Automated measurement of the increase in size of the chick area vasculosa over time

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Project Report

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Declaration

I hereby declare that, except where otherwise indicated, this document is entirely my own work and has not been submitted in whole or in part to any other university.

Signed: …… Date: ……

Abstract

Today a lot of researches are done in the field of the development of chicken embryo. There are a lot of important steps in this development but the first steps are determinant for a viable growth of the chick. The embryo starts his development in an area called area vasculosa. We will focus on the growth of the vasculosa area and the embryo over time. Researchers are particularly interested in this area for example to add drugs to improve the development of the embryo.

We will use machine Vision Techniques to extract particular features from images of this vasculosa area. In particular, the software Neatvision will be used all across the project.

The purpose of the project is to build some robust programs that would to extract all of these features.

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CHAPTER 1- Introduction

The development of chicken embryo is studied for years by researchers. They always wanted to know better all the facets of this development.

It takes about 20 days for an egg to give birth to a chick and during this time, researchers make a lot of experiments.

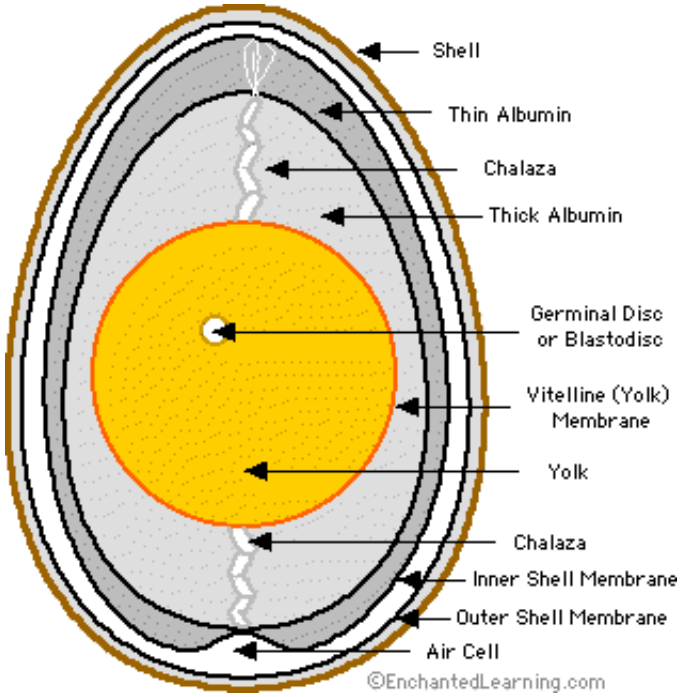


Figure 1: Cross Section of a Newly Hatched Egg [1]

The area vasculosa (also called Blastodisc) is one of the parts the most interesting in the growth of an egg as it is where the embryo grows. The area vasculosa is submitted to a lot of experiment by biological researchers in Laboratories. They are interested in evaluating new drugs, and comparing them with models. For example, the chorioallantoic membrane (CAM)[2] is widely used as a model to examine angiogenesis (after 3 days of incubation), and antiangiogenesis stage.

Other experiments such as measurement of the "Growth of the chick area vasculosa in ovo and in shell-less culture."[3], "The effect of ethanol exposure on extraembryonic vascular development in the chick area vasculosa."[4] or "The effects of lithium on vascular development in the chick area vasculosa."[5] show that these sorts of measurements are widely done in biomedical sciences researches.

We will focus on 2 main phases in the growth of the embryo: the vascologenesis (after 2 days of incubation) and the angiogenesis (after 3-4 days of incubation).
These two phases are the most interesting for researchers as it gives them a lot of information. For example, the researches on the vasculosa area "indicate that the chick area vasculosa capillaries bear similar structural and growth characteristics to those associated with tumour angiogenesis and suggest that they may prove to be a useful model system for studying the factors involved in pathological angiogenesis"[6].

All these experiments in laboratories are done manually, and the need of an automated measurement technique is needed to improve their efficiency.

CHAPTER 2- Technical Background

2.1 Previous work on image acquisition

A previous project [8] was done on the same subject, but the main objectives were to do all the part on image acquisition. In this project, the image acquisition has already been completed and a lot of problems have been resolved in this stage to achieve the best results:

- Camera selection:
  All the images were taken with a Fujifilm Finepix 6900 Zoom with a 3,3 CCD camera. The file format of the images is JPEG.

- Sample presentation:
  Instead of using a plastic hammock as it has usually been done, we use a glass container.

- Lighting conditions
  Instead of using a light box or 2 separate lamps, we use a ring light.

- Sample preparation
  We have to take care in the transfer of the samples. The lid of the sample should have a small hole. Finally the samples should be numbered.

- Vasculogenesis and angiogenesis image acquisition
  The images are taken during the Vasculogenesis and angiogenesis phases separated by a time interval of 32 hours. During the images acquisitions, a manual focusing of the digital camera is required to avoid lack of contrast. The idea of injecting ink is a possible solution but there are a lot of disadvantages like loss of important information in the images.

- The calibration

  It gives a relationship between an image pixel and real area occupied. The best technique found uses a calibration grid.

2.2 Equipment and Software

2.2.1 The images

Concerning the equipment, I already have all the support from the previous work of Adrian Gaffney (Student). He had taken all the images of the development of the embryo in the angionesis phase(72h) and vasculogenesis phase(96h).
There are 2 sets of samples: the first set of samples after 72 hours of incubation and the second set of samples after 96 hours of incubation. The first set contains 19 images and the second 13 images.

All the images, which will be processed, are in JPEG: a lossy image compression file. Moreover to have better efficiency with the process of the image, the resolution is divided by a factor of 2, so all the images processed have a resolution of 640*480. So there is loss of information, but that helps us to process the images in an easier and faster way.

2.2.2 The software

The software Neatvision version 2.1 [8][9] (www.neatvision.com) developed by the Vision System Group of DCU is used. This free software contains all the useful functions used to extract features from an image. These functions are implemented in Java, what provides high-level access to a wide range of image processing algorithms. NeatVision contains over 290 image manipulation, processing and analysis algorithms. So we will reuse all these functions and perhaps implement new ones if necessary.

2.2.3 The calibration grid

A calibration grid was build by Adrian Gaffney. He took an image with a pattern which dimensions are known. Then with a Neatvision program, the image is analysed and output a value for a millimetre-squared area per pixel.
For the images after 72 hours, the exact value for a 1280 by 960 image is: 1 mm² is 987.836 pixels and for the images after 96 hours: 1 mm² is 180.331pixels. These values will simply be reused in our program.

So after some calculation we find that:

- For the 72 hours camera calibration, the measure between two adjacent pixels in line is **0.0318168** mm. For the area, we find that 1 pixel has **0.0010123** mm².
- For the 96 hours camera calibration, the measure between two adjacent pixels in line is **0.0744672** mm. For the area, we find that 1 pixel has **0.0055453** mm².

2.3 Machine Vision techniques

The difficulty when the experiments are done, is to get quickly true results, as there are a lot a lot of images, which have to be processed in the same time. Researchers often calculate manually the area or the circumference of a feature manually. So they can make a lot of errors, as human vision isn't one hundred per cent reliable.

Machine Vision techniques can help them by automating such process and is more adapted to give quantitative information than human vision is.

Moreover, by automating such a process, a lot of information is given in only a few seconds, what help researchers not to be restricted by the number of experiments they can do.
We will describe a few techniques here that are essential in this project.

2.3.1 The key techniques

2.3.1.1 An Edge detection technique: the Canny

A few papers[10][11] study object recognition system performance as a function of the edge operator chosen at the first step of the recognition system. It compares the outputs from various edge detectors.
The conclusion is that the Canny edge detector is superior to the others compared.

- **The performances of the Canny**

The result of an edge detection can be evaluate by eyes but it is a subjective appreciation. So the performances of an edge detector like the Canny are given by:

- The detection : the operator must give a response in the neighborhood of the edge.

- The localization : the edge must localized by precision.

- The unique response : an edge must give only one response from the operator.

So Canny[12] has introduce a model that evaluate these criterions:

1. The detection of the edges: small probability not to detect a real edge,and small probability to mark a wrong edge. To do this, we have to maximize the fraction signal over noise RSB:

$$\sigma = RSB = \frac{A}{\eta_0}\Sigma = \frac{A}{\eta_0}\frac{\int_{-\infty}^{0} f(x)dx}{(\int_{-\infty}^{+\infty} f^2(x)dx)^{1/2}}$$

2. The localization of the edges: the points mark as an edge by the detector must as close as possible from the center of the real edge. This criterion corresponds at the maximization of the standard deviation (square of the variance) , so it is the inverse of the mean distance between the real edge and the detected edge:

$$\lambda = \frac{A}{\eta_0}\Lambda = \frac{A}{\eta_0}\frac{|f'(0)|}{(\int_{-\infty}^{+\infty} f'^2(x)dx)^{1/2}}$$

3. The unique response to an edge: the detector mustn't give several response to one edge. We can show that it is in fact the minimization of the expression:

$$x_{max} = \left(\frac{\int_{-\infty}^{+\infty} f'^2(x)dx}{\int_{-\infty}^{+\infty} f''^2(x)dx}\right)^{1/2}$$

The criterion of detection and of localization are paradoxical, we can combine them to maximize $\Sigma\Lambda$ with the constraint of the third criterion. At he end we obtain a differential equation, its solution is :

$$f(x) = a_1 e^{\alpha x}\sin\omega x + a_2 e^{\alpha x}\cos\omega x + a_3 e^{-\alpha x}\sin\omega x + a_4 e^{-\alpha x}\cos\omega x + c$$

Canny uses the condition at the limits:

- f(0)=0
- f(-W)=0
- f'(-W)=0
- f'(0)=s where "s" is a predetermine constant, equal to the slope at the origin of the filter.

We can then obtain: a_1, a_2, a_3 , a_4 and c an the optimal filter: f(x) and deduct $\Sigma\Lambda$ (ω, α) in different intervals m with $\alpha = m\omega$.

Based on these criteria, the canny edge detector first smoothes the image to eliminate the noise. It then finds the image gradient to highlight regions with high spatial derivatives. The algorithm then tracks along these regions and suppresses any pixel that is not at the maximum (nonmaximum suppression). The gradient array is now further reduced by hysteresis. Hysteresis is used to track along the remaining pixels that have not been suppressed. Hysteresis uses two thresholds and if the magnitude is below the first threshold, it is set to zero (made a nonedge). If the magnitude is above the high threshold, it is made an edge. And if the magnitude is between the 2 thresholds, then it is set to zero unless there is a path from this pixel to a pixel with a gradient above T2.

- **The Neatvision block**

The canny edge detector is the optimal approach for step edges corrupted by noise.
The canny returns the magnitude and the direction of the gradient.
There are 4 inputs:
- The first one is the greyscale image
- The second input is double which is the width of the Gaussian belt , it is usually between 1 and 1.5. As it increases, the noise decreases.
- The third and fourth inputs are two integer thresholds T1 and T2. The vector T=[T1 T2] containing two threshold is used to find the strong and the weak edge pixel and an edge linking is performed by including the weak pixels to the strong. So there are 2 thresholds T1 and T2. The first threshold T1 is usually set quite high and T2 is set quite low. Tracking can only begin at the point ridge higher than T1, and tracking continues in both directions out from that point until the height of that point falls below T2. However if T2 is too high noisy edge break up and if T1 is too low it increases the number of undesirable edge fragment.

2.3.1.2 A transform technique: the Hough

The transformation of Hough [13][14][15][16] is a tool for treatment of the signal whose first function is to find lines with certain properties. However a better known problem is the detection of group of dots grouped in the plan. The goal of the transformation is then to associate a line to a point in a space of two dimensions. For example, for the line of Cartesian equation y = a*x + b, we associate the point of co-ordinates (a,b).

The idea of the Hough transform is to consider all the couples of points and to calculate its mediator. We take all the couples of points and we associate to them the corresponding line in the space of Hough. For N points, it is thus necessary to calculate N(N-1)/2 lines. But at the opposite of simple object, we don't verify a non-variation by symmetry, we simply accumulate the mediators in the Hough space.

In the ideal case, that means that there is an axis of symmetry, it will be accumulated N/2 times where N is the number of points of edges. Basically, N/2 points are the symmetric of N/2 others by an axial symmetry which axis is their mediator.
The others mediators are spread in a random manner in the space of Hough. In practice, if there is an axis of symmetry, it corresponds to local maxima in the Hough space.

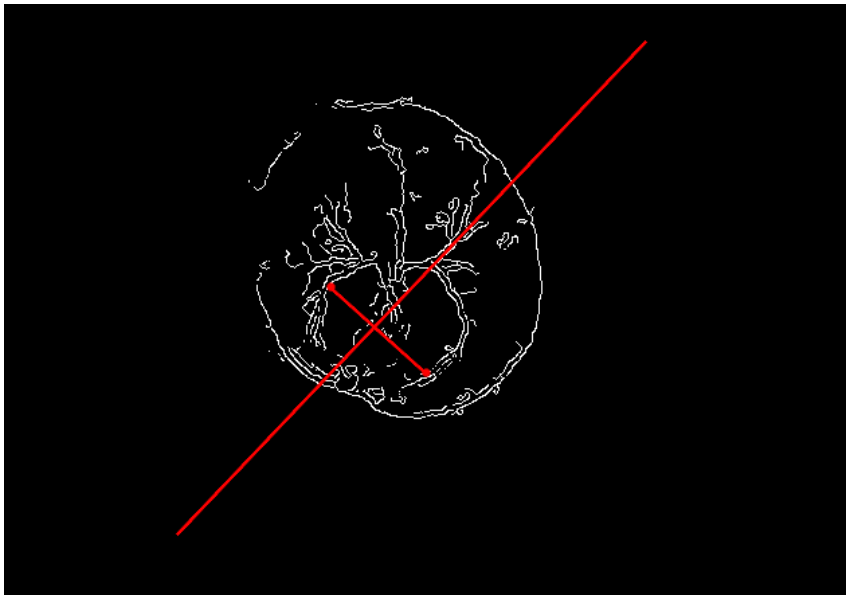


Figure 2 : A couple of 2 points on which we calculate the mediator

Each calculated mediator corresponds to one point in the space of Hough. The equation of the mediator is given by:

$$x\cos\theta + y\sin\theta = r$$

Note that, although r and θ are polar coordinates, the accumulator space is plotted rectangularly with θ as the abscissa and r as the ordinate.

2.3.2 Some usual techniques

We will here make an exhaustive list of other important techniques that are implemented under Neatvision and used all accross the project in the implementation. Note that not all the techniques being used were descibed here.

- **Utilities**

Mask

The mask returns the input image added with a black frame on the 4 edges of the image.

- **Histogram**

Hi Grey

The Hi grey block takes a grey image in input and gives an integer value at the output which is the highest grey value in the image. It is actually the global maximum of the grey histogram.

- **Processing**

Threshold

Thresholding converts a grey level image into a binary image by setting all pixel values above a threshold to 1 and all those below to zero. The threshold can either be a single threshold with one threshold of control or dual threshold with two thresholds of control.

- **Edge**

Non-maxima suppression

The magnitude of the gradient map is calculated and then input to a routine that suppresses (to zero) all but not the local maxima. The resulting map of local maxima is thresholded (small local maxima will result from noise in the signal) to produce the final edge map. It is the non-maxima suppression and thresholding that introduce non-linearities into this edge detection scheme.

Roberts

The Roberts operator performs a simple and fast, 2-D spatial gradient measurement which often correspond to edges.

This operator consists of a pair of 2×2 convolution kernels :

+1	0
0	-1

+1	0
0	-1

These kernels are designed to respond maximally to edges running at 45° to the pixel grid, one kernel for each of the two perpendicular orientations.

- **Analysis**

BlobFill

The blobFill function fills with white pixels all the blobs in the image.

BigBlob

The BigBlob function detects the biggest white blob in the image.

- **Morphology**

Dilate - Erosion

The dilation operator takes two pieces of data as inputs. The first is the image which is to be dilated. The second is a set of coordinate points known as a structuring element (4 or 8 kernel). It is this structuring element that determines the precise effect of the dilation on the input image.

The mathematical definition is given as: " Suppose that *X* is the set of Euclidean coordinates corresponding to the input binary image, and that *K* is the set of coordinates for the structuring element.

Let *Kx* denote the translation of *K* so that its origin is at *x*.

Then the dilation of *X* by *K* is simply the set of all points *x* such that the intersection of *Kx* with *X* is non-empty "

The erosion function is the dual of the dilation.

Open

A opening is defined as an erosion followed by a dilation using the same structuring element for both operations

Close

A closing is defined as an dilation followed by a erosion using the same structuring element for both operations

- **Colour**

ColourToRGB

Transform a colored image into 3 gray scale images built on the level of different coloured channels. The first output image R correspond to red channel, the second ouput G is the green channel, and the thrird output B is the blue channel.

ColourToCMY

Transform a colored image into 3 gray scale images built on the level of different coloured channels. The first output image C correspond to cyan channel, M to magenta channel and Y to yellow channel. A gray level value is given to each output depending on their coloured channel histogram.

ColourToLAB

Transform a colored image into 3 gray scale images built on the level of different coloured channels. The first output image L correspond to lighness channel, A is the difference between the red and green channels , and B is the difference between the yellow and blue channels.

2.4 Image Analysis Tasks

As we have all the images, it is now possible to move on the analysis part of these images. There are a lot of features that can be extracted from this image and a few of them were already implemented in the previous project with Neatvision 2.0 Beta. The work was only focussed on the extraction of the vasculosa area. This area was calculated on a few samples and compared with manual results. These results are quite interesting but improvements can be made on the program especially on the speed and the quality of the results.

In addition of these improvements on the vasculosa area, other useful features should be extracted. So in order of priority, there are:

1. The vasculosa area
2. The circumference of the vasculosa
3. The area of the embryo

4. The circumference of the embryo
5. The symmetry of the vasculosa area

Description of the tasks:

1. The value of the vaculosa area.
   The images that we have in input are colored image. On these images the vasculosa area is the region with all the vessels.

2. The value of the circumference of the vasculosa
   The circumference of the vasculosa area looks like a circle but this is a first approximation to calculate the circumference. So we want to have more precision, so we must find the circumference that includes exactly the vasculosa area.

3. The value of the area of the embryo
   The embryo appears after 3 days of incubation. We can note that the form of the embryo is more complex than the vasculosa area. It usually appears in the centre of the vasculosa area and it is the part of the vasculosa area that has the more red pigments.

4. The value of the circumference of the embryo.
   The circumference of the embryo is a complex task to extract as it is not well defined even by eyes. However it is possible to find the border between the red pigment of the embryo and the yolk by applying precise techniques.

5. The symmetry of the vasculosa area
   For simple geometrical objects, polygons for example, the concept of axis of symmetry is well defined. Considering a given line, it is an axis of symmetry if the image of the object by symmetry of axis of this line is

identical to itself. But a first difficulty already appears: how does one determine this line initially?

For the vasculosa area, the task is more difficult because it is in general only roughly symmetrical. Moreover, what are the "significant points" when we don't deal with a polygon?

So we have to find a measurement of this symmetry. We defined 3 ways to measure the symmetry:

- If it is the most obvious symmetry in the image that passed through the embryo, we called it the "main symmetry" (MS).
- If it is the perpendicular axis to the main symmetry that passes to the centre of the vasculosa region, we called it the "secondary symmetry" (SS).
- If it is not a relevant symmetry in the image, we called it "not apparent symmetry" (NAS)

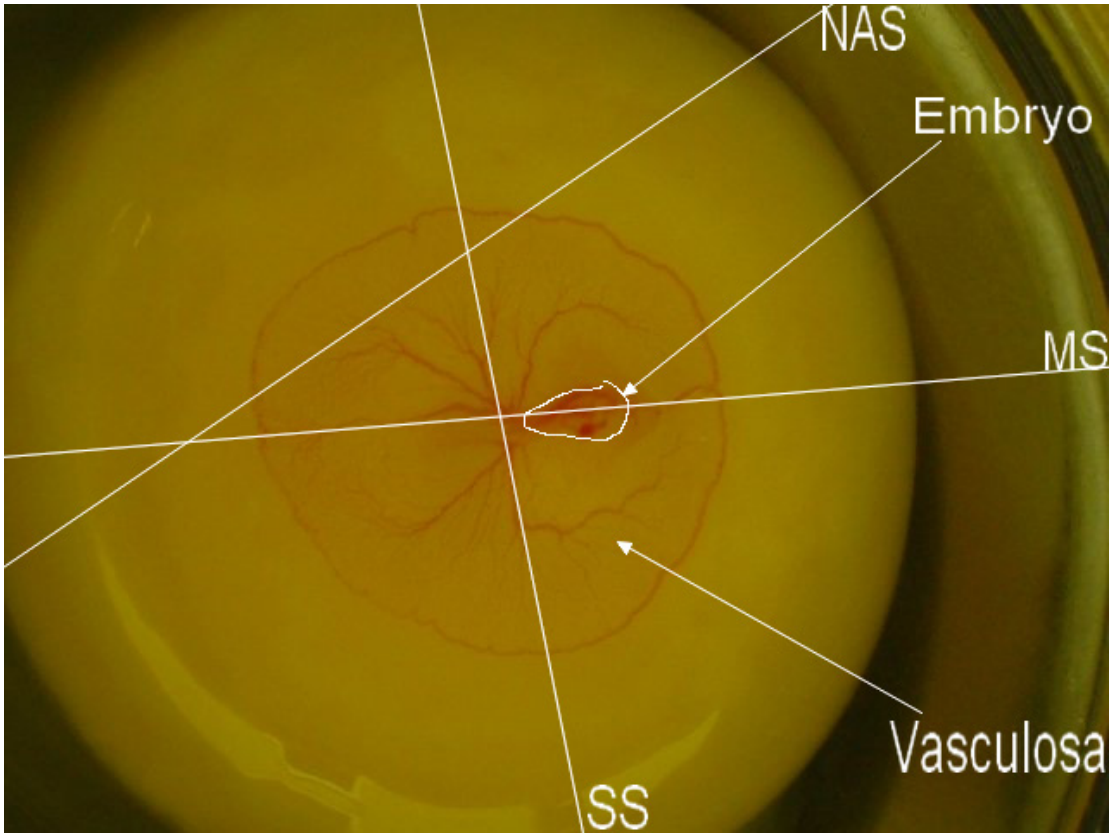


Figure 3 : Graphical description of the different features to extract

CHAPTER 3: Image Analysis implementation

This chapter treated all the software based process. Data are extracted from the images and measurements are deducted. All the functions used are implemented in Neatvision.

3.1 All the java coding blocks

3.1.1 The Neatvision block : “CircumfAreaMeasurement”

At the input, there are the grey image which represents the closed form from which we want to calculate its circumference and a double which is the length of a pixel.

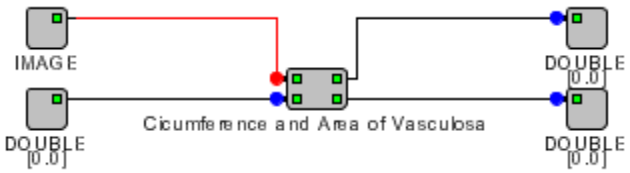


Figure 4 : The implemented Neatvision block “CircumfAreaMeasurement”

3.1.1.1 The vasculosa circumference

The input image comes from a convex hull on which we want to calculate the value of the circumference of the region vasculosa.

The input circle is split in 2, each of the input is passed through a convolution block (CONV). The convolution is done by 2 different 2 by 2 arrays:

[X X] [1 X]

[1 1] [1 X]

The 1 indicates a white pixel and X indicates a pixel in black.

As we know that the thickness of the circle is 1, and with these convolutions of 2 arrays, we can treat all the cases between two adjacent white pixels.
When one of the configurations of the arrays is founded, the convolution block put a white pixel on the output image.

It is then necessary to pass, the output of the convolution through a threshold (SINGLE block) at 255, so that the pixel indicated by the convolution is put at the grey level of 255.

We then count the number of white pixel (CWP block) and multiplied (MUL block) in each case this value by the real measure of a white pixel. This measure is given by the calibration grid (0.0318168 mm = 1 pixel for the 72 h stage and 0.0744672 = 1pixel for the 96 h stage).

We finally add (ADD block) all these measures of the 2 configurations to find the value of circumference (DOUBLE block).

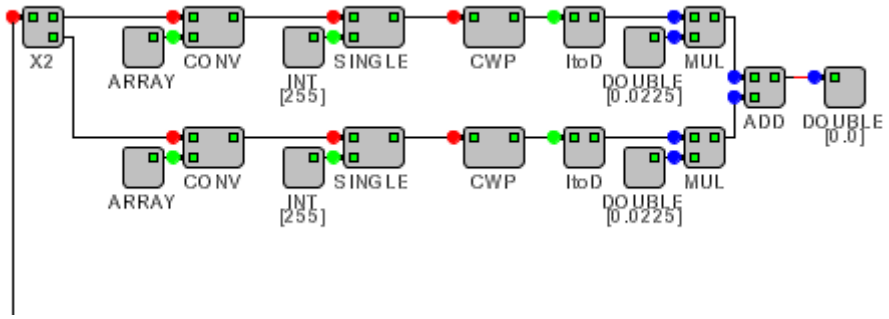


Figure 5 : Neatvision blocks to calculate the circumference of the region vasculosa

3.1.1.2 The vasculosa area

The input image comes from a convex hull on which we want to calculate the value of the area of the region vasculosa.

We start by filling the circle, and make a disk, we use the blob fill block (BLOBFILL block), it fills with white pixel all enclosed region.

We then count the number of white pixel (CWP block) and multiplied (MUL block) this value by the real measure of a white pixel value of area (DOUBLE block). This measure is given by the calibration grid.

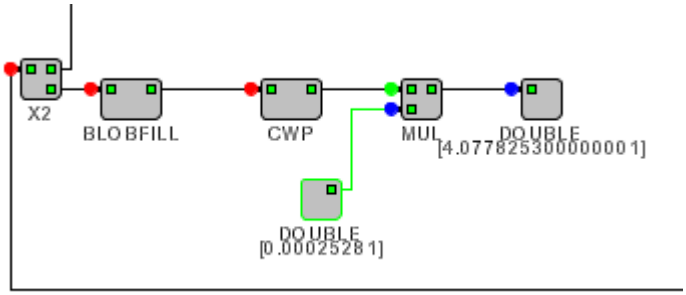


Figure 6 : Neatvision blocks to calculate the area of the region vasculosa

3.1.2 The testing block

In order to improve the speed of all the tests on the images, it is necessary to create a block that can check the robustness of a program on each image.
The “testing” block takes 34 inputs for all the sample images I have and compute in a loop, the same functions on each input image. It gives in output 34 other images processed by the program.

With this block, it is possible to call in the create method all the methods we want (here A_METHOD) , and to apply this method for all the images.

```
public Object create(DataBlock args)
{
        DataBlock returns = new DataBlock();
        for(int i=0;i<30;i++){
        Image input = (Image)args.getImage(i);
        returns.add(A_METHOD (input));
```

```
            }
            return(returns);
        }
```

The “testing” is a really efficient block for this project. It helps a lot in the implementation of a robust solution for the measures we have to find.

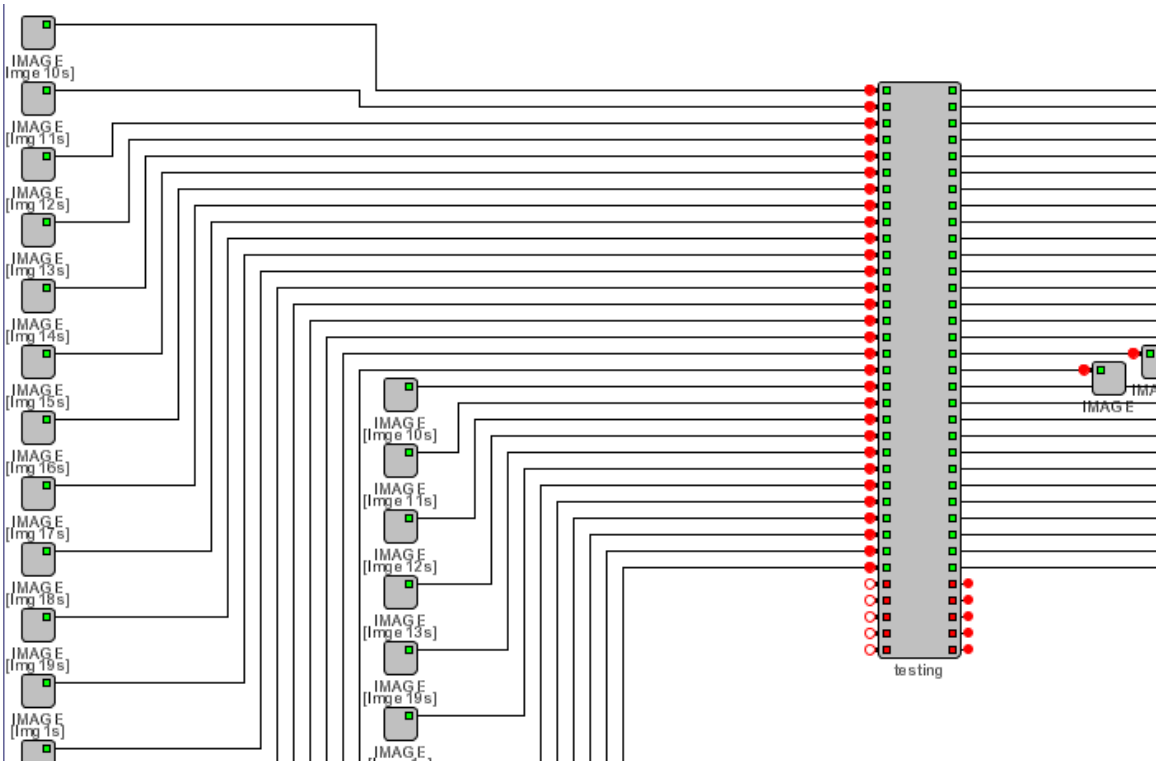


Figure 7 : The implemented Neatvision block “testing”

3.1.3 Other implemented blocks

- *The “VasculosaRegion” block*

As it will be discussed in the section 3.2, the “VasculosaRegion” block takes in input the colour image of the yolk and return in output a grey image that is the edge of the enclosed form of the vasculosa region (section 4.2.6).

- *The "FindSymmetry" block*

As it will be discussed in the section 3.4, the "FindSymmetry" block takes in input the output of a Canny operator and gives in 3 outputs : the input image with a first axis of symmetry, the input image with a second axis of symmetry, and the input image with the first and second axis of symmetry.

3.2 The area vasculosa : circumference and area

To extract the region vasculosa from the images, there are several methods possible. The difficulty here is to find one method that will solve the problem for all the images we have. The method is said "robust" when it is capable of dealing with every images.

For this part, the java block "*VasculosaRegion*" has also been implemented, it regroups the section 3.2.2 to 3.2.6.

3.2.1 2 samples sets, 2 files

The two samples sets have a different calibration grid so it is necessary to differentiate them one from another. Instead of complicating the Neatvision programme with "if" conditions, I prefer differentiating the 2 sample sets by creating one file for each sample set.

3.2.2 Coloured image to grey image

First of all, we can see that the region vasculosa is the only part of the image, that is in red. By applying a scan on the image on the region vasculosa, we can note that the red is in majority, then comes the green and finally the black and the blue

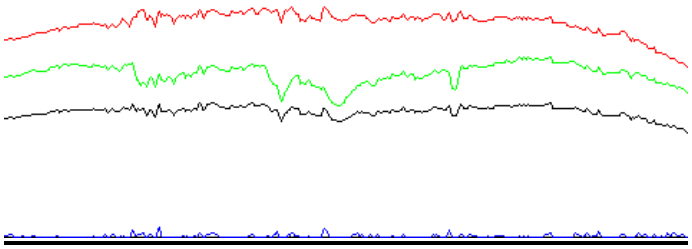


Figure 8 : Vertical scan on the region vasculosa

The idea is therefore to use a function that do a threshold based on the value of red. So the function used transform a coloured image in input in three different images in output segmented on the value of cyan, magenta and yellow. We use the magenta output that is closed to the desired red. So for each pixel a grey level is assigned to it depending on the percentage of magenta in the pixel.

Before displaying the results in an output image, the image need to pass through a normalisation block (ViewCMY).

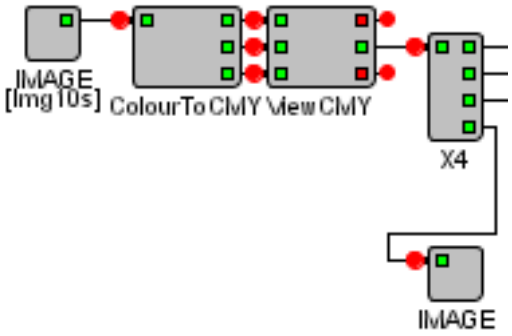


Figure 9 : Neatvision blocks for the CMY space

3.2.3 The mask

If we try to isolate the vasculosa area, we will have difficulties for some images that :

- are noisier than other
- have not the same size

- that have not the same luminosity

That's why, the idea is to find a mask in the image.

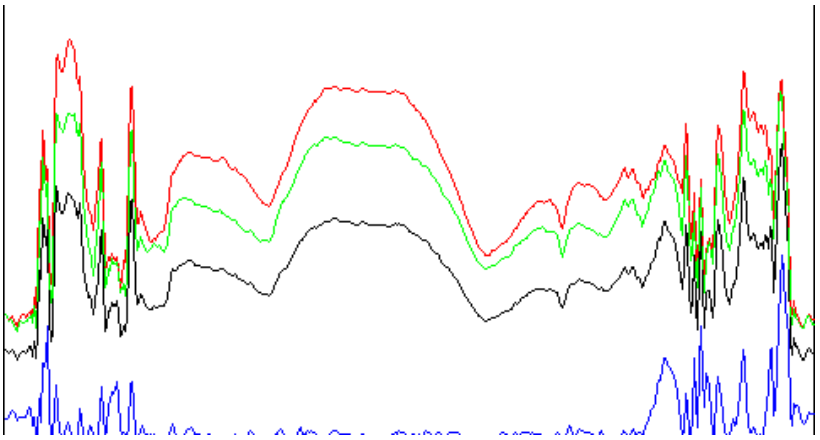


Figure 10 : Vertical scan on the region vasculosa

We can note by observing the vertical scan on the region vasculosa that the blue component is not present in this region. So the idea is to segment the image under the blue component. We will use the cyan because the blue component of the RGB block is not accurate enough and too noisy.

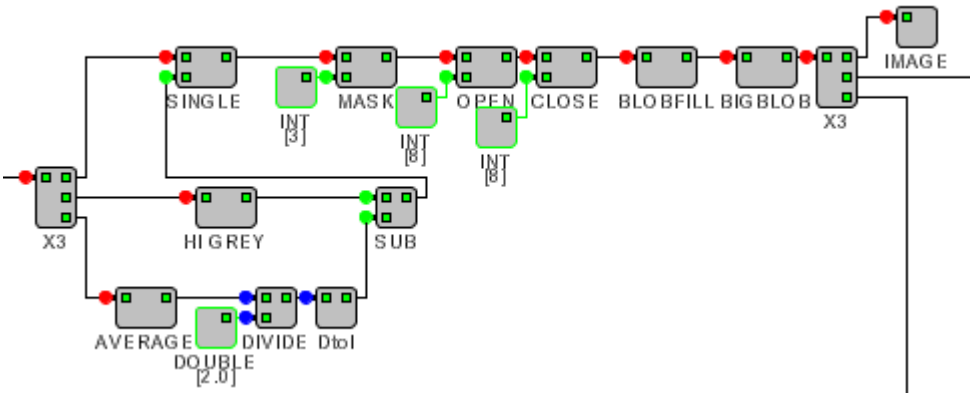


Figure 11 : Neatvision blocks to create a mask

The input image comes from the Cyan output of the ViewCMY.

We first get the highest grey value and the average grey value. We then subtract half this average value to the highest grey value in order to get a value for the threshold of the cyan. The purpose of this subtraction is to reduce the

significantly the effect of the noise as the noise is the part of the image that has the most of the cyan part.

So the effect of the threshold is to appreciatively remove the cyan noise in the 4 edges of the image, and enhance the vasculosa area.
But there is still some noise in the image that we have to remove. So we apply a mask (MASK block), and two morphological operations: one opening (OPEN block) and one closing (CLOSE block) with a structuring element of 8. These three last operations are necessary in order to enlarge the pixels so that they can be treated as blobs.

We finally fill all the blobs (BLOBFILL block) and select the biggest one (BIGBLOB block) which is the vasculosa region.

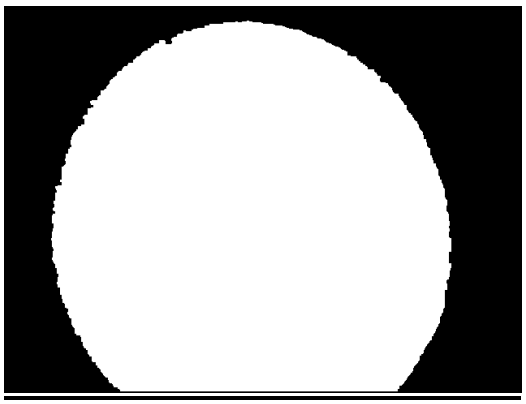
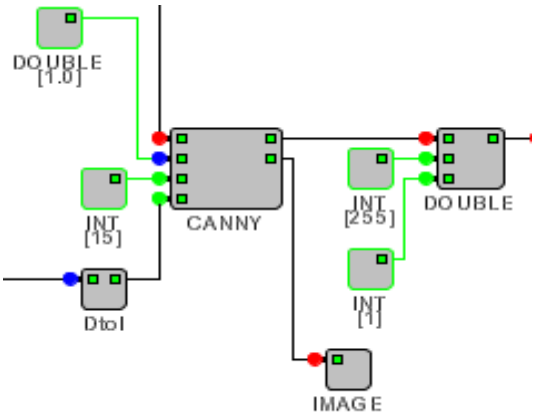


Figure 12 : The mask image

3.2.4 The edge detector

The image from part 4.1.1 is a greyscale image with 256 levels of grey. To manipulate this image, we have to reduce the number of level of grey to two (black and white).
If we only apply a threshold on the greyscale image, we can see that there is a lot of noise, so we have to apply edge detector to detect correctly the edges. The edge detector that is used is the Canny.



The canny has four inputs (image, width of gaussian, 2 thresholds) and the magnitude and the direction of the gradient in output. We will only use the magnitude

Figure 13 : Neatvision blocks for the canny edge detector

Different values of the inputs :

1. The standard deviation:

The value σ of the standard deviation of the Gaussian belt should be put at its minimum value that is **1.0**. The more this value is big, the more the noise is reduced. But in our case, we don't want to reduce the noise to much otherwise we will loose important information concerning the region vasculosa and its circumference.

2. The threshold T1:

The first threshold controls the noise in the image, but it is also correlated with the second threshold T2.
So the idea is to put a fixed value for the first threshold and to find a condition on the second threshold T2.

After several attempts with several values on all the samples, we see that if the value goes under 10, there is too much noise that corrupts the region vasculosa

and if the value goes over 20, a lot of important value is lost concerning the region vasculosa. So the first threshold T1 should be put to **15**.

2. The threshold T2:

For the second threshold, the best value is 60 and 80 for the sample set 72h and the best value is between 40 and 50 for sample set 96h. So a condition on the "how well" the vasculosa area is represented is applied on the input image to determine the threshold T2.

The method used to determine the condition is the Non Maximum suppression. We apply this operator on the coloured input image. So all the edges (the local maximum of the magnitude of the gradient) even the noise are given a grey value and the rest of the image that isn't edge are put to zero. The threshold T2 has a relationship with these grey level values, that we have to determine.

We are interested in the vasculosa area, so we add with an AND operation the mask (4.1.3) with the output the non maximum suppression block (NONMAX). The corrupted noise is that's why removed on the 4 edges of the image.

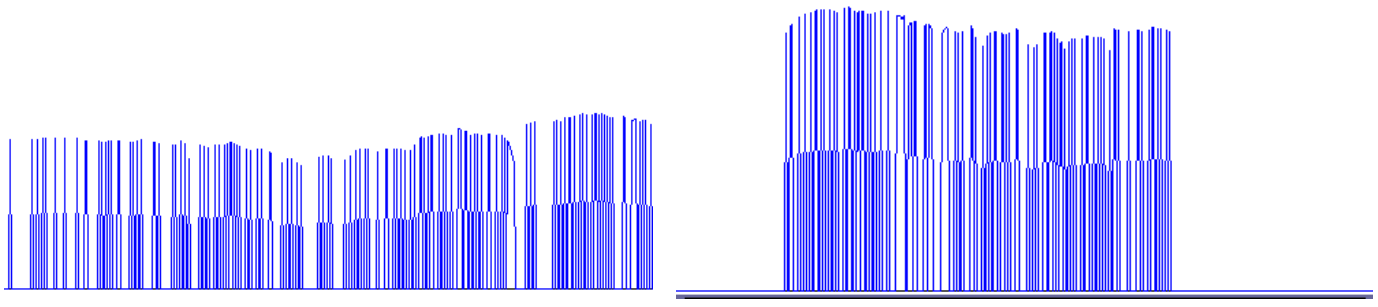


Figure 14 : Vertical histograms at the outputs of the AND operation for sample set 72h & 96 h

We then take the highest peak value (Hi grey block), that is a good approximation of the average grey value in the region vasculosa(see figure

above). After experimentation, we see that of we divide this value by 3.5, we always get a good threshold value for T2 in both of the sample set.

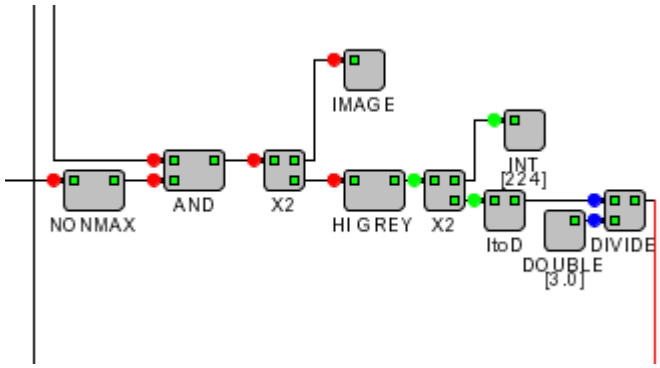


Figure 15 : Neatvision blocks for the condition that determine T2 of the Canny

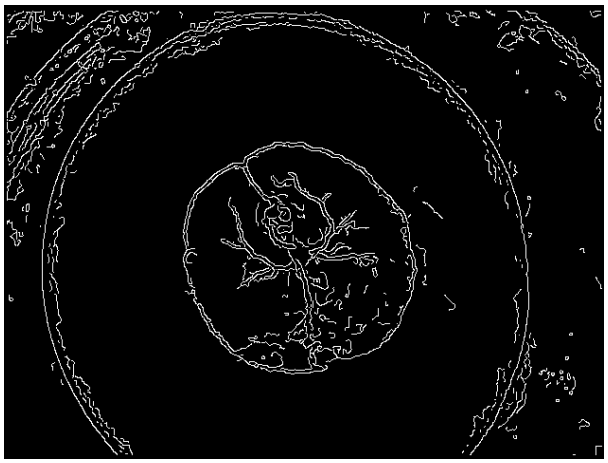


Figure 16 : The threshold output magnitude image of the canny

3.2.5 Isolation of the Vasculosa region

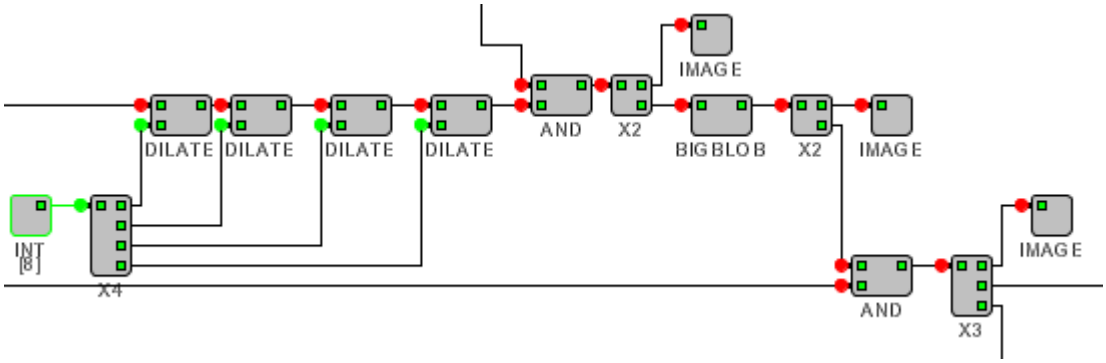


Figure 17 : Neatvision blocks to isolate the region vasculosa

In order to isolate the vasculosa region after the Canny operator, we want to delete all the noise in the border of the image.

We first start by dilated the image four times so that each relevant elements of the vasculosa region are connected each other. These four dilations are important to connect each element of the circumference and make a fully connected circumference.

We then add with an AND operation the result of the four dilation with the mask (section 4.2.3).

Figure 18 : Image output after the AND operator

It is now possible to isolate the dilated region vasculosa by applying the biggest blob operation (BIGBLOB block).

Finally we add with an AND operation this biggest blob with the output of the threshold Canny image. We get the isolated vasculosa region.

Figure 19 : Image of the region vasculosa isolated

3.2.6 The circumference image

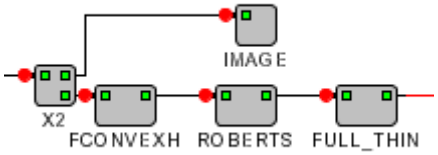


Figure 20 : Neatvision blocks to detect the circumference of the vasculosa area

The vasculosa region is now reconstructed, and there is no more noise outside of the region vasculosa. Therefore it is possible to enclose this area with a convex hull.

We use the block FCONVEXH that fill the convex hull and give a unique blob.

We apply a Roberts edge detector to detect the edge of this blob : this is the circumference of the vasculosa region.

Finally, we apply a full thin function in order to arrange the pixels in line, suppress defect on the circumference and so in order to calculate the right circumference.

3.2.7 The calculation of the circumference and the area

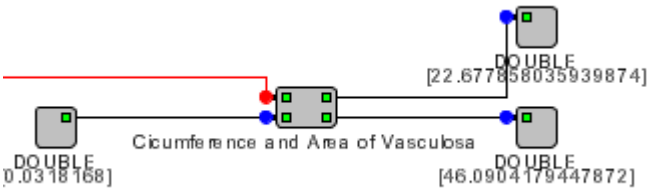


Figure 21 : Utilisation of the implemented block “Circumference and Area Vasculosa”

It is now possible to calculate the circumference of the vasculosa region by using the implemented block (section 4.1) "CircumfAreaMeasurement".
The outputs are the value of the circumference and the value of the area.

3.3 The embryo: circumference and area

3.3.1 2 samples sets, 2 files

The two samples sets have a different calibration grid so it is necessary to differentiate them one from another. Instead of complicating the Neatvision programme with “if” conditions, I prefer differentiating the 2 sample sets by creating one file for each sample set.

3.3.2. Analysing the colour image

After applying a vertical scan in the region of the embryo, we can note that the gap between the red and the green, at the particular point of the embryo, is important.
So the idea is to pass the image is the LAB space and to take the output A (red-green).

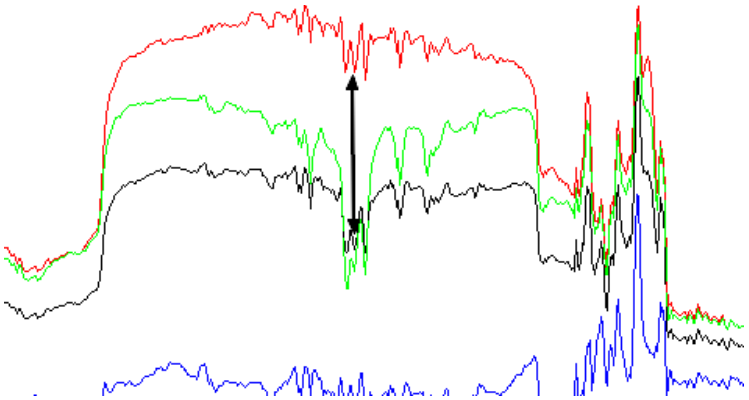


Figure 22 : Vertical scan on red, green, black and blue in the region of the embryo

However this segmentation on the red-green channel is sometimes a too brutal technique on some images on which the difference between red and green is spread along the vessels. It is therefore important to find another way to extract the embryo.

On figure 22, we can note that the difference between green and black is also relevant. If we subtract the black channel to the green channel we can extract the embryo. This technique also has his limit when the difference between green and black is not important enough or when this highlighted on the circumference of the vasculosa area rather than on the embryo.

3.3.3 Subtract green to black

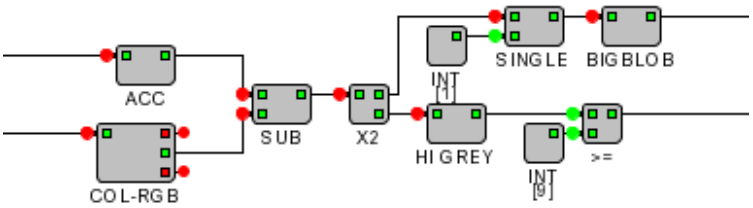


Figure 23 : Neatvision blocks to suppress the green channel to the black channel

So, the input image is first passed through the COL-RGB block that does a segmentation on the 3 channels (red, green and blue). We also pass the image through the ACC block: it transforms the colour image to a Greyscale image. So we have now our two coloured channels: green and grey. We subtract green from grey and do a threshold on this image, and finally select the biggest blob.

After the subtract block, we also apply a HIGREY block, that will calculate the value of the maximum peak. If this value is superior to 9, this method is retained to calculate the measurements on the embryo, otherwise another method with the LAB space is applied. This value of 9 was taken after several attempts on the given samples.

The sections 4.3.3 to 4.3.6 treat the case where the highest peak value is below 9.

3.3.4. The LAB space

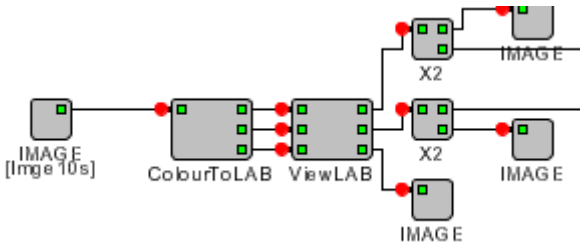


Figure 24 : Neatvision blocks for the segmentation in the LAB space

We pass the input image through a colour to LAB block. The advantage of the LAB block is to segment the image on complementary colour pairs.
The output of this block is passed through a ViewLAB block that normalises the images. We take the output A which does segmentation on a red-green channel.

3.3.5 The threshold

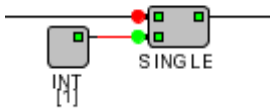


Figure 25 : The threshold to separate the embryo

In order to separate the embryo from the rest of the image, we will do a threshold on the image at 1. The value 1 of the threshold is related to the difference between red and green, and this biggest difference is noticeable at the embryo.

Figure 26 : The threshold image with the separated embryo

3.3.6 Removing the noise in the edge of the image: the mask

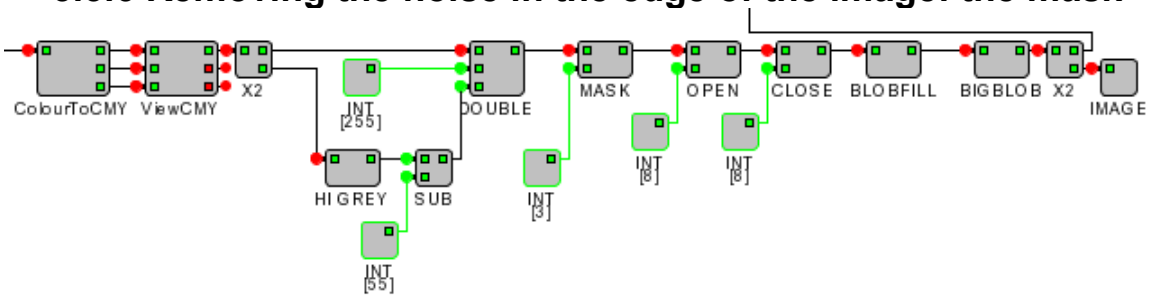


Figure 27 : Neatvsion blocks to create a mask

We now have to remove all the noise in the edge of the image in order to isolate the embryo which is in the centre of the image.

We will reuse the same blocks that we have used in the implementation of the vasculosa area (see part 4.2). So the mask will be the same than in section 3.2.3.

At the end, we add with an AND operation the mask and the threshold output.

Figure 28 : The image passed through the mask

3.3.7 Isolating the embryo

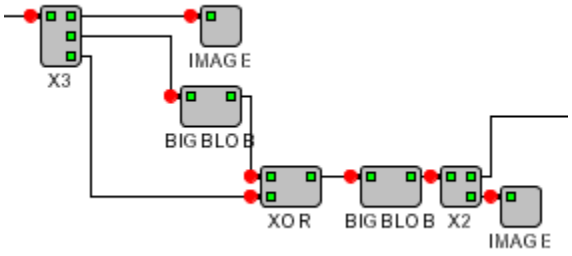


Figure 29 : Neatvision block to isolate the embryo

We can notice now as the noise at the border of the image is removed that the embryo is all of our case the second biggest blob in the image.
In order to isolate this second biggest blob, we use the BIGBLOB block. The first time in order to have the yolk and then with a Boolean XOR operation, we

suppress this blob from the image to isolate the remaining biggest blob with a second BIGBLOB block.

The embryo is now correctly isolated as a white blob.

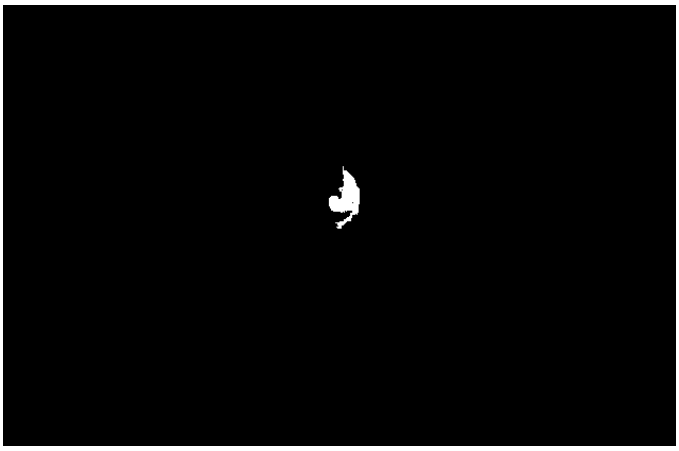


Figure 30 : The image of the isolated embryo

3.3.8 Calculating the circumference and the area

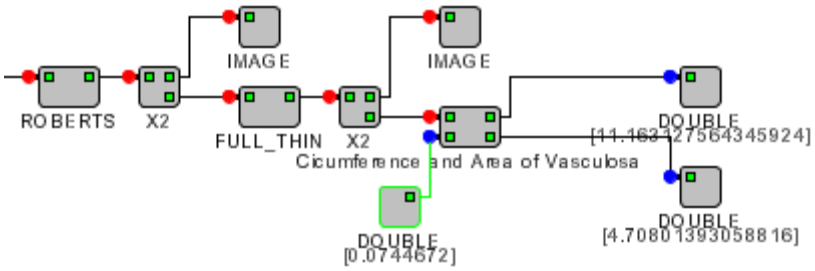


Figure 31 : Neatvision blocks to calculate the circumference and the area

The last step to get the circumference and the area of the embryo is to do the same scheme than in part 3.2.6.

So to apply first a Roberts edge detector to detect the edge of this blob, and then to apply a full thin function in order to arrange the pixel of the circumference.

Finally, we use the implemented block “Circumference and Area Vasculosa” to get the circumference and the area.

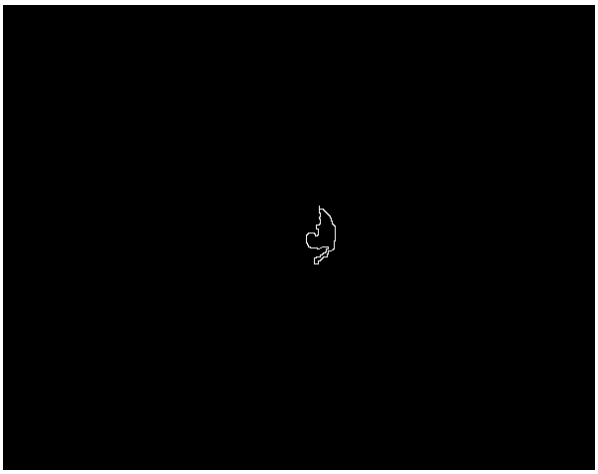


Figure 32 : The image of the circumference of the embryo

3.4 The symmetry of the vasculosa area

In this part of the implementation, we will discuss on the way to find a symmetry in the Vasculosa area. The approach taken admit that it exists an axial symmetry for the region Vasculosa. This symmetry is visible by eyes and we will autommatically the axis. To find these axis of symmetry, we will use the benefit of the powerful technique of the Hough transform through the different steps.

3.4.1 The image of the vasculosa area

We want to have an image with a lot of information about the vasculosa area and without any noise out of this area that could corrupt other relevant information.
The image we take was already the output of a block in the implementation of the vasculosa area (section 3.2). In fact we copy all the blocks used in the first five parts of the implementation of the vasculosa area and find the symmetry on their output image.

Figure 33 : Image of the region vasculosa isolated

3.4.2 The Hough space

At this stage, we take all the couples of points of the calculated edges and we determine their mediator (see section 2.4.1.2)

By eyes, it is easy to locate two significant local maxima which we can check that they correspond to two axis of symmetry in the image. On the other hand, to find them in an automatic way is less obvious (the red parts of the shape of sinusoid corresponds to the existence of points common to the many mediators ones, i.e. that mean also a centre of symmetry but we will not be interested of this here).

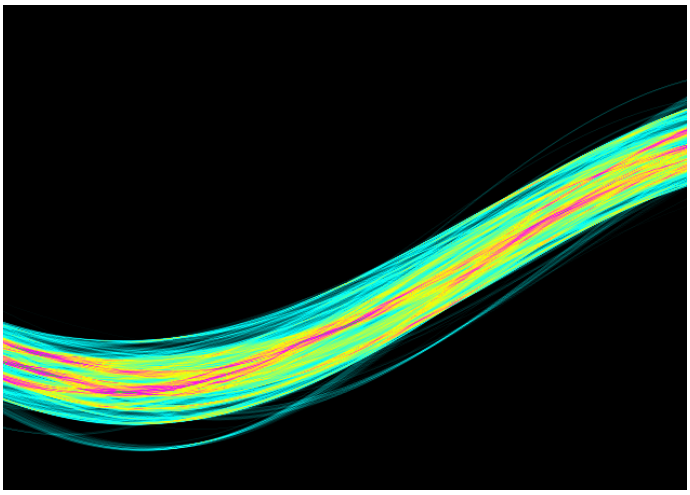


Figure 34 : The Hough space of the canny output image

3.4.3 Determination of local maxima

In practice, the accumulator obtained (such as the figure of the Hough space) presents a great number of local maxima. We want to keep only those which are statistically significant and which thus belong to regions where pass a lot of mediators.

These areas thus should be delimited, i.e. to make a classification of the transform of Hough and to extract the "significant" points.
We can use the k-means clustering but it would take ages to have results as we have to define a lot of clusters.

Another common algorithm is the isodata. It makes possible to adjust in a dynamic and automatic manner the numbers of relevant classes (of areas) whose centre is the average point. We established it just as it is, but we authorize a class of rejection at the end of the iterations, and at the end we take the maximum of each class rather than the average point. This method allow to obtain robust local maxima. ie. there are never two maximum too close corresponding to the same axis.
We do not authorize any more the regrouping of class and we use one parameter of control: DistMax. So we sort the maximum ones found and remember just those with the highest value. Moreover we reduce possibly the number of points in the space of Hough by carrying out a threshold operation on a certain proportion of these points.
The finished objects cannot have two axis of symmetry that are parallel so the angular distance θ should be enough to separate the maximum ones.

The algorithm is then the following:

- initialization: the global maximum constitutes the first class.
- repeat:

- ClasseCreated = 0
- For all the points of accumulator not null and not marked
  - Calculate the distance to all the existing classes
  - If the smallest distance is lower than DistMax
    - Place the point in the class which carries it out
    - Mark the point
  - If not
    - ClasseCreated = 1
    - The point is put in the rejection class
    - Increment the number of maximum found
- Update the center of the classes (including the class of rejection, its center is becoming the center of a true class) as long as a class is created and that the number of maximum awaited is not reached
- Return the parameters of all the local maxima calculated

This algorithm has the double advantage to locate the local maxima while reducing progressively explored space with the iterations. There are thus fast calculations giving in theory reliable axes. There are a lot of classes created in this method, but to simplying we will only create 2 class.

3.4.4 The java block to find the symmetry

In order to find the axis of symmetry, we implement a java block « FindSymmetry ». To explain the content of this block, we also design the program under the graphical interface of Neatvision.

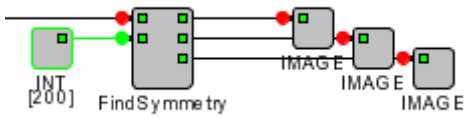


Figure 35 : The implemented Neatvision block « FindSymmetry »

3.4.4.1 Determining the first axis of symmetry

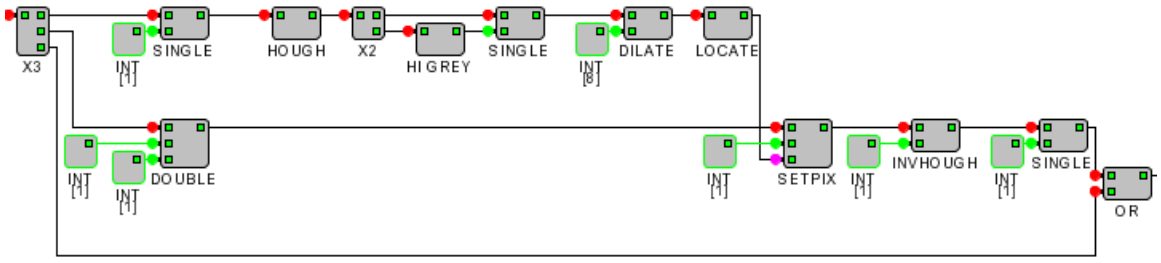


Figure 36 : Neatvision block to determine the first axis of symmetry

The input image comes from the Canny operator. It is first pass through a single threshold at 1 to make sure that the pixel value in the image is either 1 or 0.
Then we transform the image in the Hough space, and determine the maximum value of the highest peak. The coordinate of this value are the coordinate in the Hough space of the global maximum.

To determine these coordinnates, we dilate the image and pass through the « locate » block, that determine the coordinate of the first and unique white pixel in the image.

To draw the axis of symmetry, we have first to draw this pixel on a black image of the same dimensions. So, we pass the output of the canny through a dual threshold with the value of the threshold at 1. This gives us a black image on which we can draw a white pixel at the right location with the block «SetPix ».
We finally pass the image in the invHough block with a pixel value of 1, so we go back from the Hough space to the real space. We threshold the image at 1, to see the line that represents the axial symmetry and add this line with an OR operator with the image which comes from the Canny operator to get the image below.

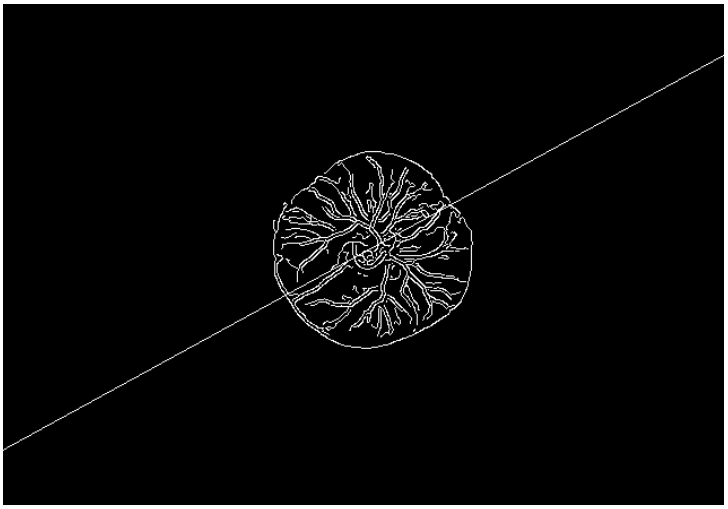


Figure 37 : Image of the first axis of symmetry

3.4.4.2 Determining the second axis of symmetry

We use the same method to determine the second axis of symmetry. But here instead of doing a single threshold on the highest grey value, we want to find the second local maximun in the image. This second maximum has to be separated from the global maximum by a value of DistMax.

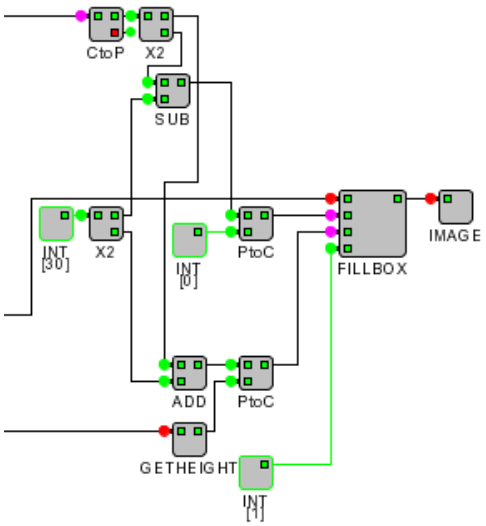


Figure 38 : Neatvision blocks to draw the first class

We first starting by defining the class of the global maximum. This class is represented by a white box around this maximum value. To draw the box, we use

the FillBox block which we have to give two coordinates in parameters. The first coordinate is the upper left corner of the box and the second coordinate is the lower right corner. The upper coordiate is (X coordinate of the global maximum – distMax, 0) and the lower coordinate is (X coordinate of the global maximum + distMax, Height of the image).

Figure 39 : Image of the filled box

To find the second local maximum, we apply a dual threshold on the output of the Hough block. The values of threshold of the dual threshold are the same, and with a loop we decrement 1 by 1 from the highest grey value till we find a pixel outside our class.

We detect that a pixel is outside of the class by adding with an AND operation the inverse of the image (« Not » block) of the filled box inversing and the dual threshold output. If there is more than one pixel (« count White pixel block superior than one), we have found the second local maximum.

Finally, like in the detection of the first axis, we locate the coordinate of this maximum, set the white pixel on a black image, and pass the image through the inverse Hough block to get the second axis.

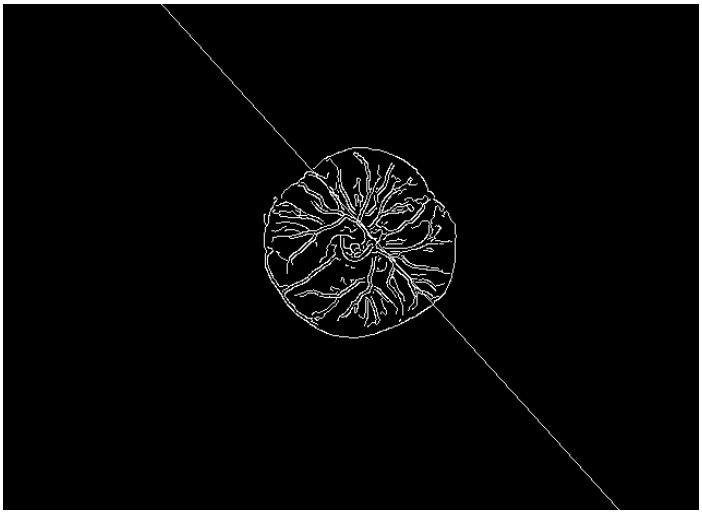


Figure 40 : Image of the second axis of symmetry

CHAPTER 4- Results and Discussion

4.1 The region vasculosa

4.1.1 Results of the values of the areas and the circumferences of the region vasculosa

Images	Area (mm^2)	Circumference(mm)
01	59.48	27.62
02	46.06	22.76
03	42.46	24.21
04	52.19	25.73
05	46.2	22.31
06	71.74	29.33
07	69.81	32.93
08	71.67	31.67
09	55.58	26.01
10	Water bubble	
11	49.25	24.38
12	55.06	27.17
13	35.21	20.69
14	63.93	29.06
15	61.05	28.7
16	56.03	29.69
18	56.24	28.88
19	52.83	27.89

Table 1 : Sample set 1 : 72 hours stage (**)

Images	Area (mm^2)	Circumference(mm)
01	176.45	49.7

02	136.6	45.28
03	115.5	36.01
04	136.31	42.96
05	*	*
06	*	*
07	*	*
08	*	*
09	*	*
10	162.61	44.86
11	135.21	46.96
12	137.13	36.22
13	97.57	28.43
14	167.89	54.34
15	*	*
16	145.24	40.21
17	129.92	36.85
19	157.33	37.49

Table 2 : Sample set 2: 96 hours stage (**)

Images	**Growth of the Area (%)**	**Growth of the Circumference(%)**
01	196.65	79.94
02	196.57	98.94
03	172.02	48.74
04	161.18	66.96
10	#	#
11	174.53	92.61
12	149.05	33.31
13	177.11	37.41
14	162.61	86.99

16	159.21	35.43
17	131.01	27.59
19	197.8	34.42

Table 3 : Percentage of growth between 72 hours and 96 hours stages

* Between the different measurement time intervals some of the embryo samples died. Of the 26 samples that were in the initial sample set 18 survived to the 72 hour stage and 12 to the 96 hour stage.
** Note that these pixel measurements refer to images with a resolution of 640 x 480

We can note that the percentage of growth of the region vasculosa for the samples that survived are closed. That show the robustness of the results.

4.1.2 Verification of the vasculosa region

To see how significant the added area is relative to the total circumference of the vasculosa, I manually outlined the circumference of six vasculosa samples of the 72 h stage on a software of image analysis (here Able Image Analyser). These results are shown in table below :

Sample No.	**Algorithm Segmentation of Circumference (mm)**	**Manual Segmentation of Circumference (mm)**	**Percentage Difference**
01	27.62	28.5	3
02	22.76	23.75	4
03	24.21	23.75	-2
04	25.73	24.75	-4
05	22.31	22.5	1
06	29.33	30.5	4

Table 4 : Comparison of manual and algorithm based segmentation of the circumference for the 72 hours stage

Sample No.	Algorithm Segmentation of Area (mm2)	Manual Segmentation of Area (mm2)	Percentage Difference
01	59.48	60	2
02	46.06	47	3
03	42.46	41	-2
04	52.19	50	-4
05	46.2	47	3
06	71.74	74	3

Table 5 : Comparison of manual and algorithm based segmentation of the area for the 72 hours stage

From tables above we can see that the algorithm gives a circumference and an area measurements that is generally within ±4% of the results that are found with we use manual methods to segment and measure the circumference and area of vasculosa.

When we look at the measurement performance of the machine vision algorithm, we can say that the algorith is a good improvement over the manual measurement techniques that are currently used and that a ±4% is an acceptable level of error for this measurement. The advantages of the algorithm are too run faster and to avoid doing huge mistakes in a manual measurement.

4.1.3 Limits of the Neatvision programme

The results given by the Neatvision program are satisfying. However, the main problem here comes from the fact that the images from the sample of 72 h and the sample of 96 h were not taken in the same condition of illumination. On the sample of 72 h, some images have their region vasculosa with not enough

contrast, compared to the sample of 96 h, where the region vasculosa is really well defined on all the images. So the main way to improve the robustness of the results is to improve the quality of the photo taken (especially here for the photos of the sample 72h).

Despite this lack of quality, the results given are satisfying because as we can not put real values of threshold in the program, we find other ways to get the right values of threshold for all the images of both of the samples. For example, the second threshold of the Canny was taken by using other techniques here the Non-maximum suppression that gives information about noise in the image. The values that are taken give good results but are certainly not the optimal ones.

4.2 The embryo

4.2.1 Results of the values of the areas and the circumferences of the embryo

Images	Area (mm^2)	Circumference(mm)
01	0.94	6.47
02	0.9	4.41
03	0.87	4.36
04	0.43	3.91
05	0.66	4.67
06	0.24	1.88
07	1.7	7.15
08	0.56	4.35
09	0.18	1.43
10	0.44	2.79
11	0.45	4.31
12	0.5	4.89
13	0.37	2.45

14	0.29	3.68
15	0.43	3.37
16	0.45	3.52
18	0.45	4.40
19	0.33	3.94

Table 6 : Results of the values of the areas and the circumferences of the embryo for the sample 72 hours stage (**)

Images	**Area (mm^2)**	**Circumference(mm)**
01	5.99	13.58
02	5.66	14.94
03	6.95	17.48
04	6.38	14.32
05	*	*
06	*	*
07	*	*
08	*	*
09	*	*
10	3.42	11.16
11	6.17	17.37
12	5.15	17.79
13	3.43	8.95
14	9.1	18.95
15	*	*
16	5.71	19.48
17	4.94	15.58
19	3.98	10.95

Table 7 : Results of the values of the areas and the circumferences of the embryo for the sample 96 hours stage (**)

Images	Growth of the Area (%)	Growth of the Circumference (%)
01	537.2	109.8
02	528.8	238.7
03	698.8	300.9
04	1383.7	266.2
10	677.2	300
11	1271.1	303.0
12	930	263.8
13	827.1	265.3
14	3037.9	414.9
16	1168.8	453.4
19	1106	177.9

Table 8 : Percentage of growth between 72 hours and 96 hours stages

4.2.2 Verification of the embryo

To see how significant the added area is relative to the total circumference of the embryo, I manually outlined the circumference and area of 3 embryos samples of the 96 h stage on a software of image analysis (here Able Image Analyser). These results are shown in table below :

Sample No.	Algorithm Segmentation of Circumference (mm)	Manual Segmentation of Circumference (mm)	Percentage Difference
01	13.58	14.6	8
02	14.94	14	-6
03	17.48	15.7	-10

Table 9 : Comparison of manual and algorithm based segmentation of the circumference for the 96 hours stage

Sample No.	Algorithm Segmentation of area (mm2)	Manual Segmentation of area(mm2)	Percentage Difference
01	5.99	6.4	7
02	5.66	6.1	9
03	6.95	6.7	-3

Table 10 : Comparison of manual and algorithm based segmentation of the area for the 96 hours stage

From tables above we can see that the algorithm gives an circumference and an area measurement that is generally within ±10% of the results that are found with we use manual methods to segment and measure the circumference and the area of the embryo.

When we look at the measurement performance of the machine vision algorithm, we can say that the algorith is a good improvement over the manual measurement techniques that are currently used and that a ±10% is an acceptable level of error for this measurement. The advantages of the algorithm are too run faster and to avoid doing huge mistakes in a manual measurement.

4.2.3 Limits of the Neatvision programme

Here again, like on the vasculosa area, we see that the images have different conditions of illumination that corrupt the implementation of the Neatvision program.

We define here two ways to extract the embryo. In each case, one method is not better than another but we approximately fix a limit where we can see that the first technique with LAB space is better than the technique with the difference

between green and black. This limit was taken after several attempts but is not here really proven.

4.3 The symmetry

4.3.1 Quality of the 2 axis of symmetry

The first and second axis can either be the main symmetry (most visible one) (**MS**), a secondary symmetry (**SS**) or it is not an apparent symmetry (**NAS**).
The quality of the symmetry is related to the number of pixels that represent the edges. So we count the number of white pixel at the output of the Canny operator.
It is also related to the distMax value that is chosen, it has to be defined by hand as it is a parameter of control.

Images	distMax	Number of pixels of edges	1st axis	2nd axis
01	50	6979	**SS**	**MS**
02	50	4733	**MS**	**SS**
03	10 to 350	3323	**MS**	**MS**
04	50	4414	**MS**	NAS
05	50	3334	**SS**	NAS
06	50	4521	NAS	NAS
07	50	4772	**SS**	**MS**
08	50	4748	**MS**	NAS
09	50	6842	NAS	**MS**
10	50	5345	**MS**	NAS
11	50	5092	**MS**	**SS**
12	150	4298	**MS**	**SS**
13	150	3462	NAS	**MS**
14	50	6142	**SS**	**MS**

15	50	6429	**MS**	**SS**
16	150	5062	**MS**	**SS**
18	150	6559	**MS**	**SS**
19	50 to 350	4610	**MS**	NAS

Table 11 : Results of the symmetry applied on the sample of 72 h

Images	**distMax**	**Number of pixels of edges**	**1st axis**	**2nd axis**
01	150	5539	**SS**	**MS**
02	50	4345	**MS**	**SS**
03	50	3635	**MS**	**SS**
04	50 to 350	4625	**SS**	NAS
10	50 to 350	4943	**SS**	NAS
11	50	4459	**MS**	**SS**
12	50 to 350	3534	**SS**	NAS
13	50	2778	NAS	**MS**
14	150	5708	**SS**	**MS**
16	50 to 350	4352	**SS**	NAS
19	50	4422	**MS**	**SS**

Table 12 : Results of the symmetry applied on the sample of 96 h

We can note that this technique for detecting 2 axis of symmetry is robust. If we were only focusing on the global maximum some of the main symmetry would not have been detected.

It is the case for the images 09 and 13 of the 72 h stage, where the first axis is not a relevant symmetry and the second axis is the main symmetry. For these two images, we note that the number of white edges pixels is either too big (too much noise) or too small (not enough information).

Hopefully, the main situation is where the main symmetry is the first axis and the secondary symmetry is the second axis. In these cases, we have to find the best distMax that give these axis.

4.3.2 Limits of the Neatvision programme

- The method is effective and detects well the axis of symmetry under good working conditions.

- It is robust to the noise because "aberrant" points of edge distribute random values in the space of Hough and disturb just a bit the segmentation by class.

- The detection of edges is the critical point of our method: if this one is not good then there is very little chance that the detected axes are good. Thus, a very noisy image giving many aberrant edges will corrupt the calculation. On the other hand, the fact that the form of the edge image is an enclosed shape or not gives very few problems if the errors on the edges have a small size.

- Lastly, it would be pleasant that the algorithm finds itself the number of relevant axes in the image. We can do this by calculating the accumulation on a neighbourhood of a local maximum in order to make a decision on the most relevant axis.

CHAPTER 5- The Planning

5.1 Main areas

As seen previously, there are mainly 2 topics that have leaded my work. The first has consisted of finding a suitable solution with Neatvision for the vasculosa area, the embryo and the symmetry. Subsequently if my first implementation has been successful, the idea was to try to transpose this solution in a suitable way so that it will be useful in laboratories.

5.2 Time Schedule : General diagram of GANTT

N°	Tasks
1	Implementation of the vasculosa area.
2	Preparation of the Colloquium in Nimes (Ecole des Mines d'Ales) (5 July)
3	Implementation of the embryo
4	Implementation of the symmetry
5	Elaboration of a suitable solution for the industry
6	Preparation of the final report and the colloquium
7	Preparation of the final colloquium

Table 13: Description of the tasks

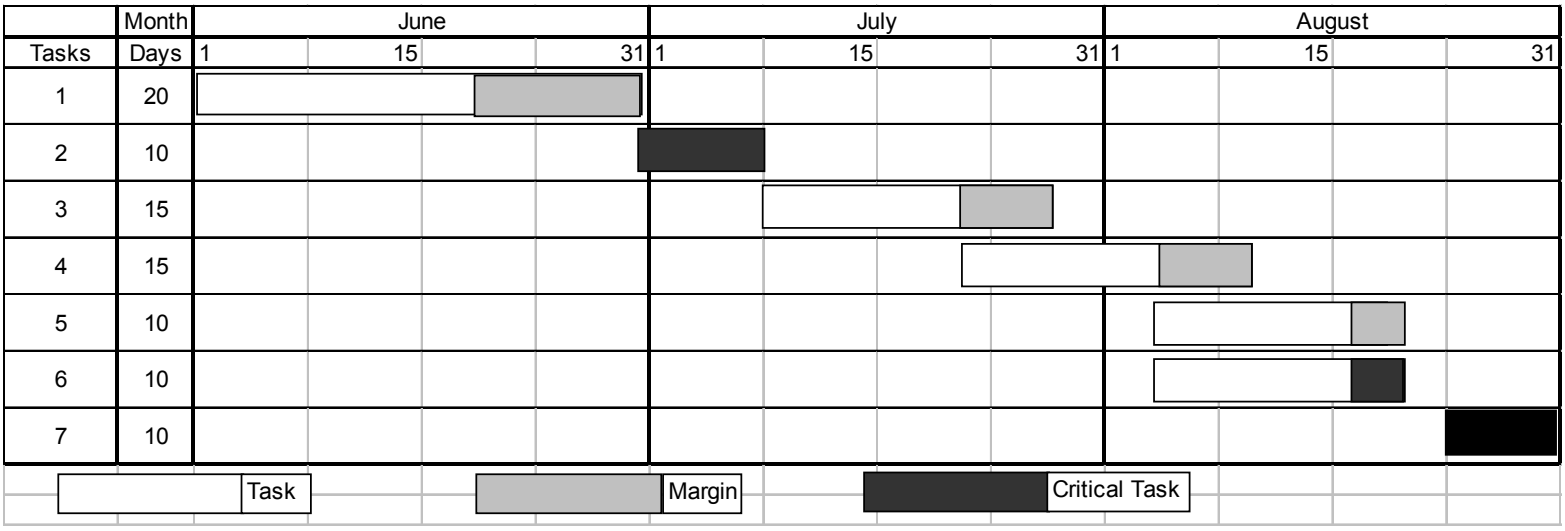


Figure 41 : General diagram of GANTT

5.3 Comments on the schedule

The schedule has been designed to be achievable as much as possible. It was respected at the exception of one task. The task 5 has not been implemented due to lack of time. But as it will be discussed in the last chapter, this tasks wasn't a critical task, it was just an improvement of the project.

Actually, the extraction of values (area & circumference of the vasculosa and the embryo) took even longer time than what is specified in steps 1) 3). But these first steps were the most important ones and have to be achieved successfully.

As it can be seen, the schedule has a lot of some margin for each tasks, this is done in order to catch up with unexpected delays that might occur as the project goes on.

In the same extend, writing the final report has actually taken me more than a week from the time I had started it, even if I was writing it as I come along the different parts.

CHAPTER 6 - Conclusions and Further Research

After the research carried on, I have acquired a good knowledge of work already done by the previous project and the importance of this project in the research. Consequently, the most challenging part of the project has therefore been to find a robust solution for all the images (in order to keep the implementation not too complex).

An implementation to find the area of the region vasculosa has already been performed in the previous project. The challenge was to analyse this previous work and to find another solution which is computationally less expensive and give more accurate results. So, a robust solution was implemented to find the area and the circumference of the region vasculosa and the embryo. 2 axis of symmetry in the region vasculosa were also found. The knowledge of these axis could, for example, to be used to find the orientation of the vasculosa area in an image and to contribute the retiming of the vasculosa area between various images.

The user interface of Neatvision has also been widely used across the project to implement new blocks that speed and facilitate the measurements on the images.

After this successful implementation, the idea is to create a graphical interface that would be more suitable in the laboratories. This interface will have to support higher resolution images than the ones we used (jpeg / 640*480). This shows promise for further development.

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Appendix

- **Implemented Java blocks**

Method	Description	Inputs	Outputs
VasculosaRegion	Extract the edge of the vasculosa region	0 : Image (in colour with the region vasculosa)	0 : GrayImage: (Edge of the vasculosa region) 1 : GrayImage (output of the Canny operator without noise)
CircumfAreaMeasurement	Calculate the circumference and the area of a region	0 :GrayImage(Enclosed form that is the edges of a region) 1: Double (calibration value that represent the measure of 1 pixel)	0 : Double (circumference of the form) 1: Double (area of the form)
FindSymmetry	Find 2 axial symmetry in an Canny output image	0 : GrayImage (comes from the Canny operator) 1 : Integer (DistMax value en degree between the 2 axis)	0: GrayImage (the first axis of symmetry) 1: GrayImage (the second axis of symmetry) 2: GrayImage (the 2 axis of symmetry)
testing	Perform the same operations on the 34 images of to test the robustness of the technique on all the images	0 to 34 : Image (all the images we have)	0 to 34: GrayImage (all the respective outputs)