**MembraneSepDist Training Set Readme**

The raw images must be processed first using the ImageSub m-file in folder S2. Then the processed images can be analyzed using the MembraneSepDist m-file.

The images in the Outlined folder have been outlined by an experienced user and are provided for reference. Note how the *inner* membrane is carefully followed. It is also perfectly acceptable to outline further from the gap junction, as the end of the perinexal outline will be cropped out in later processing steps.

The measurements of the outlined images are provided in the attached Excel file. It is strongly recommended that a new user be able to produce measurements that closely match (within ~1nm) the experienced user’s measurements in the training file before moving on to analyzing new data.