Scratch Assay protocol

Based on Scratch Wound Healing Assay (Martinotti, Ranzato)

Supplies:

- 12-Well culture plates
- Trypsin/EDTA
- Trypsin neutralizing solution
- HEPES-BSS or PBS
- 15 or 50 mL conical tube
- Fresh media (1-2 mL per each well of a 12-well culture plate)
- New flasks & media (if not using all cells for scratch assay and desire plating into new flasks).
- Cell counting:
    - Hemocytometer
    - Trypan Blue
    - Glass coverslip
    - Small Eppendorf tube
- 1 mL pipette tips
- Image J or Photoshop for analysis

Procedure:

1. Detach cells from tissue culture dish, as you would for cell passage (steps 1-12). You will need to count cells.
2. Prepare 12-well culture plate with 1-2 mL warmed media added to each well.
3. Seed cells into 12-well tissue culture plate at a density that after 24h growth, they reach 70-80% confluence.
    a. Each well of a 12-well culture plate has about 4 cm^2 of growth area.
    b. For fibroblasts, recommend plating 200k cells in each well of a 12-well plate to get confluence by the next day (50k/cm^2).
4. Once at confluence (usually after 18-24 hours), scrape cell layer in a straight line using a 1 mm pipette tip. Keep tip perpendicular to the bottom of the well. Scratch another line perpendicular to the first line to create a cross in each well.
    a. When making scratch, tip needs to maintain contact with bottom of well to remove cell layer, but pressure should not be excessive.
5. After scratch, *gently* wash cell monolayer to remove detached cells, then replenish with fresh medium.
6. Image using phase contrast microscope on 4x and 10x magnification.
    a. *Make a note of where images are taken from – ex. 3 o'clock vs. 9 o'clock. You will want to image the same spot each time!*
7. Place in incubator, and image on phase-contrast microscope every 4-8 hours until cells migrate to meet in the middle (24-48 hours).

If resources are available, cells can be imaged ideally using automatic time-lapse photography on an incubating microscope.