Preparation:

Borate buffer

Collagen solution (in acetic acid)(4.59mg/ml)

PBS

NaOH

Rhodamine solution

DMSO

Adjust collagen solution with borate buffer pH 3 to final concentration 1mg/ml.

Adjust borate buffer with NaOH to final pH 8

Procedure

1. 100μg collagen distributed over on each glass bottom dish, 37°C overnight (may be able to reduce to 45 min-1hr).

   100μl collagen solution (1mg/ml) + 200μl PBS, mix well and coat on the center of dish. This step you need to do quickly to avoid collagen gelling.
2. Remove the liquid in collagen dish, you will get the collagen gel film.
3. Incubate with borate buffer pH 8.5 for 1hr, 2ml.
4. During incubation time, make the rhodamine solution: 1mg powder in 100μl DMSO. Note that rhodamine is sensitive to condensation. So if rhodamine is stored in refrigerator, please keep it in room temperature to warm up before open the bottle.
5. After time is up, mix 7μl rhodamine-DMSO with 300μl borate buffer(pH8.5), coat on the dish, 37°C 2~3hr.
6. Wash with PBS for 5 times(last 3 times 15min each on the shaker), covered with PBS before pass cell on the dish.
7. Pass cell onto the center of dish, 37°C overnight (We can find focus and check fluorescence about 10 min after passing the cells).

   To keep the cells to the center well of the imaging dish, add 300μl cell-medium on the center of dish, add more medium ~1ml after cell adhering (30 min after passing).