## Evaluation of segmentation accuracy (SEG)

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The main purpose of SEG measure is to understand how well the segmented regions match the actual object<sup>1</sup> regions. To evaluate the measure we created gold reference segmentation annotation by manual segmentations at three different institutions by independent experts with a background in biology. For each video, we segmented a significant number of objects (at least one hundred). The objects for segmentation were randomly selected. For 3D datasets, we randomly selected a 2D slice to segment. For short videos, we randomly selected more than one slice to reach at least one hundred segmented objects. The experts could see spatial (z) as well as time context to correctly segment the datasets. Manual segmentation of each object was represented as a set of pixels (not necessarily connected) belonging to the object. The reference segmentation was obtained by a majority-voting scheme. Each manually segmented object available in the reference segmentation is called *reference object*.

The SEG measure is based on the Jaccard similarity index (J) of the sets of pixels of matching objects:

$$J(S,R) = \frac{|R \cap S|}{|R \cup S|},$$

where R denotes the set of pixels belonging to a reference object and S denotes the set of pixels belonging to its matching segmented object. A reference object R and a segmented object S are considered matching if and only if the following condition holds

$$|R \cap S| > 0.5 \cdot |R|.$$

Note that for each reference object, there can be at most one segmented object that satisfies the detection test. If there is no significant overlap with any segmented object, i.e., for the given reference set R no segmented object can be detected, we set the matching set to be empty set.

The J index is computed for each reference object in a video. It must always be in the [0, 1] interval, where 1 means perfect match and 0 means no match.

The SEG measure is represented by the mean of J indices of all reference objects in a particular video.

 $<sup>^{1}</sup>$ object = cell or cell nucleus depending on a dataset