AUTORADIOGRAPHY

DR.RUCHIKA YADU

INTRODUCTION

- 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.

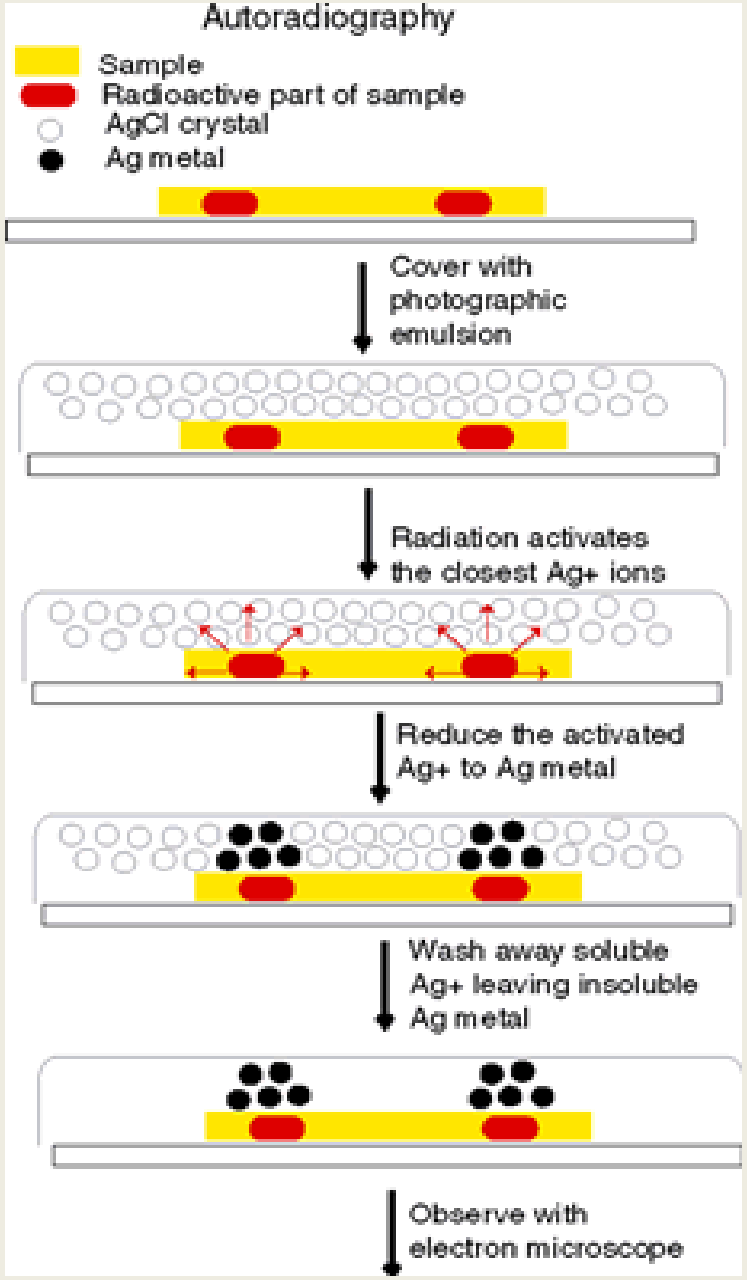
HISTORY

- The first autoradiography was obtained accidently around 1867 when a blackening was produced on emulsions of silver chloride and iodide by uranium salts observed by **Niepce de St. Victor.**
- In 1924 first biological experiment involving autoradiography traced the distribution of polonium in biological specimens.
- The development of autoradiography as a biological technique really started to happen after World war II with the development of photographic emulsions and then stripping made of silver halide.
- Radioactivity is now no longer the property of a few rare elements of minor biological interest (such as radium, thorium or uranium) as now any biological compound can be labeled with radioactive isotopes opening up many possibilities in the study of living systems.

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 (thickness of 5-50 mm) 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 into metallic silver (reduced) giving a dark color band.
- The resulting radiography is viewed by electron microscope, preflashed screen, intensifying screen, electrophoresis, digital scanners etc.

METHODOLOGY



http://lifeofplant.blogspot.com/2011/12/autoradiography.html

1. The radioactive sample is covered with the photographic emulsion

2. The radioactive part of the sample activates the silver halide crystals near by.

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.

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 preflashing the film before use.
- Preflashing needs only one hit per crystal deposited to increases sensitivity

Sequential steps of autoradiography

Brief exposure of living cells to a pulse of specific radioactive material for a variable time.

Preparation of samples are for microscopy either light or electron

Dissection of samples into sections for coverage with thin film of photographic emulsion which are then incubated in the dark for few days for radioactive decay. The exposure time depends on isotope activity, temperature and the background radiation.

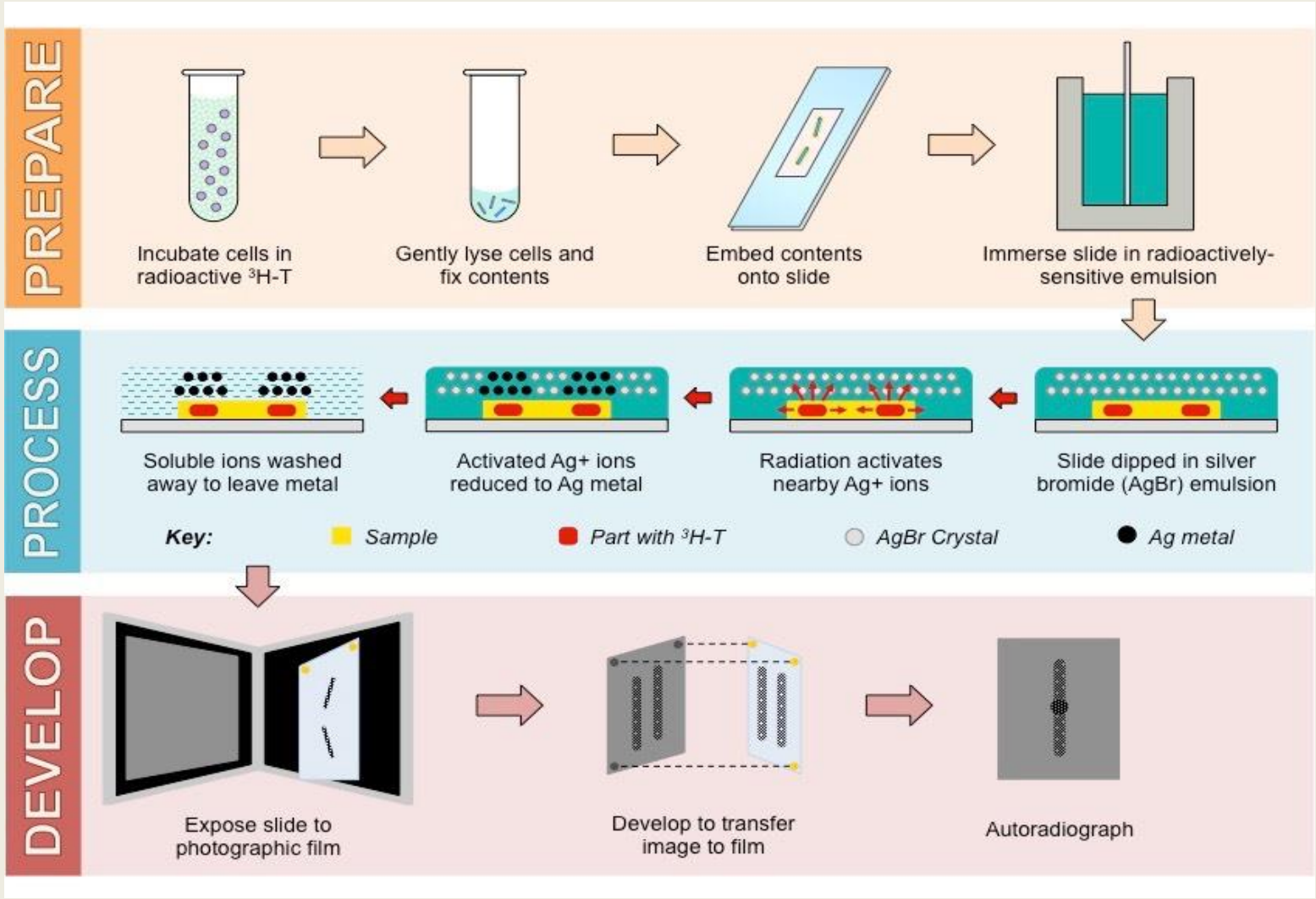
Development of photographic emulsion

Toluidine blue is used for counter staining to reveal tissue histology. Instead Osmium or dipping emulsion can be used for pre-staining of the entire tissue before exposure to the photographic emulsion to avoid for individual post- staining each slide.

Microscopy either light or electron is used to determine the relative position of the silver particles.

Generation of records in the form of autoradiographs

CAIRNS' TECHNIQUE FOR MEASURING THE LENGTH OF DNA MOLECULES BY AUTORADIOGRAPHY



1. Cells are grown in a solution containing radioactive thymidine (tritiated thymidine – 3H-T)
2. The tritiated thymidine is incorporated into the chromosomal DNA of the cell (3H-T is used as thymidine is not present in RNA)
3. The chromosomes are isolated by gently lysing the cells and fixing the chromosomes to a photographic surface
4. The surface is then immersed in a radioactively-sensitive emulsion containing silver bromide (AgBr)
5. The radiation released from the tritiated thymidine converts the Ag+ ions in silver bromide into insoluble metal grains
6. Following a period of exposure, excess silver bromide is washed away, leaving the silver grains to appear as small black dots
7. When the photographic film is developed, the chromosomal DNA can be visualised with an electron microscope

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 poorer resolution image on the film. Therefore weak b-emitting isotopes (3H, 14C and 35S) 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 far away 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 there is more availability of AgX crystals. Also concentration of gelatin should be less in emulsion as comapred 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 radiolabelled receptor,
- Non-specificity of ligands can easily cause misinterpretation of results

APPLICATIONS

Autoradiography provides qualitative as well as quantitative information regarding a specimen. Some of the following applications of this technique are given below:

- Autoradiography is used to determine receptor distribution and localization while studying neurodegenerative disorders.
- Application of autoradiography in electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets during blotting.
- To study cytogenesis of the forebrain.
- Applications in radiopharmaceutical research.
- Applications in radioimmunoelectroosmophoresis to study viruses.

APPLICATIONS (CONTINUED)

- In imaging and analyzing rock porosity
- In matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI), and secondary ion mass spectrometric imaging (SIMS-MSI) for pharmaceutical discovery and development
- In whole body imaging.
- Tool for genetic studies.
- For comparison of complex mixtures of proteins.
- Applications in microbial ecology.
- Determining gross absorption and utilization of foliar applied nutrients etc.

REFERENCES

- https://www.omicsonline.org/open-access/autoradiography-detection-and-analysis-of-radioactive-entities-2155-6180-1000361.php?aid=93116
- https://www.slideshare.net/kskuldeep1995/autoradiography-ks
- http://ib.bioninja.com.au/standard-level/topic-3-genetics/32-chromosomes/chromosome-size.html