Biological Qualification of Oocyte Maturity with the Use of the Karhunen-Loeve Transform: Computer-aided Decision for Selecting Best Oocytes Before Fertilization

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Abstract. The estimation of the *a priori* maturity degree of oocytes before fertilizing is a critical step in *In Vitro Fertilization* (IVF). This kind of analysis is currently done by the practitioner with a visual microscope inspection and is therefore subjective. In this paper, we propose the use of an image processing called Karhunen-Loeve Transform (KLT). The KLT exploits the covariance matrix and its eigenvectors for obtaining a representation of information in several images. We show that the KLT permits the determination of the oocyte maturity by examining the KL images and more precisely the eigenvalues of each. The KLT could thus be a useful tool for IVF and we intend to develop a dedicated server for all French IVF centers as a computer aided decision for fertilization.

1 Introduction

In general, the estimation of the development degree (from growing to apoptosis) of cells is very important in biology and medicine. The question is even more crucial in IVF. Indeed, the involved hormonal treatment for women is very heavy and the intervention is expensive. Practitioners have therefore to be sure that the oocyte(s) to be implemented is (are) the best one(s). Although, in some cases, the maturity is obvious and can be determined without any doubt, the decision of the practitioner is often uncertain. This gives all the interest of our research.

More precisely, there are several oocyte maturity degrees like germinative vesicle, metaphase implemented or not … Here, we will focus on three types: metaphase I, metaphase II implemented and not implemented. The aim is to discriminate automatically the development step of the gametes.

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The most used method for determining the oocyte maturity deals with a simple microscope visualization of the oocyte. This is obviously subjective and can lead to misinterpretation of the exact maturity degree. Other means have been elaborated for achieving objective determination by using digital image processing [1], [2], unconventional illumination [3] or microsystems [4]. The first one has not been completely efficient, the second remains subjective and the third is rather difficult to insert during usual act of fertilization.

We propose here a new kind of image processing, *i.e.* the use of a statistical transform (KLT), which enables an automatic determination of the oocyte maturity. After a presentation of the KLT, we will present first results obtained with pictures of oocytes which demonstrate the ability of the KLT, after pre-processing of the pictures, to separate *a priori* the various phases of oocyte development. We will then show the results related to the objective qualification with oocytes which were transferred into the uterus but which did not implant (they did not remain in the uterus). We will prove that this method permits to establish a reason for the non implantation. Finally, a blind test of the software will show the validity of the method before concluding.

2 The Karhunen-Loeve Transform and Associated Study Protocol

2.1 The KLT

The KLT is a statistical representation technique based on the changing of space. It allows the description of a phenomenon, measured in a pattern (observation) space whose elements are correlated, by means of uncorrelated parameters in another space in which the principal axes are ordered in terms of importance [5], [6] An important property of KLT is that, unlike the Fourier transform or the factorial analysis, the basic vectors are not known *a priori* but are "tailor made" for the given set of vectors. The initial step is to take into account a set of N pictures to be analyzed. The KLT needs the calculation of the covariance matrix (CM) of the picture set after resorting of the picture pixels. That is to say that you need to reshape the pixel square matrix in vectors. Next step is to compute the eigenvalues of the CM and sort them in decreasing order. To each eigenvalue is associated an eigenvector which is called KL image. The eigenvalue represents the percentage of information related to the corresponding KL image.

To illustrate the KLT, we can say that the KL image 1 (eigenvector corresponding to the higher eigenvalue) is the mean picture. The differences between pictures are more present in the KL images related to small eigenvalues which are to be quantified [7].

2.2 Associated Protocol

The chosen protocol first relies on the types of oocytes we decided to investigate. There were cells at the step of metaphase I, non implemented metaphase II and implemented metaphase II. The basic idea is to form a “basis” with one type of oocytes,

in our case 12 pictures of metaphase I oocyte whose maturity degree is obvious and does not suffer from any doubt. Then we compute the eigenvectors of the covariance matrix. The basic idea is to remove one picture of metaphase I oocyte and to replace it with another one. Next step is to analyze the variations of the eigenvalues associated to each replacement.

We decided to do this in two steps: first we replace one MI by another MI (different from the 11 remaining MI in the initial set). It has been done 12 times. Next we compute the mean and rms of the variation of the eigenvalues. This step is iterated with 12 pictures of implanted MII and the same study is applied. To find KL images which permit to separate the various maturities, we will have to find those which are the most different for the two cases described above.

This study will then be applied to not implanted MII and then with a blind test.

3 Experimental Results

In this section, we use for computing the results a 12 picture set. But for an easy understanding of figures related to pictures and KL images, a 6 pictures set will be used. It does not change the generality of the method.

3.1 Pre-processing

First we decided to apply the KLT to oocyte pictures directly obtained from a microscope analysis. It leads to the figure 1 presenting six pictures of metaphase I. In the following, the pictures are 512x512 pixels, obtained with a magnification x40. The classical size of an oocyte is 150 µm.

The direct application of the KLT to these data is given in the figure 2. It represents the six KL images, be the eigenvectors the CM. You have to keep in mind that they are not pictures but representations of the information contained in the picture set. The size is the same as those of the above pictures, be 512x512 pixels.

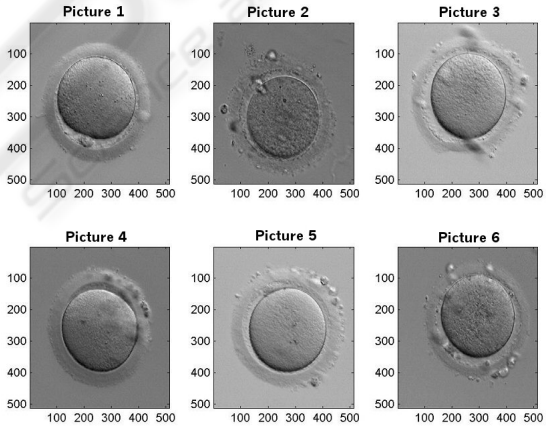

Fig. 1. Set of 6 pictures of metaphase I oocytes (512x512 pixels).

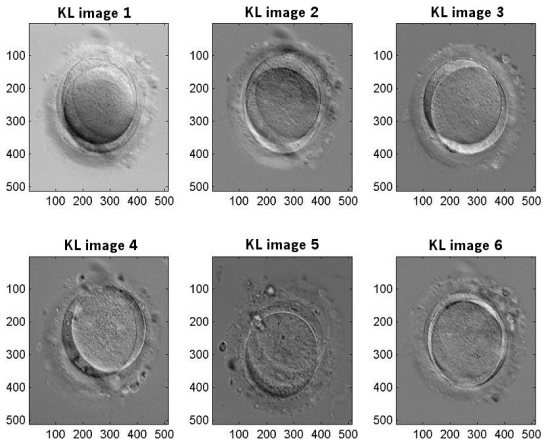

Fig. 2. KL images of the six pictures of figure 1 (512x512 pixels).

The KL images are in fact not exploitable. Indeed, they mainly exhibit the positions of the oocytes of figure 2. Putting in evidence the difference between a MI and a MII would be difficult to achieve. That is why we elaborated pre-processing for obtaining correct KL images. To do so, we first detect the center of the oocytes in the pictures by an autocorrelation of a test picture which consists of the whole cytoplasm of an oocyte extracted from the background and the zona pellucida; this test picture is done one time for all with the PC mouse. Next step is to detect the major axis of the cell (because an oocyte is slightly ovoid). A simple ray tracing from the detected center to the cytoplasm edge is used. The oocyte is then rotated to get all the time the same orientation. Finally, by using the conventional size of a cell, we isolate the useful picture. This is summarized in the figure 3.

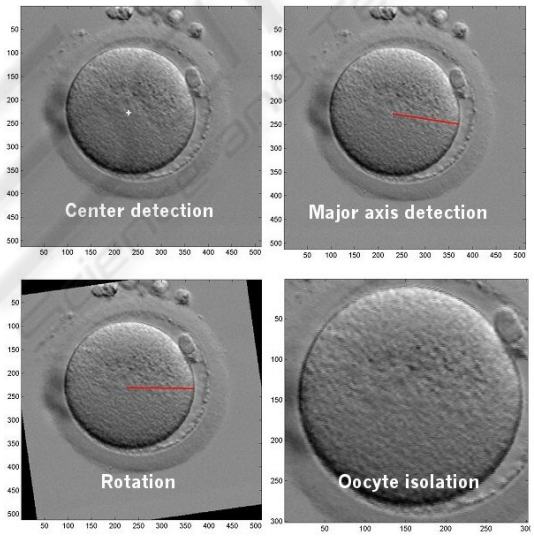

Fig. 3. Description of the various steps for obtaining suited pictures of oocyte (initial picture: 512x512 pixels, final picture: 300x300 pixels).

We have now standardized pictures which authorizes a better application of the KLT.

3.2 Results for MI and Implanted MII

Let us remind the protocol. We first initiate the study by using a set of 12 MI pictures. Then one MI picture is removed and we finally analyze the variation of each eigenvalue due to each change (in fact, we only studied the KL images from 5 to 12 because we know *a priori* that the difference, if it occurs, belongs to these KL images). We did the same with 12 implanted.

The comparison of the variation of the eigenvalues *vs.* KL images is shown in figure 4.

The issue is to select the KL images where the mean difference of eigenvalue variations between the two groups is the greater for respective rms as low as possible. It seems that the optimum is for KL images 8 and 10 as indicated by arrows in figure 4.

To examine if the KLT is suited to discriminate the types of oocytes, we plot the eigenvalue attached to each replacement of oocyte for both types in the figure 5. We can easily see that the two kinds of oocytes are separated. The signs o depicts the barycenters of the two sets and the two circles the limit of 1.4σ, σ being the dispersion. It involves a detection probability for a blind test of at least 75 %. The t-student test confirmed that the two classes were separated with an error probability less than 0.001.

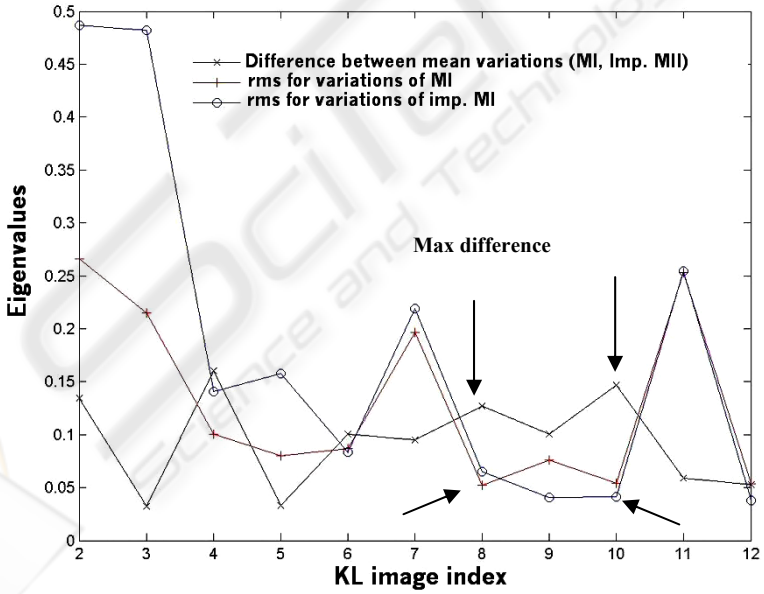

Fig. 4. Plots illustrating the variation of the eigenvalues *vs.* KL images (x: difference between the mean variations for each type of oocyte, +: rms of the variations for MI, o: rms of the variations for implanted MII).

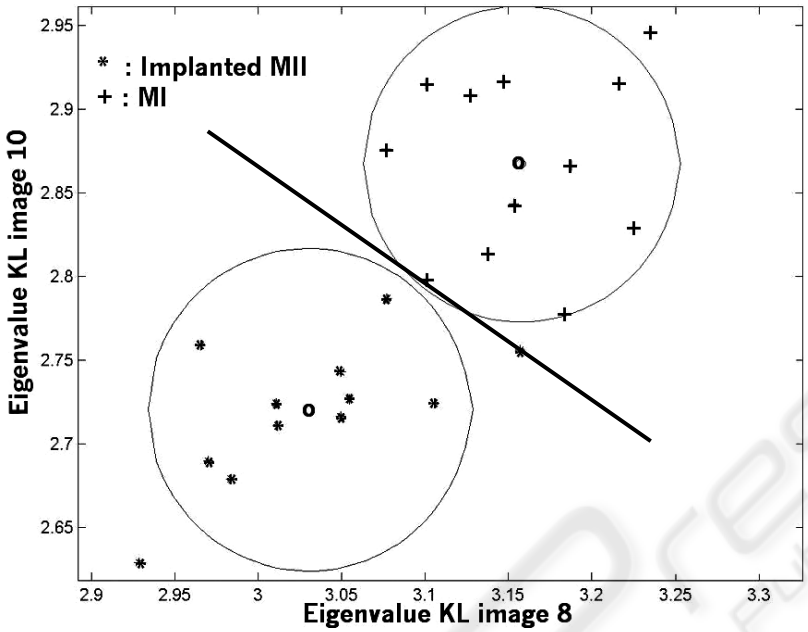

Fig. 5. Eigenvalue 10 *vs.* eigenvalue 8 for replacements with MI and implanted MII oocytes.

3.3 Results Blind Test and not Implanted MII

We used a blind test in order to evaluate the robustness of the method. The practitioner gave us seven oocyte pictures which are clearly in a clearly detectable maturity degree without telling it to us. We applied the KLT in the same conditions as previously mentioned. It led to the figure 6. By comparing the data given by the practitioner, it appeared that the classification issued from the KLT succeeded in all unknown oocyte.

Finally we analyzed the behaviour of not implanted MII (fertilized oocyte which did not lead to pregnancy). As previously, we use the KL images 8 and 10. It leads to the figure 7.

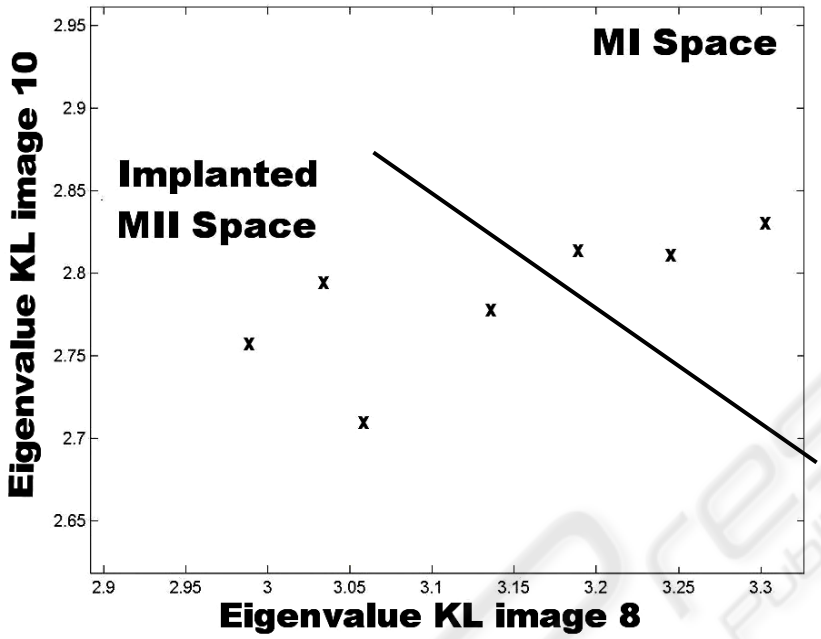

Fig. 6. Eigenvalue KL image 10 *vs.* Eigenvalue KL image 8 as a blind test for 7 oocytes.

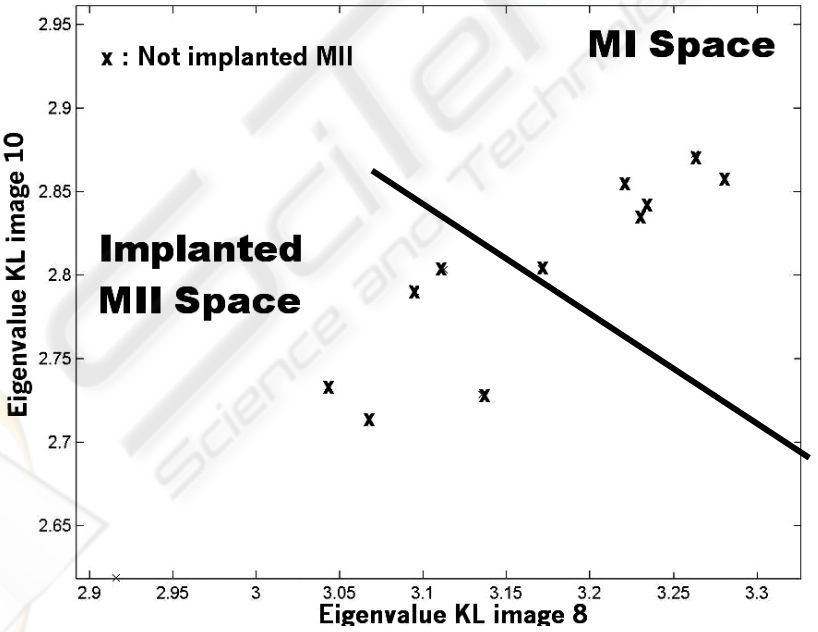

Fig. 7. Eigenvalue KL image 10 *vs.* Eigenvalue KL image 8 for not implanted oocyte.

4 Conclusions and Perspectives

The recognition of oocyte maturity degree is one of the most important steps in IVF. Choosing the best oocyte, currently done by a visual inspection of the oocyte by the practitioner and therefore very subjective, needs nowadays objective methods. We proposed here a new image processing based on the KLT for giving a computer aid to the doctor. The KLT is based on a statistical approach of a picture set and classifies information in terms of presence in one or more pictures. By creating a picture set of one type of oocytes and by replacing one picture by one to be analyzed, we showed that it was possible to discriminate the maturity degrees. We validated this concept first with oocytes which were clearly in one maturity type (MI and implanted MII), then with a blind test and finally we apply the KLT to oocytes which did not implant. The result that this was due to two reasons: non maturity of some oocytes on one hand and other problems during the process after transferring the oocyte into uterus on the other hand.

As perspectives mainly three stages are foreseen:

1. to use an initial picture set of more images,
2. to proceed with a clinical validation with a substantial number of oocytes,
3. to elaborate a server linked to other French IVF centers for offering to other practitioners a useful tool for deciding of the cell maturity in an objective manner.

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