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System of acquisition and processing of images of dynamic speckle

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Abstract. In this paper we show the design and implementation of a system to capture and analysis of dynamic speckle. The device consists of a USB camera, an isolated system lights for imaging, a laser pointer 633nm 10mw as coherent light source, a diffuser and a laptop for processing video. The equipment enables the acquisition and storage of video, also calculated of different descriptors of statistical analysis (vector global accumulation of activity, activity matrix accumulation, cross-correlation vector, autocorrelation coefficient, matrix Fujji etc.). The equipment is designed so that it can be taken directly to the site where the sample for biological study and is currently being used in research projects within the group.

1. Introduction

At present, the acquisition and processing video in real time is a field of research open. The biological tissue is one of the most complex of those present in nature. This situation is aggravated by the inherent variability present in one sample to another, which makes it more difficult the comparison of results between different samples even for the same stimulus.

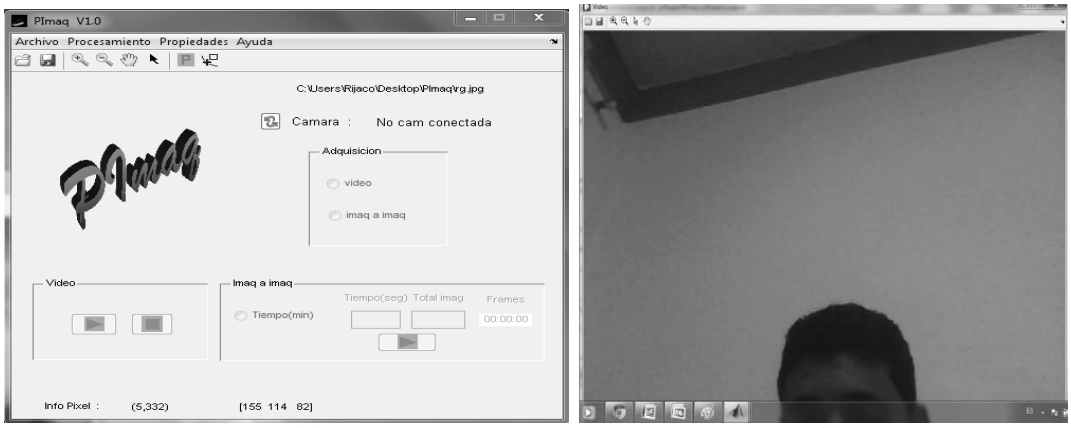
In biological materials, dynamic speckle phenomenon is also known as biospeckle and allows analysis of biological activity or image rendering in various applications such as seeds, Mushrooms, fruits, blood flow, parasites, bacteria, organic films, ice cream, roots, semen, etc. Speckle dynamic applications in non-biological materials include various, including the analysis of the dried paint, control of gels, foams, corrosion, efflorescence, etc.

The dynamic speckle has applications in many fields of current research [1], as referents have Research where they developed the first descriptors such as [2], which was obtained image of local activity in fruits, fruit bruising study [3] in 2003, evaluating viability in seeds [5], characterization paint drying processes [6]. Also been used as statistical descriptors co-occurrence matrix [4] typical of this type of study. Work is currently being developed as the measurement of blood flow velocity [9], reconstruction of audio starting biospeckle image [8] etc.

2. Software acquisition and processing video

The acquisition system and video processing, was designed with the following features: must allow the capture of video from different sources such as analog video inputs (CCD camera), digital cameras with USB output to the computer; because Nowadays high resolution USB cameras are the

order of the day. Then the system should allow the processing of images, video and apply different filters to images. Various algorithms were developed as: Filters images, color histograms, histogram equalization, line profile, conversion of pixels to micrometers according objective microscope, saved images, streaming video, histograms and export files for your particular MATLAB processing time. The graphical interface of user (GUI) of the software can observe the Fig. 1 (b).



(a) (b)

Figure 1. Graphical interface software acquisition and processing of video.

The developed software can be run on notebook computers with USB port for video capture. The software has two windows one for control and one for video view.

2.1. Algorithms developed

Were implemented algorithms for filtering the image as seen in Fig. 2. Specialized algorithms are also developed in the microparticle diameter measurement view Fig. 3 where you select the source calculation, cores optical fiber which are observed by microscopy with different objectives of different lateral raises shown in Fig. 4, statistical processing for dynamic speckle; Among these are the autocorrelation coefficient matrices see equation (1).

Figure. 2. Example filters, In order a) Original image b) Edges c) grayscale d) Gaussian e) unsharp

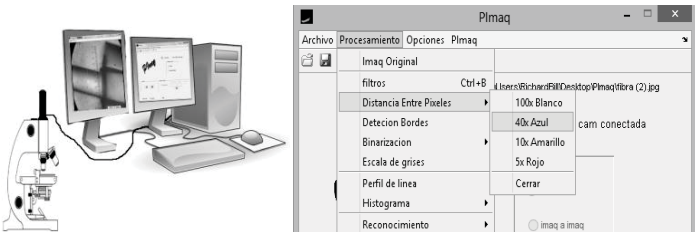


Figure 3. Selection of microscope objective for diameter calculation

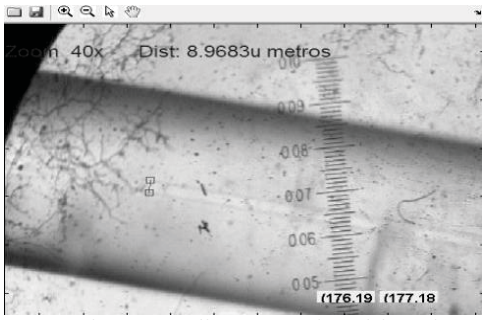


Figure 4. Measuring core diameter of multimode optical fiber

Now for compute the correlation coefficient between an image and the same image size is:

$$r = \frac{\sum_m \sum_n (A_{mn} - \overline{A})(B_{mn} - \overline{B})}{\sqrt{\left(\sum_m \sum_n (A_{mn} - \overline{A})^2\right)\left(\sum_m \sum_n (B_{mn} - \overline{B})^2\right)}} \quad (1)$$

Where $\overline{A}$ and $\overline{B}$ is Average or mean of matrix elements of (A) and (B). And (m,n) are pixel position. In this work, we used correlation coefficients for study in feeds.

3. Hardware for capturing dynamic speckle

The hardware required for applications biospeckle is specially designed to work with small objects of study, such as fruits, seeds and any biological object that can contain hardware designed.

The specific characteristics to this project were summarized as follows:

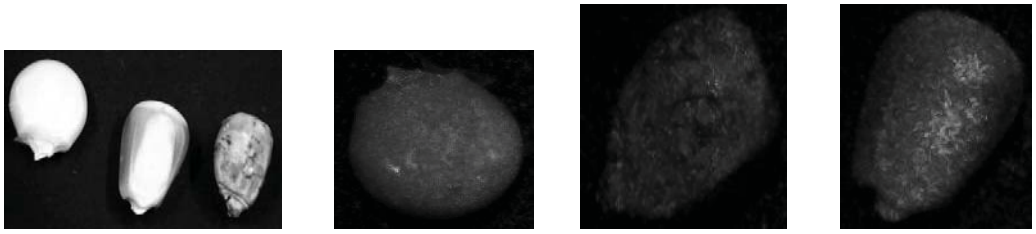
- ✓ The equipment is portable
- ✓ It has USB interface for PC and Laptops capture
- ✓ You can adapt different coherent laser sources
- ✓ The closed system prevents interference from other sources of light in the visible range
- ✓ Possesses graphical interface to capture and save
- ✓ The system can record video or save image sequence

Taking into account the required characteristics was developed a closed box, of black inner insulation for optical wavelengths in the visible range, in which to place the object of study. Also has a system movable sheets for location of the object of study, the prototype has holes to usb camera for location capture images or video, the implemented prototype can be seen in Fig. 5.

Figure 5. Prototype for capturing images of objects of study

3.1 Preliminary Catches

This work is part of a larger project wingspan which is a study of seed germination in grasses, work which is in development; In Fig. 6.a is observed the capturing a tender corn seed, in good condition, messed and their capture speckle. Where you can clearly shown the quality of images obtained in the equipment and their dynamic speckle images of maize seeds.



a) (b) (c) (d)

Figure. 6. Capturing Images with the equipment: a) Original Seeds, b) Tender Speckle seed, c) speckle seed disrepair. d) Seed Perfect condition

4. Prototype application

The equipment was initially applied in a research paper to estimate the percentage of germination of rice in Colombia, where it counted with the collaboration of Colombia Fedearroz Valledupar headquarters, which supply the seeds under study.

This work is important for the Colombian region because, in Colombia only can sell rice paddy it to germinate above 90% (Article 12 of Resolution 3034 of 1999, the Colombian agricultural institute ICA) [10]. The certification process consists initially on tests the feasibility with tetrazolium, where work has been done. Which indicates that the seed can germinate, but the final proof is the germination test samples of seed process that takes about 15 days, from sowing of the seed until the counting seeds germinated successfully. In this work is to develop a system for determining of percentage of seed germination using techniques biospeckle where will decrease the time for obtaining results from days to just minutes.

The experimental setup used for cross-correlation methods is shown in Fig. 7. and Fig. 8. It used an expanded low power He–Ne LASER (10mW, 633nm) as coherent light source, a diffuser, attenuator for decrease to avoid saturating the camera sensor; images were registered by a USB camera with focus len. Then, the images were digitized and stored as files in a host computer with

data acquisition software developed in MATLAB IDE. Care was taken so that the speckles were well resolved by the camera sensor. We used very low illuminating intensity so that the effect of the irradiation on the sample could be negligible. Laser illumination was adjusted to illuminate a broader region of the fruit during all measurements. The focus of camera was adjusted with perfect visualization of a new paper for focused capture of frame image.

The object of study was used seeds of rice variety Fedearroz 2000, the rice seed Fedearroz 2000 is a new variety that present similar to Fedearroz 50 characteristics in terms of their resistance to lodging and certain types of diseases, but their productivity levels are much higher. Fedearroz 50 yields 7 to 8 tons on average, although in certain regions of the country as the terrace of Ibague Colombia, Fedearroz 2000 has reached 13 tonnes per hectare.

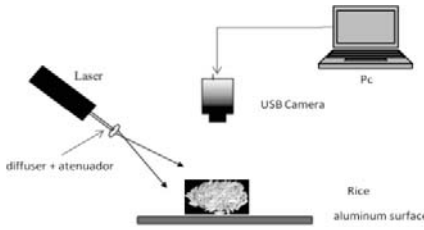


Figure. 7. Experimental setup for the Biospeckle activity analysis.

Figure. 8. Final prototype implemented for study

It is also developing a portable prototype for capturing and processed dynamic speckle images which can be observed in Fig. 2; equipment allows the capture of image on equal terms for all samples, not require a special location for its functioning; another of its main functions is to isolate samples of wavelengths in the visible range to not interfere the Laser Source thus improving the quality of the procedure; There was also the need to place seeds on an aluminium surface for better statistical processing.

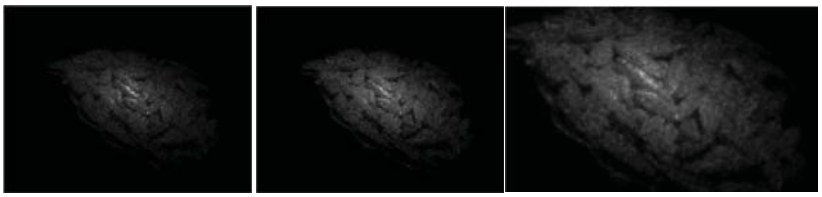
For the experiment, the recording time was equal to 60 seconds with frame rate equals to 2 seconds for a total 30 images. The objective speckle patter was obtaining by free propagation between the sample and the camera sensitive area. Data were registered and recorded on files rice seeds at different stages of latency, were obtained sample by sectional Fedearroz Valledupar of seed freshly harvested in the countryside (green paddy), Freshly processed for storage (paddy) and low latency stage (maximum germination). The samples selected by groups of 100 seeds in the same latency and grouped as shown in Fig. 9. After measurement of dynamic speckle proceeded to make them germinate and manually count how many seeds were born and how many not. These three groups of data samples, the conclusions of this work are obtained.

Figure. 9. Rice seed sample

Every point of image was essentially representative of the phenomenon and at the same instants. So, each column of these images could be used to generate THSPs that were all instantiations of the same experimental conditions. The images captured are of 640x480 pixels. Then, we proceed to convert image to grey scale 256 level and to apply a segmentation algorithm to obtain matrix NxN for analysis of interest area. The matrix of NxN is required for computing algorithm cross-correlation. The autocorrelation coefficients were obtained.

5. Segmentation and image processing

The segmentation process consist of four step, the first step is original image capture, the second step is convert the image to grayscale, the third step is to use the MATLAB function "bwlabel", this function returns a binary matrix, of the same size as image, containing labels for the connected objects in same image, then the object are removed from a binary image all connected components (objects) that have fewer than 1000 pixels, producing another binary image, BW2 using the function "bwareaopen"; next properties.
('Area','Centroid','FilledArea','MajorAxisLength','MinorAxisLength','ConvexHull') are obtained using the "regionprops" function. Now the mayor area is obtained in the properties object with your central position pixel (x, y) and its maximum row and column in the image. The final step is the segmented image obtained the original grayscale image; this image is used for biospeckle image processing. The image segmentation process is shown Fig.10.



(a) (b) (c)

Figure. 10. Image segmentation. a) Original image, b) Grayscale image c) Segmented image

The segmented image is the correlation coefficient calculation with the Equation (1) and was obtained the graphs of autocorrelation coefficients of the different groups of seeds, which can be seen in Fig. 11.

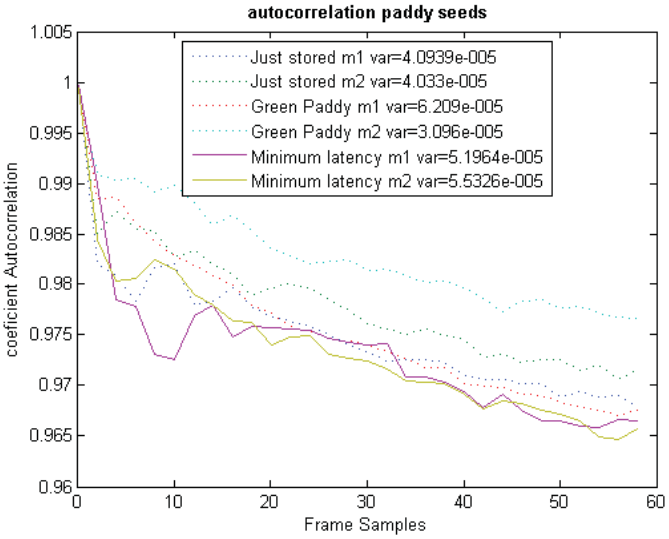


Figure. 11. Autocorrelation paddy rice seeds

In the graph we can see that the seeds have minimum latency greater slope, and having a higher cross correlation with respect to the group of seeds (green and freshly stored paddy). Table of physical evidence germination of the seeds used in the experiment can be seen in Table I.

Table 1. Percentage of seed germination

Group of sedes	% Germination	Average size cm.
Minimum latency Sample 1	98	2,7
Minimum latency Sample 2	95	2,8
Just stored Sample 1	70	1,5
Just stored Sample 2	65	1,07
Green Paddy Sample 1	80	1,4
Green Paddy Sample 2	75	1,6

Now as we have no equation of graphic for calculating the tangent line that is the speed with which the sample is decorrelates. For this resorted to use of equation (2), in which we calculated the slope between two points; for our case y_n values are the autocorrelation coefficients and the values of x_n are the time lag between samples, which are spaced every two seconds in the design of the experiment.

$$\mathbf{m} = \frac{y_i - y_f}{x_i - x_f} \tag{2}$$

Then these data proceeds to average them to obtain the average velocity decorrelation. This is summarized in equation (3)

$$\mathbf{m_{prom}} = \frac{A}{N-2}\sum_{n=3}^{N}\left|\frac{y_n - y_{n-1}}{x_n - x_{n-1}}\right| \quad (3)$$

Where N is the total number of samples (30), n is the umpteenth sample this starts at position n=3, because the first point the coefficient of autocorrelation is 1 and could affect the average overall slope and the value A is a gain value of the slope. The linearized coefficient cross correlation is shown in fig. 12.

Table 2. Slope values, with A = 1000, N = 30

Group of sedes	Average slope
Just stored Sample 1	0.4747
Just stored Sample 1	0.4738
Green Paddy Sample 1	0.3948
Green Paddy Sample 2	0.3814
Minimum latency Sample 1	0.7250
Minimum latency Sample 2	0.5076

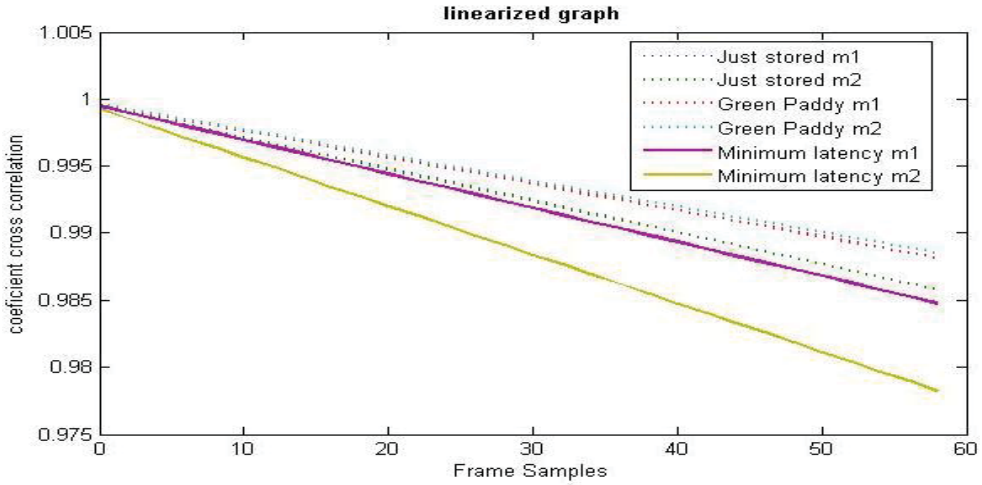


Figure. 12. Linearized coefficient cross correlation graph

As we can see in Table II and relating it to the germination percentage of the real test shown in table I, it can be seen when the cross correlation slope was above 0.5, with amplification A = 1000 and N = 30 factor, there was a 90% higher germination of seeds in physical proof of birth of the same seeds.

6. Conclusions

It was possible to develop portable equipment for acquisition and processing video in real time, the program allows capturing a number of images desired with a programmable time (tenths of a second, seconds and minutes) and then carrying out the relevant statistical processing. It was used, developed to a prototype system for estimating percentage of germination in seeds of rice, earning decorrelation slope as an indicator of germination percentage. As we can see in Table II and relating it to the germination percentage of the real test shown in table I, it can be seen when the cross correlation slope was above 0.5, with amplification A = 1000 and N = 30 factor, there was a 90% higher germination of seeds in physical proof of birth of the same seeds.

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