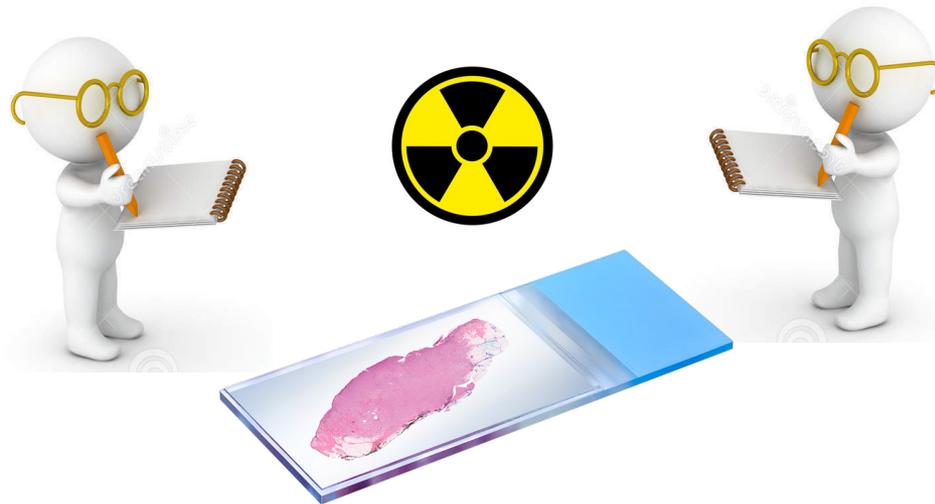


# Cellular Pathology

## Lecture #20

### Advanced Histological Techniques: Autoradiography



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# Lecture Outline

- Principle of Autoradiography
- Sequential Steps Of Autoradiography
- General Methods For Autoradiography
- Autoradiography Practical Applications
- Limitations of Autoradiography
- Types of photographic detection systems
- Problems that can occur in autoradiography



# Learning Objectives:

At the end of this lecture, the student is expected to:

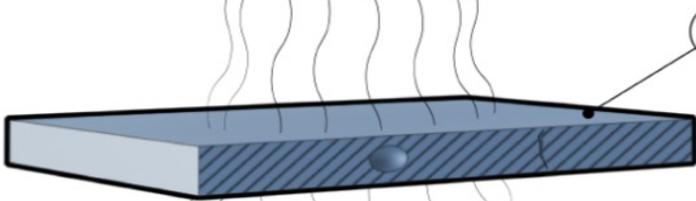
- Know and understand the principle of Autoradiography
- Appreciate how biological specimens are prepared for Autoradiography
- Compare and contrast:
  - Radiography vs. Autoradiography
  - In vivo autoradiography vs. ex vivo autoradiography
- Appreciate the practical applications of autoradiography
- Know the Limitations of Autoradiography
- Appreciate Types of photographic detection systems
- Know the common problems that can occur in autoradiography

# Synopsis

- **Radiography** is the visualisation of the pattern of distribution of radiation.
- In general, the radiation consists of X-rays, gamma or beta rays, and the recording medium (medium for converting radiation into an image) is a photographic film.
- For classical X-rays, the specimen to be examined is placed between the source of radiation and the film, and the absorption and scattering of radiation by the specimen produces its image on the film.

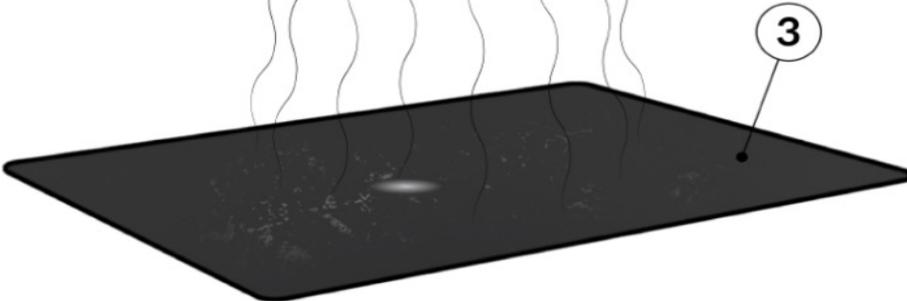


① Radiation source



② Object

Radiographic film



③

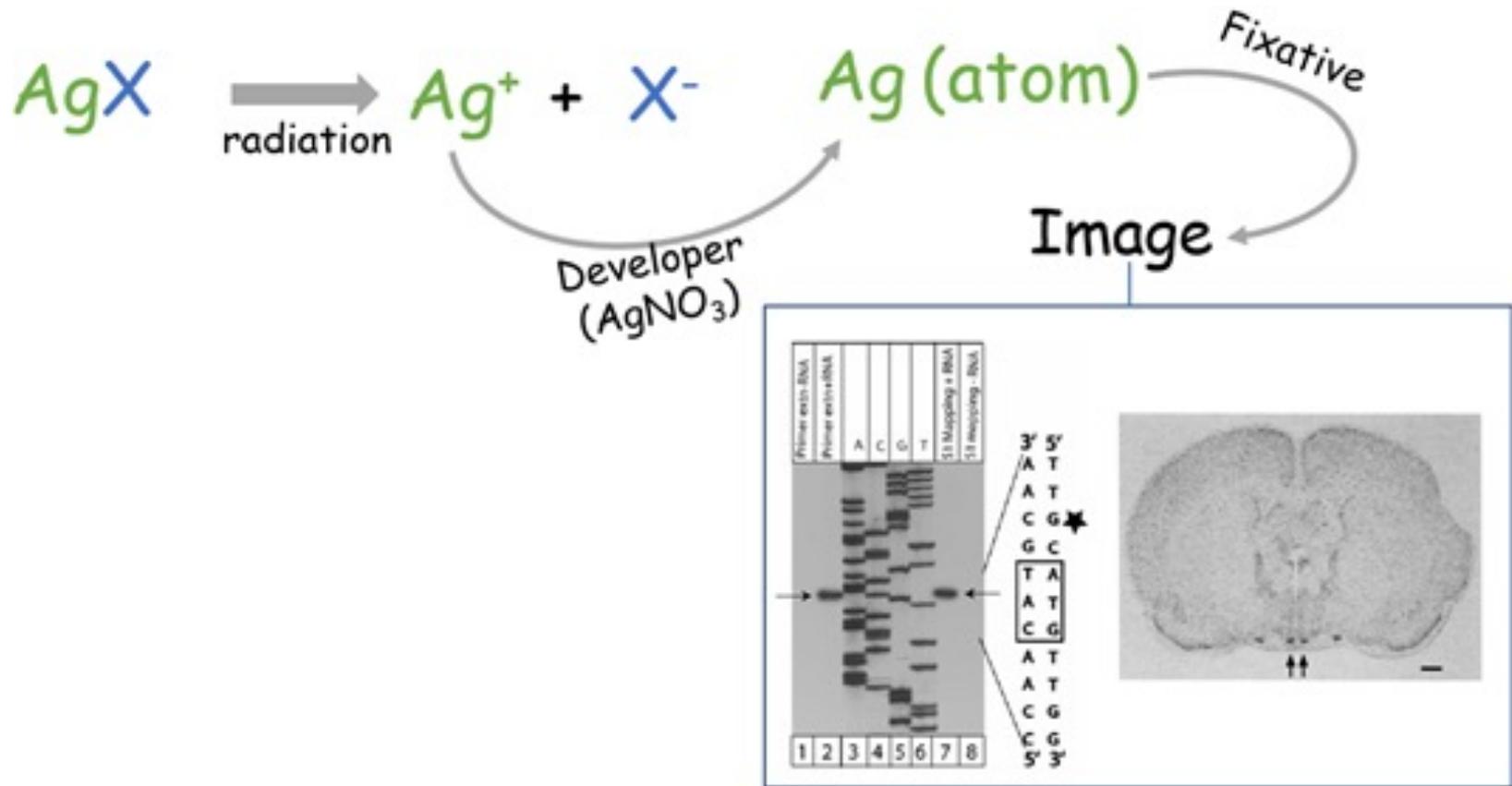


Image

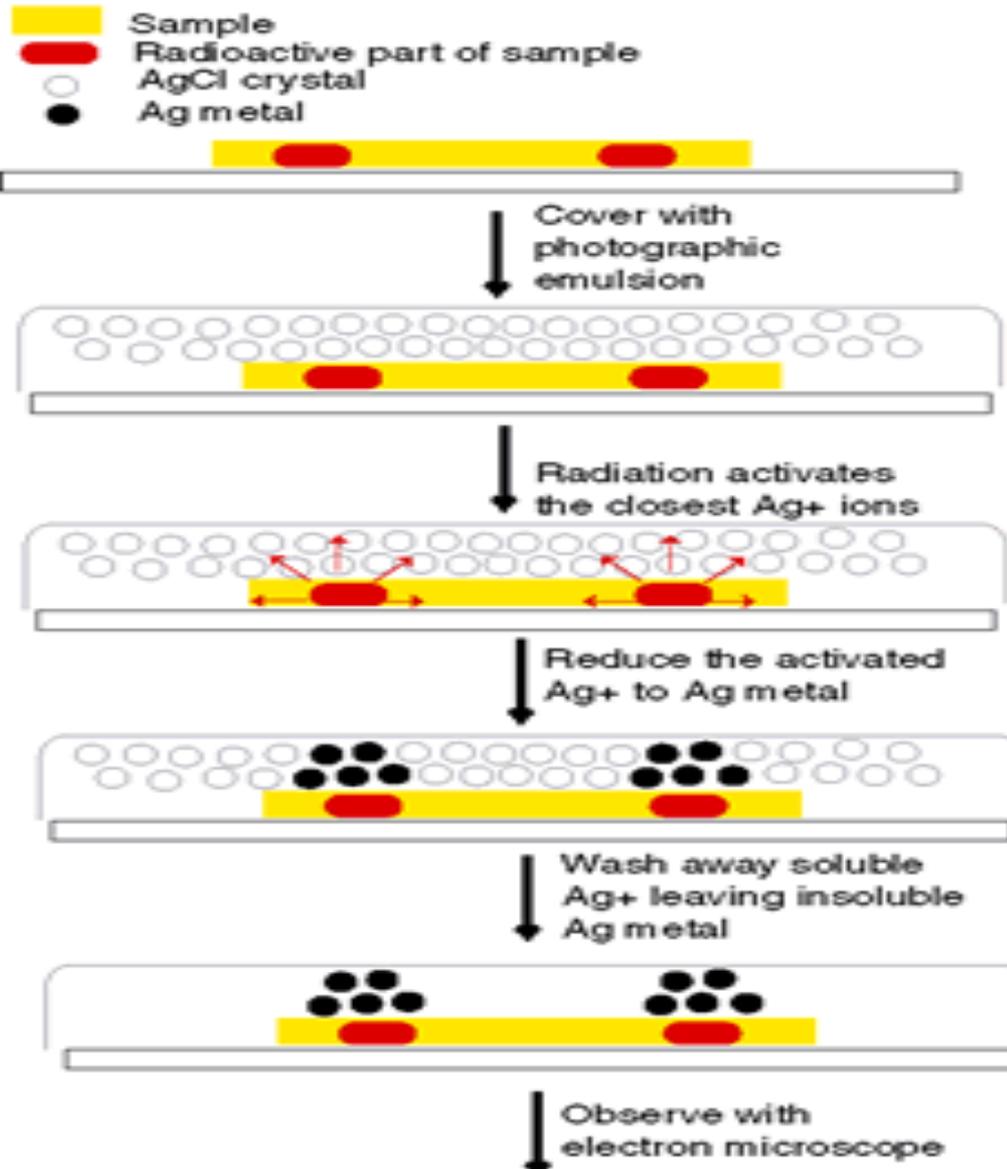
- In contrast, in **Autoradiography**, the specimen itself is the source of the radiation, which originates from radioactive material incorporated into it.
- The recording medium which makes visible the resultant image is usually, though not always, photographic emulsion.
- Therefore, Autoradiography is a specific biological tool used to detect radioactive materials by using X-ray photographic films.
- Autoradiography is employed as an important detection tool for the identification of different target receptors in various tissues to provide us with a better understanding of molecular pharmacological pathways.

# Principle of Autoradiography

- Autoradiography is based upon the ability of a 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 (5-50  $\mu\text{m}$  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 the 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 colour band.
- The resulting radiography is viewed by electron microscope, preflashed screen, intensifying screen, electrophoresis, digital scanners, etc.

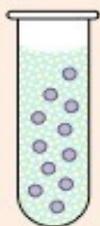


# Autoradiography



# Autoradiography Method.,

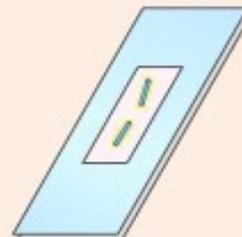
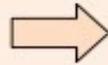
PREPARE



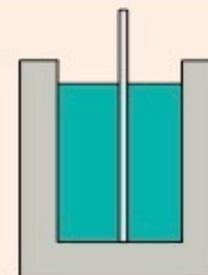
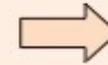
Incubate cells in radioactive  $^3\text{H-T}$



Gently lyse cells and fix contents



Embed contents onto slide



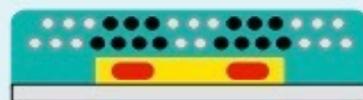
Immerse slide in radioactively-sensitive emulsion



PROCESS



Soluble ions washed away to leave metal



Activated  $\text{Ag}^+$  ions reduced to Ag metal



Radiation activates nearby  $\text{Ag}^+$  ions



Slide dipped in silver bromide ( $\text{AgBr}$ ) emulsion

Key:

■ Sample

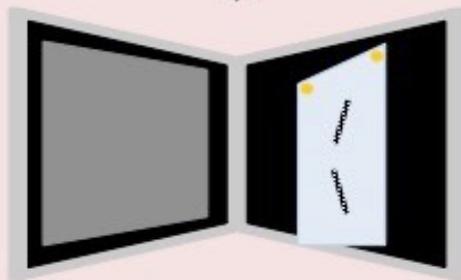
■ Part with  $^3\text{H-T}$

○  $\text{AgBr}$  Crystal

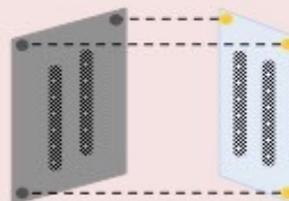
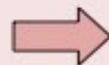
● Ag metal



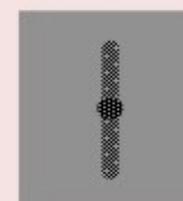
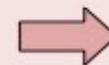
DEVELOP



Expose slide to photographic film

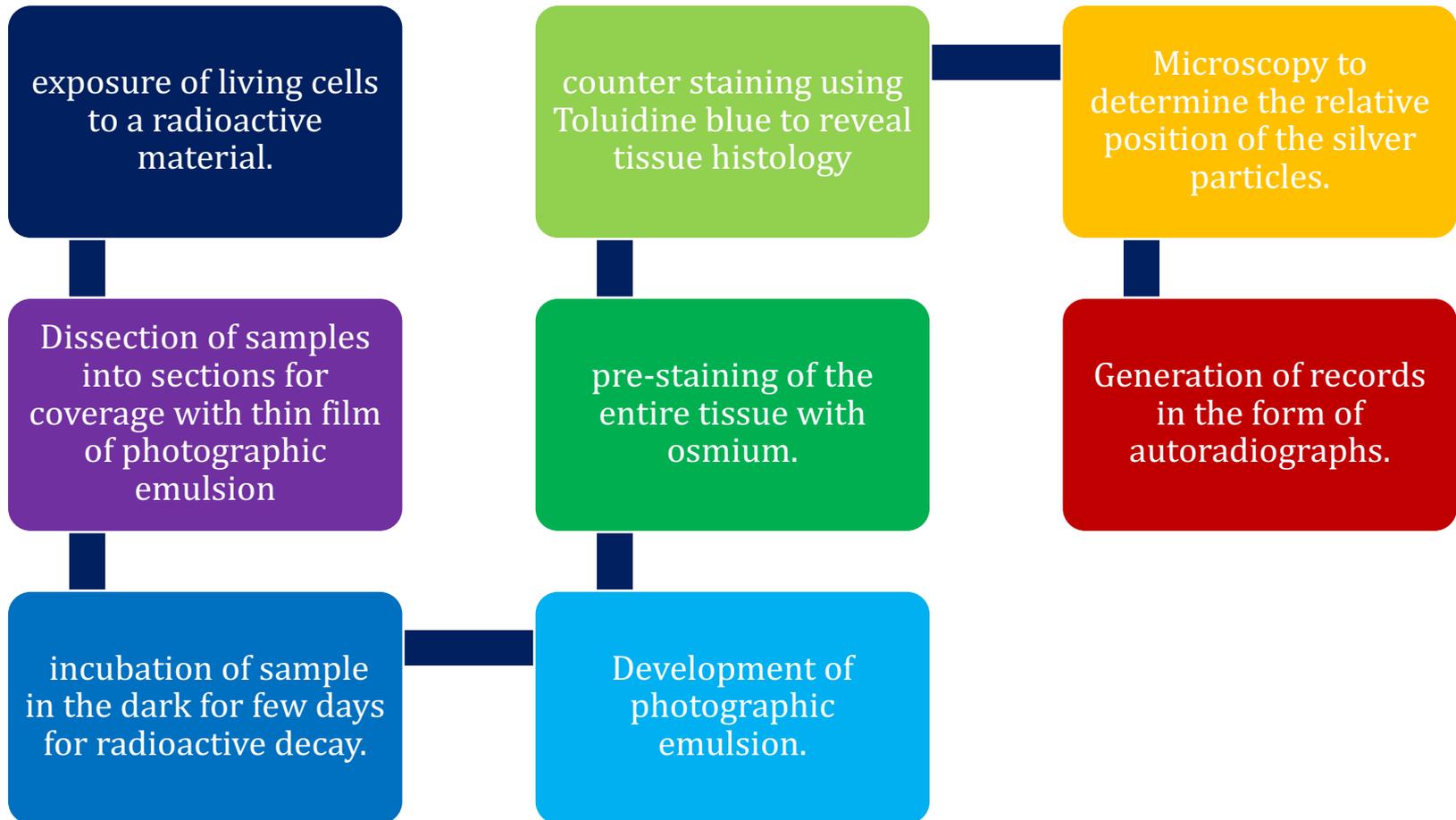


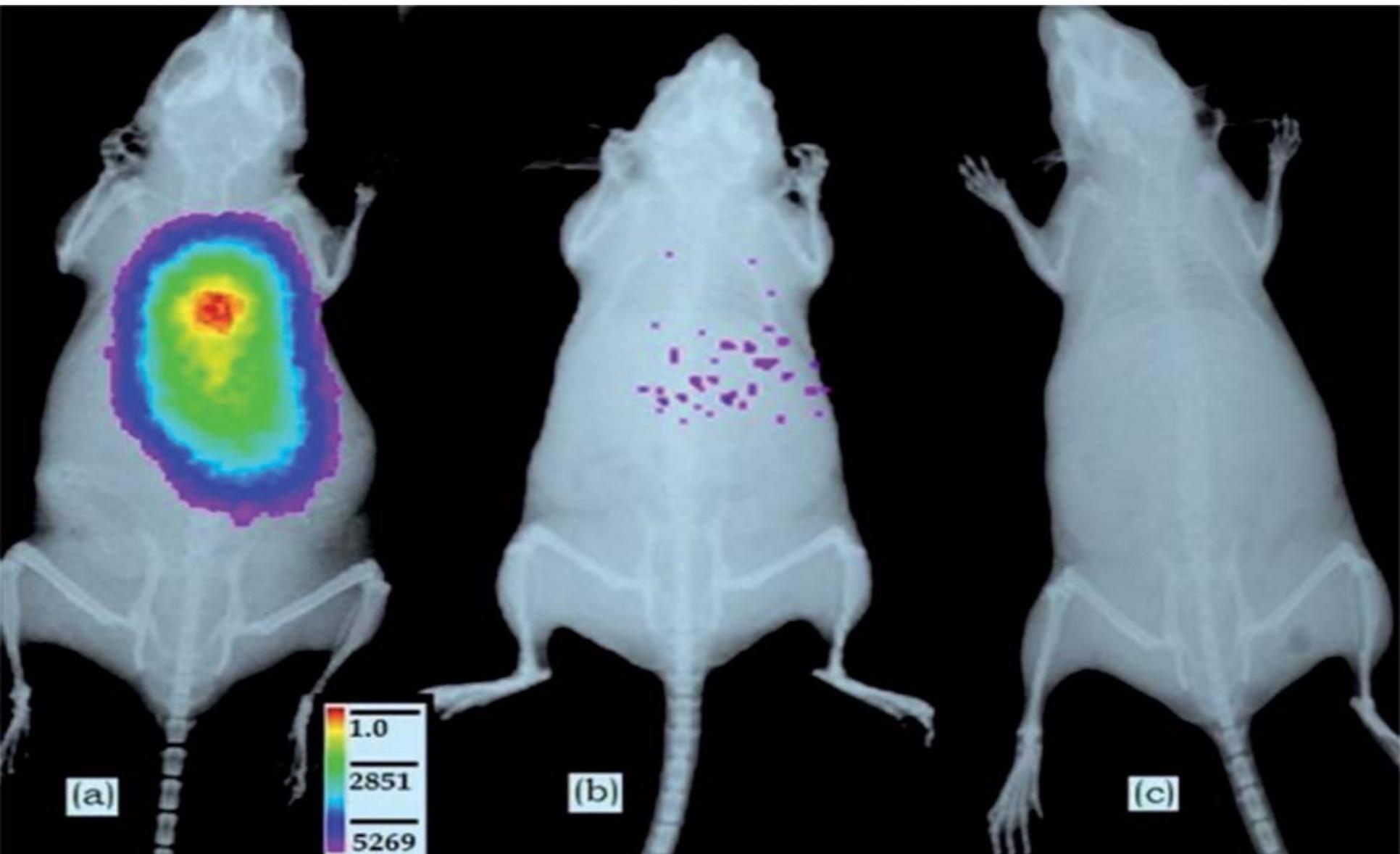
Develop to transfer image to film



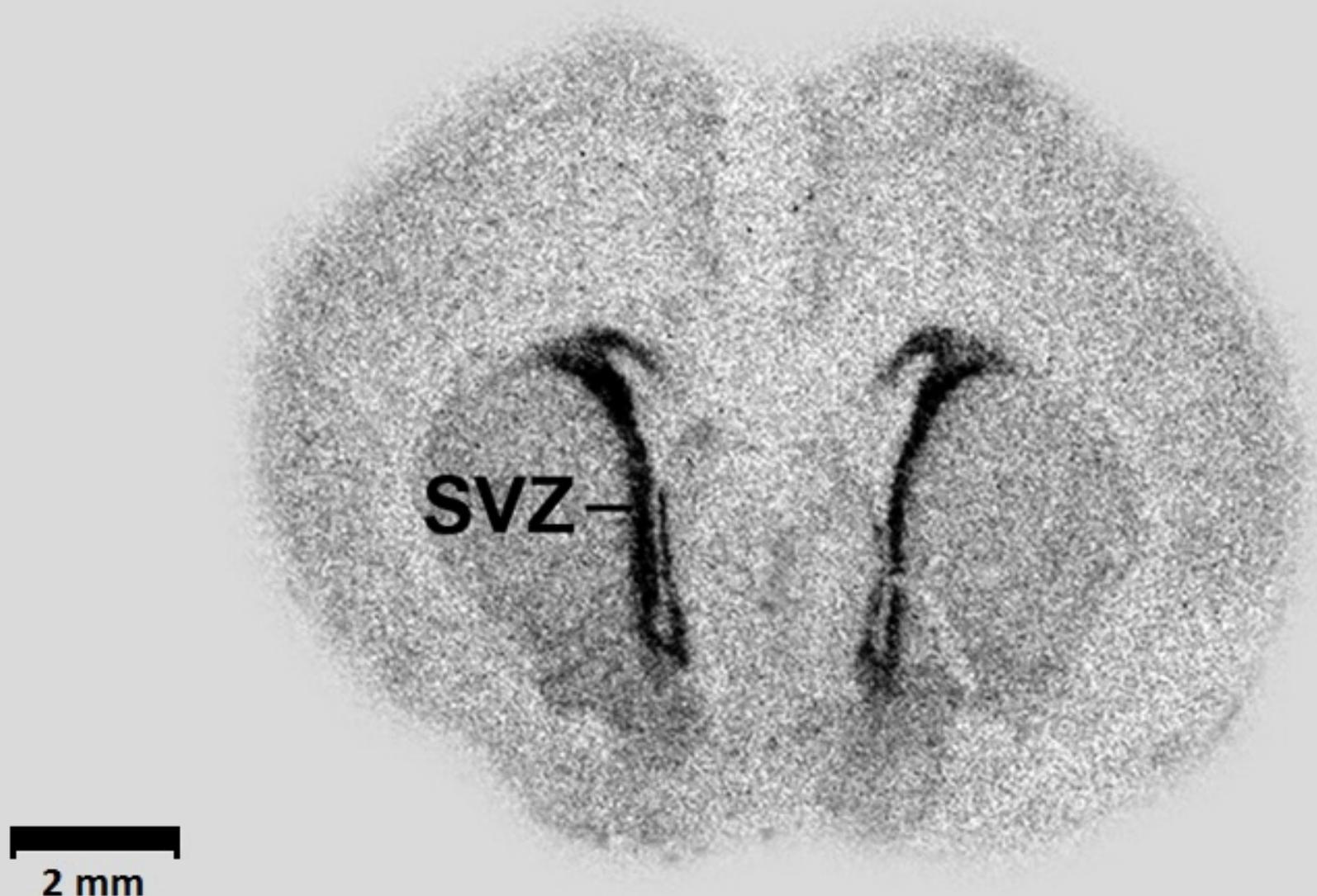
Autoradiograph

# Sequential Steps of Autoradiography





The radioautography images of  $^{125}\text{I}$ -labeled PPP. a, 5 min b, 18 h and c, 24 h after injection and merged with X-ray images.



Autoradiography of a coronal brain slice, taken from an embryonal rat. GAD67-binding marker is highly expressed in the subventricular zone

# General Methods For Autoradiography

## Two General Types of Experiments:

### 1) *In-vivo* autoradiography

- receptors are labeled in intact living tissue by systemic administration of the radioligand. Tissue is removed, processed, and visualized.

### 2) *In-vitro* autoradiography

- slide-mounted tissue sections are incubated with radioligand so that receptors are labeled under very controlled conditions

# Autoradiography Practical Applications

1. Autoradiography provides qualitative as well as quantitative information regarding a specimen.
2. It is a method of accurately following the fate of chemicals in cells and organs using radioactive isotopes (**radioisotopes**) as labels.
3. It is widely used to trace the routes of metabolism of drugs within animals.
4. it is used to Map anatomical location of radiolabeled ligands to visualize and quantify receptors in tissue
5. Trace neurons by axonal transport of radioactively labeled amino acids, certain sugars, or transmitter substances
6. Measure DNA production (e.g.,  $^3\text{H}$ -thymidine)

# Limitations of Autoradiography

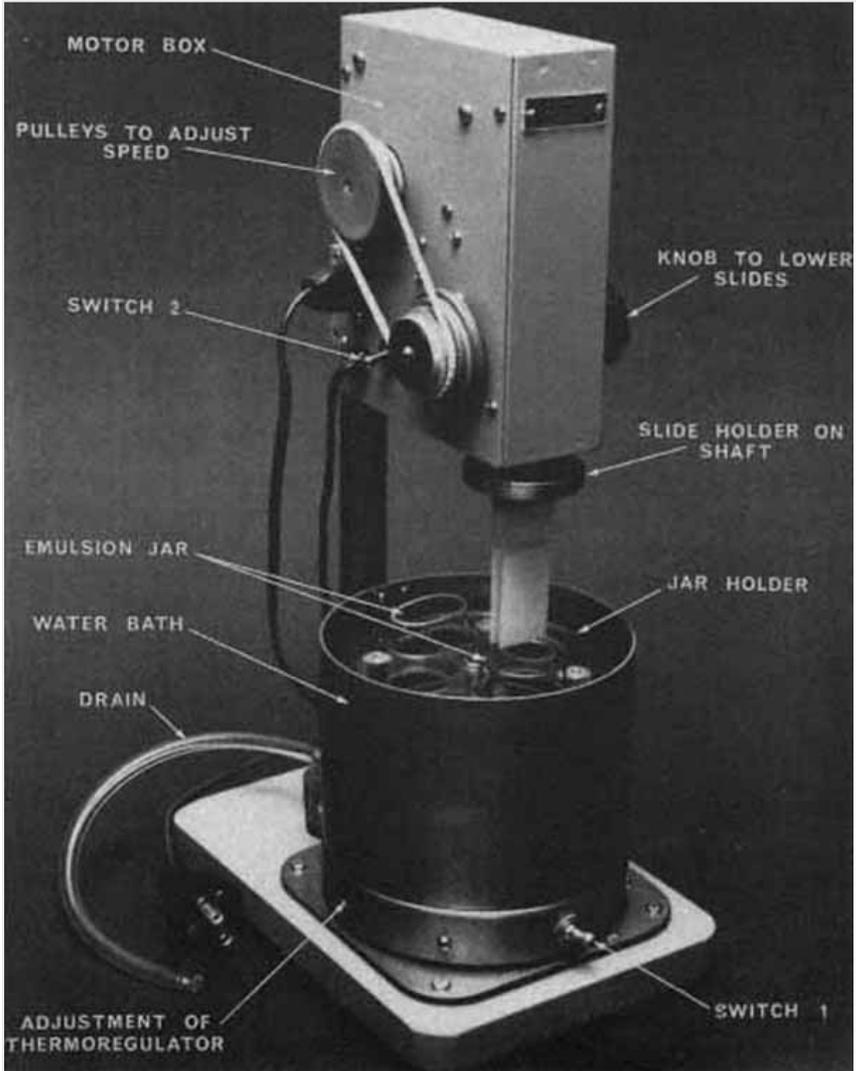
1. Everything binds to everything (easy to misinterpret results)
2. There are no biochemical or physiological criteria to assess the binding specificity (i.e., to determine whether the binding site really corresponds to an actual receptor)
3. The presence of a high-affinity radiolabelled receptor does not necessarily imply that the receptor has physiological significance
4. Ligands are not always very specific

# Types of photographic detection systems

- **Stripping film** consists of an **even layer** of photographic emulsion on a supporting gelatin membrane (e.g. Kodak AR10), it is floated on water and then wrapped around the slide and forms very close contact as it dries.
- This was once widely used but is now no longer made. It has the major advantage of uniform thickness but the disadvantage that the supporting membrane prevents counterstaining of the section and therefore the tissue block must be pre-stained before sections are coated.
- **Liquid photographic emulsion**. This is the method routinely used today.
- It is simpler and much quicker to do, but the layer of liquid emulsion (e.g. Kodak NB2) can be **slightly uneven** in thickness as it flows down to the bottom of the slide as it is withdrawn:
- for most purposes this slight variation is not important, unless the number of grains are being strictly counted and compared across one slide.



*Hypercoat Nuclear Emulsions and Hypercoat Dipping Vessel*



Commercial emulsion dipping apparatus

# Problems that can occur in autoradiography

- Although autoradiography is relatively simple to perform it requires great care and attention to detail to get good results.
- Some of the potential problems include:
  1. Incorrect localization of the radioactive site
  2. Fogging

# Incorrect localization of the radioactive site

- This may occur due to **movement of emulsion across the specimen** and will mean all the sites of radioactivity being localized at the wrong place.
- If there is a **gap between the emulsion and the section**, then the image of the radioactivity will be spread out and blurred.
- One common source of such a separation is the trapping of an air bubble under the emulsion.
- Similar spreading of the radioactivity can occur with **thick sections**.

# Fogging

- Fogging (AKA excessive background) of the film occurs when there is darkening of the film even where there is no radioactivity.
- Fogging can be due to several cause, most of which are simply lack of due care.
- If the emulsion is exposed to chemical agents capable of reducing it, then they will cause similar changes to the radiation.
- Common sources of such reactive chemicals are **fixatives**, **stains** or contamination from **fingers**.

# Fogging.,

- Exposure to **light** will also affect the emulsion. This can be due to a dark-room that lets in daylight, an unsafe 'safe-light' of the wrong type (a Wratten No. 2 safe-light is usually recommended or storage in a container that is not fully light-proof.
- Finally, fogging can be due to **storage in an area of high radioactivity**; this often happens when radiation safety officers try to keep all sources of radiation in one place.
- The worst place to store an autoradiograph while it is being exposed is in a radioactivity laboratory.
- Excessive local pressure such as finger pressure (or even more savage finger-nail pressure) will result in strong local blackening of the emulsion.

# References & Credits

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- Cook, D.J (2006). **Biomedical Sciences Explained: Cellular Pathology.** Butterworth Heinemann, Oxford. ISBN: 10: 1948 42305
- Khan NT (2017) **Autoradiography: Detection and Analysis of Radioactive Entities.** *J Biom Biostat* 8: 361. doi: 10.4172/2155-6180.1000361
- Rogers A (1979) **Techniques of autoradiography.** 3rd Edition. Elsevier, North Holland pp429.
- Sheehan D, Hrapchak B (1980), **Theory and practice of Histotechnology**, 2<sup>nd</sup> Ed, Battelle Press Ohio, pp

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# End of Lecture



## Cellular Pathology