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Automated Recognition and Measurement of Cell Morphology on Optically-induced Electrokinetic Patterning Chip*

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Abstract **- Cell behaviors including adhesion, migration, proliferation, differentiation and apoptosis, etc. deeply influence tissue formation, regeneration, and even the function of whole tissue or entity. Optically-induced electrokinetic (OEK) patterning is an efficient method to guide cells to grow and form the desired structure on a substrate and hence becomes an effective approach to the study of cell behaviors. But recognition and measurement of large number of cells are challengeable. In this study, automated recognition and measurement of cellular geometric features on the patterned substrate using the OEK method is proposed in which the recognition of cells is realized by segmenting individual cells from the microscope image using snake computation model with dynamic programming and the geometric features such as area, perimeter, semi-major axis and orientation angle are extracted for the study of cell behaviors. The relation between the geometric features and cell behaviors has been studied. The automated system can recognize and measure the geometric features of cells rapidly, accurately and efficiently, and bring more convenience for development of biology and life science.**

Index Terms - Optically-induced Electrokinetics, Automated System, Cell behavior, Image Processing, Pattern Recognition.

I. INTRODUCTION

Since the 19th century, biologists have been exploring the underlying principles and mechanisms for cells to behave as the fundamental unit of life and have achieved a large number of encouraging results [1]. As one of the main research focuses, the study of cell behaviors is attracting an increasing attention in various topics, especially the measures of behavioral phenomena including adhesion, migration, proliferation, differentiation and apoptosis, which play significant roles in tissue formation, regeneration, and even influence the function of whole tissue or entity. The significance of cell behaviors has been obviously observed *in vitro* tissue culture, tissue development and function formation [2-5]. For example, in the context of *in vitro* culture of esophageal smooth muscle cells, alignment of cells plays a crucial role in maintaining geometric structure and mimicking the native cell alignment to engineered biological tissue and even function [6]. For cell behaviors which are driven by self-organized assembly of numerous actin polymers and accessory proteins, what is challenging is how those actin polymers and accessory proteins work for cell migration, shape deformation and cluster, etc. [7]. Inner cooperation between those actin polymers and accessory proteins is complex and profound. Geometry shape however, is one of the aspects to understand the function of the actin polymers and accessory proteins. Briefly, the geometric shape deformation of cells plays an important role in understanding cell behaviors and tissue formation.

Various approaches have been developed for guiding cells to form desired shape [8-10], engineering tissue with designed geometry *in vitro* [11, 12], and cell integration with nano particle assembly [13]. However, these methods for patterning micro substrates for cell growth require complex and slow molding process. Liu et al. [14] reported a new cell patterning technique based on patterned poly(ethylene glycol) diacrylate (PEGDA) hydrogel via optically-induced electro kinetics (OEK). This technique has been demonstrated to be practical and efficient to control cell growth and cell shape on a-Si:H substrate. During the cell patterning process, many interesting phenomena which indicate the relationship between external environment and cell behaviors have been observed, for example, cells regulate their shape to adapt to external adhesive area and cells expand at a special orientation when they spread. To quantitatively analyze these relationships, amount of data about cell characteristics, such as cell shape and spreading areas, etc. are needed. Therefore, an approach which can realize rapid, accurate, adaptable and reliable recognition and measurement of cells would be significant in assessing cell growth and tissue formation for the study of cell behaviors and tissue engineering.

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Although manual measurement is reliable, the recognition and measurement of large quantity of cells in light microscope image and even video are impossible because of the following restrictions: 1) it is time consuming, 2) manual measurement is operator-dependent during long time work, 3) the processing efficiency is low. Considering those tedious and repeated tasks, various automated programs of recognition and measurement have been developed [15-19]. Based on the detailed demand for the method used to guide cell patterning, the automated systems include the following functions: 1) recognition algorithm, 2) extraction of geometric features, and 3) analysis of the geometric features.

For the OEK system, as a new method that guides cells to grow, including cell location and alignment, tedious and repeated work of recognition and measurement is time consuming. Thus, it is necessary to implement the recognition and measurement of cells automatically and quickly. In this study, an automated system is proposed to recognize and measure the breast cancer cells cultured with the patterned hydrogel on the a-Si:H substrate via the OEK approach. With this system, cell alignment and geometric characteristics can be quickly, accurately, and efficiently recognized and measured from the microscope image. Biological features which can be used to analyze cells behaviors and tissue formation process are obtained.

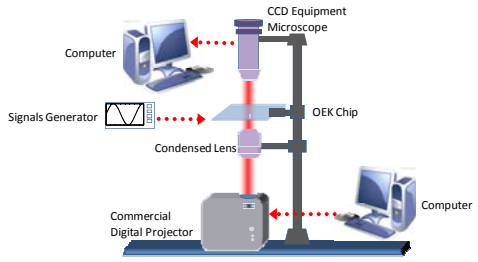


Fig. 1: the schematic illustration of the optically-induced electrokinetics (OEK) experimental setup for generating the PEGDA patterns.

Ⅱ. System Description

A. *Experimental System Setup*

Through introduction above, different manipulating system has its own characteristics based on its detailed demands [14, 15]. The manipulating system consisting of an OEK chip for cell patterning in this study is shown in Fig.1. The OEK chip is placed on a 3D movable stage embedded in the optical microscope and is connected with a programmable and high-accuracy AC signal generator to provide the alternating electric field to the OEK chip. Light patterns are projected by a projector below the OEK chip and focus on the a-Si:H surface within the OEK chip to form virtual electrodes. Light patterns are formed and their intensities are controlled by a computer aid. The existence of the virtual electrodes will induce PEGDA molecules in the solution to crosslink on the substrate and generate PEGDA patterns the same as the light ones. The cells can live only on the substrate compartment without PEDGA. Hence, we can guide cells to grow according to the pre-designed patterns by the OEK chip. The cell patterns cultured on the substrate are observed and their images are acquired with an inverse microscope (*Nikon* Ti-U).

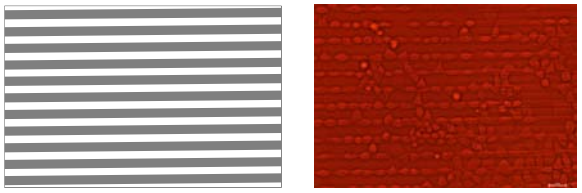


Fig. 2: The designed PEGDA pattern formed on the a-Si:H substrate with the OEK chip and the image of cell patterns guided by the PEGDA patterns. (a) The bar-grating PEGDA pattern was designed by computer aid and formed on the substrate. The grey lines are covered by the PEGDA hydrogel and the white lines are the interval spaces without the PEGDA. Cells can grow on the white interval spaces. (b) The image of cell culture according to the PEDGA patterns acquired with the microscope CCD.

In this study, the PEGDA hydrogel are patterned in the form of bar-grating on an a-Si:H substrate under the effect of the combination of electrical energy and optical energy, as shown in Fig. 2(a). The adherent cells are cultured within the interval spaces between the patterned PEGDA hydrogel film on the a-Si:H substrate, as shown in Fig. 2 (b).

B. *Automated Analysis System of Cell Patterns*

It is meaningful and essential to extract and analyze the geometric features of cells from the acquired images of cell patterns for understanding the cell behaviors and tissue formation. For a large number of cells in an image, it is tedious and time consuming to manually recognize and measure their sizes, deformation, and other geometric features. Hence, in this study, according to the detailed demands, an automated analysis system for cell patterns is proposed to recognize and measure the geometric characteristics of cells in the acquired images. Through the automated system, it becomes more accurate and time saving to analyze the image data for the geometric information of cells. The automated system satisfies the fundamental functional demands in practice including pre-processing, cells segmentation, contour recognition, geometric features extraction, feature data combination and simple statistical analysis. The schematic diagram of the automated system for cell recognition and feature extraction is shown in Fig. 3.

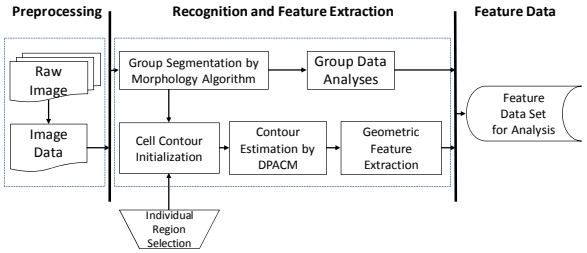


Fig. 3: the automated analysis system for cell patterns cultured on the OEK chip includes three parts: preprocessing, recognition and feature extraction and feature data combination.

The first part of the system is preprocessing, through which some blurring parts and noises will be removed to make cell segmentation and recognition easier in the following part. The second part includes image segmentation of cell region, cell contour recognition and feature extraction. In this study, cell segmentation is implemented with two modules for users

to choose: group cell segmentation and individual cell segmentation, which are followed by the contour recognition of the segmented cells and the feature extraction, including the area, perimeter and orientation angle etc. The segmentation, recognition and features extracting algorithms will be discussed in section Ⅲ and Ⅵ. The third part of the system is the combination of feature data from the second part for the following statistical analysis.

Ⅲ. Image Segmentation and Recognition

Image segmentation, which is essential for many applications such as object recognition, target tracking, reconstruction, etc., is a fundamental but still challengeable problem in image processing and computer vision[15]. For cell recognition and analysis, cell segmentation is the basic step to outline the region for individual cells, which is necessary for the cell contour cognition and feature extraction. Two main segmentation modules are adopted in this study for users to select according to the practical demands. The first segmentation module is the group segmentation in which all cells in the image are segmented together using a series of morphology algorithms without any manual intervention, and then the cells are recognized by the snake computation with dynamic programming which is discussed below. The second module is the individual cell segmentation in which the interesting cells are selected respectively for individual segmentation, followed by the latter contour recognition and feature extraction.

A. Segmentation Modules

Module 1: Considering the overlap among patterned hydrogel lines, edges and cells boundary, the whole image is segmented into many parts through a series of morphology algorithms consisting of erosion, dilation and region-based segmentation [20]. Each part contains only one individual cell. Though matching the cell contour inaccurately, each segmented part sets the initial point for the recognizing algorithm to find the contour of the target cell.

Module 2: For some interesting cells, the system will segment the interesting region through individual cell selection by users as a point and then recognize the cell contour on the basis of the initial point.

Module 1 is a fully automated segmentation operation of the system for efficiently segmenting all cells, but it is inappropriate to segment the individual interesting cells. Therefore, Module 2 is added to offer the users an option to segment the interesting cells only. Especially when the contrast between some cell's contour and the background is low, Module 2 plays a significant role in finding the cell's contour.

B. Description of Recognition Algorithm

After the segmentation, accurate recognition of cell contours is crucial for extracting geometric features. Active contour model (Snake) [21] is a significant method to recognize the object contour by optimizing an energy function. Most of active contour algorithms work under the assumption that the desired object has strong edges and the area of force searching extends beyond the desired object contour through an iterative process so that the final result of algorithm converges to real contour. One of the disadvantages of the snake model is that the model deeply relies on initial contour, and hence limits its wide application. Fortunately, Dong et al. developed an algorithm for the snake model by bringing in the score function known as the gradient inverse coefficient of variation with the assumption that the boundary of the desired object has approximately uniform edge strength, and successfully applied the algorithm to find white blood cells from the microscopic image [22]. Furthermore, Ray et al. improved Dong's algorithm with dynamic programming, in which, instead of setting initial contour, an initial point is used [23]. So in this study, Ray's version with simple initial condition is used to recognize the cell contour considering the blur between the cell contour and patterned hydrogel lines. And the initial points have been set by morphology algorithm in the group segmentation module or by cell selection in the individual segmentation module.

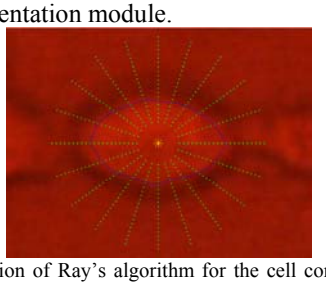


Fig. 4: The illustration of Ray's algorithm for the cell contour optimization. The yellow star is the initial point set by morphology algorithm or cell selection.. The green dotted lines represent the searching points in the algorithm, where $N = 20$ and $M = 22$. Blue circle is the initialized contour computed by the algorithm based on the yellow initial point.

A small part containing one cell cut from Fig. 2 (b) is shown in Fig.4, where the red background is the substrate on which a layer of PEGDA hydrogel was patterned with the OEK chip according to the pre-designed light pattern, the dark red area is the cell boundary together with the rim of patterned lines, the yellow star inside of the object cell is the initial point which was set by the morphological segmentation algorithm or by cell selection, and the green dotted radial lines emanating from the yellow star are the searching space for the Ray's algorithm to find optimal contour. In the process of contour optimization, the discrete radial lines will intersect with the cell contour near the blue circle which is initialized by the active contour model with dynamic programming algorithm. In Ray's algorithm, there are N lines which each has M discrete points, so the computation complexity for the contour optimization is $O(MN)$ with dynamic programming.

In the algorithm, the energy function is defined as [23]:

$$e(p_1, p_2, \cdots, p_n) = e_1(p_1, p_2) + e_2(p_2, p_3) + \cdots + e_{N-1}(p_{N-1}, p_N) + e_N(p_N, p_1)$$

where p_i is the coordinate vector for any of the M points on the i^{th} radial line, and e_i represents the partial energy associated with the point p_i, defined as,

$$e_i(p_i,p_{i+1})=\begin{cases}-g(p_i) & D(p_i,p_{i+1})<\delta\\ \infty & otherwise\end{cases},$$

in which $g_i(p_i)$ denotes the directional image gradient at location p_i, and $D(p_i,p_{i+1})$ the distance between the point p_i and p_{i+1}.

In the snake model with the dynamic programming algorithm, the score function of discrete directional gradient inverse coefficient of variation is defined as

$$G(p_1,p_2,\cdots,p_N)=\frac{\left(\text{mean}\left[g(p_1),g(p_2),\cdots,g(p_N)\right]\right)^2}{\text{var}\left[g(p_1),g(p_2),\cdots,g(p_N)\right]},$$

where "mean" and "var" respectively denote the mean and the variance of directional gradients. In the snake model, to find the optimal contour, the gradient inverse coefficient of variation score function is maximized by finding a set of points on the radial lines and the optimal set of points form the contour. So the energy function of snake evolves as below:

$$e(p_1,p_2,\cdots,p_n;t)=e_1(p_1,p_2;t)+e_2(p_2,p_3;t)+\cdots$$
$$+e_{N-1}(p_{N-1},p_N;t)+e_N(p_N,p_1;t)$$

in which e_i at the t^{th} iteration is modified by:

$$e_i(p_i,p_{i+1};t)=\begin{cases}\left(g(p_i;t)-t\right)^2 & \text{if } D(p_i,p_{i+1};t)<\delta\\ \infty & \text{otherwise}\end{cases},$$

where $i=1,2,\cdots,N$. The problems referred to the above computational algorithm are deeply analyzed in[23].

Ⅳ. Geometric Feature Extraction

Feature design is a key problem in pattern recognition and feature analysis in a certain application, because it can largely determine the performance of the following analyses. Informative features stand for the essence of patterned cells and reliable feature extraction methods facilitate a wide range of usage. And feature extraction is a task that transforms original data into representation space easy for recognition, measurement and matching etc. So a good feature should be informative, robust to noise, time saving, etc. [24]. Feature extraction includes two main approaches. One is the data driven feature extraction, which automatically discover a set of features from the massive data to alleviate the burden of designing features. The advantage of the data driven feature extraction is that some of the features will help further analysis. However, the obstacle faced by the data driven feature extraction is that the extracted features are usually difficult to explain. Another approach is manual extraction with designed features. To better understand the biological behaviors of the cells, geometric features of cell must be designed by specific meaning and rigorous definition. Therefore, the manual extraction with designed features is used in this study and the manual designed features mainly include the geometric parameters, such as area, perimeter, weight center (location), semi-major axis and growing orientation angle, etc. Each geometric feature has specific biological meaning for cell growth, migration or proliferation. The rest of this section will discuss some meaningful geometric features of cell and specific biological behavior.

A. *Area of Cell*

It's known that cells grow in 3-D space while measurement in microscope image is in 2-D plane. Area is a normal feature in scaling size of cell in different stages and states, and stands for the cell covering area in patterned chip.

B. *Perimeter*

As same as cells area, perimeter measurement is in image in 2-D space. Perimeter of cell is the length of cells contour in the image. Area and perimeter regard as two factors which scale size and deformation of cell in the microscope image.

C. *Weight Center*

Weight centre of the cell is also scaled in the image and defined as below

$$W_{ix}=\frac{1}{n}\sum_{\substack{\min x\\(x,y)\in C_i}}^{\max x} f(x,y)\cdot x,\ W_{iy}=\frac{1}{n}\sum_{\substack{\min y\\(x,y)\in C_i}}^{\max y} f(x,y)\cdot y$$

where $f(x,y)$ is pixel value at position (x,y), C_i is cell i area which was recognized in section Ⅲ, n is total number of pixel point in cell i contour, W_{ix} and W_{iy} are weight centre position in image.

D. *Semi-major axis*

Weight centre of cell is not only the centre of geometry, but concentration of organelles, nutrition, energy and etc. Semi-major axis defined as farthest point on cell contour from the weight centre. For adherent cells, the direction of the cells tentacle is a signal of migration, movement and even contact with surrounding cells. So the semi-major axis is an important factor which influences its moving direction in great possibility.

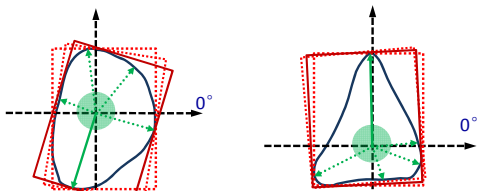


Fig. 5: Two different cells contour recognised form the microscope image. Dashed red rectangles are enclosing rectangle of cells and solid red rectangles are minimum enclosing rectangle. Horizontal axis is 0°, vertical axis is 90° and organ of coordinate is weight centre of the cell. Solid green line, the end point is farthest point in cell contour from weight centre, is semi-major axis of the cell.

E. *Growing Orientation Angle*

Orientation angle of growing cell is considered as a curial impact factor in functional physical and biological characteristics of many tissue types such as muscle, nerve, and so on. Hence, recognition of orientation angle, one of important dimensional information of cell, is significant for measuring cells behaviour, such as migration, growing and contacting trend among surrounding cells [25]. However, orientation is defined by in different ways. For example, [18] defined major axis orientation in a range between -90° and

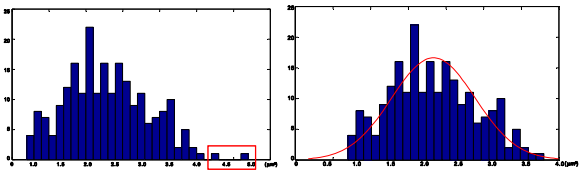
90° and [25] defined longitudinal axis of the minimal circumscribed rectangle related to the horizon axis of the image in a range between 0° and 180°. In this study, orientation angle defined as the angle between semi-major axis and horizontal direction in anticlockwise direction, in a range between -90° and 270°, shown in light green area in Fig.5.

V. Analyses of Geometric Feature

Through above discussion, fundamental features such as area, perimeter, weight centre, semi-major axis and growing orientation angle, have been extracted from image data. There are no good features unless good result got from those features. This section will discuss the method which process original geometric features data and the biological roles of those geometric features played in analysis.

Not all of geometric features data sets got from above sections are validate in analyses. In order to get validate and accurate data set, denoise original feature data is necessary and crucial. After getting data set through designed features extraction, some abnormal data should be eliminated. For instance, cells growing on the chip nearly have same size in different stages namely that the size of the cells will fluctuate around a constant. So this study makes an assumption that the size of cells follows normal distribution. There are some out lines data which were caused by inaccurate recognition of algorithm. Considering biological characters of the cells, some abnormal data of area appears in the data set, like red rectangle shown in Fig.6 (a). In normal distribution, using 3σ criterion, more than 99.7% of data is kept and some abnormal data is removed out of validate data set. Fig. 6 (a) is histogram of original data set and abnormal data is shown in red rectangle, (b) is refined data set that abnormal data has been removed. Comparing the original data, the refined data has good fitting result shown in Fig.6 (b).

Semi-major axis is a factor of scaling cells deformation in non-patterned lines in Fig.2 (b). Based on the data from above section, semi-major axis, designed as shown in Fig. 5, have extracted from microscope image.



(a) (b)

Fig. 6: Using 3σ criterion to eliminate some abnormal data in original features data set in order to avoid fault recognition caused by algorithm. X-axis is area of single cell and Y-axis is the number of cells. (a) is histogram of original data get from recognising algorithm in section Ⅲ, the column in red rectangle is abnormal data. (b) is histogram of data which have been processed by 3σ criterion eliminating abnormal data, red curve is normal fitting curve.

Orientation angle of growing cell is important factor to impact cells migration, movement and etc. Orientation feature on the chip have been extracted from image data and histogram shown in Fig. 7. According to the red fitting curve shown in Fig.7, there are two peaks appear in the histogram. Referring to the geometric patterned PEGDA hydrogel shape on the chip as shown in Fig. 2 (a), the space of cells living is restricted in horizontal patterned channels, because the radius of cells is larger than width of the channels. So the orientation of growing cells is limited in 0°, 180° or fluctuating around 0° and 180° but vary not too much. However, the histogram of orientation angles appears some unusual data around 90° and 270° which can also observed in microscope image. Red curves in Fig. 7 are fitting normal distribution with different means at 8° and 176° respectively. It is obviously that the orientation angles distribution divided into two groups in which orientation angles distribute around two concentrations. The two concentrations of growing orientation angle stand for the main direction of the growing cells.

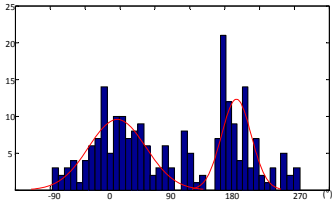


Fig. 7: Experimental and fitted results of cell orientation angles. From microscopy observation and algorithm recognition, most of the cells tend to expand toward the same direction of patterned channels. The blue bars are histogram of statistical feature data and red lines are fitted normal distribution curve based on feature data. X-axis is orientation angle in a range between -90° and 270° and Y-axis is the number of cell in different orientation angle.

Comparing the designed shape of the chip, the two main directions are similar as the direction of patterned lines though there is a little difference. In biological characteristic, because of restrictive living space which is narrower than cells radius, the cells must change their shape so that keep their bodies in a suitable living space. For some unusual orientation angles around 90° or 270°, one reason to explain this phenomenon is that breaks happened in the patterned hydrogel lines what makes cells grow to broken regions.

VI. Conclusion

In this study, automated system combining recognition and measurement of geometric features of the cells living on the patterned substrate which was produced using principle of optically-induced electrokinetics (OEK) was developed. As one of advanced methods of patterning micro structure, OEK produces customized patterns on the substrate for cells to grow. At first, for a large amount of cells, automated system was designed to segment all of cells from light microscope image and recognize cell contours by snake computation model with dynamic programming. According to the detailed demands of analyses, some geometric features such as area, perimeter, semi-major axis and growing orientation angle were designed and extracted from the contour. Finally, by those geometric features have been found, simple analyses of connection between biological features and geometric features were made. Therefore, the automated system can recognize and measure cells geometric features rapid, accurate and efficient and bring more convenience for development of biology and life science.

VII. Future Work

Although this study talked about some fundamental manual geometry features to analysis the biological features of cells, more biological features, which demand more specific biological background knowledge, needed to add into the system to rich the features set which influence cells behaviour and tissue formation. For example, mechanical properties [26-28], as one of effective label free biomarkers, are significant features of cells state. OEK system, a fast and effective pattern biomaterial method, produces a series of patterning substrates and then plant cells on the substrate. After that, an new system which combines the system in this study to recognize and measure some geometric features of cell observed in microscope image, measure mechanical property such as Young's modulus of cell membrane and imaging cells shape at nano scale using atomic force microscope (AFM), is one of meaningful tasks to deeply understand the multi biological features in cell behaviour.

Additionally, this paper use one of dynamic programming deformable model to recognize cells in microscope image while there are many other good algorithms to choose from for other different platform. Because the algorithm chose in this paper is not the best in performance of recognition but the best one of considering accuracy of cells recognition and fault tolerant ability.

In the end, it is known that manual feature design is restrictive, like complexity of designing feature and time consuming especially needed specific background and knowledge. Besides, for a large number of cells in different sorts of microscope images, features design become more and more difficult. In order to obtain effective features data, unsupervised learning data [24], find features by algorithm itself in data set, becomes a feasible method.

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