MOVIE 1

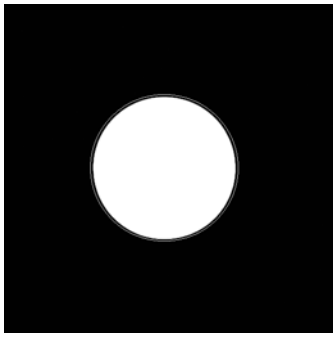
The output of the MATLAB program used to search the neighborhood of a segmented nucleus is illustrated in this movie where a disk is used as a model for the nuclear cross-section. The loop starts at the surface of the disk and then progresses outward until a predetermined number of loops are created. The diameter of the disk is 220 pixels.

SEE ATTACHED: supplemental_movie_1.avi

MOVIE 2

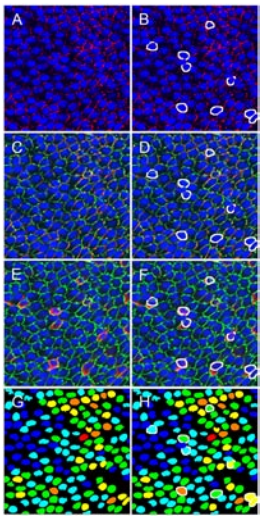
The output images obtained following the application of the loop expansion algorithm to the segmented cross-section of a nucleus in each of the ten planes in a z-stack are presented in the movie. The starting planes in the loop expansion are labeled as Plane 0, Plane 1, etc., to Plane 9.

SEE ATTACHED: supplemental_movie_2.avi



Supplemental Figure 1

The image consists of a ring concentric with the disk and separated from the disk by empty pixels. The number of pixels in the ring is 648. The diameter of the disk is 220 pixels. The contour expansion algorithm was applied to the above image after segmenting the disk. Starting from the surface of the disk, the loop expanded as demonstrated in MOVIE 1 in steps of one pixel and stopped after adding 10 loops. In doing so the neighborhood of the disk containing the ring was scanned by the expanding loops. The number of pixels in the ring calculated by the loop expansion algorithm was also 648.



Supplemental Figure 2

Figures 2A, 2C and 2E are confocal images acquired from the adult murine sub-ependymal zone from an en face perspective. The section was stained with DAPI (nuclear marker), beta-catenin (cell membrane marker), connexin 43, and GFAP (astrocyte/stem marker). To illustrate the independent use of beta-catenin and GFAP as sufficient membrane and stem markers respectively, Figures 2A depicts DAPI and connexin 43; 2C shows DAPI, connexin 43, and beta catenin; and 2E demonstrates DAPI, connexin 43, and GFAP detection. After nuclear segmentation of nine astrocytic cells using FARSIGHT, the contour expansion algorithm was applied to each. The ten contours associated with each of them are represented in white in figures 2B, 2D and 2F. In all three cases, connexin expression is correctly detected and assigned by our algorithm (figure 2G). Additionally, it confirms that the nine astrocytic cell types express connexin 43, consistent with previous reports describing connexin 43 as an astrocytic connexin (Pannasch et al., 2011) (figure 2H). Furthermore, we applied the algorithm to all 162 DAPI stained nuclei segmented by FARSIGHT. Figure 2G portrays the color coded nuclei based on their connexin expression; where blue and red correspond to the lowest and highest frequency of connexin detection respectively.