



Supplementary Figure 1 (a): input image sample used for test segmentations. All tools are executed with standard parameters, results are shown in (b)-(f). (b): CellProfiler with the *IdentifyPrimaryObjects* function in mode *Auto*. The input was inverted before analysis. (c): Microbetracker; multiple configuration were found to yield similarly results. (d): molyso automatically rotates the input image. (e): Schnitzcells with the *e.coli* preset. (f): TLM-Tracker with the Chan-Vese active contours method as one example of multiple, similar performing segmentation techniques.