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Digital Image Techniques for the Comet-FISH Assay in the Search for DNA Damage and Repair

Mehmet Can
International University of Sarajevo,
Faculty of Engineering and Natural Sciences,
HrasnickaCesta 15, Ilidža 71210 Sarajevo,
Bosnia and Herzegovina
mcan@ius.edu.ba

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Abstract

Studies worldwide have demonstrated that the Comet-FISH assay can detect DNA damage and repair in a number of genes, gene regions and loci. Any gene could be detected if a suitable probe is available in theory. Assay's relative speed and sensitivity make it a very handy laboratory technique for studying the cellular response to damage. It has also the advantage of being able to study specific genes and gene regions of interest, particularly those associated with disease. Its speed and sensitivity also makes it versatile for use in a clinical setting whereby data can be quickly supplied from patient cell samples. The test helps to get information about the development of treatment. This is proved to be particularly beneficial in cancer management, where increasing emphasis is being placed on personalized medicine. Until recently, no research group has yet reported the successful use of a reliable software package for accurately counting hybridization signals from comet slides. They prefer counting signals manually. Manual work is both time consuming and laborious, and also brings in user subjectivity. This study is an attempt in computerization of the process. In a case study, the comet image is processed by the help of digital image handling techniques, and parameters that will help to decide about the DNA damage are derived.

1. INTRODUCTION

Single cell gel electrophoresis is a technique for the detection of DNA damage and repair at the level of single cells, which is also called The Comet Assay. Assay is one of the most advanced techniques introduced to the life sciences in recent years.

The single-cell gel electrophoresis (SCGE) assay was first reported by Ostling and Johanson in 1984 (Ostling, and Johanson, 1984) as a technique for visualizing the migration of DNA containing strand breaks in individual agarose-embedded cells under electrophoretic conditions. A few years later, Singh et al. (Singh et al. 1988) modified the technique to use highly alkaline (pH413) conditions that encourage unwinding of DNA around a strand break. In both studies, the underlying principle of the assay is that when DNA is subjected to an electric current, DNA containing strand breaks will migrate through an agarose gel due to relaxation of the DNA supercoils, whilst unbroken DNA remains immobile.

Following the staining of DNA with a fluorescent DNA-specific dye, the resulting image is visualized and

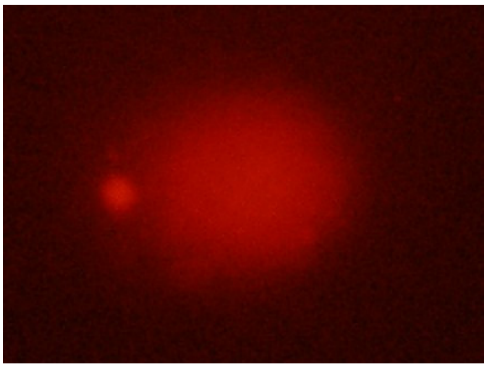
resembles a comet, with undamaged DNA forming a head and damaged DNA forming a tail, an observation that has led this technique to be more commonly called the Comet assay (Olive et al. 2000).

Since then, it has become a widely accepted and versatile method for measuring a variety of DNA lesions in individual cells (Fairbairn, et., al. 1995, Tice al. 2000, Collins, 2004). Its versatility stems from various modifications to the original Comet assay that have been developed in order to measure different types of the DNA damage, including single- and double strand breaks, cross linking and oxidative damage, as well as DNA breaks associated with replicating DNA and DNA repair.

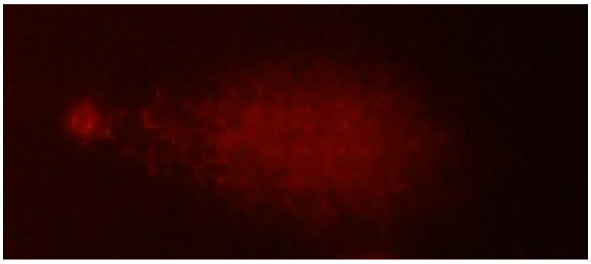
2 METHOD

Analysis of cells involves examining both the distribution of total genomic DNA in the comet, together with the number of hybridization signals, and the location of each signal. Overall DNA distribution can be measured using standard Comet assay analysis software, whereby the amount of DNA in the head and tail of each selected cell is assessed using a number of parameters, including % tail DNA and Olive tail moment, the two preferred measurements of DNA damage in Comet assay experiments. Then, in the same selected cells, the number of signals and the position of each hybridization signal in the head or tail of the comet may be recorded, thereby giving an indication of whether it lies in, or close to, a region of damaged DNA.

The appearance of hybridization signals in the comet tail generally indicates that the region of DNA within, or around, the probe contains strand breakage. Information about where exactly the DNA breakage occurs in relation to the probed region is obtained by counting the frequency distribution of signals in each comet. Increase in signal number would suggest the probed region itself contains strand breakage, since the probe will bind to each broken DNA fragment from the target region. Of course, control cells must always be included to give an indication of baseline damage for both overall DNA and hybridisation signals. Figure 1. shows representative examples of images from Comet-FISH experiments.



(a)



(b)

Figure 1. Representative images of cells processed using the Comet-FISH protocol. (a) Unirradiated control cell showing little DNA damage as evidenced by the absence of a comet tail. One hybridisation signal, bright spot is clearly visible in the intact head. (b) Immediately after exposure to g-radiation a large comet tail is visible, reflecting the extent of DNA damage. *(With the courtesy of Murat Dikilitas, Department of Plant Protection, Harran University, Faculty of Agriculture, 63300, S. Urfa, Turkey).*

3. SCORING

To diagnose the DNA damage or repair, by the use of digital image processing techniques following parameters can be estimated:

1) The Percentage of DNA in the head (H-DNA, %)
2) The percentage of DNA in the tail (% of migrated DNA)
3) Tail length (TL, μm) and
4) Tail moment (TM expressed in μm, which is the fraction of migrated DNA multiplied by the tail length divided by 100) are easily measured.
5) Head-tail ratio
6) OTM (Olive tail moment), which was calculated with the following formula;
   %Tail DNA x [(tail CoG-center of gravity) – (head CoG-center of gravity)].

Although tail moment and/or tail length measurements are the most commonly reported, the use of percent DNA in tail is recommend, since it gives a clear indication of the appearance of the comets and in addition, is linearly related to the DNA break frequency over a wide range of levels of damage (Gichner et al., 2008; Collins and Harrington, 2002).

Commercial softwares which, linked to a closed circuit digital camera mounted on the microscope, automatically analyses individual comet images. The programs are designed to differentiate comet head from tail and to measure a variety of parameters including cell area, comet area, % head DNA, % tail DNA, TL, TM (Dikilitas, et. al 2009).

It is also possible to analyze comets quantitatively without image analysis software. The human eye can discriminate comets representing different levels of damage; therefore,

visual scoring is performed due to its speed and simplicity. Comets must be selected without bias and must represent the whole gel, so it is important to scan the whole gel either in computer-based analysis or visual scoring. The migration of DNA could be categorized according to its head and tail shape by visually.

For this, a generally accepted DNA damage-index has been used in many cited articles (Kobayashi et al., 1995; Gichner et al., 2003; Kocyigit et al., 2005). According to this; different levels of DNA damage is classified from 0 (no tail) to 4 (almost all DNA in tail). The scale used is as follows:

0 = no cometting;
1 = comet < 0.5 times the width of nucleus;
3 = Comet greater than width of nucleus;
4 = Comet > twice the width of the nucleus.

Scoring cells in this manner has been shown to be as accurate and precise as using computer image analysis (Dikilitas, et. al 2009).

4. COMPUTER IMAGE ANALYSIS

Using the above mentioned approach, several interesting papers have been published over the past decade in which the Comet-FISH assay has been utilized to investigate the cellular response following DNA damage. To contribute to those efforts, we propose a computerized digital image technique in the use of Comet-FISH Assay in DNA damage and repair.

4.1. Rotate the Image to Make the Comet Axis Horizontal

To fit a circle to the head of the comet, and an ellipse to the body, we rotate the image to make the comet axis horizontal. The angle of the comet axis to horizontal is found through a least squares fit

$$\text{line} = \text{Fit}[\text{pck}, \{1, x\}, x]$$

$$87.5419373944438 + 0.0709971242441607 4x \quad (1)$$

ArcTan[0.070997]=0.07 radians = 4 degrees

Using a picture editor, image in Figure 1.b. is rotated by 4 degrees to obtain the horizontal comet mage in Figure 2.

Figure 2. Image in Figure 1.b. is rotated by 4 degrees to obtain the horizontal comet mage.

A second least squares fit gives

$$\text{line} = \text{Fit}[\text{pck}, \{1, x\}, x]$$

$$35.19 + 0.00\, x$$

Which means that, axis of the rotated comet is horizontal.

4.2. Find the Profile

First, colored image in Figure 2, is transformed into a gray mage. Then gray levels along the comet axis y=35.19 are plotted.

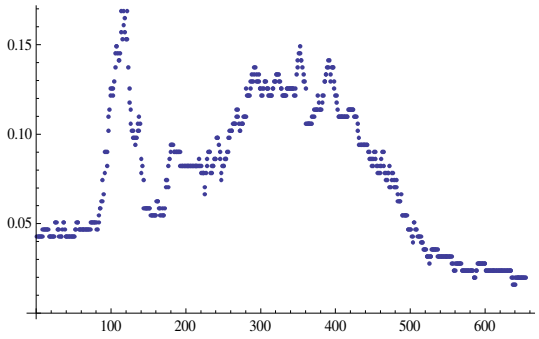


Figure 3. Gray level profile of the comet, along the comet axis.

From the profile, it is seen that the gray level of the background is 0.05 near head, and 0.02 near tail. A threshold of t=0.05 is adopted and pixels whose gray levels are more than this threshold are picked. These pixels supply a black-white profile of the comet as in Figure 4.

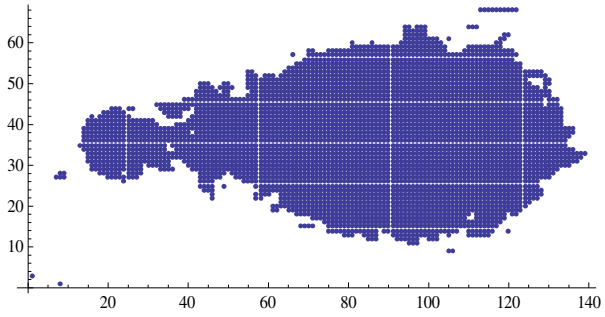


Figure 4. Profile of the comet

To fit a circle to the head, we chop the head, and find the center of the gravity of pixels in the chopped image as the center of the circle on the comet axis. Radius of the circle is found such that 90% of the pixels in the chopped image will remain in the circle.

For ellipse, center of the gravity of pixels in the body is taken as the center of the ellipse on the comet axis. Big and small radii of the ellipse are found such that 90% of the pixels in the black-white image of the comet body will remain in the ellipse. When circle and ellipse are plotted together with the comet profile, the picture in Figure 5 is obtained.

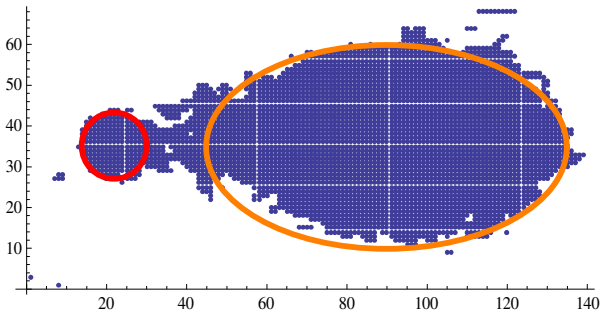


Figure 5. Circle of the head and ellipse of the body are plotted together with the comet profile.

Computations gave the geometric properties of the circle and the ellipse as follows:

Circle = $\{\{21.83, 35.19\};\ 8.12\}$
Ellipse= $\{\{90, 35.19\}, \{45, 25\}\}$ (2)
R1=8.12
R2=45
R3=25

The six parameters in Section 3 can be formalized as follows (Dikilitas, et. al 2009):.

Parameters:

1) The percentage of DNA in the head (H-DNA, %),
$HDNA = R_1^2/(R_1^2 + 4 * R_2 * R_3)$ (3)
2) The percentage of DNA in the tail (% of migrated DNA),
$TDNA = 4 * R_2 * R_3/(R_1^2 + 4 * R_2 * R_3)$ (4)
3) Tail length (TL, μm)
$TL = 2R_2$ (5)
4) tail moment (TM expressed in μm, which is the fraction of migrated DNA multiplied by the tail length divided by 100)

$TM = HDNA * TL/100$ (6)

5) Head/Tail ratio
$TL = R_1/(2R_2)$ (7)
6) OTM (Olive tail moment),
%Tail DNA * [(tail CoG-center of gravity) – (head CoG-center of gravity)].

Using the radii lengths in (2) the above six parameters are computed.

Table 1. Parameter values for the given image

Parameter	Values
HDNA	0.0001%
TDNA	99.99%
TL	90
TM	0.0001
H/T	0.09
OTM	0.007

The parameter values in Table 1, clearly reveals that the sample cell has a double strand damage.

5. CONCLUSION

Over the past decade, the Comet-FISH assay has proven a rapid and relatively simple procedure for measuring DNA damage and repair in both gene-specific loci and whole chromosomes in a variety of different cell types. The versatility of the assay means it offers great potential as a method for assessing DNA damage in specific gene regions, as well as the overall genome, in individual cells in response to many damaging agents, with clear implications for both basic science research and clinical application. To contribute to these efforts, we propose a computerized digital image technique in the use of Comet-FISH Assay in DNA damage and repair. It is seen that there is a future for computerized tools in Comet-FISH assay applications.

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