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Application of Computer-Controlled, Real-time TV Single-Object Tracking in Behavioural Biology and Rehabilitation Medicine

Yu. KAMINSKY, I. KREKULE

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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Summary
A PC-based system with TV input for automatic tracking of a single and contrast object in 2D in a homogeneous and stationary environment has been developed and applied to Morris water maze experiments. Further development of the system aimed at broader support of experiments, reduction of requirements on the stationarity and homogeneity of the scene background and on multiple-object tracking is discussed. The computer control of active light markers of the tracked object applicable to multiple-objects tracking in a time-sharing regime is also mentioned in the conclusion. The latter extension of the system can be applied to kinematic studies in biomechanics, sport and rehabilitation medicine.

Key words
TV tracking – PC – TV – Morris water maze – Real-time imaging

Introduction

Automatic, computer-based tracking of animals in 2D is required in numerous behavioural studies under various conditions, e.g. in a maze alone and in combination with exploration of activity of specific tissues, e.g. neurones in the hippocampus (Muller *et al.* 1987). Though a number of suitable systems are commercially available (e.g. ETHOVISION 1994; SMART 1992; VIDEOMEX-V 1988, VIDEOMEX-X 1992; VIDEO TRACKER 1989), the development and design of such a system can still be accomplished in a biomedical engineering laboratory, especially if the requirements on the system are limited to single object tracking on an optically homogeneous and stable background. The wide variety of "add-on" cards available for PCs nowadays significantly simplifies the design of such systems.

Automatic, TV-based, computer-controlled tracking of a single object in 2D is simplified even more if the object image has a high contrast with respect to the background. It is simplified even further if the background is optically homogeneous and stationary in time (illumination, movement, etc). In such a case, a current PC enhanced by a frame grabber can be applied to this task in real time if the spatial and time resolutions of the TV camera applied (e.g. 512x512 pixels, 25 frames/s) suit the requirements of the experiment. The system could be simplified even more if the tracked object is recognizable by its videosignal amplitude, since the grabber can then be replaced by a trivial, custom-made PC board, consisting of i) a TV synchronization pulses separator and ii) a timer controlled by the video signal comparator.

Alphabetically arranged list of abbreviations:
B/W = black/white;
HW = hardware;
I/O = Input/Output;
LED = light emitting diode;
MWM = Morris Water Maze;
PC = personal computer;
SW = software;
TV = television;
V-RAM = dual port part of the computer random access memory applied simultaneously to the input and to the output of graphics/image data;
2D = two dimensional;
3D = three dimensional.

Such a system has already been designed and implemented with various PCs, including an IBM PC enhanced by an universal I/O board (Kaminsky and Krekule 1994). The system which was applied to the automation of Morris water maze experiments is discussed in detail in the following paragraph. Further improvements of the system are described in the second part of this contribution.

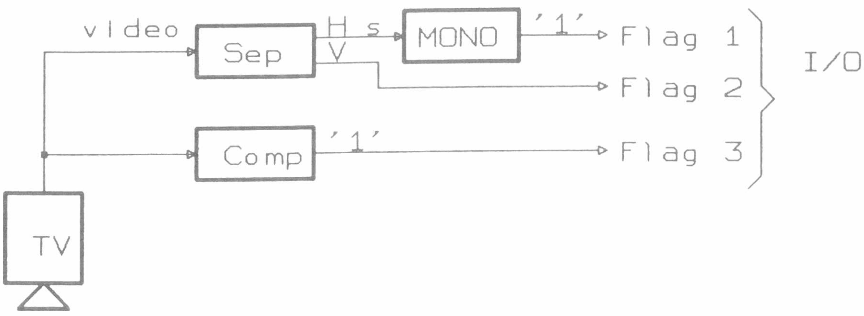

Fig. 1. *Schematics of the minimal HW implementation of the simple, single object detection procedure based on evaluation of the videosignal level. The HW consists of: Comp = comparator which detects the videosignal amplitude value above the threshold level, i.e. background; MONO = monostable flip-flop which generates pulses the duration of which corresponds to a horizontal scanning line (s = set); Sep = separator of TV synchronization pulses: H – horizontal (line) and V – vertical (frame) synchronization. The SW part consists of a clock and two counters (X, Y) the contents of which correspond to the position (coordinates) of the centre of the tracked object: Xc and Yc. The object detection routine functions as follows: the vertical (frame) synchronization impulse (V) which is transformed into the logical signal (Flag 2) is read by the initial part of the frame processing routine of the programme. Whenever Flag 2 is detected, the counter Y and the Flag 2 itself are reset and the frame evaluation loop is entered. Each TV line synchronization pulse (H) is transformed by the monostable circuit into the logical signal, i.e. Flag 1, the duration of which corresponds to the exploited length of the TV line. Whenever the front edge of Flag 1 appears, the contents of the counter X is stored as Xc (i.e. the horizontal coordinate of the centre of the tracked object), the counter X is reset and the contents of the counter Y is incremented by +1. During the time period when the Flag 1 is on, the clock pulses (i.e. programme loop) are counted by the counter X. The output signal of the comparator transformed into Flag 3 activates division of the frequency of the above clock by two. Moreover, the trailing edge of the Flag 3 signal inhibits the clock impulses till the end of the TV line. In this way, the contents of X at the end of empty lines is constant, equalling the length of these lines. Moreover, if an object is detected, i.e. if the "true" ("1", "ON") output of the comparator is generated (Flag 3), the contents of the X counter corresponds to the centre of the TV line intercept with the binary picture of the object. In case the object is detected in a pair of consecutive TV lines, the longest intercept or the median of these intercepts is considered to be the intercept passing through the centre of the object, so that the contents of counter X correspond in the above case to the Xc coordinate.*

Simple algorithm for single object detection, its implementation and application to Morris water maze experiments

The Morris water maze (MWM) (Morris 1984, Brandeis *et al.* 1989), i.e. a water tank with an underwater escape platform, is used in studies of the spatial memory of animals. During these experiments, a computer-controlled TV system automatically tracks, records and evaluates locomotion (swimming) of an animal, e.g. a rat attempting to find and reach an invisible escape platform situated below the water surface. The position of the platform with respect to remote landmarks was learned by the animal beforehand. The image contrast between the tracked object (rat) and the background (water and tank) can be increased by colouring the water, e.g. white colour for black rats, or by using black bottom and walls of the tank when experimenting with albino rats. The homogeneity of the image background is predominantly achieved by uniform illumination of the maze and by elimination of the peripheral areas (e.g. walls of the water tank) from the TV camera field of vision (i.e. TV frame).

a) Simple algorithm for single object TV-tracking in 2D

The discussed simple tracking algorithm is based on evaluation of the amplitude of the TV camera videosignal, since its value above a preset level (i.e. image background) indicates the presence of object (Galik *et al.* 1985, Buresova *et al.* 1988). The position of the object with respect to the TV frame (pixel matrix) is determined according to the time instant of the object detection with respect to the videosignal synchronization pulses (i.e. according to the ordinal number of the relevant lines and columns of the pixel matrix). Whenever the threshold is crossed by a growing videosignal, the detection of the left edge (with respect to the direction of the scanning) of an object image is assumed (see Fig. 1). The length of the intercept of the scanning line with the object image (width) is determined by the time during which the comparator output stays at the high level (i.e. "ON"). Coordinates of the centre of the detected object, (i.e. Xc and Yc) are evaluated as follows. The Yc coordinate is determined by the ordinal number of the scanning line of the TV frame (i.e. the line of the pixel matrix) which hits the centre of the object. The centre is determined as the median of the set of scanning lines consecutively intercepting the object image. The horizontal coordinate of the object centre, i.e. the Xc is evaluated as the mean value of that intercept which hits the centre of the object. In practice, the position of each scanned pixel in the line is determined by the time of its read-out measured from the beginning of the scanning line, i.e. by counting clock pulses starting with the line synchronization pulse. Whenever the object is detected, the clock frequency is halved. The clock is further switched-off by the right edge of the object image. In this way the count of clock pulses at the end of every scanning line corresponds either to the centre of the intercept of the object image or to the length of the scanning line, if the tracked object was not intercepted.

The main disadvantage of the above system is its susceptibility to false detection of objects due to the noise. To achieve some level of noise protection, at least against isolated noise peaks, the horizontal and vertical dimensions of the picture of the detected object should exceed a preset number of consecutive pixels. In practice, this means that the object picture must cover a preset number of lines and/or of consecutive pixels in the intercept passing through the centre of the object image. The noise protection can be further improved by a suitable choice of the scanning rate (i.e. frequency of analyzed TV frames) with respect to the speed of movement of the tracked object. The rate/speed should satisfy the following condition: the maximal distance between object centre positions in consecutively analyzed frames should be less than an *a priori* chosen value, which means that the probability of finding the object in a given neighbourhood of its previous position will be high enough. Therefore, the search for intercepts in the next TV frame can actually be limited to the neighbourhood of an already detected centre (e.g. Spooner *et al.* 1994). This latter rule is important whenever the object is not detected in a frame for whatever reason, so that only the neighbourhood of its last detected place should then be searched during the following frame. The trajectory of the tracked object is then interpolated to keep the track continuous and smooth.

b) Implementation of the simple detection algorithm

The trade-off between the hardware (HW) and software (SW) implementation of the described detection algorithm depends on the power (processing speed) of the computer and the speed of locomotion of the tracked object, i.e. the required frequency of analyzed TV frames. The higher the speed of the object or the lower the power of the computer, the greater part of the detection routine should be implemented by a custom-made HW to achieve on-line performance of the system. Furthermore, the higher the requirements on simultaneous experiment support (e.g. monitoring, trajectory plotting etc), the higher are the demands on the HW.

The minimum HW, implementing the above simple tracking algorithm (under the assumptions of an homogeneous and stationary image background) consists of:

i) a separator of both TV synchronization pulses (i.e. vertical: frame synchronization (V) and horizontal: line synchronization (H));

ii) an amplitude comparator of the videosignal which can be replaced by a fast, i.e. flash A-D converter.

In this minimum HW system (see Fig. 1), an SW implemented clock and counter are used to measure the length (duration) of every scanning line and to check the presence of comparator output-flag. When this flag is "ON", only every second clock pulse is counted and moreover, the counting is terminated when the flag is "OFF" again. Assuming the real-time measurement with all interrupt sources disabled, the SW clock with a pulse period in the range of 1.6 μs can be achieved (e.g. IBM PC, Pentium 100 MHz; 33 MHz busclock). This yields the Xc resolution (minimum intercept) in the range of 3 % of the x-length of the picture matrix (scanning line) if the duration of the standard scanning line is about 50 μs. The counts, i.e. the evaluated lengths of the each scanning line are stored in a buffer and the Yc and Xc coordinates can be evaluated from its contents during the following frame synchronization time interval of about 50 μs.

However, the vertical resolution, determined by the number of scanning lines employed (e.g. 256 per half-frame) is < 0.5 % of the vertical span of the TV frame. To diminish the difference in attainable horizontal and vertical resolutions of the tracking system, the SW clock should be replaced by an HW clock-counter, the stability and frequency of which determine the resolution of the Xc measurement. The horizontal resolution limited by the TV camera pixel matrix, e.g. 1/256, can be achieved easily by using such HW solution (see Fig. 2).

On-line monitoring of the experiment requires at least display of the trajectory of the object simultaneously with its tracking. However, the speed of the display system still represents the Achilles heel of any IBM PC. Therefore, a time interval necessary for the display update and for accomplishing other routines supporting and controlling the experiment (e.g. checking whether the animal has already reached the target, i.e. the escape platform or another zone of interest, on-line evaluation of the instant speed and direction of locomotion, distance to the target etc. (see Fig. 3, e.g. Burešová *et al.* 1985, 1988, Chirardi *et al.* 1992, Fenton *et al.* 1994, Arolfo *et al.* 1994) should be provided. Therefore, the time corresponding to one or n-subsequent TV frames must be fully assigned to these routines, so that only a fraction of all available TV frames is analyzed.

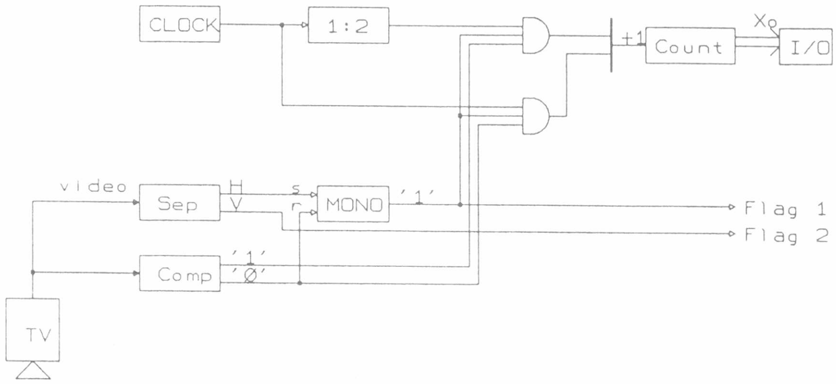

Fig. 2. *Schematics of the full HW implementation of the simple, single object detection procedure based on evaluation of the videosignal amplitude. The additional HW (compared with the schematics in Fig. 1) consists of: clock, divider (1:2), pair of AND gates, one OR gate and the counter X (Count) with parallel I/O. The additional HW functions correspond to that of the SW described in Fig 1.*

Further enhancement of the tracking system

The above simple tracking algorithm is so advantageous that it is used even if all prerequisites for its application are not met. Two problems will be discussed: a) optical inhomogeneity of the background and b) tracking of multiple objects simultaneously.

a*) Object detection on a inhomogeneous picture background*

It may be difficult in practice to achieve an optically homogeneous background (e.g. due to light reflections from the water surface, inhomogeneous illumination) necessary for the application of the discussed simple algorithm for object detection.

In case that the inhomogeneity of the background is constant in time, i.e. stationary, it can be compensated for in two ways, depending on its location and size. An inhomogeneity located somewhere at the periphery of the TV frame, should be masked out, i.e. excluded from videosignal analysis. The simplest solution represents an optical diaphragm which usually masks-out edges of the water tank. The diaphragm should be positioned close to the lenses of the TV camera and its aperture should limit the TV frame to the homogeneous part of the background. Moreover, the illumination of the diaphragm itself should result in a videosignal amplitude similar to that of the background.

Inhomogeneities can also be masked-out by an SW controlled diaphragm, i.e. an user defined (irregular) area (a polygon) of the TV frame which limits the videosignal evaluated. The SW controlled diaphragm can be applied to inhomogeneities situated both at the periphery or in the central part of the TV frame. However, to deal with the dot-like inhomogeneity in the central part of the TV frame, the above diaphragm should function in the opposite way, so that the videosignal inside its borders (i.e. including the inhomogeneities), will not be analyzed. The object will be excluded (not detected) inside the polygon in this case, however, it trajectory passing through the polygon should be interpolated. The polygon, i.e. the SW controlled diaphragm should be supported by an additional pair of preset counters and an SW routine providing read-out of specific presets of these counters for each scanning line (see Fig. 4). To generate the polygon, a pair of numbers, i.e. integers from the interval 0 − x, (where x stays for the number of pixels in the picture matrix line, e.g. 255) representing the left and right edges of the polygon should be assigned to each scanning line. The first number equals the clock counts separating the onset of scanning on a given line from the line synchronization pulse. The second number determines the length of the active line, i.e. terminates scanning by resetting the output of the comparator till the end of the line. Both these two numbers must be loaded into a pair of counters, the signals of which gate (activate or inhibit) the output of the videosignal comparator, before starting the scan of the line. Therefore, the introduction of the polygon requires a more powerful PC or decreases the speed of TV frame analysis.

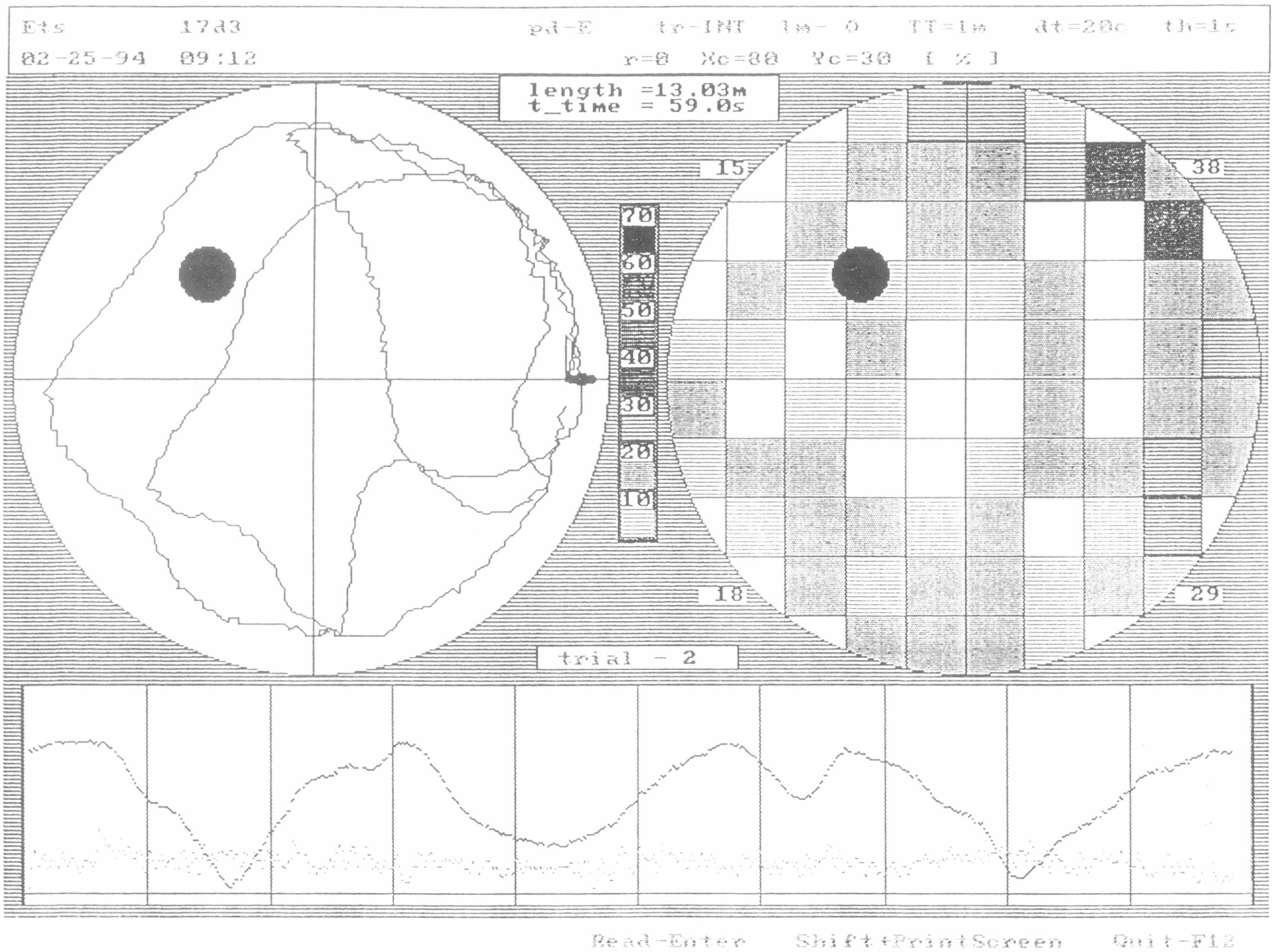

Fig. 3. *Picture of the display screen depicting the full-scale monitoring of a Morris water maze experiment (illustrating unsuccessful searching). The upper 2 lines contain the data identification label, i.e. file name, date, time. The left circle shows the animal's trajectory with the start and target marked distinctly. The right circle shows the histogram of the overall time spent by the animal in different areas of the water maze. Numbers outside the right circle represent the percentage of time spent by the animal in each quadrant of the maze. The horizontal rectangular window at the bottom presents the distance of the animal from the target (heavy dotted line) and its instantaneous speed (dotted curve) of movement in time. Vertical bars mark time intervals of 6 s (the whole window = 1 min).*

The inhomogeneity of the background can in general be compensated by the background subtraction. In practice, the HW of the system should be extended by a frame grabber, i.e. a dual port video RAM (V-RAM) capable to write-in and read-out an image in the TV frequency. The rapid spread of frame grabbers is nowadays stimulated by progress in multimedia. Picture of the background alone is stored in the V-RAM and the corresponding videosignal is read-out in synchronism with the real-time video signal from the TV camera (e.g. ETHOVISION 1994). The subtraction can be implemented by connecting the output of the V-RAM synchronized with the TV camera to a reference input of the videosignal comparator. If the background is stationary, the above V-RAM is loaded only once. In case the background is slowly changing in time, the V-RAM contents should be updated in appropriate time intervals. The limiting case represents subtraction of the output of the V-RAM which presents the previous frame compared with the current one.

b) Simultaneous tracking of multiple objects

In some experiments (e.g. studies of the social behaviour of animals), a pair of objects should be tracked simultaneously. The simple, videosignal amplitude based, single object tracking routine can also be used in case of simultaneous tracking of a pair of objects, assuming they will either never approach each other closely enough to be detected as a single object or if this happens, the objects should be recognizable by some of their image features afterwards. In this case, the Xc counter should be doubled to handle the case when intercepts of two different objects appear on the same scanning line. The scheme shown in Figure 5 can evaluate Xc coordinates of two objects with respect to the beginning of the scanning line. The scheme can be simplified if the Xc coordinate of the second object is evaluated with respect to the first one. The number of simultaneously tracked objects in this manner can be increased at the expense of additional Xc counters and more sophisticated distribution of clock pulses.

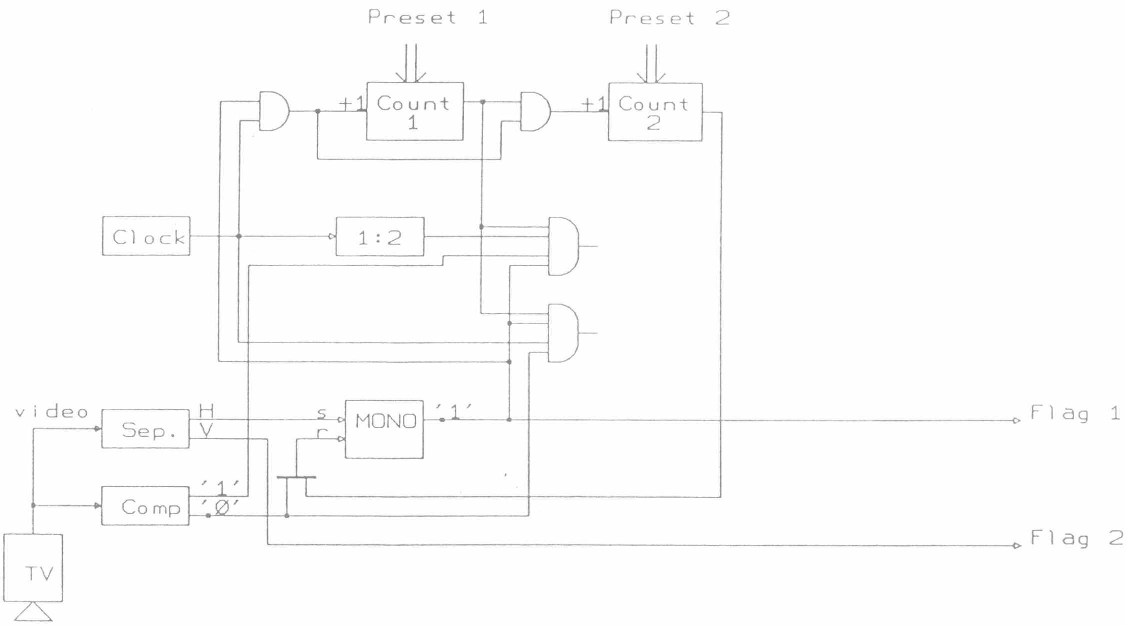

Fig. 4. *Schematics of the full HW implementation of the simple object detection procedure already shown in Fig. 2, however, extended by the SW controlled diaphragm with an active field inside the polygon. The signal of the comparator is not considered outside the polygon, because the input clock pulses are inhibited there. The active field is determined by its left margin and its width on each TV line. The discussed extension consists of two preset counters of the clock pulses: Count 1 (determines the left margin of the polygon at the given line) and Count 2 (the width of the active field). The preset values of both counters must be loaded before the start of line scanning, i.e. by the front edge of the Flag 1 signal at the latest. Both counters terminate the counting up on reaching the preset value. The OR circuit and the Xo counter are not shown in Fig. 4 for the sake of simplicity.*

If a pair of objects is automatically tracked on-line, their differentiation by using markers seems to be the most appropriate. The colour, size or shape are the most obvious features exploited by the markers. The latter requires more sophisticated image processing for pattern recognition, however, it allows a higher number of simultaneously tracked objects when compared with the application of colour. On the other hand, the colour base differentiation of tracked objects makes it possible to exploit the discussed simple detection procedure based on the evaluation of videosignal amplitude. Simultaneous tracking of up to three objects by using a colour TV camera is an example of this solution. Each of the tracked objects is painted with one of the basic colours (RGB) and up to three decoded (RGB) videosignals, each representing one of the 3 different objects, are processed simultaneously. The simple, videosignal amplitude based detection routine can be applied to each separate channel. Further extension of the number of simultaneously tracked objects can be achieved by using markers coloured with pairs of basic colours and by following the rules for simultaneous tracking of a pair of objects and exploiting the simple videosignal amplitude based detection procedure. The system VIDEOMEX-X (1992) tracking up to 6 animals, serves as an example of the application of colour markers with basic colours and their combinations, though it uses a more sophisticated processor.

The number of objects which can be tracked simultaneously by using a B/W TV camera and the simple object detection routine can also be extended by introducing active (light) markers and a time sharing regime of their activation. Each of the objects bears a light marker, i.e. a miniature light source, e.g a LED. The markers are switched on and off under control of the TV frame synchronization pulses in such a manner, that only one marker is "ON" at any time (see Fig. 6). The markers assigned to individual objects are switched-on in turn, one marker per frame, so that each of the n objects is tracked with a period of F/n, where F is the TV frame frequency, e.g. 50 TV frames/s.

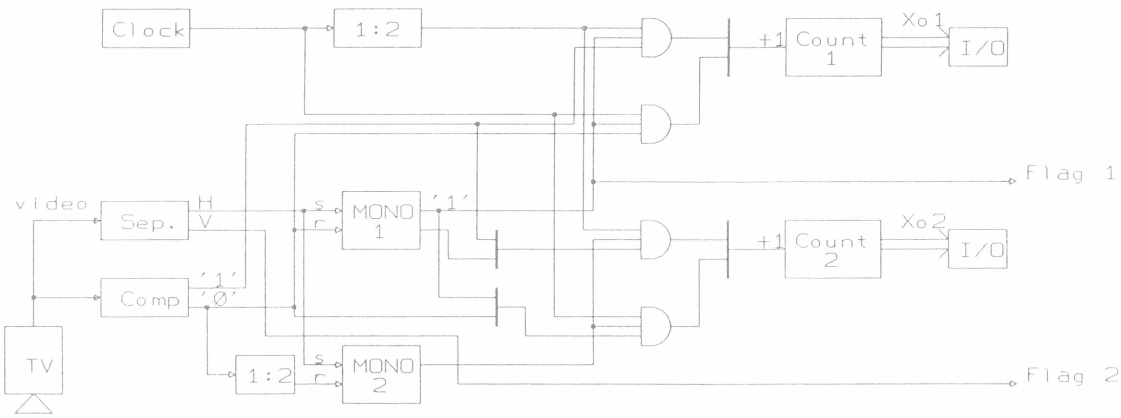

Fig. 5. *Extension of the schematics shown in Fig. 2 designed for the full HW implementation of the simple detection routine for tracking a pair of objects simultaneously even on the same TV line, i.e. for evaluation of Xo1 and Xo2. The extension is accomplished by doubling the X counter and by appropriate distribution of clock pulses. The latter is achieved by doubling the above combination of AND and OR gates at the input of the counters and by adding a divider (1:2) to the output of the comparator, a monostable circuit (MONO 2) and a pair of OR circuits connected with the input gates of counter 2. Objects are discriminated by "OFF" ("0") pulses of the comparator fed directly or through the divider, which reset the monostable circuits (MONO 1,2) the outputs of which control the input of the X counters. The output of the second counter corresponds either to the length of the scanning line (if no second object is detected) or to the centre of the intercept of the TV line with the picture of the second object on the TV line.*

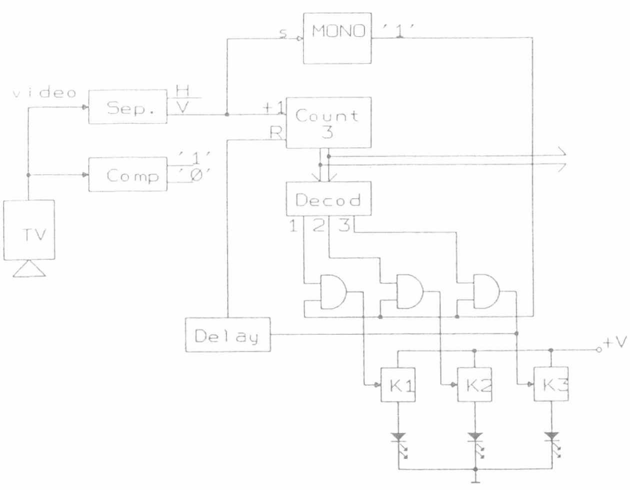

Fig. 6. *Schematics of the HW extension which implements control of the illumination of markers in the time-sharing regime. It consists of the counter (Count 3) which is incremented by frame synchronization pulses. Its output is decoded by the decoder (Decod) the outputs of which are connected with inputs of particular AND gates controlling switches (K1 .. Kn) of individual LED markers. The other input of the above AND gates is fed by the output pulse of the monostable (MONO 4) which is triggered by the frame synchronization impulse. To avoid the requirement for a preset counter (preset = number of markers), Count 3 is reset by the delayed (Del) impulse of the last LED of the set used. The information about the state of Count 3 is somewhat redundant from the point of view of the programme flow, though it simplifies its control.*

Moreover, the duration and timing of the light marking pulse should correspond to the frame (vertical reset) black-out pulse of the TV camera. This timing, i.e. scene sampling, improves the precision of tracking in time and space which is one of the advantages of the above time-sharing system. Moreover, the tracking frequency of individual objects can be adjusted with respect to their mobility allowing for more precise adaptive tracking. The light markers activated in synchronism with the TV camera have already been applied to motion analysis, however, with a special TV camera (e.g. SELSPOT II 1992).

The capacity of the above time-sharing system can be increased by applying colour differentiation of the markers. The time-sharing tracking system with active light markers can also be applied in biomechanics, in sport and rehabilitation medicine for on-line recording and study of movements of the human body or of its parts (i.e. study of gait, posture, grip etc.) (e.g. KINEMETRIX 1993).

The described system has already been tested in pilot experiments aimed at recording human arm movements. Three light markers were fixed on different points (joints) and tracked in the time-sharing regime. The reconstructed trajectories of the marker points described the arm movement. The reconstruction (interpolation) of the trajectories and their analysis was simplified by considering the constant, *a priori* known distances between the markers (Krekule and Kaminsky 1992, Chrásková *et al.* 1995).

Software support

Software supporting all described applications as well as pilot tests was developed and implemented for IBM PC/AT in MS DOS environment by using the compiled Basic language (MS Basic Professional Development System V.7.10). Specific I/O operations were controlled *via* flags (i.e. state bits of specific I/O registers) for the sake of simplicity of the programme flow. An user friendly software package was developed for MWM and similar experiments which consists of three parts. The first one serves the set-up of the system including its HW, i.e. TV videosignal checking and object threshold setting, evaluation of the homogeneity of the maze illumination, choice of working windows (diaphragm setting) and island positioning as well as its SW, i.e. parameters of the experiment, e.g. the paradigm of the experiment such as its duration, length of the series of experiments, starting positions of tracked animals, data file names, acoustic signalization control etc. These HW and SW settings are usually stored in a specific file. Experiments are controlled by the second part of the SW package, which deals with the collection and storage of experimental data as well as with on-line display of the basic results (e.g. see Fig. 3). The third part of the package provides experimental data correction (if necessary), e.g. interpolation of missing coordinates of the object trajectory and experimental data analysis (e.g. length of the trajectory, maximal speed attained etc.) which is accomplished for each trial of the series separately. The third part of the SW also makes it possible to export data into a current statistical SW package (e.g. Statgraph, Excel). All three parts of the developed SW package for MWM experiment control and evaluation represent about 80 kB of the machine code.

Conclusion

Despite the ever increasing computing power of PCs and the growing number of suitable HW devices for on-line videosignal processing, the application of the simple, single object detection routine based on the instant videosignal amplitude evaluation will dominate in both the laboratory and commercially designed systems for an automatic tracking of laboratory animals in behavioural studies, e.g. Morris water maze in the future. It was shown that this basic routine can potentially also be applied in multiple objects tracking and in tracking in an optically inhomogeneous background.

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Reprint Requests

I. Krekule, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic.