
ACCURACY OF DIGITAL MEASUREMENTS IN OPTICAL MICROSCOPY IMAGING.

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The quantitative analysis of images is a general procedure involving several steps from image acquisition to pixel classification (segmentation) and feature analysis. Therefore, the accuracy of a measurements performed with the image analyser must be regarded as the result of the combined influence of the acquisition, segmentation and analysis parameters. Because of the complexity of most materials imaged under the microscope, it is impossible to predict the accuracy of a given measurement with respect to a simple image operator like thresholding. However, raising (or lowering) the thresholding level within reasonable limits most often results in a broad scattering of experimental results.

The approach taken in this paper is empirical and limited to reflected light optical microscopy, but results can easily be extended to other imaging techniques. We first investigate the operating conditions in optical microscopy by checking the light source (intensity, stability and centering), the optical path (diaphragm, centering, focus) and finally the specimen (levelling, polishing). We consider the scattering of basic measurements like area and perimeter. We then investigate the influence of the camera and electronic devices (stability, thermal drift, linearity of the photon response). At last, we look at the influence of segmentation techniques from simple thresholding to nearest neighbour multivariate classifications. As a conclusion, we try to derive some important practical recommendations for getting more accurate measurements out of digital images. It is important to recognize that without such investigations it is often illusory to try comparing results of similar materials imaged under different conditions and measured with different image analysers.