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Building an Open-Source Robotic Stereotaxic Instrument

--Manuscript Draft--

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Abstract:	This protocol includes the designs and software necessary to upgrade your existing stereotax to a computer numeric controlled (CNC) stereotax for around \$1000 (excluding a drill), using industry standard stepper motors, and CNC controlling software. Each axis has variable speed control, a resolution of ~10µm, and may be operated simultaneously or independently. Our robot's flexibility and open coding system (g-code) make it capable of performing custom tasks that are not supported by commercial systems. Its applications include, but are not limited to, drilling holes, sharp edge craniotomies, skull thinning, and lowering electrodes or cannula. In order to expedite the writing of g-coding for simple surgeries, we have developed programs that allow individuals to design a surgery with no knowledge of programming. However, for users to get the most out of their motorized stereotax, it would be beneficial to be knowledgeable in Matlab® and G-Coding (simple programming for CNC machining).
Author Comments:	I have opted for standard access so far, but I will be discussion that with the lab to see if we have the funds to publish it Open Source. Also, I will email you a copy of the manuscript with figures embedded (I think it will be helpful for story-boarding).
Additional Information:	
Question	Response

March 25th, 2013

Allison Diamond

Journal of Visualized Experiments
17 Sellers Street
Cambridge, MA 02139

Dear Ms. Diamond:

Enclosed please find a manuscript entitled “Building an Open-Source Robotic Stereotaxic Instrument” submitted as an original report for possible publication in the *Journal of visualized experiments*.

The aforementioned report includes the designs and software necessary to upgrade your existing stereotax to a robotic (computer numeric controlled; CNC) stereotax for around \$1000. We also compare and contrast this technique with other comparable methods and enumerate the advantages and shortcomings of the preparation.

All co-authors provided significant contributions to the manuscript and were instrumental in developing the protocol in its present form. David Barker, Kevin Coffey and Sisi Ma prepared and edited the manuscript and figures. Dr. Mark West provided oversight for the project and edited the manuscript for content. All coauthors have seen and approve of the contents of the manuscript. There are no financial interests to be disclosed nor is this article currently under review by another journal. Please do not hesitate to contact us if further information is needed concerning this manuscript.

Thank you for your consideration.

Sincerely,

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Building an Open-Source Robotic Stereotaxic Instrument

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Keywords: Steoreotactic Surgery; Replicability; Open-Source; Robotic Rodent Surgery; CNC; G-Code;

Short Abstract: This protocol includes the designs and software necessary to upgrade an existing stereotaxic instrument to a robotic (computer numeric controlled; CNC) stereotaxic instrument for around \$1000 (excluding a drill).

Long Abstract: This protocol includes the designs and software necessary to upgrade an existing stereotaxic instrument to a robotic (CNC) stereotaxic instrument for around \$1000 (excluding a drill), using industry standard stepper motors and CNC controlling software. Each axis has variable speed control and may be operated simultaneously or independently. The robot's flexibility and open coding system (g-code) make it capable of performing custom tasks that are not supported by commercial systems. Its applications include, but are not limited to, drilling holes, sharp edge craniotomies, skull thinning, and lowering electrodes or cannula. In order to expedite the writing of g-coding for simple surgeries, we have developed customscripts that allow individuals to design a surgery with no knowledge of programming. However, for users to get the most out of the motorized stereotax, it would be beneficial to be

knowledgeable in mathematical programming and G-Coding (simple programming for CNC machining).

The recommended drill speed is greater than 40,000rpm. The stepper motor resolution is 1.8°/Step, geared to .346°/Step. A standard stereotax has a resolution of 2.88um/step. The maximum recommended cutting speed is 500um/s. The maximum recommended jogging speed is 3500um/s. The maximum recommended drill bit size is HP 2.

Introduction:

Stereotactic rodent surgery is used in a wide variety of neuroscience applications, including lesioning¹, iontophoresis², microwire implantation³, stimulation⁴, and thin skull imaging⁵. However, there are major hurdles facing those who wish to apply these techniques, including the steep learning curve for performing accurate stereotactic surgery and the high probability of human error. Human errors include measurement and calculation failures, as well as the low accuracy and replicability of human movements. In an effort to reduce these confounding errors, stereotactic surgeons would benefit from a system that ensures that all surgical procedures are performed identically across subjects. The reduction of errors is also one method by which investigators can minimize the use of animal subjects, a primary goal of the National Institutes of Health for animal experimentation⁶. In an ideal world, all stereotactic surgeries would be perfectly replicable within experiments, and between labs. To address this issue, companies have developed new ultra-precise stereotaxics, and digital displays for reading measurements. To remove human movement errors, motorized micro manipulators and stereotaxics were produced commercially, but their high cost can be prohibitive to a laboratory with a limited budget. Also, their software is fully proprietary, and cannot be modified by the researcher to accommodate a new type of surgery.

An affordable solution to the human error problem is to build a robotic stereotax from a lab's existing model, using industry standard CNC equipment. Because of a burgeoning CNC hobbyist community, the materials are significantly less expensive than scientific equipment. This allows one to build an accurate CNC stereotaxic instrument, which is also highly flexible and inexpensive. With a basic knowledge of CNC machining and G-Code, individuals can program any stereotactic surgery that they imagine, without the limitations of proprietary software. And, in order to expedite the production of g-code for simple surgeries, this protocol includes software that allows the user to design surgeries (sharp edge craniotomy, thin skull windowing, hole drilling & implant lowering) within point and click menus. These programs output a completed g-code that may be run directly from CNC software.

In all, a motorized stereotaxic upgrade is ideal for those who have an interest in increasing the accuracy and replicability of surgeries, while retaining the flexibility and low cost of an open source platform.

Procedure:

1. Wire the bipolar stepper motors by screwing the wires into the connectors supplied with the driver board. Wire colors on bipolar stepper motors are standardized (figure 1).

Note: The described stepper motors have a resolution of $1.8^\circ/\text{step}$, geared to $.346^\circ/\text{step}$. A standard stereotax has $3\text{mm}/360^\circ$ of travel. The final resolution is $2.88\mu\text{m}/\text{step}$. The motors are also capable of fractional stepping.

1.1. Connect the green wire to A+, connect the black wire to A-, connect the red wire to B+ and connect the blue wire to B-.

2. Slide the couplers over the stepper motors, being careful to align the mounting holes, and secure them using **12x** M3 Socket Head Screws (20mm) (figure 2).

2.1. Ensure that the couplers are firmly attached to the motors.

Note: The 3D models do not include threads. The parts are labeled with thread size, but they must be tapped after they are manufactured.

3. Remove the set screws from the thumb grips on all 3 axes of the stereotaxic instrument using a small hex key. The thumb grips are threaded, so turn them counter-clockwise for removal. Keep the PTFE washers in place on the arm (figure 3).

4. Screw the threaded end of the collars onto the threaded rod of the stereotaxic instrument's arms (figure 3).

4.1. Ensure there is no gap between the collars and PTFE O-rings. This guarantees that coordinates are maintained when the robot changes directions.

4.2. Secure the collars onto the threads of the stereotaxic arms using **3x** NF10-32 (1/4") cup point set screws.

4.3. Slide each motor and coupler over the collars & stereotaxic arms. Ensure that the motors sit flush with the arms, and the set screw holes on the collars line up with the flat portion of the motor shafts (figure 4).

4.4. Secure the couplers to the stereotax using the mounting holes and **6x** NF10-32 (1/2") cup point set screws (figure 4).

4.5. Secure the collars to the motor shafts using **3x** NF10-32 (1/4") set screws (figure 4).

5. Prepare the CNC driver board by setting each of the controller pins to half stepping.

Note: This stepper motor driver comes as an exposed circuit board. A case may be built, although it is not necessary. Also, a number of different bipolar stepper motor drivers may be used. If so, ensure that the setup instructions are followed for the specific board purchased.

5.1. Align all 6 pins per stepper motor in the same way. Half-stepping allows for double the step resolution in Degrees/step (figure 5).5.2. Flip pin 1 to the **on** position, pin2 to the **off** position, pin 3 to the **on** position, pin 4 to the **off** position, pin5 to the **on** position, and pin 6 to the **off**position(figure 5).

6. Plug the motors (X - Y - Z) into the stepper motor driver, along with the 12v power supply. The correct placement is marked on the driver. Also, attach the stepper driver to a computer's serial port using a DB25cable (figure 6).

7. Install CNC milling software onto a personal computer (this will need to be located in asurgical area) following the default instructions. Once installed, open the software to begin configuration.

7.1. Configure the software to communicate with the stepper motors.

Note: The following instructions are intended for use **only** with the **TB6560** stepper motor driver.

7.2. Click through the software's menus as follows. Open → Config → Ports and Pins → Output Signals. Fill in the prompt to match figure 7 and hit apply.

7.3. Click through the software's menus as follows. Open → Config → Ports and Pins → Input Signals. Fill in the prompt to match Figure 8 and hit apply.

7.4. Click through the software's menus as follows. Open → Config → Ports and Pins → Motor Outputs. Fill in the prompt to match figure 9 and hit apply.

7.5. Click through the software's menus as follows. Open → Config → Motor tuning. Fill in the prompt to match figure 10 and click **Save Axis Settings**. Repeat the previous step for all 3 axes using the same values.

8. Calibrate the stereotax to the scale of the CNC software.

Note: The software is designed for standard milling machines, so its unit of measure will not be proportional to the travel of a stereotaxic instrument.

8.1. Set the motors' velocity to 1 inch per minute, and "jog" the stereotaxic instrument with the arrow keys (X & Y) or PgUp/PgDn (Z) to the nearest millimeter.

Note: The maximum recommended jogging speed is 3500um/s and the maximum recommended cutting speed is 500um/s.

8.2. Record how far the CNC software travels per 1 mm of stereotax travel. This value is the "Scaling Constant" for the stereotaxic. Multiply skull coordinates (mm) by this value to determine their g-code position.

8.3. Perform random tests of all 3 axes by programming them to travel some known distances, and ensure the movements are accurate. If the stereotax travel is too far or short, modify the scaling constant accordingly.

Note: Once scaling is complete, the included custom scripts may be used to generate g-code for surgeries. However, it is highly recommended that users become familiar with g-code before attempting to auto-generate surgeries. This is imperative for troubleshooting and modifying automated surgeries.

9. Attach the micro motor drill to the stereotaxic instrument using the extra large probe holder. Note: The minimum recommended drill bit speed is 40,000rpm.

10. Auto-generate G-Code for a sharp edge craniotomy with 3 skull screw holes.

10.1. Place all of the custom scripts from the software table into a single folder on a PC.

10.2. Open the script "SharpEdgeCraniotomy.m" and run the code.

10.3. Select **Both** to the prompt "What Type of Surgery Will You be Performing?" (figure 11).

10.4. Select **Custom**, to define the corners of the skull window. Fill in each prompt to match figure 12.

10.5. Define the X and Y positions of the craniotomy corners. Each coordinate must be entered in correct order, according to the example in figure 13.

10.6. Enter 3 in the prompt to produce 3 skull holes (figure 14).

10.7. Select **Define Using Coordinates**, and enter the coordinates of each hole from the template in figure 15.

Note: If precise coordinates are not important, there is an option to point and click the holes' positions onto an image of a rat skull. Positions will be automatically generated.

10.8. Define the drilling parameters. For the first tests surgery, accept the default values.

Note: These values are dependent on the stepper motors, and the animal's skull. Every rat breed and target location has a slightly different skull thickness. For the initial few surgeries using this device, be prepared to test the drilling depths and remove any remaining skull pieces manually. The values can then be modified for future surgeries (figure 16).

10.9. Name the g-code; it will be automatically generated and saved to the working directory.

11. Load the g-code into the CNC milling software and a test skull into the stereotaxic instrument's ear bars.

11.1. Manually jog the drill bit to Bregma using the arrow keys. Use a slow jog speed (~5"/m) to ensure accuracy.

11.2. Start the drill bit rotating at greater than 38,000 rpm.

11.3. Press **CycleStart**; the stereotax will perform many passes of the same cut, at different depths. Between each pass, the stereotax will pause, so the surgeon may continue or abort cutting. Press **Continue Cycle** (Alt-R) to continue cutting passes.

Example Results

The end result of the surgery designed in the methods will be a rat skull with a sharp edge craniotomy, and 3 skull holes (Figure 17). Note that the skull used to demonstrate the surgery was much wider than the prototypical rat skull. The sharp edge craniotomy may be used to insert a microwire array into the brain, for high density neural recordings. The CNC stereotax may also be used to lower the array with great precision. Software is included in this protocol that allows the surgeon to define the parameters of a microwire, or cannula lowering. Sharp edge craniotomies could also be used to uncover large portions of motor cortex, for sensorimotor mapping studies.

The skull holes may be used to insert skull screws (figure 18). These can be used as anchors for dental cement when affixing a head stage to the animal. The holes may also be used to insert single electrodes into the brain. These electrodes may be used for anesthetized or chronic recordings. However, when using the stereotax for anesthetized recording, ensure the power to the motors is off before collecting data. The motors electrical properties may induce noise in the recording.

The CNC stereotax may also produce thin skull windows with a great deal of accuracy (figure 19). These windows may be used for in-vivo optical imaging in anesthetized animals. The uniformity of the thin skull allows for even light penetration, which is necessary for comparative or quantitative analysis of optical imaging data.

Discussion

The use of automated surgery equipment helps to eliminate some of the most common problems in neuroscience research. First, the tool paths are 100% reproducible. Every cut is guaranteed to be in the same location relative to Bregma. Second, it should reduce experimenter error. Although many researchers are highly skilled surgeons, it takes an exceptional amount of practice to become even a competent surgeon. This device will allow new students to quickly and easily perform highly accurate surgeries. Third, motorized surgery devices should reduce the number of animals needed to perform an experiment⁶. Surgeons will need less training, and mistakes will be made less frequently. Finally, the motorized stereotaxic is capable of making more precise and accurate movements than the human hand, allowing for more resolution in coordinate choice.

In today's neuroscience climate, there has been a push to increase the accuracy of surgical methods and techniques. It is no longer enough to target a brain region as a whole

when it is clear that smaller sub-regions exist, and that they could be functionally distinct. One example comes from research focusing on microinjections into hippocampus. Not only do individuals desire to target sub-regions, like CA1 and CA3, but they wish to study dorsal and ventral subfields within these regions⁷. However, targeting these regions with a manual surgical technique is exceedingly difficult. The benefit of the motorized stereotaxic approach is that, once the correct coordinates of a target region are identified, every future surgery may be directed to the identical location. However, it's important to note that morphological differences in the skull and brains of animals will still contribute some error to surgeries.

Another field that would benefit from automating surgeries is chronic microelectrode implantations. Some labs are attempting to lower electrodes into sub-regions of nuclei, such as the sub-regions of globus pallidus or ventral pallidum⁸. Lowering electrodes with a motorized stereotaxic instrument will not only increase accuracy, but should increase recordable neuron yield. This is due to the fact that microwires cause damage as they are lowered. The robot is capable of lowering the electrodes slower than human hands and at a constant rate, minimizing damage to axons or neurons that very well may be afferent to the target region.

The robot's level of accuracy should also be beneficial to those who are imaging through skulls, to obtain quantitative measures of optical density⁹. The amount of light that may enter and exit through the skull is dependent on the skull thickness. Our motorized stereotax is capable of ensuring that the entire surface area of the thin skull window has identical depth. This helps light penetrate the window equally across its entire surface.

It is important to note the limitations of the included surgery generating software. First, all corners will be rounded off to the radius of the drill bit. For the hole drilling code, the diameter of each hole is dependent on the drill's diameter. For the thin skull window and craniotomy codes, the center of the drill bit will follow the cutting path. This means that size of the window will increase on all sides by the radius of the drill bit. This may be remedied by subtracting the radius of the drill bit from the corner dimensions. Also for the thin skull window and craniotomy codes, the depth of drilling is static on each pass. This means that the entire window will be drilled to the same depth, regardless of the curvature of the skull. This is especially important to consider at extremely lateral coordinates, where the skull curves ventrally. However, there are no such limitations to the hardware, and researchers need not use the included software. With proper understanding of g-code, users may create surgeries from scratch which perfectly match the contour of the specific skull being used. Also, any hole larger than the current bit diameter may be made using simple circle interpolation. Movement in 3 dimensions is only constrained by the travel of the stereotax, and the user's proficiency at g-coding.

In all, automating surgeries provides a number of benefits for a modest cost, and as such, is becoming an increasingly popular technique^{10, 11}. But it is important to recognize that the accuracy of the robot depends on quality machining, proper setup, and proper understanding of how CNC machines operate. As long as researchers are willing to take the time to understand the functioning of this motorized stereotaxic instrument, they may perform surgeries more accurately, with better replicability, and with less training. This makes integrating a motorized stereotaxic instrument into experiments a smart choice for any lab that performs a large number of surgeries.

Figure Captions:

Figure 1.Wiring diagram for stepper motor plug.

Figure 2.Assembly diagram for attaching the couplers to the stepper motors.

Figure 3.Assembly diagram for attaching the collars to the stereotaxic arm.

Figure 4.Assembly diagram for attaching the motors/couplers to the collars/stereotaxic arm.

Figure 5.Switch diagram for setting the stepper motor driver to ½ steps.

Figure 6.Wiring diagram for stepper motor driver.

Figure 7.Configuration diagram for output signals in CNC software.

Figure 8.Configuration diagram for input signals in CNC software.

Figure 9.Configuration diagram for motor outputs in CNC software.

Figure 10.Configuration diagram for motor tuning in CNC software.

Figure 11.Prompt for choosing surgery type in the “surgery designing” software (sharpedgecraniotomies.m).

Figure 12.Prompt for choosing custom or preset target coordinates in the “surgery designing” software.

Figure 13.Prompt for entering window coordinates (4 corners) in the “surgery designing” software.

Figure 14.Prompt for choosing the number of skull holes in the “surgery designing” software.

Figure 15.Prompts for choosing the method of placing skull holes, and for entering the hole coordinates in the “surgery designing” software.

Figure 16.Prompt for defining the drilling parameters in the “surgery designing” software.

Figure 17.A skull showing the end result of running the previously designed surgery.

Figure 18.A skull showing skull screws inserted into a hole produced during the example surgery. The drill bits size will determine the hole’s diameter and consequentially the screw size.

Figure 19. Shows the skull from the example surgery, containing a thin skull window, indicated by the arrows. Note that in the right panel, (skull lit from within) light seems to penetrate the thin skull window uniformly. Also note that the window does not need to be square, or contain 90° corners.

Acknowledgments:

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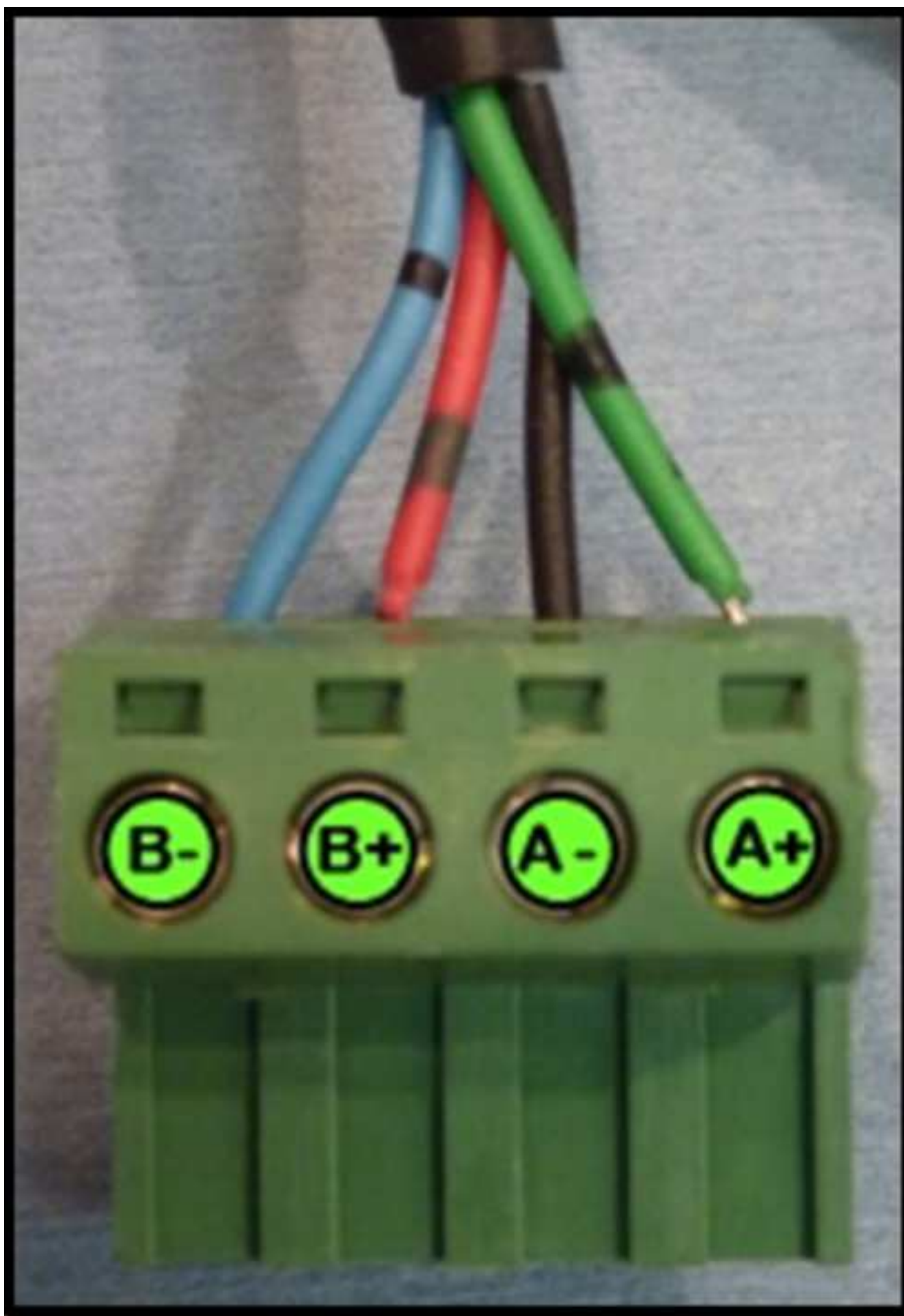
Disclosures: The authors have no competing financial interests to disclose.

References:

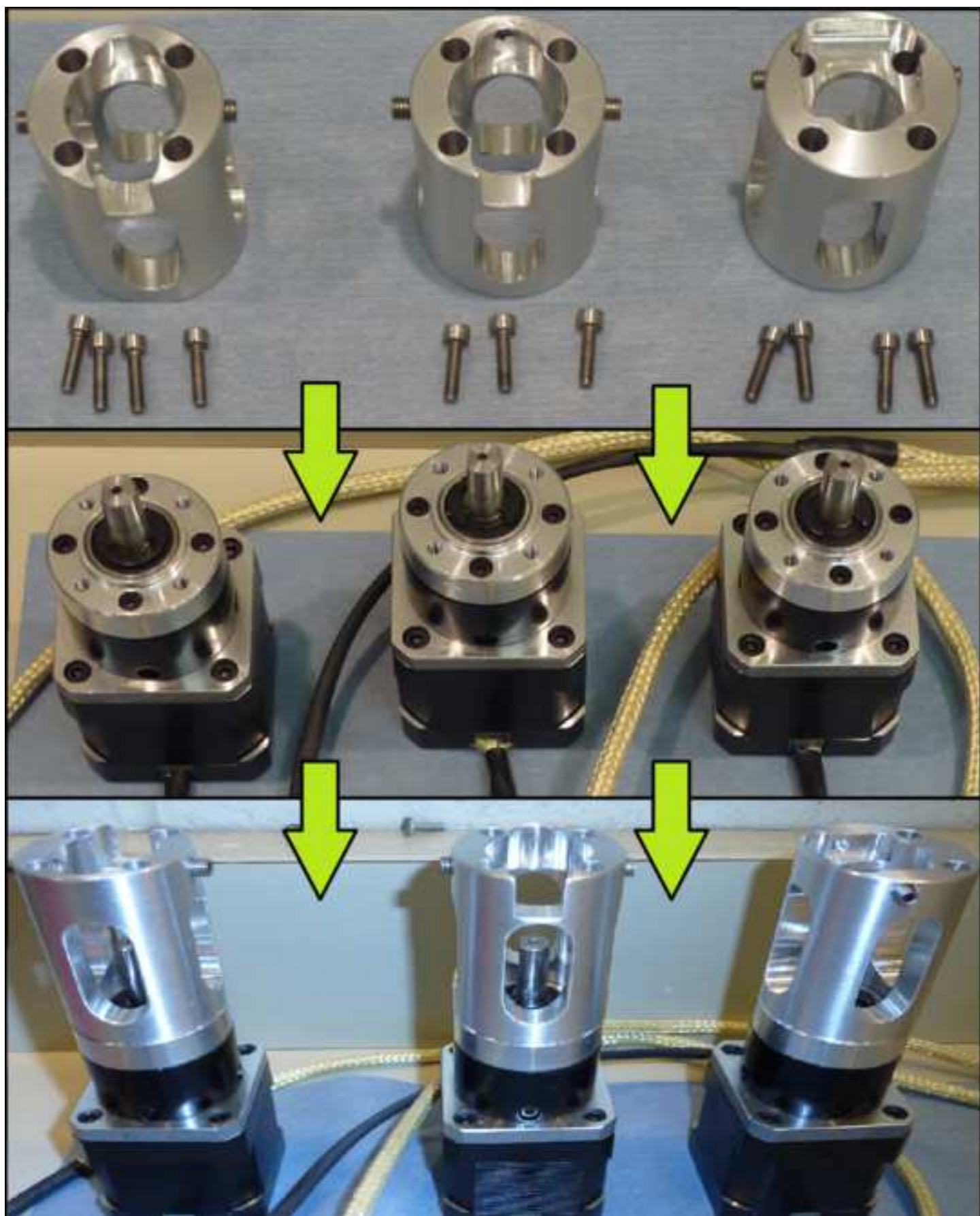
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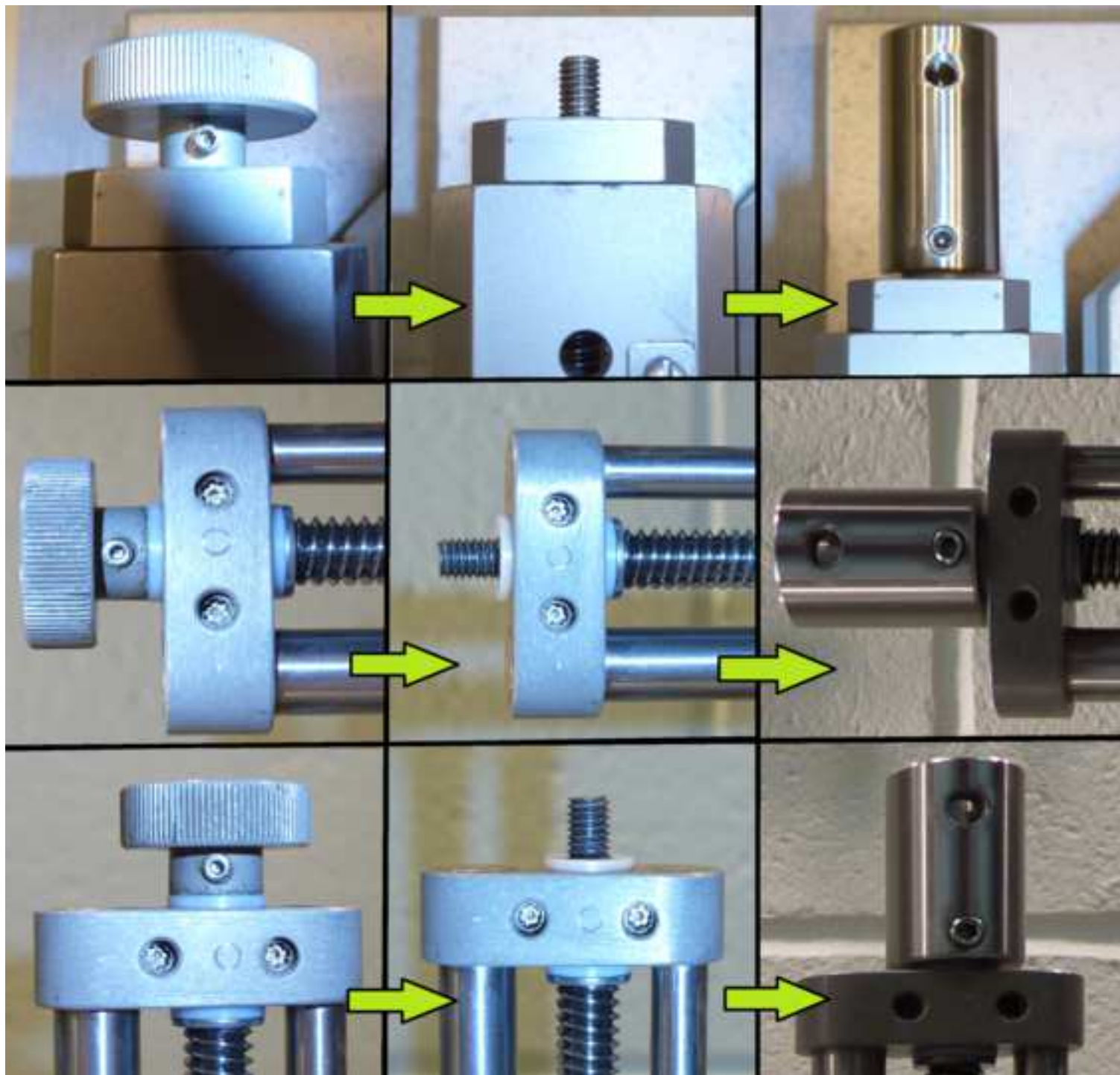
*Figure 1
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*Figure 2
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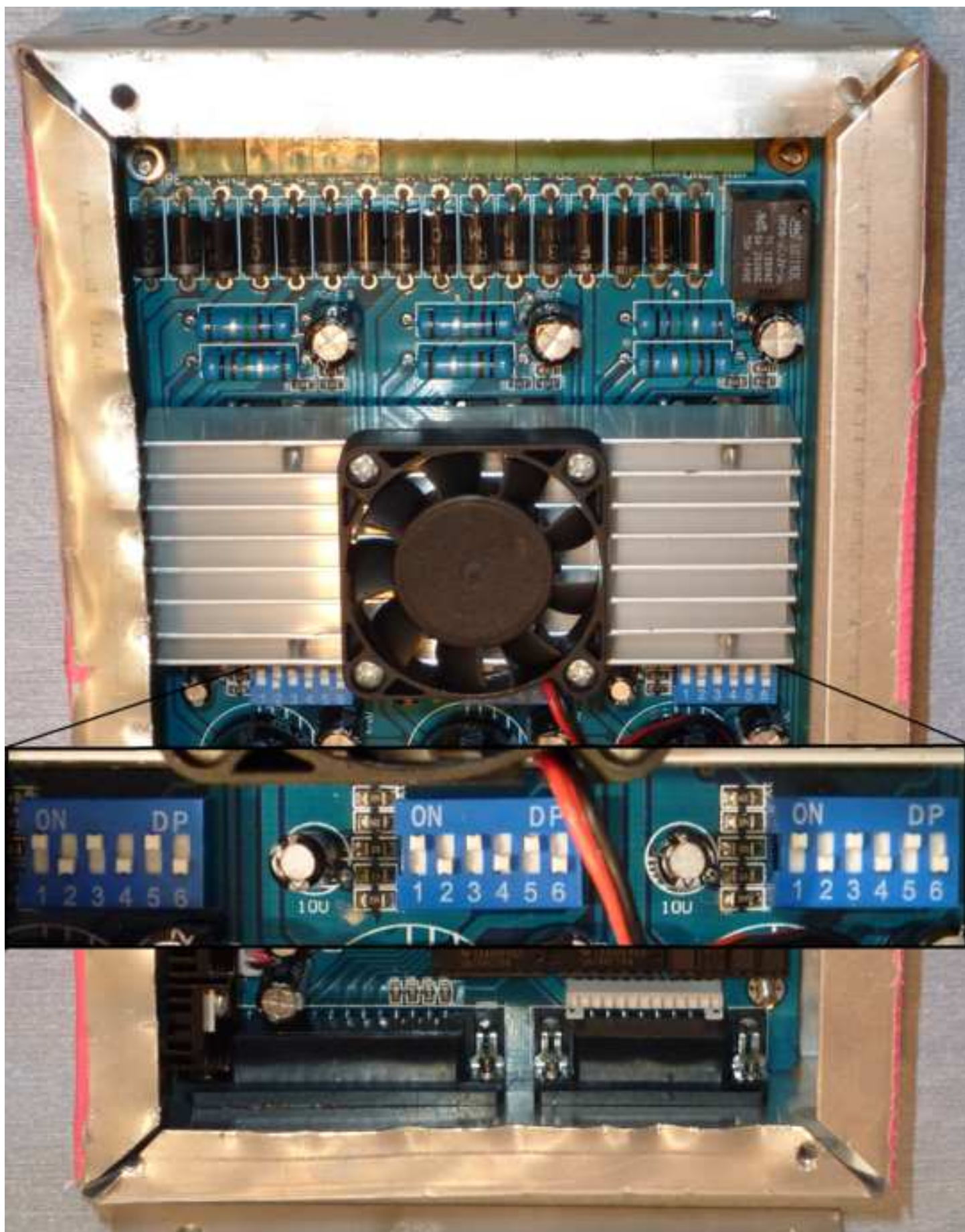
*Figure 3
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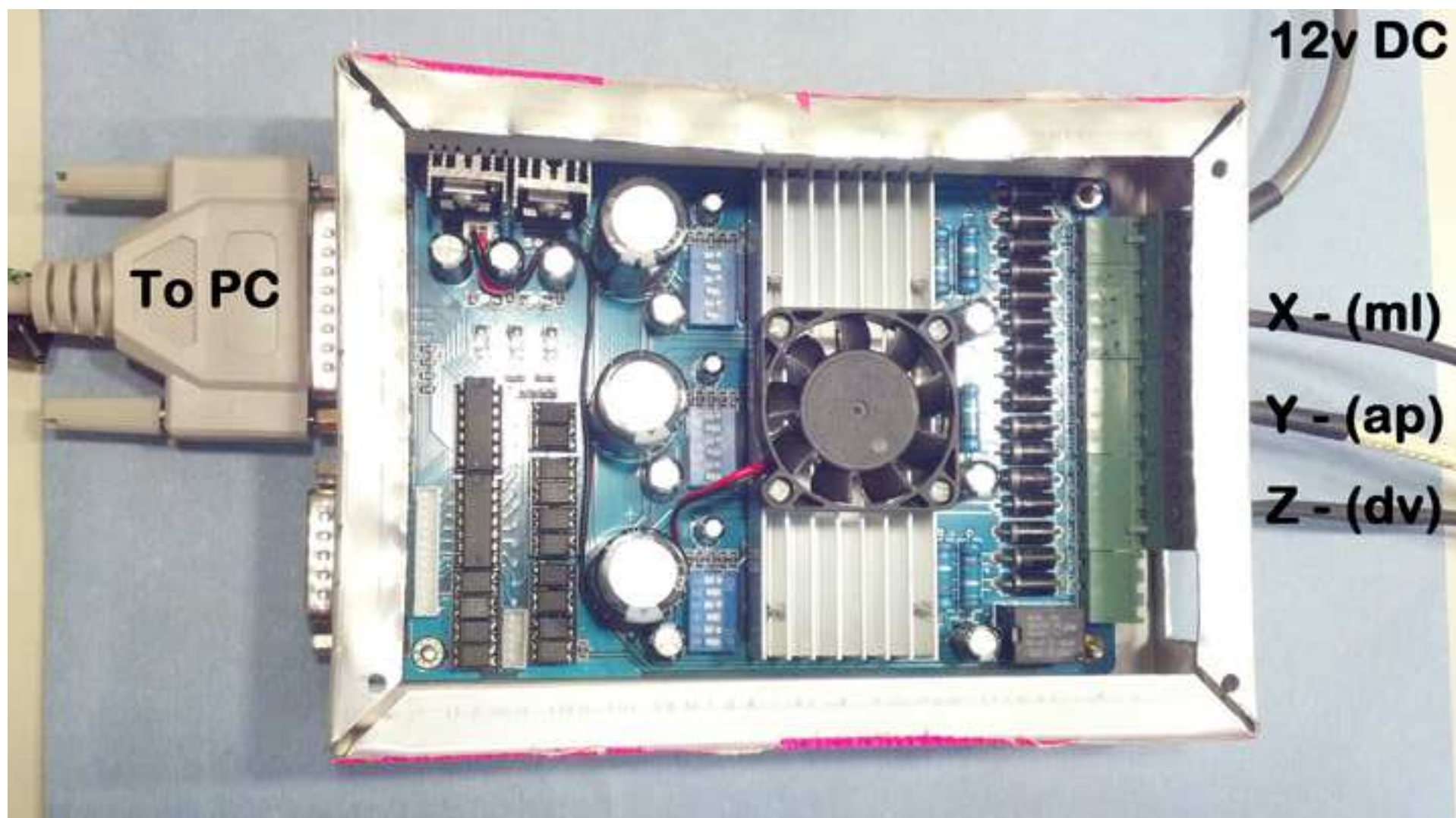
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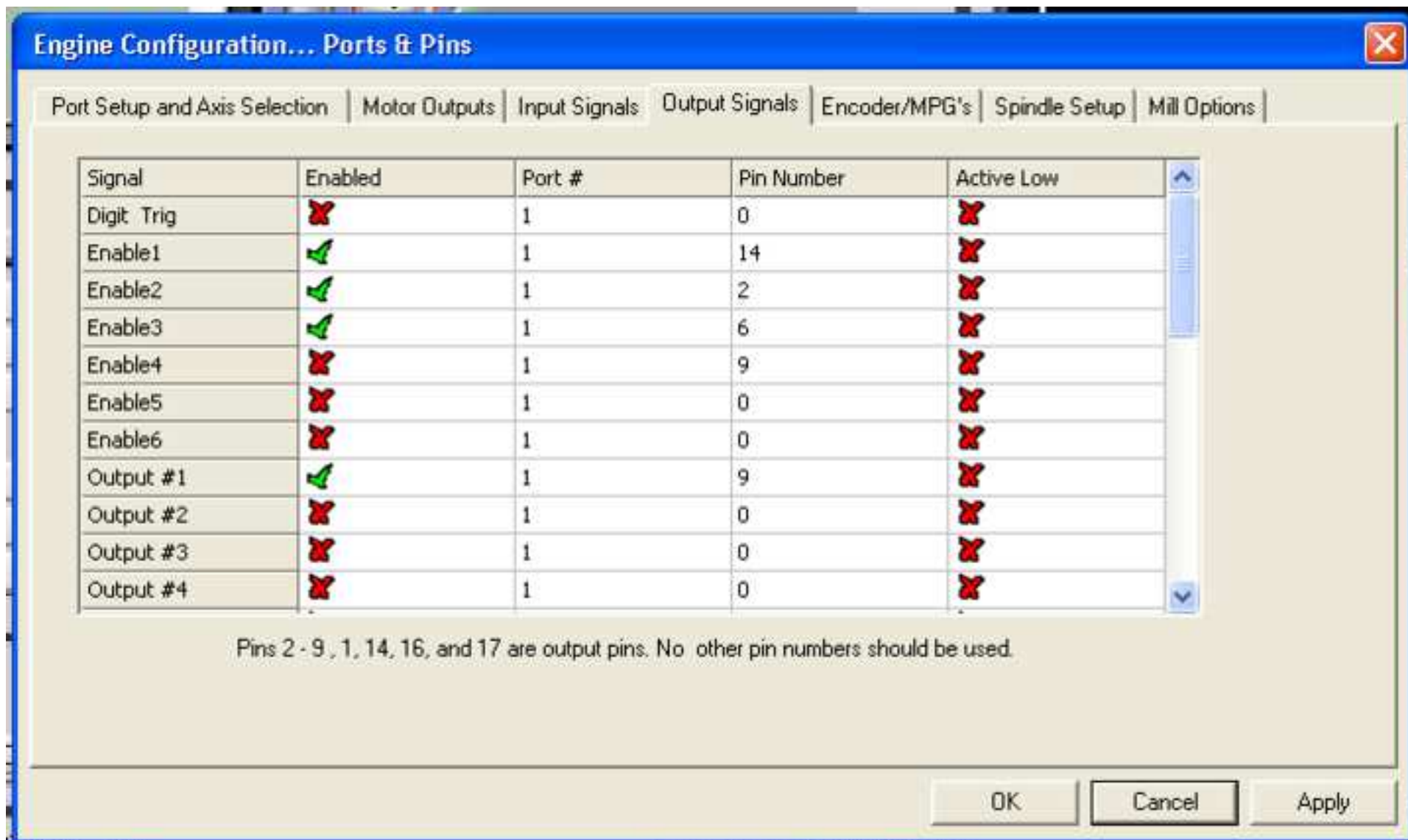


*Figure 6
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*Figure 7

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The image shows a software dialog box titled "Engine Configuration... Ports & Pins". It has a blue title bar with a close button (X) in the top right corner. Below the title bar is a tabbed interface with six tabs: "Port Setup and Axis Selection", "Motor Outputs", "Input Signals", "Output Signals", "Encoder/MPG's", "Spindle Setup", and "Mill Options". The "Port Setup and Axis Selection" tab is currently selected. Inside this tab is a table with five columns: "Signal", "Enabled", "Port #", "Pin Number", and "Active Low". The table lists various signals and their configurations. A vertical scrollbar is on the right side of the table. Below the table, there is a text note: "Pins 2 - 9, 1, 14, 16, and 17 are output pins. No other pin numbers should be used." At the bottom right of the dialog are three buttons: "OK", "Cancel", and "Apply".

Signal	Enabled	Port #	Pin Number	Active Low
Digit Trig		1	0	
Enable1		1	14	
Enable2		1	2	
Enable3		1	6	
Enable4		1	9	
Enable5		1	0	
Enable6		1	0	
Output #1		1	9	
Output #2		1	0	
Output #3		1	0	
Output #4		1	0	

Pins 2 - 9, 1, 14, 16, and 17 are output pins. No other pin numbers should be used.

OK Cancel Apply

*Figure 8
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Engine Configuration... Ports & Pins

Port Setup and Axis Selection | Motor Outputs | Input Signals | Output Signals | Encoder/MPG's | Spindle Setup | Mill Options

Signal	Enabled	Port #	Pin Number	Active Low	Emulated	HotKey
X ++		1	10			0
X --		1	10			0
X Home		1	10			0
Y ++		1	11			0
Y --		1	11			0
Y Home		1	11			0
Z ++		1	12			0
Z --		1	12			0
Z Home		1	12			0
A ++		1	0			0
A --		1	0			0

Pins 10-13 and 15 are inputs. Only these 5 pin numbers may be used on this screen

Automated Setup of Inputs

OK Cancel Apply

*Figure 9
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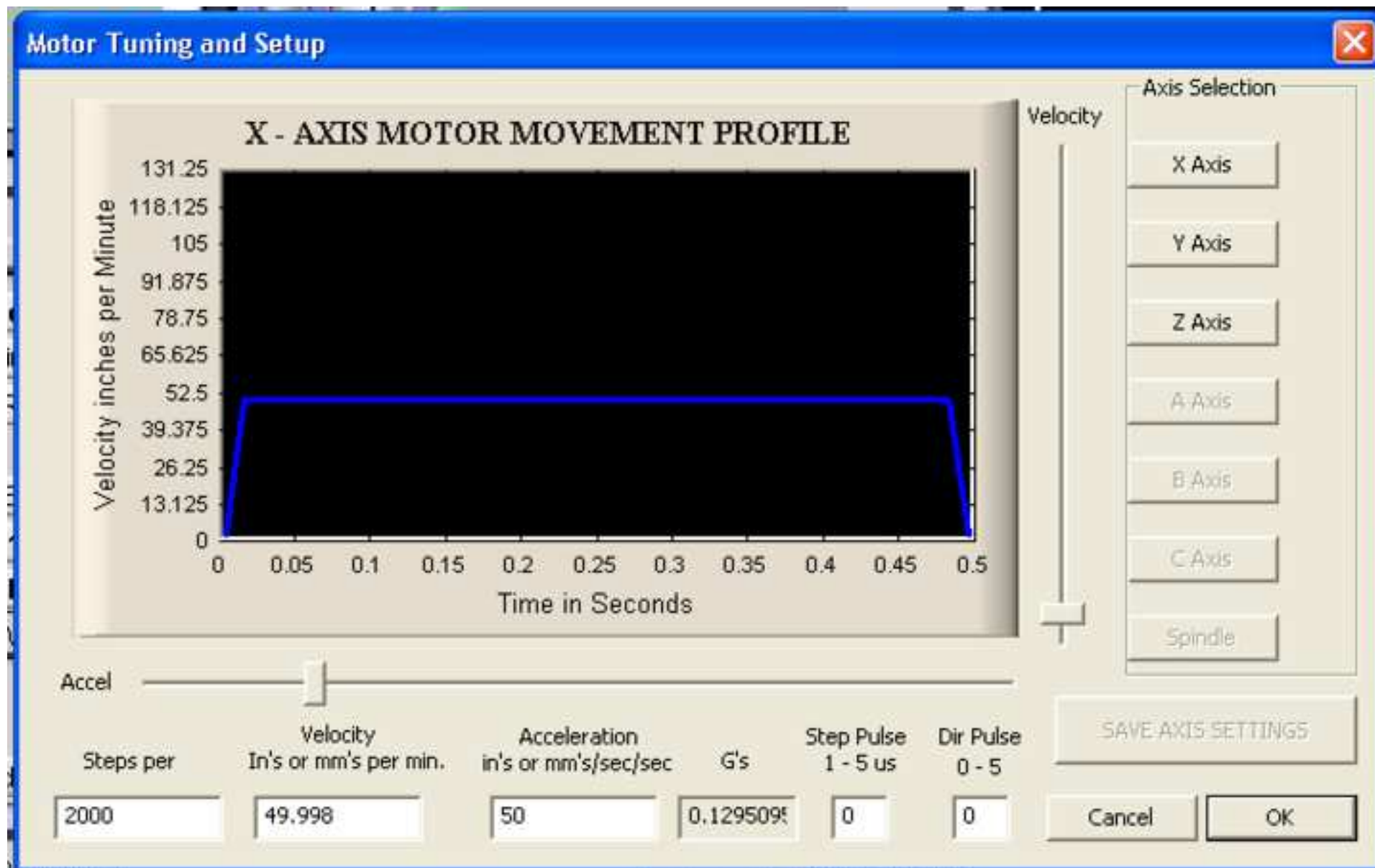
Engine Configuration... Ports & Pins

Port Setup and Axis Selection | Motor Outputs | Input Signals | Output Signals | Encoder/MPG's | Spindle Setup | Mill Options

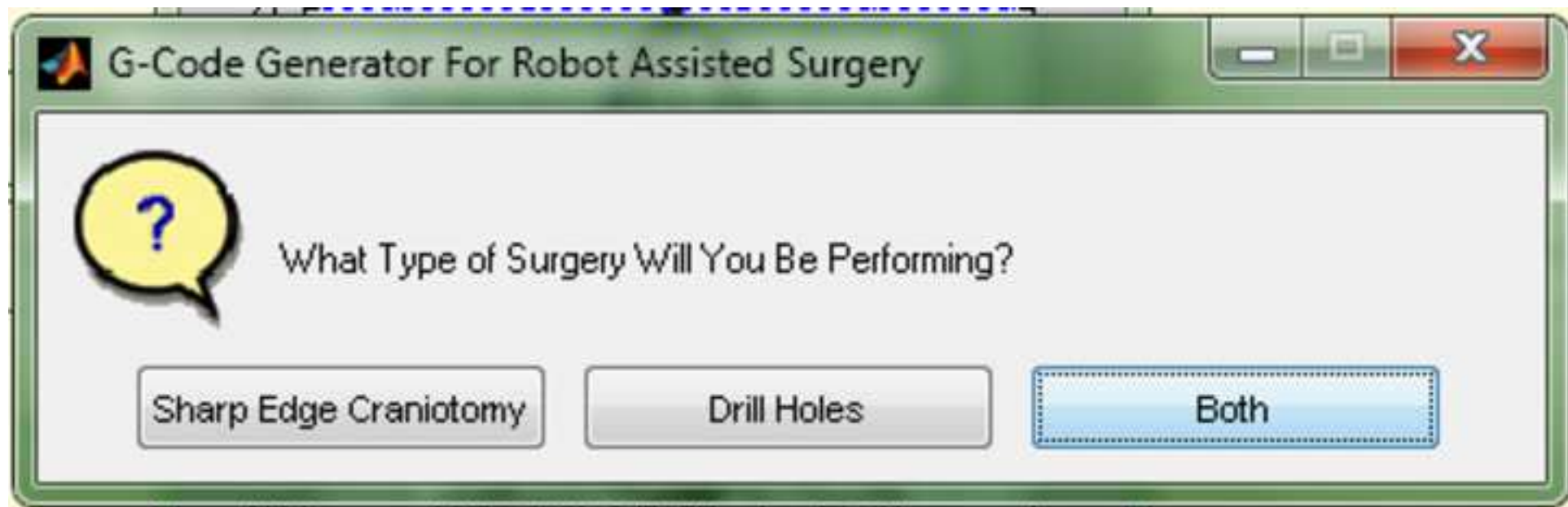
Signal	Enabled	Step Pin#	Dir Pin#	Dir LowActive	Step Low Ac...	Step Port	Dir Port
X Axis		1	7			1	1
Y Axis		8	3			1	1
Z Axis		5	4			1	1
A Axis		5	9			1	1
B Axis		0	0			0	0
C Axis		0	0			0	0
Spindle		0	0			0	0

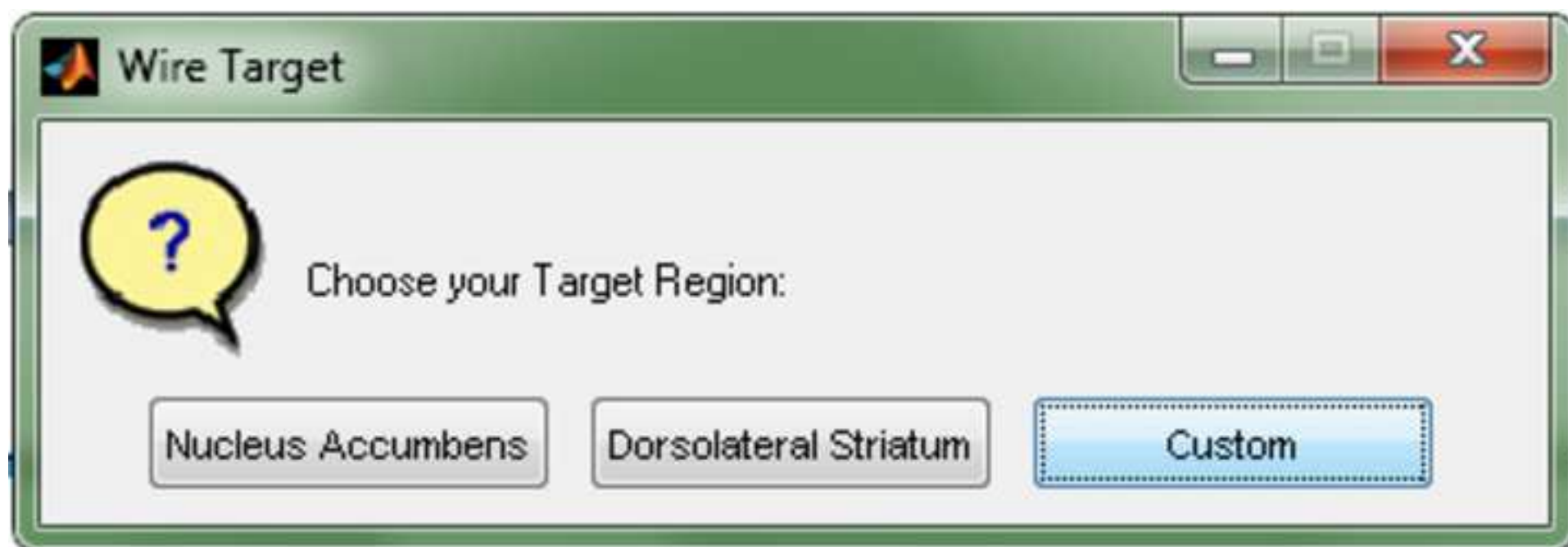
OK Cancel Apply

*Figure 10
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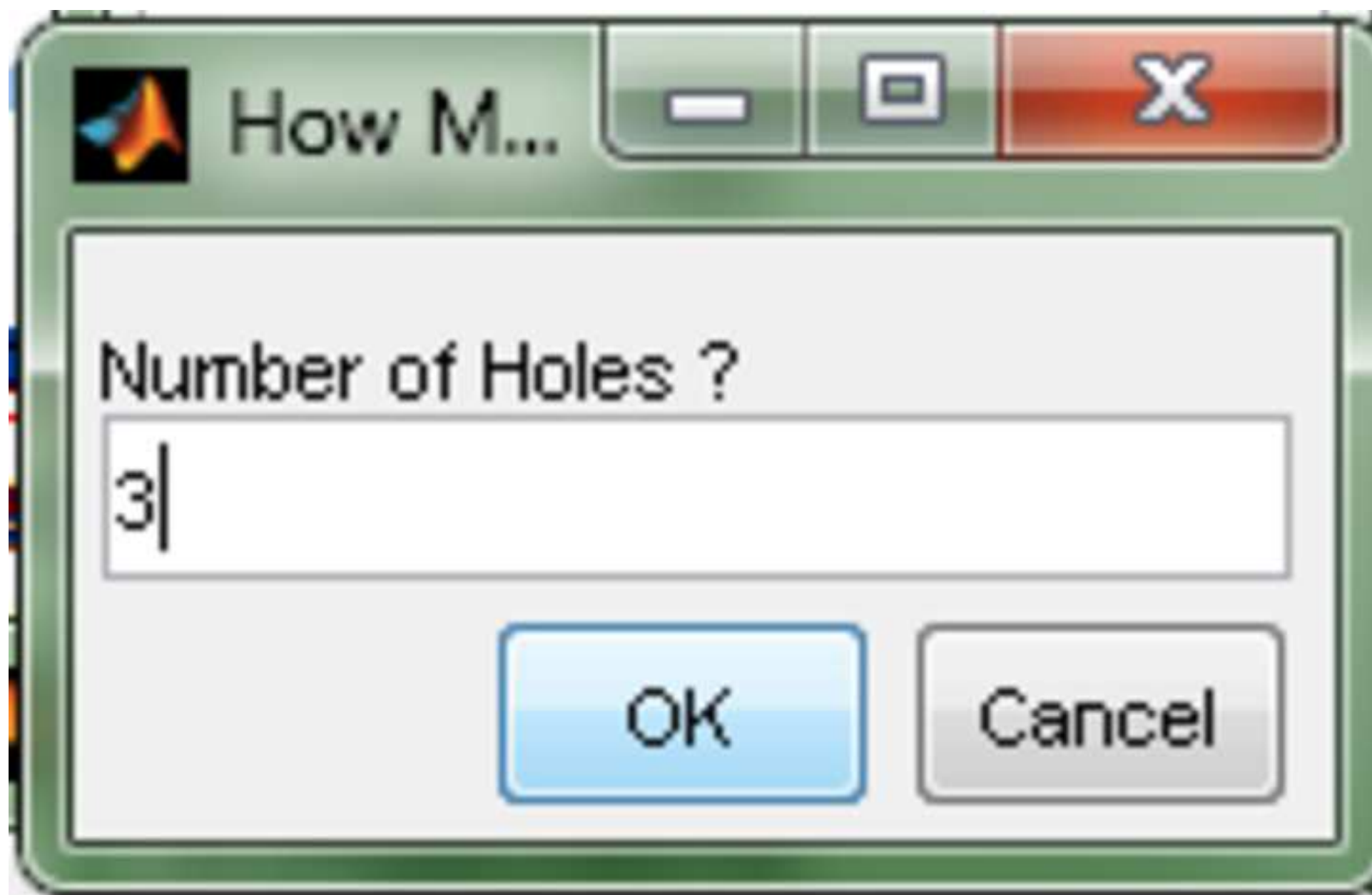


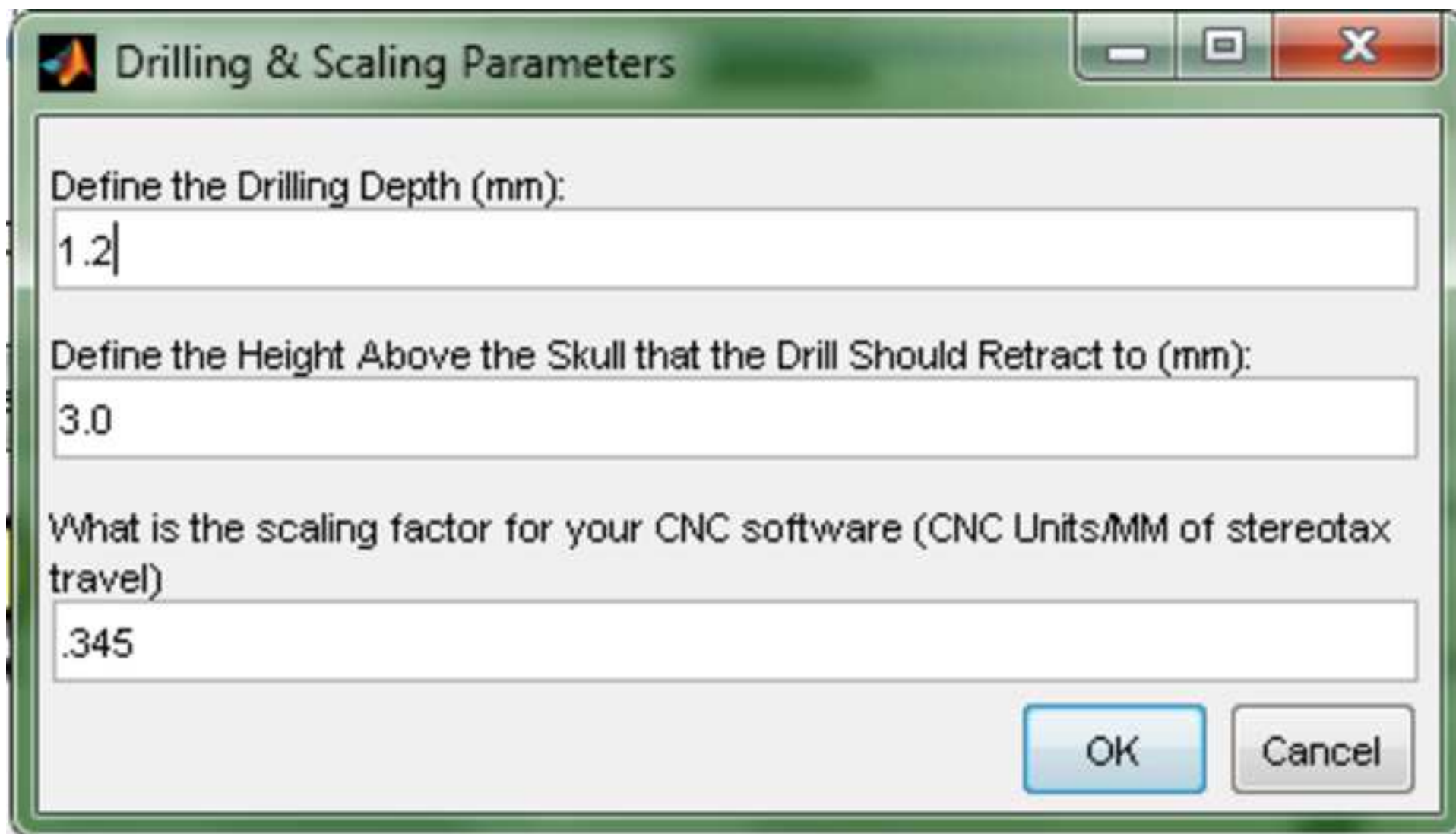
*Figure 11
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*Figure 14
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A screenshot of a software dialog box titled "Drilling & Scaling Parameters". The dialog box has a green title bar with a small icon on the left and standard window controls (minimize, maximize, close) on the right. The main area is light gray and contains three text input fields. The first field is labeled "Define the Drilling Depth (mm):" and contains the value "1.2". The second field is labeled "Define the Height Above the Skull that the Drill Should Retract to (mm):" and contains the value "3.0". The third field is labeled "What is the scaling factor for your CNC software (CNC Units/MM of stereotax travel)" and contains the value ".345". At the bottom right of the dialog box are two buttons: "OK" and "Cancel".

Drilling & Scaling Parameters

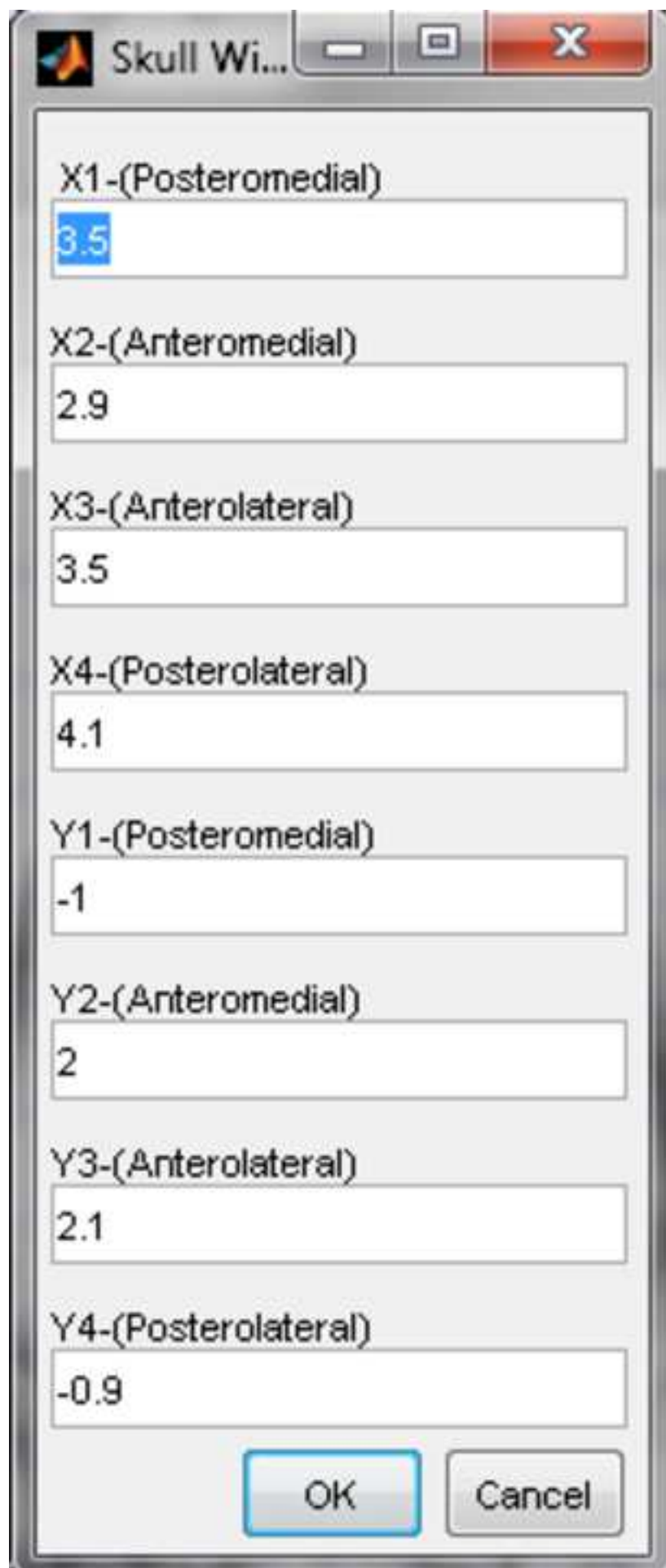
Define the Drilling Depth (mm):
1.2

Define the Height Above the Skull that the Drill Should Retract to (mm):
3.0

What is the scaling factor for your CNC software (CNC Units/MM of stereotax travel)
.345

OK Cancel

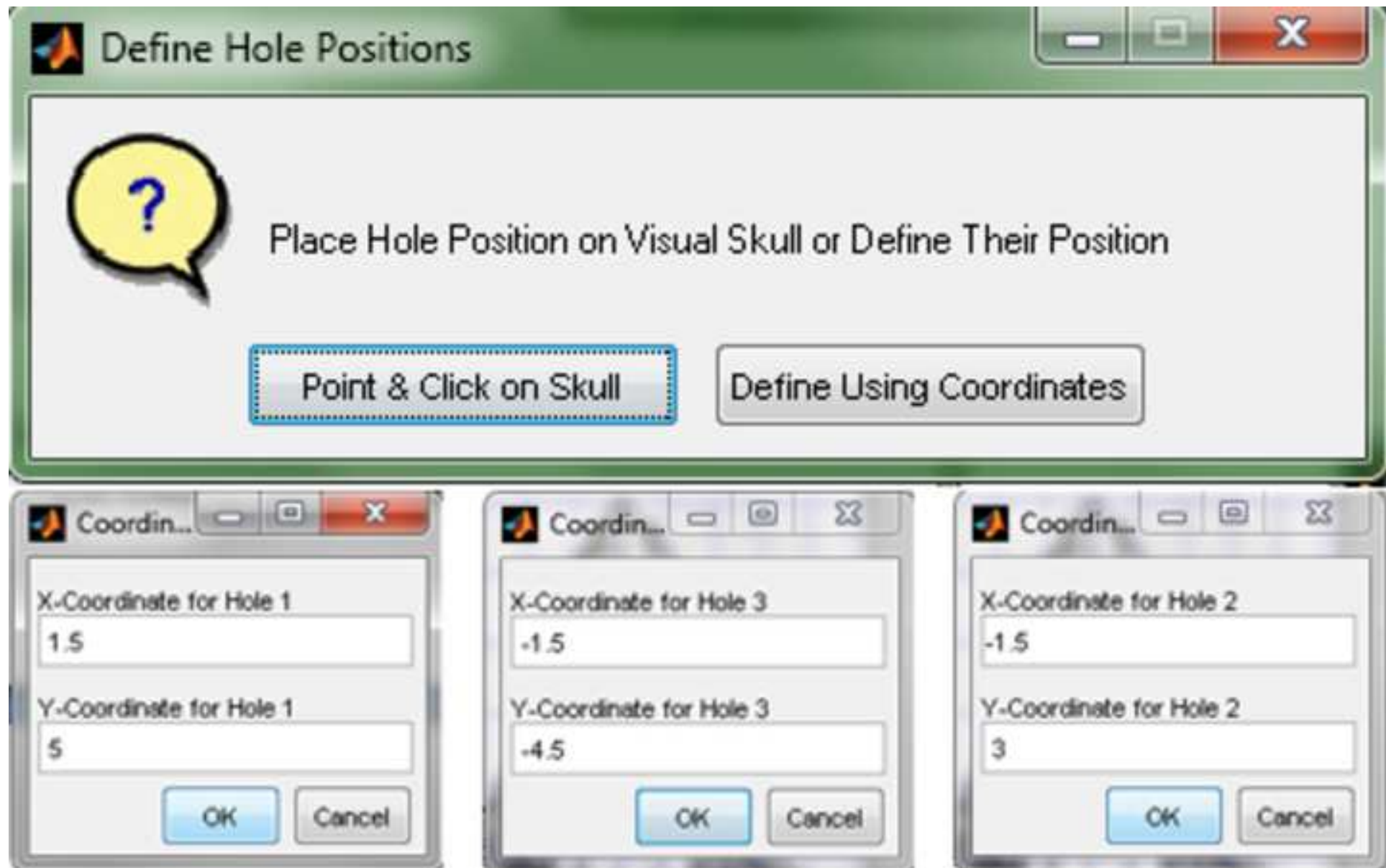
*Figure 13
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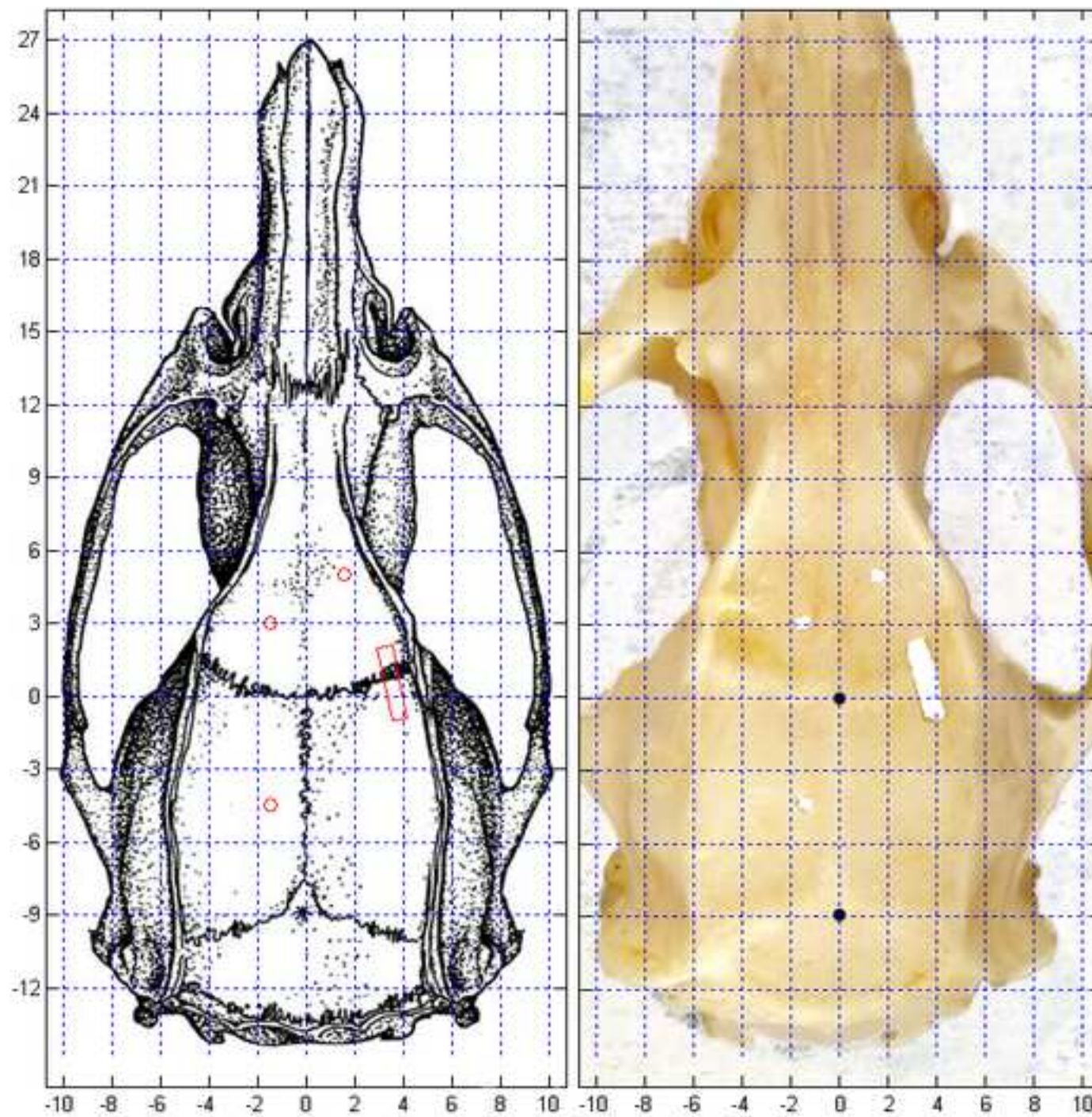
A screenshot of a software dialog box titled "Skull Wi...". The dialog box contains eight input fields arranged in four pairs. Each pair consists of a label and a text input field. The labels are: X1-(Posteromedial), X2-(Anteromedial), X3-(Anterolateral), X4-(Posterolateral), Y1-(Posteromedial), Y2-(Anteromedial), Y3-(Anterolateral), and Y4-(Posterolateral). The values entered in the input fields are: 3.5, 2.9, 3.5, 4.1, -1, 2, 2.1, and -0.9. At the bottom of the dialog box are two buttons: "OK" and "Cancel".

Label	Value
X1-(Posteromedial)	3.5
X2-(Anteromedial)	2.9
X3-(Anterolateral)	3.5
X4-(Posterolateral)	4.1
Y1-(Posteromedial)	-1
Y2-(Anteromedial)	2
Y3-(Anterolateral)	2.1
Y4-(Posterolateral)	-0.9

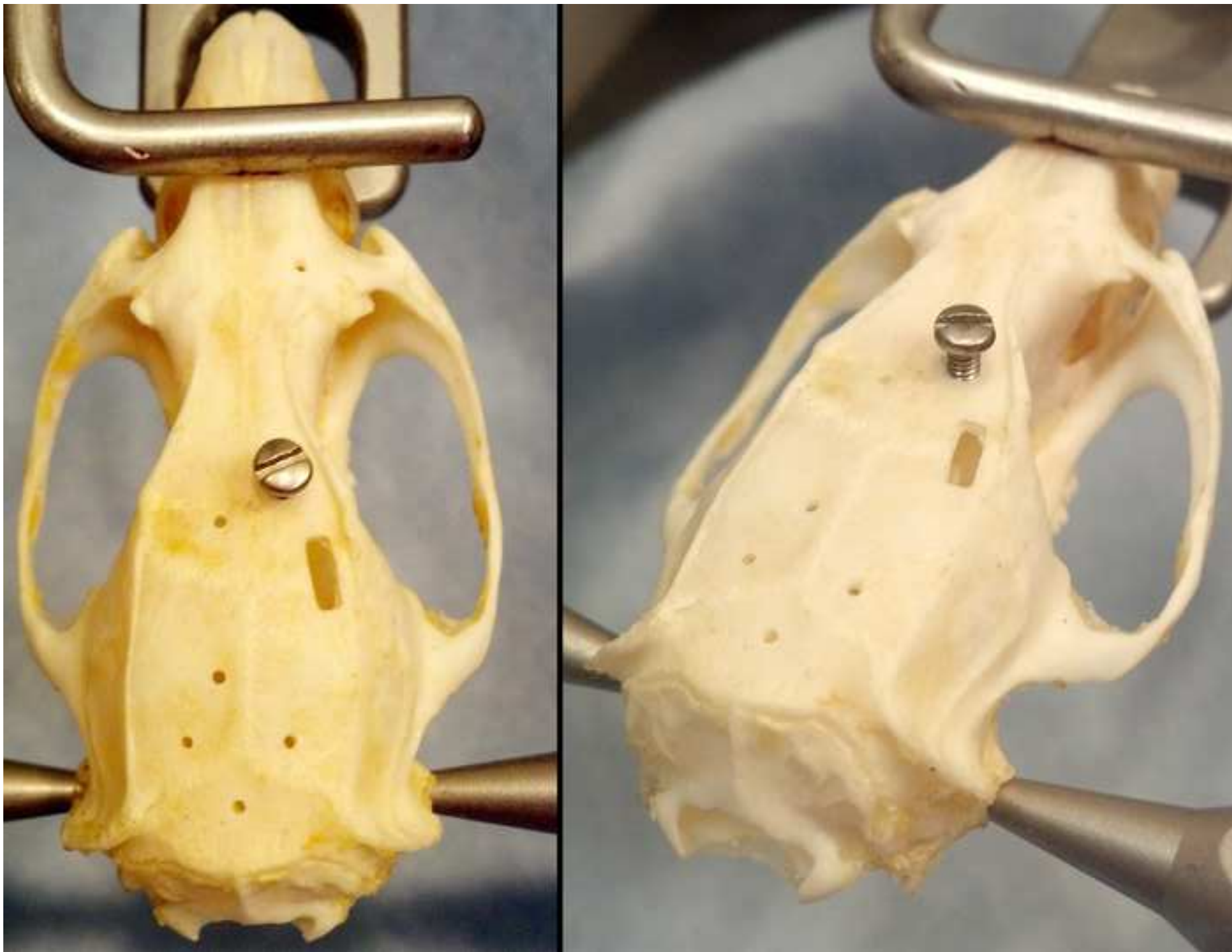
*Figure 15
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*Figure 17
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*Figure 18
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*Figure 19
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Name of Part	Company	Purchase Link	Comments
1 x Standard U Frame Stereotax	Kopf	Kopf	This protocol should work with most existing stereotaxic devices.
3 x 12V, 1.6A, 233 oz-in Geared Bipolar Stepper Motor	Phidgets	Robot Shop Mechanical Drawing	Any High torque geared stepper motor should do.
1 x 3 Axis CNC Stepper Motor Driver Board Controller	Toshiba	Ebay	Any 3 Axis CNC driver should do. Linked Item includes Mach3 CNC software.
2 x Arm Couplers: medial-lateral (ML) & dorsal-ventral (DV)	Custom Machined	Part Drawings View in Browser	These must be machined by your local machine shop. (costs will vary)
1 x anterior-posterior (AP) Coupler	Custom Machined	Part Drawings View in Browser	These must be machined by your local machine shop. (costs will vary)

3 x Motor to Stereotax Collar	Custom Machined	Part Drawings	These must be machined by your local machine shop. (costs will vary)
		View in Browser	
12 x NF10-32 Cup Point Set Screws	McMaster Carr	1/2" Length	You will need 6 of each.
		1/4" Length	
12 x M3 Socket Head Screws (20mm)	McMaster Carr	20mm Length	You will need 4 for each motor
1 x Micro-Motor Drill	Buffalo Dental	X50	Any Micromotor drill will work. At least 38,000 RPM recommended
1 x 12v DC Power Supply	12 Volt Adapters	12v DC – 7 Amp	Any 12v DC PSU should work (ensure amperage rating is higher than the sum of the motors' amperage)
1 x Extra Large Probe Holder	Stoelting	Stoelting	
1 x Grade B Rat Skull	Skulls Unlimited	Skulls Unlimited	

*Table of Reagents/ Materials Used
[Click here to download Excel Spreadsheet- Table of Materials/Equipment: Software.xlsx](#)

Name of Software	Company	Purchase Link	Comments
Mach 3 Mill	ArtSoft USA	Trial Download Fully Functioning (Limited to 500 line g-codes)	Any Standard CNC controlling software should work.
Surgery Designer	Kevin Coffey & David Barker	MATLAB File Exchange	These codes are available to modify. We accept no responsibility for your use or modification of code.



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Author(s): Kevin R. Coffey; David S. Barker; Sisi Ma; Mark O. West

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AUTHOR:

Name:

Kevin Coffey

Department:

Psychology

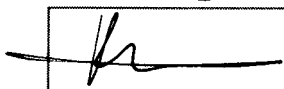
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MS # (internal use):

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Editorial comments:

1) Please adjust the page margins to 1 inch on all sides and use single-spaced text throughout the manuscript.

The margins have been adjusted.

2) Please adjust the formatting of your protocol section so that all text is aligned to the left margin with no indentations. There must also be a space between each protocol step.

Everything is aligned to the left margin, and there is a space between each protocol step.

3) Please revise the protocol text to avoid the use of any pronouns (i.e. "we", "you", "our" etc.). If you feel it is very important to give a personal example, you may use the royal "we" sparingly and only as a "NOTE:" after the relevant protocol step. Please also minimize the use of pronouns in the remainder of the manuscript.

Pronouns have been removed from the manuscript, and the notes have been properly formatted.

4) Please add more details to your protocol steps and make sure that each step is written in complete sentences. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

The protocol has undergone significant revisions. All steps have been clarified, and written in complete sentences, with the exception of the directions for clicking through software menus. 8.2 through 8.5 are more comprehensible with arrows. Let me know if this is a problem.

5) Please revise step 2 as follows:

2. Attach Couplers to your stepper motors using the 12 x NF10-32 Cup Point Set Screws (figure 2).

2.1. Ensure that the couplers are firmly attached to the motors.

Note: The 3D models do not include threads. The parts are labeled with thread size, but they must be tapped after they are manufactured.

Please also revise step 8.1 in a similar way so that the step does not comprise of only a "Note:"

All instances of "Note:" have been properly formatted to meet requirements.

6) After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section and ensure that it complies with JoVE's protocol length guidelines. Please see JoVE's instructions for authors for more information.

The protocol is formatted correctly, and less than 3 pages in length.

7) The text in your Representative Results section contains steps that are more suited to the Protocol. Please move the Representative Results text to the protocol and please add at least one paragraph of results text that explains your representative results in the context of the technique you describe; i.e. how do these results show the technique, suggestions about how to analyze the outcome etc. This text should be written in paragraph form under a "Representative Results" heading and should discuss the results of all figures.

8) Figure 17 is missing from the uploaded Figures. Please upload Figure 17 to the Editorial Manager.

Figure 17 has been uploaded.

9) Please modify your references section to comply with JoVE instructions for authors, specifically when there are more than 6 authors, list only the first author then "et al."

References have been edited to meet JoVE's criteria.

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All trademarked or copyrighted language has been removed from the manuscript. They still remain in the materials & software tables.

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Editorial comments:

Thank you for your revisions, we have accepted them. However, the following points still need to be addressed:

- 1) Please use the attached updated manuscript for all future revisions
- 2) Please revise the protocol text to avoid the use of any pronouns (i.e. "we", "you", "our" etc.). If you feel it is very important to give a personal example, you may use the royal "we" sparingly and only as a "NOTE:" after the relevant protocol step. Please note that although the pronoun "we" may be used sparingly, "you" and "your" should be completely avoided in the protocol text. For example, the "Note:" following step 5 may be revised as:

Note: This stepper motor driver comes as an exposed circuit board. A case may be built, although it is not necessary. Also, a number of different bipolar stepper motor drivers may be used. If so, ensure that the setup instructions are followed for the specific board purchased.

Please revise step 7 and the "Notes" following steps 8.3, 10.7 and 10.8 accordingly.

All personal pronouns have been removed from the notes, and step 7. I also used **find** to ensure there weren't any remaining.

- 3) Please revise step 5.1 so that it is written in imperative tense, such as:

5.1. Align all 6 pins per stepper motor in the same way. Half-stepping allows for double the step resolution in Degrees/step (figure 5).

This step has been re-written.

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I have removed all of the trademark and copyright symbols from the tables.

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Please move the [YEAR] to the end of the citation.

I have reformatted the references.

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- a) Use only a skull and not a live/euthanized animal in your protocol. In this case, please remove all reference to an animal from your protocol steps.

b) Use an animal in the protocol. In this case, please refer to the attached Animal Care Guidelines and address all of the points regarding humane animal treatment in your protocol.

For simplicities sake, all mention of live animals has been removed from the protocol.

CC: allison.diamond@jove.com

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- 1) All of your previous revisions have been incorporated in to the most recent version of the manuscript. Please download this version of the Microsoft word document from the "file inventory" to use for any subsequent changes.
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Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This paper by Coffey et al. provides a protocol for designs and software to economically transform an existing stereotaxic to a robotic stereotaxic. The system is designed to reduce the human error normally associated with stereotaxic surgery performed by laboratory personnel (i.e., humans). This is a well-written paper from a team of researchers highly-skilled in the stereotaxic surgery, and with the technical knowledge necessary to automatize these skills. The protocol will be of great use to researchers wishing to automate surgical protocols, particularly those with some background mechanical and software skill.

STRENGTHS:

- 1) The advantages of this technology are clearly described in the Introduction.

- 2) The step-by-step instructions are described in a straightforward manner.
- 3) The illustrations are clear and useful.
- 4) The potential value of this technology for the many laboratories that employ stereotaxic surgery as a research tool is high.

Major Concerns:

None.

Minor Concerns:

None

Additional Comments to Authors:

N/A

Reviewer #2:

Manuscript Summary:

a cnc mill is attached to a stereotax for more precise hole drilling relative to the skull

Major Concerns:

N/A

Minor Concerns:

limitations are not fully described. performance, speed, depth, size of holes, accuracy is not fully described. these should be listed in the abstract and explained in a couple of sentences each in the text

Technical specifications have been added to the Abstract [46-52], as well as to the appropriate methods sections. [89-91; 165-166; 181]. Also, a paragraph describing the limitations of the software has been added to the discussion [279-292].

Additional Comments to Authors:

N/A

Reviewer #3:

Manuscript Summary:

The manuscript by Coffey et al. provides a clever, inexpensive and novel solution to lack of accuracy, repeatability and variations between surgeons. Such an inexpensive, easy to implement and efficient solution will certainly attract attention and usability from a big audience among biomedical scientists.

Major Concerns:

The authors would probably like to mention and consider how the apparatus will perform or what adjustments will be required in order to perform thin skull windows at high medio-lateral coordinates, where the skull curvature is different than at more medial areas.

I have included a complete paragraph with technical limitations. The limitations of the included software are also discussed, and possible ways to remedy those limitations are included [279 - 293]. There is no physical limitation on cutting extremely laterally (angled or curved surfaces), but the user needs to be proficient at g-coding to design the surgeries from scratch.

Minor Concerns:

N/A

Additional Comments to Authors:

Background and technique are explained. The steps presented should not become a problem for most of biomedical scientists. Limitations are also mentioned and addressed.

Your revision is due by Jun 04, 2013.

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Sincerely,

Sephorah Zaman, Ph.D.

Science Editor

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