# AN AUTOMATED ASSAY FOR THE ASSESSMENT OF CARDIAC ARREST IN FISH EMBRYO


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## *PROBLEM STATEMENT : IS A FISH EMBRYO DEAD OR ALIVE ?*

Fish embryos are widely used in toxicology and ecotoxicology screening. Such tests require trained operators and the manipulation of a large number of samples. To reduce the time needed for data processing and improve objectivity, we propose to automate the mortality assessment.
Here, we present an efficient image processing pipeline for heartbeat detection in Medaka *(Oryzias latipes)* embryos. We have designed a 2D acquisition device to record 1 second long videos of each embryo. After a pre-processing step, the heart is identified as the area presenting the highest pixel intensity variation in the thorax region of the embryo. As a result, based on a set of more than 2000 videos, we report an accuracy greater than 98%.

## VIDEOS ACQUISITION AND PROTOCOL

The protocol is the following :

- Embryos are analyzed 9 days post fertilization;
- Observations are made in 24-well plate;
- Each well contains one embryo in 57µL of anaesthetic solution (125mg/L Tricaïne);
- The plates are placed on a light table;
- The acquisition camera moves above each well and records a sequence of 30 images over 1 second.

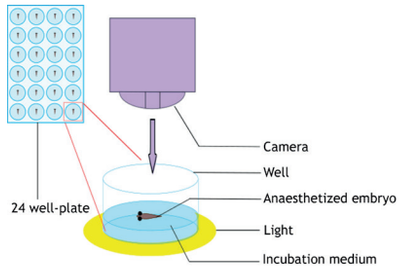


**Fig 1.** Scheme of the acquisition set-up

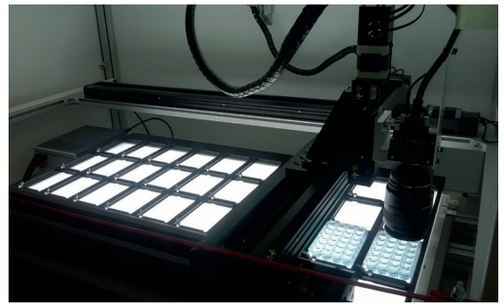

**Fig 2.** Video acquisition device

## PRE-PROCESSING

**Localization of the embryo:**
- In the first image, the embryo is segmented using morphological operators based on pixels intensity, geometry and area analysis;
- All images of the video are cropped around the embryo to improve speed and to reduce memory usage.

**Segmentation of the inner parts of the embryo:**
- A refined segmentation of the inner parts is obtained on the cropped image.
- Eyes are identified as the one or two most proeminent and darkest areas, then are subtracted to the segmentation.

**Fig 3.** Segmentation to locate the embryo in the well.

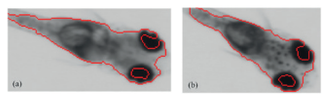

**Fig 4.** Inner parts segmentation on two embryos.

## HEARTBEAT DETECTION

**Motion detection in inner parts:**
- We rely on intensity variations, analyzed on several sub-sequences;
- We identify motion areas which are present over the entire sequence. Artifact motion due to noise is rejected.

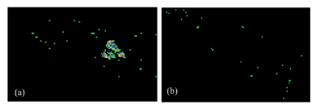

**Fig 5.** False color rendering of the temporal variation. (a) is alive and (b) is dead.

**Is the embryo dead or alive ?**
- We binarize the variance rendering then measure its area;
- If no area is detected, the embryo is considered dead, if not, it is considered alive.

**Fig 6.** Segmentation of motion areas. (a) is alive and (b) is dead.

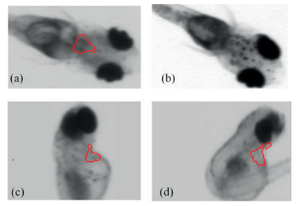

**Fig 7.** Heartbeat detection in (a) living embryo, (b) dead embryo, (c) edema and (d) axial malformation.

## GENERAL IMAGE PROCESSING PIPELINE

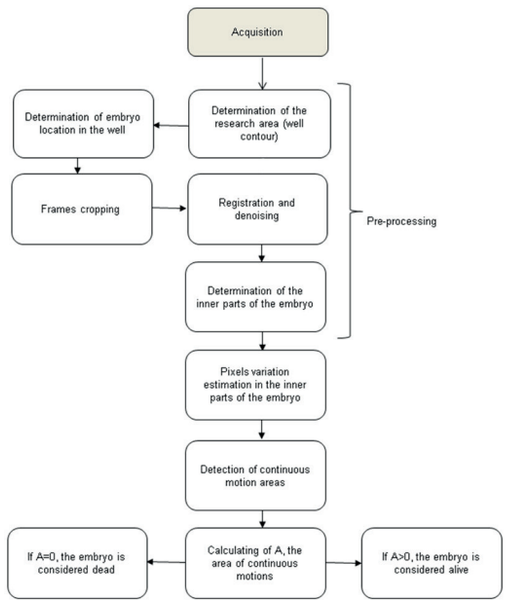


**Fig 8.** Flowchart of the embryo mortality assay

## *RESULT AND CONCLUSION*

**Results on total dataset:**
- The error rate was calculated using video-based ground truth.
- The total set of 3192 videos was observed by 1 expert (expert A).

| | |
|---|---|
| Initial number of videos | 3192 |
| Number of processed videos | 2537 |
| Errors on total processed set | 45 |
| **Error rate** | **1,77%** |

**Subjectivity assessment:**
- A subset of 200 usable videos was observed by 3 experts (experts A, B and C) and a consensus was reached between them, leading to a set of consensus ground truth;
- To assess the subjectivity rate, we compare the consensus to each expert observation and to the program results.

| Experts | A | B | C | Program |
|---|---|---|---|---|
| **Error rates between consensus and experts observations** | 1.5% | 2.5% | 1.5% | 1.5% |

We conclude that the subjectivity rate is near 1.5% and the computer program has a similar error rate to humans.

**Visual VS videos analysis:**
- To assess the reliability of the entire procedure, we compare these results to careful visual inspections of embryos under a microscope;
- On the full dataset, we noticed a discrepancy in 282 cases, for a rate of 11% due to images acquisition limitations. We are working on improving the current acquisition procedure.

## CONCLUSION

We propose a simple and effective image analysis pipeline to detect wether a fish embryo is alive or dead, by detecting the presence or absence of motion in its heart region.


**Références:**
- An automated assay for the assessment of cardiac arrest in fish embryo. E Puybareau, D Genest, E Barbeau, M Léonard, H Talbot. Computers in Biology and Medicine 81 (2017) 32-44, Elsevier
- L. Najman, H. Talbot (Eds.), Mathematical Morphology: from Theory to Applications, ISTE-Wiley, London, UK, 2010, ISBN 978-1848212152.


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