

Deep learning for high-throughput label-free cell classification

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Label-free cell analysis is essential to personalized genomics, cancer diagnostics, and drug development as it avoids adverse effects of staining reagents on cellular viability and cell signaling. However, currently available label-free cell assays mostly rely only on a single feature, namely on cell size or dielectric constant. Also, the sample size that can be analyzed by them is limited due to low throughput. Here, we integrate high-throughput quantitative imaging enabled by the photonic time stretch with feature extraction and deep learning, achieving record high accuracy in label-free cell classification. Our system captures quantitative optical phase and loss images in a flow-through microscope and extracts multiple biophysical features of individual cells, such as morphological attributes, optical loss characteristics, and protein concentration indicators. These biophysical measurements form a hyperdimensional feature space in which supervised deep learning is performed for cell classification. A novel deep learning pipeline is introduced by adopting evolutionary global optimization of receiver operating characteristics. As a validation of the enhanced classification sensitivity and specificity of our method, we show binary classification of *OT-II* white blood T-cells against *SW-480* colon cancer cells. Additionally, we show classification of lipid accumulating algal strains for biofuel production. We demonstrated and compared various learning algorithms including support vector machine, artificial neural network, logistic regression, linear discriminant analysis as well as our new deep learning model trained on AUC by genetic algorithm. This system opens up a new path to data-driven genotype-trained phenotypic diagnosis and better understanding of the heterogeneous gene expressions in cells.

Conventional flow cytometry is a powerful tool for large-scale cell analysis due to its ability to measure anisotropic elastic light scattering of millions of individual cells as well as emission of fluorescent labels conjugated to cells [1, 2]. However each cell is represented with single values per detection channels (forward scatter, side scatter, and emission bands), requiring labeling with specific biomarkers for acceptable classification accuracy [1, 3]. Imaging flow cytometry [4, 5] on the other hand captures images of cells revealing significantly more information about the cells. For example, it can distinguish clusters and debris that would otherwise result in false positive identification in a conventional flow cytometer based on light scattering [6].

In addition to classification accuracy, another critical specification of a flow cytometer is its throughput. Indeed high throughput, typically 100,000 cells per second, is needed to screen a large enough cell population to find rare abnormal cells that are indicative of early stage diseases. However there is a fundamental trade-off between throughput and accuracy in any measurement system [7, 8]. Additionally, imaging flow cytometers face

a throughput limit imposed by the speed of the CCD or the CMOS cameras, a number that is approximately 2000 cells/s for present systems [9]. Higher flow rates lead to blurred cell images due to the finite camera shutter speed. Many applications of flow analyzers such as cancer diagnostics, drug discovery, biofuel development, and emulsion characterization require classification of large sample sizes with a high-degree of statistical accuracy [10]. This has fueled research into alternative optical diagnostic techniques for characterization of cells and particles in flow.

Recently, our group has developed a label-free imaging flow-cytometry technique based on coherent optical implementation of the photonic time stretch concept [11]. This instrument overcomes the trade-off between sensitivity and speed by using Amplified Time-stretch Dispersive Fourier Transform (ADFT) [12–14]. In time stretched imaging [15], the object's spatial information is encoded in a spectrum of laser pulses within a pulse duration of sub-nanoseconds (Fig. 1). Each pulse representing one frame of the camera is then stretched in time so that it can be digitized in real-time by an electronic analog-to-digital converter (ADC). The ultra-fast pulse illumination freezes the motion of high-speed cells or particles in flow to achieve blur-free imaging. Detection sensitivity is challenged by the low number of photons collected during

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the ultra-short shutter time (optical pulse width) and the drop in the peak optical power resulting from the time stretch. These issues are solved in time stretch imaging by implementing a low noise-figure Raman amplifier within the dispersive device that performs time stretching [8, 11, 15]. In the coherent version of the instrument, the time stretch imaging is combined with spectral interferometry to measure quantitative phase and intensity images in real-time and at high throughput [16]. Integrated with a microfluidic channel, coherent time stretch imaging system in this work measures both quantitative optical phase shift and loss of individual cells as a high-speed imaging flow cytometer, capturing 36 million images per second in flow rates as high as 10 meters per second, reaching up to 100,000 cells per second throughput.

On another note, surface markers used to label cells, such as EpCAM [17], are indispensable tools for cell classification. However, they are unavailable in some applications; for example, melanoma or pancreatic circulating tumor cells (CTCs) as well as some cancer stem cells are EpCAM-negative and will escape EpCAM-based detection platforms [18]. Furthermore, large-population cell sorting opens the doors to downstream operations, where the negative impacts of labels on cellular behavior and viability are often unacceptable [19]. Cell labels may cause activating/inhibitory signal transduction, altering the behavior of the desired cellular subtypes, potentially leading to errors in downstream analysis, such as DNA sequencing and subpopulation regrowth. In this way, quantitative phase imaging (QPI) methods [20–22] that categorize unlabeled living cells with high accuracy are needed. Coherent time stretch imaging is a method that enables quantitative phase imaging at ultrahigh throughput for non-invasive label-free screening of large number of cells.

In this work, the information of quantitative optical loss and phase images are fused into expert designed features, leading to a record label-free classification accuracy when combined with deep learning. Image mining techniques are applied, for the first time, to time stretch QPI to measure biophysical attributes including protein concentration, optical loss, and morphological features of single cells at an ultrahigh flow rate and in a label-free fashion. These attributes differ widely [23–26] among cells and their variations reflect important information of genotypes and physiological stimuli [27]. The multiplexed biophysical features thus lead to information-rich hyper-dimensional representation of the cells for label-free classification with high statistical precision.

We further improved the accuracy, repeatability, and the balance between sensitivity and specificity of our label-free cell classification by a novel machine learning pipeline, which harnesses the advantages of multivariate supervised learning, as well as unique training by evolutionary global optimization of receiver operating characteristics (ROC) [28–30]. To demonstrate sensitivity, specificity, and accuracy of multi-feature label-free flow

cytometry using our technique, we classified (1) *OT-II* hybridoma T-lymphocytes and *SW-480* colon cancer epithelial cells, and (2) *Chlamydomonas reinhardtii* algal cells (herein referred to as *Chlamydomonas*) based on their lipid content, which is related to the yield in bio-fuel production. Our preliminary results show that compared to classification by individual biophysical parameters, our label-free hyperdimensional technique improves the detection accuracy from 78.1% to 95.5%, or in other words, reduces the classification inaccuracy by about five times.

I. RESULTS

A. Time Stretch Quantitative Phase Imaging

The application of time stretch quantitative phase imaging (TS-QPI) to imaging flow cytometry has been recently demonstrated in our group [11]. Broadband optical pulses from a mode-locked laser were firstly conditioned in fiber optics and then spatially dispersed in free-space optics with a pair of reflection diffraction gratings creating 1-D “rainbow flashes” (Fig. 1). Each one of rainbow flashes was composed of all the wavelength components distributed laterally over the field of view. These flashes illuminated the target as in traditional photography, but in addition, rainbow flashes targeted different spatial points with distinct colors of light, resulting in space-to-spectrum encoding. Rainbow pulses were then split into the two arms of a Michelson interferometer. Different wavelength components of the rainbow flash traveled parallel to each other but individually focused on the mirror in the reference arm or on the reflective substrate of a microfluidic device in the sample arm. In the sample arm, the cells in the microfluidic channel were hydrodynamically focused [31, 32] into the rainbow’s field of view and flowed perpendicular to the rainbow flash. Reflected pulses from the microfluidic device and the reference arm were recombined and coupled back into the fiber, optically amplified and linearly chirped through Raman-amplified time-stretch dispersive Fourier transform (TS-DFT) system. An amplified time-stretch system that utilizes a low-noise distributed Raman amplifier within dispersive fiber with a net optical gain of approximately 15 dB enables high-sensitivity detection at high speeds. An ultrafast single-pixel photodetector transformed instantaneous optical power into an electrical signal and subsequently, an analog-to-digital converter (ADC) samples and quantizes the signal. Acquired data are passed down to processing stages for big data analytics. The interference between time-shifted linearly chirped pulses create a beat (fringe) frequency, which can be adjusted via the interferometer arm length mismatch. Details of the demonstration system can be found in Section IIIA.

The photodetected time-stretched pulses, each representing one line-scan, are converted to analytic signals

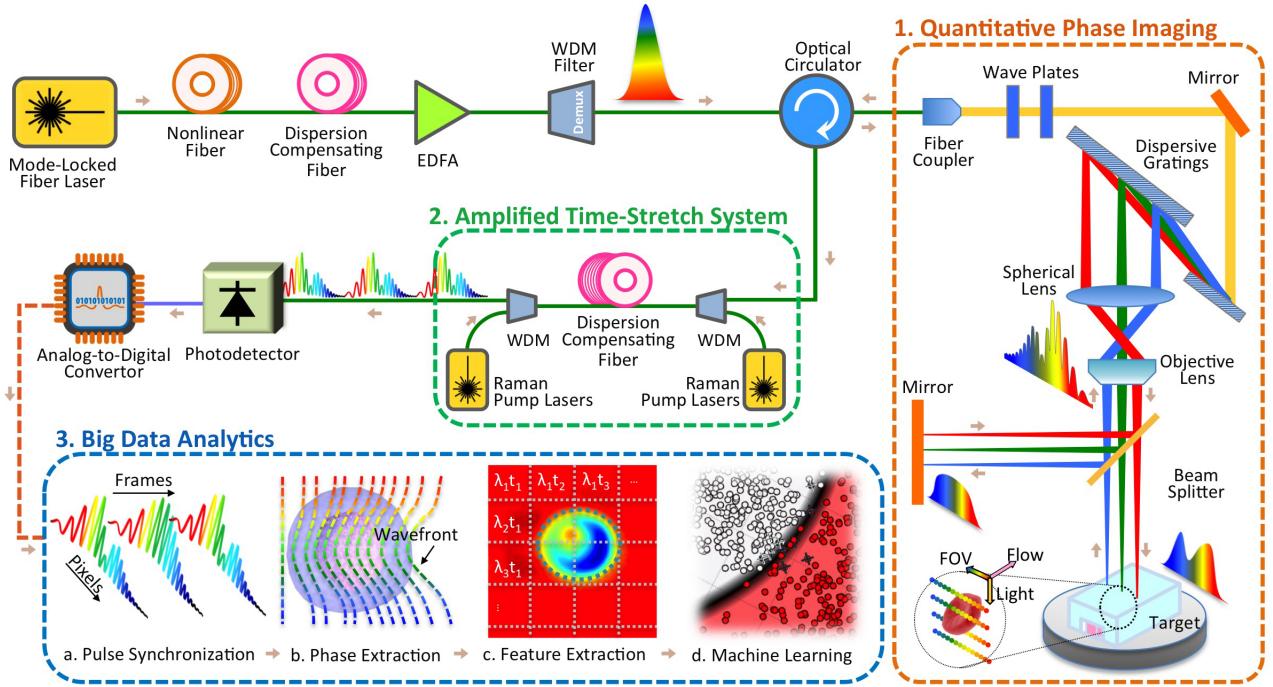
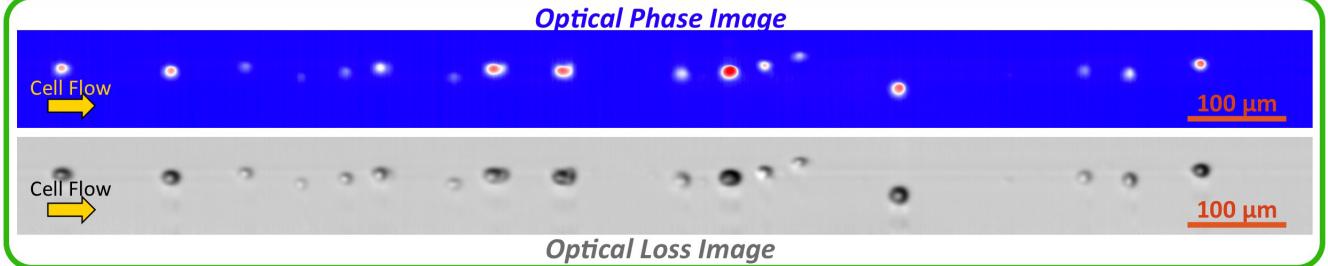


FIG. 1. Time stretch quantitative phase imaging (TS-QPI) and analytics system; A mode-locked laser followed by a nonlinear fiber, an erbium doped fiber amplifier (EDFA), and a wavelength-division multiplexing (WDM) filter generate and shape a train of broadband optical pulses. Box 1: The pulse train is spatially dispersed into a train of rainbow flashes illuminating the target as line scans. The spatial features of the target are encoded into the spectrum of the broadband optical pulses each representing a one-dimensional frame. The ultra-short optical pulse illumination freezes the motion of cells during high speed flow to achieve blur-free imaging with a throughput of 100,000 cells/s (limited by the flow channel). The phase shift at each location within the field of view is embedded into the spectral interference patterns using by a Michelson interferometer. Box 2: The interferogram pulses were then stretched in time so that spatial information could be mapped into time through time-stretch dispersive Fourier transform (TS-DFT), and then captured by a single pixel photodetector and an analog-to-digital converter (ADC). The loss of sensitivity at high shutter speed is compensated by stimulated Raman amplification during time stretch. Box 3: (a) Pulse synchronization; the time-domain signal carrying serially captured rainbow pulses is transformed into a series of one-dimensional spatial maps, which are used for forming line images. (b) The biomass density of a cell leads to a spatially varying optical phase shift. When a rainbow flash passes through the cells, the changes in refractive index at difference locations will cause phase walk-off at interrogation wavelengths. Hilbert transformation and phase unwrapping are used to generate the spatial information of phase shift. (c) Decoding the phase shift in each pulse at each wavelength and remapping it into a pixel reveals the protein concentration distribution within cells. The optical loss induced by the cells, embedded in the pulse intensity variations, is obtained from the amplitude of the slowly varying envelope of the spectral interferograms. Thus, quantitative optical phase shift and intensity loss images are captured simultaneously. Both images are calibrated based on the regions where the cells are absent. Cell features describing morphology, granularity, biomass, etc are extracted from the images. (d) These biophysical features are used in a machine learning algorithm for high-accuracy label-free classification of the cells.

using Hilbert transformation [33] and the intensity and phase components are extracted. The phase component is a fast oscillating fringe (carrier frequency), caused by the interference of the linearly chirped pulses arriving from the reference and signal arms in the Michelson interferometer. Acting as a radio-frequency (RF) carrier whose frequency is set by the user adjusted arm length mismatch, the fringe frequency is modulated when the optical path length in the sample arm is changed by the arrival of a cell. This frequency shift and the accompanying phase change are used to measure the optical path length of the cell (see Section IIIB). Since the phase varies over a wide range (much larger than 2π radians),

an unwrapping algorithm is used to obtain the continuous phase profile. The phase profile contains an increasing term with time, corresponding to the fringe (beat) frequency and the phase shift induced by the cell. By eliminating the background phase component, the cell-induced phase shift is extracted. The second component in the waveform is a lower frequency envelope corresponding to temporal shape of the optical pulse. The amplitude of this envelope provides information about optical loss caused by transparency, surface roughness, and inner organelle complexity (Section IIIC).

White Blood Cell Line (*OT-II*)



Colon Cancer Cell Line (*SW-480*)

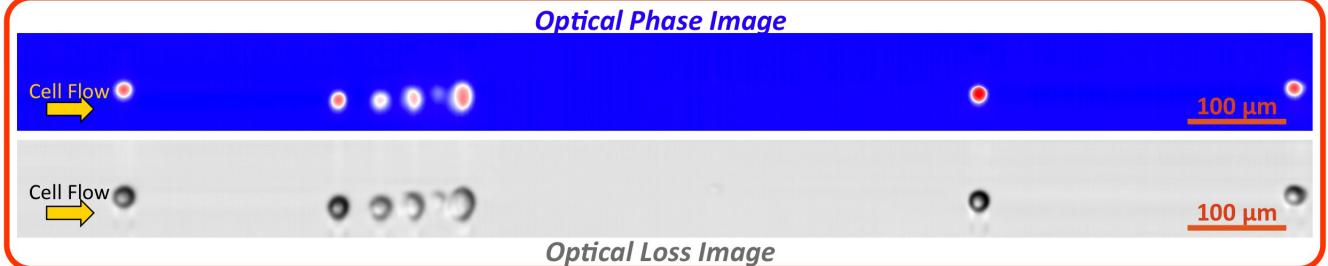


FIG. 2. Quantitative phase and optical loss images of *OT-II* (blue) and *SW-480* (green box) cells; The optical loss images of the cells are affected by the attenuation of multiplexed wavelength components passing through the cells. The attenuation itself is governed by the absorption of the light in cells as well as the scattering from the surface of the cells and from the internal cell organelles. The optical loss image is derived from the low frequency component of the pulse interferograms. The optical phase image is extracted from the analytic form of the high frequency component of the pulse interferograms using Hilbert Transformation, followed by a phase unwrapping algorithm. Details of these derivations can be found in Section III. Also, Videos 1 and 2 show measurements of cell-induced optical path length difference by TS-QPI at four different points along the rainbow for *OT-II* and *SW-480*, respectively.

B. Feature Extraction

The decomposed components of sequential linescans form pairs of spatial maps, namely, optical phase and loss images (see Section IIID). These images are used to obtain biophysical fingerprints of the cells [8, 34]. With domain expertise, raw images are fused and transformed into a suitable set of biophysical features, listed in Table I, which the deep learning model further converts into learned features for improved classification.

The feature extraction operates on optical phase and loss images simultaneously, including object detection, segmentation, and feature measurement, as well as clump identification, noise suppression, etc. As an example of the expert designed features, the average refractive index, used as a measure of protein concentration [35], is obtained by dividing the integral of the optical path length by the cell volume. Since cells in suspension relax to a spherical shape (due to surface tension) [36, 37], an independent measure of cell diameter can be obtained from its lateral dimension for volume estimation.

In feature extraction, one of the most important advantages of optical loss and phase fusion, is its robustness and insensitivity to axial defocusing [38] caused by the limited depth-of-focus of the objective lens and variations of the cell alignment in microfluidic channel. Diffracted

photons have little chance to be influential in phase images. This makes the size measurements in optical phase images relatively accurate and consistent, more suitable than direct size measurements in optical loss images for extraction of scattering and absorption features. Among different features, size measurement is particularly important as it is used by itself in many technologies [26, 39–41].

The large data set captured by TS-QPI provides sufficient statistical characteristics for cell analysis based on biophysical features. Since cells from even the same line or tissue exhibit variations in size, structure, and protein expression levels [42–44], high accuracy classification can only be achieved by a model tolerant to these intrinsic variations. On the other hand, the feature extractor must reflect the intricate and tangled characteristics caused by extrinsic variations, e.g. drug treatment [27], cell cycles, rare cell types, labeling, and transcription rate [45].

A total of 16 features are chosen among the features extracted from fusion of optical phase and loss images of each cell. Features that are highly correlated do not provide unique information. Pairwise correlation matrix among these features is shown as a heat map in Fig. 3a. Diagonal elements of the matrix are correlation of each feature with itself, i.e. the autocorrelation. The subset of the features in Box 1 shows high correlation among morphological features. Also, the subset features in Box 2

and 3 are correlated as they are mainly related to optical phase shift and optical loss, respectively.

As a representation of our biophysical features in classification, Fig. 3b shows classification accuracy based on each single feature arranged in descending order. The features are color coded into three categories: morphology, optical phase, and optical loss, to describe the main type of information provided by each. The figure provides valuable insight into the relative importance of each category of cell features and suggests that morphological features carries the most information about cells, but at the same time, significant additional information is contained in optical phase and loss measurements.

C. Machine learning

Neural networks are a flexible and powerful bioinspired learning model, which perform layers of nonlinear feature transformations, learned from the training data [46–48]. The transformations morph the input data with weighted sums and nonlinear activation functions into feature spaces more suitable for classification. Shown in Fig. 4 is a unique feedforward neural network learning model that is globally trained by the objective of improving receiver operating characteristic (ROC). The learning algorithm introduced here maximizes the area under ROC curve (AUC), which is a global indicator of the classifier performance on the entire training dataset. The global training of the neural network, although computationally costly, results in a classifier more robust, repeatable, and insensitive to imbalance among classes. For the purpose of end-to-end supervised learning with AUC whose gradient is not well-behaved, we employed the heuristic genetic algorithm (GA), which is resilient to discontinuities of the cost function and being trapped in local minima during optimization.

The network is composed of multiple hidden layers, which automatically learn representations of the data at different levels of abstraction, and thus, is considered a form of deep learning [49, 50]. Each layer performs a linear combination on its inputs from the previous layer and operates a nonlinear function on the weighted sums. The output of the node j in layer $l+1$, denoted by $z_j^{(l+1)}$ is generated from inputs x_1, x_2, \dots, x_N as

$$z_j^{(l+1)} = h(a_j^{(l+1)}) = h\left(\sum_{i=1}^{N_l} \omega_{ji}^{(l)} x_i^{(l)} + x_0^{(l)}\right) \quad (1)$$

$$i = 1, \dots, N_l; \quad j = 1, \dots, N_{l+1}; \quad l = 0, \dots, L$$

Here $a_j^{(l+1)}$ is the linear combination of inputs, and $\omega_{ji}^{(l)}$ are the weights of the linear combination. The summation runs over N_l , the total number of nodes in the layer l , and L is the total number of hidden layers. The constant term in the summation, $x_0^{(l)}$, is the bias node in layer l . Some popular choices for the nonlinear activation function $h(\cdot)$ include logistic sigmoid function

$h(a) = 1/(1 + \exp(-a))$, hyperbolic tangent function $\tanh(a)$, and commonly used in deep learning, rectified linear unit (ReLU) $h(a) = \max(0, a)$. In our learning model, we use ReLU, which typically speeds up the supervised learning process of deep neural network by inducing sparsity and preventing gradient vanishing problem.

For a trained classifier in hyperspace, receiver operating characteristics (ROC) curve describes the sensitivity and specificity of a classifier collection that includes nonlinear classifiers scaled in the direction of their normal vector field. In a deep learning network, this is equivalent to shifting the value of the bias node in the last hidden layer. ROC highlights the trade-off between sensitivity and specificity (Fig. 4), and the area under ROC (AUC) provides a quantitative robust measure of classifier performance [51–54]. Choosing a large value for the bias node results in high sensitivity, however this sacrifices the specificity leading to large number of false positives. As a way to visualize the impact of the threshold on classification accuracy, a classifier that accurately separates the classes will have an ROC curve that approaches the upper left corner. Conversely, a random guess, corresponding to accuracy of 50% in binary classification will have an ROC that is a diagonal line. The AUC parameter serves as an effective analysis metric for finding the best classifier collection and has been proven to be advantageous to the mean square error for evaluating learning algorithms [55].

D. Demonstration in Classification of *OT-II* and *SW-480* Cells

□ We used hyper-dimensional scatter plots as a tool for high content cell analysis. To demonstrate application in circulating cancer cell (CTC) detection, we used a white blood cell line (*OT-II* hybridoma T cell) and a cancer cell line (*SW-480* epithelial colon cancer). Fig. 6 shows the 3D scatter plot based on size, protein concentration, and attenuation of a large number of *OT-II* (black dots) and *SW-480* (red dots) cells. The attenuation is a parameter describing the optical intensity loss caused by cell absorption (Absorption-1 feature in Table I). The 2D projections on the three orthogonal planes are also shown. It is clear that certain pairs of features are better able to differentiate the two populations.

Classification of cells based on size alone in a label-free manner has been previously reported by several groups [26]. However, in contrast to these single parameter (size) approaches, our multi-parameter classification, enabled by the time stretch QPI, offers considerable improvements in detection sensitivity and accuracy for cancer diagnosis and selection of algal species. To demonstrate the effectiveness of the machine learning plus time stretch QPI imaging system for cancer cell detection, we used *OT-II* hybridoma T cells as a model for normal white blood cells and *SW-480* colon cancer cells. A 16 feature



FIG. 3. (a) Pairwise correlation matrix visualized as a heat map; The map depicts the correlation coefficient between all major 16 features extracted from the quantitative images. Diagonal elements of the matrix represent correlation of each parameter with itself, i.e. the autocorrelation. The subset in box 1, box 2, and box 3 show high correlation because they all measure morphological, optical phase, and optical loss features, respectively. This suggest that the dataset can be adequately represented by a smaller set that excludes highly correlated parameters. (b) Ranking of biophysical feature performance based on the accuracy in 1-D classification; The value is the area under receiver operating characteristics curve of each individual parameter. Blue bars show performance of the morphological parameters, which includes diameter along the interrogation rainbow, diameter along the flow direction, tight cell area, loose cell area, perimeter, circularity, major axis length, orientation, and median radius. As expected, morphology contains most information, but other biophysical features can contribute to improved performance of label-free cell classification. Orange bars show optical phase shift features i.e. optical path length differences and refractive index difference. Green bars show optical loss parameters representing scattering and absorption by the cell. Red boxes show the best performed feature in these three categories.

data set was measured using the imaging system and feature extraction technique described previously. By sliding the detection limit along the direction perpendicular to the optimum classification plane, a receiver operating characteristic (ROC) curve for multi-dimensional data is generated (Fig. 5).

Blue curves in Fig. 5 show the ROC for classification using all sixteen biophysical features extracted from the time-stretch QPI images. The gray diagonal line shows a random guess classifier with no discrimination skill. For generating the ROC curves, we employ the normal unit vector of a decision boundary in n-sphere and use genetic algorithm for optimization. At step $k+1$ a trial set of weights (children) is randomly generated in the neighborhood of the present set (parents). Each generation produces new ROC curves. The genetic algorithm randomly selects from this population and uses them as parents to produce the children for the next generation by

mutation and crossover. Over successive generations, the population evolves toward an optimal solution that maximizes the AUC. The area under the ROC curve (AUC) was calculated using a non-parametric method based on constructing trapezoids under the curve.

As a way to visualize the improvement in classification by hyperdimensional feature space of TS-QPI, we also show the ROC curves of classification based on several single features: diameter along the interrogation rainbow, integral of cell's optical path length difference, and cellular absorption in near-infrared window. These three biophysical parameters individually perform the highest accuracy among morphology, optical phase, and optical loss parameter groups respectively. But multivariate analysis based on TS-QPI shows significant improvement in classification. For each type of classification we show the ROC for 10 fold cross validation data set. As the curves approach random guess line, the 10 fold cross val-

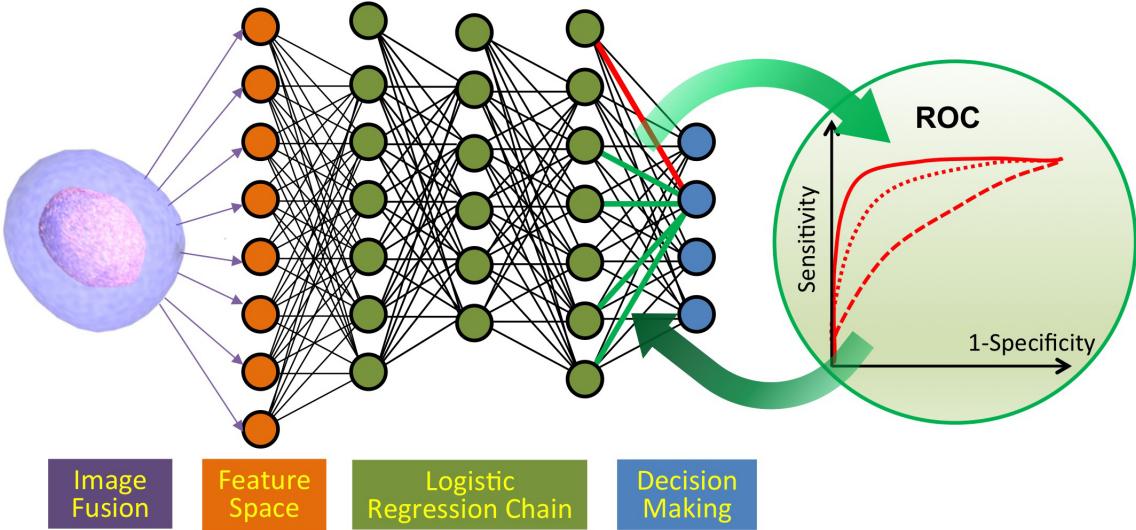


FIG. 4. Neural network architecture and training via area under the curve (AUC) of the receiver operating characteristics (ROC); Multivariate features of each measurement (cell) are feed into the neural network and the output shows classification labels, i.e. cell types. We train the weights of the last layer perceptron by optimizing the area under ROC curve. Each ROC curve corresponds to a set of weights for connections to an output node, generated by scanning the bias weight. AUC is proven to be statistically consistent and more discriminating than accuracy.

ification curves deviate more from each other. Additionally purple curves shows the classifier optimized with the first principal component in PCA feature space.

E. Demonstration in Algae Lipid Content Classification

Microalgae are considered one of the most promising feedstock for biofuels [56]. The productivity of these photosynthetic microorganisms in converting carbon dioxide into useful carbon-rich lipids greatly exceeds that of agricultural crops. Worldwide, research and demonstration programs are being carried out to develop the technology needed to expand algal lipid production as a major industrial process. Selecting high yield microalgae with fast growth factors are essential in biofuel production industry. Because algae differ greatly in size and structure, cell size alone provides insufficient information for cell classification. Here we show that adding optical phase and loss data, obtained by the phase contrast time stretch imaging flow cytometer, to size data enables cells to be distinguished on the basis of lipid content.

To test our apparatus for its ability to separate high and low-lipid containing cells we exploited the starch-null *sta6* strain of *Chlamydomonas*. This strain is deleted for *sta6* [57] (encoding the small subunit of ADP-glucose-pyrophosphorylase), and when nitrogen-deprived accumulates more lipid than wild-type [58–61]. Comparison of the two strains therefore provides an ideal setup to test our ability to distinguish lipid-content phenotypes.

Fig. 7a shows the 3D scatter plot showing the three

principle physical features for the two algae populations. Also shown is the scatter plot obtained using a conventional flow cytometer. It is readily apparent that the TS-QPI capture much more information about individual cells and is better suited for differentiation of low- and high-lipid content algae species. In Fig. 7b, we show ROC curves for binary classification of these populations. Blue curve shows the classifier performance using all 16 physical features extracted from the time-stretch QPI images. Red, green, and orange curves show the classifier decision made using only the three major biophysical features: diameter for morphology (Diameter-RB in Table I), optical path length difference for optical phase (OPD-1 in Table I), and absorption for optical loss (Absorption-2 in Table I). We observe that while biophysical parameters, in particular cell diameter, is an effective feature for classification, hyperdimensional classification using all 16 parameters offers the best accuracy.

II. CONCLUSION

Time-stretch quantitative phase imaging (TS-QPI) is capable of capturing images of flowing cells with minimal motion distortion at unprecedented rates of 100,000 cells/s. TS-QPI relies on spectral multiplexing to capture simultaneously both phase and intensity quantitative images in a single measurement, generating a wealth of information of each individual cell eliminating the need for labeling with undesirable biomarkers. Here, we summarized the information content of these images in a set of 16 parameters for each cell, and performed classifica-

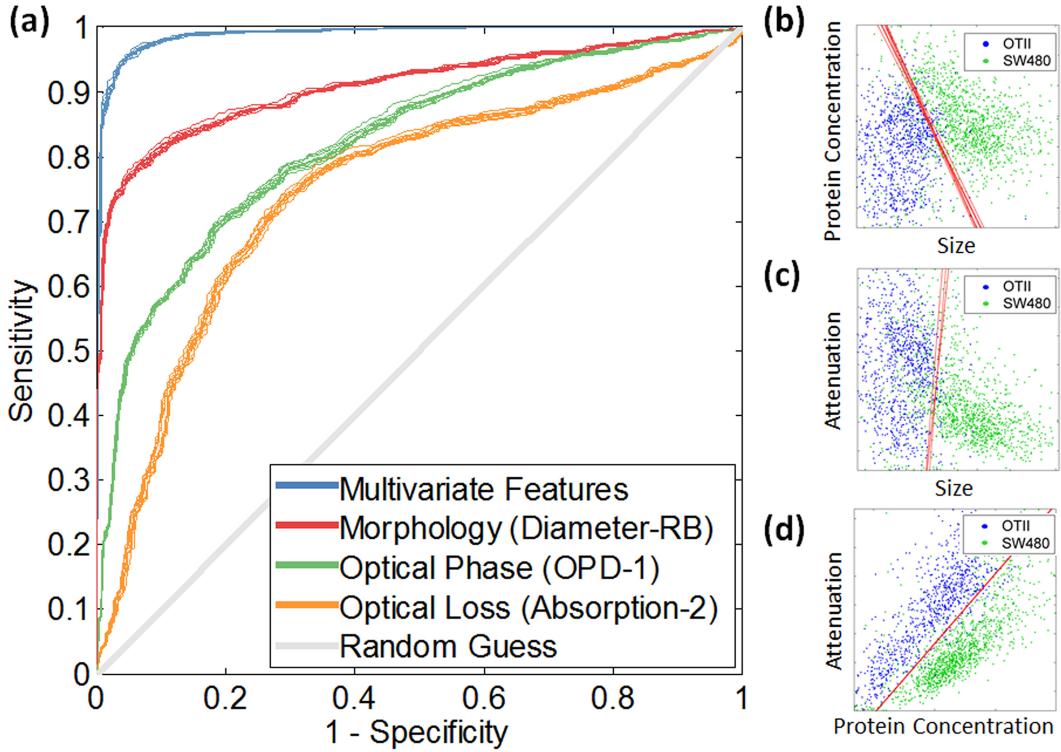


FIG. 5. Consistency in binary classification of white blood cells (*OT-II*) and cancer cells (*SW-480*) by TS-QPI label-free features; (a) For each classifier, we show 10 receiver operating characteristic (ROC) curves demonstrating ten-fold cross validation of the dataset. Blue curves show the classifier performance using all 16 biophysical features extracted from the TS-QPI quantitative images. We also show ROC for classification by three single parameters (red, green, and orange curves) representing the decision performance of the classifiers using only the best biophysical feature in each category: morphology (Diameter-RB in Table I), optical phase (OPD-1 in Table I), and optical loss (Absorption-2 in Table I), respectively. Multivariate analysis based on TS-QPI images (blue curves) shows significant improvement in classification sensitivity and specificity. The gray diagonal line shows results of random guess classification. In the ten-fold cross validation, the total data set is split into 10 equal subsets and each time the test is performed for one part while the other nine parts are used for training. The fact that the classifiers remain almost unchanged during the ten runs of cross validation shows consistency and robustness of the dataset and the classifier. (b-d) Decision boundaries (red lines) in two-parameter scatter plots trained by ten-fold cross validation. They also show the consistency and robustness of supervised cell classification by TS-QPI data.

tion in the hyperdimensional space of this dataset. Our classifier learning method is based on evolutional optimization of the area under the receiver operating characteristic curve for a single-layer binary-classifier neural network. The results from two experimental demonstrations, one on detection of cancerous cells among white blood cells, and another one on identification of lipid-rich algae, shows that classification accuracy by using the TS-QPI hyperdimensional space is more than 17% better than the conventional size-based techniques. Our system opens up a new path to data-driven genotype-trained phenotypic diagnosis and better understanding of the heterogeneous gene expressions in cells and thus is expected to be a valuable tool for high-throughput label-free cell analysis in medical, biotechnological, and research applications.

III. METHODS

A. Time Stretch Quantitative Phase Imaging (TS-QPI) System

Broadband optical pulses from a mode-locked laser (center wavelength = 1565 nm, repetition rate = 36.128 MHz, pulse width ~100 fs) are further broadened using a highly nonlinear fiber to approximately 100 nm with a spectral range up to 1605 nm. These broadband pulses are then linearly chirped to 1.2 ns pulse width by a short dispersion compensating fiber (DCF) of 60 ps/nm, so that an erbium doped fiber amplifier (EDFA) can amplify them with minimal distortion. A coarse wavelength division multiplexer (WDM) filters the pulses from 1581 nm to 1601 nm, where the spectrum is reasonably flat. Therefore, the total bandwidth of the pulses interrogating the cells in our setup is less than 20 nm centered at 1591 nm, giving a negligible fractional bandwidth of

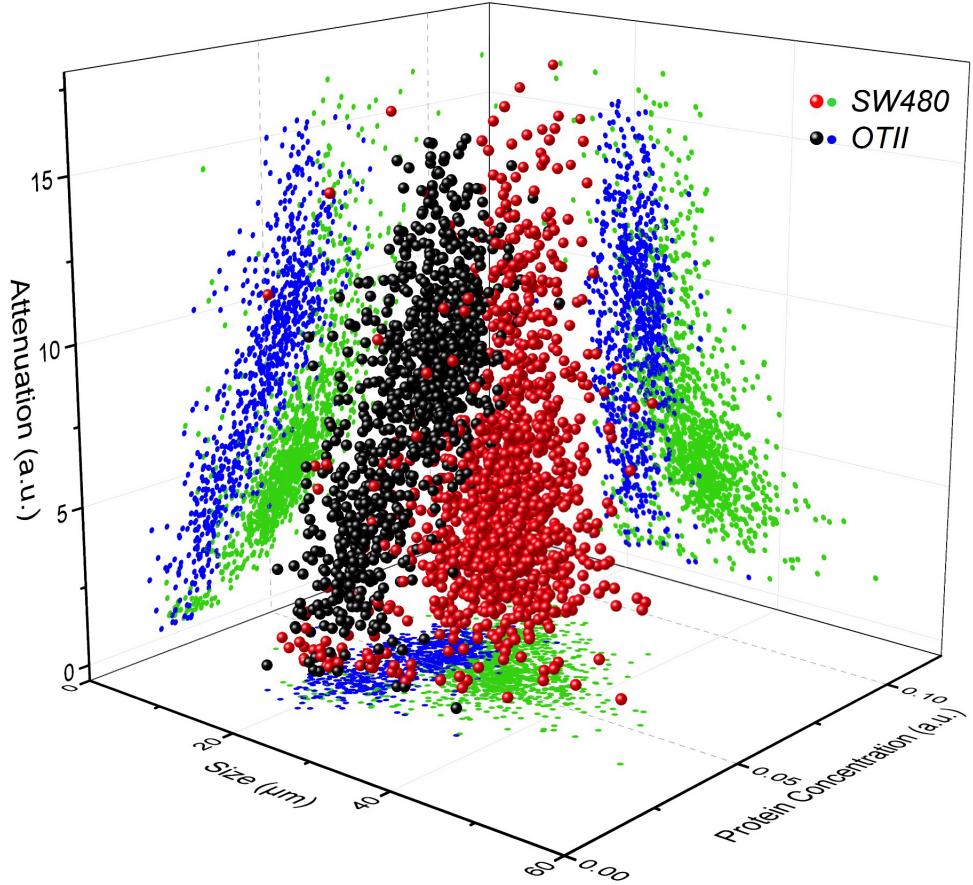


FIG. 6. Three-dimensional scatter plot based on size, protein concentration, and attenuation of *OT-II* and *SW-480* cells measured by TS-QPI; The green and blue dots are two-dimensional (2-D) projections of cell data points on the planes containing only two of the biophysical parameters. The cell protein concentration corresponds to the mean refractive index difference of the cell (Refractive index feature in Table I). The attenuation is a parameter describing the optical intensity loss caused by cell absorption (Absorption-1 feature in Table I). Comparison of 2-D scatter plots reveals that additional biophysical parameters (in this case protein concentration) serve to classify the cell types more accurately.

1.3%. These filtered pulses then pass through an optical circulator and are coupled to free-space with a fiber collimator.

Free-space laser pulses were linearly polarized with quarter- and half-wave plates, and then spatially dispersed with a pair of reflection diffraction gratings, so that each wavelength component of the collimated beam was positioned at a different lateral point similar to a line flash rainbow. A beam reducer shrank the rainbow beam 6 times with a pair of 90 degree off-axis parabolic gold-coated mirrors with reflected focal lengths as 152.4 mm and 25.4 mm, respectively. Next, a 15 degree off-axis parabolic gold-coated mirror with 635 mm reflected focal length and a long working-distance objective lens with 0.4 numerical aperture further shrank the rainbow to about 130 μm in width, i.e. field of view. Reflective optics with parabolic gold-coated mirrors is used in our experimental demonstration to minimize loss, aberration, and polarization sensitivity. The rainbow flashes were then split into the two arms of a Michelson inter-

ferometer by a beam splitter. In the sample arm, the rainbow pulses pass through the cells and are reflected by the reflective substrate of the microfluidic device. In the reference arm, a dielectric mirror reflected the rainbow with a length mismatch with the sample arm causing spectral interference fringes (Fig. 8a). Cells are hydrodynamically focused at the center of the channel flow at a velocity of 1.3 m/s. The reflected pulses from reference and sample arms were recombined at the beam splitter, compressed by the two diffraction gratings and coupled back into the fiber. These return pulses were spectrally encoded by the spatial information of the interrogation field of view. Then they were redirected by the optical circulator to a Raman-amplified time-stretch dispersive Fourier Transform (TS-DFT) system followed by a 10 Gb/s photodetector (Discovery Semiconductors DSC-402APD). An analog-to-digital converter (Tektronix DPO72004C) with a sampling rate of 50 GS/s and 20 GHz bandwidth is used to acquire the output signal of the photodetector, which is a series of spectral

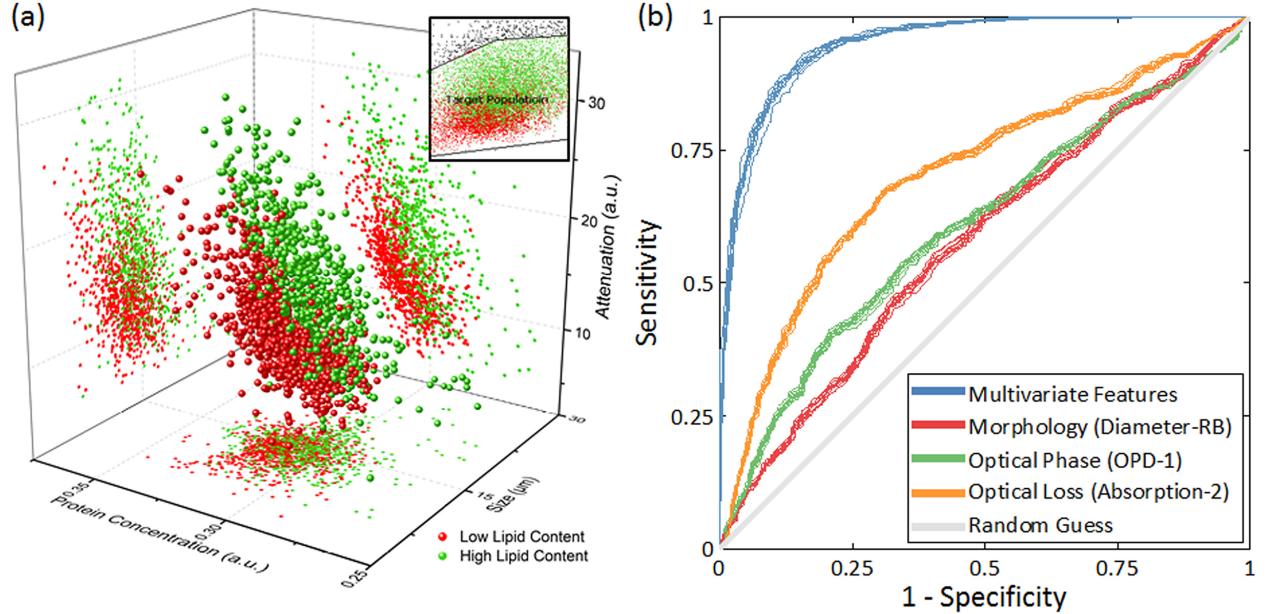


FIG. 7. Classification of algae cells (*Chlamydomonas reinhardtii*) based on their lipid content by TS-QPI; (a) Three-dimensional scatter plot based on size, protein concentration, and attenuation of the cells measured by TS-QPI, with 2D projections for every combination of two parameters. Inset: Conventional label-free flow cytometry using forward scattering and side scattering is not enough to distinguish the difference between high-lipid content and low-lipid content algae cells. Time-stretch QPI is much more effective in separating the two algae populations. (b) ROC curves for binary classification of normal and lipid-rich algae species using ten-fold cross validation; blue curve shows the classifier performance using all 16 biophysical features extracted from the TS-QPI quantitative images. Red, green, and orange curves show the classifier decision performance using only the best biophysical feature in each category: morphology (Diameter-RB in Table I), optical phase (OPD-1 in Table I), and optical loss (Absorption-2 in Table I), respectively. Additionally, we show ROC curves in purple for classification based on the first principal components in PCA space. The diagonal line shows results of random guess classification. Clearly, the label-free cell classification performance improves as more biophysical features are employed.

interferogram mapped into time (Fig. 8b).

B. Coherent Detection and Phase Extraction

Unlike in conventional heterodyne detection, which uses a narrowband continuous-wave signal as the local oscillator or reference, the coherent detection in our time stretch system uses an unmodulated copy of the original optical input, which is a broadband optical pulse train [62, 63].

Since the spectrum is mapped into space by diffraction gratings, the complex field at any specific spatial location within the field of view is a narrowband optical wave. As the envelope of the optical wave varies slowly in time compared to the period of the optical electromagnetic wave and the time mismatch between the reference arm and the sample arm, we employ slowly varying envelope approximation in our analysis. The complex envelope of the input electric field, $\tilde{E}_{in}(\omega, t_p)$, is split into two arms of the Michelson interferometer at the beam splitter. Here, ω is the optical frequency of the input signal, which corresponds to the spatial location x being interrogated by the optical wave at this frequency (i.e. spectral encod-

ing of the object image). t_p specifies the time when each rainbow flash reaches the beam splitter, corresponds to the p -th incoming pulse. Note that $\tilde{E}_{in}(\omega, t_p)$ can be simplified as $\tilde{E}_{in}(\omega)$ when pulse shape is stable from pulse to pulse. The light split into the two arms of the Michelson interferometer can be expressed as

Into the sample arm:

$$\tilde{E}_s(\omega, t_p) = \sqrt{T_b} \tilde{E}_{in}(\omega, t_p) \quad (2)$$

Into the reference arm:

$$\tilde{E}_r(\omega, t_p) = i\sqrt{1 - T_b} \tilde{E}_{in}(\omega, t_p)$$

where T_b is the power transmission ratio of the beam-splitter. Optical intensity in the sample arm will be altered by the absorption and scattering of imaged cells, as well as that of the microfluidic channel and buffer solution. Not only the electric field amplitude after passing through semitransparent objects will be modulated by the optical attenuation in the sample arm, but also the optical path length difference will lead to a phase shift, $\Delta\varphi_c(x, t_p)$, induced by refractive index change from the object along the interrogation beam. Thus, the complex fields of the light waves coming back to the beam splitter

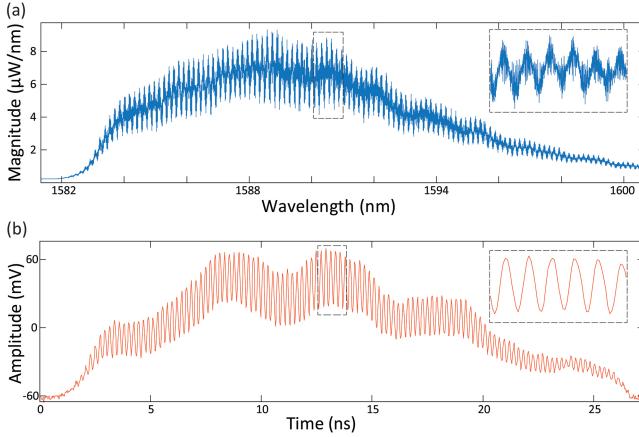


FIG. 8. Comparison of the interferograms measured by optical spectrum analyzer and time-stretch dispersive Fourier Transform; (a) Optical spectrum of the signal after quantitative phase imaging (box 1 in Fig. 1) and before it enters the amplified time-stretch system (box 2 in Fig. 1). The interference pattern in spectral domain is measured by an optical spectrum analyzer. (b) With time stretch, the interference pattern in spectral domain is linearly mapped into time. The baseband intensity envelope is slightly modified by the wavelength-dependent gain profile of the Raman amplifier. The inserts in panels a and b show the zoomed-in spectrum and waveform in the dashed black boxes, respectively. Clearly, the single-shot interferogram measured by Raman-amplified time-stretch dispersive Fourier Transform has a higher signal-to-noise ratio compared to that captured by optical spectrum analyzer.

become

From the sample arm:

$$\tilde{E}_s(\omega, t_p) = T_s(\omega)T_c(x, t_p + t_d)\sqrt{T_b(\omega)R_m(\omega)}\tilde{E}_{in}(\omega, t_p) \cdot \exp\left\{i\left[-\frac{\omega}{c} \cdot 2(L + \Delta L) - \Delta\varphi_c(x, t_p)\right]\right\}$$

From the reference arm:

$$\tilde{E}_r(\omega, t_p) = i\sqrt{1 - T_b(\omega)}\sqrt{R_m(\omega)}\tilde{E}_{in}(\omega, t_p) \cdot \exp\left(-i\frac{\omega}{c} \cdot 2L\right) \quad (3)$$

where L is the length of reference arm, and ΔL is the arm length mismatch between two arms. $R_m(\omega)$ is the wavelength-dependent reflectance of the reflective substrate of the microfluidic channel. t_d is the time delay during which rainbow flash travels from the beam splitter to the sample cell, $t_d = \frac{L_0 + \Delta L}{c}$. $T_s(\omega)$ is power transmittance of the surrounding buffer solution and microfluidic channel, and $T_c(x, t_p + t_d)$ is spatial power transmittance of cells at location x along the rainbow when being illuminated at time $t_p + t_d$. Both $\sqrt{T_s(\omega)}$ and $\sqrt{T_c(x, t_p + t_d)}$ affect the optical field twice as each rainbow flash passes through the cell twice. Since the t_d is much smaller than the time scale of the envelope variations caused by the cell flow, we can approximate $T_c(x, t_p + t_d)$ to be $T_c(x, t_p)$

to synchronize with $\tilde{E}_{in}(\omega, t_p)$ without sacrificing accuracy.

The total electric field at each wavelength or optical frequency after two arms of the interferometer recombine at the beam splitter becomes

$$\begin{aligned} \tilde{E}_o(\omega, t_p) &= T_s(\omega)T_c(x, t_p)T_b(\omega)\sqrt{R_m(\omega)}\tilde{E}_{in}(\omega, t_p) \\ &\cdot \exp\left\{i\left[-\frac{\omega}{c} \cdot 2(L + \Delta L) - \Delta\varphi_c(x, t_p)\right]\right\} \\ &- [1 - T_b(\omega)]\sqrt{R_m(\omega)}\tilde{E}_{in}(\omega, t_p)\exp\left[-i\frac{\omega}{c} \cdot 2L\right] \\ &= \sqrt{R_m(\omega)}\tilde{E}_{in}(\omega, t_p)\exp\left(-i\frac{2\omega L}{c}\right) \\ &\cdot \left\{T_s(\omega)T_c(x, t_p)T_b(\omega)\exp\left\{i\left[-2\frac{\omega}{c}\Delta L - \Delta\varphi_c(x, t_p)\right]\right\} - [1 - T_b(\omega)]\right\}. \end{aligned} \quad (4)$$

Based on the spectral encoding setup, we know spatial information has been encoded into spectrum,

$$T_c(x, t_p) \Rightarrow T_c(\omega, t_p) \quad (5)$$

$$\Delta\varphi_c(x, t_p) \Rightarrow \Delta\varphi_c(\omega, t_p) \quad (6)$$

The intensity envelope then becomes

$$\begin{aligned} \tilde{I}_o(\omega, t_p) &\propto \tilde{E}_o^*(\omega, t_p)\tilde{E}_o(\omega, t_p) \\ &= \left\{[1 - T_b(\omega)]^2 + T_b^2(\omega)T_c^2(\omega, t_p)T_s^2(\omega)\right\}R_m(\omega) \\ &\cdot |\tilde{E}_{in}(\omega, t_p)|^2 - 2[1 - T_b(\omega)]T_c(\omega, t_p)T_b(\omega)R_m(\omega) \\ &\cdot T_s(\omega)|\tilde{E}_{in}(\omega, t_p)|^2\cos[2\omega\Delta L/c + \Delta\varphi_c(\omega, t_p)] \end{aligned} \quad (7)$$

During time stretch, each frequency component ω , or wavelength λ will be one-to-one mapped into time domain. We define the relative time delay of λ compared to the central wavelength, λ_c , as t_i , which is usually called intrapulse time delay. Written in terms of λ , Eq. 7 can be simplified as

$$\tilde{I}_o(\lambda, t_p) \propto I_b(\lambda, t_p) + I_i(\lambda, t_p)\cos\left[\frac{4\pi\Delta L}{\lambda} + \Delta\varphi_c(\lambda, t_p)\right] \quad (8)$$

where $I_b(\lambda, t_p)$ is the background or baseband intensity envelope, and $I_i(\lambda, t_p)$ is the interference or intermediate intensity envelope:

$$\begin{aligned} I_b(\lambda, t_p) &= \left\{[1 - T_b(\lambda)]^2 + T_b^2(\lambda)T_c^2(\lambda, t_p)T_s^2(\lambda)\right\} \\ &\cdot R_m(\lambda)|\tilde{E}_{in}(\lambda, t_p)|^2 \end{aligned} \quad (9)$$

$$\begin{aligned} I_i(\lambda, t_p) &= -2[1 - T_b(\lambda)]T_c(\lambda, t_p)T_b(\lambda)R_m(\lambda) \\ &\cdot T_s(\lambda)|\tilde{E}_{in}(\lambda, t_p)|^2 \end{aligned} \quad (10)$$

Linear time stretch maps frequency domain into time domain by

$$t_i = D(\lambda - \lambda_c)L_f. \quad (11)$$

where λ_c is the central wavelength and L_f the length of dispersive fiber. D is the group velocity dispersion, that is, the temporal pulse spreading, Δt_i , per unit bandwidth, $\Delta\lambda$, per unit distance traveled. Thus the temporal samples of the energy flux absorbed at the photodetector are the intrapulse concatenation of spectral samples followed by interpulse concatenation of pulse waveforms:

$$\begin{aligned} \tilde{I}_{PD}[t] = & \\ & \text{cat}_\rightarrow \text{cat}_\downarrow \left(\begin{array}{cccc} \tilde{I}_o(t_i^{(1)}, t_p^{(1)}) & \tilde{I}_o(t_i^{(1)}, t_p^{(2)}) & \cdots & \tilde{I}_o(t_i^{(1)}, t_p^{(m)}) \\ \tilde{I}_o(t_i^{(2)}, t_p^{(1)}) & \tilde{I}_o(t_i^{(2)}, t_p^{(2)}) & \cdots & \tilde{I}_o(t_i^{(2)}, t_p^{(m)}) \\ \vdots & \vdots & \ddots & \vdots \\ \tilde{I}_o(t_i^{(n)}, t_p^{(1)}) & \tilde{I}_o(t_i^{(n)}, t_p^{(2)}) & \cdots & \tilde{I}_o(t_i^{(n)}, t_p^{(m)}) \end{array} \right) \\ \rightleftharpoons & \text{cat}_\rightarrow \text{cat}_\downarrow \left(\begin{array}{cccc} \tilde{I}_o(\lambda_1, t_p^{(1)}) & \tilde{I}_o(\lambda_1, t_p^{(2)}) & \cdots & \tilde{I}_o(\lambda_1, t_p^{(m)}) \\ \tilde{I}_o(\lambda_2, t_p^{(1)}) & \tilde{I}_o(\lambda_2, t_p^{(2)}) & \cdots & \tilde{I}_o(\lambda_2, t_p^{(m)}) \\ \vdots & \vdots & \ddots & \vdots \\ \tilde{I}_o(\lambda_n, t_p^{(1)}) & \tilde{I}_o(\lambda_n, t_p^{(2)}) & \cdots & \tilde{I}_o(\lambda_n, t_p^{(m)}) \end{array} \right) \end{aligned} \quad (12)$$

where cat_\rightarrow and cat_\downarrow mean horizontal and vertical concatenations, respectively. Each $\tilde{I}_o(t_i^{(n)}, t_p^{(m)})$ expresses the n th spectral (spatial) pixel at the m th pulse (line image). Applying Eq. 11 to Eq. 8,

$$\begin{aligned} \tilde{I}_o(t_i^{(n)}, t_p^{(m)}) \propto & I_b(t_i^{(n)}, t_p^{(m)}) + I_c(t_i^{(n)}, t_p^{(m)}) \\ & \cdot \cos \left[\frac{4\pi\Delta L \cdot DL_f}{t_i^{(n)} + D\lambda_c L_f} + \Delta\varphi_c(t_i^{(n)}, t_p^{(m)}) \right] \end{aligned} \quad (13)$$

Therefore, the time stretched temporal waveform corresponding to each line scan image consists of two features [16]: One is $I_b(t_i^{(n)}, t_p^{(m)})$, a temporal envelope of the time-stretched optical pulse at baseband frequencies. The amplitude of this envelope corresponds to the temporal shape of the optical pulse and its deviations caused by the object transmission as in brightfield microscopy. It provides information about optical loss, i.e. light absorption and scattering caused by surface roughness, granularity, and inner cell organelle complexity.

The second term in Eq. 13 (with cosine component) is a fast oscillating fringe, caused by the spectral interference of the pulses multiplexed between the sample and the reference arms in the Michelson interferometer. This term can be separated by a bandpass filter, and its envelope can be derived by a nonlinear envelope detection technique. Here we used a moving minimum/maximum filter to extract the envelope. After normalization to the envelope, the cosine component

$$I_c(t_i^{(n)}, t_p^{(m)}) = \cos \left[\frac{4\pi\Delta L \cdot DL_f}{t_i^{(n)} + D\lambda_c L_f} + \Delta\varphi_c(t_i^{(n)}, t_p^{(m)}) \right] \quad (14)$$

is used for calculation of the object phase shift, $\Delta\varphi_c(x, t_p)$. The first term in cosine causes the interferogram fringe pattern. Since $t_i \ll D\lambda_c L_f$, it can be

approximated as

$$\frac{4\pi DL_f \Delta L}{t_i + D\lambda_c L_f} \approx -f_i t_i + \varphi_{i0} \quad (15)$$

where φ_{i0} is an initial phase constant, f_i is the fringe frequency:

$$f_i \approx \frac{4\pi\Delta L}{\lambda_c^2 DL_f} \quad (16)$$

As seen in Fig. 8b, the fringe frequency, f_i , in our setup is about 4.7 GHz determined by the optical path length mismatch between the interferometer arms.

The instantaneous phase of $I_c(t_i^{(n)}, t_p^{(m)})$ can be readily retrieved from its analytic representation given by Hilbert transform, \mathcal{H} :

$$\begin{aligned} \angle I_c(t_i^{(n)}, t_p^{(m)}) &= \arg \left[I_c(t_i^{(n)}, t_p^{(m)}) + j \cdot \mathcal{H}\{I_c(t_i^{(n)}, t_p^{(m)})\} \right] \\ &= \frac{4\pi DL_f \Delta L}{t_i^{(n)} + D\lambda_c L_f} + \Delta\varphi_c(t_i^{(n)}, t_p^{(m)}) \end{aligned} \quad (17)$$

Here \arg means the argument of a complex number. A one-dimensional phase unwrapping algorithm followed by background phase removal gives the object phase shift,

$$\Delta\varphi_c(t_i^{(n)}, t_p^{(m)}) = \text{unwrap}\{\angle I_c(t_i^{(n)}, t_p^{(m)}) - \angle I_c(t_i^{(n)}, t_p^{(\text{empty})})\} \quad (18)$$

where $t_p^{(\text{empty})}$ corresponds to an empty pulse when no cell is in the field of view, i.e. background phase. The unwrapping algorithm used in our processing acts when the absolute phase difference between two consecutive samples of the signal is greater than or equal to π radians, and adds multiples of 2π to the following samples in order to bring the consecutive samples phase difference in the acceptable range of $-\pi$ to π .

To perform combined quantitative phase and intensity imaging, the phase derived by Hilbert transformation should be corrected to eliminate the artifacts caused by the intensity variations induced by the passing cells. Most cells of interest in clinical or industrial applications have a diameter 3–40 μm , when suspended in fluid. Given the field of view and the period of the interrogation rainbow pulses are 130 μm and 27 ns, respectively, the time duration of the instantaneous intensity change induced by the single cells in each laser pulse is about 0.6–8.3 ns, which will bring in frequency components up to about 1.2 GHz. Compared to the higher frequency components at 4.7 GHz corresponding to the interference fringes, the frequency of intensity variations is small (<1.2 GHz), and in this scenario, our method remains robust to separate the two electrical spectral components for optical loss and phase.

C. Cell Transmittance Extraction

One of the greatest advantage of TS-QPI is its ability to extract the cell transmittance, $T_s(\lambda)$, without prior knowledge of the transmittance of the solution, $T_s(\lambda)$, that of the beam-splitter, $T_b(\lambda)$, and the reflectance of substrate of the microfluidic channel, $R_m(\lambda)$. During measurements when there is no cell in the field of view (empty frames), Eq. 10 becomes

$$I_i(\lambda, t_p^{(empty)}) = -2[1 - T_b(\lambda)]T_b(\lambda)R_m(\lambda) \cdot T_s(\lambda)|\tilde{E}_{in}(\lambda, t_p^{(empty)})|^2 \quad (19)$$

In addition, the signal from only the reference arm can be recorded by blocking the sample arm:

$$I_r(\lambda, t_p) = [1 - T_b(\lambda)]^2 R_m(\lambda) |\tilde{E}_{in}(\lambda, t_p)|^2 \quad (20)$$

Combining Eq. 9, 19, and 20, and assuming that the input electric field pulse shape, $|\tilde{E}_{in}(\lambda, t_p)|$, is invariant to t_p , the cell transmittance can be derived as

$$T_c(\lambda) = \frac{-2\sqrt{I_r(\lambda, t_p) \cdot (I_b(\lambda, t_p) - I_r(\lambda, t_p))}}{I_i(\lambda, t_p^{(empty)})} \quad (21)$$

Please note that the values of $I_r(\lambda, t_p)$, $I_b(\lambda, t_p)$, and $I_i(\lambda, t_p^{(empty)})$ are directly measured by TS-QPI, and no prior knowledge of $T_b(\lambda)$, $T_s(\lambda)$, $R_m(\lambda)$, and $|\tilde{E}_{in}(\lambda, t_p)|$ is needed to calculate the cell transmittance.

D. Image Reconstruction

We reconstruct both quantitative brightfield and phase-contrast images simultaneously from single-shot frequency-multiplexed interferometric measurements. The envelope and phase of the time-domain signal $\tilde{I}_o(t_i^{(n)}, t_d^{(m)})$ was firstly mapped into series of spatial information $\tilde{I}_o(x^{(n)}, t_p^{(m)})$, forming a line scanning bright-field image and phase contrast image, illuminated by the optical pulse at time t_p (Fig. 2b). This is because within each optical pulse, the spatial information is mapped one-to-one into spectral domain, $x^{(n)} \rightarrow \lambda_n$, and spectrum is stretch in time, $\lambda_n \rightarrow t_i^{(n)}$, where $t_i^{(n)}$ is the relative group delay time of each frequency component within a pulse with respect to the central wavelength. These line-scan images based on $\tilde{I}_o(x, t_p^{(1)})$, $\tilde{I}_o(x, t_p^{(2)})$, $\tilde{I}_o(x, t_p^{(3)})$, ... were then cascaded into a two dimensional image corresponding to $\tilde{I}_o(x, y)$, where the second dimension y is the spatial mapping of time elapse based on object flow speed.

The optical path length difference image can be calculated by the phase shift line scans as

$$OPD(x^{(n)}, y^{(m)}) = \frac{\lambda(t_i^{(n)}, t_p^{(m)})}{2\pi} \Delta\varphi_c(t_i^{(n)}, t_p^{(m)}) \quad (22)$$

On the other hand, if the axial thickness of the cell at reconstructed image pixel (x, y) is $d(x, y)$,

$$OPD(x, y) = 2[n_{cell}(x, y) - n_{solution}(x, y)] \cdot d(x, y) \quad (23)$$

in which n_{cell} and $n_{solution}$ are the refractive indices of the cell and the surrounding buffer solution, respectively. The factor 2 is to account for the fact that each wavelength component passes the cell twice in Michelson interferometer.

If we integrate Eq. 23 over the area of the cell, we can derive an average refractive index contrast for the cell, which corresponds to the average protein concentration of the cell:

$$\overline{\Delta n_{cell}} = \overline{n_{cell} - n_{solution}} = \frac{\iint_{cell} OPD(x, y) dx dy}{2V} \quad (24)$$

where $\iint_{cell} t(x, y) dx dy$ is the volume of the cell obtained from its diameter, d , as $V \approx \pi d^3/6$.

The coefficient of from each cell corresponding to the intensity

The unit net change of intensity envelope variations induced by the cell is obtained from the amplitude of the slowly varying envelope feature of the interferogram as

$$\Delta I_b(\lambda, t_p) = \frac{T_b(\lambda)^2(1 - T_c(\lambda, t_p))}{[1 - T_b(\lambda)]^2 + T_b(\lambda)^2 T_s(\lambda)} \quad (25)$$

It gives the temporal and spatial information of the combined effects from absorption and scattering.

E. Big data analytics pipeline

The high-content image analysis and cell screening pipeline is implemented by combining multiple informatics tools, namely CellProfiler for image processing [6, 64], MySQL/MongoDB for database, Matlab for machine learning, and Javascript for interactive visualization. Feature extraction from quantitative phase and intensity images leads to multiplexed hyperdimensional analysis. When monitoring a large amount of cells, it is not initially trivial which features are statistically significant for cell classification. However, once the learning is performed, a ranking of the significance of the parameters can be helpful for future tasks on the same type of samples.

In our pipeline first of all, image noise reduction and smoothing have been performed. Smoothing can remove artifacts that are smaller than optical resolution limit. Then for object segmentation, which is one of the most challenging steps in image processing and highly influences the downstream classification, we use the Otsu's thresholding method. Since the ratio of the number of foreground (objects or cells) pixels to that of the background pixels is small, and it normally does not vary substantially from image to image), the histogram of pixel gray levels is fitted into two classes, corresponding to the background and foreground pixels. The optimum threshold is calculated so that the total weighted variance within both classes is minimized or intra-class variance is maximized. Once objects are identified in the image, morphology of each single cell can be described by area,

diameter, uniformity, aspect ratio, perimeter, number of surrounding clumped cells, etc.

The capability to identify clumped cells from single large cells greatly reduces the misclassification rate in imaging flow cytometry compared to traditional flow cytometry. Intensity peaks of pixel brightness within each object are used to distinguish clumped objects. The object centers are defined as local intensity maxima in the smoothed image. Retaining outlines of the identified objects helps validate and visualize the algorithm. In the next step, we discard the objects touching the borders of the image, i.e., the edges of the field of view and data acquisition time window. However, the chance of cells showing up at the edges is very low due to hydrodynamic focusing. We are also capable of excluding dust, noise, and debris by neglecting the objects that are too small or their aspect ratio is too extreme to be a cell. Size measurements are performed along both the rainbow direction and the cell flow direction. Measurement of the diameter along the rainbow direction is primarily used for cell classification since it helps to eliminate measurement inaccuracies caused by fluctuations of flow speed.

The intensity variations caused by cell absorption and scattering can be extracted from the pixel intensities in the optical loss images, and the phase shift caused by the cell protein concentration can be extracted from the pixel intensities in the phase contrast images. Due to limited optical resolution of the setup, the edges of bead or cell are blurred, generating the tails of point spread functions in phase and intensity signals outside of the diameter boundaries. In order to maximize the accuracy in morphological, phase, and loss measurements, after

object segmentation, we expanded the object boundaries by 2.5 μm (optical resolution of the setup measured by knife-edge method), which serve as loose boundaries, indicating the area within which the pixel intensities are measured and integrated in phase and transmission images. To calculate the refractive index, the axial diameter along the interrogation optical beam is assumed to be equal to the lateral diameter along the rainbow direction, since cells in suspension relax to a spherical shape due to surface tension.

F. Calibration of size measurement

To calibrate the imaging system and image processing pipelines for size measurement, 5 μm polystyrene beads (from Polysciences, Inc.) with NIST traceable particle size standards were analyzed. Size measurement of the polystyrene beads had a distribution with 5.06 μm expected mean and 0.5 μm standard deviation. The broadened standard deviation was within the range of optical resolution limit and was caused mainly by performing object recognition on resolution limited images.

G. Data cleaning

Data cleaning includes two steps. Firstly, Hotelling's T-squared distribution is calculated and top 2% of the extreme data was set as outliers due to experimental errors or object recognition errors. Secondly, debris discrimination is performed; any data point with negative phase shift was considered as either air bubble, flow turbulence, or object recognition errors.

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- [1] Howard M Shapiro, *Practical flow cytometry* (John Wiley & Sons, 2005).
 - [2] James V Watson, *Introduction to flow cytometry* (Cambridge University Press, 2004).
 - [3] Stephen P Perfetto, Pratip K Chattopadhyay, and Mario Roederer, "Seventeen-colour flow cytometry: unravelling the immune system," *Nature Reviews Immunology* **4**, 648–655 (2004).
 - [4] David A Basiji, William E Ortyn, Luchuan Liang, Vidya Venkatachalam, and Philip Morrissey, "Cellular image analysis and imaging by flow cytometry," *Clinics in laboratory medicine* **27**, 653–670 (2007).
 - [5] David A Basiji and William E Ortyn, "Imaging and analyzing parameters of small moving objects such as cells," (2001), uS Patent 6,249,341.
 - [6] Anne E Carpenter, Thouis R Jones, Michael R Lamprecht, Colin Clarke, In H Kang, Ola Friman, David A Guertin, Joo H Chang, Robert A Lindquist, Jason Moffat, *et al.*, "Cellprofiler: image analysis software for identifying and quantifying cell phenotypes," *Genome biology* **7**, R100 (2006).
 - [7] Behzad Razavi, *Principles of data conversion system design*, Vol. 126 (IEEE press New York, 1995).
 - [8] Ata Mahjoubfar, Keisuke Goda, Gary Betts, and Bahram Jalali, "Optically amplified detection for biomedical sensing and imaging," *JOSA A* **30**, 2124–2132 (2013).
 - [9] Keisuke Goda, Ali Ayazi, Daniel R Gossett, Jagannath Sadasivam, Cejo K Lonappan, Elodie Sollier, Ali M Fard, Soojung Claire Hur, Jost Adam, Coleman Murray, *et al.*, "High-throughput single-microparticle imaging flow analyzer," *Proceedings of the National Academy of Sciences* **109**, 11630–11635 (2012).
 - [10] Fabian Zanella, James B Lorens, and Wolfgang Link, "High content screening: seeing is believing," *Trends in biotechnology* **28**, 237–245 (2010).
 - [11] Ata Mahjoubfar, Claire Chen, Kayvan R Niazi, Shahrooz Rabizadeh, and Bahram Jalali, "Label-free high-throughput cell screening in flow," *Biomedical optics express* **4**, 1618–1625 (2013).
 - [12] K Goda and B Jalali, "Dispersive fourier transformation for fast continuous single-shot measurements," *Nature Photonics* **7**, 102–112 (2013).
 - [13] DR Solli, S Gupta, and B Jalali, "Optical phase recovery in the dispersive fourier transform," *Applied Physics Letters* **95**, 231108 (2009).

- [14] Keisuke Goda, Daniel R Solli, Kevin K Tsia, and Bahram Jalali, "Theory of amplified dispersive fourier transformation," *Physical Review A* **80**, 043821 (2009).
- [15] K Goda, KK Tsia, and B Jalali, "Serial time-encoded amplified imaging for real-time observation of fast dynamic phenomena," *Nature* **458**, 1145–1149 (2009).
- [16] A Mahjoubfar, C Chen, KR Niazi, S Rabizadeh, and B Jalali, "Label-free high-throughput imaging flow cytometry," in *SPIE LASE* (International Society for Optics and Photonics, 2014) pp. 89720F–89720F.
- [17] Olivier Gires, Christoph A Klein, and Patrick A Baeuerle, "On the abundance of epcam on cancer stem cells," *Nature Reviews Cancer* **9**, 143–143 (2009).
- [18] Jim Kling, "Beyond counting tumor cells," *Nature biotechnology* **30**, 578–580 (2012).
- [19] Sophie E Boddington, Elizabeth J Sutton, Tobias D Henning, Alexander J Nedopil, Barbara Sennino, Anne Kim, and Heike E Daldrup-Link, "Labeling human mesenchymal stem cells with fluorescent contrast agents: the biological impact," *Molecular Imaging and Biology* **13**, 3–9 (2011).
- [20] Takahiro Ikeda, Gabriel Popescu, Ramachandra R Dasari, and Michael S Feld, "Hilbert phase microscopy for investigating fast dynamics in transparent systems," *Optics letters* **30**, 1165–1167 (2005).
- [21] Gabriel Popescu, *Quantitative phase imaging of cells and tissues* (McGraw Hill Professional, 2011).
- [22] Hoa V Pham, Basanta Bhaduri, Krishnarao Tangella, Catherine Best-Popescu, and Gabriel Popescu, "Real time blood testing using quantitative phase imaging," *PloS one* **8**, e55676 (2013).
- [23] Ofer Feinerman, Joël Veiga, Jeffrey R Dorfman, Ronald N Germain, and Grégoire Altan-Bonnet, "Variability and robustness in t cell activation from regulated heterogeneity in protein levels," *Science* **321**, 1081–1084 (2008).
- [24] Alex Sigal, Ron Milo, Ariel Cohen, Naama Geva-Zatorsky, Yael Klein, Yuvalal Liron, Nitzan Rosenfeld, Tamar Danon, Natalie Perzov, and Uri Alon, "Variability and memory of protein levels in human cells," *Nature* **444**, 643–646 (2006).
- [25] Moritz Friebel, Klaus Do, Andreas Hahn, Gerhard Mu, *et al.*, "Optical properties of circulating human blood in the wavelength range 400–2500 nm," *Journal of biomedical optics* **4**, 36–46 (1999).
- [26] Giovanna Vona, Abdelmajid Sabile, Malek Louha, Veronique Sitruk, Serge Romana, Karin Schütze, Frédérique Capron, Dominique Franco, Mario Pazzaglia, Michel Vekemans, *et al.*, "Isolation by size of epithelial tumor cells: a new method for the immunomorphological and molecular characterization of circulating tumor cells," *The American journal of pathology* **156**, 57–63 (2000).
- [27] Sabrina L Spencer, Suzanne Gaudet, John G Albeck, John M Burke, and Peter K Sorger, "Non-genetic origins of cell-to-cell variability in trail-induced apoptosis," *Nature* **459**, 428–432 (2009).
- [28] Andrew P Bradley, "The use of the area under the roc curve in the evaluation of machine learning algorithms," *Pattern recognition* **30**, 1145–1159 (1997).
- [29] David Martin Powers, "Evaluation: from precision, recall and f-measure to roc, informedness, markedness and correlation," (2011).
- [30] Jin Huang and Charles X Ling, "Using auc and accuracy in evaluating learning algorithms," *Knowledge and Data Engineering, IEEE Transactions on* **17**, 299–310 (2005).
- [31] James B Knight, Ashvin Vishwanath, James P Brody, and Robert H Austin, "Hydrodynamic focusing on a silicon chip: mixing nanoliters in microseconds," *Physical Review Letters* **80**, 3863 (1998).
- [32] Gwo-Bin Lee, Chih-Chang Chang, Sung-Bin Huang, and Ruey-Jen Yang, "The hydrodynamic focusing effect inside rectangular microchannels," *Journal of Micromechanics and Microengineering* **16**, 1024 (2006).
- [33] Frederick W King, *Hilbert transforms*, Vol. 2 (Cambridge University Press Cambridge, UK, 2009).
- [34] Meghan K Driscoll, Jason L Albanese, Zheng-Mei Xiong, Mitch Mailman, Wolfgang Losert, and Kan Cao, "Automated image analysis of nuclear shape: What can we learn from a prematurely aged cell?" *Aging (Albany NY)* **4**, 119 (2012).
- [35] R Barer, KF Ross, and S Tkaczyk, "Refractometry of living cells," *Nature* **171**, 720 (1953).
- [36] JP Revel, Pamela Hoch, and D Ho, "Adhesion of culture cells to their substratum," *Experimental cell research* **84**, 207–218 (1974).
- [37] P Whur, H Koppel, CM Urquhart, and DC Williams, "Substrate retention of fractured retraction fibres during detachment of trypsinized bhk21 fibroblasts," *Journal of cell science* **24**, 265–273 (1977).
- [38] Ingrid Spadinger, Steven SS Poon, and Branko Palcic, "Effect of focus on cell detection and recognition by the cell analyzer," *Cytometry* **11**, 460–467 (1990).
- [39] André A Adams, Paul I Okagbare, Juan Feng, Matuesz L Hupert, Don Patterson, Jost Gottert, Robin L McCarley, Dimitris Nikitopoulos, Michael C Murphy, and Steven A Soper, "Highly efficient circulating tumor cell isolation from whole blood and label-free enumeration using polymer-based microfluidics with an integrated conductivity sensor," *Journal of the American Chemical Society* **130**, 8633–8641 (2008).
- [40] Sunitha Nagrath, Lecia V Sequist, Shyamala Maheswaran, Daphne W Bell, Daniel Irimia, Lindsey Ulkus, Matthew R Smith, Eunice L Kwak, Subba Digumarthy, Alona Muzikansky, *et al.*, "Isolation of rare circulating tumour cells in cancer patients by microchip technology," *Nature* **450**, 1235–1239 (2007).
- [41] Daniel R Gossett, Westbrook M Weaver, Albert J Mach, Soojung Claire Hur, Henry Tat Kwong Tse, Wonhee Lee, Hamed Amini, and Dino Di Carlo, "Label-free cell separation and sorting in microfluidic systems," *Analytical and bioanalytical chemistry* **397**, 3249–3267 (2010).
- [42] Mads Kærn, Timothy C Elston, William J Blake, and James J Collins, "Stochasticity in gene expression: from theories to phenotypes," *Nature Reviews Genetics* **6**, 451–464 (2005).
- [43] Narendra Maheshri and Erin K O'Shea, "Living with noisy genes: how cells function reliably with inherent variability in gene expression," *Annu. Rev. Biophys. Biomol. Struct.* **36**, 413–434 (2007).
- [44] Thomas A Zangle, Michael A Teitell, and Jason Reed, "Live cell interferometry quantifies dynamics of biomass partitioning during cytokinesis," *PloS one* **9**, e115726 (2014).
- [45] Iain G Johnston, Bernadett Gaal, Ricardo Pires das Neves, Tariq Enver, Francisco J Iborra, and Nick S Jones, "Mitochondrial variability as a source of extrinsic

- cellular noise,” PLoS computational biology **8**, e1002416 (2012).
- [46] Yaser S Abu-Mostafa, Malik Magdon-Ismail, and Hsuan-Tien Lin, *Learning from data* (AMLBook, 2012).
- [47] Christopher M Bishop *et al.*, *Pattern recognition and machine learning*, Vol. 4 (springer New York, 2006).
- [48] Lynne Boddy, CW Morris, MF Wilkins, GA Tarran, and PH Burkhill, “Neural network analysis of flow cytometric data for 40 marine phytoplankton species,” Cytometry **15**, 283–293 (1994).
- [49] Yann LeCun, Yoshua Bengio, and Geoffrey Hinton, “Deep learning,” Nature **521**, 436–444 (2015).
- [50] Jürgen Schmidhuber, “Deep learning in neural networks: An overview,” Neural Networks **61**, 85–117 (2015).
- [51] James A Hanley and Barbara J McNeil, “The meaning and use of the area under a receiver operating characteristic (roc) curve.” Radiology **143**, 29–36 (1982).
- [52] Charles X Ling, Jin Huang, and Harry Zhang, “Auc: a statistically consistent and more discriminating measure than accuracy,” in *IJCAI*, Vol. 3 (2003) pp. 519–524.
- [53] Corinna Cortes and Mehryar Mohri, “Auc optimization vs. error rate minimization,” Advances in neural information processing systems **16**, 313–320 (2004).
- [54] Zhenqiu Liu and Ming Tan, “Roc-based utility function maximization for feature selection and classification with applications to high-dimensional protease data,” Biometrics **64**, 1155–1161 (2008).
- [55] Herman Verrelst, Yves Moreau, Joos Vandewalle, and Dirk Timmerman, “Use of a multi-layer perceptron to predict malignancy in ovarian tumors,” Advances in Neural Information Processing Systems , 978–984 (1998).
- [56] Sabeeha S Merchant, Janette Kropat, Bensheng Liu, Johnathan Shaw, and Jaruswan Warakanont, “Tag, you’re it! chlamydomonas as a reference organism for understanding algal triacylglycerol accumulation,” Current opinion in biotechnology **23**, 352–363 (2012).
- [57] Christophe Zabawinski, Nathalie Van Den Koornhuyse, Christophe D’Hulst, Ralf Schlichting, Christoph Giersch, Brigitte Delrue, Jean-Marie Lacroix, Jack Preiss, and Steven Ball, “Starchless mutants of chlamydomonas reinhardtii lack the small subunit of a heterotetrameric adp-glucose pyrophosphorylase,” Journal of Bacteriology **183**, 1069–1077 (2001).
- [58] Victoria H Work, Randor Radakovits, Robert E Jinkerson, Jonathan E Meuser, Lee G Elliott, David J Vinyard, Lieve ML Laurens, G Charles Dismukes, and Matthew C Posewitz, “Increased lipid accumulation in the chlamydomonas reinhardtii sta7-10 starchless isoamylase mutant and increased carbohydrate synthesis in complemented strains,” Eukaryotic Cell **9**, 1251–1261 (2010).
- [59] Yantao Li, Danxiang Han, Guongrong Hu, David Dauvillee, Milton Sommerfeld, Steven Ball, and Qiang Hu, “Chlamydomonas starchless mutant defective in adp-glucose pyrophosphorylase hyper-accumulates triacylglycerol,” Metabolic engineering **12**, 387–391 (2010).
- [60] Ursula Goodenough, Ian Blaby, David Casero, Sean D Gallaher, Carrie Goodson, Shannon Johnson, Jae-Hyeok Lee, Sabeeha S Merchant, Matteo Pellegrini, Robyn Roth, *et al.*, “The path to triacylglyceride obesity in the staf strain of chlamydomonas reinhardtii,” Eukaryotic cell , EC-00013 (2014).
- [61] Ian K Blaby, Anne G Glaesener, Tabea Mettler, Sorel T Fitz-Gibbon, Sean D Gallaher, Bensheng Liu, Nanette R Boyle, Janette Kropat, Mark Stitt, Shannon Johnson, *et al.*, “Systems-level analysis of nitrogen starvation-induced modifications of carbon metabolism in a chlamydomonas reinhardtii starchless mutant,” The Plant Cell Online **25**, 4305–4323 (2013).
- [62] Brandon W Buckley, Asad M Madni, and Bahram Jalali, “Coherent time-stretch transformation for real-time capture of wideband signals,” Optics express **21**, 21618–21627 (2013).
- [63] Peter TS DeVore, Brandon W Buckley, Mohammad H Asghari, Daniel R Solli, and Bahram Jalali, “Coherent time-stretch transform for near-field spectroscopy,” IEEE Photonics Journal **6**, 3300107 (2014).
- [64] Lee Kamensky, Thouis R Jones, Adam Fraser, Mark-Anthony Bray, David J Logan, Katherine L Madden, Vebjorn Ljosa, Curtis Rueden, Kevin W Eliceiri, and Anne E Carpenter, “Improved structure, function and compatibility for cellprofiler: modular high-throughput image analysis software,” Bioinformatics **27**, 1179–1180 (2011).

SUPPLEMENTARY

Supplementary methods.

Supplementary movies:

Video 1: video of *OT-II* hybridoma T-lymphocytes flowing in a microfluidic channel; The cells are aligned at the center of the channel by hydrodynamic focusing. Optical path difference measured at four of the interrogation points on the rainbow flash is shown as a function of time in the right panels.

Video 2: video of *SW-480* colon cancer epithelial cells flowing in a microfluidic channel; The cells are aligned at the center of the channel by hydrodynamic focusing. Optical path difference measured at four of the interrogation points on the rainbow flash is shown as a function of time in the right panels.

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AUTHOR CONTRIBUTIONS STATEMENT

C.L.C and A.M. conceived the classification method; A.M. and C.L.C. designed TS-QPI system and performed experiments; C.L.C. and A.M. collected the data, processed the signals, and analyzed the results; A.M., L.C.T. and A.H. designed and fabricated the microfluidic channels. I.K.B. provided algal cell samples; K.R.N. provided *SW-480* and *OT-II* cell samples; C.L.C., A.M., B.J., I.K.B., and K.R.N. prepared the manuscript.

ADDITIONAL INFORMATION

The authors declare no competing financial interests.

TABLE I. List of extracted features

Feature Name	Description	Category
Diameter-RB	Diameter along the interrogation rainbow. It is insensitive to flow rate fluctuation. For higher accuracy, it is calibrated by the spatial nonuniform distribution of rainbow wavelengths.	Morphology
Diameter-FL	Diameter along the flow direction. It is sensitive to flow rate fluctuation, but can be a candidate parameter for monitoring flow speed and channel condition.	Morphology
Tight Area	Total number of pixels in the segmented region in the phase image	Morphology
Perimeter	Total number of pixels around the boundary of each segmented region	Morphology
Circularity	$4\pi \text{Area}/\text{Perimeter}^2$	Morphology
Major Axis	Considering the cell as elliptical in lateral imaging plane, the length of the major axis of the ellipse with a normalized second central moment same as the cell.	Morphology
Orientation	Angle between the flow direction and the major axis of the cell elliptical shape	Morphology
Loose Area	Total number of pixels in the expanded segmented region for measurement of the pixel intensities	Morphology
Median Radius	The median distance of any pixel in the object to the closest pixel outside of the object.	Morphology
OPD-1	Integrated optical path length difference within the entire segmented area (cell), calibrated by the power distribution within different wavelength components of the incident laser pulses.	Optical Phase
OPD-2	Integrated optical path length difference within the entire segmented area (cell). In addition to the calibration of OPD-1, it is calibrated by the pulse-to-pulse fluctuations within a 1 μs detection window.	Optical Phase
Refractive index	The mean refractive index difference between the object and the surrounding liquid (buffer solution), which is calculated based on OPD-2 and size measurement (see detail in Section III). Refractive index difference for cells is proportional to their protein concentration.	Optical Phase
Absorption-1	Mean absorption coefficient within the entire segmented area (cell). It is calibrated by the power distribution within different wavelength components of the incident laser pulses and by the pulse-to-pulse fluctuations within a 1 μs detection window. This parameter corresponds to an absorption-dominant model for the cell.	Optical Loss
Absorption-2	Mean absolute absorption coefficient within the entire segmented area (cell). It is calibrated by the power distribution within different wavelength components of the incident laser pulses and by the pulse-to-pulse fluctuations within a 1 μs detection window. This parameter corresponds to an absorption-dominant model for the cell.	Optical Loss
Scattering-1	Mean optical loss within the entire segmented area (cell). It is calibrated by the power distribution within different wavelength components of the incident laser pulses and by the pulse-to-pulse fluctuations within a 1 μs detection window. This parameter corresponds to a scattering-dominant model for the cell.	Optical Loss
Scattering-2	Mean absolute optical loss within the entire segmented area (cell). It is calibrated by the power distribution within different wavelength components of the incident laser pulses and by the pulse-to-pulse fluctuations within a 1 μs detection window. This parameter corresponds to a scattering-dominant model for the cell.	Optical Loss