Content: Protocol for: 1) aldehyde derivitisation of glass slide surface and 2) attachment of cells

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Notes:
1. I usually do silanisation in 10-gap glass slide holders– they hold about 100ml of solution and 19 slides (zig-zagged). **Do not use plastic racks for silanisation (not resistant to HCl or acetone!!).**
2. Silanisation is same as for production of positive-charged adhesive slides (i.e. PCF cells), but with 4% organosilane instead of 2%.
3. Alternatively, commercially-available silane-coated or lysine-coated slides can be used (for example: – the technique is applicable to any surface with available primary amines. In this case, skip Part 1 of the protocol and move straight to Derivatisation.
4. When using aldehyde-derivatised slides **washing your cells <u>well</u> is crucial**. The presence of protein in the s/n will immediately block the reactive sites and prevent your cells from sticking. If they don't stick, you've probably not washed sufficiently.
5. As well as settling live, cells can be fixed in medium, <u>washed</u> and then attached to slides. However, if you do this bear in mind that:
   a) medium will quench reactive fixatives (HMI-9 w/ 15% FCS will mop up ~0.7-1% FA).
   b) the buoyancy of cells in medium+fixative is different from those in normal medium so centrifugation steps may need to be altered accordingly.

<!> Glutaraldehyde is v. powerful cross-linker and should be used in fume hood. **Do not throw down sink** until you have neutralised:
- add Na bisulph<u>ite</u> (**not –ate**) to <u>twice</u> the % concentration of Glutaraldehyde
- incubate 15' and dispose down sink

3-aminoalkylmethoxysilane – will react with atmospheric moisture: **reclose bottle promptly**

see COSSH forms particularly for: Glutaraldehyde, HCl, (3-Aminopropyl)triethoxysilane

Aldehyde-derivatised slides

1. Silanisation

- clean slides 10' in 1M HCl <!>
- rinse several times ddH2O
- wash 1' acetone to remove H2O
- silanise 5' with 4% amino-alkylsilane in acetone (I use 3-Aminopropyl triethoxysilane from Sigma (A3648), but other amino-alkyl silanes can also be used).

  (<!> water sensitive reagent – slides must be free of moisture)
- wash 1' acetone and air dry o/n at room temp or at 60°C for 1h.

2. Derivatisation

- **<u>In fume hood</u>** derivatise slides 5' in 5% glutaraldehyde in 0.1M $NaHCO_3$ pH8.5

  (Glutaraldehyde soln. is kept at 4°C and may be used several times, if you can salvage it. Alternatively, place slides <u>face-down</u> on 50ul drop of 5% glutaraldehyde on parafilm sheet.)
- rinse 2x 1' ddH2O and air dry

  (if required, can be speeded up by heating)

3. Attachment of cells

- spin down and wash twice/three-times in PBS (or PSG)

  (Cells **can not be settled in medium** as serum – or any other protein/amines – will block reactive sites. I find 2x washes sufficient for serum removal, but if you are more "generous" with leaving s/n on the pellet, then more will be required.)

- spin down again, resuspend in PBS (or PSG) at ~$2x10^7$ cells/ml

  ($2x10^7$ cells/ml gives a good spacing for most IF – if you don't get a good number of cells attached at this density then there is a problem.)

- allow to settle on slide 2' (live cells) or 10' (fixed cells)

  (Live BSF cells do not like being attached to slides for long periods of time, so for best results do not settle for >2mins. Fixed cells can be left for longer. Attachment only takes a few second, but only cells in contact with the surface will stick!! There is no point in putting on a large volume – it will make no difference. 10-15ul easily covers a 1x1cm square.)

- fix in style of your choosing

  (Cells fixed in medium are usually post-fixed at this stage to ensure good attachment.)

nb: before immunofluorescence, you should block unreacted aldehyde groups for 5' in 0.1% (w/v) Glycine/PBS. This can be incorperated into your rehydration/wash steps.