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The Image Analysis, Tools for Measuring Quality Criteria of Apples by Characterization of its Cellular Structure

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ABSTRACT

The measure of the quality of fruits is an economic stake and of human health. The tools for estimating the quality of fruit are still limited. Each tool provides a partial approach; it is the case of the colorimeter, the sound level meter, the penetrometer, etc. The assessment of the quality of fruit resulted from the combination of qualitative and quantitative measures. Histology and image analysis open up new fields of investigation by examining the internal structures of fruits and accurately locating the compounds involved in the development of quality. The work undertaken aims to develop methods for measuring the thickness of the cuticle, size and density of cells and cell walls of apple. We show here that the image analysis of histological sections is full of potential solutions allowing to better understanding the differences in texture and firmness or crispness among the three varieties of apples are examined as Braeburn, Fuji and the Golden Delicious. The materials and methods used to demonstrate are described. The results are presented and analyzed.

Key words: Color images, segmentation, 3D compact histogram, apple texture, firmness, cell surface, cell wall surface, cell wall thickness, cuticle thickness, interpolation

INTRODUCTION

In many fields such as medicine, food, robotics, security systems,...; the acquisition and the analysis of images are essential processes for taking decisions, as it is often difficult to perform them manually, it is necessary to automate certain tasks by computing. Thus in this study, we present, first a new original segmentation method based more particularly on the 3D compact histograms and then we apply this segmentation method to histological sections of apples for studying their textures and their firmnesses.

The apple is one of the most cultivated and consumed fruits in the world. There are different species; a preference over another is related to their eating quality, farmers know this quality empirically but they have no formal means to justify it.

The works of previous research on fruits have shown that their quality is related to their rheological properties which are frequently estimated by an unique measurement: the firmness value (Harker *et al*., 1997). This value is obtained by penetrometric tests which record the maximal force needed to penetrate the apple (with or without skin) until a determined depth (Hoehn *et al*., 2003; Johnston *et al*., 2001).

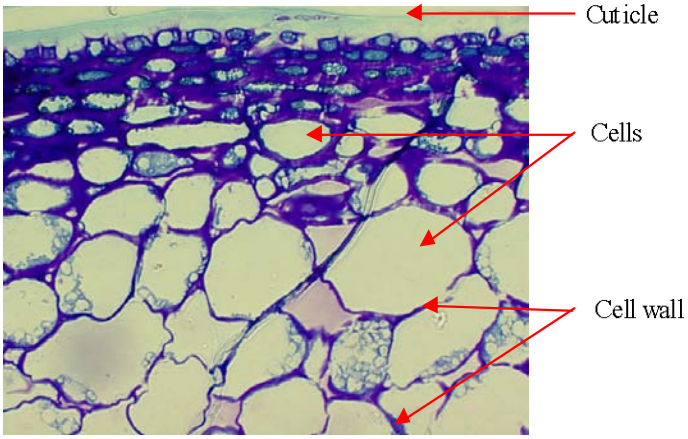

Fig. 1: An example of histological section of apples

Zhu and Melrose (2003) have supposed that the rheological properties of apples depend on different characteristics of inner fruit texture like cell morphology, cell-to-cell adhesion and cell wall composition (Camps *et al*., 2005). They have also assumed that a function of water status of fruits and thus could be influenced by the cuticle thickness and composition (Morice and Shorland, 1973; Baur *et al*., 1996).

The aim of this work is to differentiate certain varieties of apples by characterizing their inner structure after segmentation of their histological sections and then we prove their firmness. The varieties of apples concerned are the Braeburn (B), the Fuji (F) and the Golden (G); the images to be processed are colour images of optical microscopy obtained from preparations coloured by toluidine blue.

An apple has two sides, a coloured side noted c, which is exposed to sun and the other denoted n; For example a histogical section of apple Braeburn with a coloured side will be denoted Bc for the prefix and Bn for the second side. To differentiate apples of the same variety and the same type of side, a numerical or string suffix is added; for example Bc13 which means the coloured face of histological section of Braeburn apples number 13. Figure 1 shows an example of histological section of apples with an annotation highlighting components to be quantified in this study.

Tor partition the images in three classes of interest (Fig. 1), we developed a vectorial unsupervised method of segmentation based on the analysis of the 3D compact histogram (Clement and Vigouroux, 2001) inspired by the works of Zoueu *et al*. (2009) and Clement and Vigouroux (2003) which is a bi-marginal method using a classic 2D histogram which does not take into account all the correlation between the components of the color image.

SEGMENTATION OF COLOR IMAGES

The classification of colours is carried out in two steps: the learning step and the decision step:

The learning step is a hierarchical decomposition of populations in the 3D compact histogram. For each level of population p_m, peaks P_i are identified by a Connected Components Labelling (CCL) algorithm of nD (here n=3) compact histograms (Ouattara and Clement, 2007), which retains the connected components whose populations are greater than or equal to p_m. Each peak is then iteratively decomposed into narrower peaks, beginning from population 0. A peak is labelled as

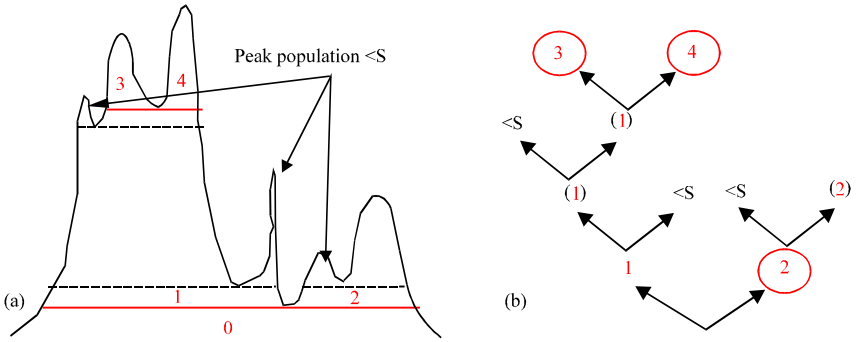

Fig. 2: An example of hierarchical decomposition. The circled leaves (part b) correspond to significant peaks as obtained at the end of the iterative decomposition (solid lines in part a), whereas leaves marked < S (part b) correspond to insignificant peaks (dotted lines in part a)

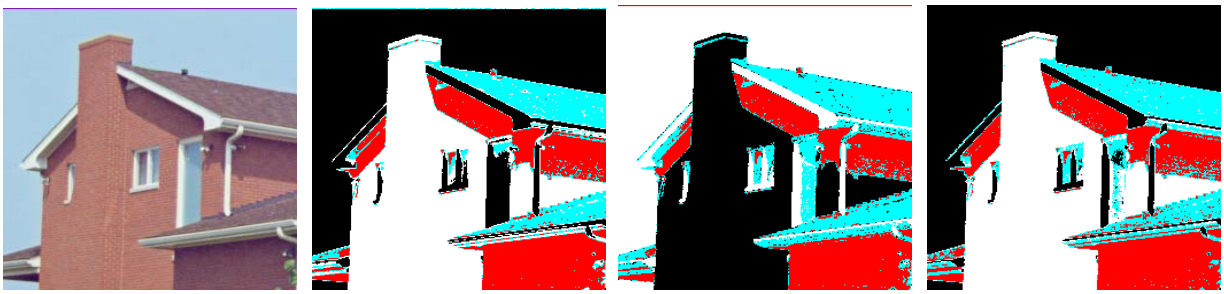

Fig. 3: Image House (a) classified in 4 color classes by analysis of the RGB (b) or RG (c) or RB (d) histogram

significant if it represents a population greater than or equal to a threshold S (expressed in percent of the total population in the 3D compact histogram). The procedure is illustrated in Fig. 2 (drawn in one dimension for clarity). We shall name kernels K_i the peaks corresponding to circled leaves in part b of Fig. 2. In other words, kernels are significant peaks (part a of Fig. 2) without descendants in the hierarchical decomposition tree (part b of Fig. 2) (for example, Fig. 2 shows five significant peaks P_i (i = 0 to 4) and three kernels K_i (i = 2, 3, 4)). The number of classes N_c is taken equal to the number of kernels (the class corresponding to kernel K_i is noted C_i). Therefore N_c depends on the threshold S, i.e., on the precision the image colors are analyzed with.

In the decision step, the mass center $\mu(K_i)$ of each kernel K_i is calculated in the color space. Let us denote by β the color corresponding to the point of coordinates (r, g, b) in the color space. Two cases can appear: if (r, g, b) belongs to K_i, color β is attributed to class C_i; if not, let us denote by P_k the peak which belongs to (r, g, b). Color β is attributed to class C_i corresponding to kernel K_i, link to the peak P_k, such that $d[\mu(K_i), (r, g, b)]$ is minimum, where d[a, b] is the Euclidean distance between a and b.

A performance illustration of the classification by analysis of the 3D compact histogram is shown in Fig. 3, where image House (Fig. 3a) has been segmented according to 4 classes of colors by analyzing the RGB, or the RG, or the RB histogram (Fig. 3b, c and d, respectively). The blue door is associated with the sky by using RGB histogram (which seems natural for a standard

Table 1: Unsupervised evaluation of segmentation, the maximum value of the parameter of Zeboudj indicates the best segmentation

Criterion of unsupervised evaluation	The three segmented images of house in Fig. 2		
	RGB (b)	RG (c)	RB (d)
Zeboudj	0,7847	0,7733	0,7713

observer) and it is also confirmed as being the best segmentation in Table 1 by a criterion of unsupervised evaluation of segmentation (Zeboudj, 1988; Chabrier *et al*., 2006), while it is associated with the roof and the wall by using RG or RB histograms.

HISTOLOGICAL SECTIONS STUDY OF APPLES

General objective: It is a question of characterizing the varieties of apples, i.e., of carrying out a histological analysis by image processing to determine if there are measurable differences between varieties and coloured and not coloured sides.

Specific objectives: To evaluate a certain number of parameters which are:

- Number of cells
- Minimum and maximum size of cells
- Changes in cell size cumulated when you move away from the cuticle
- Changes in cell size when you move away from the cuticle
- Quantity of cell walls
- Changes in cell walls quantity when you move away from cuticle
- Changes in the thickness of cell walls when you move away from cuticle
- Size of the cuticle
- Thickness of the cuticle

MATERIALS

Eighty apples of each cultivar, Braeburn, Fuji and Golden, are harvested at commercial maturity in the orchards of the Moriniere-CTIFL. The fruits are stored under controlled atmosphere (2.5% of O_2, 1.5% of CO_2, at 0.5-1°C) during 4-5 months and thereafter they was stored in a normal atmosphere during two weeks at 4°C. Three batches, each consisting of eight half-apples are freeze-dried and used for biochemical analyses.

Pieces of apples (8 mm×2 mm×2 mm of thickness) are removed from the equator of half-apples and included in a methacrylic resin (Cadot *et al*., 2006). Sections of 3 μm thickness are coloured by toluidine blue to highlight the cell walls and phenolic compounds (Camps *et al*., 2005). Two stereotypes taken from each image of apple have been photographed through an Olympus BH2 microscope with a lens of 40X. the resolution of each colour image is 760×574.

METHODS

Five apples per variety are used for these analyses, with the aim of automating the histological sections study of apples; firstly our segmentation method based on unsupervised 3D vectorial classification is applied to images (Fig. 1) in colorimetric space Lab to segment them into two classes in order to differentiate the cells and the cell walls, thereafter we compute the eccentricity of cells

to insulate the cuticle and we subsequently developed an algorithm using mathematical morphology to close the open cells and finally a certain number of parameters were computed to characterize apples. The various algorithms have been developed with Matlab.

Segmentation of histological sections of apples: The segmentation is applied to five images per variety and for a type of side (Braeburn and Fuji), including 10 images per variety; however for the Golden, treatment relates to only five images for the not coloured sides. We have segmented into two classes the images with our 2D and 3D segmentation methods in RGB and Lab spaces but the best segmentations are obtained with the 3D method in Lab space; this can be explained by the following reasons:

- Difficulty in justifying the relevance of the colorimetric plans chosen in 2D segmentation
- The 2D segmentation methods do not take into account the concept of three-dimensional color unlike 3D methods
- An important colorimetric variability of the images in RGB space does not permit to obtain best segmentation; hence the use of Lab space fostering uniformity of color in the images makes it possible to obtain the desired segmentation

For all images treated in Lab space, a threshold S ranging from 2 to 12% of the total number of pixels in the image allowed to segment images in two classes in order to separate the cell surfaces and the cell walls (Fig. 4b).

The ideal solution would be to have a segmentation in 3 classes, the third would correspond to the cuticle, which is impossible because the cuticle and the cells have the same color in the original image (Fig. 4a).

Extraction of the cuticle: Figure 4b shows that the cuticle is confused with the cells in the same class. A post-segmentation has been realized to differentiate them.

Separation consists to label the region in white (Fig. 4b), processing the eccentricity (Eq. 1) and the Major axis length (we suppose that their geometrical forms can be assimilated to ellipses) of connected components, using initially morphometry tools in Matlab.

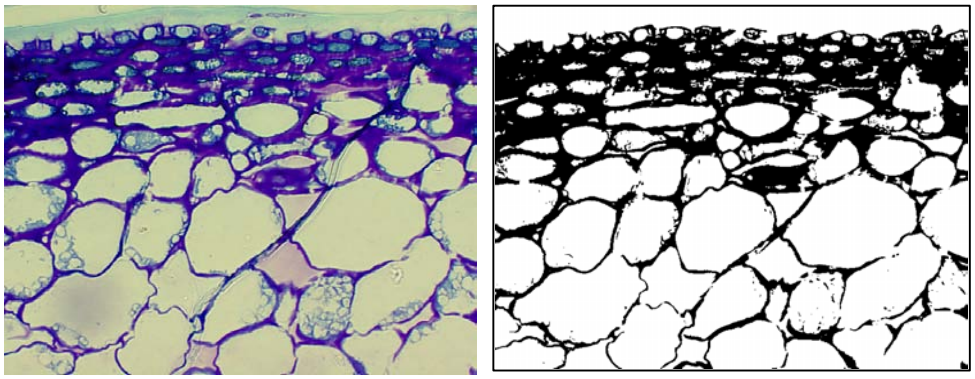

Fig. 4: Example of segmentation: (a) image Bc8 in RGB space and (b) image segmented in two classes

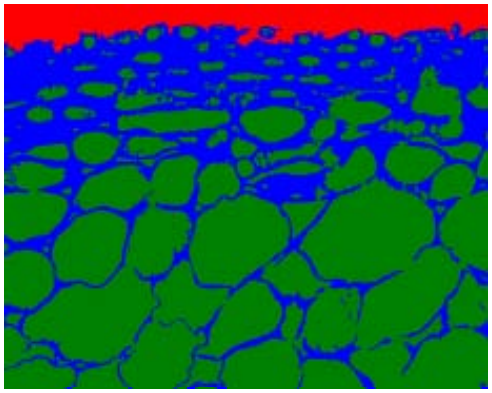

Fig. 5: Bc8 image segmented in 3 classes, in red the cuticle, in green the cells and in blue the cell walls

$$\text{Eccentricity} = \frac{\sqrt{a^2 - b^2}}{a} \quad (1)$$

where, a and b are denoted respectively Major and minor axis lengths and perimeter of a connected component.

From natural way, choosing the connected component of a high Major axis length and a high eccentricity, we manage to isolate the cuticle, as shown in the Fig. 5.

Contours closing of the cells: Figure 5 shows that the contours of certain cells are open, yet in the next section we must count the cells of segmented images; thus that would largely distort calculations if we preserve these anomalies which are related mainly to the acquisition of images even if we can find some little error of segmentation.

To close these contours, we have developed an algorithm in which a first step confused the cuticle with the cell walls in order to produce a binary image, see the image below in Fig. 6a. The second step consists to label the binary images in order to extract the connected components and to detect the open cells checking the following conditions after training:

- Compactness ≤ 0.3
- Size of connected components $\geq 0.6\%$ of the total number of pixels in the image

The compactness is defined by the following equation

$$\text{Compactness} = \frac{P_i^2}{S_i} \quad (2)$$

where, S_i and P_i are denoted respectively to the surface and perimeter of the connected component of number i.

The open cells detected are insulated and closed sequentially by an adaptive and a supervised algorithm. It is adaptive because the number of morphological erosion occurrences is different from the number of morphological dilation. A treated cell is integrated in initial binary image by using

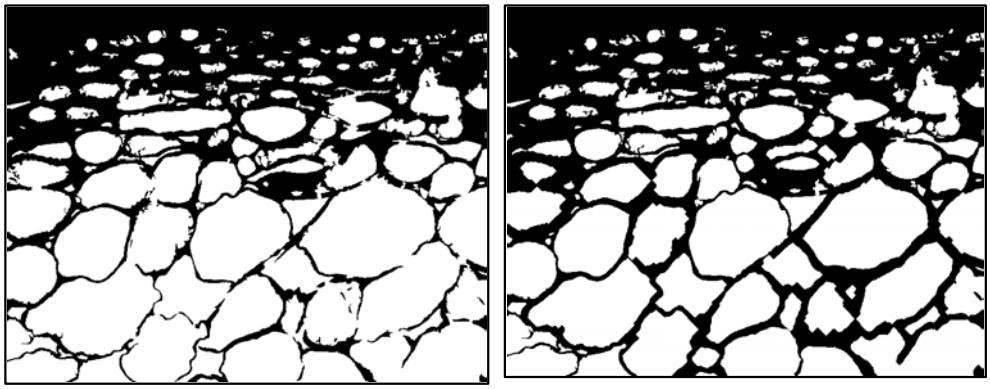

Fig. 6: (a) Bc8 binary image: cuticle confused with cell walls and (b) Bc8 binary image with closed cells

a logical AND between the open cell and the closed cell. Figure 6b shows a binary image with closed cells contrary to the initial binary image (Fig. 6a).

Interpolation of the gradient of the surface cells and the surface cell walls: The purpose of this part is to explain the interpolation of changes in the size of cells when away from the cuticle, that we call gradient of surface cells, this gradient is also interpolated for the cell walls. The gradient is calculated in relation to the depth which corresponds to the direction of the vertical axis of the image. It is directed top (cuticle) to the bottom and its origin corresponds to the first pixel of image (Fig. 1).

The binary image (Fig. 6b) which contours of cells were closed, a labeling of connected components is performed on each image to identify the different cells; then surfaces and spatial centers of gravity of cells are estimated, one of the coordinates describing the depth compared to cuticle.

We rather interpolated the gradients of cells cumulated surfaces and the cell walls for 25 treated images. An interpolation of representative gradients is estimated by merging data from 5 tests images for the same variety and the same type of side. Regarding the cell surfaces gradients and cell walls thickness, scatter plots are drawn to deduce the firmness of apples.

After learning sessions of interpolation, we have selected those which minimize the Mean Square Error (MSE) with MSE <6%.

RESULTS AND DISCUSSION

Quantification of components of histological sections of apples: The results illustrating the estimation of the proportions of the different components of the histological sections of apples are given in the synthetic Table 2. They are in general standardized compared to the total number of pixels in image.

Clouds points of the gradients of the cells surface and cell walls surface and their interpolation: The results of our plotting concern:

Table 2: Summarizes in a synthetic way the mean values of the estimated parameters of the different constituents of apples per variety and per type of side in order to facilitate the comparisons

	Braeburn		Fuji		Golden
Apples variety	Bc	Bn	Fc	Fn	Gn
Mean total surface of cells (%)	56.03	65.33	60.66	66.38	65.68
Mean total surface of cell walls (%)	35.25	24.71	32.37	23.78	24.09
Mean total surface of cuticle (%)	8.72	9.96	6.96	9.84	10.23
Number of cells (pixels)	200.00	132.00	166.00	137.00	134.00
Mean thickness of cuticle (%)	66.60	73.40	52.60	75.00	92.00
Mean maximum size of cells (%)	4.99	5.34	4.16	6.06	4.80

Table 3: Interpolation functions of cumulated cells surface gradients for merging data of apples per variety and per type of side

	Interpolation functions			
Groups	S_1	S_2	P_0	MSE (%)
Bc	0.87	-1.04	517.80	0.70
Bn	2.14	-2.48	583.47	3.50
Fc	1.13	-1.27	424.07	3.85
Fn	1.67	-1.97	486.42	5.62
Gn	2.28	-2.63	589.71	5.55

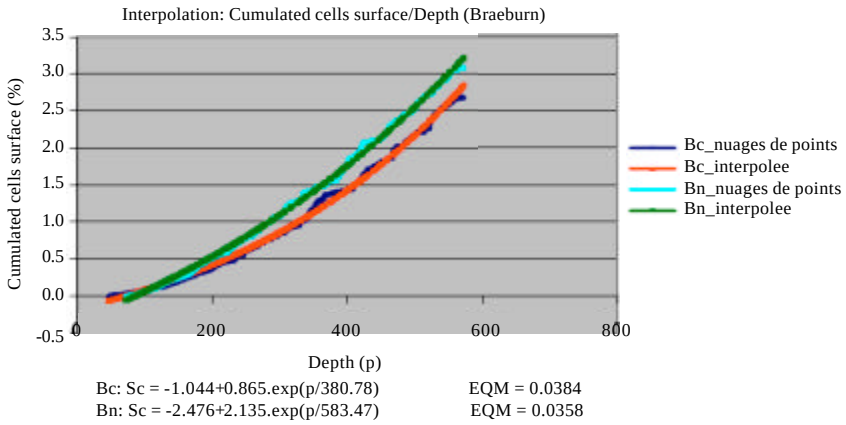

Fig. 7: Point cloud and curves interpolation of cumulated cells surface gradients for merging data of Braeburn apples

- Clouds points and curves interpolation of the changes of cumulated cells surface (S) according to the depth (P) per variety and for a type of side (obtained by data merging of 5 images), Fig. 7 shows an example of interpolation for Braeburn apples and Table 3 summarizes the interpolation coefficients for all varieties
- Clouds points of the mean value of the cells surface and the thickness of cell walls variations according to the depth (Fig. 8-10)

Knowing that the interpolation function are the form $Sc = S_2+S_1.\exp(P/P_0)$, where Sc and P are respectively the cumulated cells surface and the depth and S_1, S_2 and P_0 are coefficients to calculate, Table 3 shows the estimated interpolation functions coefficients.

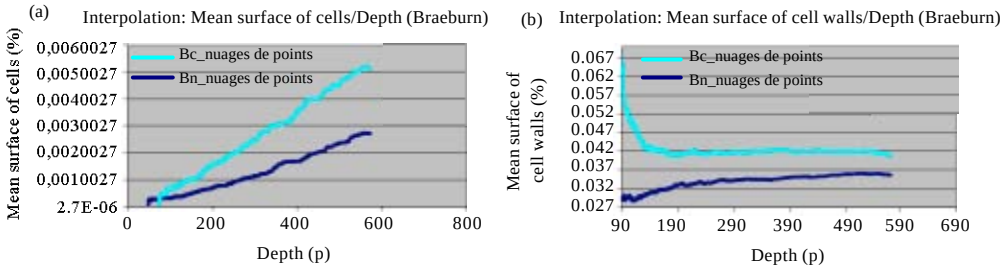

Fig. 8: (a) Gradient of cells surface and (b) gradient of cell walls thickness of apples Braeburn

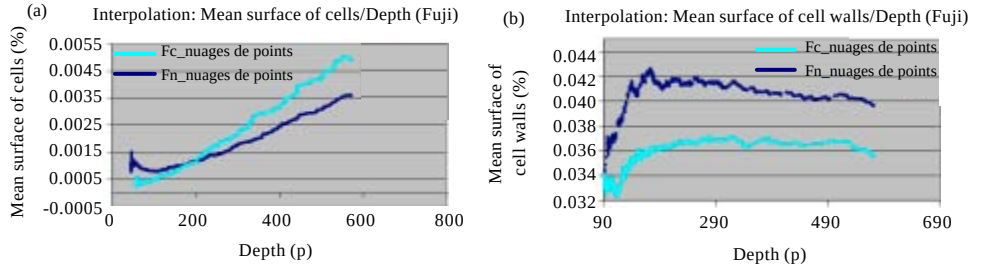

Fig. 9: (a) Gradient of cells surface and (b) gradient of cell walls thickness of apples Fuji

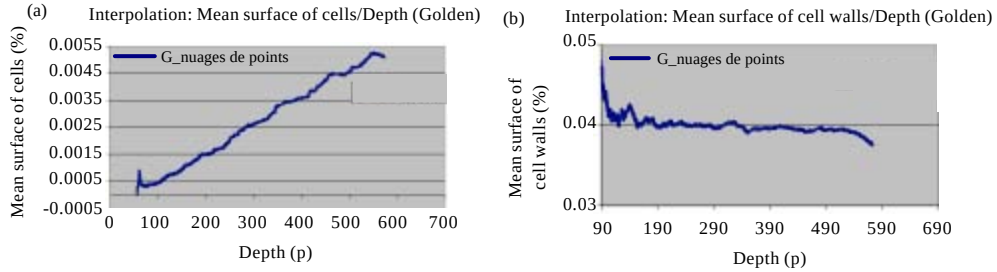

Fig. 10: (a) Gradient of cells surface and (b) gradient of cell walls thickness of apples Golden

Our final objective is not only to determine the cumulated cells surface gradient (Sc) but to determine the gradient of cells surface (S) and the gradient of thickness cell walls (T). The gradient of cells surface and thickness of cell walls are not directly estimable for that we have estimated Nc denotes the cumulated number of cells for a depth level (Fig. 8-10 for the different plotting). Thus an estimate of gradients can be obtained through the following formulas:

$$S = \frac{Sc}{Nc}$$

$$T = \frac{Tc}{Nc} \quad (3)$$

where, Tc is cumulated surface of cell walls.

Figure 8-10 the gradient of cell surface and the gradient of cell walls thickness per variety and by type of sided.

Interpretation of results: The automatic results are compared with those of the direct analysis i.e. a manual measurement with the ruler on the photos. For the moment, there is no existing results provided by other researchers that can compare our results, reason for which we proceed here by manual measurements. Many properties on texture of histological sections of apples can be deduced from the results obtained in previous sections:

- The non-colored side of the various varieties of apples has on average a proportion of cell internal surface higher than that colored, however the area occupied on average by the cell walls of colored side is greater than non-colored side for different varieties according Table 2 and Fig. 8-10. These results are in agreement with direct measurements
- The proportion on average of cell walls for the non-colored is practically the same for the 3 varieties of apples (Table 2)
- The proportion on average of the cuticle is higher in non-colored side than that colored for all varieties and this is true for the cuticle thickness on average (Table 2). By direct measurements, this result is not true for the Fuji because of the irregular surface of the cuticle. To solve this problem, we can use a black Sudan B colorant which makes it possible to better discriminate the cuticle; we may referer to work of Camps
- Cuticle thickness of the Golden on average is higher than the other apples varieties (Table 2). It is not true for Fuji because of the uneven surface of the cuticle
- Cells number on average is practically the same in non-colored side for all apples varieties (Table 2). This result is the same for manual measurements
- Cells size of Fuji on average in non-colored side is higher than the other varieties (Table 2). This result is the same for manual measurements
- Cells number of Braeburn on average in non-colored side is higher than the other varieties (Table 2)
- The gradient interpolations of cumulated surface of cells and cumulated thickness cell walls describe an exponential function (Fig. 7). For Braeburn, the differences in the intra-cellular surfaces between the not-colored and colored faces are mostly in the first cell layers, i.e., in the epidermis and hypodermis
- From Fig. 8-10, we deduce that:
  - The non-colored side of apple Braeburn is firmness than that colored (Fig. 8)
  - The colored side of apple Fuji is firmness than that non-colored (Fig. 9). But it is not true for Fuji because of the uneven surface of the cuticle
  - The firmess level in the Golden is constant (Fig. 10)
  - The mean maximum firmness level in the various varieties is the same (Fig. 8-10)

CONCLUSION

We are satisfied with the developed segmentation method because it shows its performance for the different applications treated in this study.

We prove the firmness of studied apples and the characterization of their different textures. To estimate correctly the cuticle, we will have made recourse to black sudan B colorant. Our method of segmentation can apply to all images with three components; some works are underway to

validate the segmentation of multispectral images with a number of components larger than 3. In a future article, we will present a general methodology for characterize the inner texture of certain fruits.

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