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SYSTEMS-LEVEL QUALITY IMPROVEMENT

Classification and Recognition of Ovarian Cells Based on Two-Dimensional Light Scattering Technology

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Abstract

Ovarian cancer is a very insidious malignant tumor. In order to detect ovarian cancer cells early, the classification and recognition of ovarian cancer cells is mainly studied by two-dimensional light scattering technology. Firstly, a single-cell two-dimensional light scattering pattern acquisition platform based on single-mode optical fiber illumination is designed to collect a certain number of two-dimensional light scattering patterns of ovarian cancer cells and normal ovarian cells. Then, the HOG (Histogram of Oriented Gradient) algorithm is used to extract shaving anisotropy feature of two-dimensional light scattering pattern. The results show that the accuracy of classification and identification of ovarian cancer cells by two-dimensional light scattering technology is 90.81%, which suggests that the specificity of cancer cells and normal cells can be characterized by two-dimensional light scattering technology.

Keywords Ovarian cancer cell detection · Two-dimensional light scattering patterns · HOG feature extraction · Machine learning

Introduction

Light scattering analysis of cells is an important development direction of unlabelled cell analysis. Because scattered light is extremely sensitive to the internal structure of cells and can detect the subtle differences within cells, it has great potential in the analysis of cell label-free pathology. The results show that one-dimensional cell light scattering technology can further analyze more properties of cells. For example, the label-free detection of oxidative stress-induced mitochondrial swelling is realized and has also been used to differentiate subclasses of macrophages [1]. This series of studies show that the collection and analysis of scattered light from multiple angles is conducive to the further study of cells. In addition to one-dimensional light scattering technology, two-dimensional light scattering technology also plays an important role in label-free analysis of cells [2]. In the analysis of two-dimensional light scattering pattern, there are methods based on spot size and spot area distribution, average light intensity, entropy calculation and pattern recognition [3, 4].

When a single cell is irradiated by laser, the complex distribution of different organelles results in the anisotropy of scattered light. In one-dimensional scattering light technology, the anisotropic information of one-dimensional scattering light has been used to reveal the biophysical characteristics of blood cells and bacteria [5]. This method enlightens people that the anisotropic information of two-dimensional scattering light may also reveal some specific information of cells, such as characterizing the specificity of cancer cells and normal cells and realizing their unlabelled classification and recognition [6]. Based on this, a HOG (Histogram of Oriented Gradient) analysis method is proposed to extract the anisotropic characteristics of two-dimensional light scattering patterns. And then, combined with the machine learning method of SVM (Support Vector Machine), it is used to establish the relationship between two-dimensional light scattering and cell types, so as to realize the differentiation between ovarian cancer cells and normal ovarian cells.

Feature extraction using HOG algorithm has been widely used in pedestrian recognition and face recognition. The application of this method in two-dimensional light scattering pattern analysis is based on the fact that it can well carry out

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multi-directional statistics on the anisotropic information of two-dimensional light scattering pattern. In the process of HOG feature extraction, the gradient information of the image is counted into several histograms in different directions, and the scattered light information in each direction of the two-dimensional light scattering pattern is characterized by the direction histogram. Therefore, the anisotropic characteristics of the two-dimensional light scattering pattern can be quantitatively described. This quantitative feature can be well processed by SVM algorithm for classification and recognition.

Feature extraction and platform construction of two-dimensional light scattering patterns

Image feature extraction

Features are attributes that distinguish one class of objects from others. Each image has different characteristics or attributes from other images. Some attributes can be perceived intuitively, such as edge, texture, brightness and color, while others are difficult to be perceived by human eyes, which need to be processed by computer, such as gray histogram. In order to facilitate computer processing, these sets of attributes corresponding to the image are given in the form of numerical vectors in the computer. These numerical vectors are usually called image eigenvectors.

In an image, if the feature of a certain kind of object can be represented by a single numerical value, then the dimension of the image feature vector is 1. If n numerical representations are needed, then the n numerical values are the n-dimensional feature vector of the image. The feature vectors can be input into the training system or classifier, and the classifier can be obtained through training or the class label can be obtained from the classifier. In fact, objects with n-dimensional characteristics can be abstracted as points in n-dimensional space, and the classification of objects is equivalent to partitioning points in n-dimensional space.

In order to distinguish the target image from other images and realize image classification, it is necessary to select features that can describe the uniqueness of the image as much as possible. In addition, it is also expected that the selected features will have greater similarity in the same class samples and smaller similarity with other different classes.

HOG feature extraction of two-dimensional light scattering pattern

A two-dimensional light scattering pattern of a cell is used to demonstrate the process of feature extraction of two-dimensional light scattering pattern using HOG algorithm. The process of feature extraction consists of four steps: image pre-processing, gradient calculation, cell histogram calculation and combination of image feature vectors, as shown in Fig. 1.

Image pre-processing Two-dimensional light scattering pattern is collected from CMOS (Complementary Metal-Oxide-Semiconductor) sensor and then transferred to computer for feature extraction and classification recognition. Before extracting HOG features, images need to be pre-processed. Image pre-processing includes two main steps: image color space conversion and image denoising. The color space includes device-related color space and device-independent color space. The color space conversion of the collected image usually involves the conversion between the RGB format of the acquisition device and the standard CIE XYZ format, as well as the conversion between the standard CIE XYZ format (CIE-XYZ is a class of CIE (color system) spectral tristimulus values) and the different display RGB formats [7]. Image denoising is the basis of image processing and the key research content of image restoration. The main method to eliminate noise is to use filters. Before filtering noise, different filters need to be designed according to different noises.

Gradient calculation The image data stored in two-dimensional space can be regarded as a two-dimensional scalar field. The gradient direction of a point in the scalar field indicates the direction of the fastest change of the scalar field at that point, and the gradient size of the point indicates the speed of the change of the scalar field at that point. Therefore,

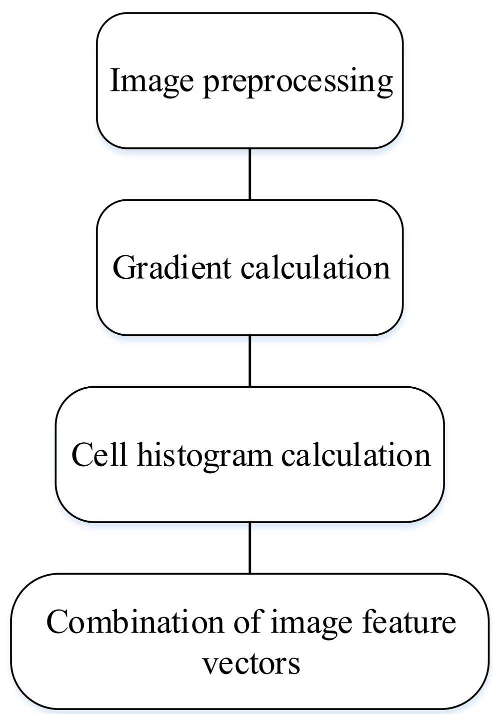

Fig. 1 HOG feature extraction process of two-dimensional light scattering pattern

after gradient calculation, the gray value change rate and direction of each point in the image can be obtained. Because the edge is perpendicular to the gradient direction, the calculation of gradient also reflects the edge or texture information in the image, as shown in Fig. 2. Edges and gradients exist in places that vary from 0 to 1.

From the above three formulas, it can be seen that the gradient calculation only needs to obtain the partial derivatives of the image in the x and y directions. In computer, the image is stored in the form of discrete data, so when calculating image gradient, the derivative is generally approximated by differential operation. The commonly used approximate expressions are as follows, where f (x, y) represents the gray value of the pixel in the image (x, y):

$$\frac{\partial f(x,y)}{\partial x} \approx G_x = f(x+1,y) - f(x-1,y) \quad (1)$$

$$\frac{\partial f(x,y)}{\partial x} \approx G_y = f(x,y+1) - f(x,y-1) \quad (2)$$

In gradient calculation of HOG algorithm, the gradient component G_x of x direction (horizontal direction, right direction is positive) is obtained by convolution operation of gradient operator [−1,0,1] on the whole image. The gradient component G_y in the y direction (vertical direction, positive upward) is obtained by convoluting the whole image using the vertical gradient operator $[1,0,-1]^T$ [8]. In order to keep the size of the processed image consistent with that of the original image, the original image expands one pixel outward by copying edge pixels before using gradient operator.

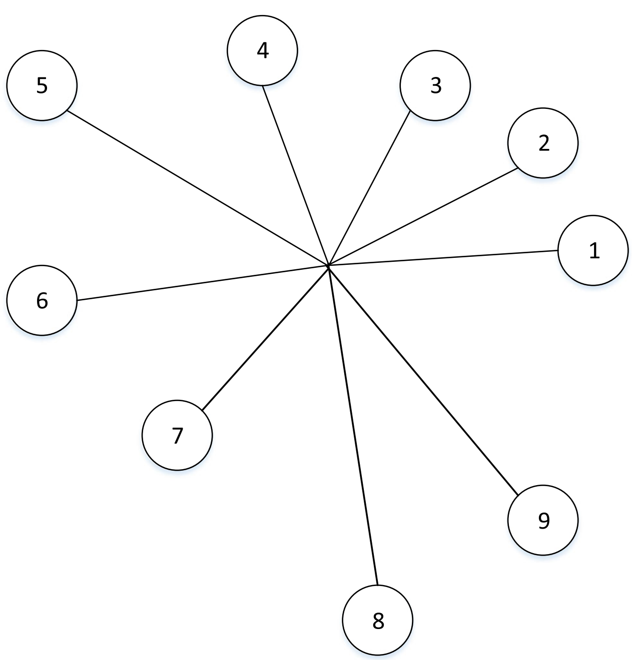

Fig. 2 Histogram star type

Histogram calculation Histogram statistics are often used in image processing. Different types of image objects usually have unique histogram features, which often have better results in image recognition and matching. Because the different positions and angle changes of the object in the image have little influence on the histogram, the recognition method based on histogram usually does not have obvious difference because of the spatial position and direction of the object. In addition, histogram can greatly reduce data redundancy and maximize the contribution of data points to different categories. The key step of the HOG algorithm is to calculate the gradient, then calculate the histogram according to the gradient and get the HOG features.

Histograms are computed separately in each cell. Therefore, the whole image needs to be segmented in a certain way before statistical histogram. For the convenience of demonstration, a two-dimensional light scattering pattern is divided into 36 cells using a 6*6 mesh. Here "6" is the adjustable configuration parameter of the algorithm. By adjusting this parameter, the influence of different mesh generation methods on the subsequent classification can be analyzed, and then the optimal segmentation method can be selected. When partitioned into 6*6 meshes, each cell will be 160*160 pixels in size. Table 1 shows an enlarged view of a small area, and the normalized gradient size is numerically marked.

The number of histogram channels determined by this example is 9, and the histogram of one channel is established every 40 degrees. Taking the cells marked by dotted boxes as an example, 160*160 pixels in a dotted box vote on 9 histogram channels according to their gradients. In order to display the final statistical results in different directions more intuitively, the histogram is usually represented as a star, as shown in Fig. 2.

Combine of image feature vectors. In the example above, the histogram statistics results of each cell are a 9-dimensional vector. When the histogram statistics of all cells are completed and combined, there are vectors of 6*6*9 = 324 dimensions. This is the HOG feature in this example, whose dimension n = number of cells * number of channels. Thus, it is known that the two HOG configuration parameters mentioned above, one determines the number of cells and the other provides the number of channels, which together determine the dimension of the final HOG eigenvector. By changing these two parameters, the number of features finally output can be adjusted.

Table 1 Gradient size after processing

0.66	0.59	0.49	0.34
0.52	0.41	0.27	0.10
0.29	0.17	0.03	0.17
0.12	0.10	0.25	0.42

Construction of two-dimensional light scattering experimental platform

The experimental acquisition platform of two-dimensional light scattering pattern consists of three parts: illumination light path part, signal acquisition part and computer processing system. The part of illumination light path is built on the optical platform, including laser used for cell excitation and related devices for light path conduction, such as dimmer filter, objective, triaxial displacement table and optical fiber. The signal acquisition part is mainly mounted on the microscope to stably carry out scattered light acquisition and control the acquisition process. This part includes a liquid-based chip for loading static cell suspension, acquisition objective and CMOS optical sensor. After laser irradiation, scattered light passes through the objective lens and is recorded by CMOS optical sensors. The two-dimensional light scattering patterns collected by CMOS sensors are transmitted to the computer system for processing. The computer system is mainly composed of a DELL computer.

In the experiment, the optical fibers are inserted into the slot of the liquid-based chip to illuminate the target cells. The distance between the target cell and the end of the optical fiber is adjusted to be more than 5 mm. At the observation target, the illumination area of single mode optical fibers is a spot with diameter greater than 344 μm. In this case, considering that the diameter of the cell is about 10–20 μm, the irradiation of the cell can be approximated to plane wave irradiation. The experiment is carried out in dark environment to reduce the light interference in the peripheral environment. In the two-dimensional light scattering pattern acquisition of ovarian cancer and normal ovarian cells, 40 times long working distance objective with NA value of 0.6 is used to collect scattered light signals. The acquisition objective operates in defocusing mode to obtain two-dimensional pattern of speckle state, and the uniform defocusing distance is adjusted to 90 μm for single cell two-dimensional light scattering pattern acquisition.

Compared with natural light, laser has some outstanding advantages, such as good monochrome, strong coherence and high directivity. It is a good light source for stimulating cells or micro-particles in experiments and for scattering light analysis. The laser is mainly composed of three parts: pump source, laser working substance and resonator. In the experiment, the laser is a single longitudinal mode laser (Frankfurt Laser Company, 100 mW, Germany) of FPYL-532-100 T-SLM type. The output of the laser is a 532-nm continuous wave green laser with a maximum output power of 100 mW. The laser is coupled to a single-mode fiber after beam splitting and a dimming filter.

Lastly, single-mode optical fibers with S405-XP core diameter of 3.0 μm and numerical aperture of 0.12 μm are selected to guide laser irradiation of ovarian cancer cells and normal ovarian cells to collect two-dimensional light scattering patterns (Table 2).

The two-dimensional light scattering pattern acquisition device for text uses objective lenses in two places, and laser beams are coupled into optical fibers in one place by coupling objective lenses. The other is to isolate other scattered light interference by using the acquisition objective when acquiring two-dimensional light scattering pattern, so that the scattered light in a specific angle range can enter the sensor. Coupled objective lens, the laser used in the experiment does not have a self-contained optical fiber collision. If lasers want to enter the optical fiber, they need to making coupling through external equipment. A long working distance lens (LUCPLFLN 40XPH, Olympus) with a magnification of 40, a numerical aperture of 0.6 and a working distance of 3.3–4.2 mm is selected to collect two-dimensional light scattering patterns of ovarian cancer cells and normal ovarian cells.

Two-dimensional light scattering patterns are collected by planar optical sensors. There are two common types of light scattering patterns: CCD (Charge Coupled Device) and CMOS, and they are integrated in different ways. The former is made of semiconductor single crystal, while the latter is based on metal oxide semiconductor technology. Compared with CMOS, the early CCD optical sensors have many advantages, such as high permeability, good color restoration and accurate exposure. However, the process of CCD is complex and the prices is expensive, and only a few sensor manufacturers can produce it. After the development of semiconductor technology and the progress of optical sensing technology in recent years, in fact, the imaging effect of these two types of optical sensors tends to be the same, and the imaging difference between them is getting smaller and smaller. Moreover, CMOS has the advantages of low power consumption and small size, which can also achieve random, high frame rate and no tail reading. Based on these advantages, CMOS sensors have been widely used in various shooting terminals in recent years. The CMOS sensor of Canon SSOD camera is used in the acquisition device here Sensor resolution is 5184*3456 pixels, analog-to-digital conversion is 16 bits, and sensor size is 22.3*14.9 mm.

Table 2 Parameters of S405-XP single-mode fiber

The fiber core diameter	3.0 μm
The cladding diameter	125.0 ± 1.0 μm
Diameter of coating layer	245.0 ± 15.0 μm
Numerical aperture	0.12
The mode field diameter	3.3 ± 0.5 μm@405 nm 4.6 ± 0.5 μm@630 nm

Experiment and result analysis of two-dimensional light scattering of ovarian cancer cells

Cell allocation experiment

Epithelial ovarian cancer cell line A2780 and normal epithelial ovarian cell line HOSEpiC are used in the experiment. In the process of cell allocation, A2780 cells and HOSEpiC cells are cultured in RPMI1640 medium with 10% fetal bovine serum, 100 Unit/mL penicillin and 100 μg/mL streptomycin for two generations. In order to obtain cell suspension, monolayer cells in the previous step are treated with trypsin preheated at 37°Cin phosphate buffer solution (PBS) until the cells are observed to shrink into a round shape. Subsequently, the cells are centrifuged under a centrifuge at a rate of 1000 rpm for 5 min and then suspended in 1*PBS solution. In order to operate the cells safely in later experiments, the cells are centrifuged again and suspended again for 30 min at room temperature with 75% ethanol. After washing, immobilization and centrifugation, the cells are suspended in 1*PBS solution. The cells are finally diluted into a cell suspension with a concentration of about 3000cells/mL to facilitate the acquisition of two-dimensional light scattering patterns of single cells.

Acquisition experiment operation

The acquisition operation of two-dimensional light scattering pattern mainly includes before acquisition operation and acquisition process operation. Before acquisition, the adjustment of optical fiber coupling, optical fiber cutting and acquisition system and other experimental operations are needed. The acquisition process is mainly completed under a microscope.

In the experimental system, the green laser emitted by a diode-pumped solid-state laser enters the liquid-based chip through a single-mode optical fiber, and stimulates the scattering light of the suspended single cell in the chip trough. Before configuring the cell suspension, the whole optical system should be adjusted to ensure that the laser can accurately combine from the objective lens to the single-mode optical fiber. The smoothness of the incision at the end of the optical fiber also affects the facula. Good laser collision requires regular, smooth and clean laser inlet and outlet. The acquisition part of the system is mounted on the microscope. The acquisition process is mainly completed by operating the microscope. The specific operation steps of the microscope are as follows:

Rotate the brightness adjusting knob to make sure that the brightness of the microscope lamp is the smallest. Turn on the main switch, make the liquid-based chip and place on the microscope carrier. Insert the optical fiber into the cell suspension in the chip trough and fix it by the optical fiber. Observe under the low power objective (l0 *) and adjust the brightness until appropriate, adjust the single mode fiber head to the middle of the field of vision, and adjust the focus until the fiber head can be clearly seen. Convert to a 40-fold long working distance objective, observe the optical fiber head and focus again. Turn on the laser switch, move the field of view of the microscope along the direction of light propagation for about 5 mm, and turn off the light of the microscope. Look for a single cell in the field of vision and focus on it. Note the fine-tuning knob scale when focusing, then defocus 90 μm, observe the brightness of speckle pattern when defocusing, and adjust the speckle to the appropriate brightness by adjusting the dimming filter in the optical path. And debug shooting and determine the camera's shooting parameters according to these shooting. Take two-dimensional light scattering patterns of cells. Repeat the above steps to obtain a certain number of two-dimensional light scattering patterns without adjusting the parameters of the filter and camera. Replace the cell type and repeat the above operation to obtain a certain number of two-dimensional light scattering patterns.

Two-dimensional light scattering patterns and dark field primitive patterns of cells

Typical two-dimensional light scattering patterns of ovarian cancer cells and dark field images of the cells are shown below. Figures 3 and 4 are obtained without labelling, and the image sizes of both are 960*960 pixels. Figure 3 is a two-dimensional light scattering pattern collected from the experimental device. It can be seen from the figure that the two-dimensional light scattering pattern is mainly composed of complex light spots, which represent different intensity information in different scattering directions, i.e. the anisotropy information of scattered light. These complex speckle patterns may be due to the inhomogeneous distribution of organelles

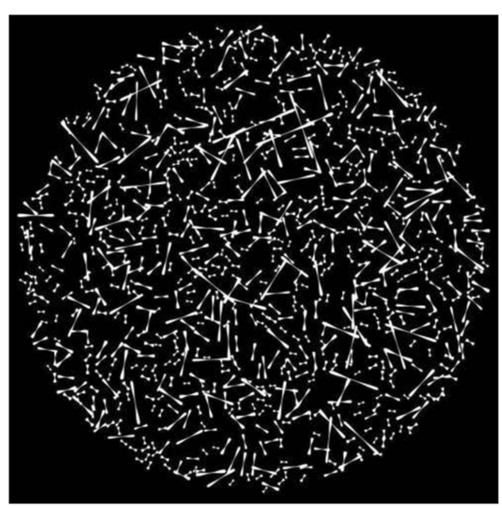

Fig. 3 Scattering pattern

Fig. 4 Corresponding dark field map

such as mitochondria within cells. Figure 4 shows the image of the cell in dark field and focusing state. In static suspension, the cells are transparent and spherical without staining, and the internal organelles and their structures are difficult to distinguish. Therefore, it is difficult to classify cells based on this. In addition, compared with two-dimensional light scattering patterns of the same pixel size, the original dark field image is smaller, while the speckle pattern occupies a larger area of the image and may extract more information.

HOG processing of two-dimensional light scattering patterns

The HOG algorithm is used to extract speckle features. HOG feature dimension can be flexibly changed according to needs. The extracted features can usually have tens of thousands of dimensions, which are expected to be used to specifically characterize a certain kind of cells. HOG algorithm counts the histogram according to the different gradient directions of cells, which is the anisotropy statistics of two-dimensional light scattering patterns. Because the method is based on gradient and is less affected by the absolute brightness of the speckle, it can effectively avoid the instability caused by the fluctuation of the excitation light source. Two-dimensional light scattering patterns of 148 ovarian cancer cells and 148 normal ovarian cells are conducted with HOG processing.

In the process of HOG feature extraction, the whole pattern is divided into 144 cells by 12*12 mesh. The gray gradient information of each cell is counted into the histogram of nine channels. The histogram counted by cells directly represents the statistical results of each direction of cell in the form of stars. The length of the pointer in the star structure represents the gray gradient statistics of the pointer direction. Therefore, HOG features can quantitatively characterize the anisotropic information of two-dimensional light scattering patterns from the local area of speckles. HOG features can be trained and classified as input of SVM.

Classification results of linear SVM

SVM, also known as support vector network, is a general learning algorithm in statistical learning theory. This algorithm is proposed by Vapnik and Cortes for the first time and has been widely used in machine learning [9]. According to statistical learning theory, if the output of the computer is as close as possible to the ideal output for input data subject to a certain distribution, the machine algorithm should follow the principle of structural risk minimization (SRM). SVM algorithm is established based on SRM principle. Compared with traditional algorithm, it can overcome over-fitting and dimension disasters in the learning process, and has good learning ability. Compared with the traditional artificial neural network, it has the advantages of strong generalization ability and simple structure. At present, SVM algorithm has been applied in many fields. This algorithm will be used to classify two-dimensional light scattering patterns after feature extraction.

In the process of classification, two-dimensional light scattering patterns of HOSEpiC cells are labelled as positive group, and two-dimensional light scattering patterns of A2780 cells are labelled as negative group. In the verification phase, 10-fold cross validation is used to obtain the classification accuracy of SVM. In this process, 296 two-dimensional light scattering patterns are randomly mixed and then divided into 10 non-overlapping subsets, each of which has 29 or 30 images. A 10-fold cross validation is processed 10 times, each time using 9 subsets for training, and the remaining subset for testing, so that the cycle is 10 times. Finally, according to these 10 times processing, the accuracy, sensitivity and specificity of classification are counted.

In order to validate the classification results more reliably, five independent 10-fold cross validation processes are carried out, and then five times of accuracy, sensitivity and specificity as well as their average are obtained. Results are shown in Table 3, and the accuracy of five independent 10-fold cross validation results is over 90.20%, with an average of 90.81%. The highest accuracy rate is 91.55%, the maximum sensitivity is 95.95% and the average is 94.05%, and the average specificity is 87.57%. The results show that HOG features can specifically characterize ovarian cancer cells and normal ovarian cells. Gradient anisotropy of two-dimensional light scattering patterns can be used as a marker-free method to distinguish the two kinds of cells. The two-dimensional light scattering technique is expected to achieve high accuracy in clinical identification of ovarian cancer cells.

Table 3 SVM classification results

Random test	Accuracy	Sensitivity	Specificity	Diagnostic table	
1	91.55	93.24	89.86	138 (TP)	15 (FP)
				10 (FN)	133 (TN)
2	90.54	93.92	87.16	139 (TP)	19 (FP)
				9 (FN)	129 (TN)
3	90.20	93.24	87.16	138 (TP)	19 (FP)
				10 (FN)	129 (TN)
4	90.20	93.92	86.49	139 (TP)	20 (FP)
				9 (FN)	128 (TN)
5	91.55	95.95	87.16	142 (TP)	19 (FP)
				6 (FN)	129 (TN)
Average	90.81	94.05	87.57	–	–

Accuracy analysis under different HOG parameters

Two-dimensional light scattering patterns are divided into grids with different densities. The grid settings range from 4*4 to 48*48, and the cell size varies from 240*240 pixels to 20*20 pixels due to the 960*960 pixels of the collected two-dimensional light scattering pattern, as shown in the horizontal axis of Fig. 5. The number of channels in the histogram varies from 6 to 24. The two sets of parameters cooperate with each other to extract HOG features for the configuration parameters of HOG algorithm. All the features obtained by each combination are classified into the same SVM algorithm to obtain the accuracy under the configuration parameters. The height of each column in the histogram in Fig. 5 represents the accuracy of SVM processing under a set of configuration parameters. Figure 5 shows that cell segmentation has a great influence on the results, and the number of channels used will also affect the results. When the number of channels is 9, the accuracy of 6*6 mesh segmentation is the highest, and the accuracy is 92.57%.

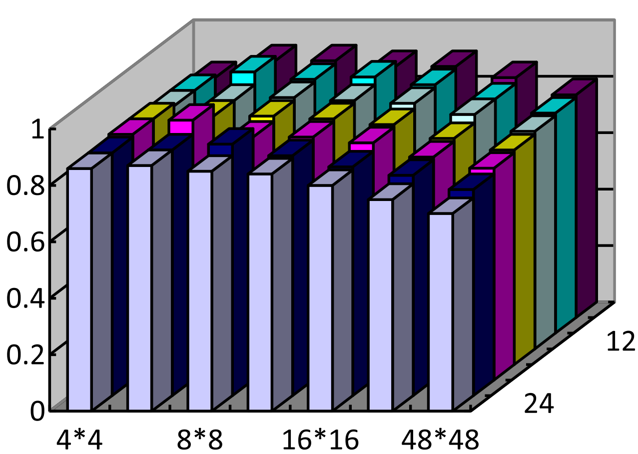

Fig. 5 Classification results under different HOG parameters

Conclusion

A two-dimensional light scattering pattern acquisition system based on single-mode optical fiber illumination is built, and two-dimensional light scattering patterns of ovarian cancer cells and normal ovarian cells are collected. Then, HOG feature extraction algorithm is used to extract gradient anisotropy features of two-dimensional light scattering patterns, and realize automatic classification of ovarian cancer cells and normal ovarian cells through SVM algorithm. This method provides a label-free analysis method for ovarian cancer screening.

The results show that the specific information of ovarian cancer cells and normal ovarian cells can be characterized by gradient anisotropy of two-dimensional light scattering patterns. This suggests that the gradient anisotropy of two-dimensional light scattering patterns of different kinds of cells may be unique. It is expected that HOG algorithm can be used to extract and classify cells. Two-dimensional light scattering pattern label-free cell recognition has important application prospects.

Compliance with ethical standards

Conflict of interest Author Qi Chen declares that he has no conflict of interest. Author Jianling Zhang declares that he has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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