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POSSIBILITIES OF KINETIC IMAGING IN THE OBSERVATION OF BIRD EMBRYOS IN NON - INVASIVE ECOTOXICOLOGICAL STUDIES

[KINETIKUS KÉPALKOTÁS LEHETŐSÉGEI A MADÁREMBRIÓK NEM INVAZÍV ÖKOTOXIKOLÓGIAI VIZSGÁLATAINAK KERETÉBEN]

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Abstract. In order to make ecotoxicological studies using bird embryos simpler and more cost effective, kinetic imaging, already proven in human medicine, was tested. In the study, a total of 8 still-living embryos were examined non-invasively with low-intensity (50 keV) X-rays. During the measurement, we tried to assess how the embryos react if they are not stored at the temperature of 38 °C required for hatching for 1 hour.

Keywords: X-ray, image series, eggs, non-destructive examination, vital signs

Introduction

Bird embryos in ecotoxicological studies

Pollutants are a great health threat to every living organism due to their uptake and translocation in different tissues and organs. Ecotoxicological effects of these pollutants are important topics nowadays, including research with bird embryos. A Chinese research group introduced a method for hatching without eggshells, in order to examine the effect of microplastics on bird embryos. The bird egg is a complete development and nutrition system, and the entire embryo development occurs in the eggshell (Wang et al, 2021). Therefore, a direct record of bird embryo development under the stress of pollutants is highly limited by the opaque eggshell in traditional hatching which is intended to treat the disadvantage of cracking the egg and terminating the embryo at each exam. Which is crucial when we want to study endangered, vulnerable or threatened species. (Ágh et al, 2018) This makes these examinations less effective, because it is impossible to check an exact embryo multiple times, during the development, and also means additional costs to the research. Bird embryos are widely used for ecotoxicological research projects, for decades, these examinations also deal with elements that affect human health directly, like selenium (Weech et al, 2011), and cadmium (Wayland et al., 2011).

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Scope of the study

In this proof of concept study we would like to validate kinetic imaging as an alternative method to cost effective or destructive testing methods on eggs, the current study is intended to prove that we can gain information about the embryos vital signs without destructing the eggs.

Materials and methods

X-ray device

Images were recorded using an X-ray device developed for research and diagnostic purposes. Recordings were handled and stored according to the Dicom standard. The Dicom standard is an international standard for medical imaging devices, it is intended to harmonize medical data management formats (Dicom Standard Committee, 2020 INTERNET1) since 1983.
The device used for the measurements: GE OEC 9800 (GE Healthcare, Little Chalfont, UK). A C-arm X-ray used for human angiography, at the Városmajor Heart and Vascular Centre large animal laboratory. The advantage of this device is that it is suitable for measuring larger samples, the keV range is wider (it includes protocols between 40-120keV). The instrument contains preloaded protocols which can be altered by a service engineer authorized to modify them.

Kinepict Medical Imaging Tool 2.2

Kinepict Medical Imaging Tool 2.2 is a medical software tool intended to be used in human angiography procedures. It is a practical and effective add-on to the original imaging methods. A colour look-up table was used to enhance the movement of the embryos.

Fiji Software

We used Fiji software for image analysis and statistics.

Chicken embryos

We used chicken embryos (*Gallus gallus domesticus*) from Bábolna hatchery, there were 10, 13, 14, 16 days old embryos, which we stored at 38 °C and the images were taken within 3 hours after the eggs were removed from the hatcher. We chose these because these are the most popular ages of the commercial embryos to purchase.

X-ray image series of the eggs were recorded at 50 KeV, with 0.5 fps. One set of eggs was incubated at room temperature for 1 hour before the examination (cold group), and the other set was removed from the heater (warm group).

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Results

Comparing the individual X-ray image (Figure 1), and the kinetic image generated from the same image series (Figure 2), shows the eggs with the embryos inside.
Both images contains the embryos ages ascending from top to bottom: day 10, 13, 14 and 16, the left hand side column contains the group which were removed from the heater 1 hour prior the examination (cold group), and the right hand side were removed right before the image was taken (warm group).
Although the individual X-ray image shows some shadows and the air cells, they do not provide information about the embryos themselves. However the Kinetic image shows visible movements which were enhanced by colour. The brighter colours represent the movement inside of the eggs. The brighter the colour, the more intense the movement is. We can detect significant movement even on day 13 (second row on Figure 1-2).

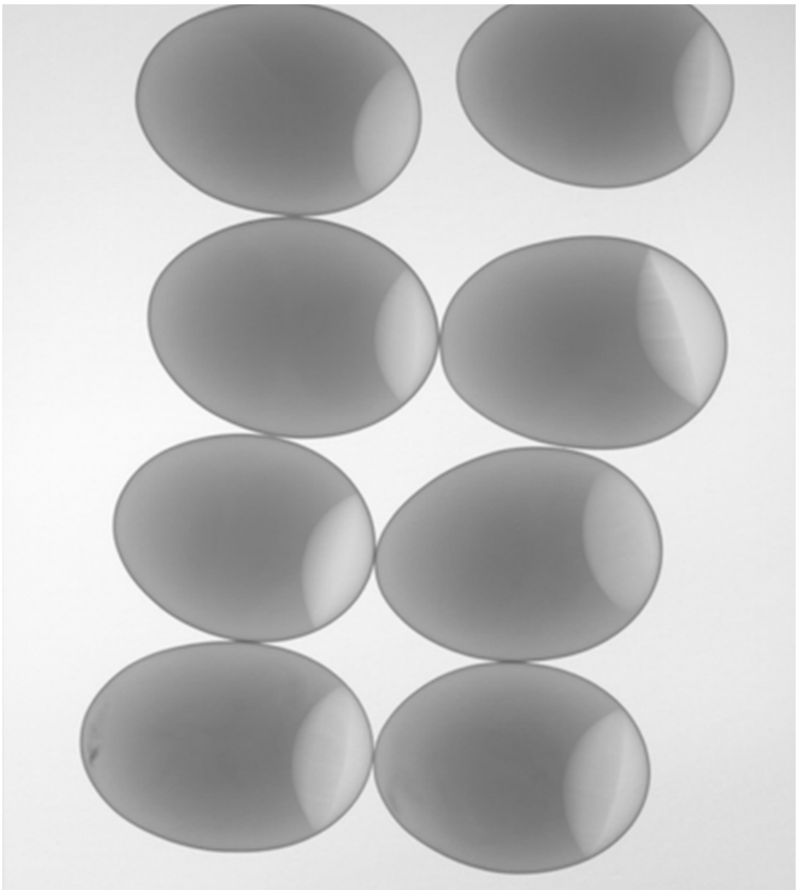

Figure 1: *Original individual X-ray image of the eggs*

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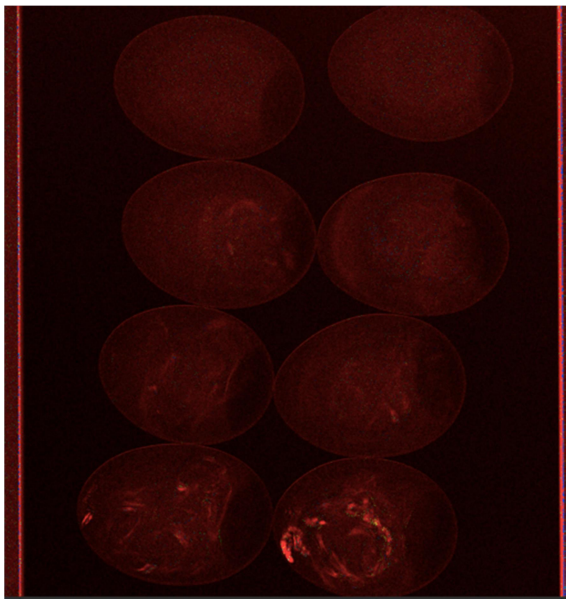

Figure 2: *Kinetic image of the embryos from ages 10-16 days. Eggs represented in the, left hand side column were removed from the heater an hour before the examination (cold group).*

Statistics of the kinetic images

We detected movement from every embryos, we detected the highest pixel intensity on the 16 days old embryos (Figure 4) and the lowest on the 10 days old embryos (Figure 3), which aligns with the development of the embryos. There was no detectable difference in pixel intensity between the cold and warm groups.

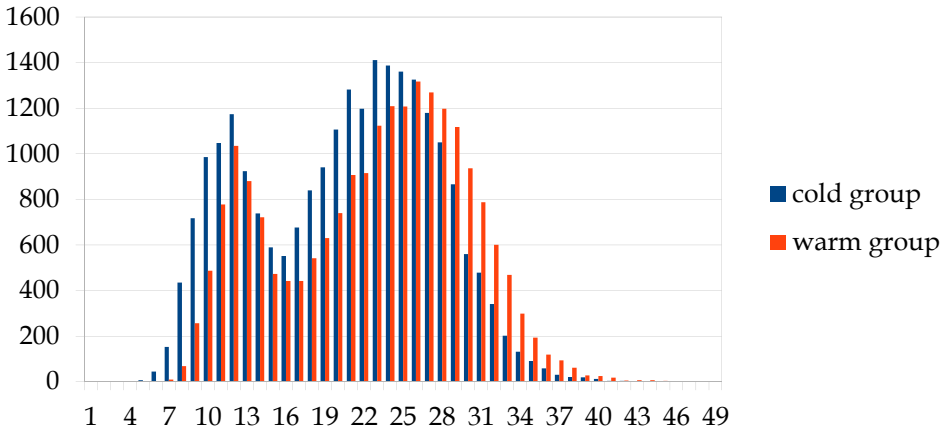

Figure 3: *Histograms of the 10 days old embryos (cold and warm group)*

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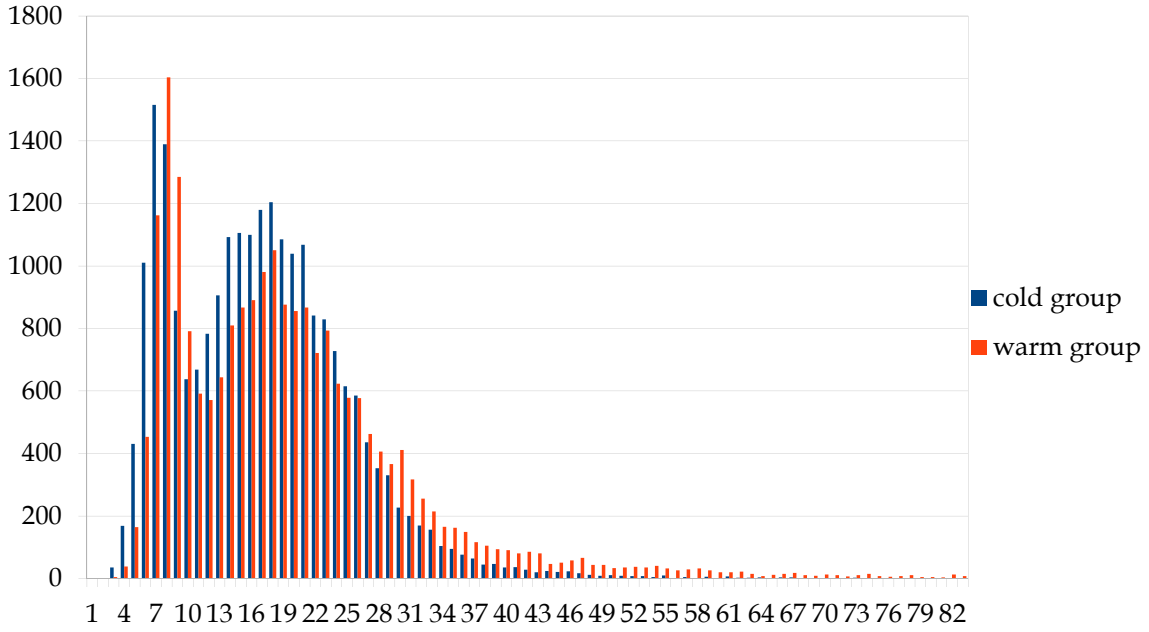

Figure 4: *Histograms of the 16 days old embryos (cold and warm group)*

Discussion

Kinetic Imaging

Kinetic imaging is a technology developed and patented by Krisztián Szigeti and Szabolcs Osváth (2015). It works with imaging procedures based on penetrating radiation, one of which is ultrasound and certain types of electromagnetic radiation, such as infrared, visible light, and also X-rays. The image created by these modalities is by a wave passing through the sample. The radiation is attenuated by the sample and the intensity of the radiation is measured, i.e. how much of the radiation reached from the source to the detector. In these imaging methods, the total detected radiation during the exposure time and the amount of energy is the key information, but the intensity of the beam during the exposure does not provide information about dynamic changes, i.e. the resulting image is an average image that is not suitable to represent moving elements.

During the kinetic imaging process, instead of a single long exposure, there are multiple, shorter exposures recorded, i.e. multiple underexposed images instead of a single, well-exposed image are prepared, which can already carry information about the functional and physiological movements that happen during imaging. The intensity change over time in each pixel is a statistical variable, which can be characterized by several parameters. These parameters can be used to constract parametric images which give more detailed information about the bird embryo. The average (which gives a "static image" very similar to the usual one), and the variance or the standard deviation (this can be called a “kinetic image”) images were found.

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The image representing the average is actually the same as the well-exposed image, but it is only calculated differently in its way. The other images contain new information. This paper deals with a "kinetic image" representing variance.

The mathematical background of kinetic imaging

To understand kinetic imaging, one must start from the beam attenuation law, which describes that the relationship between the change in intensity and the X-ray density is exponential:

$$J=J_0 \times e^{-D}$$

Where:
J: the intensity of the X-ray after absorption,
J0: the X-ray intensity from the radiation source before absorption,
D: X-ray density.

During X-ray imaging, the degree of absorption, i.e. D, is determined by measuring J and J_0 in each pixel. Digital detectors measure J by the number of X-ray photons, which is denoted by K. If the measurement is repeated several times, with that phenomenon we can see that the measured photon numbers fluctuate. There are two sources of such fluctuations: random measurement noise and changes in X-ray absorption, e.g. physiological movements of the bird embryo. Kinetic imaging can isolate random noise and visualize absorption as a random kind of change, which usually means some kind of movement.
As a first step, we make the assumption that the fluctuation of D follows a normal distribution: this is true for the majority of biological variables and phenomena in life that depend on many parameters. The measurement results show that this approximation is not completely accurate, the model, despite this, can be used well to isolate movement and random noise.
Our second reason: if the noise caused by the measuring instrument is negligible and the radiation source is also taken to be of constant intensity over time, in that case, the measured number of photons result from Poisson-distributed quantum noise and possible time-dependent absorption, i.e., depends on the movement within the examined object. In the case of time-constant absorption, quantum noise caused by fluctuation dominates the measurements. Let us assume that several underexposed images are recorded instead of a single, well-exposed image. If the measurement is carried out according to the above conditions, then the expected value of the number of detected photons is described by the following formula:

$$E(K_0)e^{\frac{Var(D)}{2}-E(D)}$$

The variance of the detected photons is given by the following equation:

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$$Var(K) = E(K_0)e^{\frac{Var(D)}{2}-E(D)}\left[1 - E(K_0)e^{\frac{3Var(D)}{2}-E(D)} + E(K_0)e^{\frac{Var(D)}{2}-E(D)}\right]$$

where Var(K) is the temporal variance of the photon number, E(K): expected number of X-ray photons after absorption. $E(K_0)$: expected number of X-ray photons before absorption. Var(D): the density its variance. E(D): the expected value of the density.
Based on the previous two equations, E(D), is the "static image", and Var(D), can be determined as the "kinetic image" (Szigeti et al, 2014):

$$E(D) = ln\left(E(K_0)\frac{\sqrt{Var(K) - E(K) + E(K)^2}}{E(K)}\right)$$

$$E(D) = 2ln\left(\frac{\sqrt{Var(K) - E(K) + E(K)^2}}{E(K)}\right)$$

Kinetic imaging can be used in any area where internal hidden moving elements need to be visualised without disassembling these constructions.
This method is widely used in the medical field as practical addition to X-ray angiography. The method was also used to detect and monitor the activity of hidden lifestyle arthropods' (Keszthelyi et al, 2020), but this method is not yet widely spread.

Conclusion

We believe this examination can be a first step for towards developing a new method which can make ecotoxicological researches more reliable and cost effective. Based on this study we believe that the method should be investigated further with different bird species, stressors, modalities, light sources and most important, bigger sample sizes. Our concept that we can gain information without destructing the egg is proved. Kinetic imaging also has the possibility to perform multiple exams on the same embryos even carried to hatching therefore we can see the effects of different stressors on grown birds.

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Absztrakt. A madárembriókat is felhasználó ökotoxikológiai vizsgálatok egyszerűbbé és költséghatékonyabbá tétele érdekében kipróbálásra került a humán gyógyászatban már bizonyított kinetikus képalkotás. A vizsgálatban összesen nyolc még élő embriót vizsgáltunk nem invazív módon, alacsony fotonenergiájú (50 keV) röntgensugárral. A mérés alatt megpróbáltuk felmérni, hogy hogyan reagálnak az embriók, ha egy órán keresztül nem a keltetéshez szükséges 38 °C-os hőmérsékleten tároljuk őket.