Protocol graciously provided by: Dr. Mariano Viapiano, Viapiano Lab protocols (© 2006-2014)

K. Measuring axial cell dispersion on aligned nanofibers

1. Seed individual, fluorescently-labeled spheroids on aligned-nanofiber plates and let them attach as indicated in Protocol *F. preparation and culture of cell spheroids on nanofibers*.
2. Using a fluorescent microscope take pictures corresponding to t=0h. Spheroids are easily detected by brightfield microscopy at this time, therefore they can also be imaged by phase brightfield microscopy
3. At the desired intervals, image the spheroids using fluorescence microscopy.It is essential that the spheroids are fluorescently labeled because dispersed cells on nanofibers cannot be reliably imaged using brightfield microscopy *(Figure reproduced from Agudelo-Garcia et al., Neoplasia (2011)13: 831-840, Suppl. Fig S1)*
4. To calculate a *Cell Dispersion Index* at t=x measure the ratio $Fmax_{tx}/Fmax_{t0}$, where Fmax= Feret diameter of the cell population as shown in the Figure above (do not use total area because it is not a reliable measure depending solely on cell dispersion)

   Please Note:

   a. Cell migration experiments should be performed for 24-48 h. Longer migration times can introduce artifacts due to cell proliferation and loss of fluorescent dye.
   b. If addition of Cell-Tracker to the initial spheroids is a concern then the following protocol can be performed:
      i. Seed unstained aggregates and image them at t=0 using brightfield microscopy
      ii. At the desired times post-migration add 1 μM Calcein-AM (Invitrogen) to each well and incubate it for 20 min at 37C.
      iii. Rinse the wells once with culture medium and image the dispersed cells using fluorescence microscopy (Calcein-stained cells will be bright green)
   c. **Do NOT** add Cell-Tracker dyes or nuclear dyes (DAPI, Hoechst) to unstained aggregates once they are on nanofibers. The dyes will bind the nanofibers resulting in very high background for imaging

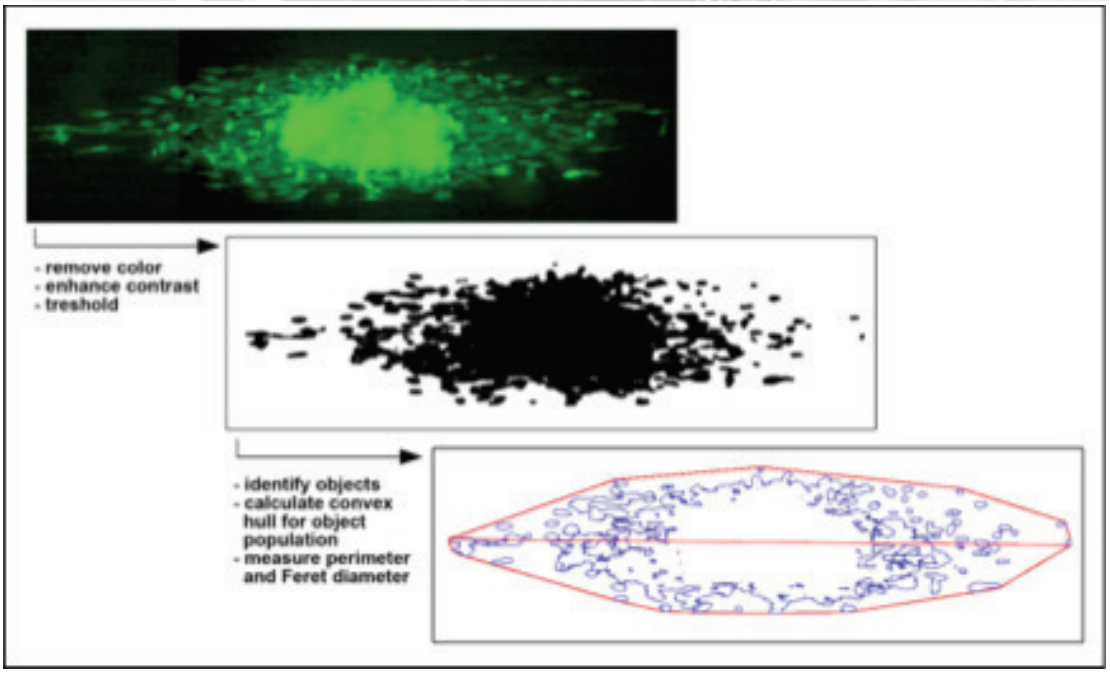

Figure reproduced from Agudelo-Garcia et al., Neoplasia (2011)13: 831-840, Suppl. *Fig S1*