AUTOMATIC CLASSIFICATION OF CELLS AND INTERCELLULAR SPACES OF APPLE TISSUE

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Abstract

A procedure was developed for the automatic identification of cells and intercellular spaces of apple tissue from microscopic images. Two cultivars were used to develop the method: ‘Golden Delicious’ and ‘Champion’. Images of tissue microstructure were obtained using a confocal scanning laser microscope (CSLM). The area, perimeter, elongation, convexity, circularity, rectangularity, shape roughness and angularity of objects were automatically segmented from CSLM images and then analysed. These geometric features were used to classify objects into two groups by means of linear discriminant analysis (LDA). Expert-based classification was used as the reference data for the LDA model. Using "step forward" analysis and the elimination of redundant variables, four geometric parameters: area, perimeter, circularity and shape roughness, were found to be the most important for the classification of cells and intercellular spaces.

Key words: apple, object identification, multivariate analysis.

1. Introduction

The presence of intercellular spaces has a significant influence on the physical properties of tissues and the whole fruits. The proportion of such spaces varies from 1% for tissues that contain densely-packed polygonal cells like potato and carrot parenchyma, to over 30% in the case of apples. The intercellular spaces fraction can change during ripening due to decrease of cell-cell adhesion and in consequence increase of tissue porosity. This is associated with decaying of firmness and crispness and emphasizing of the mealiness sense (Harker et al., 1993). From mechanical point of view, the loosening of cell-cell adhesion causes change of cracking propagation mode from cell wall fracturing to cell-cell debonding (Niklas, 1992). The material structure is also important to supply and remove the required gasses (O₂ and CO₂) for intercellular respiration (Mebatsion et al., 2008). Commonly used methods for the study of tissue morphology are based on use of the microscopes and following image processing and analysis. Cross-sections of samples, viewed under microscope provide clear insight into anatomical structure of plant tissues. Since at present there is no standard method for performing quantitative evaluation of cell and intercellular spaces within tissue, a procedure was developed for the automatic identification of both classes of objects within apple tissue from microscopic images.

2. Material and Methods

Two cultivars were used to develop the method: ‘Golden Delicious’ and ‘Champion’. Images of tissue microstructure were obtained using a confocal scanning laser microscope (CSLM). The images were processed using a protocol developed by Zdunek et al. (2004) in Aphelion v.3.2 (Adcis, France) image analysis software. The procedure consisted of a series of standard morphological operators such as reconstruction, dilation, skeletonization and watershed segmentation. Set of eight parameters was chosen to describe both the size and shape of the cross-sections of cells and intercellular spaces in the images. The area, perimeter, elongation, convexity, circularity, rectangularity, shape roughness and angularity
of objects were calculated from CSLM images and then analysed. These geometric features were used to classify objects into two groups by means of linear discriminant analysis (LDA). All regions separated from the microscopic images were manually investigated and divided into two groups: cells and intercellular spaces. The expert-based classification was considered as the reference data for the planned automatic classification models. In this study, two thirds of the data from the expert-based classification was used as the training set. In order to test the performance of the models, validation by a test set with one third randomly-chosen objects was used. The most important variables for discrimination were determined by “step forward” analysis.

### 3. Results and conclusions

Using "step forward" analysis and the elimination of redundant variables, four geometric parameters: area, perimeter, circularity and shape roughness, were found to be the most important for the classification of cells and intercellular spaces. The performance of this method was assessed by comparison with manual classification carried out by an expert. The resulting classification functions allowed for very accurate identification of cells and intercellular spaces in the images from CSLM, with an overall success rate of 97.51% for Golden Delicious apples, and 97.73% for Champion. A range of error below 5% ensured that the results are comparable to those obtained by manual classification. The practical application of the final classification functions is presented on figure 1.

![Figure 1: Example of manual and automatic classification method applied to the same microscopic images.](image-url)
The automatic classification enables a large number of images to be processed in a relatively short period of time. This creates the possibility of many applications for this method such as the evaluation of texture changes during storage or improving the geometric models used in, for example, the finite analysis method. This approach should be studied more in the future on a larger set of cultivars to find an answer for the question whether the classification intercellular spaces vs. cells must be a single-cultivar or could be common for many apples cultivars.

References: