# A Single Cell Gel Electrophoresis of Its Optical Image Analysis*

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**Abstract** A single cell gel electrophoresis was used to measure DNA damage in individual cells. The extent of DNA damage was quantified by the various parameter which can be done with computerized image analysis system. It was shown that: as a rapid, sensitive and simple tool that can be utilized to nucleus DNA damage test, cell genetics, environmental monitoring.

**Keywords** Comet assay; Single cell gel electrophoresis; Image analysis
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## 0 Introduction

Single cell gel electrophoresis (SCGE), also called comet assay, was firstly introduced by Östling and Johanson as a method of assessment of DNA damage in individual cells in 1984[1], which was based on electrophoresis of cells embedded and lysed in agarose on a microscope slide, and further developed by Singh in 1988[2] and Olive in 1990[3]. As a quick, sensitive and convenient method to detect the DNA damage, this assay is widely applied in radiation biology genetic toxicology, apoptosis, etc.

Östling and Johanson used neutral electrophoresis at their experiments[4]. Under neutral condition DNA can keep its bifilar helix structure that accidental single strand breaks doesn't effect it's continuity and the obtained comet tail seemed to consist of DNA loops[4].

## 1 Experiment

### 1.1 Mechanism

The mechanism of SCGE is that DNA is organized in a supercoil structure, which will be partly collapsed into pieces by strand breaks in the DNA. And these fragments will be migrate out by electrophoresis Under the indication of fluorescent dye, the resulting images named 'comet' for their appearance can be measured to determine the extent of the DNA damage.

Under alkaline condition, the double strands are separated that the single strand breaks could be released and stretched out by electrophoresis. The obtained tail consists of fragments, which migrate more freely than loops in neutral electrophoresis. The comet image parameters for analysis: "tail length", "tail DNA" (fluorescence intensity) and "tail moment" are directly correlated with the frequency of DNA strand breaks. The assessment of DNA damage could be given after the parameters coming out.

### 1.2 Material and method

Microscope slide was firstly pretreated by spreading 50 μL of NMA (0.5%) evenly on its surface and letting it air-dry. 75 μL cell suspension was mixed with 75 μL LMA (1%) kept at 37 ℃. Then, piped 75 μL of the mixture onto the pretreated slide and covered it with a 18 × 18 mm coverslip. After immediately gelling for 10 m at 4 ℃, the coverslip was gently removed. And the third layer of 75 μL NMA (0.5%) was added and gelled both as the way referred just before.

Coverslips were removed away and slides were immersed in lysis solution (2.5 M NaCl, 100 mM Tris, 1% tritonX-100, NaOH was added to pH 10) and kept at 4 ℃ for 2h. Then, the slides were put into a electrophoresis tank filled with buffer (0.3 M NaOH, 1 mM EDTA (pH 13)) and kept for 20 m. Electrophoresis was carried out at 25V and 300 mA for 20 m. Finally, slides were neutralized in 0.4 M Tris-HCl (pH 7.4) for 30 m twice. Following that, slides was stained with a fluorescent DNA binding dye for image analysis. Two fluorescent dye prevail; ethidium bromide (10 μg/mL) and acrodine orange (100 μg/mL).

## 2 Image analyses

After the ending of the experiment, photos of the comet was taken through a fluorescence microscope. A typical comet image distinctly consists of "head" and "tail". Following parameters were used to assess the images in different way.

### 2.1 Simple parameters

1) Shape

The DNA fluorescent images were classified into two groups: the comet groups, with its images

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distinctly have "head" and "tail", and the non-comet group. Calculating the rate of the comet amount of these two groups, extent of DNA damage generally comes out.

2) Length

"Tail length" (from the center of the comet head, along the electrophoresis direction, to the outlying edge of the comet tail), "comet length (the length of the tail along the electrophoresis direction)" can be got directly from Microscope photographing. These parameters described the action of DNA fragments during electrophoresis. Especially these numerical values are linearly dependent on the damaging dosage goodly, as the damaging factors are heavily dosed with.

3) Intensity

Using the fluorescent intensity rate of the comet head to the tail to assess the damage extent.

## 2.2 Synthetic parameters

1) "Tail moment"

"Tail moment" (TM), was defined as the product of tail length, which is from the center of the head to the center of the tail, and the rate of the tail DNA to head DNA. And it was conformed to be a superior parameter with the author's experiments.

2) "Comet moment"

Comet image was divided up into many small pieces from the center of the comet head along the electrophoresis direction. Every piece's fluorescence intensity ($D_i$), total fluorescent intensity ($D_t$) and the distance ($X_i$) from the center of the head to the center of the small piece was measured. CM was defined as following:

$$\mathrm{CM} = \sum \left( \frac{D_i \times X_i}{D_t} \right) \tag{1}$$

3) "Tail inertia" (inertia)

"Tail inertia (TI)" was used with the assistance of professional software and equipment. Similar to CM comet was firstly divided into two parts: head and tail. And the tail was divided into many small pieces. To every piece, take the product of the fluorescent intensity ($D_i$) and the area ($S_i$) as DNA amount. Then TI was defined as following

$$\mathrm{TI} = \sum \frac{S_i D_i X_i^2}{S_n D_n + S_t D_t} \tag{2}$$

$S_n$ denotes the total area of the comet head; $D_n$ denotes the total fluorescent intensity of the comet head; $S_t$ denotes the total area of he comet tail.

Some details were discussed. 1) How to distinguish the "head" and "tail" exactly, as the comet assay was applied on cells of different tissue and under different experiment condition, which would make the obtained images were various. 2) As the center of the "head" was set to be the origin to calculate the distance (referred before), in fact, the "head" was not the original "head" any more; it just consisted of residual DNA after the DNA fragments migrating out.

For reducing the subjective influence during the analysis, a resolution with common mathematic software MATLAB 6.0 was supplied to complete a specific analysis. Based on "comet inertia", a new parameter "excursion" was suggested for describing the whole excursion of DNA under electrophoresis.

In this way, images were digitalized and read into computer. With the help of MATLAB, digital images were read in matrix format for next calculating. Then a function can be written for computing the objective value ($E$). The calculating method is described below

$$E = \sum \frac{(x_i - x_0)^2 d_i}{D} \tag{3}$$

As show in Fig. 1, $x_i$ denotes the distance from the image edge to the point $P(i,j,d_i)$; $x_0$ denotes the distance from the image edge to the base line; $d_i$ denotes the fluorescent intensity of point $P(i,j,d_i)$; $D$ denotes the total fluorescent intensity of comet.

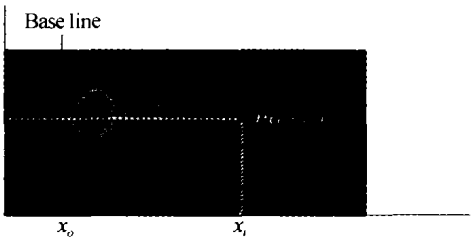


Fig. 1 The image of single cell gel electrophoresis

Procedures: ①Input a digital comet image (200 × 100 pix) into a computer; ②Read the digital image to variable I in MATLAB workspace (use function misread, help can be get in image processing toolbox); ③Call variable $I$, it will display in matrix format. The element of the matrix can be denoted in mathematic as $P(i, j, d_i)$, and the number in the matrix is the value of $d_i$; ④Run function for calculating and get a value $E$.

Analyses a group of twenty or more fine "comets" to get an average of moment of "head" and "tail", which was set to compare, and takes the ratio to assess the DNA damage

In this method, a tangent of the "head" (perpendicular to the electrophoresis direction) was chosen, as the base line, for it's easier to be located exactly than distinguish "head" and "tail" exactly with a dividing line. And we took the "comet" as a whole to calculating that it is no necessary to have some

misgivings about the fitness of the analysis method to the shapes of various "comet".

## 3 Conclusion

SCGE is frequently applied in radiation biology, DNA damage and repair, genetic toxicology, apoptosis and environment inspection. On the other side, some problems still existed. The exact mechanism of SCGE is not very clear. Analysis parameters needs to be standardized, factors effect the results were too many and difficult to control. But, with the development of technique, SCGE will be applied more widely and the problem will be solved without doubt.

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# 单细胞凝胶电泳其及光学图像分析


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**摘 要** 采用单细胞凝胶电泳检测 DNA 损伤度,运用光学图像处理软件对彗星试验得到的参数进行图像分析,结果表明:该方法快捷,灵敏度高,能够较好地应用于 DNA 损伤度的修复、细胞遗传、环境检测等方面.

**关键词** 彗星试验;单细胞凝胶;图像分析

**Zhang Wei** was born in Shaanxi in 1974. He received his B. S. degree in 1997 from Northwest University. Now, he is studying for M. S. degree in Institute of Photonics & Photon-Technology of Northwest University. His major is Biophotonics.