Investigation into Low Energy Beta Imaging Using CCD Imaging Sensor

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Abstract

Autoradiography is an extensively used technique to create a record of the radioactivity dispersion throughout the biological samples. Conventionally, this technique was performed using film. Spatial resolution and sensitivity are the main parameters of interest in developing new digital autoradiography systems. In conventional autoradiography, the images that have been documented display an excellent spatial resolution but at the same time show lack of sensitivity, very limited dynamic range and non-linear response. To overcome the disadvantages of this method, digital autoradiography systems have been developed to address the major drawbacks of the former technique.

This thesis is focussed in a method of imaging the beta emissions from radiolabelled tissue specimens that has been developed by using a CCD imaging sensor. Most of the work involving autoradiography techniques is conducted using beta emitters such as ^{3}H and ^{14}C because of their occurrence in abundance in organic materials. Thus, the work in this project is to develop a method for imaging these very low energy emissions for ^{14}C where the end point energy around 156 keV. Preliminary calculations suggest that by using scintillator paper, then such an approach would open up the way for dual tracer imaging. It also involves learning aspects of applying CCD imaging system to compare the images acquired from ^{14}C and ^{241}Am sources either using scintillator paper or without it. Finally, preliminary results demonstrate that this approach may will be feasible based on the perceived images from the experiment.

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.....To my family

For your encouragement and support

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Chapter 1

Introduction

1.1 Overview

Autoradiography was originated by the discovery of uranium nitrate and tartrate blackens silver chloride and silver iodide emulsions. The incident was noticed by Niepce de St. Victor in 1867 and similarly by Henri Becquerel in 1869, when he observed that opaque paper placed between the uranium nitrate and the emulsion experienced the same darkening effect **[GAM, 2004]**.

The autoradiography technique initiated from this incident when Becquerel discovered the plates were darkened in the regions where radioactive materials were located. This agrees with the basic principle of autoradiography: the location of radioactivity specifically in biological materials is observed through dark spots on an emulsion film.

Autoradiography consists of the following parts: the biological samples, the radionuclide that is used as a tracer to label the sample, and the detecting medium which is a film in traditional method. Gradually, this conventional technique of autoradiography is being replaced by digital detectors. One of the most popular detector in digital autoradiography which will be described in this dissertation is Charge-Coupled Device (CCD) sensor.

1.2 Components of Autoradiography

1.2.1 Specimen / Sample

There are many types of specimen that are used in autoradiography either from biological organisms, chromatography or electrophoresis. The prepared samples should be very thin (~few μm) whenever the main interest is high resolution. The samples can be acquired from the entire organism (plant seeds up to animal) or just take slices of tissue.

Typically, a microscope is not necessary for the work of preparing specimens. The preparation of the samples in light microscopy (LM) autoradiography is about ~0.5-5μm in thick. Electron microscopes are only used in ultra-thin sections, which is typically about 40-80 nm of plastic embedded material **[JTO, 1999]**.

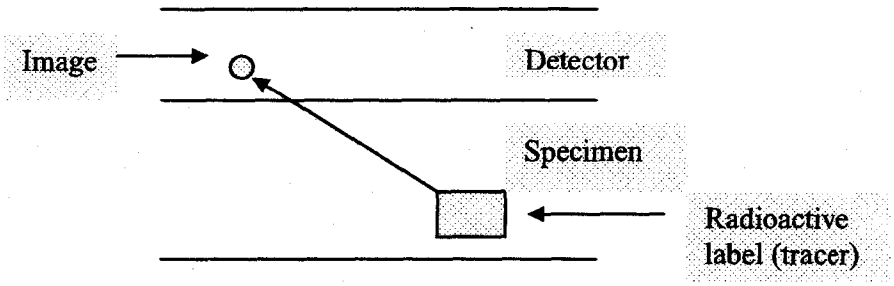

Figure 1.2.1:1: Autoradiography process involves specimen, tracer and detector to form the image [BAK, 1989].

1.2.2 Radionuclides

The main considerations that need to be taken into account when choosing the radioisotopes to perform autoradiography are the characteristics of the radiation emitted from the biological aspect. The most common radioisotopes used are ^{3}H and ^{14}C because both of them can easily be incorporated into nearly any organic molecules. However, the radioisotopes sometimes deemed unsuitable due to their low energy (E_{max} ~ 18-156 keV), where self-absorption in the sample of specimen can reduce the ability to detect radiation emission **[GAH, 1972]**.

ISOTOPE	Half-life ($T_{1/2}$)	Decay	Energy
Tritium	12.35 years	β	18.6 keV (Max); 5.7 keV (Average)
Carbon-14	5760 years	β	156 keV (Max); 50 keV (Average)
Sulphur-35	87.4 days	β	167 keV (Max); 49 keV (Average)
Phosphorus-32	14.3 days	β	1.71 MeV (Max); 0.70 MeV (Average)
Phosphorus-33	25.5 days	β	248 keV (Max); 77 keV (Average)
Calcium-45	165 years	β	250 keV (Max)
Chlorine-36	3.03×10^5 years	β	714 keV (Max)
Strontium-90	28 years	β	540 keV (Max)
Iron-55	2.7 years	Auger e^-	5.5 keV
Iodine-125	60 days	Auger e^-	2.9 keV; 0.8 keV (X-ray present)
Cobalt-57	270 days	Auger e^-	14 keV (γ-ray present)
$^{99}Tc^m$	6.02 hours	γ	140 keV

Table 1.2.2:1: The Beta radioisotopes used with its various characteristic: half-life, mode of decay and energy. The top five are commonly used in beta autoradiography whereas some radionuclides emitting Auger electrons and gamma as well [JTO, 1999].

Instead of the half-life of the radioisotope, important parameters are the energy and type of particles emitted. Half-life is a measurement of the probability that a certain radioisotope will spontaneously decay to become half of its initial activity and emit particles. For the autoradiography purposes, the radioisotope's half-life should be long enough so that sufficient radioactivity still remains after sample processing but not too long for enough exposure.

1.2.3 Film

The film for autoradiography consists of three main layers: emulsion, base and protective coating. The emulsion layer usually 10-20μm in thick and composed of silver halide grains (AgI, AgBr, AgCl) dispersed within gelatine. The larger grains show better

sensitivity but display low resolution. Conversely, smaller grains will produce the reverse effect.

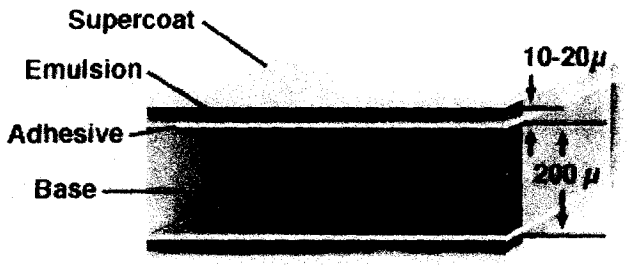

Figure 1.2.3:1: Cross-section of double coated photographic emulsion illustrating the base, emulsion and supercoat. The emulsion is attached to the base by a thin layer of adhesive [GAM, 2004].

The latent image formation is due to the oxidization of the bromine atoms, causing the release of free electrons. The free electrons travel through the crystal until it becomes trapped in one of the imperfection in the lattice. The electrons attract the silver ions, which reduce to silver atom and form clumps called latent image centers **[ROG, 1967]**.

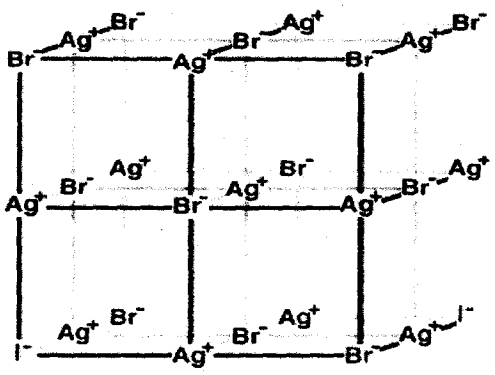

Figure 1.2.3:2: Silver halide crystal lattice structure showing the relative locations of Br^-, Ag^+ and I^- ions [GAM, 2004].

1.3 Techniques of Conventional Autoradiography

Most of the autoradiography experiments can be carried out in different ways according to the type of study performed and information required. Briefly, autoradiography is divided into

two subdivision, DNA sequencing and thin tissue imaging. In the early years, thin tissue autoradiography was performed either as macroautoradiography or microautoradiography. Over a few decades, light and electron microscopy autoradiography was introduced.

Autoradiography is a useful biological tool where organisms are screened to monitor the distribution of a wide variety of compounds including drugs, hormones and growth substances. It requires the entire organisms, or slices of whole organisms such as bone or leaves placed in close contact with film to produce an autoradiograph. The resolution level required by the macroautoradiography is less than microautoradiography. Macroautoradiography provides a spatial resolution of approximately 30μm **[GAH, 1972]**.

In all types of autoradiography, there are different techniques of getting an exposure. The oldest and simplest method is through the direct contact method in which the specimen is just pressed directly onto the radiographic film or plate. After exposure, the film is separated from the sample and being developed. A factor that affects the quality of the images obtained is the uniformity of pressure between the sample and photographic material as well as the uniform thickness of the sample **[VMR, 1966]**.

The other technique is by coating the sample with a liquid emulsion. This has added an advantage of autoradiography to be conducted on curved surface samples. By this technique, a melted photographic emulsion is poured onto the specimen, which means the emulsion will shape itself to the sample. Typically a layer of preparative coating is poured onto the specimen before the liquid emulsion, in order to give better adherence of the emulsion layer **[BAK, 1989]**.

Other techniques used are base-free emulsion layer or more often recognized as the 'stripping film' technique and direct mounting method. In electron and light microscopy autoradiography, the samples thickness should be very thin to get higher resolution. The darkened grains have to be observed under an electron microscope or light microscope. Usually electron microscope is used in detecting the tracks of particles since these tracks are generally very short and small.

Resolution is defined as the capability to distinguish two nearest objects as separate. Basically, an increase in resolution means a decrease in sensitivity. The factors which may influence the resolution are isotope selection, distance from the source to the emulsion, the thickness of emulsion layer, exposure time, size of silver halide crystals and the ability of emulsion to detect emitted wavelength **[GAM, 2004]**.

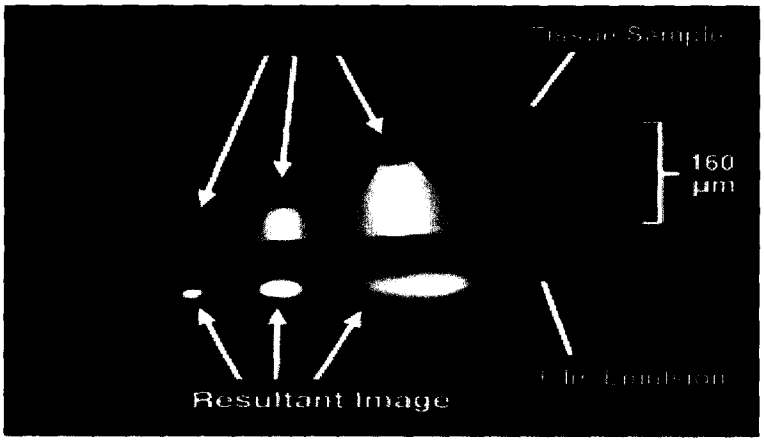

Figure 1.3:1: The effect of slice thickness on resolution. Emission sources that are distant from the photographic film (as in thick slices) may expose a broader area of the emulsion than sources that are close [GAM, 2004].

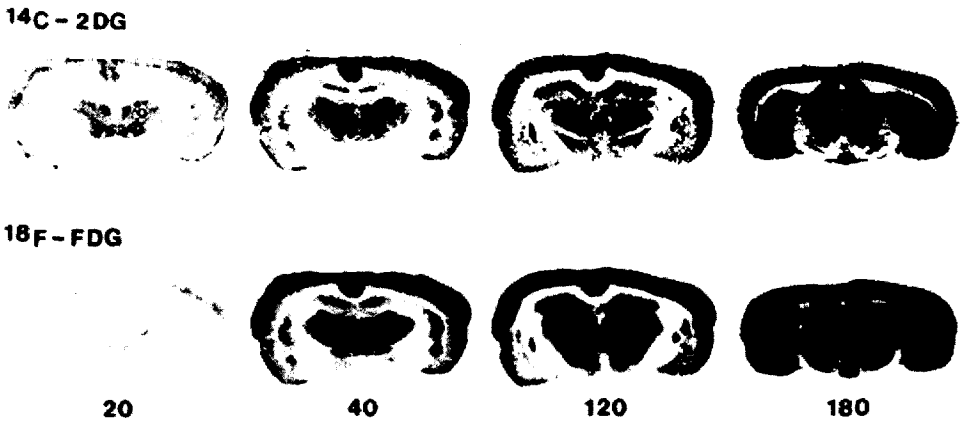

Figure 1.3:2: The effect of energy and tissue thickness on resolution. The top row of sections uses the low-energy emitter ^{14}C as a tracer; the bottom row uses the higher-energy emitter ^{18}F. Thicknesses of the sections in both rows are 20, 40, 120, and 180 µm from left to right. Resolution is degraded for the higher-energy emitter and diminishes as tissue thickness increases [GAM, 2004].

Achievable resolution is a function of electron energy (see figure 1.3:1) and slice thickness (see figure 1.3:2). High energy emissions will travel farther from their point of origin, and emissions from deep within a thick tissue section will subtend a greater angle as they reach the film. Since higher-energy emissions are attenuated less through thick slices than low energy emissions, resolution drop-off due to increasing slice thickness is more pronounced for high-energy emitters.

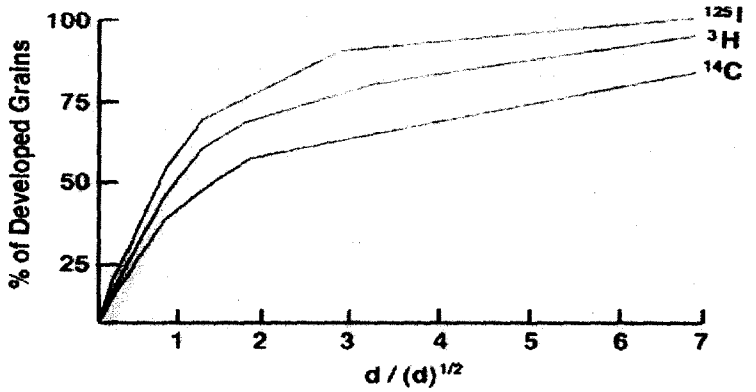

Figure 1.3:3: Distribution of tracks versus energy for microautoradiography. $d / (d)^{1/2}$ is the distance between a developed grain and its radioactive source divided by the median distance of all developed grains. The graph implies that it is more difficult to determine the origin of high-energy electron emitters than low-energy electron emitters due to longer tracks and wider track scattering [GAM, 2004].

Sensitivity can be improved by increasing the thickness of section. In addition, the larger the silver grains, meaning that more light will be absorbed. However, this is not applicable in microautoradiography, where number of grains per unit area is concerned. Therefore, sensitivity is assisted by smaller and more densely packed grains **[GAM, 2004]**.

1.4 Advantages and disadvantages of Conventional Autoradiography

Conventional autoradiography is less expensive technique available which supplies an excellent spatial resolution of the images down to approximately 0.1 μm. As a result, it can be applied to produce the image even at sub-cellular levels.

Besides a better spatial resolution, this technique suffers from poor sensitivity due to the low X-ray / beta particles detection efficiency. Integration period up to several months is required to exhibit satisfactory outcomes. Moreover, this technique also has a limited dynamic range (maximum 10^2) that may lead to under exposure or over exposure some sections of the image, comprising quantification of the absolute range of tracer concentration within the image **[RJO, 2000]**.

The other drawbacks of conventional autoradiography are high background noise level ~ 10^5 events/mm^2, non-linear response of the film and does not provide any information for real time imaging. The need of chemical development of film could also be considered as a problem since many factors (e.g. temperature of developing solution) may transform the quality of the image.

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