

water/sodium chloride can be used as the coolant in the tandem water traps. (Dry ice/acetone is a slightly more efficient trap than water/sodium chloride, but a great deal more troublesome because of the problem of condensation and freezing of atmospheric moisture on the outside of the traps when they are removed from the freezing mixture to be weighed.) At the beginning of the procedure, valve 1 is set so as to connect the reservoir with the homogenizer (that is, as it appears in the figure). Valve 2 is clamped closed. The styrofoam-supported part of the apparatus is then removed from the baths, disconnected from the pump, dried off and weighed (accurate to 0.01 g) on a top-loading balance. The water traps are allowed to hang down on one side of the weighing pan, the homogenizer and reservoir hang down on the other side. The apparatus is then returned to its support (a ring stand is convenient) and the tubes replaced in the hot water bath and the contents of the Dewar flask respectively. The vacuum pump is reconnected (a water aspirator is sufficient) and turned on just before the rat is to be killed.

The rat is decapitated, and with a second stroke of the guillotine, the skull is split lengthwise and the brain or brain parts removed by aspiration with the brain extrac-

tor. Valve 2 is quickly turned so that the hot buffer in the reservoir is open to atmospheric pressure. The brain extractor is then inserted into the tubing above valve 2, thus sucking the buffer from the reservoir through the brain extractor tubing and washing the brain into the homogenizer. The aperture of the brain extractor can be chosen to give the proper combination of accuracy and speed. (The inverted stopper shown inside the homogenizer does not touch the inside walls of the tube; its purpose is to minimize upward splattering of the buffer. Its optimal position in the homogenizer must be determined by trial and error.)

With the brain suspended in the hot buffer, the apparatus is weighed again, the weight of the extracted brain being equal to the difference between the first and second weighings. The brain suspension is allowed to sit in the boiling water bath for a total of 5 min to insure complete enzyme denaturation. Either before, after, or during those 5 min, the homogenizer can be opened and its contents further homogenized with a teflon plunger. We have found wet weights obtained by this method are accurate to  $\pm 2\%$ . Adenyl cyclase (and presumably other enzymes as well) was over 90% denatured within 30 sec after decapitation.

## PRO EXPERIMENTIS

### Continuous registration of X, Y-coordinates and angular position in behavioural experiments

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**Summary.** An electronic camera and 2 simple additional circuits for the registration of the X,Y-coordinates or the angular position of animals in behavioural experiments are described.

In behavioural experiments, the problem of recording continuously the X,Y-coordinates or the angular position of an animal frequently occurs. For this purpose, electronic cameras are particularly suitable. To demonstrate their manifold possible applications, 2 simple circuits will be discussed which allow the registrations of the X,Y-coordinates of a running beetle and the rotation of a crab's eyestalks.

**The output signal of the electronic camera.** The camera objective Ob projects a white stripe S on a black background (figure 1) on to the semiconductive layer Sl of the image converter tube. An electron beam scanning the image on the layer Sl converts the light distribution line by line into a positive, analogue voltage called video signal. The temporal course of the video signal for 1 line corresponds to the distribution of the light intensity of the image on this line. In order to synthesize a TV-image by means of a monitor, the information about the beginning of the image and the lines has to be added to the video signal. The vertical synchronization pulse (negative, duration 110  $\mu$ sec) signals the beginning of the image and causes the flyback of the vertical deflection. When the first horizontal synchronization pulse (negative, duration 4.5  $\mu$ sec) reaches the monitor, the horizontal deflection starts and the light intensity distribution of the first line, corresponding to the video signal voltage, is displayed. The following horizontal synchronization pulses trigger the display of the subsequent lines. The video signal is blanked from about 3  $\mu$ sec before to 3  $\mu$ sec after the horizontal synchronization pulse, and for 1 msec from the beginning of the vertical synchronization pulse in order to make the flyback of the electron beam invisible. The

signal received by the monitor therefore contains 3 components: Video-Blanking-Synchronization-signal (VBS-signal).

According to the CCIR-norm, 1 line is scanned in 64  $\mu$ sec so that the whole-image consisting of 625 lines is presented in 40 msec. The whole-image is divided into 2 successive half-images, each consisting of 312.5 lines and being displayed in 20 msec. The consecutive parts of the whole-image are interlaced, i.e. the lines of the first half-image are positioned exactly between the lines of the second half-image. At the border of the image, a few lines are suppressed by blanking and synchronisation. Therefore, only 296 lines per half-image are available for further analysis.

**Registration of the X,Y-coordinates.** In this section, a description of a basic circuit is given which allows the X,Y-coordinates of the upper corner of the white stripe S to be recorded (figure 1). The distance of this corner from the upper border of the image (Y-coordinate) is determined by line k. In this line, and for the first time in this half-image, the scanning electron beam reaching the corner of the white stripe causes the video signal voltage to rise. The X-coordinate is measured by counting the pulses of a fast clock from the beginning of the line (i.e. from the leading edge of the horizontal synchronization pulse) until the video signal reaches a given threshold. For electronic realization of the registration method (figure 2, a) discriminator D2 (passive filter + transistor) transforms the vertical synchronization signal to a short pulse resetting counter ZY. The latter counts the reshaped horizontal synchronization pulses provided by discriminator D3. Additionally, each output pulse D3 resets

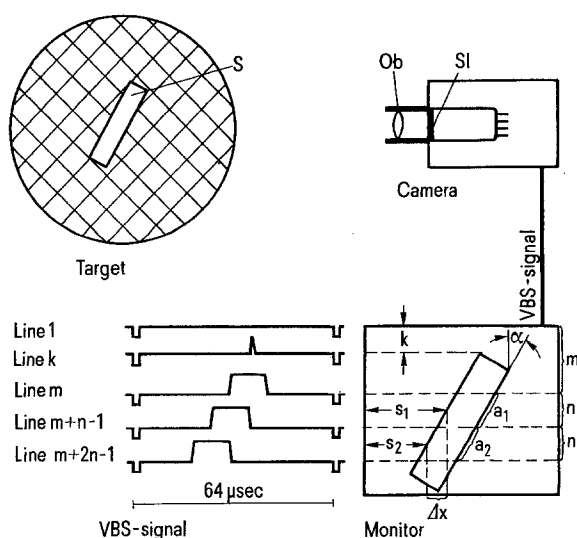


Fig. 1. A white stripe *S* on a black background is projected by a camera objective on to the semiconductive layer *SI* of the image converter tube. This image is scanned by an electron beam and converted to VBS-signal which is fed into the monitor. Left to the monitor, a few lines of the VBS-signal are drawn for illustration. For further details, see text.

counter *ZX* which continuously receives pulses from a 10-MHz-clock. Whenever the video signal of line *k* becomes greater than a given threshold, a 50-nsec-pulse occurs at the output of discriminator *D1* once in the present half-image. This pulse causes the counts in *ZX* and *ZY* to be stored in the digital memories *SX* and *SY*. The contents of *SX* and *SY* are converted to analogue voltages by means of digital-to-analogue-converters (DAC). These voltages are proportional to the *X*, *Y*-coordinates of the upper left corner of the white stripe (figure 1).

A further problem remains to be solved. The lines of the 2 half-images are interlaced. Alternately the first line of the first half-image starts 32  $\mu$ sec after the end of a vertical synchronization signal, and the first line of the second half-image starts 64  $\mu$ sec after the end of the following vertical synchronization signal. Therefore, discriminator *D1* signals the corner of the white stripe either in line *k* + 1 during the first half-image or in line *k* during the second half-image. Thus, the output may oscillate with 25 cps and an amplitude corresponding to 1 line distance. This oscillation is suppressed by AND-gate *G* and flip-flop *F* acting as frequency divider. By this method, only every second half-image is analyzed. The sample rate is restricted to 25 per sec and the resolution of the *Y*-direction is only 296 lines. Nevertheless, this is sufficient for many applications, e.g. for continuous registra-

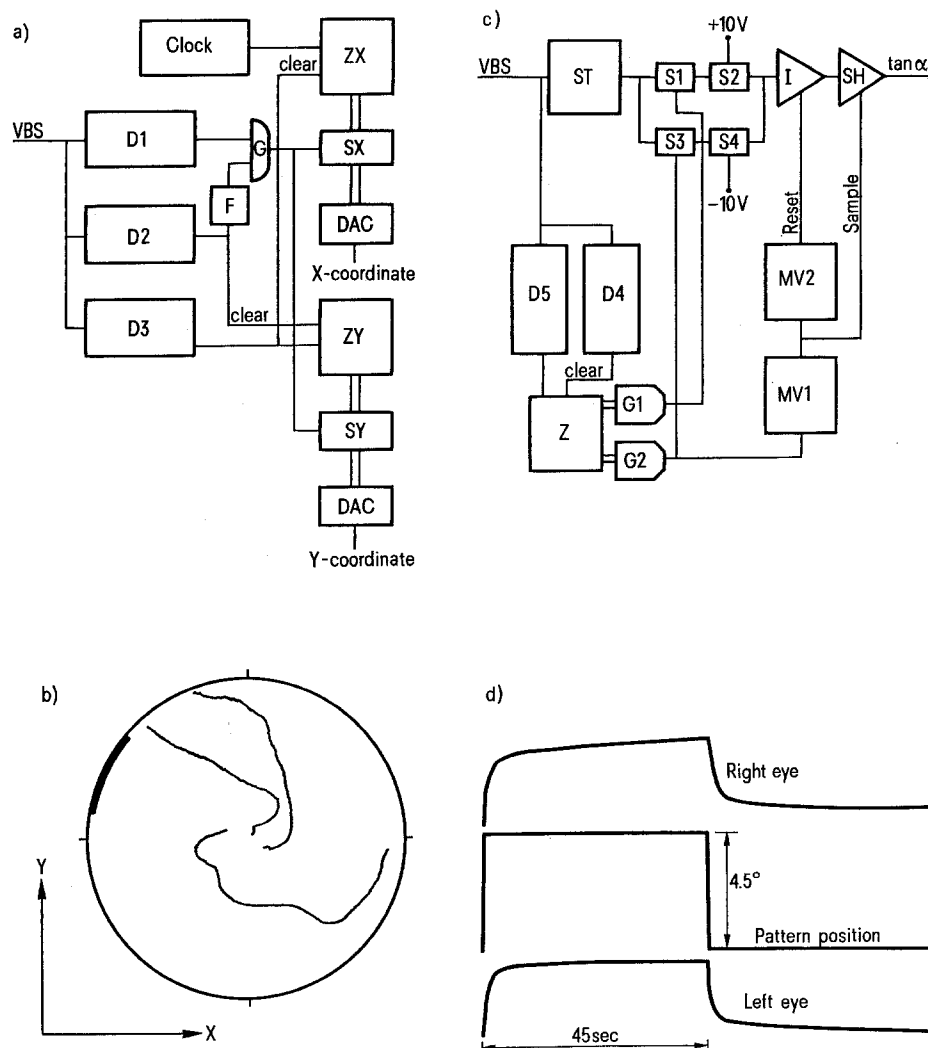


Fig. 2. Basic electronic circuits for analyzing the VBS-signal and examples of registration. *a* Electronic circuit for the registration of the *X*, *Y*-coordinates. *b* The registration example shows 3 traces of a running beetle (*Tenebrio molitor*) in a circular arena, carrying a black stripe, 30° wide in azimuth, on its inner wall (heavy black line). The diameter of the arena is 1 m. The traces start at the centre and end at the border of the arena. Applying the method discussed in the text, the resolution in *Y*-direction is 4 mm and in *X*-direction 2 mm. The measured values of the coordinates were stored by computer and the traces plotted OFF-line.

*c* Electronic circuit for the registration of the angular position. *d* The registration example shows the turning reaction of both eyestalks of a crab (*Carcinus maenas*), fixed in the centre of a striped cylinder and stimulated by a step-like rotation. For registration of the eye movement, very small stripes of white paper were glued to the eyestalks. The pattern consisted of 12 equidistantly spaced vertical black stripes upon a white background, 15° wide in azimuth each. The calculation of  $\alpha$  from  $\tan \alpha$  and the plotter graphic was also done by means of a computer.

tion of the X,Y-coordinates of a running beetle (figure 2, b).

**Registration of the angular position.** In order to determine the angular position of the white stripe S, the registration process starts at line  $m$  (figure 1). In the following  $n$  lines, the distance between the left image border and the edge of the stripe is measured and summed up. Thus, except for a factor of proportionality, the distance  $s_1$  between the left image border and the centre of the straight line  $a_1$  is determined. The distance  $s_2$  can be measured in a similar way. With  $f$  as calibration factor, the following equation holds:

$$s_1 - s_2 = \Delta x = f \cdot \tan \alpha. \quad (1)$$

For electronic realization of this method (figure 2, c), the VBS-signal triggers a Schmitt-trigger ST during  $2n$  lines, starting at line  $m$ . At the beginning of the horizontal synchronization pulse, the output of ST jumps to  $+5$  V and drops to 0 V when the positive step of the video signal voltage caused by the edge of the white stripe exceeds a given threshold value.

The vertical synchronization pulse, reshaped by discriminator D4, resets counter Z. This counts the horizontal synchronization pulses which are reshaped by discriminator D5. The output of gate G1, constructed of a few NAND-gates, is only positive for counts between  $m$  and  $m + n - 1$ . Similarly, the output of G2 is only positive for counts between  $m + n$  and  $m + 2n - 1$ . The positive output of G1 closes the switch S1 (transistor). Thus, for  $n$  lines, the output of Schmitt-trigger ST is connected to switch S2 (field-effect-transistor). When the output voltage of ST is positive, then switch S2 is conductive and  $+10$  V are integrated by integrator I from the beginning of line  $m$  until the scanning electron beam reaches the edge of the white stripe. After the  $n$ -th repetition of this process, the output voltage  $U_1$  of integrator I is proportional to  $s_1$ . Subsequent to line  $m + n - 1$ , the positive output voltage of G2 closes switch S3 (transistor), and

during the following  $n$  lines, the output of ST is connected to switch S4 (field-effect-transistor). Integrator I thereafter integrates  $-10$  V while the output voltage of ST is positive. This integration produces a negative voltage  $U_2$  that is proportional to  $s_2$ . Finally, after  $m + 2n - 1$  lines being scanned, the output voltage of the integrator I is proportional to  $\Delta x$ . The equation

$$\Delta x = e(U_1 - U_2) \quad (2)$$

holds, with  $e$  as a factor of proportionality. From equation 1 and 2 follows

$$\tan \alpha = e(U_1 - U_2)/f. \quad (3)$$

In order to clear integrator I for successive half-images, the final output voltage is stored by a sample-and-hold circuit triggered by a pulse of monovibrator MV1, and afterwards integrator I is reset by a pulse of monovibrator MV2.

This method for registration of the angular position allows for a sample rate of 50 per sec. Analyzing  $2n = 128$  lines as in the present case, the noise amplitude of the output signal corresponds to  $\alpha = 0.03^\circ$ . The number of lines limits the measurable range of angle  $\alpha$ , since all  $2n$  lines have to be crossed by the white stripe. In the case discussed here, the maximal measurable angle amounts to  $\pm 70^\circ$ . As an example figure 2, d, shows the registration of the eye movement of a crab stimulated by a step-like rotation of the optic surrounding.

The applied electronic camera (Philips LDH 0051) is provided with a special camera tube, Silicon-Diode Array Vidicon (RCA type 4532 or Valvo type XQ 1400). Using this tube, all registrations can be favourably performed with infrared target illumination about 1000 nm, avoiding interference with optical stimulation of the animals. The digital electronic elements are available as integrated circuits (Texas Instruments: counter SN74193, digital storage circuit SN7475; Hybrid-Systems: digital-to-analogue converter DAC V 10).

## CONGRESSUS

### Italy

#### **EUCHEM Conference on Structure, Synthesis and Biosynthesis of Mono- and Sesquiterpenoids**

*in Varenna (Lake Como), 25-31 August 1977*

About 12 plenary lectures will be given by invited speakers and a limited number of short communications will be accepted from the participants. Further information by: Conference on Mono- and Sesquiterpenoids, Laboratorio di Chimica Organica dell'Università, via C. Saldini 50, I-20133 Milano, Italia.

### France

#### **Protons and ions involved in fast dynamic phenomena**

*30th international meeting of the Société de Chimie physique, Paris, 28 November-2 December 1977*

Contributions and requests for information should be addressed to the general secretary of this 30th meeting: Dr. C. Troyanowsky, 10, rue Vauquelin, F-75231 Paris Cédex 05 (France).

### France

#### **7th international congress of pharmacology**

*in Paris, 16-21 July 1978*

Prof. Paul Lechat, president. Secretarial office: 21, rue de l'Ecole de Médecine, F-75006 Paris (France).

### The Netherlands

#### **International symposium on metathesis**

*Noordwijkerhout, 19-21 September 1977*

The symposium covers all aspects of metathesis, including: homogenous and heterogenous reaction systems, mechanism and kinetics, studies on the nature of active sites, ring opening polymerisation, influence of functional groups, applications.

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