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**A GASEOUS DETECTOR FOR HIGH-ACCURACY AUTORADIOGRAPHY
OF RADIOACTIVE COMPOUNDS WITH OPTICAL READOUT
OF AVALANCHE POSITIONS**

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ABSTRACT

We describe a detector based on the imaging of avalanches in gases initiated by ionization electrons produced by β -particles entering an amplifying gap limited by two parallel electrodes. The avalanches are made visible by the addition of an efficient light-emitting vapour to noble gases such as argon or xenon. Imaging of the avalanches with an image intensifier and a CCD coupled to a computer permits accuracies of the order of 50 μ m for the β -rays emitted by tritium. Application of the method to biological tissue slices labelled with tritium shows a gain in time, with respect to the photographic method, by a factor close to 100. Images of gels labelled with ^{32}P or ^{35}S show accuracies better than 0.5 mm (FWHM). The linearity and large dynamic range in intensity measurements are far superior to those of the standard autoradiography method.

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1. INTRODUCTION

The imaging of β -emitting radionuclides is a routinely used technique in biophysics, biochemistry, pharmacology and clinical medicine. Depending on the methods of separation of chemical compounds one has to deal with one-dimensional or two-dimensional imaging. Commonly used tracers are ^3H , ^{14}C , ^{35}S , ^{32}P , ^{125}I .

The differences in the energy spectra of the electrons emitted by these various nuclei result in differences in the detection conditions. The most commonly used method consists of exposing the chromatography plates or the biological slices to photographic films (autoradiography).

Compounds labelled with ^{32}P or ^{125}I may yield satisfactory pictures after a few hours, whilst with ^3H it may take several months to obtain an adequate image. In this last case the range of the low-energy electrons of maximum energy 18 keV limits the useful emitting layer to a thickness of the order of a micron.

After an image has been obtained on a film, time-consuming and expensive methods have to be used to obtain a quantitative estimate of the intensity distribution.

Many attempts have been made in the last two decades to design electronic systems that would detect and localize the β -rays. Wire chambers have led to several commercial instruments with an advertised accuracy ranging from 1 to 3 mm (FWHM). Restricting ourselves only to gaseous detectors, one of the main difficulties encountered is the large range of the β -rays in the gases, at atmospheric pressure. One way to overcome this has been the use of the multistep avalanche chamber [1, 2]. In this device a wire chamber localizes a cloud of electrons from an avalanche produced in the uniform electric field created between two parallel electrodes. The sample with the radioactive compound is placed against the cathode, either outside the gap or so that its surface is the cathode plane. The anode of the gap is a grid, transparent to electrons, made for instance of a mesh of $50\text{ }\mu\text{m}$ wires spaced by $500\text{ }\mu\text{m}$. This introduces a significant improvement in the positional accuracy for the following reason: when a long-range β -particle penetrates such a gap, it produces ionization electrons all along the track, but the multiplication of the electrons is not constant. It is proportional to $e^{\alpha X}$, where X is the distance of the electron to the anode and α is the number of ionizing collisions per centimetre, produced in the gas by an electron experiencing ionizing collisions under the influence of an electric field.

Thus the ionizing electrons produced upon the entrance of the β -particle give rise to the largest amplification. This reduces very much the error due to the finite range of the β -particle, if the position of the maximum of the charge distribution produced by the avalanches is measured.

Accuracies of the order of 0.5 mm (FWHM) had been obtained with β -rays from ^{14}C and ^{35}S [2]. Using a similar design, other groups have obtained two-dimensional images with an accuracy of the same order, with ^3H and ^{35}S -labelled compounds [3, 4]. In all these attempts, the localization of the avalanches in the last step chamber is obtained by various electronic methods giving the electrical centroid of the avalanches. Another group, using only one step of amplification between parallel electrodes, has also illustrated the advantages of parallel electrodes for the amplification [5].

In this article we present a new method. It exploits the advantage introduced by the electron amplification due to avalanches in a uniform electric field in the gas, between parallel electrodes; but the readout method, based on the optical imaging of the atoms excited in an avalanche, leads to a substantial improvement in the accuracy, bringing it to better than $100\text{ }\mu\text{m}$ (FWHM). Not only is this method a very accurate one for measuring the centroid of an avalanche, but the detailed picture it gives of the distribution of the charges in the avalanche may lead to further improvement in the accuracy of the position measurement of the first electron produced in the gap by the entering β -ray.

2. OPTICAL READOUT OF ELECTRON AVALANCHES

When avalanches are produced by multiplication of electrons in gases under the effect of strong electric fields, a great variety of processes lead to the emission of photons with a spectrum ranging from vacuum ultraviolet (VUV) to visible. The intensity may vary over a considerable range from gas to gas and as a function of the electric field. Several years of research permitted us to find the optimum conditions for the emission of light in a wavelength range convenient for optical imaging, with the maximum amount of photons per charge produced in an electron avalanche at the largest charge gains. The initial goal was to visualize the position of charged-particle tracks in gases [6] or to image the VUV photons produced by Cherenkov light. It was concluded that the optimum structures for the maximum amount of light utilize, for the amplification, parallel-grid electrodes and not wires. It was found that addition, to the noble gases, of vapours of triethylamine (TEA) [7] or tetrakis(dimethylamino)ethylene (TMAE) [6, 8], results in copious emission of fluorescence photons, spectra peaked at 280 nm and 480 nm, respectively; the second is obviously more convenient for optical imaging, but TMAE is not easy to handle, because of its low vapour pressure and strong oxidation. Gooch et al. have reached independently the same conclusion [9] on the advantages of TEA for optical imaging of avalanches initiated by Cherenkov VUV photons, and Sauvage et al. have made a detailed study [10] of the light emission of TMAE and TEA in avalanche chambers. It was also found that it is easy to convert the photons of the first spectrum into visible light on appropriate thin plastic wavelength shifters^{*)} or by a thin layer of sodium salicylate deposited on the Mylar foil and placed in contact with the anode grid [6]. The average intensity of photons produced in avalanches is close to one per electron. We found it easy to image single electrons produced by the absorption of VUV Cherenkov photons in a multistep structure made of two successive parallel-grid gaps [11, 12]. We applied this technique to the imaging of the β -ray-emitting compounds labelled with ^3H , ^{32}P , and ^{35}S .

3. THE EXPERIMENTAL SET-UP

The schematics of the experimental set-up is shown in Fig. 1, which displays two versions of the detector that we have used for the imaging of β -emitters. The principle of detection is the same in all cases: light emitted by atoms excited during the electron multiplication in the gas is detected by means of an image intensifier coupled to a CCD camera.

It has been observed that the yield of photons that can be obtained, for a given charge gain, from electron avalanches between parallel electrodes [13] is larger than that in the multiwire chamber where multiplication occurs near the thin wires. This fact led us to using crossed-wire meshes for the construction of the charge-amplifying structures. They have the advantage of being transparent to light, and to electrons if multiplication in successive structures is required. A mesh of wires of 50 μm diameter and 500 μm pitch was employed.

The installation of the radioactive sample inside the active volume of the detector is necessary in the case of tritium sources, where the very low energy β -particle ($E_{\text{max}} = 18.6 \text{ keV}$) would be absorbed by even the thinnest plastic window. We studied two versions of the detector for the ^3H -labelled sample tested: sample behind a cathode mesh, in contact with it (Fig. 1a), and sample on a glass plate covered by a 200 Å thick layer of gold constituting the cathode of the amplifying gap (Fig. 1b). Such a thin layer of gold is transparent for the major fraction of the electrons emitted by tritium and yet mechanically strong enough to withstand occasional sparking.

For most uses it is, however, more convenient to have the sample separated from the gas volume of the detector; we tested a version with aluminized Mylar of about 6 μm thickness serving as the

^{*)} 100 μm of doped polystyrene, made by M. Bourdinaud, DPhPE, CEN-Saclay, France.

window and the cathode of the chamber (Fig. 1c). Such a configuration can be used only for isotopes emitting β -particles of higher energy such as ^{14}C ($E_{\text{max}} = 156 \text{ keV}$), ^{35}S ($E_{\text{max}} = 168 \text{ keV}$), and ^{32}P ($E_{\text{max}} = 1700 \text{ keV}$). Optimal position resolution implies a minimal distance between the source and the amplifying gap.

The use of multistep structures (Fig. 1d) permits the gating of the image intensifier and the triggering of the data-acquisition system by the information from the first preamplification stage, since it is detected before the preamplified charge reaches the main amplification gap. The delay of a few hundred nanoseconds due to the drift time in the transfer gap is sufficient for this purpose.

The optical information is detected by the image intensifier coupled to a CCD camera through the Aclar^{*)} 22A window, 50 μm thick, which is transparent in the TEA emission region.

Two gas mixtures were used: argon and xenon, with a few per cent TEA admixture.

A UV-sensitive photomultiplier was installed close to the camera; light pulses in coincidence with charge signals from the chamber were used for triggering the image digitization and storing system.

4. THE OPTICAL READOUT SYSTEM

A CCD camera equipped with an image intensification system read out the information carried by the photons emitted by the charge avalanches in the chamber.

The CCD (Thomson 7852 CDA-80) camera is a matrix of 144×208 pixels of total area $4.32 \times 5.82 \text{ mm}^2$, which allows a high granularity of the detector surface readout. The associated electronics produces the chain of electric pulses corresponding to the contents of the pixels for each image detected. This signal is fed to the digitizer card (Data Translation DT 2851) which interfaces with the IBM-PC/AT computer. The digitizer has a precision of 8 bits, which determine the dynamic range of the light intensity measurement in every pixel.

The impact point of the β -particle is reconstructed by calculating the centre of gravity of the detected luminous spot for ^3H , where the range of the β is small, and by calculating the centroid of the avalanche within a determined range around the maximum of intensity for ^{32}P and ^{35}S .

The amount of light emitted by the avalanches is not sufficient to be directly detected by the CCD. Therefore the use of an image intensifier coupled to it is indispensable.

Two different chains of image intensification were used:

- i) Camera A: a proximity-focused image intensifier of 25 mm diameter, with a bialkali photocathode deposited on the fused silica window and an X3 phosphor screen (100 ns decay time) on the fibre optics plate, served mainly as a wavelength converter because of its small photonic gain. The second-stage multichannel-plate image intensifier, of the same diameter, was optically coupled to the exit window of the first one. The image size was then reduced from 25 mm to 7 mm (CCD diagonal length) by the fibre-optics taper.
- ii) Camera B: a multichannel-plate gateable image intensifier, of 18 mm diameter, with an S20 photocathode deposited on the sapphire window and on the P36 screen as a first stage (RTC XX 1410/SP 41721.160). The DEP electrostatic magnifying tube reducing the image size from 18 mm to 7 mm was optically coupled to the exit window of the first stage and followed by the CCD.

Both modules were operated at the total gain of about 3000 W/W for the peak wavelength of the incident photons: 280 nm.

With our CCD and the associated electronics, a maximum of 50 images per second could be transferred to the digitizing card in the PC. The rate of data taking in our initial instrument was limited, by the speed of the digitizer and of the analysing and storing system, to about 3 images per second. It has now increased to 20 images per second.

*) Polychlorotrifluoroethylene (PCTFE).

An efficient light collection is one of the most important parameters of the optical readout system. We used a reflective-type UV objective 90 mm f/1.1 (Lyman-Alpha II of NYE).

5. IMAGING OF ^3H -LABELLED COMPOUNDS

In order to test the accuracy of the method we imaged a structure (produced by Amersham) commonly used in biology laboratories as a calibration standard to analyse autoradiographs quantitatively.

It is made of thin plastic strips divided in rectangular spots labelled with ^3H , with variable intensities. We placed the sample behind the cathode grid (Fig. 1a). The position accuracy of our method can be demonstrated in a convenient way by the modulation in intensity introduced by the grid of wires (of 50 μm diameter, spaced every 500 μm) that serves as a collimator for the low-energy electrons. The width of the amplifying gap was 4 mm.

With the Ar + TEA (2%) filling gas we tested the performance of our detector using Camera A for light detection. Figure 2 shows the intensity distributions of the pixels of maximum light intensity in the detected spots and the integrated intensity of these spots. Both distributions have a peaked shape, making it easy to set the criteria for the spot recognition and the analysis by software. The physical size of the single luminous spot was about 1.5 mm total width, and the pixel of maximum intensity contained on average about 10% of the integrated intensity of a spot. A high granularity of the CCD readout results in a good precision of the impact point.

The image of the Amersham calibration standard is displayed in Fig. 3. The two parallel arrays of the variable-intensity radioactive bars are visible. The ratio of intensity of the strongest bar (top left) to the weakest one (bottom right) is about 1000. The structure appearing between the two arrays comes from contamination by residual radioactivity of the substrate, caused by temporary contact with the sample. Unfortunately the dynamic range of our display was smaller than that of the local source rate; this results in the apparent saturation of the most intense part of the image. This region, magnified by a factor of two, is shown in Fig. 3b. One can observe the structure of the crossed-wire grid. The accuracy is of the order of 100 μm (FWHM). Figure 4 shows the distributions of intensity in the most intense array of rectangular spots. The separation is excellent, and the relative intensity of the spots coincides with the one given by the manufacturer.

We also tested slices of biological specimens from a kidney and the brain of a rat, using the chamber of the configuration shown in Fig. 1b filled with a gas mixture of Xe (96.5%) + TEA (3.5%). The image of the kidney (Fig. 5a) clearly displays the renal ducts, which have a physical width of 30 to 50 μm , as exhibited by the autoradiography, obtained in a 3-month exposure (Fig. 5b), of the adjacent slice. The presented image was obtained in 40 h, with only 40% efficiency of the data-acquisition system, caused by the computer dead-time that we have now reduced by a factor of 10. It can nevertheless be analysed for quantitative information. Figure 5a also shows the intensity distribution along the line crossing the image displayed on the screen of an Apollo DN580, which has a resolution of 1280×1024 pixels. The images are displayed as matrices of 256×256 pixels, each pixel being represented by 8 bits. Images for which the dynamic range of the data exceeds 8 bits can be displayed with different positions of the 8-bit window, i.e. the 8 bits of the display screen can be mapped onto any 8 bits of the input image. Multiple 8-bit images are therefore used to display the full dynamic range of the data. The single pixel of the CCD corresponded to $180 \times 180 \mu\text{m}^2$ area in the detector plane. Such an image can thus be obtained in about 20 h, and this has to be compared with the three months of exposure time for the autoradiography. A gas filling containing mainly xenon is most favourable, because of the small range of the low-energy β -particles emitted by tritium: this is close to 100 μm for the average electrons of 6 keV. Among the other factors determining the position resolution is the statistics of light emitted by a single avalanche and the spread of the avalanche in the image plane, allowing dense sampling of the light spot by the CCD. From this point

of view, the situation is slightly better with argon mixed with TEA, for which higher charge gain is achievable with a lateral charge diffusion larger than with Xe + TEA. All these parameters have to be optimized by choosing the adequate detector geometry, pressure of the gas, and optical readout granularity. With argon at atmospheric pressure, the accuracy is slightly worse than with xenon, because of the larger range of the β -particles.

6. IMAGING OF ^{32}P - AND ^{35}S -LABELLED COMPOUND

The imaging of ^{32}P and ^{35}S tracers gives more freedom in the detector construction, because these offer the possibility of using thin windows between the samples and the detector. On the other hand, the position resolution may be degraded because of the long range of the β -rays. The measurements with these sources were performed in different versions of the chamber filled with an Ar + TEA (2%) gas mixture and by means of the Camera B system.

We have tested a chromatography gel with compounds labelled by ^{32}P placed against the grid (see Fig. 1a). The effect of the parallel gap multiplying preferentially the ionization electrons liberated in the gas close to the entrance point is visible on the resulting image (Fig. 6); this shows an accuracy of about 0.5 mm whereas the gap thickness was 6 mm and the range of the β -particles emitted by this isotope is much larger. The algorithm used for the reconstruction of the entrance points, from the light spots measurement, was the same as in the ^3H experiment, with a small modification, since the structure of the events is very different, the spots being elongated because of the long path of the electrons in the amplifying gap. This illustrates the potential of the parallel-plate detector, with such an imaging device, for the determination of the entrance point of the β -particles. We limited the calculation of the centroid to 9×9 pixels, centred around the pixel of maximum intensity. In principle, a measurement of this pixel alone should allow the optimal precision, but since it is subject to various sources of fluctuations, we found that the accuracy was best when we took the centroid of the pixels around it.

The compound labelled with emitters such as ^{32}P and ^{35}S can be separated from the chamber working volume by a thin plastic foil, transparent to these energetic β -rays. This avoids damaging the sample by the organic compounds in the gas mixture or by accidental sparks; it avoids the chamber pollution by products evaporating from the gel and facilitates the change of the gel under test. The separating foil should serve as the entrance window as well as the cathode plane, in order to exploit the parallel-plate amplification advantage of favouring the detection of the ionization electrons close to the cathode. We have tested this type of chamber configuration (Fig. 1c), using aluminized Mylar of 6 μm thickness as the cathode and a ^{35}S -doped bidimensional gel placed in contact with the foil. The gap thickness was 4.5 mm in this measurement. The resulting image of the pattern is shown in Fig. 7. There was no significant degradation of the detection efficiency from the β -rays absorption in the window. The detector worked well for a few days of data taking, under conditions of a high charge gain and high light output, without damage from accidental sparks. Permanent sparking points must be avoided, for example at the edge of the electrode, because they may ultimately lead to chamber damage, through aluminium evaporation from the Mylar window. We have measured the position accuracy of ^{35}S emitters by imaging linear sources of 150 μm width, separated by a distance of 2 mm. We obtained images with a width of 500 μm [FWHM] (Fig. 8).

The use of a multistep chamber for radiography offers several advantages. The signal from the first stage may be used for the image intensifier gating and for a short-time increase in the light gain of the second stage by pulsing. In this case, a larger total charge gain and larger intensity of photons can be obtained. However, we expect a small degradation of the position resolution, caused by diffusion of the electron cloud in the transfer gap and its distortion during its passage through the successive meshes separating the regions of strong and weak electric field. We have tested this using

the chamber shown schematically in Fig. 1d and using the same ^{35}S -labelled pattern as in the measurement described above. Figures 9a and b show the vertical slices of the central, intense spot from Fig. 7, recorded in the single and multistep detector, respectively. The readout granularity from these measurements was $340\text{ }\mu\text{m}$ per pixel and $317\text{ }\mu\text{m}$ per pixel, respectively. The measured width of the spot is 1.71 mm (FWHM) for the single-step detector and 1.99 mm (FWHM) in the multistep case. Although the conditions of operation and the geometry of the multistep detector were not optimized, all significant spots are clearly distinguishable on the detected image.

7. DISCUSSION: THE LIMITS OF ACCURACY

The description of the results obtained with some radioactive samples routinely used in biological applications shows that the accuracy reached at present should lead to useful practical applications. The gain in time for the imaging of radioactive samples is two orders of magnitude, compared to photographic film. The resolution is still not as good, but it is sufficient for many applications, and the results are obtained directly in a digitized form, with a much better linearity.

The width of the avalanches is, physically, of the order of 0.5 mm (FWHM) in the case of a ^3H source. The accuracy in the position of the centroid is improved if the image is distributed over many CCD pixels. It is possible to widen the avalanches either by using a single gap with larger electrode spacing, or by using a multistep chamber. In this case one could also obtain a much larger intensity of photons. In order to achieve the best resolution, the charge gain of the first stage should be maximal.

The accuracy is limited by the physical extension of the β -ray track. This is the reason why, with ^3H , the accuracy is better with Xe than with Ar as the main gas component in the chambers. An increase in pressure could lead to improvements. We intend to investigate this further, since any significant progress in the accuracy we have currently reached may lead to new applications.

In the measurements with ^{32}P and ^{35}S we used an Ar + TEA (2%) gas mixture. In argon, the mean distance between ionizing collisions of a minimum ionizing particle is $300\text{ }\mu\text{m}$, thus limiting the position resolution. It is not easy to find more favourable gas mixtures: they have not only to give a larger primary ionization but also to be compatible with the emission of light. Choosing a reconstruction algorithm better than the centroid of charges around the maximum may lead to some improvement in accuracy, but the one that we obtain already with ^{35}S sources, $200\text{ }\mu\text{m}$ (r.m.s.), is close to the optimum and is convenient for most applications.

One attractive feature of the method is that it allows the user to follow in a continuous way the build-up of the image and thus to take, if necessary, action before any preset time limit. This should also lead to the use of less intense sources for the routine work in biology laboratories.

The linear response and large dynamic range of the detector should permit a better quantitative evaluation in the interpretation of complex images.

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Figure captions

- Fig. 1 Charge-amplifying structures and geometrical coupling of radioactive sources. a) Parallel-grids amplifying gap with the radioactive sample placed in contact with a cathode mesh. b) The ^3H -labelled surface is covered by about 200 Å of gold and is part of the cathode. c) For emitters of more penetrating β -particles, such as ^{32}P or ^{35}S , the radioactive samples are separated from the gap by a thin conductive cathode. d) Multistep parallel-plate structure. The avalanches produced against the anode mesh emit light, which is imaged through Aclar windows that are transparent to UV photons. The light is transmitted through lenses to an image intensifier and a CCD camera, read out by an IBM PC/AT digitizer.
- Fig. 2 Pulse-height distributions of the pixels at maximum light intensity in the detected spots (a) and the integrated intensity of spots (b). Tritium-labelled sample detected by parallel-plate chamber filled with Ar + 2% TEA.
- Fig. 3 a) Image of ^3H -labelled Amersham calibration standard. b) Enlarged view of the four most intense bars of the pattern; the bars are 1.4 mm wide and 1 mm apart. Single parallel-plate amplifying gap; in Ar + 2% TEA.
- Fig. 4 Intensity distribution along the array of most intense bars of ^3H -labelled Amersham calibration standard; same conditions as in Fig. 3.
- Fig. 5 a) Images of the tritiated rat-kidney slice obtained with a gaseous detector: 256 grey level window centred at three different levels, in order to display details of very different intensity. Details of the renal ducts of about 50 μm are visible. Gas filling Xe + 2.5% TEA. Top right is the intensity distribution along the oblique line in the top-left picture, with a screen pixel width of 40 μm in the plane of the sample. b) Autoradiography. Image of the rat kidney slice, adjacent to the preceding one, to which a photographic emulsion was apposed during 3 months.
- Fig. 6 Image of a portion of a chromatography gel, with compounds labelled with ^{32}P , with the intensity distribution along the line. Gas filling Ar + 2.5% TEA. Screen pixels 40 μm . Detector structure of Fig. 1a.
- Fig. 7 Image of an electrophoresis gel with compounds labelled with ^{35}S . Portion of a two-dimensional gel, with the intensity distribution along the oblique line. Gas filling Ar + 2.5% TEA. Detector structure of Fig. 1c.
- Fig. 8 Localization accuracy with ^{35}S . Image of 5 slits, 150 nm, 2 mm apart, placed on a non-uniform source. Resolution 500 μm (FWHM). Gas filling: Ar + 2.5% TEA.
- Fig. 9 Position distribution in the vertical cross-section of the central intense structure seen in Fig. 7, measured with two versions of the detector: a) Single parallel-plate detector of the structure shown in Fig. 1c; and b) Multistep parallel-plate chamber (Fig. 1d). Gas filling Ar + 2.5% TEA.

Imaging
camera

Fig. 1

Fig. 2 a)

Fig. 2 b)

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9 a)

Fig. 9 b)