PERGAMON

Water Research 36 (2002) 807

WATER RESEARCH

www.elsevier.com/locate/watres

Author's response

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While we do not argue with much of what Professor Baveye has to say about image segmentation methods in general, his characterization of our work has some inaccuracies.

Our conclusions are not that iterative selection is an optimal automatic thresholding algorithm, simply that it performed better than the other algorithms we used to threshold a specific type of images, those derived from biofilm (light) microscopy. Any conclusion that this result is generally applicable or an encouragement for replacing all manual thresholding lies with the reader. Our goal was to screen available algorithms searching for those that generated reproducible results tending toward the average generated by human operators. Unavoidably, we assumed that the average generated by human operators is the appropriate value, and that reproducibility is a reasonable measure of effectiveness. Our experience with automatic thresholding found reproducibility to be a significant problem and we believe that using an algorithm that consistently generates results similar to those generated by human operators is superior to using an algorithm generating thresholds that vary unpredictably from one image to another.

It is not true that all the biofilm images we used had unimodal histograms, and we said explicitly that "many biofilms have unimodal histograms". We deliberately chose a wide range of image types, including those with unimodal histograms and images of both high and low contrast to achieve the most general results. As you might expect in any image segmentation problem, we had varying results, and multimodal histograms gave better, more reproducible results. We would like all of our images to have clearly defined segment boundaries, but the real world doesn't always oblige. Professor Baveye suggests the use of unsharp masking to strengthen the gray-scale gradients, and those methods were part of our experimentation. However, these methods also rely on human choices for parameters and provide a confounding factor in image analysis, which effectively reduces their objectivity.

We cannot follow Professor Baveye's suggestion that we retain the color information in our images because all of these images were collected in native mode 256 gray scale. Obviously more information would allow greater flexibility in selecting methods. While we are familiar with various staining methods for microbes, these stains often have negative side-effects on biofilms that may need to be avoided for a number of reasons. To avoid these complications, we thresholded gray scale images of biofilms produced by light microscopy. We often quantify heterogeneity in living, fully hydrated biofilms, and this dramatically limits the choice of available stains. For this reason, to quantify biofilm structure, typically we do not stain the biofilms.

Even though Professor Baveye is obviously unimpressed with our use of a panel of experts to create a reference for measuring the efficacy of automatic thresholding methods, we still find this procedure acceptable. While a mechanical or theoretical reference would be preferable, none exists, and using a panel of experts is universally accepted as a reasonable method given proper constraints and careful design. After all, manual methods rely on a human to make the decision as to the proper threshold value. We can only assure Professor Baveye and the readers that our experts were all familiar with biofilm structure and that the methods used were designed by a statistician to remove potential bias. We did not find universal consensus among our panel members, nor did we find that they tended to split the image into two equal parts.

We appreciate Professor Baveye's interest and obvious knowledge in this area. He has suggested a number of alternative methods and algorithms and we hope that his comments will encourage others to extend our work.

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0043-1354/02/$ - see front matter
PII: S0043-1354(01)00416-X