**3D Segmentation and analysis of Arabidopsis hypocotyl epidermis cells**

Prior to analysis, seedlings were fixed, cleared and stained as previously described with minor modifications. Briefly, five-day-old seedlings grown on half MS-agar (0.8 % w/v) supplemented with 1 % sucrose, were fixed using 4 % Paraformaldehyde in PBS pH 6.9 while applying mild vacuum (100 mBar). Fixed samples were washed 3 times with PBS and then incubated in ClearSee solution (25 % Urea (w/v), 15 % Sodium deoxycholate (w/v), 10 % Xylitol (w/v)). Clearing was performed for at least 2 weeks while changing ClearSee every second day. Seedlings were stained using 0.1 % (v/v) SR2200 stain in ClearSee for 1 h, then washed with fresh ClearSee for at least 30 minutes. For imaging, samples were mounted in ClearSee and imaged on a Zeiss LSM880 confocal laser scanning microscope, equipped with a C-Apochromate 40x/1.2W Autocorr M27 water objective. SR2200 was excited with a 405 nm diode laser and emission detected between 413 nm-472 nm. Z-stacks acquisition was performed with an interval size of 0.3 μm to obtain cubic voxels (pixel size 0.3 μm x 0.3 μm). To analyze whole hypocotyls, z-stacks from one seedling were combined in Fiji using the stitching plug-in. 3D segmentation and analysis were carried out in MorphoGraphX. Hypocotyl stacks were blurred using Gaussian Blur Stack with values between 1 μm-1.5 μm. Automated 3D segmentation was carried out using the Watershed Auto Seeded process with a threshold ranging from 800 – 1000. Segmentation was manually corrected, and cells not fully represented in the stack deleted. The 3D mesh was created using Marching Cubes 3D process with a cube size of 2 and 3 smoothing steps. To achieve optimal cell axis, a Bezier cord was formed according to hypocotyl shape and custom cell axis created with the process Create Bezier Grid Directions. Measurements were exported as CSV files and index of anisotropy calculated as follows: Cell Length/Cell Width = Index anisotropy.