Quantitative size and biomass distributions from particle images: An improved algorithm applied to IFCB observations

Heidi M. Sosik[1], Emily E. Peacock[1], E. Taylor Crockford[1], Kevin Archibald[1], Bethany Fowler[1], Alexi Shalapyonok[1] and Collin S. Roesler[2]

Introduction and Summary

Automated imaging is a powerful approach for detailed characterization of plankton and other particles that impact ocean optical properties, structure food webs, and influence carbon cycling and export processes. Quantitative interpretation of images is essential to produce size and biomass estimates that are unbiased and consistent across different particle types and different measurement systems. Here we show step-by-step evaluation of a new algorithm for analysis of Imaging FlowCytobot (IFCB) data and show that it produces quantitative particle sizes that are consistent with independent assessments. We recommend that this new algorithm (ifcb-analysis, v4) should replace the current standard (v2) in the IFCB user community for applications where quantitative particle sizing is a priority. All IFCB data collected during EXPORTS and SPIROPA cruises in 2018 have been reprocessed and new products are available from the IFCB dashboard (https://ifcb-data.whoi.edu/EXPORTS; https://ifcb-data.whoi.edu/SPIROPA).

IFCB analysis workflow and data access

The IFCB analysis workflow (Fig. 1) begins with fully-automated in situ image acquisition on the IFCB and continues onshore with automated image processing, feature extraction, and classification. The classifier is trained with annotations produced by human experts and produces identifications that are combined with image processing features to produce per-taxon time series of abundance and biomass. All EXPORTS IFCB images and image products are accessible via web services and through the IFCB dashboard (Fig. 2).

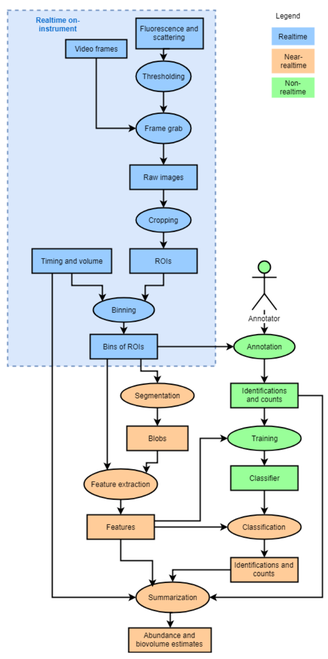

Figure 1. Overview of the IFCB-analysis workflow including steps that occur in real-time onboard the instrument (blue regions), automated post-processing steps for characterization and classification of images (orange regions). Human input is required for initial training of supervised machine learning algorithms (green).

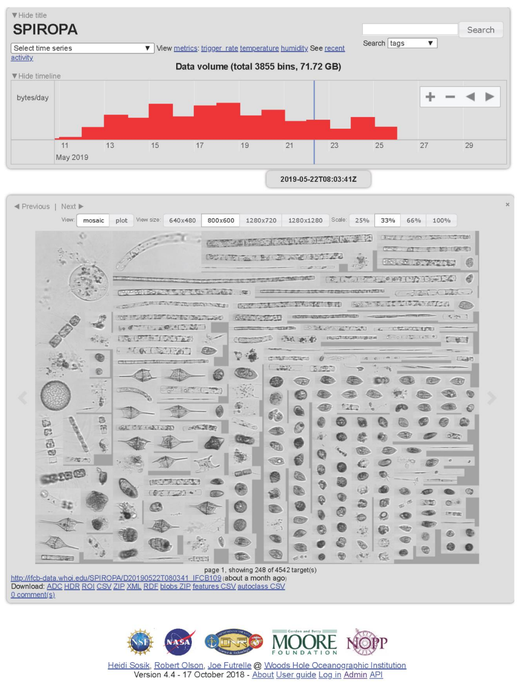

Figure 2. All IFCB images and image products from EXPORTS and SPIROPA cruises are available through the IFCB Dashboard hosted at WHOI:
https://ifcb-data.whoi.edu/SPIROPA
https://ifcb-data.whoi.edu/EXPORTS

Image segmentation, size metrics, and biovolume

Estimation of geometric characteristics from images begins with segmentation to identify which pixels correspond to the target of interest. The standard IFCB-analysis pipeline includes automated algorithms for this step (Fig. 3; Sosik and Olson 2007; https://github.com/hsosik/ifcb-analysis/), as well as subsequent computation of size metrics and estimation of biovolume (Fig. 4).

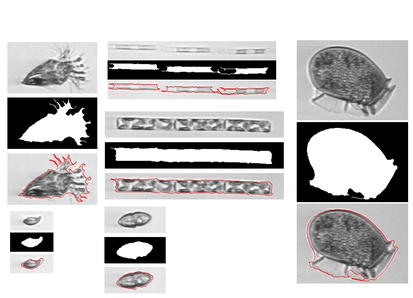

Figure 3. Example original images, results of segmentation ("blob" image, v2), and corresponding target perimeter. Features computed after this analysis include measures of cross-sectional area, perimeter, minimum and maximum Feret diameter, surface area, and biovolume (see Fig. 4).

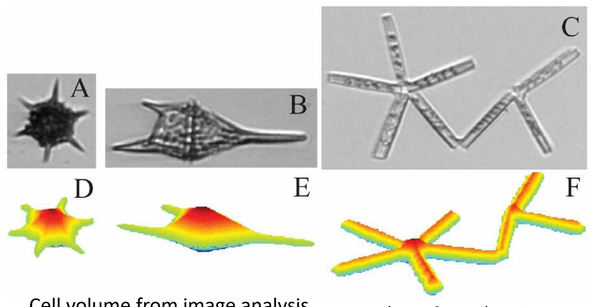

Figure 4. An automated custom algorithm makes it possible to estimate the biovolume of each imaged target even if the shapes are convoluted. Example original images of plankton (A, B, C) and their distance map representations in "step-pyramid" form (D, E, F) as described in Moberg and Sosik (2012). (A,D) *Dictyocha* sp. (B,E) *Ceratium* sp. (C,F) *Thalassionema* sp. Target biovolume is represented by the space enclosed by the 3D distance map (yellow to red colors indicate larger distance above the image plane, green to blue colors larger distance below the image plane).

Calibration of pixel size with standard beads

Calibration of pixel size should be straightforward, but sizing of a wide variety of natural particles requires a calibration that can be applied systematically regardless of size and shape and without biases that may be introduced by the segmentation.

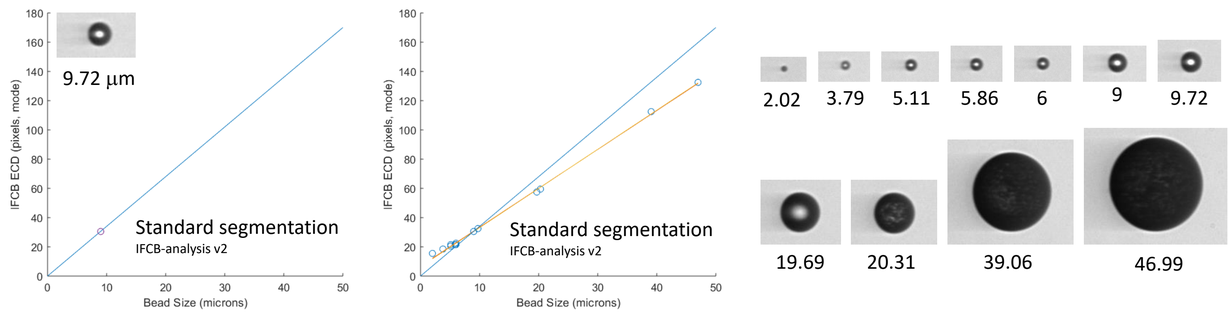

Figure 5. Computing geometric features from segmented images (blobs) requires precise calibration of the pixel size. Standard IFCB processing has used a single point calibration from IFCB images of a standard bead and comparing equivalent circular diameter in pixels to the known size of the bead (left panel). Our recent more extensive evaluation with a wide range of bead size (right panel) highlights systematic biases in this approach (center panel), including a non-zero intercept that traces to the segmentation algorithm.

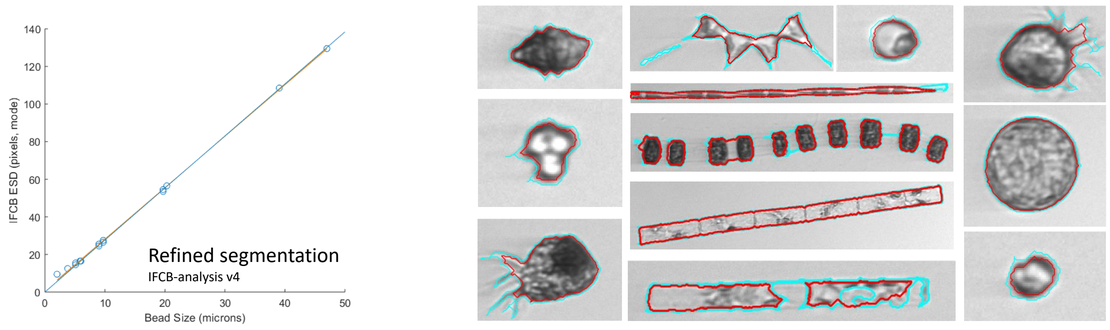

Figure 6. Refinement of the segmentation algorithm (ifcb-analysis v4 products) and reanalysis of the same bead data as in Fig. 5 produces a new calibration (left panel) where the intercept is not significantly different from zero and the slope is reduced compared to the original one-point calibration (Fig. 5 left). The v4 segmentation algorithm results in systematically smaller blobs, though with fairly subtle differences in target perimeter, as evident for various example particle types and sizes (right panel), comparing the v2 (cyan) and v4 (red) perimeters; images not displayed to scale).

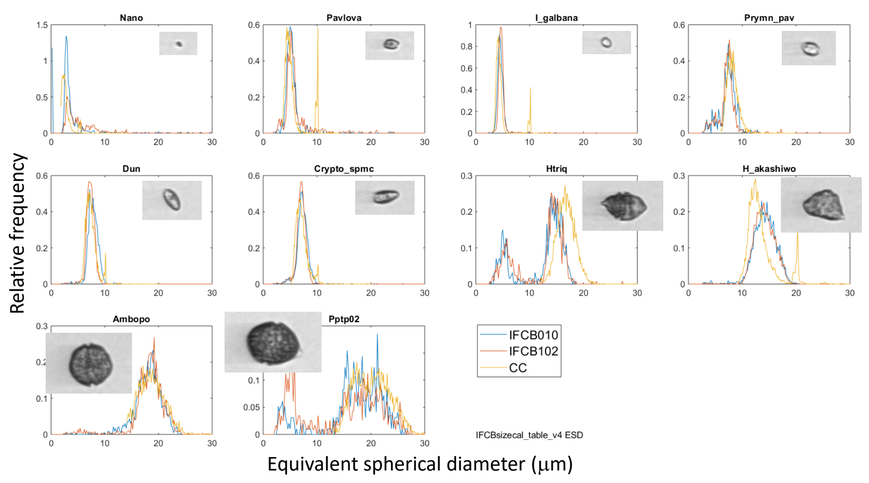

Figure 7. Independent evaluation of the new segmentation algorithm and pixel size calibration for a range of cultured phytoplankton. For each culture, size distributions were determined from images collected with two different IFCB instruments (blue and red lines) and completely independently with a Coulter Counter (CC, orange lines). This confirmation is important to verify that edge detection approach optimized for bead images (Fig. 6 left) is also appropriate for images of cells.

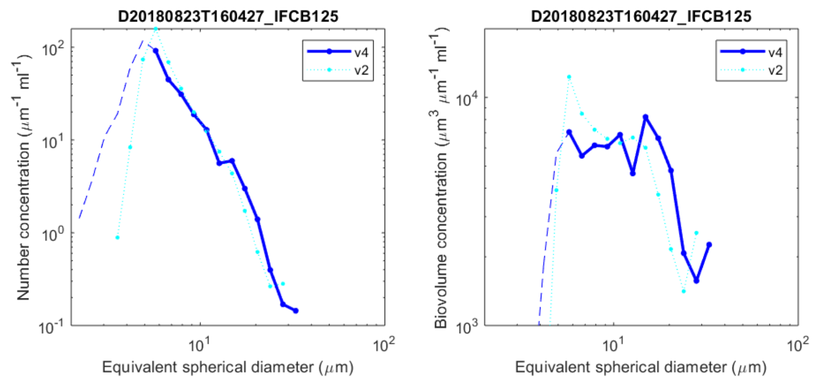

Figure 8. Example size distributions from IFCB images acquired during underway sampling on 23 August 2018 (16:04 UTC) on the survey ship (R/V Sally Ride), comparing the standard v2 (cyan) processing and the new (recommended) v4 (blue) processing. As expected from the evaluation described above, the new results provide number and biovolume spectra with shallower slopes and relatively more contribution in the microplankton size range. (Particles smaller than ~4 µm are not reliably detected by IFCB, as evident from the dashed blue line where measured numbers decline).

[1]Woods Hole Oceanographic Institution
[2]Bowdoin College

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