a desired geometrical shape. For example, as shown, the molded component is typically molded to define inlet holes 38, outlet holes 40, and gutters 48 which surround the central portion of each of the chambers. The gutters typically facilitate filling of the chambers with the bodily sample, by allowing air to flow to the outlet holes, and/or by allowing the bodily sample to flow around the central portion of the chamber.

For some applications, a sample carrier as shown in Figs. 2A-C is used when performing a complete blood count on a blood sample. For some applications, a first portion of the blood sample is placed inside first set 52 of chambers (which are used for performing microscopic analysis upon the sample), and a second portion of the blood sample is placed inside second set 54 of chambers (which are used for performing optical density measurements upon the sample). For some applications, first set 52 of chambers includes a plurality of chambers, while second set 54 of chambers includes only a single chamber, as shown. However, the scope of the present applications includes using any number of chambers (e.g., a single chamber or a plurality of chambers) within either the first set of chambers or within the second set of chambers, or any combination thereof. The first portion of the blood sample is typically diluted with respect to the second portion of the blood sample. For example, the diluent may contain pH buffers, stains, fluorescent stains, antibodies, spherizing agents, lysing agents, etc. Typically, the second portion of the blood sample, which is placed inside second set 54 of chambers is a natural, undiluted blood sample. Alternatively or additionally, the second portion of the blood sample may be a sample that underwent some modification, including, for example, one or more of dilution (e.g., dilution in a controlled fashion), addition of a component or reagent, or fractionation.

For some applications, one or more staining substances are used to stain the first portion of the blood sample (which is placed inside first set 52 of chambers) before the sample is imaged microscopically. For example, the staining substance may be configured to stain DNA with preference over staining of other cellular components. Alternatively, the staining substance may be configured to stain all cellular nucleic acids with preference over staining of other cellular components. For example, the sample may be stained with acridine orange reagent, Hoechst reagent, and/or any other staining substance that is configured to preferentially stain DNA and/or RNA within the blood sample. Optionally, the staining substance is configured to stain all cellular nucleic acids but the staining of DNA and RNA are each more prominently visible under some lighting and filter conditions, as is known, for example, for acridine orange. Images of the sample may be acquired using imaging conditions that allow detection of cells (e.g., brightfield) and/or imaging conditions that allow visualization of stained bodies (e.g. appropriate fluorescent

illumination). Typically, the first portion of the sample is stained with acridine orange and with a Hoechst reagent. For example, the first (diluted) portion of the blood sample may be prepared using techniques as described in US 2015/0316477 to Pollak, which is incorporated herein by reference, and which describes a method for preparation of blood samples for analysis that involves
5 a dilution step, the dilution step facilitating the identification and/or counting of components within microscopic images of the sample.

Typically, prior to being imaged microscopically, the first portion of blood (which is placed in first set 52 of chambers) is allowed to settle such as to form a monolayer of cells, e.g., using techniques as described in US 9,329,129 to Pollak, which is incorporated herein by reference. For
10 some applications, the microscopic analysis of the first portion of the blood sample is performed with respect to the monolayer of cells. Typically, the first portion of the blood sample is imaged under brightfield imaging, i.e., under illumination from one or more light sources (e.g., one or more light emitting diodes, which typically emit light at respective spectral bands). Further typically, the first portion of the blood sample is additionally imaged under fluorescent imaging.
15 Typically, the fluorescent imaging is performed by exciting stained objects (i.e., objects that have absorbed the stain(s)) within the sample by directing light toward the sample at known excitation wavelengths (i.e., wavelengths at which it is known that stained objects emit fluorescent light if excited with light at those wavelengths), and detecting the fluorescent light. Typically, for the fluorescent imaging, a separate set of light sources (e.g., one or more light emitting diodes) is used
20 to illuminate the sample at the known excitation wavelengths.

It is noted that, in the context of the present application, the term monolayer is used to mean a layer of cells that have settled, such as to be disposed within a single focus field of the microscope. Within the monolayer there may be some overlap of cells, such that within certain areas there are two or more overlapping layers of cells. For example, red blood cells may overlap
25 with each other within the monolayer, and/or platelets may overlap with, or be disposed above, red blood cells within the monolayer.

As described with reference to US 2019/0302099 to Pollack, which is incorporated herein by reference, for some applications, chambers belonging to set 52 (which is used for microscopy measurements) have different heights from each other, in order to facilitate different measurands
30 being measured using microscope images of respective chambers, and/or different chambers being used for microscopic analysis of respective sample types. For example, if a blood sample, and/or a monolayer formed by the sample, has a relatively low density of red blood cells, then measurements may be performed within a chamber of the sample carrier having a greater height

(i.e., a chamber of the sample carrier having a greater height relative to a different chamber having a relatively lower height), such that there is a sufficient density of cells, and/or such that there is a sufficient density of cells within the monolayer formed by the sample, to provide statistically reliable data. Such measurements may include, for example red blood cell density measurements, measurements of other cellular attributes, (such as counts of abnormal red blood cells, red blood cells that include intracellular bodies (e.g., pathogens, Howell-Jolly bodies), etc.), and/or hemoglobin concentration. Conversely, if a blood sample, and/or a monolayer, formed by the sample, has a relatively high density of red blood cells, then such measurements may be performed upon a chamber of the sample carrier having a relatively low height, for example, such that there is a sufficient sparsity of cells, and/or such that there is a sufficient sparsity of cells within the monolayer of cells formed by the sample, that the cells can be identified within microscopic images. For some applications, such methods are performed even without the variation in height between the chambers belonging to set 52 being precisely known.

For some applications, based upon the measurand that is being measured, the chamber within the sample carrier upon which to perform optical measurements is selected. For example, a chamber of the sample carrier having a greater height may be used to perform a white blood cell count (e.g., to reduce statistical errors which may result from a low count in a shallower region), white blood cell differentiation, and/or to detect more rare forms of white blood cells. Conversely, in order to determine mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), red blood cell distribution width (RDW), red blood cell morphologic features, and/or red blood cell abnormalities, microscopic images may be obtained from a chamber of the sample chamber having a relatively low height, since in such chambers the cells are relatively sparsely distributed across the area of the region, and/or form a monolayer in which the cells are relatively sparsely distributed. Similarly, in order to count platelets, classify platelets, and/or extract any other attributes (such as volume) of platelets, microscopic images may be obtained from a chamber of the sample chamber having a relatively low height, since within such chambers there are fewer red blood cells which overlap (fully or partially) with the platelets in microscopic images, and/or in a monolayer.

In accordance with the above-described examples, it is preferable to use a chamber of the sample carrier having a lower height for performing optical measurements for measuring some measurands within a sample (such as a blood sample), whereas it is preferable to use a chamber of the sample carrier having a greater height for performing optical measurements for measuring other measurands within such a sample. Therefore, for some applications, a first measurand within

a sample is measured, by performing a first optical measurement upon (e.g., by acquiring microscopic images of) a portion of the sample that is disposed within a first chamber belonging to set 52 of the sample carrier, and a second measurand of the same sample is measured, by performing a second optical measurement upon (e.g., by acquiring microscopic images of) a portion of the sample that is disposed within a second chamber of set 52 of the sample carrier. For some applications, the first and second measurands are normalized with respect to each other, for example, using techniques as described in US 2019/0145963 to Zait, which is incorporated herein by reference.

Typically, in order to perform optical density measurements upon the sample, it is desirable to know the optical path length, the volume, and/or the thickness of the portion of the sample upon which the optical measurements were performed, as precisely as possible. Typically, an optical density measurement is performed on the second portion of the sample (which is typically placed into second set 54 of chambers in an undiluted form). For example, the concentration and/or density of a component may be measured by performing optical absorption, transmittance, fluorescence, and/or luminescence measurements upon the sample.

Referring again to Fig. 2A, for some applications, chambers belonging to set 54 (which is used for optical density measurements), typically define at least a first region 56 (which is typically deeper) and a second region 58 (which is typically shallower), the height of the chambers varying between the first and second regions in a predefined manner, e.g., as described in WO 17/195205 to Pollack, which is incorporated herein by reference. The heights of first region 56 and second region 58 of the sample chamber are defined by a lower surface that is defined by the glass sheet and by an upper surface that is defined by the molded component. The upper surface at the second region is stepped with respect to the upper surface at the first region. The step between the upper surface at the first and second regions, provides a predefined height difference Δh between the regions, such that even if the absolute height of the regions is not known to a sufficient degree of accuracy (for example, due to tolerances in the manufacturing process), the height difference Δh is known to a sufficient degree of accuracy to determine a parameter of the sample, using the techniques described herein, and as described in US 2019/0302099 to Pollack, which is incorporated herein by reference. For some applications, the height of the chamber varies from the first region 56 to the second region 58, and the height then varies again from the second region to a third region 59, such that, along the sample chamber, first region 56 defines a maximum height region, second region 58 defines a medium height region, and third region 59 defines a minimum

height region. For some applications, additional variations in height occur along the length of the chamber, and/or the height varies gradually along the length of the chamber.

As described hereinabove, while the sample is disposed in the sample carrier, optical measurements are performed upon the sample using one or more optical measurement devices 24. Typically, the sample is viewed by the optical measurement devices via the glass layer, glass being transparent at least to wavelengths that are typically used by the optical measurement device. Typically, the sample carrier is inserted into optical measurement unit 31, which houses the optical measurement device while the optical measurements are performed. Typically, the optical measurement unit houses the sample carrier such that the molded layer is disposed above the glass layer, and such that the optical measurement unit is disposed below the glass layer of the sample carrier and is able to perform optical measurements upon the sample via the glass layer. The sample carrier is formed by adhering the glass sheet to the molded component. For example, the glass sheet and the molded component may be bonded to each other during manufacture or assembly (e.g. using thermal bonding, solvent-assisted bonding, ultrasonic welding, laser welding, heat staking, adhesive, mechanical clamping and/or additional substrates). For some applications, the glass layer and the molded component are bonded to each other during manufacture or assembly using adhesive layer 46. For some applications, due to tolerances in the manufacturing process, the absolute heights of the sample chambers are not known. As described above, the step between the upper surface at the first and second regions, provides a predefined height difference Δh between the regions, such that even if the absolute height of the regions is not known to a sufficient degree of accuracy (for example, due to tolerances in the manufacturing process), the height difference Δh is known to a sufficient degree of accuracy to determine a parameter of the sample. Alternative or additional variations in height (e.g., stepped variations in height or gradual variations in height) along the length of the chamber may be used as an alternative or in addition to the step between the first and second regions, for example, using the techniques described herein, and as described in US 2019/0302099 to Pollack, which is incorporated herein by reference.

For some applications, a portion of sample carrier 22 is configured to fluoresce, at least under certain conditions. For example, the portion of the sample carrier may be configured to fluoresce when exposed to light emitted by optical measurement device 24 (e.g., brightfield light or fluorescent light that is emitted by a microscope system). Or the portion of the sample carrier may be configured to fluoresce when placed within optical measurement unit 31 in which optical measurement device 24 is housed. As described hereinabove, for some applications, sample carrier 22 includes adhesive layer 46. For some applications, the adhesive layer, or a portion

thereof, is configured to fluoresce in the above-described manner (e.g., by an adhesive material within the adhesive layer being configured to fluoresce, by the adhesive layer containing an additional material that is configured to fluoresce, and/or by the adhesive layer being coated with such a material). For some applications, the adhesive layer is a pressure-sensitive adhesive, at least a portion of which is configured to fluoresce. For example, the pressure-sensitive adhesive may be an acrylic-based pressure-sensitive adhesive, at least a portion of which is configured to fluoresce.

In accordance with some applications of the present invention, various techniques are performed (typically by the computer processor), in order to determine whether various errors have occurred, and optionally to identify the source of the error if an error has occurred. Such errors may result from the preparation of all of the sample. For example, the sample may have been left in the sample carrier for too long before the measurements were performed (which may result in the sample become degraded, and/or which may result in stains that were mixed with one or both portions of the sample from becoming overly-absorbed by entities within the sample). Alternatively, errors may result from the preparation of a particular one of the portions of the sample. For example, there may have been an error in the preparation of the first portion (which is typically diluted and is placed within the first set 52 of chambers to be analyzed microscopically), such as an error in the dilution of the first portion of the sample, entry of air bubbles into the first set 52 of chambers or contamination of that portion of the sample. Alternatively or additionally, there may have been an error in the preparation of the second portion (which is typically undiluted and is placed within the second set 54 of chambers to be analyzed via optical density measurements), such as contamination of that portion, or entry of air bubbles into the second set 54 of chambers.

Alternatively or additionally, errors may result from an error with the sample carrier (such as the material of the sample carrier itself (e.g., the substrate) being unclean, and/or dirt or spilled blood on the sample carrier), and/or an error with the microscope system itself, such as lighting (e.g., a light emitting diode that used for the brightfield imaging, and/or a light emitting diode that is used during fluorescent imaging of the sample), and/or errors associated with the optical path, and/or motor and stage components or controllers, and/or errors resulting from the environment in which the device is placed (such as, relative humidity, temperature, pressure, particulate concentration, or any other environmental factor). Further alternatively or additionally, there may be an inherent problem with the sample (such as a very low count or a very high count of a certain entity, or too much time having elapsed since the sample collection was performed), which means

that the computer processor is unable to perform certain measurements with a sufficient degree of accuracy, and/or which means that the computer processor should flag this to the user.

For some applications, in response to identifying an error, the computer processor outputs a message indicating the error and/or indicating the source of the error. For some applications, the computer processor does not perform certain measurements upon the blood sample in response to identifying an error. For some applications, in response to identifying the error, the computer processor does not perform any measurements on the sample, and/or flags that the sample is invalid to the user, and/or instructs the user to repeat the sample preparation with a new test kit and/or to re-collect the blood sample. Alternatively or additionally, certain parameters of the blood sample are determined by the computer processor by calibrating measurements that are performed upon the blood sample, in order to account for the error.

For some applications, one or more of the following errors is accounted for, e.g., in one or more of the above-described ways:

Errors in the microscopy device, such as:

- steps-loss in a motor that moves the microscope stage
- changes in backlash of the movement of the microscope stage (e.g., as described in further detail hereinbelow)
- changes in timing between the microscope camera and the microscope stage (e.g., as described in further detail hereinbelow)
- alignment between microscope camera and the microscope stage (e.g., due to relative rotation between these elements)
- issues with the optical system (for example, changes in focus quality over time, e.g., caused by samples, or caused by a piece of the microscope stage (e.g., a scratched piece), e.g., as described in further detail hereinbelow)
- levelling changes in the microscope system
- change in expected focus location along the z-axis (i.e., the optical axis)
- loss of communication between elements
- changes in the camera linear response

Errors caused by environmental factors, such as:

- device outside of allowable temperature, humidity, elevation, etc.
- specific components outside of target values

General errors, caused by factors such as:

- time of scan
- time of device startup
- available working/storage memory

Some examples of the techniques for identifying errors and accounting for such errors are described hereinbelow.

As described hereinabove, typically, the first portion of the blood sample is analyzed microscopically, while disposed inside first set 52 of sample chambers. Typically, prior to being imaged microscopically, the first portion of blood (which is placed in first set 52 of chambers) is allowed to settle such as to form a monolayer of cells, e.g., using techniques as described in US 9,329,129 to Pollak, which is incorporated herein by reference.

For some applications, the computer processor is configured to determine whether one or more cell types (e.g., red blood cells) within the sample chamber had already settled within the sample chamber, prior to the sample carrier being placed into the microscopy unit by acquiring one or more microscopic images of the sample after the sample carrier has been placed into the microscopy unit, and analyzing the one or more images.

Typically, if it is determined that all of the red blood cells had already settled prior to the microscopic images being acquired, this is an indication that the blood sample was left within the sample chamber for too long prior to the sample carrier placed into the microscopy unit. It is noted that, although the analysis of the microscopic images is typically performed with respect to the monolayer of settled cells, it is nevertheless desirable that the sample carrier be placed into the microscopy unit while some red blood cells are still settling, since this indicates that the sample was not left in the sample carrier for too long, prior to being placed into the microscopy unit. Conversely, if all of the red blood cells (or a sufficiently great proportion of the red blood cells) had already settled, then the sample may have become degraded, and/or the stains may have become overly-absorbed by entities within the sample. Thus, the extent to which the cells are still settling may be used as a measure of how recently the sample was drawn from the subject and/or how recently the sample was placed in the sample carrier or sample chamber (i.e., the freshness of the sample). Therefore, for some applications, in response to determining that more than a threshold amount of red blood cells within the sample chamber had already settled within the sample chamber, prior to acquisition of the one or more microscopic images, the sample (or a portion thereof) is invalidated from being used for performing at least some measurements upon the sample.

For some applications, an indication of the age of the blood sample is determined, at least partially based upon determining whether one or more cell types within the sample chamber had already settled within the sample chamber, prior to acquisition of the one or more microscopic images. For some applications, an indication of the age of the blood sample is determined, at least
5 partially based upon an amount (or a proportion) of one or more cell types within the sample chamber that had not yet settled within the sample chamber, prior to acquisition of the one or more microscopic images.

For some applications, a generally similar analysis to that described in the above paragraphs (for determining how recently the sample was drawn from the subject and/or how
10 recently the sample was placed in the sample carrier or sample chamber) is performed with respect to the second portion of the sample (which is typically placed into second set 54 of chambers in an undiluted form).

It is noted that, typically, after the computer processor has determined an indication of how recently the sample was drawn from the subject and/or how recently the sample was placed in the
15 sample carrier or sample chamber (e.g., using the above-described analysis technique), the sample carrier is left in place within the microscopy unit for several minutes (for example, between 2 and 10 minutes, e.g., approximately 5 minutes) before further imaging of the first portion of the blood sample is performed (the further imaging being performed for the purpose of microscopic analysis of the blood sample). This is in order to allow time for the first portion of the blood sample to
20 settle into a monolayer.

For some applications, measurements that are performed upon the sample are calibrated, in response to determining that more than a threshold amount of red blood cells within the sample chamber had already settled within the sample chamber, prior to acquisition of the one or more
microscopic images. For example, such measurements may be calibrated to account for an amount
25 of staining that entities within the blood sample underwent as indicated by more than the threshold amount of red blood cells within the sample chamber having already settled within the sample chamber, prior to acquisition of the one or more microscopic images. For some applications, measurements that are performed upon the sample are calibrated, based upon an amount (or a proportion) of one or more cell types within the sample chamber that had not yet settled within the
30 sample chamber, prior to acquisition of the one or more microscopic images.

For some applications, microscopic images of red blood cells within the blood sample are acquired, while the red blood cells within the blood sample are settling within the sample chamber,

and a settling-dynamics characteristic of the blood sample (e.g., the red blood cell sedimentation rate) is determined by the computer processor by analyzing the images. Typically, the settling-dynamics characteristic of the blood sample (e.g., the red blood cell sedimentation rate) is determined by the computer processor in real-time with respect to the settling of the red blood cells (i.e., while the red blood cells are still settling). This is in contrast with other techniques for determining the settling-dynamics characteristic of the blood sample (e.g., the red blood cell sedimentation rate), in which the total time that it takes for red blood cells to settle is measured. It is noted that, if such measurements are performed on a diluted portion of the blood sample, the effect of the proteins on the settling time of the red blood cells is diluted. Therefore, for some applications, such measurements are performed on an undiluted portion of the blood sample.

For some applications, the above-described analyses for determining an indication of how recently the sample was drawn from the subject and/or how recently the sample was placed in the sample carrier and/or for determining settling-dynamics characteristics are corrected using per sample information such as red blood cell or platelet mean cell volume, mean cell hemoglobin concentration, or other such sample-indicative measures. Alternatively or additionally, the analyses for determining an indication of how recently the sample was drawn from the subject and/or how recently the sample was placed in the sample carrier and/or for determining settling-dynamics characteristics are corrected using information on a single-cell level (i.e., by extracting data relating to individual cells and correcting the determined settling-dynamics characteristics based upon these data).

For some applications, in response to determining that the concentration of a given entity within the blood sample passes a threshold, the computer processor determines a cause of the concentration of the given entity passing the threshold by comparing a parameter determined from the microscopic images of the first portion of the blood sample to a parameter determined from the optical density measurements performed on the second portion of the blood sample. For example, in response to detecting that the first portion of the sample (which is typically diluted and placed inside first set 52 of chambers) has a very high red blood cell count or a very low red blood cell count, the computer processor may perform the comparison in order to determine whether it is the case that the blood sample inherently has a very high red blood cell count or a very low red blood cell count, or whether it was caused by an error in the preparation of the portion of the sample (e.g., the dilution of the first portion). The computer processor typically determines that the blood sample itself is the cause of the concentration of the given entity within the sample passing the threshold by determining that a concentration of the entity as indicated by the

microscopic images is similar to the concentration of the entity as determined from the optical density measurements. Further typically, the computer processor determines that preparation of the portion of the blood sample is the cause of the concentration of the given entity within the sample passing the threshold by determining that a concentration of the entity as indicated by the
5 microscopic images is different from the concentration of the entity as determined from the optical density measurements.

For some applications, a similar analysis to that described in the above paragraph is performed using parameters of the sample other than concentration (e.g., mean cell volume, mean cell hemoglobin, etc.). For some applications, in response to identifying that there is a difference
10 between parameters as measured upon the respective portions of the sample, it is determined that the respective portions of the sample are likely to be portions of two different samples (e.g., from two different patients).

For some applications, the parameters as determined from the respective portions of the sample are used to correct each other. For example, if the values of a parameter as measured in
15 each of the sample portions are different from each other, but it is determined that there is a third value (or range of values) of the parameter that lies within the range of error for both of the values as measured in each of the sample portions, then it may be determined that the third value (or range of values) is likely to be correct.

For some applications, in response to one or more parameters of the sample being outside
20 of the normal range, the computer processor compares these parameters to other parameters of the sample. In response to all of the parameters being outside of the normal range (or even erroneous in a manner that correlates with the error in the one or more parameters), then the computer processor may determine that this is due to an error in a calculation that affects all of these parameters.

25 For some applications, the computer processor is configured to identify echinocytes, spherocytes, and/or crenate red blood cells within the microscopic images of the first portion of the sample. Typically, the presence of such entities is an indication of the portion of the sample having become degraded due to age and/or sample storage conditions. For some such applications, the computer processor measures a count of such entities, and invalidates at least the portion of
30 the blood sample from being used to perform at least some measurements upon the blood sample, at least partially based upon the count of the selected type of entity passing a threshold. For some applications, the computer processor generates an indication of the count, and/or an associated

clinical condition, to a user. Alternatively or additionally, the computer processor determines an indication of an age of the portion of the sample, at least partially based upon the count. For some applications, in order to determine a parameter of the sample, a measurement is performed upon the microscopic images and the measurement is calibrated based upon the determined indication of the age of the portion of the sample, and/or based upon the count of the aforementioned entities. For some applications, a parameter of the sample is determined by performing optical density measurements upon the second portion of the blood sample (which is typically disposed inside second set 54 of sample chambers), and calibrating the optical density measurements based upon the determined indication of the age of the portion of the sample, and/or based upon the count of the aforementioned entities. (For some applications, optical density measurements are calibrated based upon one or more other factors, e.g., red blood cell morphology, red blood cell volume, count of platelets, level of debris within the sample, and/or presence or amount of any object or attribute that may affect scattering.)

For some applications, the computer processor estimates the volumes of the echinocytes, spherocytes, and/or crenate red blood cells. For some such applications, the volumes of such cells is incorporated into an overall measure of the mean red blood cell volume within the sample.

Reference is now made to Figs. 3A and 3B, which are brightfield microscopic images, acquired, respectively, under violet and green LED illumination in accordance with some applications of the present invention. As described hereinabove, typically microscopic images of the first portion of the blood sample are acquired and a monolayer of cells within the first portion is analyzed. Typically, spherification techniques are not applied to the red blood cells in the sample prior to the portion of the sample being imaged. The inventors of the present application have discovered that when the portion of the sample is microscopically imaged using the techniques described herein, some hemolyzed red blood cells are visible in the microscopic images. Typically, after the sample is stained by Hoechst reagent (or any fluorescent or non-fluorescent stain that has affinity to the cell membrane), under brightfield imaging, the cell outline is visible, but the rest of the cell appears like the background of the image. This effect may be observed in Figs. 3A and 3B, in which red blood cells 60 are visible, and the outlines of hemolyzed red blood cells 62 are visible, but the interior of the cells appear similar to the background, such that the outlines appear as "empty" cells. The "empty" cells typically have generally similar shapes and sizes to red blood cells.

Reference is also made to Fig. 3C, which is a fluoroscopic microscopic images, acquired in accordance with some applications of the present invention. The image was recorded after a

blood sample had been stained with a Hoechst reagent, and excited using UV illumination centered around approximately 360 nm. It may be observed that, unlike red blood cells 60, which appear faintly as darkened circles, the hemolyzed red blood cells 62 appear as bright circles having generally similar shapes and sizes to the red blood cells. It is hypothesized that the hemolyzed red blood cells become stained by the Hoechst reagent binding to remnants that remain on the membrane of the hemolyzed red blood cells. This gives rise to the hemolyzed red blood cells appearing as "empty" cells in the brightfield images, and/or appearing as bright circles in the fluorescent images. It is further hypothesized that the reason why the red blood cells appear as darkened circles in the fluorescent image is because there is background emission of free Hoechst reagent throughout the sample, but this emission is attenuated by the hemoglobin in the red blood cells, such that they appear darker than the background.

Therefore, in accordance with some applications of the present invention, hemolyzed red blood cells are identified within a non-spherificated blood sample. Typically, the sample is stained with a stain such as Hoechst reagent (or any fluorescent or non-fluorescent stain that has affinity to the cell membrane). The hemolyzed red blood cells are identified within brightfield images of the stained sample, by identifying cells the outlines of which are visible, but the interiors of which appear generally similar to the background (such that the outlines appear as "empty" cells). Alternatively or additionally, the hemolyzed red blood cells are identified within fluorescent images of the stained sample. Typically, the hemolyzed red blood cells have generally similar shapes and sizes to the red blood cells within the images.

For some applications, the hemolyzed red blood cells that are visible constitute only a fraction of the total number of hemolyzed red blood cells that are present within the portion of the sample, since it is typically the case that a portion of the hemolyzed red blood cells are not visible. For some applications, the computer processor identifies the visible hemolyzed red blood cells, and measures a count of the identified hemolyzed red blood cells within the portion of the sample. Typically, based upon the count of the identified hemolyzed red blood cells, the computer processor estimates a total count of hemolyzed red blood cells within the portion of the sample that is greater than the count of the identified hemolyzed red blood cells. Alternatively or additionally, based upon the count of the identified hemolyzed red blood cells, the computer processor estimates a ratio of hemolyzed red blood cells to non-hemolyzed red blood cells within the sample. Typically, this ratio is calculated by estimating a total count of hemolyzed red blood cells within the portion of the sample that is greater than the count of the identified hemolyzed red blood cells. For some applications, the computer processor outputs an indication of the estimated

total count of hemolyzed red blood cells within the blood sample, or of the ratio of hemolyzed red blood cells to non-hemolyzed red blood cells, to a user. Alternatively or additionally, the computer processor invalidates the sample from being used for performing at least some measurements upon the sample, at least partially based upon the count of the identified hemolyzed red blood cells
5 passing a threshold, or based upon the aforementioned ratio exceeding a threshold. For some applications, the invalidation of the sample is based upon estimating a total count of hemolyzed red blood cells within the portion of the sample that is greater than the count of the identified hemolyzed red blood cells.

For some applications, in order to identify a given entity within the blood sample (such as
10 platelets, red blood cells, white blood cells, etc.) the computer processor first identifies candidates of the given entity within the blood sample, by analyzing the microscopic images of the first portion of the blood sample. Subsequently, the computer processor validates at least some of the candidates as being the given entity, by performing further analysis of the candidates.

For some applications, the computer processor compares a count of the candidates of the
15 given entity to a count of the validated candidates of the given entity, and invalidates at least the portion of the sample from being used for performing at least some measurements upon the sample, at least partially based upon a relationship between the count of candidates and the count of validated candidates. For example, if the ratio of the count of validated candidates to the count of candidates exceeds a maximum threshold, the computer processor may invalidate at least the
20 portion of the sample from being used for performing at least some measurements upon the sample, as this is indicative of too many candidates having been validated, indicating an error. Alternatively or additionally, if the ratio of the count of validated candidates to the count of candidates is lower than a minimum threshold, the computer processor may invalidate at least the portion of the sample from being used for performing at least some measurements upon the sample,
25 as this is indicative of too few candidates having been validated, indicating an error. For some applications, if the ratio of the count of validated platelets to the count of platelet candidates is lower than a minimum threshold, the computer processor invalidates at least the portion of the sample from being used for performing a platelet count, as this is indicative of too few platelet candidates having been validated as platelets, indicating an error. For example, this error may be
30 caused by debris (or other contaminating bodies) having been wrongly identified as platelets or as platelet candidates. It is noted that the source of the error may be in the preparation of the portion of the sample, in the sample carrier, in portions of the microscopy unit, and/or in the blood itself. For some applications, similar techniques are performed with respect to red blood cells, white

blood cells, and/or other entities within the sample (e.g., anomalous white blood cells, circulating tumor cells, red blood cells, reticulocytes, Howell-Jolly bodies, etc.).

For some applications, the computer processor is configured to identify white blood cell candidates within the blood sample, and is then configured to validate at least some of the white blood cell candidates as being given types of white blood cells (e.g., neutrophils, lymphocytes, eosinophils, monocytes, blasts, immature cells, atypical lymphocytes, and/or basophils), by performing further analysis of the white blood cell candidates. For some applications, the computer processor compares a count of the white blood cell candidates to a count of the white blood cell candidates validated as being the given types of white blood cells, and invalidates at least the portion of the sample from being used for performing at least some measurements upon the sample, at least partially based upon a relationship between the count of the white blood cell candidates and the count of the white blood cell candidates validated as being given types of white blood cells. For example, if the ratio of the count of white blood cell candidates validated as being given types of white blood cells to the count of white blood cell candidates exceeds a maximum threshold, the computer processor may invalidate at least the portion of the sample from being used for performing at least some measurements upon the sample, as this is indicative of too many candidates having been validated, indicating an error. Alternatively or additionally, if the ratio of the count of white blood cell candidates validated as being given types of white blood cells to the count of white blood cell candidates is lower than a minimum threshold, the computer processor may invalidate at least the portion of the sample from being used for performing at least some measurements upon the sample, as this is indicative of too few candidates having been validated, indicating an error. It is noted that the source of the error may be in the preparation of the portion of the sample, in the sample carrier, in portions of the microscopy unit, and/or in the blood itself.

For some applications, the computer processor is configured to identify debris (or other contaminating bodies) within the sample, by identifying stained objects having irregular shapes (e.g., fibrous shapes, non-circular shapes, and/or elongate shapes). As described hereinabove, typically, the computer processor performs a count of one or more entities disposed within the sample, by performing microscopic analysis upon the sample. For some applications, the computer processor invalidates regions of the sample disposed within given distances from the identified debris (or other contaminating bodies) from being included in the count. Typically, debris are stained by stains, and further typically debris are stained by both acridine orange and Hoechst reagent. For some applications, the computer processor identifies the debris (or other

contaminating bodies) by identifying objects that have irregular shapes and/or that are stained by a stain (e.g., by both the acridine orange and Hoechst reagent).

Reference is now made to Fig. 4, which is a graph showing normalized light logarithm-of-transmission-intensity profiles measured along the length of a sample chamber belonging to second set 54 of sample chambers, in accordance with some applications of the present invention. As described hereinabove, typically the computer processor is configured to perform optical measurements (e.g., optical density measurements) on the second portion of the sample, which is placed into second set 54 of sample chambers. For some applications, light (e.g. light from an LED) is transmitted through a chamber at a spectral band at which hemoglobin absorbs light, and the intensity of the transmitted light is detected by a photodetector. The amount of light that is absorbed is interpreted to be indicative of the concentration of hemoglobin within the second portion of the sample, in accordance with the Beer-Lambert law.

For some applications, the computer processor determines that one or more air bubbles are present within one of the second set of sample chambers, based upon the optical measurements. For example, due to the regions having different heights within the sample chamber the normalized logarithm-of-transmission-intensity profile (which is related to the hemoglobin absorption profile) along the length of the chamber is expected to have a given shape, e.g., regions along which the light logarithm-of-transmission-intensity is substantially constant with differences between the regions (due to steps in the height of the chamber). This is indicated by the solid curve 70 in Fig.4, which is a combination of the normalized logarithm-of-transmission-intensity recorded along the length of a sample chamber for several different samples. As shown, along the minimum height region 56 of the sample chamber, logarithm-of-transmission-intensity is substantially constant. (In fact, there is a slight slope along the length of this region, which is due to height variation along the length of the region due to tolerances in the manufacture of the sample carrier, as described hereinabove.) There is then a drop in the logarithm-of-transmission-intensity as the distance along the sample chamber transitions to the medium height region 58 (at which hemoglobin absorption is greater, and light transmission is therefore lower), before there is a further drop as the distance along the sample chamber transitions to the maximum height region 59 (at which hemoglobin absorption is greater still, and light transmission is therefore even lower). It is noted that the way in which the transmission was normalized was by taking the mean of the value of the logarithm of light transmission intensity within a given portion of the maximum height region and assigning this a value of 1, taking the mean of the value of the logarithm of light transmission intensity within a given portion of the minimum height region and assigning this a second value, and then

normalizing other values with respect to these two values. (In some cases, the value of the logarithm of light transmission intensity within the given portion of the minimum height region is assigned a value of Euler's number (i.e., 2.718), although in the example shown in Fig. 4, this is not the case.) One would expect the normalized profile of any sample that fills the sample chamber
5 to have a similar profile, irrespective of absolute hemoglobin absorption of the sample, since the shape of the profile is dependent upon the relative absorption along the different regions of the sample chamber.

Thin curve 72 in Fig. 4 shows the normalized logarithm-of-transmission-intensity recorded along the length of a sample chamber for a given sample. It may be observed that within the
10 minimum height region, there is a relatively flat portion of the curve (which is below curve 70) and then a peak (which is above curve 70). In addition, along the medium height region, curve 72 is below curve 70. Typically, such a profile is indicative of the fact that there is a bubble (e.g., an air bubble, and/or the presence of a different substance) within the minimum height region. At the location of the bubble, the light transmission is greater, causing there to be a peak in curve 72 at
15 this location. At other locations (e.g., within other portions of the minimum height region) and along the entire medium height region, the presence of the bubble within the minimum height region causes the normalized logarithm-of-transmission-intensity values to be lowered relative to those of a sample that does not contain a bubble. Similarly, when there are bubbles within other regions of the sample chamber (or along an entire region), this will give rise to a different
20 normalized logarithm-of-transmission-intensity profile. For example, if there would be a bubble along the entire minimum height region, this would cause the normalized logarithm-of-transmission-intensity within the medium height region to be lowered relative to that of a sample that does not contain a bubble. For some applications, the height of the chamber varies in a different manner, but generally similar techniques are performed, *mutatis mutandis*.

25 Therefore, in accordance with some applications of the present invention, in addition to measuring absolute values of a parameter that is indicative of light transmission along the length of the sample chamber, normalized values of a parameter that is indicative of light transmission (e.g., normalized logarithm of light transmission intensity) are determined along the sample chamber. Based upon the normalized values of the parameter, the computer processor determines
30 that there is likely to be a bubble (e.g., an air bubble or presence of a different substance) within the sample chamber. For some applications, in response to determining that there is likely to be a bubble within the sample chamber the computer processor generates an output. For example, the computer processor may generate an error message (e.g., a message indicating that the sample

chamber should be refilled), may invalidate the sample, and/or may invalidate a portion of the measurements that are performed upon the sample.

For some applications, the computer processor performs optical absorption measurements, but only uses the regions of the chamber in which there are no bubble present, for doing so. Thus, for some applications, based upon an absolute value of a parameter that is indicative of light transmission at at least some of regions within the sample chamber, the computer processor calculates hemoglobin concentration within the sample. In addition, the computer processor normalizes the parameter as measured at the respective regions within the sample chamber with respect to each other. At least partially in response to the normalized parameter, the computer processor determines which of the regions to use for calculating hemoglobin concentration within the sample. Alternatively, the computer processor may invalidate the sample from being used for calculating hemoglobin concentration within the sample, and/or may generate an error message (e.g., a message indicating that the sample chamber should be refilled).

Along the width of the chamber, the sample is expected to have an absorption profile that is substantially constant. For some applications, the computer processor is configured to interpret an unexpectedly low level of absorption (which does not conform with the above-described profiles) as being indicative of the presence of an air bubble. For some applications, in order to determine whether there is a presence of air bubbles, one or more optical absorption measurements are performed at a wavelength at which hemoglobin does not absorb light (e.g., using green light). Alternatively or additionally, a camera (e.g., the CCD camera or CMOS camera of the microscope) is used to image the second portion of the blood sample, and the computer processor determines whether there is a presence of air bubbles based upon the image(s).

Typically, one or more parameters of the sample are determined by the computer processor, based upon the optical measurements. For some applications, at least some of the optical measurements are invalidated from being used to determine the one or more parameters of the sample, based upon determining that the one or more air bubbles are present within the chamber. For some applications, the computer processor generates an output indicating that the portion of the blood sample has been invalidated from being used to perform at least some measurements upon the sample, based upon determining that the one or more air bubbles are present within the sample chamber.

For some applications, generally similar techniques are performed with respect to the first portion of the blood sample, which is placed in first set 52 of sample chambers. For example, the

computer processor may be configured to identify a region in which the sample is not present, within a microscopic image of the sample chamber, and to invalidate at least the identified region from being used in microscopic analysis of the portion of the sample. For some applications, the computer processor is configured to identify such a region by identifying an interface between a wet region and a dry region upon the base surface of the sample chamber. Typically, in identifying such a region, the computer processor is configured to distinguish between regions in which the sample is not present and regions in which the sample is present and the sample has a low cell density.

For some applications, the computer processor is configured to identify a presence of dirt (e.g., spilled blood) on an outer surface of the sample carrier. As described hereinabove, typically, at least one microscope image is acquired of a monolayer of cells that has settled on a base surface of the sample carrier. Typically, the image is acquired while the microscope is focused on a monolayer focal plane, with the monolayer of cells being disposed within the monolayer focal plane. For some applications, the computer processor identifies that dirt is disposed on the top cover of the sample carrier or on an underside of the base surface by identifying a region in which an entity is visible at a focal plane that is different from the monolayer focal plane. Alternatively or additionally, the computer processor identifies a region in which a background intensity of the microscope image (which was acquired at the monolayer focal plane) is indicative of dirt is disposed on the top cover or on an underside of the base surface. For some applications, in response thereto, the computer processor invalidates at least the identified region from being used in microscopic analysis of the portion of the sample.

As described hereinabove, typically, the first portion of the blood sample is imaged under brightfield imaging, i.e., under illumination from one or more brightfield light sources (e.g., one or more brightfield-light-emitting-diodes, which typically emit light at respective spectral bands). Further typically, the first portion of the blood sample is additionally imaged under fluorescent imaging. Typically, the fluorescent imaging is performed by exciting stained objects within the sample by directing light toward the sample at known excitation wavelengths (i.e., wavelengths at which it is known that stained objects emit fluorescent light if excited with light at those wavelengths), and detecting the fluorescent light. Typically, for the fluorescent imaging, a separate set of one or more fluorescence-light-emitting-diodes is used to illuminate the sample at the known excitation wavelengths.

For some applications, the computer processor analyzes light that is emitted by the brightfield light source, and determines a characteristic of the brightfield light source. For

example, the computer processor may periodically determine whether the spatial distribution of the illumination by the brightfield light source has changed since a previous measurement (e.g., whether the spatial uniformity of the illumination has changed or whether the spatial location has changed since a previous measurement), whether vignette effects of the illumination by the brightfield light source has changed since a previous measurement, whether the spectral distribution of the illumination by the brightfield light source has changed since a previous measurement, and/or whether the intensity of the illumination by the brightfield light source has changed since a previous measurement. As described hereinabove, typically, the computer processor determines a parameter of the blood sample by performing measurements upon entities that are identified within the microscopic images of the blood sample. For some applications, such measurements are normalized based upon the determined characteristic of the brightfield light source. For some applications, at least some measurements are invalidated from being performed upon the blood sample, based upon the determined characteristic of the brightfield light source.

For some applications, in order to determine the characteristic of the brightfield light source, the computer processor analyzes light that is emitted by the brightfield light source in the absence of the sample carrier. Alternatively or additionally, the computer processor analyzes light that is emitted by the brightfield light source that passes through intercellular regions within the blood sample.

For some applications, the computer processor analyzes light that is emitted by the fluorescent light source, and determines a characteristic of the fluorescent light source. For example, the computer processor may periodically determine whether the spatial distribution of the illumination by the fluorescent light source has changed since a previous measurement (e.g., whether the spatial uniformity of the illumination has changed or whether the spatial location has changed since a previous measurement), whether vignette effects of the illumination by the fluorescent light source has changed since a previous measurement, whether the spectral distribution of the illumination by the fluorescent light source has changed since a previous measurement, and/or whether the intensity of the illumination by the fluorescent light source has changed since a previous measurement. As described hereinabove, typically, the computer processor determines a parameter of the blood sample by performing measurements upon entities that are identified within the microscopic images of the blood sample. For some applications, such measurements are normalized based upon the determined characteristic of the fluorescent light source. For some applications, at least some measurements are invalidated from being performed upon the blood sample, based upon the determined characteristic of the fluorescent light source.

For some applications, in order to determine the characteristic of the fluorescent light source, the computer processor identifies one or more fluorescent regions that are visible within a microscopic image that is acquired under lighting by the fluorescent light source, other than the stained cells, and determines a characteristic of the fluorescent light source, based upon the identified fluorescent regions. For example, such fluorescent regions may include intercellular regions within the blood sample, one or more fluorescent regions of the microscopy unit, and/or one or more fluorescent regions of the sample carrier (e.g., the pressure-sensitive adhesive of the sample carrier, described hereinabove). For some applications, the sample carrier is placed upon a stage within the microscopy unit, and the stage includes fluorescent regions for performing the above-described measurements. Alternatively or additionally, the sample carrier includes a fluorescent region (e.g., a fluorescent patch), for performing the above described measurements.

For some applications, the computer processor is configured to detect whether portions of the microscopy unit (such as the CCD camera, the CMOS camera, the lenses, and/or the stage for holding the sample carrier) have dirt or scratches on them, by acquiring microscopic images in the absence of any sample carrier and identifying dirt or scratches in such images. For some applications, such images are acquired periodically (e.g., at fixed intervals in time), in order to determine whether the microscopy unit whether portions of the microscopy unit have dirt or scratches on them. For some applications, in response to detecting such dirt and/or scratches, this is flagged to the user. Alternatively or additionally, regions within images that correspond to the locations at which the dirt and/or scratches are present are not included in at least some the image analysis of the samples. Further alternatively or additionally, measurements that are performed upon regions within images that correspond to the locations at which the dirt and/or scratches are present are calibrated, in order to account for the dirt and/or scratches.

For some applications, the computer processor is configured to detect whether portions of the sample carrier have dirt or scratches on them, using generally similar techniques to the aforementioned techniques. For example, the sample carrier may be imaged in the absence of a sample therein. For some applications, the computer processor is configured to detect whether portions of the sample carrier fluoresce irregularly, e.g., by imaging the sample carrier in the absence of a sample therein.

For some applications, in response to detecting that there is an error in at least some of the fluorescent microscopic images, the computer processor classifies the source of the error as follows. In response to detecting that the error was introduced from a given point in time, the computer processor identifies the stain as being the source of the error, since this indicates that the

error was introduced as a result of a new batch of the stain being used. In response to detecting that the error gradually increased over time, the computer processor identifies dirt within the microscopy unit as being the source of the error, since such dirt typically causes a gradual degradation over time. In response to detecting that the error is present only in images acquired
5 under illumination by a given one of the light sources (e.g., light emitting diodes), the computer processor identifies the given one of the light sources as being the source of the error.

For some applications, the optical measurement unit includes a microscope that includes a microscope stage upon which the sample carrier is typically placed. Typically, the computer processor drives the microscope stage to move using one or more motors that are used to drive
10 movement of mechanical elements. In some cases, there is a degree of backlash associated with the movement of the mechanical elements. For example, when the direction of the movement is initiated or reversed, there may be a delay between the computer-implemented instructions being delivered to the motors and the movement of the mechanical elements being implemented. For some applications, the computer processor accounts for this effect when performing any
15 movement (e.g., by assuming that there is a given fixed delay, or by periodically measuring the delay and accounting for the delay as most-recently measured). For some applications, the amount of backlash is quantified using the microscopic system, and the amount of backlash is interpreted as being indicative of the condition of the mechanical elements and/or the motor(s). For example, the amount of backlash may be quantified by observing how many motor steps are required to
20 create a discernible movement in the microscope system, and/or by repeating the same measurement in two different directions of motions and correlating between images or metrics extracted from images associated with the motion in the two different directions. For some applications, based upon the above-described measurements, the condition of the mechanical elements and/or the motor(s) is determined. For some applications, an output is generated in
25 response to the determined condition of the mechanical elements and/or the motor(s). For example, at least some images of a sample (and/or data relating to the sample) may be rejected from being analyzed, the microscope and/or other portions of the optical measurement unit may be locked such that they cannot be used, and/or an alert may be generated (e.g., an alert indicating that servicing is required and/or an alert indicating that preemptive servicing is advisable may be
30 generated).

For some applications, microscopic images (and/or other signals) are acquired during movement of the stage. For some such applications, there may be timing mismatches between the reported or interpolated location of the stage at a given time and the actual timing of the camera

or sensor acquisition at that location. This may lead to an error in the assumed position where a given image was (or data were) acquired and may subsequently lead to additional error if this position is used for other purposes (e.g., if this image (or these data) and the corresponding location are used as an input for focusing the microscope). For some applications, such a timing mismatch is measured by using two measurements of the same target, for example, as follows. A first image of the target (or set of data associated with the target) is acquired using a static acquisition along the mechanical axis and a second image of the target (or set of data associated with the target) is acquired using acquisition during movement of the stage. Differences between images or metrics extracted from images (and/or differences between the two sets of data) are detected, if such differences are detected, the computer processor uses this as an input for determining that there is a timing mismatch, and/or for correcting the timing mismatch. For some applications, such measurements are made periodically, and are used as an input for determining the state of portions of the optical measurement system (such as portions of the microscope system, including the image-acquisition portion, mechanical elements, and/or motors). For some applications, in response to detecting that there is a timing mismatch and/or a difference relative to a previous measurement, an output is generated. For example, at least some images of a sample (and/or data relating to the sample) may be rejected from being analyzed, the microscope and/or other portions of the optical measurement unit may be locked such that they cannot be used, and/or an alert may be generated (e.g., an alert indicating that servicing is required and/or an alert indicating that preemptive servicing is advisable may be generated).

For some applications, the status of the optical-measurement unit (e.g., a microscope of the optical measurement system, and/or an optical-absorption measurement portion of the optical-measurement unit) is estimated by imaging a target that is installed in the optical-measurement unit itself (or is routinely inputted into the optical-measurement unit). For example, a scratched glass surface, a printed glass surface, one or more fluorescent or non-fluorescent beads, pinholes, or any other resolution target may be used. For some applications, by acquiring and analyzing images of (and/or data relating to) such a target, the computer processor derives optical attributes of the optical-measurement unit, such as aberration, resolution, contrast, scatter and attenuation. For some applications, the computer processor performs such an analysis periodically, and compares the determined attributes to predetermined values. For some applications, in response to detecting that there is a difference between one or more of the determined attributes and the predetermined values, an output is generated. For example, at least some images of a sample (and/or data relating to the sample) may be rejected from being analyzed, the microscope and/or

other portions of the optical measurement unit may be locked such that they cannot be used, and/or an alert may be generated (e.g., an alert indicating that servicing is required and/or an alert indicating that preemptive servicing is advisable may be generated). For some applications, based upon the determined attributes, the computer processor corrects extracted image features, measured values (in case of a non-imaging measurement), and/or measurands of a sample. For example, absorption measurements may be corrected due to change in apparent contrast in the target.

For some applications, the sample as described herein is a sample that includes blood or components thereof (e.g., a diluted or non-diluted whole blood sample, a sample including predominantly red blood cells, or a diluted sample including predominantly red blood cells), and parameters are determined relating to components in the blood such as platelets, white blood cells, anomalous white blood cells, circulating tumor cells, red blood cells, reticulocytes, Howell-Jolly bodies, etc.

In general, it is noted that although some applications of the present invention have been described with respect to a blood sample, the scope of the present invention includes applying the apparatus and methods described herein to a variety of samples. For some applications, the sample is a biological sample, such as, blood, saliva, semen, sweat, sputum, vaginal fluid, stool, breast milk, bronchoalveolar lavage, gastric lavage, tears and/or nasal discharge. The biological sample may be from any living creature, and is typically from warm blooded animals. For some applications, the biological sample is a sample from a mammal, e.g., from a human body. For some applications, the sample is taken from any domestic animal, zoo animals and farm animals, including but not limited to dogs, cats, horses, cows and sheep. Alternatively or additionally, the biological sample is taken from animals that act as disease vectors including deer or rats.

For some applications, similar techniques to those described hereinabove are applied to a non-bodily sample. For some applications, the sample is an environmental sample, such as, a water (e.g. groundwater) sample, surface swab, soil sample, air sample, or any combination thereof. In some embodiments, the sample is a food sample, such as, a meat sample, dairy sample, water sample, wash-liquid sample, beverage sample, and/or any combination thereof.

Applications of the invention described herein can take the form of a computer program product accessible from a computer-usable or computer-readable medium (e.g., a non-transitory computer-readable medium) providing program code for use by or in connection with a computer or any instruction execution system, such as computer processor 28. For the purposes of this

description, a computer-usable or computer readable medium can be any apparatus that can comprise, store, communicate, propagate, or transport the program for use by or in connection with the instruction execution system, apparatus, or device. The medium can be an electronic, magnetic, optical, electromagnetic, infrared, or semiconductor system (or apparatus or device) or
5 a propagation medium. Typically, the computer-usable or computer readable medium is a non-transitory computer-usable or computer readable medium.

Examples of a computer-readable medium include a semiconductor or solid state memory, magnetic tape, a removable computer diskette, a random-access memory (RAM), a read-only memory (ROM), a rigid magnetic disk and an optical disk. Current examples of optical disks
10 include compact disk-read only memory (CD-ROM), compact disk-read/write (CD-R/W) and DVD.

A data processing system suitable for storing and/or executing program code will include at least one processor (e.g., computer processor 28) coupled directly or indirectly to memory elements (e.g., memory 30) through a system bus. The memory elements can include local
15 memory employed during actual execution of the program code, bulk storage, and cache memories which provide temporary storage of at least some program code in order to reduce the number of times code must be retrieved from bulk storage during execution. The system can read the inventive instructions on the program storage devices and follow these instructions to execute the methodology of the embodiments of the invention.

20 Network adapters may be coupled to the processor to enable the processor to become coupled to other processors or remote printers or storage devices through intervening private or public networks. Modems, cable modem and Ethernet cards are just a few of the currently available types of network adapters.

Computer program code for carrying out operations of the present invention may be written
25 in any combination of one or more programming languages, including an object-oriented programming language such as Java, Smalltalk, C++ or the like and conventional procedural programming languages, such as the C programming language or similar programming languages.

It will be understood that algorithms described herein, can be implemented by computer program instructions. These computer program instructions may be provided to a processor of a
30 general-purpose computer, special purpose computer, or other programmable data processing apparatus to produce a machine, such that the instructions, which execute via the processor of the computer (e.g., computer processor 28) or other programmable data processing apparatus, create

means for implementing the functions/acts specified in the algorithms described in the present application. These computer program instructions may also be stored in a computer-readable medium (e.g., a non-transitory computer-readable medium) that can direct a computer or other programmable data processing apparatus to function in a particular manner, such that the instructions stored in the computer-readable medium produce an article of manufacture including instruction means which implement the function/act specified in the flowchart blocks and algorithms. The computer program instructions may also be loaded onto a computer or other programmable data processing apparatus to cause a series of operational steps to be performed on the computer or other programmable apparatus to produce a computer implemented process such that the instructions which execute on the computer or other programmable apparatus provide processes for implementing the functions/acts specified in the algorithms described in the present application.

Computer processor 28 is typically a hardware device programmed with computer program instructions to produce a special purpose computer. For example, when programmed to perform the algorithms described herein, computer processor 28 typically acts as a special purpose sample-analysis computer processor. Typically, the operations described herein that are performed by computer processor 28 transform the physical state of memory 30, which is a real physical article, to have a different magnetic polarity, electrical charge, or the like depending on the technology of the memory that is used.

The apparatus and methods described herein may be used in conjunction with apparatus and methods described in any one of the following patent applications, all of which are incorporated herein by reference:

US 2012/0169863 to Bachelet;

US 2014/0347459 to Greenfield;

US 2015/0037806 to Pollak;

US 2015/0316477 to Pollak;

US 2016/0208306 to Pollak;

US 2016/0246046 to Yorav Raphael;

US 2016/0279633 to Bachelet;

US 2018/0246313 to Eshel;

WO 16/030897 to Yorav Raphael;

WO 17/046799 to Eshel;

WO 17/168411 to Eshel;

WO 17/195205 to Pollack;

5 US 2019/0145963 to Zait; and

WO 19/097387 to Yorav-Raphael.

It will be appreciated by persons skilled in the art that the present invention is not limited to what has been particularly shown and described hereinabove. Rather, the scope of the present invention includes both combinations and subcombinations of the various features described
10 hereinabove, as well as variations and modifications thereof that are not in the prior art, which would occur to persons skilled in the art upon reading the foregoing description.