Autoradiography

Effects on Photography Plates

Autoradiography

- Autoradiography is the bio-analytical technique used to visualize the distribution of radioactive labeled substance with radioisotope in a biological sample
- It is a method by which a radioactive material can be localized within a particular tissue, cell, cell organelles or even biomolecules
- It is a very sensitive technique and is being used in a wide variety of biological experiments
- Autoradiography, although used to locate the radioactive substances, it can also be used for quantitative estimation by using densitometer

Principle

- Autoradiography is based upon the ability of radioactive substance to expose the photographic film by ionizing it
- In this technique a radioactive substance is put in direct contact with a thick layer of a photographic emulsion (5-50mm thick) having gelatin substances and silver halide crystals
- This emulsion differs from the standard photographic film in terms of having higher ratio of silver halide to gelatin and small size of grain

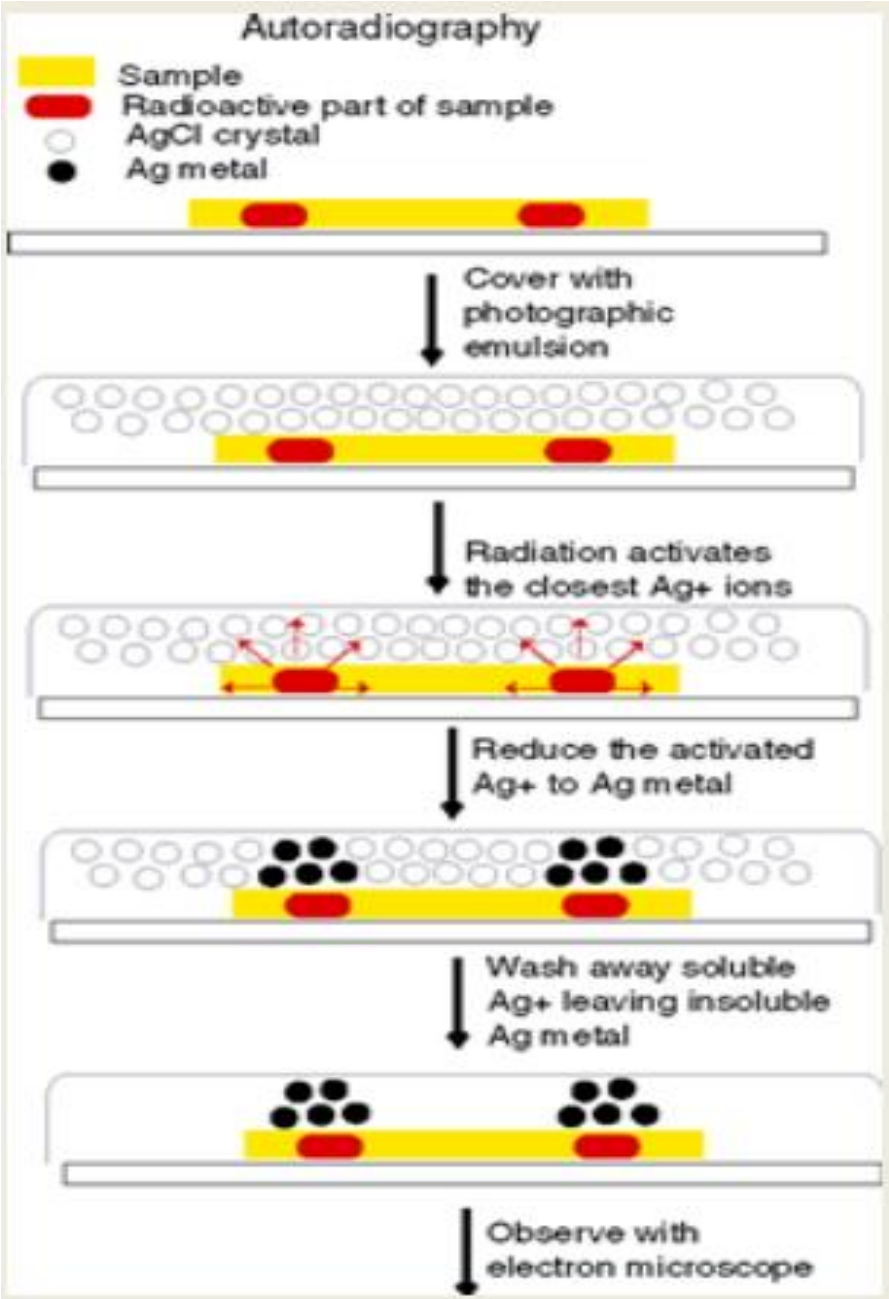
- **It is then left in dark for several days for proper exposure**
- **The silver halide crystals are exposed to the radiation which chemically converts silver halide in to metallic silver(reduced) giving a dark color band**
- **The resulting radiography is viewed by electron microscope, pre-flashed screen, intensifying screen, electrophoresis, digitals canners etc.**

Basic Mechanism

- Penetration of negatively charged beta particles emitted by radioactive salts through silver halide film emulsion causes activation of silver present in the emulsion
- Activated silver crystals are very unstable therefore quickly reduced to black silver particles which is easily detectable
- Autoradiography sensitivity is improved by carrying the detection process at 70°C and pre-flashing the film before use
- Pre-flashing needs only one hit per crystal deposited to increases sensitivity

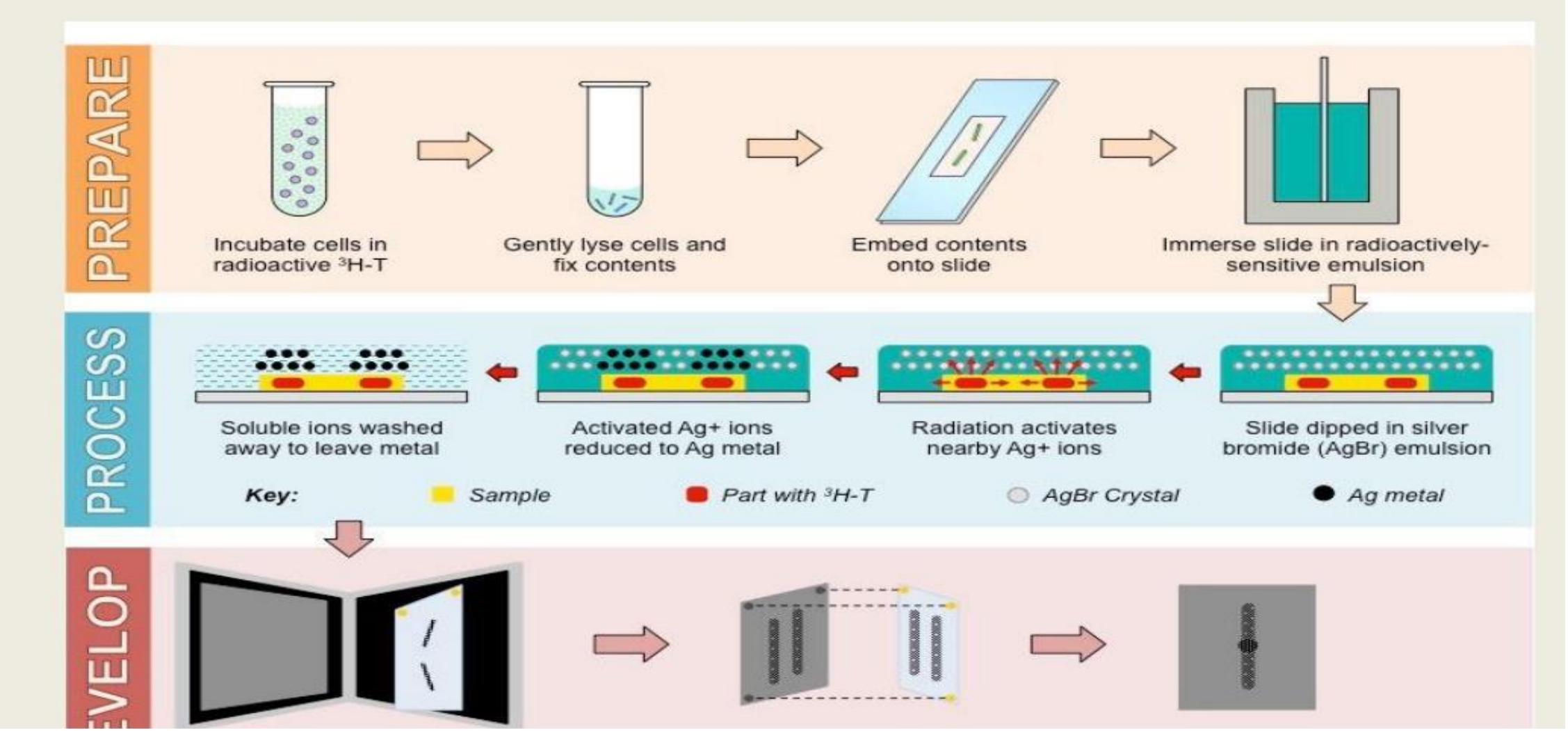
Procedure

1. Preparation of section – frozen or paraffin sections of soft tissues fixed by fixative depending upon the nature of isotope or tissue component to be studied (Bouins fluid, Neutrol formalin, alchoho may be used for protein bound isotopes)
2. Replacement of tissue components with radioactive isotope (Labelling)
3. Covering the samples with photographic emulsion
4. Activation of silver halide crystals by radioactive compound
5. Reduction of Ag+ to Ag atoms , leaving dark colour bands
6. Observation of radiogram under microscope



- 1. The radioactive sample is covered with the photographic emulsion
- 2. The radioactive part of the sample activates the silver halide crystals nearby
- 3.This results in reduction of Ag+ ions to Ag atom leaving dark color bands
- 4.The slide is then washed away by fixers to get insoluble Ag atom only
- 5.The autoradiogram can further be viewed and observed under the microscope

Cairns' technique for measuring the length of DNA molecules by Autoradiography



Factors Affecting Efficiency of Autoradiography

1.Energy of emitter: Higher the energy longer is the track length and so it's difficult to localize the points in the low density region of the same track. Further very low energy radiation also creates a poor image on the film. Therefore weak b-emitting isotopes (3H,14Cand35S) are most suitable because the energy of radiation is in between g and a radiations

2.Distance and Thickness of sample: If either the sample is very thick or the sample is faraway from the emulsion film, resolution will be lost

3.Grain size and amount of silver halide crystals: The grain size should be smaller so that the availability of AgX crystals is more. Also concentration of gelatin should be less in emulsion as compared to AgX crystals

4. Thickness of emulsion: The emulsion thickness affects the efficiency of autoradiography with different emitters. For b-emitters the thickness of the emulsion should be less

5. Exposure time : An autoradiogram must be exposed for a sufficiently long time for proper exposure to view pattern of the track length

Advantages

- Technically easy not much expertise required
- Highly specific detection tool
- Unlike tissue bath preparations, pharmacologically characterize and localize receptors in tissues
- Enables characterization of receptors in different tissues in different animals or brain regions

Disadvantages

- Lack of assessment criteria to determine whether the binding site really corresponds to an actual receptor
- Non-physiological significance of high affinity radio labelled receptor
- Non-specificity of ligands can easily cause misinterpretation of results

Applications

- To study the property of DNA
- To studying neurodegenerative disorders
- In whole body imaging
- To study the location and amount of a particular substance within a cell or tissue
- In electrophoretic transfer of proteins during blotting
- In imaging and analysing rock porosity
- Applications in microbial ecology
- Determining gross absorption and utilization of foliar applied nutrients etc.