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Label-free optical technique for identifying lymphocyte types using 3D quantitative phase imaging and machine learning

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Dear Editor:

We thank the Editor for processing, formatting, and editorially reviewing our submission (JoVE58305, Yoon *et al.*). After carefully studying the editorial comments, we revised the manuscript by addressing all concerns raised by the Editor. In particular, we thoroughly revised the protocol section to provide detailed experimental steps as required by the Editor.

Below we address the editorial comments and the list of changes that we have made to our manuscript according to the concerns. The original editorial comments are provided in **black**, whereas our answers are given in **blue**. We believe that these modifications have strengthened the protocol and hope that it is now suitable for publication in *Journal of Visualized Experiments*.

Sincerely,
YongKeun (Paul) Park,
On behalf of all co-authors

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TITLE:

Label-Free Identification of Lymphocyte Subtypes using Three-Dimensional Phase Imaging and Machine Learning

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KEYWORDS:

Quantitative phase imaging, optical diffraction tomography, holotomography, holographic microscopy, lymphocyte identification, immune cell, immunology, machine learning, label-free imaging

SUMMARY:

We describe a protocol for the label-free identification of lymphocyte subtypes using quantitative phase imaging and a machine learning algorithm. Measurements of 3D refractive index tomograms of lymphocytes present 3D morphological and biochemical information for individual cells, which is then analyzed with a machine-learning algorithm for identification of cell types.

ABSTRACT:

We describe here a protocol for the label-free identification of lymphocyte subtypes using quantitative phase imaging and machine learning. Identification of lymphocyte subtypes is important for the study of immunology as well as diagnosis and treatment of various diseases.

Currently, standard methods for classifying lymphocyte types rely on labeling specific membrane proteins via antigen-antibody reactions. However, these labeling techniques carry the potential risks of altering cellular functions. The protocol described here overcomes these challenges by exploiting intrinsic optical contrasts measured by 3D quantitative phase imaging and a machine learning algorithm. Measurement of 3D refractive index (RI) tomograms of lymphocytes provides quantitative information about 3D morphology and phenotypes of individual cells. The biophysical parameters extracted from the measured 3D RI tomograms are then quantitatively analyzed with a machine learning algorithm, enabling label-free identification of lymphocyte types at a single-cell level. We measure the 3D RI tomograms of B, CD4+ T, and CD8+ T lymphocytes and identified their cell types with over 80% accuracy. In this protocol, we describe the detailed steps for lymphocyte isolation, 3D quantitative phase imaging, and machine learning for identifying lymphocyte types.

INTRODUCTION:

Lymphocytes can be classified into various subtypes including B, helper (CD4+) T, cytotoxic (CD8+) T, and regulatory T cells. Each lymphocyte type has a different role in the adaptive immune system; for example, B lymphocytes produce antibodies, whereas T lymphocytes detect specific antigens, eliminate abnormal cells, and regulate B lymphocytes. Lymphocyte function and regulation is tightly controlled by and related to various diseases including cancers¹, autoimmune diseases², and viral infections³. Thus, the identification of lymphocyte types is important to understand their pathophysiological roles in such diseases and for immunotherapy in clinics.

Currently, methods for classifying lymphocyte types rely on antigen-antibody reactions by targeting specific surface membrane proteins or surface markers⁴. Targeting surface markers is a precise and accurate method to determine lymphocyte types. However, it requires expensive reagents and time-consuming procedures. Furthermore, it carries risks of the modification of membrane protein structures and the alteration of cellular functions.

To overcome these challenges, the protocol described here introduces the label-free identification of lymphocyte types using 3D quantitative phase imaging (QPI) and machine learning⁵. This method enables the classification of lymphocyte types at a single-cell level based on morphological information extracted from label-free 3D imaging of individual lymphocytes. Unlike conventional fluorescence microscopy techniques, QPI utilizes refractive index (RI) distributions (intrinsic optical properties of live cells and tissues) as optical contrast^{6,7}. The RI tomograms of individual lymphocytes represent phenotypic information specific to subtypes of lymphocytes. In this case, to systemically utilize 3D RI tomograms of individual lymphocytes, a supervised machine learning algorithm was utilized.

Using various QPI techniques, the 3D RI tomograms of cells have been actively used for the study of cell pathophysiology because they provide a label-free, quantitative imaging capability⁸⁻¹³. Also, the 3D RI distributions of individual cells can provide morphological, biochemical, and biomechanical information about cells. 3D RI tomograms have been previously utilized in the fields of hematology¹⁴⁻¹⁷, infectious diseases¹⁸⁻²⁰, immunology²¹, cell biology^{22,23}, inflammation²⁴, cancer²⁵, neuroscience^{26,27}, developmental biology²⁸, toxicology²⁹, and microbiology^{12,30-32}.

Although 3D RI tomograms provide detailed morphological and biochemical information of cells, the classification of lymphocyte subtypes is difficult to achieve by simply imaging 3D RI tomograms⁵. To systematically and quantitatively exploit the measured 3D RI tomograms for the cell type classification, we utilized a machine learning algorithm. Recently, several works have been reported in which quantitative phase images of cells were analyzed with various machine learning algorithms³³, including the detection of microorganisms³⁴, classification of bacterial genus^{35,36}, rapid and label-free detection of anthrax spores³⁷, automated analysis of sperm cells³⁸, analysis of cancer cells^{39,40}, and detection of macrophage activation⁴¹.

This protocol provides detailed steps to perform label-free identification of lymphocyte types at the individual cell level using 3D QPI and machine learning. This includes: 1) lymphocyte isolation from mouse blood, 2) lymphocyte sorting via flow cytometry, 3) 3D QPI, 4) quantitative feature extraction from 3D RI tomograms, and 5) supervised learning for identifying lymphocyte types.

PROTOCOL:

Animal care and experimental procedures were performed under the approval of the Institutional Animal Care and Use Committee of KAIST (KA2010-21, KA2014-01, and KA2015-03). All the experiments in this study were carried out in accordance with the approved guidelines.

1. Lymphocyte Isolation from Mouse Blood

1.1. Once a C57BL/6J mouse is euthanized via CO₂ inhalation, insert a 26-G needle into the mouse heart and collect 0.3 mL of blood. Directly put blood into a tube with 100 U/mL heparin solution diluted with 1 mL of phosphate-buffered saline (PBS).

NOTE: Alternatively, lymphocytes from the spleen can be isolated.

1.2. Centrifuge the tube at 400 x g for 5 min at 4 °C.

1.3. Add 0.5 mL of ammonium-chloride-potassium lysing buffer to the tube and gently invert it a few times to mix the solution.

1.4. Incubate the tube at room temperature (RT) for 5 min.

1.5. Wash the cells by adding 4.5 mL of PBS and centrifuging at 400 x g for 5 min at 4 °C, twice.

1.6. Remove the supernatant and resuspend the cell pellet in 100 µL of fresh RPMI-1640 medium with 10% fetal bovine serum (FBS).

1.7. Add 0.1 µg of CD16/32 (2.4G2) antibody to the tube for blocking.

1.8. Keep the tube on ice.

2. Flow Cytometry and Sorting of Lymphocyte Subtypes

Note: Sorting lymphocytes depending on cell type is essential for establishing the ground-truth (*i.e.*, correct) cell type labels to train and test a cell type classifier in supervised learning. Flow cytometry, a gold standard method, is used to identify and separate lymphocytes⁴².

2.1. Make a mixture of surface marker staining antibodies in 100 μ L of fresh RPMI-1640 medium [with 10% FBS, 0.1 μ g of CD3e (17A2), CD8a (53-6.7), CD19 (1D3), CD45R (B220, RA3-6B2), and NK1.1 (PK136)] and 0.25 μ g of CD4 (GK1.5) antibodies to target B, CD4⁺ T, and CD8⁺ T lymphocytes.

2.2. Add 100 μ L of the antibody mixture to the cell suspension (obtained in step 1.8).

2.3. Incubate for 25 min on ice.

2.4. Wash the cells by adding 5 mL of PBS and centrifuging at 400 x g for 5 min at 4 °C, twice.

2.5. Resuspend the cell pellet in 5 mL of fresh RPMI-1640 medium with 10% FBS and 2.5 μ g of DAPI (4,6-diamidino-2-phenylindole).

2.6. Collect each lymphocyte type separately with flow cytometry using the fluorescence levels of the markers described above. Simultaneously exclude dead cells using the DAPI signals.

Note: Detailed protocols regarding flow cytometry-based cell sorting have been described previously⁴².

3. 3D Quantitative Phase Imaging

3.1. Keep the sorted lymphocytes on ice throughout the imaging procedures, which should be completed within 5 h (since lymphocyte isolation from the mouse) to avoid cell damage and biochemical alterations.

3.2. Select a sorted cell type (among B, CD4⁺ T, and CD8⁺ T lymphocytes) and dilute the sample (obtained in step 2.6) to 180 cells/ μ L for optimal imaging condition (*i.e.*, one cell per single field-of-view).

3.3. Load 120 μ L of the diluted sample into an imaging chamber by slow injection. Thoroughly check on the presence of bubbles in the imaging chamber with the sample. If there are bubbles, carefully remove them, as they will compromise the quality of the measurements.

3.4. Acquire 3D RI tomograms using a commercial 3D quantitative phase microscope, or holotomography, and its imaging software.

Note: Detailed information about the experimental setup can be found in the original

manuscript⁵.

3.4.1. Place a drop of distilled water on top of the objective lens of the microscope.

3.4.2. Place the imaging chamber with the sample on the translation stage of the microscope and adjust its location so that the sample aligns with the objective lens.

3.4.3. Adjust the axial positions of the objective and condenser lenses by clicking **Focus** and **Surface**, respectively, on the “Calibration” tab of the “Microscope” perspective of the imaging software.

3.4.4. Align the objective and condenser lenses by clicking **Auto Mode**. Alternatively, use **Scanning Mode** and manually adjust the lenses so that the illumination patterns are localized at the central region of the field-of-view.

3.4.5. Return to **Normal Mode** and adjust the translation stage to locate a cell in the field-of-view.

3.4.6. Find the focal plane by adjusting the axial position of the objective lens. Perfect focusing makes the sample boundary visualized in the screen almost invisible.

3.4.7. Adjust the translation stage to find a location without a cell.

3.4.8. Click **Calibrate** to measure multiple 2D holograms with varying illumination angles.

3.4.9. Adjust the translation stage to locate a cell at the center of the field-of-view.

3.4.10. Move to the “Acquisition” tab and click **3D Snapshot** to measure the holograms of the cell with the identical illumination angles as done in step 3.4.8.

3.4.11. When the acquired data is presented on the “Data Management” panel, right-click on the acquired data and click **Process** to reconstruct a 3D RI tomogram from the holograms measured in steps 3.4.8 and 3.4.10, using the diffraction tomography algorithm^{9,10} implemented in the imaging software.

3.4.12. Repeat steps 3.4.5-3.4.11 to measure more than 100 cells to ensure statistical power for machine learning.

3.5. Repeat steps 3.2-3.4 to measure 3D RI tomograms of all lymphocyte subtypes.

4. Quantitative Morphological and Biochemical Feature Extraction from 3D RI Tomograms

4.1. Place all the tomographic data measured above in a single folder. Split the cell types in the subfolders of this folder. Prepare each tomogram to be a single *.mat* file.

221 4.2. Open **Supplementary File 1** (written for an image processing software).

222
223 4.3. Edit line 14 to designate the tomogram folder prepared in step 4.1.

224
225 4.4. Edit line 15 to designate a folder to save the extracted feature data.

226
227 4.5. Optionally, edit line 17 to adjust the RI threshold parameters for feature extraction. The
228 default option is 20 RI thresholds from 1.340-1.378, with an increment of 0.002 as described
229 previously⁵.

230
231 4.6. Execute the code. For every tomogram in the dataset, the code calculates five features:
232 surface area (SA), cellular volume (CV), sphericity (SI), protein density (PD), and dry mass (DM),
233 per RI threshold. The detailed algorithms for feature extraction are described elsewhere⁵.

234
235 4.7. In order to monitor the feature extraction, during the execution check on the screen
236 visualizing RI threshold-based cell segmentation.

237
238 4.8. Check on the extracted feature data, as *.mat* file per tomogram, saved in the folder
239 designated in step 4.4.

240 241 **5. Supervised Learning and Identification**

242
243 5.1. Randomly split the feature data obtained in step 4.8 to training (70%) and test (30%) sets
244 with separate folders.

245
246 5.2. Open **Supplementary File 2** (written for an image processing software).

247
248 5.3. Edit line 14 to designate the training set prepared in step 5.1.

249
250 5.4. Edit line 16 to designate a folder to save the trained classifier.

251
252 5.5. Edit line 17 to set a file name for the classifier.

253
254 5.6. Optionally, edit line 19 to select the features for training. The default option, as specified
255 previously⁵, was used to obtain the representative results below.

256
257 5.7. Execute the code. Using the selected features of the training set, the code trains a classifier
258 with the *k*-nearest neighbor algorithm (*k*-NN; *k* = 4) and then saves the classifier (as named in
259 step 5.5) in the folder designated in step 5.4.

260
261 5.8. Check on the screen visualizing the classifier performance and the cross-validation accuracy.

262
263 5.9. Optionally, train multiple classifiers with different feature combinations by repeating steps
264 5.5-5.7. Then choose the classifier with the highest cross-validation accuracy.

265
266 5.10. Open **Supplementary File 3** (written for an image processing software).

267
268 5.11. Edit lines 14-15 to designate the trained classifier to be tested.

269
270 5.12. Edit line 17 to designate the test set prepared in step 5.1.

271
272 5.13. Execute the code. The classifier described above identifies the cell types of the individual
273 lymphocytes in the test set.

274
275 5.14. Check on the screen visualizing the identification performance and the test accuracy.

276 277 **REPRESENTATIVE RESULTS:**

278 **Figure 1** shows the schematic process of the entire protocol. Using the procedure presented here,
279 we isolated B (n = 149), CD4+ T (n = 95), and CD8+ T (n = 112) lymphocytes. To obtain phase and
280 amplitude information at various angles of illumination, multiple 2D holograms of each
281 lymphocyte were measured by changing the angle of illumination (from -60° to 60°). Typically, 50
282 holograms can be used to reconstruct a 3D RI tomogram, but the number of 2D holograms can
283 be adjusted considering the imaging speed and quality. Amplitude and phase information of the
284 measured holograms are retrieved using a field retrieval algorithm based on Fourier
285 transform^{43,44}. The 3D RI tomogram of each lymphocyte was reconstructed from multiple 2D
286 retrieved phase and amplitude information at various angles of illumination using optical
287 diffraction tomography algorithm. Details of image process and 3D RI tomogram reconstruction
288 method can be found elsewhere^{21,45}.

289
290 **Figures 2A-2C** shows representative 3D rendered RI tomograms of B, CD4+ T, and CD8+ T
291 lymphocytes by allocating different color schemes according to RI values via the imaging
292 software. From the RI values, quantitative morphological (SA, CV, and SI) and biochemical (PD
293 and DM) features were calculated (**Figures 2A-2C**). This result clearly demonstrates that 3D RI
294 distribution enables quantitative analysis of morphological as well as biochemical information of
295 lymphocytes.

296
297 Supervised machine learning was exploited to identify lymphocyte types at a single-cell level. The
298 measured 3D RI tomograms were randomly split into 70% and 30% of training (B: 104, CD4+ T:
299 66, and CD8+ T: 77) and test (B: 45, CD4+ T: 29, and CD8+ T: 35) datasets, respectively. We
300 optimized the classifiers to maximally utilize the cell-type-specific fingerprints encoded in the
301 feature space. The total accuracy, sensitivity (true positive), and specificity (true negative) were
302 calculated by comparing the classifier-predicted results and ground-truth cell types.

303
304 In order to demonstrate proof-of-concept of the proposed protocol, we performed supervised
305 machine learning on three different cases: binary classification of (i) B and T lymphocytes and (ii)
306 two T lymphocyte subtypes (CD4+ and CD8+), and (iii) multiclass classification of all lymphocyte
307 types.
308

Figure 3 shows identification performance of optimized classifiers for training and test stages. The accuracy of the T and B lymphocyte classification was 93.15% and 89.81% for the training and test cases, respectively. The CD4+ and CD8+ T lymphocytes were statistically classified, and the accuracy was 87.41% and 84.38% for the training and test sets, respectively. Lastly, the accuracy of the multiclass cell type classifier was 80.65% and 75.93% for the training and test stages, respectively.

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic diagrams of the label-free identification of lymphocyte types exploiting 3D quantitative phase imaging and machine learning.

Figure 2: Representative 3D rendered RI tomograms of each lymphocyte cell type with quantitative morphological and biochemical features. (A) B cell, (B) CD4+ T cell, and (C) CD8+ T cell. Scale bar = 2 μm . SA, surface area; CV, cellular volume; SI, sphericity; PD, protein density; DM, dry mass. This figure is modified with permission⁵.

Figure 3: Identification of individual lymphocyte types via supervised machine learning (A) binary classification of B and T cells, (B) binary classification of CD4+ and CD8+ T cells, and (C) multiclass classification of all three lymphocyte cell types; for both training and test sets. Note the small difference between the training and test cases, suggesting nice generalization of the established classifiers. The numbers below the names of each cell type indicate the number of cells used. This figure is modified with permission⁵.

Supplementary File 1: Feature extraction code. Extracting features (SA, CV, SI, PD, and DM) after RI threshold-based segmentation of each tomogram. Implemented in an image processing software.

Supplementary File 2: Training code. Training a k -NN classifier training based on selected features. Implemented in an image processing software.

Supplementary File 3: Testing code. Testing a trained k -NN classifier for a new dataset (*i.e.*, test set). Implemented in an image processing software.

DISCUSSION:

We present a protocol that enables the label-free identification of lymphocyte types exploiting 3D quantitative phase imaging and machine learning. Critical steps of this protocol are quantitative phase imaging and feature selection. For the optimal holographic imaging, the density of cells should be controlled as described above. Mechanical stability of the cells is also important to obtain a precise 3D RI distribution because floating or vibrational cellular motions will disturb hologram measurements upon illumination angle changes. We, therefore waited several minutes until the sample became stable and static in the imaging chamber before measuring holograms. Lastly, bubbles inside the imaging chamber are problematic when measuring holograms due to RI differences between air and the sample; thus, the sample should be carefully loaded to the imaging chamber.

Feature extraction and selection help determine the identification performance of the classifier. We calculated 5 quantitative morphological (CV, SA, SI) and biochemical (PD, DM) features from 3D RI distribution at 20 different RI threshold values; thus, we extracted a total of 100 features. We exhaustively searched optimal feature and classifier combinations, which show that the best cross-validation accuracy was selected. We tested 6 different machine learning algorithms, including k -NN ($k = 4$ and $k = 6$), linear discrimination analysis, quadratic discrimination analysis, naïve Bayes, and decision tree, and we found that k -NN ($k = 4$) showed the best identification performance. However, there is a chance to improve identification accuracy using other machine learning methods, including support vector machine and neuronal networks.

This protocol measures intrinsic optical properties via 3D quantitative phase imaging in order to identify lymphocyte types; thus, it does not require a labeling process based on antigen-antibody reactions used in fluorescence or magnetic bead-based cell-sorting techniques, which have risks of altering cellular function by modifying membrane protein structures. Moreover, the present method measures 3D RI distribution and provides 3D morphological and biochemical information about the cell, which cannot be obtained by a single-shot holography method⁴⁶; therefore, the identification performance of the protocol is more accurate due to high-dimensional information.

A minor limitation of this protocol is the manual adjustment of the sample stage and required labeling process for supervised machine learning. We searched a lymphocyte by adjusting the manual translational stage and measured holograms, which are the most time-consuming steps. This limitation would be improved by employing an automated motorized stage or microfluidic channel devices. Regarding supervised learning, the known lymphocyte types are required to establish the optimal classifier; thus, we had to first isolate and identify lymphocyte cell types based on the antigen-antibody-based sorting technique. Nonetheless, this protocol still uses the intrinsic optical contrast of lymphocytes, and the labeling agents used to specify antibodies have negligible effects on the measured 3D RI signal. Therefore, the established classifier may be used for identifying lymphocytes in a label-free manner.

Although this protocol mainly utilizes phenotypes of lymphocytes by measuring 3D RI tomograms of individual cells, these 3D RI data can also be used in combination with other modalities addressing genotypes or proteomic information for better classification of subtypes. Recently, correlative microscopy techniques combining fluorescence imaging and QPI have been introduced⁴⁷⁻⁴⁹. The approach presented in this protocol can also be extended to these correlative imaging methods.

Label-free identification of lymphocyte types can be applied to studying pathophysiology or diagnosing disease by detecting abnormal lymphocytes or ratios among lymphocyte types. Furthermore, this protocol can be applied to whole blood analysis by identifying various cells including red blood cells, platelets, and white blood cells.

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DISCLOSURES:

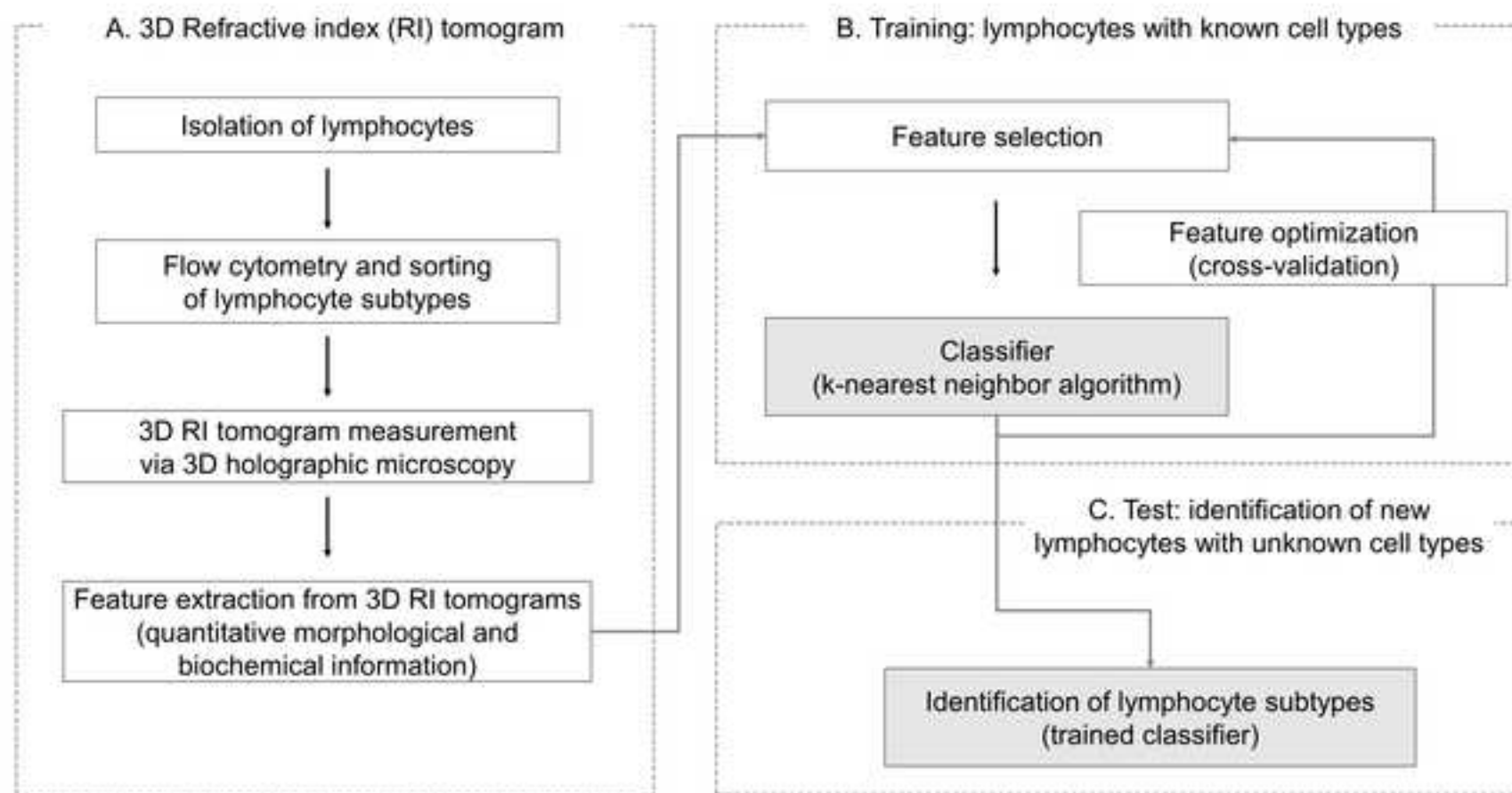
Prof. Y. Park, Y. Jo, Y. S. Kim, and S. Lee have financial interests in Tomocube, Inc., a company that commercializes optical diffraction tomography and quantitative phase imaging instruments and is one of the sponsors of the work.

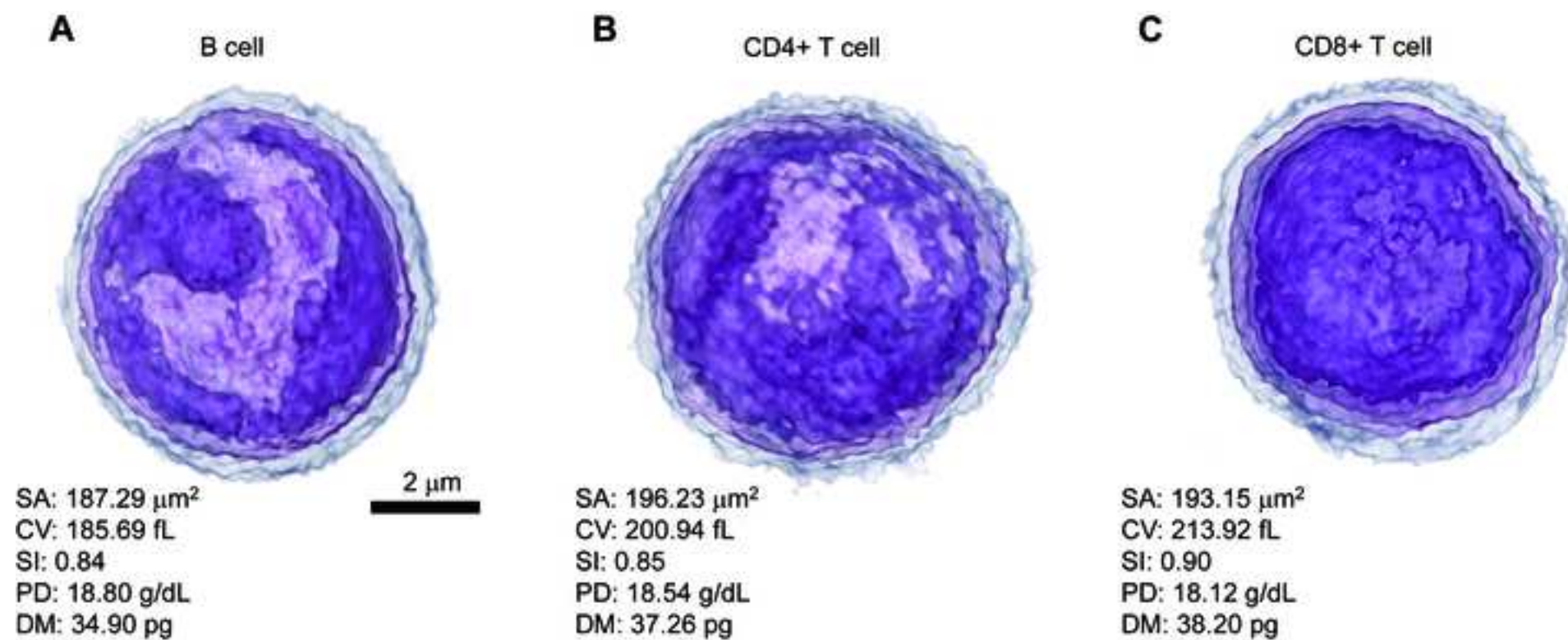
REFERENCES:

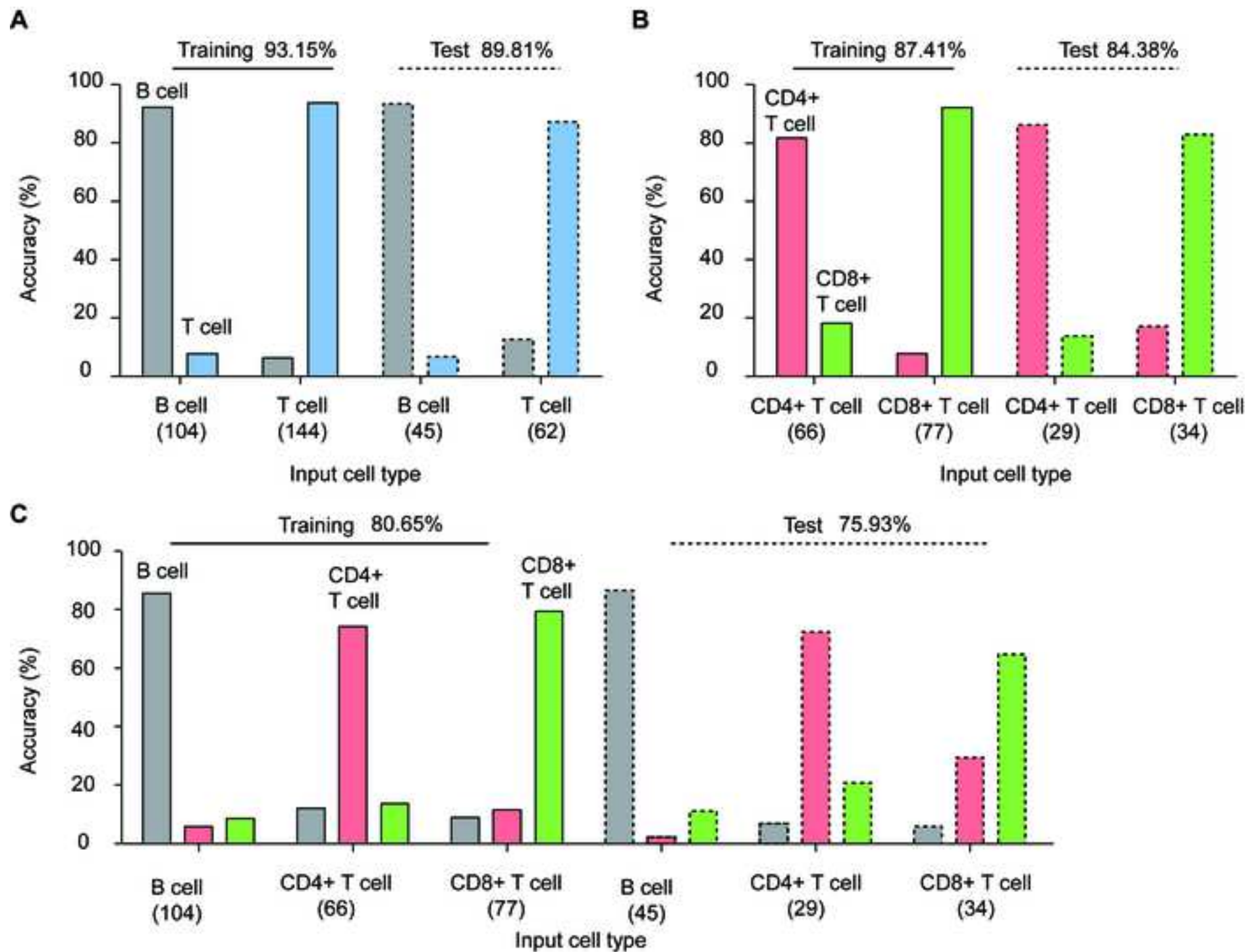
- 1 Alizadeh, A. A., *et al.* Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. **403** (6769), 503 (2000).
- 2 Von Boehmer, H., Melchers, F. Checkpoints in lymphocyte development and autoimmune disease. *Nature Immunology*. **11** (1), 14 (2010).
- 3 Sáez-Cirión, A., *et al.* HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. *Proceedings of the National Academy of Sciences*. **104** (16), 6776-6781 (2007).
- 4 Fischer, K., *et al.* Isolation and characterization of human antigen-specific TCR $\alpha\beta$ + CD4-CD8-double-negative regulatory T cells. *Blood*. **105** (7), 2828-2835 (2005).
- 5 Yoon, J., *et al.* Identification of non-activated lymphocytes using three-dimensional refractive index tomography and machine learning. *Scientific Reports*. **7** (1), 6654 (2017).
- 6 Popescu, G. Quantitative phase imaging of cells and tissues. (McGraw Hill Professional, 2011).
- 7 Lee, K. *et al.* Quantitative phase imaging techniques for the study of cell pathophysiology: from principles to applications. *Sensors*. **13** (4), 4170-4191 (2013).
- 8 Kim, D., *et al.* Refractive index as an intrinsic imaging contrast for 3-D label-free live cell imaging. *bioRxiv*. 106328 (2017).
- 9 Kim, K., *et al.* Optical diffraction tomography techniques for the study of cell pathophysiology. *Journal of Biomedical Photonics & Engineering*. **2** (2), (2016).
- 10 Wolf, E. Three-dimensional structure determination of semi-transparent objects from holographic data. *Optics Communications*. **1** (4), 153-156 (1969).
- 11 Kuś, A., Dudek, M., Kemper, B., Kujawińska, M., Vollmer, A. *SPIE - the international society for optics and photonics*. 8.
- 12 Kim, T., *et al.* White-light diffraction tomography of unlabelled live cells. *Nature Photonics*. **8** (3), 256 (2014).
- 13 Simon, B., *et al.* Tomographic diffractive microscopy with isotropic resolution. *Optica*. **4** (4), 460-463 (2017).
- 14 Kim, Y., *et al.* Profiling individual human red blood cells using common-path diffraction optical tomography. *Scientific Reports*. **4**, (2014).
- 15 Park, H., *et al.* Measuring cell surface area and deformability of individual human red blood cells over blood storage using quantitative phase imaging. *Scientific Reports*. **6**, (2016).
- 16 Lee, S., *et al.* Refractive index tomograms and dynamic membrane fluctuations of red blood cells from patients with diabetes mellitus. *Scientific Reports*. **7**, (2017).

- 441 17 Merola, F., *et al.* Tomographic flow cytometry by digital holography. *Light-Science &*
442 *Applications*. **6**, (2017).
- 443 18 Park, Y., *et al.* Refractive index maps and membrane dynamics of human red blood cells
444 parasitized by *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences*.
445 **105** (37), 13730-13735 (2008).
- 446 19 Park, H., *et al.* Characterizations of individual mouse red blood cells parasitized by *Babesia*
447 *microti* using 3-D holographic microscopy. *Scientific Reports*. **5**, 10827 (2015).
- 448 20 Chandramohanadas, R., *et al.* Biophysics of malarial parasite exit from infected
449 erythrocytes. *Public Library of Science ONE*. **6** (6), e20869 (2011).
- 450 21 Yoon, J., *et al.* Label-free characterization of white blood cells by measuring 3D refractive
451 index maps. *Biomedical Optics Express*. **6** (10), 3865-3875 (2015).
- 452 22 Kim, K., *et al.* Three-dimensional label-free imaging and quantification of lipid droplets in
453 live hepatocytes. *Scientific Reports*. **6**, 36815 (2016).
- 454 23 Kim, D., *et al.* Label-free high-resolution 3-D imaging of gold nanoparticles inside live cells
455 using optical diffraction tomography. *Methods*. (2017).
- 456 24 Lenz, P., *et al.* Multimodal Quantitative Phase Imaging with Digital Holographic
457 Microscopy Accurately Assesses Intestinal Inflammation and Epithelial Wound Healing.
458 *Journal of Visualized Experiments*. (115), (2016).
- 459 25 Huang, J., Guo, P., Moses, M. A. A Time-lapse, Label-free, Quantitative Phase Imaging
460 Study of Dormant and Active Human Cancer Cells. *Journal of Visualized Experiments*.
461 (132), (2018).
- 462 26 Yang, S. A., Yoon, J., Kim, K., Park, Y. Measurements of morphological and biochemical
463 alterations in individual neuron cells associated with early neurotoxic effects in
464 Parkinson's disease. *Cytometry Part A*. **91** (5), 510-518 (2017).
- 465 27 Cotte, Y., *et al.* Marker-free phase nanoscopy. *Nature Photonics*. **7** (2), 113-117 (2013).
- 466 28 Nguyen, T. H., Kandel, M. E., Rubessa, M., Wheeler, M. B. & Popescu, G. Gradient light
467 interference microscopy for 3D imaging of unlabeled specimens. *Nature Communications*.
468 **8** (1), 210 (2017).
- 469 29 Kwon, S. *et al.* Mitochondria-targeting indolizino [3, 2-c] quinolines as novel class of
470 photosensitizers for photodynamic anticancer activity. *European Journal of Medicinal*
471 *Chemistry*. **148**, 116-127 (2018).
- 472 30 Bennet, M., Gur, D., Yoon, J., Park, Y., Faivre, D. A Bacteria-Based Remotely Tunable
473 Photonic Device. *Advanced Optical Materials*. (2016).
- 474 31 Kim, T. I., *et al.* Antibacterial Activities of Graphene Oxide–Molybdenum Disulfide
475 Nanocomposite Films. *ACS Applied Materials & Interfaces*. **9** (9), 7908-7917 (2017).
- 476 32 Bedrossian, M., Barr, C., Lindensmith, C. A., Nealson, K., Nadeau, J. L. Quantifying
477 Microorganisms at Low Concentrations Using Digital Holographic Microscopy (DHM).
478 *Journal of Visualized Experiments*. (129), (2017).
- 479 33 Jo, Y., *et al.* Quantitative Phase Imaging and Artificial Intelligence: A Review. *arXiv preprint*
480 *arXiv:1806.03982*. (2018).
- 481 34 Javidi, B., Moon, I., Yeom, S., Carapezza, E. Three-dimensional imaging and recognition of
482 microorganism using single-exposure on-line (SEOL) digital holography. *Optics Express*. **13**
483 (12), 4492-4506 (2005).
- 484 35 Jo, Y., *et al.* Label-free identification of individual bacteria using Fourier transform light

- scattering. *Optics Express*. **23** (12), 15792-15805 (2015).
- 36 Jo, Y., *et al.* Angle-resolved light scattering of individual rod-shaped bacteria based on Fourier transform light scattering. *Scientific Reports*. **4**, 5090 (2014).
- 37 Jo, Y., *et al.* Holographic deep learning for rapid optical screening of anthrax spores. *Science Advances*. **3** (8), e1700606 (2017).
- 38 Mirsky, S. K., Barnea, I., Levi, M., Greenspan, H., Shaked, N. T. Automated analysis of individual sperm cells using stain-free interferometric phase microscopy and machine learning. *Cytometry Part A*. **91** (9), 893-900 (2017).
- 39 Roitshtain, D., *et al.* Quantitative phase microscopy spatial signatures of cancer cells. *Cytometry Part A*. **91** (5), 482-493 (2017).
- 40 Lam, V. K., Nguyen, T. C., Chung, B. M., Nehmetallah, G., Raub, C. B. Quantitative assessment of cancer cell morphology and motility using telecentric digital holographic microscopy and machine learning. *Cytometry Part A*. (2017).
- 41 Pavillon, N., Hobro, A. J., Akira, S., Smith, N. I. Noninvasive detection of macrophage activation with single-cell resolution through machine learning. *Proceedings of the National Academy of Sciences*. 201711872 (2018).
- 42 Basu, S., Campbell, H. M., Dittel, B. N., Ray, A. Purification of Specific Cell Population by Fluorescence Activated Cell Sorting (FACS). *Journal of Visualized Experiments*. (41), e1546 (2010).
- 43 Takeda, M., Ina, H., Kobayashi, S. Fourier-transform method of fringe-pattern analysis for computer-based topography and interferometry. *Journal of the Optical Society of America*. **72** (1), 156-160 (1982).
- 44 Debnath, S. K., Park, Y. Real-time quantitative phase imaging with a spatial phase-shifting algorithm. *Optics Letters*. **36** (23), 4677-4679 (2011).
- 45 Kim, K., *et al.* High-resolution three-dimensional imaging of red blood cells parasitized by Plasmodium falciparum and in situ hemozoin crystals using optical diffraction tomography. *Journal of Biomedical Optics*. **19** (1), 011005 (2013).
- 46 Vercruysse, D., *et al.* Three-part differential of unlabeled leukocytes with a compact lens-free imaging flow cytometer. *Lab on a Chip*. **15** (4), 1123-1132 (2015).
- 47 Kim, K., *et al.* Correlative three-dimensional fluorescence and refractive index tomography: bridging the gap between molecular specificity and quantitative bioimaging. *Biomedical Optics Express*. **8** (12), 5688-5697 (2017).
- 48 Shin, S., Kim, D., Kim, K., Park, Y. Super-resolution three-dimensional fluorescence and optical diffraction tomography of live cells using structured illumination generated by a digital micromirror device. *arXiv preprint arXiv:1801.00854*. (2018).
- 49 Chowdhury, S., Eldridge, W. J., Wax, A., Izatt, J. A. Structured illumination multimodal 3D-resolved quantitative phase and fluorescence sub-diffraction microscopy. *Biomedical Optics Express*. **8** (5), 2496-2518 (2017).







Name of Material/Equipment	Company
Mouse	Daehan Biolink
Falcon conical centrifuge tube	ThermoFisher Scientific
Phosphate-buffered saline	Sigma-Aldrich
Ammonium-chloride-potassium lysing buffer	ThermoFisher Scientific
RPMI-1640 medium	Sigma-Aldrich
Fetal bovine serum	ThermoFisher Scientific
Antibody	BD Biosciences
Antibody	BD Biosciences
Antibody	Biolegnd
Antibody	BD Biosciences
Antibody	BD Biosciences
Antibody	BD Biosciences
Antibody	eBioscience
DAPI	Roche
Flow cytometry	BD Biosciences
Imaging chamber	Tomocube, Inc.
Holotomography	Tomocube, Inc.
Holotomography imaging software	Tomocube, Inc.
Image professing software	MathWorks

Catalog Number	Comments/Description
C57BL/6J mice	gender and age-matched, 6 – 8 weeks
14-959-53A	15 mL
806544-500ML	
A1049201	
R8758	
10438018	
553140 (RRID:AB_394655)	CD16/32 (clone 2.4G2)
555275 (RRID:AB_395699)	CD3 ϵ (clone 17A2)
100734 (RRID:AB_2075238)	CD8 α (clone 53-6.7)
557655 (RRID:AB_396770)	CD19 (clone 1D3)
557683 (RRID:AB_396793)	CD45R/B220 (clone RA3-6B2)
552878 (RRID:AB_394507)	NK1.1 (clone PK136)
11-0041-85 (RRID:AB_464893)	CD4 (clone GK1.5)
10236276001	4,6-diamidino-2-phenylindole
Aria II or III	
TomoDish	
HT-1H	
TomoStudio	
Matlab R2017b	

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