STEREOTAXIC INSTRUMENT FOR RODENTS

A Final Year Project Report

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In Partial Fulfillment
of the Requirements for the Degree of
Bachelors of Mechanical Engineering

by

Mohid Muneeb Khattak
Maaz Ahmad

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EXAMINATION COMMITTEE

We hereby recommend that the final year project report prepared under our supervision by:

MOHID MUNEEB KHATTAK NUST201432158BSMME11114F

MAAZ AHMAD NUST201432397BSMME11114F

Titled: "STEREOTAXIC INSTRUMENT FOR RODENTS" be accepted in partial fulfillment of the requirements for the award of MECHANICAL ENGINEERING degree with grade ____

Supervisor: Dr. Nabeel Anwar, HOD BME	Dated:
Committee Member: Dr. Emad, Assistant Professor	Dated:
Committee Member: Dr. Zaib Ali, Assistant Professor	Dated:

______________________ ___________

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ABSTRACT

Stereotaxic instruments are being used around the globe to examine brain activities of rodents. While these are commercially available, but their cost and complexity do not enable them to be used locally in labs even though the need to use them for research purposes in labs is profound. In addition, the holder of such instruments incorporates one tool at a time which becomes a hurdle for surgeries with limited time constraints as well as additional work for the stereotaxic user. Most of these instruments offer a trade-off between precision and price, while none of them provide a holder with option of multiple tooling. The most precise ones are too expensive to be bought for local use while the cheap ones readily available are imprecise to experiment with and obtain viable results. Companies like Kopf instruments, Leica, Stoelting, etc. manufacture these commercially available stereotaxic instruments but none of them provide an efficient yet comprehensive solution to the aforementioned consideration. Finally, none of these companies cater for the additional time and work required in replacing the tools, prolonging surgeries and complications in such an intricate process. This thesis discusses the design, modelling and fabrication of such an instrument as well as the provision of a solution to be used locally in labs.

PREFACE

This thesis is presented to the NUST School of Mechanical and Manufacturing Engineering (SMME), Islamabad in partial fulfilment of the requirement of the degree BE Mechanical Engineering for the student authors and describes in detail all the efforts that led to completion of their Final Year Project titled “Stereotaxic Instrument for Rodents”. This thesis elaborates extensively all the stages that this project went through from conception phase leading to the finalization phase and also sheds some light on the methodology and calculations that were adopted in order to design the instrument. While organizing this thesis, care has been taken to strictly keep it in accordance with the recommended format provided by the SMME. The authors have devised a conscious methodology making use of simple and lucid diction for a model inclusive of the commercially available standard and provisions for further proficiency. Visual aids like pictures, drawings, tables and graphs etc. have been used wherever necessary to add to the overall clarity of the report. Furthermore, design calculations and formulae have also been written. A dedicated chapter at the end identifies certain aspects that might address a room for improvement and prowess in the field of stereotaxy, listing and explaining the potential future prospects. This thesis aims at the design and modelling of a stereotaxic instrument which is to be used in the local labs. Universities in Pakistan are in dire need of a precise stereotaxic instrument to conduct lab experiments on rodents in order to understand neurodegenerative diseases and cognition. This instrument will help them perform in depth analysis of brain both invasively and non-invasively. The small head size of the rodents makes it difficult to use other instruments as an alternative or to use imprecise cheaper versions. While commercially available stereotaxic instruments accomplish this objective, it exceeds the budget. Encompassing precision, electro-mechanical control and a unique holder design as its deliverables, the scope of this report attempts to neutralize the economic and ergonomic limitations by designing and modelling such an instrument to be fabricated for local use.

ACKNOWLEDGMENTS

We would like to express our deepest appreciation to our Creator and Sustainer, Allah Almighty, The Most Beneficent and The Most Merciful, who provided us the possibility to complete the report by helping and guiding us through each and every step of the project. Indeed nothing is possible without His divine decree. Furthermore, we would also like to acknowledge with much appreciation the crucial role of our parents and siblings who lent us their valuable support through all the ups and downs of our life. We are highly indebted to our Project Supervisor Dr. Nabeel Anwar for his guidance and constant supervision as well as for providing necessary information regarding the project and also for his support in completing the project. He has been a source of continuous support and assisted us wherever possible. Finally, we would like to thank all the individuals who helped in the completion of this project in one way or the other. Some of them include Mr. Haris Shahzad, Mr. Ramish Durab Khan, Mr. Talha Yousaf and Mr. Muhammad Naeem.

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ABBREVIATIONS

E	Modulus of Elasticity
UTS	Ultimate Tensile Strength
YS	Yield Strength
HB	Brinell Hardness
S/W	Strength to Weight Ratio
mm	Millimeter
MPa	Mega Pascal
GPa	Giga Pascal
F.S	Factor of Safety

NOMENCLATURE

α	Step Angle
p	Pitch of Power Screw
δ	Deflection of Beam
F	Force due to Weight
l	Length of Power Screw
E	Modulus of Elasticity
I	Area Moment of Inertia
ω_n	Natural Frequency
ω	Excitation Frequency
m_m	Mass of Motor
m_s	Mass of Structure
k	Spring Constant
c	Damping Constant
ζ	Damping Ratio
R	Center of Gravity Offset from Motor’s Rotational Axis

CHAPTER 1

INTRODUCTION

1.1 What is Stereotaxy?

A precise surgical procedure which makes use of three dimensional co-ordinate axis to locate non-visualized anatomic structures, usually in the brain or spinal cord, and to perform operations on them such as lesions, biopsy, injection, stimulation, etc.

Farlex Partner Medical Dictionary defines stereotaxic surgery as:

"*An apparatus attached to the head, used to localize precisely an area in the brain by means of coordinates related to intracerebral structures*." [1]

Theoretically, stereotaxic surgery can be applied to any organ or system inside the body but it has been limited to brain surgeries in modern times due to the complexities that arise while designing a frame structure for various organs within the body as well as locating reliable reference points in the bones or tissues that make the surgery more complicated. Otherwise, stereotaxy has been prevalent for brain biopsies or breast surgeries.

1.2 Aim of Stereotaxy:

The basic goal related to stereotaxic surgeries is to remove abnormalities in the brain. We know that CT scans and MRI can successfully detect problems such as tumors, etc. in the brain. These modern techniques assist to a great extent in stereotaxic surgeries by locating such abnormalities and helping surgeons locate the safest way around the brain for removal of as much abnormal tissue as possible while avoiding to damage the surrounding healthy tissue.

1.3 Operation:

To summarize, stereotaxic surgeries are performed by integration of an image (commonly known as the brain atlas) with a computer device. This atlas is generated through CT scans and MRI procedures. The image is then transferred to the system which gives a detailed view of the operation zone in the brain by generating a two or three dimensional view of the brain. This precisely locates the abnormal area to be operated on, such as removal of the abnormal tissue.

1.4 Working:

A stereotaxic surgery is performed on the basis of three main components:

1. Brain atlas, image guidance, computer integration, etc.
2. Stereotaxic instrument
3. Localization of target abnormality or area of operation

The apparatus uses a system of referencing in the orthogonal reference frame which encompasses a three dimensional co-ordinate system which is analogous to the Cartesian axis system but is different in some ways, as explained later on. The device has a base where clamps and ear bars are available to clamp the head of the rodent in a precise position with reference to the co-ordinate system available. In small animals such as rodents, bone structures are set as reference points or 'landmarks' for the successful targeting of abnormality, as explained later on in the report. Guide bars are used in the apparatus which allow the user to locate the probe with high precision over the target region by either using Vernier scales or computer generated co-ordinate commands.

1.5 Types of Stereotaxic Cages:

Stereotaxic cages vary on the basis of structure of their frames as well as the method of probe insertion. Generally these types [2] are described as follows:

1. *Simple Orthogonal Stereotaxic System:*
   As the name suggests, this system allows the probe (electrode or injection) to be inserted perpendicularly in the brain with reference to a base plate attached to the skull. With three guide bars in the apparatus, this system provides a three degree of freedom movement.
2. *Burr Hole Mounted Stereotaxic System:*
   The system utilizes a fixed entry point and hence limits the range of target brain insertion points in the intracranial region. Hence it becomes a two degree of freedom system with depth control along the fixed entry point.

3. *Arc-Quadrant Stereotaxic System:*

   In this system, an arc is made to rotate about the vertical axis whereas a quadrant is rotated about the horizontal axis. The probe is inserted tangentially to this arc and the quadrant. Due to this arc-quadrant, the probe always arrives at the center of the sphere where the depth of probe equals the radius of the sphere.

4. *Arc-Phantom Stereotaxic System:*

   A simulation is used in this system where an aiming bow attached to the brain is utilized to align the probe over the desired target in 3D co-ordinates. The aiming bow is, then, transferred from the simulation ring (phantom ring) to the actual base ring where the depth is adjusted so the probe reaches the target.

1.6 Commercially Available Instruments:

Commercially, many companies make various types of stereotaxic instruments. These variants differ in their ability to precisely monitor the target, digital integration, electro-mechanical variations, etc. A few leading companies which undertake the design and fabrication of such instruments are:

- Stoelting Co.
- Neurostar
- Kopf Instruments
- Leica Biosystems
- ASI-instruments

1.7 Problem:

Aforementioned instruments are available and operational globally. These are majorly used in large research laboratories or medical camps in hospitals where thorough research is being conducted to study brain activities of small animals. The real issue arises when the need to conduct such research is hindered by the non-availability of these instruments. Local labs cannot purchase such instruments since they are fairly expensive, prices ranging higher than $2500, as found through the market survey. As a result, small local or university labs cannot afford such an instrument for further research. Moreover, the holder of commercially available instruments allows placement of one tool at a time. This not only adds to the surgery time but also demands additional effort from the user to constantly remove and replace tools on the holder. Increased surgery time can be fatal in many cases where rodents are to be analyzed afterwards hence they have to be kept alive throughout the duration of surgery.

1.8 Objective:

The aim of this project is to account for all the above mentioned problems. This encompasses the design, development and fabrication of an electromechanical stereotaxic instrument (for more high precision) with a unique holder design. The project was assigned by our supervisor for local use in the human systems lab at SMME, hence the project will attend to the need of department; along with cheaper availability in market as a future prospect. The objectives can be enlisted as follows:

- The instrument would incorporate Small Animal Stereotaxic frame designed for rodents (rats/mice)
- The fabrication of the product would be economical in comparison to the commercially available stereotaxic instrument in relation to the aforementioned paragraph
- Prospective Atlas Integration on computer with software configuration for choice of mouse or rat installed, currently being worked on by a postgraduate student as her thesis
- A revised instrument over the traditional stereotaxic cage in terms of precision and design

1.9 Approach:

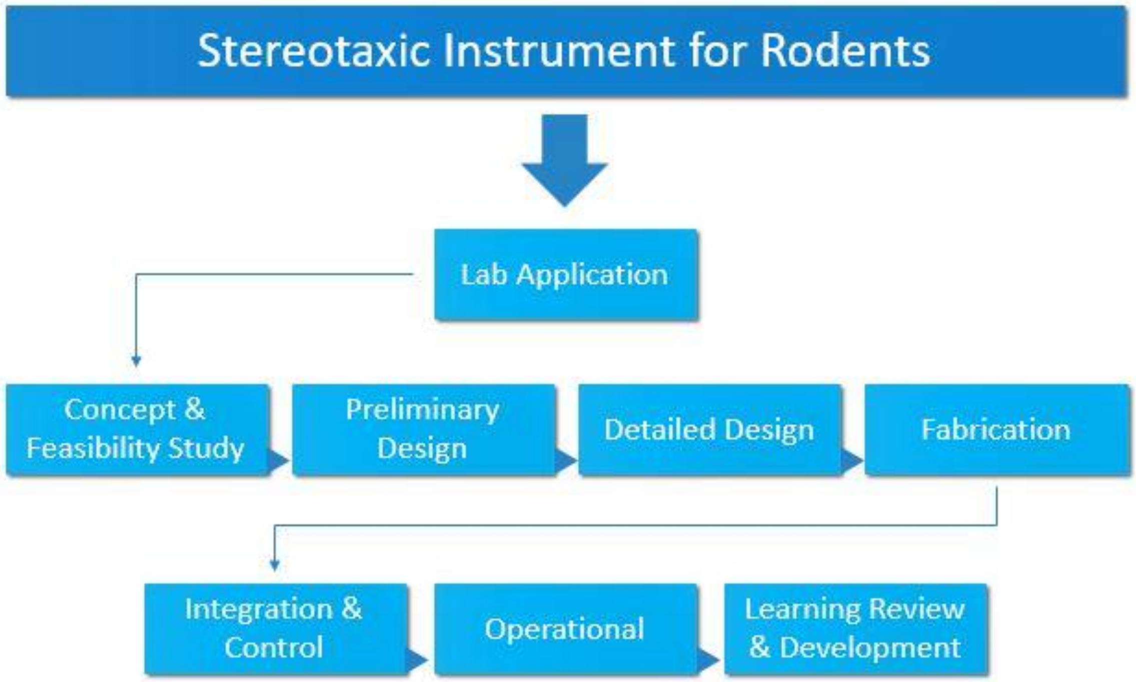


Figure 1 - Approach towards the project

CHAPTER 2

LITERATURE REVIEW

2.1 Small Animals:

Small animals, in particular rodents, have long since been used for neurobehavioral experiments due to a wide variety of options and convenience that they bring along with them. Their small size allows to easily care and manage them. To a great degree, the availability of pure breeds of mice and rats helps even further in the cause of experimentation; this majorly explains their popularity as lab animals. Human brain, therefore, has been modelled using a rodent's brain in numerous researches and has successfully aided in treating different diseases and understanding the behavior. Stereotaxic surgeries are also available for humans, as well as large animals including monkeys; but due to the profound usage of small animals (squirrels, mice, rats, etc.) as experimental specimens, most stereotaxic instruments are aimed towards small animal surgeries.

To begin with, there is a pre-operative phase which deals with a checklist that includes the rodent's health, sterilization of equipment, anesthetic methods and procedures. Furthermore, this follows a detailed protocol to carry out the surgery as well as complex post-operative procedures for a complete successful autopsy.

2.2 Atlas:

The stereotaxic surgeries are carried out by using an atlas for the said specie under experimentation. This atlas is specific to each animal being operated. These surgeries require knowledge on different regions of the brain in order to successfully operate on the target area. Brain atlases help in this regard by providing a layout or a map of the brain through theoretical co-ordinates for the successful insertion of electrodes, implantation of cannulas, etc.

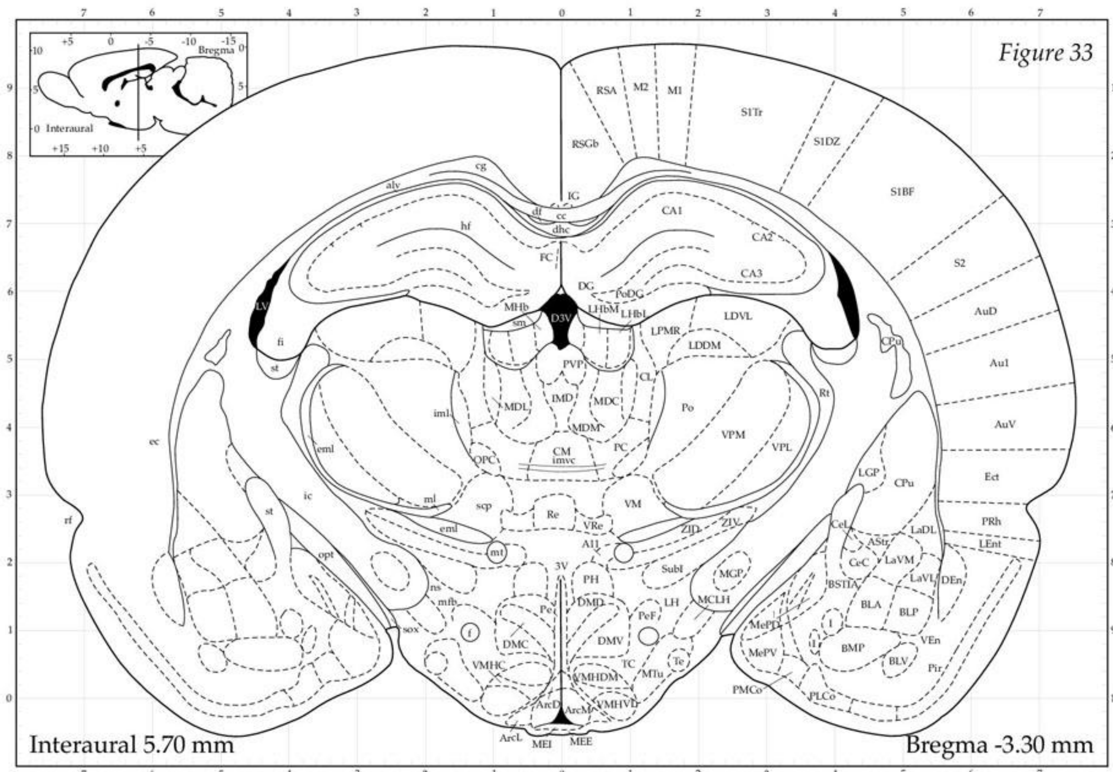


Figure 2 - An Example of a Rat Atlas

Multiple atlases have been published for various species which take into account the differences in sex, age, size, weight, etc. These parameters must be taken into account while choosing the right atlas for the animal under operations. A few of these [3] are listed below:

2.2.1 Stereotaxic Atlases for Mice:

- Atlas of the Mouse Brain and Spinal Cord - Sidman RL, Angevine JB Jr, Taber PE
- A Stereotaxic Atlas of the Diencephalon and Related Structures of the Mouse - Montemurro DG, Dukelow RH
- Atlas Stereotaxique du Cerveau de la Souris - Lehmann A, Gautier M, Ghilini G, Henry J, Langlois R, Laplante S
- Atlas of the Prenatal Mouse Brain - Schambra UB, Lauder JM, Silber J
- The Mouse Brain in Stereotaxic Co-ordinates - Paxinos G, Franklin KBJ

2.2.2 Stereotaxic Atlases for Rats:

- Atlas of Histology of the Juvenile Rat - George A Parker, Catherine A. Picut
- The Rat Brain in Stereotaxic Co-ordinates - Paxinos, George, Charles Watson
- Atlas of the Postnatal Rat Brain in Stereotaxic Co-ordinates - Roustem Khazipov, Dilyara Zaynutdinova, Elena Ogievetsky, Guzel Valeeva, Olga Mitrukhina, Jean-Bernard Manent, Alfonso Represa

2.3 Co-ordinate System:

A particular issue that occurs during stereotaxic surgeries is the inability to correctly identify the target region for operation. For this purpose, a special method was devised which utilizes the relative position of landmarks in the bones and their spatial relationship in the brain. This relationship is described using three co-ordinates, namely:

1. Anterior-Posterior (AP)
2. Medial-Lateral (ML)
3. Dorsal-Ventral (DV)

This is highly analogous to the Cartesian co-ordinate system whereby any point can be located using three points of the axes. However, there are some visible differences between the two:

- Cartesian system uses a single reference point i.e. the origin whereas this system makes use of different references
- Cartesian system has a single accepted orientation however, this system has the freedom of different orientations

2.4 Reference Points and Orientation:

As mentioned before, the stereotaxic co-ordinate system does not have a single fixed reference point. It varies from specie to specie and is dependent upon bone structure and landmark position. In rodents however, three most common points which are used as reference points are:

1. Bregma
2. Lambda

3. Interaural Line/Point (IAL/IAP or Stereotaxic Zero)

Bregma and Lambda are the intersection points of bones of the skull of rodents, on the dorsal surface. [4]

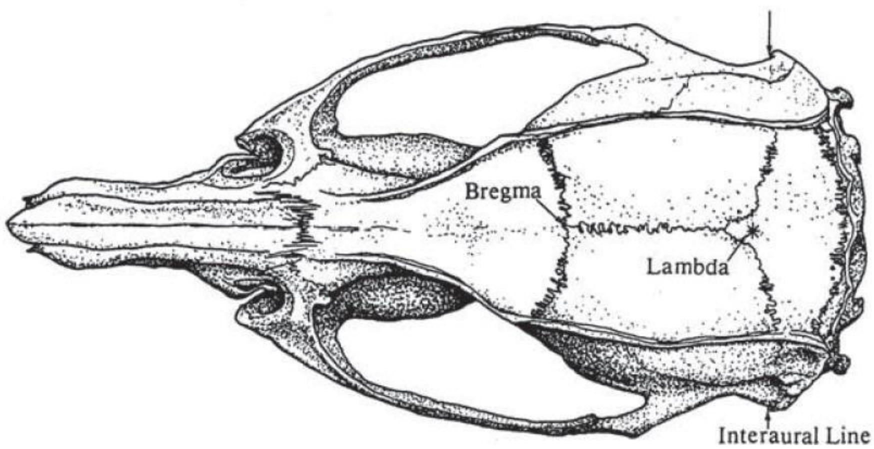


Figure 3 - Top View of Rat Skull

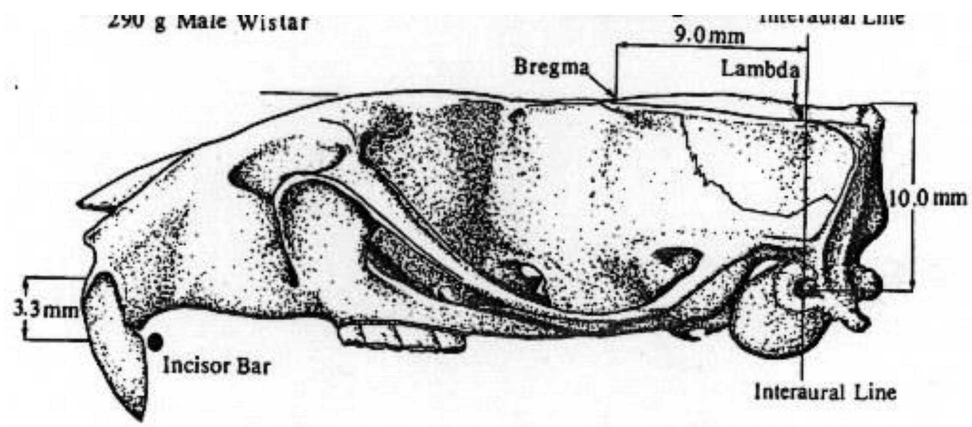


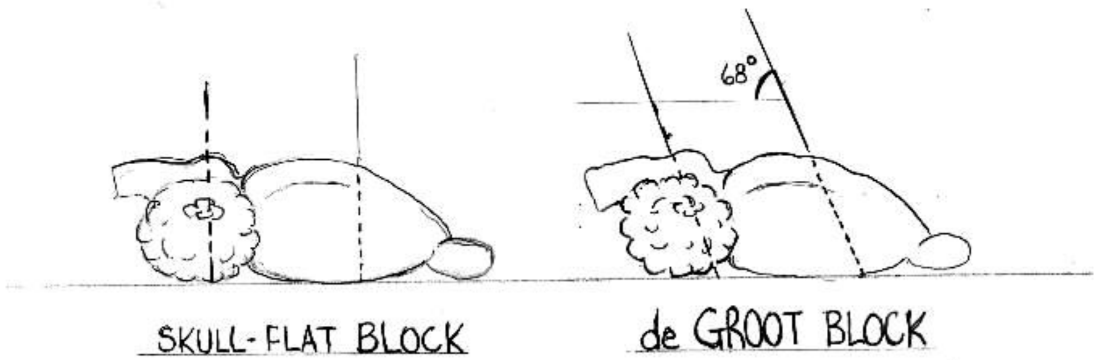
Figure 4 - Side View of Rat Skull

The IAL reference point is not located anatomically but is found through the stereotaxic instrument.

As mentioned before, there is no fixed orientation for the stereotaxic co-ordinate system. The two most commonly used orientations are:

1. Plane of De Groot

2. Skull Flat Plane



3.

Figure 5 - Stereotaxic Co-ordinate System Orientation

2.5 Stereotaxic Instrument:

The main body of the apparatus consists of a frame that is fixed upon a base plate. Three guide rails are placed that allow for a successful attachment of the holder along the three axes i.e. sideways, forwards-backwards and up-down. These help in precisely locating the holder. This can either be mechanically actuated using Vernier scales or electro-mechanically operated using stepper motors. The base plate contains ear buds and incisor bar. The ear buds can be laterally adjusted and are parallel to the AP axis while the incisor bar helps adjust the inclination of the rat's skull along the plane in the flat-skull position. These together help fix the exact position of the rat's skull. Bregma and lambda, the reference points on the skull, are kept in the same horizontal plane. [3]

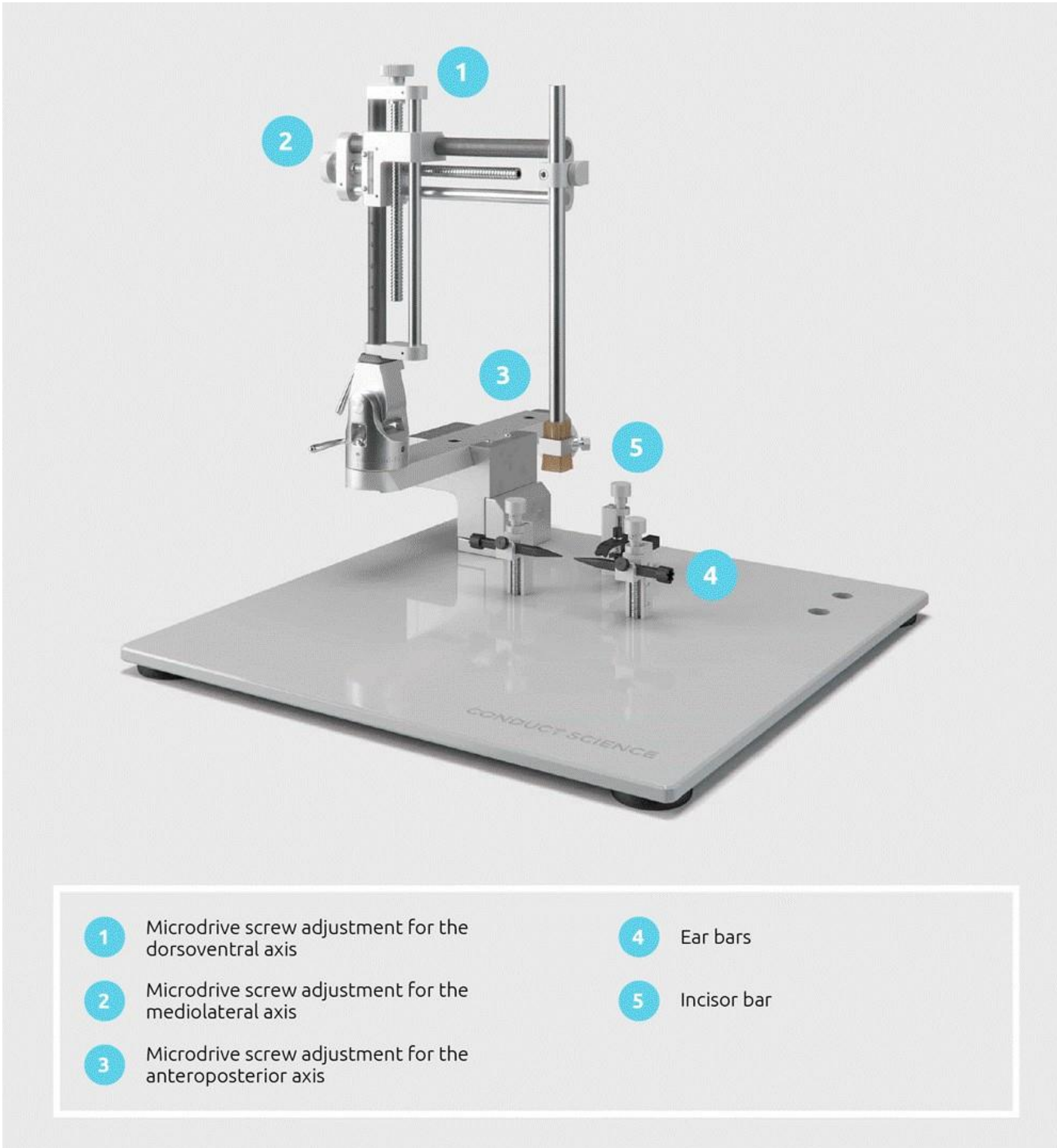


Figure 6 - Parts of a Stereotaxic Apparatus

2.6 Designs:

From figure 6, it can be seen that the instrument is mechanically actuated as it has no motors to operate it. This mechanical actuation is done by mechanical knobs available at the end of each guide rail. Commercially available are similar in design to this instrument and mostly are mechanically actuated. This limits its precision to the least count of the Vernier scale and induces human error in the results of experiment. Following are some commercially available stereotaxic instruments made by Stoelting and RWD instruments.

Figure 7 - Commecially Available Instruments

Various designs of stereotaxic instruments are available with different variants of each but all of them lack in some category. The most common issue with each one is their price. As an example, Kunal D. Chaniary, Mark S. Baron, Pete Robinson along with three other people have designed the following apparatus:

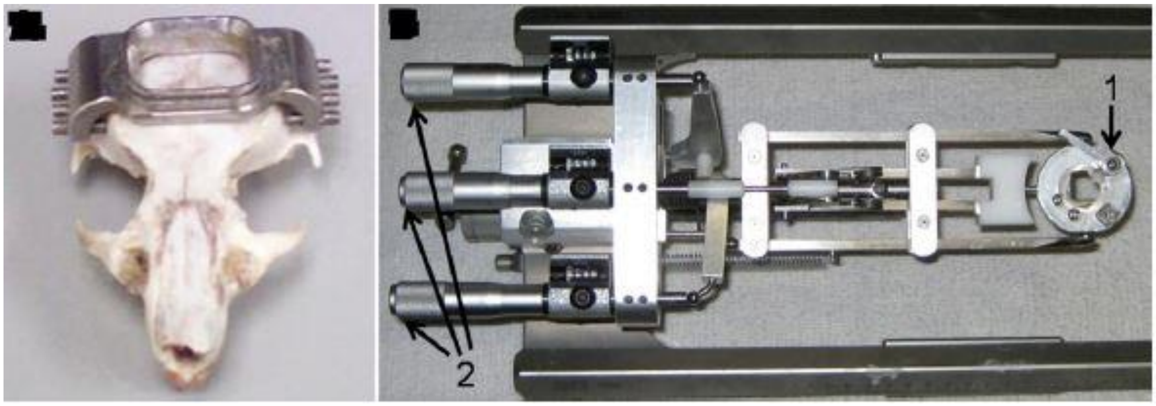


Figure 8 - A Novel Stereotaxic Apparatus for Neuronal Recordings

This design [5] gave a new technique to record brain activities in awake rats with their heads restrained. This setup makes use of an 8 point fixation method without any anesthesia. The fixation helps in high stability of the head. It also allows for accurate localization for recording over multiple sessions while reducing the time of operation, considerably.

Similarly another design by Cunningham and McKay [6] is illustrated as below:

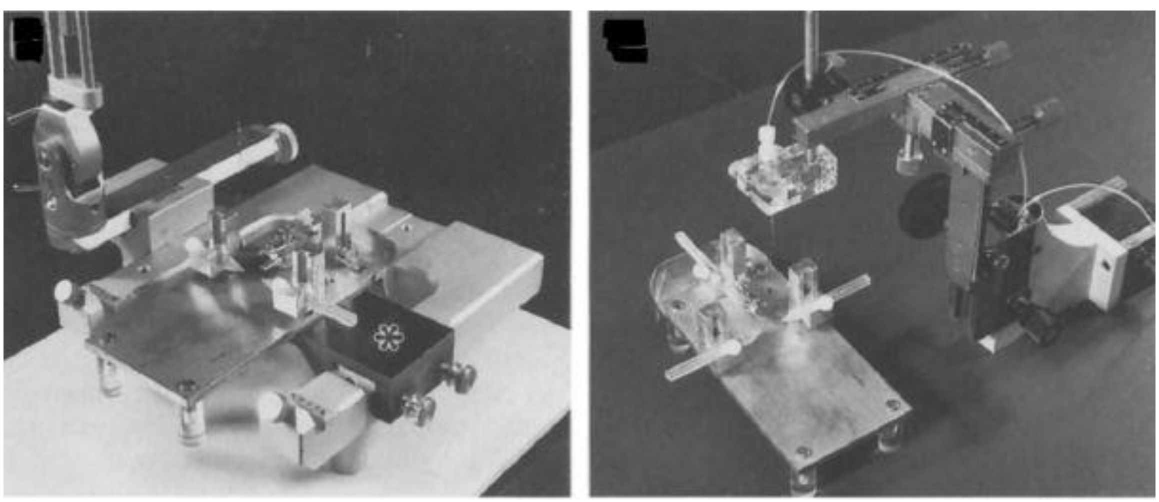


Figure 9 - A Hypothermic Miniaturized Stereotaxic Instrument

This instrument allows for an accurate identification of the localization sites in the brain of rodents. It uses various methods for anesthesia insertion and the design helps in precisely location the sites in horizontal and vertical planes. This allows to accommodate a large range of animal sizes and also gives the freedom to use the device as a stand-alone system or with combination of orthodox adult stereotaxic frames.

Another kind of design by Greer and Yowell [7] can be seen in figure 10 as follows:

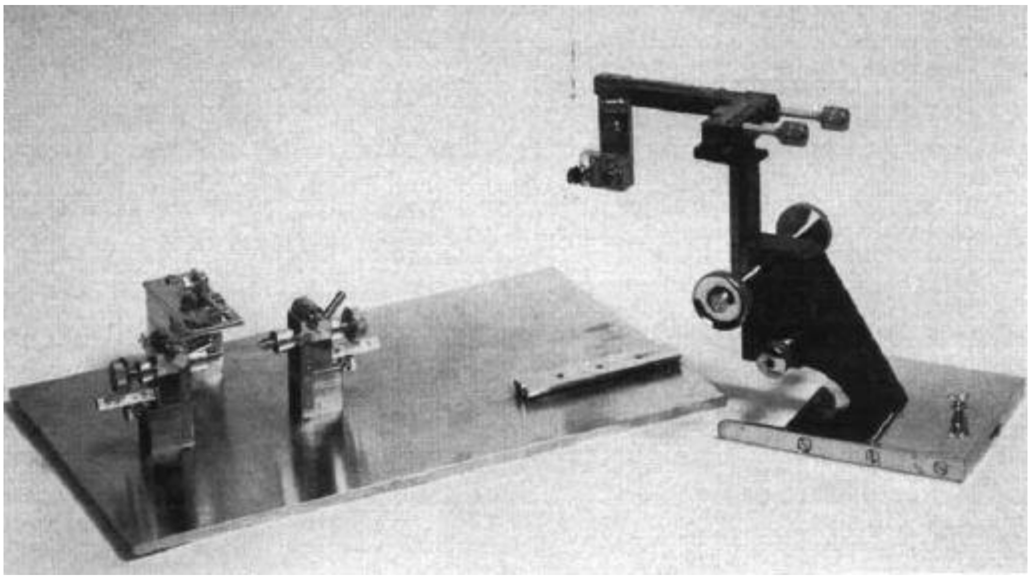


Figure 10 - An Improved Stereotaxic Instrument for Small Animals

This instrument is advantageous as it allows for surgery over a wide range of brain sizes, from mice to Guinea pigs. With adjustable height of incisor bar and ear bars, the instrument can utilize a range of stereotaxic atlases. It also helps in keeping the skull level and stabilized for insertion of electrodes.

Lohman and Peters also published their design [8] in Brain Research journal. It is as follows:

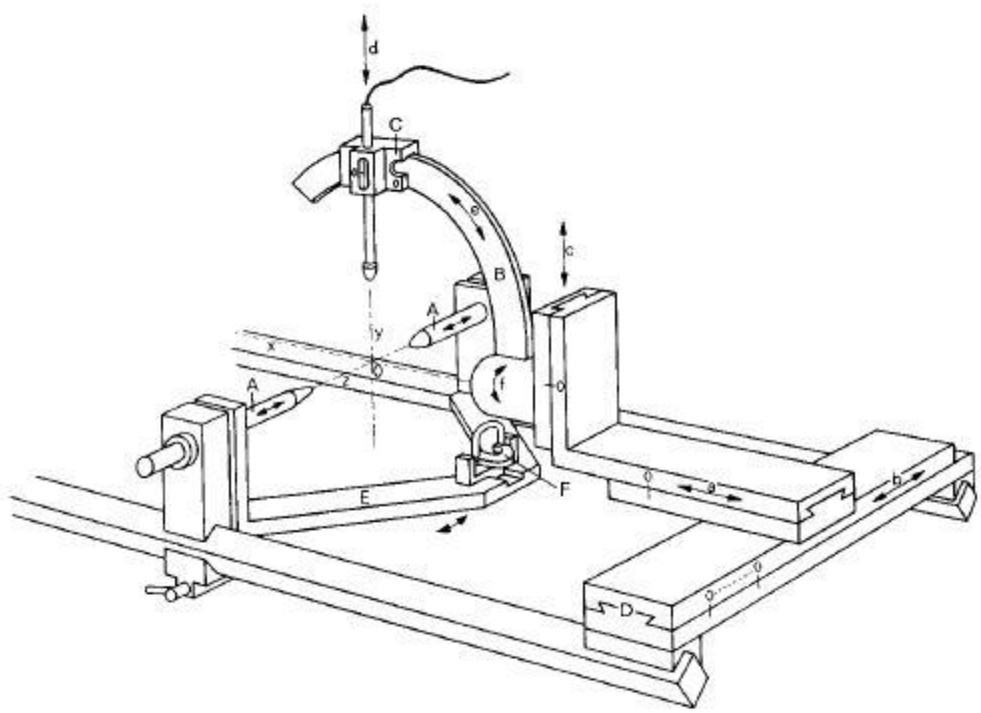


Figure 11 - A New Stereotaxic Instrument

An advantage of this instrument is that it makes use of phantoms that allow to follow the contour of the brain for insertion of electrodes at an angle, along with the conventional perpendicular approach. The electrode holder moves along a traverse bar and can be rotated 30º towards the free end while 50º towards the other. The arms carrying the holder itself can rotate 90º laterally.

Sutton and Quigley [9] have also published their own design in Physiology and Behavior journal. It can be seen as follows:

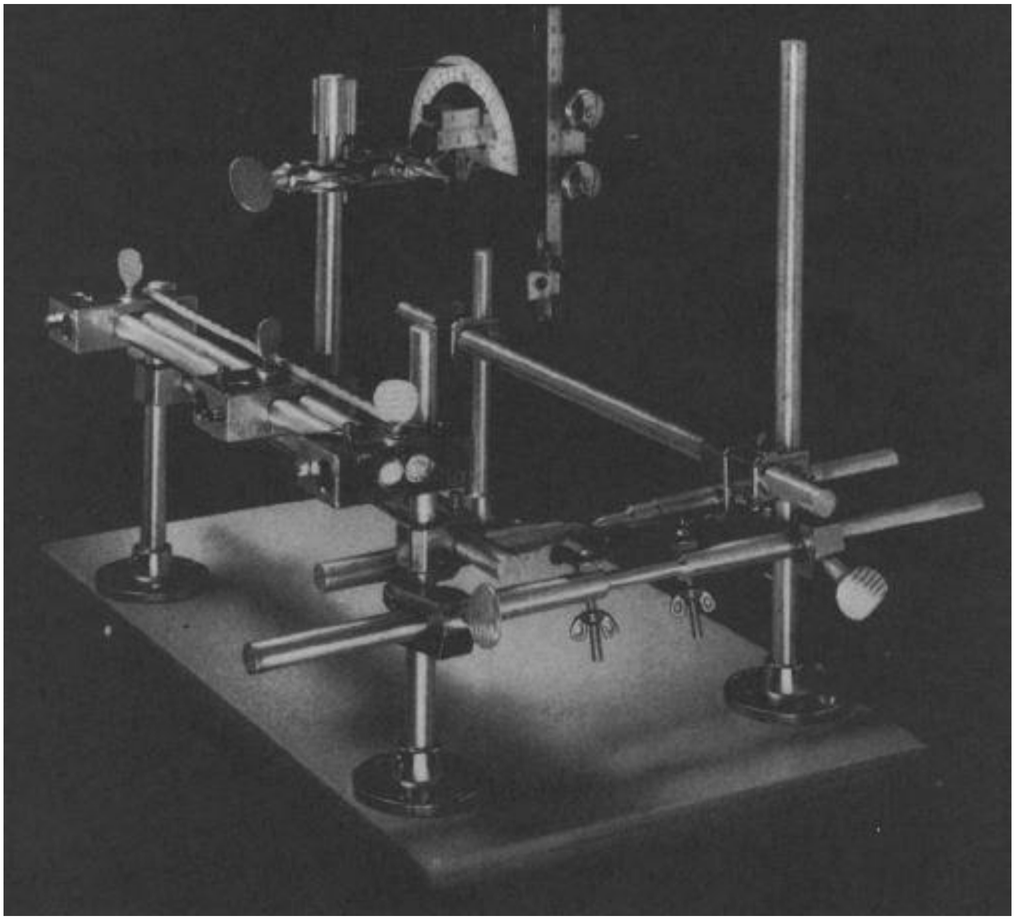


Figure 12 - The Sutton and Quigsley Design

This design is made cheap from commercially available parts and can provide a precision of up to 0.3 mm. It is most suitable for laboratory work and local use. The instrument has the feature of allowing the head to be tilted about the interaural axis. The ear bars are convertible to accommodate small animal head sizes while its design allows for an unobscured field of view.

All of these designs vary in one way or the other but collectively it can be seen that all of these are mechanically actuated which requires more effort and puts precision at risk. An apparatus with electro-mechanical integration not only will help in precise navigation but will also be able to readjust accordingly through computer systems. Hence the need for such an instrument that can be used locally but is cheap and electromechanically actuated is indeed, high. All of this literature has been kept in mind and the designs been taken into consideration while development of a suitable apparatus for local lab use. Furthermore, a holder design has also been aimed at, as can be seen from various designs, to incorporate an all-in-one system where the need to replace the tools can be eradicated. This approach has not been since undertaken in any design and will act as a novelty in our approach. The methodology and design is explained in the forthcoming section.

CHAPTER 3

METHODOLOGY

3.1 Concept Design:

The concept design and preliminary model depicted in Figure 12 comprises of a rectangular base, two base supports and power/lead screw with dual rail shaft support on either side. The assembly is somewhat replicated in the vertical direction equipping a cantilever in order to sustain the triangular holder provision incorporating the drill, syringe and electrode. There are three different motors for the horizontal, vertical and operational movement of the holder. The measurements of the instrument were taken using a Vernier caliper using the model available in the lab. For measurements regarding the holder and operational area, the animal lab available in NUST was visited. The head sizes for rats and mice were recorded for both male and female, and an average value was used for the design.

This designed originated after consideration of a multiple other designs available in the market or in research papers, as explained in the literature review section. One design was available at the human systems lab at SMME and it used dual support by rail shafts for movement in x-axis. This eliminated the need of having a cantilever beam at the top with supports in either side. The design was rather more compromising with precision as an objective since deflection of the beam was no longer an issue. But the same design wasn’t adopted due to the following reasons:

1. The already available lab instrument used synchronous stepper motors instead of one. These two operated power screws individually but were synchronized for a cumulative movement in x-axis. This synchronization, however, cannot be perfected since there will always be a delay (even in milliseconds) when both of them are programmed to operate simultaneously. This does not meet the required precision goal. Even if a timing belt was used to provide for the synchronization imprecision, the inherent property of belts to slip will still cause a delay. [10]

2. Additional material is to be used while designing a dual support system. This not only increase the weight of the instrument but also would add to the cost, which is not to be compromised upon.
3. The design provides for a precision of under 1 mm, as mathematically and theoretically calculated. Its minimalistic design helps for an easy operation and localizing of surgery sites.
4. The use of stepper motors eliminates the human error that occurred while manually aligning the tools over the brain of small animals. Electromechanical operation helps in visualizing and magnifying the small brain on computer and moving it through pre programmed codes. All this will be achievable after computer integration.
5. The unique design of the holder allows for a pre-assembly of a set of tools that need not to be changed during operation. This removes considerable surgery time, which if prolonged, can prove to be fatal for these animals.

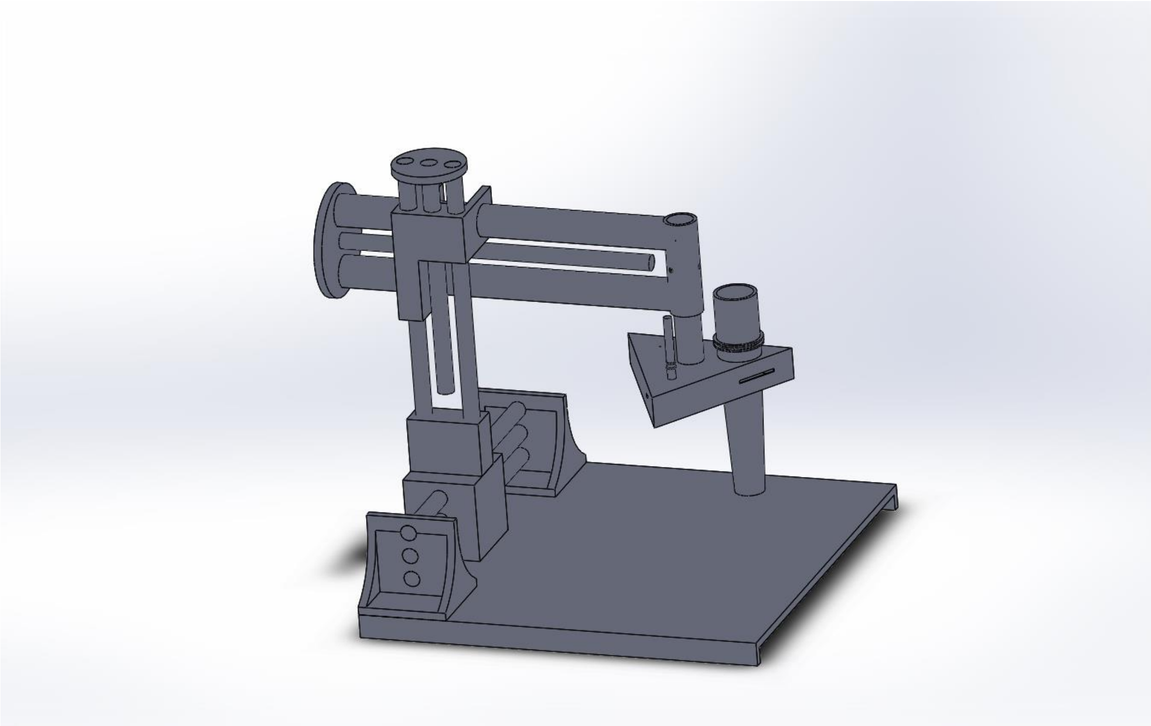


Figure 13 - Concept design

3.2 Material:

The material thought to be used is the 6061-T6 aluminum alloy. While 1018 AISI mild steel can also be used, the aluminum alloy provides approximately same strength while reducing considerable weight (which will decrease the deflection of cantilever beam). Its hardness values are not much different from its steel counterpart. Also it is cheap and has high weldability. While the 7075-T6 variant of aluminum can also be used, it is more expensive than the commercially available 6061 aluminum. Though it has higher strength values, for our application, strength can be compromised over and the ultimate strength values provided by 6061 Al are more than enough to satisfy our cause. However, both of these materials are always interchangeable with applications in vehicle frames, aircrafts, etc. A comparison of their properties [11] [12] [13] are as follows:

Table 1 - Material Comparison

Properties/Material	6061-T6 Al	7075-T6 Al	AISI 1018 MS
E	68.9 GPa	71.7 GPa	205 GPa
UTS	310 MPa	572 MPa	440 MPa
YS	276 MPa	503 MPa	370 MPa
HB	95	150	126
S/W	115 kN-m/kg	196 kN-m/kg	-

3.3 Mathematical Modelling:

3.3.1 Precision in Movement:

To keep the instrument precise and under the 1 mm cap, a commercially available ball screw with 5 mm pitch and lead was considered. Having a stepper motor with 1.8^{o} of step angle, we have the following:

p = 5 mm

$\alpha = 1.8^{o}$

Total number of degrees in a revolution = 360^{o}

No. of steps of stepper motor = $\frac{360}{1.8}$ = 200 steps

Movement along one step over the power screw = $\frac{pitch}{no.\ of\ steps} = \frac{5}{200}$ = 0.025 mm

Hence, theoretically, a precision of 0.025 mm can be obtained while using a 5 mm pitch power screw with a stepper motor having a step angle of 1.8^{o}, which is well below our upper limit.

3.3.2 Deflection of Cantilever Beam:

The cantilever beam at the top will cause a deflection due to the weight at the end and a fixed support at the other end. This can greatly affect the precision objective and hence its calculations are a compulsion. These are as follows:

Deflection of cantilever beam is given by:

$$\delta = \frac{Fl^3}{3EI}$$

We have:

m = 5 kg (an upper limit taking the factor of safety in account)

F = 5 x 9.81 = 49.05 N

l = 100 mm

For I, we have:

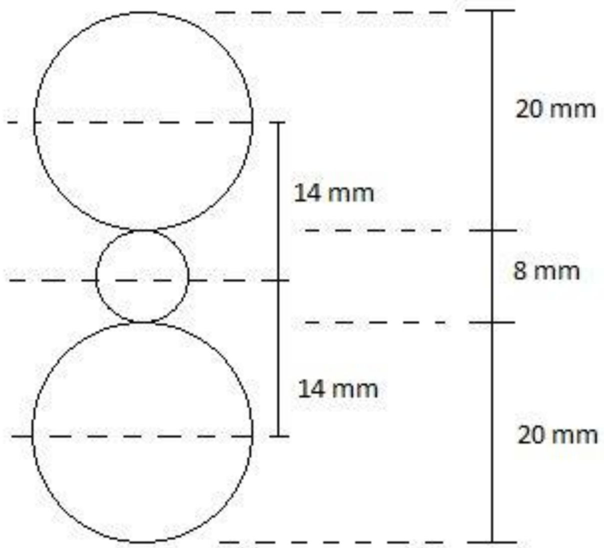


Figure 14 - Moment of Inertia Calculations

Assume there is no gap between the ball screws and the rail shafts to calculate for the maximum area moment of inertia. Also assuming that all three of them are made of the same material since that will cause for maximum deflection. We have:

$$I_t = I_1 + 2I_2$$

$$I_t = \frac{\pi}{4}r_1^4 + 2(\frac{\pi}{4}r_2^4 + Ad^2)$$

$$I_t = \frac{\pi}{4}(0.004)^4 + 2[\frac{\pi}{4}(0.01)^4 + \pi(0.01)^2(0.014)^2]$$

$$I_t = 1.39 \text{ x } 10^{-7} \text{ m}^4$$

Using E = 68.9 GPa and solving for deflection, we have:

$$\delta = \frac{Fl^3}{3EI}$$

$$\delta = \frac{49.05 * (0.1)^3}{3 * 68.9 * 10^9 * 1.39 * 10^{-7}}$$

$$\delta = 1.707 * 10^{-6}\ m$$

$$\delta = 0.001707\ mm$$

This small deflection using 6061-T6 Al shows that deflection of cantilever beam is not that much even when the maximum weight is kept 5 kg including upper limits. Hence the deflection is well thought out for and will not hinder the design considerations.

For MS, this deflection comes out to be 0.00578 mm while for 7075-T6 Al, it is 0.00164 mm. In both cases, the deflection is not interfering with our objective.

3.3.3 Raising Torque:

Raising torque calculations are compulsory to determine the stepper motor model to use in our design. This can be attained as below: [10]

$$T_R = \frac{Fd_m}{2}\left(\frac{p + \pi\mu d_m}{\pi d_m - \mu p}\right) + \frac{F\mu_c d_c}{2}$$

Here,

F = 49.05 N (for m = 5 kg)

p = 5 mm

d_m = 12 mm

μ = 0.15 (maximum coefficient of friction for an oiled stainless steel screw with brass nut)

Assuming no collar friction, but to cover for the torque due to collar factor, the value at the end will be multiplied by a factor if safety (F.S) of 1.5 [14] [15] (collar specifications will be determined at the time of purchase and fabrication).

$$T_R = \frac{49.05 * 0.012}{2}\left(\frac{0.005 + \pi * 0.15 * 0.012}{\pi * 0.012 - 0.15 * 0.005}\right)$$

$$T_R = 0.084866\ Nm$$

$$T_R = 0.084866 * 1.5\ Nm$$

$$T_R = 127.299\ Nmm$$

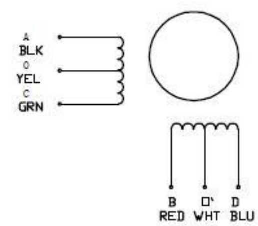
$$T_R = 1.298\ kgcm$$

Hence we will use a stepper motor will holding torque greater than 1.298 kgcm. Commercially, a NEMA 23 stepper motor is a suitable model for our design. As shown in the figure below:

STEPPING MOTORS TYPE SM57HT51-3006A.

GENERAL SPECIFICATIONS		ELECTRICAL SPECIFICATIONS	
STEP ANGLE	1.8°±5%	VOLTAGE	2.25V
PHASE	4	CURRENT	3.0A
INSULATION RESISTANCE	100MΩ(500VDC)	INDUCTANCE	1.0±20%mh
CLASS OF INSULATION	B	RESISTANCE	0.75±10% Ω
WEIGHT	0.65Kg	HOLDING TORQUE	7.2Kg.cm

WIRING DIAGRAM



Units:mm

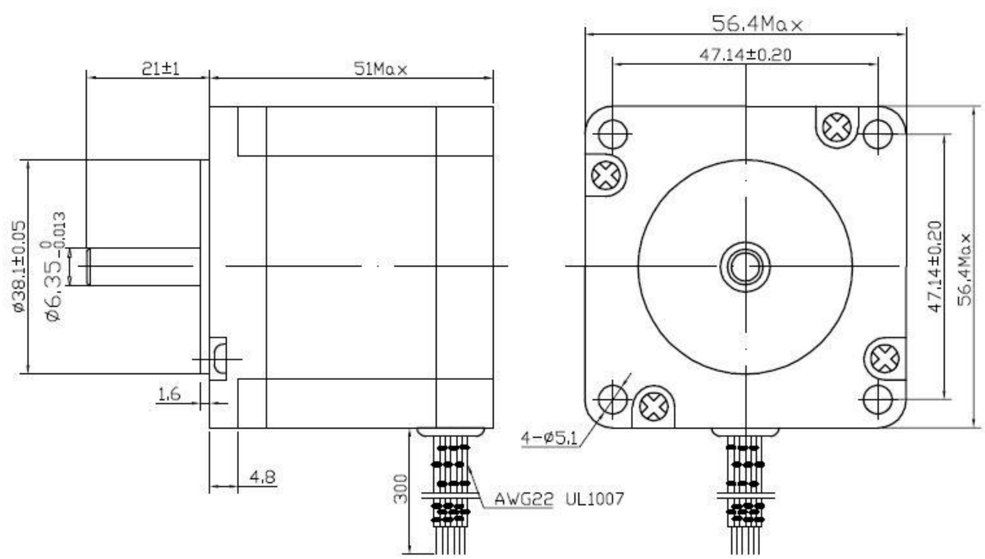


Figure 15 - A NEMA 23 Stepper Motor Specification Sheet with Dimensions

3.4 System Modelling:

Analysis of the stereotaxic cage was done on Ansys software to obtain system parameters needed for determination of various aspects of the design. Nodal analysis was done and natural frequency was obtained for the instrument.

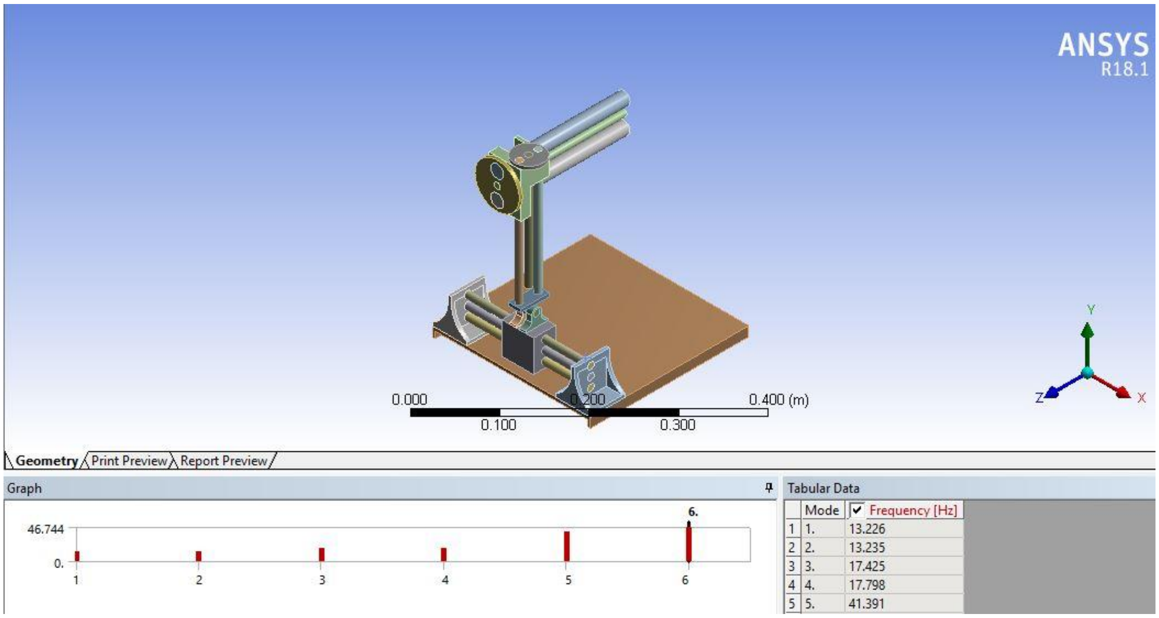


Figure 16 - Nodal Analysis for determination of natural frequency

Details of "Geometry"	
Definition	
Source	C:\Users\Mohid Muneeb Khatta...
Type	SolidWorks
Length Unit	Millimeters
Element Control	Manual
Display Style	Body Color
Bounding Box	
Properties	
Volume	8.0397e-004 m^3
Mass	2.4076 kg
Scale Factor Value	1.
Statistics	
Basic Geometry Options	
Advanced Geometry Options	

Figure 17 - Geometry Properties

The vibrational analysis of a potential area of interest in the Stereotaxic Instrument was carried out for successful estimation and demonstration of the viability of the design and model in terms of precision. Since we have a drill at the end of our cantilever beam in our design which moves at high RPM, its vibrational analysis became a compulsion to know how much vibrations it would cause in the design. This is discussed as below:

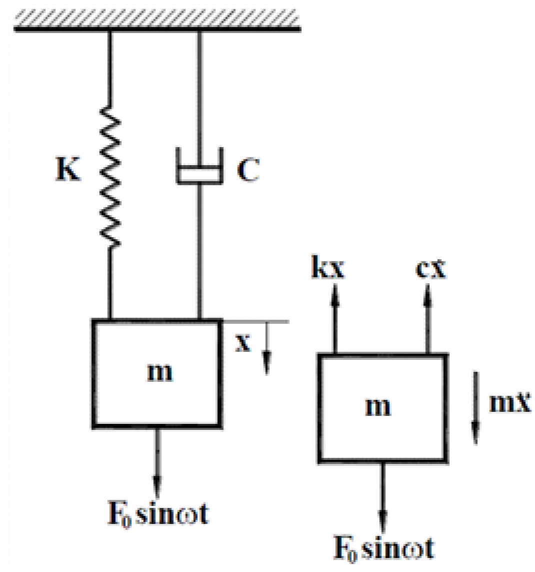


Figure 18 - The model can be demonstrated by the following Free Body Diagram, it comprises of a spring, damper and a harmonic forcing function, representing the drill

3.4.1 Governing Equation and Mathematical Modelling:

$$mx'' + cx' + kx \ = \ Fcos\theta$$

The equation for the system model has the following final form after mathematical manipulation:

$$x'' = (\frac{k}{m})x + (\frac{c}{m})x' + (\frac{m_m}{m_s})R\omega^2 sin\omega t$$

Using,

f = 13.226 Hz

$\omega_n = 2\pi f = 83.35$ rad/s

$m_s = 2.4076$ kg

k = 16726.13 N/m

m_m = 1.4 kg (measured)

R (three cases) = 1mm, 1 micron, 1 nanometer

c (three cases, demonstrated in Analysis)

$\omega = 2\pi f$ where f = 30000rpm/60 (at maximum RPM)

3.4.2 Modelling in Simulink MATLAB:

Modelling was done in Simulink to solve the equation for vibration. As demonstrated in Figure 19, the sine wave function depicts the harmonic forcing function and the results are demonstrated and discussed in the Analysis section with three different values of 'R'.

The three cases of damping are demonstrated with a step input in order to determine validity of the system and the settling time.

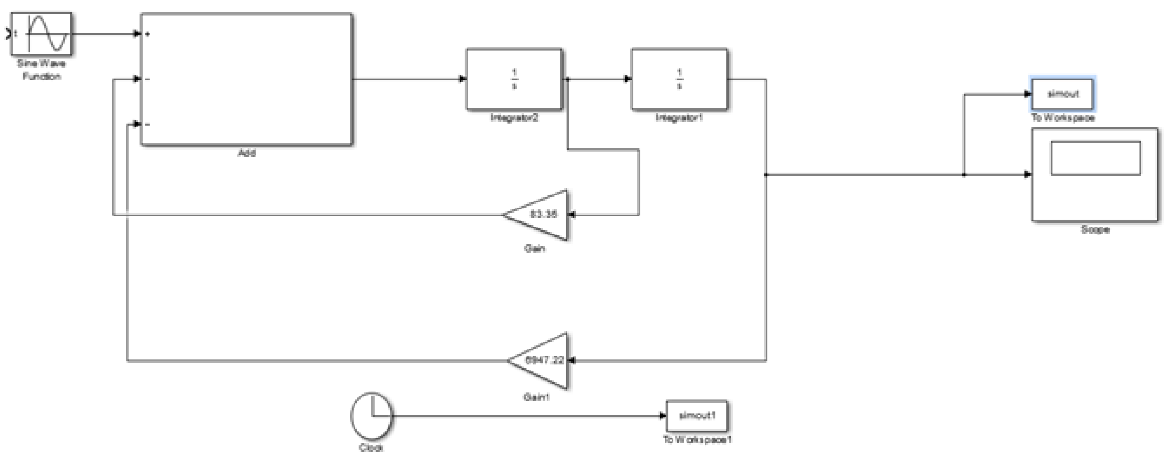


Figure 19 – Simulink Model

The case displayed by Figure 20 is for critical damping with the graph shown in the analysis section.

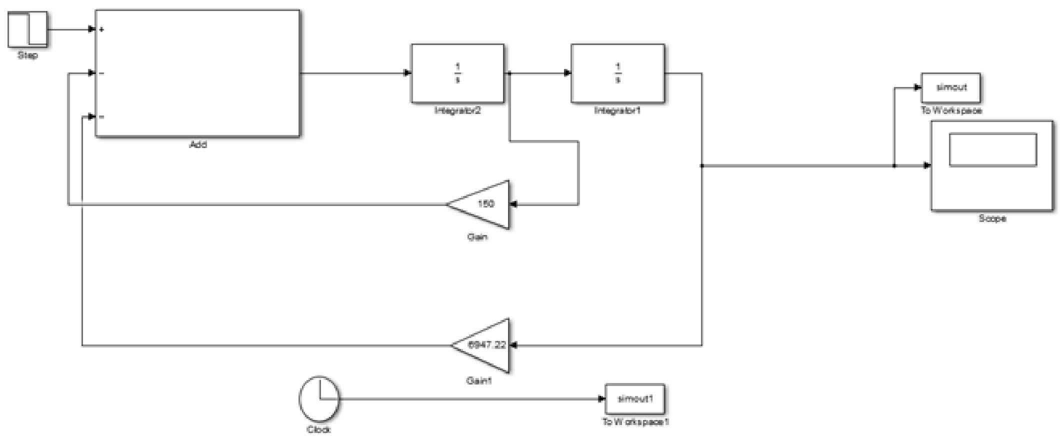


Figure 20 – Critical Damping

3.5 Analysis:

The three cases of the relevant deflection and its implication on imprecision for center of gravity offset from motor's rotational axis are discussed as follows:

<u>Case 1:</u> Figure 21 demonstrates the maximum deflection value in the range of 12 mm for the value of R = 1mm with amplitude of deflection on y-axis in meters and the scaled time in intervals of 0.02 seconds on x-axis.

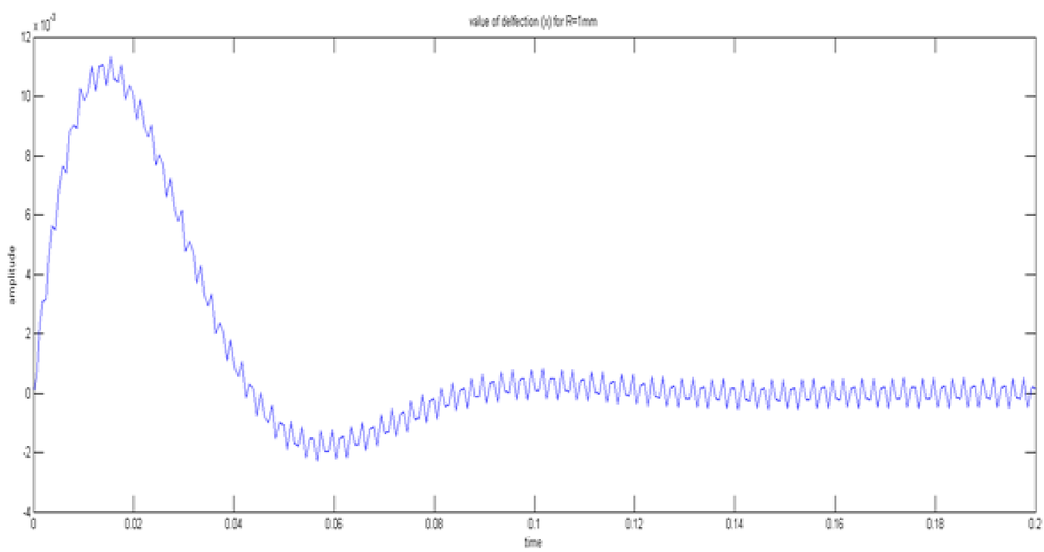


Figure 21 - Case 1

Case 2: Figure 22 indicates the maximum deflection value in the range of $12*10^{-3}$ mm for the value of R = 1 micron with amplitude of deflection on y-axis in meters and the scaled time in intervals of 0.02 seconds on x-axis.

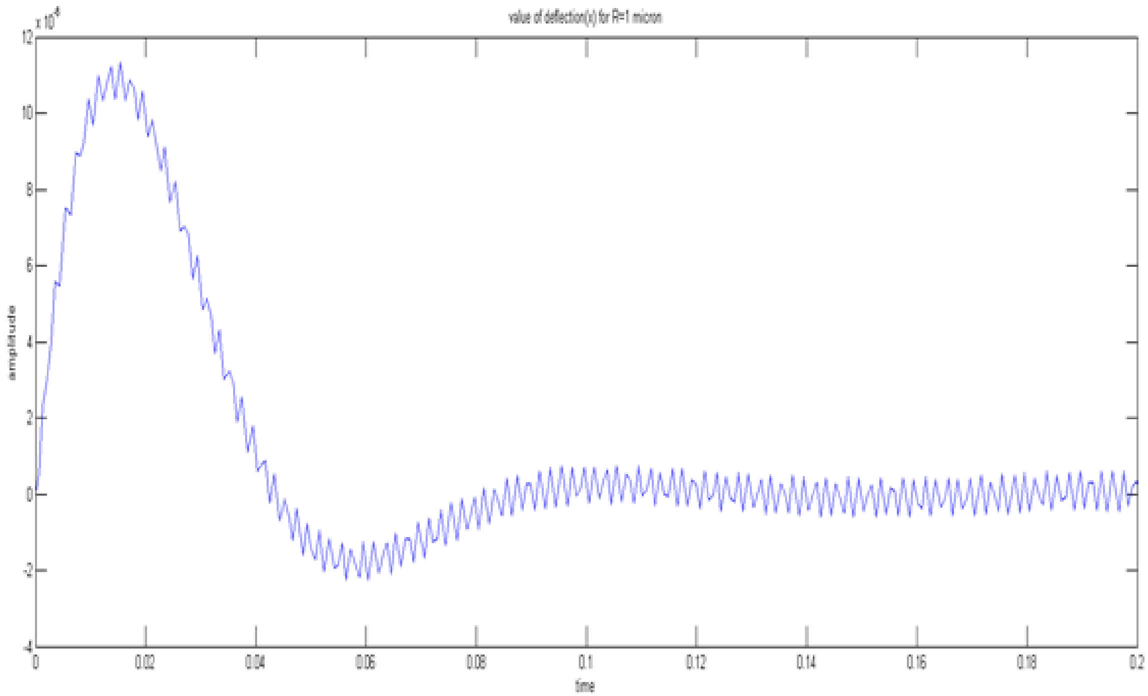


Figure 22 - Case 2

Case 3: It can be seen from Figure 23 that the maximum deflection value is in the range of $1*10^{-4}$ mm for R = 1 nanometer.

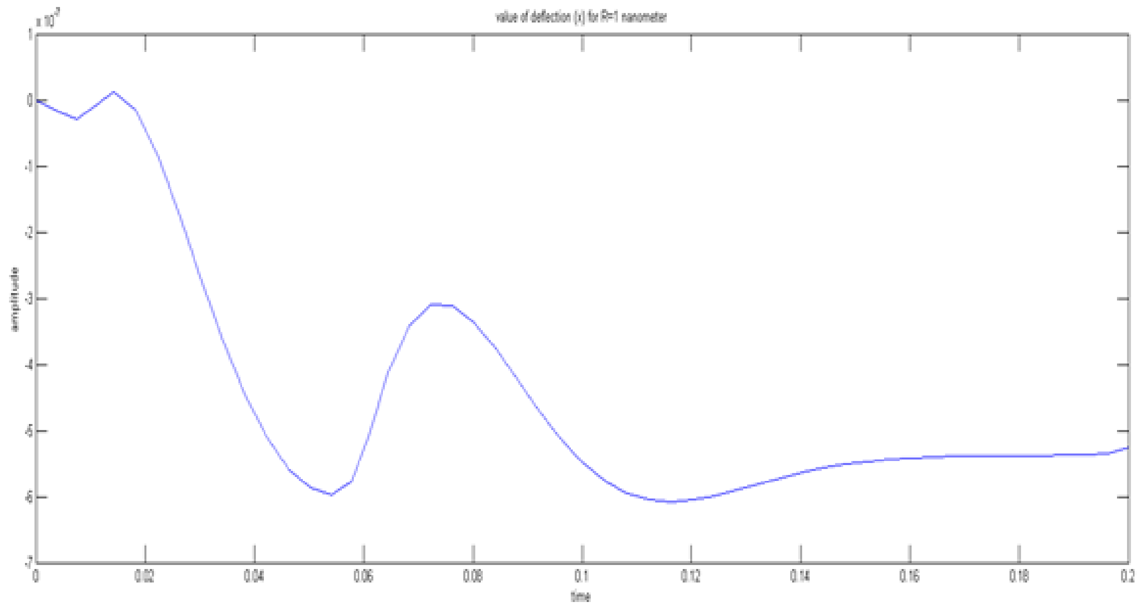


Figure 23 - Case 3

The three cases of the relevant deflection and its implication on imprecision for damping constant are discussed as follows:

Case 1: The settling time as seen by Figure 24 for the case of critical damping ζ = 1 can be observed to be in the range of 0.06 seconds with time on x-axis and the amplitude of deflection on y-axis.

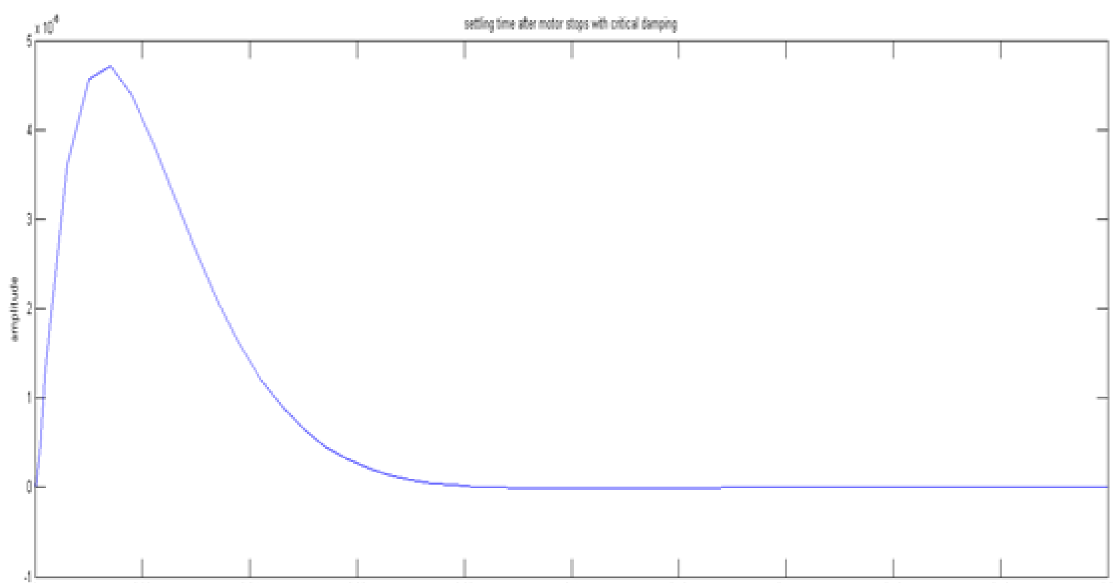


Figure 24 - Case 1, ζ = 1

Case 2: Figure 25 demonstrates the underdamped case for ζ = 0.05.

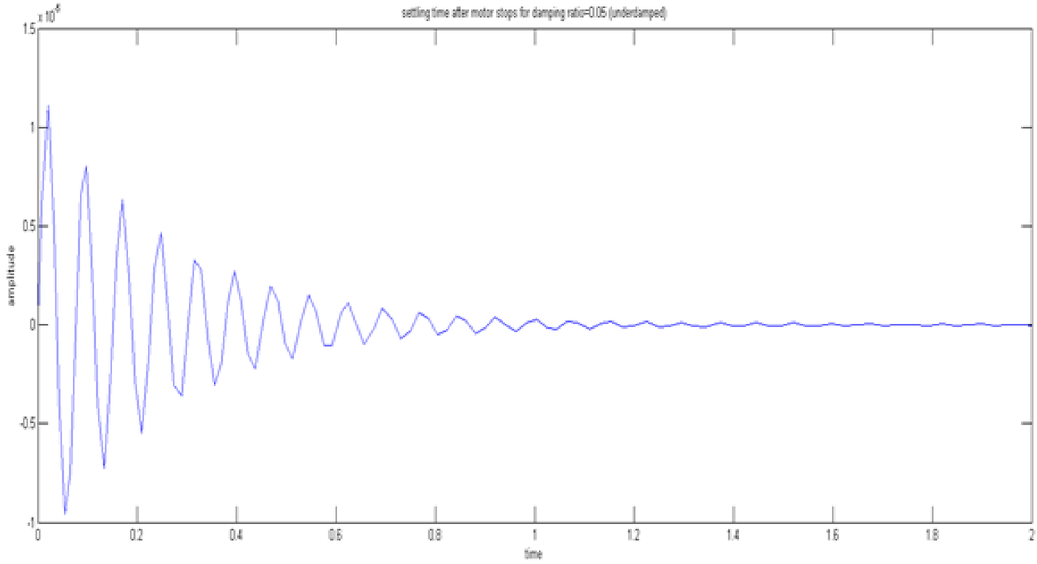


Figure 25 - Case 2, ζ = 0.05

Case 3: The settling time as seen by Figure 26 for the case of overdamped system, damping ratio ζ = 2 can be observed to be in the range of 0.15 seconds which is more than that for the critically damped case as expected.

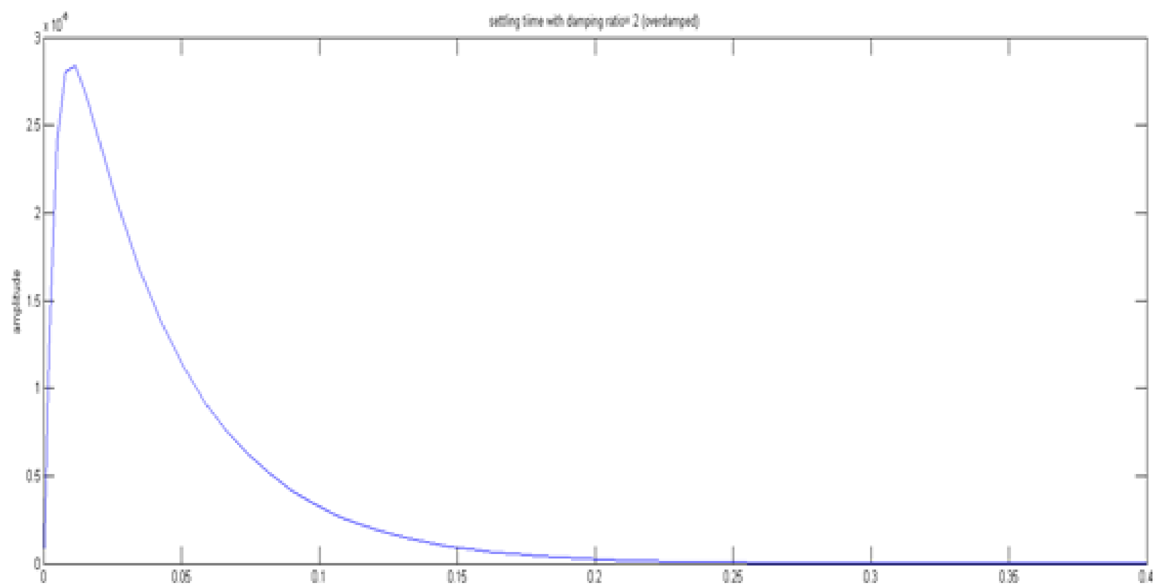


Figure 26 - Case 3, ζ = 2

3.6 Fabrication:

Considering the conception and verification of a preliminary working model, as we move towards fabrication, the task at hand is primarily to minimize complications resulting from local machining since the precision and efficiency of the Stereotaxic Instrument cannot be compromised. As a result of careful council and assessment, the shortlisted guiding mechanisms include,

- Lead screw and dual rail shaft support as portrayed in the concept design. This mechanism is relatively cheaper, lead screw is self-locking and better for vertical applications. However, it is less efficient and any sort of machining error related to permanent fastening with other components would render it rather imprecise. Furthermore, important considerations of lead screw include the higher friction, temperature and the need to be replaced more frequently when it comes to continuous or long cycle time. [15] [10] [16]
- An upgrade on the aforementioned design would incorporate a lead screw and slide rail coupled via clamp and stepper motor results in high co-axiality and precision (Figure 27). Furthermore, the model has exceptional transmission efficiency, large thrust and operational stability. The sliding table does not have drivability and the drawbacks associated with the use of a lead screw are applicable, as already mentioned.

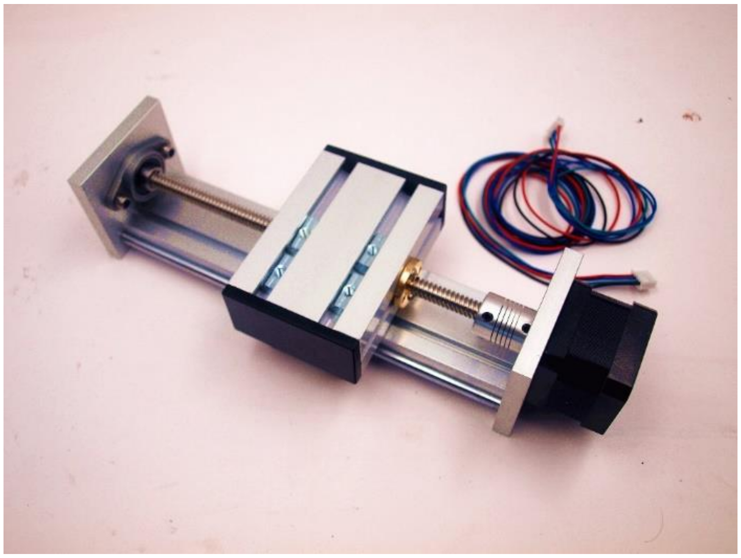


Figure 27 – Slotted Table Clamp CNC Rail

- A pre-engineered, easily mounted, ready-to-run solution would be to use a ball screw (positional accuracy up to ±0.05mm) driven XYZ stage. It comprise of three linear guides for X, Y, Z motion and connecting panels (Figure 28). Ball screws provide higher precision with little friction as compared to lead screw. Furthermore, they can be easily controlled at high efficiency and precision in the range of microns. However, ball screws might get back-driven due to low internal friction and they are bulkier in comparison to lead screws.

  In conclusion, the efficient problem solving of this mechanism makes up for the significantly higher cost associated with it.

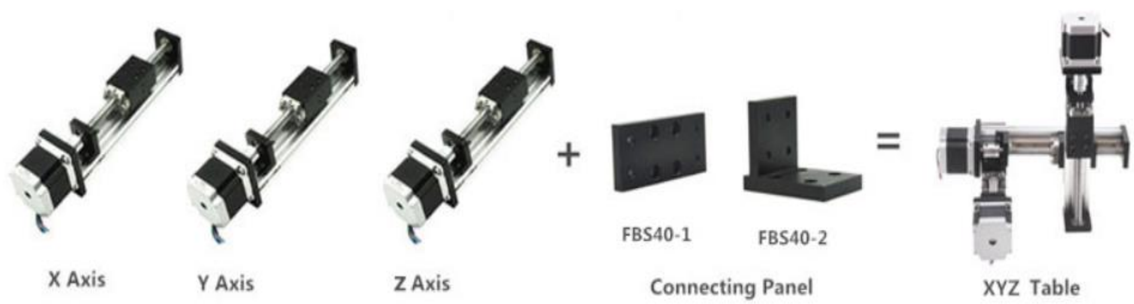


Figure 28 - Ball Screw Type CNC Rail

3.6.1 Practical Application:

After careful consideration, the third bullet point in the aforementioned heading was rendered suitable for use and hence a 3 axis linear guide rail was ordered keeping our parameters in check. It consists of an Aluminum body which has pre-fixed ball screws of 5 mm pitch, 16 mm diameter and 100 mm travel. Three stepper motors are fixed to each axis (NEMA 23 model) and are coupled with the ball screws. The actuator consists of mountings required to hold each rail in the desired axis while that of x-axis is free with spaces made for screws in order to mount the holder on it. This can be seen in the figure as below:

Figure 29 - 3 axis CNC linear rail set

According to the dimensions of this rail set, the novel holder was redesigned for the provision of all the three tools simultaneously. It was first designed using SolidWorks, analyzed upon and then a drawing file was made using which it was manufactured with the help of CNC. Mild steel was chosen as the material for its fabrication since it not only had to hold the instruments but also had internal threads for screws (M8) which were used to position the tools. Aluminum cannot be threaded nor is it resistant to bending as compared to its MS counterpart under weight of the tools. It was made in two parts, a front plate and a side plate. The two plates were then welded together at 90 degrees using tacks. A drawing of the holder and its corresponding fabricated part can be seen in figures below:

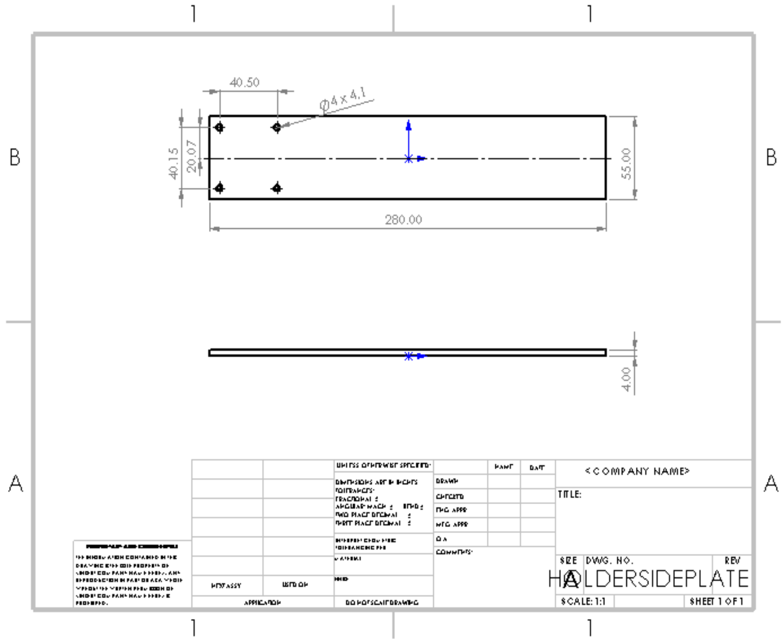


Figure 30 - Holder Side Plate

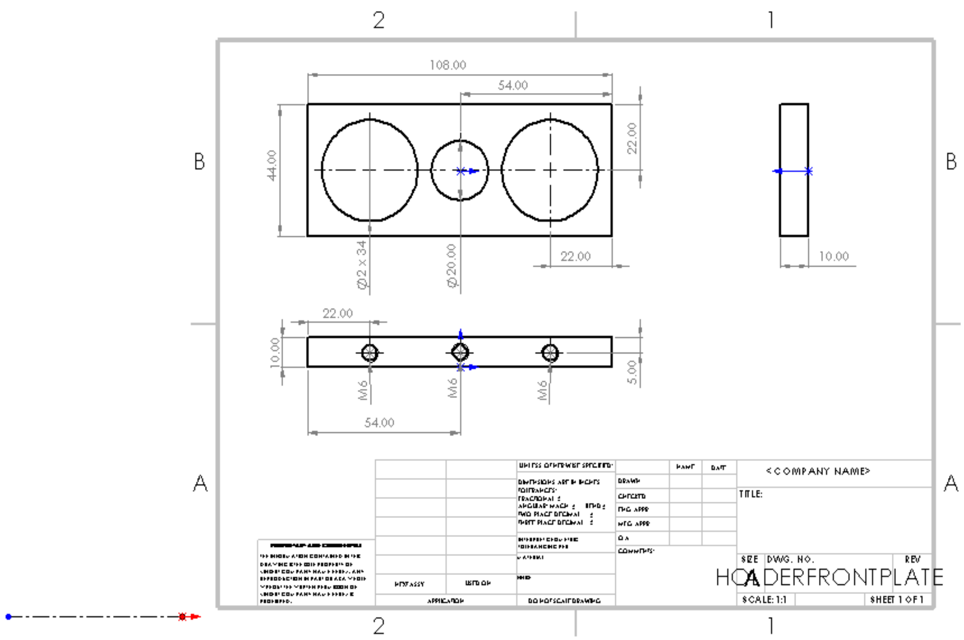


Figure 31 - Holder Front Plate

The final part took the form as below:

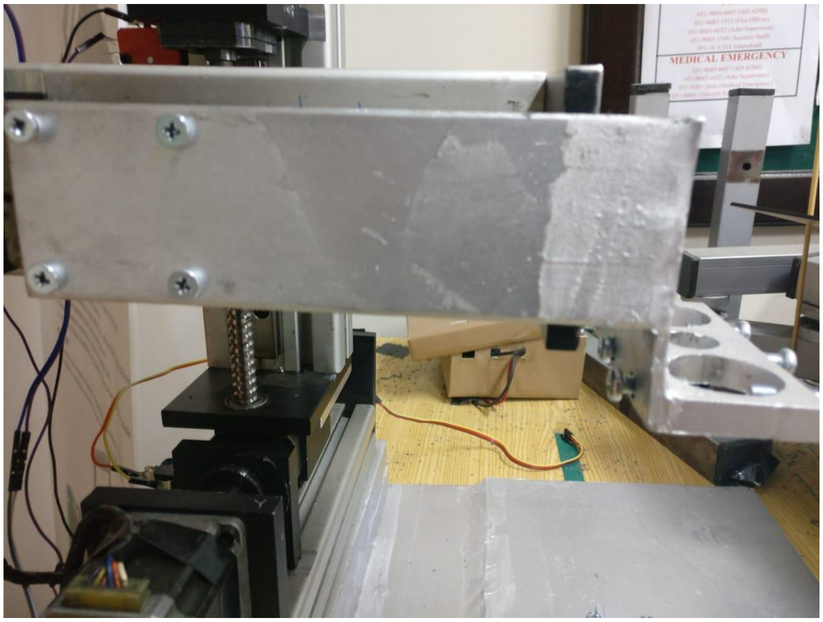


Figure 32 - Holder from the side

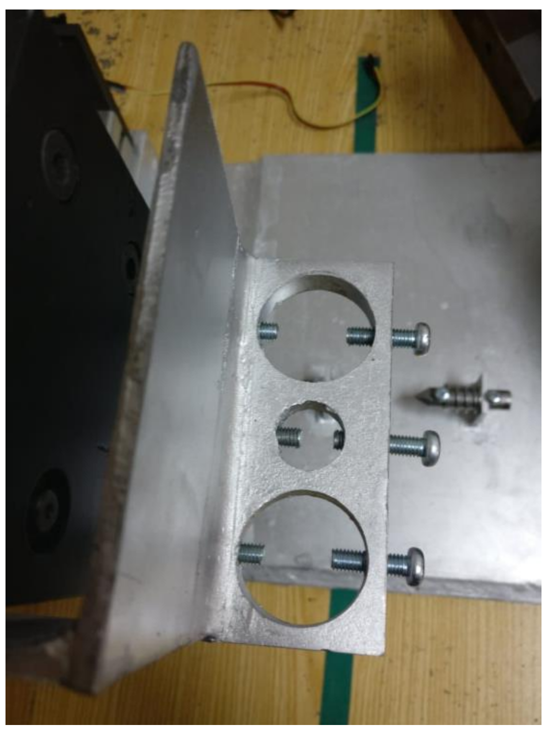


Figure 33 - Holder from the top with corresponding CNC holes and assembled screws

The whole part was then assembled and placed on a MS base, the dimensions of which were measured according to the linear rail set and the holder position. A sample space of 10 rats was taken at the NUST animal lab and rat head sizes were measured for all of them. An average of the data was taken and compared with data available online to accurately manufacture ear bars for head placement. The novelty in ear bars was the way they were actuated. Commercially available ones are set up using screws where as our design incorporated spring actuation. Just by changing the spring constant for these springs, the ear bars can be adjusted for all kinds of head size. And since they not only clamp the head but force it in place due to the spring force, the ear bars are sufficient enough to clamp the rat in place. The average head size of the rats are as follows:

Male rat – width: 26.35 mm, length: 27.89 mm

Female rat – width: 23.64 mm, length: 23.82 mm

The correct gap between the ear bar plates, their height as extracted using the research paper by M. Zubrzycka, J. Fichna, A. Janecka [17], which are as follows:

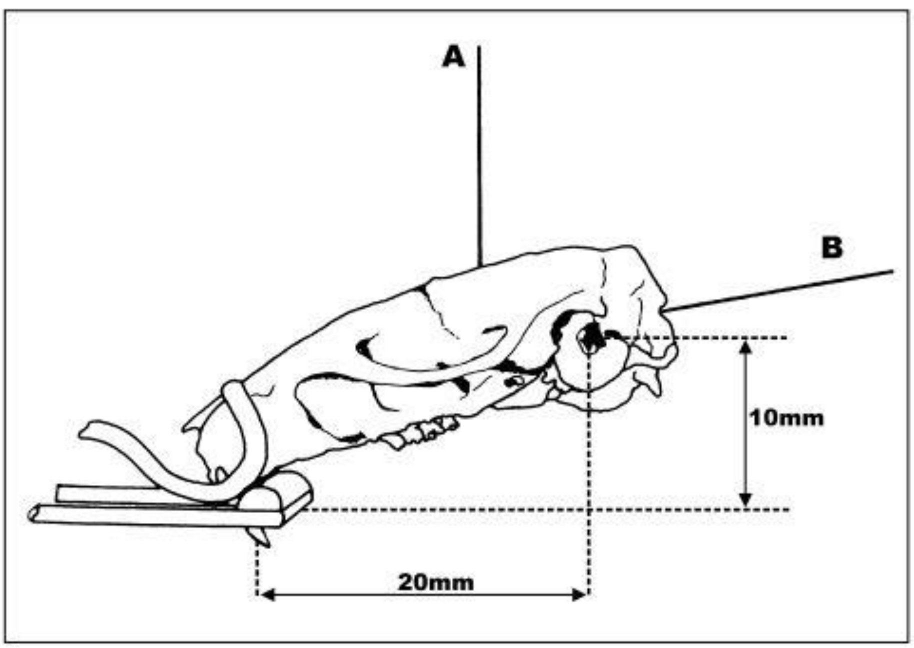


Figure 34 - Rat head position dimensions for ear bars

Hence the manufactured ear bars can be seen in the following figure:

Figure 35 - Ear Bars

Accordingly after thorough study, it was found out that since the skull of the rat is paper thin, it only needs an RPM of about 30000 to 35000 to drill through it. We bought a corresponding dental drill with variable RPM, the maximum of which was 45000. This drill had an independent power supply and could be easily fixed in out holder.

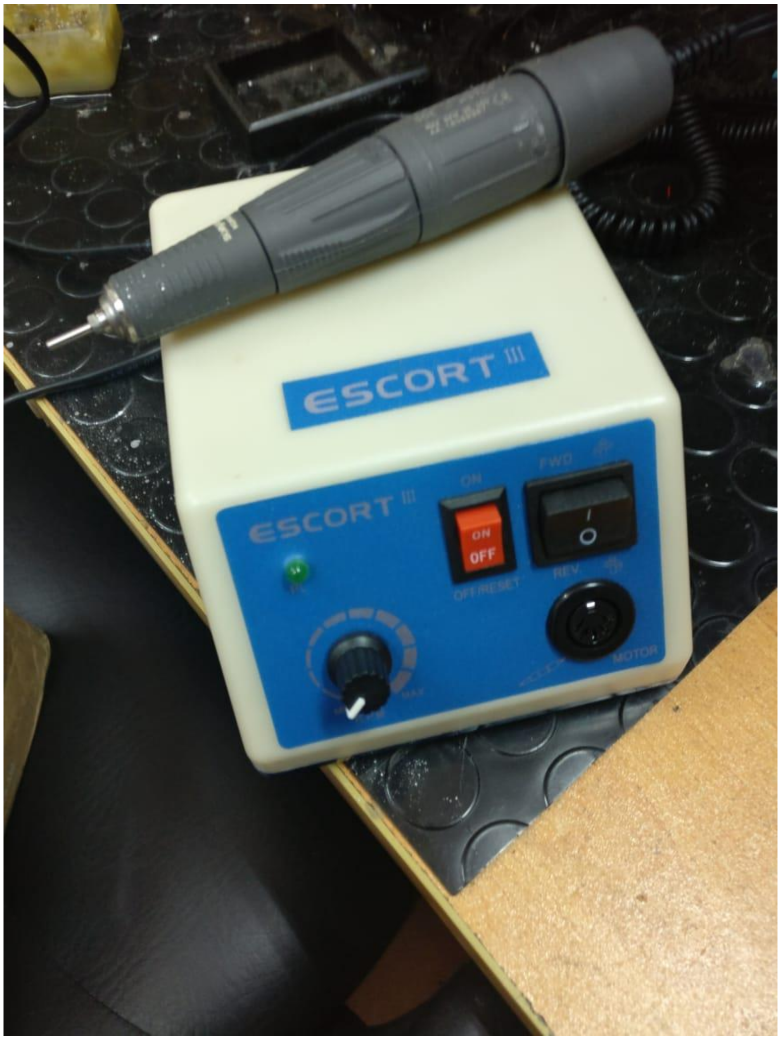


Figure 36 – Drill

The final mechanical assembly was as shown below:

Figure 37 - Final Assembly

3.7 Computer Integration:

Computer integration was done afterwards for a complete electro-mechanical model instrument. This allowed to control the motors as well as allow the movement of the drill to accurately follow the atlas of the rodent as well as insert electrodes and drill inside it. This was accomplished using an Arduino Uno, a CNC shield mounted over the microcontroller and three stepper motor drivers (DRV 8825) one for each axis.

The DRV 8825 driver was used because its specifications matched the parameters of our motors such as max current and voltage. It also had a small heat sink attached to it in order to cool it if the current drawn exceeds its maximum limit. [18]

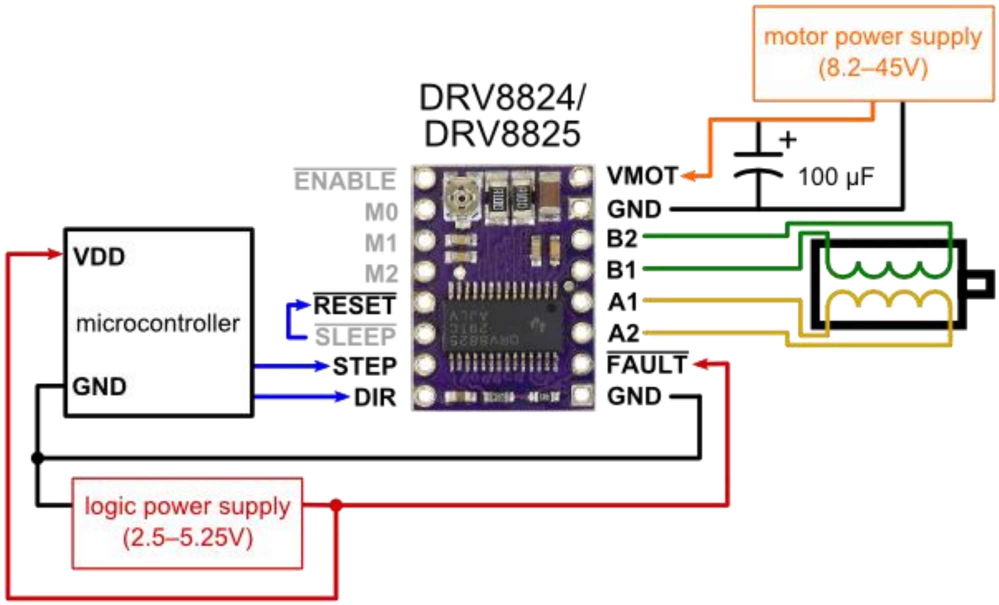


Figure 38 - DRV 8825 driver and its connections

The CNC shield used was of Protoneer (figure 39) and had place for 4 axis in case one wants to incorporate another axis. Its configuration and connections were done using schematics as shown in figure 38 and as displayed on their website. [19]

The drivers were placed over the CNC shield and the Arduino was connected to the laptop which configured the CNC shield. An Arduino library known as "GRBL" was used which contained pre-programmed code to run a 3 axis CNC. A software called "Universal G-code Sender" was then installed which ran on Java. This software read the library burned on Arduino and produced G-codes (as commanded by the user), linked them to the specific library and produced the corresponding output. Three limit switches were also used on one side of each axis which helped the machine to get back to its reference point. There are two kinds of limits for the GRBL library:

3.7.1 Hard Limit:

These limits used physical normally open switches to control the actuator. They performed two functions. It stopped the actuator to extend beyond its length of travel so that the motors do not burn, and secondly it helped them to find their starting point and hence accurately position themselves to machine zero every time the machine starts.

3.7.2 Soft Limit:

Soft limits help to check the machines from going beyond their maximum travel limit by not accepting commands for coordinates that are in excess to the defined limit. For example if the max travel is 50 mm then a command of 51 mm would trigger a soft limit. Extension of actuator on the other side of axis was controlled by this limit.

Every time the code $H was entered, the machine referenced itself to machine zero point by configuring all three axis using the hard limit switches. This point was then used to calculate the respective distances of the bregma, lambda and interaural line of the encapsulated rat, and hence correspondingly from the rat Atlas, was then used to move the machine using G-odes to the area of inspection. This is the first of its kind Stereotaxic instrument that is easy to operate using basic CNC G-codes and is ultraprecise.

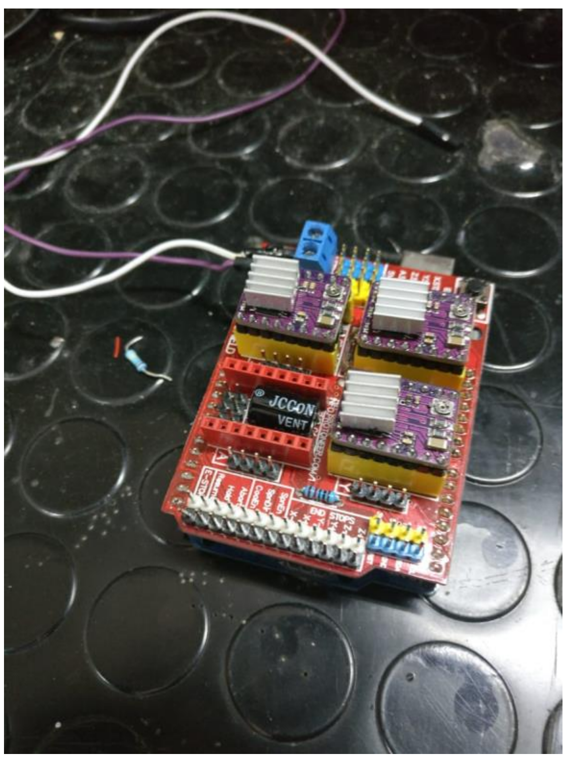


Figure 39 - CNC Shield placed over arduino along with the three drivers

The GRBL library was first configured using the Universal G-code Sender Software and then was set up accordingly. The GRBL settings [20] can be seen in Appendix 1.

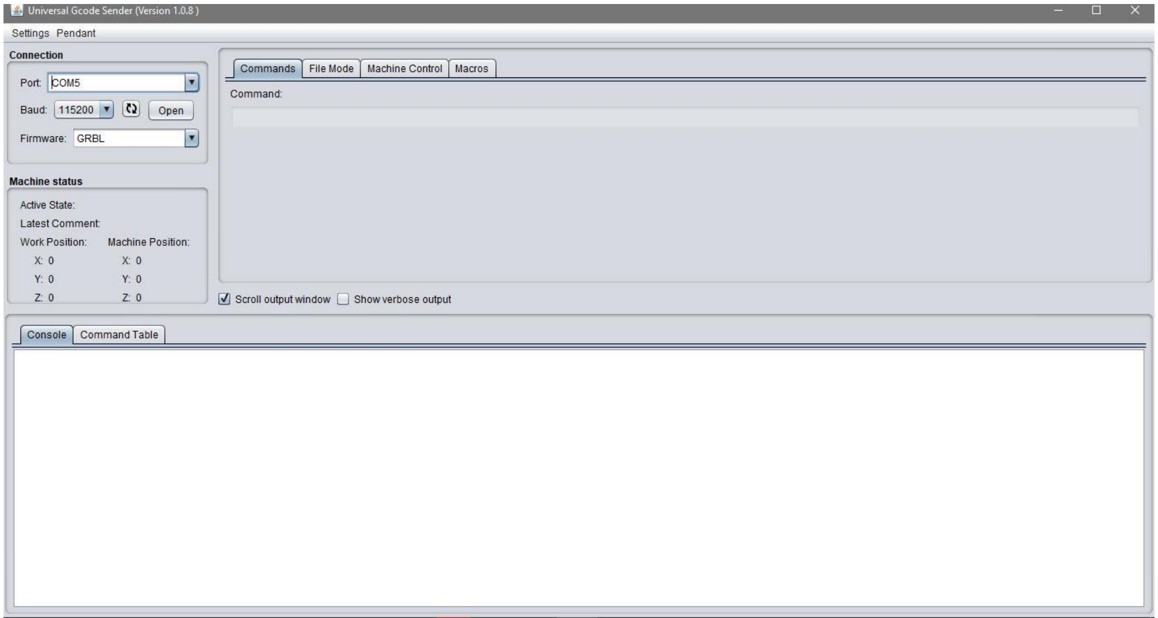


Figure 40 - Universal G-code Sender Interface

The final complete working assembly along with the power supply and a Teflon rat head model and connected drill can be seen in the figure attached below:

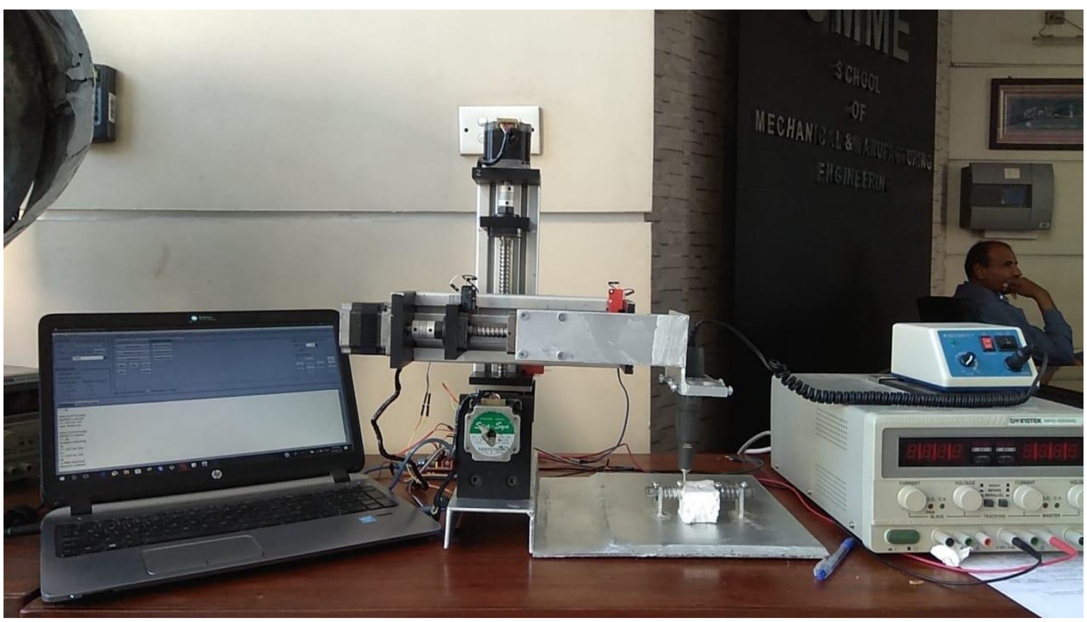


Figure 41 - Complete working prototype

CHAPTER 4

RESULTS AND DISCUSSIONS

- It can be observed that changing the R value for the forcing function case in Simulink the deflection as a result of vibrations can be minimized by decreasing values of R. Since we have a pre-manufactured dental drill with an already analyzed motor, vibrations due to its high RPM are negligible. Nonetheless, the analysis shows that deflection due to vibrations will not compromise on our objective.
- Minimizing settling time is of major concern for a Stereotaxic instrument and it is accomplished effectively by the aforementioned electromechanical integration.
- The deflection due to weight of the cantilever beam does not interfere with the precision of the instrument, confirmed by the practical operational repeatability of the instrument.
- Raising torque of the motor helps in selection of the correct NEMA motor, while the factor of safety caters for the assumptions involved.
- The instrument provided precision below 1 mm and as accurately as half of a millimeter. This was measured using a scale placed behind the drill bit and observing the movement of the bit when commanded to move 1 mm on the corresponding axis. It can be observed in the figure below:

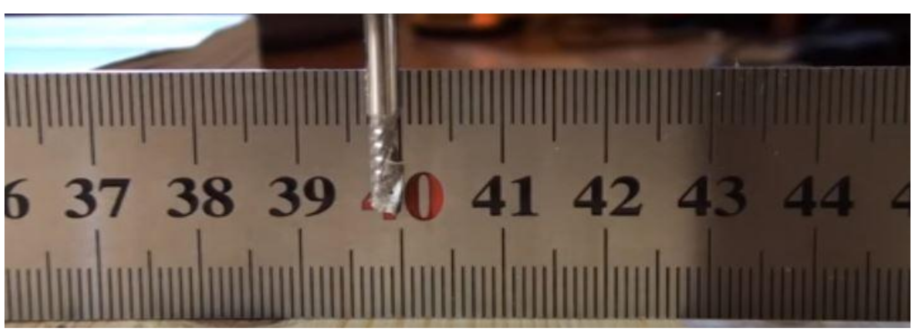


Figure 42 - Accuracy measurement in movement

- In order to eliminate the parallax in the above mentioned method, a Vernier caliper was used to marked 1 mm marks on the Teflon head of the rat and the instrument was made to move from one mark to another. This way the precision in movement was accurately measured.

- Repeatability is defined as:

  "*Repeatability or test–retest reliability is the closeness of the agreement between the results of successive measurements of the same measureand carried out under the same conditions of measurement" – Wikipedia*

  According to this, a measurement made successively should be the same as recorded the first time it was observed. To measure this, a drill was made on the sample Teflon rat head and the machine was brought back to its reference point. It was then switched off and turned on, given the exact same commands so that it drills on the same spot. As a result the drill was made at exactly the same place and this was ensured by measuring the diameter of the drilled hole repeatedly in order to know of the error in each measurement. This method was carried out 10 times and an error of 0.003 mm was recorded. This ensured the deliverable of precision and consequently, the electro-mechanical integration.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion:

- The feasibility of the Stereotaxic Instrument considering the modelled analysis is of paramount importance, the safety and optimal working of a stereotaxic instrument during operation depends upon the precision and smooth settling time. In order to attain a precision of 1 mm as a limiting case, the analysis depicts that only values in the range of 1 micron for the center of gravity offset from the motor's rotational axis are acceptable since a value of 1 mm results in the deflection of 12 mm which is not acceptable for ultraprecision of the stereotaxic instrument.
- As we increase the value of the damping ratio from 1 to 2, the settling time ranges from 0.06 to 0.15 seconds. Minimizing the settling time is our second major concern and a system in close proximity to the critically damped case would accomplish the task at hand.
- Minimizing the deflection of cantilever beam is another factor of paramount importance which is tackled effectively through use of different material.
- Removing play between components and keeping them stable and free of vibrations allow us to use aluminum extrusions. This can be done after fabrication in order to maximize stability in highest vibrational area.
- Alignment of lead screws along with the shafts of stepper motors are important in order to allow them to move in the required axis without a component of force hindering the moving along the said axis. This will be done either through precise CNC manufacturing or purchase of pre-aligned CNC 3D printer rails which allow for a much more precise instrument as discussed above.
- The CNC manufactured rails consisting of ball screws ensured minimal deviation from the set precision target. Step idle delay of the motors accounted for the low friction in ball screws in order to prevent back drive and the "zero" back lash of the ball screws enabled a precise movement in each axis without loss of steps.

- The driver used and the CNC shield both provided an additional option of micro-stepping for the stepper motors in order to further increase precision by compromising torque. This can be measured experimentally and iteratively regarding how much micro-stepping is needed. As a rule of thumb, one should stop once the target precision is met. That being said, our model meets the required needs without the incorporation of micro-stepping.
- Aluminum extrusion supports can be utilized to further decrease the deflection in cantilever beams by converting them into simply supporting beams. This will also aid in decreasing vibrations of the beam during its movement.
- The Universal G-code Sender software incorporates an additional feature where the rat atlas can be fed to it and the machine can be moved to the specific location with respect to the reference point by simply clicking on the location on the atlas.

5.2 Future Recommendations:

The future prospects of the stereotaxic instrument are widespread and appeal to a range of utilities depending upon the work environment. Considering application in the local lab, some of the provisions that would contribute to the practicality of the instrument have been discussed as follows:

- Surgical positioning incorporating a user- interface software for accurate movement. The integration of the software with brain atlas of the rodent results in an image-guided operation. The 3D atlas integration with the software for navigation provides real time visualization and calculation which is of paramount importance in terms of planning before the operation and guidance during operation.
- Provision of anesthesia on site at the head stabilizing mechanism. The idea is to perform the stereotaxic operation, stabilize the head of the rodent, while minimizing pain and complications at the same time. One way to accomplish this is the use of polydimethylsiloxane (PDMS). This PDMS device is heat and water tolerant making it possible to maintain sterile working environment. Moreover, since the skull of rodents is

very soft, this device can successfully make the required cut and hole. Another advantage of its applicability is that it is compatible with a wide range of stereotaxic designs. [21]

- Development of a stage for simultaneous operation on two rodents with separate head clamping apparatus. Depending upon the degree of application, another clamping mechanism as a future prospect would reduce the time of operation and provide simultaneous performance leading to better accessibility with the same holder.
- Adjustable ear bars could be manufactured in order to incorporate various small rodents including squirrels, mice, etc. so that each head size can be fixed between the ear bars.

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APPENDIX I:

Table 2 - GRBL library settings

Settings and sample values	Description
$0=10	Step pulse, microseconds
$1=25	Step idle delay, milliseconds
$2=0	Step port invert, mask
$3=7	Direction port invert, mask
$4=0	Step enable invert, boolean
$5=0	Limit pins invert, boolean
$6=0	Probe pin invert, boolean
$10=1	Status report, mask
$11=0.010	Junction deviation, mm
$12=0.002	Arc tolerance, mm
$13=0	Report inches, boolean
$20=1	Soft limits, boolean
$21=1	Hard limits, boolean
$22=1	Homing cycle, boolean
$23=3	Homing dir invert, mask
$24=25.000	Homing feed, mm/min
$25=500.000	Homing seek, mm/min

$26=250	Homing debounce, milliseconds
$27=3.000	Homing pull-off, mm
$30=1000.	Max spindle speed, RPM
$31=0.	Min spindle speed, RPM
$32=0	Laser mode, boolean
$100=40.000	X steps/mm
$101=40.000	Y steps/mm
$102=40.000	Z steps/mm
$110=500.000	X Max rate, mm/min
$111=500.000	Y Max rate, mm/min
$112=500.000	Z Max rate, mm/min
$120=450.000	X Acceleration, mm/sec^2
$121=450.000	Y Acceleration, mm/sec^2
$122=450.000	Z Acceleration, mm/sec^2
$130=100.000	X Max travel, mm
$131=100.000	Y Max travel, mm
$132=100.000	Z Max travel, mm

Step Idle Delay = Hold position

Direction Invert = Invert direction of axis

Limit Pins Invert = Invert action of limit switches on either side of the axis

Homing = Bring back to reference point

Homing Direction Invert = Invert homing direction for each axis

Homing Pull off = the distance actuator draws back after closing a limit switch.

Acceleration = Acceleration of actuator on each axis. The more the acceleration, the faster the actuator initiates. (Measured experimentally and iteratively)

Speed = Velocity of actuator on each axis. Lesser the velocity, more the loss in steps. (Measured experimentally and iteratively)

Steps/mm = 200 steps per revolution and 5 mm of travel in one revolution. Hence steps per mm should be 40 (200/5).