# IN SITU MEASUREMENT

OF CHICK EMBRYO

## HEART RATE

by

Nicolas Salamina Bioengineering Department

Submitted in Partial Fulfillment of the Requirements of the University Undergraduate Fellows Program 1983-1984

.

Approved by:

Yon Hunter

April 1984

\*Format and Style: Annals of Biomedical Engineering

#### ABSTRACT

Finding the most suitable and non-invasive way for recording the chick embryo's heart rate is the principal objective of this research. The primary rationale for this project is to develop an animal model for evaluating the effects of physical or chemical agents on normal fetal development, to hopefully reveal potential causes of spontaneous abortion and genetic disorders.

The heart rate of the chick embryo has been recorded in the past using various techniques - microelectrodes, <sup>12,24</sup> chemical compounds,<sup>8,9</sup> ultrasound<sup>15</sup> and others. In this research, a determination of the chick embryo's heart rate was performed using a light-reflective system. This technique was developed to minimize the effects of environmental influences on the embryo's heart rate. A stable model is essential if this model is to be used to assess the influence of different factors on the development of the embryo's heart.

i

## ACKNOWLED GEMENTS

I wish to express my appreciation to Dr. Jon Hunter for his advice and collabaration, and for supplying all the equipment necessary for this research. I also wish to express my appreciation to Miss Carmen Martinez for her assistance in drawing the descriptive figures of this thesis. Also, I wish to thank the department of Veterinary Physiology and Pharmacology for letting me use their laboratory facilities.

# TABLE OF CONTENTS

Section Pag	е
Abstract	i
Acknowledgements	i
Table of Contentsii	i
List of Tables	٧
List of Figures	v
Introduction	1
Review of Literature	2
Photoplethysmography Technique for Monitoring Chick Embryo's Heart Rate	8
Materials and Methods	C
Signal Processing	5
Results	8
Discussion	3
Conclusions	ō
References	3
Appendix 1	1
Appendix 2	4
Appendix 3	9
Vita	1

# LIST OF TABLES

Table		Page
1	Frequency of Selected Genetic Disorders	4
2	Data of Chick Embryos Heart Rate	24
3	Average of Heart Rate (After 10 Minutes of Recording)	31

# LIST OF FIGURES

Number	Title	Page
1	Chick Embryo	5
2	Microelectrodes Connected Inside the Embryo	6
3	Microelectrodes <code>Placed</code> Outside the <code>Embryo</code> $\ldots$ $\ldots$	7
4	Photoplethysmography Technique	9
5	Photoplethysmography Technique in Eggs	10
6	60 Hz Noise Recorded Using Fiber Optic- Germanium Detector Design	12
7	Emitter-Detector Attachment	13
8	Recording Technique Using a Moveable Alligator Clip	14
9	Circuit Diagram	16
10	Input and Output Characteristics	17
11	Experimental Set Up	18
12	Human Heart Rate Using Second Design	19
13	Experimental Recording Without Signal Filtering Outside the Incubator	20
14	Experimental Recording With Signal Filtering Outside the Incubator	21
15	Experimental Recordings Inside the Incubation	22
16	3 Day-old Chick Embryo's Heart Rate Recordings	25
17	4 Day-old Chick Embryo's Heart Rate Recordings	26
18	5 Day-old Chick Embryo's Heart Rate Recordings	27
19	6 Day-old Chick Embryo's Heart Rate Recordings	28
20	7 Day-old Chick Embryo's Heart Rate Recordings	29
21	Artifacts	30

Number	Title	Page
22	Heart Rate vs. Incubation Time	31
23	Effect of Opening the Incubator Door	32
24	Effect of Incubation Temperature on Heart Rate	33
25	Range of Detection by the Infrared Transistor $$ .	34
26	Heart Rate as a Function of Incubation Time (7 Day-old Embryo)	36

#### INTRODUCTION

Alterations in the normal development of the human embryo/fetus can cause spontaneous abortion or malfunctioning in the principal organs of the embryo/fetus. One system particularly susceptible to early environmental influences on the fetus is the cardiovascular system. By recording the fetus or embryo's heart rate a determination of abnormal fetal or embryonic development may perhaps be made. For this study, a teratogenic model with a development similar to that of the human embryo/fetus will be used. The model chosen for this experiment is the chick embryo, shown in figure 1.

Development of the chick embryo's heart has been the subject of numerous studies during the past 50 years. Most of these studies have been qualitative in nature; however, during the past 10 years, quantitative indices of cardiac function have been obtained.<sup>1</sup> The most common technique for recording heart rate has been to visually observe the beating heart. This technique requires opening of the egg and exposing the embryo to a foreign environment. The altering of the embryo's natural environment will affect the function of its organs; temperature, humidity, microorganisms, chemical contaminants, and oxygen/carbon dioxide content will certainly influence the development of the embryo. Therefore, recordings should be made in such a way as to minimize these environmental factors.

During the past 2 years, some researchers have developed new techniques which attempt to minimize the effects of environmental factors on heart rate. However, these new techniques don't provide a reliable, quantitative measurement of heart rate. These studies have generally violated one of the principles of physiological measurements - Kelvin's law, which states that the measuring instrument should not affect the event being measured.

A new method that more closely obeys Kelvin's law and also minimizes the influence of external environmental factors is described in this report. This method is based on a light-reflective heart beat detection system.

# REVIEW OF LITERATURE

The Need for an Animal Model to Study the Physiological Development of the Heart

The cardiovascular system is one of the first organ systems of the embryo to physiologically mature. This system is essential for the continued well being of the embryo and fetus. Early spontaneous abortions in humans can be caused by a malfunctioning of this.

Spontaneous abortion, also called miscarriage, is defined by the World Health Organization as a premature delivery of a nonviable fetus before the 28th week of gestation.<sup>2</sup> The incidence of spontaneous abortion during early pregnancy has been estimated as 10%, but this percentage is probably much higher since many early abortions are not detected or are manifested as delayed menstruation.<sup>3</sup> Some studies realized by Sentrakul and Potter, show that 12.3% of all pregnancies ended in abortion before 20 weeks of gestation. Most likely from 15 to 20% of all pregnancies terminate in spontaneous abortions; this is about 300,000 miscarriages occurring each year in the United States.<sup>2</sup>

The percentage of spontaneous abortion increases with age.<sup>2</sup> Also, spontaneous abortion is more common during the first weeks of pregnancy and decreases appreciatively as the gestational age increases.<sup>5,6</sup>

The causes of spontaneous abortion are often multiple and include both fetal and maternal factors. A specific reason for spontaneous abortion cannot always be established because of the difficulty in analyzing all causative factors.<sup>3</sup> The most common factors causing spontaneous abortion can be enumerated as follows:

- a) Abnormalities in the ovum and spermatozoa.<sup>5,14</sup>
- b) Chromosomal abnormalities of many different types: abnormal meiosis, translocation of genes.
- c) Congenital disorders (enumerated in table 1),<sup>7</sup> and
- d) Such others influencing factors as: teratogenic factors (radiation, viruses, chemicals), maternal diseases, infections, endocrine defects, abnormalities in endocrine organs and others.

All these factors can cause abnormalities in the normal development of the human embryo/fetus, and may cause malfunctioning in the principal organs of the embryo/fetus. Some of these factors and genetic disorders may affect the development of the heart of the embryo/fetus. As shown in table 2, cardiac defects are present in approximately 0.5% of all births.<sup>7</sup> By monitoring heart rate of the embryo/fetus, the effects of radiation, chemicals and other potential teratogenic factors may possibly be detected. Since teratogenic

# Table 1\*

	Frequency of Selected Genetic Disorder	S
Genet	tic_type_	Number per million births
Ι.	Single gene	17412
II.	Chromosomal abnormalities	5000
III.	Complex malformations	30000
	- Cardiac defects	5000
IV.	Incompatibilities	4200
۷.	Polygenic traits	850000

<sup>\*</sup>Maternal and Child Health Practices, p. 290.

experiments cannot be performed in humans, suitable animal models must be developed for experimental studies.

# Chick Embryo Model for Heart Rate Recording

The domestic chick embryo, in figure 1, is readily available and relatively accessible for experimentation. Being a higher vertebrate, its cardiac physiology is comparable with that of humans, particularly during the embryonic stages of development of the cardiovascular system. Compared to other experimental animals, the chick embryo is a relatively simple teratogenic model.

In developing a physiological measurement system to record heart rate, Kelvin's law has to be strictly obeyed; that is, the





measuring instruments must have minimal effect upon the event being measured (i.e., heart rate of the chick embryo). Many researchers have recorded the chick embryo's heart rate. However, these investigators have used methods which violate the principle of Kelvin's law.

Techniques that have been used to measure heart rate, include:

a) The Hamburguer and Hamilton technique: This procedure involves opening the shell of the egg and introducing a micropipete into the veins; injecting parafin oil and then recording heart rate using a Bolex Reflex Camera.<sup>1,10,11</sup> This technique has been used by researchers to determine the effects of typan-blue, isoproterenol and other teratogenic agents on heart rate.<sup>8,9</sup> In this model, the recordings are affected by external environmental factors and the insertion of instruments into the embryo.

b) Microelectrodes technique: This method uses  $25 \ \mu m$  diameter microelectrodes inserted into the embryo to record the electrocardiogram (figure 2).<sup>12,24</sup> In Hunter's studies, approximately half of



Figure 2 Microelectrodes Connected Inside the Embryo

the embryos died during the instrumentation or within seven days following instrumentation, thus one can reasonably postulate that the heart rate was affected by this procedure.

A variation of this technique consists in placing the microelectrodes in the amniontic fluid surrounding the embryo (figure 3). This technique is non-invasive, but an electrocardiogram cannot be recorded because of the extremely small level of electrical signal, < 2  $\mu$  volts.<sup>26</sup>



Figure 3

Microelectrodes Placed Outside the Embryo

c) Ultrasonic radiation: This is a non-invasive procedure, that uses a pulse-echo mode of recording.<sup>15</sup> However, this technique subjects the embryo to total body radiation. Some studies have demonstrated that this type of radiation may possibly affect the heart of the embryo and damage some endothelial and red blood cells.<sup>16,17</sup>

This project was directed toward finding a technique to measure heart rate which does not violate Kelvin's law or in itself causes teratogenic effects.

# PHOTOPLETHYSMOGRAPHY TECHNIQUE FOR MONITORING CHICK EMBRYO'S HEART RATE

Photoplethysmography is a simple, non-invasive technique for studying peripheral circulation in human subjects.<sup>18</sup> The technique is based detecting a difference in the intensity of transmitted or reflected light as blood flows through a subject's finger (figure 4 (a)). A light source and a phototransistor can be placed either in a backscattered (figure 4(b)) or transilluminated mode at the recording site. The emitted light is absorbed, reflected and scattered by the vascular bed with a small fraction of the emitted light received by the photodetector.<sup>19</sup> Blood volume changes in the vascular bed producing variation in the incident light measured by the photodetector; thus permiting the detection of the pulse. This technique is shown in figure 10(a); the emitter here is a LED gallium arsenide infrared emitting diode and the detector is a silicon phototransistor.<sup>20</sup> Since the amount of absorption of light by the hemoglobin and oxyhemoglobin will influence the light intensity received by the photodetector, it is important to restrict the emission of light between 600 and 805 nanometers (range of light absorption for blood).<sup>19</sup>

It was postulated that this technique could be used to measure the heart rate of the chick embryo. For this application, a small opening must be made in the shell in the vicinity of the air sac



a

emitter

b



Photoplethysmography Technique

a) LED-transistor photoplethysmograph in humans

b) Backscattered mode

and a fiber optic and photodetector positioned perpendicular to the embryo (figure 5). The photoelectric detector, which is designated to measure the amount of light absorbed by changing its internal resistance,<sup>21</sup> will detect the backscattered light intensity which should change as the chick embryo's heart beats. To minimize noise associated with ambient light, a detector should be selected which is sensitive to those wavelengths most affected by blood (600-805 NM).<sup>19</sup>



Photoplethysmography Technique in Eggs

#### MATERIALS AND METHODS

Approximately 250 fertile white leghorn chicken eggs were used in this study. The eggs were incubated at 37.5°C and 50-55% relative humidity. The period of incubation varied from 72 hours to 168 hours and heart rate recordings were obtained at 3, 4, 5, 6 and 7 days. A standardized technique was used in opening all eggs; 1 cm diameter hole was created in the shell and and shell membrane.<sup>22</sup>

# First Design Approach

In initial studies, a fiber optic guide (diameter =  $0.64 \text{ mm}^2$ ) was used to transmit light<sup>23</sup> and a germanium infrared photodiode was used as the sensing element. A filter was placed over the end of the fiber optic guide to provide a cold, red/infrared light source. Cold light was used to avoid any heating of the embryo

and subsequent alteration of heart rate. (Appendix 1 shows the characteristics of the fiber optic and light source used.)

A 1 mm diameter germanium infrared photodiode was used as a detector. This transducer was sensitive to wavelengths of light between 800 nm to 1800 nm. As shown in Appendix 2, the sensitivity of this device is 50% at 800 nm.

The set up for this light-reflective system is shown in figure 5 and the circuitry used to process the signal obtained by the infrared germanium detector is shown in Appendix 2 (see photoconductive diagram). The output signal was displayed on an oscilloscope. Unfortunately, heart rate could not be detected using this method (around 30 eggs were used). The signals that were recorded, figure 6, consisted of 60 Hz noise.

The possible causes for failure of this technique were that:

- The filter used for the fiber optic was inappropriate and blocked infrared radiation.

- The sensitivity of the detector in the 600-800 nm range was too low to detect changes in reflected light from the embryo.

- Electrical noise was too high compared to the amplitude of the signal.

#### Second Design Approach

After the failure of the first design, a second attempt was made, using a commercially available transducer. This is the same technique that is used for LED-transistor photoplethysmography in





humans.<sup>20</sup> The emitting element was an XC-880 gallium aluminum arsenide infrared emitting diode. The output peak is centered around 880 nm (Appendix 3-A). The detector used was the TIL414 infrared NPN silicon phototransistor, with high photosensitivity (Appendix 3-B). To simplify orientation of the emitter and detector, these devices were attached together separated by a plastic tubing (figure 7-a).

Two methods were used to position this transducer relative to the chick embryo. The first technique, shown in figure 7-b uses a set of moveable wires to physically hold and allow movement of the



а

b



a- Emitter-Detector Attachment

b- Recording Technique Using Moveable Wires

transducer (similar to the wiring used to cover the champagne bottles).

The second method for positioning the transducers is shown in figure 8. Here the transducer is attached to a moveable alligator clip (wrapped with a rubber tubing to provide electrical insulation).



Recording Technique Using a Moveable Alligator Clip

Figure 8

This technique provided the stable method to control positioning of the transducer.

The transducer (emitter-detector) was oriented perpendicular to the embryo and positioned very close to it to obtain the best results.

#### SIGNAL PROCESSING

Two separate circuits were used in the measurement system - one for the emitter and one for the detector (Figure 9). For the XC-880-A, a 75  $\Omega$  in series with the emitter is necessary to limit the current to less than 60 mA (Appendix 3). Since the input voltage is 5 V-dc, the current will be (I) = 50 mA.

Taking into consideration the electrical characteristics of the T1414 infrared phototransistor (Appendix 3) and the necessity to amplify the small signal obtained from the change in light reflection, a differentiator circuit was used. This circuit is good for wave peaking or wave-shaping type of detection.<sup>27</sup> The characteristics of input and output voltages of this circuit are shown in figure 10. The gain of 33 was selected and it proved to be enough to provide a suitable signal associated with the chick embryo's heart beat. Besides a differentiator circuit, a differential amplifier could also be used to process the signal.

The output signal from the differentiator was displayed on an oscilloscope. Later a high-pass filter was added to eliminate 60 Hz noise.



Circuit Diagram



Input and Output Characteristics



A diagram of the experimental set up is presented in figure 11.

Figure 11

Experimental Set Up

#### RESULTS

#### Experimental Studies

First, experimental studies were conducted to record chick embryo's heart rate - proving the functionality of the second design.

Experimental studies were conducted as follows:

\* The transducer was first used in humans to prove that it could pick up digital pulses. The result, a large signal (600 mV amplitude), was easily recorded (figure 12).





\* Next, recordings were obtained with the egg located outside the incubator, with the membrane surrounding the embryo removed and without the signal being filtered (figure 13). It became apparent that transducer positioning was critical in these studies.

\* Next, recording were obtained with the egg outside the incubator, without the membrane, but with signal filtering. The results are presented in figure 14. The filter removed noise from the signal and thereby improved the signal to noise ratio.

\* Finally, recordings were made with the membrane covering the embryo and with eggs either outside or inside the incubator. The recorded signals are shown in figure 15 (a) (unfiltered) and figure





15 (b) (filtered). It was found that the embryonic membranes decreased the amount of reflected light to about half. So, the signal amplitude obtained with the membrane was approximately half of that obtained without the membranes intact. Since the membranes help protect the embryo from external environment, the recordings were always attempted first with the membrane intact and then with the membranes removed if the first recordings were unsuccessful.

The protocol used in these studies is outlined below:

 $\star$  Eggs were moved outside the incubation and opened by the windowing technique.  $^{22}$ 





Experimental Recording with Signal Filtering Outside the Incubator

\* Eggs were placed back inside the incubator.

\* The transducer and circuitry were moved inside the incubator.

\* The transducer was oriented perpendicular to the embryo (figure 8).

\* The transducer was positioned over the embryo until a signal was obtained on the oscilloscope. (If the signal could not be obtained, the membranes covering the embryo were removed).

\* Determination of the heart rate was performed immediately after and ten minutes after the signal was first obtained.



 $(10^{-3})$  Volts b 150 75 0 -75 -150 1 2 3 4Seconds



Experimental Recordings Inside the Incubator

a - with the membrane b - with the membrane, filter cut off at 10 Hz

Recordings were made on embryos of the following ages: (78-83) hours (~3 day-old) embryos, (100-105) hours (~4 day-old) embryos, (124-129) hours (~5 day-old) embryos, (149-154) hours (~6 day-old) embryos, and (173-178) hours (7 day-old) embryos. Every recording obtained came from a different embryo.

Table 2 presents the data recorded in this study: the number of embryos used, the heart rates measured with and without the membranes covering the embryo and the average heart rate for each age group of embryos. Figures 16 through 20 illustrate typical signals that were recorded.

Many times the results were not as clear as others because sometimes artifacts were induced mainly by the movement of the embryo (figure 21). Table 3 presents the average heart rate for each age group of embryos after 10 minutes of recording. The results show a linear increment in heart rate as a function of embryo age.

#### DISCUSSION

Tables 2 and 3 show the results obtained in this research. From these tables of results and from the way the recording were made, some of the advantages and disadvantages using the light (infrared)reflective technique can be discussed.

The results obtained using light-reflective technique can be compared with the results obtained using microelectrodes (Figure 22). With microelectrodes, only one egg was used to record the heart rate

Table 2

_
Minute
Per
(Beats
Rate
Heart
Embryos'
Chick
of
Data

Embryo		3 day	-old			4 day-	plo.		5 day-ol	**D		6 day-	old			7 day-	plo	
Number	wit	ų	with	out	with	ſ	witho	nt			with		witho	ut	with		witho	ut
	memb	ranes	memb	ranes	membr	ranes	membr	anes	without m	nembranes	membr	anes	membr	anes	membr	anes	membr	anes
	Init.	After	Init.	After	Init.	After .	Init.	After	Initial	After	Init.	After	Init.	After	Init.	After	Init.	After
	Record.	10 min.	Record.	10 min.	Record.	10 min.	Record.	10 min.	Recording	10 minutes	Record.	10 min.						
-	108	114	66	96	144	144	140	140	168	192	192	221	061	190	225	228	204	216
2	108	Ξ	96	96	146	146	156	156	180	186	192	204	221	216	220	224	216	216
3	112	114	60	60	157	160	146	152	176	180	204	209	204	204	228	234	216	220
4		Ξ	105	96	156	156	140	144	192	188	204	204	185	190	228	240	216	204
5	105	107	105	93	'	'	ŀ	ı	192	196	'	'	204	200	234	240	216	216
9	1	1	ı	I	ī	ı	ı	1	192	192	ı	,	'	ı	1	,	ı	,
Avg. of Heart Rate	f 109	Ξ	66	94	151	152	146	148	183	189	198	210	201	200	227	233	214	214

\*\* Studies without the membranes were the only ones done in 5 day-old embryos.





3 day-old Chick Embryo's Heart Rate Recordings

a - with the membrane, filter cut off at 20 Hz b - without the membrane, filter cut off at 20 Hz  $\,$ 





4 day-old Chick Embryo's Heart Rate Recordings

a - with the membrane, filter cut off at 20 Hz b - without the membrane, filter cut off at 20 Hz  $\,$ 



Figure 18

5 day-old Chick Embryo's Heart Rate Recording (without the membrane)





6 day-old Chick Embryo's Heart Rate Recording a - with the membrane, filter cut off at 20 Hz b - without the membrane, filter cut off at 20 Hz  $\,$ 





# Figure 21 Artifacts

as a function of embryo age.<sup>24</sup> With light-reflection, a different embryo was used in every recording as a function of embryo age. As shown in figure 22, using microelectrodes a better and more accurate heart rate recording was obtained. The principal reason for the low heart rate output using light (infrared)-reflective techniques was the cooling down of the embryo due to the opening of the incubator door. Figures 23 shows the effect of opening the incubator door for only 60 seconds.<sup>24</sup> Since the incubator door was opened several times

# Table 3

# Average of Heart Rate

# (After 10 minutes of recording)

Days Old	With Membrane (BPM)	Without Membrane (BPM)
3	111	94
4	152	148
5		189
6	210	200
7	233	214





Heart Rate vs. Incubation Time

--- Using microelectrodes

– Using light-reflective technique



(60 seconds)

to position the transducer at the right place (to be able to obtain the recordings), the heart rate of the embryo was 30 or more beats/ minutes less than the normal rate. To illustrate this problem better, figure 24 shows the change in heart rate as a function of the change of temperature in the incubator. From this graph, a decrease in temperature decreases the heart rate of the chick embryo. When a recording was obtained, the incubator temperature had dropped from 37.5°C to around 34°C; so, the results obtained were greated affected by the changes in temperature.





Effect of Incubation Temperature on Heart Rate

Positioning the transducer at the right place was a problem found in 3 and 4 day-old embryos. Only one out of five embryos' heart rate were recorded. This was mainly due to the size of the embryo in comparison to the size of the transducer. Figure 25 shows a possible reason for this problem. In figure 25-a, the transducer is placed perpendicular to the embryo; the range of reflection is too big and covers the entire embryo; the recordings cannot be obtained because the difference in intensity of reflected light from the embryo's vessels cancels out due to total body reflection (being detected by the infrared phototransistor. The way to obtain the recordings for younger embryos is shown in figure 25-b, where the transducer was placed at one side away from the embryo and very close to the amniontic fluid surrounding the embryo. In this way, only one portion of the embryo's vessels is reflected back to the transistor, making possible the recording of the heart rate. This problem didn't affect older embryos (6 or 7 days old), where the range of detection of the transducer only covered one portion of the embryo (figure 25-c).





С

#### Figure 25

Range of Detection by the Infrared Transistor

a and b - 3-4 day-old embryos

c 6-7 day-old embryos

To obtain a clear and readable signal, the transducer had to be very close to the embryo. Due to this fact, an artificial membrane was not used, making impossible an effective  $0_2$ ,  $C0_2$  and water vapor exchange. Also, the transducer used was not sterilized, being a possible source to cause infection.

Taking in consideration effects of all the factors mentioned above (temperature changes, lack of artificial membrane, no sterilization), the chick embryos' heart rate recordings were available for a limited time, before they dried out - dying eventually. So, a continuous recording for several days, using the same embryo, was difficult to obtain. These effects are shown for a 7 day-old embryo in figure 26, where the heart rate stayed fairly constant the first 20 minutes, but dropped 32 beats/minute in one hour and 32 beats/minute more in the next 2 hours.

#### CONCLUSIONS

The light-reflective technique, for recording the heart rate of chick embryo, obeys Kelvin's law better than any other method of recording; but some adjustments need to be made in order to decrease the external environmental factors (temperature, sterilization, dryness of the embryo).

Some improvements are enumerated as follows:

\* Attach to transducer a colummeter to reduce the area of reflection to a very small spot of the embryo. This eliminates the



Heart Rate as a Function of Incubation Time (7 day-old embryo)

a)	At	10	minutes;	heart	rate	=	237	BPM
b)	At	1	hour;		HR	Ξ	205	BPM
c)	At	3	hours;		HR	=	174	BPM

problem presented in figure 23 for detecting the signal in a shorter time for younger embryos (3-4 days old).

\* Use of a thermally sealed incubator to decrease the effects of temperature changes on heart rate (figure 22).

\* Use of an artificial, transparent membrane or an artificial membrane adapted with a colummeter to provide  $0_2$ ,  $C0_2$  and water vapor exchange.

All these improvements may possibly help to obtain better recordings of the same embryo for several days. So by using these improvements a more reliable data may possibly be obtained to be used as a base model for future studies.

This research was restricted for recordings obtained in a backscattered mode; future studies may use a trans-illuminated technique (placing the emitting source on the bottom of the egg).

This new method for recording the heart rate of the chick embryo could be used to study the effects of drugs and radiation on the embryo's heart. For example, this technique may be used to determine the effects of ultrasonic radiation in the chick embryo's heart, in order to determine a more reliable data regarding the effects of ultrasound.<sup>25</sup>

#### REFERENCES

- Ruckman, R. N., R. J. Cassling, E. B. Clark, and G. C. Rosenquist. "Cardiac Function in the Embryonic Chick." <u>Perspective in Cardio-vascular Research</u>. Volume V. New York: Tomas Pexieder, Raven Press, 1981, p. 407.
- 2) Pizer, H. and C. Palinske. <u>Coping with a Miscarriage</u>. New York, New York: The Dial Press New York, 1980, pp. 10-16, 43-61.
- 3) Willson, R. J., C. T. Beechan, and E. R. Carrington. <u>Obstetrics</u> <u>and Gynecology</u>. Fifth Edition. Saint Louis: The C. V. Mosby Company, 1975, pp. 181-186.
- 4) Potter, E. L., and J. M. Craig. <u>Pathology of the Fetus and the</u> <u>Infant.</u> Third edition. Chicago, <u>Illinois: Year Book Medical</u> <u>Publishers</u>, Inc., 1975, pp. 62-64.
- 5) Pritchard, J. A., and P. C. Macdonald. <u>Williams Obstetrics</u>. Fifteenth Edition. New York, New York: Appleton-Century Crafts, 1976, pp. 483-488.
- 6) Hellman, L. M., and J. A. Pritchard. <u>Williams Obstetrics</u>. Fourteenth Edition. New York, New York: <u>Appleton-Century-Crofts</u>, 1971, pp. 493-509.
- 7) Wallace, H. M., E. M. Gold, and A. C. Oglesby. <u>Maternal and child health practices</u>: problems, resources, and methods of delivery. Second Edition. New York: Wiley, 1982, p. 290.
- 8) Rajala, J. M., and S. Kaplan. "The Formation of Caudal Hematomes in Trypan Blue-Treated Chick Embryos as a Function of Morphological Stage at Treatment." Teratology. 21:265, 1980.
- 9) Gilbert, E. F., H. J. Bruyre, S. Ishikawa, L. M. Foulke, and S. R. Heismann. "Role of Decreased Cardiac Output in Isoprotenerol-Induced Cardiovascular Teratogenesis in Chick Embryos." Teratology. 21:209, 1980.
- 10) Gilbert, E. F., H. J. Bruyere, S. Ishikawa, M. O. Cheung, and R. J. Hodach. "The Effect of Pratodol and Butoxamine on Aortic Arch Malformation in Beta Adrenoreceptor Stimulated Chick Embryos." Teratology. 15:317, 1977.
- 11) Gilbert, E. F., H. J. Bruyere, S. Ishikawa, and M. O. Cheung. "Aortic Aneurysm Associated with Cardiac Defects in Theophylline Stimulated Chick Embryos." Teratology. 18:23, 1978.

- 12) Cheung, M. O., E. F. Gilbert, H. J. Bruyere, S. Ishikawa, and R. J. Hodach. "Chronotropism and Blood Flow Patterns Following Teratogenic Doses of Catecholamines in 5 day-old Chick Embryos." Teratology. 16:337, 1977.
- 13) Burrow, N. G. and T. F. Ferris. <u>Medical Complications During</u> <u>Pregnancy</u>. Philadelphia: W. B. Saunders Company, 1975, pp. 814-819.
- 14) Morison, J. E. Foetal and Neonatal Pathology. Third Edition. New York: Appleton-Century-Crofts, 1970, pp. 161-164.
- 15) Fry, F. J. "Biological Effects of Ultrasound A Review." IEEE Proc. 67:604, 1977.
- 16) Dyson, M., J. B. Pond, B. Woodward, and J. Broadbent. "The Production of Blood Cell Stasis and Endothelial Damage in the Blood Vessels of Chick Embryos Treated with Ultrasound in a Stationary Wave Field." Ultrasound Med. Biol. 1:133-134, 1974.
- 17) Dyson, M., B. Woodward, and J. B. Pond. "Flow of Red Blood Cells Stopped by Ultrasound." Nature (London). 232, 572, 1971.
- 18) Weinman, J., A. Hayat, and G. Raviv. "Reflection photoplethysmography of arterial-blood-volume pulses." Med. & Biol. Eng. & Comput. 15:22-23, 1977.
- 19) Tahmoush, A. J., R. Jennings, A. L. Lee, S. Camp, and F. Weber. "Characteristics of a Light Emitting Diode-Transistor Photoplethysmograph." (Instrumentation). Psychophysiology. 13:357-360, 1976.
- 20) Lee, A. L., A. J. Tahmoush, and J. R. Jennings. "An LED-Transistor Photoplethysmograph." IEEE Transactions on Biomedical Engineering. May:248-250, 1975.
- 21) Silonex Inc. "Handout of semiconductor sensitive devices." Plattsburgh, New York, 1983.
- 22) Oppenheim, R. W., H. L. Levin, and M. S. Harth. "An Investigation of Varios Egg-Opening Techniques for Use in Avian Behavioral Embryology." Developmental Psychobiology, Vol. 6, No. 1, 1973, pp. 53-68.
- 23) Kao, C. "Optical Fiber Technology, Vol. II." Physics Today. March:66-67, 1982.
- 24) Hunter, J. Unpublished Research Data. (Personal Communication).

- 25) Lele, P. P., J. W. Sofia, and P. Svetz. "Studies on the Effects of Ultrasound on Cell Growth and Division." Lab. for Med. Ultrasonics, Massachusetts Institute of Technology, Cambridge, Mass. 02139, pp. 96-97.
- 26) Adolph, Anne. Unpublished Report. (Personal Communication).
- 27) Johnson, D. E., and V. Jayakumar. <u>Operational Amplifier Circuits</u> <u>Design and Application</u>. New Jersey: Prentice-Hall, Inc., Englewood Cliffs, 1982, pp. 77-81.

Appendix 1

# AO<sup>°</sup> Flexible Light Guides

# Standard Single Light Guides

AO flexible light guides carry light around bends and corners to provide illumination precisely where it is required. Light guides are particularly useful in illuminating remote or inaccessible areas that are normally difficult or impossible to inspect. They can be used to transmit signals or sense light, and are also useful in detection and control systems.

American Optical manufactures light guides in a range of lengths and diameters. A new, larger diameter light guide, the LGM-4, is protected with PVC-covered steel monocoil for added durability. All AO light guides have stainless steel end tips.

The numerical aperture of the fiber is 0.56, and the acceptance angle of light is 60°. Fresnel reflection and effective fiber core account for about 30% loss of light at the ends. Light is also attenuated in the fiber at the rate of 8% per foot.

AO light guides transmit through the visible spectrum as shown in Figure I, and in the near infrared out to a wavelength of 1.5 microns, including the critical 900 NM region.

# **Branch Light Guides**

AO can supply standard two-branch light guides for use in applications where the subject must be illuminated from two directions to eliminate shadows. Branches are joined at a common end, designed for insertion into the AO II-80 Illuminator. Light guide branches are available in lengths of 18", 24" and 36". Branches are protected with flexible gooseneck type sheathing that can be pre-formed to meet a wide variety of positioning requirements.

#### Ultraviolet Transmitting Light Guides

UV light guides are used when it is necessary to transmit UV light down to about 350 NM. The numerical aperture is 0.44. UV light guides are available in the same sizes as the standard LGM light guides.

#### **Special Light Guides**

Custom light guides can be designed and fabricated according to customer specifications, including diameter, length, end tip configuration and type of sheathing.





#### LIGHT GUIDE SPECIFICATIONS (NOMINAL)

			B	end		End Tip Diameters			Fiber	Tip		
Cat. Number	Sheathing	g	Ra	adius		Max. O	.D.	Tip O.D.	O.D.	Length		
LGM-1	PVC		3/	8 in. 9.5 mm		0.032 0.81	2 in. mm	0.032 in. 0.81 mm	0.02 in. 0.5 mm	0.38 in. 9.5 mm		
LGM-2	PVC		5/ 15	8 in. 5.9 mm		0.160 4.0 דו	) in. 1m	0.110 in. 2.8 mm	0.062 in. 1.9 mm	0.5 in. 12.7 mm		
LGM-3	PVC		3/ 19	4 in. 9.0 mm		0.250 7.9 m	) in. 1m	0.167 in. 4.2 mm	0.125 in. 3.2 mm	0.5 in. 12.7 mm		
LGM-4	PVC/Mor	10	1- 38	1/2 in. 3.1 mm		0.375 9.5 m	5 in. nm	0.250 in. 6.3 mm	0.177 in. 4.5 mm	0.5 in. 12.7 mm		
LGM-5	PVC			1-1/2 in. 31.7 mm		1-1/2 in. 31.7 mm		0.406 in. 10.3 mm		0.316 in. 8.0 mm	0.250 in. 6.4 mm	0.5 in. 12.7 mm
Standard Lengths	s Available											
	Inches CM	12 30.5	24 61.0	36 91.4	48 121.9	60 152.4	72 183.0					

For ACMI style end fitting, order with #402119 end tip (1). Tip O.D. is 0.299 in. (7.6 mm), tip length is 0.320 in. (8.1 mm).

# Image Conduit

AO<sup>®</sup> image conduit transmits an image from one end of a rigid fiber optic rod to the other end. The image is displayed on the face of the rod.

Image conduit can be bent in almost any shape to fit almost any space requirement. The small diameter fibers can be bent to very small radii with a minimum loss of light transmission. Bend it as you would any other glass rod by heating over a Bunsen burner or in a glass oven while applying pressure.

Image conduit is supplied with ground and polished ends.

In addition to the sizes listed here, custom lengths and cross sections can be manufactured to customer specifications.

Cat. Number	Cross Sec. (inches)	Fiber Size (microns)	Length (inches)
IC-50-6	1/16 round (1.6 mm)	25	6 (15.2 cm)
IC-50-12	1/16 round (1.6 mm)	25	12 (30.5 cm)
IC-100-6	1/8 round (3.2 mm)	50	6 (15.2 cm)
IC-100-12	1/8 round (3.2 mm)	50	12 (30.5 cm)
IC-200-6	1/4 round (6.35 mm)	100	6 (15.2 cm)
IC-200-12	1/4 round (6.35 mm)	100	12 (30.5 cm)

# Clad Rod

Rigid clad rod is the simplest and most economical form of fiber optics for light transmission.

The rods are used to isolate or remotely locate photosensors and light sources.

Clad rod is supplied with ground and polished ends and can be bent with heat.

Custom diameters and lengths can also be fabricated.

Cat. Number	Diameter (inches)	Length (inches)
CR-05-6	1/16 (1.6 mm)	6(15.2cm)
CR-05-12	1/16 (1.6 mm)	12 (30.5 cm)
CR-1-6	1/8 (3.2 mm)	6 (15.2 cm)
CR-1-12	1/8 (3.2 mm)	12 (30.5 cm)
CR-2-6	1/4 (6.35 mm)	6 (15.2 cm)
CR-2-12	1/4 (6.35 mm)	12 (30.5 cm)

Specifications are subject to change without notice

SBLGM 1/79



write or call:

American Optical SCIENTIFIC INSTRUMENT DIVISION

FIBER OPTICS • SOUTHBRIDGE, MA 01550 (617) 765-9711 Ext. 2445



Appendix 2



## PHOTOVOLTAIC DETECTOR CIRCUIT



1) Calibrated Light Source

- 2) Chopper
- 3) Distance (d) source to detector
- 4) Feedback resistor  $R_{F}$
- 5) Oscilloscope
- 6) Wave analyzer

Shetland Industrial Center, Building 4 / York Street / Box 65 / Shawsheen Village Station Andover, Massachusetts 01810 / Telephone (617) 475-5982 / Telex 94-7150 GPD ANDR

## GERMANIUM PHOTODIODES

GM Series: Photodiodes may be used in photovoltaic or photoconductive mode. Applications include optical communications, optical power measurements, spectrometers, fiber attenuation, etc.

## TYPICAL ELECTRICAL CHARACTERISTICS



Andover, Massachusetts 01810 / Telephone (617) 475-5982 / Telex 94-7150 GPD ANDR

#### GERMANIUM PHOTODIODE

Specifications Active dia.	.3 mm
Spectral Response	.5 - 1.8 um
Peak Response	1.5 um
Responsivity	.7 A/W
Temperature	-10 to +75°C

# TYPICAL ELECTRICAL CHARACTERISTICS AT 25°C.

Breakdown Voltage	25 V
Dark Current (@ 10 V)	1.0 uA
Capacitance (@ 6 V)	3.0 pF
N.E.P. (1.5, 500, 1)	0.8 x 10 <sup>-12</sup> W/ HZ
Cutoff Frequency	350 MHz
Rise Time	l n sec

#### TYPE: GM/4



#### ANODE MARKED RED





Shetland Industrial Center, Building 4 / York Street / Box 65 / Shawsheen Village Station Andover, Massachusetts 01810 / Telephone (617) 475-5982 / Telex 94-7150 GPD ANDR

#### GERMANIUM PHOTODIODE

Active dia.	1 mm
Spectral Response	.5 - 1.8 um
Peak Response	1.5 um
Responsivity	.7 A/W
Temperature	-10 to +75°C

TYPE: GM/5



# TYPICAL ELECTRICAL CHARACTERISTICS AT 25°C.

Breakdown Voltage	25 V
Dark Current (@ 10 V)	5.0 uA
Capacitance (@ 6 V)	50 pF
N.E.P. (1.5, 500, 1)	3.3 X 10 <sup>-12</sup> W/ HZ
Cutoff Frequency	35 MHz
Rise Time	l0 n sec

ANODE MARKED RED

# TYPICAL CIRCUIT CONFIGURATIONS



Shetland Industrial Center, Building 4 / York Street / Box 65 / Shawsheen Village Station Andover, Massachusetts 01810 / Telephone (617) 475-5982 / Telex 94-7150 GPD ANDR Appendix 3



 $\triangleleft$