**Fiducialisation**

* Take out 1 ml of gold fiducials (beads sizes vary) and place in Eppendorf tubes
* Leave out the beads on the bench overnight for natural sedimentation
* Alternatively, put the beads in the mini spin centrifuge and spin for 15 minutes at the lowest speed (0.8 rpm) (Pipette out 1 ml of distilled water as balance)
* Once spinning is complete, take out 950 µL of the supernatant from the fiducials out and leave 50 µL (This method should be used for tissues or cells not grown in DMEM).
* Alternatively, if cells were grown in DMEM, take out as much supernatants as you can take out and leave the lump of gold particles only.
* Then add 30 – 50 µL of DMEM+serum to the beads
* Take fiducials to the vortex and vortex at speed 10 continuously to de-clump them
* During plunge freezing, add 2 µL of beads to each grid

**Discharging grids**

This deposits charges on the grids so that they become cell-friendly and accept adherence of cells to them

* Place grids (carbon side up) on a parafilm-wrapped glass slide
* Turn on the PELC Easiglow equipment
* Place glass slides on the metal stand
* Press auto for auto run
* Leave this for about 10 minutes

**Coating grids in serum**

Grids can also be pre-coated in FBS 8 – 24 hours before cells are seeded on them. To do this:

* Dip grids in 70% ethanol and place in 6-well plates containing serum
* Leave this for 8 – 24 hours before seeding cells unto them

**Coating grids with Poly-L-lysine (on the day of cell seeding)**

Grids can also be charged by discharging as described above and then soaking in Poly-L-lysine.

* Dip grids in ethanol for sterilisation
* Place grids in poly-L-Lysine for about 10 minutes
* Place grids in serum for about 5 minutes
* These grids are ready for cells to be seeded unto them