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Detection of DNA Damage Using Comet Assay Image Analysis

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ABSTRACT

Reactive species such as free radicals are constantly produced in vivo and DNA is the very important target of oxidative stress. Oxidative DNA damage is considered as a predictive biomarker to monitor the risk of development of many diseases. The comet assay is widely used for specifying oxidative DNA damage at a single cell level. The analysis of comet assay output images, however, poses considerable challenges. The comet assay, also known as single-cell gel electrophoresis (SCGE), is a simple, sensitive and reliable method for studying DNA damage caused by physical and chemical agents. So the comet assay is a well-established, simple, versatile, visual, rapid, and sensitive method used extensively to assess DNA damage quantitatively and qualitatively at single cell level. The comet assay is most frequently used to analyze white blood cells or lymphocytes in human bio monitoring studies. So through the analysis of comet assay image we can detect edge of damaged DNA comet isolating it from undamaged DNA.

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I. Introduction

A. Basics of DNA:

Deoxyribonucleic acid (DNA) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses. DNA is a nucleic acid; alongside proteins and carbohydrates, nucleic acids forms the three major macromolecules necessary for all known forms of life. Most DNA molecules have two biopolymer strands coiled around each other forming a double helix. The two DNA strands are known as polynucleotides since they are made up of simpler units called nucleotides. Each nucleotide is composed of a nitrogen-containing nucleobas that are either guanine (G), adenine (A), thymine (T), or cytosine (C) as well as a monosaccharide sugar called deoxyribose and a phosphate group.

The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, forming an alternating sugar-phosphate backbone. According to base pairing rules (A with T and C with G), hydrogen bonds bind the nitrogenous bases of the two isolated polynucleotide strands to form double-stranded DNA.

DNA is well-suited for biological information storage. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. Biological information is replicated as the two strands are isolated. A major portion of DNA (more than 98%

for humans) is non-coding, meaning that these sections do not serve a function of encoding proteins. The two strands of DNA run in opposite directions to each other and are hence anti-parallel. Attached to each sugar is one of four types of nucleobases (informally, bases). It is the sequence of these four nucleobases along the backbone that encodes biological information. Under the genetic code, RNA strands are modified to specify the sequence of amino acids within proteins. These RNA strands are created using DNA strands as a template in a process called as a transcription.

The basic comet assay method is simple. Cells are embedded in agarose and lysed, which is followed by lectrophoresis. Upon electrophoresis, undamaged DNA in a super coiled state remains intact while the damaged DNA reveales strand breaks.

B. Background of Comet Assay method:

Detection of deoxyribonucleic acid (DNA) damage at the level of an individual eukaryotic cell indicates high significance in the fields of toxicology, pharmaceuticals, genotoxicity testing, environmental/ human bio-monitoring, diagnosis of genetic disorders etc. Single cell gel electrophoresis (SCGE) or the comet assay is a versatile, sensitive yet simple and economical technique used to measure DNA damage and repair in individual cells. The comet assay helps to measure the single/ double-strand DNA breaks. In 1984, Ousting and Johnson demonstrated migration of DNA strands from nuclei which were exposed to an electric field under neutral conditions. Later, in 1988, Singh and his co-workers modified and optimized this procedure using alkaline conditions which substantially increased its specificity and reproducibility. Since then SCGE has achieved huge popularity and evolved as a standard technique for calculation of DNA damage/repair. There has been constant modification and innovations in the protocol which has led to an array of comet assay variants. A wide variety of samples including peripheral blood, cultured cells, buccal mucosal cells, solid tumor, cancer cells, sperm, yeast cells, bacteria etc., can be subjected to SCGE which makes the assay more versatile. The most widely used method for assessment of DNA damage is the alkaline comet assay.

II. EVALUATION OF DNA DAMAGE USING SINGLE-CELL GEL ELECTROPHORESIS (COMET ASSAY)

The comet assay, also known as single-cell gel electrophoresis (SCGE), is a simple, sensitive and reliably efficient method for studying DNA damage induced by physical and chemical agents .The basic principle of the comet assay method is simple. Cells are embedded in agarose and lysed, followed by electrophoresis. Upon electro-phoresis, undamaged DNA in a supercoiled state remains intact while damaged DNA strand breaks are revealed. These relaxed loops of damaged DNA extend to the anode that forms a comet-shaped structure. Comets can then be visualized by staining with a DNA-binding dye using fluorescence microscopy. To assess the level of DNA damage, the comet size, shape and the amount of DNA within it needs to be measured. Ostling and Johanson introduced the microgel electrophoresis method to measure DNA strand breaks under neutral pH condition[1].

Comet assay is one of the most popular tests for the detection of DNA damage at single cell level. In this study, an algorithm for comet assay analysis has been proposed, that has an important step of comet identification via and its edge detection via Fuzzy Inference System (FIS) .The algorithm has been evaluated using comet images from human leukocytes treated with a commonly used DNA damaging agent. The proposed approach with a commercial system has been shown and results show that fuzzy inference systems are able to assess the images and the work can be further extended for isolating damaged and undamaged DNA.

III. COMET FINDING USING FUZZY INFERENCE SYSTEM:

The first task in finding comets is to separate meaningful objects from the background .These objects then need to be either accepted as comets or discarded. Our approach involves extracting for the objects of interest shape parameters using a set of fuzzy rules. We show that the shape parameters can be used to obtain edges of comets. To obtain regions of interest ,the edge detection method using fuzzy Logic image processing (Particularly a Mamdani FIS System is proposed).

A. Fuzzy Logic Image Processing:

A fuzzy inference system (FIS) is a system that uses fuzzy set theory to map inputs (features in the case of fuzzy classification) to outputs (classes in the case of fuzzy classification). A rule based fuzzy-approach system based on the concept of fuzzy logic is applied to the edge point detection. In this approach, a set of fuzzy rules, membership functions, are formulated and developed and then are implemented in MATLAB. With these steps, the system is capable of detecting the edge points.

B. Edge detection

In digital image, the edge is a collection of the pixels whose gray value has a step or roof change, and it also refers to the part where the brightness of the image local area changes significantly. The gray profile in this region can generally be seen as a step. That is, in a small buffer area, a gray value rapidly changes to another whose gray value is largely different with it. Edge widely exists between objects and backgrounds, objects and objects, primitives and primitives. The edge of an object is reflected in the discontinuity of the gray. Therefore, the general method of edge detection is to study the changes of a single image pixel in a gray area, use the variation of the edge neighboring first order or second-order to detect the edge.

C. The FIS Structure

The FIS structure is the MATLAB object that has all the fuzzy inference system information. This structure is provided inside each GUI tool. Access functions such as getfis and setfis make it very easy to examine this structure.

All the information for a given fuzzy inference system is contained in the FIS structure, including variable names, membership function definitions, and so on. This structure can itself be thought of as a hierarchy of structures.

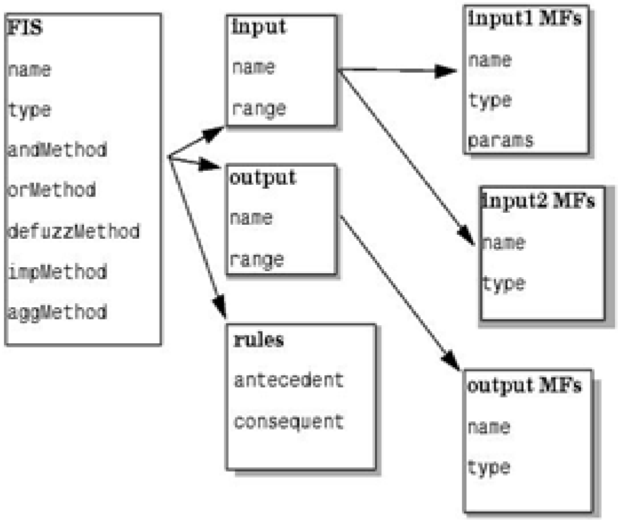

Fig 1 .General FIS Structure

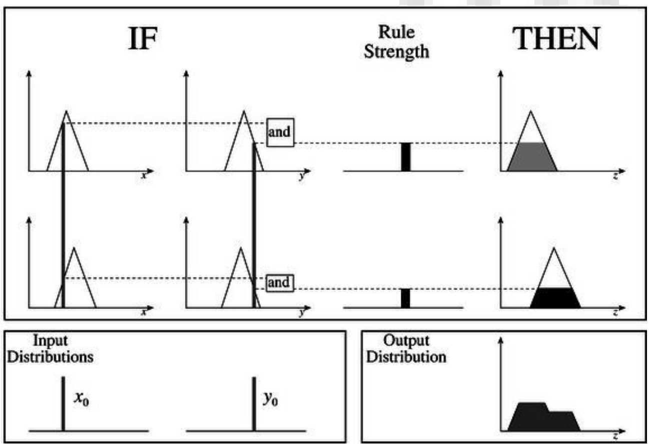

Fig 2: A two input, two rule Mamdani FIS with crisp inputs

We can create custom membership and inference functions as described in representing custom membership Functions and indicating custom interference systems and specify them for building fuzzy inference systems at the command line. Mamdani Fuzzy Model is an important technique in Computational Intelligence (CI) study[4]. An example of a Mamdani inference system is shown in figure.

To compute the output of this FIS given the inputs, we must follow these six steps:

- Determining a set of fuzzy rules
- Fuzzifying the inputs using the input membership functions,
- Combining the fuzzified inputs according to the fuzzy rules to establish a rule strength,
- Finding the consequence of the rule by combining the rule strength and the output membership function,
- Combining the consequences to get an output distribution, and
- Defuzzifying the output distribution (this step is only if a crisp output (class) is needed).

C. FIS Evaluation:

To evaluate the output of a fuzzy system for a given input, we use the function evafis .For example, the following script evaluates tipper at the input if a = readfis('tipper') and if we put function evalfis([1 2], a) then it will display the answer as 5.5586 This function can be also used for multiple collections of inputs, because different input vectors are shown in different parts of the input structure. If we consider an example evalfis([3 5; 2 7], a).This would display the answer as 12.2184 and 7.7885. 5 while edge FIS will evaluate edges in image.

D. Implementation

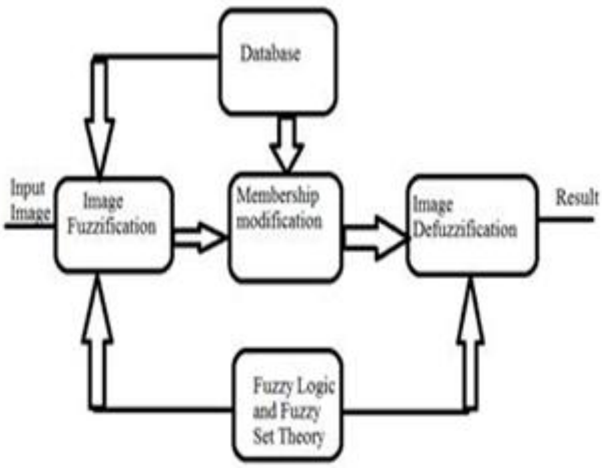

Fig 3. Fuzzy Logic Image Processing

For the implementation we adopt following procedure:

- Import RGB Image and Convert to Grayscale.
- Convert Image to Double-Precision Data.
- Obtain Image Gradient.

- Define Fuzzy Inference System (FIS) for Edge Detection.
- Specify FIS Rules and Evaluate FIS and finally plot the results.

IV. EXPERIMENTAL RESULTS:

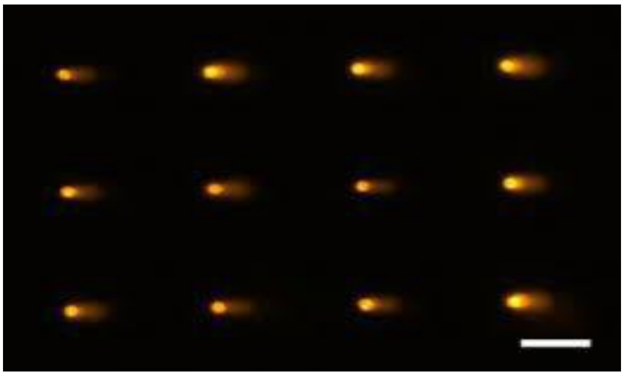

Fig 4. Comet Input Image

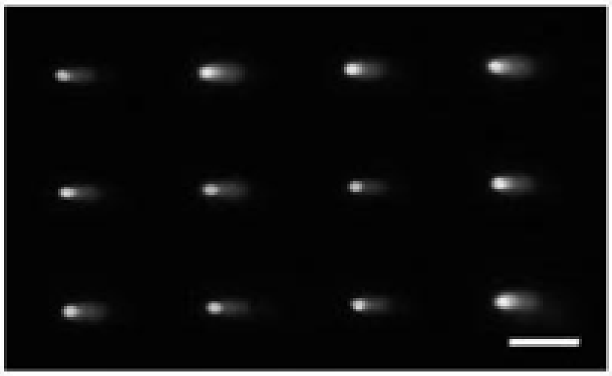

Fig 5.Input Image in Greyscale

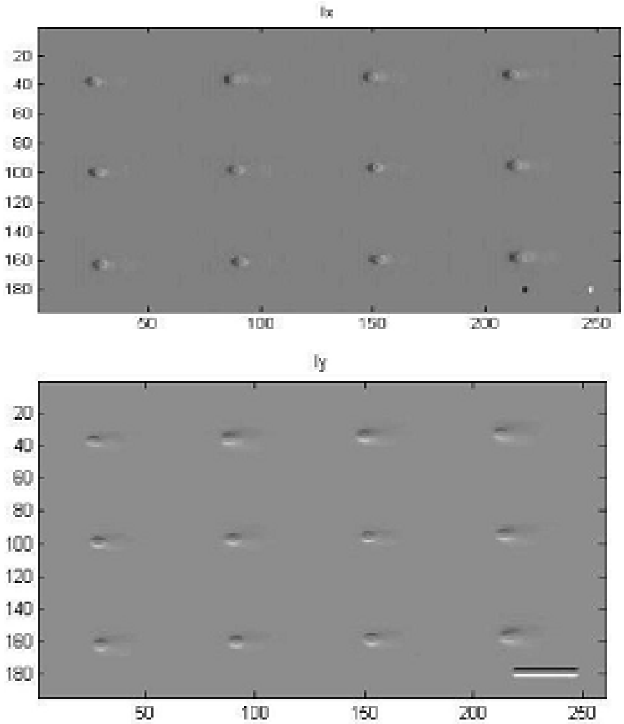

Fig 6 .Image Gradients Ix and Iy

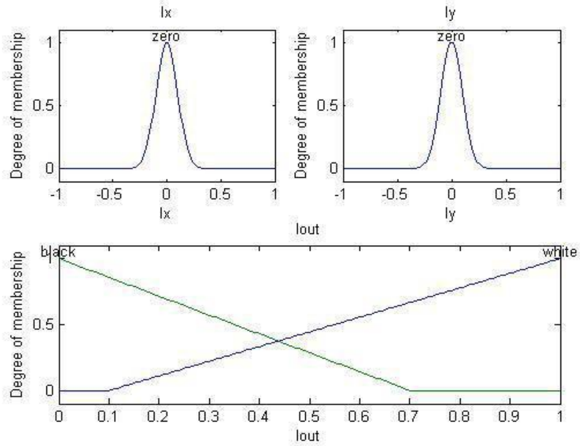

Fig7 .Membership functions of the inputs/outputs of edge FIS.

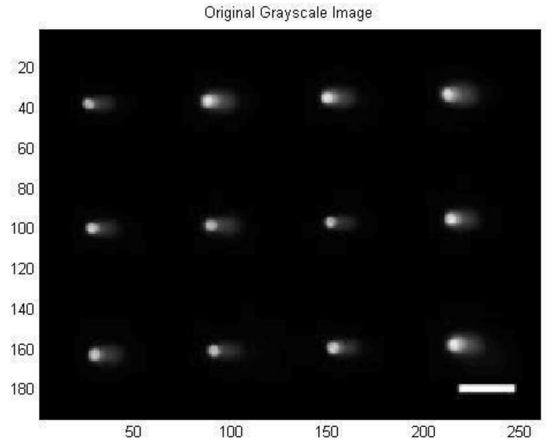

Fig 8 .Original Grey Scale Image

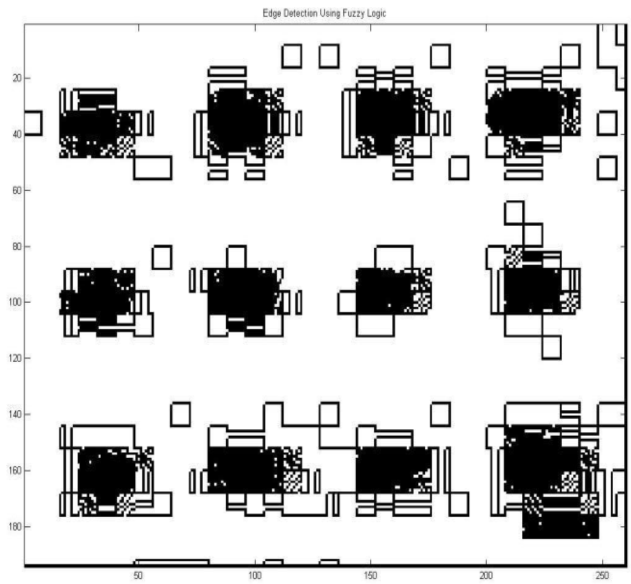

Fig 9.Edge Detection using fuzzy Logic

V. APPLICATIONS OF COMET ASSAY IMAGE ANALYSIS

SCGE (Single Cell Gel Electrophoresis) has a wide range of applications such as genotoxicity testing, toxicological studies, drug evaluation, molecular epidemiology, ecological monitoring, human bio- monitoring, occupational exposure to genotoxic chemicals and radiation, nutritional studies, assessment of DNA damage in certain genetic disorders, and assessing background levels of DNA damage.

VI. CONCLUSION

Fuzzy Logic Image Processing is a powerful tool in which fuzzy sets provide a framework for incorporating human knowledge in the solution of problems whose formulation is dependent on imprecise concepts. This algorithm based on FIS system is able to detect edges of various images .The algorithm developed here exhibits huge scope of application in image analysis. FIS is a very simple and efficient method; identify the edge without determining a threshold value. So here FIS gives single output value corresponding to multiple input values and identifying the an edge pixel. So here Fuzzy inference system in MATLAB environment has been developed which gives the output result of the input values used as membership functions. Thus with the help of this analysis algorithm the unique representation along with the edge detection of comet assay image is performed and further work can be extended to isolate damaged and undamaged DNA detection.

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