Ultrasonic Cleaning of Slides

1. Once slides have been stripped of old sample, they need to be cleaned by sonication. Place slides in a plastic slide holder (up to 5 slides per holder).
2. Pick out coverslips that are appropriate for the slides you are using (i.e. use a 24X60mm for a 5 lane slide or a stop-flow diagonal channel). For the prism TIRF, always use the 1.5mm thick coverslips. It is a good idea to clean twice the amount of coverslips as slides because they are easily broken.
3. Fill both slide and coverslip holders with 2% micro90 solution so that slides are covered.
4. Close the holder and wrap with parafilm so that it does not leak.
5. Place slide holder in floater in water-filled ultrasonic bath, Sonicate at RT for 1 hour.
6. Pour out 2% micro90 and rinse with MiliQ water in slide holder at least six times.
7. Repeat sonication with 200 proof Ethanol
8. Pour out 200 proof Ethanol and rinse with MiliQ water in slide holder at least six times.
9. Repeat sonication with 1M KOH
10. Pour out 1M KOH and rinse with MiliQ water in slide holder at least six times.
11. Pull out one slide with clean forceps and hold gently by edges. Make sure to use clean gloves. Be extra careful not to touch middle of the slides in the sample lane area.
12. Carefully dry slides with Nitrogen air gun (less than 60psi). Thoroughly dry all slides and cover slips on all sides.
13. Place dried slides and cover slips in a new clean, dry holder

Slide loaded in slide holder

Parafilm sealed slide holders in ultrasonic water bath

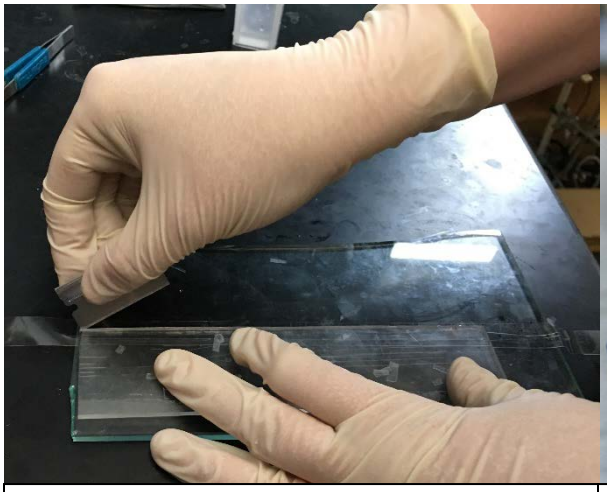
Silanization & PEGylation of Slides

1. Start with ultrasonically cleaned, dried slides
2. In a 50ml falcon tube, combine 20-25 ml Acetone and a Vectabond (aminosilane/APTES) aliquot (300 µL, premeasured aliquots can be found in the desiccator in cupboard below slide prep bench in Room 2228).
3. Pour Vectabond/acetone solution into slide holder with dried slides and let sit in a drawer at RT for 10 minutes. Retain 50ml falcon tube for next step. While waiting place a damp paper towel in the bottom of slide box, also prepare sodium bicarbonate solution now (step 2 of PEGylation) if time allows.
4. After incubation, quickly pour off and save Vectabond solution into the saved 50ml Falcon tube and replace with 200 proof ethanol. The aminosilane should not be exposed to air for long periods of time.
5. Rinse with ethanol 5-6 times. Leave slides and coverslips under ethanol until each is ready to be dried with Nitrogen air gun. Keep pressure below 60 psi.
6. Thoroughly dry slides with Nitrogen air gun and place in a different clean, dry holder; alternatively keep additional slides or coverslips in EtOH until needed to be dried.
7. Place one cleaned, silanized slide in a slide box. Careful to not let the middle of the slide touch any surface. Use label tape on short end of the slide to affix to the box, leaving room for coverslip placement in subsequent steps.

Clean Silanized slide taped in box

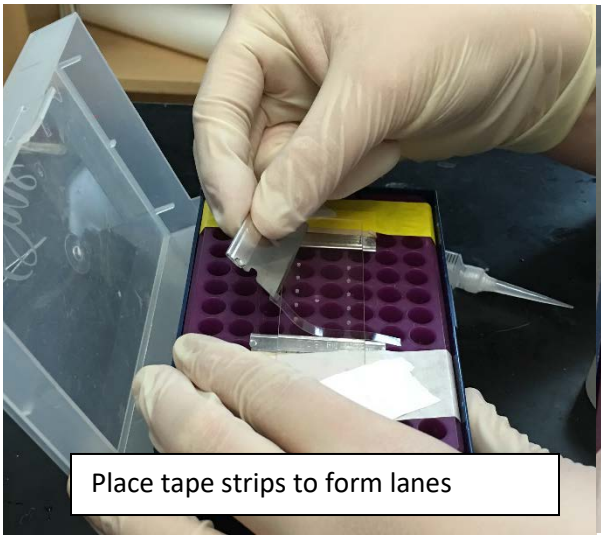
Creation of Lanes/Channels on Slides for Prism

1. Use a razor blade to cut thin strips of double sided Scotch tape and carefully place them on the slide to form lanes. See images below.



One method to cut strips of double sided tape

One method to cut strips of double sided tape



Place tape strips to form lanes

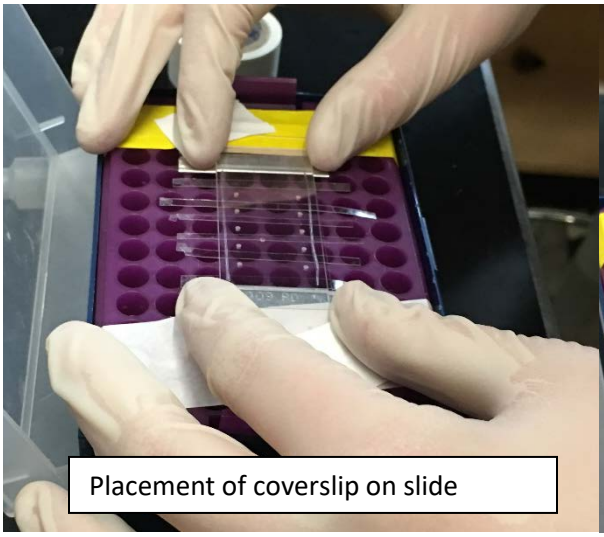
A slide with all lanes laid out

2. Seal the ends of the lanes with a small amount of vacuum grease. If using epoxy, place coverslip on first and then epoxy edges to seal the lanes.

Beads of vacuum grease seal the lanes

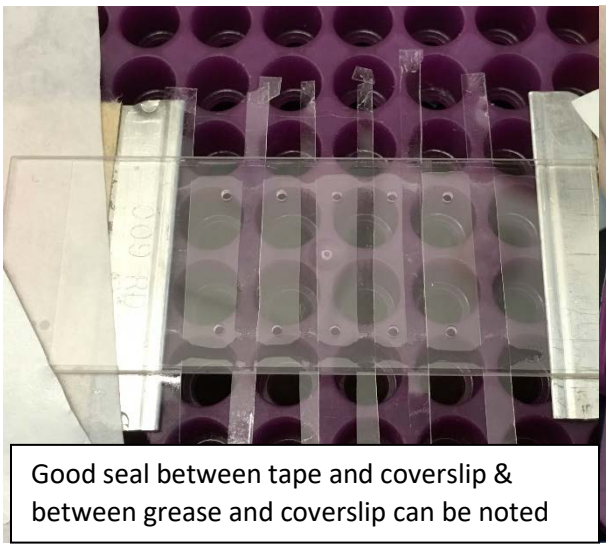
Taped lanes and grease beads on slide are ready for coverslip placement

3. Carefully place a clean, dry coverslip over the lanes and gently push down to adhere the coverslip to the tape. Be careful not to break the coverslip on top of the slide.



Placement of coverslip on slide

Gently press edge of syringe plunger on coverslip to make seal



Good seal between tape and coverslip & between grease and coverslip can be noted

Cut off overhanging tape with a razor

4. Turn slide over and tape in place.

5. Repeat lane making with all other slides (steps 1-5).

PEGylation / Biotinylation of slides

1. Take an aliquot of mPEG (close to 40-50mg) and PEG-biotin (about 2mg) from the -20 freezer.

2. Make a fresh 100mM sodium bicarbonate stock and sterile filter it:

   Weight out 84mg of sodium bicarbonate, resuspend in 10mL MiliQ water, filter with 0.22µm PES syringe tip filter.

3. Spin down the PEG-biotin (about 2mg) and add 200 µL of 100mM sodium bicarbonate per every 1 mg of PEG-biotin. Vortex to dissolve.

4. Add 2 times the weight of mPEG aliquot (40-50mg) in µLs of PEG-biotin solution made in step 3. (e.g. add 84 µL of PEG-biotin solution to 42 mg of PEG) Spin down and vortex the mPEG/PEG-biotin solution. This solution is only good for about an hour, so work relatively speedily.

5. Flow on 20-40 µL of the PEG/PEG-biotin solution into a channel on the slide. Don't push too hard in the hole of the slide because it may unseal the coverslip/tape. Slowly add the solution, and wick up the excess with a folded filter paper triangle at the other hole. Be wary of air bubbles in your pipet and wicking away too much solution on the other side. The entire channel should be filled with the solution with no air bubbles.

6. Fill all lanes with this solution and place the slides in their slide boxes. Place an extra drop of the solution over each hole, forming a slight dome, or cap, of solution to help protect the lane from drying out from evaporation.

7. Put a wet paper towel in the base of the slide box to keep the slide environment humid (if not done in earlier step already).

8. Place slide box in a drawer (dark conditions) at RT and leave it overnight (slides will be ready after 3-4 hours and can proceed with conjugation of sample – next page).

9. PEGylated slides are best when they are used the next day, but can be saved for a few days (week at maximum).

Immobilization of Sample on the slide

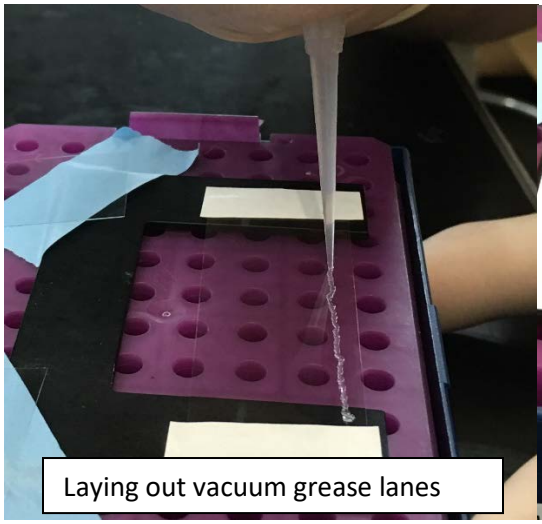
1. For a PEGylated slide, use 1X PBS sterile filtered buffer solution.
2. Wash the mPEG/PEG-biotin using 1X PBS. Flow on 20 µL of the PBS using a 20 µL tip. The solution is viscous and takes some time to wash it out. Wick up the excess using filter paper.
3. Wash the channel with an additional 200 µL of 1X PBS. This can be done with a 200 µL tip by adding the solution on top of the slide hole and sucking up the solution at the other end of the channel with filter paper. Watch out for air bubbles. If you get an air bubble and can't flow any more buffer through, use a smaller tip to add buffer directly into the slide hole until the air bubble is pushed through the channel.
4. Wash all the channels on all the slides planned for use.
5. Make a 1:40 - 1:50 dilution of Streptavidin from the 10 µL aliquot in the -80^{o}C freezer. (10 µL into 400 µL PBS final)
6. Flow on 60 µL of the Streptavidin solution and let it incubate on the slide for 2 minutes.
7. Wash away the Streptavidin with 1X PBS. Flow on 20 µL first to get rid of most of it quickly, and then thoroughly wash with 200 µL of 1X PBS.
8. Prepare sample to be in the range of 10-100 pM of the fluorescent molecule.
9. Prepare a wash buffer solution with oxygen scavenging solution (OSS). Dilute each component of the OSS (aliquots of Glucose Oxidase and Catalase are 100x stocks stored in the -80^{o}C, 2.5M glucose solution stored at RT) in 1X PBS. I usually make a 200 µL stock and add 2.0 – 3.0 µL of each. It is important to add the OSS to wash the sample, because OSS helps to reduce photobleaching by singlet oxygen. Sometimes it is also useful to add Trolox (also in -80^{o}C, 1:100 dilution from 100mM stock -- 3.0 µL into 100 µL, ~3mM) to reduce blinking.
10. Flow on 60 µL of the sample (or enough to fill the channel), again wick away excess with filter paper.
11. Let it sit for 2 minutes at RT.
12. Wash away with 200 µL of 1X PBS + OSS.
13. Repeat sample conjugation and washing with all lanes. Remember to do at least two negative controls to assess nonspecific binding.
14. Slides are ready to put on the microscope!

Cleaning Used Quartz Slides for Storage until Next Use

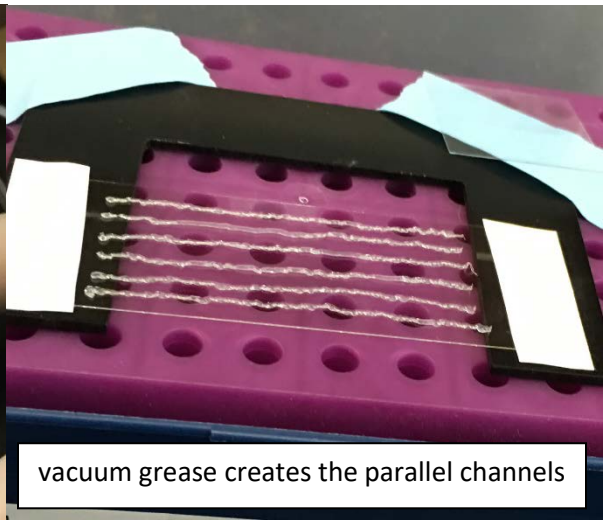
1. Used slides with coverslips can be stored submerged in water at RT for a while to loosen tape/coverslip interface, but follow these steps to prepare slides for next use:
2. Gently pull off coverslip and tape with a razor blade. Be careful not to scratch the surface of the slide.
3. Combine Alconox powder with a few drops of water in a weighing boat to make a paste.
4. Gently scrub the slide with the Alconox paste using either your fingers or a toothbrush. Be careful not to drop the slide or scratch the surface.
5. Scrub and rinse the slide in DI water several times. It is important to get rid of all the proteins/RNA that are still bound to the slide.
6. Thoroughly rinse the slide with MiliQ water after Alconox scrubbing.
7. Place slides on the edges of a clean 250 ml beaker so that no slides are touching and that the entire slide is exposed.
8. In the fume hood, prepare a solution of 3:1 ammonium hydroxide to hydrogen peroxide in the slide beaker. Fill up until slides are completely covered (should need about 250ml).
9. Boil the solution at 95^{o}C so that you can see bubbles emerging around the slides.
10. Let the slides boil in this solution for 1 hour.
11. Place slides into a clean weigh boat. Careful not to touch the middle of the slide.
12. Sterilize slides by flaming them with the blowtorch and forceps. Let slide cool down (hold it for 30 seconds) before placing it a container.

Slide Preparation for CoSMoS

1. Tape down clean, dry 22 x 60 mm coverslip (using half width tape strips) – as close to open end of "Π" bracket as possible.
2. Create lanes by laying down thin parallel beads of vacuum grease.

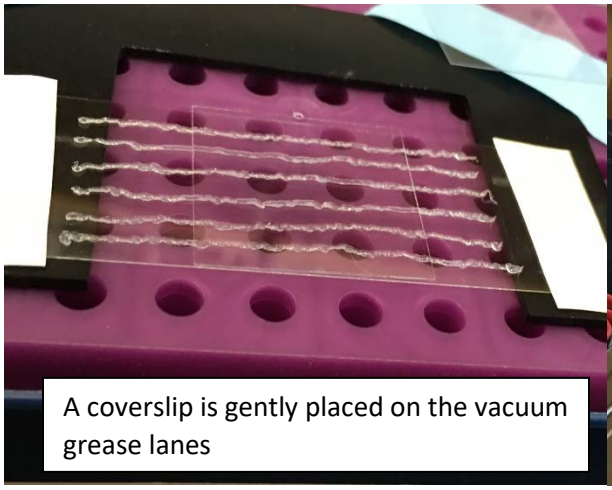


Laying out vacuum grease lanes



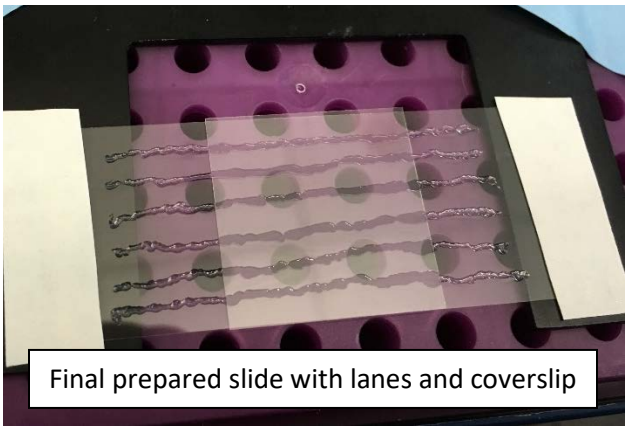
vacuum grease creates the parallel channels

3. Carefully place a clean, dry 25 x 25 mm coverslip over the lanes and gently push down to evenly adhere the coverslip to the grease. Be careful not to break the coverslip on top of the slide.



A coverslip is gently placed on the vacuum grease lanes

Gentle pressure is applied to the coverslip to seal the lanes



Final prepared slide with lanes and coverslip

Materials

Cover slips:

Gold Seal Cover Glass: 24 x 60 mm No 1.5 Reorder No 3423 (used as cover slide for prism and as slide for CoSMoS)

Gold Seal Cover Glass: 22 x 22 mm No 1.5 Reorder No 3406 (generally not used)

Corning Cover Glass: 25 x 25 mm x 1.5 mm Cat No 2890-25 (used as cover slide for CoSMoS)

Quartz Slides:

Quartz Custom Slides 1" x 3" x 1mm quartz microscope slides with 10 predrilled 0.039" holes per print. G. Finkenbeiner, Inc, Waltham, MA, USA. www.finkenbeiner.com

Acetone, HPLC grade, Fisher Scientific (or comparable), Cat No. A949-1, 1 Liter, www.fishersci.com

Ethanol, Ethyl Alcohol, 200 Proof, Absolute, Anhydrous, ACS/USP Grade, Pharmco-Aaper (or comparable), Cat No. 111000200, 1 Pint, www.pharmcoaaper.com, Brookfield, CT, USA

High Vacuum Grease, Dow Corning, Cat No. 1597418, www.dowcorning.com, Midland, MI, USA

Permanent Double Sided Tape, Scotch, 3M, Cat No. 665, 1/2IN x 900IN (25YD), 12.7mm x 22.8m, www.scotchbrand.com, St. Paul, MN, USA

5-Slide Mailer, End-opening, Natural Color 25/pk HS15986

Micro-90 Concentrated Cleaning Solution, 1 Liter, International Products Corporation, Cat No. M-9050-12, www.ipcol.com, Burlington, NJ, USA

Vectabond Reagent, (mostly 3-Triethoxysilylpropylamine, APTES) Cat No. SP-1800, unit size 7mL, www. vectorlabs.com, Burlingame, CA, USA. "Chemically modifies the surface of the glass with positive charges to form a highly adherent surface"

mPEG-SVA, Methoxy poly(ethylene glycol) succinimidyl valerate, MW 5000 – 1 g. Item No MPEG-SVA-5000-1g, Laysan Bio, www.laysanbio.com, Arab, AL, USA

Biotin-PEG-SVA, MW 5000 - 100 mg. Item No. Biotin-PEG-SVA, MW 5000-100mg. Laysan Bio, www.laysanbio.com, Arab, AL, USA

mPEG and Biotin-PEG-SVAs are also available as a kit. Item No. BIO-PEG-SVA-5k-100mg & MPEG-SVA-5K-1g. Laysan Bio, www.laysanbio.com, Arab, AL, USA