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(54) Title: ANALYZING DIGITAL HOLOGRAPHIC MICROSCOPY DATA FOR HEMATOLOGY APPLICATIONS

(57) Abstract: A method for analyzing digital holographic microscopy (DHM) data for hematology applications includes receiving a DHM image acquired using a digital holographic microscopy system and identifying one or more erythrocytes in the DHM image. For each respective erythrocyte included in the one or more erythrocytes, a cell thickness value for the respective erythrocyte using a parametric model is estimated, and a cell volume value is calculated for the respective erythrocyte using the cell thickness value.

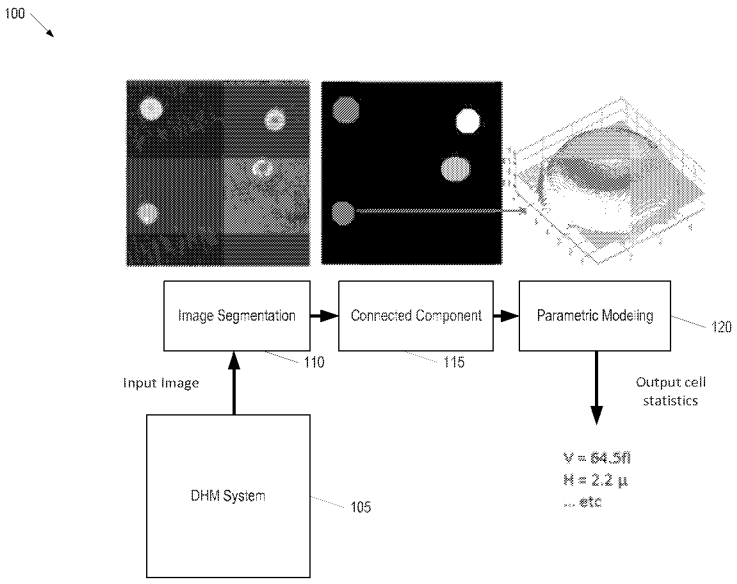

Fig. 1

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ANALYZING DIGITAL HOLOGRAPHIC MICROSCOPY DATA FOR HEMATOLOGY APPLICATIONS

CROSS REFERENCE TO RELATED APPLICATIONS

[1] This application claims the benefit of U.S. Provisional Application Serial No. 62/012,636 filed June 16, 2014, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[2] The present disclosure relates generally to analyzing digital holographic microscopy (DHM) for hematology applications. The various systems, methods, and apparatuses described herein may be applied, for example, red blood cell (RBC) volume measurement and white blood cell (WBC) differential (cell type classification) applications.

BACKGROUND

[3] Digital holographic microscopy (DHM), also known as interference phase microscopy, is an imaging technology that provides the ability to quantitatively track sub-nanometric optical thickness changes in transparent specimens. Unlike traditional digital microscopy, in which only intensity (amplitude) information about a specimen is captured, DHM captures both phase and intensity. The phase information, captured as a hologram, can be used to reconstruct extended morphological information (such as depth and surface characteristics) about the specimen using a computer algorithm. Modern DHM implementations offer several additional benefits, such as fast scanning/data acquisition speed, low noise, high resolution and the potential for label-free sample acquisition.

[4] Conventional cellular analysis techniques such as volume measurement and classification rely on two-dimensional cellular images that lack topographical information. Thus, while these techniques may analyze a cell based on information such as intensity, their accuracy is limited due to a lack of knowledge of the size and shape of the cell. Accordingly, it is desired to provide cellular analysis techniques which are applicable to imaging modalities such as DHM that provide more detailed information regarding cellular structure.

SUMMARY

[5] Embodiments of the present invention address and overcome one or more of the above shortcomings and drawbacks, by providing methods, systems, and apparatuses related

to analyzing digital holographic microscopy (DHM) for hematology applications. Additionally, as explained in further detail in the disclosure, the technology described herein may be applied to other clinical applications as well.

[6] The ability of DHM to achieve high-resolution, wide field imaging with extended depth and morphological information in a potentially label-free manner positions the technology for use in several clinical applications. For example, in the area of hematology DHM may be used for red blood cell (RBC) volume measurement, white blood cell (WBC) differential (cell type classification). For urine sediment analysis DHM allows for scanning a microfluidic sample in layers to reconstruct the sediment (possibly without waiting for sedimentation); improving the classification accuracy of sediment constituents. DHM may also be used for tissue pathology applications through utilization of extended morphology / contrast of DHM (e.g. to discriminate cancerous from healthy cells, in fresh tissue, without labeling). Similarly, for rare cell detection applications may utilize extended morphology /contrast of DHM (e.g. to differentiate rare cells such as circulating tumor / epithelial cells, stem cells, infected cells, etc.).

[7] According to some embodiments of the present invention, a method for analyzing digital holographic microscopy (DHM) data for hematology applications includes receiving a DHM image acquired using a digital holographic microscopy system and identifying one or more erythrocytes in the DHM image. Next, for each respective erythrocyte included in the one or more erythrocytes, a cell thickness value is estimated for the respective erythrocyte using a parametric model and a cell volume value is calculated for the respective erythrocyte using the cell thickness value. In some embodiments, the method further comprising performing a complete blood cell (CBC) test using the cell thickness value and the cell volume value associated with each respective erythrocyte.

[8] Various enhancements, modifications, or additions may be added to the aforementioned method in different embodiments of the present invention. For example, in some embodiments, the method further comprises performing a segmentation on the DHM image to generate a segmentation mask including boundaries for the one or more erythrocytes. This segmentation may be performed, for example, by minimizing a piecewise constant energy functional in a combinatorial optimization framework. Following the segmentation, a connected component analysis may be performed on the segmentation mask

to label the one or more erythrocytes. In some embodiments, the parametric model represents each of the one or more erythrocytes using Cassini ovals. In other embodiments, the parametric model represents each of the one or more erythrocytes using a cell diameter value, a minimum dimple thickness value, a maximum thickness value and a circle diameter value representative of maximum cell thickness. The parameters used in the parametric model may be determined, for example, by minimizing a sum squared error between a depth map estimated from the parametric model and a depth observed from the DHM image.

[9] According to another aspect of the present invention, as described in some embodiments, an article of manufacture for analyzing DHM data for hematology applications comprise a non-transitory, tangible computer-readable medium holding computer-executable instructions for performing the aforementioned method. This article of manufacture may further include instructions for any of the additional features discussed above with respect to the aforementioned method.

[10] A system for analyzing DHM data for hematology applications comprises a networking component and a modeling processor. The networking component is configured to communicate with using a DHM system to retrieve a DHM image. The modeling processor is configured to identify one or more erythrocytes in the received DHM image. The modeling processor is further configured to estimate a cell thickness value (using a parametric model) and calculate a cell volume for each respective erythrocyte included in the one or more erythrocytes.

[11] Additional features and advantages of the invention will be made apparent from the following detailed description of illustrative embodiments that proceeds with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[12] The foregoing and other aspects of the present invention are best understood from the following detailed description when read in connection with the accompanying drawings. For the purpose of illustrating the invention, there is shown in the drawings embodiments that are presently preferred, it being understood, however, that the invention is not limited to the specific instrumentalities disclosed. Included in the drawings are the following Figures:

[13] FIG. 1 provides an illustration of a framework for processing DHM images for erythrocyte volume computation, according to some embodiments;

[14] FIG. 2 provides an example illustration of segmentation of erythrocytes in optical path difference DHM (OPD-DHM) images, as may performed in some embodiments;

[15] FIG. 3 shows a geometry model of a normal erythrocyte, as may be utilized in some embodiments;

[16] FIG. 4 provides an example of the parametric modeling of the erythrocytes which illustrates the regularization;

[17] FIG. 5 provides an illustration of a process for calculating cell volume, according to some embodiments;

[18] FIG. 6 provides an illustration of a pre-processing framework for classifying white blood cells, as it may be applied in some embodiments;

[19] FIG. 7 shows a sample of the white blood cells used in training and testing, according to some embodiments;

[20] FIG. 8A provides a table showing an example of a data set that may be used for training and testing for each cell type, as may be used in some embodiments;

[21] FIG. 8B shows pairwise classification results obtained using texton features and KNN classifier for this sample case, as may be obtained according to some embodiments;

[22] FIG. 9 provides an example of local image feature sampling, as it may be applied in some embodiments;

[23] FIG. 10 shows a complete binary search tree structure which represents a vocabulary dictionary structures;

[24] FIG. 11 provides a visualization of the workflow for the transformation from local image features (dense SIFT descriptor) to a global image features (vocabulary histogram), as may be applied in some embodiments;

[25] FIG. 12 shows Pairwise Classification results using SIFT and SVM classification, obtained according to some embodiments;

[26] FIG. 13 shows the structure of a feed-forward neural network with one hidden layer that may be utilized in some embodiments;

[27] FIG. 14 shows a GUI which provides for results visualization and facilitates user interaction and correction, according to some embodiments;

[28] FIG. 15 illustrates an exemplary computing environment within which embodiments of the invention may be implemented

DETAILED DESCRIPTION

[29] The following disclosure describes the present invention according to several embodiments directed at methods, systems, and apparatuses related to analyzing digital holographic microscopy (DHM) for hematology and other clinical applications. Briefly, the techniques discussed herein comprise of three principle ideas that may be applied in different configurations in the various embodiments described herein. First, in some embodiments, red blood cell (RBC) volume estimation is performed using parametric modeling of erythrocytes to regularize the raw DHM data to match physical models of the erythrocytes, in an attempt to eliminate any distortion caused during the image acquisition process. Second, in some embodiments, label-free differentiation of white blood cells (WBCs) captured by DHM is performed using a machine learning algorithm which extract characteristic topological changes from a set of training samples DHM images for each sub-type of WBCs (namely, monocytes, neutrophils, basophils, lymphocytes, eosinophils). The machine learning algorithm then classifies a new cell into one of the categories automatically based on the learnt DHM image based features. Third, in some embodiments, "digital staining" or "pseudo-coloring" of the classified DHM images of the cells is performed to resemble conventional staining techniques. The digital staining method described herein uses a matched pair of DHM and stained images of a set of cells, and a regression function is learnt to map the DHM image pixel (representing the topology of the cell constituents to a RGB color scheme of conventional staining techniques).

[30] FIG. 1 provides an illustration of a framework 100 for processing DHM images for erythrocyte volume computation, according to some embodiments. Briefly, a DHM

System 105 is used to acquire one or more input images. The Digital Holographic Microscopy System 105 may be any system known in the art capable of acquiring DHM images. An Image Segmentation Component 110 performs segmentation of the erythrocytes in the acquired input images. Next, a Parametric Modeling Component 115 develops a model of the erythrocytes (e.g., using Cassini ovals). Then, a Parametric Modeling Component 120 calculates thickness information of each erythrocyte which, in turn, can be used to determine the erythrocyte's volume.

[31] Various segmentation techniques may be applied to the input image to segment the erythrocytes. For example, in some embodiments, the segmentation problem performed by the Image Segmentation Component 110 is formulated as an energy minimization problem that minimizes the piecewise constant Mumford-Shah energy functional in a combinatorial optimization framework. The segmentation energy can be described as follows. Given an image u and an image of interest u: $\Omega \rightarrow$ R, where Ω is an open bounded subset in R2 that comprises several connected components Ω_i and bounded by a closed boundary C = $\partial\Omega$, find a piecewise constant approximation such that u is constant within the components Ω_i and sharp in the transition across the boundary C. This is formulated as:

$$F(x1, x2, \ldots, xn) = \mu \sum_{epq \in E} w_{pq} \ |x_p - x_q| + \sum_p |u(p) - c_1|^2 \ x_p + \sum_p |u(p) - c_1|^2 \ (1 - x_p) \quad (1)$$

where x_p is a binary variable that indicates whether a particular pixel p = (x, y) belongs to the cell or to the background. A binary variable x_p for each pixel p = (x, y) $\in \Omega$ is set such that

$$x_p = \begin{cases} 1 & if\ p \in \bigcup_i \Omega_i \\ 0, & otherwise \end{cases} \quad (2)$$

[32] The constants c_1 and c_2 represent the piecewise constant approximation of the input DHM image, w_{pq} represents the weights of the Euclidean length regularization, and μ is a weighting coefficient that defines the contribution of the length regularization to the segmentation energy. A relatively high value for mu may be used to avoid separating the nucleus from the rest of the cell.

[33] FIG. 2 provides an example illustration of Segmentation of erythrocytes in optical path difference DHM (OPD-DHM) images. Image 205 shows the input image, while

image 210 shows the delineation (represented by a dotted line) of the erythrocyte boundaries is imposed on the image 205.

[34] A normal erythrocyte is generally shaped as a biconcave disk to achieve a large surface area -to-volume ratio. For example, FIG. 3 shows a geometry model 300 of a normal erythrocyte. There are four main parameters that affect the shape of the cell (shown in FIG. 3): the diameter of the cell (D), the minimum dimple thickness (t), the maximum thickness (h) and the diameter of the circle that determines the maximum thickness (d).

[35] Using the geometric understanding presented in FIG. 3, there are several bi-concave models for the erythrocyte surface representation that may be applied by Parametric Modeling Component 120 to characterize the shape and geometry of each erythrocyte. For example, some embodiments utilize the Evans-Fung Model technique generally known in the art which utilizes the following equation:

$$z(\rho) = \pm\sqrt{1-\left(\frac{\rho}{R}\right)^2}\left[c0 + c1\left(\frac{\rho}{R}\right)^2 + c2\left(\frac{\rho}{R}\right)^4\right] \quad (3)$$

where R is the radius of the cell (R = D/2) and ρ is the horizontal distance from the center of the cell. To estimate the model parameters c0, c1 and c2 we minimize the sum squared error between the depth map as estimated from the parametric model and the depth observed from the DHM. The sum squared error of the thickness profile is expressed as:

$$SSE_{thickness} = \sum_p \left(z(\rho) - \frac{u(\rho)}{2}\right)^2 \quad (4)$$

[36] In some embodiments, the parametric modeling of the cell regularizes the cell surface to match the physical models of the erythrocytes and eliminate any distortion caused during the image acquisition process. FIG. 4 provides an example of the parametric modeling of the erythrocytes which illustrates the regularization. In Image 405, the cell surface is depicted as observed from the DHM image. Image 410 shows the cell surface as characterized by the Evans - Fung model.

[37] After performing the segmentation and the parametric modeling for each cell, the volume of each RBC may be computed. The RBC volume may then be used as a clinical

measurement in and of itself, or it may be used to derive additional clinically significant values. For example, the calculation of RBC volume may be used in determining mean cell volume (MCV) which, in turn, is a critical parameter in a complete blood count (CBC).

[38] FIG. 5 provides an illustration of a process 500 for calculating cell volume, according to some embodiments. At step 505, an OPD-DHM image of erythrocytes is received. Next, at step 510, the DHM image is segmented to extract all the erythrocytes from the background. Segmentation may be performed, for example, using the combinatorial piece wise constant Mumford-Shah energy functional or any similar technique generally known in art. At step 515, connected component analysis is applied to the segmentation mask to label the independent erythrocytes.

[39] Continuing with references to FIG. 5, at steps 520 – 530 a measurement process is performed for each erythrocyte. At step 520, the cell surface is approximated, for example, using the Evans-Fung technique. The sum squared differences in Equation 4 are optimized and the model parameters C0, C1 and C2 are computed. Next, at step 525, the model is used to estimate the updated cell thickness $z(\rho)$ using Equation 3. Then, at step 530, the updated cell thickness is used to calculate the cell volume. The output of the process 500 is a volume for each erythrocyte.

[40] The process 500 can be extended to provide the mean hemoglobin content of the cell as well. For this, a conventional hematology analyzer may be used for calibration of mean cell volume and conversion of optical density map based volume to the average hemoglobin content.

[41] Differential blood tests measure the percentage of each type of white blood cell type in a blood cell sample. Identifying each type of white blood cell is a crucial preliminary step to blood differential analysis that is used to diagnose diseases such as infections, anemia and Leukemia. To address this step, the system described herein may be applied to differentiate among the different white blood cell types. Specifically, the described system aims to differentiate five different types of white blood cells, namely: monocytes, basophils, neutrophils, eosinophils and lymphocytes. Briefly, a pipeline of processing the white blood cells comprises the following three steps. The first step is pre-processing where various types of white blood cells are identified and isolated. The second step is training where a classifier

is trained from the extracted cells. The third step is classification where a classifier is used to categorize unseen cells. Each of these steps will now be discussed in detail.

[42] FIG. 6 provides an illustration of a pre-processing framework 600 for classifying white blood cells, as it may be applied in some embodiments. During the pre-processing step, a pipeline is used to prepare the training patches for the classifier training step. Initially, a threshold is applied to an input image 605 to capture the bright spots (that are highly likely to be cells). After thresholding, a connected component analysis is applied to the thresholded image 610. Then, as illustrated in image 615, the component size is calculated for each connected component. A component is rejected if its size is below or above predefined thresholds t1 and t2. The rejected components are excluded from training and testing. The patches (patch is a rectangular box including the connected component), including the remaining components are used to train the classifier (e.g., a K-Nearest Neighbor classifier).

[43] Following the pre-processing framework 600, various machine learning algorithms may be used for performing five part differential on DHM images of white blood cells. For example, in some embodiments, a texton-based approach is used, where the textural characteristics of DHM image of cells are used as the main discriminating feature. In other embodiments, a more data driven approach is employed, where a dictionary of image patches is learned and used to represent the entire image as a histogram. The histogram is then used as the main feature for classification between various cell sub-types. In other embodiments, classification is based on a convolutional neural network with multiple layers to perform feature extraction, selection, and classification at once with a multi-layer network. Each of these different approaches is described in detail below.

[44] As is generally understood in the art, image classification algorithms are trained using a data set of representative images. One example of a training data set is illustrated in FIG. 7. In this example, cell images are labelled in one of four categories: monocyte, basophil, neutrophil, and lymphocytes. It should be noted that this training set is but one example of a data that may be used in training the classifier. For example, other training sets employed in some embodiments may include additional categories (e.g., eosinophils). Additionally (or alternately), the categories could have a finer level of granularity.

[45] In embodiments where the texton-based approach is used for classification, the extracted patches that include the preprocessed cell images are used to train a K-Nearest

Neighbor (K-NN) classifier. The classifier utilized texton based texture features extracted from each patch. Textons are the representation of small texture features by a collection of filter bank responses. Texton based texture classifiers use the histograms of the textons to characterize the texture by the frequency of the textons in a particular patched as compared to the textons of the training data set. The texton features are used, in particular, because it is pathologically proven that the granularity of cell nuclei differ in the different cell types and this can be captured using texture features.

[46] To illustrate the texton-based approach, consider a combination of the texton based texture feature representation with a simple K-NN classifier. FIG. 8A provides a table showing an example of a data set that may be used for training and testing for each cell type. The classification rate obtained for this example dataset in all the pairwise classifications varies from 75% to 95%. FIG. 8B shows pairwise classification results obtained using texton features and KNN classifier for this sample case.

[47] To provide a multi-label classifier using the previous pairwise classification, one or more pairwise classifiers may be combined using voting. Ideally four classifiers should agree on the class label and 6 classifiers would provide random labeling. For example, if 10 classifiers are denoted as C1 to C10, the final class label L for an unknown sample S can be represented as the mode (most frequent value) of the labels of the 10 classifiers. In other words, the majority vote of the 10 pairwise classifiers is labeled for the multi-label classification problem. Combining all the pairwise classification to one multi-label classifier yielded a 76.5% correct classification rate for the example dataset set presented in FIG. 8A.

[48] In some embodiments, a bag of visual words (BOW) approach may be used to solve this multi-class based image classification problem. In the BOW approach, the global image features are represented by vectors of occurrence counts of visual words (a histogram over a vocabulary dictionary based on local image feature). These global image features are then used for classification. The pipeline may be divided into three stages: offline vocabulary learning, image classification training and testing. In the offline vocabulary learning stage, a visual vocabulary dictionary may be trained using hierarchical k-means and SIFT (scale-invariant feature transform) descriptor as local image feature. For classification, one against one n-label supporting vector machine (SVM) may be utilized. To avoid an over-fitting problem, two approaches may be employed. First, each training image may be perturbed at

random degree. Secondly, SVM parameters may be determined for the kernel using cross validation on the training data set.

[49] SIFT descriptor describes invariant local image structure and capturing local texture information. Dense SIFT descriptors may be computed for every n_s pixels of each image ($w \times h$, where w and h are image width and height respectively. For example, a 128 dimension SIFT descriptor may be used and, thus, there are about $(\frac{w}{n_s}) \times \left(\frac{h}{n_s}\right) \times 128$ local image features per image. FIG. 9 provides an example of local image feature sampling, as it may be applied in some embodiments. In this example, each white dot represents a location where 128 dimension SIFT descriptor is computed.

[50] In some embodiments, a modified vocabulary tree structure is utilized to construct a visual vocabulary dictionary. The vocabulary tree defines a hierarchical quantization using a hierarchical k-means clustering. FIG. 10 shows a complete binary (k=2) search tree structure which represents a vocabulary dictionary structures. The node 1005 is a visual cluster center. 2^{n_d} leaf nodes are finally used as visual vocabulary words. n_d is the depth of the binary tree. In the vocabulary tree learning stage, first the initial k-means algorithm is applied on to the training data (a collection of SIFT descriptors derived from training data set) and then partitioned into 2 groups, where each group comprises SIFT descriptors closest to the cluster center. This process is then recursively applied until tree depth reaches n_d. In the online stage, a SIFT descriptor (a vector) is passed down the tree by each level via comparing this feature vector to the 2 cluster centers and choosing the closest one. The visual word histogram is computed for all the dense SIFT descriptors on each image.

[51] FIG. 11 provides a visualization of the workflow for the transformation from local image features (dense SIFT descriptor) to global image features (vocabulary histogram). The results obtained by combining SIFT features with SVM classification for an example dataset are depicted in FIG. 12. Combining these pairwise classifiers yielded an 84% correct classification rate for the 5-type classification problem.

[52] In some embodiments, a Deep Learning (DL) architecture is used to perform the 5-type classification. While SVM is mainly formulated for binary classification, DL is inherently a multi-label classifier. To illustrate the DL classification technique, the

convolutional network was directly trained for the multi-label classification problem. 500 cells were used for training, 100 for each category and the rest of the cells were used for testing.

[53] For this example DL application, an auto-encoder convolutional neural network (CNN) was used to train the marginal space learning (MSL) classifier. FIG. 13 shows the structure of a feed-forward neural network with one hidden layer (also referred to as an "AE"). Ignoring the bias term (the nodes labeled as "+1" in FIG. 13), the input and output layers have the same number of nodes. The objective of such auto encoders is to learn a transfer function between the input layer (features that represent the images) and the output layer (the labels for the image). If the hidden layer has a size equal or larger than the input layer, potentially, an AE may learn an identity transformation. To prevent such a trivial solution, previously, an AE is set up with a hidden layer with fewer nodes than the input. Recently, denoising auto-encoder (DAE) was proposed to learn a more meaningful representation of the input. A certain percentage (e.g., 50%) of input nodes are randomly picked to be disturbed (e.g., set the value to zero) and the DAE is required to reconstruct the original input vector given a contaminated observation. With DAE, the hidden layer may have more nodes than the input to achieve an over-complete representation. After training an AE, the output layer is discarded and another AE is stacked using the activation response of the already trained hidden layer as input to the new AE. This process can be repeated to train and expand a network layer by layer. After pre-training, the output of the hidden layers can be treated as high-level image features to train a classifier. Alternatively, we can add one more layer for the target output and the whole network can be refined using back-propagation.

[54] After the classification is done, the various categories of white blood cells may be presented to the pathologist in a graphical user interface (GUI) for final confirmation. The probability of a given cell belonging to a certain sub-type may be calculated and presented numerically or graphically to the pathologist during the final check.

[55] FIG. 14 shows a GUI which provides for results visualization and facilitates user interaction and correction, according to some embodiments. As shown in the FIG. 14, the pathologist will have a chance to make a modification based on the DHM image of the cells. The results of the automatic blood differential is depicted at the top two rows 1405 displaying

the percentage of each blood cell type and the possible diagnosis based on these percentages. The bottom part of the figure 1410 shows how the user can modify the results. The system shows the top cell candidates for user interactions. These cells are chosen and sorted based on the difference of the top two probabilities. The system displays the extracted cell (extracted by our preprocessing pipeline) and displays the probability of it belonging to each WBC type. The user can accept the label or choose a new label by simply marking a check box. If the user changes the label, the system may automatically update the counts, the percentages and the possible diagnosis, which changes the top row and displays the new results.

[56] Additionally, in order for pathologist to best be able to review the images, in some embodiments, a pseudo-colorization scheme is utilized which converts the DHM images into an image with a color pattern similar to cell images stained for conventional staining methodologies such as the Wright and Giemsa methodologies. For example, Giemsa-Wright stains use solutions which include eosin Y, azure B, and methylene blue for staining. These solutions bind to the constituents of the cells including nucleus and granules differently and thereby provide a more pronounced coloring which is essential for visual inspection of the cells. From a DHM image of a cell, we obtain different optical density patterns for the cell constituents and that could be used as a feature to perform colorization. The method that we envision is based on having matched pairs of cells which are imaged both with DHM and Giemsa staining respectively. In some embodiments a simple regression function is used, which can be implemented using a machine learning technique such as a convolutional neural network to map optical density maps from DHM to the RGB color scheme consistent with Giemsa-Wright staining protocol. The regression map could work either on the single pixel or groups of pixels (i.e., image patches). In addition, a Markov random field based regularization may be utilized to make sure that the neighboring pixels with similar optical density will be colored similarly. Aside from Giemsa and Wright stains, other staining protocols can be learned based on set of matching pairs of cellular images and the stained version can be digitally reproduced from the DHM images.

[57] In some embodiments, the mean cell volume computation for RBC described herein and/or the WBC five part differential may be used as key measurements for a Complete Blood Count (CBC) test. As is understood in the art, a CBC test evaluates an individual's overall health and may be used in the detection of blood-related disorders such as

anemia, infection and leukemia. So, for example, abnormal increases or decreases in particular WBC counts may be used as an indicator of an underlying medical condition that calls for further evaluation.

[58] Various other extensions, enhancements, or other modifications may be made or added to the techniques described herein to provide additional functionality. For example, in some embodiments, the five part differential methodology for white blood cell sub-typing is applied on raw fringe pattern images prior to the reconstruction as well. In order to increase the overall accuracy of either RBC volume or WBC classification, multiple images of the same cell could be used and the results could be averaged. The systems and methods described herein may also be applied to clinical applications outside the cell classification space. For example, the size calculation and classification of various objects based on the methodology described can be extended for urine analysis applications, where the size of urine sediments are measured and various kinds are identified by analyzing the DHM images using the methodology described. Additionally, given the latest advancements in DHM technology – particularly reductions in size, complexity and cost - these (and other) applications could be performed within a clinical environment or at the point of care (in a decentralized manner).

[59] FIG. 15 illustrates an exemplary computing environment 1500 within which embodiments of the invention may be implemented. For example, this computing environment 1500 may be configured to execute one or more of the components of the framework 100 for processing DHM images illustrated in FIG 1. Additionally (or alternatively), this computing environment 1500 may be configured to perform one or more of the processes described herein (e.g., the process 500 for calculating cell volume shown in FIG. 1. The computing environment 1500 may include computer system 1510, which is one example of a computing system upon which embodiments of the invention may be implemented. Computers and computing environments, such as computer system 1510 and computing environment 1500, are known to those of skill in the art and thus are described briefly here.

[60] As shown in FIG. 15, the computer system 1510 may include a communication mechanism such as a bus 1521 or other communication mechanism for communicating information within the computer system 1510. The computer system 1510 further includes

one or more processors 1520 coupled with the bus 1521 for processing the information. The processors 1520 may include one or more central processing units (CPUs), graphical processing units (GPUs), or any other processor known in the art.

[61] The computer system 1510 also includes a system memory 1530 coupled to the bus 1521 for storing information and instructions to be executed by processors 1520. The system memory 1530 may include computer readable storage media in the form of volatile and/or nonvolatile memory, such as read only memory (ROM) 1531 and/or random access memory (RAM) 1532. The system memory RAM 1532 may include other dynamic storage device(s) (e.g., dynamic RAM, static RAM, and synchronous DRAM). The system memory ROM 1531 may include other static storage device(s) (e.g., programmable ROM, erasable PROM, and electrically erasable PROM). In addition, the system memory 1530 may be used for storing temporary variables or other intermediate information during the execution of instructions by the processors 1520. A basic input/output system (BIOS) 1533 containing the basic routines that help to transfer information between elements within computer system 1510, such as during start-up, may be stored in ROM 1531. RAM 1532 may contain data and/or program modules that are immediately accessible to and/or presently being operated on by the processors 1520. System memory 1530 may additionally include, for example, operating system 1534, application programs 1535, other program modules 1536 and program data 1537.

[62] The computer system 1510 also includes a disk controller 1540 coupled to the bus 1521 to control one or more storage devices for storing information and instructions, such as a hard disk 1541 and a removable media drive 1542 (e.g., floppy disk drive, compact disc drive, tape drive, and/or solid state drive). The storage devices may be added to the computer system 1510 using an appropriate device interface (e.g., a small computer system interface (SCSI), integrated device electronics (IDE), Universal Serial Bus (USB), or FireWire).

[63] The computer system 1510 may also include a display controller 1565 coupled to the bus 1521 to control a display 1566, such as a cathode ray tube (CRT) or liquid crystal display (LCD), for displaying information to a computer user. The computer system includes an input interface 1560 and one or more input devices, such as a keyboard 1562 and a pointing device 1561, for interacting with a computer user and providing information to the processor 1520. The pointing device 1561, for example, may be a mouse, a trackball, or a

pointing stick for communicating direction information and command selections to the processor 1520 and for controlling cursor movement on the display 1566. The display 1566 may provide a touch screen interface which allows input to supplement or replace the communication of direction information and command selections by the pointing device 1561.

[64] The computer system 1510 may perform a portion or all of the processing steps of embodiments of the invention in response to the processors 1520 executing one or more sequences of one or more instructions contained in a memory, such as the system memory 1530. Such instructions may be read into the system memory 1530 from another computer readable medium, such as a hard disk 1541 or a removable media drive 1542. The hard disk 1541 may contain one or more datastores and data files used by embodiments of the present invention. Datastore contents and data files may be encrypted to improve security. The processors 1520 may also be employed in a multi-processing arrangement to execute the one or more sequences of instructions contained in system memory 1530. In alternative embodiments, hard-wired circuitry may be used in place of or in combination with software instructions. Thus, embodiments are not limited to any specific combination of hardware circuitry and software.

[65] As stated above, the computer system 1510 may include at least one computer readable medium or memory for holding instructions programmed according to embodiments of the invention and for containing data structures, tables, records, or other data described herein. The term "computer readable medium" as used herein refers to any medium that participates in providing instructions to the processor 1520 for execution. A computer readable medium may take many forms including, but not limited to, non-volatile media, volatile media, and transmission media. Non-limiting examples of non-volatile media include optical disks, solid state drives, magnetic disks, and magneto-optical disks, such as hard disk 1541 or removable media drive 1542. Non-limiting examples of volatile media include dynamic memory, such as system memory 1530. Non-limiting examples of transmission media include coaxial cables, copper wire, and fiber optics, including the wires that make up the bus 1521. Transmission media may also take the form of acoustic or light waves, such as those generated during radio wave and infrared data communications.

[66] The computing environment 1500 may further include the computer system 1510 operating in a networked environment using logical connections to one or more remote computers, such as remote computer 1580. Remote computer 1580 may be a personal computer (laptop or desktop), a mobile device, a server, a router, a network PC, a peer device or other common network node, and typically includes many or all of the elements described above relative to computer system 1510. When used in a networking environment, computer system 1510 may include modem 1572 for establishing communications over a network 1571, such as the Internet. Modem 1572 may be connected to bus 1521 via user network interface 1570, or via another appropriate mechanism.

[67] Network 1571 may be any network or system generally known in the art, including the Internet, an intranet, a local area network (LAN), a wide area network (WAN), a metropolitan area network (MAN), a direct connection or series of connections, a cellular telephone network, or any other network or medium capable of facilitating communication between computer system 1510 and other computers (e.g., remote computer 1580). The network 1571 may be wired, wireless or a combination thereof. Wired connections may be implemented using Ethernet, Universal Serial Bus (USB), RJ-11 or any other wired connection generally known in the art. Wireless connections may be implemented using Wi-Fi, WiMAX, and Bluetooth, infrared, cellular networks, satellite or any other wireless connection methodology generally known in the art. Additionally, several networks may work alone or in communication with each other to facilitate communication in the network 1571.

[68] As one application of the exemplary computing environment 1500 to the technology described herein, consider an example system for analyzing DHM data for hematology applications which includes a network component, a modeling processor, and a GUI. The networking component may include network interface 1570 or some combination of hardware and software offering similar functionality. The networking component is configured to communicate with a DHM system to retrieve DHM images. Thus, in some embodiments, the networking component may include a specialized interface for communicating with DHM systems. The modeling processor is included in a computing system (e.g. computer system 1510) and is configured with instructions that enable it to train a classifier for cell types present in cell images extracted from DHM images received via the networking component. The modeling processor may include additional functionality, as

described in this disclosure, to support this task (e.g., segmentation, identifying connected components, etc.). The modeling processor is further configured to use the classifier to determine the probability that new cell images belong to one of the types used to train the classifier. The GUI may then be presented on a display (e.g., display 1566) for review by a user.

[69] The embodiments of the present disclosure may be implemented with any combination of hardware and software. In addition, the embodiments of the present disclosure may be included in an article of manufacture (e.g., one or more computer program products) having, for example, computer-readable, non-transitory media. The media has embodied therein, for instance, computer readable program code for providing and facilitating the mechanisms of the embodiments of the present disclosure. The article of manufacture can be included as part of a computer system or sold separately.

[70] While various aspects and embodiments have been disclosed herein, other aspects and embodiments will be apparent to those skilled in the art. The various aspects and embodiments disclosed herein are for purposes of illustration and are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

[71] An executable application, as used herein, comprises code or machine readable instructions for conditioning the processor to implement predetermined functions, such as those of an operating system, a context data acquisition system or other information processing system, for example, in response to user command or input. An executable procedure is a segment of code or machine readable instruction, sub-routine, or other distinct section of code or portion of an executable application for performing one or more particular processes. These processes may include receiving input data and/or parameters, performing operations on received input data and/or performing functions in response to received input parameters, and providing resulting output data and/or parameters.

[72] A graphical user interface (GUI), as used herein, comprises one or more display images, generated by a display processor and enabling user interaction with a processor or other device and associated data acquisition and processing functions. The GUI also includes an executable procedure or executable application. The executable procedure or executable application conditions the display processor to generate signals representing the GUI display images. These signals are supplied to a display device which displays the image for viewing

by the user. The processor, under control of an executable procedure or executable application, manipulates the GUI display images in response to signals received from the input devices. In this way, the user may interact with the display image using the input devices, enabling user interaction with the processor or other device.

[73] The functions and process steps herein may be performed automatically or wholly or partially in response to user command. An activity (including a step) performed automatically is performed in response to one or more executable instructions or device operation without user direct initiation of the activity.

[74] The system and processes of the figures are not exclusive. Other systems, processes and menus may be derived in accordance with the principles of the invention to accomplish the same objectives. Although this invention has been described with reference to particular embodiments, it is to be understood that the embodiments and variations shown and described herein are for illustration purposes only. Modifications to the current design may be implemented by those skilled in the art, without departing from the scope of the invention. As described herein, the various systems, subsystems, agents, managers and processes can be implemented using hardware components, software components, and/or combinations thereof. No claim element herein is to be construed under the provisions of 35 U.S.C. 112, sixth paragraph, unless the element is expressly recited using the phrase "means for."

CLAIMS

1. A method for analyzing digital holographic microscopy (DHM) data for hematology applications, the method comprising:

receiving a DHM image acquired using a digital holographic microscopy system;

identifying one or more erythrocytes in the DHM image;

for each respective erythrocyte included in the one or more erythrocytes,

estimating a cell thickness value for the respective erythrocyte using a parametric model, and

calculating a cell volume value for the respective erythrocyte using the cell thickness value.

2. The method of claim 1, wherein the one or more erythrocytes in the DHM image are labeled by a process comprising:

performing a segmentation on the DHM image to generate a segmentation mask including boundaries for the one or more erythrocytes; and

performing a connected component analysis on the segmentation mask to label the one or more erythrocytes.

3. The method of claim 2, wherein the segmentation is performed by minimizing a piecewise constant energy functional in a combinatorial optimization framework.

4. The method of claim 1, wherein the parametric model represents each of the one or more erythrocytes using Cassini ovals.

5. The method of claim 1, wherein the parametric model represents each of the one or more erythrocytes using a cell diameter value, a minimum dimple thickness value, a maximum thickness value and a circle diameter value representative of maximum cell thickness.

6. The method of claim 1, further comprising:

performing a complete blood cell (CBC) test using the cell thickness value and the cell volume value associated with each respective erythrocyte included in the one or more erythrocytes.

7. The method of claim 1, wherein parameters used in the parametric model are determined by minimizing a sum squared error between a depth map estimated from the parametric model and a depth observed from the DHM image.

8. An article of manufacture for analyzing digital holographic microscopy (DHM) data for hematology applications, the article of manufacture comprising a non-transitory, tangible computer-readable medium holding computer-executable instructions for performing a method comprising:

receiving a DHM image acquired using a digital holographic microscopy system;

identifying one or more erythrocytes in the DHM image;

for each respective erythrocyte included in the one or more erythrocytes,

estimating a cell thickness value for the respective erythrocyte using a parametric model, and

calculating a cell volume value for the respective erythrocyte using the cell thickness value.

9. The article of manufacture of claim 8, wherein the one or more erythrocytes in the DHM image are labeled by a process comprising:

performing a segmentation on the DHM image to generate a segmentation mask including boundaries for the one or more erythrocytes; and

performing a connected component analysis on the segmentation mask to label the one or more erythrocytes.

10. The article of manufacture of claim 9, wherein the segmentation is performed by minimizing a piecewise constant energy functional in a combinatorial optimization framework.

11. The article of manufacture of claim 8, wherein the parametric model represents each of the one or more erythrocytes using Cassini ovals.

12. The article of manufacture of claim 8, wherein the parametric model represents each of the one or more erythrocytes using a cell diameter value, a minimum dimple thickness value, a maximum thickness value and a circle diameter value representative of maximum cell thickness.

13. The article of manufacture of claim 8, wherein the method further comprises:

performing a complete blood cell (CBC) test using the cell thickness value and the cell volume value associated with each respective erythrocyte included in the one or more erythrocytes.

14. The article of manufacture of claim 8, wherein parameters used in the parametric model are determined by minimizing a sum squared error between a depth map estimated from the parametric model and a depth observed from the DHM image.

15. A system for analyzing digital holographic microscopy (DHM) data for hematology applications, the system comprising:

a networking component configured to communicate with a digital holographic microscopy system to retrieve a DHM image;

a modeling processor configured to:

identify one or more erythrocytes in the DHM image;

for each respective erythrocyte included in the one or more erythrocytes,

estimate a cell thickness value for the respective erythrocyte using a parametric model, and

calculate a cell volume value for the respective erythrocyte using the cell thickness value.

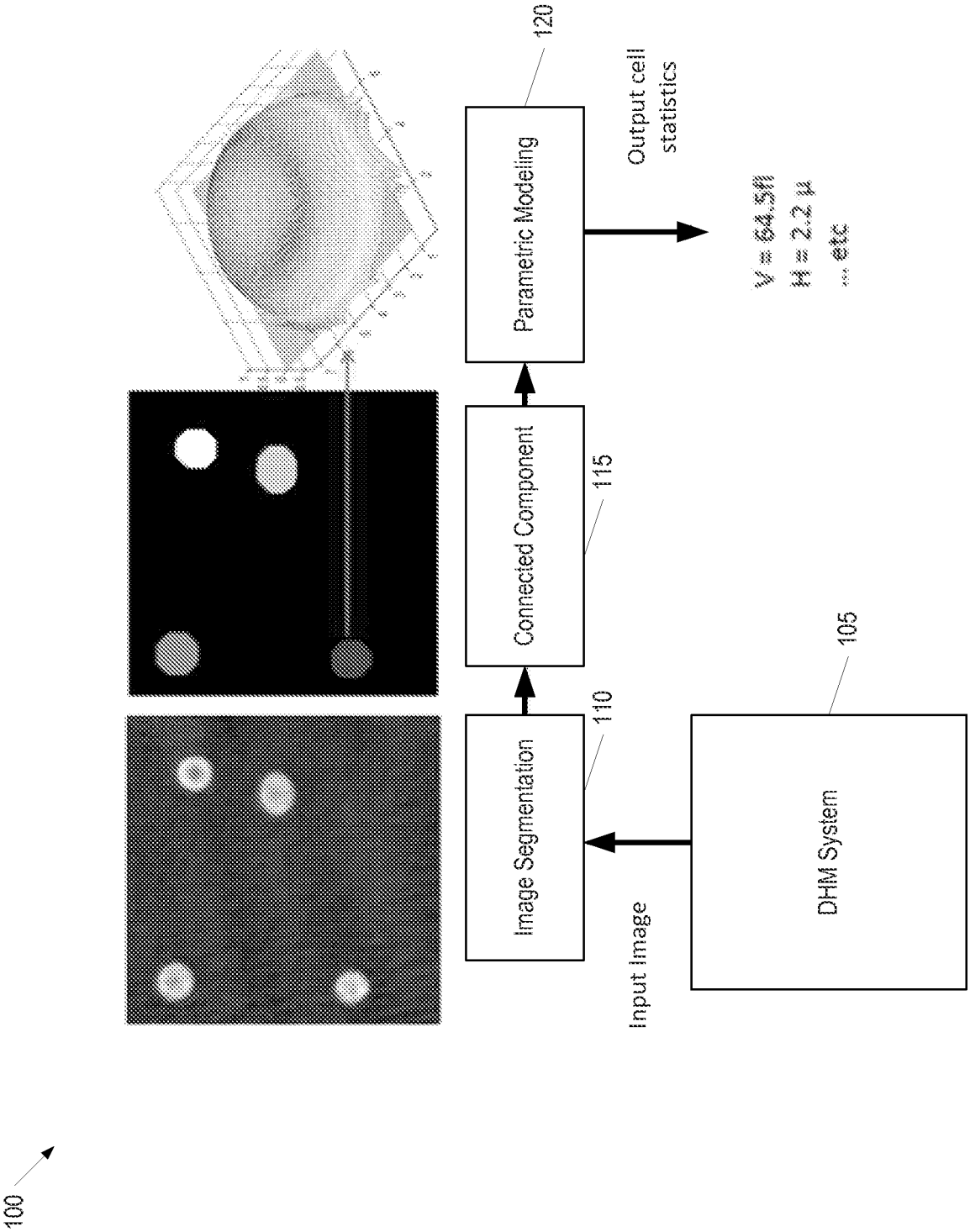
100
DHM System
105
Input Image
Image Segmentation
110
Connected Component
115
Parametric Modeling
120
Output cell statistics
V = 64.3fl
H = 2.2 μ
... etc

Fig. 1

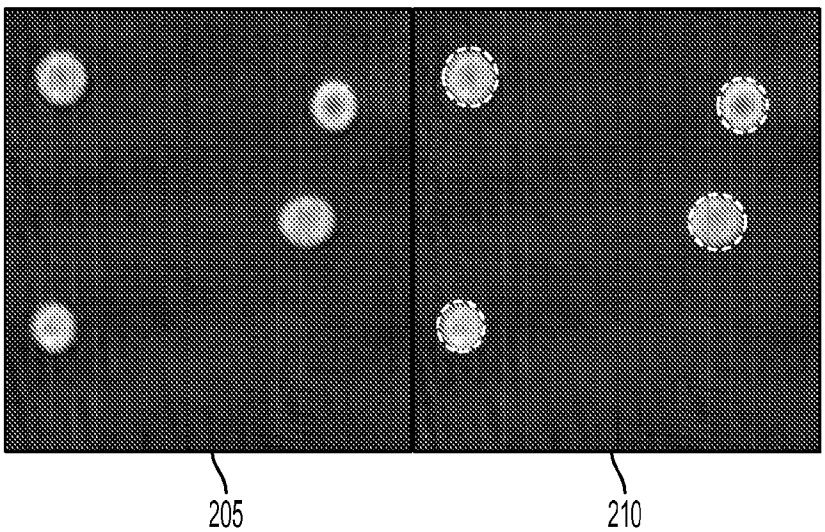

FIG. 2

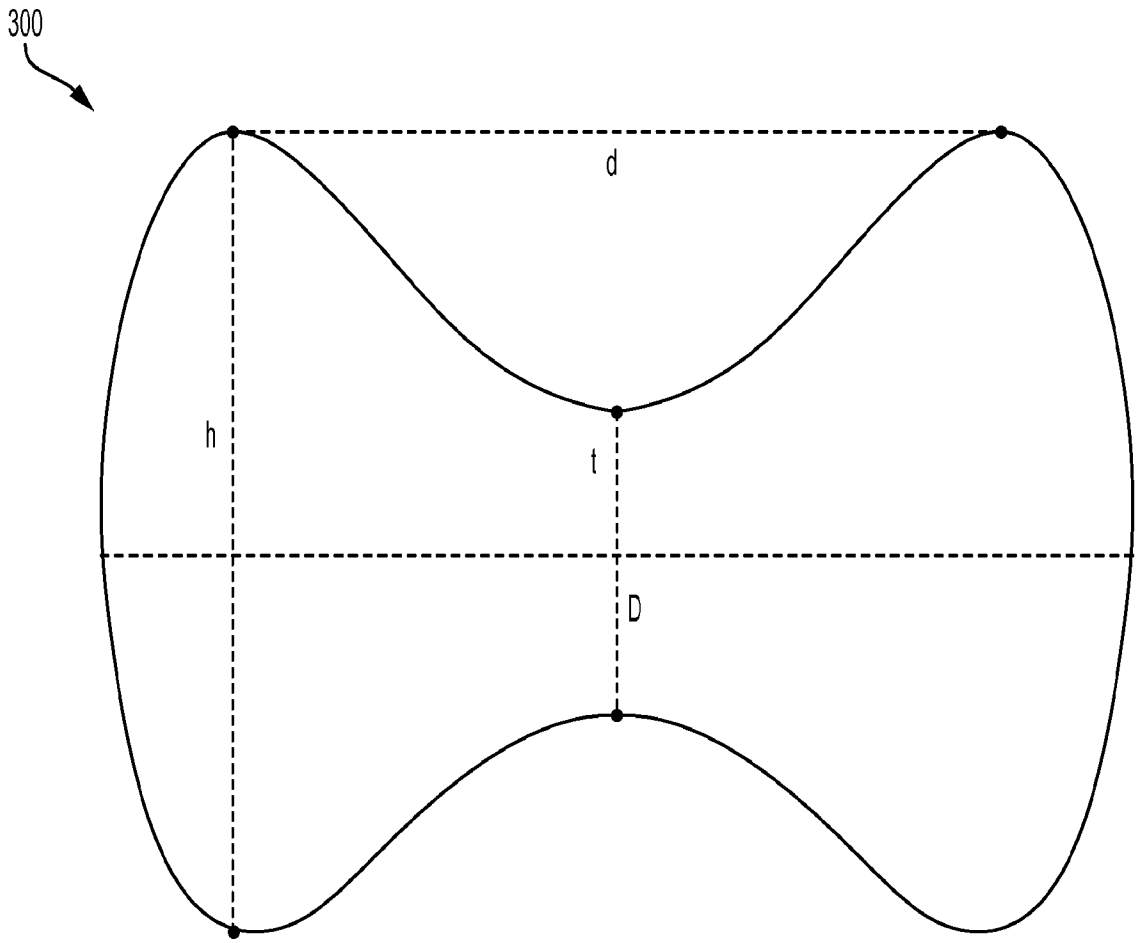

FIG. 3

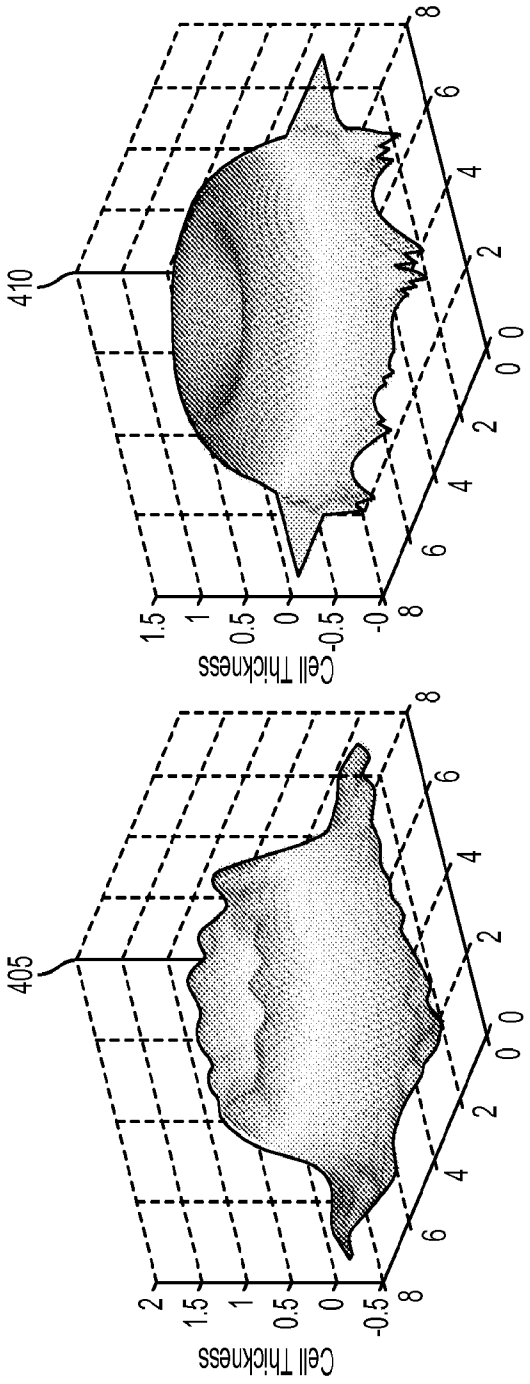
410
405
Cell Thickness
Cell Thickness
1.5
1
0.5
0
-0.5
2
8
6
4
2
0
0
2
4
6
8

FIG. 4

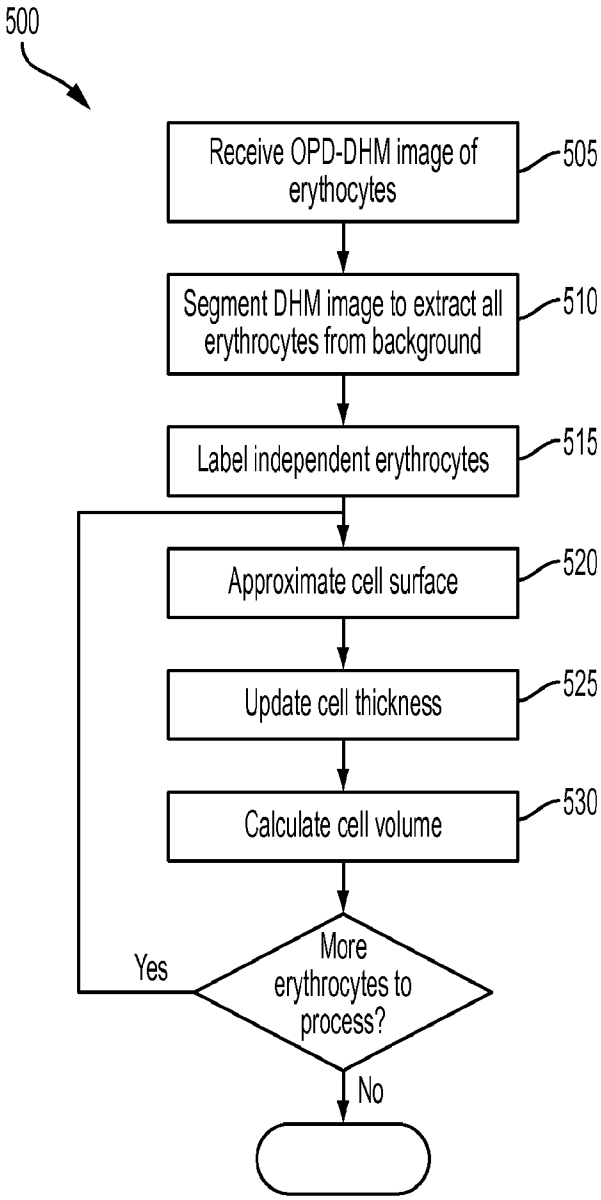
500
Receive OPD-DHM image of erythocytes
505
Segment DHM image to extract all erythrocytes from background
510
Label independent erythrocytes
515
Approximate cell surface
520
Update cell thickness
525
Calculate cell volume
530
More erythrocytes to process?
Yes
No

FIG. 5

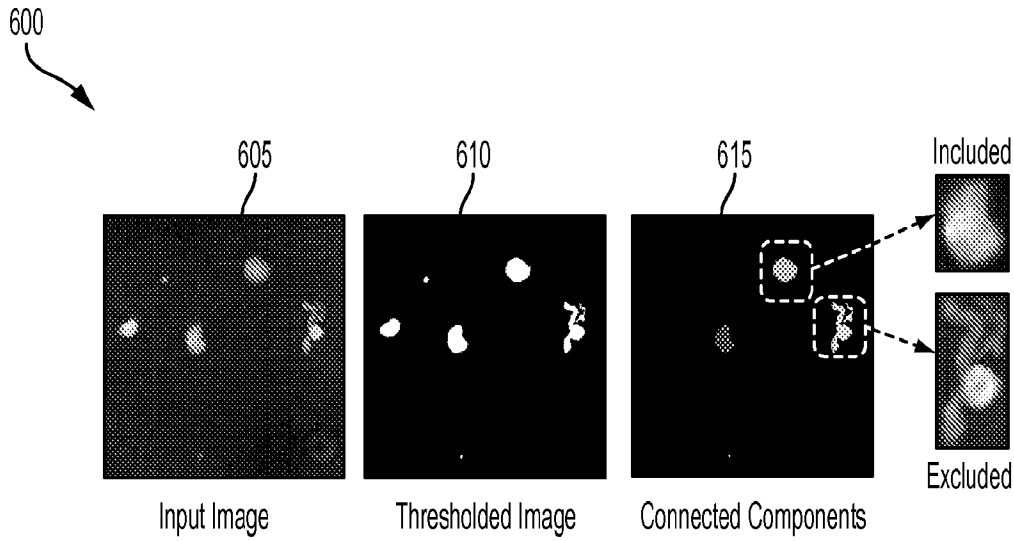
600
605
610
615
Included
Excluded
Input Image
Thresholded Image
Connected Components

FIG. 6

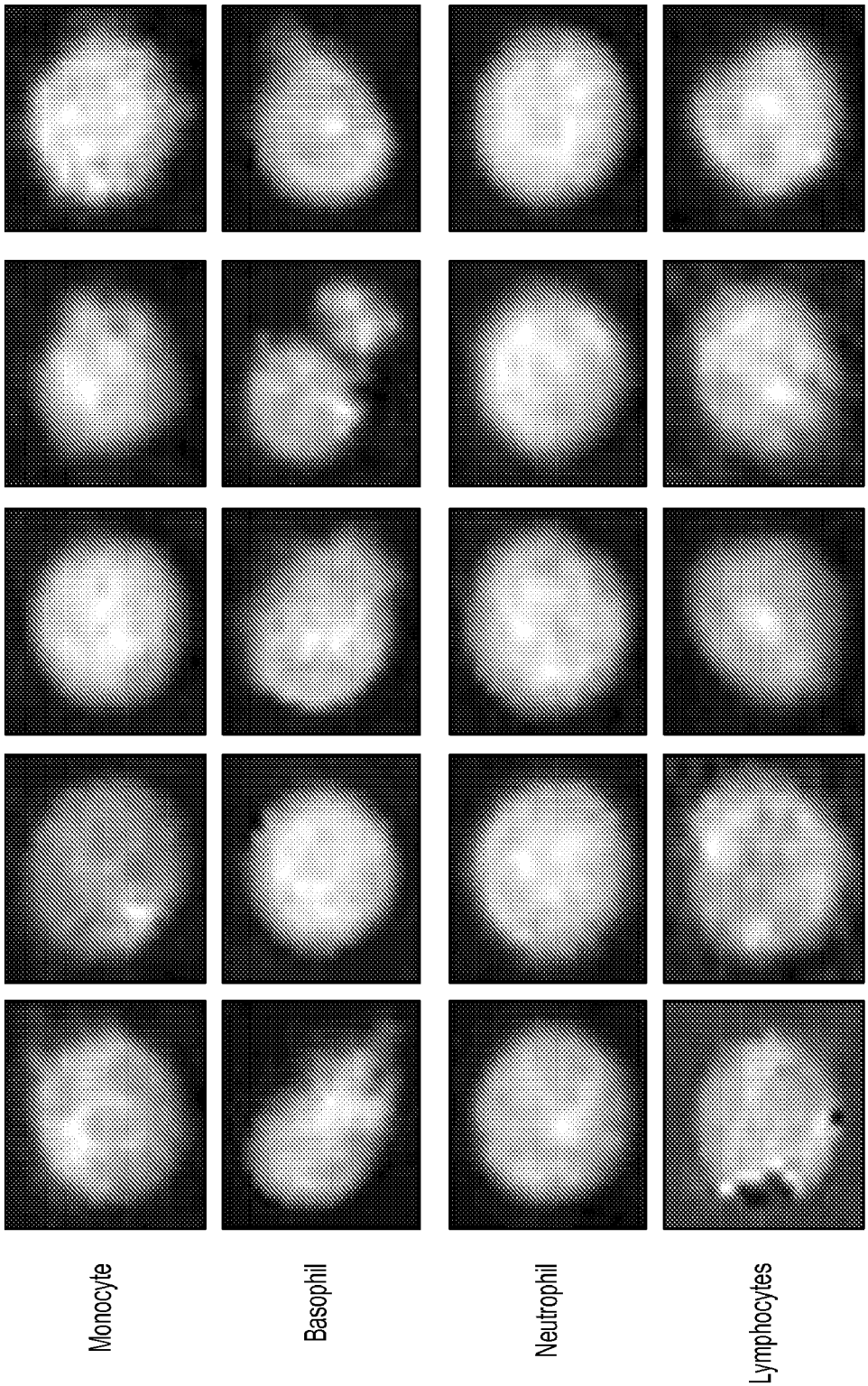

FIG. 7

Cell Type	Nr. of Images	Nr. Cells	Training Cells	Testing Cells
Monocyte	110	191	100	91
Neutrophil	117	155	100	55
Basophil	129	140	100	40
T Cell	110	406	100	306

FIG. 8A

Classifier Type1-Type2		Correct Classification rate for Type 1	Correct Classification rate for Type 2	Average Correct Classification Rate
Monocyte	Neutrophil	81.32%	74.55%	77.93%
Monocyte	Basophil	89.01%	100%	94.51%
Monocyte	T Cell	90.11%	95.42%	92.77%
Basophil	Neutrophil	95%	92.73%	93.86%
Basophil	T Cell	80%	89.54%	84.77%
Neutrophil	T Cell	90.91%	94.44%	92.68%
Monocyte	Eosinophil	84.62%	82.42%	83.52%
Neutrophil	Eosinophil	80%	68%	74%
Basophil	Eosinophil	100%	96.7%	98.35%
TCell	Eosinophil	93.46%	98.9%	96.18%

FIG. 8B

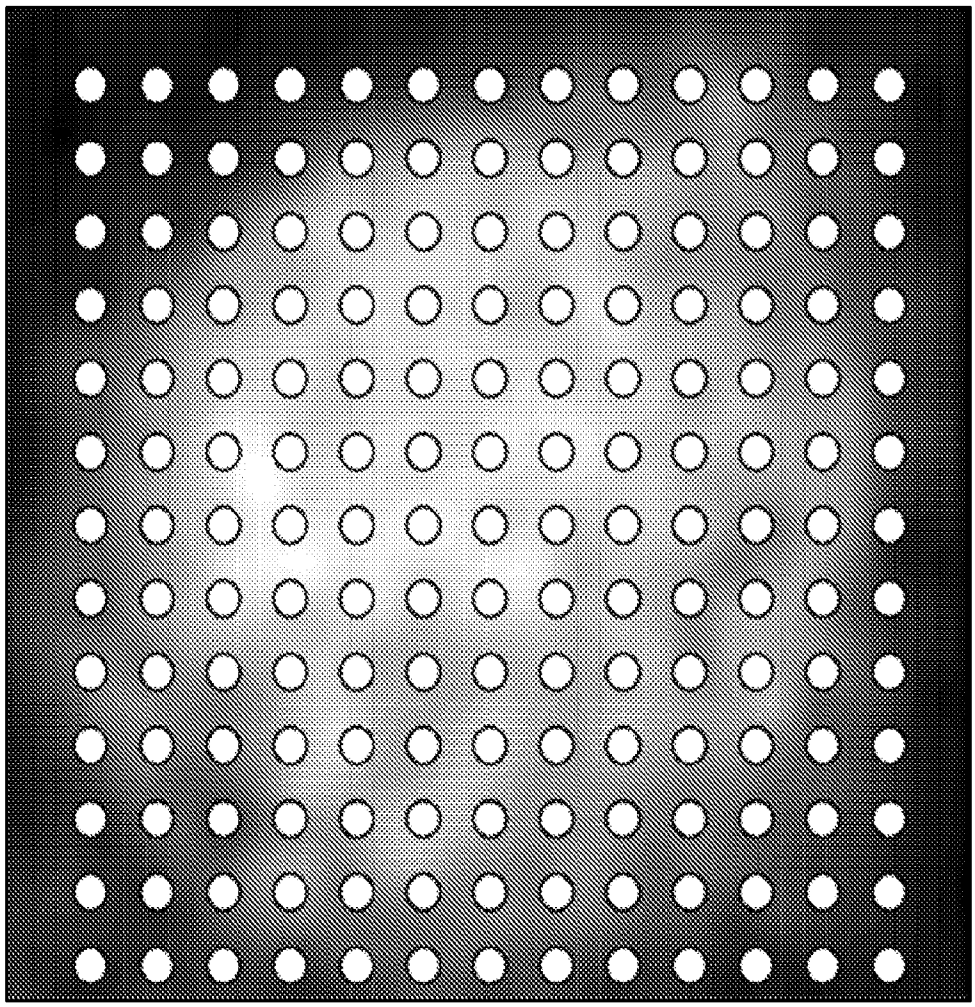

FIG. 9

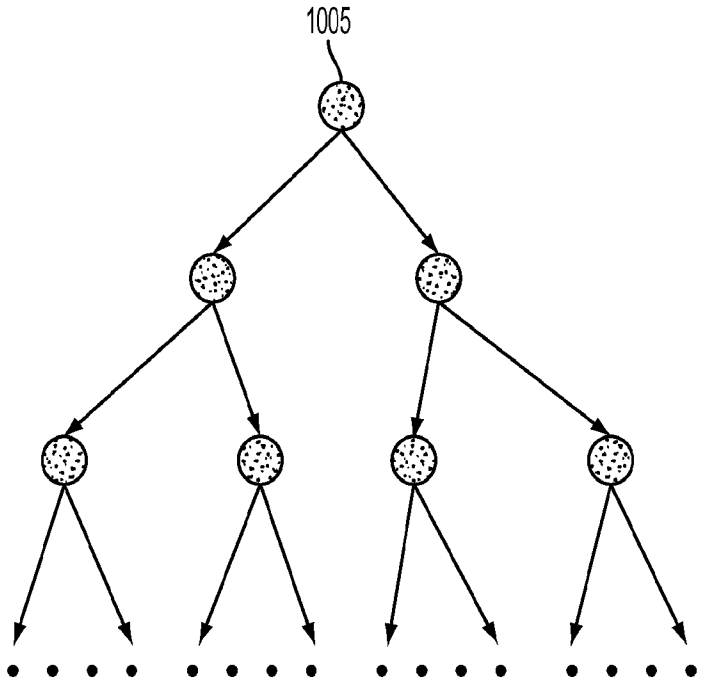

FIG. 10

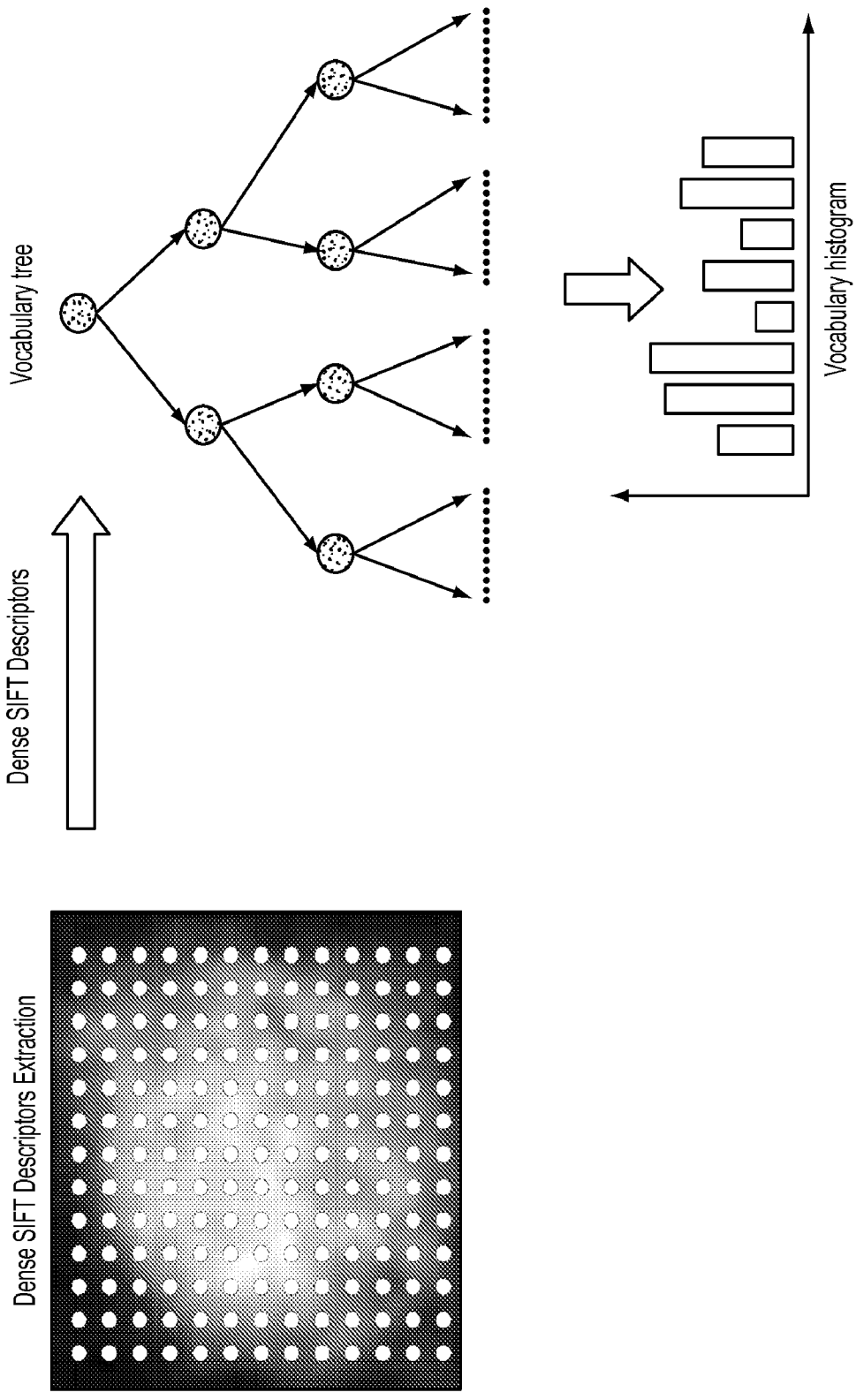
Dense SIFT Descriptors Extraction
Dense SIFT Descriptors
Vocabulary tree
Vocabulary histogram

FIG. 11

Classifier Type1-Type2		Correct Classification rate for Type 1	Correct Classification rate for Type 2	Average Correct Classification Rate
Monocyte	Neutrophil	84.6%	70.9%	79.4%
Monocyte	Basophil	96.7%	96.7%	97.7%
Monocyte	T Cell	92.3%	96.7%	95.7%
Basophil	Neutrophil	100%	96.3%	97.8%
Basophil	T Cell	97.5%	91.8%	92.5%
Neutrophil	T Cell	94.54%	95.7%	95.5%
Monocyte	Eosinophil	96.7%	97.8%	97.2%
Neutrophil	Eosinophil	72.7%	82.4%	82.4%
Basophil	Eosinophil	100%	100%	100%
TCell	Eosinophil	91.8%	97%	92.5%

FIG. 12

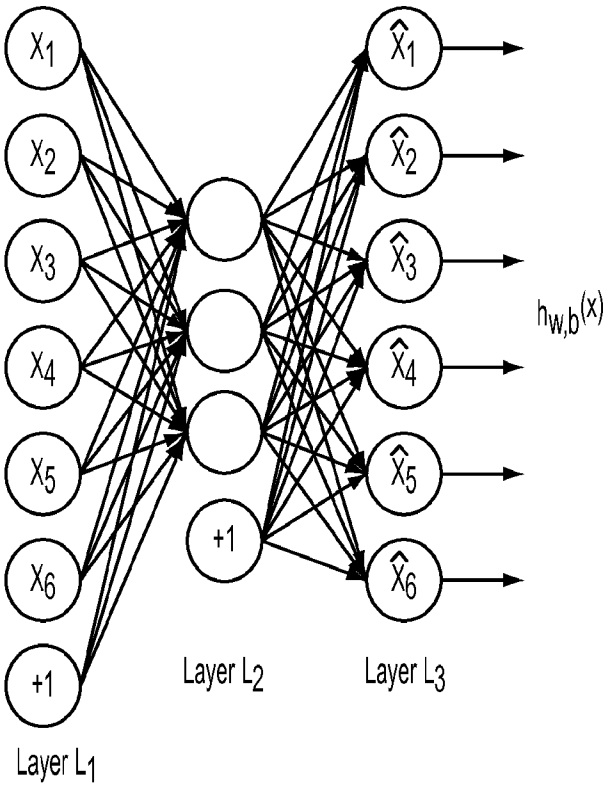
x_1
x_2
x_3
x_4
x_5
x_6
+1
Layer L_1
+1
Layer L_2
$\hat{x}_1$
$\hat{x}_2$
$\hat{x}_3$
$\hat{x}_4$
$\hat{x}_5$
$\hat{x}_6$
Layer L_3
$h_{W,b}(x)$

FIG. 13

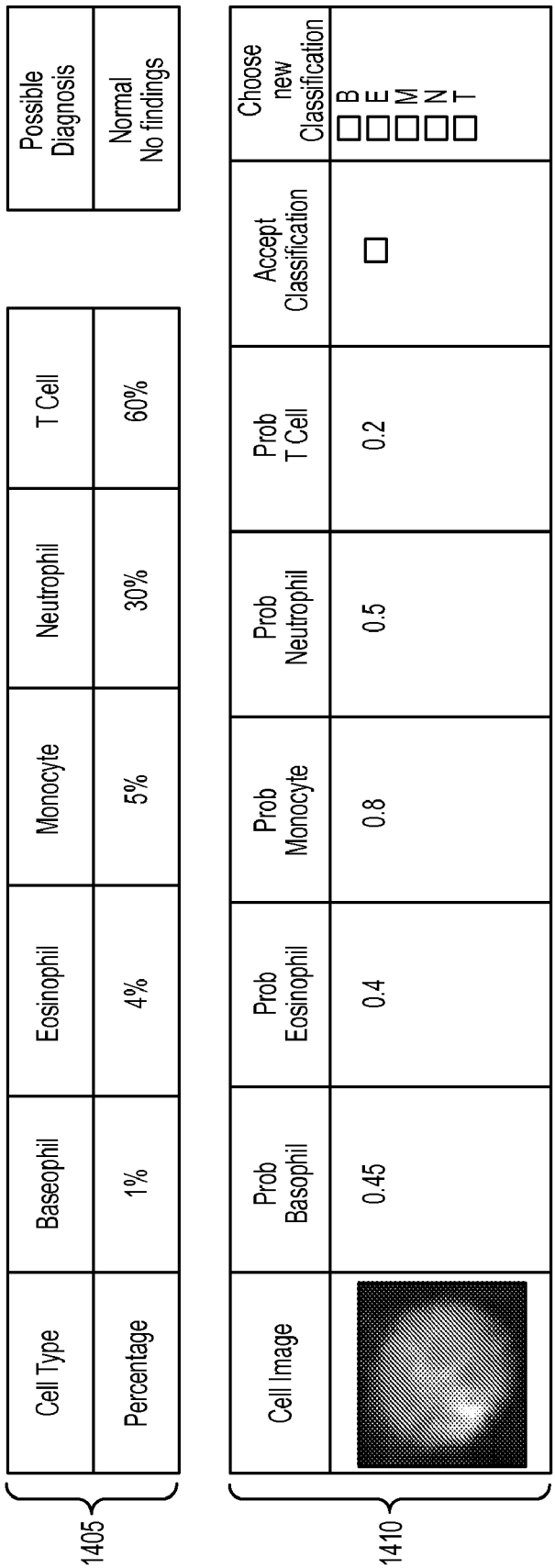
Possible Diagnosis
Normal No findings
Cell Type
Baseophil
Eosinophil
Monocyte
Neutrophil
T Cell
Percentage
1%
4%
5%
30%
60%
1405
Cell Image
Prob Basophil
Prob Eosinophil
Prob Monocyte
Prob Neutrophil
Prob T Cell
Accept Classification
Choose new Classification
B
E
M
N
T
0.45
0.4
0.8
0.5
0.2
1410

FIG. 14

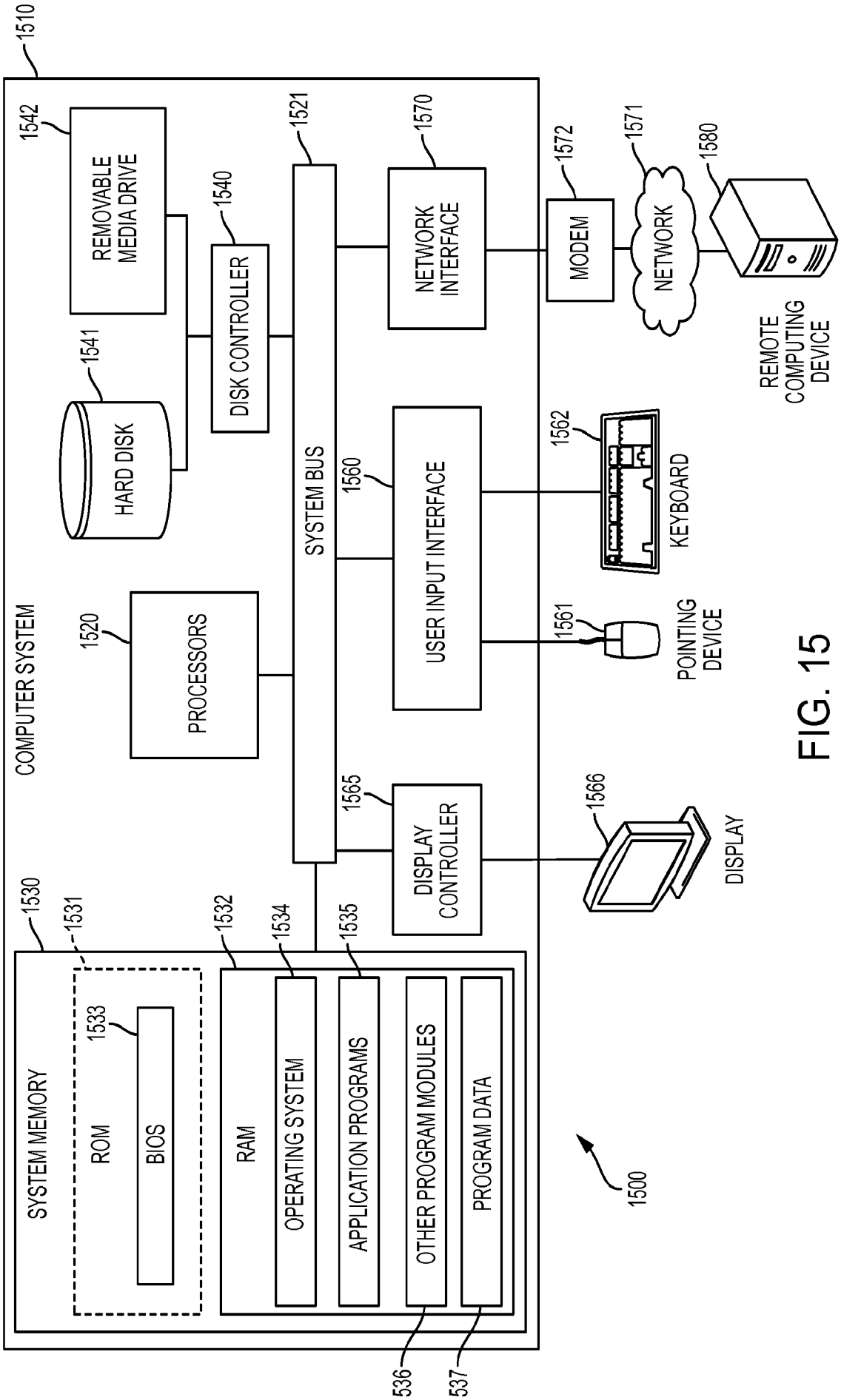
1510
COMPUTER SYSTEM
1542
REMOVABLE MEDIA DRIVE
1540
DISK CONTROLLER
1541
HARD DISK
1521
SYSTEM BUS
1570
NETWORK INTERFACE
1572
MODEM
1571
NETWORK
1580
REMOTE COMPUTING DEVICE
1560
USER INPUT INTERFACE
1562
KEYBOARD
1561
POINTING DEVICE
1520
PROCESSORS
1565
DISPLAY CONTROLLER
1566
DISPLAY
1530
SYSTEM MEMORY
1531
ROM
1533
BIOS
1532
RAM
1534
OPERATING SYSTEM
1535
APPLICATION PROGRAMS
OTHER PROGRAM MODULES
PROGRAM DATA
1536
1537
1500

FIG. 15

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/035999

A. CLASSIFICATION OF SUBJECT MATTER
INV. G06K9/00 G01N15/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G06K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J O RICARDO ET AL: "Digital holography microscopy in 3D biologic samples analysis", JOURNAL OF PHYSICS: CONFERENCE SERIES, vol. 274, 1 January 2011 (2011-01-01), page 012066, XP055209796, DOI: 10.1088/1742-6596/274/1/012066 abstract sections 1-7 figures 1-10 ----- -/--	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 August 2015	08/09/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Nilsson, Martin

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Inkyu Moon ET AL: "Automated statistical quantification of three- dimensional morphology and mean corpuscular hemoglobin of multiple red blood cells filtering for zero-order and twin-image elimination in digital off-axis holography", Opt. Express, 23 April 2012 (2012-04-23), pages 163-168, XP055209826, Retrieved from the Internet: URL:https://www.osapublishing.org/DirectPDFAccess/4F14B849-0DD7-18C1-CB67F824BF45684A_232081/oe-20-9-10295.pdf?da=1&id=232081&seq=0&mobile=no [retrieved on 2015-08-27] abstract sections 1-5 figures 1-4 -----	1-15
X	BENJAMIN RAPPAZ ET AL: "Comparative study of human erythrocytes by digital holographic microscopy, confocal microscopy, and impedance volume analyzer", CYTOMETRY PART A, vol. 73A, no. 10, 1 October 2008 (2008-10-01), pages 895-903, XP055032058, ISSN: 1552-4922, DOI: 10.1002/cyto.a.20605 page 896, left-hand column, line 18 - page 901, right-hand column, line 29; figures 1-4 -----	1-15

1