Published in final edited form as:

J Biophotonics. 2019 April ; 12(4): e201800291. doi:10.1002/jbio.201800291.

Integration of diffraction phase microscopy and Raman imaging for label-free morpho-molecular assessment of live cells

Rishikesh Pandey^{1,*}, Renjie Zhou^{2,3,4,*}, Rosalie Bordett¹, Ciera Hunter¹, Kristine Glunde⁵, Ishan Barman^{5,6}, Tulio Valdez⁷, and Christine Finck^{1,8}

¹Connecticut Children's Innovation Center, University of Connecticut School of Medicine, Farmington, Connecticut ²Department of Chemistry, Laser Biomedical Research Center, George R. Harrison Spectroscopy Lab, Massachusetts Institute of Technology, Cambridge, Massachusetts ³Laser Metrology and Biomedicine Lab, Department of Biomedical Engineering, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China ⁴Shun Hing Institute of Advanced Engineering, The Chinese University of Hong Kong, Shatin, Hong Kong, SAR, China ⁵The Johns Hopkins University School of Medicine, The Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland ⁶Department of Mechanical Engineering, Johns Hopkins University, Baltimore, Maryland ⁷Department of Otolaryngology, Stanford University, Palo Alto, California ⁸Department of Surgery, Connecticut Children's Medical Center, Harford, Connecticut

Abstract

Label-free quantitative imaging is highly desirable for studying live cells by extracting pathophysiological information without perturbing cell functions. Here, we demonstrate a novel label-free multimodal optical imaging system with the capability of providing comprehensive morphological and molecular attributes of live cells. Our morpho-molecular microscopy (3M) system draws on the combined strength of quantitative phase microscopy (QPM) and Raman microscopy to probe the morphological features and molecular fingerprinting characteristics of each cell under observation. While the common-path geometry of our QPM system allows for highly sensitive phase measurement, the Raman microscopy is equipped with dual excitation wavelengths and utilizes the same detection and dispersion system, making it a distinctive multi-wavelength system with a small footprint. We demonstrate the applicability of the 3M system by investigating nucleated and nonnucleated cells. This integrated label-free platform has a promising potential in preclinical research, as well as in clinical diagnosis in the near future.

Graphical Abstract

***Correspondence:** Rishikesh Pandey, Connecticut Children's Innovation Center, University of Connecticut School of Medicine, Farmington, CT 06032. pandey@uchc.edu, Renjie Zhou, Laser Metrology and Biomedicine Lab, Department of Biomedical Engineering, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China. rjzhou@cuhk.edu.hk.

CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Keywords

cellular imaging; label-free imaging; quantitative phase microscopy; Raman spectroscopy

1 | INTRODUCTION

Unlabeled cells have negligible scattering and absorption under visible light illumination, thus resulting in poor image contrast under bright field microscopy. To improve the image contrast, phase contrast microscopy (PCM) has been developed which translates small variations in optical path length differences into corresponding changes in intensity [1, 2]. However, PCM does not offer quantitative information—either chemical, functional or morphological. Fluorescence microscopy has made a stunning progress in cellular imaging by introducing targeted reporters, especially the unprecedented advances in genetically encoded fluorescent proteins [3]. Yet, adding exogenous contrast disturbs the native cell function, dyes are susceptible to photobleaching, and label-free imaging remains highly desirable [4]. While autofluorescence microscopy is a promising contrast-free method but its scope is limited in visualizing or monitoring only certain metabolites in biological samples [5, 6]. Chemical imaging techniques based on vibrational spectroscopy, such as infra-red (IR) and Raman microspectroscopy (RM), have engendered significant attention due to its ability to obtain abundant molecular and functional information without dye or contrast agent labeling [7–10]. RM, in particular, which is based on inelastic scattering of light called Raman effect [11], has been widely used as a nonperturbative analytical tool to determine molecular composition [12–14]. RM has also recently acquired substantial traction in molecular phenotype profiling of pathologic conditions [15–17], and is an upcoming technique for live cell imaging, as the acquired spectral fingerprints are the expression of cellular biochemistry and structure [18–22]. While RM provides molecular fingerprinting attributes, it lacks morphologic information and is limited by sampling speed.

Cell phenotype, as an important functional characteristic in morphology, is known to change during cellular processes such as in response to extracellular cues and pharmacologic perturbations [23]. Quantitative phase microscopy (QPM), which measures the optical path length differences at each sample point, is capable of quantifying nanometer scale axial motions in a wide-field fashion with millisecond scale image acquisition time. While QPM has gained acceptance as an analytical tool to quantify biophysical processes at the cellular level, most of the studies have been limited to understanding the morphology (volume, surface area and sphericity) and biomechanics (membrane fluctuation) of erythrocytes [24, 25]. For instance, using QPM, investigators have reported that membrane deformability of diabetic red blood cell (RBCs) is significantly lower than that of healthy, nondiabetic RBCs; and glycation of hemoglobin and membrane proteins of RBCs by hyperglycemia significantly compromises RBC deformability in diabetic patients [26]. Although QPM provides well-needed quantitative morphologic attributes and mechanical properties of a live cell, the deficiency of molecular information content limits its widespread application.

Hence, by combining RM with QPM, we can gain access to both molecular and morphological information in a label-free manner. An attempt has been made to integrate both modalities in the past; however, the combined system utilized the noncommon-path QPM geometry and was limited to a single excitation wavelength [27]. Here we demonstrate a morpho-molecular microscopy (3M) system that merges diffraction phase microscopy (DPM) with RM, and is equipped with multiple excitation wavelengths. DPM, a common-path QPM technique, has a well-proven attribute of measuring the phase with high sensitivity [28–30]. Further, the availability of multiple excitation wavelengths in the RM system allows one to carefully exploit the resonance condition, thereby increasing sensitivity and molecular selectivity [31] and at the same time balancing photodamage and Raman cross-section. In the resonance condition, the intensities of certain Raman bands of the scattering molecule are enhanced by several orders of magnitude relative to their off-resonance values. In addition, resonance excitation enables high selectivity for vibrations of interest because excitation can be localized within specific chromophoric groups in a sample, such as in particular sidechains in specific proteins. This is of profound importance when investigating complex biological systems. The Raman measurements performed in both resonance and off-resonance conditions provide valuable information for peak assignments. While 532 nm excitation is better suited for aforementioned reasons, the undesirable fluorescence and photobleaching limit its use for wide varieties of biological specimens. Therefore, having a Raman system equipped with both laser wavelengths is essential to study diverse biological specimens. Importantly, since QPM requires single-shot acquisition, thus its morphological screening speed is only limited by the camera speed. Several biological problems are being studied in our laboratory to utilize the unique capability of the system. For instance, we are currently using this system to develop a reagent-free platform for rapid blood cancer diagnosis. In such a study, we first employ QPM to identify the leucocyte type and subsequently acquire Raman measurement from the screened leukocyte. The combined platform will be highly desirable for several other important biological applications. For instance, in studying blood disorders such as *Plasmodium falciparum*-infected RBC and sickle cell disease, QPM can be used as a tool for high-throughput screening of morphologically abnormal suspicious RBCs. Subsequently,

Raman spectra can be measured on screened cells to obtain the cellular level biomolecular insights of different RBC pathologies such as hemozoin [32, 33] and HbS [34] which are unique to malaria infection and sickle cell disease, respectively. Our integrated system can also find applications in studying cell physiology such as RBC aging by offering access to complementary mechanical and biochemical information from individual RBCs, which is not possible with any single one. Our 3M system can measure comprehensive information of live cells, including cell height, membrane fluctuation, quantitative morphology and molecular profiling. In this paper, we present experimental results on imaging blood cells and breast cancer cells using our 3M system, which establishes this system as an important imaging tool for biomedical research in the future.

2 | MATERIALS AND METHODS

To first validate our 3M system, we performed quantitative phase imaging and RM of standard polystyrene microspheres. We subsequently applied our 3M system to image nonnucleated cells (RBCs), as well as nucleated cells that were suspended (leukocytes) and adherent (breast cancer cells), which demonstrated the potential of our system for cell physiology studies and cancer diagnoses.

2.1 | Polystyrene microsphere imaging

The polystyrene microspheres (Duke Standards, Thermo Fisher Scientific, Waltham, Massachusetts) were diluted and dispersed in water 1:50 (vol/vol) before spreading onto a quartz cover glass (Alfa Aesar, Ward Hill, Massachusetts) and carefully dried. Then index matching liquid was dropped on the quartz cover glass and a second cover glass was used to cover the sample. After the sample was placed onto the sample chamber, we first imaged it with QPM using 532 nm laser illumination. Phase images of microspheres and background (without any microspheres) were acquired.

2.2 | RBC imaging

For RBC measurement, 10 μ L of ethylenediaminetetraacetic acid (EDTA) containing fresh whole blood was added in 1 mL of phosphate buffered saline (PBS). The mixture was centrifuged twice at 100 rcf at 25°C for 10 minutes and each time re-suspended in 1 mL of PBS. The supernatant was removed, and the pellet was re-suspended in 200 μ L of PBS. A secure-seal spacer (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts) was attached on a coverslip and 10 μ L of cell suspension was added at the center of the chamber. The chamber was then sealed by placing a second quartz coverslip on top of the glue from the spacers.

2.3 | Nucleated cell imaging

Nucleated cells namely, leukemia and breast cancer cell lines, were also imaged. The leukemic MN60 cell line was purchased from DSMZ (Germany) and grown in medium consisting of 90% minimum essential medium (MEM), 10% fetal bovine serum, 50 mg/mL pen/strep and 2 mM L-glutamine (Thermo Fisher, Waltham, Massachusetts). The cells were cultured at a density of 1×10^6 cells/mL prior to taking them out for measurement. Normal B-cells were isolated from whole peripheral blood obtained from a healthy donor using

EasySep negative selection kit (Stemcell Technologies Inc, Vancouver, British Columbia, Canada) as per the manufacturer instructions. To test adherent nucleated cells, we choose breast cancer cell lines derived from MDA-MB-231. The breast cancer cell lines were obtained from Prof. Kristine Glunde's lab at Johns Hopkins University, Baltimore as published previously [35]. The breast cancer cells were grown on UV-treated cover glasses in six-well plates using RPMI-1640 growth media supplemented with 10% fetal bovine serum (FBS), 100 mg/mL penicillin, and 100 mg/mL streptomycin (all purchased from Invitrogen Corporation). For blood cancer measurements, 10 μ L of cells suspended in the medium were taken directly from the culture flask in sterile conditions. The cells were then placed on a coverslip sample holder for imaging similar to the RBC measurement mentioned above.

3 | RESULTS AND DISCUSSION

The schematic of the optical set-up is illustrated in Figure 1. The 3M system consists of an epi-illumination Raman microscope system and a transmission-mode DPM system. Both systems use the same objective lens to collect Raman scattering and elastic scattering signal (containing sample phase information), thereby avoiding any image registration issues. The Raman system is based on a custom-built inverted microscope that is equipped with two excitation wavelengths, that is, 785 and 532 nm. The 785 nm light was achieved by a compact laser diode (LM series volume holographic grating-stabilized model from Ondax, Monrovia, CA) with a clean-up filter (LL01-785-12.5; Semrock, Rochester, NY). The 532 nm wavelength illumination comes from a solid-state laser (Gem 532, Laser Quantum, Stockport, United Kingdom). Both the near IR and green laser beams were expanded such that they can just fill the back aperture of the objective. With the help of mirrors and dichroic beam splitters, the expanded beams are directed and focused onto the sample through a water immersion objective lens (Olympus, LUMFLN60XW, 60X/1.1, Shinjuku, Tokyo, Japan). The scattered signal retraces the same excitation beam path until the dichroic mirror. The Raman signal is filtered using an edge filter before being focused onto the fiber. The backscattered light is collected by a multimode fiber (100 μ m core diameter; Andor, Belfast) which serves as the confocal pinhole in an optically conjugate plane in the detection arm, and then the light was delivered to a spectrograph (Shamrock 193i, Andor) attached to a TE-cooled CCD Camera (iDus DU420A-BEX2-DD, Andor). Of note, the F/# of the focusing lens was matched with the F/# of the fiber. Micropositioning stage (Optiscan 2, Prior Scientific, Cambridge, United Kingdom) was used for spectroscopic mapping in *X-Y* direction, whereas a one-axis high precision translation stage (Thorlabs, Newton, New Jersey, United States) was used to focus the objective. For wide-field imaging, we used 532 nm excitation and a CMOS camera (Mightex, SME-B050-U, Toronto, Ontario, Canada). This camera is also used to check the position and focus of the Raman excitation beam onto the sample plane. A custom built removable metal stage insert holds the quartz coverslip or petri dish. The conversion of one excitation wavelength to the other was seamlessly performed using flip mirrors, appropriate mountable lenses, dichroic mirror, and filters.

The DPM system was integrated with the above scanning confocal Raman microscope for quantitative phase imaging at 532 nm. A flip mirror placed just after a collimating lens next to 532 nm laser opening enables laser excitation change from QPM set-up to Raman. Unlike

Raman, the excitation for QPM is obtained through a single-mode (SM) fiber-coupled system. The fiber launch system is configured just after the flip mirror. The collimated light is allowed to illuminate the sample from the top side. A flip mirror placed after the tube lens is used to direct the light towards QPM arm. QPM experiment is performed on the same live cell scanned under Raman by a flip mirror. This is ensured by a pair of cameras—one placed in the QPM arm and the other in the Raman arm. The collimated light after the tube lens passes through the grating and generates multiple diffraction orders. The diffraction orders are subsequently focused onto the pinhole of 10 μm diameter through a $2f$ system formed by Fourier lens 1 (FL1). The light from with and without the pinhole goes through another $2f$ system formed by Fourier lens 2 (FL2). Both beams spatially overlap on the imaging plane, where a camera (Pointgrey, Flea3, Richmond, British Columbia, Canada) is used to capture the interference fringes. The actual magnification of the DPM system, double checked with a known size of polystyrene microsphere, is ~ 100 . The theoretical lateral resolution is calculated to be 590 nm.

To test the phase imaging accuracy of the current set-up we measured standard 10 μm diameter polystyrene microspheres (Thermo Fisher Duke Standard). The phase is retrieved from the raw interferogram (Figure 2A) by using the Fourier transform method [29]. The raw interferogram is first Fourier transformed, and the +1 order signal is chosen and shifted to the center. An inverse Fourier transform of the +1 order produces the complex field that contains the sample optical phase delay map. To calibrate the sample phase delay map measurement, we discretely measure the reference phase delay without a sample present in the field of view and subtract it from the phase delay for the sample. Figure 2B illustrates typical fringe contrast of our set-up. The phase image $\phi(x, y)$ of a single microsphere is shown in Figure 2C. The surface height profile $h(x, y)$ can be calculated from $\phi(x, y)$ through the following equation:

$$h(x, y) = \frac{\Delta \phi(x, y) \lambda}{2\pi(n_s - n_m)}, \quad (1)$$

where λ is the wavelength of light in free space, n_c and n_m are the average refractive indices of the specimen and the dispersion medium, respectively. As the refractive index difference ($n_s - n_m$) is known to be around 0.03, one can calculate the surface height of the sphere from the above equation. As presented in Figure 2D, the height of the sphere calculated from the phase measurement is $\sim 10 \mu\text{m}$ which reflects high phase measurement accuracy. Next, we performed Raman measurements on the same sphere with both 785 and 532 nm excitation wavelengths. The Raman spectrum, acquired from a single polystyrene microsphere at 785 nm, is shown in Figure 2E. The spectrum which has a very high signal to noise ratio, consists of typical bands of polystyrene reported earlier [36]. Subsequently, we switched over to 532 nm and recorded the Raman spectrum (Figure 2F). As evident from the figure, the peak positions are consistent with both the excitations and the difference is smaller than the wavenumber resolution of the spectrometer. The measurements of standard microspheres using QPM and Raman systems at different wavelength demonstrate multimodal attribute of our 3M system.

After calibration testing, we then performed measurements on RBCs using our 3M system. Owing to a relatively homogeneous internal structure and surface membrane dynamics, RBCs have been an important specimen for QPM measurement [25, 37]. The RBC phase image acquired from the 3M system is shown in Figure 3A. Assuming that the RBC is primarily made up of hemoglobin, the phase value can be directly converted into RBC thickness using Equation (1), where we assumed the refractive indices of PBS and RBC to be 1.337 and 1.410, respectively. The calculated surface height profile of RBCs is presented in Figure 3B. The height calculated was $\sim 2.5 \mu\text{m}$ which is typical of a healthy RBC. Further, we measured the RBC membrane fluctuations to elucidate the system capability for cellular dynamics imaging (Supporting Information Video S1). The Raman spectrum obtained at 785 nm from a single RBC is given in Figure 3C. The spectral shape is akin to the Raman spectrum of RBC [38, 39] and prominent peaks observed at 1614, 1601, 1542, 1125, 1001 and 754 cm^{-1} could be ascribed primarily from the hemoglobin. Interestingly, the Raman spectrum of the RBC acquired at 532 nm (Figure 3D) is considerably different from 785 nm spectrum. This difference in spectral features is attributable to the resonance enhancement of hemoglobin at this wavelength.

Finally, we carried out experiments on nucleated cells and chose leukemia and breast cancer cells as representative examples of suspended and adherent cells, respectively. The phase images of circulating tumor cell (CTC) and lung metastatic (LM) cell along with the parental MDA-MB-231 cell as described earlier [35] are shown in Figure 4A-C with their corresponding raw interferograms (D-F). As evident from the figures, the phase images show high contrast with detailed spatial information. Importantly, one can calculate the dry mass (nonaqueous content) of the live cell from surface integral of the optical phase shift [40]. Cell dry mass, being an important morphometric quantity, can be utilized for various motives including phenotyping and growth dynamics [41]. Using the respective phase information from Figure 4A-C, the dry masses of the parental, CTC and LM cells were calculated as 897.3, 433.4 and 399.0 pg, respectively. The QPM images of blood cancer cell (MN60) and normal B-cell are shown in Figure 4G,H, respectively. The difference in phase values is indicative of their different morphologic attributes. We are currently investigating an approach in which the quantitative morphological parameters can be utilized for identification and classification of B acute lymphoblastic leukemia. The Raman spectra of these cell lines are provided in Supporting Information Figure S1. Further, the Raman spectra of malignant MN60 and normal B-cell show subtle, but consistent, differences (Figure 4I). These variations are correlated to the compositional contributors in the two types of cells and by employing multivariate analysis of the recorded spectral profiles one could discern between these two cell types. The spectra with sharp and identifiable peaks have Raman frequencies values which are in line with a recent exciting study by Managò et al. [42] The observed bands are characteristics of protein/lipids (amide I, 1655 cm^{-1}), protein (phenylalanine, 1004 cm^{-1}), nucleic acids (DNA, 784 cm^{-1}), etc.

Taken together, our investigation with an exploratory 3M system shows the feasibility of obtaining both the molecular and morphologic information in situ without the use of any contrast agent. By leveraging the wealth of molecular information encoded in the Raman spectroscopic data and morphological characteristics from QPM, one can obtain complementary information for phenotype profiling of cells.

4 | CONCLUSION

We have built a unified label-free multi-modal imaging system consisting of common-path quantitative phase imaging and Raman imaging to investigate live cells in situ. The combination of both intrinsic contrast imaging modalities has enabled us to achieve quantitative morphologic and molecular information without necessitating any exogenous dye. While the QPM system in common-path configuration allows phase measurement with high sensitivity, integration of the multi-wavelength Raman, with the same detection and dispersion system makes this a unique multi-modal system. We have demonstrated the applicability of the system in biomedical studies by measuring live RBCs and leukemia and breast cancer cells. This is an important step towards linking the biochemical and morphologic information to obtain integrated insight into live cell biology. We envision that this newly developed system can be used for a large variety of cellular studies in the near future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This research was partially supported by Connecticut Children's innovation center seed money, the National Institute of Biomedical Imaging and Bioengineering (9P41EB015 871-26A1), Shun Hing Institute of Advanced Engineering (project BME-p3-18) and the Direct Research Grant of The Chinese University of Hong Kong.

Funding information

The Chinese University of Hong Kong Direct Research Grant; Shun Hing Institute of Advanced Engineering, Grant/Award Number: BME-p3-18; Connecticut Children's Innovation Center Seed Money; National Institute of Biomedical Imaging and Bioengineering, Grant/Award Number: 2-P41-EB015871-31; National Institute of General Medical Sciences, Grant/Award Number: DP2GM128198

REFERENCES

- [1]. Zernike F, Physica 1942, 9, 686.
- [2]. Zernike F, Physica 1942, 9, 974.
- [3]. Ettinger A, Wittmann T, in Methods in Cell Biology (Eds: Waters JC, Wittman T), Academic Press, Cambridge, Massachusetts 2014, p. 77.
- [4]. Christiansen EM, Yang SJ, Ando DM, Javaherian A, Skibinski G, Lipnick S, Mount E, O'Neil A, Shah K, Lee AK, Goyal P, Fedus W, Poplin R, Esteva A, Berndt M, Rubin LL, Nelson P, Finkbeiner S, Cell 2018, 173, 792. [PubMed: 29656897]
- [5]. Rodrigues RM, Macko P, Palosaari T, Whelan MP, Toxicol. Lett 2011, 206, 281. [PubMed: 21864658]
- [6]. Valdez TA, Pandey R, Spegazzini N, Longo K, Roehm C, Dasari RR, Barman I, Anal. Chem 2014, 86, 10454. [PubMed: 25226556]
- [7]. Kwak JT, Kajdacsy-Balla A, Macias V, Walsh M, Sinha S, Bhargava R, Sci. Rep 2015, 5, 8758. [PubMed: 25737022]
- [8]. Varma VK, Kajdacsy-Balla A, Akkina SK, Setty S, Walsh MJ, Kidney Int 2016, 89, 1153. [PubMed: 26924056]
- [9]. Qianru J, Ming L, Beril P, Santosh KP, Aimee D, Amy Z, Jayson VP, Ishan B, David HG, Angew. Chem. Int. Ed 2017, 56, 3822.
- [10]. Huang S, Pandey R, Barman I, Kong J, Dresselhaus M, ACS Photonics 2018, 5, 2978.

- [11]. Raman CV, Krishnan KS, Nature 1928, 121, 501.
- [12]. Pandey R, Paidi SK, Valdez TA, Zhang C, Spegazzini N, Dasari RR, Barman I, Acc. Chem. Res 2017, 50, 264. [PubMed: 28071894]
- [13]. Azan A, Untereiner V, Gobinet C, Sockalingum GD, Breton M, Piot O, Mir LM, Sci. Rep 2017, 7, 40448. [PubMed: 28102326]
- [14]. Majzner K, Kochan K, Kachamakova-Trojanowska N, Maslak E, Chlopicki S, Baranska M, Anal. Chem 2014, 86, 6666. [PubMed: 24936891]
- [15]. Pandey R, Zhang C, Kang Jeon W, Desai Parind M, Dasari Ramachandra R, Barman I, Valdez Tulio A, J. Biophotonics 2018, 11, e201700259. [PubMed: 29232053]
- [16]. Kong K, Kendall C, Stone N, Notingher I, Adv. Drug Deliv. Rev 2015, 89, 121. [PubMed: 25809988]
- [17]. Matousek P, Stone N, Chem. Soc. Rev 2016, 45, 1794. [PubMed: 26455315]
- [18]. Palonpon AF, Ando J, Yamakoshi H, Dodo K, Sodeoka M, Kawata S, Fujita K, Nat. Protoc 2013, 8, 677. [PubMed: 23471112]
- [19]. Smith R, Wright KL, Ashton L, Analyst 2016, 141, 3590. [PubMed: 27072718]
- [20]. Zhang D, Wang P, Slipchenko MN, Cheng J-X, Acc. Chem. Res 2014, 47, 2282. [PubMed: 24871269]
- [21]. Lu F-K, Basu S, Igras V, Hoang MP, Ji M, Fu D, Holtom GR, Neel VA, Freudiger CW, Fisher DE, Xie XS, Proc. Natl. Acad. Sci. U. S. A 2015, 112, 11624. [PubMed: 26324899]
- [22]. Galler K, Requardt RP, Glaser U, Markwart R, Bocklitz T, Bauer M, Popp J, Neugebauer U, Sci. Rep 2016, 6, 24155. [PubMed: 27063397]
- [23]. Gordonov S, Hwang MK, Wells A, Gertler FB, Lauffenburger DA, Bathe M, Integr. Biol 2016, 8, 73.
- [24]. Hosseini P, Abidi SZ, Du E, Papageorgiou DP, Choi Y, Park Y, Higgins JM, Kato GJ, Suresh S, Dao M, Yaqoob Z, So PTC, Proc. Natl. Acad. Sci. U. S. A 2016, 113, 9527. [PubMed: 27512047]
- [25]. Park H, Lee S, Ji M, Kim K, Son Y, Jang S, Park Y, Sci. Rep 2016, 6, 34257. [PubMed: 27698484]
- [26]. Lee S, Park H, Kim K, Sohn Y, Jang S, Park Y, Sci. Rep 2017, 7, 1039. [PubMed: 28432323]
- [27]. Kang JW, Lue N, Kong C-R, Barman I, Dingari NC, Goldfless SJ, Niles JC, Dasari RR, Feld MS, Biomed. Opt. Express 2011, 2, 2484. [PubMed: 21991542]
- [28]. Popescu G, Ikeda T, Dasari RR, Feld MS, Opt. Lett 2006, 31, 775. [PubMed: 16544620]
- [29]. Bhaduri B, Edwards C, Pham H, Zhou R, Nguyen TH, Goddard LL, Popescu G, Adv. Opt. Photon 2014, 6, 57.
- [30]. Zheng C, Zhou R, Kuang C, Zhao G, Yaqoob Z, So PTC, Opt. Lett 2017, 42, 1448. [PubMed: 28362789]
- [31]. Clark RJH, Dines TJ, Angew. Chem. Int. Ed. Engl 1986, 25, 131.
- [32]. Wood BR, Langford SJ, Cooke BM, Glenister FK, Lim J, McNaughton D, FEBS Lett. 2003, 554, 247. [PubMed: 14623074]
- [33]. Frosch T, Koncarevic S, Zedler L, Schmitt M, Schenzel K, Becker K, Popp J, J. Phys. Chem. B 2007, 111, 11047. [PubMed: 17718555]
- [34]. Filho ACB, Silveira L, Yanai ALSA, Fernandes AB, Lasers Med. Sci 2015, 30, 247. [PubMed: 25217409]
- [35]. Rizwan A, Cheng M, Bhujwala ZM, Krishnamachary B, Jiang L, Glunde K, Npj Breast Cancer 2015, 1, 15017. [PubMed: 28721370]
- [36]. Bankapur A, Zachariah E, Chidangil S, Valiathan M, Mathur D, PLoS One 2010, 5, e10427. [PubMed: 20454686]
- [37]. Evans AA, Bhaduri B, Popescu G, Levine AJ, Proc. Natl. Acad. Sci. U. S. A 2017, 114, 2865. [PubMed: 28242681]
- [38]. Lemler P, Premasiri WR, DelMonaco A, Ziegler LD, Anal. Bioanal. Chem 2014, 406, 193. [PubMed: 24162820]

- [39]. Atkins CG, Buckley K, Blades MW, Turner RFB, Appl. Spectrosc 2017, 71, 767. [PubMed: 28398071]
- [40]. Popescu G, Park Y, Lue N, Best-Popescu C, Deflores L, Dasari RR, Feld MS, Badizadegan K, Am. J. Physiol. Cell Physiol 2008, 295, C538. [PubMed: 18562484]
- [41]. Park K, Millet LJ, Kim N, Li H, Jin X, Popescu G, Aluru NR, Hsia KJ, Bashir R, Proc. Natl. Acad. Sci. U. S. A 2010, 107, 20691. [PubMed: 21068372]
- [42]. Managò S, Valente C, Mirabelli P, Circolo D, Basile F, Corda D, De Luca AC, Sci. Rep 2016, 6, 24821. [PubMed: 27089853]

FIGURE 1. Schematic illustration of our home-built multi-wavelength multi-modal imaging system. This system consists of confocal Raman imaging and transmission common-path diffraction phase microscopy; and uses the same objective to collect signal from the sample. This system uses 532 and 785 nm wavelengths for Raman excitation. The sample is spatially scanned by a nano-positioning stage. DM, dichroic mirror; FL, Fourier lens; FM, flip mirror; L, lens; LLF, laser line filter; NF, Notch filter; OI, optical isolator; SM, static mirror; TL, tube lens

FIGURE 2.

A, Raw interferogram used for phase imaging (scale bar = 10 μm). B, Magnified portion showing fringe contrast. C, Phase image of a single 10 μm polystyrene microsphere. D, Height calculated from the phase image; Raman spectra of the polystyrene bead with (E) 785 nm and (F) 532 nm excitations. For both Raman spectra, the integration time was 1 second and three accumulations were averaged. For acquiring phase images, the camera exposure time was set to be ~ 7 ms. The power at the sample was ~ 30 mW

FIGURE 3.

A, Phase image of live healthy RBCs in PBS (scale bar = 10 μm). B, Surface height profile calculated from the phase image of RBCs. Representative Raman spectrum of a single RBC at (C) 785 nm excitation and (D) at 532 nm excitation. Each acquired spectra consisted of three accumulations each with an integration time of 10 seconds. For acquiring phase images, the camera exposure time was set to be ~ 7 ms. The power at the sample was approximately 15 mW

FIGURE 4.

Phase images of adherent breast cancer cells: A, MDA-MB 231-parental. B, MDA-MB 231-LM-121. C, MDA-MB 231-CTC with corresponding bright field images (D-F). The phase images have better contrast and have higher information content. The bottom panel represents the QPM and Raman data obtained from cells in suspension. Phase images of (G) leukemic cell (MN60), (H) healthy B cell. Representative Raman spectra acquired from single live blood cells both healthy and malignant are shown in bottom right panel (I). For both the spectra, three accumulations each with an integration time of 20 seconds were recorded at 785 nm excitations. For acquiring phase images, the camera exposure time was set to be ~7 ms. The power at the sample was approximately 25 mW