Demonstration of Nucleic Acids

Introduction

- Nucleic acids are molecules that store information for cellular growth and reproduction.

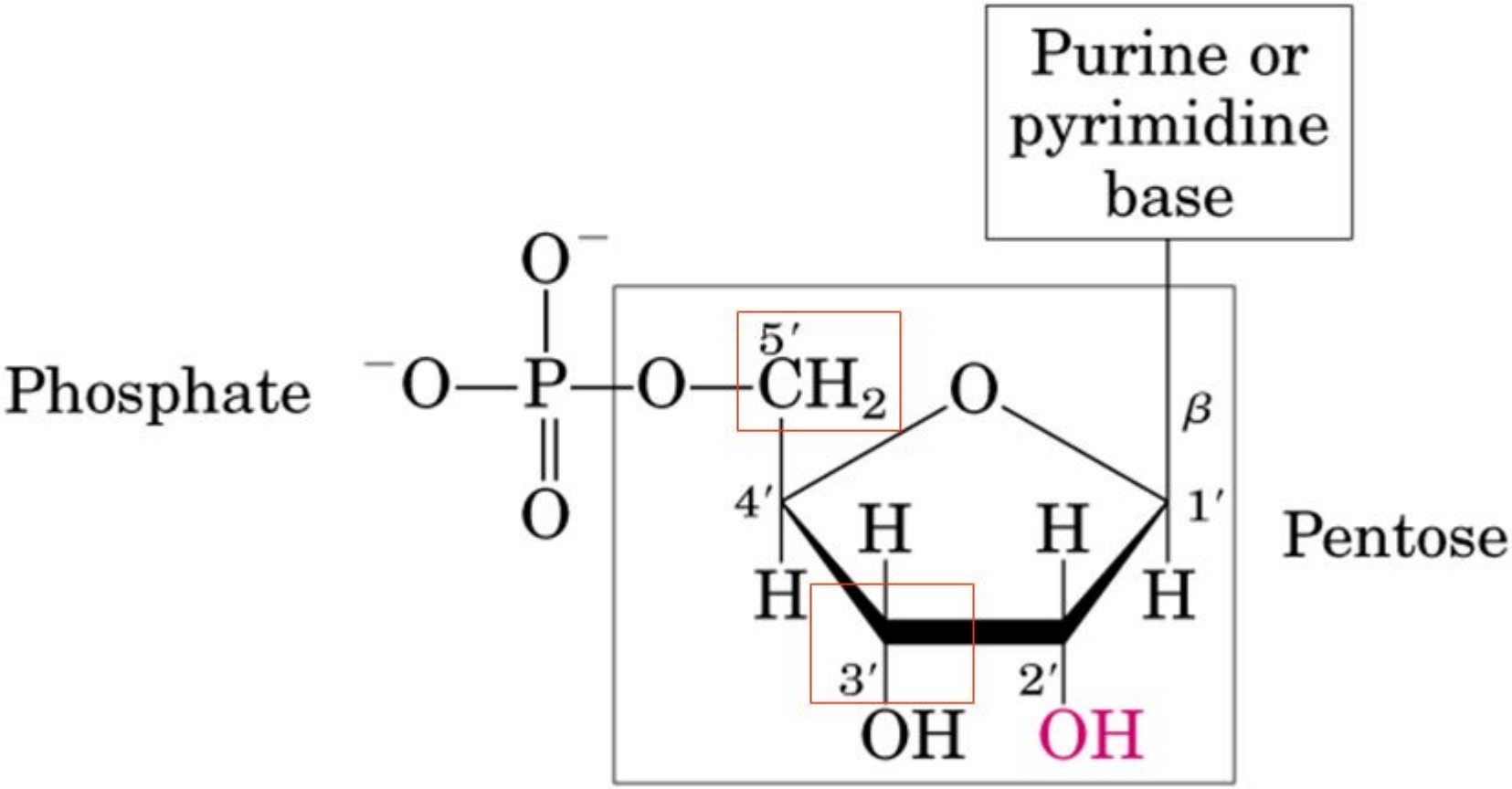
- Two nucleic acids are:
  1. DNA
  2. RNA

- They consist of : sugar (Deoxyribose/Ribose), phosphate and nitrogenous base.

Chemical Structure of DNA vs RNA

Ribonucleotides have a 2'-OH

Deoxyribonucleotides have a 2'-H



Staining Techniques for NAs

- NAs are strongly basophilic and will stain with almost any basic dye.

- This is fine for morphological purposes but basophilia is not limited to NAs and needs controlling in order to get specific identification.

Feulgen Technique

Purpose

- Used for the identification of DNA in paraffin-embedded tissue or cell specimens.

- The method is specific for DNA and will not stain RNA.

Feulgen Technique

Principle of the Feulgen Method

- HCl hydrolyze the deoxyribose sugar of DNA into an aldehyde.

- Results in the cleavage of the nitrogen bases.

- Aldehyde reacts with Schiff's reagent, which specifically binds to aldehydes.

Feulgen Technique

- RNA is not hydrolysed by the HCl treatment, and, thus the reaction is DNA specific

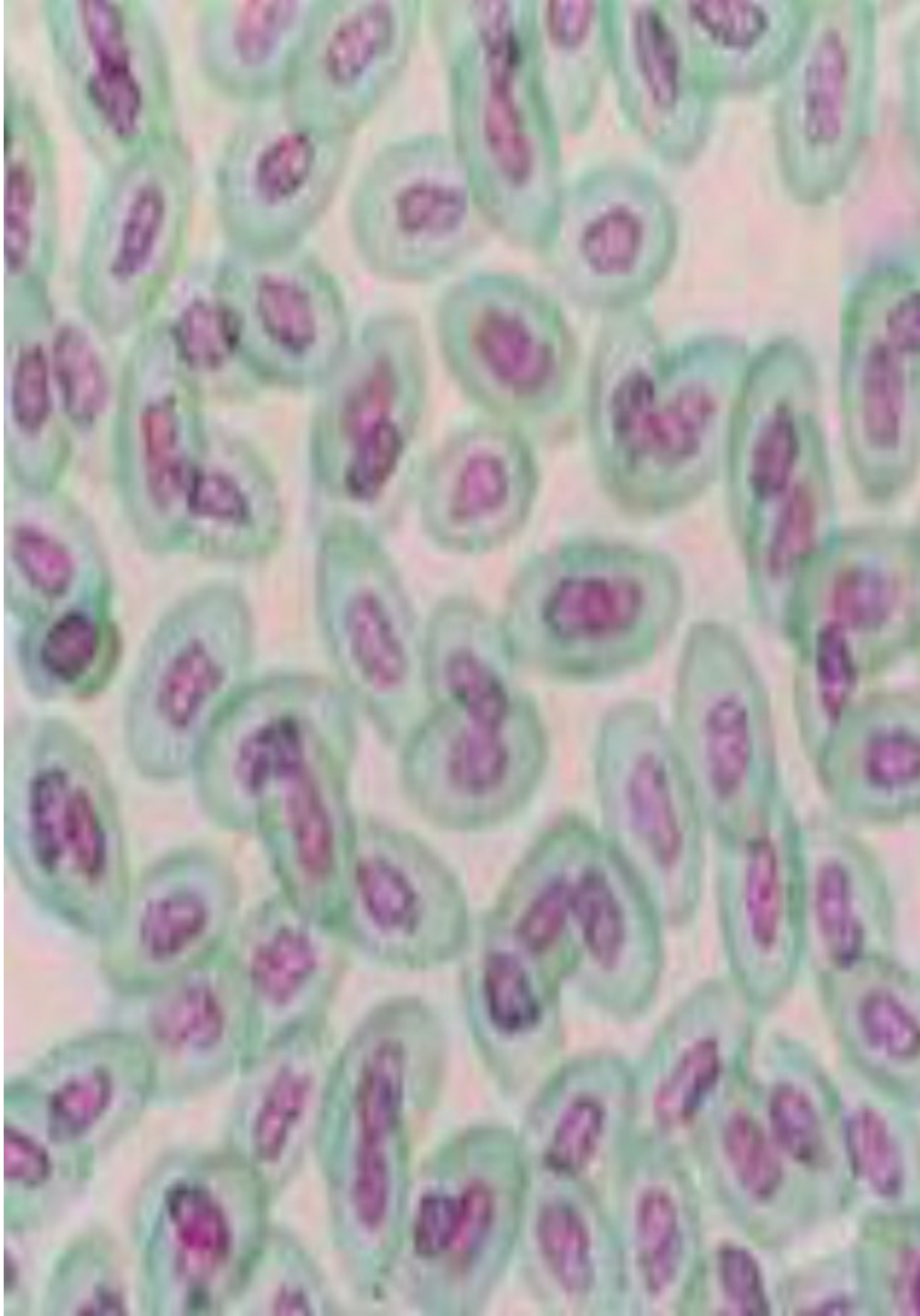
- A light green counterstain is often applied to the tissue section to achieve a better contrast.

Feulgen Technique

- Is a semi-quantitative technique
  - If the only aldehydes remaining in the cell are produced from hydrolysis of DNA
  - DNA in each cell can be measured by microdensitometry.

Results

DNA……Red-Purple
Cytoplasm…..Green



Methyl Green-Pyronin Technique

Purpose

- For demonstration of both DNA and RNA

Principle

- The method uses two different basic dyes to stain the different NAs and distinguish DNA and RNA.
  - Methyl green (a triaryl methane dye) = DNA
  - Pyronin Y(a xanthene dye) = RNA

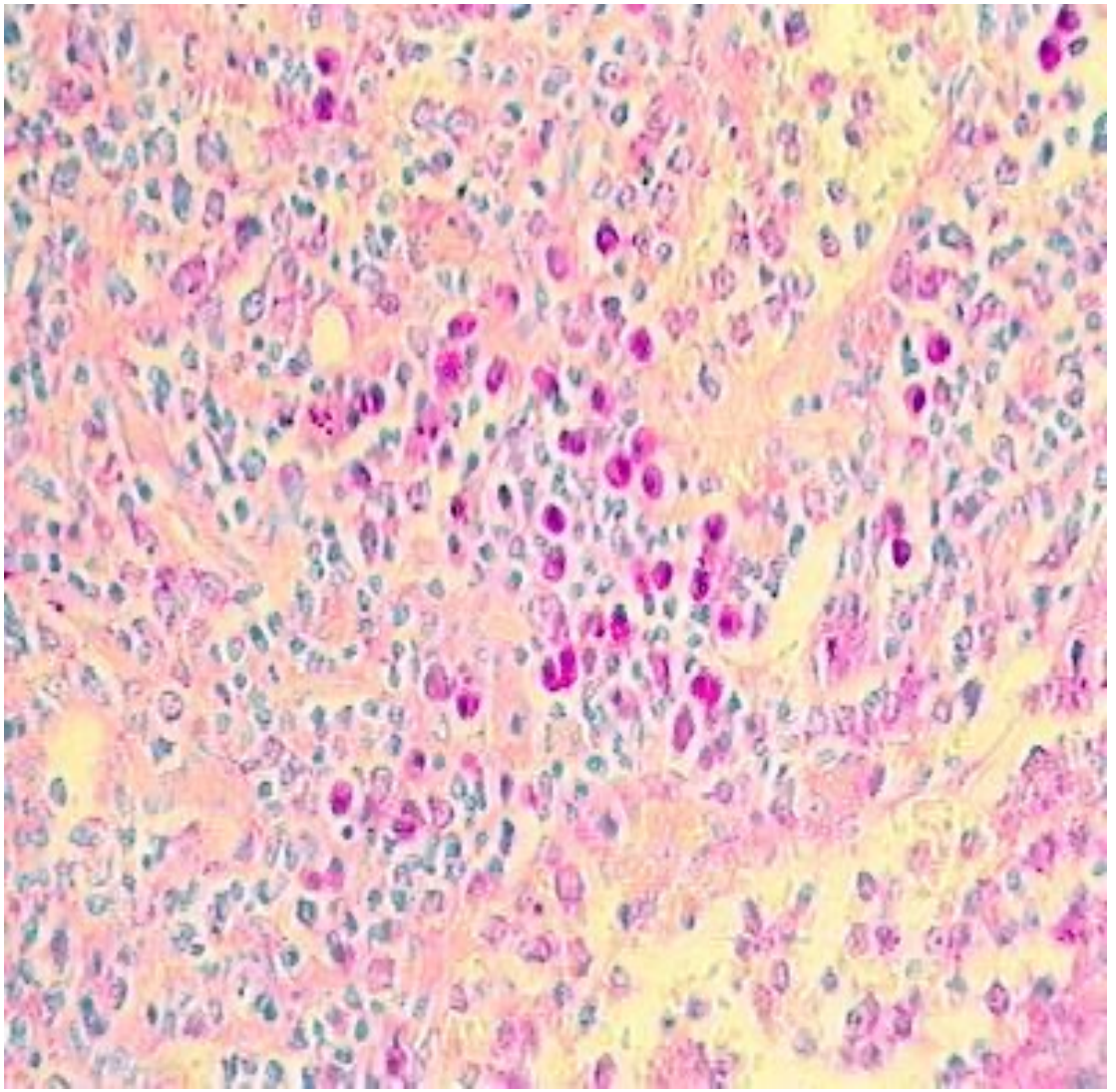
Methyl Green-Pyronin Technique

- The reason that two basic dyes bind preferentially is suggested to be related to the different degrees of polymerization observed in DNA and RNA.

Methyl Green-Pyronin Technique

When used at pH 4.6 the dyes compete for binding to the NAs

DNA → green colour
RNA → red colour



Gallocyanin-Chrome Alum Method

- Stains both DNA and RNA and does not distinguish between them.
- Used at pH 1; it is specific for NAs
- But if it is used at pH of 1.64; Gallocyanin also stains non-NA materials

Principle:

- Method relies upon the combination of the phosphoric acid residues of the NAs with Gallocyanin at an acidic pH.

- Extraction techniques can be used to identify either RNA or DNA if necessary.

Results:

- DNA and or RNA……Blue

Fluorescent Stains

- A number of fluorescent dyes can be used to demonstrate NAs.

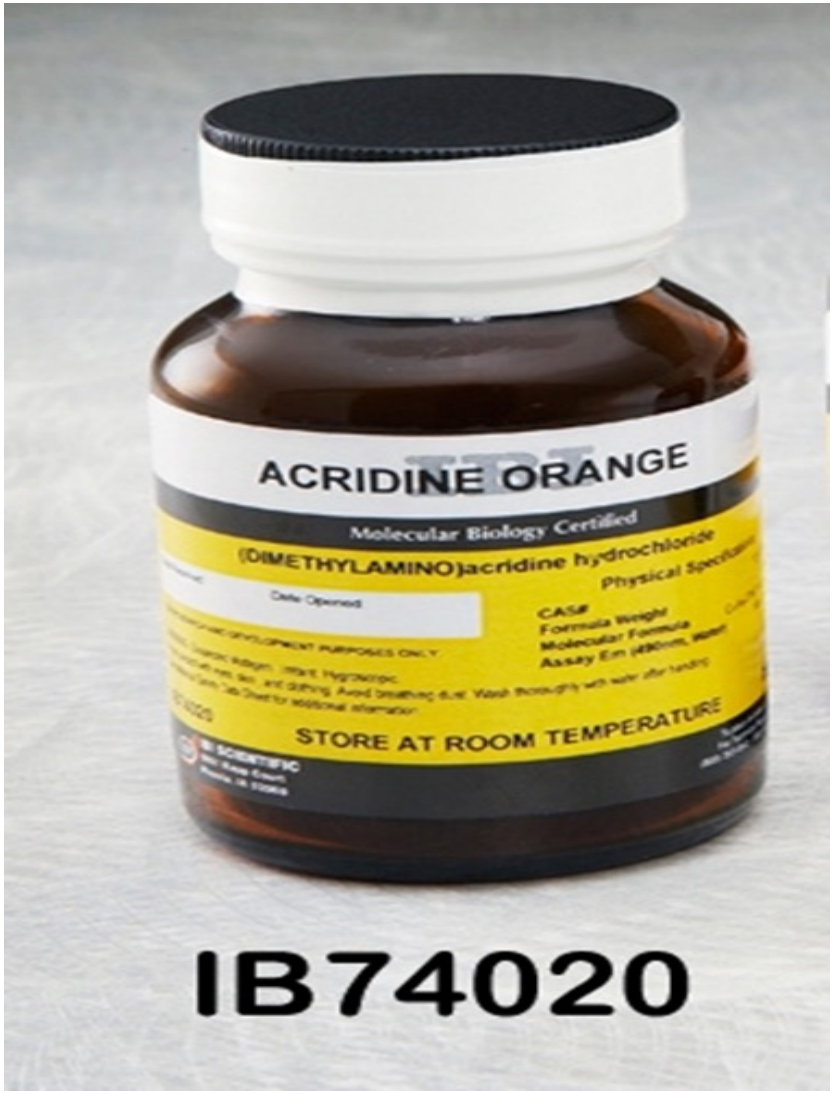
- These dyes include:
  - Acridine Orange
  - Ethidium Bromide
  - Quinacrine

Acridine Orange

Uses

- Used to detect single and double stranded NAs

- Is a very informative reagent when determining the structure of NAs that have undergone electrophoresis



Principle

- Acridine Orange is a metachromatic stain that interacts with polynucleotides either by:
  - Intercalation between stacked bases of dsDNA

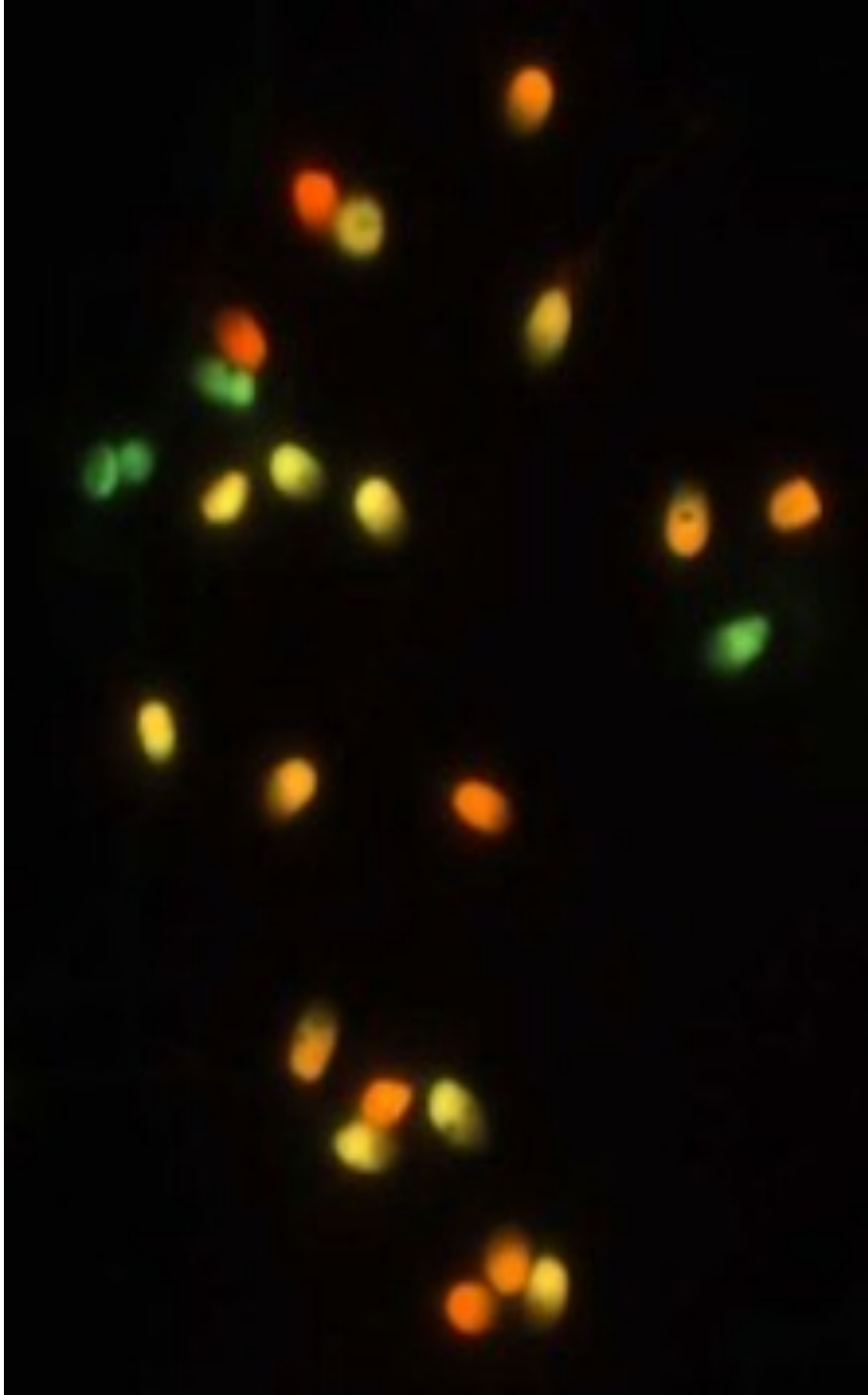
  or
  - Electrostatically to the phosphate backbone when the polynucleotide is predominantly single-stranded.

- Emits green fluorescence when bound to dsDNA (at 530nm).

- If the stain binds to the single-stranded NAs, it will fluoresce orange-red at 640nm.

DNA damage to human sperm assessed by Acridine Orange staining.

Green sperm heads show normal DNA while Yellow to Red sperm heads show damaged DNA



Ethidium Bromide (EtBr)

Uses

- The most convenient and commonly used method to visualize DNA in agarose gel.

- EtBr can be used to detect both single- and double-stranded nucleic acids (both DNA and RNA).

Principle

- It intercalates between NAs bases and provides opportunity to easily detect NA fragments in gels

- NAs running on an electrophoresis can be detected by staining with a dye and visualized under 300-nm UV light.

- Exposing DNA to UV fluorescence should be performed rapidly.


Agarose gel with UV illumination - Ethidium bromide stained DNA glows orange

Quinacrine

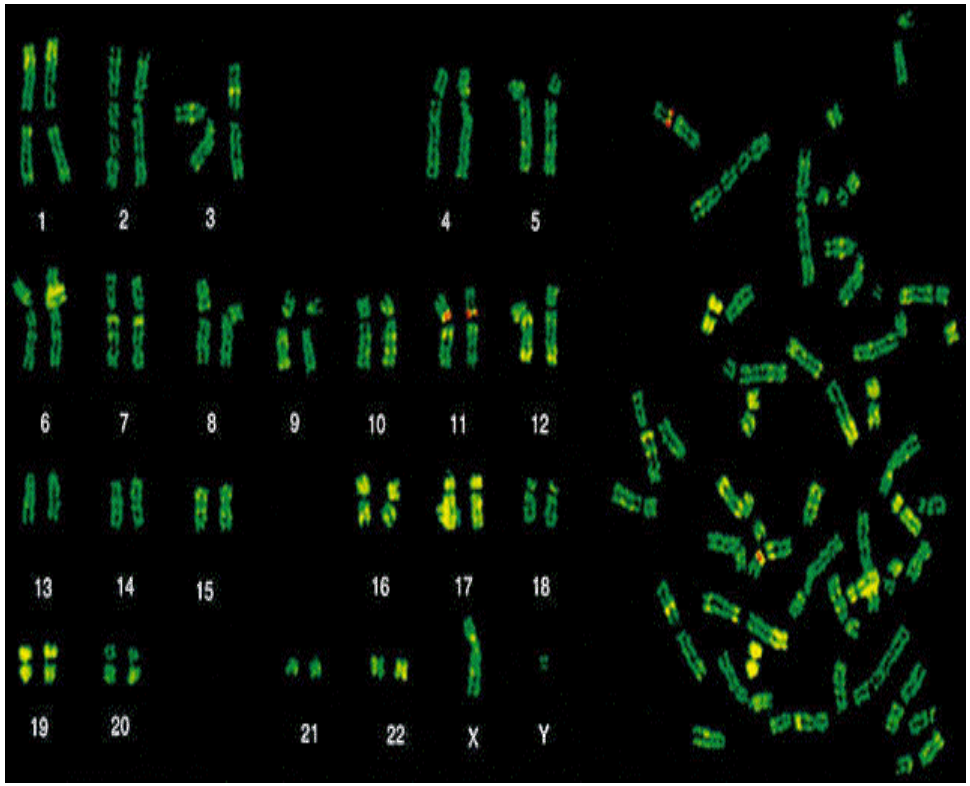
- Used to stain DNA and can be used as vital stains to show DNA in living cells.

- Act by intercalating into NAs.

- Demonstrate chromosome bands (so-called q-banding) in the identification of chromosome structure and the diagnosis of chromosome disorders

Quinacrine

- It produces bright fluorescence in adenine/thymine-rich regions and less fluorescence in guanine/cytosine-rich regions.



- Quinacrine gives a banding pattern of bright and dim transverse bands.

Summary – Histochemistry of Nucleic Acids

Stain or Dye	Demonstration	Staining Characteristics
Feulgen	DNA	DNA - magenta
Methyl Green-Pyronin Y	DNA & RNA	DNA – green RNA – red
Gallocyanin-Chrome Alum	DNA & RNA	DNA & RNA – blue
Ethidium Bromide	DNA & RNA	DNA & RNA – orange
Acridine Orange	DNA & RNA	dsNA (at 530nm) - green ssNAs (640nm) - orange-red
Quinacrine	DNA	Bright and dark bands

References

- Bancroft J.D. & Stevens A, (2013) **Theory & Practice of Histological Techniques**, 7th edition, Churchill Livingstone, NY, U.S.A.
- Culling CFA, **Handbook of Histopathological and Histochemical Techniques**, 3rd Ed.
- Suvarna, S.K, Christopher L, Bancroft J.D, **Bancroft`s Theory & Practice of Histological Techniques**, 7th edition, Churchill Livingstone, NY, U.S.A.