Your New Default Thresholding Method?
A robust global gray-level thresholding method based on object features

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Abstract—We present a new robust method for global gray-level thresholding. The method is based on object features and the input is an interval of the expected object sizes. It is especially suitable for biomedical microscopy applications where objects often vary in number, but have limited variation in size. Our vision is that this method should be the first thresholding method you try when designing a pipeline for object segmentation, and thus your new default method.

Index Terms—Robust global gray-level thresholding

I. INTRODUCTION

Global gray-level thresholding is a very useful and widely used method for image segmentation. It is especially powerful in combination with preprocessing steps like background correction, top hat filtering etc., where the aim is to make the object and background classes well separated in grayscale.

There are a vast number of global gray-level thresholding methods available, see some surveys in [1] [2]. Most of them are based on the gray-level histogram. However, the gray-level histogram provides no information about the spatial distribution of the pixels in the image. Here we present a global thresholding method based on object features. The details are submitted for publication elsewhere [3], but here we include some additional example applications and show its extension to 3D (see Fig. 1). For objects with similar size and intensity we show that a very stable thresholding method can be implemented based on object features. The only input is an interval of the expected object sizes, which in many applications is known a priori and often required as input parameter for pre- and postprocessing purposes, e.g. in IdentifyPrimaryObjects in CellProfiler. The method is not very sensitive to the size interval settings. If the image is dominated by clusters of objects instead of well separated objects, then the interval can be set to include the typical cluster size, but otherwise the interval should correspond to the typical size of a single object.

An additional advantage of this method is that it is robust also if the images do not contain any objects within the given interval, and thus avoids identifying noise as false objects.

One application field where this has great potential is high throughput analysis of microscopy images of cell nuclei or fluorescent spots, where the background and number of objects may vary significantly, but object size is fairly constant.

The segmentation is quite fast, about 0.3s on an 8 bpp 1350×1030 image in our ImageJ implementation on a standard laptop computer and it works equally well in 2D and 3D.

II. RESULTS

We have compared the proposed method to all standard global thresholding methods available in ImageJ and CellProfiler on images of cell nuclei and synthetic data sets mimicking fluorescent spots. The proposed method was more robust than all the other methods. In fact, the proposed method was the only one that performed well on all the tested data sets.

REFERENCES


Fig. 1. Examples of applications with the corresponding segmentation results: a,b) fluorescent cell nuclei, c,d) phase contrast image of E-coli bacteria, e,f) fluorescent spots, g,h) macrophages in zebrafish vessels (one slice from the 3D volume), with result as rendered 3D volume.