

## Image Analysis in Whole-body Autoradiography (WBA)

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### INTRODUCTION

Image processing by computers has become increasingly important for a wide variety of fields, ranging from aerial and space photography to medical diagnosis – e.g. images assembled from X-ray data or from positron emission tomography (PET).

During the last five years, computer-assisted image analysis has also been used for different kinds of autoradiographic applications. Most of these studies, however, deal with various aspects of the brain. Thus, quantitative receptor autoradiography has been used in *in vitro* studies on the distribution of transmitter receptors in the central nervous system (5-7, 12, 13). Also, quantitative autoradiographic *in vivo* techniques, based on studies of brain functions and using computerized densitometry, have been described (3, 4, 11).

WBA (9, 10) is a well-established radiotracer technique for studying the distribution of labelled compounds in the entire animal. Practically all tissues, even very small ones, can be studied. In pregnant animals, the concentration of radioactivity in the fetal tissues may be compared with that of the mother. Information about metabolism and tissue binding may be obtained by different applications of WBA (for review, see ref. 1).

The extraction of all information from such studies is very tedious and time-consuming. Earlier, densitometric data were obtained by a manual densitometer and a semiquantitative staircase of radioactive standards, or by direct impulse counting of punched tissue pieces (2). Recent development of computer-assisted image analysis has now greatly improved the possibilities to measure and describe visual information obtained from the various WBA techniques.

In the following, some essential steps involving computer-assisted image analysis will be briefly discussed. First, a short description will be given of the entire image analysis system<sup>i</sup>, and the image pick-up procedures<sup>ii</sup>, and briefly some main processing commands<sup>iii</sup> used in analyzing whole-body autoradiograms. Finally, some of these procedures will be exemplified.

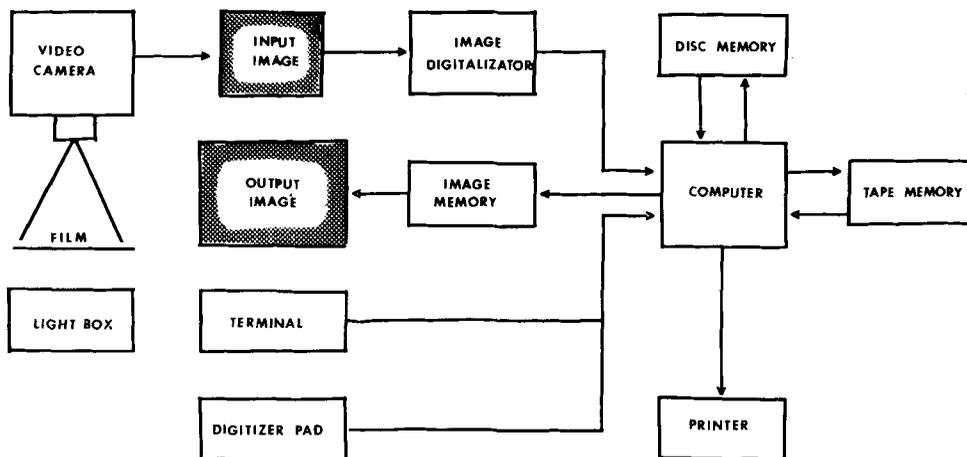


Fig. 1. Block diagram of the image analysis system.

### IMAGE ANALYSIS SYSTEM

The structure of the image analysis system is shown in Figure 1. The autoradiographic film is transilluminated by a reostat-controlled light-box. By proper choice of the camera lens and its distance to the video camera, almost any size of autoradiogram can be analysed. A microcomputer with a disc memory for the storage of pictures (up to 120 pictures can be stored in the memory) receives input data from the video camera via an image digitizer. The output data in the form of processed images are stored in the image memory, where up to four processed pictures can be alternatively displayed on the screen (output image).

To the computer, a digitizer pad allows the operator to indicate objects or regions of interest in the image, and the position, the mean optical density, the standard deviation, and the area size are displayed on the terminal, or could be printed on paper. In addition to the disc memory, the system is also complemented with a tape memory for storage and back-up of the programs.

### IMAGE PICK-UP SYSTEM

We have chosen a video camera for the image pick-up system, because it has the advantage of being fast, sensitive, and rather cheap. The image in the camera tube is divided by the video digitizer into an array of 384 horizontal columns and 241 vertical rows, thus producing 92 544 equal and discrete squares (pixels). The pixel is the basic element of the picture and its size also defines the optical resolution of the system. Each pixel is then assigned a value based on the gray level on that minute portion of the picture which it encompasses. The digitizer provides 256 different gray levels.

A series of gray filters from known optical densities calibrate the gray levels to optical density (OD), and then a second calibration may be performed, using standards with known amounts of radioactivity, thus converting the optical densities to real concentration values.

## IMAGE PROCESSING PROCEDURES IN WBA

In order to extract as much information as possible from different WBA techniques, a number of image-enhancement functions can be generated. These have been especially adapted for the analysis of whole-body autoradiograms. A detailed description of basic image-enhancement procedures (8) and pseudo-colour coding (3) has been reported earlier.

An autoradiographic investigation includes various levels of the body, and thus most tissues and fluids can be scanned. It is possible to observe whether the radioactive level is present in small structures as the muscle layer of the intestinal tract, or in the mucosa. In the eye, the radioactivity can be traced to e.g. the cornea, retina, pigment layers, or aqueous humour. Small endocrine organs will show up, such as the pituitary, thyroid and different layers of the adrenal cortex. When investigating pregnant animals, the activity can be compared in both the maternal and the fetal tissues. Up to now, the quantitation of such small structures has been very hard and time-consuming. However, by suitable image processing procedures – as magnification, histogram generation, and object classification – even the quantitation of such small structures can be performed very rapidly and with high accuracy.

Magnified picture. There are two methods of performing magnification with this system. A zoom lens on the video camera can be used to magnify optically the image (Fig. 2 b,c,d), or the image can be magnified in the computer (Fig. 2 h) by software operation. When double magnification is performed, each pixel of a certain gray level is replaced by 2 x 2 pixels of equal value.

Gray-level histogram plot. The histogram is a graphical plot of the gray-scale distribution in the picture. The horizontal axis represents the gray-scale, starting with 0 (white) and ranging through 255 (black), and the vertical axis represents the number of pixels (Fig. 2 e,f,g).

Gray-level window measurement. The histogram produced as above can be analyzed by specifying, with the digitizer pad, any two gray levels, which represent a window (region) of interest. This program will comprise the number of pixels (the selected area within the picture) falling within this gray-level window, and it will point out the mean density, standard deviation, the selected gray-levels in the window, and the total number of pixels (the area) within the window. The selected pixels will then immediately show up on the image display (Fig. 2 b,c,d). This procedure can be used interactively by the operator.

Object classification by gray-level windows. By inspection of the histogram of a picture, one may guess where the thresholds should be located for a classification of the image into its different parts. To do this, one assumes that each peak of the histogram corresponds to a certain anatomical structure of the autoradiogram. Classification of an image on the basis of its gray-level histogram has been shown to be especially useful

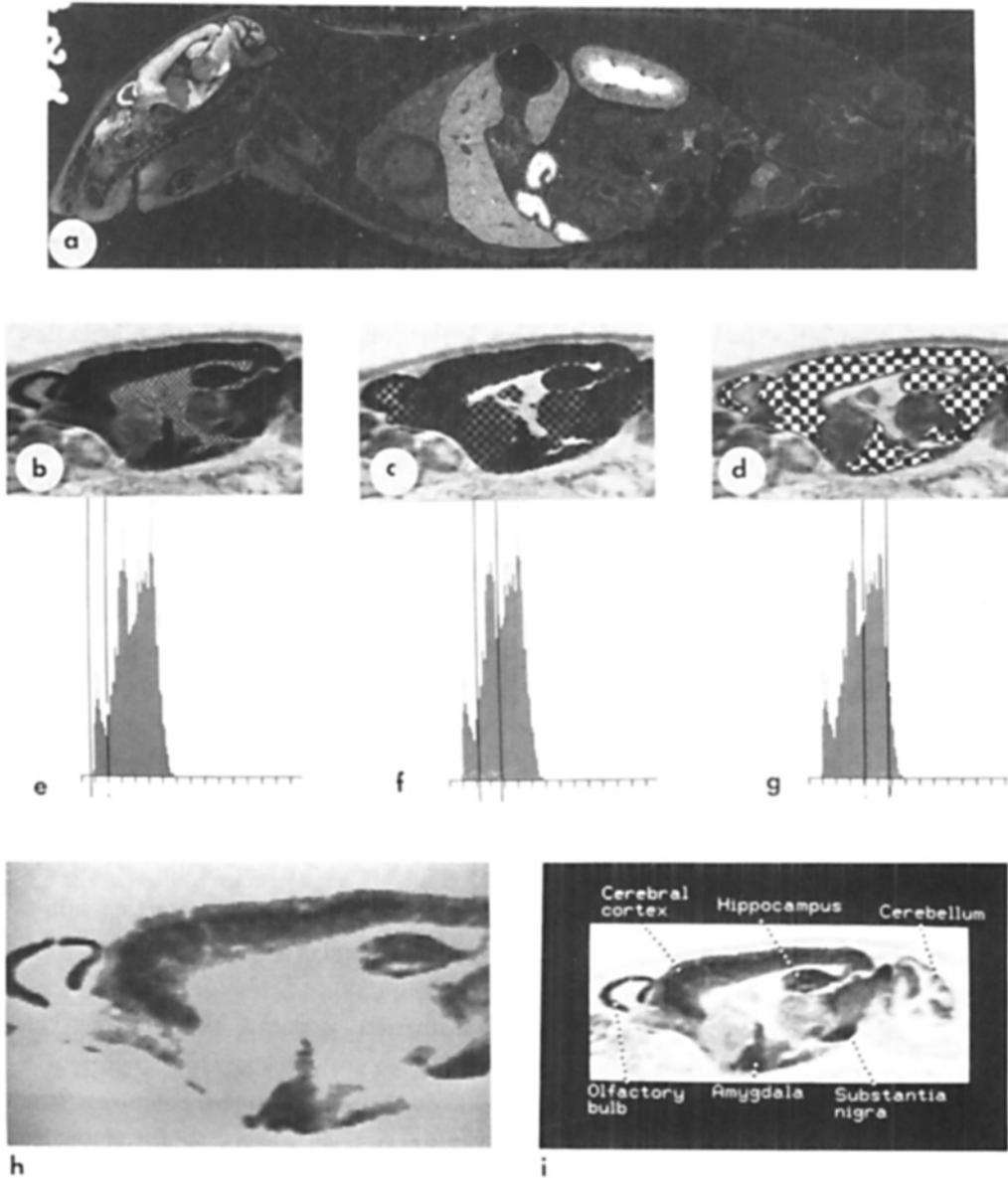


Fig. 2 a-i.

A sequence of pictures from the image display, illustrating some main image processing procedures in WBA. – In (a), the distribution of a benzodiazepin antagonist (RO 15-1788) is shown in the original autoradiogram. Note the high activity in the brain (white area). In (b-d), optically magnified and digitized pictures of the brain, selected from the original, and their corresponding gray-level histograms, are shown just below (e), (f), and (g). By selection of different gray-level windows in (e), (f), and (g) – drops in the histogram –, a classification of various brain areas is displayed as a chess pattern in (b), (c), and (d). Fig. 2 h illustrates a computer magnification routine, and exclusively the selected gray levels obtained from the brain classification in (d). Fig. 2 i illustrates some computer routines, such as "retouch", "text-writing", plus "arrows" to mark tissues.

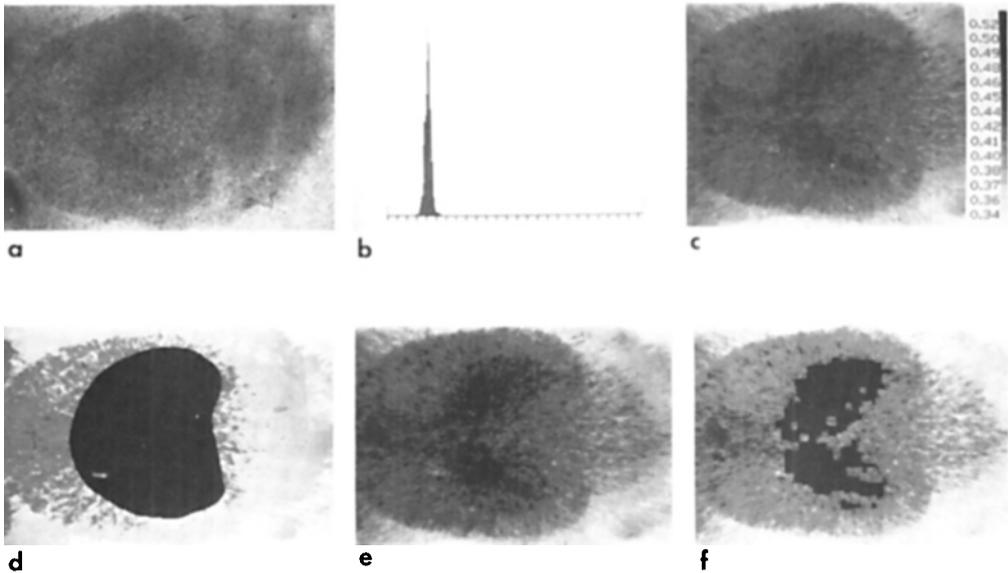


Fig. 3 a-f.

Sequence of pictures illustrating ordinary contrast enhancement (a, b, c), and another computer routine (d, e, f) that brings out details and contours, which appear diffuse in the original picture. The sequence illustrates the distribution of  $^{11}\text{C}$ -labelled deprinyl in a rat brain. Fig. 3 c shows the result of contrast enhancement, when the gray levels obtained from the narrow histogram (b) of the original autoradiogram (a) are spread over a wider range.

In (f), the contours of the nucleus caudatus / putamen are made clear by the use of the highest gray-level pixels (cf. b). These are marked in black (e) and selected from the region of interest (d).

in measuring complicated structures as, e.g., the intestinal mucosa, the various portions of the brain (Fig. 2 h), and different parts of the spleen (red and white pulp).

This procedure might also be used to exclude activity in blood vessels or bile ducts, thus measuring the activity of the liver parenchyme exclusively. Each class of gray levels (a certain anatomical structure) can then also be given a certain colour.

## CONCLUSIONS

A single low-cost image analysis system has been described. The image processing system explained in this report has been especially adapted to WBA. It has largely increased the possibilities to measure and describe very small and heterogenous structures. Contrast enhancement routines have also been illustrated, thus giving the observer further information.

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