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TITLE: Modification of a High-Throughput Automatic Microbial Cell Enumeration System for Shipboard Analyses

AUTHOR: ['Christin Bennke', 'Greta Reintjes', 'Martha Schattenhofer', 'Andreas Ellrott', 'Jörg Wulf', 'Michael Zeder', 'Bernhard M. Fuchs']

ABSTRACT:

In the age of ever-increasing "-omics" studies, the accurate and statistically robust determination of microbial cell numbers within often-complex samples remains a key task in microbial ecology. Microscopic quantification is still the only method to enumerate specific subgroups of microbial clades within complex communities by, for example, fluorescence in situ hybridization (FISH). In this study, we improved an existing automatic image acquisition and cell enumeration system and adapted it for usage at high seas on board an oceanographic research ship. The system was evaluated by testing settings such as minimal pixel area and image exposure times ashore under stable laboratory conditions before being brought on board and tested under various wind and wave conditions. The system was robust enough to produce high-quality images even with ship heaves of up to 3 m and pitch and roll angles of up to 6.3°. On board the research ship, on average, 25% of the images acquired from plankton samples on filter membranes could be used for cell enumeration. Automated enumeration was highly correlated with manual counts ($r(2) > 0.9$). Even the smallest of microbial cells in the open ocean, members of the alphaproteobacterial SAR11 clade, could be confidently detected and enumerated. The automated image acquisition and cell enumeration system developed here enables an accurate and reproducible determination of microbial cell counts in planktonic samples and allows insight into the abundance and distribution of specific microorganisms already on board within a few hours.IMPORTANCE In this research article, we report on a new system and software pipeline, which allows for an easy and quick image acquisition and the subsequent enumeration of cells in the acquired images. We put this pipeline through vigorous testing and compared it to manual microscopy counts of microbial cells on membrane filters. Furthermore, we tested this system at sea on board a marine research vessel and counted bacteria on board within a few hours after the retrieval of water samples. The imaging and counting system described here has been successfully applied to a number of laboratory-based studies and allowed the quantification of thousands of samples and FISH preparations (see, e.g., H. Teeling, B. M. Fuchs, D. Becher, C. Klockow, A. Gardebrecht, C. M. Bennke, M. Kassabgy, S. Huang, A. J. Mann, J. Waldmann, M. Weber, A. Klindworth, A. Otto, J. Lange, J. Bernhardt, C. Reinsch, M. Hecker, J. Peplies, F. D. Bockelmann, U. Callies, G. Gerdts, A. Wichels, K. H. Wiltshire, F. O. Glöckner, T. Schweder, and R. Amann, Science 336:608-611, 2012, http://dx.doi.org/10.1126/science.1218344). We adjusted the standard image acquisition software to withstand ship movements. This system will allow for more targeted sampling of the microbial community, leading to a better understanding of the role of microorganisms in the global oceans.

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