**Protocol 12-87 The cortical representation of taste**

**Amendment to Bitter Chow for cortical mapping**

During the last 2 months we, in collaboration with Ed Boyden’s group at MIT, have been developing an automated surgical platform capable of doing craniotomies automatically. Our primary interest in this work has been to better the creation of imaging windows over the insular cortex- a hard to reach area located on the lateral portion of the skull in mice. Our hope is to optimize the usage of experimental animals so that we need to perform fewer surgeries and primarily reduce attrition rates due to bone regrowth.

Our systems are independently designed and implemented, so for the rest of the document we will describe of the Janelia version of the robot and in turn provide a manual that the MIT group wrote for their respective implementation.

The system is comprised of a [Sutter micromanipulator stage (MP-285)](http://www.sutter.com/MICROMANIPULATION/mp285.html), which supports electric [Foredom drill running at 38K rpm](http://www.foredom.net/k1090.aspx). The mouse sits on a stereotaxic stage positioned below the Foredom drill, fixed to a Narishige stereotaxic head mount. We have machined a set aluminum adapters in order to fit both drill to the Sutter stage as well as the Narishige adapter to the Thor breadboard support structures. These adapters support interchangeable parts (not only the Foredom drill) that we plan on running on this stage in the future. Powering the drill is a 5A, 0-60V BK Precision power supply- limited to 2A (the max current draw of the drill), coupled with a 3.1A fuse (on the drill). Please refer to the image below for a picture of the physical system.

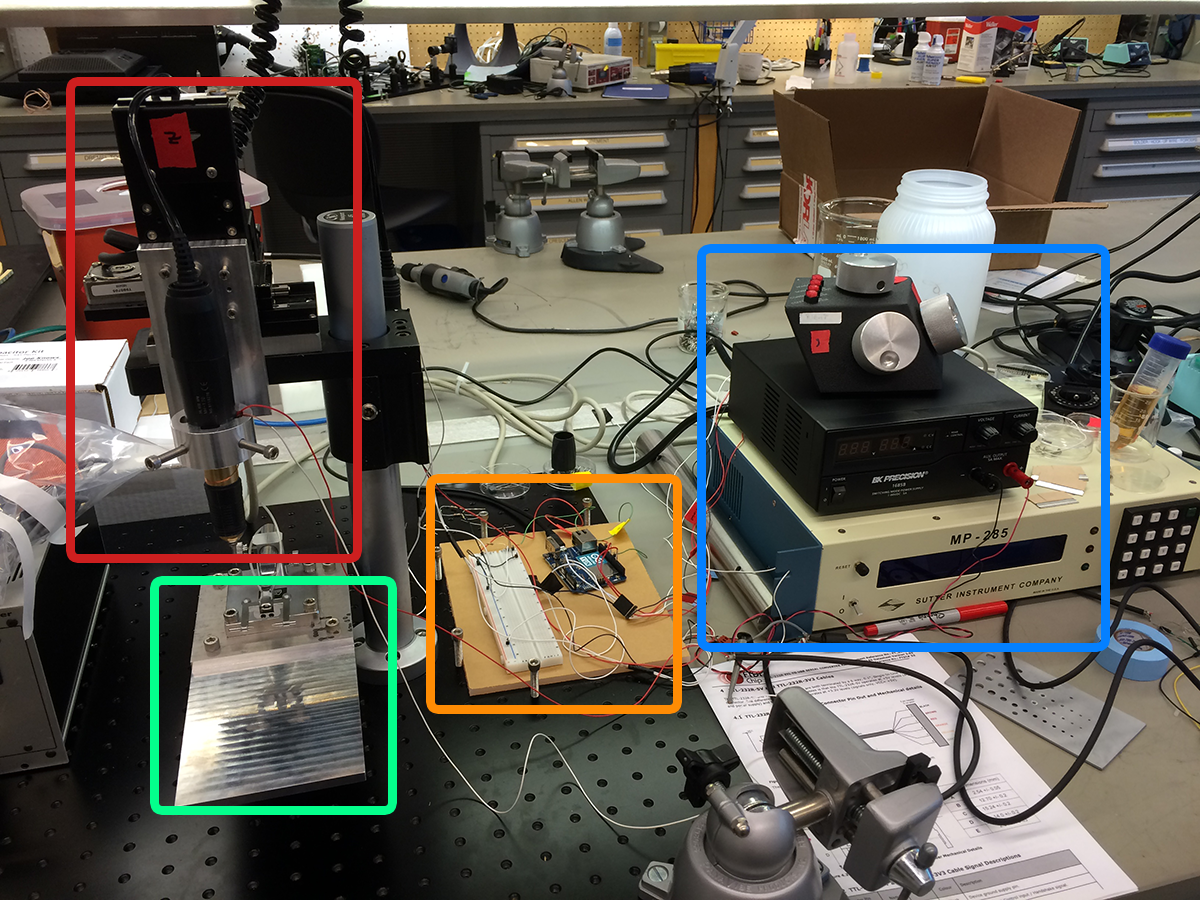


Fig 1. This is an image of the current robot with all components visible apart from the pc that runs in.

On the left side we see the stereotaxic stage with the machined connectors (green). The drill siting in its connector to the Sutter stage is also visible (red). On the right we see the BK precision power supply, the Sutter MP285 controller (blue) , and the Arduino microcontroller (orange).

In order to detect when the drill bit has pierced skull, we have developed a small circuit that aims to pass a sinusoidal wave pulse through the mouse with ultra-low currents (1-10pA). A circuit diagram is provided below. We utilize a NI-DAQ (USB 6009) to provide the output and record the input signals coming through the mouse. As we are dealing with very low currents, we have aimed to minimize noise by standard techniques - using shielding and reducing the length of wiring and doing passive low-pass filtering. Our DAQ outputs a 1-10mA 100Hz signal, and records the incoming signal through a standard analog input. Our software then performs Fourier analysis on the power spectrum to detect the signal based on an empirical threshold that accounts for various parasitic losses. Please see the schematic below.

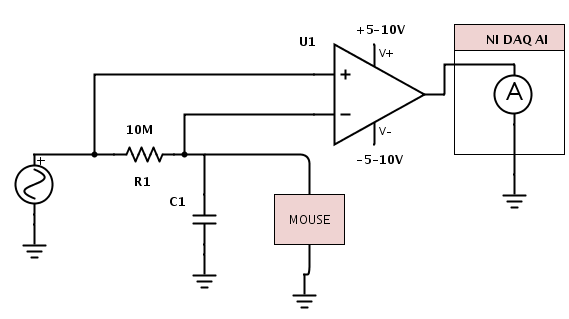


Fig 2. Schematic representing the sensing circuit. Current output at the power source will vary from 1-10mA. C1 represents the parasitic capacitances of the circuit. R1 is the 10M sense resistor. The amplifier in the diagram is an instrument amp ([AD621ANZ from DigiKey](http://www.digikey.com/product-detail/en/AD621ANZ/AD621ANZ-ND/750968)).

In its entirety the system is linked through a set of drivers to a Matlab control loop that we developed, running on a PC and communicating over TTL. An Arduino micro-controller is used to set the current and voltage provided to the drill, also through a Matlab/Arduino driver, which gives us control of both speed and the milling power of the drill (through the current draw, a critical parameter to minimize fracturing of the bone during milling). We have implemented full control points for all parameters of the drilling system including, moving in all 3 axes, speed and shear control of the drill. We had to implement a timed loop in order to create a 100hz signal as the low end NI board does not provide us with a continuous output signal functionality (an non-issue on higher end NI-DAQ boards). We have also implemented a Fourier signal decomposition function in order to analyze the frequency spectrum of the signal that is passed through the mouse.

The drilling protocol that we have coded implements the following logic. The user provides a set of points drawn from the perimeter of a shape. The robot then drills these control points with a settable step size (now set at 5 microns). At every step the robot evaluates if it detects the 100hz pulse, if not it continues, otherwise stops and moves on to next control point or finishes. The software then interpolates a path between those points and feeds it as position coordinates to the robot, which then drills non stop along the path with a settable speed for the stage and the drill speed.. This in turn ensures an optimal interpolation between points along any of the axis, most importantly the z-axis that represents the thickness of bone and varies throughout the skull. We will use robot for circular windows, but the system has already been expanded to support any set points along the perimeter of any shaped surface, where it uses Bezier spline interpolation in order to find the optimal path between the full set of points.

Our robot has 2 functional operation modes: depth probing and milling run in that order. Initially, the robot takes the user provided points as control points to probe the depth of the skull. It then drills test holes according to these points and stores the depths at which the skull was pierced, based on the signal detection described previously. Once the the first pass is complete, the robot switches into milling mode and interpolates (using Bernstein functions) the path between each pair of control points and mills accordingly. This represents the optimal path between the attributed point sets (where 2 points will always reduce to a line path). We usually aim to give 20 – 50 micron error threshold to minimize any local bone depth variations, meaning we just subtract that distance in the z direction. There is no signal detection while the robot is running in the milling mode.

We have also developed a real-time representation of the actions of the robot, which we plan to extend to a interactive GUI in the future, that will enable real-time interactions with.

At any time during the activity of the robot we have implemented a number of safety controls. We have functions that kill the drilling; the stage and that disconnect all systems from the computer. If for any reason during a procedure there is a bug/ error we can trigger these stops. Our initial testing will always be supervised, but we hope to extend this so that the system works independently including error catching and handling.

We have already tested the robot/ drilling protocol on various materials of similar Young modulus to bone, including wood and acrylic. We are currently performing test for the electrical properties of mice in order to acquire the proper signal parameters for our electronic circuit. Once these parameters are acquired the robot should be ready for animal testing.

We plan to set up the robot inside the vivarium within our surgical room, on the table by our surgical bench.

Animals during craniotomies will be held under isoflurane anesthesia according to the previously described procedures in the protocol. All preparatory steps for the craniotomies will also be the same. We plan to perform initially terminal craniotomies (approx. 5) to calibrate the parameters for milling the bone and ensure that the size match the inputted values. Once that is done we will move to perform windows implants following craniotomies and quantify how much the robot improves our attrition rates/imaging qualities.