

Chapter 20

Microscope Volume Segmentation Improved through Non-Linear Restoration

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ABSTRACT

An efficient segmentation technique based on the use of a modified k-Means algorithm and the Otsu's thresholding method is improved through a non-linear restoration of microscope volumes. An algorithm is proposed to automatically compute the k value for the clustering k-Means method. The unsupervised algorithm is used in the context of segmentation by considering regions as clusters. A comparison between the segmentation results before and after restoration is presented. The evaluation of the region segmentation included the root mean squared error and a normalized uniformity measure. Results showed significant improvement of segmentation when using the non-linear restoration method based on prior known information, such as the imaging system and the noise statistics.

INTRODUCTION

Image segmentation methods are widely used in image processing based applications, such as remote sensing, biomedical image, precision agriculture. Techniques of image understanding,

analysis, and computer vision systems often require a segmentation step. A class of image compression algorithms also included color and texture segmentation as a part of the method (Wei, 2002).

The most common segmentation methods are based on the detection of boundaries, edges, and lines (Castleman, 1996). However, clustering

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algorithms can also be explored to achieve automatic segmentation with good results (Jeon et al., 2006). Methods such as *k*-Means, ISODATA and Fuzzy *c*-Means has particularly interesting characteristics, including: a) simplicity, b) no need for previous knowledge, c) developed to handle large amounts of data (Duda, 2000). These methods are not novel, but are frequently used as basis on the development of new solutions. The *k*-Means algorithm was used by Luo et al. (2003) to obtain segmentation using spatial constraints. The works of Jeon et al. (2006) and Samma and Salam (2009) have also applied a unsupervised learning method on an image segmentation task.

Some images are severely degraded after acquisition by an imaging system. Effects as blurring, noise, and geometrical aberrations can hamper the possibility of using those images as an input to a machine vision system. Optical microscopy is one of the imaging equipments that cause degradation, especially when used to obtain three-dimensional data (Olympus, 2008; Wu, 2008). Biological and medical applications require a segmentation of cells and microscopic structures. However, the undesirable effects may lead the analysis algorithms to produce wrong outputs. In this paper, the effect of restoring the data to be segmented is addressed, and a modified clustering algorithm is used to perform segmentation in volumes acquired by an optical microscope. An unsupervised method is applied with a non-linear restoration adapted for microscope volumes.

OPTICAL MICROSCOPE VOLUMES

Volumes acquired through conventional optical microscopes have two main sources of degradation: a) frequencies cut-off that works as a low-pass filter, which causes a blurring effect especially throughout the *z*-axis (Goodman, 1996), and b) photon-count noise, a signal-dependent noise that can be well modeled by a Poisson distribution,

due to the nature of light based sensors, such as the CCD (charged-coupled devices) (Snyder & Miller, 1991).

Restoration on Microscope Volumes

Pre-processing steps in computer vision applications uses often linear and smoothing filters to improve image condition and the posterior analysis (Colicchio et al., 2005). However, these linear filters and smoothing operators such as Gaussian filters, as well as non-linear morphological opening or closing operators, may remove important structures present in images (Agard, 1984). Then, restoration methods that are based on image degradation and formation can improve the results. By using the theoretical model of a point-spreading function (PSF) of the microscope developed by Gibson and Lanni (1991), and using the noise statistics knowledge, it is possible to apply non-linear restoration algorithms that are best suited to the problem.

SEGMENTATION AND EVALUATION METHODS

Pre-Processing

The restoration method used is the iterative Maximum-Likelihood Expectation Maximization (ML-EM) (Conchello, 1998), assuming a Poisson distribution. This algorithm is also known as the Richardson-Lucy iteration (Lucy, 1974) (Richardson, 1972). The algorithm is given by:

$$\hat{i}_{n+1}(\mathbf{x}) = \left[\frac{g(\mathbf{x})}{\hat{i}_n(\mathbf{x}) * h(\mathbf{x})} * h(\mathbf{x}) \right] \cdot \hat{i}_n(\mathbf{x}), \quad (1)$$

where $\mathbf{x} = (x, y, z)$, \hat{i}_n is the estimate of the restored image on the n^{th} iteration, g is the observed

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