FUJIFILM

Application Note

SCIENCE IMAGING SYSTEMS

Application Note No. 7

Film Method and CCD Method for Chemiluminescence Detection

LAS-1000

LAS

Introduction

The chemiluminescence method of detecting DNA or protein on blotted membrane filters is widely used. Until recently, this method depended almost exclusively on x-ray film for detection of the chemiluminescent emission. This is now being changed by dramatic advances in cooled CCD camera technology. Today, the traditional x-ray film system is gradually giving way to high-sensitivity cooled CCD camera systems.

Both systems have the same purpose - to detect the emitted light - but they differ totally in principle. The film method records the pattern of the light by a chemical reaction while the CCD method converts the light into an electrical signal. The difference in principle leads to different image features. The CCD method is superior in a number of points: the digital image it produces is broader in dynamic range, has better linearity and therefore more accurate.

This Application Note compares the light detection principles of the film method and the CCD method and explains the imaging processes.

Contents

Summary

- The film and CCD methods are the ones generally used for chemiluminescent imaging.
- The digital image obtained by the CCD method is ideal for quantitative analysis.
- With the CCD method, imaging is simple and optimum exposure conditions are easy to determine.
- When equipped with the URF20L (f0.85) lens, the LAS-1000 provides sensitivity equal to, or better than, the film method.

1 Light Detection and Imaging

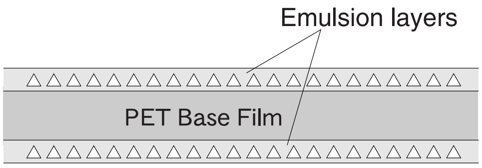
Film Method

The chemiluminescent light forms an image on x-ray film by reducing the silver halide grains in the film's emulsion layers followed by development procedure.

Silver halide
A compound of silver (Ag) and a halogen. AgCl, AgBr and AgI are typical silver halides.
General symbol: AgX.

Emulsion
Short for "photographic emulsion," a suspension of photosensitive silver halide grains in an aqueous gelatin solution.

(1) Layer structure of the film



Both surfaces of the x-ray film base are coated with silver halide grain emulsion layers.

Fig.1-1

Fig.1-1 Simplified diagram of x-ray film layers

Development
To obtain a visible image. Silver halides are reduced to silver metal by a reducing compound such as phenidone or hydroquinone.

Fixing
For stabilizing the developed image. A weak acidic solution of sodium thiosulfate is used to dissolve undeveloped silver halide grains.

(2) Film imaging process

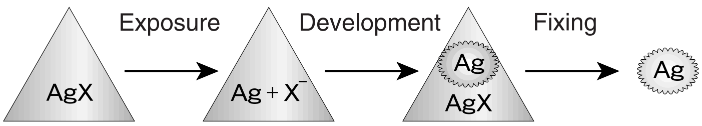


Fig.1-2

Fig.1-2 Schematic diagram of the film imaging process

Exposure to the image-carrying light generates development centers in the emulsion layers in the pattern of the image. Development produces a visible image by reduction of the silver halides near the development centers to silver metal.

CCD Method

The CCD camera has a matrix of over one million light-receiving elements each corresponding to one image pixel. The elements (photo pixels) accumulate electrons in proportion to received light and then convert the electrons to an electrical signal.

Gate
Voltage is applied to this electrode to transfer the accumulated electrons as image data.

Oxide film
A thin, transparent layer of silicon dioxide for insulating the gate from the substrate.

Silicon substrate
Most CCD image sensors are fabricated on a base of silicon crystal.

(1) CCD photo element

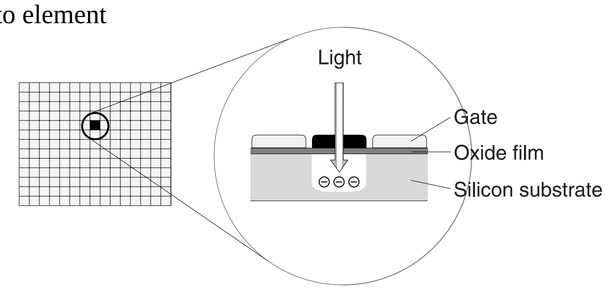


Fig.1-3

Fig.1-3 Simplified, conceptual diagram of CCD element (photo pixel)

When light enters a CCD element, the element generates and accumulates electrons.

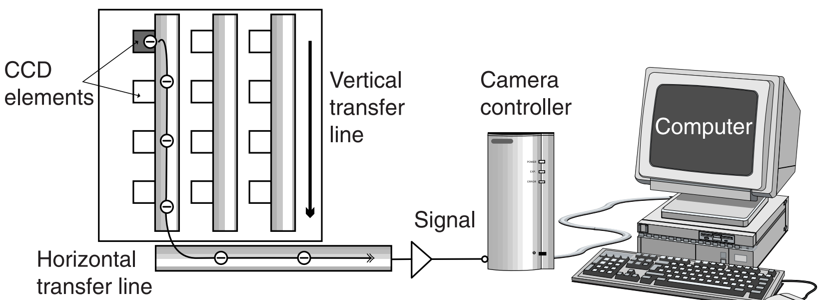


Fig.1-4

Fig.1-4 CCD imaging process
The electrons accumulated on the CCD elements (photo pixels) are sent to an output section through transfer lines and then used as image data by a computer.

2 Comparison of the Film Method and CCD Method

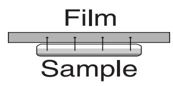
Light Emission and Image-capture

In the film method, the sample is placed in direct contact with the film. This maximizes the exposure to light, however not all of the photons entering the film are used to reduce the halide grains to silver. For instance, several photons are needed to form a development center.

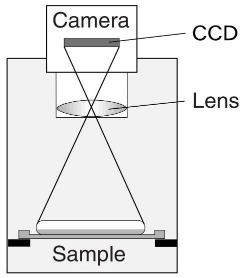
In the CCD method, light from the sample is collected through a lens. This decreases the light emission entering the CCD. However, the CCD is very efficient. That it generates and stores an electron for almost every photon received.

Obviously, the detection sensitivity of the CCD method depends greatly on how effectively the light is collected. That is why the high-sensitivity Fujinon URF20L (f0.85) lens has been made available for use with the LAS-1000.

Film method exposure



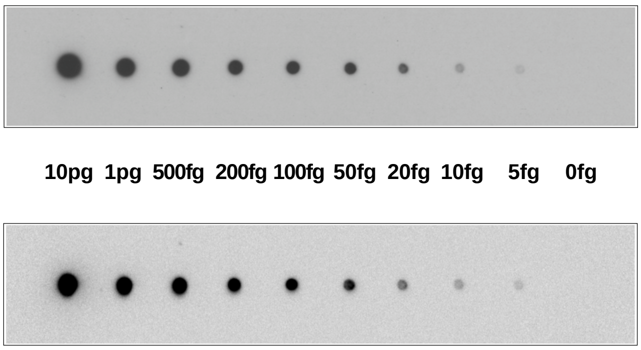
CCD method exposure



Sensitivity

The LAS-1000 was used to detect extremely weak chemiluminescence [photon flux: approx. 5 x 10^5 photons/sec•cm2 (equivalent to 5 fg spot in the below image.)]. These results are comparable to those obtained with film in the same concept.

Film method
(3 min. exposure)



CCD method
(2 min. exposure)

Fig.2-1

Fig.2-1 Detection of chemiluminescence by film method and CCD method

Sample : pBR328 dot blotted membrane filter
Reagent : CDP-*Star*®
Film : FUJI newRX
Detection system : LAS-1000
Lens : URF20L (f0.85)

Buffer was applied as negative control. (0fg)

Imaging Process

(1) Film method

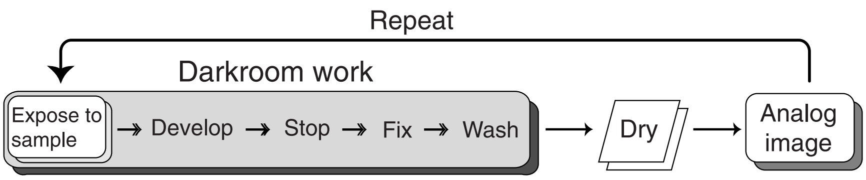


Fig.2-2

Fig.2-2 X-ray film imaging process

In the film method, the steps from exposure through development are conducted in a darkroom.

(2) CCD method

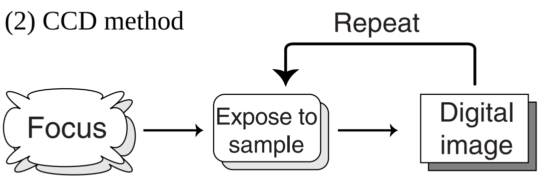


Fig.2-3

Fig.2-3 CCD imaging process

In the CCD method, a digital image can be obtained in a light room by conducting the exposure in a special, light-tight darkbox like that in the LAS-1000.

Quantitative Image Analysis

Fig.2-4

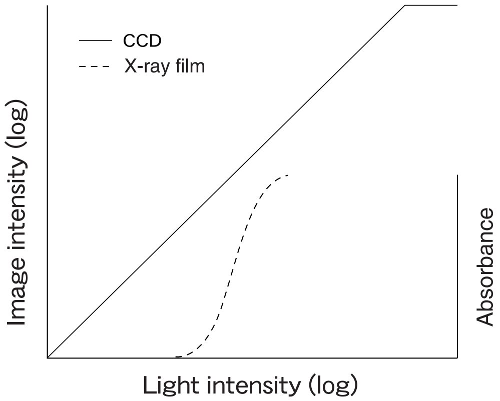


Fig.2-4 Light intensity vs image intensity with x-ray film and CCD

The density of the image by the film method is not directly proportional to the light intensity and only a segment of the curve can be treated as linear.

The intensity of the image by the CCD method is directly proportional to the light intensity. Quantitative analysis is greatly simplified.

Sample Size

The largest sample that can be imaged by the LAS-1000 is determined by the tray height. Since the CCD has fixed 1384 x 922 available pixels, the resolution of the image is determined by the tray height. LAS-1000 is designed to get enough resolution for practical recognition of separate bands on the blotted membrane.

Tray height vs. max. sample size in LAS-1000

Tray height	Max. sample size (cm)
1	7 x 11
2	9 x 14
3	12 x 18
4	14 x 21
5	17 x 25
6	20 x 25
7	25 x 25

Tray height

The sample tray of the LAS-1000 can be set at any of seven levels.

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