Segmentation results of two images of the same field of view at 50 hours (left column) and 130 hours (right column). Both images were background corrected by the machine-learning based method used in our pipeline. (A) Raw bright field out of focus images. (B) Segmentation with Otsu method clearly fails at 50 hours (most likely due to violation of the bimodality assumption of the intensity histogram). The image at 130 hours is segmented very well. (C) By applying a threshold correction factor of 1.17, the segmentation on the first time point improves slightly, while in the later timepoint artifacts appear. (D) A threshold correction factor of 1.3 results in good segmentation of the first time point, and a complete failure at the second time point. (E) MSER performs well on both images with the same parameter setting.