Label-Free Assessment of the Drug Resistance of Epithelial Ovarian Cancer Cells in a Microfluidic Holographic Flow Cytometer Boosted through Machine Learning

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ABSTRACT: About 75% of epithelial ovarian cancer (EOC) patients suffer from relapsing and develop drug resistance after primary chemotherapy. The commonly used clinical examinations and biological tumor tissue models for chemotherapeutic sensitivity are time-consuming and expensive. Research studies showed that the cell morphology-based method is promising to be a new route for chemotherapeutic sensitivity evaluation. Here, we offer how the drug resistance of EOC cells can be assessed through a label-free and high-throughput microfluidic flow cytometer equipped with a digital holographic microscope reinforced by machine learning. It is the first time that such type of assessment is performed to the best of our knowledge. Several morphologic and texture features at a single-cell level have been extracted from the quantitative phase images. In addition, we compared four common machine learning algorithms, including naive Bayes, decision tree, K-nearest neighbors, support vector machine (SVM), and fully connected network. The result shows that the SVM classifier achieves the optimal performance with an accuracy of 92.2% and an area under the curve of 0.96. This study demonstrates that the proposed method achieves high-accuracy, high-throughput, and label-free assessment of the drug resistance of EOC cells. Furthermore, it reflects strong potentialities to develop data-driven individualized chemotherapy treatments in the future.

1. INTRODUCTION

The platinum-based combined chemotherapy is the important adjuvant treatment for epithelial ovarian cancer (EOC). However, after primary chemotherapy, nearly 25% of EOC patients will be native resistant to chemotherapy, and the remaining 75% will suffer from relapsing, which eventually develops into drug resistance. According to the National Comprehensive Cancer Network (NCCN) standard-based platinum-free interval (PFI), the recurrent EOCs are classified into two types: patients with PFI longer than 6 months are regarded as “Platinum sensitive”, while those with PFI shorter than 6 months are regarded as “Platinum resistant”. Currently, once drug resistance is developed, recurrent EOC will be suffered from different side effects, high cost, and low curative for single-agent chemotherapy using liposome adriamycin, albumin-bound paclitaxel (Abraxane), or docetaxel. Therefore, drug resistance of EOC will seriously impact the survival rate and prognosis of patients, and it will be of great importance to accurately evaluate drug sensitivity and drug resistance before chemotherapy for patients and the implementation of individualized therapy. Nevertheless, an effective evaluating system for chemotherapeutic sensitivity is still lacking at present.

The current clinical methods for evaluating the chemotherapeutic sensitivity of EOC patients are mainly based on clinical auxiliary examinations and detection such as tumor marker detection and diagnostic imaging test, which include functional imaging technology such as position emission tomography (PET-CT). Based on the differences in carbohydrate intake between tumor tissues and normal tissues, PET-CT could track the process of metabolism and proliferation of tumor tissues, but it has a relatively lower imaging resolution. The biological model of tumor tissues is another common method to access chemotherapeutic sensitivity. In this method, tumor tissues or purified tumor cell lines are transplanted subcutaneously into nude mice, and the chemotherapeutic sensitivity of primary tumors is accessed by observing the reactions of tumors in nude mice to

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chemotherapy drugs. However, the clinical application of this method is constricted for its low success rate of modeling, high cost, and being time-consuming.

It is well known that the development of drug resistance in cancer cells is accompanied by unique morphological changes because cancer cell morphology (MP) is closely related to their phenotype and activity. It has been observed that the drug-resistant breast cancer cells (MCF7) will be larger with less defined irregular, round-shaped, and contained nuclei in the cytoplasm compared to drug-sensitive breast cancer cells. Moreover, during the development of cisplatin resistance, the breast cancer cells change with the enlargement of cell size, the increase of nucleolus and cytoplasm ratio, and the increase in the number of microvascular and cytoplasmic granules. It was found that there was a correlation between the topological structure of the membrane and the drug resistance of colon cancer cells (HCT8), that is, the more diffusive the shape, the more resistant the cancer cells. There was a significant difference in the MP of parental lung cancer cells (A549) and etoposide-resistant cells (A549RT-eto). The A549 cells show an epithelioid-like shape, whereas the MP of A549RT-eto cells changed to a spindle-like shape, varying in size. The investigation revealed morphological differences between the Adriamycin-resistant leukemia cells (P388/ADR) and Adriamycin-sensitive (P388/0) cells. P388/ADR cells showed longer villus-like protrusions and large folding of the plasma cell membrane, whereas P388/0 cells had only short membrane protrusions. It was confirmed that the drug-sensitive pancreatic cancer cells were uniform in shape, whereas the drug-resistant cells were more irregular in shape. It was demonstrated that for two different types of breast cancer cells (MDA-MB-231 and MCF-7), the morphological parameter, such as the area ratio of the nucleus and cytoplasm, correlated intimately with the drug sensitivity. The drug resistance is increased with a decrease in the proportion. The drug-resistant EOC cells (ACRP) have an elongated, irregular fibroblastoid MP. In contrast, the drug-sensitive EOC cells (A2780) had a round shape, such as an epithelial cobblestone appearance. These results support the idea of following a cancer cell transition toward a drug-resistant phenotype through morphological monitoring. Therefore, the shape analysis of cell MP brings important information about drug resistance.

Microscopy techniques are usually employed in the cell MP investigation, including Zernike phase contrast microscopy (ZPCM), differential interferometric contrast microscopy (DICM), fluorescence microscopy (FM), electron microscopy (EM), atomic force microscopy (AFM), and flow cytometry (FC). Since live cells are transparent and difficult to observe under a normal bright-field microscope, ZPCM and DICM can produce high-contrast images of living cells by translating the phase difference into amplitude difference. However, these two techniques are not inherently quantitative, thus presenting limitations for minute signal variations in terms of the corresponding cellular morphometry. FM, such as confocal fluorescent laser scanning microscopy, can identify cells and sub-microscopic cellular components with a high degree of specificity by applying fluorescent probes. However, the use of fluorophores is associated with undesirable side effects which damage biological cells. EM, including transmission EM (TEM) and scanning EM (SEM), can examine the structure of cells down to molecular detail by using an electron beam. However, EM cannot observe live cells because the samples have to be placed in high-vacuum conditions. AFM enables the production of cell topographic images with a resolution on the order of a nanometer. However, due to time-consuming scanning procedures, it has prevented applications for real-time imaging of cells. FC is a widespread and powerful technique employed in cell counting, biomarker detection, and cell sorting in a high-speed manner. In general, this technique is developed to measure multiple scatter or fluorescence parameters. The light scattered from a single cell can indicate the relative size and granularity of the cell and other simple morphological information. However, the scattered light intensity (both forward and side) is strongly dependent on the position of the cells in the microchannel. Hence, the intensity of scattered light will dramatically change when the cells locate the different depths, which would seriously affect the measurement reliability. Therefore, some external force would have to act orthogonally to the main flow direction, for example, from magnetic and electric fields, acoustic waves, and hydrodynamic shear flows. However, all of these strategies require extra components in the microfluidic device and complex channel geometries. In another work, the author reported a purely viscoelastic focusing method to achieve 3D focusing in a square-shaped microchannel without extra components. However, viscosity of the suspending fluid, flow rate, and channel dimension should be strictly taken into account. On the other hand, if instead the cells are labeled with chemical markers, the fluorescent light can be emitted and detected to investigate specific cellular substances. For investigating the drug resistance of cancer cells, specific fluorescent protein markers are necessary. Unfortunately, for assessing the drug resistance of EOC cells, the clinical protein marker is still under study, and consequently, a specific fluorescent antibody must also be developed. At the current state of the art, FC cannot be employed to assess the drug resistance of EOC cells.

Digital holographic (DH) microscopy is a quantitative phase imaging technique that can provide rich intracellular information non-invasively by the changing cell’s intrinsic properties. It can identify label-free cells based on the refractive index (RI) contrast, without using exogenous contrast agents. The RI of the cell is an inherent optical parameter that describes the optical path difference of the light passing across the cell, which is correlated with cell biophysical properties. Holographic imaging allows retrieving the rich information of the phase-contrast map (PCM), which makes it substantially different from most of the other sensors that collect and retrieve the sole light intensity. Quantitative PCMs supply the whole intracellular dry mass and concentration of the cell besides valuable information about the MP, thus allowing the identification of all-optical fingerprints directly connected with biophysical parameters. The ability of numerical refocusing also makes DH a valuable microscopy tool being unique and optimal when there is a need for imaging cells flowing in microfluidic channels, that is, a 3D volume. DH allows a posteriori sharp focusing also in the case of cells being captured out of focus. Therefore, the combination of DH microscopy with FC principles can enable significant enhancement of automatic label-free single-cell assessment in cell analysis based on morphological cues. Chen et al. classified label-free white blood T-cells against colon cancer cells with high-throughput quantitative imaging enabled by photonic time stretch. Roitshtain et al. applied stain-free imaging flow cytometry to identify live healthy and
cancerous cells, primary tumor cells, and metastatic cancer cells using both machine learning and deep learning. Min et al. analyzed two types of pancreatic tumor cells by combining a DH microscopy system with a common analyzed two types of pancreatic tumor cells by combining a DH microscopy system with a common flow cytometer. Lee and Ugele et al. developed FC-based quantitative phase imaging platform to classify multiple types of label-free human leukemic cells. Nissim and Dudaia et al. used FC based on DH microscopy and machine learning to classify untreated cancer cells in blood. Singh et al. introduced an in-line DHM approach for label-free analysis of liquid-biopsy samples, which can enrich tumor cells in blood. Bianco et al. designed a pocket holographic slide that allows building an interferometer directly on the microfluidic chip without bulky optical components. Merola et al. demonstrated that it could achieve single-cell interferometric tomography for red blood cells by exploiting the rolling of cells while they flow along a microfluidic channel, while Villone et al. proofed in-flow tomography also for cancer breast cells. On the other side, it is foreseen that artificial intelligence can be a valuable tool for analyzing data from FC and, more in general, for Lab-on-Chip devices.

In this study, for the first time, we detect the drug resistance of EOC cells by combining FC and DH microscopy by boosting the quantitative phase imaging (QPI) feature of DH thanks to machine learning methods. We demonstrate the possibility to classify with high classification accuracy cells thanks to morphological biomarkers, that is, quantitative features about MP parameters. The high-accuracy, high-throughput, and label-free classification ability of this approach can greatly simplify the sensitivity detection process of cancer cells with different drug resistance. In this assessment, holograms of living cells flowing in a microfluidic chip are dynamically acquired by an off-axis DH system. Then, the phase maps of each cell are reconstructed digitally from these holograms. The MP and texture features are calculated based on these quantitative phase maps of cancer cells (A2780 and A2780CisR cells) and act as biologically independent information for identifying cancer cells with different drug resistance. With the achievement of high-accuracy, high-throughput, and label-free classification of cancer cells with different drug resistances, our approach is expected to provide an adjuvant evaluation of clinical chemotherapy in the near future.

2. METHOD

2.1. Sensitive and Resistant EOC Cell Preparation. Two types of EOC cells, A2780 cells and A2780-CisR cells, were studied in this work. A2780 cells were from the ATCC (American Type Culture Collection) and preserved in the Obstetrics and Gynecology Laboratory of Peking University People’s Hospital. The drug-resistant characteristic of A2780-CisR cells was obtained by exposing A2780 cells to different concentrations of platin for 10 months. A2780 and A2780-CisR cells were cultured and grown in 1640 (RPMI Medium 1640 basic 1X, GibCO, China) supplemented with 1-glutamine, 15 mM HEPES, and 10% fetal bovine serum (Gibco 10099-141, Australia). Cells were seeded in a 100 mm glass Wilson dish at a density of $1.7 \times 10^6$ cells for 24 h with 5% CO₂ and 37 °C. After the treatment, the cells were collected from wells using trypsin digestion, washed in phosphate-buffered saline (PBS) to dilute to half the concentration (about $5 \times 10^5$), and then suspended in 500 μL of 1x binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl₂, pH 7.4) with 10 μL of annexin conjugate and propidium iodide (PI).

2.2. DH FC System Based on Microfluidics. We built a DHFC system to capture the wave field of cancer cells rapidly. As shown in Figure 1, we used a Mach–Zehnder imaging interferometer with an off-axis configuration. This system enables the reconstruction of the object’s complex wave front from a single camera exposure, making it suitable for the acquisition of rapid dynamics of flowing cells. A coherent laser (532 nm, 100 mW) acts as a source and an output with a single-mode fiber. The plane wave is produced by a collimating lens (L) and then split into the object beam and the reference beam by a polarizing beam splitter (PBS). The half-wave plate (HPW1) in front of the PBS is used to adjust the intensity ratio between the two beams. There is another half-wave plate (HPW2) in the reference arm to make the polarization direction of the reference wave consistent with the object wave. The infinity-corrected microscopic imaging configuration is arranged in the object arm. In this optical configuration, the object is placed on the front focal plane of an infinity-corrected microscopy object (MO, 20x, NA = 0.5), and a tube lens (TL, $f = 180$ mm) is placed after the MO, and a real image is created at its focal plane. In this case, the magnification is the ratio of the focal length of TL and that of MO. Moreover, a condenser lens (CL) is added to focus the illumination beam to prevent parasitic diffraction for high-magnification objectives. It is worth noting that we finely adjust the relative

![Figure 1. (a) DHFC system setup. (b) Cell-flowing devices of the microfluidic chip and the syringe pump.](https://doi.org/10.1021/acsomega.1c04204)
positions between the CL, MO, and TL to make the wave after the TL a plane wave. Specifically, the distance between the CL and the MO is slightly less than the sum of their focal lengths and the front focus of the TL is between the MO and its focus. In this arrangement, the object wave is a quasi-plane wave, and its curvature matches with the reference wave. The object wave is then combined with the reference wave in the beam splitter (BS) at a small angle. The holograms are recorded using a CCD camera (5.86 μm, 1024 × 1024, PointGrey, Canada) controlled by the FlyCapture Software exploited by FLIR Systems Company. The off-axis hologram has approximate straight fringe interference pattern, and the camera sampling capacity is exploited to the maximum.

In this system, a microfluidic PMMA chip (Microfluidic-chip shop, Fluidic 144, Germany) is utilized to flow the cell solution. The straight channel on the chip is 100 μm in width and depth and 58.5 mm in length. The cell solution flows in the channel from the left (the inlet) to the right (the outlet) driven by a syringe pump system. Before flowing through the channel, the solution is first stored in a syringe with a volume of 15 mL, and the observed cells are collected by a test tube. To ensure that every single cell is imaged in the camera and reduce as much as possible duplicate cell imaging, the speed of the pump is set as 1 μL/min, the frame rate of the camera is 25 fps, and the exposure time is 2 ms. As the concentration of cell solution is about 2 × 10^6/mL, the cell throughput is about 200 cells/min.

2.3. Holographic Reconstruction and Features’ Extraction. Once the digital holograms have been recorded, the cell phase image can be digitally retrieved by numerical reconstruction. First, two pre-processing operations are implemented, consisting of hologram apodization and spatial filtering. The hologram apodization is to avoid diffraction ripples on the wave front due to the non-infinite nature of the CCD chip. It is achieved by multiplying the hologram by a two-dimensional cubic spline interpolation function. The hologram spatial filtering is applied for isolating the virtual image and zero-order image in the off-axis hologram. It is achieved by multiplying the hologram spatial frequency spectra by a Fourier mask acting as a band-pass spatial filter. Second, the angle spectrum reconstruction algorithm is employed to implement the numerical propagation, keeping the object image size constant regardless of the propagation distance. Furthermore, to obtain an in-focus and sharp object image, an optimal propagation distance needs to be found by automatic focus. In this process, the propagation calculation is repeated with a variation of the propagation distance. At the same time, a criterion coefficient is calculated to search the optimal distance by determining the maximization of the image sharpness. It needs to note that due to cells located at different depths when flowing in the microchannel, each cell has a unique propagation distance of in-focus imaging. Therefore, the searching process is implemented for each cell in the field of view, which can be recognized and segmented using the image processing algorithm. Third, to obtain the absolute phase image of cells, the phase aberrations caused by the microscope object, the imperfections of optical components, and the construction of the experimental system have to be eliminated from the reconstructions. Here, a numerical method based on numerical parametric lenses (NPL) is employed to compensate for all these aberrations. The NPL shape describes these aberrations and can be defined by Zernike polynomial models. The polynomial coefficients can be automatically determined by performing a two-dimensional phase fitting in background (no object) areas of reconstructions. The phase map only associated with the object is retrieved by multiplying the reconstruction by the conjugation of NPL. Due to the phase value reconstructed from the hologram constrained between −π and π, the continuous phase map of the object can be retrieved by phase unwrapping and used for the following morphological analysis. The software of Matlab R2020b is employed to program for implementing holographic reconstruction on a personal computer (CPU: i7-11700K, 3.6 GHz, Intel). On average, it consumes 0.25 s for reconstructing a phase image of a single cell.

The optical phase images contain the biophysical fingerprints of living cells. Thus, a feature extraction operation is used to calculate the biophysical features of single cells. After that, every cell is represented by several independent features as the basis of data analysis. Due to the digital focusing ability of DH, each cell from different depths of the microchannel can be reconstructed precisely so that the feature data of cell phase images are extremely accurate, which is an important advantage for cell classification.

All the 20 features extracted from cell phase images are shown in Table 1. There are three types of features extracted from the optical phase images, and the information provided by each of them is different. Morphology represents the outline shape feature of the suspended cells. Besides, due to the three-dimensional quantitative imaging ability of DHM, the optical length distribution of cells can be calculated from the phase image. Thus, OPL refers to the statistical characteristics of the optical path length (OPL) within the cell area. In addition, the statistics based on the gray-level co-occurrence matrix (GLCM) are used to describe the texture features of the cell phase. Different classification performances of these feature parameters are researched.

2.4. Machine Learning Algorithms for Cell Classification. Machine learning is a method of data analysis that automates model building through experience. So far, it has progressed dramatically and is employed in many applications. The classification process categorizes a given set of data into classes called labels or categories. The classification model is established by approximating a mapping function from input variables to output labels. Many machine learning algorithms have been proposed to solve the classification problem. To find the optimal algorithm for the assessment of drug resistance of cancer cells, the four commonly used machine learning algorithms are investigated in this work, such as naive Bayes (NB), decision tree (DT), K-nearest neighbors (KNN), support vector machine (SVM), and fully connected network (FCN).

NB is one of the statistical learning algorithms which provide the probability of an input data set belonging to each class, rather than just a class label. This classifier learns the conditional probability of each sample given the class label C from training data and then applies the Bayes rule to compute the probability of C. The final predicting class possesses the highest probability. In practical applications, the NB model assumes that a particular feature variable is independent of any other variables and uses the maximum likelihood method for parameter estimation. The advantage of NB is that it only requires a small number of training data to estimate the parameters for classification.
DT algorithm is a logic-based technique. The structure of the DT is top-down recursive, like a flowchart. The elements of a DT are nodes and branches. The nodes include the root node that divides the tree into two or more subsets, internal nodes representing one of the possible choices within the tree structure, and the final leaf nodes representing the tree’s decisions. Each branch represents one of the courses of action available at that point. The path from the root nodes through internal nodes to leaf nodes indicates the classification rule. As DT is non-parametric, it can cope with large data sets without imposing a complicated parametric structure. In this work, the boosted tree method is used to achieve classification by building multiple DTs.

KNN is a lazy-learning algorithm in which it delays the induction or generalization process until classification is performed. Lazy-learning algorithms require less training time during the learning process than eager-learning algorithms, such as NB. The classification of KNN is based on the principle that the samples within the same data set are generally close to other samples with similar properties. The class label of a test sample can be determined by observing the class of its nearest neighbors and identifying the most common class label. In the classification phase of KNN, the normalization of training data can improve its accuracy dramatically.

SVM is another statistical learning algorithm, and it has become an exceedingly popular machine learning method. An SVM model maps the samples as points in space, and the goal of the SVM is to find a hyperplane in space that distinctly separates the two groups of points. As the data are linearly separable, the separating hyperplane is easy to find, and data points closest to the hyperplane are called support vectors, which allow maximizing the margin of the classifier. However, most real-world data are non-separable. Thus, no hyperplane exists that successfully separates the two data points. In this case, SVM can efficiently perform a non-linear classification by applying the kernel trick to map the data into a higher-dimensional feature space and find a separating hyperplane.

FCN consists of a series of fully connected layer, input layer, output layer, and multiple hidden layers, which is the most basic neural network structure. Each node of the fully connected layer is connected to all nodes of the previous layer. The fully connected layer was mainly used to extract features and classification in the early stage. However, as all outputs and inputs of the fully connected layer are connected, the parameters are generally large, which requires a considerable amount of storage and computing space. Because of the redundancy of parameters, FCN is seldom applied to complex scenes and is mainly used to classify extracted features. To avoid overfitting, the hidden layer of FCN used in this paper is 3 and the number of epochs is 300.

In this work, the performance of these four types of classifiers will be tested. Classification accuracy, specificity, sensitivity, and the area under the curve (AUC) of the receiver operating characteristic (ROC) curve are utilized to evaluate the performances of machine learning classifiers.

\[
\text{Accuracy} = \frac{TP + TN}{TP + FP + TN + FN} \\
\text{Specificity} = \frac{TN}{TP + FN} \\
\text{Sensitivity} = \frac{TP}{TP + FN}
\]

### Table 1. Features Extracted from Quantitative Phase Images of Single Cells

| feature name | description | category |
|--------------|-------------|----------|
| area | total number of pixels in the segmented region in the phase image | MP |
| perimeter | total number of pixels around the boundary of each segmented region | MP |
| major axis | the length of the major axis of the ellipse with a normalized second central moment | MP |
| minor axis | the length of the minor axis of the ellipse with a normalized second central moment | MP |
| equivalent diameter | distance of diameter corresponding to the area of the region | MP |
| max OPL | the max phase value of the pixel within the region in the phase image | OPL |
| mean OPL | the mean phase value of the pixel within the region in the phase image | OPL |
| median OPL | the median phase value of the pixel within the region in the phase image | OPL |
| OPL variance | the variance of all phase values in the phase image | OPL |
| OPL gradient | average of gradient obtained by successive subtraction of image phase | OPL |
| contrast | \[ \sum_{i=1}^{N} \sum_{j=1}^{N} (i - j)^2 p(i, j) \] | GLCM |
| correlation | \[ \sum_{i=1}^{N} \sum_{j=1}^{N} \left( \frac{j - \mu_y}{\sigma_y} \right) \left( \frac{i - \mu_x}{\sigma_x} \right) p(i, j) \] | GLCM |
| difference entropy | \[ -\sum_{k=0}^{N-1} p_{xy}(k) \log p_{xy}(k) \] | GLCM |
| difference variance | \[ \sum_{k=0}^{N-1} (k - \mu_{xy})^2 p_{xy}(k) \] | GLCM |
| dissimilarity | \[ \sum_{i=1}^{N} \sum_{j=1}^{N} \left| i - j \right| \beta p(i, j) \] | GLCM |
| energy | \[ \sum_{i=1}^{N} \sum_{j=1}^{N} p(i, j)^2 \] | GLCM |
| entropy | \[ -\sum_{i=1}^{N} \sum_{j=1}^{N} p(i, j) \log p(i, j) \] | GLCM |
| homogeneity | \[ \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{p(i, j)}{1 + (i - j)^2} \] | GLCM |
| inverse difference | \[ \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{p(i, j)}{1 + (i - j)} \] | GLCM |
| sum entropy | \[ -\sum_{k=2}^{M} p_{xy}(k) \log p_{xy}(k) \] | GLCM |

### 3. EXPERIMENTAL RESULTS

#### 3.1. Phase Images of EOC Cells from a DH Microscope

Figure 2a shows a typical hologram of 5 A2780 cells. To obtain the single-cell phase images needed for cell drug resistance classification, a series of processing steps are applied to the holograms. The angular spectrum method is utilized to reconstruct the object wave from the hologram. As shown in Figure 2b, the reconstructed phase image shows phase aberrations introduced by the setup of the off-axis DH system. The aberration in the microchannel area is eliminated by the background fitting method. Meanwhile, the unwrapping...
algorithm is also used to obtain a continuous phase image. Clearly, in Figure 2c, most of the cells are unfocused, and the regions around each of them are recognized as the basis of the refocusing procedure. Figure 2d shows the focused phase image of all five cells. There are not many complex objects in the phase image. Therefore, we used the OTSU segmentation method to identify and segment individual cells from the QPI background for extracting features of a single cell. The Supplementary Movies (SM1 and SM2) show the phases of flowing cells of A2780 and A2780CisR, respectively.

Figure 3 shows the single-cell phase images of A2780 cell and A2780CisR cell. Apparently, there was not a significant difference in the phase images between sensitive EOC cells and cisplatin resistance EOC cells that could be observed by human eyes.

3.2. Features’ Analysis. A total of 2139 cells are acquired by two experiments, 1038 images of A2780 cells and 1101 images of A2780CisR cells. The multiple features of all cells are extracted for conducting a statistical analysis. These calculations are programmed with Matlab R2020b, where it costs about 0.002 s for extracting single-cell features. Figure 4 shows the statistical histograms of 20 feature parameters between the two types of EOC cells. As shown in these histograms, each feature is isolated to varying degrees between the two groups of parameters. In general, the more the overlapping area of a histogram, the less the unique information features possess. Clearly, the isolation of the GLCM feature is relatively larger than the other two features, suggesting that GLCM parameters may be adequate to distinguish cancer cells. To further investigate the different classification abilities of these 20 features, the classification accuracy of a single feature is calculated (using an SVM classifier). Figure 5 shows the sort of single-feature performance based on classification accuracy, and the features of MP, OPL, and GLCM groups are displayed in different colors. The orange bars show the performance of MP features, in which the equivalent diameter and perimeter perform better than the other three parameters. OPL gradient with a green bar exhibits extremely high classification accuracy among all the OPL features. As a whole, the GLCM feature has better classification performance, which is the same as the histograms present.

3.3. Classification Result. Multiple classifiers and feature settings are investigated in this section. The total number of analyzed cells is 2139, while the number of features is 20 for an individual cell. Thus, the data set for machine learning is a matrix with about $2139 \times 20$ data points. There is an essential pre-processing of the feature data set before putting it into the classifier. It can be seen from the histograms in Figure 4 that the scale of different feature data varies greatly. Therefore, a linear normalization method is used to standardize each feature data before putting it into the classifier. It can be seen from the histograms in Figure 4 that the scale of different feature data varies greatly. Therefore, a linear normalization method is used to standardize each feature data. A 10-fold cross-validation method is applied in this work. In this method, the total data set is split into 10 sets, including a validation set and a test set. Each set is chosen as a training set, validation set, and test set one by one. Table 2 shows the classification accuracy of various feature combinations classified by NB, DTs, KNN, SVM, and FCN. The values present in Table 2 are the average classification accuracy of the 10 test sets.

The optimal criteria for each classification algorithm are different. Accordingly, when achieving the best classification performance, each feature set corresponds to a different classifier. As shown in Table 2, when GLCM features and the multi-features group is selected, the SVM classifier achieves the highest accuracy. However, the best performance for MP and OPL features are random trees and FCN. Among all the classifier and feature settings, the best combination is the...
Figure 4. Histograms of cell features extracted from single-cell quantitative phase image: (a) MP, (b) OPL, and (c) GLCM.

Figure 5. Sorting the accuracy of single-feature classification.
multi-feature and SVM classifier with the highest accuracy of 92.2%.

The performances of the four classification models are compared and shown in Table 3. Then, the ROC curves of the true-positive rate (sensitivity) versus the false-positive rate (1-specificity) are used to further estimate the classifiers, as shown in Figure 6 with different colors. The gray line is the random guess result. Although the best accuracy of DTs is higher than the NB classifier, its AUC is relatively low. The SVM classifier still shows the best performance with an AUC of 0.96. Although the training time of the SVM classifier is much longer than others, its predicting time is acceptable.

Figure 7 shows the two-dimensional visualization of the classification result of test samples, which is presented by the t-distributed stochastic neighbor embedding (t-SNE) method. The red circles and blue circles are the A2780 and A2780CIRS cells that are classified correctly. Incorrectly classified sample cells are represented by “×”. The green line in the figure represents the SVM decision boundary for classification reduced from high dimension.

4. DISCUSSION AND CONCLUSIONS

Chemotherapy is one of the primary therapies for cancer patients. However, drug resistance is a well-known phenomenon that results when cancer cells become tolerant to chemotherapy drugs during chemotherapeutic treatment. Therefore, the drug resistance of cancer cells’ assessment plays a pivotal role in the optimal selection of chemotherapeutic strategies for cancer patients in a personalized manner. For EOC patients, the cisplatin-based chemotherapy regimen is the most important postoperative treatment. Thereby, platinum resistance severely impacts the prognosis of patients. Nowadays, the assessment of chemosensitivity of EOC cells is based on biomarker detection and diagnostic imaging, which makes it depend deeply on the exploration of expressed proteins in drug-resistant cancer cells. In this work, we proposed a new method to achieve label-free, high-throughput, high-precision assessment of drug resistance of EOC cells. This work is a vital breakthrough to distinguish drug-sensitive EOC cells and drug-resistant EOC cells. In this approach, thousands of single-cell phase images are quantitatively acquired with high throughput by combining DH microscopy and microfluidics technology. Then, multiple feature parameters of the individual cell are extracted from the reconstructed phase image. Finally, the cancer cellular heterogeneity is accurately revealed with drug sensitivity and drug resistance using machine learning classification algorithms. The experiments demonstrate that the classification accuracy of the cisplatin-sensitive EOC cells (A2780) and the cisplatin-resistant EOC cells (A2780CisR) achieve with a specificity of 94% and a sensitivity of 91%. The significant advantage of the proposed approach is the label-free, high-throughput, and high-precision single-cell detection ability. It allows a minimal amount of biological samples for automatic

Table 2. Classification Accuracy of Multiple Machine Learning Algorithms and Feature Settings

| model | accuracy | MP (%) | OPL (%) | GLCM (%) | multi-features |
|-------|----------|--------|---------|----------|----------------|
| NB    | 66.5     | 69.8   | 81.3    | 78.8     |                |
| RT    | 67.5     | 76.1   | 81.7    | 82.9     |                |
| KNN   | 63.6     | 77.1   | 84.5    | 84.7     |                |
| SVM   | 55.9     | 76.7   | 87.2    | 92.2     |                |
| FCN   | 67.4     | 78.6   | 86.2    | 90.9     |                |

Table 3. Performances of the Classification Models with the Best Classification Accuracy

| model    | specificity | sensitivity | AUC       | training time (ms) | predicting time (ms) |
|----------|-------------|-------------|-----------|--------------------|---------------------|
| NB       | 0.84        | 0.74        | 0.88      | 16.21              | 4.93                |
| RT       | 0.82        | 0.84        | 0.83      | 2.59               | 0.18                |
| KNN      | 0.86        | 0.83        | 0.91      | 6.60               | 0.74                |
| SVM      | 0.91        | 0.94        | 0.96      | 56.48              | 0.31                |
| FCN      | 0.92        | 0.88        | 0.94      | 4.76               | 0.046               |

Figure 6. ROC curves of different classifiers with the best performance.
assessment of drug resistance of cancer cells with many sample sets and feature sets. It is worth noting that there are three types of features employed for cell classification, that is, morphology (MP) features, OPL features, and gray texture (GT) features. Here, the MP features included five parameters that describe the outline of the cell. OPL features also included five parameters that refer to the first-order statistical characteristics of the OPL within the cell area. The considered GT features describe the higher order texture features of cell image, where difference entropy reflects the randomness of neighbor pixel’s difference, contrast reflects the local intensity variation, and correlation represents the linear dependency of cell phase image. Moreover, it is found that these features have a greater difference and lower correlation between the neighborhood phase of drug-resistant cells than drug-sensitive cells. It may suggest that drug-resistant cells possess more irregular structures. Besides, to find optimal machine learning algorithms, we compared four common algorithms, NB, RT, KNN, and SVM. The results show that SVM achieved the best classification performance at classification accuracy, AUC, and training and predicting time. This is because it applies the maximum-margin hyperplane to separate space data points and introduces the kernel function to map non-separable data to higher-dimensional space. In fact, SVM has been proved successful in various biological applications.41,46,76

In conclusion, this work demonstrates the successful label-free, high-throughput, high-precision assessment of the drug resistance of EOC cells in the microfluidic holographic flow cytometer. This is the first step of an attempt to distinguish cancer cells from drug sensitivity and drug resistance. Furthermore, for most cancer patients, cancer cells’ drug resistance is gradually induced by chemotherapy drugs during chemotherapy. In this case, chemotherapy drugs are often effective at the beginning but gradually become ineffective or even ineffective after some time. If the drug resistance of cancer cells can be quantitatively assessed and monitored in the chemotherapy process, the treatment strategy can be opportunistically adjusted according to the assessment results. Thus, the treatment efficacy can be improved, and the side effects of chemotherapy will be reduced. Because the research results prove that the development of drug resistance of cancer cells is accompanied by unique morphological change, we believe that the proposed method can implement the quantitative assessment of drug resistance based on the cell MP analysis. It would be noted that the relatively low throughput of 200 cells/min is mainly due to the currently employed instrumentation in this paper. In principle, a high throughput could be achieved if a fast liquid flux and a high-speed camera were employed in the experiments. For instance, a throughput approaching about 20 000 cells/min has been demonstrated using holographic technology.79 In the future, more cells’ features will be extracted, and more advanced algorithms and fast instruments will be employed in experiments to achieve high-accuracy, high-throughput, and label-free assessment of the drug resistance of EOC cells.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04204.

Phase image of the flowing A2780 cell (AVI)
Phase image of the flowing A2780CisR cell (AVI)

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Notes
The authors declare no competing financial interest.

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