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# **Optical Sensing to Measure Chicken Embryo Cardiac Rhythm**

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OVERALL: <https://doi.org/10.21061/IntroBiosystemsEngineeringII>

INDIVIDUAL CHAPTER: [https://doi.org/10.21601/IntroBiosystemsEngineeringII/Sensing\\_Chicken\\_Embryos](https://doi.org/10.21601/IntroBiosystemsEngineeringII/Sensing_Chicken_Embryos)

How to cite this chapter:

Khaliduzzaman, A., Ogawa, Y., & Zhang, R. (2022). Optical Sensing to Measure Chicken Embryo Cardiac Rhythm.

In Holden, N. M., Wolfe, M. L., Ogejo, J. A., & Cummins, E. J. (Ed.), Introduction to Biosystems Engineering II.

<https://doi.org/10.21061/IntroBiosystemsEngineeringII/SensingChickenEmbryos>

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## KEY TERMS

Cardiac rhythm  
Informatics

Fast Fourier Transform (FFT)  
Egg incubation

Next generation hatcheries  
Animal welfare  
Digital signal processing

## Variables

$f$  = heartbeat frequency

$f_n$  = Nyquist frequency

$f_s$  = sampling frequency

$HR$  = heart rate

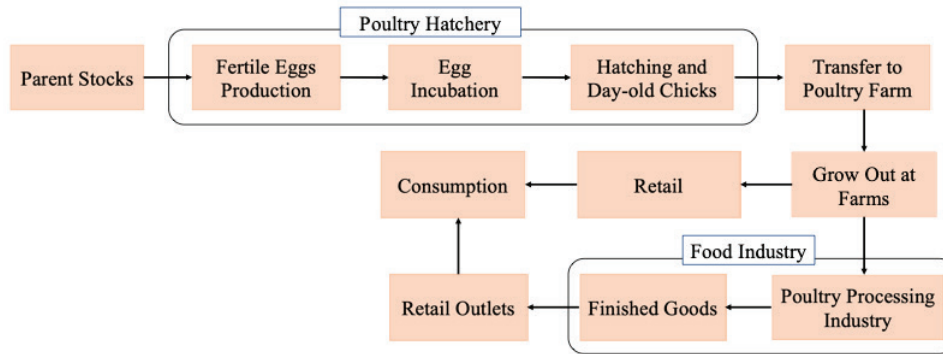
$k$  = constant, converts from beats per second to beats per minute  
(60 s min<sup>-1</sup>)

## Introduction

Poultry is the largest source of global meat production, so efficient management is needed to ensure sustainable production and food security. Merging engineering with science offers potential for precise, smart poultry production systems. The basic concepts in this chapter are the foundations for precision poultry hatchery practices integrating both the engineering and the biological aspects of the system.



A poultry hatchery is a space where eggs are incubated and hatched under specified temperature and humidity conditions to produce and supply day-old chicks. The growth, health, and egg output of chicks on farms partly depend on how they were managed in the hatchery. The continuous improvement of hatchery practices and management through monitoring individual eggs and embryos with advanced technology will significantly enhance the economic, environmental, and welfare performance of a poultry hatchery. A typical and complete value chain (i.e., farm to fork) showing the place of a hatchery near the beginning of the poultry production and consumption system is shown in figure 1.



**Figure 1.** Flow diagram showing the stages of the poultry production system, processing, and consumption value chain.

Considering the need to increase egg output efficiency while meeting animal welfare requirements in poultry production, it is imperative to merge engineering and poultry production sciences to develop precision management systems. To maximize egg production, healthy, non-defective, high-grade chicks need to be identified at the embryo stage in the hatchery.

A clear heartbeat signal from a chicken embryo is an indicator of a high-grade chick and its future health status. Small deviations from the normal patterns of fetal cardiac activity can have long-lasting consequences post-hatching (Sival, 1993). Using a non-destructive measurement method during incubation minimizes human and mechanical disturbance of the chicken embryo. Optical sensors can observe the heartbeat non-destructively with minimal disturbance by measuring the intensity of transmitted light affected by the pulsing blood and mechanical activity of the heart during blood pumping.

This chapter presents some basic biology and engineering approaches that are fundamental to managing chicken eggs during incubation: key physiological changes during the incubation period, the hatch window, the hatching process, the basic cardiac cycle / heartbeat, key components of optical sensors to measure heartbeats, digital signal processing, the calculation of heart rate from the embryonic activity signal, and the interpretation of the heartbeat signal. It is important to measure the heart rate of individual embryos during incubation as an indication of health status (by detecting heart rate anomalies). Equipment and machinery manufacturers are helping producers move to automation, precision hatchery practices, and smart poultry production systems. It is important for them to consider measuring heartbeat as part of equipment specifications and designs to support the future of the poultry production industry.



## Outcomes

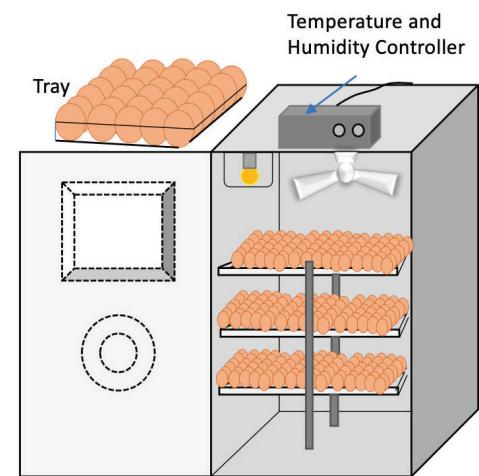
After reading this chapter, you should be able to:

- Describe the chicken egg incubation and hatching process
- Explain the physiological changes in a developing embryo
- Describe signal acquisition using an optical sensor and the necessary digital signal processing to calculate heart rate from a frequency-domain signal
- Evaluate the cardiac performance of an embryo during incubation

## Concepts

### *The Incubation Process*

A typical incubator is shown in figure 2. Incubation is a complex biological and physiological process where an embryo passes a series of developmental stages before hatching. In general, the incubation period varies by bird species, but on average, it takes 20 to 21 days depending on genetic factors, egg size, shell structure, egg dimension, internal contents, and other factors such as egg-turning frequency, relative humidity (RH), and temperature. Hatch rate (the percentage of eggs that successfully hatch) depends on how the RH and incubation temperature are managed. A temperature of 37.8°C and RH of 55% are usually maintained during the first 18 days of incubation, as these are ideal for efficient cell growth and development, water vapor management, gas exchange (mass transfer) through the eggshell, and embryo development. Around day 18, the RH in the incubator is increased to 60% when the eggs are transferred to the hatcher tray. Increasing the humidity around the hatcher tray reduces the evaporation rate of the eggs, preventing the embryos from drying during the pipping period (when the chicks start to break out of their eggs) and hatching (when the chicks are free of their eggs) processes.



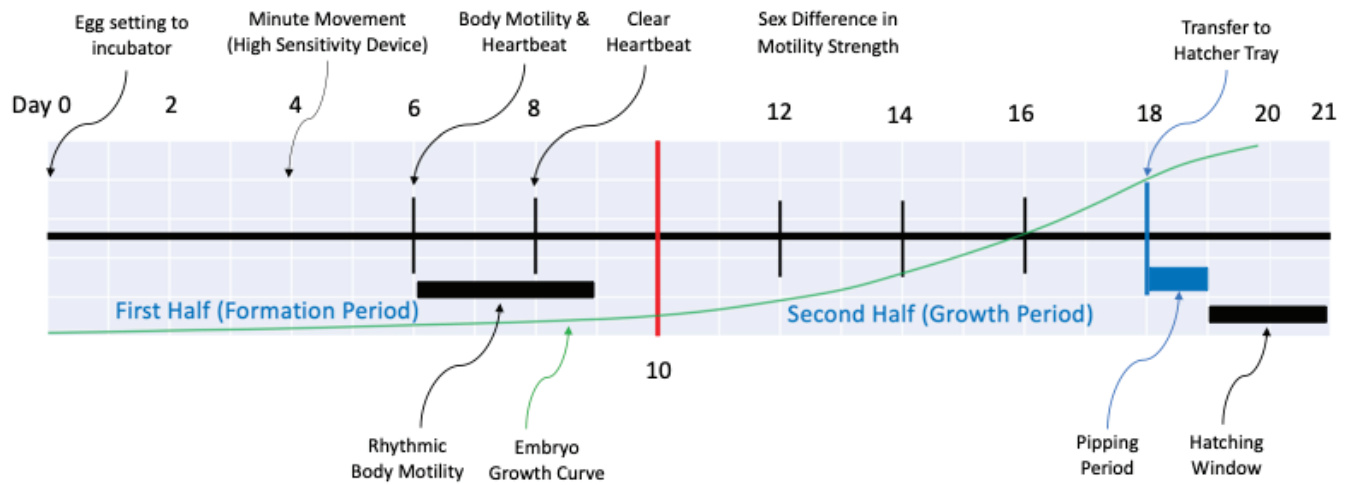
**Figure 2.** Schematic diagram of a typical egg incubator.

### *The Developmental Stages of Embryos during Incubation*

The embryos go through biological and physiological changes before hatching (as presented in figure 3). Byerly (1932) studied the fundamentals of chicken embryonic growth and showed that organs form in the first half of the incubation period, days 1 to 10 (the embryo formation period), to create the structures shown in figure 4. From day 11 to hatching (the growth period), an exponential increase in the size of the embryo occurs.

In the formation period, the allantois appears on day 4 and gains maximum weight between days 9 and 11. The yolk sac grows peripherally until it almost surrounds the yolk. The yolk increases rapidly in size during the first four days of incubation due to the absorption of water from the albumen. From day 6, the weight of the yolk sac steadily increases, surpassing all other components





**Figure 3.** Major biological and physiological events during the incubation process of chicken eggs.

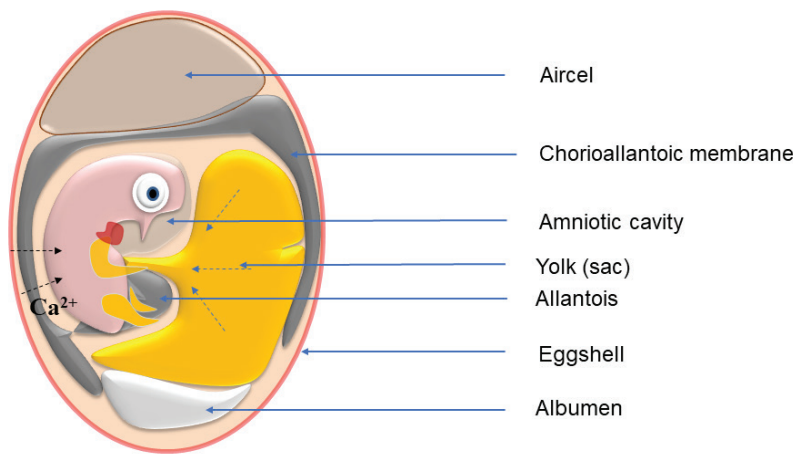
**Table 1.** The physiobiological development of a chicken embryo (adapted from Hamburger & Hamilton, 1951).

Age (h)	Age (d)	Developmental Event
6–7	1	Primitive streak begins
32–36	2	Bending and contraction of heart begin
38–49	2	Hemoglobin synthesis begins, heart starts becoming S-shaped
65–72	3	Allantois is now distinct, embryo is surrounded by amnion
84	4	First active movements of head and neck occur
108–120	5	Trunk movements begin, four-chambered heart is formed
120	5	Amnion begins to contract rhythmically
144	6	Limbs now participate in whole body movements Active transport of amino acids by yolk sac membrane
150–156	6	Independent limb movements, sexual differentiation
180–192	8	Mineralization of bone begins
192–206	9	Chorioallantois becomes fixed to shell
216–240	10	Whole body movements become jerky and random
252	11	Amniotic contractions much reduced
264–288	12	Albumen absorption, calcium absorption from shell starts
288–312	13	Length and amount of activity phases reach a peak
312–336	14	Body begins to assume position along axis of egg
360–384	16	Embryo capable of respiratory movements
384–408	17	Co-ordinated and stereotyped movements begin
420	18	First behavioral response to light
432–456	19	Absorption of allantoic fluid completed, yolk sac withdrawal begins, internal pipping starts
456–480	20	Begins to breathe and vocalize, shell externally pipped, withdrawal of yolk sac completed

by the middle (days 10 to 11) of the incubation period. The function of the yolk sac is to regulate the delivery of energy and nutrients from the yolk to the embryo. The yolk sac should have a fairly constant thickness during the process of yolk enclosure. During days 0 to 16, the yolk decreases in size due to the excretion of water into the allantois. A new mode of growth of the yolk sac starts with the formation of radiating lamellae invading the yolk. During days 16 to 21, the yolk and yolk sac both decrease in size. The yolk becomes more hydrated, and finally, both are drawn into the body of the embryo. The typical physiobiological changes of a chicken embryo during incubation are summarized in table 1.

The respiration process of birds during incubation differs from that of placental mammals. In the case of a developing chicken embryo, the respiration process (i.e., the exchange of oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ )) during the first 15 to 16 days of incubation takes place through the eggshell pores and chorioallantoic membrane. During the last few days





**Figure 4.** Schematic diagram of embryonic components.

of incubation, the lungs gradually become active and start working. In mammals, these services are provided by a physical connection to the mother through the placenta.

### ***The Cardiac Rhythm of a Chicken Embryo***

The hearts of birds are morphologically similar to mammals—that is, they contain full atrial and ventricular septums (Cook et al., 2017). The basic cardiac cycle of birds, like other vertebrates, consists of systole and diastole (figure 5). The heart rate can be defined as the number of heartbeats given by the chicken embryo per minute. One heartbeat is equal to one complete cardiac cycle (i.e., consists of systole and diastole). The heart rate can also be expressed in terms of frequency, as hertz (Hz), which indicates beats per second (bps). The heart of the chicken embryo begins to form at the end of day 2. The heart is very small and S-shaped with tiny movement. Researchers have reported cardiovascular movement in chicken embryos in the range of 180 to 320 beats per minute (bpm) throughout the incubation period (Khaliduzzaman et al., 2019b). In each cardiac cycle of a chicken embryo, ventricular systole and diastole (including the isovolumic phases) persist at about 80 ms and 120 ms, respectively, as detailed in figure 5 (Khaliduzzaman et al., 2019a).

Arrhythmia, or an irregular heartbeat, is a common cardiovascular disorder in birds. Arrhythmia can be classified into sinoatrial arrhythmia, atrioventricular arrhythmia, and ventricular arrhythmia depending on where it originates in the heart (Olkowski & Classen, 1998). The atrium, atrioventricular node, and ventricle are three different places in the heart. With the currently available technologies or sensors, it is impossible to detect the origin of arrhythmia in a chicken embryo heart. The arrhythmia can also be classified according to the heart rate: bradyarrhythmia, when the heart rate is below the normal range, and tachyarrhythmia, when the heart rate is above the normal range. Relatively little is known about these disorders in birds in general chiefly because of the difficulty of diagnosis (Vostarek et al., 2014; Sedmera et al., 2015). Although arrhythmia is quite a rare event in laboratory experiments, it is important

Each component of the egg has a specific function:

**Aircel:** A pocket of air in the egg. Prior to hatching, the fully developed embryo will break the membrane to this air pocket before breaking the eggshell.

**Chorioallantoic membrane:** A site for gaseous exchange ( $O_2$  and  $CO_2$ ); transports calcium ions from the eggshell; a protective layer that prevents microbial contamination.

**Amniotic cavity:** The space that provides a stable fluid environment for the embryo to develop.

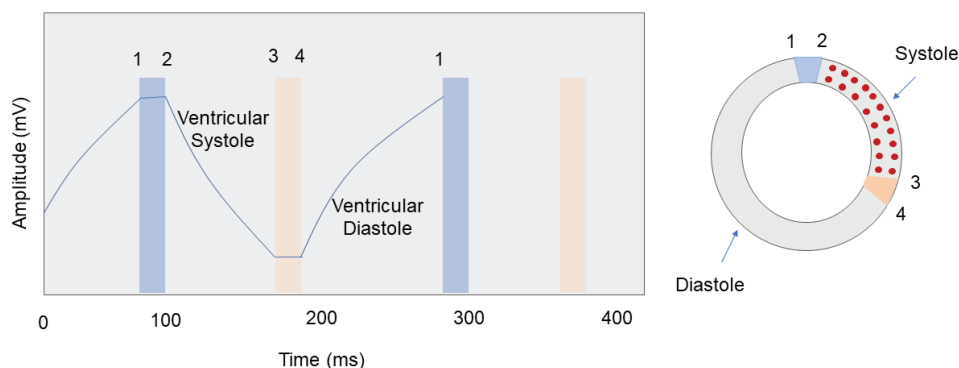
**Yolk:** The fat-rich, main nutrient source for the developing embryo.

**Allantois:** A membrane that regulates gas diffusion to the growing embryo and removes wastes.

**Eggshell:** A porous protective cover that regulates gas exchange (predominantly  $O_2$  in;  $CO_2$  and  $H_2O$  out) and provides minerals (calcium) for the developing embryo.

**Albumen:** A protein-rich food important for embryo growth (e.g., muscle formation).





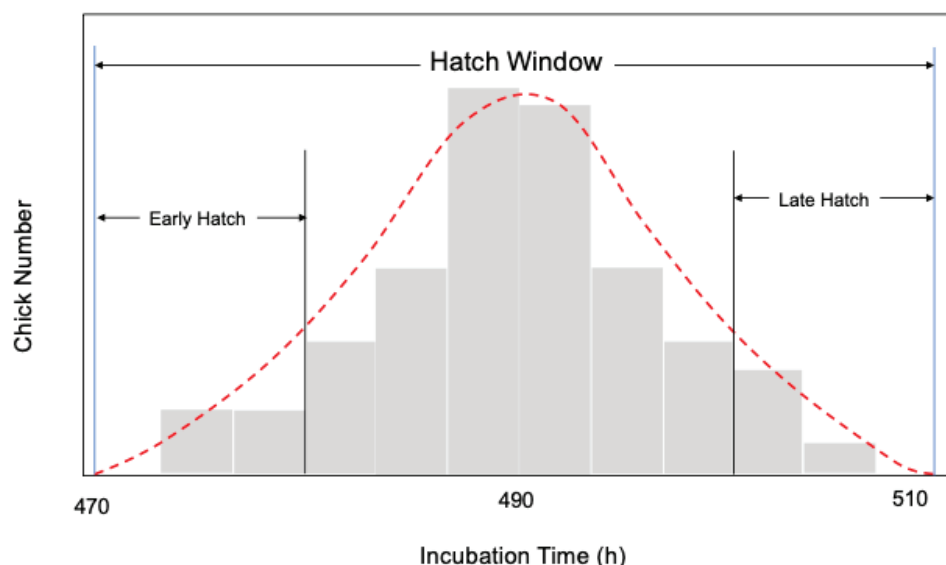
Single Cardiac Cycle: 200 ms (80 ms systole + 120 ms diastole) = 5 bps

1–2: Isovolumic Ven. Contraction phase (~25 ms) 3–4: Isovolumic Ven. Relaxation phase (~25 ms)

2–3: Ventricular Systole (~55 ms)

4–1: Ventricular Diastole (~95 ms)

**Figure 5.** Cardiac cycle signal pattern of a normal chicken embryo.



**Figure 6.** A typical hatch window of chicken embryos.



**Figure 7.** Image of chicks hatching—that is, emerging from their shells.

for industrial mass production because of the number of birds involved.

The change of cardiac rhythm influences the growth of the embryo and subsequent hatching time. Chicken embryos stay in their eggs for a fixed period, and all embryos have more or less constant heartbeats during their incubation span (Ar & Tazawa, 1999). Thus, the development of the embryonic heart is a good indicator of embryonic growth and maturity. Superior cardiac activity during incubation may shorten the total incubation time, so having the means to monitor embryos' heart rates offers the opportunity for precision hatchery management.

### The Hatch Window and Hatching Process

The hatch window refers to the time between the first and the last chick hatched in a batch of incubated eggs (figure 6). It can vary from 24 to 72 hours depending on incubated egg sizes and dimensions, incubation tem-

perature, RH, and genetic factors.

The hatching process is defined as a chick emerging from the egg by breaking the eggshell (figure 7). This includes internal and external pipping, the onset of respiration with the lungs, and complete emergence from the eggshell.

The supply of high-quality and uniform batches of chicks is considered one of the most important challenges for breeders and poultry farmers (Decuypere et al., 2001). However, the homogeneity of the day-old chick cohort is frequently compromised by a wide hatch window with chicks that have had too-short or too-long



incubation periods (Tona et al., 2003; Bergoug et al., 2013), which negatively affects the post-hatch performance of the chicks. Furthermore, late-hatching chicks have an inferior production quality—for example, a low growth rate, high mortality rate, and increased susceptibility to disease (Løtvedt et al., 2014)—and cause downstream complications for chick sorting, feeding and water supply, vaccination, and maintenance of the rearing environment. Therefore, a narrow hatch window is desirable for efficient production.

## Fundamentals of Optical Sensing for Embryo Heartbeat

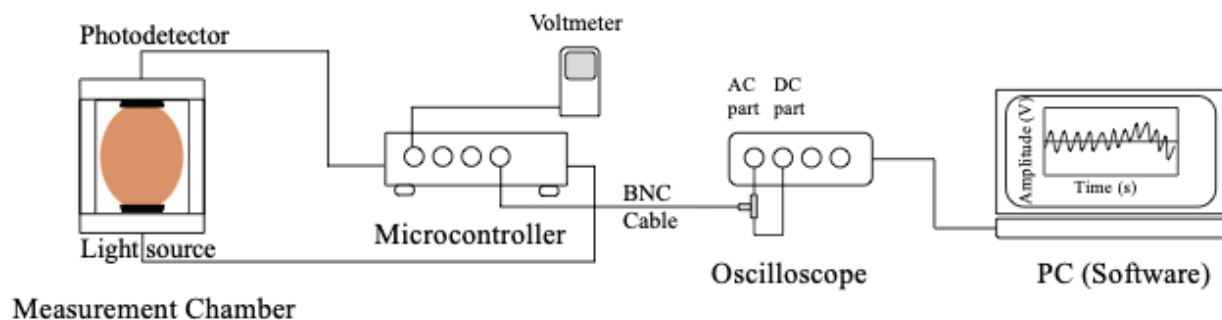
Optical sensors offer non-invasive, non-destructive means of measuring embryonic heart rate with minimum disturbance to the eggs. The intensity of transmitted light is affected by the blood volume moved by the heart and its mechanical activity during the cardiac cycle or rhythm (i.e., systole and diastole). The major components of an optical sensor system (figure 8) are a light source (e.g., light-emitting diodes, LED), a photodetector (e.g., photodiode) with a built-in signal amplifier (i.e., transistor), a microcontroller or control box, an oscilloscope, a computer, and software. The light is directed through the egg, and its transmission is recorded by the photodetector. As the embryo grows, the input current needs to be increased to account for the greater absorbance by the egg and to ensure a suitable output voltage. The amplified voltage signal is connected to an oscilloscope using BNC cables. The oscilloscope is then connected to the computer, usually through a USB connector so that a user-friendly interface can be deployed. The pumped blood volume and mechanical activity of the heart cause a changing pattern of transmitted light intensity reflected in small oscillations of the photodetector signal, which can be interpreted to represent heartbeats.

### Attributes of high-quality chicks

1. Good and clean condition of navel
2. No visible defects in legs, beak, and other parts of the body
3. Short reflex time
4. No cardiac abnormalities
5. Withdrawal yolk sac is not too large (just before hatching)
6. Hatched in the early or middle segment of the hatch window

## Basic Signal Processing

Signal processing includes analyzing, modifying, filtering, decomposing, and/or synthesizing signals, such as optical or sound data, to serve a specific purpose. The purpose of digital signal processing here is to identify components of interest in the embryonic activity or heartbeat signal. The biological response (heartbeat) can be extracted from the optical or electrical voltage signal (digital or analog). The digital signal is the discrete time-sampled analog signal. The heart



**Figure 8.** Schematic diagram of an optical sensor for heartbeat signal acquisition with a vertical egg optical configuration (i.e., the egg was placed vertically, with the large end at the top).



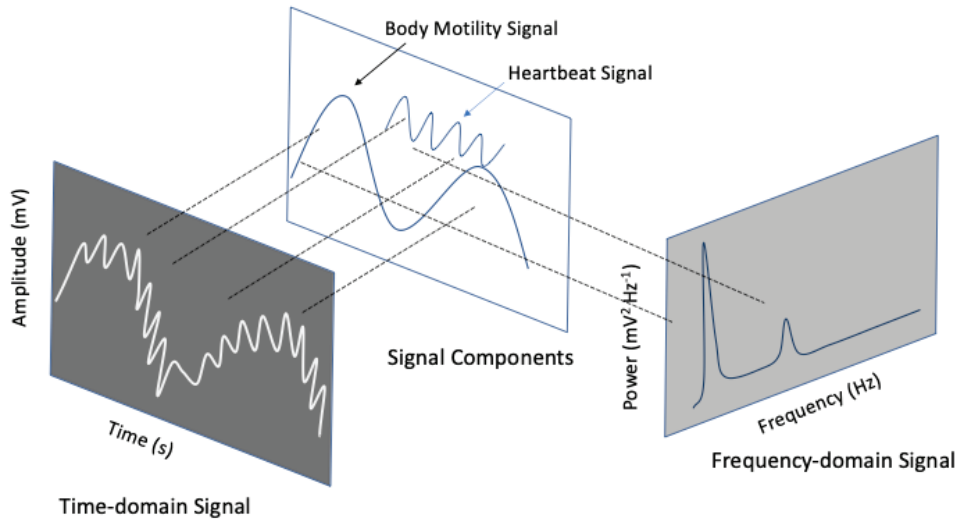
rate provides a certain frequency in the embryo activity signal (a regular change in voltage per unit time). To detect the change in output from the photodetector, it is necessary to sample the signal more frequently than the change that is to be measured. The sampling must be at a higher frequency than the maximum expected heart rate, or it will not be possible to measure accurately. The heart rate of chicken embryos typically varies from 180 to 320 bpm (3.0–5.33 Hz). The heart rate is usually lower in the first half of incubation (developmental stage) compared to the second half (exponential growth stage).

The sampling rate or sampling frequency ( $f_s$ ) is defined as the number of samples per second, or the number of discrete data points picked up per second from a continuous time-domain signal. The period between two sampling points is called the sampling interval or sampling period and is inversely related to the sampling rate. The calculation of a suitable sampling frequency is necessary for an optical system design for heart rate measurement. There are two components to the calculation of the correct sampling frequency. In signal processing, the Nyquist frequency ( $f_n$ ) refers to the conversion of a continuous signal into a discrete sequence. Its value (cycles per second) is half of the sampling rate or sampling frequency (i.e., sampling points per second). It is important to consider when designing the sensor sampling rate. The recommended Nyquist frequency is greater than or equal to 4 times the frequency to be measured, the heartbeat frequency ( $f$ ) in this case:

$$f_n (\text{minimum}) = 4 \times f \quad (1)$$

Next, the Nyquist frequency can be used to estimate the necessary sampling frequency:

$$f_s = 2 \times f_n \quad (2)$$



**Figure 9.** The time-domain and corresponding frequency-domain signal of a chicken embryo motility consisting of two different frequency components that can be observed using an FFT.

For example, to measure a heart rate of 4 Hz (4 bps, 240 bpm), the Nyquist frequency of a sensor would be 16 Hz, which means the sampling frequency should be 32 Hz (32 samples per second).

The signal from the embryo within an incubated fertile egg contains both body motility (a typically low frequency signal associated with the embryo moving about inside the eggshell) and heartbeat (a higher frequency signal) components. Motility can be separated from the heartbeat signal, as shown in figure 9. The data can be



analyzed in the time domain or the frequency domain. Since the time-domain data of embryonic activity contains both movement components and other noise overlaid in the signal, it is common to transform the data from the time domain to the frequency domain to calculate the heart rate of the embryo. These two components can be separated based on frequency analysis, where each peak represents a dominant frequency within the time series. A Fast Fourier Transform (FFT) algorithm can be used (equation 3) to convert the discrete time signal to the frequency domain; thus, the processing is referred to as Discrete Fourier Transformation (DFT). The FFT is widely used in many fields in engineering, music, science, and mathematics to compute the transformation of values faster than a Fourier Transform (FT).

$$Y = \text{FFT}(X, N) \quad (3)$$

where  $Y$  = Fourier transformed signal or frequency-domain signal ( $\text{mV}^2 \text{Hz}^{-1}$ )

$X$  = amplitude value in time-domain signal ( $\text{mV}$ )

$N = 2^n$  = total sampling points for transformation using FFT

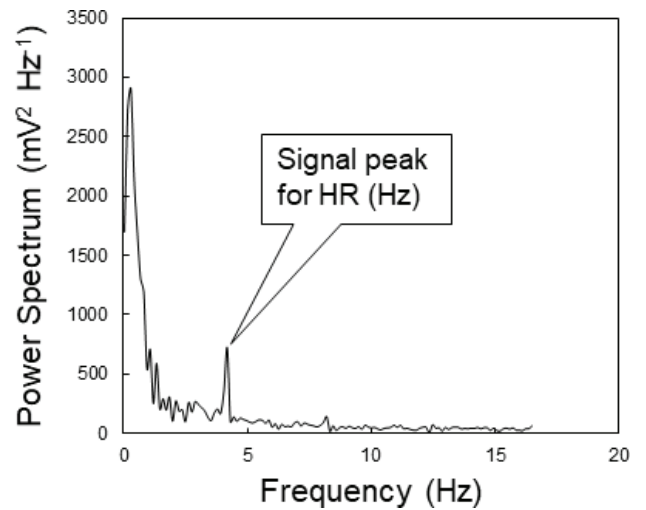
The parameter  $n$  determines the time over which the sample is taken and is chosen based on using the smallest value that will capture a representation of the data in the time series. If  $n = 8$ , the total sampling points would be  $N = 2^n = 2^8 = 256$ , which would result in a 7.68 second period for a signal sampled at 33.3 Hz but less than 4 seconds for a signal sampled at 66.6 Hz. The sample size,  $2^n$ , is used for an efficient and faster conversion of the time-domain data into frequency-domain data, which is the ultimate purpose of using the FFT algorithm. Understanding the root of unity in number theory can provide clarity on the performance of FFT or DFT.

### Heart Rate Calculation

From the frequency-domain signal of embryonic activity, the heart rate can be estimated directly in Hz from a clear peak, as shown in figure 10. The lower frequency peak ( $<1 \text{ Hz}$ ) represents body motility, while the next dominant peak ( $\sim 4.5 \text{ Hz}$ ) represents the heartbeat. The heart rate can be calculated as follows (equation 4):

$$HR = f_p \times k \quad (4)$$

where  $HR$  is heart rate (bpm) and  $k (= 60 \text{ s min}^{-1})$  is a constant to convert from beats per second to beats per minute.



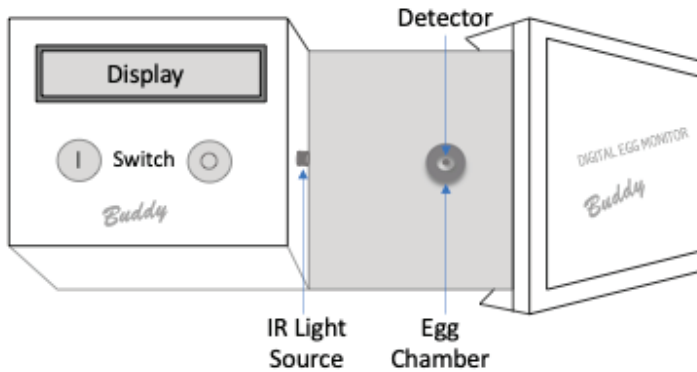
**Figure 10.** A frequency-domain signal with a clear  $HR$  peak.



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## Applications

A major challenge in the coming decades will be efficient chicken production, which is needed to ensure a sustainable, secure food supply. Chicken products start with eggs, and the improved monitoring and management of incubated hatching eggs start with heartbeat measurement. There are some commercial devices for heartbeat measurement, such as the Buddy Digital Egg Monitor (Avitronics, United Kingdom) and the Embryonic Vital Scope (EVS; NABEL Co., Ltd., Japan). The Buddy was invented for hobbyists to monitor parrot eggs. It



**Figure 11.** The Buddy Digital Egg Monitor (schematic view), which can be considered a first-generation device for the heart rate monitoring of avian eggs.

works based on the diffuse transmission of infra-red light striking the egg surface (figure 11). This portable egg monitor is suitable for single-egg operations only. A limitation of this device is that it has a high probability of including embryonic motility (outer organ or body movement) in the heart rate measurements, since it allows for frequencies from 50 bpm and cannot separate body movement, making the technology unsuitable for scaling to commercial applications. The EVS could be considered a second-generation update of the Buddy. It can capture both the heartbeat and the body motility signals during days 6 to 20 and has been used commercially for detecting live embryos in poultry hatcheries in Japan and other countries.

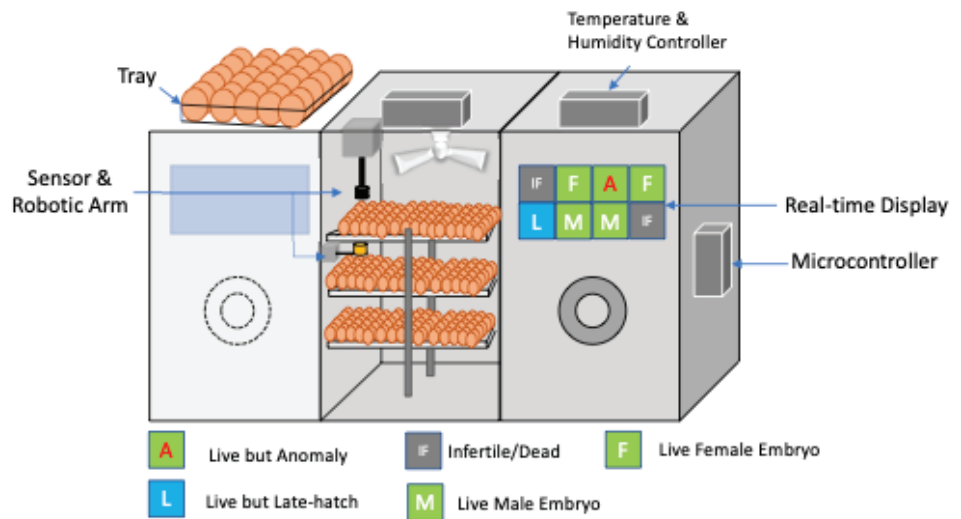
The next generation poultry production systems and improved hatchery practices for egg and poultry industries need to be developed. Biosystems engineering graduates and other stakeholders in the sector are now working to implement innovative incubation management technologies rather than considering post-hatch management only. Precision management during the incubation period can reduce or eliminate many complications (e.g., space, energy, and labor) during post-hatch (i.e., day-old chicks) management that occur due to keeping unwanted chicks in the production line. Currently, precision poultry farming systems are becoming more automated and machine oriented (e.g., semi-automated day-old chick grading, automated water and feeding systems). Non-destructive technologies, including automation and informatics, can be applied during the incubation period for individual egg/embryo management. The supergrade embryos (i.e., those with healthy heartbeats) can substantially increase the productivity, profitability, and environmental performance of the poultry sector and thus contribute to achieving global animal-based food security. Grading based on abnormalities, fertility, viability, and gender could be done in industrial situations by measuring and detecting the heartbeat patterns of chicken embryos. For example, an incubator with a built-in optical sensor, robotic arm, real-time display, and operation control system based on big data, artificial intelligence, and Internet of Things could potentially be used in future smart poultry hatchery practices (figure 12). Thus, the knowledge in this chapter could help biosystems engineers generate ideas to develop new kinds



of devices for inspecting hatching eggs (e.g., detecting abnormal embryos, fertile and infertile eggs, gender, dead embryos, supergrade chicken embryos) in the future.

Similar engineering principles and protocols could be applied for the non-destructive monitoring of eggs from other domestic fowl such as quail, turkeys, and ducks (Lierz et al., 2006) toward diversifying global meat production. This kind of technology can also contribute to other fields of the biological sciences, including animal ecology and evolutionary biology (e.g., physiological traits), and could open many new research areas in avian and reptile protocols (Aubret et al., 2016).

Culling weak and abnormal chicks is a major ethical concern worldwide. The early detection of abnormal chicken embryos during incubation may minimize the intensity of the debate. Biosystems engineers can minimize many problems regarding poultry welfare and ethical issues by applying their knowledge of automation and control together with informatics for egg and poultry industry management. It has been reported that chicks with lower cardiac performances hatch late and, hence, show low post-hatch performances (Khaliduzzaman et al., 2018). The current practice of chick grading in poultry hatcheries is performed only after hatching, which cannot eliminate or minimize the many ethical problems, such as discarding the inferior (abnormal or small size) chicks and killing those of a less preferred gender (i.e., gender-biased chick selection). These problems could be minimized if the farmers or other relevant personnel apply non-destructive methods (e.g., heartbeat-based grading) for assessing hatching eggs or chicken embryos during incubation (i.e., before hatching). For those interested in this topic, the ethics of poultry production have been reviewed by Macer (2019).



**Figure 12.** Schematic diagram of a future smart egg incubator with an optical sensor and real-time display to monitor individual egg conditions. This could be used in grading eggs/embryos inside an incubator.

## Examples

### Example 1: Minimum appropriate sampling frequency

#### Problem:

The maximum heart rate of a chicken embryo on day 14 of incubation is 320 bpm. Determine the minimum appropriate sampling frequency to design an optical sensor for measuring the heartbeat of the embryo.



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### Solution:

Estimate the minimum Nyquist frequency to measure the maximum heart rate using equation 1:

$$f_n (\text{minimum}) = 4 \times f$$

$$f_n = 4 \times (320 \text{ bpm} / 60 \text{ s min}^{-1}) = 21.33 \text{ Hz}$$

The sampling frequency is twice the Nyquist frequency (equation 2):

$$f_s = 2 \times 21.33 \text{ Hz} = 42.66 \text{ Hz}$$

### Example 2: Calculation of sampling frequency

#### Problem:

The average heart rate of a chicken embryo on day 10 of incubation is 280 bpm. Choose the most appropriate sampling interval from the two options below to design an optical sensor for measuring the heartbeat signal of chicken embryos:

- (a) Sampling interval of 10  $\mu\text{s}$
- (b) Sampling interval of 10 ms

State the reason(s) for your choice.

#### Solution:

To select the appropriate option, first calculate the minimum Nyquist frequency to measure the given heart rate and the Nyquist frequency of each sampling interval option. Then, compare the Nyquist frequencies to determine the more suitable sampling interval.

From equation 1,

$$f_n (\text{minimum}) = 4 \times f = 4 \times (280 \text{ bpm} / 60 \text{ s min}^{-1}) = 18.67 \text{ Hz}$$

Solve equation 2 for the Nyquist frequency of each sampling interval, noting that the sampling frequency is the inverse of the sampling interval:

$$\text{Option a: } f_n = \frac{1}{2} \times f_s = \frac{1}{2} \times (10 \mu\text{s})^{-1} \times (10^6 \mu\text{s s}^{-1}) = 50,000 \text{ cycles s}^{-1} = 50,000 \text{ Hz} \\ = 50 \text{ kHz}$$

$$\text{Option b: } f_n = \frac{1}{2} \times f_s = \frac{1}{2} \times (10 \text{ ms})^{-1} \times (10^3 \text{ ms s}^{-1}) = 50 \text{ cycles s}^{-1} = 50 \text{ Hz}$$

Both options exceed the computed minimum Nyquist frequency needed to measure the heartbeat, so to determine which option is more suitable, other factors are considered. The resolution of the sampling points of option a would be much greater than option b, giving a much shorter sampling interval for option a. However, as the minimum Nyquist frequency is met by option b, the resolution is sufficient. The longer sampling interval of and fewer samples to be collected and analyzed using option b would reduce technical complexity



and cost. Thus, the more suitable sampling interval is 10 ms (option b).

### Example 3: Heartbeat signal

#### Problem:

The frequency-domain activity signal of an embryo on incubation day 10 showed two distinct peaks, at 0.6 Hz and at 3.8 Hz (figure 13). Calculate the heart rate of the embryo in bpm.

#### Solution:

Generally, the heart rates of chicken embryos vary from 180 to 320 bpm. First, check which of the distinct frequency peaks falls within the range of a normal heart rate using equation 4:

$$HR = (3.8 \text{ Hz} \times 60 \text{ s min}^{-1}) = 228 \text{ bpm}$$

$$HR = (0.6 \text{ Hz} \times 60 \text{ s min}^{-1}) = 36 \text{ bpm}$$

The peak <1 Hz is unlikely to relate to heart rate and is more likely to be due to embryo motility. The peak at 3.8 Hz falls within the normal heart rate range of a chicken embryo. Thus, the embryo's heart rate is most likely to be 228 bpm on day 10 of incubation.

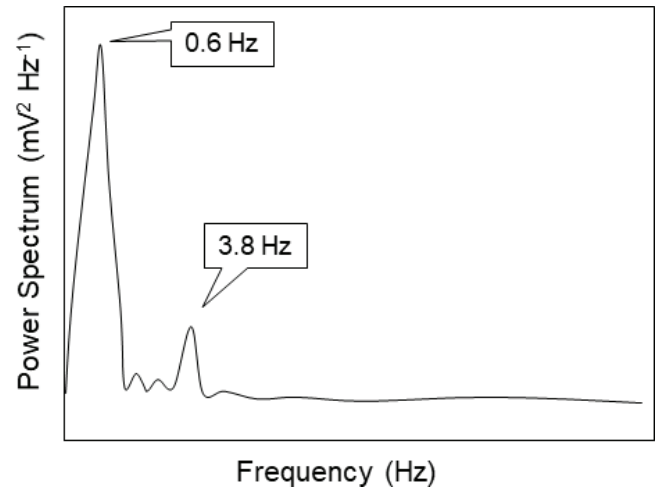
### Example 4: Detection of abnormal cardiac activity

#### Problem:

The time-domain dataset (128 data points, equivalent to 3.84 s) of a chicken embryo (on day 18) is given in table 2. Graph the data and then calculate the approximate heart rate from the graph. Also explain the condition of the embryo's heartbeat signal (normal/abnormal).

#### Solution:

First, plot (i.e., scatter plot) the data where time (s) is on the x-axis and amplitude (mV) is on the y-axis. Next, calculate the frequency of the heartbeat (bps) from the graph manually. Peak-to-peak distance is called one cardiac cycle (i.e., one beat). In one second, there are about 4.0 cycles (i.e., 4.0 Hz). Therefore, the heart rate of the embryo is  $4.0 \text{ bps} \times 60 \text{ s min}^{-1}$ , or 240 bpm, which is within the normal range.



**Figure 13.** Frequency-domain activity signal of a chick embryo on incubation day 10.



**Table 2.** Time-domain dataset of a chicken embryo on day 18 of incubation.

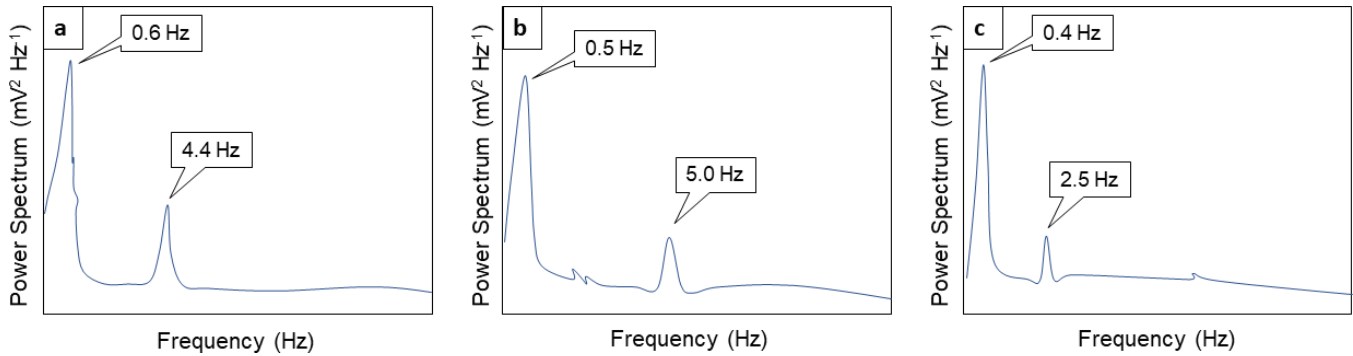
Time (s)	Amplitude (mV)	Time (s)	Amplitude (mV)	Time (s)	Amplitude (mV)	Time (s)	Amplitude (mV)
0.03	16.0243	0.99	11.0243	1.95	16.0243	2.91	21.0243
0.06	16.0243	1.02	8.5243	1.98	11.0243	2.94	21.0243
0.09	18.5243	1.05	8.5243	2.01	8.5243	2.97	16.0243
0.12	18.5243	1.08	11.0243	2.04	8.5243	3.00	13.5243
0.15	21.0243	1.11	16.0243	2.07	8.5243	3.03	13.5243
0.18	21.0243	1.14	16.0243	2.10	8.5243	3.06	13.5243
0.21	18.5243	1.17	18.5243	2.13	13.5243	3.09	16.0243
0.24	16.0243	1.20	18.5243	2.16	16.0243	3.12	16.0243
0.27	13.5243	1.23	11.0243	2.19	16.0243	3.15	18.5243
0.30	13.5243	1.26	8.5243	2.22	11.0243	3.18	18.5243
0.33	13.5243	1.29	8.5243	2.25	8.5243	3.21	18.5243
0.36	16.0243	1.32	11.0243	2.28	8.5243	3.24	13.5243
0.39	18.5243	1.35	13.5243	2.31	8.5243	3.27	11.0243
0.42	21.0243	1.38	13.5243	2.34	11.0243	3.30	11.0243
0.45	21.0243	1.41	16.0243	2.37	13.5243	3.33	11.0243
0.48	16.0243	1.44	16.0243	2.40	13.5243	3.36	16.0243
0.51	11.0243	1.47	13.5243	2.43	13.5243	3.39	18.5243
0.54	11.0243	1.50	8.5243	2.46	13.5243	3.42	18.5243
0.57	13.5243	1.53	8.5243	2.49	8.5243	3.45	18.5243
0.60	13.5243	1.56	8.5243	2.52	8.5243	3.48	13.5243
0.63	16.0243	1.59	11.0243	2.55	8.5243	3.51	11.0243
0.66	18.5243	1.62	13.5243	2.58	11.0243	3.54	11.0243
0.69	18.5243	1.65	13.5243	2.61	13.5243	3.57	11.0243
0.72	16.0243	1.68	16.0243	2.64	16.0243	3.60	16.0243
0.75	11.0243	1.71	16.0243	2.67	16.0243	3.63	16.0243
0.78	11.0243	1.74	11.0243	2.70	16.0243	3.66	16.0243
0.81	11.0243	1.77	6.0243	2.73	13.5243	3.69	16.0243
0.84	13.5243	1.80	6.0243	2.76	11.0243	3.72	11.0243
0.87	13.5243	1.83	8.5243	2.79	11.0243	3.75	8.5243
0.90	16.0243	1.86	11.0243	2.82	11.0243	3.78	3.5243
0.93	16.0243	1.89	13.5243	2.85	13.5243	3.81	1.0243
0.96	16.0243	1.92	16.0243	2.88	18.5243	3.84	1.0243



## Example 5: Detection of abnormal cardiac activity

### Problem:

Figure 14 shows frequency-domain curves for three day-12 chicken embryos' activities (both body movement and cardiac rhythm). Which embryo has a cardiac abnormality, and why? What is this abnormality called?



**Figure 14.** Frequency-domain curves for three day-12 chicken embryos' activities.

### Solution:

Using equation 4,

$$HR \text{ (chick a)} = 4.4 \text{ Hz} \times 60 \text{ s min}^{-1} = 264 \text{ bpm}$$

$$HR \text{ (chick b)} = 5.0 \text{ Hz} \times 60 \text{ s min}^{-1} = 300 \text{ bpm}$$

$$HR \text{ (chick c)} = 2.5 \text{ Hz} \times 60 \text{ s min}^{-1} = 150 \text{ bpm}$$

Chick c has bradycardia, or bradyarrhythmia, indicating an unusually slow heartbeat.

## Acknowledgements

Alin Khaliduzzaman acknowledges the late Mr. Shinichi Fujitani of NABEL Co., Ltd., who was a co-researcher during his PhD studies at Kyoto University. Mr. Fujitani invented the commercial EVS discussed in this chapter and was the source of much practical, technical knowledge about optical sensor design.

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