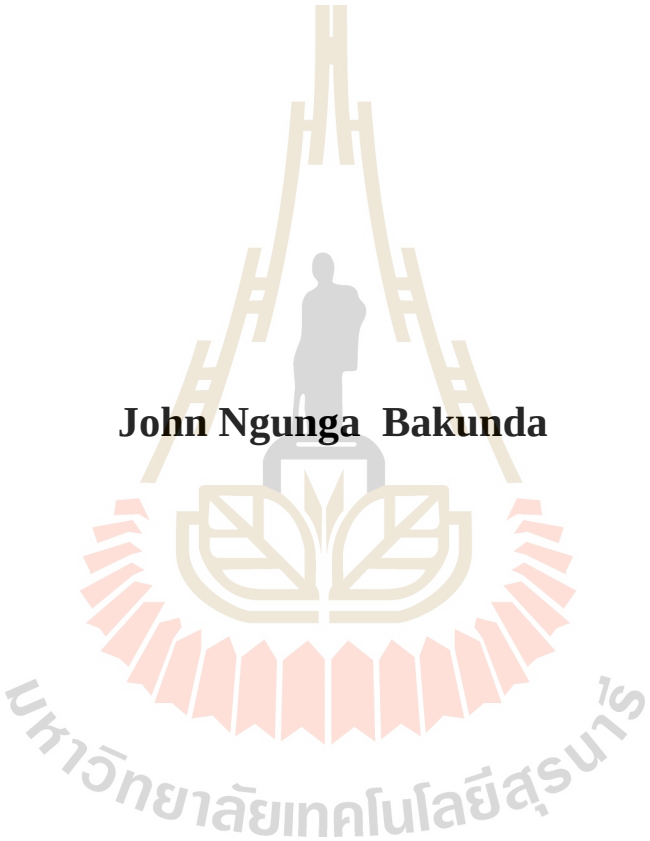
NON-CONTACT EMBRYONIC HEART RATE MEASUREMENT OF MULTIPLE EGGS USING A DIGITAL CAMERA

John Ngunga Bakunda



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วิธีการวัดแบบไม่สัมผัสสำหรับการวัดอัตราการเต้นของหัวใจของลูกไก่ในไข่หลายฟองพร้อมกัน

นายจอห์น นกุนกา บากุนดา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
สาขาวิชาวิศวกรรมไฟฟ้า
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ปีการศึกษา 2561

NON-CONTACT EMBRYONIC HEART RATE MEASUREMENT OF MULTIPLE EGGS USING A DIGITAL CAMERA

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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นายจอห์น นกุนกา บากุนดา : วิธีการวัดแบบไม่สัมผัสสำหรับการวัดอัตราการเต้นของหัวใจของลูกไก่ในไข่หลายฟองพร้อมกัน (NON-CONTACT EMBRYONIC HEART RATE MEASUREMENT OF MULTIPLE EGGS USING A DIGITAL CAMERA) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.บุญส่ง สุตะพันธ์, 118 หน้า.

ปัจจุบันอุตสาหกรรมการเลี้ยงไก่ การฟักไข่เพื่อผลิตลูกไก่จะใช้ตู้ฟักไข่ซึ่งจะทำให้ผลิตลูกไก่ได้ปริมาณมากและมีต้นทุนต่ำ การคัดแยกไข่ไก่มีเชื้อพร้อมฟักและไม่มีเชื้อจะใช้เทคนิคการส่องไข่ เทคนิคการส่องไข่เป็นเทคนิคที่ทำได้ง่ายและไม่ใช้เครื่องมือที่มีราคาแพง อย่างไรก็ตามเทคนิคการส่องไข่ไม่สามารถจำแนกระหว่างไข่มีลูกไข่ตายกับลูกไก่ที่มีชีวิตอยู่ภายในไข่ได้ เนื่องจากไข่ทั้งสองกรณีจะทึบแสงใกล้เคียงกัน งานวิจัยนี้จึงได้พัฒนาต้นแบบระดับปฏิบัติการที่สามารถวัดอัตราการเต้นหัวใจของลูกไก่ในไข่แบบที่สามารถวัดพร้อมกันได้หลายฟองได้ เทคนิคการวัดที่พัฒนาขึ้นเป็นเทคนิคที่ไม่ส่งผลกระทบต่อลูกไก่และไม่สัมผัสกับไข่โดยตรง

ในงานวิจัยนี้ได้ใช้ต้นแบบที่พัฒนาขึ้นในการวัดอัตราการเต้นหัวใจของลูกไก่ในไข่ทั้งหมด 115 ฟอง โดยวัดในช่วงวันที่ 0-18 ที่ไข่อยู่ตู้ฟักไข่ เครื่องมือที่พัฒนาขึ้นสามารถวัดอัตราการเต้นหัวใจของลูกไก่ได้ตั้งแต่วันที่ 3-18 ของการฟัก อัตราการเต้นของหัวใจของลูกไก่มีค่าเพิ่มขึ้นตามระยะเวลาในการฟัก โดยมีอัตราการเต้นของหัวใจเฉลี่ยอยู่ในช่วง 154-310 ครั้งต่อนาที การยืนยันผลการวัดของต้นแบบที่พัฒนาขึ้นจะใช้เครื่องวัดอัตราการเต้นหัวใจแบบใช้ไฟฟ้า (Electrocardiography) พบว่าผลการวัดอัตราการเต้นของหัวใจมีค่าใกล้เคียงกันสำหรับทั้งสองวิธี

เครื่องมือที่พัฒนาขึ้นในงานวิจัยนี้สามารถนำไปใช้ประโยชน์ทั้งในอุตสาหกรรมการเลี้ยงไก่และการศึกษาวิจัยด้านเทคโนโลยีการผลิตสัตว์ อุตสาหกรรมการเลี้ยงไก่สามารถใช้เครื่องมือนี้ในการจำแนกไข่มีเชื้อพร้อมฟักออกจากไข่ไม่มีเชื้อพร้อมฟัก และสามารถใช้แทนเทคนิคการส่องไข่ได้ นักวิจัยสามารถเทคโนโลยีการผลิตสัตว์สามารถใช้วัดอัตราการเต้นของลูกไก่ใข่ระหว่างการฟัก เพื่อศึกษาความสัมพันธ์ระหว่างอัตราการเต้นของหัวใจกับสุขภาพของไก่เลี้ยงหรือเพศของลูกไก่ได้

สาขาวิชา วิศวกรรมอิเล็กทรอนิกส์ ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2561 ลายมือชื่ออาจารย์ที่ปรึกษา _____

JOHN NGUNGA BAKUNDA : NON-CONTACT EMBRYONIC HEART RATE MEASUREMENT OF MULTIPLE EGGS USING A DIGITAL CAMERA. THESIS ADVISOR : ASST. PROF. BOONSONG SUTAPUN, Ph.D., 118 PP.

HEART RATE DETECTION/CHICKEN EMBRYO/NON-CONTACT MEASUREMENT/ FERTILE EGGS/ INFERTILE EGGS/DIGITAL CAMERA.

Artificial incubation of chicken eggs is the economic procedure in the poultry industry. Early detection of infertile and dead embryos could add value to the poultry industry. Candling is widely used to check for the incubated multiple eggs' fertility and embryos development. It cannot precisely sort out the dead embryos especially when the embryo is large and dark. In this work, we designed the lab-scale noninvasive and non-contact optical equipment that could measure the embryonic heart rate of the multiple incubated chicken eggs at the same time by using a digital camera.

We measured the embryonic heart rate of 115 incubated chicken eggs in total for days 0−18 of incubation period. We could recover the embryonic heart rate of multiple incubated eggs from day 3−18 of the incubation period. The measured embryonic heart rate ranged from 151−315 beats per minute. The electrocardiography method was used to validate the results from our optical equipment. The two methods showed good agreement in their embryonic heart rate results.

The developed system could offer good benefits to both, the poultry industry and animal science researchers. The poultry industry might use this system to precisely identify hatching eggs from non-hatching eggs. This system could replace the candling method. The animal science researchers might also use this system for studying

purposes. For instance, they may research the usefulness of the embryonic heart rate data to the health status and sex of the hatched chicks. Such information could help the poultry farmers decide which chicks to keep or discard to gain more economic advantages.

School of Electronic Engineering

Academic Year 2018

Student's Signature

Advisor's Signature

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John Ngunga Bakunda

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

SYMBOLS AND ABBREVIATIONS

ACG	=	Acoustocardiogram
AVI	=	Audio Video Interleave
BCG	=	Ballistocardiogram
BPM	=	Beats Per Minute
CMOS	=	Complementary Metal-Oxide Semiconductor
DFT	=	Discrete Fourier Transform
ECG	=	Electrocardiograph
FFT	=	Fast Fourier Transform
HD	=	High Definition
HDD	=	Hard Disk Drive
HR	=	Heart Rate
ICG	=	Impedance Cardiograph
LabVIEW	=	Laboratory Virtual Instrument Engineering Workbench

CHAPTER I

INTRODUCTION

The chicken embryo is a fundamental early stage of the chick development within the chicken egg. The chicken embryo has been a widely used experimental animal in physiological and developmental biology research (Nowak-Sliwinska, Segura, & Iruela-Arispe, 2014;T. Sugiyama, H. Miyazaki, K. Saito, H. Shimada, & K. Miyamoto, 1996). It is often considered as a useful model for studying physiological responses and adaptation to the altered environments as well as for neurobiology research (Youssef, Viazzi, Exadaktylos, & Berckmans, 2014).

A fertile chicken embryo can either develop and hatch into a chick within 20–21 days of incubation or die before hatching due to several reasons such as failure to manage the incubation parameters, poor eggs storage, poor hygiene and bacterial attacks, improper turning of eggs during incubation, long time power failure or poor ventilation. The infertile and dead chicken embryos cannot hatch at all. The process of timely monitoring of fertility and hatchability status of artificially incubated chicken eggs could be essentially and of economic value to the poultry industry; hence it must be given higher priority. Measuring and monitoring the embryonic HR of incubated chicken eggs, using a method that offers affordable and accurate solution to assess the hatchability status of the artificially incubated chicken eggs could add value poultry industry practices. Normally, incubated fertile and hatching chicken eggs will have detectable embryonic HR pulses while dead and infertile eggs will have not.

Sorting out infertile and dead incubated chicken eggs (no-hatching eggs) from

the incubator is a crucial step during the artificial incubation processes as we can save incubator's space as well as ensure safe hatching environment. Sometimes, eggs whose embryos are dead can explode in the incubator, spread infection to the good eggs, hatchlings, incubator, hatching baskets and cause unpleasant smell throughout the environment.

Traditionally, "candling" of eggs is the popular and widely used method throughout the poultry industry to assess the fertility status and development of the embryos among incubated chicken eggs. This process is a dark-field illumination similar to that shown in Figure 1.1. Light absorption, reflection and transmission from the internal structure of eggs could help the experienced observer to identify hatching and non-hatching incubated chicken eggs by visual inspection. During candling, all the source's light must reach the observer's eyes after passing through the eggs. It is expected that; fertile eggs would cast visible shadows under dark-field illumination while infertile eggs will not. Important note is that, the observed shadow inside an egg does not surely inform whether the embryo is alive or dead. It is even harder to tell during the last week of incubation period where the embryo is larger and darker. Some eggs may require more time and experienced observer to predict for their fertility and hatchability status, which keep them outside the incubator for a long time. This may affect the embryos' development, growth, increase hatching time or cause embryo's death. However, candling is an effective method and can assess many eggs in a short time. Candling is also used at Suranaree university of Technology poultry farm.

Apart from candling, there are reported scientific methods that utilized sensors available in the market to measure the embryonic HR of an incubated chicken egg.

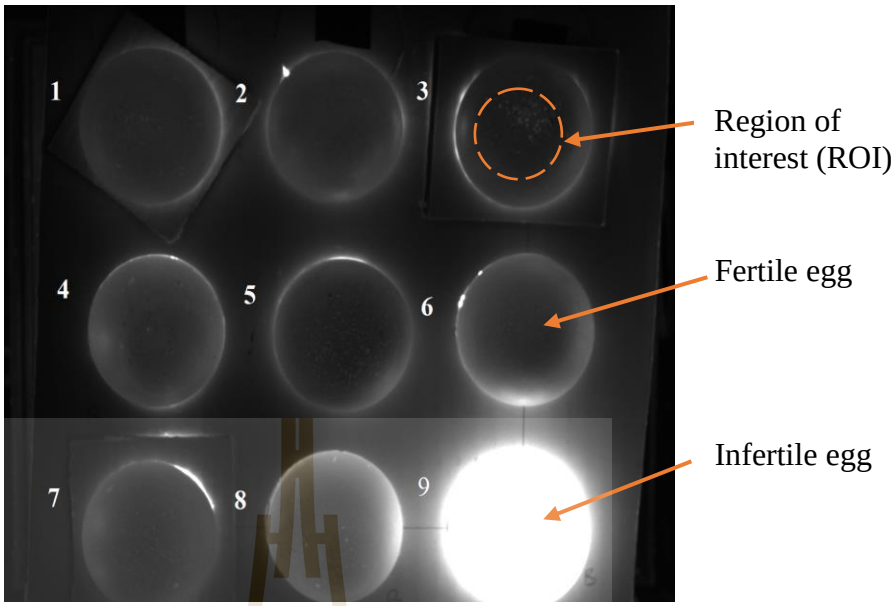


Figure 1.1 Dark-field illumination of incubated chicken eggs

This was a good step towards finding a sure indicator of hatching incubated eggs. The available sensors fall into two major categories, invasive and noninvasive. Invasive sensors fall into two subgroups, purely invasive and semi-invasive.

Popularly reported invasive methods for detecting cardiogenic signals and measure the embryo's HR include catheterization and arterial blood pressure (Haque, Watanabe, Ono, Sakamoto, & Tazawa, 1994;Hochel et al., 1998) which involved cutting the embryo's blood vessels and connecting the measurement instrument. Implementation and embryonic HR measurement using these methods is possible for one egg at a time, but the embryo dies a few hours after the experiment. The methods are not practical for multiple embryos HR measurement in the poultry industry where eggs must be examined in multiscale and must remain alive after the experiment.

The reported semi-invasive methods for embryonic HR measurement of the chicken eggs included electrocardiography (ECG) (Bhuiyan, Sevcencu, Struijk,

Kanters, & Graff, 2016; E. et al., 2004; Habermann, Feske, & TÖNhardt, 2008; Haque et al., 1994; Hoff, Kramer, DuBois, & Patten, 1939; Lazzarini Jr & Bellville, 1956; Periasamy, Lindroth, & Thompson, 1988; Pirow, Bilsing, Nichelmann, & Höchel, 1995; T. Sugiyama et al., 1996; Wang, Butler, & Banzett, 1990), impedance cardiogram (ICG) (Haque et al., 1994; Hebrank & De Pauw, 1998b) and pulse oximetry (POM) (Hebrank & De Pauw, 1998a; Lewin, Dörner, & Tönhardt, 1997). These methods involved partial removal of the eggshell and required a dedicated equipment to measure the embryonic HR of one egg at a time. The removed part of the eggshell exposed the developing embryo to the risk of bacterial infection after the experiment. The semi-invasive methods are impractical to the poultry industry since they cannot be used for multiscale embryos examination and they pose bacterial infection risk the examined developing embryos.

The noninvasive methods included acoustocardiogram (ACG) (Rahn, Poturalski, & Paganelli, 1990), ballistocardiogram (BCG) (A. E. Aubert, C. Leribaux, F. Beckers, D. Ramaekers, & D. Berckmans, 2000; Hashimoto, Narita, & Tazawa, 1991; H Tazawa, Hashimoto, Takami, Yufu, & Whittow, 1993), laser imaging (Liu & Ngadi, 2013; Smith, Lawrence, & Heitschmidt, 2008; Hiraguchi Tazawa, Hiraguchi, Asakura, Fujii, & Whittow, 1989) and photoplethysmography (Hashemzadeh & Farajzadeh, 2016; Lierz, Gooss, & Hafez, 2006; Youssef et al., 2014). ACG and BCG methods involved placing an egg in isolated chambers with an appropriate sensor and could be used to measure the embryonic HR of one egg at a time. These methods required expensive equipment for successful embryonic HR measurement implementation, making them impractical for multiscale HR measurements as well as the poultry industry.

Laser speckle contrast imaging (Yang et al., 2013;Yeo, Park, Lee, & Song, 2016) could confirm for the fertility of an incubated egg based on the recorded pictorial map of the blood vessels vasculature. The method could not measure the embryonic HR of the developing chick. It could only detect the fertility in the early days of incubation. It processed one egg at a time. It is not suitable for multiscale embryos examination especially after the first week of incubation.

The photoplethysmograph method is composed of a light source and a photo detector. The digital egg monitor (Lierz et al., 2006) utilized this principle of light absorption and measured the embryonic HR of one egg. The Buddy digital egg monitor is a commercial product. Also, Hashemzadeh and Farajzadeh (Hashemzadeh & Farajzadeh, 2016) built a mechatronic device, which classified fertile and infertile eggs basing on machine vision algorithm. The system detected the fertility of one egg at a time but could not measure the HR of the developing embryo. Both methods are not suitable for multiple embryos HR measurements hence impractical in poultry industry implementation.

Even though, the reported non-invasive methods solved the problem of risking the examined embryo to bacterial infection but still none of them has been reported capable of examining multiple chicken embryos at the same time.

Generally, methods of monitoring the embryonic HR of chicken eggs should be readily affordable, offer low risk of bacterial infections, applicable to multiple eggs at once and easy to perform. All the reported methods do not offer these benefits in a good scale. The use of digital camera makes the experimental setup implementation of multiple embryonic HR measurement of chicken eggs easy to perform at affordable cost.

The objective of our work is to design and implement a noninvasive and non-contact equipment that is capable of measuring the embryonic HR of multiple incubated chicken eggs using a digital camera. Sequential images were captured by CMOS camera, saved in AVI file format in a computer memory for post analysis. Signal processing techniques including moving average, detrend and bandpass filtration were employed to suppress the background noise and the zero frequency (DC) components. Fast Fourier transform method was employed to easy the frequency components analysis and for heart rate extraction of each examined egg. Embryonic HR measurement results of the multiple examined chicken eggs from the designed optical system were compared to the ones obtained from the ECG method for validation purpose.

The designed lab-scale equipment could offer some useful applications to the poultry industry as well as the animal science researchers. The system would accurately help the poultry industry to sort out hatching from non-hatching incubated chicken eggs, save the incubation space and assessment time. The system would help animal science researchers to further the study about the usefulness of the measured embryonic HR information to the health status of the hatched chicks.

CHAPTER II

LITERATURE REVIEW

2.1 Incubation and embryo development

Incubator is basically a box that holds eggs while at the same time maintaining appropriate incubation parameters such as temperature, humidity, and oxygen level that favors the embryo growth until hatching. Incubators have varying capacities and adapters for eggs from different species. For example, incubators found in the SUT poultry farm have capacity of holding 9600−38400 chicken eggs.

Successful hatching of artificially incubated eggs starts from proper storage and careful selection of eggs. Environmental conditions, handling, sanitation, and record keeping are all important factors when it comes to artificial incubation and hatching of eggs. Fertile eggs must carefully be collected and properly stored until they are incubated. Collected eggs should be stored for less than 10 days, at the temperature range of 10−18°C (in a refrigerator), humidity 75% and turned at least once a day. After 7 days of storage, hatchability could decrease by 0.5−1.5% per day. Each day in storage might add one more hour to the incubation time (Boleli, Morita, Matos Jr, Thimotheo, & Almeida, 2016).

Incubation must not include cracked, misshapen, soiled, or unusually small or large eggs. These types of eggs could rarely hatch and may potentially contaminate the good eggs. The stored eggs should be allowed to warm at room temperature for 4-10 hours before setting them into the warm incubator to avoid suffocation.

Incubation temperature must be set to 37.5±0.1 ℃ and humidity range of 55-75%. Incubated eggs should be turned at least five times every 24 hours to avoid suffocation (turning eggs after every hour could be much better). Near the end of incubation period, the eggshell is nearly filled with the embryo and a full incubator requires large amounts of oxygen (Boleli et al., 2016).

The most common causes of embryo mortality before hatching are related with the breeder or storage-incubation problems. Breeder problems are related to the nutritional deficiencies, infectious diseases, management errors and feed toxicity among others. Storage-incubation problems are related to incorrect storage, eggs handling, incubation practices, incubation humidity, incubation temperature, bacterial contamination and genetic abnormalities. A bacterial or fungal infection could be avoided through eggs from clean nest boxes as well as clean incubators. Improving incubation practices could be one of the most controllable ways to increase hatchability. Maintaining humidity and temperature is another difficult part of home incubation (Kasielke, 2007).

During incubation time, the embryo could change in shape and size (growth). Stages of embryo development could help poultry farmers and researchers characterize in which stage of incubation a specific change would occur in an embryo. This information could be very helpful for either studying embryo's behavior or correct problems. Embryo development stages could also be informative for the best days to measure embryonic HR based on the method to be used. Table 2.1 shows some of the more easily identifiable features of each development stage; there are many more subtle changes, but these should help identify the embryo within a day or two of development.

Table 2.1 Embryo growth changes during incubation period

Days	Identifiable Features	Reference
1-3	Blood circulation system, head, brain and trunk.	(Hamburger & Hamilton, 1992)
4-5	Front limbs with differentiable fingers.	(Hamburger & Hamilton, 1992)
6-9	neck, beak, first feather follicle	(Christ & Ordahl, 1995)
10-13	eyelids, egg tooth, claws and leg scales	(Christ & Ordahl, 1995)
14-18	Down covers entire body, head on pipping position, albumen entirely absorbed.	(Hamburger & Hamilton, 1992)
19-21	Beak below air cell, piercing shell, chick emerges from shell.	(Christ & Ordahl, 1995)

2.2 Candling

Candling is highly recommended for small scale poultry farming because it lets the farmer judge how many eggs could be fertile and discard eggs that are infertile or dead. The white-shelled eggs are easiest, but any egg could be candled using either a candler or a high-powered flashlight. The best times to candle eggs are at 7, 10 and 18 days of incubation (David, 2015). These are times when it is easiest to differentiate an infertile egg from a fertile egg and identify any embryos that may have died. A 7-day incubated egg should have blood vessels clearly visible against the shell and a dark spot the size of a quarter where the embryo is in the center of the egg. A 10-day incubated egg would still have visible blood vessels against the shell, but the embryo would be larger. An 18-day incubated egg should be entirely dark

except for the air cell. Blood vessels might still be apparently in some areas or not visible due to the embryo filling the egg and blocking the light. The growing chick might even move in response to the light. An egg that is entirely clear at day 7 is infertile and should be discarded. An egg that has a red blood ring around the bottom 1/3 is considered a dead embryo, no matter the stage of incubation (David, 2015). Other signs of death could be the gas bubbles below the air cell, the dark embryo sticking to the shell on one side of the egg or lack of growth between candlings. Not discarding infertile or dead eggs might result in "exploders" that could explode in the incubator due to bacterial growth and gases. Exploders could coat other eggs with those bacteria. This could result in more infected eggs and a difficulty to clean incubator. It is always advised to break open the dead embryos and look at the contents. This might give important information of why the embryo died and if there is something abnormal (Boleli et al., 2016; Liu & Ngadi, 2013; Roux, 2002). Candling method needs experienced personnel to sort out dead embryos from fertile developing embryos. This type of sorting is by trial and error. There is no assurance of picking up the real eggs whose embryos are dead. We need assurance of sorting out dead embryos by measuring the embryonic HR of the multiple incubated eggs. Example of candling practice is shown in Figure 2.1.

Figure 2.1 Candling of chicken eggs (Roller Julie, 2016)

2.3 Embryonic HR monitoring techniques

During the incubation process, embryonic HR measurement using different methods has been reported starting from day 4 of incubation until hatching as it has been shown table 2.1. Even though, many methods have been reported to recover the embryonic HR of an incubated chicken egg after the first week of incubation.

The non-hatching eggs among the incubated eggs may reach as high as about 10% (Boleli et al., 2016). The rate depends on many factors including incubation environment, eggs' fertility and/or eggs' handling. Non-hatching eggs may be infertile or dead embryos. Many scientific works have considered developing methods to measure embryonic HR of the incubated eggs. Most of these works concentrated on the embryonic HR measurement of one egg at a time; hence making them impractical to the real poultry industry implementation which demands processing hundreds to thousands of eggs. The developed scientific methods are invasive or noninvasive.

2.3.1 Invasive Methods

Invasive methods involved either partial or total sensor installation beneath the eggshell. These methods gave accurate embryonic HR results, but they destroy the eggshell and expose the embryo to the risk of bacterial infection. Some invasive methods could leave the embryo dead a few hours after the experiment.

(a) Blood pressure (BP): This is totally an invasive method. The allantoic artery and vein, floating in the allantoic fluid, are catheterized through a small hole opened on the eggshell. The embryonic HR is measured by a conventional pressure transducer. The embryonic HR has been reported in chicken embryos by day 3 until hatching. Recordings could only be performed over a short period before the embryo dies (Haque et al., 1994). This method is not practical in the real poultry industry because it could not examine multiple embryos at once (every egg needs a dedicated equipment) and an embryo dies after the experiment.

(b) Electrocardiography (ECG): This is a partially invasive method. The content of the egg is an electrically conductive mass. The electrical excitation generated by the heart propagates through the embryo body, the extraembryonic fluids and the egg contents toward the eggshell. This electrical signal (ECG) could be detected with electrodes piercing the eggshell. ECG is reported to detect the embryonic HR starting from week 2 of incubation (Haque et al., 1994; Leribaux et al., 1999; Pirow et al., 1995; Wang et al., 1990). ECG method was used as a golden standard of comparison and validation of our designed optical system.

(c) Impedance Cardiograph (ICG): This is also a semi invasive method. It detected the embryonic HR of an incubated egg from the electrical impedance variation generated by the cardiac activities starting from day 3 through 9 of

incubation (Haque et al., 1994; Hebrank & De Pauw, 1998a). A dedicated equipment is used to measure the embryonic HR of one egg at a time, making it impractical for examining multiple eggs and implementation in the poultry industry where hundreds or thousands of eggs must be examined at once.

(d) Pulse Oximetry (POM): This is also a semi invasive optical method which is reported effective from day 12 of incubation until hatching. It used the LED as a light source and the photodetector lied beneath the eggshell to record the embryonic HR from the variations of the transmitted light intensity through an egg (Hebrank & De Pauw, 1998a; Lewin et al., 1997). Like other semi-invasive methods, it utilized a dedicated equipment to record the embryonic HR of an egg. This method is not practical to implement for multiple embryos HR measurements as well as to the poultry industry.

Generally speaking, invasive methods are applicable for one egg at a time. They are not practical for multiple embryos HR measurements. None of which could be implemented to the poultry industry practice.

2.3.2 Noninvasive with Contact Methods

These methods could not involve the removal of the eggshell, but the sensor touched the eggshell to record the cardiogenic signals. Acoustocardiogram (ACG) is a good example for methods falling in this group. ACG is a noninvasive contact method used for long term embryonic HR measurements. It used the effect of the pulsatile air movement (pressure) across the eggshell, to record the embryonic HR of an embryo. This pressure variation could be detected by microphone (R Akiyama, Ono, Höchel, Pearson, & Tazawa, 1997; Rahn et al., 1990) or differential pressure transducer (Wang et al., 1990). The development of this technique involved placing

an egg in a tightly sealed vessel containing a condenser microphone. It is possible to measure the instantaneous heart rate (IHR) in chicken embryos using ACG method from day 12 till hatching (Ryuichi Akiyama, Matsuhisa, Pearson, & Tazawa, 1999; Rahn et al., 1990). Equipment required for acoustocardiography is expensive, and a special audio-isolated room is necessary for measurement, making it impractical for the poultry industry implementation.

2.3.3 Non-Invasive with Non- Contact Methods

There are methods that could measure the embryonic HR of an incubated egg noninvasively and without touching the embryo. These methods solved the problems introduced to the eggshell by the invasive methods. Non-invasive and noncontact methods could not destroy the natural habitat of the growing embryo and could not pose the risk of bacterial infection to the examined embryo.

(a) Ballistocardiogram (BCG): This is a noninvasive and non-contact method used for long term embryonic HR measurements as shown in Table 2.2. The fertile egg experienced minute movements each time during the cardiac cycles, thus producing ballistic movements of the egg. This method is based on recording slight movements or vibrations of the eggshell, caused by contraction of the embryonic heart, which could be detected by piezoelectric sensors (Ryuichi Akiyama et al., 1999; A. E. Aubert et al., 2000; H Tazawa et al., 1993) or optically via laser interference (Hutchins, 2003; Lawrence et al., 2007; Hiraguchi Tazawa et al., 1989). Since the ballistic movements are minute and easily contaminated by external vibrations, the egg and the transducer should be placed on a floating platform which acts as an anti-vibrator. The costly equipment required for this modality makes this method impractical for the poultry industry. BCG could measure embryonic HR of a

chicken egg starting from day 7 until hatching (Leribaux et al., 1999; Sakamoto, Haque, Ono, Pearson, & Tazawa, 1995; Suzuki, Musashi, & Tazawa, 1989).

(b) Laser Speckle Contrast Imaging (LSCI): This method used for detecting the vital signs of an egg, such as fertility of a chicken egg in the early stages of incubation. The system was based on the measurement of intensity fluctuations of speckles caused by the embryo's blood flow in the intact egg under laser light illumination as shown in Figure 2.2. The system is useful for examining the vasculature in the egg and confirming its fertility from day 2−5 of incubation. Laser speckle contrast imaging could tell the information about the eggs fertility from the acquired blood flow map of the examined eggs. This method could not measure the chicken embryo HR and it also used to examine one egg at a time. Hence, it is not suitable for practical poultry industry as it could not examine multiple embryos at once (Liu & Ngadi, 2013; Smith et al., 2008; Yang et al., 2013).

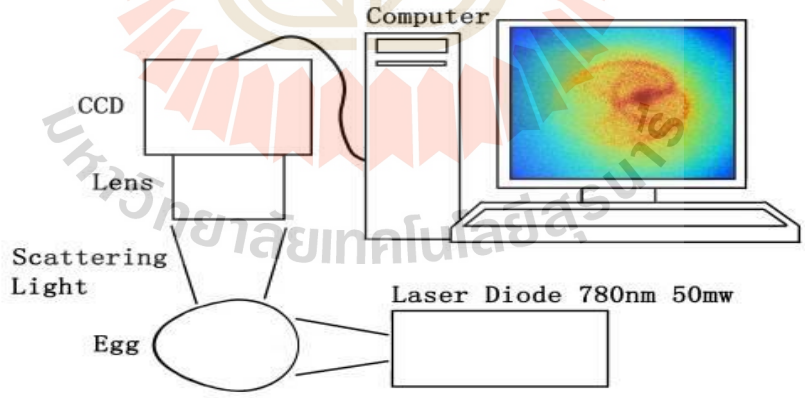


Figure 2.2 Laser speckle imaging system (Yang et al., 2013)

(c) Digital Egg Monitor: This is a method used to monitor the embryonic HR of a chicken egg by measuring the amount of infrared light absorbed by the embryonic blood. It is commercially designed for breeders to investigate the status of

an embryo during the incubation period. If an embryo's HR is reduced, the device could immediately react to alter incubation parameters. The digital egg monitor is as shown in Figure 2.3. The chicken egg embryonic HR was detected starting from day 6 of incubation until hatching (Lierz et al., 2006). The device could not be used for multiple embryos HR measurement.

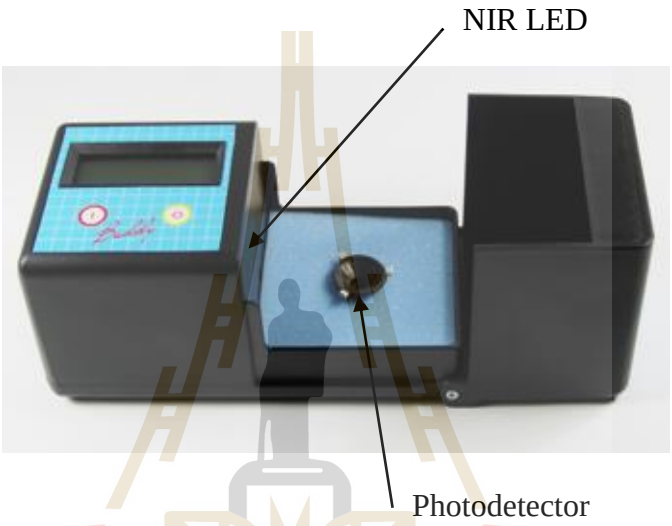


Figure 2.3 Buddy Digital Egg Monitor (Lierz et al., 2006)

We can summarize that; different scientific methods to measure the embryonic HR of the incubated chicken eggs have been reported. The reported methods could measure the embryonic HR measurement of one egg at a time. To the best of our knowledge, no attempt to measure the embryonic HR of multiple incubated eggs has been reported so far. We are hoping that our attempt to measure the embryonic HR of multiple incubated chicken eggs using non-invasive and noncontact machine vision algorithm could stimulate more research works. Table 2.2 shows the reported methods in the literature.

Table 2.2 Methods for embryonic HR measurement for chicken embryos

Name	Characteristic	Accuracy	Application	Reference
BP	Invasive	High	Embryonic HR days 2−21, one egg	(Haque et al., 1994)
ECG	Semi-invasive	High	Embryonic HR days 11−21,one egg	(Leribaux et al., 1999)
ICG	Semi-invasive	High	Embryonic HR days 3−9, one egg	(Haque et al., 1994)
POM	Semi-invasive	High	Embryonic HR days 12−21, one egg	(Lewin et al., 1997)
ACG	Non-invasive	Low, unaffected by embryo activities	Embryonic HR days 12−21, one egg	(Ryuichi Akiyama et al., 1999)
BCG	Non-invasive	Low, affected by embryo activities	Embryonic HR days 7−21, one egg	(A. E. Aubert et al., 2000)
LSCI	Non-invasive	High	Checks fertility days 2−5, one egg	Yang et al., 2013
MV	Non-invasive	High, affected by embryo activities.	Embryonic HR days 3−21, one/multiple eggs	Youssef et al., 2014

2.4 Principles of HR Measurement using Optical Methods

Photoplethysmography (PPG) is a non-invasive method of monitoring the HR by measurements of temporal variation in the blood volume using digital camera. The PPG is composed of a light source and a photodetector, specimen is placed between the dedicated light source and the sensor. PPG waveform is formed by measuring the amount of light passing through the specimen and represent the changes in the shape of the pulse. This phenomenon is caused by absorption of light by the capillaries. PPG signal is composed of a pulsatile component (ac) and nonpulsatile component (dc). ac is synchronized with the heart and related to arterial pulsation, while dc is related to light absorption in the tissue, vein, and diastolic arterial blood volume. Beer–Lambert law explains the mechanisms of PPG (see Figure 2.4). The ac part of PPG signal is generated by the varying optical path length that changes with the refilling of volume in blood vessels as they pulsate (Aldrich, Moosikasuwan, Shah, & Deshpande, 2002).

$$I = I_0 e^{-\varepsilon_\lambda cd} \tag{2.1}$$

Where I is the light intensity of the signal after passing through the medium, I_0 is initial light intensity of the signal, ε_λ is the extinction coefficient or absorptivity, c is fluid concentration and d is light's path length.

The time varying signal could originate from the amount of light intensity absorbed due to the optical path length variation resulted from the blood volume in the vessels during systole and diastole cycles. During systole cycle, blood vessels are filled, optical path length increases, and more light is absorbed. In diastole cycle, blood vessels have less volume of blood, path length decreases, and less light

intensity is absorbed. The sawtooth like variations seen in Figure 2.4 represent the light intensity absorption as a result of optical path length variations. These variations due to light absorption also appear in the transmitted portion of light.

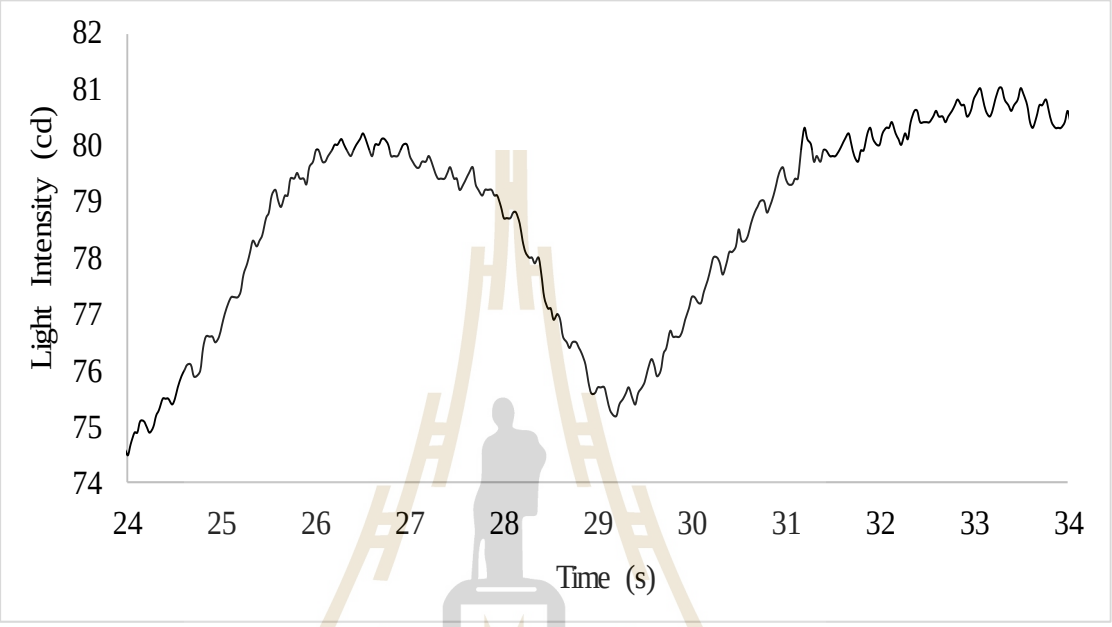


Figure 2.4 Sample zoomed time varying signal

The PPG waveforms obtained from video recording, has been shown to be used for estimating the embryonic HR of the artificially incubated chicken eggs (Youssef et al., 2014). It has also been shown possible to detect the HR from the human face using the digital camera by detecting the subtle color changes on the skin due to blood flow after ambient or fluorescent light illumination (Alghoul, Alharthi, Al Osman, & El Saddik, 2017; Poh, McDuff, & Picard, 2010; Scalise, Bernacchia, Ercoli, & Marchionni, 2012; Wieringa, Mastik, & van der Steen, 2005).

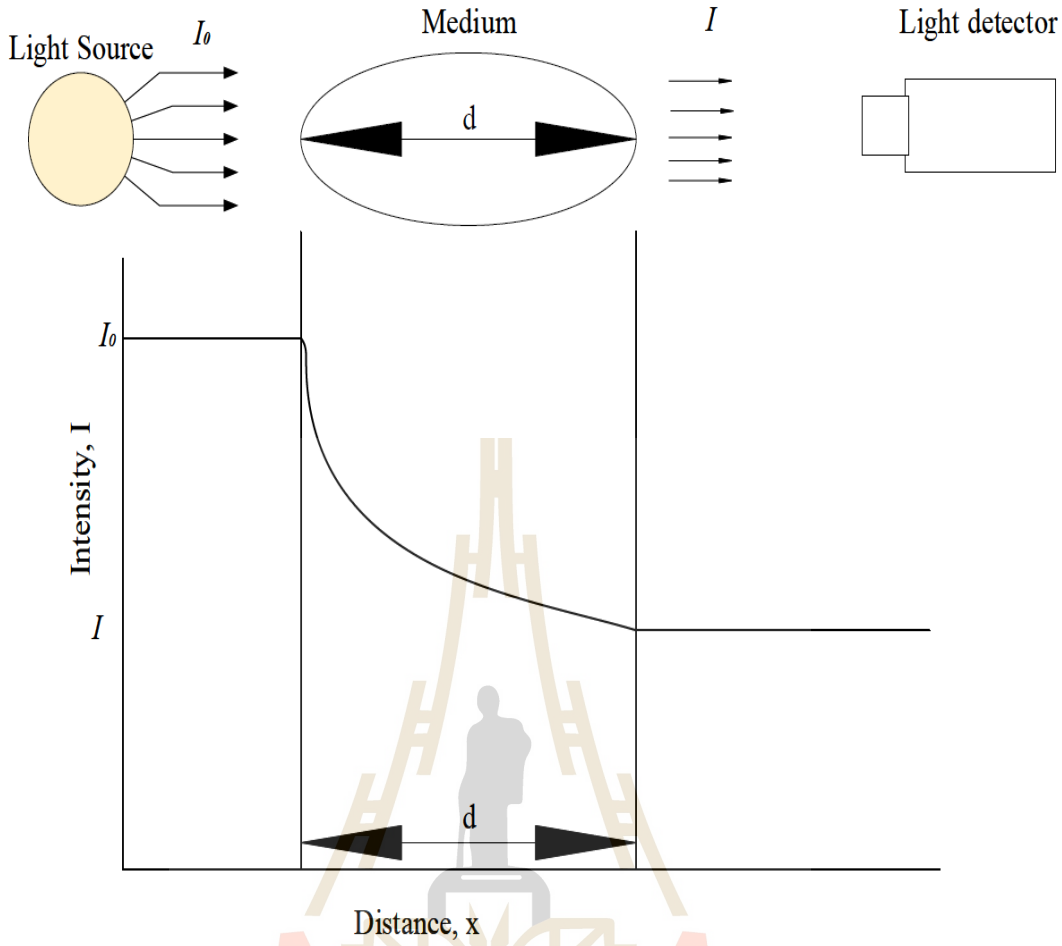


Figure 2. 5 Illustration of Beer Lambert's law

The heartbeat is a periodic activity, so has a measurable frequency. The heartbeat activity has the highest amplitude to all other embryonic activities within the developing embryo. Other periodic embryonic activities include respiration, but it has smaller amplitude than the embryonic HR.

This research work designed a simple non-contact method that allowed identifying and sorting out infertile and dead eggs from the pool of incubated chicken eggs using the principle of light absorption under dark-field illumination. This technique could be used to measure the HR of the live incubated eggs. The detected and measured embryonic HR indicates life of the developing embryo within an egg.

The choice of the light source should consider the transmission spectra of the chicken of the eggshell as well as the absorption characteristic of the blood. The chicken egg transmission spectra are a function of egg size, eggshell thickness, pores distribution, density, conductance, color pigment and genetic makeup of the bird. The eggshell transmits longer wavelength much better than shorter wavelengths. For example, the chicken egg can transmit up to 0.12% of the 1075−nm light and absorb about 99.8% (Shafey, Al-mohsen, Al-sobayel, Al-hassan, & Ghnnam, 2002). The low pole transmits more than the equator and the wide pole regions. For visible spectrum, the spectra transmission is less than 0.1% (Abdel-Nour, Ngadi, Prasher, & Karimi, 2011; Bamelis et al., 2008; Bamelis, Tona, De Baerdemaeker, & Decuypere, 2002; Liu & Ngadi, 2013; Shafey et al., 2002).

Our light sources produced white light (wavelength 400−700 nm). These light sources are commercially available and have various applications in our daily life since they could offer strong light intensity at low power consumption. We selected a white light source because it was already available, and we could predict fertility by visual inspection as well.

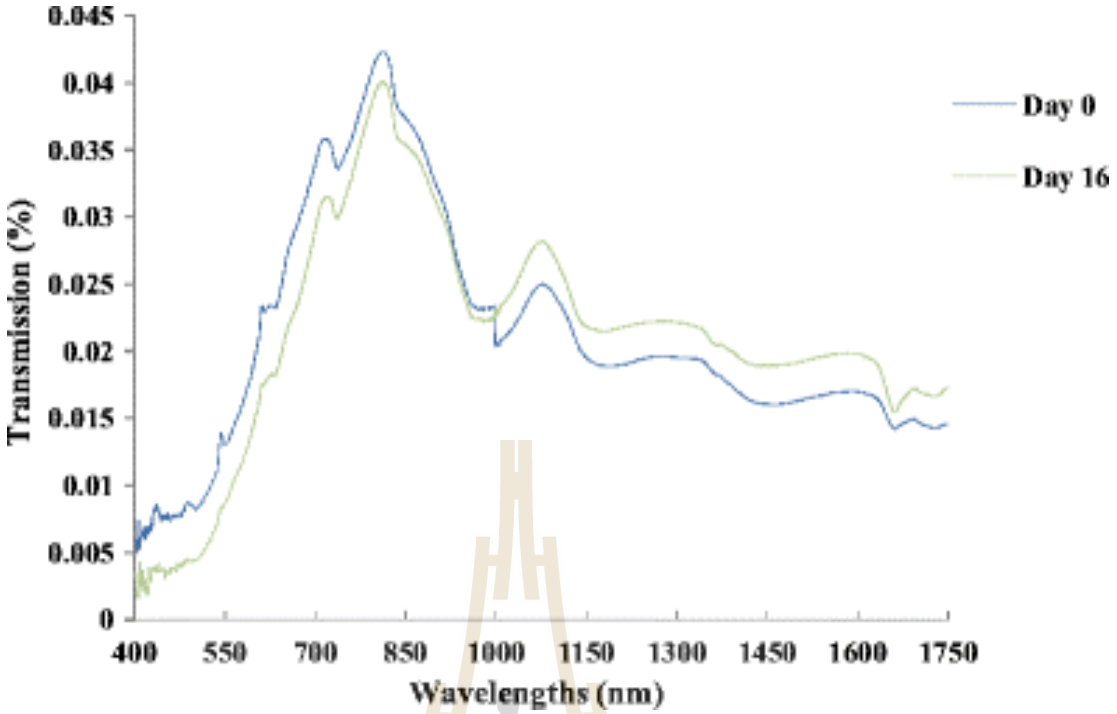


Figure 2.6 Mean transmission spectra (Abdel-Nour et al., 2011)

CHAPTER III

EXPERIMENTATION

3.1 Samples

Samples used in our experiments were collected from the Suranaree University of Technology (SUT) poultry farm. Samples were the incubated chicken eggs from Korat chicken breeder, whitish brown by color. Samples consisted of fertile and infertile eggs. In total we examined 115 incubated chicken eggs, 100 fertile and 15 infertile eggs. At the SUT farm, samples were artificially incubated at the temperature of 37.6±0.1 ℃ and relative humidity of 60%. Eggs were turned automatically every hour to avoid the known adverse effects of lack of turning on embryonic oxygen consumption, growth and embryonic HR (Moriya, Pearson, Burggren, Ar, & Tazawa, 2000). Samples were timely transferred from the SUT poultry farm to the electronic laboratory and stored in the incubator ready for examination. In our laboratory incubator, incubation parameters were set to a temperature of 37.2±0.2 ℃ and relative humidity of 47.4%. These parameters were maintained throughout the experimentation until hatching. Due to the limited space in our laboratory incubator and some experimental control practices, samples were brought in our laboratory incubator in batches.

Each of the first, second and third batches consisted of 15 eggs, 12 fertile eggs and 3 infertile eggs. The first batch was examined starting from day 10−19 of incubation period, while the second batch was examined from day 7-18 of the incubation period and the third batch examined from day 11−18 of incubation period. The fourth batch

consisted of 40 fertile eggs examined from day 0−18 of incubation period. The fifth batch consisted of 30 fertile eggs and examined from day 0−18 of the incubation period. Table 3.1 shows the summary of the examined samples distribution in each batch of experiments.

Table 3.1 Samples characteristics in each experimental batch.

Batch number	Number of eggs		Incubation days
	Fertile	Infertile	
I	12	3	10−18
II	12	3	7−18
III	12	3	11−18
IV	40	0	0−18
V	30	0	0−18

3.2 Setup

The setup used to measure the embryonic HR of multiple incubated eggs based on optical measurement techniques. It consisted of light sources and camera. The light sources used were composed of uniformly distributed LEDs and a camera was equipped with the CMOS sensor. The setup assembly consisted of iron sheet base, aluminum poles, plastic eggs' holders and black covers. Light sources were fixed on top of plastic square box mounted which was mounted on the iron sheet base. Eggs plastic holders were fixed on top of the light sources. The camera was fixed to the top aluminum pole facing the light sources. Each light source was made of white light emitting diodes

module (Lamptan 7W MR16), each light source could accommodate one specimen (chicken egg) only. Our setup had nine light sources in total. Each light source has an internal AC to DC converter driving it. The LEDs in our light sources could give strong dark-field illumination. We chose these bright light sources due to the fact that, the eggshell could only transmit less than 0.12% of the total incident light intensity (Yu, Li, & Pan, 2016). Eggs holders were fitted on top of the fixed light sources. Each egg lied vertically, with its air cell facing up, on top of each light source.

Light sensor made of a CMOS camera (Basler acA1300–60gmNIR). The camera was equipped with an imaging lens (Edmund Optics, 16mm/F1.4). The camera supported the frame rate up to 60 frames per second (fps) and binning up to 4×4. The camera was fixed right on top of the light sources using aluminum poles. The camera has the Gigabit Ethernet port which interfaced it with the computer. The camera was interfaced to the computer for recording the video files of the examined eggs.

Computer was installed with LabVIEW software. The sequential images/samples of the examined eggs were recorded and stored on the computer memory in AVI file format for later analysis. LabVIEW software was used for recording the video frames and performed analyses to extract the embryonic HR information for each examined egg.

The used lab-scale optical setup equipment examined 9 eggs (3×3) at once. The setup was covered completely all sides using black materials, which ensured that the ideal dark-field illumination was achieved throughout the experimentation process. The typical arrangement of the setup used is shown in Figure 3.1. The light sources were approximately 54 cm away from the camera. This was the minimum distance required to capture the frames of all nine (3×3) specimens under examination.

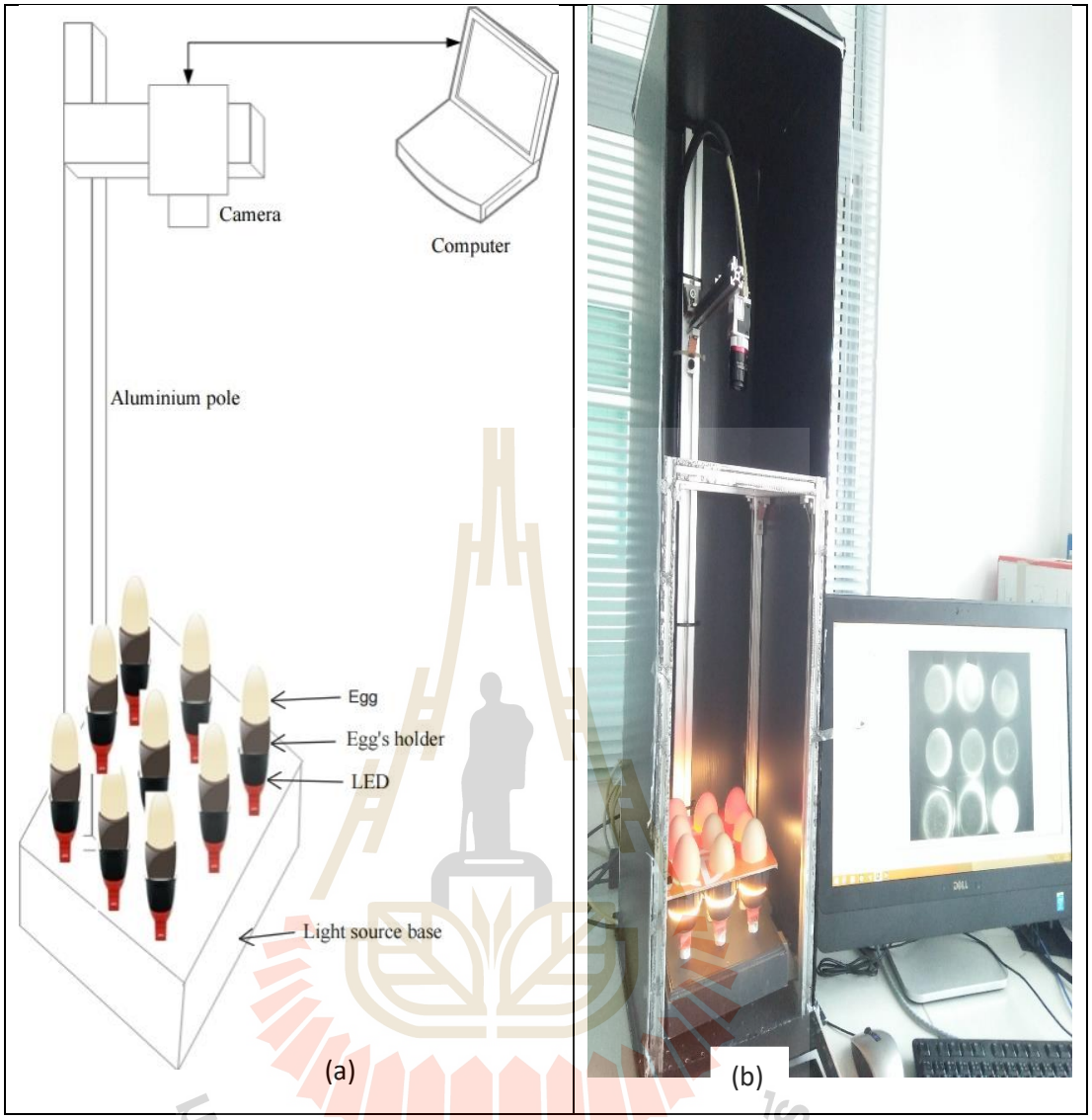


Figure 3.1 Experimental setup (a) setup sketch (b) setup picture

3.3 Experimental

This subsection has described in brief the procedures and the basic settings used to record the video frames in AVI format. This part also highlighted on some experimental materials, conditions and methods necessary used for the accomplishment of the embryonic HR extraction and measurement of the examined multiple incubated chicken eggs.

The experimental setup was arranged as shown in Figure 3.1 and the equipment used as well explained in Section 3.2. The camera was set at 2×2 binning and summing mode which resulted to the frames whose horizontal and vertical sizes are 636 by 510 respectively. The set binning mode was chosen because it could increase the transmitted light intensity. This setting mode could sum up four pixels (2×2) into one pixel. The camera's exposure time was set to 8000-μs and the acquisition frequency to 30 frames per second. The resulted camera's acquisition time 1/fs was 33333-μs. The exposure time set (8000-μs) was below one fourth of the acquisition time which could sufficiently allow for the capture, transmission and storage of the acquired frame/image before an interrupt to capture the next image is issued.

The computer used was installed with the LabVIEW software release 15.0.1. The software was used for recording the video files. The recorded video files were stored in a computer memory in AVI uncompressed file format. The stored video files were post analyzed using LabVIEW software to extract the embryonic HR information of the examined chicken eggs.

Samples used were incubated chicken eggs collected from the Korat chicken breeder as explained in Section 3.1. Samples were examined from day 0−19 of incubation period. Specimens were not examined during days 20−21 of incubation period because within these days pipping started, and hatching occurred. Unhatched eggs after 21 days of incubation were recorded for HR measurements and open broken to eye witness whether they are dead or otherwise.

The experiments were conducted at a room temperature in our laboratory under dark-field illumination. All recordings were done at the fixed acquisition rate of 30 fps for 60 seconds. We expected to collect around 1800 frames in each experimental

recording. But due to several reasons, such as unstable acquisition frame rate and initialization time, our recording acquired and saved between 1728−1765 frames. Our experiments were done once a day (morning time) to reduce disturbances to the incubation process.

In each experiment, nine (3×3) incubated chicken eggs were examined. Light was shone through each egg. The egg was vertically lied, on a holder, top of light source, with its wider end (air cell side) facing up and the subsequent video files in AVI uncompressed format recorded and stored in a computer memory. The AVI uncompressed format was chosen because of its high ability to retain the high quality of the stored videos files even if compressed. Our video files were not compressed. After recording the video files, followed the computer-based frames analysis to extract the embryonic HR information of the examined chicken eggs from the stored videos.

CHAPTER IV

EMBRYONIC HEART RATE ANALYSIS

4.1 Time domain waveforms extraction

The process of embryonic HR measurement starts with the extraction of time domain waveforms from the stored sequential images in the form of AVI video files. Stored video files were read and region of interests (ROIs) were created by user in the middle of the specimen. The varying light intensities within defined ROIs, between consecutive images/frames were quantified and registered at the rate of 30 fps (the same rate was used to record the video files) with the help of the software. The mean quantified light intensities represented the mean blood pulsatile waveforms. IMAQ ROIToMask 2 vi and IMAQ Quantify 2 vi found in LabVIEW software were used to perform this task. The IMAQ ROIToMask 2 vi created space for the ROIs coordinates in the computer memory during processing while the IMAQ Quantify 2 vi was responsible for quantifying the mean light intensities within ROIs with respect to the rest parts of the images. We used the mean value because the light intensity transmission characteristics of an egg varies from the equatorial part to the poles (Abdel-Nour et al., 2011). Figure 4.1 shows three sample 60-s extracted waveforms from 13-days old incubated eggs.

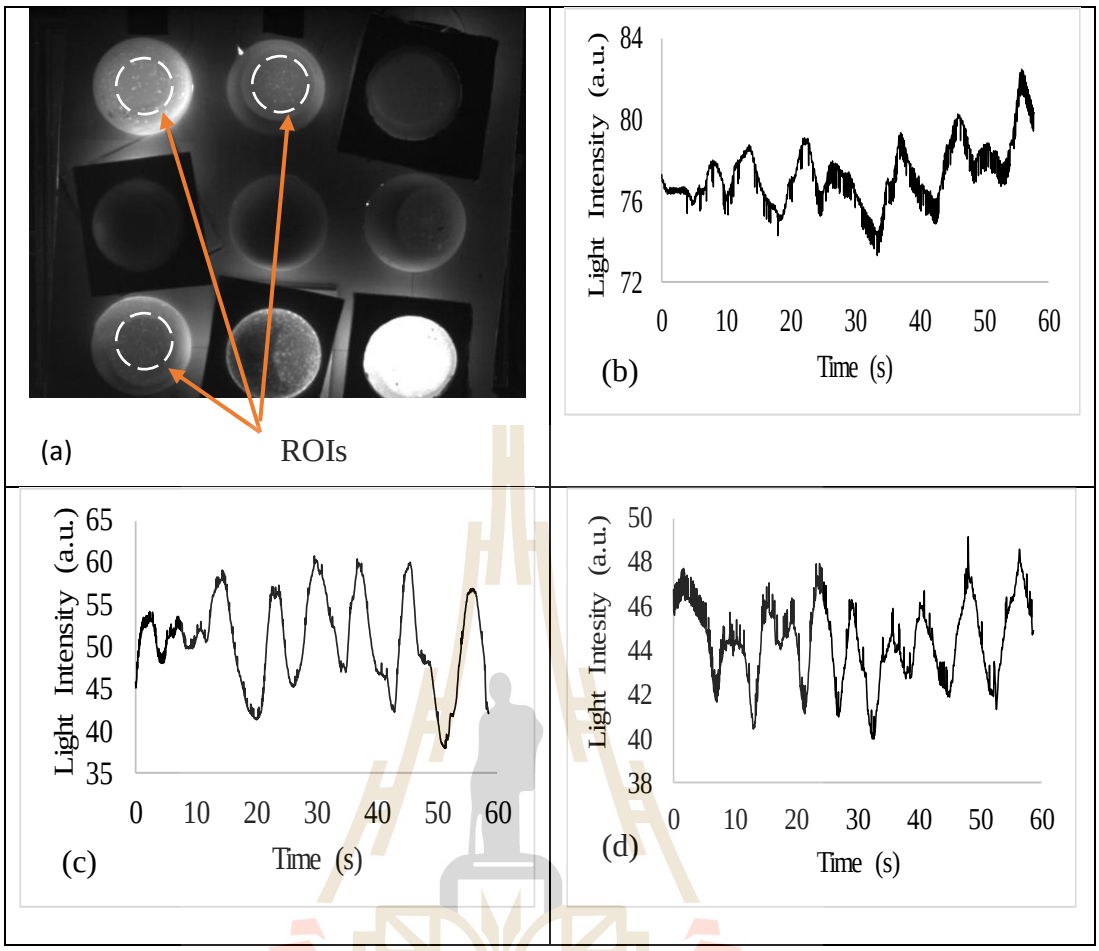


Figure 4.1 Sample waveforms from a 13-days egg (a) Created ROIs on the analyzed egg (b-d) Extracted waveforms from the ROIs

The extracted waveforms were contaminated with other unwanted signals (noises). The noises corrupted the waveform signals and it was not easy to observe the HR variations. Signal processing techniques were then used to minimize the effect of unwanted signals as well as to smooth the extracted waveforms.

The waveform graph for the extracted waveforms consisted of the recorded samples number (x-axis) against the transmitted light intensity values (y-axis) for every sample. The x-axis scale was made to display the real measurement time by diving the samples number at each point on the graph by the acquisition frame rate.

4.2 Preprocessing techniques

The digital signal processing techniques are the collection of mathematical, analysis and computational tools that enable the optimization and extraction of an analog or digital signal from the mixture of a variety of signals by using the computer processor. Several signal processing algorithms could be introduced to reduce the noise effect added to the original recorded signals by a digital camera. The noise added to the required signal normally corresponds to different illumination levels during the video recording, motion artifact added to the signal due to the embryonic movements or the background noise in the environment during the recording. In this section, we provided an overview of the different signal preprocessing techniques used to reduce noise, smooth the extracted signals in preparation to the embryonic HR analysis. Preprocessing of waveforms involved moving average, detrending and background subtraction, windowing and filtration. Finally, preprocessed signals were analyzed by fast Fourier transform to extract the embryonic HR frequency.

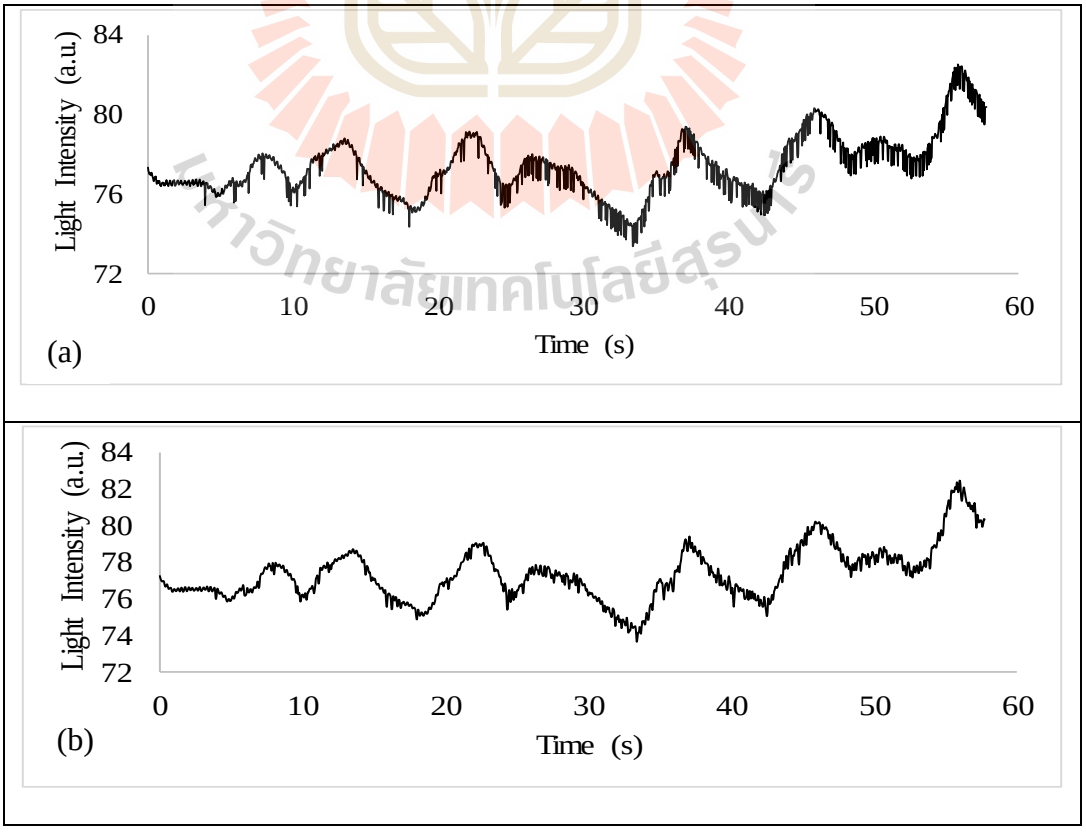
4.2.1 Moving average

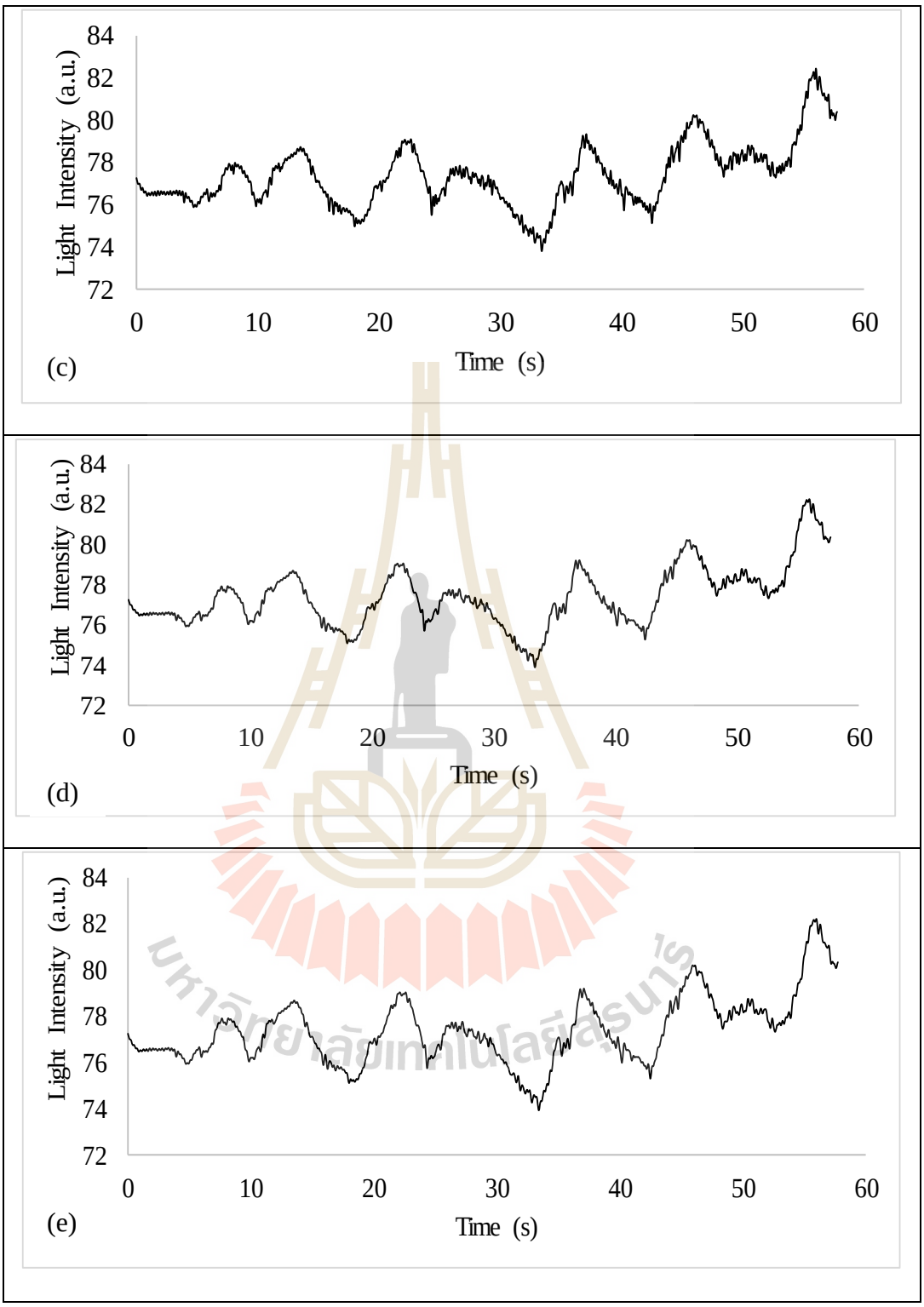
Moving average is an optimum low pass finite impulse response filter (FIR) used for reducing random noise and minimizing degradation of step impulse response. The moving average filter operates by averaging a number of points from the input signal to produce each point in the output signal. Increasing the number of points (M) in the filter could increase the smoothness of the output, whereas the sharp transitions in the data are made increasingly blunt. This implies that this filter has excellent time domain response but a poor frequency response. This filter could provide the lowest possible noise level for a given sharpness of the edges. The possible amount of noise reduction is equal to the square-root of the number of points in the average. In

mathematical notation, this filter with length M, input vector represented by x and output vector by y could be represented by equation 4.1. The filter length *M*, could be an even or odd number, but for symmetrical averaging it is advised to be an odd number.

$$y[i]=\frac{1}{M}\sum_{j=0}^{M-1}x[i+j] \tag{4.1}$$

In this work, the moving average was implemented using the TSA Moving Average.vi block found in LabVIEW software. This vi offers filter lengths of 7, 9, 13, 15 and 23 points. We chose weighted filter length of 23 terms/points designed by Henderson as it could give more smoother output which resulted to higher peaks in the power spectrum analysis. Comparisons of the resulted waveforms after applying moving averages with different number of points was shown in Figure 4.2.




Light Intensity (a.u.)
84
82
80
78
76
74
72
0
10
20
30
40
50
60
Time (s)
(c)
Light Intensity (a.u.)
84
82
80
78
76
74
72
0
10
20
30
40
50
60
Time (s)
(d)
Light Intensity (a.u.)
84
82
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74
72
0
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Time (s)
(e)

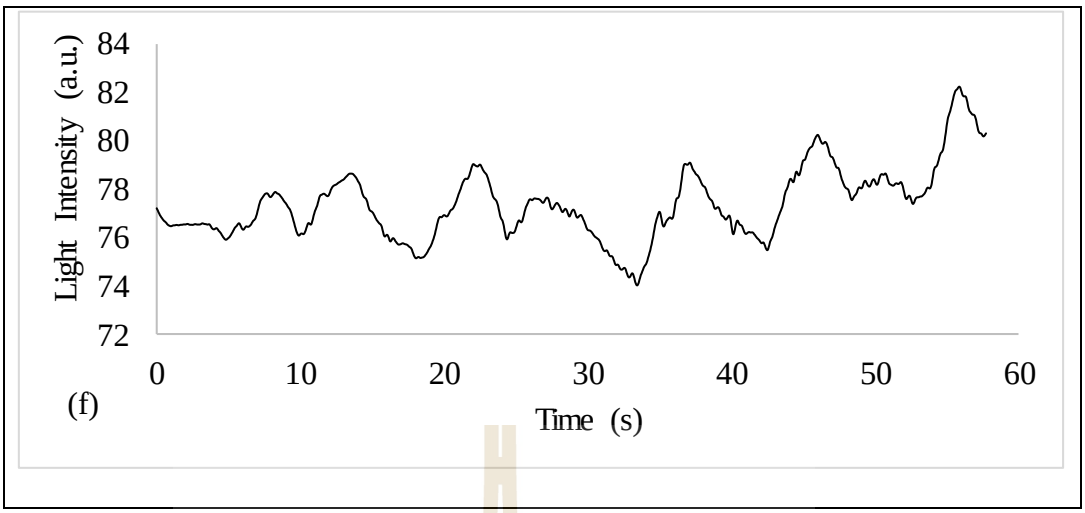


Figure 4.2 Resulted waveforms after applying different filter lengths (a) original waveforms (b) 7 points filter (c) 9 points filter (d) 13 points filter (e) 15 points filter and (f) 23 points filter

Resulted waveforms after moving average of 23 filter points is much better and smooth as seen in Figure 4.2. These waveforms must be detrended to focus the power spectrum analysis. Due to the variety nature of our resulted signals, we applied the moving average filter of 23 points length throughout our analysis.

4.2.2 Detrending and background subtraction

Detrending is the statistical operation of removing trend from the series. It is aimed to remove irregular fluctuations from the data series or remove a feature thought to distort the relationships of interest. Irregular fluctuations may be caused by nonuniform illumination of the source, power line fluctuations, camera frame rate adjustment or movements of the embryo. Detrend prepare time series for analysis by methods that assume stationarity such as power spectrum analysis. We performed detrending by subtracting the moving average signal calculated as explained in section 4.2.1 from the original waveforms. Detrended signal is shown in Figure 4.3.

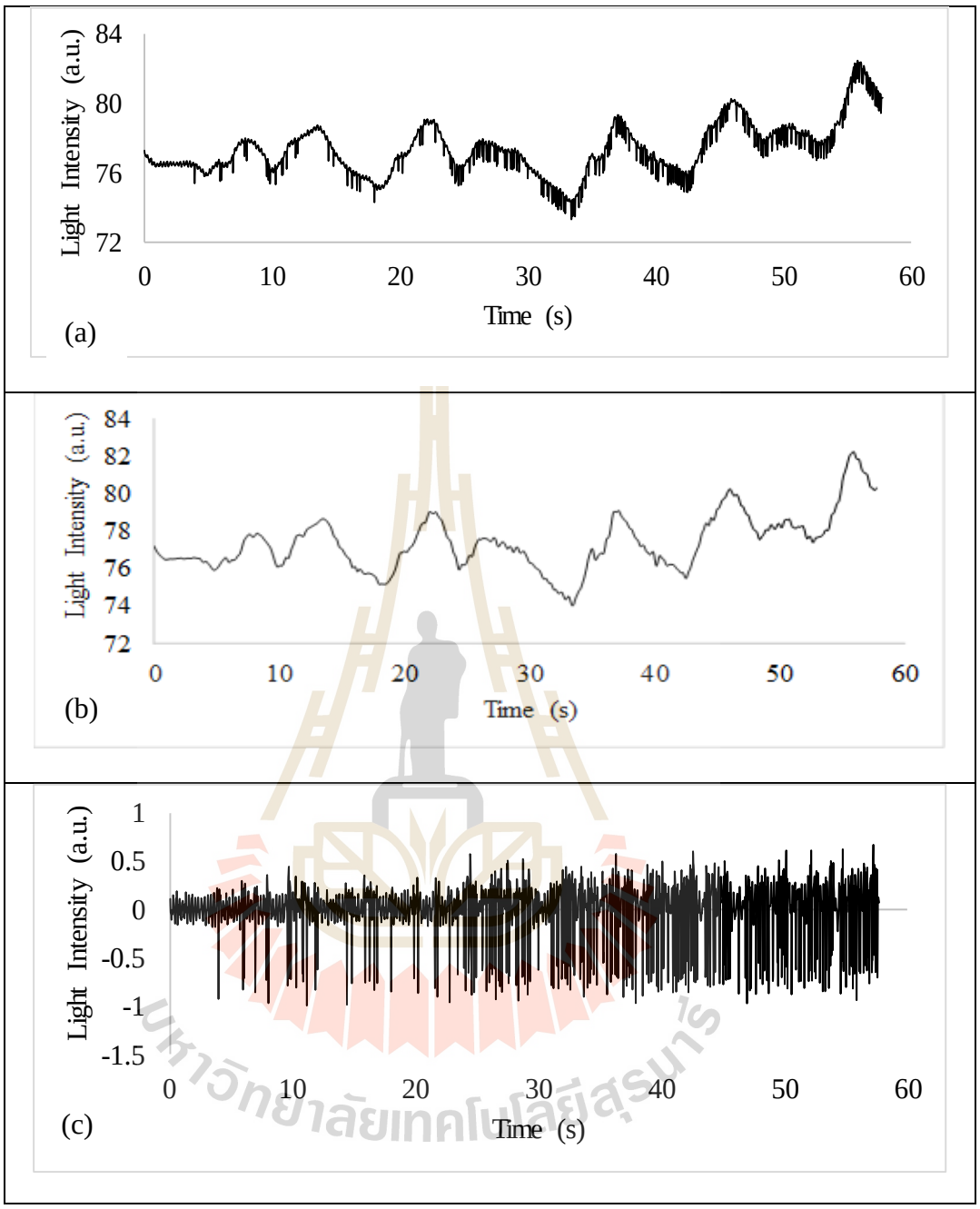


Figure 4.3 Detrending of a signal (a) original signal (b) moving averaged signal and (c) detrended signal

Detrending a signal by subtracting its moving average from the original signal introduced another problem, the impulse changes and other noises removed in the moving average process, reappeared again as it can be seen in Figure 4.3. We had

to refilter the signal to remove the frequency components outside the band of interest. Filtration followed the time scale window process. The power spectrum analysis for a detrended and without detrended signal is shown in Figure 4.4. No detrended signal cannot be processed by FFT analysis because it is wandering (not stationary) contrary to the stationarity assumed by the FFT analysis.

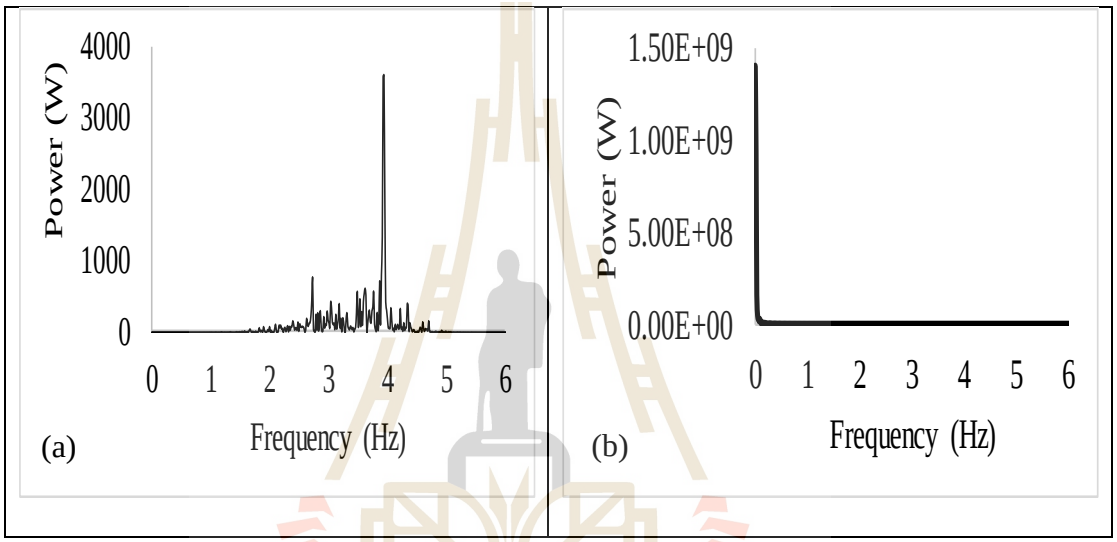


Figure 4.4 Power spectrum analysis (a) with and (b) without detrending

4.2.3 Time scale window

Most digital signals are infinite, or sufficiently large that the dataset cannot be manipulated as a whole. Large signals are also difficult to analyze statistically, because statistical calculations require all points to be available for analysis. In order to avoid these problems, engineers typically analyze small subsets of the total data, through a process called windowing.

In signal processing, a window function (also known as an apodization or tapering function) is a mathematical function whose value is zero outside of its

chosen interval. For instance, a function that is constant inside the interval and zero elsewhere is called a rectangular window, which describes the shape of its graphical representation. When another function or waveform is multiplied by a window function, the product is also zero-valued outside the defined interval: all that is left is the part where they overlap, the "view through the window". In typical applications, the window functions could be non-negative, smooth, "bell-shaped" curves, rectangle, triangle or any other functions.

The fast Fourier transform algorithm takes the small amount of captured data and repeats it, in order to perform the Fourier transform and produce a frequency spectrum. It assumes this signal repeats for all time. When the signal is periodic the resulting frequency spectrum will have no leakage. When the number of periods in the acquisition is not an integer multiple, the endpoints of the signal are discontinuous and non-periodic, which causes the fine spectral lines to spread into wider signals. This phenomenon is called spectral leakage. We can minimize the effects of spectral leakage by performing an FFT over a noninteger number of cycles by using a technique called windowing. Windowing reduces the amplitude of the discontinuities at the boundaries of each finite sequence of the acquired signal. Windowing is done by multiplying the time record by a finite-length window with an amplitude that varies smoothly and gradually toward zero at the edges. This makes the endpoints of the waveform meet and, therefore, results in a continuous waveform without sharp transitions. Figure 4.5 shows an example of overlapped captured discontinuous waves which leads to spectral leakage.

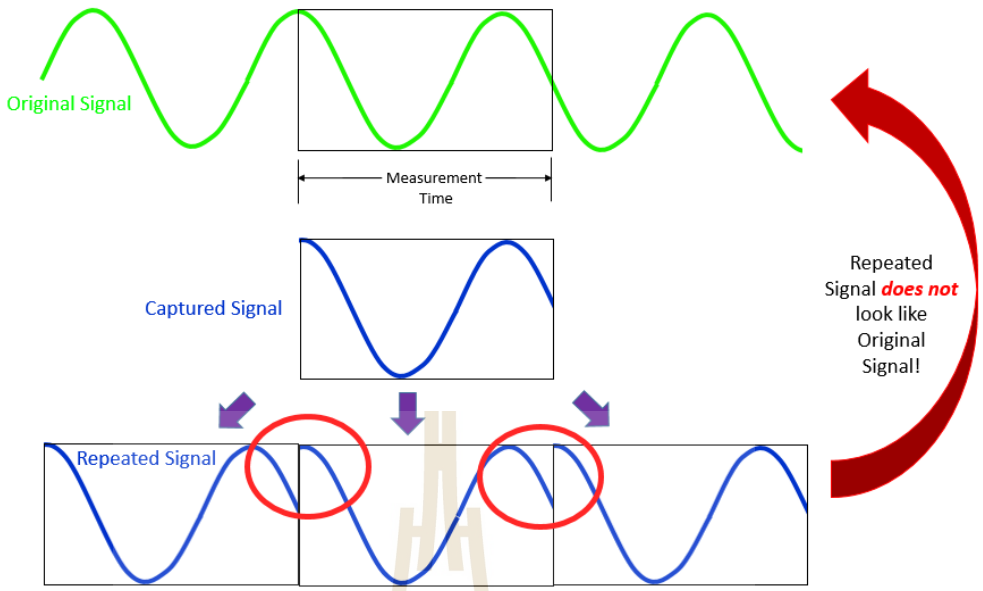


Figure 4.5 Discontinuous captured waveform (Siemens Legend, 2017)

There are several types of window functions, such as, rectangular window, triangular window, Hanning window, hamming window, flat-top window, Welch window, Kaiser window and many others that can be applied. These window functions can be applied in a time or frequency domain depending on the application. In this work we used the Kaiser window because it can give good results for small frequencies resolution in both frequency and amplitude (Chavan, Agarwala, & Uplane, 2006; Seydnejad & Kitney, 1997). Kaiser window of duration and shape determinant factor could be implemented by using the Bessel function shown in equation (4.2).

$$\omega_\alpha(t)=\begin{cases}\dfrac{I_0\left[\alpha\sqrt{1-\left(t/\tau\right)^2}\right]}{I_0|\alpha|}, & |t|\le\tau,\\ 0, & |t|>\tau\end{cases} \tag{4.2}$$

We applied the windowing technique to minimize the spectral leakage in case it could occur as in real data acquisition perfect periodic signals rarely occur

4.2.4 Filtration

Filter or shaper is a system that performs mathematical operations on a signal to reduce or enhance certain aspects of that signal. Filters are used in circuits that require separation of signals according to their frequencies. Filter can be used to pass lower, mid-range or higher frequencies of a frequency range of interest. Filters help to optimize signal to noise ratio, throughput optimization and signal conditioning (Oppenheim, 1999).

Band-pass filter is a filter that passes the band of frequencies and attenuates those above or below that band. It can attenuate the DC component and higher frequency noises. In this work we utilized the 5th order Chebyshev filters to pass the frequencies in the range of 0.001–6 Hz which included to the embryonic HR of the incubated chicken eggs in the range of 2.5−5 Hz (E. et al., 2004; Lierz et al., 2006; Youssef et al., 2014). We set the lower range to 0.01 Hz so that we could resolve the DC frequency which appeared dominant in dead and infertile eggs.

Filter could affect the power spectrum analysis as shown on Figure 4.6. Power spectrum of unfiltered signal showed all the frequency components below fs/2 while that of the filtered signal showed values within the range of interest. It could be easy for the power spectrum of unfiltered noisier signal to mislead into judgements especially when the higher peaks appear in the higher frequency components signals. Filtration could add more advantages to the power spectrum of a noisier signal than a less noisy signal. Less noise signal can successfully be analyzed without filtration.

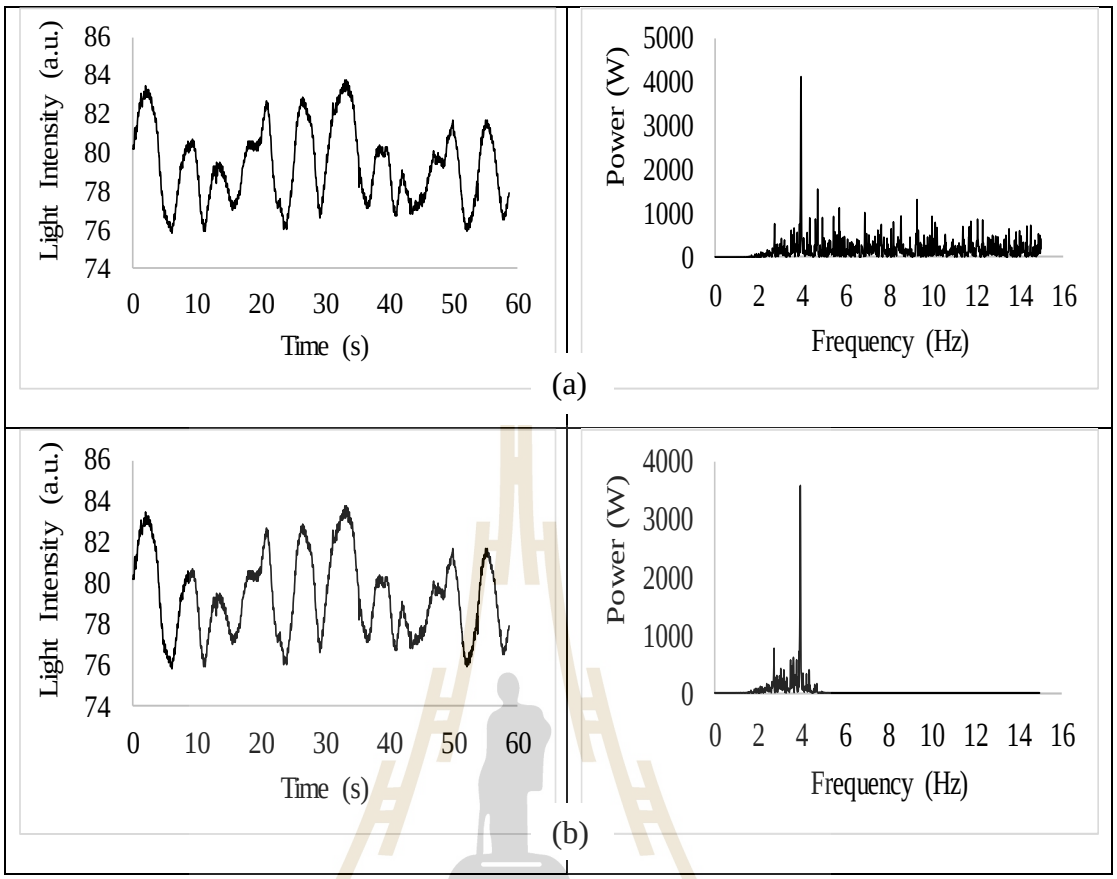


Figure 4.6 Waveforms and power spectrum (a) without (b) with filter applied

4.3 Fast Fourier transform (FFT)

The Fourier transform (FT) is a mathematical transformation employed to transform signals between time domain and frequency domain. The Fourier transform mathematical equation is given by:

$$X(\omega) = \int_{-\infty}^{+\infty} x(t) e^{-j\omega t} dt \qquad (4.3)$$

Where is the frequency components of the signal and is the time domain signal. Equation (4.3) defines the spectrum of which consists of components at all frequencies over the range of which it is nonzero.

For many signals, Fourier analysis is useful because the signal's frequency content is important. But Fourier analysis has a drawback of information loss while transforming the nonlinear and nonstationary/transient time domain signal into frequency domain. It is only valid under general conditions which assumes linear, periodic and stationary signal.

Fourier transform can be used to analyze a small set of signals at a time. This approach is called Short-time Fourier transform (STFT). The STFT is obtained from the usual FT by multiplying time domain signal x(t) by an appropriate sliding time window ω(t) (Garrido, 2016; Su & Wu, 2017). Thus, equation (4.3) is modified as shown in equation (4.4).

$$X(\tau,\omega) = \int_{-\infty}^{+\infty} x(t)\omega(t-\tau)e^{-j\omega t}dt \tag{4.4}$$

Where ω(t-τ) is the time window applied to the signal.

In practice, the transforms often occur within discrete samples of a signal, and the transform happens for only a discrete set of frequencies. For this thesis, the recorded grayscale frames were discrete. Thus, Discrete Fourier transform (DFT) was employed. The fast Fourier transform (FFT) is the preferred fast algorithm for implementing the DFT (Lyons, 2004; Oppenheim, 1999). The DFT transform formula for N samples is defined by the equation:

$$F[n] = \sum_{k=0}^{N-1} f[k]e^{-j\frac{2\pi}{N}nk} \tag{4.5}$$

Where n is 0,1, 2,…, N-1, F[n] denotes DFT sequence of f[k] and f[k] denotes the N

discrete samples of continuous signal f[t]. F[n] in equation 4.5 is the nth frequency "bin" in Hertz (Hz). This frequency could be calculated using equation:

$$f = n \times \Delta\omega = n \times \frac{f_S}{N} \tag{4.6}$$

where fs is the sampling frequency. In this work, we used the sampling frequency of 30 fps.

There are two major effects introduced into the computation of the DFT which are aliasing and leakage (Lyons, 2004). The DFT values might be corrupted by aliasing, if the sampling frequency were smaller than the highest frequency component in the underlying function. Aliasing effect could be reduced by sampling at least at the rate twice the highest frequency component value in the analyzed signal. Spectral leakage could be minimized by applying the Kaiser window as explained in section 4.2.3.

The NI_NASM_FFT.Ivlib: Multi-channel FFT.vi found in LabVIEW software was employed to perform the 2048-points FFT size power spectrum analysis of every stored frame in a video file. The samples were acquired at 30 fps for 60 seconds (s), acquiring approximately 1800 frames. The FFT multi-channel.vi could analyze all examined specimen simultaneously. A 512-FFT size was not used as it offered lower amplitude resolution and broader frequency resolution hence inaccurate frequency resolution. A 1024- FFT size gives a much better processing gain as compared to a 512-FFT size analysis. A 1024-FFT size provided a lower but close frequency resolution to a 2048-FFT size analysis. A 2048-points FFT size was chosen since it gives much better frequency resolution and higher processing gain as shown in Figure 4.7. The wandering

of the signal shown in Figure 4.7 (d) is caused by the embryo's movements. In our measurements, frequency accuracy in measurement is our primary goal.

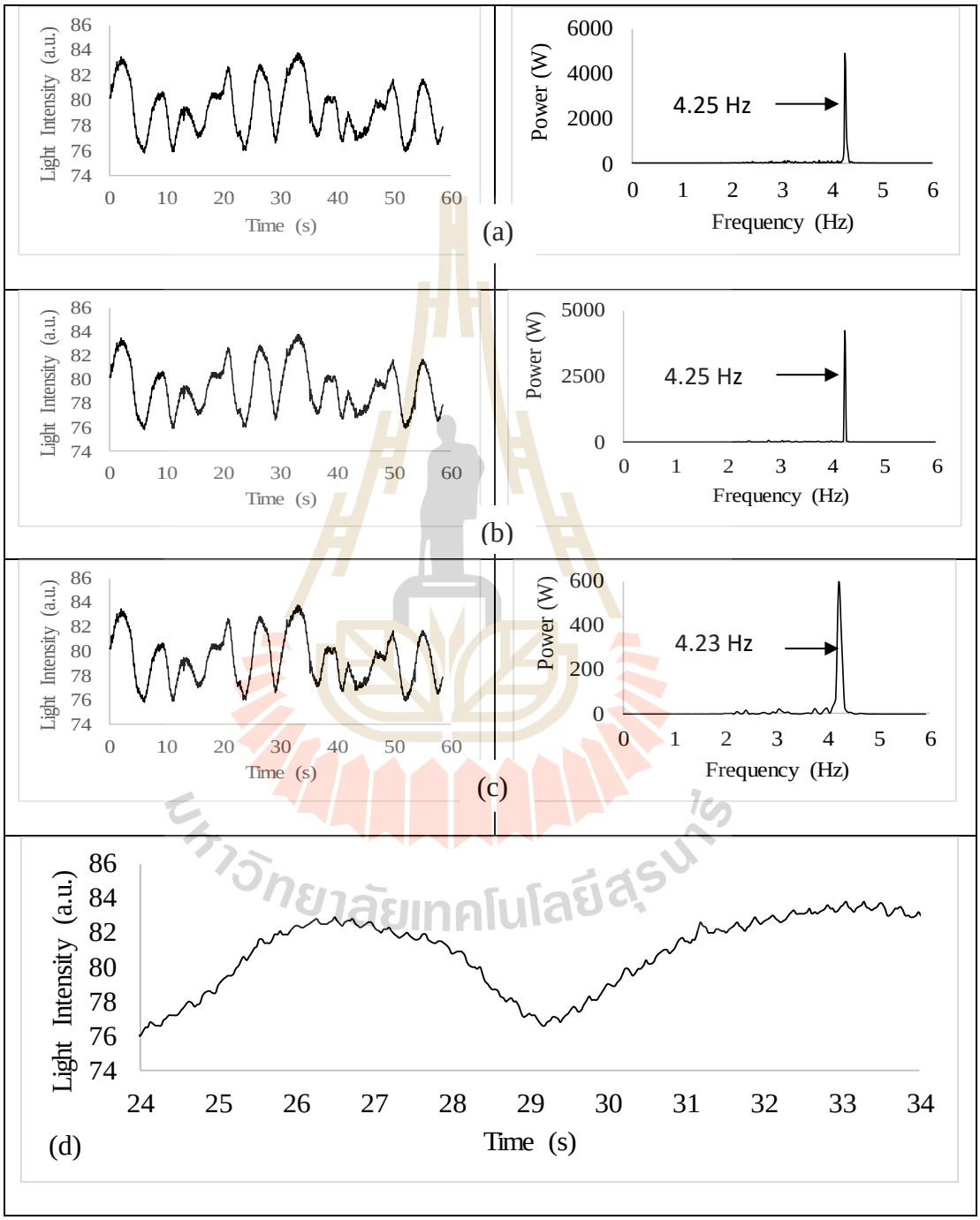


Figure 4.7 Waveforms and power spectrum (a) 2048 (b) 1024 and (c) 512 FFT size. (d) Zoomed in waveforms to show HR of 12-days embryo from batch IV

The power spectrum graphs represented the number of frequency components "bins" (x-axis) and the power (y-axis) carried on each signal. The x-axis was adapted to display frequency in Hertz by multiplying the "bins" number and the ratio of sampling frequency to the FFT size.

4.4 Embryonic HR information extraction

The HR information of the incubated chicken eggs could be extracted from the time domain signal, frequency domain signal or both. Once the signal is recovered from the stored video files, it could be possible to recover the HR information. But from the practical point of view, most of the extracted time domain signals could not show HR variations clearly; hence difficulty in recovering the embryonic HR in time domain. For the time domain signal that showed the HR variations well, the embryonic HR could easily be determined by taking the reciprocal of the period (T), of that signal but for the signal that does not show well the HR variations with time, further processing to extract the HR frequency using Fourier transform was used. To simplify our work, all signals were analyzed using fast Fourier transform method to extract and measure the embryonic HR frequency. Normally, the frequency whose amplitude is the highest in the power spectrum analysis correspond to the embryonic HR of the developing examined embryo in Hertz (Akselrod et al., 1981; Youssef et al., 2014).

4.5 Multiple eggs HR measurements

Regions of interests (ROIs) were created in the middle of images stored in AVI file format of all nine examined eggs to extract the waveforms. The For loop ensured the waveforms processing of all ROIs before executing the next stage. Once the waveforms were retrieved, a preprocessing stage for all ROIs followed. Preprocessing

involved moving average, detrending, windowing and filtration. Finally, the power spectrum analysis was performed for all created ROIs simultaneously. The peaks detection and embryonic HR calculation performed after FFT process. The extraction automatically halted after all images for each created ROI have been processed. The procedures involved for the embryonic HR extraction of all examined multiple chicken eggs whose ROIs were created are shown in Figure 4.8.

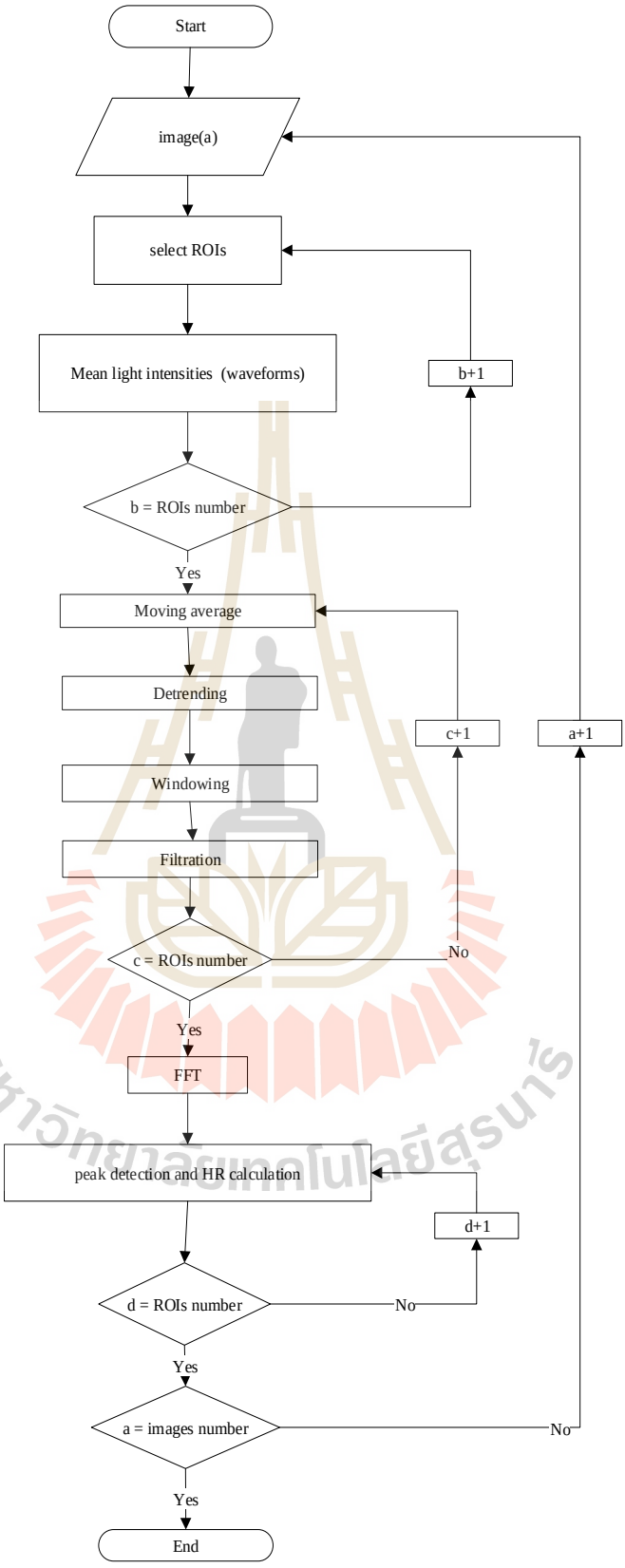


Figure 4.8 Flow charts showing methods to analyze embryonic HR of multiple eggs

CHAPTER V

RESULTS AND DISCUSSIONS

5.1 Embryonic HR and power spectrum analysis

Before discussing the overall embryonic measurement results from our optical system, we should answer one important question, “Does the frequency of the highest peak signal in the power spectrum analysis represent the embryonic HR of the specimen”?

We let a few live embryos to die by keeping them at room temperature (outside the incubator). The room temperature was controlled by air condition and temperature set to 25℃. We recorded and analyzed video files after every 20 minutes and observe any changes that may occur to the amplitude and HR frequency.

The assumption was that, the embryo could not survive the temperature change of 2℃ from the recommended incubation temperature (36−39℃) for a long time. Keeping the embryo at 25℃ for a long period of time, the embryo would die. Towards death, the embryo lose energy and its HR frequency would gradually decrease in value and amplitude. We observed the gradual decrease in frequency HR and amplitude with time as shown in Figure 5.1. We were sure that the frequency of the highest amplitude in the power spectrum analysis represented the embryonic HR of the examined embryo as also stated in the literature (Youssef et al., 2014). Plots are in different amplitude scales to easy peak visibility.

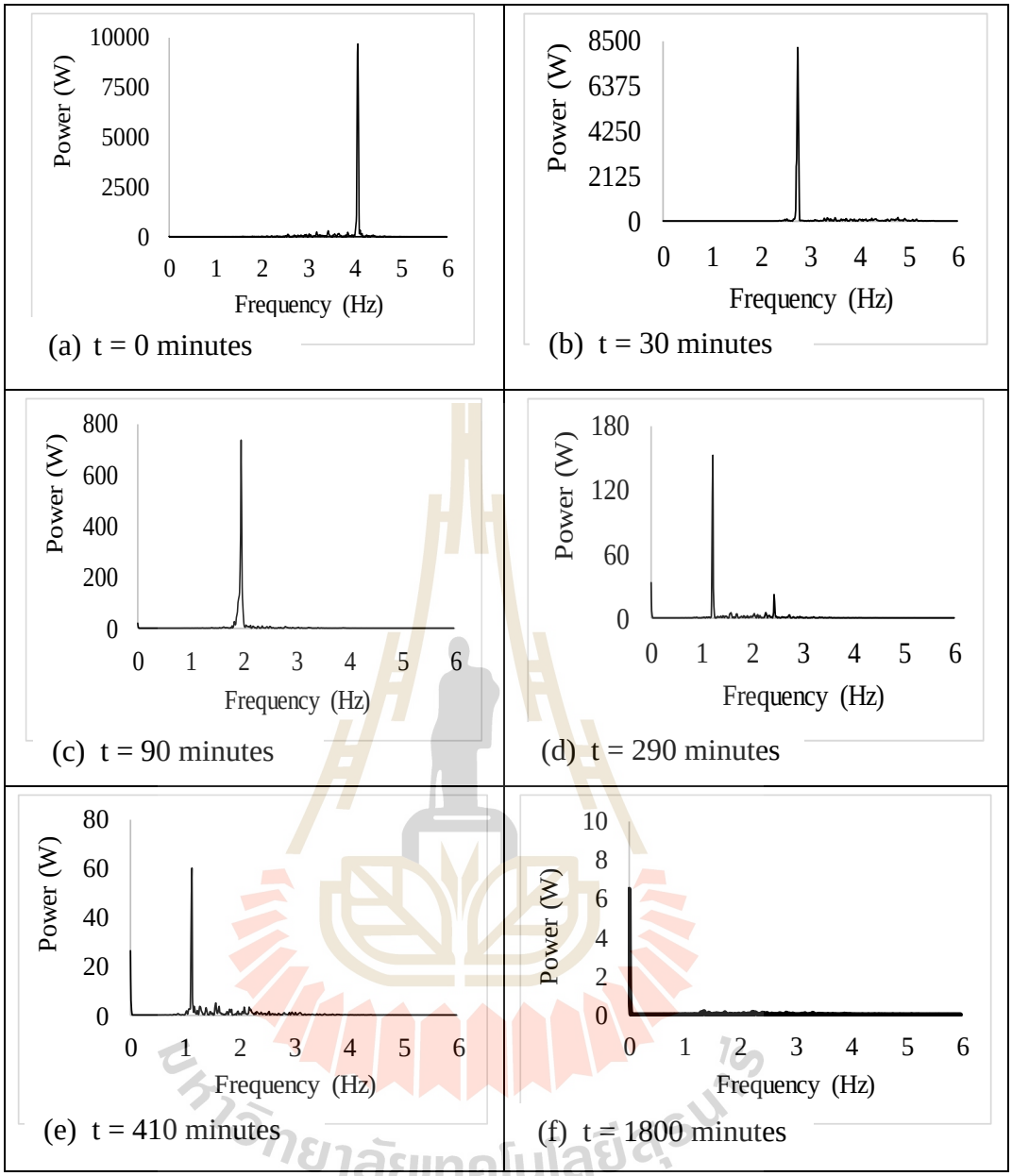


Figure 5.1 HR of a 10-days embryo at room temperature. (a) taken out of incubator (b) after 30 minutes (c) after 90 minutes (d) after 290 minutes (e) after 410 minutes and (f) after 1800 minutes out of the incubator

When the egg was taken out of the incubator, the embryonic HR was measured to be 244 BPM. After keeping it outside of the incubator, we observed faster decrease of embryonic HR in the first 60 minutes. The HR was measured to be 76 BPM after

300 minutes of the experiment. The embryo was strong and struggled to survive by increasing its HR to 90 BPM during the 1100 minutes, but it finally died 1800 minutes since it was taken outside the incubator. The plot of embryonic HR variation with time until the embryo died is shown in Figure 5.2.

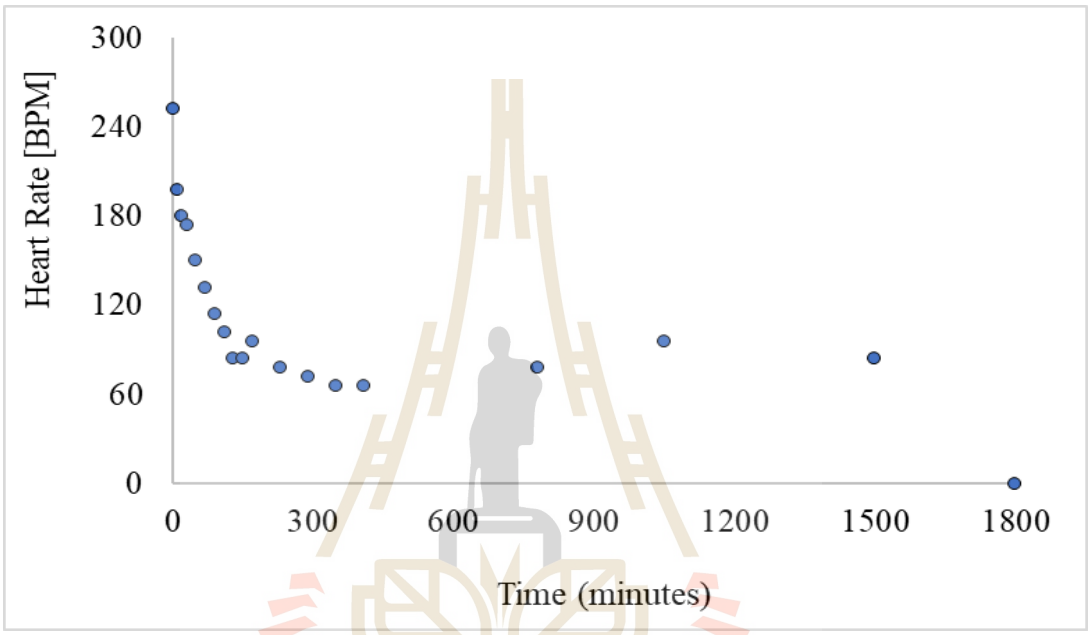
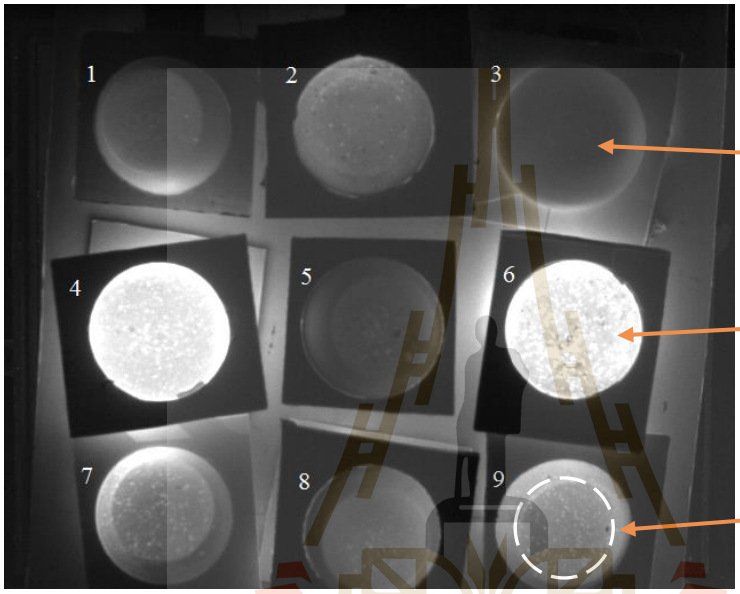


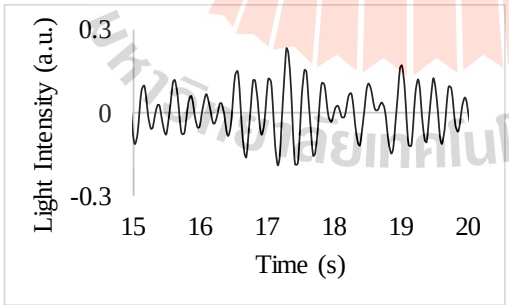
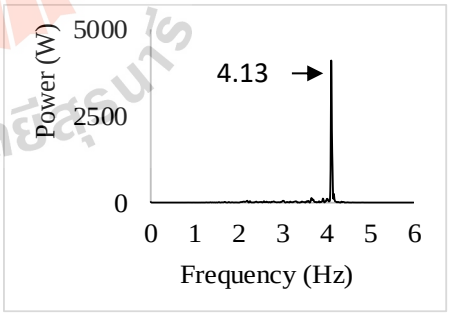
Figure 5.2 Embryonic HR variation to death

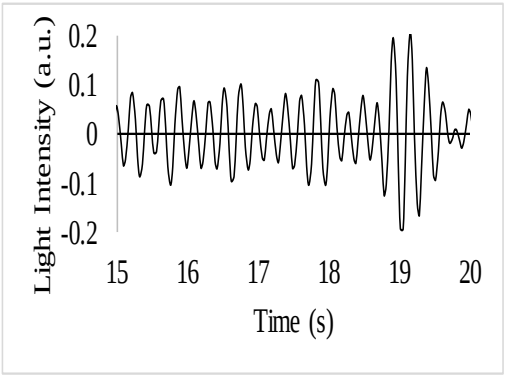
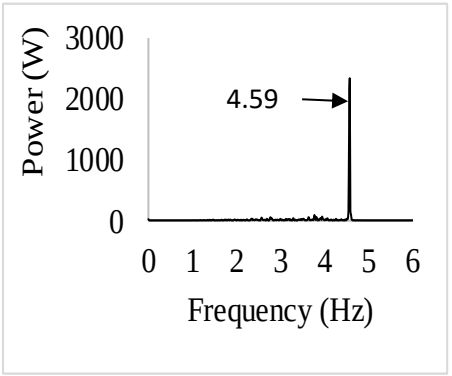
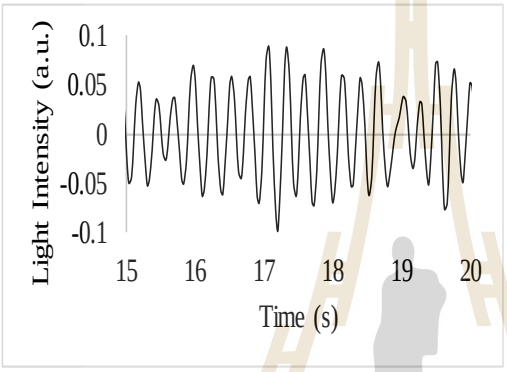
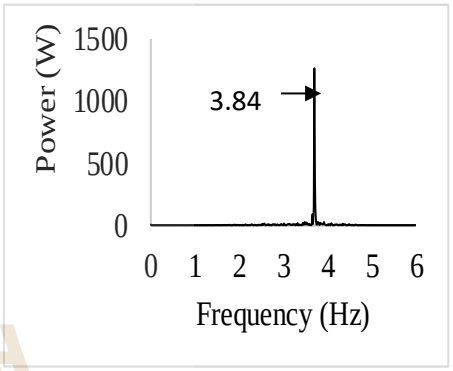
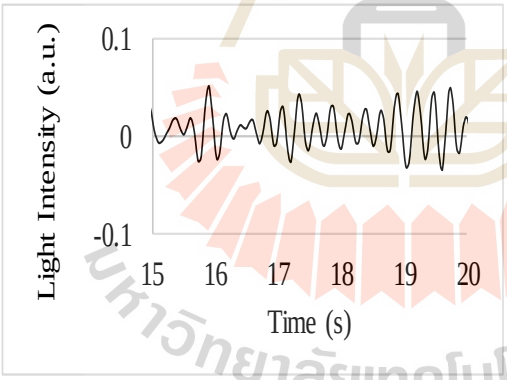
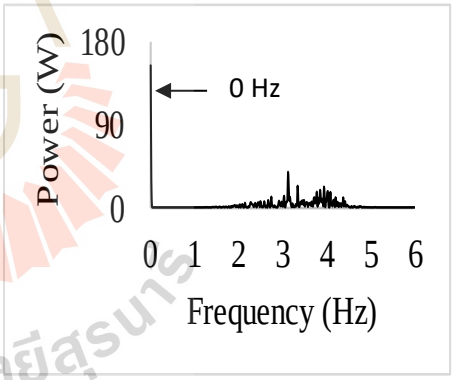
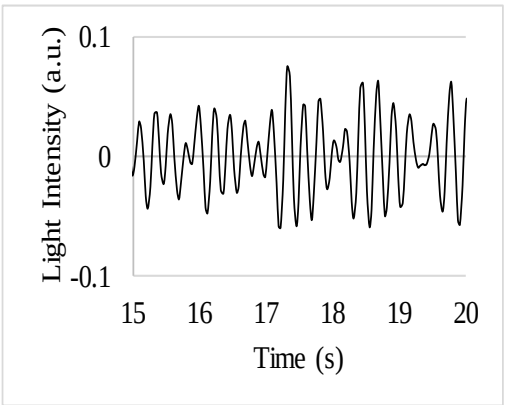
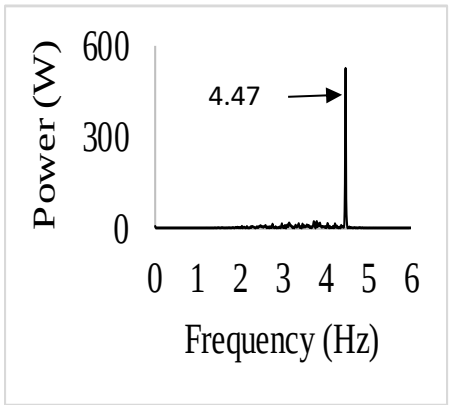
5.2 Fertile and infertile embryos

The captured images, waveforms and power spectrum analysis for fertile alive eggs and infertile incubated eggs were different. The fertile alive embryo could show a dark image under dark-field illumination (less light transmitted because more light blocked by the embryo), sawtooth like varying waveforms and a distinct highest amplitude embryonic frequency in its power spectrum analysis, whereas infertile egg showed bright image under dark-field illumination (more light transmitted because less light is scattered by the egg's content with no embryo), minor variations of waveforms

(random and non-sawtooth) and power spectrum analysis dominated by DC component. Figure 5.3 shows sample results for preprocessed and power spectrum analysis from nine, 12-days old incubated embryos which were simultaneously examined. Plots are different scales to easy peaks visibility.



Egg	Prepossessed waveforms	Power spectrum
1		

2		
3		
4		
5		

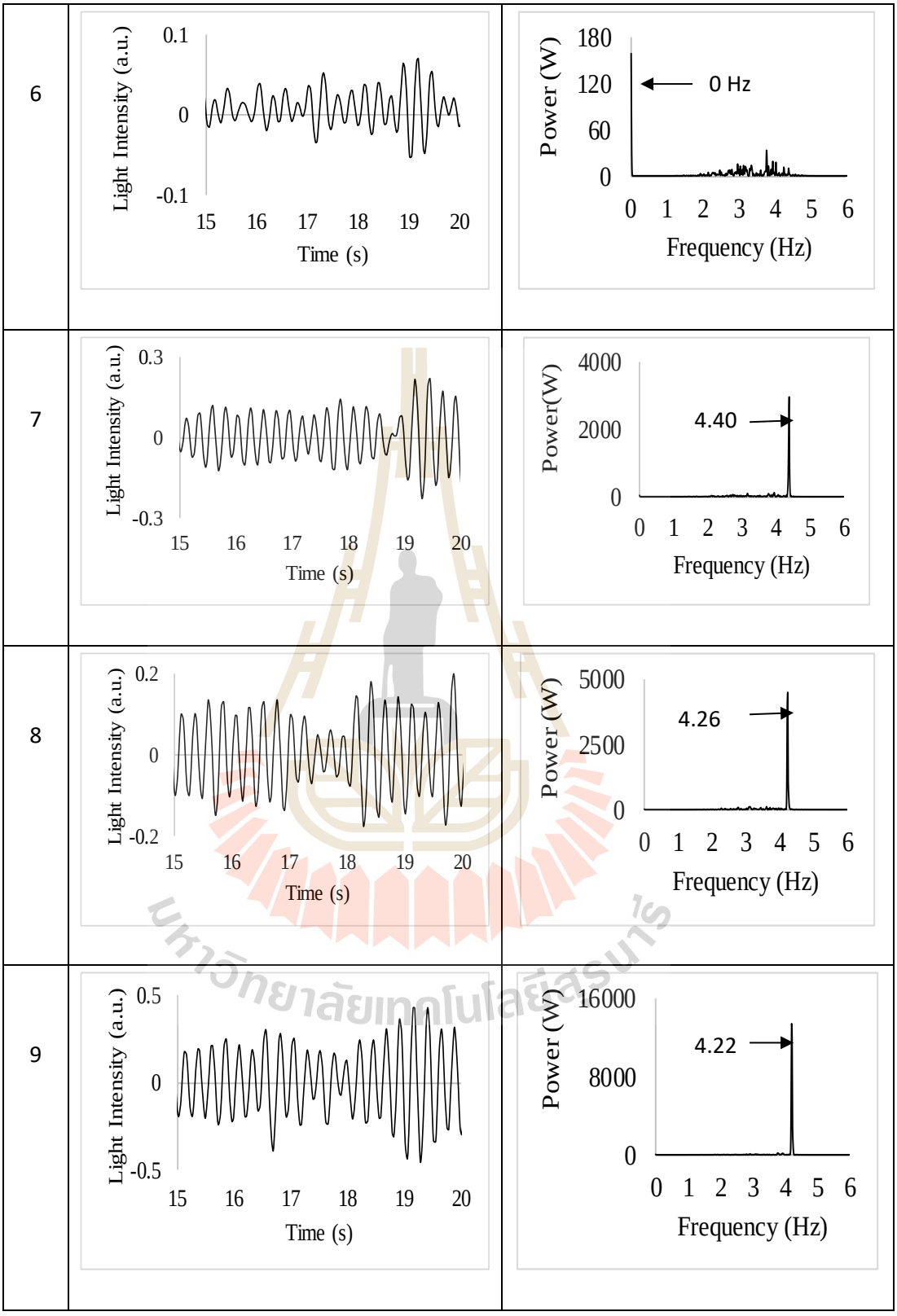


Figure 5.3 Nine simultaneously examined eggs at day 12 of incubation of batch IV

Some eggs did not show good time domain signals as well as clear power spectrum results due to noises, most presumably the embryos' movements (motion artifacts). Embryonic HR measurements using optical methods are susceptible to noise from motion artifacts due the fact that the cardiac activities signals are very weak. There are advanced signal processing techniques such as independent component analysis (ICA) which have proved success to minimize the effect of motion artifacts for embryonic HR measurements using optical techniques in human subjects (Alghoul et al., 2017; Zhang, Wu, Zhang, He, & Lv, 2017). Other researchers could apply sleeping drugs to embryos to minimize motion artifact and get a more stable embryonic ECG signal (T. Sugiyama, H. Miyazaki, K. Saito, H. Shimada, & K.-i. Miyamoto, 1996). We did not employ such techniques in this work, yet our results were good.

Mean embryonic HR from days 3−18 of incubation period ranged between 154−310 BPM for the examined eggs. Similar embryonic HR results for chicken eggs were reported between 175−287 BPM using BCG and ECG methods for 11−20 incubation days (A. Aubert, C. Leribaux, F. Beckers, D. Ramaekers, & D. Berckmans, 2000), 252−276 BPM for 13−19 incubation days using optical technique described in (Youssef et al., 2014) and 169−267 BPM for 6−21 incubation days using optical method described in (Lierz et al., 2006). The embryonic HR observed to increase and decrease with increasing incubation days. The decreased embryonic HR with increasing incubation day could be influenced by many factors including the room temperature and elapsed time for recording video files. Mean embryonic HR results obtained from our optical method for all examined eggs from day 0−18 of incubation are shown in Table 5.1. The corresponding heart rate variability (HRV) with incubation days is shown in Figure 5.4.

Table 5.1 Embryonic HR results with incubation days

Batch / Day	HR [BPM] Batch I	HR [BPM] Batch II	HR [BPM] Batch III	HR [BPM] Batch IV	HR [BPM] Batch V	HR [BPM] Mean
0	NA	NA	NA	undetected	undetected	undetected
1	NA	NA	NA	undetected	undetected	undetected
2	NA	NA	NA	undetected	undetected	undetected
3	NA	NA	NA	153±0.1	162±10	158±5
4	NA	NA	NA	165±11	176±10	171±6
5	NA	NA	NA	155±4	166±10	161±6
6	NA	NA	NA	162±12	179±18	171±9
7	NA	189±8	NA	174±10	182±16	182±6
8	NA	210±15	NA	180±16	179±13	190±14
9	NA	212±7	NA	196±5	182±6	197±12
10	248±20	208±41	NA	197±16	225±38	220±19
11	245±8	215±20	201±20	213±5	228±28	220±15
12	247±12	217±8	227±6	249±29	256±4	239±15
13	243±14	216±27	203±18	258±9	251±9	234±21
14	248±24	219±7	215±21	237±21	259±7	236±17
15	253±14	236±34	230±9	273±26	244±26	247±15
16	230±10	232±14	246±12	272±2	236±7	243±15
17	255±44	232±12	241±4	245±5	264±26	247±11
18	259±41	262±23	254±32	279±27	242±30	259±12

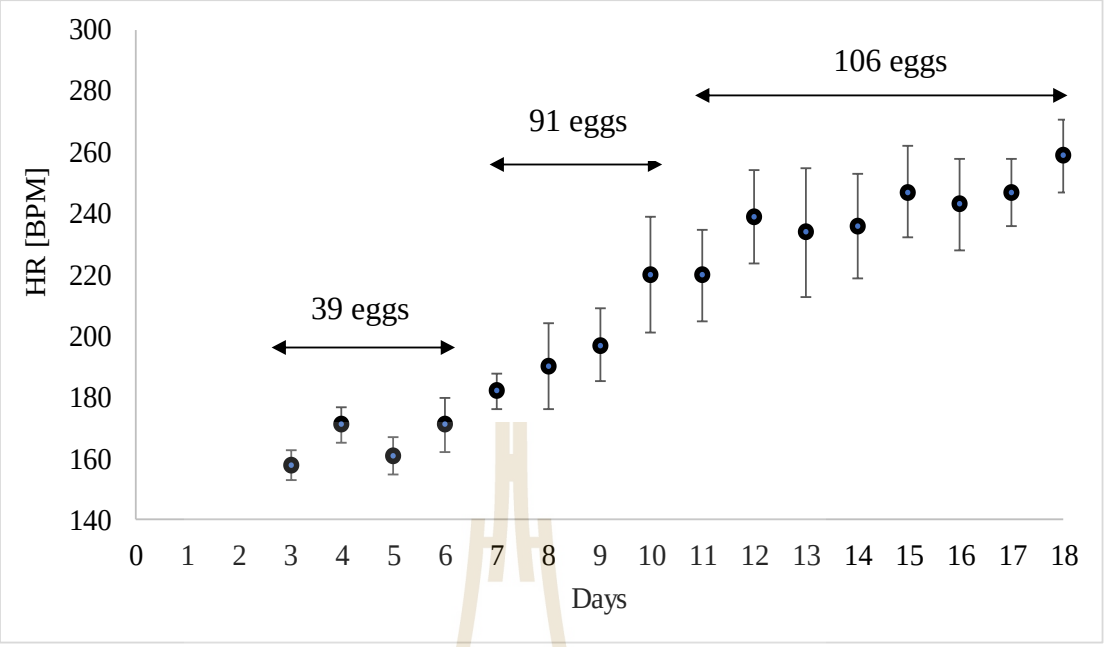


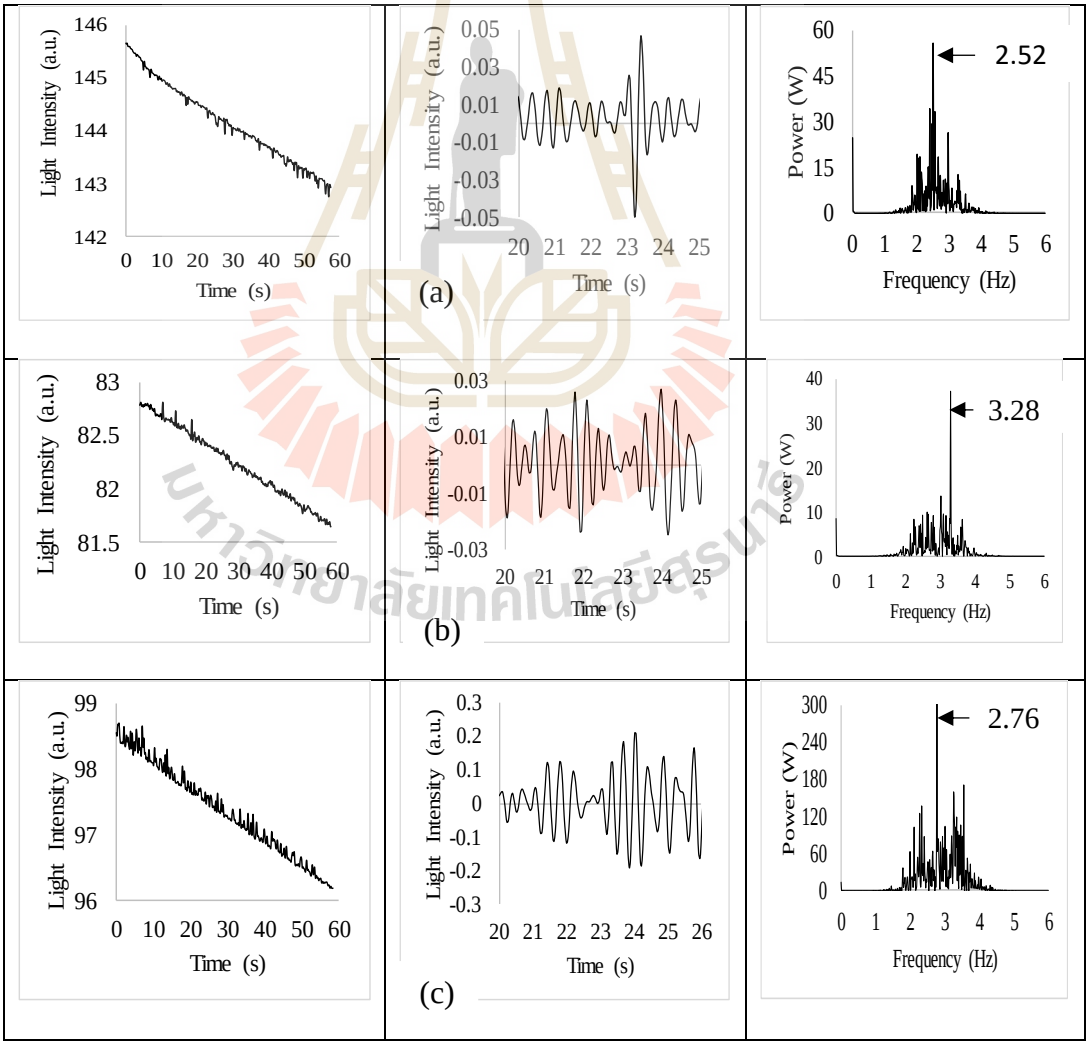
Figure 5.4 Embryonic HRV with incubation days

5.3 Earliest and latest days for embryonic HR measurements

In the early incubation days (first week of incubation), the embryo is small and largely composed of fluids. Higher light intensities from the source could be transmitted through an egg. It is easier to detect embryonic HR in these days than in the late incubation days. The heart of the incubated chicken embryo could be formed and function within 2−3 days of incubation (Youssef et al., 2014). Using our designed noninvasive and non-contact lab-scale optical measurement system, we were able to extract and measure the embryonic HR of the incubated chicken eggs starting from day 3 of incubation. In (Youssef et al., 2014), the embryonic HR of two incubated chicken eggs were measured starting from day 4 of incubation by windowing technique using two microscopic digital cameras installed within the incubator. Also, embryonic HR has been reported starting from day 3 of incubation using invasive techniques such as

blood pressure and catheterization technique (Haque et al., 1994). In our experiments, 2 out of 9 eggs could not show HR by day 3 of incubation and instead showed HR by day 5 of incubation.

In the late incubation days (third week of incubation), the chicken embryo is large and nearly fills the egg. Only a small fraction of the light from the source could be transmitted through an egg. Our optical measurement system was able to measure the embryonic HR until day 18 of incubation. Sample waveforms and power spectrum analysis results for day 3 and 18 of incubation are as shown in Figure 5.5.



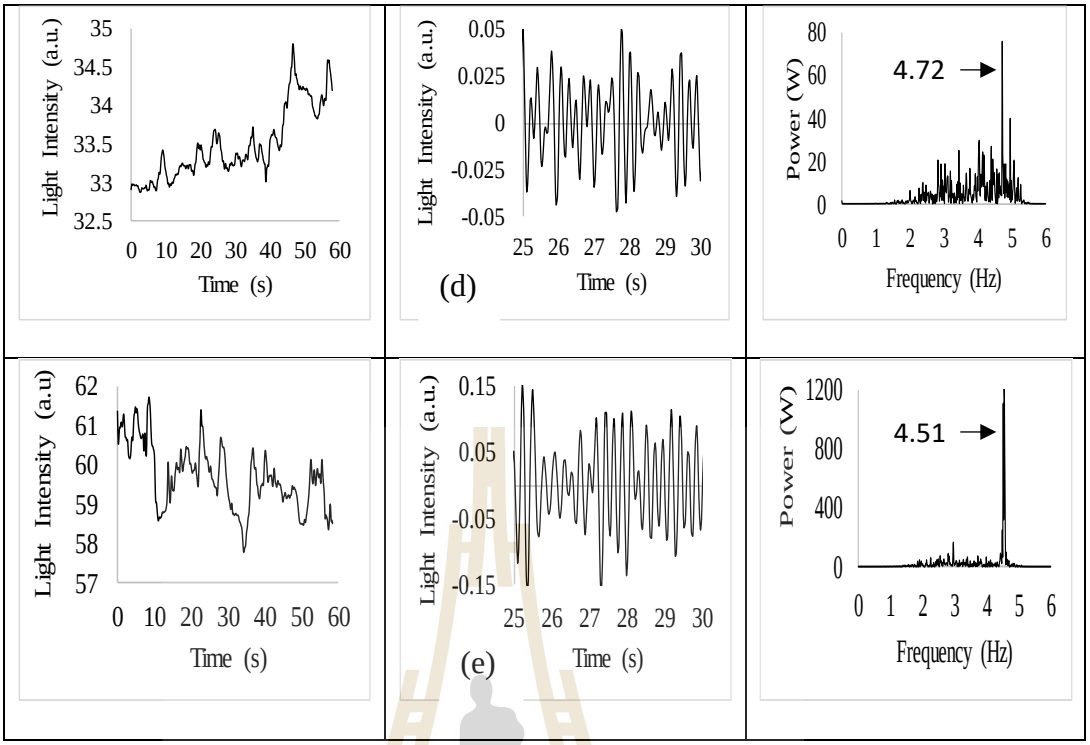


Figure 5.5 Sample waveform, preprocessed waveform and power spectrum analysis results for (a-c) day3 and (d-e) day 18 of incubation

5.4 Dead embryos

During the incubation period, some fertile eggs died due to several reasons. Some of the reasons include; failure to manage the incubation parameters, poor storage, poor hygiene and bacterial attacks, improper turning, power failure, poor ventilation to mention a few.

The dead embryo could show dark image under dark-field illumination (blocks transmitted light), slightly changing waveforms due to scattering of light and the power spectrum dominated by the zero-frequency (DC) component as seen in Figure 5.6.

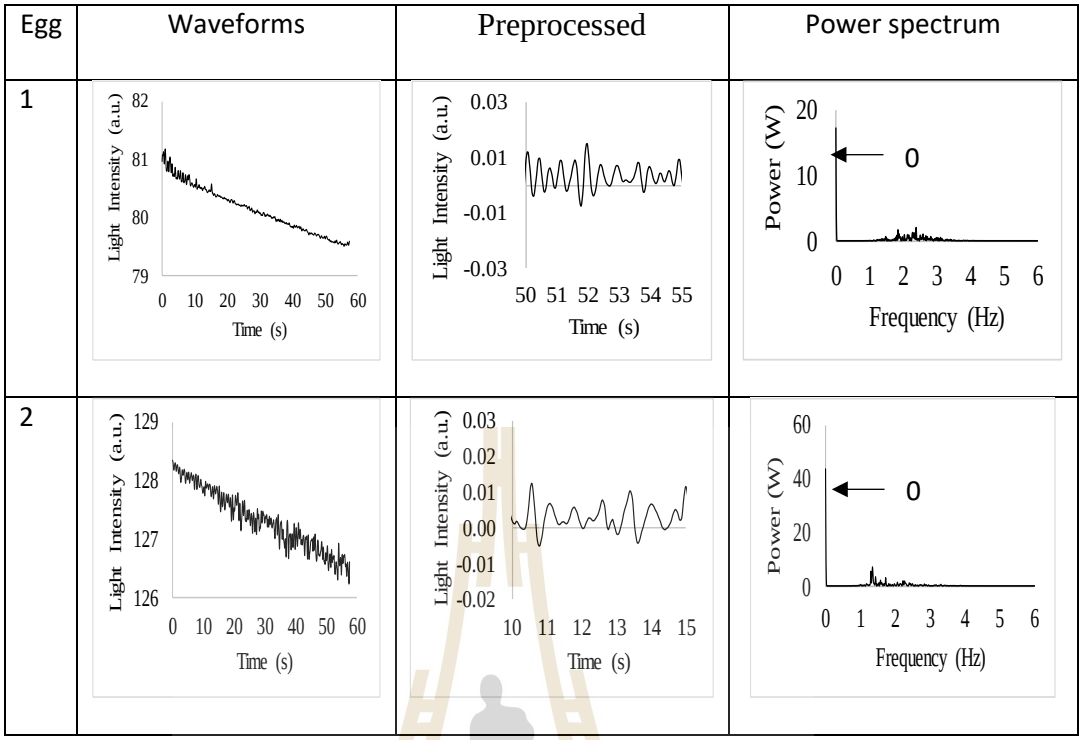
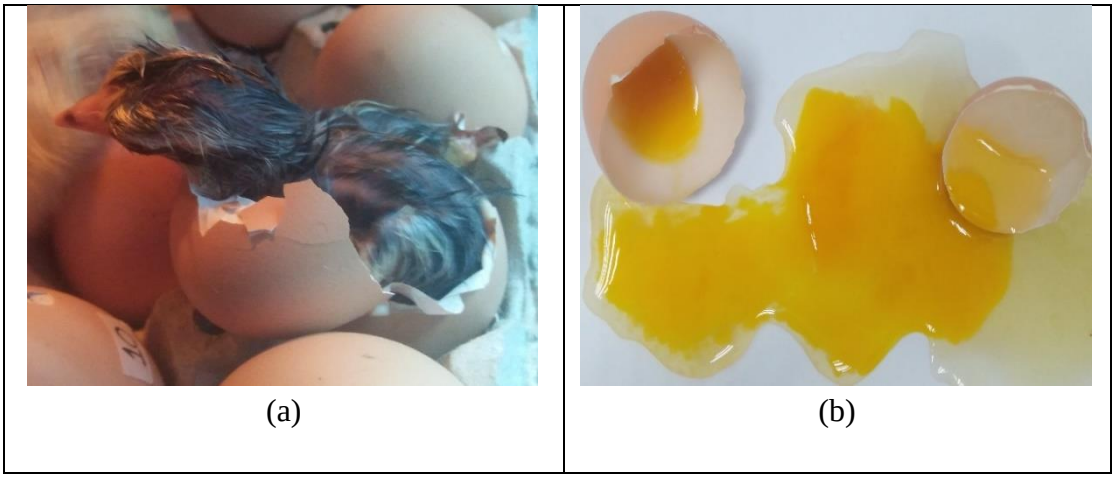


Figure 5.6 Waveforms, preprocessed and power spectrum for the dead embryos

Non-hatching incubated eggs at the end of incubation were open cracked to eye witness the embryos within them. Infertile eggs appeared fresh after 21 days of incubation. Some embryos died before incubation and a few of them turned into exploders. Figure 5.7 shows pictures of open cracked eggs at the end of incubation.



(a) (b)

(c) (d)

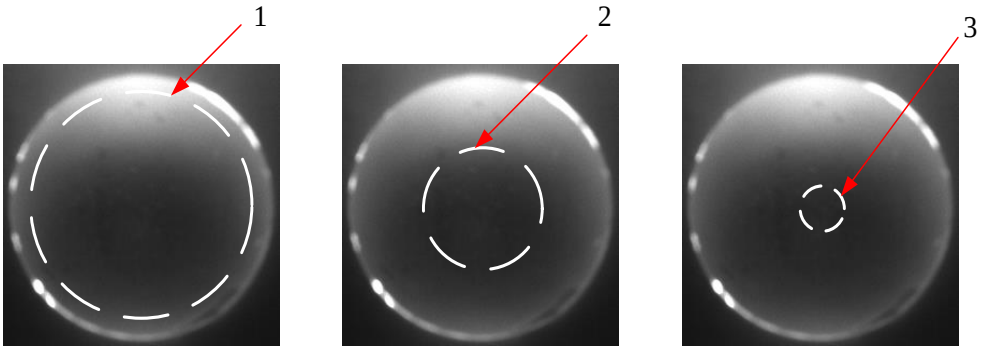
Figure 5.7 Open broken eggs at the end of incubation (a) hatched chick (b) infertile egg (c) dead embryo and (d) exploder (rotten) egg

Results from our optical measurement equipment have shown the potential of this method to measure HR of multiple embryos simultaneously. So far, the method could be used for research purposes because the 3x3 scale could not suit the commercial context demand.

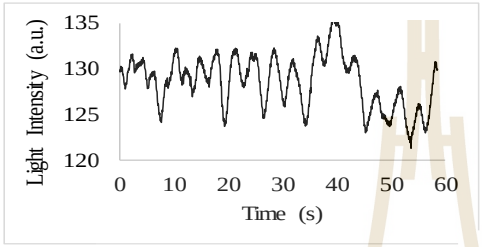
5.5 The role of ROI's position and size

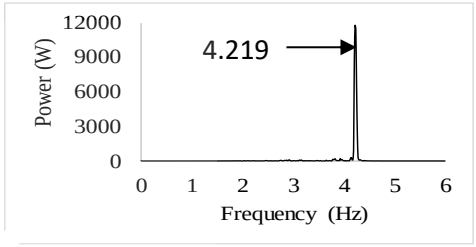
The ROI is an important practice in signal processing techniques. It is created by setting boundaries of an object isolating a segment of the image/dataset to concentrate the analysis to a specific region containing relevant information of interest.

In this work, ROIs manually created by user to extract time waveforms. ROIs of three different sizes, 130, 121 and 80 pixels at three different positions tested. The results showed slight difference in the transmitted light intensity, similar waveforms and the same embryonic HR results for three samples as shown in Figure 5.8. We can say, ROI size and position has negligible influence on the final embryonic HR results.

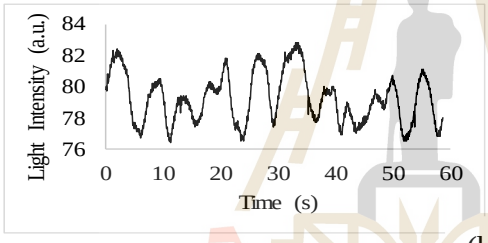


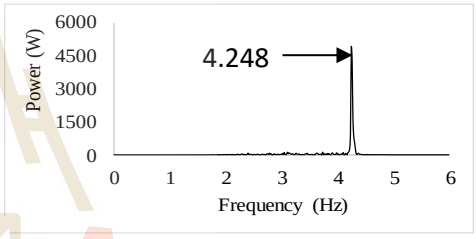
1



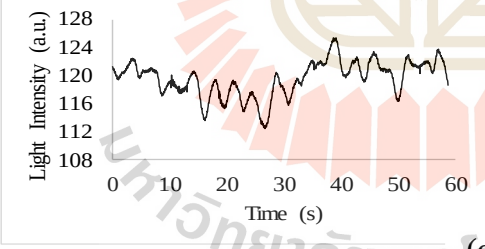


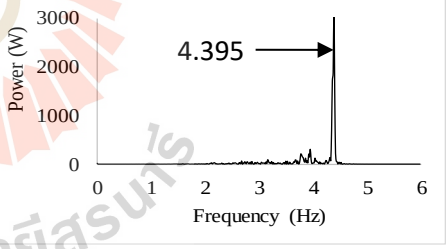
(a)





(b)





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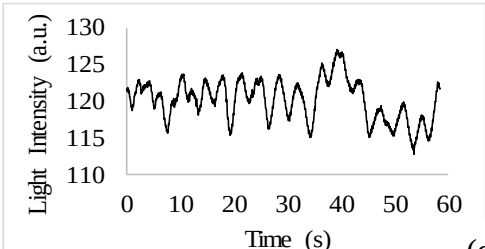

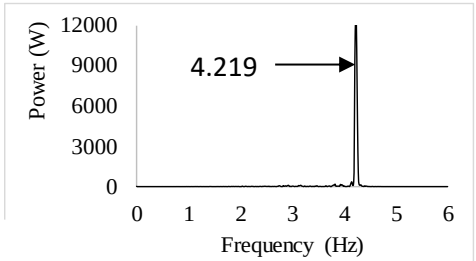

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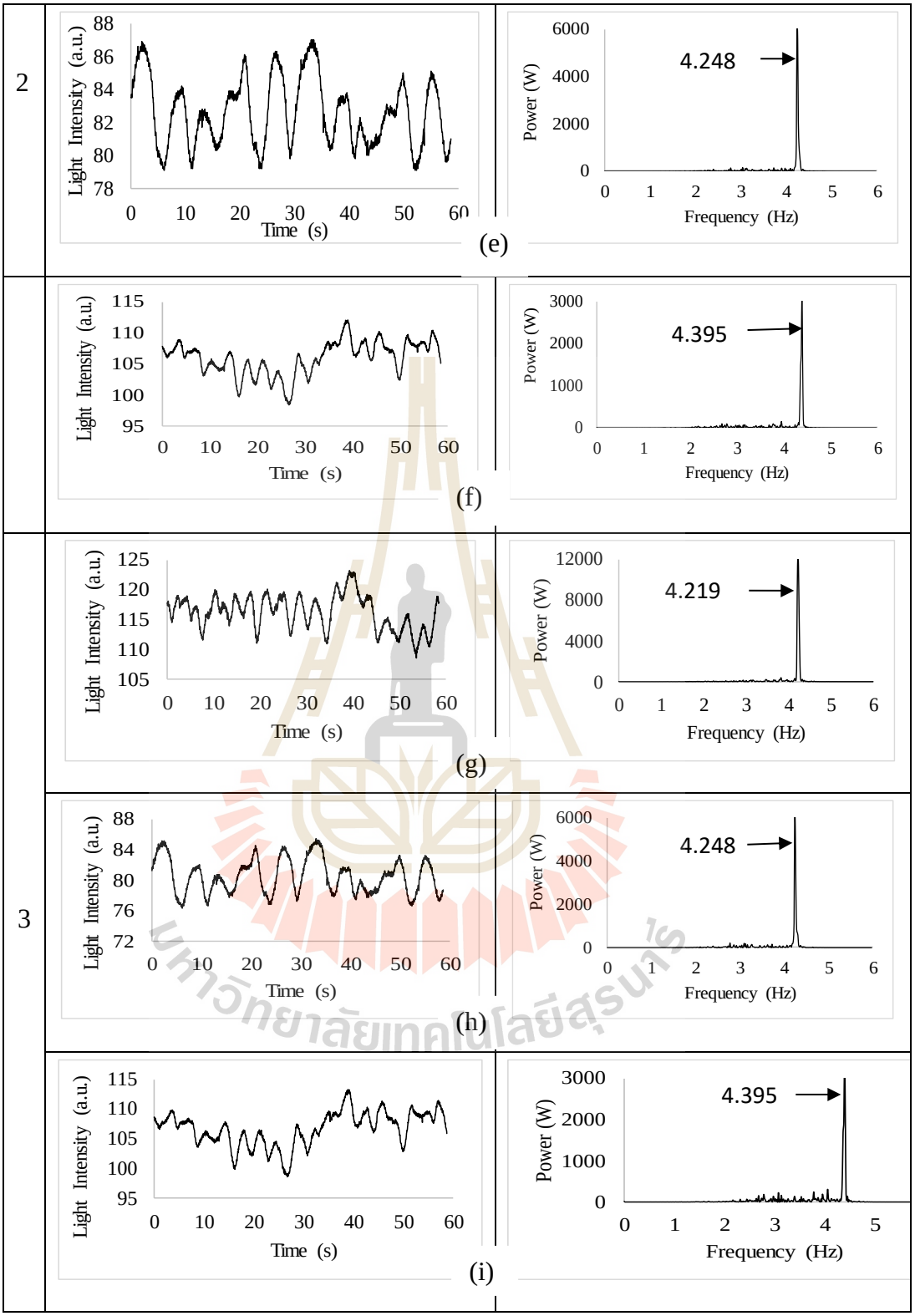


Figure 5.8 Influence of ROI size and position on HR measurements (a,d and g) first sample (b, e and h) second sample and (c, f and i) third sample

5.6 The influence of window functions to HR measurements

The window functions help to process large (infinitely long) data sets by using small data sets. They help to minimize spectral leakage by minimizing the amplitudes near the end of the discontinuous signal as explained in Section 4.2.3. Window functions are designed to perform different tasks. Table 5.2 shows a summary of some window functions and their applications.

Table 5.2 Commonly used windows ((Essick, 2013)

Window	Optimal Application
Hanning	General-purpose applications
Hamming	Resolve closely spaced sine waves
Flat Top	Accurate amplitude measurement of isolated frequency
Kaiser	Resolve two closely spaced frequencies with widely differing amplitudes
Rectangle	Resolve two closely spaced frequencies with almost equal amplitudes

The embryonic periodic activities involve heart beats and breathing. The heart beat frequency is the superior of the breathing frequency. The breathing frequency for chicken embryos is reported to fall between 1.2−1.5 Hz which is below the embryonic HR range of 2.5−5 Hz (E. et al., 2004). Breathing is evident starting from day 19 of incubation as shown in Table 2.1. Therefore, in our analyses we have only one dominant frequency to resolve. The depicted results in Figure 5.9 shows no difference in the HR results for two tested samples using various window functions. Throughout our analyses we used the Kaiser window as it concentrates energy in the main lobe.

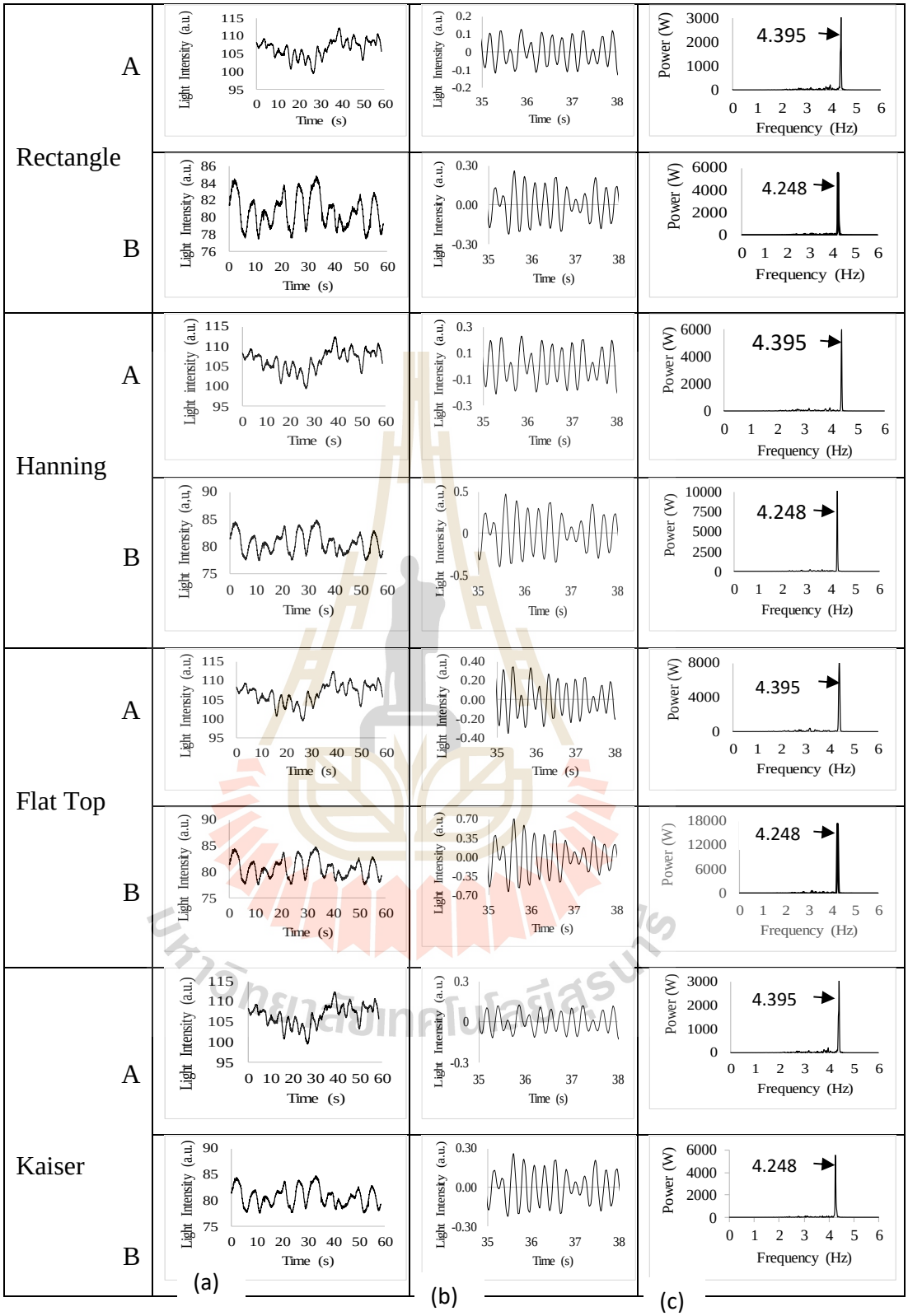


Figure 5.9 Windowing effect (a) waveforms (b) preprocessed and (c) power spectrum analyses of (A) first sample and (B) second sample

5.7 The effect of detrending to HR measurements

We discussed the detrending of the signal and its role in signal processing in Section 4.2.2. We mentioned that detrending removes the wandering as well as the DC part of the signal (non-stationarity) and introduces new trend of stationarity ready for power spectrum analysis. The detrending was done by subtracting the moving average of the time domain signal from the time domain signal itself.

This mathematical approach does not remove all the DC component but minimizes it to the level that is far below the rectified embryonic HR analysis. When there are no embryonic HR waveforms contained in the extracted signal (the case infertile and dead) eggs, the weaker DC part becomes evident and dominant. Figure 5.10 shows the weaker DC part from two infertile eggs emerging after the power spectral analysis. The DC part for non-detrended signal was shown in Figure 4.4 (b) and its rectified power spectrum peak is in the order of billions of Watts square.

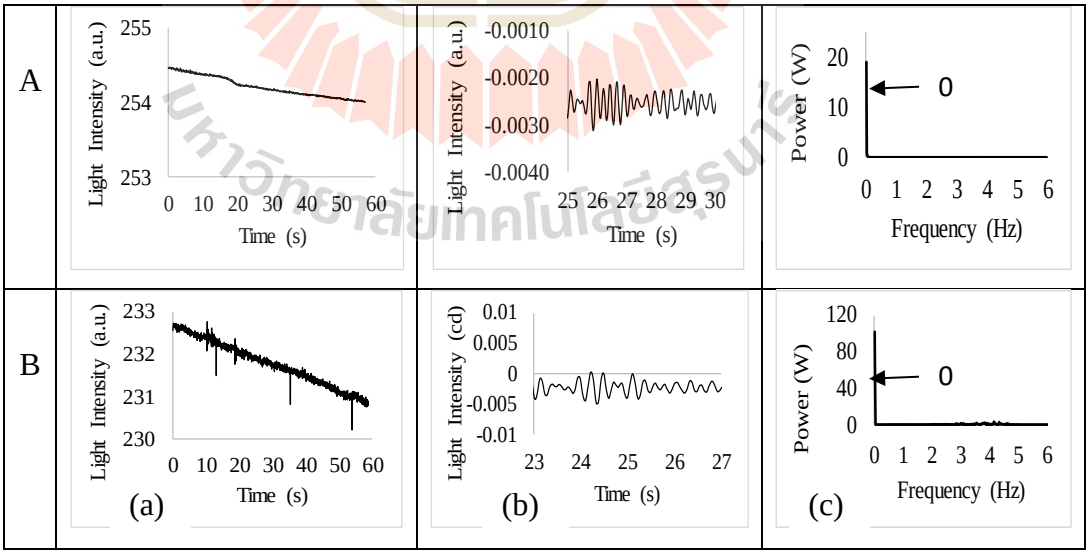


Figure 5.10 Effect of detrending (a) waveforms (b) preprocessed and (c) power spectrum of (A) first sample and (B) second sample

5.8 Scaling up our design

There is a booming desire to expand the equipment's examination scale to meet the poultry industry demand. So far, our designed equipment is in a lab-scale. Our primary goal in this work was to test the idea of multiple embryos HR measurement from incubated chicken eggs. We evidently beyond doubt proved the idea to be to true.

The poultry industry demand is to examine hundreds to thousands of eggs at once. This is a new challenge to our design so that this equipment to meet this demand. With our setup, it may take a little effort to expand the scale from 3×3 to say 10×15 (150 eggs). The 9 LEDs must be replaced by a single uniformly distributed light source or strips of light sources covering more than 10 eggs at once. The light must sufficiently cover the whole area holding the eggs under examination. The camera must carefully be selected to be able to capture good images of all examined eggs at once. The design must take care of the maximum optimum distance between the camera and the light source. Increasing distance between light source and camera lowers the signal to noise ration and hence difficulty to resolve the embryonic HR. Eggs' holders must carefully be selected to block all the leaking light from reaching the camera. The change is subject to hardware part only. Similar pair of codes may be used for recording and analysis. Figure 5.11 summarizes the scaling up idea.

Apart from advancing the main components of the setup, care must also be included to other parameters to make our measuring instrument good and reliable. Teste to examine if the designed system is good must be carried on. The test must cover parameters like validity, reliability, objectivity and administrability of our designed optical system. Instrument's measurement capacity, accuracy, resolution and efficiency tests are mandatory.

One major advantage offered by multiple embryos HR measurement system using a camera over other optical instruments is its minimal cost when scaling up. Only a few hardware parts of the setup like camera's lens and/or light source may need to be changed while all other sections of the measurement system remain the same. For other optical measurement systems such as digital egg monitor where a single egg requires a separate measuring equipment, it is even harder with higher cost to scale up from one egg to hundreds of eggs.

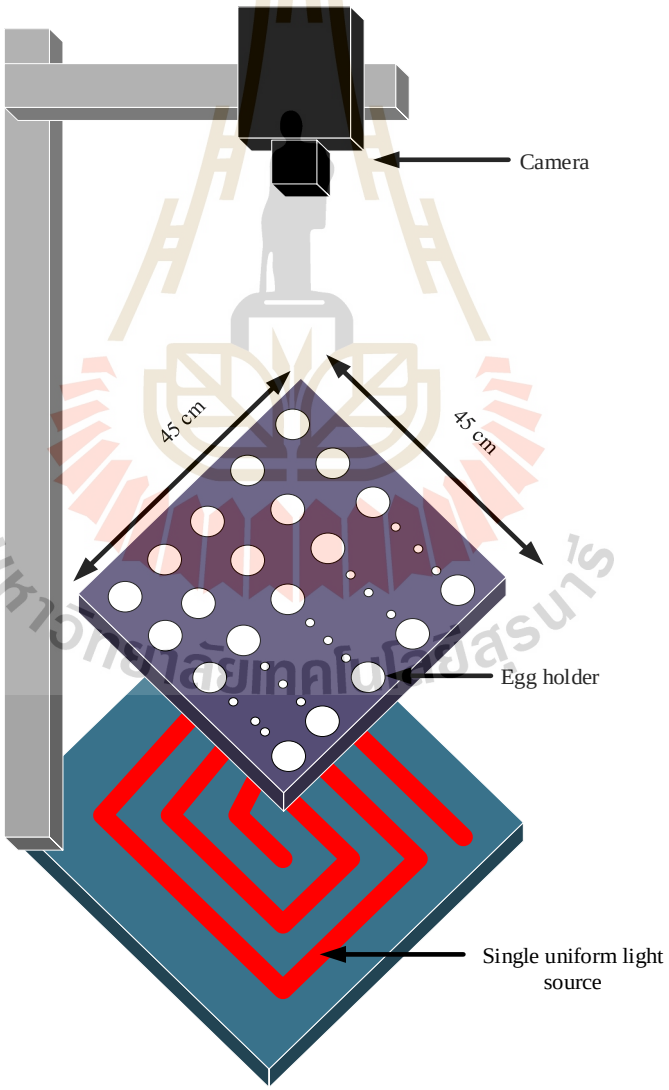


Figure 5.11 Sample scaled up measurement system

CHAPTER VI

EMBRYONIC HEART RATE MEASUREMENT BY ELECTROCARDIOGRAPHY (ECG)

6.1 Basic definitions

Electrocardiography (ECG or EKG) is the process of recording the electrical activity of the heart over a period by means of electrodes. The electrodes could be placed on the skin for humans or inserted beneath the skin or eggshell for avian/birds or eggs respectively. Electrodes could be conductive pads (for humans) or thin conductive wires such as silver, lead or cupper for incubated eggs. These electrodes could detect tiny electrical changes that might arise from the heart muscle's electrophysiologic pattern of depolarizing and repolarizing during each heartbeat. Important parts of the ECG signal and their inference are the P which represents the atrial systole contraction pulse, Q which is a downward deflection immediately preceding the ventricular contraction, R which is the peak of the ventricular contraction, S which is the downward deflection immediately after the ventricular contraction and T which is the recovery of the ventricles as shown in Figure 6.1. In chicken embryos, ECG rises due to imbalance of potassium ions (K+). The cardiac works causes the permeability of the cells membranes to vary. These permeability variation lead to exchange of potassium ions across the cell and hence arising of ECG signals.

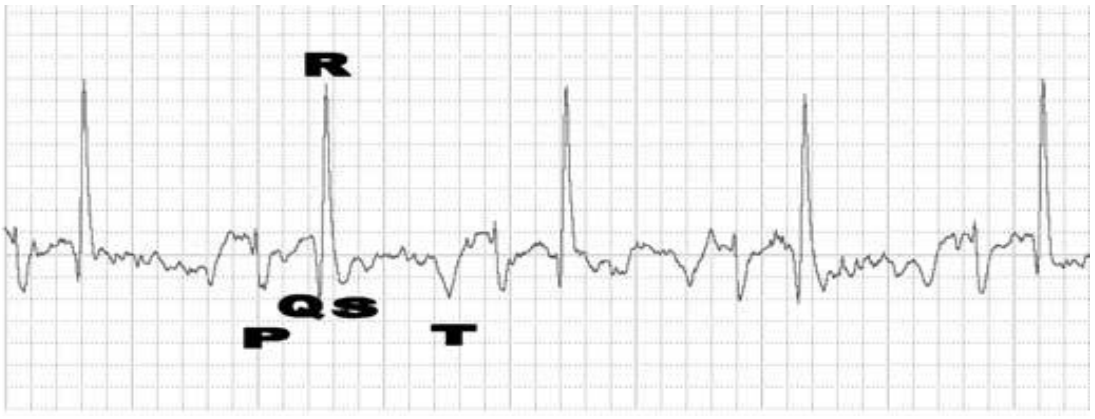


Figure 6.1 Sample ECG signal (E. et al., 2004)

Electrocardiograph is the tool used to perform electrocardiography and produces electrocardiogram. The main component of the electrocardiograph is the instrumentation amplifier, which amplifies the weak cardiac generated signal (voltage difference between electrodes) ready for HR extraction. Electrodes are also important in the electrocardiograph, they help collect ECG signals and lead them to the measurement system. The embryonic ECG signal for chicken eggs ranges from 10−100 μV (Aubert, Leribaux, Beckers, Ramaekers, & Berckmans, 2000). The nature of ECG signal is weak (small amplitude), susceptible to noises and hence hard to measure. Recording good ECG signal could require high precaution starting from electrodes choice, implantation, amplifier used, and software used for recording and analysis. Electrocardiography, like any other invasive methods provide good accuracy and is regarded as the golden method for HR measurements. In this work we used ECG method to measure the embryonic HR of incubated chicken eggs to validate similar results from our designed non-contact and noninvasive optical system. Validation measurements were taken simultaneously for both systems of measurements to acquire real time signals for both methods.

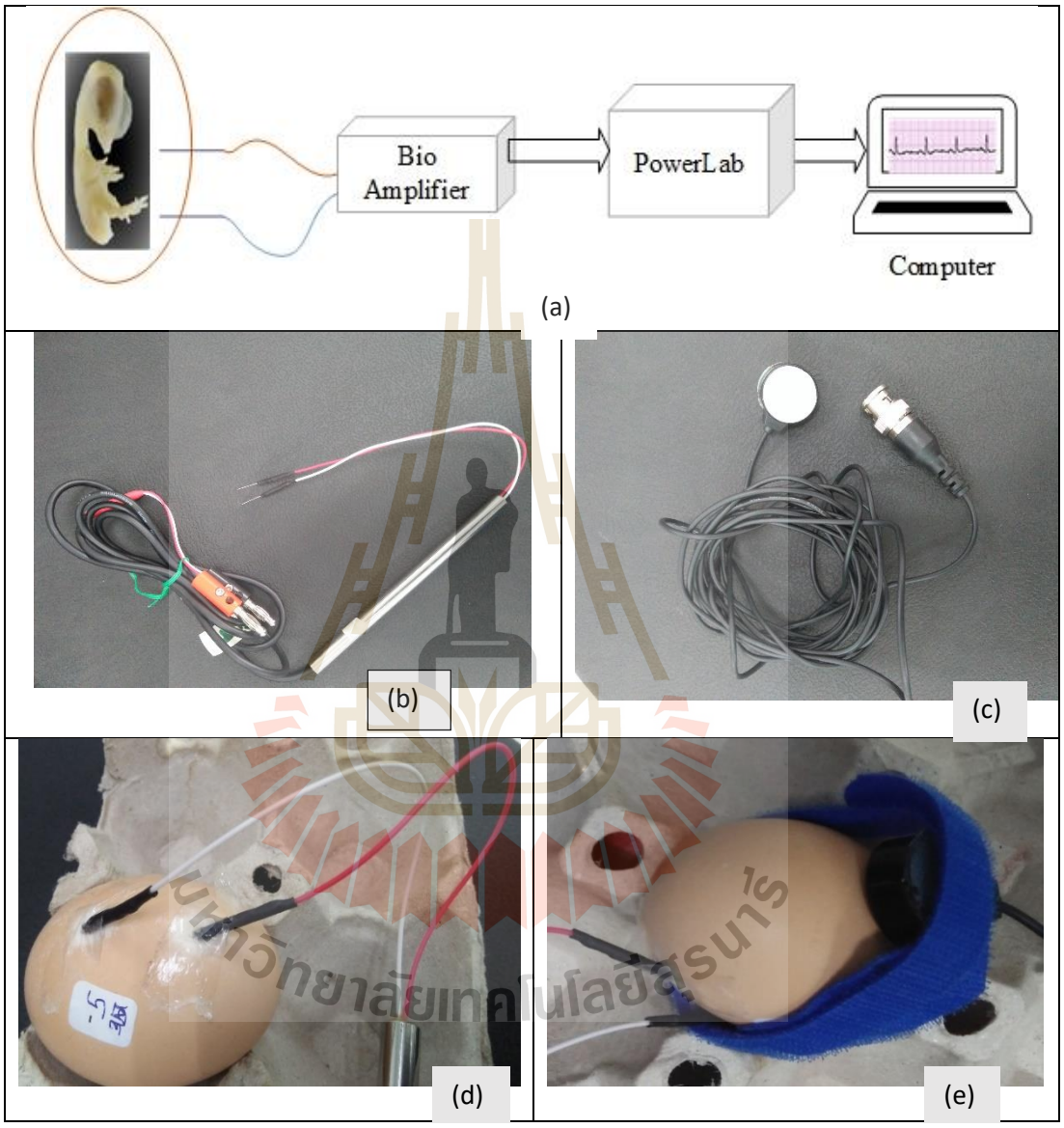
6.2 ECG Setup

Three eggs which gave active embryonic HR signal with our optical measurement system were candled to confirm the embryo's position and to mark the air cell. ECG electrodes formed from two thin silver wires, 10−11 mm long and 0.5 mm in diameter were used. Two locations were marked on the eggshell, one in the middle and the other near the wide end (air cell side). A tiny piece of eggshell was removed by a sharp blow with the tip of a sharp knife, which pierced the shell membranes and the chorioallantoic membrane. The holes were covered by croco tape glue. The eggs with created tiny holes were placed in a still-air incubator for stabilization before experiments. The tips of the silver electrodes were inserted into the holes, and the electrodes were fastened on the eggshell using croco tape during the experimentation time. Fastening of the electrodes was necessary to reduce unnecessary movements which could affect the acquired signal.

The two electrode leads were connected to a differential amplifier (AD Instruments, Bio Amps, FE132) input. This amplifier is designed for recording wide variety of biomedical signals such as ECG, EMG and EEG from animals. The bio amplifier has a six pin (DIN/MS) socket that fit 3-lead cable, input ranges of ±5-μV to ±100-mV, accuracy of ±1.5% and the bandwidth of 0.1 Hz to 5 kHz. Its frequency bandwidth is sufficient to measure the embryonic HR of incubated chicken eggs which is between 2.5 Hz to 5 Hz.

Data acquisition board (DAQ) is another component necessary for computer-based measurements. We used the AD Instrument's PowerLab (8/30 series, 6-channels) to acquire ECG signals from the examined chicken embryos. PowerLab was interfaced with the LabChart software to acquire, store and process the ECG signals. The sensors

used, sensors placement and full validation setup arrangement for recording embryonic HR for the examined samples is shown in Figure 6.2.



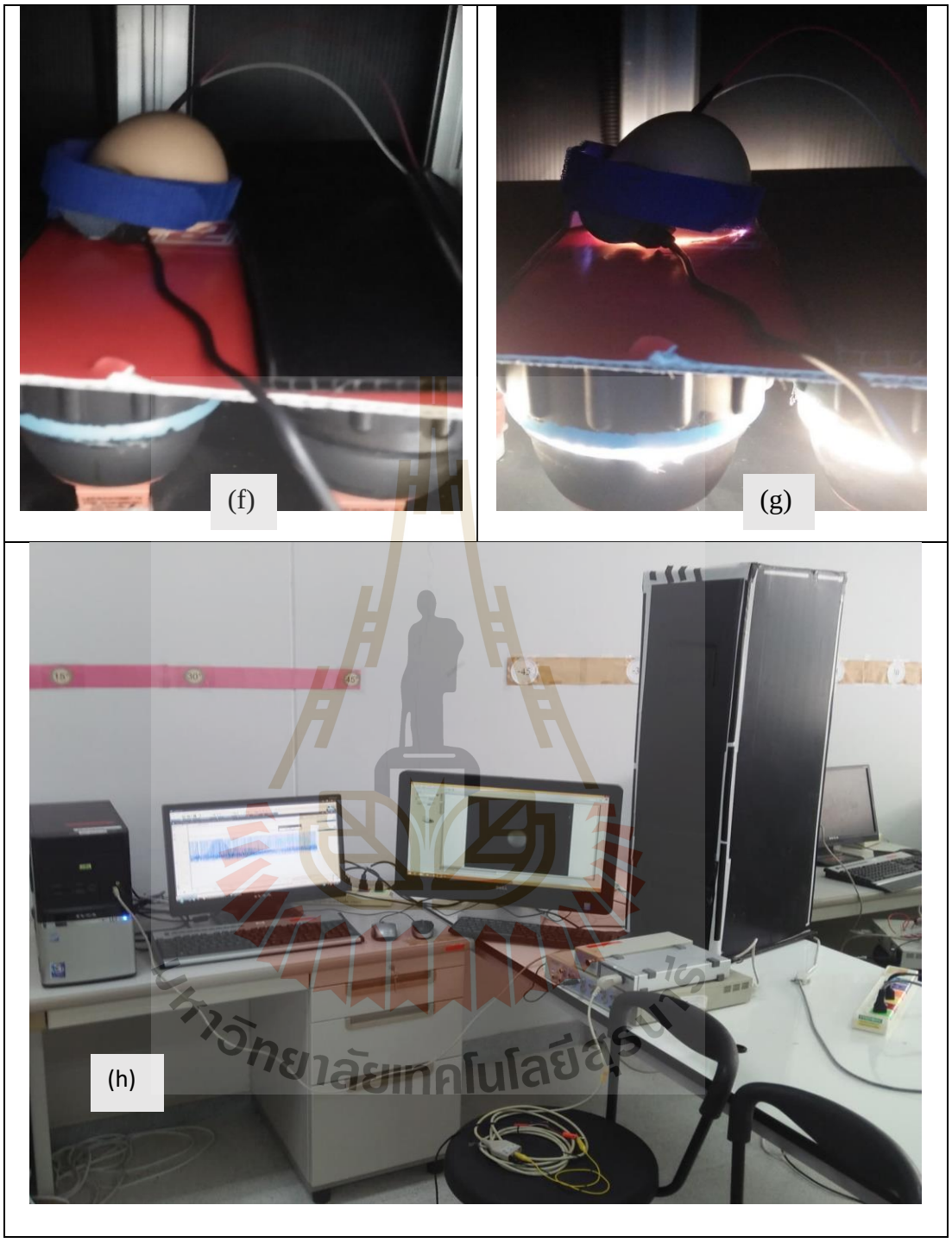


Figure 6.2 Validation setup (a) basic ECG setup (b) ECG silver probes (c) ECG pulse transducer (d) ECG silver probes inserted to an egg (e) ECG silver probes and pulse transducer set to an egg (f) setup before recording (g) setup during recording and (h) full experimental setup.

6.3 ECG Experimental

The content of the egg is an electrically conductive mass. The electrical excitation generated by the heart propagates through the embryonic body, the extra-embryonic fluids and the egg contents towards the eggshell. This electrical signal (ECG) can be detected with electrodes piercing the eggshell (E. et al., 2004; Kato, Moriya, Dzialowski, Burggren, & Tazawa, 2002; Sugiyama, Miyazaki, Saito, Shimada, & Miyamoto, 1996). The position of the embryo was confirmed, and experimental setup arranged as explained in Section 6.2. The electrodes were cleaned in 70% v/v alcohol solution to prevent bacterial formation. The electrode leads were connected to bio amplifier and then to the PowerLab. The electrode placed in the middle of the egg was connected to the live wire while the other to the ground of the amplifier as shown in Figure 6.2. The pulse simulator transducer was also placed as shown in Figure 6.2 for simultaneous recording.

ECG signals acquisition was done at 1000 Hz. Continuous 3-minutes ECG signals were recorded at room temperature from each of the three. All eggs returned to the incubator after experimentation. All the recordings were taken simultaneously for the three methods involved.

6.4 ECG system results

Generally, the recorded ECG signal is often contaminated by noise and artifacts that could be within the frequency band of interest and manifest with similar characteristics as the ECG signal itself. In order to extract useful information from the noisy ECG signals, it is needed to process the raw ECG signals.

ECG signal processing could be roughly divided into two stages by functionality: preprocessing and feature extraction. The preprocessing stage suppressed noise from the raw ECG signal and the feature extraction stage extracted embryonic HR information from the ECG signal. With AD Instrument's LabChart software or any other related tool such as Biomedical Toolkit, one could conveniently process acquired ECG signal by baseline wandering removing, noise cancellation, QRS complexes detection, HR extraction and so forth. ECG signal processing and features extraction processes are shown in Figure 6.3.

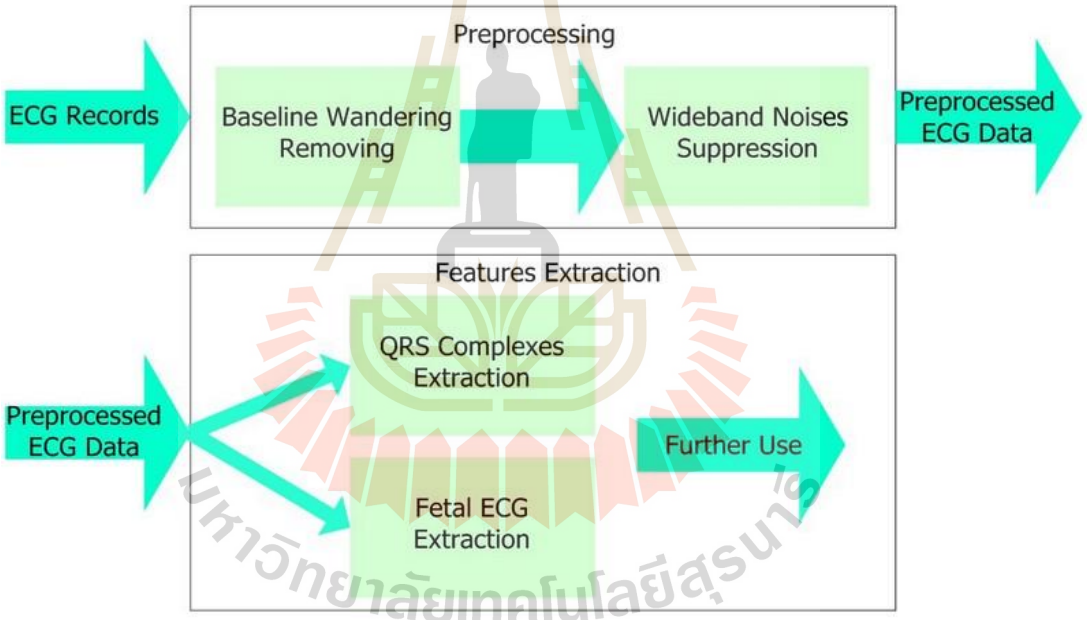


Figure 6.3 ECG signal processing steps (National Instruments, 2017)

6.4.1 Preprocessing ECG signals

Preprocessing ECG signals helped to remove power line interference, motion artifacts, electromyographic (EMG) noise and baseline wandering contaminants from the ECG signals. Among these noises, the power line interference and the baseline

wandering (respiration and motion artifacts noises) are the most significant and can strongly affect ECG signal analysis. Except for these two noises, other noises may be wideband and usually a complex stochastic process which also distort the ECG signal. The power line interference is narrow-band noise centered at 50 Hz (or 60 Hz) with a bandwidth of less than 1 Hz. Usually the ECG signal acquisition hardware with proper grounding can remove the power line interference. However, the baseline wandering, and other wideband noises are not easy to be suppressed by hardware equipments. Instead, the software scheme is more powerful and feasible for offline ECG signal processing.

Baseline wandering is highly contributed by the respiration or motion artifacts of the embryo within an egg. Baseline wandering noise could be removed by using the digital filter or wavelet transform approaches. The LabChart removed the baseline wandering noise automatically during ECG recording.

After removing baseline wandering, the resulting ECG signal is more stationary and explicit than the original signal. However, some other types of noise might still affect feature extraction of the ECG signal. The noise may be complex stochastic processes within a wideband, so cannot be removed by using traditional digital filters or wavelet transform. Removing the wideband noises, we could use the wavelet denoise process. Wavelet denoise process first decomposes the ECG signal into several sub-bands by applying the wavelet transform, and then modifies each wavelet coefficient by applying a threshold function, and finally reconstructs the denoised signal. This process can be performed automatically by the LabChart software.

6.4.2 Feature extraction on ECG signals

This section mainly discusses how to perform ECG feature extraction. ECG features from the preprocessed ECG data, includes QRS intervals, QRS amplitudes, PR intervals, QT intervals, etc. These features provide information about the HR, the conduction velocity, the condition of tissues within the heart as well as various abnormalities.

Embryonic HR was determined from the filtered ECG signals with the aid of a computer. From the length of a heart beat on the ECG trace, one can be able to calculate the HR. Embryonic HR (BPM) was then calculated from the time between adjacent QRS complexes (RR interval). Measurements were made in noise free segments. Figure 6.4 shows a sample of a 12-seconds ECG waveform obtained from a 14-days old embryo. By visual inspection, the RR intervals are not uniform, hence we could determine the embryonic HR of an egg by considering the whole section. Mean embryonic HR (beats min-1) could be obtained by multiplying the total number of R-peaks by 60 and dividing by the time interval between the first and last R-peak. We assumed the R-peaks are all peaks with amplitude above 1.4 units. We counted 38 R-peaks. Mean Embryonic HR is (38×60) ÷12 or 190 BPM.

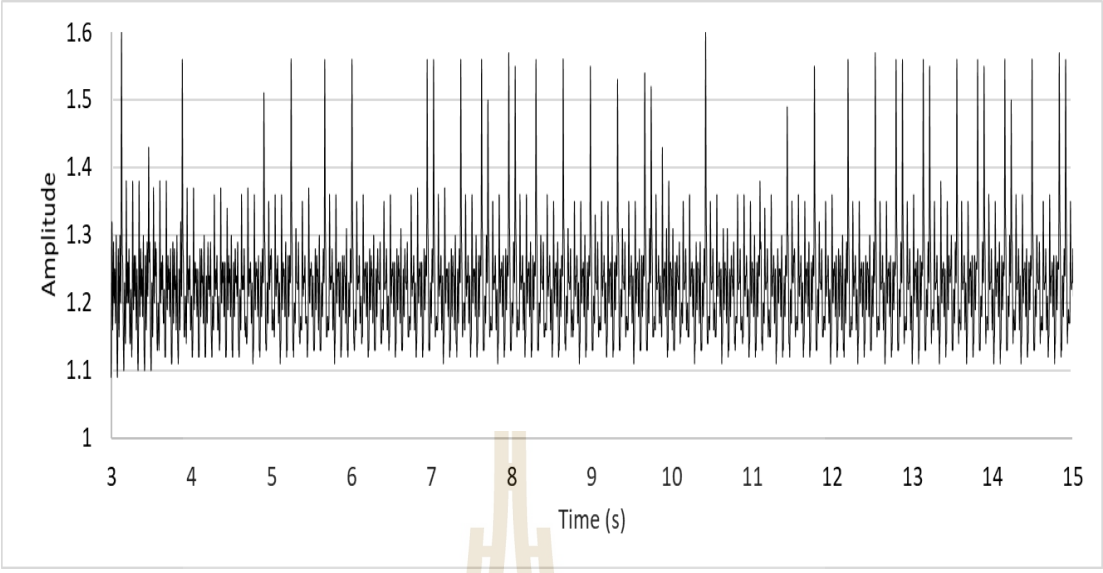
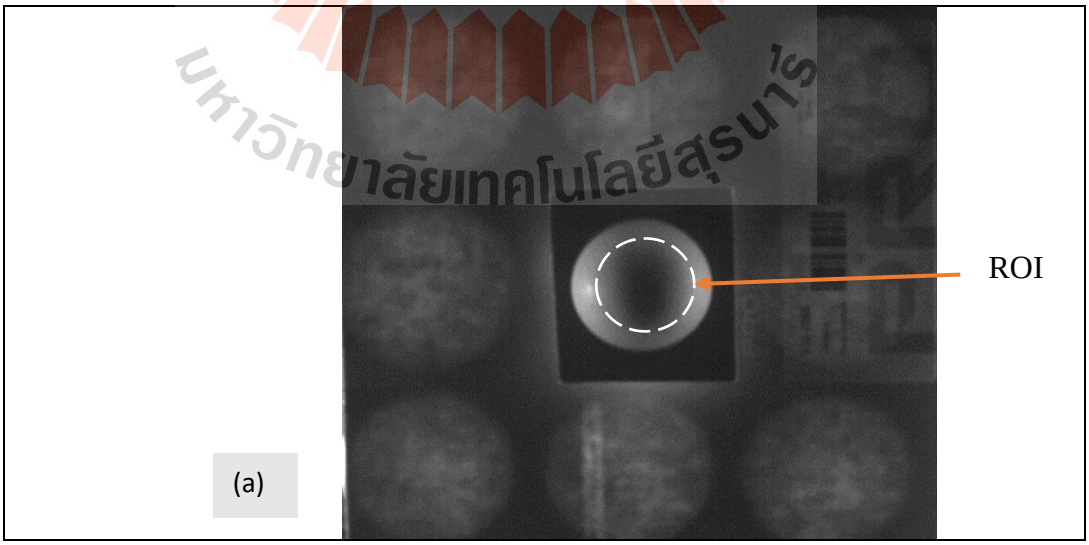


Figure 6.4 Sample ECG result

6.5 Optical system results

The same egg was simultaneously recorded using our optical system. The embryonic HR was measured to be 3.15 Hz or 189 BPM, as shown in Figure 6.5.



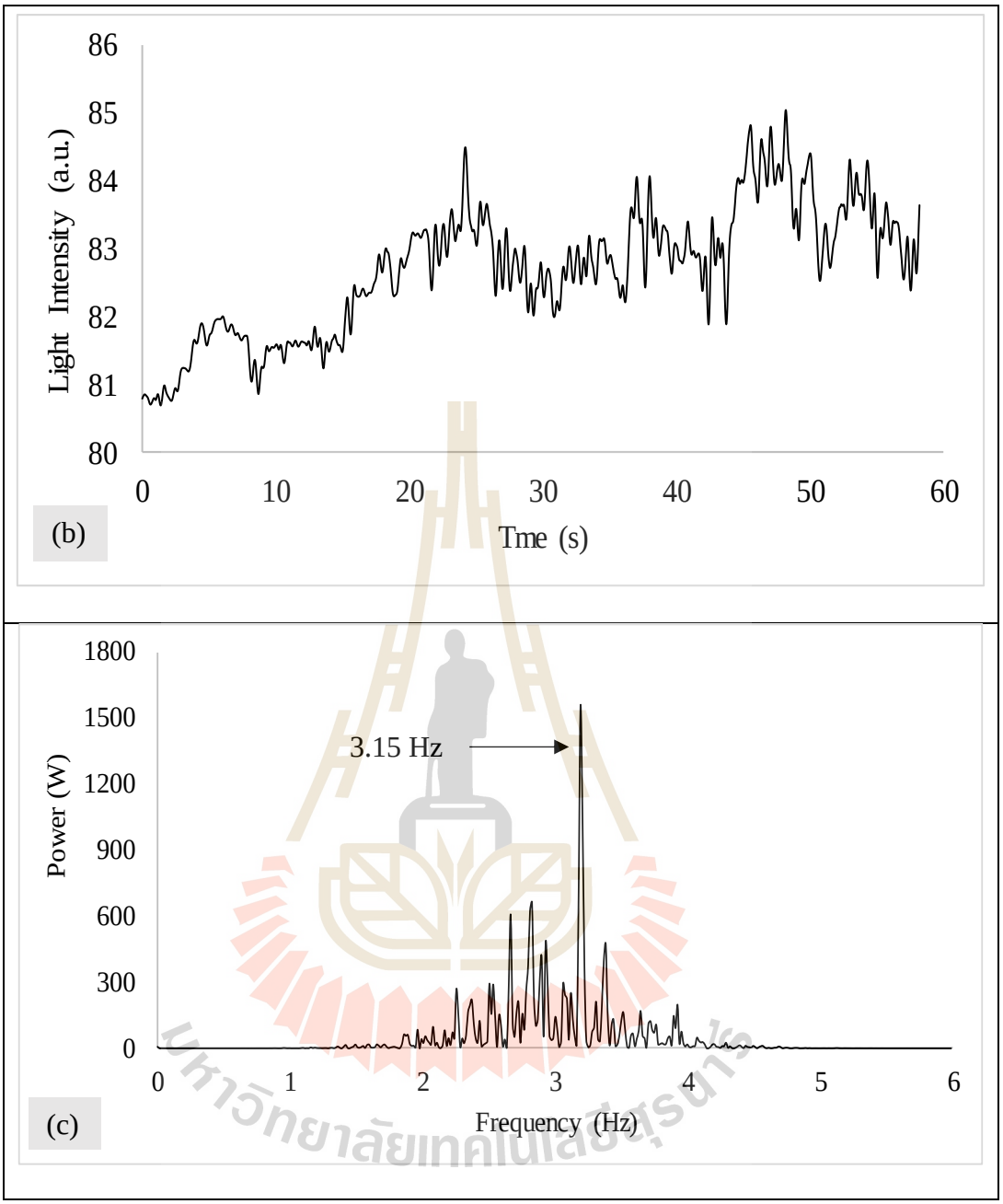


Figure 6.5 Optical system result (a) recording capture (b) waveforms (c) power spectrum analysis

6.6 Results Comparison

Both ECG system and optical system showed a very good agreement for the measured embryonic HR frequency. The frequency coherence was in the range 0-1 Hz.

Since the invasive ECG method is regarded as the golden standard for measurement of embryonic HR. We are confident that our optical measurement system has higher accuracy and can be used for embryonic HR measurements of the incubated chicken eggs. The optical system percentage error in embryonic HR measurement could be calculated by ((189-190)/190) ×100% which gives -0.52%.

The embryonic HR measurement of chicken egg using pulse transducer (TN1012/ST, ADInstruments), could not give a good ECG signal. This transducer uses piezo-electric element to convert force applied to the active surface of the transducer into an electrical analog signal. The pulse transducer connects directly to a PowerLab input and is automatically recognized by the LabChart software. Blood pressure changes will change the finger circumference (expansion and contraction), which will then change the force applied to the active surface of the transducer. Therefore, changes in the electrical analog signal correspond to these force changes and can then be used to determine heart rate. The typical output is 50–200 mV. The pulse transducer could be used for monitoring of finger peripheral pulse as well as small animal respiratory activity. The transducer is sensitive to movement. To avoid large motion or artifacts and obtain good recordings, the transducer must be kept still.

We thought that, we could not get a good signal because the eggshell is very hard, and the embryonic activities might have exerted weaker pressure the eggshell which was hard to detect with this transducer. We also thought that, we need an isolated chamber that could not absorb the vibrations arising due to the cardiac activities.

CHAPTER VII

CONCLUSION

We have developed a lab-scale instrument that can measure the embryonic HR of the nine incubated chicken eggs. The instrument is composed of a light source and a digital camera as a light sensor. The system first captured sequential images of the examined eggs under dark-field illumination and saved them in .AVI format. Secondly, the saved video files were analyzed, and the embryonic HR extracted and measured. We examined a total of 115 incubated chicken eggs, 106 fertile eggs and 9 infertile eggs. We could measure the HR of multiple embryos from day 3−18 of the incubation period. The measured HR ranged between 150−315 BPM.

Our noninvasive optical system was able to identify alive, infertile and dead embryos among examined incubated chicken eggs from earlier to late incubation days by measuring the embryonic HR. This ability could give flexibility to the user to examine the life status of the incubated eggs at any incubation day of choice. Early identification of infertile and dead embryos could help the poultry industry save incubator space, learn and fix problems leading to infertility and premature embryo's deaths, run activities more economical and could motivate new practitioners start the business. Identifying dead embryos in the late incubation days could help remove the eggs which could turn into the exploders and affect the good eggs or chicklings or environment.

Similar results from simultaneous recordings of both noncontact, noninvasive Optical equipment and invasive ECG methods for the examined embryos were

observed. The Optical method offers more advantages than the invasive ECG method as, first it is noncontact and noninvasive method which does not affect the natural habitat of the growing embryo and secondly its ability to measure embryonic HR at early days of embryo development.

Our accomplishment as for now is still in a lab-scale, we could examine and recover the embryonic HR of nine incubated chicken eggs at once. This idea requires further development to examine larger number of eggs at the once to meet the real poultry industry demands where hundreds to thousands of eggs must be examined at once.

Some of the benefits that could be offered from using our non-invasive and noncontact optical embryonic HR measurement system. Researchers could use this system to studying embryonic HR of incubated eggs to identify and predict for the sex of the developing embryo. This could help farmers decide which eggs to keep or discard from the incubator in their economic favors. It might also help researchers explore more about the influence of external incubation parameters such as temperature, humidity and illumination during the incubation process and to optimize them.

Multiple embryonic HR measurement of incubated chicken eggs using a digital camera worth special attention as compared to other optical systems such as using a photodiode. The use of a digital camera makes the measurement process easier to adapt the equipment to any scale of measurement with minimal or no hardware nor software change. This flexibility makes it more useful and promising in the future to replace the widely used candling method.

Next step of this work should consider expanding scale to examine hundreds

of eggs at once, be checked for resolution and accuracy to attract the commercial context. To achieve better results, it may also make use of the NIR light source (wavelength 800 −1000 nm) as they offer higher transmission of light through the chicken egg which is an important requirement to achieve good results in optical measurement systems.

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APPENDIX A

LABVIEW CODES

I. Recording codes

This was the first main part of acquiring video files of the examined chicken eggs. This piece of codes consisted of subsections like acquisition, storage and clear buffer after writing an image. Figure A.1 shows full recording piece of codes used.

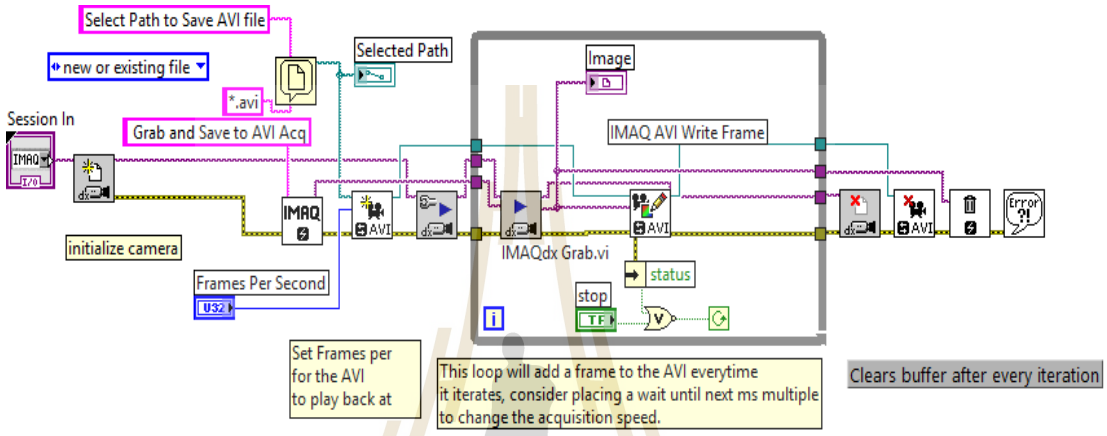

Figure A.1 Codes for recording video files

II. Embryonic HR Extraction and measurements codes

This piece of codes extracted and measured embryonic HR of the examined chicken embryos from the offline stored AVI video files. This piece of code involved, reading the AVI video file, creating and getting coordinates of the regions of interests (ROIs), quantification of mean pixel intensities for each image, preprocessing of acquired waveforms, perform power spectral analysis, extract and measure embryonic HR and clears the buffer after every iteration. The outer FOR loop managed the examined frames while inner FOR loops ensured the processing of each recorded frame before the next frame.

The LabVIEW AVI VIs read the stored AVI files. The IMAQ create VI created

space on the computer RAM for every read image. Sample codes shown in Figure A.2.

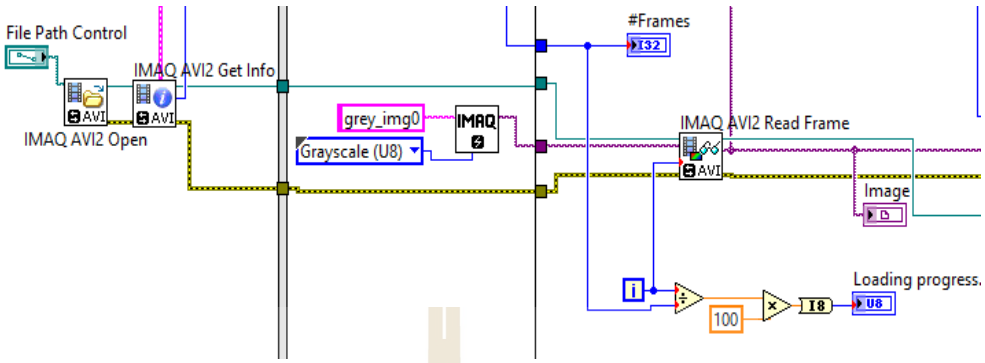

Figure A.2 Example of codes to read offline AVI file

Image create ROI, IMAQ ROIToMask 2, IMAQ Quantify 2 and IMAQ create VIs created ROI, acquired ROI coordinates, quantified pixels mean intensities (waveforms) for each image and stored ROI coordinates on computer memory for each iteration respectively. Figure A.3 shows a used code for ROI creation and image pixels quantification. The FOR loop ensured each created ROI is processed.

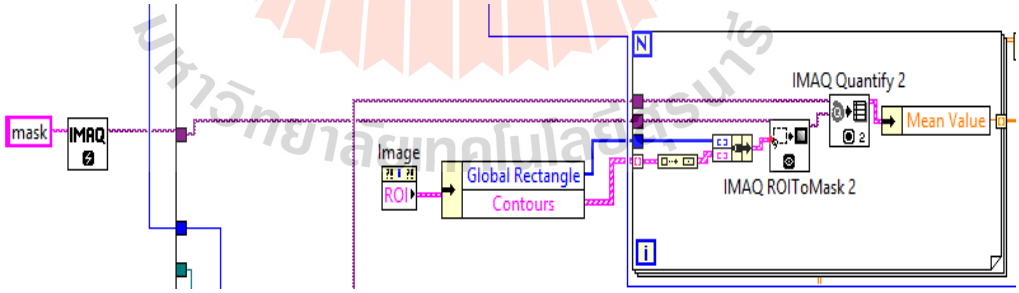

Figure A.3 Example of code used to create ROI and extract the waveforms

The waveforms obtained from each created ROI were preprocessed ready for embryonic HR measurements. Preprocessing involved the moving average, detrending and

background noise subtraction, time scaled Kaiser window and filtration as explained in Section 4.2. The FOR loop helped the preprocessing of waveforms from each image. Sample codes used are shown in Figure A.4.

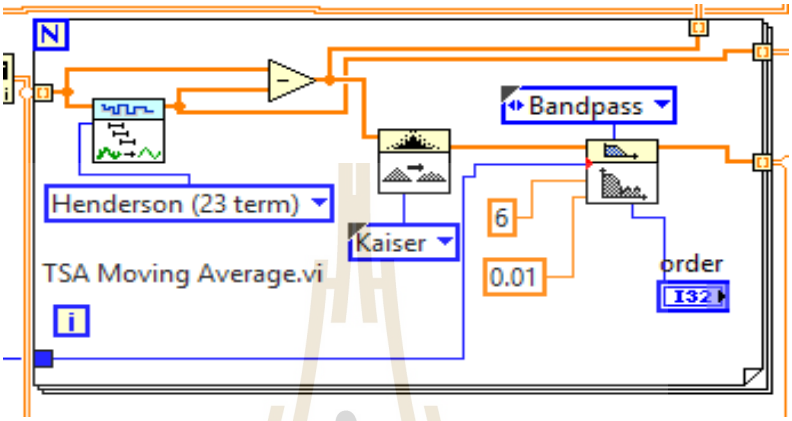

Figure A.4 Example of preprocessing codes

The HR feature extraction came after the power spectrum analysis using the Multi-channel FFT VI. Array subset VI isolated values for each ROI representing an egg. The absolute and the square functions ensured the display of positive values and clear visibility of the resolved highest power frequency respectively. Figure A.5 shows sample codes used to perform DFT using FFT.

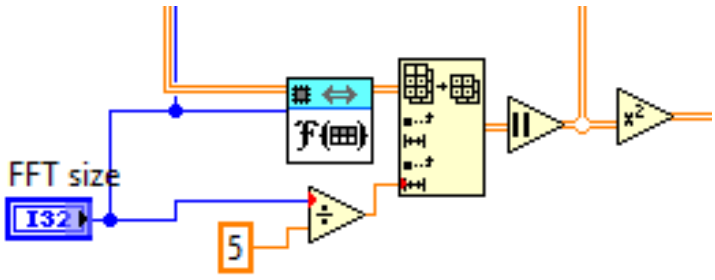

Figure A.5 Sample codes used to perform FFT

The embryonic HR for each egg was extracted and measured using the peak detection VI. The position (HR frequency) of the highest energy signal was detected and embryonic HR measured. Sample codes used to perform this task is shown in Figure A.6.

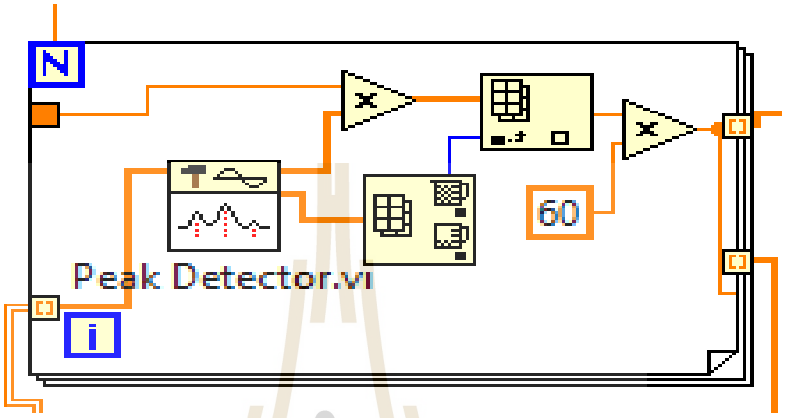

Figure A.6 Sample codes used to extract and measure HR of chicken eggs

Result for each examined egg was displayed by using the Index Array VI as shown in Figure A.7. This VI isolated values corresponding to each examined egg.

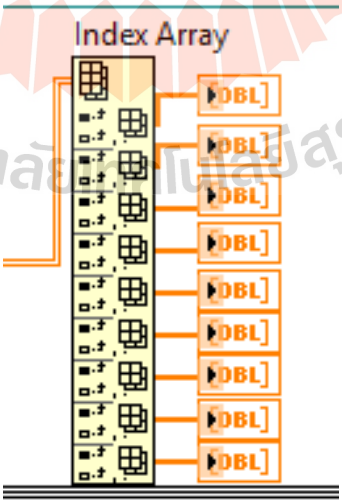

Figure A.7 Display each result for each examined egg

APPENDIX B

PUBLICATION

List of Publications

Bakunda, J.N. Pongchalee, P. and Sutapun, Boonsong. (2018). Live embryo detection using a non-contact heart rate measurement of chicken eggs. **Proceedings Volume 10821, Advanced Sensor Systems and Applications VII: Photonics Asia, Beijing, China.**

Live embryo detection using a non-contact heart rate measurement of chicken eggs

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ABSTRACT

Artificial incubation of chicken eggs is the economic procedure in the poultry farms. Eggs candling method is widely used to check for the embryo fertility among the incubated chicken eggs. At late incubation period, fertilized eggs appear darker than unfertilized eggs under dark-field illumination. However, the method cannot precisely differentiate between live eggs and non-viable eggs at the late incubation days since both types of eggs appear dark. Infertile eggs could be exploded in the hatcher baskets and cause infection to newborn chicks. In this work, we proposed a non-contact and non-invasive method that can precisely checked for live embryos by extracting the heart rate of multiple incubated eggs using a digital camera. System consisted of light emitting diode as light source and CMOS camera for recording video images. Digital processing techniques followed by fast Fourier transform algorithm were employed to extract embryonic HR information. A total of 30 chicken eggs including 24 fertile and 6 infertile eggs were examined from days 7-16 of incubation. The proposed technique may offer benefits to both poultry industry and animal science researchers. Poultry industry may use this system to confirm for chicken embryos' fertility as well as sorting out dead eggs from live eggs whereas animal science researchers may use this system to further the study about the development of chicken embryos.

Keywords: Chicken embryo development, heart rate detection, non-contact measurement, egg fertility.

1. INTRODUCTION

Chick embryos have been used as an experimental animal to study developmental physiology [1,2]. Detectable embryonic heart rate (HR) is a sure sign for the alive and developing embryo within an incubated egg. Traditionally, hatching and non-hatching eggs are identified by a candling method [3-5]. Hatching eggs cast dark shadows starting from day 7 of incubation under dark illumination. Candling method is effective but cannot accurately distinguish the dead eggs from live eggs, especially during late incubation days. Dead eggs can explode and infect the hatchling basket and flocks; hence, they should be removed from an incubator before transfer to the hatchery baskets.

There are several methods that can be used to study embryonic HR from incubated chicken eggs. These methods are either invasive or noninvasive. Electrocardiogram (ECG), impedance cardiogram (ICG) and pulse oximetry (POM) are referred as semi-invasive methods. ECG uses at least two electrodes inserted into the tissues surrounding the embryo to record the electrical signals generated by the cardiac work [1,6,7]. However, ECG can measure the HR starting from the second half of incubation until hatching. ICG can detect the embryonic HR of the incubated egg from the electrical impedance variations generated by the cardiac activities [8]. ICG is useful for embryonic HR estimation between days 3–9. POM, an invasive optical method, consists of a light emitting diode and a photodetector lied beneath the eggshell to record the embryonic HR of the incubated eggs from the variations of the transmitted light intensity [9]. POM is effective for HR measurement from days 12–20. These semi-invasive methods are useful for HR measurement of one egg at a time and they leave a hole on the eggshell, risking the developing embryo to bacterial infection. These methods are not practical for multiple embryo HR measurement.

To overcome the mentioned challenges, other researchers have developed noninvasive methods to measure the embryonic HR of the incubated eggs. Reported noninvasive methods for embryonic HR measurements of incubated eggs include ballistocardiograph (BCG) which measures the HR from minute ballistic movements caused by the cardiac activities which can be detected by piezoelectric sensors [10-11] or by an optical laser interference [12] and by acoustocardiography (ACG) which involves detecting the pulsatile air variations (pressure) using a microphone or a differential transducer. The setup requires placing an egg in a sealed and isolated vessel containing condenser

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microphone [13,14]. BCG can measure the HR starting from day 7 until hatching while ACG can measure from day 12 until hatching. BCG and ACG can measure the HR of one egg at a time and require expensive equipment to implement, making them not practical for multiple embryo HR measurement.

Non-contact techniques based various optical methods for embryonic HR measurement and fertility detection have been developed [2,15-18]. In Ref. 2, a windowing technique was used to allow for long-time microscopic camera recording of the embryonic cardiac activities. This semi-invasive method allowed embryonic HR measurement starting from day 4 until hatching, but it measured the HR of one egg at a time. Also in Ref. 15, an imaging processing system for detecting the fertility of an egg was developed. The system used machine vision algorithm to process the captured eggs images to classify fertile eggs from infertile eggs. However, this system cannot detect embryonic HR of the egg. In Ref. 16, an optical and noninvasive egg monitor was developed. The system consisted of the light source and the photodetector and measured the embryonic HR from the transmitted light signal through the sample. The method is not applicable for multiple embryonic HR measurements. In Ref. 18, a hyperspectral imaging system was developed to detect fertility of an incubated egg between days 2-5. This method cannot detect the HR of an embryo. None of the reported methods so far can be used for noninvasive multiple embryonic HR measurements.

We propose a non-invasive and non-contact optical method for multiple embryonic HR measurements. The proposed system using a digital camera consisted of an LED as a light source and a digital camera as a light detector. Signal processing techniques including moving average and bandpass filtration were employed to suppress the background noise and the zero frequency (DC) components. Fast Fourier transform method was employed to analyze the frequency components and for the heart rate extraction. We were able to extract and measure the embryonic HR of multiple incubated eggs simultaneously. Infertile and dead eggs can be detected. The proposed system could offer benefits to the poultry industry as well as the animal science researchers.

2. MATERIALS AND METHODS

2.1 Samples

Experiments examined a total of 30 chicken eggs of which 24 were fertile and 6 were infertile. These eggs were artificially incubated at the Suranaree University of Technology (SUT) poultry farm. Eggs were incubated at 38.5 °C and 60% relative humidity. The eggs were automatically turned every hour. The eggs collected were from Native Korat chickens. Eggs were transferred from SUT farm to the testing laboratory at the incubation day 7. During the course of experiments, the eggs were stored in a laboratory incubator at 37.2 °C and 47.4% humidity until hatching.

2.2 Imaging system

Figure 1(a) shows the imaging setup for the embryonic HR measurement. The main hardware components consisted of a light source, a camera and a sample holder. Light source is made of a white-light emitting diode module (Lamptan MR16). Each specimen (chicken egg) had its own dedicated light source. The camera is a monochrome CMOS camera (Basler, acA1300-60gmNIR) equipped with an imaging lens (Edmund Optics, 16mm/F1.4). Sample holders fitted on top of the fixed light sources. Each egg lied vertically with air cell facing up (wide end of the egg facing up) on top of the light source. The video file was recorded for 60 s at the frame rate of 30 fps and was stored on the computer memory for further analysis. LabVIEW 2016 software (National Instruments) was used for recording the video frames and performed data analyses to extract the HR information. The setup was covered completely all sides using black sheets to isolate an external light sources influence. The eggs were immediately placed back to the incubator after finishing each experiment. This setup can examine 9 eggs simultaneously (Figure 1(b)).

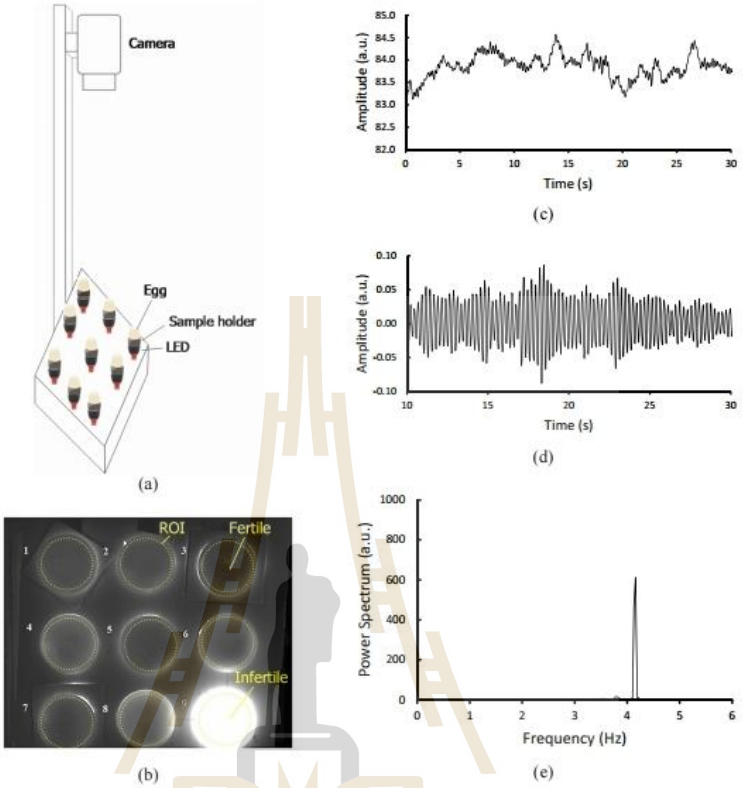

Figure 1. (a) Experimental setup. (b) Captured image of 9 eggs at day 9. ROIs (dotted circles) are shown. One egg with high brightness is infertile and the rest of the eggs are fertile. (c) A typical time-varying signal obtained from a fertile egg at day 9 (d) The signal after the detrending and filtering processes. (e) The corresponding power spectrum showing the peak representing the HR of the embryo.

2.3 Experimental

All of 30 eggs had been examined for every days starting from day 7. After day 16, the fertile eggs appeared very dark and the transmitted light intensity was low. The HR rate cannot be extracted from these eggs. The eggs were imaged for 60 s and 9 eggs were examined simultaneously for each measurement. All experiments were conducted at room temperature. Eggs taken out of the incubator during the experimentation were immediately returned to the incubator. The stored video files were analyzed later using a custom program written based on LabVIEW software to detect embryonic HR. After day 20, eggs were left in the hatching basket. Fertile eggs are verified by a successful hatching. Infertile eggs and dead eggs are broken and verified by visually observation.

To simulate a dead egg, one of fertile eggs was left at room temperature after day 10 until dead. The egg was examined using the setup right after taken out of the incubator and after the embryo was dead. The dead egg was broken for a visual verification.

2.4 Heart rate extraction

Extraction of embryonic HR information for chicken embryos followed the waveforms recovery from every stored specimen's video file. The signal is derived from the recorded pixel values that correspond to the variation of the light intensity transmitted through the eggs. Stored AVI video frames are in a grayscale mode (an 8-bit image). Regions of interest (ROIs) were carefully selected and defined by the user in the LabVIEW software. ROIs were created at the middle of the eggs. Mean values of the light intensity of each image frame for every ROI was analyzed and the results for each egg was plotted over time. Time interval of the plot can be determined from the sampling frequency (f_s) ($\Delta t = 1/f_s$). Sampling rate of 30 fps used to capture the video frames. It satisfies Nyquist criteria ($f_{max} = 30\ Hz$) which is sufficient to extract the embryonic HR of chicken eggs in the range of 2.5–5 Hz.

The HR waveform obtained from fertile eggs is relatively noisy and is not easy to observe. Large variations of the waveform at low frequencies are usually found due to the movement of the embryo (see Figure 1(c)). To minimize the movement artifacts and suppress background noises, the recovered waveforms were detrended using a moving average algorithm (Henderson method, 23 terms) and filtered using a 4^{th}-order inverse Chebychev filter (2 – 6 Hz, 60dB attenuation, available in the LabVIEW software) (see Figure 1(d)). Discrete Fourier transform (DFT) analysis employed to represent each frequency component of the time-varying signal with respect to their relative amplitudes. Fast Fourier transform (FFT) is an effective and efficient method to implement DFT. 1,000 data-point size was used to extract the embryonic HR information of all signals. The multivariate FFT function found in the LabVIEW software successfully served the purpose. Normally, the most energetic and periodic activity within the live embryo is the cardiac work, pumping blood throughout the body of the embryo. The frequency component corresponding to the most energetic embryonic activity represents the embryonic HR [2]. Finally, a peak detection technique is used to locate the highest peak which is the HR of the embryo (Figure 1(e)). Figure 2 shows the flow chart summarizing the calculation procedures to extract the embryonic HR of the chicken eggs.

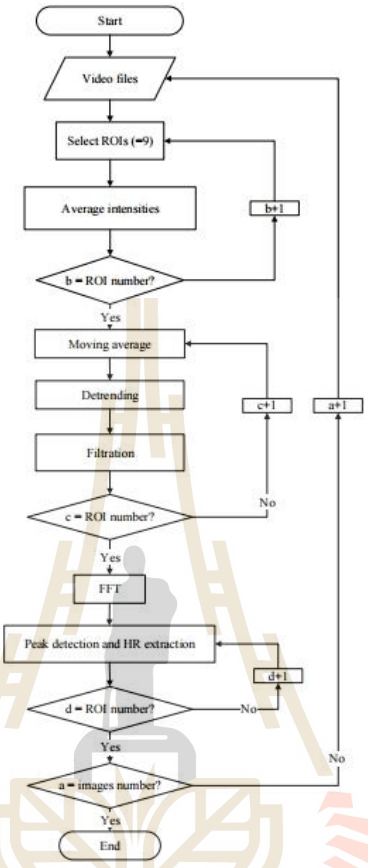

Figure 2. Calculation procedures to extract the heart rates from multiple eggs from the recorded video files.

3. RESULTS AND DISCUSSION

Figure 3 shows the signal waveforms and their corresponding power spectrum obtained simultaneously from 9 eggs at the incubation day 11. There were 8 fertile eggs and one infertile egg (Figure 1(b)). A fertile egg has a transmitted light intensity lower than that of an infertile egg due to light blockage by a developing embryo. Therefore, a fertile egg appears darker than an infertile egg. The raw signal waveforms from fertile eggs have a large fluctuation over time with a low frequency range 0.1–0.3 Hz. These fluctuations occur due to the movement of the live embryo. After detrending and filtering, the signal waveforms from fertile eggs were clearly a periodic signal. The corresponding FFT spectra from fertile eggs show a dominant peak. For an infertile egg (egg 9), the high image brightness is saturated the camera

response and its respect power spectrum contains no dominant peaks except at zero frequency is high and (a DC component).

Some fertile eggs (eggs 5 and 8) did not show a good time domain signals and a clear power spectrum. Results in Figure 3 show that the light intensities are lower for these eggs compared to the intensities obtained from the other eggs. The different size and position an embryo inside an egg may vary among each egg resulting in different transmitted light intensities. The large fluctuations of the waveforms were contributed by embryo movements at a frequency close to the heart rate. Embryonic HR measurements using optical methods are susceptible to noise from motion artifacts due the fact that the cardiac activity signal is very weak. Such movements could be minimized by applying sleeping drugs as shown in Ref. 19. No drugs were applied during our experiments.

We hypothesize that a fertile egg kept at the temperature below an incubation specified value, the embryonic HR will eventually decrease in both amplitude and frequency. Figure 4 show the successive power spectra of a fertile egg at day 10 after the egg was taken out of incubator and kept at room temperature (25°C) until the embryo was dead. The egg had a dominant HR peak at 250 BPM at the beginning of the measurements. After 1.5 h, the HR peak decreased to 120 BPM and the dominant peak amplitude drops significantly. The HR was stable in the range of 70–100 BPM for 30 h until dead. Dead embryo showed no cardiac activity and the corresponding power spectrum has no dominant peaks. The HR peak from the power spectrum changes the peak position to lower values and its amplitude drops as a function of time as expected. Decreased amplitude and HR frequency caused by loss of embryo's energy. Towards death, embryo decreased cardiac activities to conserve energy.

Mean embryonic HR for all 23 fertile eggs from days 7–16 of the incubation period ranged between 157–310 BPM Similar embryonic HR results for chicken eggs are reported between 175–250 BPM for days 11–14 using BCG and ECG methods [10], 152–275 BPM for days 13–14 using an optical imaging technique [1] and 210–277 BPM for days 10-14 using a near infrared optical method [16].

All 23 fertile eggs hatched successfully between day 20 and 21 of incubation and all 6 infertile eggs did not hatch. Note that one fertile egg left to die at day 10 to simulate a dead egg. Non-hatching eggs were broken at the end of incubation to eye witness the embryos within them. The results have shown the potential of our technique to measure multiple embryonic heart rates simultaneously in just one minute. The proposed technique is non-invasive and non-contact; therefore, it is suitable for long-time measurement of heart rate in chicken eggs. The image and signal processing algorithms have been developed for real time implementation. At the present, the setup can be used to measure the embryo heart rate up to 9 eggs. However, this technique can be designed to accommodate much larger number of eggs by using a proper detection unit and a strong light source.

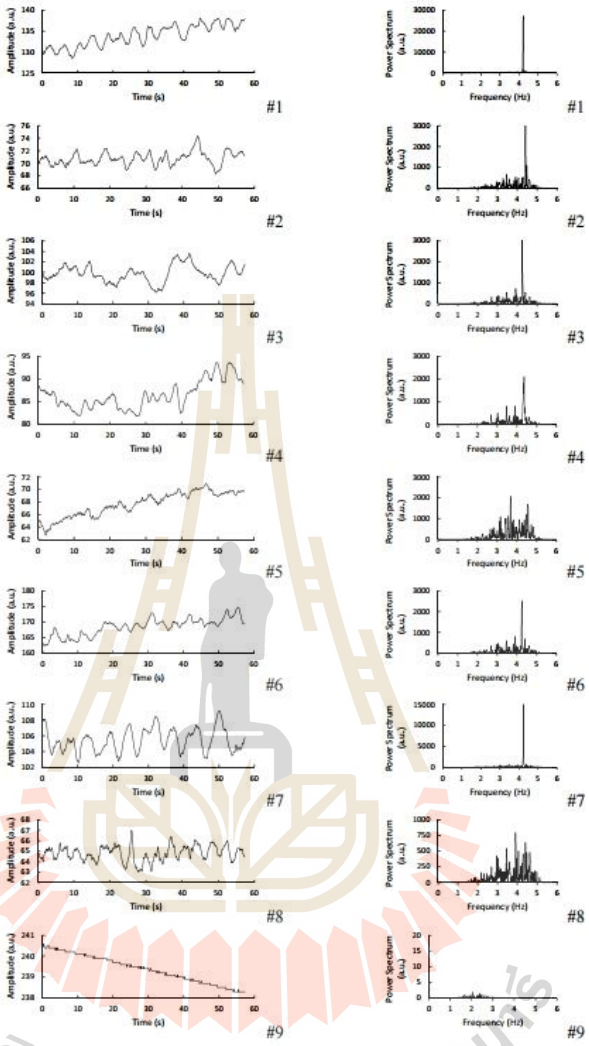

Figure 3. Time-varying signals (left) and their respective power spectra (right) from 9 eggs (eggs in Figure 1(b))

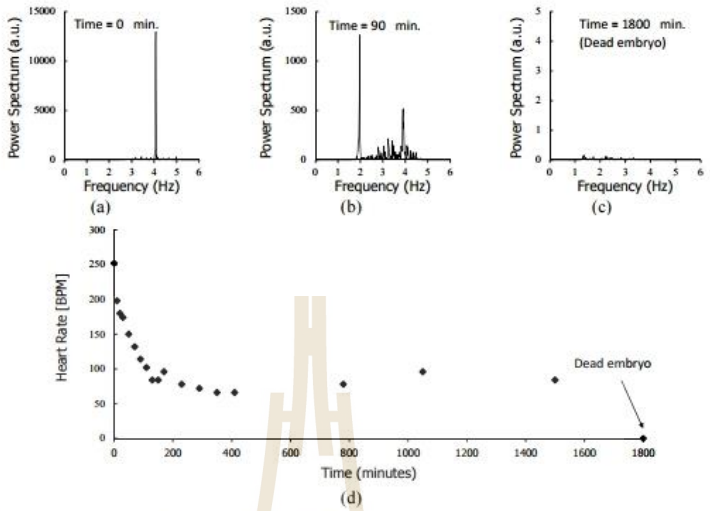

Figure 4. Power spectrum of a fertile egg at day 10 of incubation (a) the egg just removed from the incubator, (b) the same egg but left at room temperature for 1.5 h and (c) the egg left at room temperature for 30 h and the embryo was later confirmed dead at this stage. (d) Embryo heart rate plotted as a function of time the egg left outside the incubator.

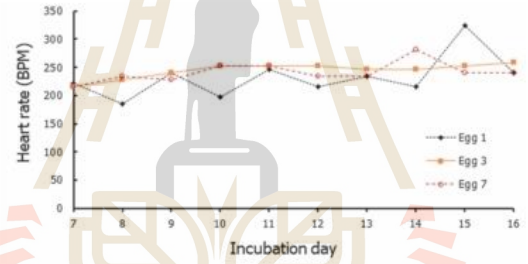

Figure 5. Variation of HR with the incubation day from days 7-16. No measurements have been performed for the eggs before day 7. After day 16, the embryonic HR cannot be extracted due to low transmitted light intensity.

4. CONCLUSION

A non-contact and non-invasive optical technique for measuring embryonic heart rate of multiple chicken eggs has been developed. System consisted of a light emitting diode as light source and a CMOS camera for recording video images. Digital processing techniques followed by the fast Fourier transform algorithm were employed to extract HR information from each egg.

Embryonic heart rates obtained from this technique are within a range reported in the literature. The proposed technique has an ability to sort infertile eggs and dead eggs during days 7–16 from fertile eggs before transfer to the hatchery.

The current setup can examine 9 eggs simultaneously in less than one minute. However, it can be expanded to accommodate hundreds of to bring more benefits to the poultry industry. It can also benefit animal science researchers to study the developing chicken embryos.

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BIOGRAPHY

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