# Shuttle Drive Protocol

## Printing Pieces:

* There are three types of pieces that need to be printed:
* The STL file is in Jays github repo called ‘SpikeGadgetsShuttleDrive, but it can also be found on the Solidworks printer in Data/Jay.

1. The drive body
   1. This is printed in CLEAR v4. It is called ShuttleDriveBodyV4
   2. Print at 50 micron height. Clean thoroughly with alcohol, including the holes at the top AND bottom of each shuttle lane. Wash in the IPA bath under vortex for 10 minutes in the mirky bath and 10 minutes in the cleaner bath.
   3. Let the IPA dry off of the print before curing, then cure for about 30-45 minutes to properly cure
2. The shuttles
   1. These are printed in GREY or GREY PRO or Tough. The STL file will be a rack of about 40. Print one or two racks at a time.
   2. These can be printed at 50-100 microns, make sure the hook is perpendicular to the build plate.
   3. Grey needs less time in the alcohol bath (5-10 minutes in each bath) but the hooks and through holes will need to be hand cleaned (I use a 30Ga needle or cannula).
   4. Try to vortex a bit more vigorously for these, there are small through holes and very small inside features that need to be cleared
   5. Do not exceed 15 total minutes in IPA, the small features will swell and become soft
   6. Cure grey for 30 minutes
3. The guide for the tetrode cannulae:
   1. These will depend on your application, but building them will always be the same. I use clear v4, and try to print them at the same time as the drive body under the same IPA and curing parameters.

## Shuttles and screws

* The next step is to install the shuttles and screws into the drive and glue the screws in place.

1. Use a pair of forceps to insert a shuttle into the bottom of each lane. The shuttles should have an intact hook, the screw hole must be cleared already, and it should fit snug. The hollow lane in the shuttle allows the walls to pinch in by the drive lane. This minimizes the lateral movement of the shuttle when it is moving up and down. The shuttle is tall enough so that it won’t bend in the vertical direction.
2. Once the shuttles are in place, you can hand place about five screws into the top of the drive at a time. Then use a turning tool to turn each screw down to the top of its shuttle. When the leading tip of the screw gets to the shuttle, aim it into the through-hole and drive it through. The screw will create a threaded fitting through the hole so that when you keep driving it will lift the shuttle towards the top of the drive. When you are driving make sure you don’t hear a ‘crack’ as this might be the through hole cracking, ruining the threaded fit.
3. Once the shuttle has ascended about half way up, make sure the leading tip of the screw has found its hole in the bottom of the drive. Don’t be scared to apply a little bit of pressure to make sure that the bare end is hidden in the drive. This traps the screw at the top and bottom so that there is no lateral movement when the drive is in use.
4. Once you have gone around the drive and installed every screw and shuttle, make sure each shuttle is intact, and make sure that the leading tip of EVERY SCREW is buried. After the next step it is difficult to remove the screws and shuttles.
5. Finally, you will glue the screws in place with 5-minute epoxy.
   1. First gather a handful of 25 ga needles and crack the beveled head off with a pair of pliers (the beveled tips go in the sharps).
   2. Then gather a handful of 1 ml luer lok syringes.
   3. Mix about two kidney bean sized dollups of the epoxy together with a toothpick for about 10 seconds, and draw the mix up into a syringe slowly (make sure there are no air bubbles).
   4. Attach a 25g tip to the syringe and start pushing epoxy into the cup at the neck of the screw via the hole on the outer ring of the drive (off of the side). Once the cup is half filled, fill the rest through the top via the center side slot. There will be bubbles, but they will settle, if there remains air bubbles, wait and you can return to that shuttle later.
   5. Work quickly, as the epoxy will cure in the syringe. Once the syringe is seized, repeat the above steps until the drive is done.

## Guide Cannulae

* While the targeting of the tetrodes may vary per drive, the principles for Positioning the polyimide and polymicro guides remain the same.
* The polyimide is flexible, larger in diameter and comes in 1 foot increments
* The polymicro is thinner, more rigid and comes on a reel

1. Cut guide cannulae (both):
   1. About 1 revolution of the polymicro will slide into 1 foot of the polyimide. Slide the polymicro into the polyimide, and make sure it sticks out both ends evenly, and by about 5 cm each end.
   2. Bisect the polyimide-polymicro with scissors four times, making sure the polymicro sticks out evenly through both sides EACH time.
   3. You will need to do the above two steps about five times to get a total of 80 guides. This will leave you with about 16 extra cannulae, in the event that some get crimped or are the wrong height.
2. Insert guide cannulae into drive:
   1. Prep the guide by taping it down on the flat side to a flat surface (one of the blue screw lifts works well). You can either tape it down this way, or you can tape it down the other way by fitting a cut 5 ml syringe, or a cut 5 ml falcon tube around the neck of the guide.
      1. Alternatively, fit a 25 ml falcon tube around the neck of the drive, cut the bottom of the tube to the height such that the cannulae, even with the end of the falcon tube will themselves stick out of the drive body about 3-4 mm.
   2. Grab a handful of polyimide-polymicros and even the end of all tubes on one side. I do this by holding the bundle gently and pushing against a flat surface. Slide your fingers towards the surface allowing the even cannulae to stay put but those that haven’t reached the surface to slide towards it. The goal here is to have the polymicros even with the polyimides at that even surface.
   3. You can iterate through this by pushing the polymicros out a tiny bit with your hands and then pushing them back on the flat surface.
   4. Drop the cannulae into one of the guide holes even side down, making sure they fall all the way to the flat surface. They should fall freely, as you haven’t packed this hole tight yet.
   5. Once the last one has fallen freely, use forceps to insert a few extras one and make sure they descend to the flat surface.
   6. Pack this tight so that the cannulae don't move when you lift the drive.
   7. The top should now look like it has many uneven polyimides sticking up, and each one a polymicro sticking further up, also uneven. However, the bottom will have all perfectly even length polyimides AND polymicros.
      1. Bottom if you have falcon tube at bottom will mean that sticking out (into brain) will be even and the right length)
   8. Untape the guide, and examine the cannule going into the drive. They should all be even out this end. One trick I use is to flatten them out like a fan to see if I can find any that are low.
      1. If you are examining out the bottom of the drive (the head we’ll call it) you can use a microscope
   9. Pull each bundle up towards the drive about 3-4 mm, to check the height of these, drive down (lefty loosey) four shuttles at opposite ends of the drive. The cannulae should just reach under a shuttle that is driven all the way down.
      1. ONLY IF NEEDED
   10. Now you will glue these shuttles.
   11. You will use quik epoxy to glue the bottom. I use viscous epoxy because we don’t want the glue to bead up towards the tips via capillary action.
   12. Dab a tiny bit on the tubes as they exit the bottom of drive and on the drive around the exit point. Be wary of getting glue past the end of any of the polyimides, this will glue the polymicro to the polyimide.
   13. You can also clean the excess epoxy off the bottom with a cotton applicator or a tooth pick.
   14. Next, you can glue the cannulae on the inside with zap-a-gap on with a toothpick, and make sure you get zap-a-gap inbetween the tubes, you can push them around to make sure. Try not to glue these too far up in the direction of the shuttles, they will be bent, and glued again later.

## Attaching cannulae to the shuttles

* This is where fine motor skills become important

1. Glue the cannula-guide to the drive body. Be sure to get the axis right, I try to get the orientation of the cannulae perpendicular to the two tabs on the drive, but you can also make sure that one bank of EIB holes lines up with one bank of cannulae. You can use zap-a-gap directly out of the tube to glue this in, as the key-lock between the drive and guide is pretty tight already.
2. Now you need to cut the polyimide to just below the height of a shuttle when it is in the down position. The concept here is so that when the shuttle is in the down position, almost 100% of the polymicro is encased in the polyimide.
3. To do this drive a few spread out shuttles to the down position (this might also be a good time to test the epoxy that is trapping the neck of the antrin screws). Then you can use microdissection scissors to cut the polyimide. When you do this, make sure the polymicro has been pushed down so that the top of the polymicro is flush with the top of the polyimide. (this makes sure you dont cut more polymicro then is necessary, and prevents your scissors from crimping the polyimide.)
4. Once they have all been cut, you can push the polymicro back up into the drive, and loop them around the shuttle hooks.
5. Key here is planning. Make sure the polymicros arent crossing over each other to get to the shuttles, they should fan out evenly, and they should be grouped by brain region.
6. As you fit polymicros around the hooks, be sure to turn your shuttles all to the down position, this will be important later
7. Once all shuttles have a polymicro fitting nicely in its hook, you will need to use epoxy to glue all of the polyimides in place. The polyimide is flimsy, and needs to be rigid so that pushing the shuttles does not bend the polyimide and polymicro, rather than making the polymicro slip inside the polyimide.
8. To do this, I like to epoxy each bundle of polyimides from the drive head all the way up to the shuttles as close as possible. This provides a rigid frame so that the polyimides have very little room to bend (only where they are bare between the shuttle and the polyimide that is now encased in epoxy).
9. I generally start at the bottom and pull glue up towards the top slowly with a tooth-pick, so as not to dab the glue on the shuttles or where the polyimide ends.
10. Let this dry, and make sure its nolonger tacky.
11. Now you can drive all the shuttles to about half-up position, and push the top of the polymicros so that they stick only a mm or two above the top of the shuttle. Then, glue each with zap-a gap.

## Loading Tetrodes

* Before loading, you will need to get the drive in the jig, and be comfortable moving it in the jig
* You will also have to have a nice pool of spun tetrodes, you can either use our multi-puller, or you can do it your own way.

1. A picture is probably most helpful here: