— INSTRUMENTATION & TECHNIQUES —

A computerized infrared monitor for following movement in aquatic animals

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Reliable monitoring of the movement of small aquatic animals presents some unique problems. In the case of the larval salamander, the animal may be less than an inch long and move in sporadic short bursts. A device is described that utilizes infrared emitters and wide-band sensors interfaced to a computer for monitoring location and movement of animals between light and dark environments over extended periods of time. The system employs a multiplexed digital input, is inexpensive, and can be easily expanded to deal with a range of monitoring problems that may occur with a wide range of both aquatic and terrestrial species.

In a recent set of experiments (Schneider, 1989) concerned with the establishment of a reliable behavioral measure of neural regeneration in the optic nerve, we were faced with the problem of measuring phototactic responses in larval salamanders approximately an inch or less in length. The larval salamander presents a particular challenge for the observer in the laboratory because it is small and it moves rarely and only in short bursts (Schneider, 1968). Cognizant of this fact, our initial determinations utilized a time-lapse video system to monitor the movement of animals to the dark side or illuminated side of an alley over a 24-h period. This method had a number of limitations. For example, at normal tape speed, it required approximately 1 h and 15 min to observe and score the number of crossings from dark to light during a 24-h period. This proved to be a very tedious procedure and suffered from a lack of interrater reliability. In addition, a great deal of valuable information was lost because we could not observe the animals when they were in the dark, and there was no good reliable measure of activity on either the light or dark side of the alley. Therefore, we sought to circumvent these problems by developing an infrared monitoring system that would interface with a computer and capture movement of our animals over small distances, thus eliminating the need for a human observer and increasing reliability.

INFRARED MONITOR AND COMPUTER INTERFACE

We found the most reliable method for capturing an animal at any point in space was through the use of collimated infrared emitters and wide-band photometric sensors. Whenever an object passes between an infrared emitter and its receiver, a signal is generated and can be counted. Therefore, we constructed an alley (18 in. long and 2 in. wide) containing black Plexiglas on one half and white on the other. Seventeen infrared emitters and wide-band photometric sensors (Hewlett Packard, c/o Pioneer-Standard, 259 Kappa Drive, Pittsburgh, PA) were placed equidistant the length of the alley. Half of the alley was covered and remained dark, while the other half was illuminated. The sensors and the emitters were mounted in ¼-in. brass tubes (¾ in. long) in order to restrict the field of view of both. This design limited the response of the sensor to the infrared emitter directly across the alley. In addition to the inhibition of cross talk between adjacent sensor and emitter pairs, the narrow field of view also enhanced the rejection of ambient light. A removable Lexan liner, which did not inhibit the transmission of infrared and which fit snugly into the alley, prevented exposure of the brass tubing to salt solutions that might create a galvanic action between the sensor and emitter cases, which are different potentials. Careful attention was paid to keeping the solution free of particulate matter, because each sensor requires a minimum number of photons for activation. A cross section of the alley is illustrated in Figure 1.

The sensor circuit employs a General Instrument MTH360 Phototransistor; the sensor threshold is set by a three-transistor amplifier using 2n3094s to drive the corresponding bit on the hex tristate inverter. Threshold adjustment is provided for each sensor circuit and can be

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Figure 1. Cross section of the alley showing infrared emitter and sensor mountings.

determined by observing a corresponding LED assigned to each circuit. When the LED is on, it indicates that the sensor cannot see the opposite emitter, which, during a normal test run, would indicate the presence of an object

between the emitter and the sensor. A schematic diagram of the interface and infrared circuit is shown in Figure 2.

All of the alleys are tied in parallel back to the computer distribution panel. Each alley decodes its own unique address via 1 of 8 and 1 of 16 decoders on the sensor board. This design permits 16 alleys to be connected to one parallel bus. Two decoders are used to specify the alley and sensors within that alley that are to be read by a digital input word sampled by the computer (see below). Each alley decodes its own address via a 1-of-16 decoder, allowing up to 16 alleys to be connected to one parallel bus and to be utilized simultaneously. The two sets of eight sensors and the one additional sensor in each alley decode their own addresses via a 1-of-8 decoder, permitting a single 8-bit digital input word to sample sequentially all 17 sensors.

The power supply is an important consideration since each infrared emitter draws approximately 50 mA of current $\times$ 17 emitters per alley. In addition, there are the power requirements of the sensors and the interface. We used a Power/Mate (Model EVS-5F) switching power supply rated at 100 W (5 V at 20 A) to run three alleys (Power/Mate, 514 S. River St., Hackensack, NJ).

Figure 2. Schematic diagram of the infrared circuit and interface described in the text.

However, this power source could safely support 12 alleys. The power supply was placed in a separate enclosure with forced air cooling, using a box fan (Allied Electronics Model BS2107FL 1000). The forced air cooling is critical for reliable operation of the switching power supply.

COMPUTER OPERATION

The software used to acquire data from the sensors is written in the ASYST language (Copyright 1984-1989 ASYST Software Technologies, Inc.). It is implemented on an IBM PC-XT computer (ASYST Versions 3.0 and above require 640K RAM, a hard disk drive, and a math coprocessor) equipped with an IBM PC Data Acquisition and Control Adapter. ASYST code for acquiring data from a single alley is given in Appendix 1 in order to illustrate the simplicity afforded by the electronics.

The desired sampling period in milliseconds and the number of samples to be taken during the session are entered on the keyboard. Following a "go" signal from the keyboard, the state (on/off) of each of the 17 sensors is updated during each sampling period in the following manner. A single 8-bit input word is used, so that three samples must be taken for each alley during each sampling period (1 bit represents the state of each of the 17 sensors). A single 8-bit digital output word is used to choose the alley and sensors within that alley that are to be sampled. Setting Bit 0 specifies Sensors 0-7; setting Bit 1 specifies Sensors 8-15; setting both Bit 0 and Bit 1 specifies Sensor 16. Bits 2-5 specify the alley (0001-1000).

For each alley, a masking algorithm codes the state of all 17 sensors in a single variable, called SENSOR, so that the value of SENSOR on each sampling period can be used as the index of counting variables that specify the number of times that various states occur. Each input word is masked so that each bit, corresponding to a sensor, is set if and only if that sensor has its beam interrupted. For Sensors 0-7, SENSOR is equated to $1 + \text{the digital value of the 8-bit word input from the digital port}$, so that SENSOR takes on the value $2^N + 1$, where N is the number of the sensor (from 0-7). For Sensors 8-15, $256 (2^8)$ is added to the digital port input value, so that SENSOR takes on the value $2^8 + 2^{N-8} + 1$, where N again is the number of the sensor. For Sensor 16, SENSOR is set equal to 707. Finally, at the end of each sampling period, SENSOR is equated to the sum of the index values. Since SENSOR has a maximum value of 3,281 (if all 17 sensors simultaneously have their beams interrupted), the number of times that the state of all 17 sensors specified in SENSOR appears during an entire session can be stored in an array having 3,281 elements. Each element of this array, called ALL.COUNT in our implementation, then gives the number of times that each state of the 17 sensors occurs during a given session. A 17-element array called OUT.SINGLE.ALL is constructed from ALL.COUNT and specifies the number of times that the animal was located at each

of the 17 sensors. A second array with 3,281 elements, called CROSS.COUNT, is updated only if the current state of SENSOR is different from that during the previous sampling period. Thus, CROSS.COUNT contains the number of times that a particular state of the 17 sensors results when the animal moves from its position on the previous sampling period. A 17-element array called OUT.SINGLE.CROSS is constructed from CROSS.COUNT and specifies the number of times that the animal crossed the path of each of the 17 sensors. Finally, two variables, DARK.COUNT and LIGHT.COUNT, store the cumulative number of SENSOR states that indicate interruption of sensors located in dark and light sides of the alley, respectively.

DISCUSSION

We have used the infrared system to monitor the phototactic responses of a wide array of aquatic animals (fishes and amphibians) and have found it to be highly reliable. A recent investigation (Schneider, Marquette, & Pietsch, 1990) requiring an 8-h-per-day run 6 days a week for 18 months was completed without a single system failure. It is apparent that the system we have described could be used to monitor the movement of mammals in an open field under ambient light with only the sensors arrayed under a Plexiglas floor or with a horizontal grid created by collimated sensors and emitters placed on the periphery of the field. A single overhead infrared source with sensors arrayed under Lexan could be used to monitor movement in the dark. The horizontal grid could also be used to track movement in several individuals simultaneously, with the obvious limitation that specific individuals could not be identified. This extends its utility to some social interactions (e.g., grouping behavior).

There are several advantages of this computerized infrared monitoring system. The multiplexed digital input makes it possible to use different numbers and combinations of animal chambers simply by making changes in the software. The availability of cheap, high-level laboratory languages allows rapid, cost-effective development of a variety of configurations of observation units. Different strategies can be employed in order to increase sampling speed or to locate an animal within a large array of sensors, without the necessity of time-intensive assembly-language programming. Our application uses a 4.77-MHz processor and achieves a sampling period of 120 msec for each set of three input words (i.e., three digital outputs to the multiplexer and three digital inputs from the multiplexer during each 120-msec period). A faster computer would provide an increase in sampling speed that is at least proportional to the difference in processor speeds. More efficient code would also decrease the effective sampling time. For example, the computer program might use the memory of which set of sensors contained a sensor with an interrupted beam on the previous sample in order to select where in a large array the

next sample will be taken. An animal's location within a rectangular chamber with sensors on two perpendicular walls could be rapidly followed in this manner, precluding the necessity of sampling every sensor.

It is also relatively easy to detect hardware problems using the data-collection software. For example, the availability of information on the number of interruptions and crossings of each sensor beam (ALL.COUNT and CROSS.COUNT) will make apparent a malfunctioning emitter or sensor. Finally, the system as designed is inexpensive. Although the DACA board that we used is no longer available, a number of low-cost data acquisition and control boards are available (e.g., Keithley-Metrabyte

Model PIO-12, \$125). The infrared transmitter and sensor are \$5 a pair, the power supply is \$100, and Plexiglas and Lexan are between \$5 and \$6 per square foot.

REFERENCES

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APPENDIX 1

```
\ Statements preceded by a backslash "\" are comments.
\ This program reads digital input bits 0-7 from sensor
\ inputs.
\ It counts the total number of times that a sensor does not
\ receive an infrared beam.
\ It only works for Alley (Track) #2.
\ SAMPLING.PERIOD contains the time in milliseconds between
\ whole-track samples.
\ SAMPLE.COUNT contains the number of whole-track samples.
\ ALL.COUNT is a 3281 element array containing the number of
\ samples of each possible sensor, and sensor combinations.
\ CROSS.COUNT is a 3281 element array containing the number of
\ non-consecutive samples of each possible sensor and of each
\ possible combination of sensors.
\
\ OUT.SINGLE.ALL contains the total number of samples during
\ which each of the 17 sensors read an interrupted beam, and
\ OUT.SINGLE.CROSS contains the number of samples during which
\ each of the 17 sensors read an interrupted beam AND did not
\ have its beam interrupted during the previous sampling period
\ (i.e., actual crossings of the sensor as opposed to both
\ crossings and remaining in place.
\
\ DARK.COUNT and LIGHT.COUNT contain the total number of samples
\ of sensors 0-7, and 9-16, respectively.
\
\ Declare I/O interface hardware.
```

IBM.DACA

\ Array and scalar variable declarations.

```
DP.INTEGER DIM[ 3281 ] ARRAY ALL.COUNT
          DIM[ 3281 ] ARRAY CROSS.COUNT
          DIM[ 17 ] ARRAY OUT.SINGLE.ALL
          DIM[ 17 ] ARRAY OUT.SINGLE.CROSS
```

```
SCALAR SAMPLING.PERIOD
SCALAR SENSOR
SCALAR LAST.ONE
SCALAR SAMPLE.COUNT
SCALAR FIRST.SAMPLE
SCALAR DARK.COUNT
SCALAR LIGHT.COUNT
SCALAR LIGHT.DARK.INDEX
```

```

\
\ #CHECK ensures valid number input.
\
: #CHECK
  BEGIN
    #INPUT NOT
  WHILE
    CR ." Not a valid number; reenter: "
  REPEAT
;

\
\ ALLEY contains the loop that reads the digital input.
\

0 DIGITAL.TEMPLATE 1ALLEY
BINARY 11111111, DIGITAL.MASK
DECIMAL
: ALLEY
  1ALLEY DIGITAL.INIT
  SYNCHRONIZE
  3 1 DO
  SAMPLE.COUNT 2 / 1 + 1 DO
    1 SENSOR :=
      BINARY
      8, WRITE.BITS READ.BITS           \ Alley #2, low-order byte.
      MASK># SENSOR + SENSOR :=
      9, WRITE.BITS READ.BITS           \ High-order byte.
      MASK># DUP
      DECIMAL 0 <> IF 256 + SENSOR + SENSOR :=
      THEN
      BINARY 10, WRITE.BITS READ.BITS 1, AND \ Top byte.
      MASK># DUP
      DECIMAL 1 = IF 707 SENSOR + SENSOR :=
      THEN

\ SENSOR now contains a number that uniquely specifies
\ the state of all 17 sensors.

\ Update ALL.COUNT, CROSS.COUNT, LIGHT.COUNT and DARK.COUNT.

  ALL.COUNT [ SENSOR ] 1 + ALL.COUNT [ SENSOR ] :=
  \ There is a crossing if they are not equal.
  LAST.ONE SENSOR <>
  IF
    CROSS.COUNT [ SENSOR ] 1 + CROSS.COUNT [ SENSOR ] :=
  THEN
    SENSOR DUP LAST.ONE :=
    \ Calculate #samples when in light & when in dark.
    \ Have any samples in light or dark occurred?
    1 FIRST.SAMPLE = IF \ If false, no samples have yet occurred.
    SENSOR DUP 194 <= 1 > AND
    IF \ If true, we are in the dark.
      DARK.COUNT 1 + DARK.COUNT :=
      0 LIGHT.DARK.INDEX :=
    THEN
    SENSOR 259 >=
    IF \ If true, we are in the light.
      LIGHT.COUNT 1 + LIGHT.COUNT :=
      1 LIGHT.DARK.INDEX :=
    THEN

```

```

    SENSOR 1 =
    IF \ We are between sensors.
        LIGHT.DARK.INDEX 0 =
        IF \ If true, we are still in the dark.
            DARK.COUNT 1 + DARK.COUNT :=
        ELSE
            LIGHT.COUNT 1 + LIGHT.COUNT :=
        THEN
    THEN
    THEN \ No samples have been taken.
    258 <> IF 1 FIRST.SAMPLE := THEN
    SYNCHRONIZE
    STACK.CLEAR
LOOP
LOOP
;

\ SALLY is the main routine.

DECIMAL

: SALLY
    DECIMAL
    STACK.CLEAR
    0 FIRST.SAMPLE := 0 DARK.COUNT := 0 LIGHT.COUNT := 0
    LIGHT.DARK.INDEX :=
    BEGIN
        CLS ." Enter desired sampling period (ms) > "
        #CHECK SAMPLING.PERIOD :=
        CR ." Enter number of samples > "
        #CHECK SAMPLE.COUNT :=
        CR CR ." It will take "
        SAMPLE.COUNT SAMPLING.PERIOD * 1000. / . ." seconds."
        CR ." Is this what you want?(Y/N) >"
        KEY DUP EMIT 89 =
    UNTIL
    SAMPLING.PERIOD SYNC.PERIOD
    -1 LAST.ONE := \ Initialize variables.
    0 ALL.COUNT :=
    0 CROSS.COUNT :=
    ALLEY
    BELL
    9 1 DO
        ALL.COUNT [ 2 I 1 - ** 1 + ] OUT.SINGLE.ALL [ I ] :=
        ALL.COUNT [ 256 2 I 1 - ** + 1 + ] OUT.SINGLE.ALL [ I 8 + ] :=
        CROSS.COUNT [ 256 2 I 1 - ** + 1 + ]
        OUT.SINGLE.CROSS [ I 8 + ] :=
        CROSS.COUNT [ 2 I 1 - ** 1 + ] OUT.SINGLE.CROSS [ I ] :=
    LOOP
    ALL.COUNT [ 708 ] OUT.SINGLE.ALL [ 17 ] :=
    CROSS.COUNT [ 708 ] OUT.SINGLE.CROSS [ 17 ] :=
;

```

APPENDIX 2
Device Data for Alleys

PHOTOTRANSISTOR DATA

NPN SILICON

Collector - Emitter Voltage	30	V
Emitter - Collector Voltage	7	V
Collector - Base Voltage	70	V
Collector - Emitter (V sat)	.4	V

Flat Lens for Wide Acceptance Angle	60	Deg
-------------------------------------	----	-----

Light Current	1 mA	VCE = 5 V	Ee = 5 mV/cm ²
Dark Current	100 nA	VCE = 10 V	Ee = 0 mV/cm ²

Ee=Radiant Incidence

Light Source is an Unfiltered Tungsten Lamp at 2870° K Color.

A Ga As Source of 1.7 milliwatt/cm² is Approx Equivalent to
a Tungsten Source of 5 milliwatt/cm² at 2870° K. K=deg Kelvin.

INFRARED EMITTER DATA

ALUMINUM GALLIUM ARSENIDE DIODE

Output Flux of 10 mW at 100 mA
 Axial Intensity of 35mW/SR at 100 mA
 Continuous Forward Current 100 mA
 Reverse Voltage 3 Volts

Total Output Power	10 mW	If = 100 mA DC
Peak Emission Wavelength	880 nM	If = 20 mA
Spectral Line Half Width	40 nM	If = 20 mA
Forward Voltage	1.8 V	If = 100 mA

Included Angle Between Half Radiant Intensity Points 20 Degrees

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