COMET ASSAY PRINCIPLES & APPLICATIONS

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Comet assay

Single cell gel electrophoresis (SCGE)

Advantages:

1. It is a sensitive and rapid technique for quantifying and analyzing DNA damage in individual cells.

2. Collection of data at the level of individual cell.

3. Requirement for small number of cells per sample.

4. Any nucleated cell is amenable to analysis.

What does Comet Assay measure:

- **Comet assay detects**:

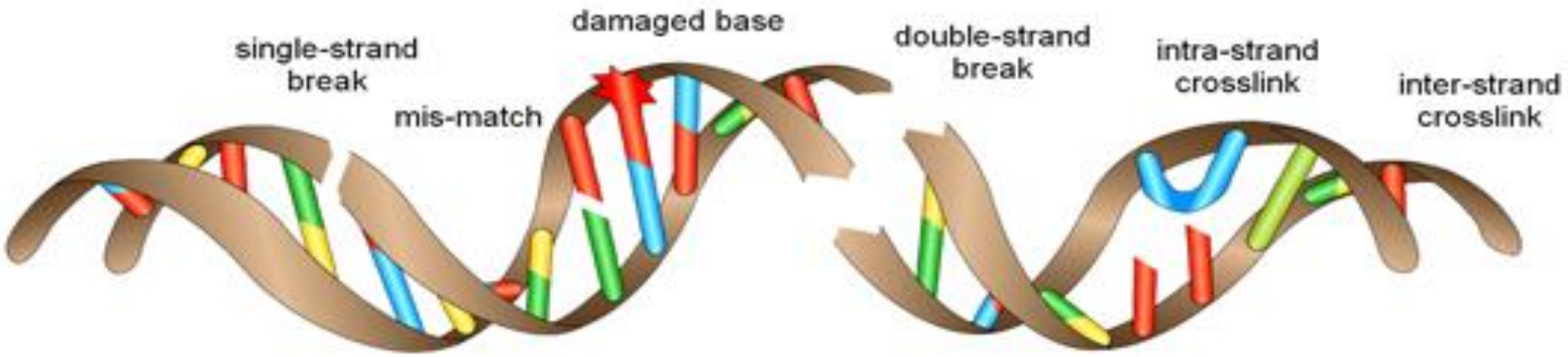
✓ **Single strand breaks (SSBs),**

✓ **Double strand breaks (DSBs),**

✓ **Alkali labile sites, Ap sites,**

✓ **Oxidative DNA base damage,**

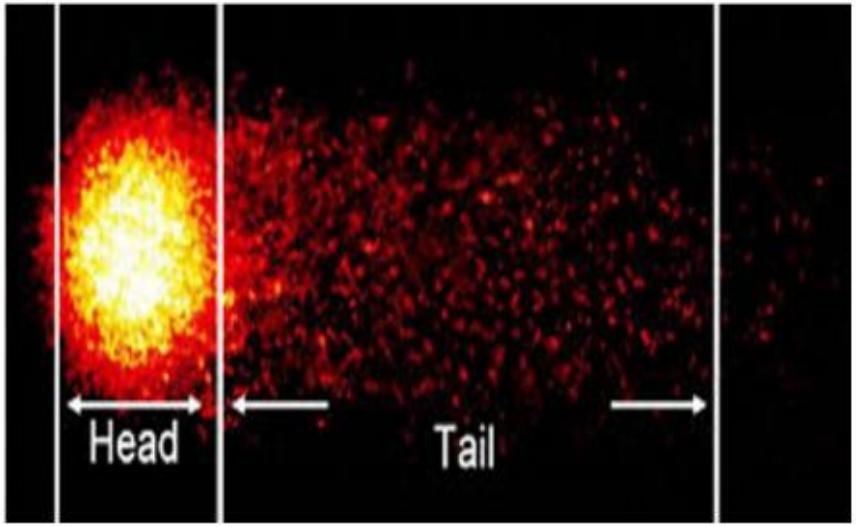
✓ **DNA-DNA cross link and DNA-protein and Drug cross linking & DNA repair.**

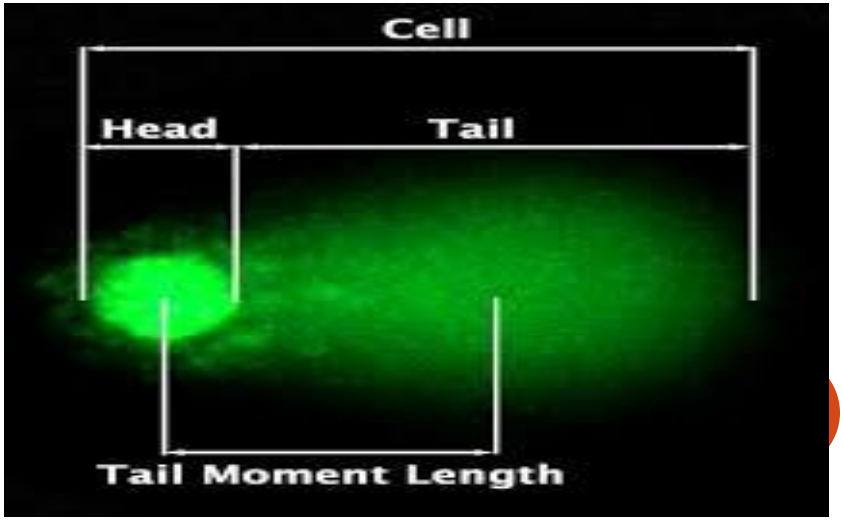


PRINCIPLE:

- **Individual cells are embedded in a thin agarose gel on a microscope slide (frosted slide).**
- **All cellular proteins are then removed from the cells by lysing.**
- **The DNA is allowed to unwind under alkaline/neutral conditions.**
- **Following the unwinding, the DNA undergoes electrophoresis, allowing the broken DNA fragments or damaged DNA to migrate away from the nucleus.**
- **After staining with a DNA-specific fluorescent dye such as ethidium bromide, the gel is read for amount of fluorescence in head and tail and length of tail.**

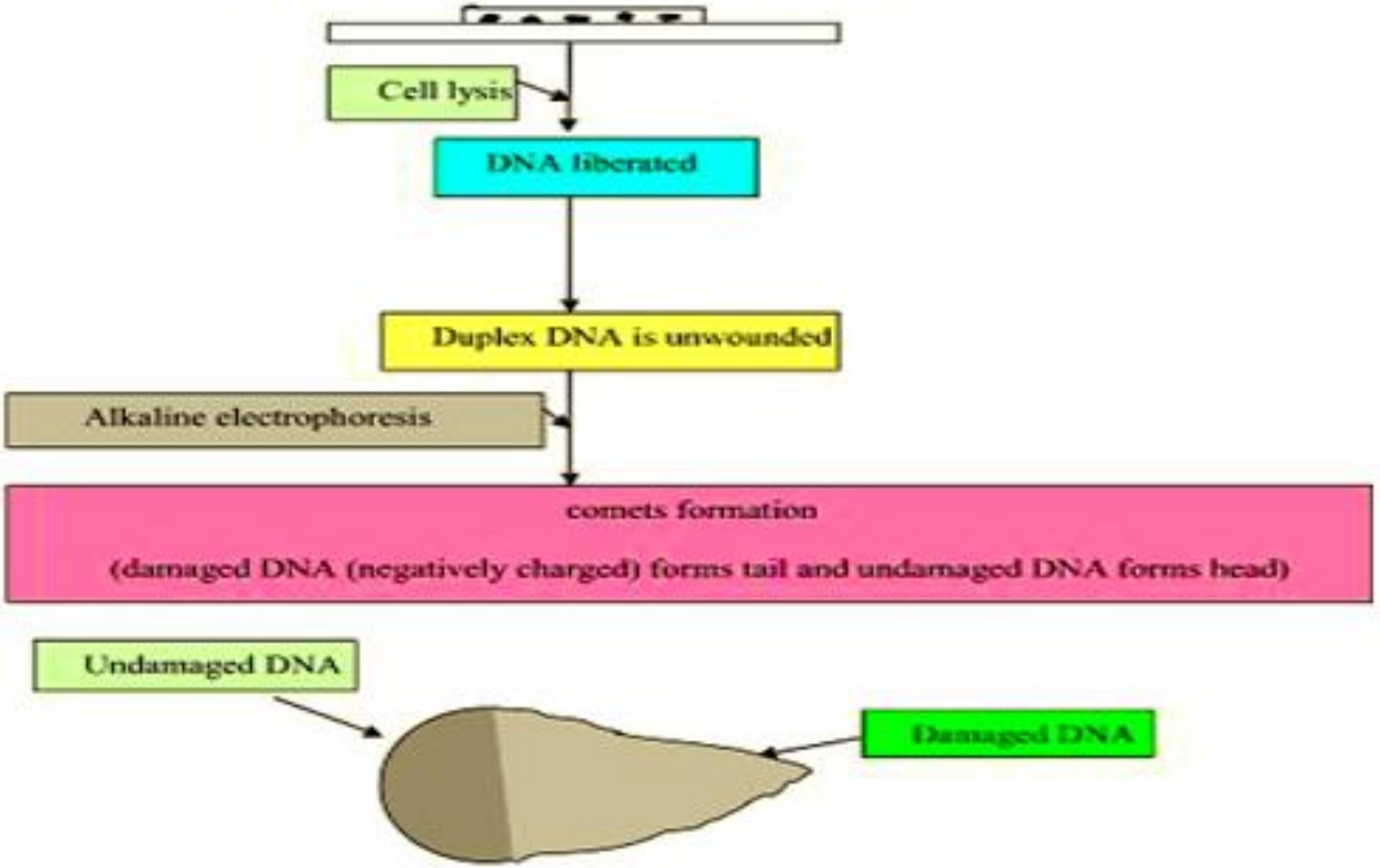
- **The results appear as structures resembling comets observed by fluorescence microscopy.**
- **Comet contains a distinct head and tail.**
- **The head is composed of intact DNA, while the tail consists of damaged or broken pieces of DNA.**
- **The extent of DNA liberated from the head of the comet is directly proportional to the amount of DNA damage.**





Methodology

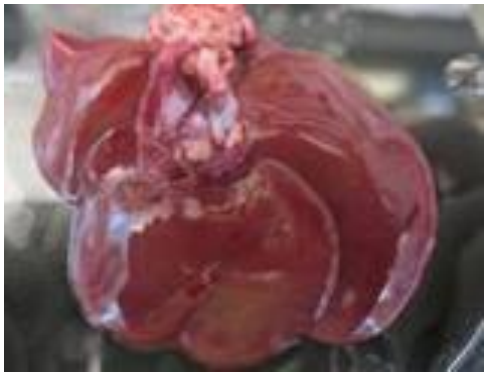
Cells are embedded in agarose gel on a slide



A- Sample preparation

1- homogenization

Tissue sample (liver, kidney, brain, testis….) homogenize in a **chilled** homogenizing buffer (0.075 M NaCl, 0.024 M Na_2EDTA, pH 7.5) using automatic homogenizer.



Tissue sample

Homogenizer

2- Centrifugation

To obtain the nuclei, the homogenate should be centrifuged at 1500 rpm for 10 min. at 0°C, using cooling centrifuge.

Cooling Centrifuge

3- Slide Preparation:

1- **Fully frosted slides** are layered twice with 100 µL **1% GP-42** agarose (normal agarose).

2- **Mix 75 µL** of nuclear suspension (supernatant) with **75 µL of 2% LGT** agarose (low melting point) . Cover the slide with another slide and leave to solidify.

3- **Finally** 100 µl of agarose GP-42 1% was quickly layered on the surface and covered with another slide and allowed to gel.

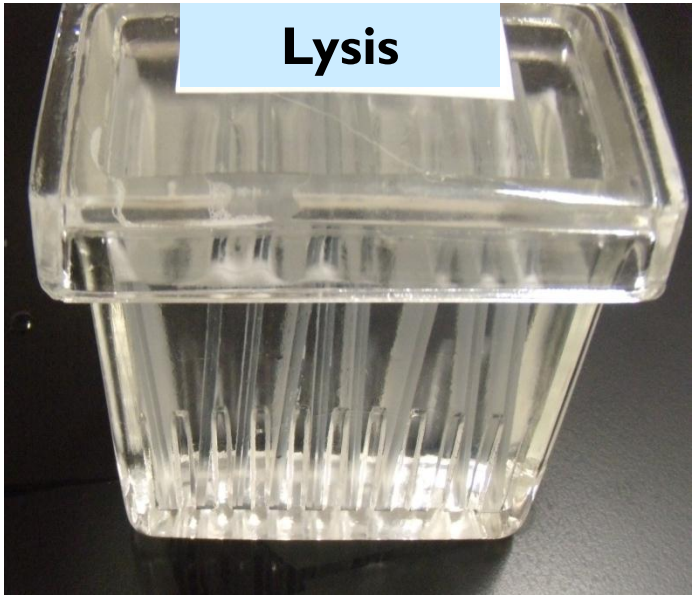
Water bath with shaking

4- Lysis

to remove membranes, cytoplasm, and most nuclear proteins.

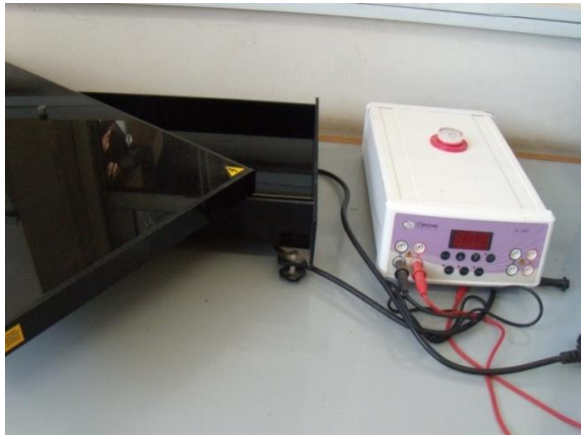
- **Immerse slides in chilled lysing solution:**

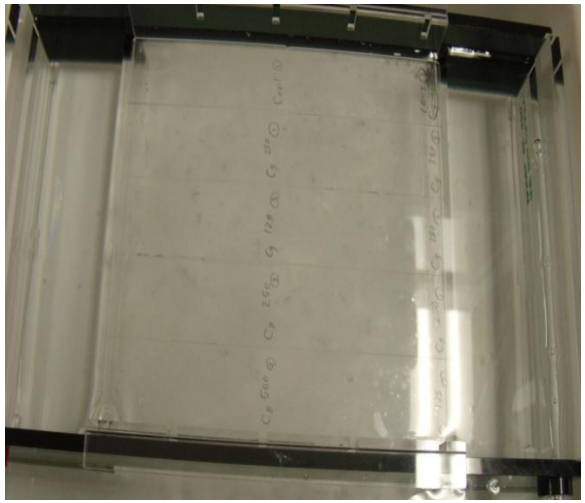
(2.5 M NaCl, 100mM Na_4EDTA, 10mM Tris base, 1% sarcosinate, 10% dimethyl sulfoxide, and triton X-100) and keep at 4°C in the dark for 1-24 hours.



5- Unwinding & Electrophoresis

The slides are placed on a horizontal gel electrophoresis platform and covered with chilled alkaline solution (300 mM NaOH and 1mM Na_2 EDTA, pH 13) in the dark at 0°C for 20 min, (OFF)
then electrophoresis is conducted (25 V, 300mA) (ON) at 0°C in the dark for 20 min.

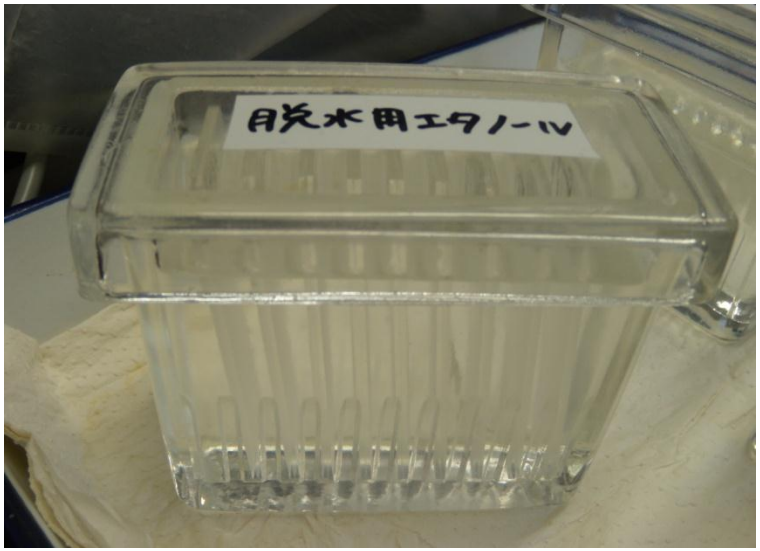




✓**Under electrophoresis: broken DNA is pulled towards the anode, forming a comet-like tail when stained and examined under fluorescence microscopy.**

6- Neutralization and dehydration

- **Immerse slides in neutralizing solution (400 mM Tris buffer pH 7.5) for 7 minutes.**
- **Dehydrate slides in ethanol for 5 minutes**
- **Allow slides to dry at room temperature.**



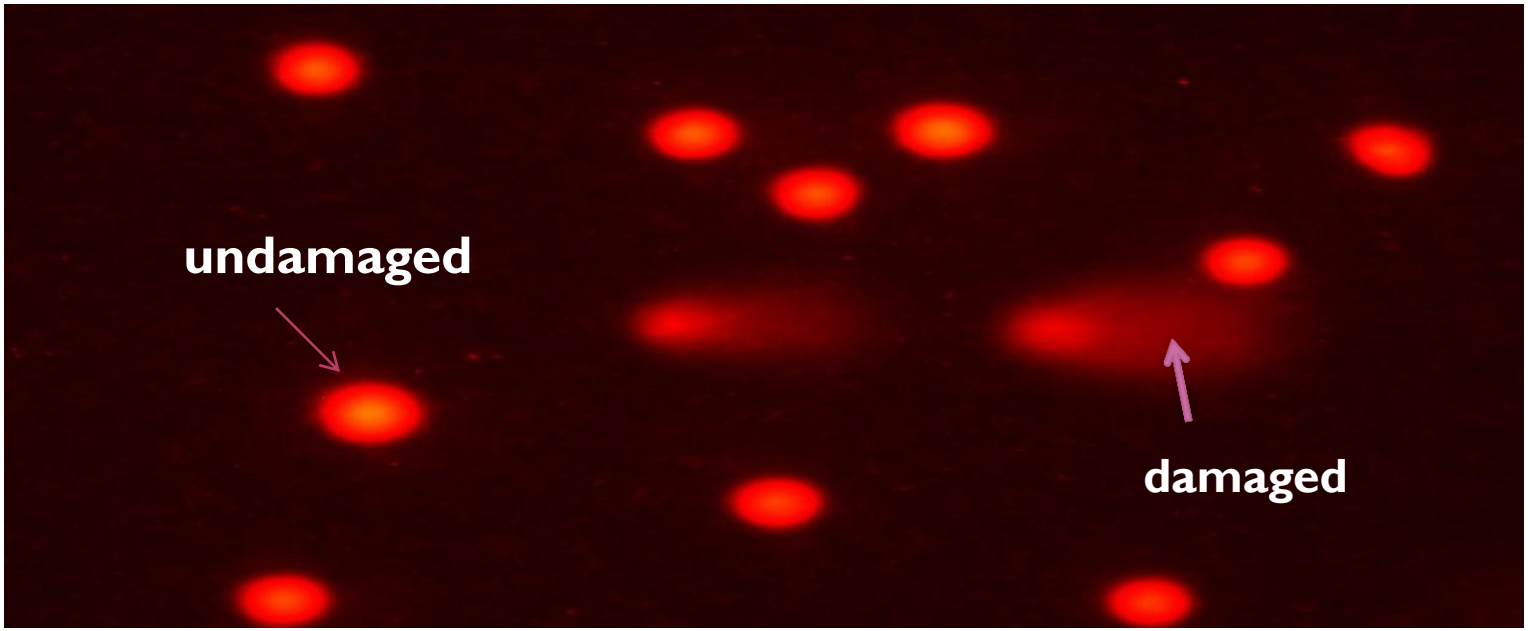
Ethanol

Dry slides at room temperature.

7- Staining and analysis

- **Stain dry slides with fluorescent stain: Ethidium bromide or sybr green stain.**

- **Examine microscopically using fluorescent microscope with green filter.**



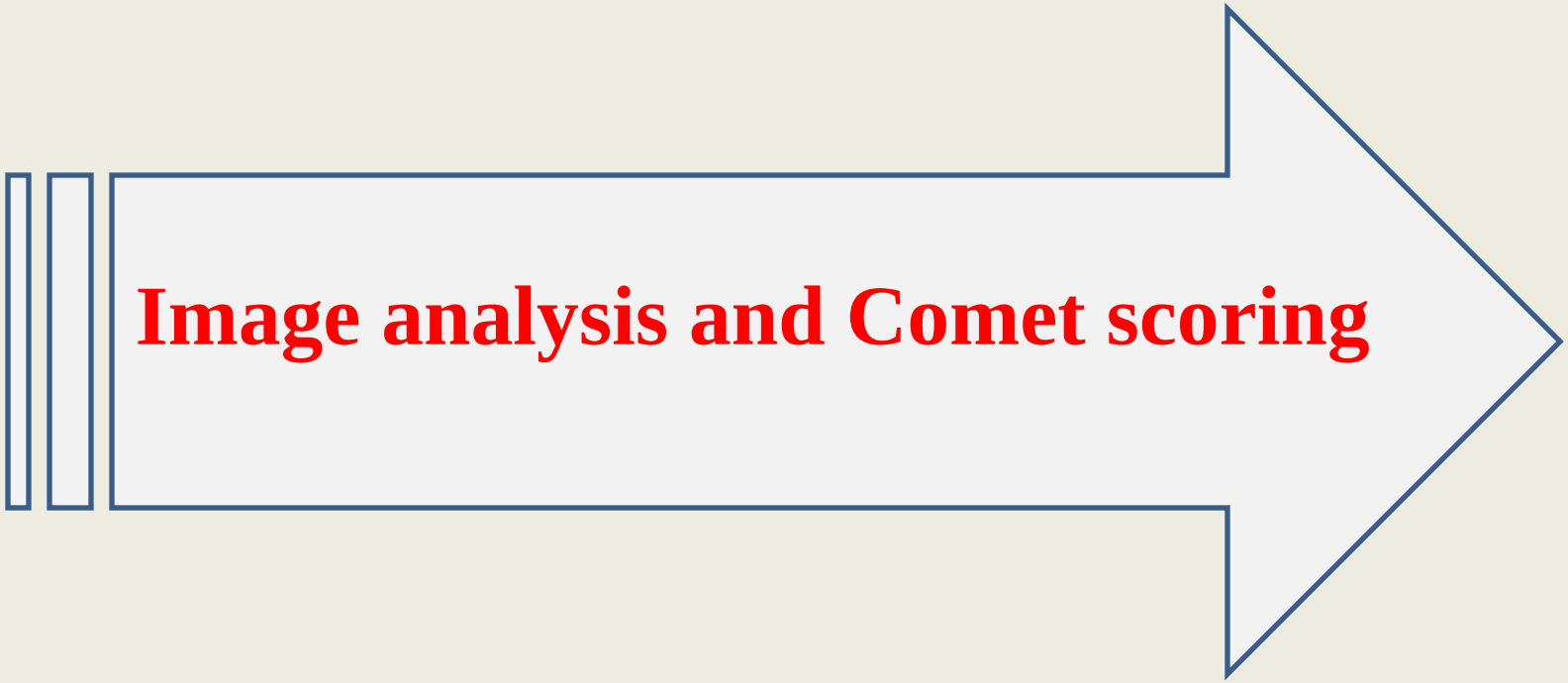
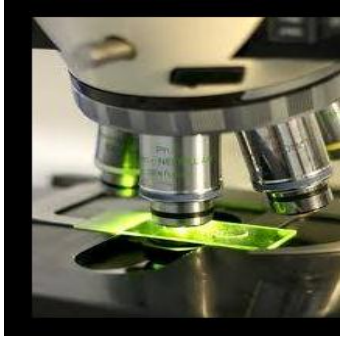
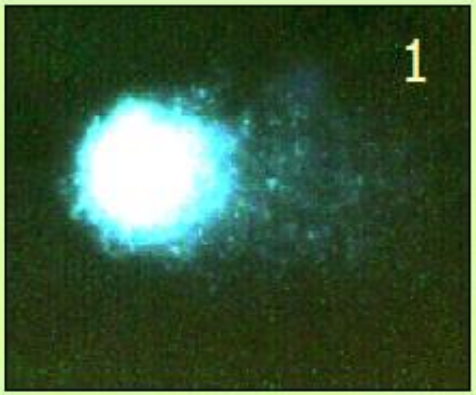
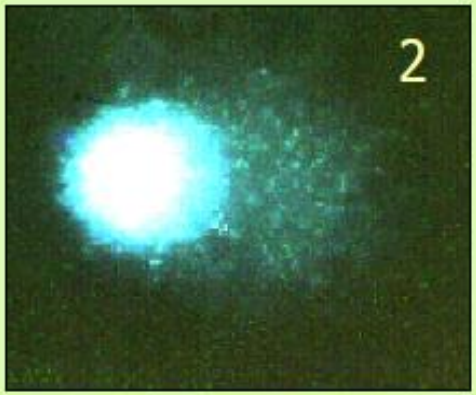


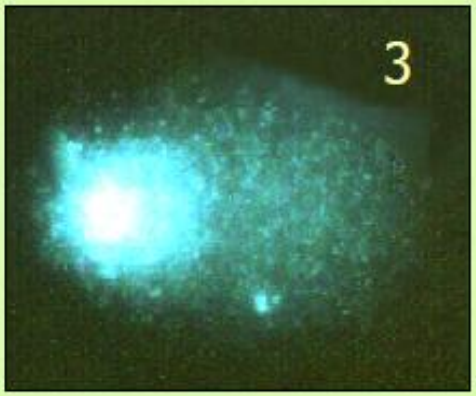
Image analysis and Comet scoring

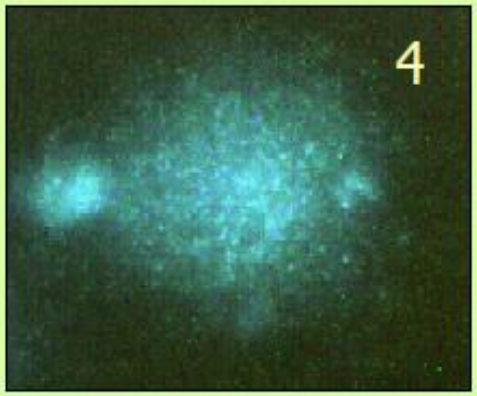
1- Visual scoring:



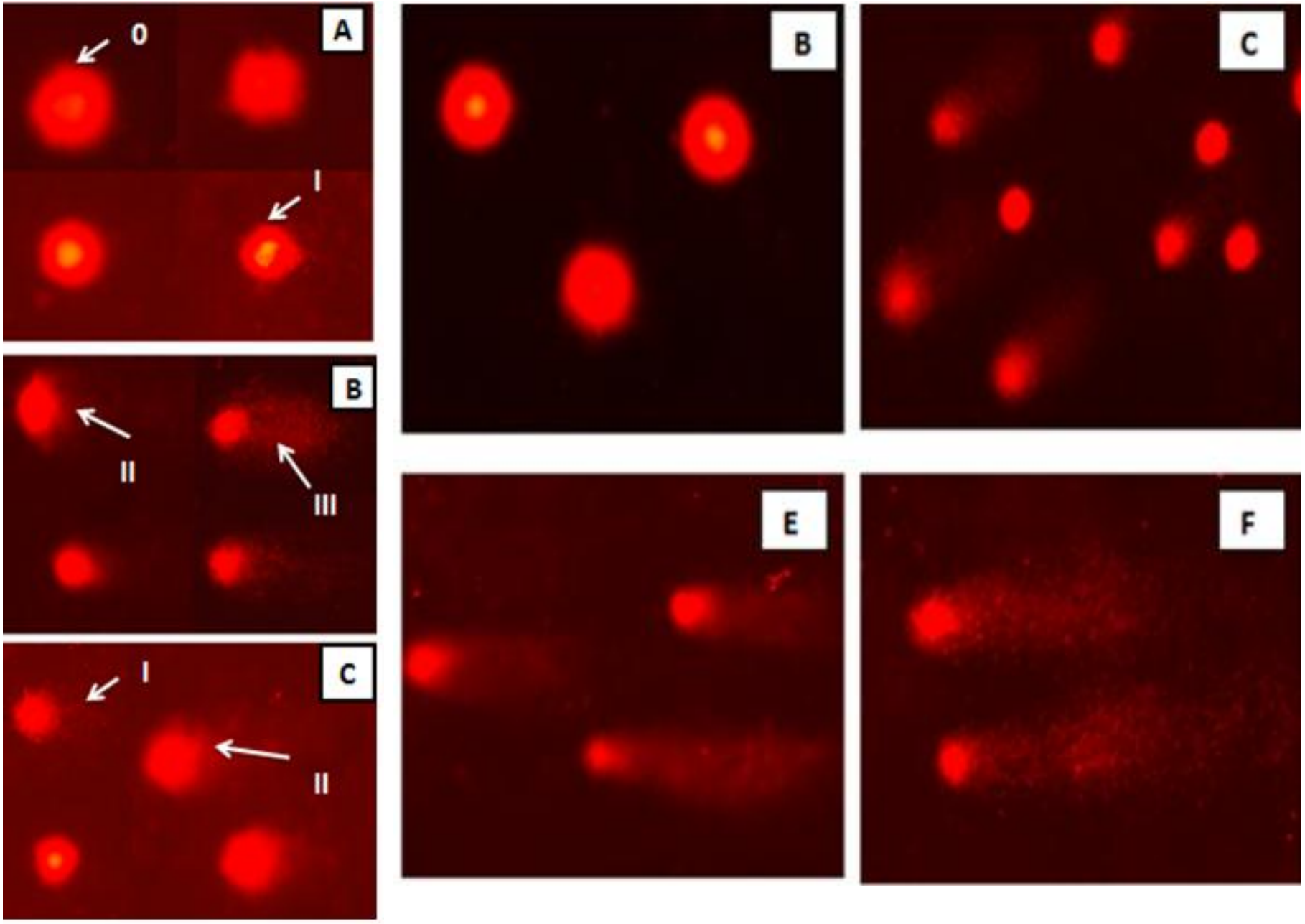


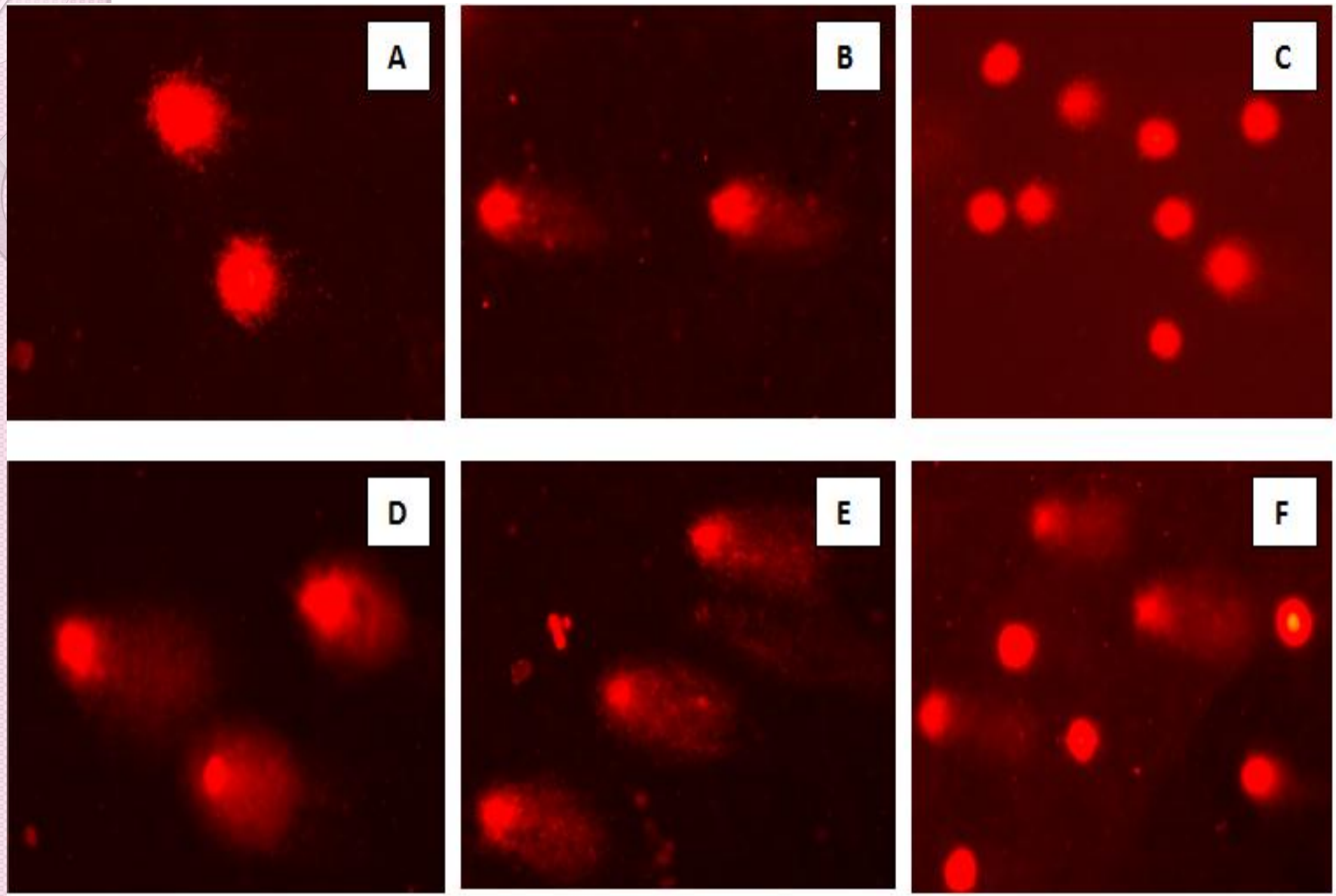




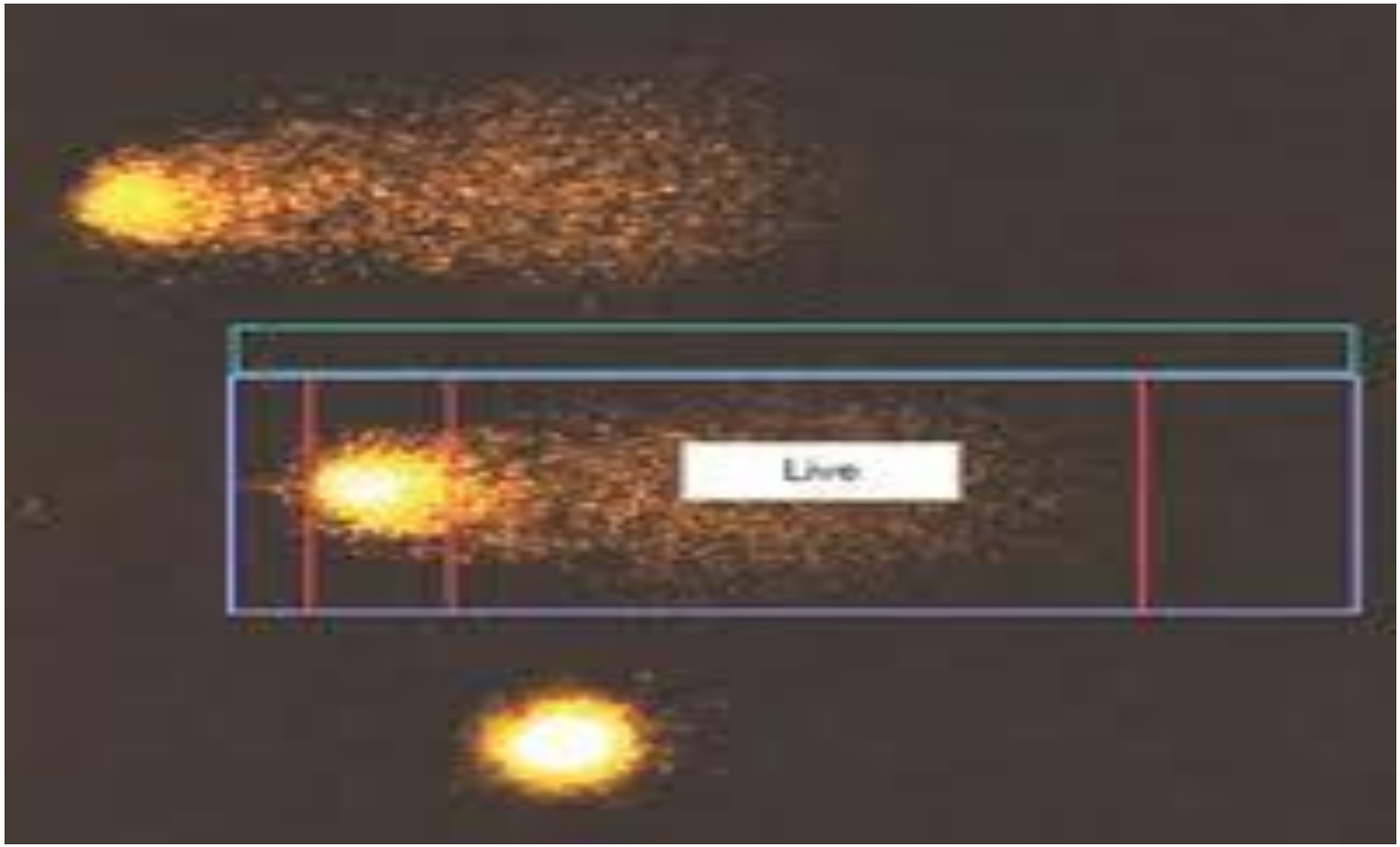


Classify comets according to extent of tail DNA and give value 0-4;

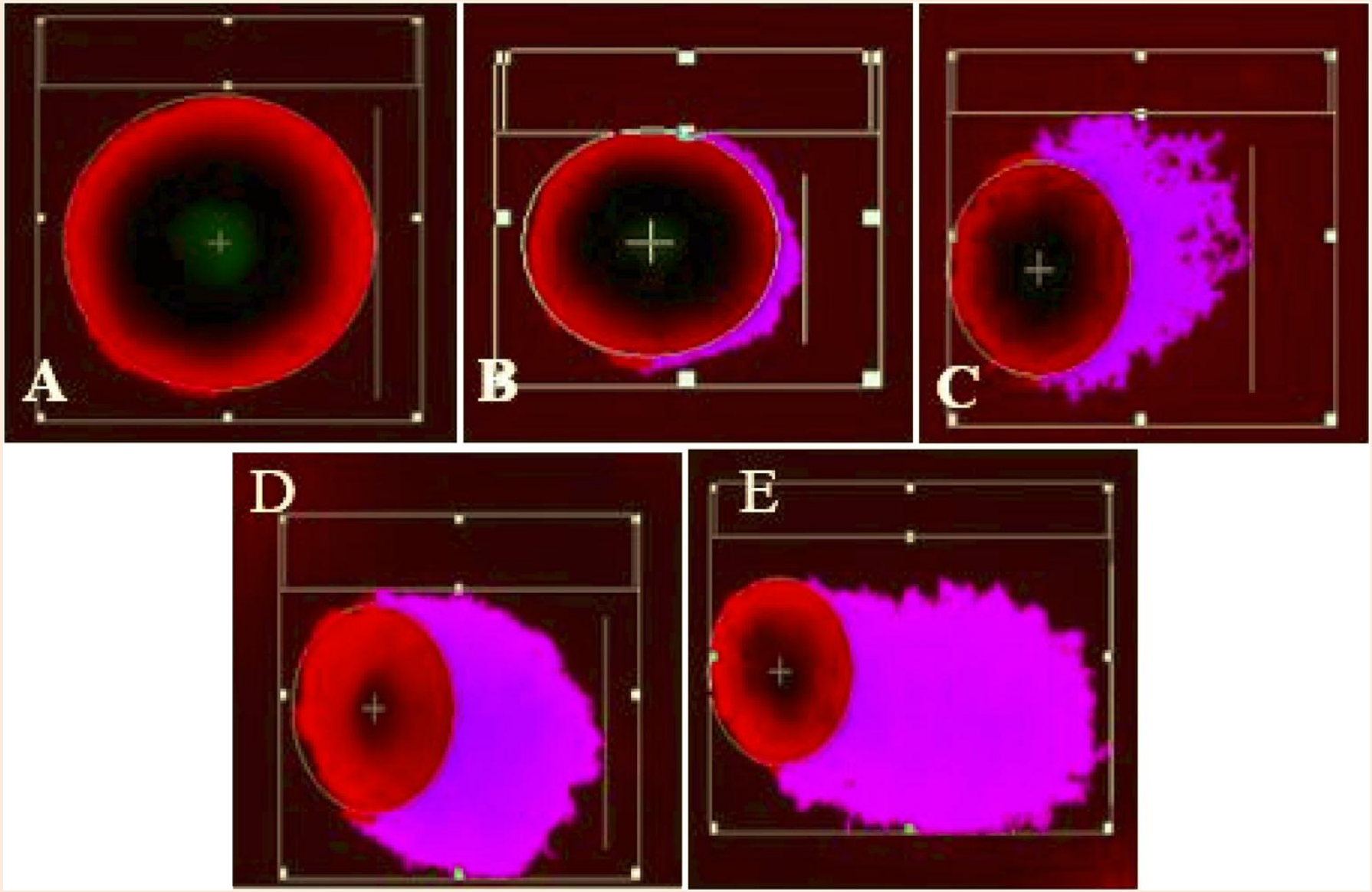

A
0
I
B
II
III
C
I
II
B
C
E
F


A
B
C
D
E
F

2- Using computer image analysis (Software):



At least 50 nuclei are analyzed per slide


A
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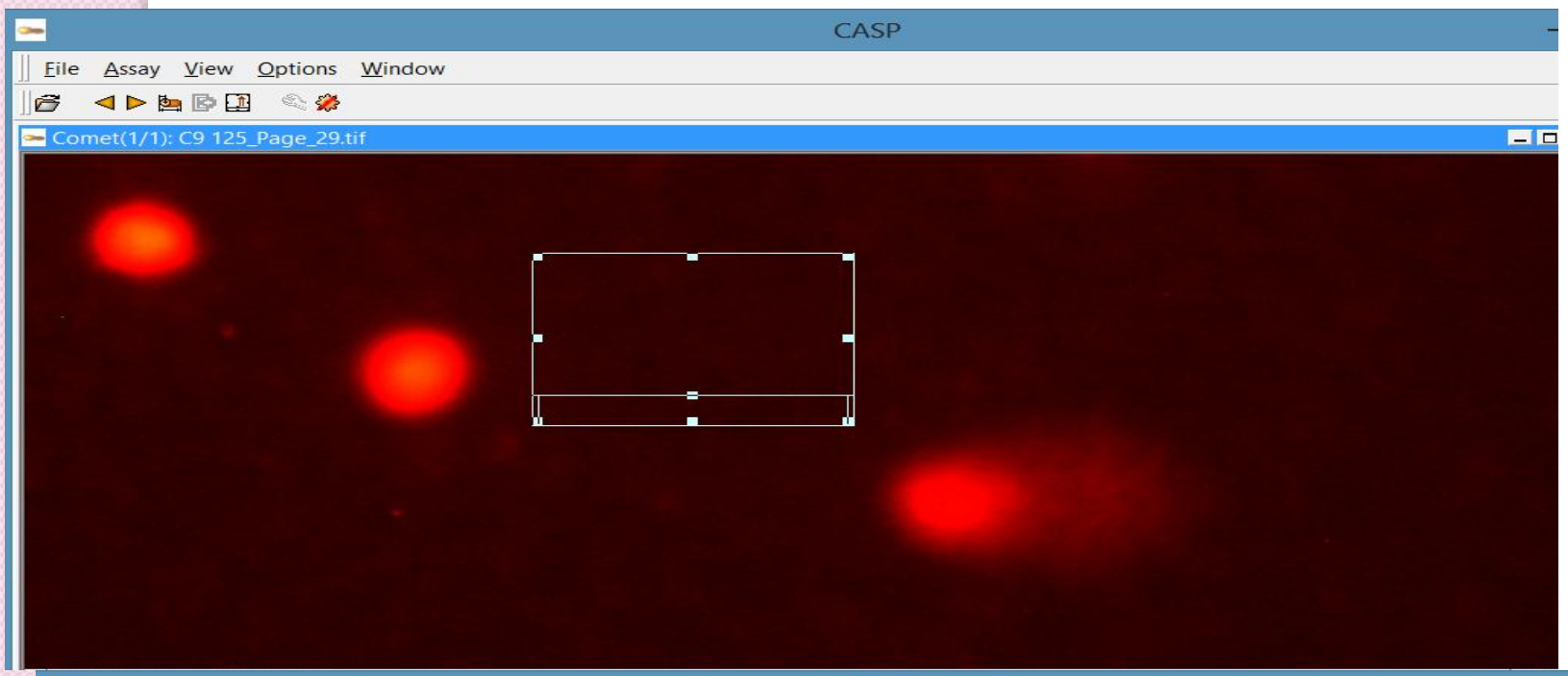
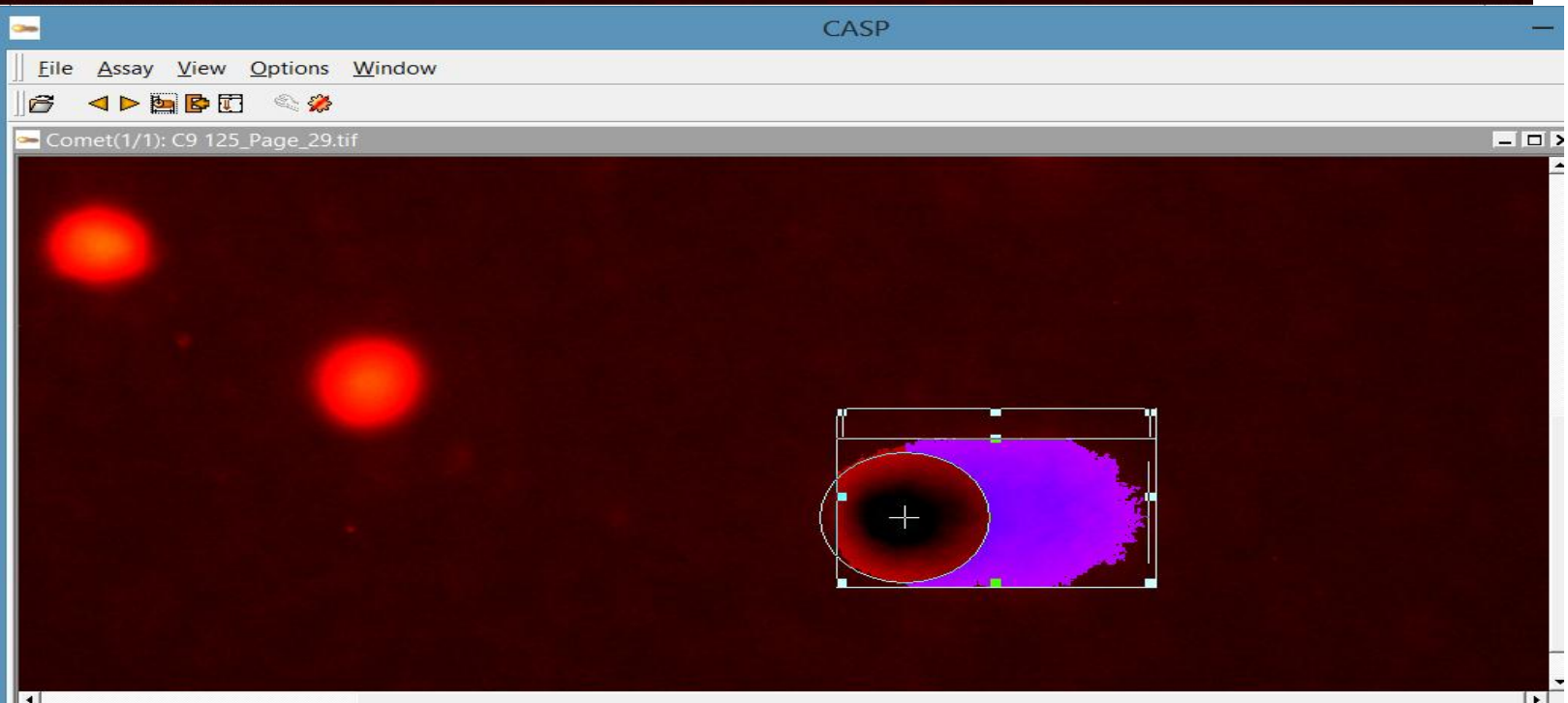

CASP
File Assay View Options Window
Comet(1/1): C9 125_Page_29.tif


CASP
File Assay View Options Window
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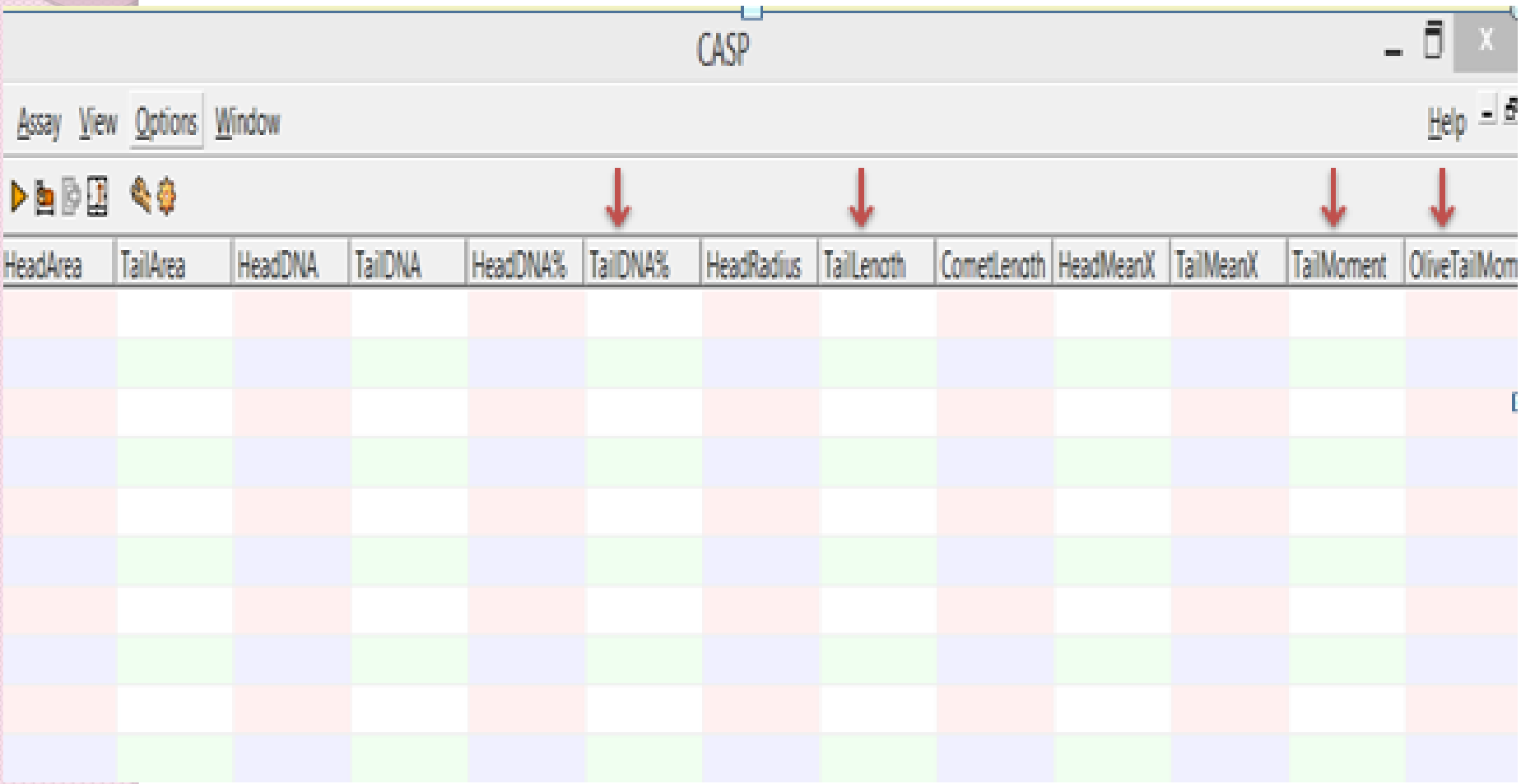
CASP-lab (Comet Assay Software Project)



Parameters:

1- **Tail length (DNA migration):** indicate initial DNA damage and confirm exposure to a genotoxicant.

✓**2- Tail moment: indicates the intensity of damage.**

Tail moment = tail length x % DNA in the tail

3- Olive tail moment =

(Tail.mean - Head.mean) X Tail%DNA/100.


THANK YOU

Applications of comet assay

- **Genotoxicity testing:**
- It provides a set of information about the safety and genotoxicity of newly developed pharmaceuticals and chemicals.

- Study of the protective effect of some phytochemicals on cells when exposed to some genotoxic insults.

- It is one of the techniques used in the area of cancer research for the evaluation of genotoxicity and effectiveness of chemotherapy.

- **genotoxicity of nanoparticles.**

- **Monitoring environmental contamination with genotoxins:**
- **<u>Human biomonitoring including:</u>**
- Monitoring occupational exposure to genotoxic chemicals or radiation.
- Assessment of oxidative stress associated with various human diseases.
- Detection of DNA damage associated with smoking.

- **Nutritional Studies :**
- Comet assay is ideal for investigating nutrient or micronutrient effects at the level of DNA damage in humans.

Measuring DNA Repair

- Comet assay is an important determinant of individuals capacity for DNA repair and their susceptibility to cancer.