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

D. Seeding Cell aggregates on nanofiber plates

1. Aggregates must be manually picked one by one using a dissecting microscope (preferably with epifluorescence illumination, but a simple epi-illumination works well). Because this scope is rarely inside a culture hood, it must be located in a relatively isolated and very clean area of the laboratory. Use lab coat, hairnet, gloves and mouth cover to avoid contamination of the aggregates. Surround the microscope with clean bench paper and sterilize the whole area with 70% ethanol. You can also keep a lit Bunsen burner close to the microscope to generate a continuous sterile area.
2. Using the dissecting scope, carefully pick up one stained aggregate from the 35mm dish in 1 µl of medium, using a 10 µl pipette with a 10 µl tip.
3. Transfer the aggregate to a well in the nanofiber plate under the dissecting scope, carefully "seeding" the aggregate onto the fibers in the center of the well. It is best to bring the pipette tip as close to the fibers as possible -without touching them- before ejecting the aggregate. This prevents the aggregate from floating away towards the edge of the well.
4. After adding the aggregates, cover the plate and allow aggregates to attach without movement for 20-25 min.
5. Complete the culture medium in the wells to 500 µl by adding 250 µl medium with or without test compounds. This allows the addition of test compounds without disrupting the aggregates.
6. Image the aggregates in a fluorescence microscope (usually at 4X and/or 10X). This will be the t=0h images. Further imaging can be done at different times (usually t=8h or t=24h), or by time-lapse microscopy.