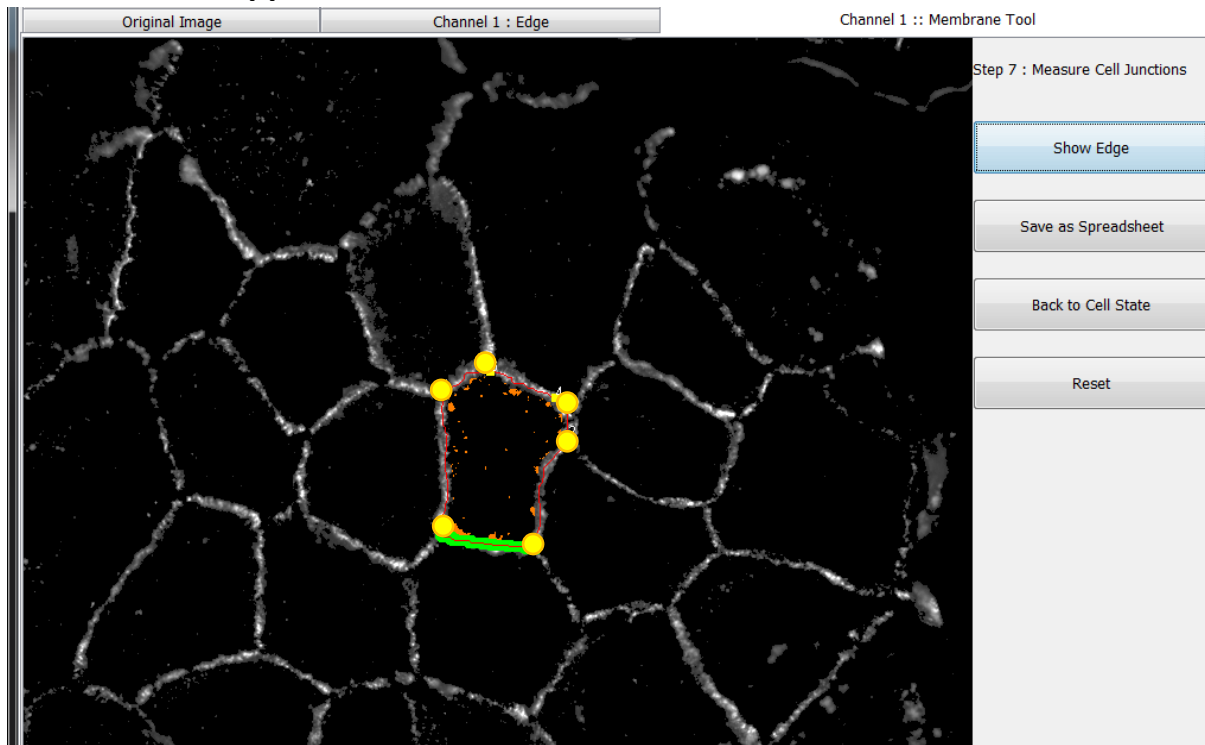










## A - Junction Mapper



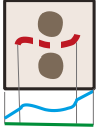


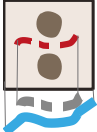


● corners      — skeleton      — dilated area

## B - Primary parameters.

#	Name	Units	Description	Details
1	Interface Contour 	[pixels]	Distance between two corners of the defined cell edge	Algorithm walks along the defined edge from corner to corner and sums the pixel to pixel distance. This is measured by the Euclidian distance between neighbouring pixels along the edge (as diagonally connected pixels distance = approx. 1.4, whilst straight connected pixels = 1). The red line on the image represents the defined cell edge.
2	Straight-line Interface Length 	[pixels]	Straight line distance between two corner points	Euclidian distance between the two corner pixels defined for the edge. The corner pixels are the last pixels at either end of the red line on the image.
3	Fragmented Junction Contour 	[pixels]	Sum of stained fragments along the single pixel edge	Sum of pixel to pixel distance measured by Euclidian distance between neighbouring pixels along the defined cell edge where the staining intensity exceeds the threshold set. The distance in between individual fragments is NOT calculated. Isolated single above threshold pixels are not included in this measurement.

4	Dilation Cycles	[unitless]	Number of cycles used to dilate the defined edge	Number of times the binary image dilate algorithm is used to expand the defined edge. Essentially one dilate cycle changes a single pixel line to a three pixels-wide line. Two dilation cycles make the line five pixels-wide, etc.
5	Interface Area 	[pixels <sup>2</sup> ]	Area in pixels of the dilated edge area between two corners	Number of pixels within the dilated edge area defined by two consecutive corners. The dilated area is represented by the green area on the image. Pixels on the red line are also included in this area.
6	Junction marker 1 Area 	[pixels <sup>2</sup> ]	Area covered by cadherin staining (junctional protein) within the interface area	Total number of pixels within the interface area defined in 5 where the junction marker 1 staining has intensities exceeding the threshold set.
7	Junction marker 1 Intensity 	[A.U.]	Sum of cadherin (junctional protein) Intensity within the interface area.	Sum of junction marker 1 intensities in the interface area defined in 6. This measurement only applies to pixels in which marker intensity is above the selected threshold. Pixels below the threshold are set to zero.
8	Junction Contour 	[pixels]	Sum of pixel to pixel distances between the first and last cadherin (junctional protein) pixels along the interface contour	Sum of pixel to pixel distance measured by Euclidian distance between junction marker 1 pixels along the interface contour defined in 1. Distance is measured between the first and last above-threshold pixel, but in between these points all pixels (above and below threshold) are considered; i.e. the distance in-between junction marker 1 fragments (gaps) is also included.
9	Straight-line Junction Length 	[pixels]	Euclidian distance from first to the last pixel of junction marker 1 on the interface contour	Euclidian distance between the first pixel of Junction marker 1 above threshold encountered along the interface contour defined in 1 and the last junction marker 1 pixel above threshold on the edge defined in 1.

## C - Secondary parameters.

#	Name	Units	Description	Details
10	Interface Linearity Index 	Ratio [unitless]	Ratio of 'Interface Contour' to 'Straight-line Interface Length'	Measurement 1 / Measurement 2
11	Coverage Index 	[%]	Ratio of 'Fragmented Junction Contour' to 'Interface Contour'	Measurement 3 / Measurement 1
12	Interface Occupancy 	[%]	Ratio of Junction marker 1 Area to Interface Area	Measurement 6 / Measurement 5
13	Junction marker 1 Intensity per Interface Area 	[A.U./pixel <sup>2</sup> ]	Ratio of 'Junction marker 1 Intensity' to 'Interface Area'	Measurement 7 / Measurement 5
14	Cluster Density 	[A.U./pixel <sup>2</sup> ]	Ratio of 'Junction marker 1 Intensity' to 'Junction marker 1 Area'.	Measurement 7 / Measurement 6
15	Junction Linearity Index 	Ratio [unitless]	Ratio of 'Junction contour' and 'Straight-line junction length'	Measurement 8 / Measurement 9