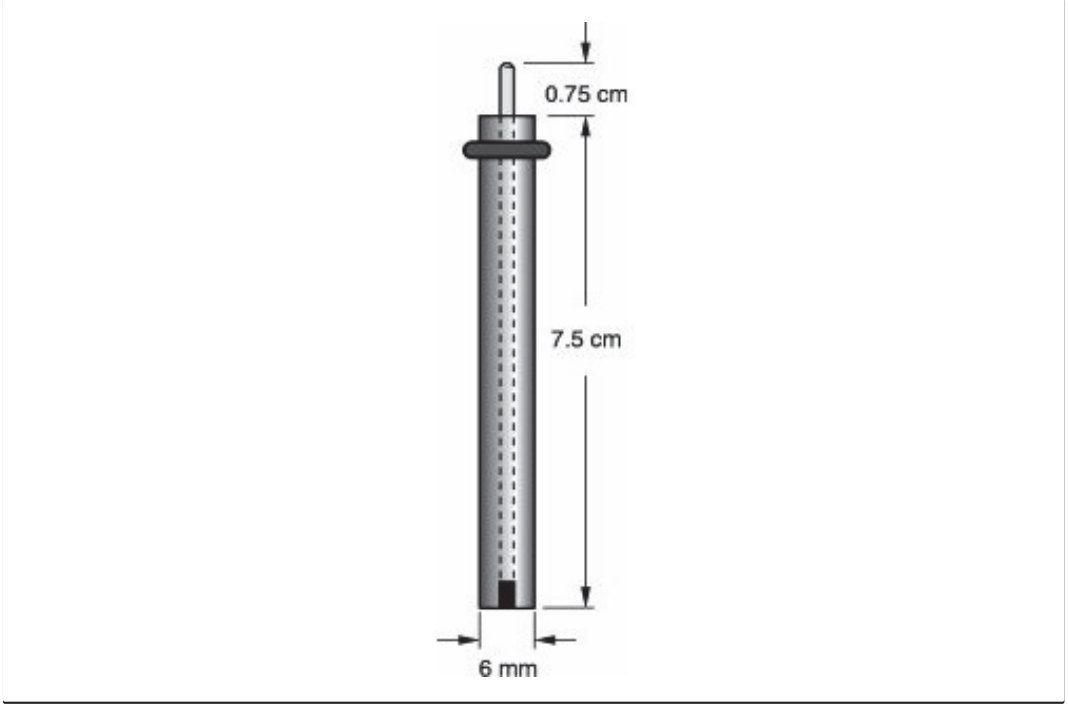
Electrochemical Cell Counting

Conditions:

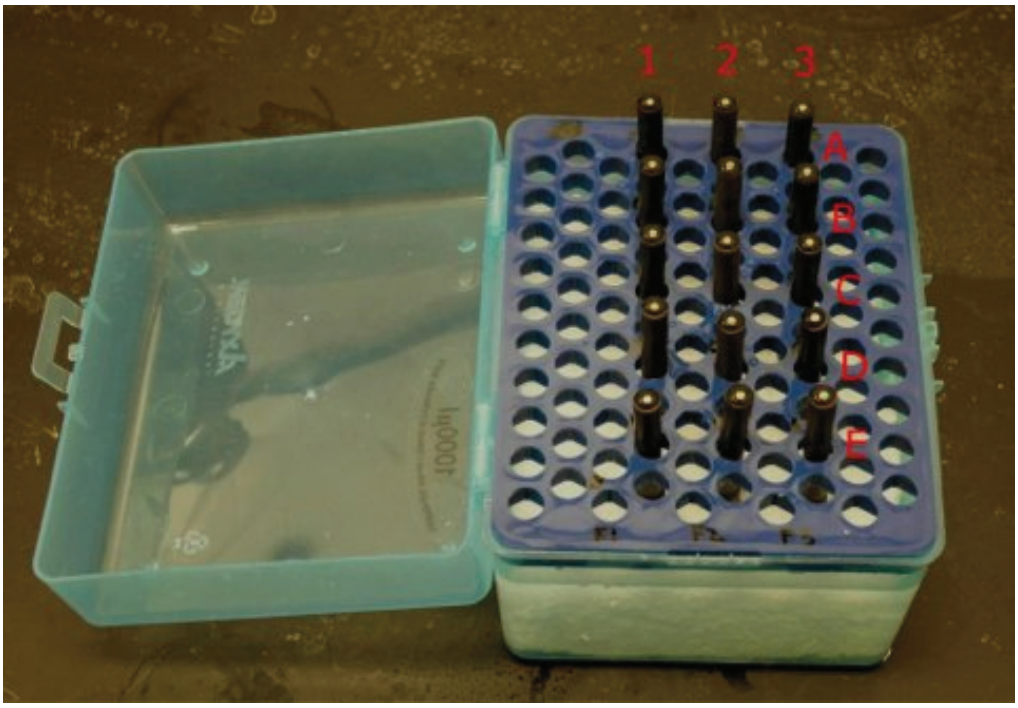
- A: blank PBS scan (EpCAM antibody)
- B: 1, 000, 000 cells/mL @ 40 uL of U937 cells in PBS (EpCAM antibody)
- C: 1, 000, 000 cells/mL @ 40 uL of DU145 cells in PBS (IgG antibody)
- D: 1, 000, 000 cells/mL @ 40 uL of DU145 cells in PBS (EpCAM antibody)
- E: 500, 000 cells/mL @ 40 uL of DU145 cells in PBS (EpCAM antibody)
- F: 250, 000 cells/mL @ 40 uL of DU145 cells in PBS (EpCAM antibody)

Experimental Setup:

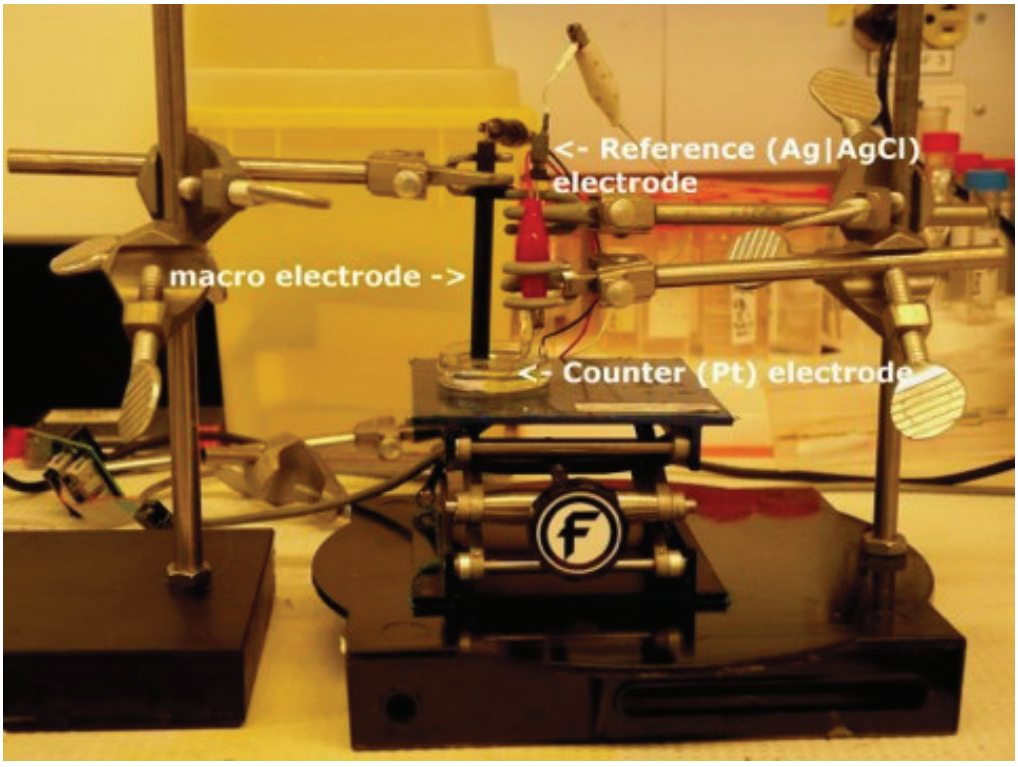
- electrode specs (macro electrode from BASi)



- experimental setup for incubation



- experimental setup for scans



Protocol:

1. Find 18 macro electrodes for the experiment (6 conditions; 3 repeats)
2. Place in a beaker with DI H2O and sonicate for 5 minutes
3. Use Buehler micropolish II powder with water on a pad rub in figure 8 motion for 1

minute
    - two electrodes at a times
4. Rinse with DI H2O and place in a new beaker
5. Sonicate with Acetone for 5 minutes; Sonicate with Methanol for 5 minutes
6. Rinse with DI H2O very carefully
7. Perform Acid Scan in 1 M H2SO4 (only do this every month or so)
    - 0 mV to 1500 mV for 10 Segments (the rest of the parameters leave as default)
    - Scan rate at 100 mV/s
8. Rinse with water and label electrode with condition and trial number
    - Ex: Condition A, trial 2 will be A2
9. Make 10 mM cysteamine solution
10. Place water at the bottom of the box
11. Place all electrodes upside down in a 1000 uL pipette box and styrofoam at the bottom to stabilize
12. Pipette 30 uL of 10 mM cysteamine on each electrode
13. Incubate for 10 minutes
14. Pipette out cysteamine
15. Pipette 60 uL of DI H2O on each electrode and wash for 3 minutes
16. Repeat step 2: wash again
17. Prepare 10% GA solution in DI H2O (defrost alliquote from freezer)
18. Pipette out the liquid and pipette 30 uL after taking out liquid from each aparature
19. Incubate for 30 minutes
20. Pipette out GA solution and pipette 60 uL of DI H2O (wash twice 3 minutes)
21. Defrost EpCAM antibody and IgG antibody
22. Dry device using air stream until no water is left in aparatures
23. Dilute to 100 ug/mL concentration and pipette 10 uL of antibody according to experimental conditions
24. Pipette out antibody and put 60 uL PBS on each electrode (wash twice 5 minutes)
25. Make 1% BSA solution in PBS and 1% BSA in 5% pluronic 68
26. Add 30 uL of 1% BSA to each aparature and incubate for 1 hour except F where you add 1% BSA + 5% Pluronic 68
27. Pipette 60 uL of PBS and wash 5 minutes 3 times
28. Add target as specified in experimental conditions and incubate for 20 minutes
29. Remove liquid from each aperture
30. Place 60 uL of PBS on EACH electrode
    - Pipette each up and down to wash cells (5 times pipette up down and replace solution 3 times)
    - After every 5 minutes replace PBS solution on all electrodes (wash proteins away)
31. Leave electrode in PBS until scan
32. Remove PBS from an electrode and place scanning solution (2 mM Ferro/Ferri in 1X PBS)
33. Do before DPV scan and record all results:

- initial potential: 0 mV
- final potential: 400 mV
- quiet time: 5 sec
- step E: 5 mV
- pulse width: 50 ms
- pulse period 100 ms
- pulse amplitude: 50 mV
- pulse type: Voltometry
- sample period: 1 line period

34. After scanning place in PBS wash bath
35. Image all electrodes using DIC and count number of cells