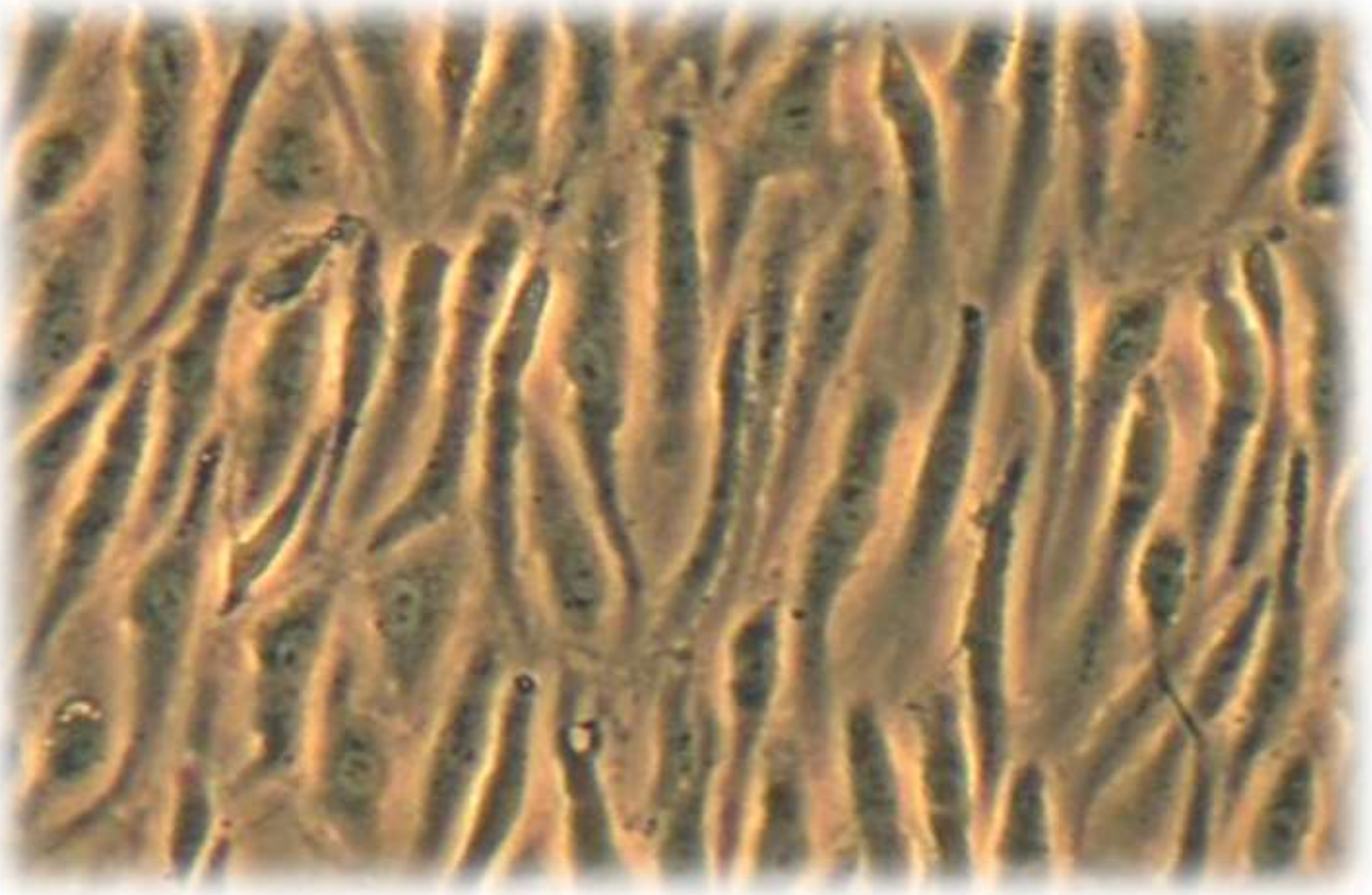
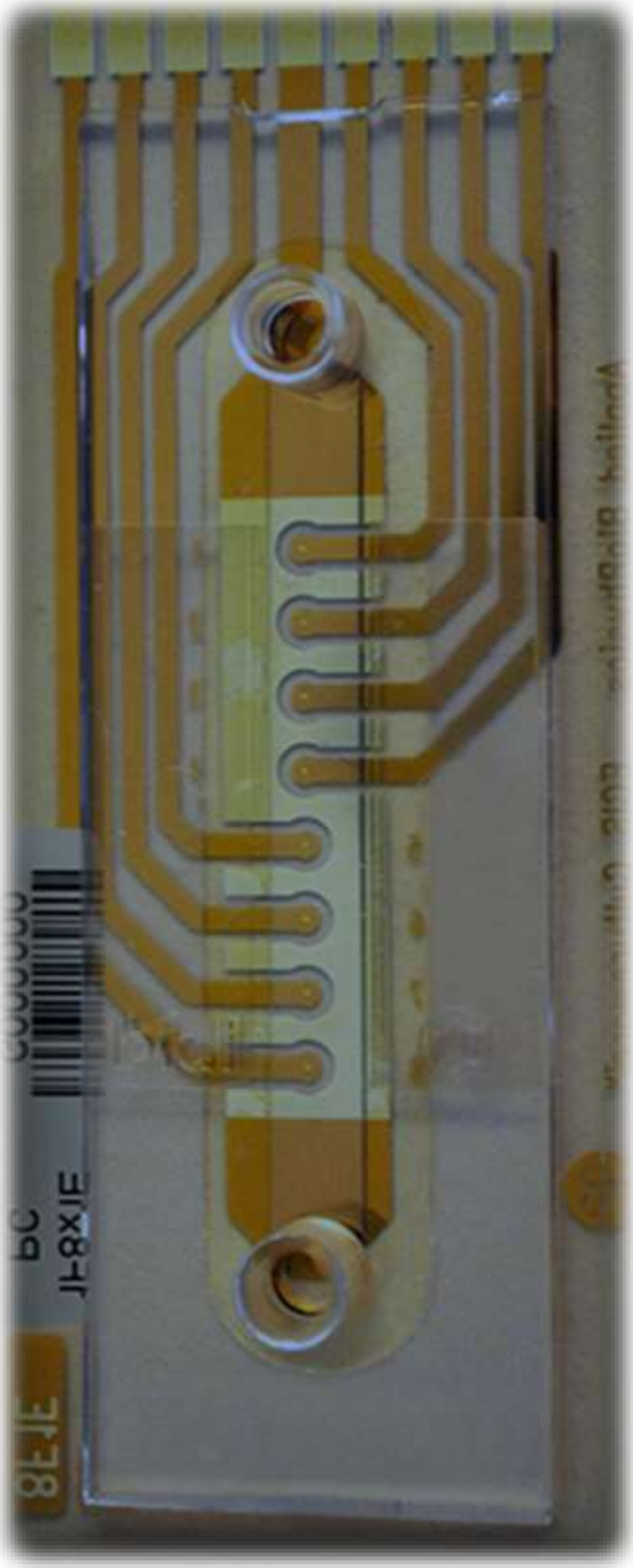


Applied Biophysics

FLOW NOTES

Ibidi Flow Arrays

Flow Application Note



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FLOW ARRAY SPECIFICATIONS

Array Type	Electrodes per Channel	Channels per Array	Electrode Area (mm^2)	Maximum Number of Cells Measured	Max Well Volume Channel/Reservoirs (µL)	Channel Height (µm)
1F8x1E PC	8 x 1	1	0.049	50-100	90/60	360
1F8x10E PC	8 x 10	1	0.49	500-1000	90/60	360
6F1E PC	1	6	0.049	50-100	50/60	660
6F10E PC	10	6	0.49	500-1000	50/60	660
1F2Y8x10E PC	8 x 4*	2**	0.49	500-1000	180/60	660

*Four electrodes are equal in size to 8W10E

** Array can be used on 2 different sides. (30 & 45-degree bifurcated sides)

INNOCULATION OF ARRAYS

The p-Flow module allows one to study cells under flow condition while simultaneously making ECIS measurements. The heart of this system is the ECIS Flow array that has ECIS electrodes on the floor of a flow channel.

The suggested protocol listed below is that suggested by **ibidi Integrated BioDiagnostics**, the manufacturer of the channel used with the ECIS flow arrays.

- Prepare your cell suspension at the desired concentration. Depending upon your cell type, application of 100,000 cells per cm^2 growth area should result in a confluent layer within the channel in 2-3 days. When seeding cells, fill only the correct channel volume into the channel. Avoid surplus cell suspension in the reservoirs.
- Refer to the table below for recommended seeding protocols for each type of flow array.
- If desired, the floor of the channel can be pre-coated with protein following the information provided in the appendix of this manual.

Array Type	Growth Area per Channel	Channels per Array	Recommended Cells per Channel	Recommended Seeding Density	Max. Well Volume Channel/Reservoirs (µL)	Channel Height (µm)
1F8x1E PC	2.5 cm^2	1	110,000 – 250,000	1.2 – 2.5 $x10^6$	90/60	360
1F8x10E PC	2.5 cm^2	1	110,000 – 250,000	1.2 – 2.5 $x10^6$	90/60	360
6F1E PC	0.6 cm^2	6	30,000 – 60,000	0.7 – 1.4 $x10^6$	50/60	660
6F10E PC	0.6 cm^2	6	30,000 – 60,000	0.7 – 1.3 $x10^6$	50/60	660
1F2Y8x10E PC	2.8 cm^2	2**	140,000 – 280,000	0.8 – 1.6 $x10^6$	180/60	660

** Array can be used on 2 different sides. (30 & 45 degree bifurcated sides)

ADDING CELL SUSPENSION

The suggested protocol listed below is that suggested by **ibidi Integrated BioDiagnostics**, the manufacturer of the channel used with the ECIS flow arrays.

- Method 1: Apply cell suspension ***(suspension volume should be = to the maximum channel volume for array type)*** into the end of the channel allowing capillary action to fill the narrow space. Quick dispensing helps to avoid trapped air bubbles. Inject 60µl of cell-free medium simultaneously into each reservoir at the ends of the channel to avoid flushing out the cells; avoid tilting the flow slide to prevent unwanted removal of cells from the channel.

- Method 2: Apply cell suspension ***(suspension volume should be = to the maximum channel volume for array type)*** and then place the flow slide in the incubator for the time required for cells to attach to the surface. Once the cells are in place, add 60µl of medium into each reservoir.

Use the following medium exchange protocol:

- Empty the reservoirs completely without emptying the channel.
- Inject the new media solution into one reservoir and remove the old solution from the other side. Make sure the old solution is completely replaced. For a ~99% exchange add about 3 x the channel volume from one side.

NOTE: In order to avoid air bubbles inside the channels, fill the channels by putting the pipet tip directly to the channel's inlet. Apply the volume with a constant and swift flow. When using very small volumes, it may be necessary to fill the channel with a low volume syringe (1.0 - 2.5 ml).

MEDIUM EXCHANGE

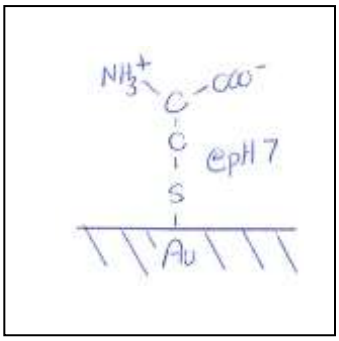
The suggested protocol listed below is that suggested by **ibidi Integrated BioDiagnostics**, the manufacturer of the channel used with the ECIS flow arrays.

- Method 1: **Continuous Medium Exchange - Recommended in Standard Use**
    - For continuous medium changing, remove the old medium from the reservoirs first. Then, add an appropriate amount of fresh medium into one reservoir and aspirate from the other reservoir at the same time. Carefully use a cell culture aspirator. We recommend a volume that is at least 3 times the channel volume. Refill the reservoirs.
- Method 2**: Complete Medium Exchange - Recommended for Expensive Liquids Only**
    - For replacing only the channel volume, empty the reservoirs first. Then, put the pipet tip right on the channel inlet and aspirate the liquid out of the channel carefully. Use a cell culture aspirator to completely remove all liquid. To refill the channel, inject fresh medium directly into the channel using the channel volume. Avoid trapping air bubbles. Refill the reservoirs.

CYSTEINE TREATMENT

Our preferred way to clean the electrodes is with the amino acid **L-cysteine**. This treatment cleans and modifies the electrode surfaces enhancing experimental repeatability with minimum variation between ECIS wells.

To accomplish this, the well should be flooded with a 10 mM sterile solution of L-cysteine in distilled H_2O. The solution can easily be prepared in your laboratory and filter sterilized, or the solution can be purchased from Applied BioPhysics*****. After exposure to the cysteine, the solution can be rinsed out of the wells, proteins adsorbed if desired, and the wells inoculated with cells. The time for the cysteine exposure depends upon the history of the array, for relatively newly etched arrays often the reaction is completed in only a few minutes, but to be certain the reaction has gone to completion, an hour-long exposure is recommended for all arrays.



The cysteine will form a covalent sulfur-gold linkage with the electrode surface likely displacing any unwanted small molecules that have adsorbed to gold surface over time and stabilizing the impedance. This cysteine layer provides a hydrophilic substrate that is excellent for protein adsorption and ultimately cell attachment and spreading.

Flow arrays: Add 100ul for 15 minutes

NOTE: You can purchase Electrode Stabilizing Solution in 50mL or 250mL bottles.

- ***50mL – Catalog Number: C-001***
- ***250mL – Catalog Number: C-002***

COATING WITH PROTEINS

ECIS electrodes shipped from Applied Biophysics have no macromolecular coatings. When culture medium is added to the arrays, proteins and other large molecules in the medium immediately adsorb to the very wettable gold surface as they do with any uncoated tissue culture dish. Often one wishes to define the substrate by coating it with proteins – for example collagen, gelatin, fibronectin or other ECM proteins.

We suggest using the following protocol: Prepare a solution of the desired protein at 100 micrograms per ml or more in 0.15M NaCl. We have found that the use of phosphate buffer (PBS) can seriously interfere with the adsorption of some proteins, so if a buffer is required, a mild Tris solution (e.g. 0.01M) is recommended. The electrode arrays are stable under acidic conditions, so when coating with collagen there is no problem using solutions containing acetic acid.

To coat the electrode, place the protein solution in the bottom of the well for 10 minutes or more. If the protein is valuable, lower protein concentrations can be used with increased times for adsorption.

Once adsorption has taken place, an approximate mono-molecular layer of the protein will coat the surface. You can safely rinse the protein solution from the well with sterile medium, saline or water without concern of removing the adsorbed layer. Medium can now be added and inoculation begun.

Remember, the first macromolecules the substrate is exposed to will be the final coating that the cells first encounter.

NOTE: We do not recommend drying the protein solutions in place as it can foul or damage the electrodes.