Automatic Positioning Device for Cutting Three-Dimensional Tissue in Living or Fixed Samples. Proof of Concept

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***Abstract*— The study and analysis of tissues has always been an important part of the subject in biology. For this reason, obtaining specimens of tissue has been vital to morphological and functionality research. Historically, the main tools used to obtain slices of tissue have been microtomes and vibratomes. However, they are largely unsatisfactory. This is because it is impossible to obtain a full, three-dimensional structure of a tissue sample with these devices.**

This paper presents an automatic positioning device for a three-dimensional cut in living or fixed tissue samples, which can be applied mainly in histology, anatomy, biochemistry and pharmacology. The system consists of a platform on which the tissue samples can be deposited, plus two containers. An electromechanical system with motors and gears gives the platform the ability to change the orientation of a sample. These orientation changes were tested with movement sensors to ensure that accurate changes were made.

This device paves the way for researchers to make cuts in the sample tissue along different planes and in different directions by maximizing the surface of the tract that appears in a slice.

I. Introduction

A microtome is one device used to cut slices through tissue, but it has a fixed blade. A vibratome, on the other hand, is similar to a microtome but has the advantage of a vibrating blade. The amplitude and speed of vibration, and the angle of the blade, can all be controlled in current models of vibratomes; however, the sample orientation cannot [1].

Vibratomes are mainly used in histology [2–4], anatomy [5], biochemistry [6–8] and pharmacology [9]. Furthermore, they are key in neuroscience [10], because they are useful tools which allow researchers to obtain fresh tissue slices.

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In the last two decades the use of vibratomes has increased in neuroscience laboratories [11-13]. Vibratomes are used to obtain relatively thin sections of tissue (hundreds of microns thick) without the need to process this tissue. As a result, they are used to simplify the three-dimensional complexity of brain function and to investigate the anatomical connectivity of selected neuronal groups. Therefore, this equipment permits *in vitro* electrophysiological studies; for instance, the investigation of electrical and functional coupling between neuronal populations and the fine details of the structural connectivity.

The drawback with existing commercial vibratomes is that they only permit cuts in a single plane which is defined by the user [14]. Moreover, once the cut has begun, the plane cannot be altered. In addition, these planes are subject to axial and longitudinal directions. Because of the three-dimensional nature of the brain, it is impossible to study most of its information processing pathways. The most interesting tracts in the brain do not exist in a two-dimensional plane.

Physiological studies in local neuronal populations using conventional vibratomes are limited in the usefulness. This is in sharp contrast to the requirements of a three-dimensional structure such as the brain. With our current three-dimensional positioning device, the position and orientation of the sample can be changed while the vibratome is in use. Therefore, it is possible to perform a tomographic slice of any plane following a three-dimensional curve path as shown in Fig. 1. As a result, long distance circuits can be dissected and studied *in vitro* for electrophysiological experiments.

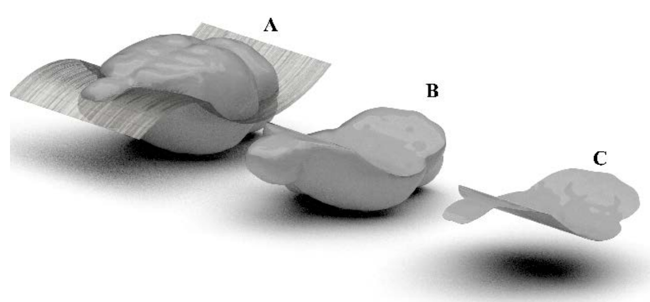

Figure 1. Three-dimensional (3D) model of a mouse brain. The figure shows a simulation process where the curved plane represents the path followed by the cutting blade. A) Tissue sample to be cut and cutting plane. B) Bottom part of the tissue sample after slicing. C) Desired three-dimensional slice.

II. Materials and methods

A. System Concept

The automatic positioning device for cutting three-dimensional tissue is based on a commercial vibratome. The original vibratome has two containers, which sit one inside the other. The outer container is used to keep ice close to the inner container and to maintain temperature of the tissues. The outer container is made of plastic and the base is rectangular. It is 145 mm wide, 235 mm long and 40 mm high. The inner container is used to hold the sample and buffered liquid. It is 90 mm wide, 90 mm long and 35 mm high.

The automatic positioning device for cutting three-dimensional tissue in living or fixed samples consists of two containers with dimensions approximately equal to those mentioned above. Moreover, it was redesigned to contain a mechanical system, as it is shown in Fig. 2. The mechanical system is composed of a pair of interconnected toothed gears, which allow the tissue samples platform to rotate.

Another mechanical system inside the *Gearbox* consists of a worm gear and a toothed gear. This gives the platform the ability to change the angle at which the microtome blade penetrates sample tissue. In addition to this flexibility, the device enables multidirectional histological sections. All parts of this system were designed and modelled with Unigraphics NX 9 (Siemens PLM software, Plano, TX, USA) as shown in Fig 3.

The part of the system which controls the mechanics consists of an Arduino (Smart Projects, Turin, Italy & SparkFun Electronics, Boulder, CO) and a set of electronic drivers; these drivers control two stepper engines that move the gears described above. By combining these engines, the control system and the gears, the device was able to accomplish rotation and inclination movements. A specific firmware was designed to control and coordinate the whole system.

The system was powered by a power supply of 12V (2A) which provides the necessary voltage range for the Arduino system and steppers motors.

B. System Parts

The automatic positioning device for cutting three-dimensional tissue (Fig. 4) contains the *Samples platform* on which tissue samples can be deposited. It was necessary in the design process to ensure that the platform was centered in the *Inner container*.

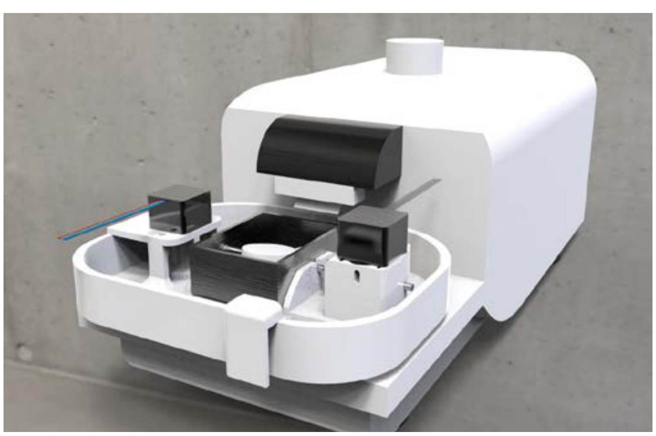

Figure 2. Automatic positioning device for cutting three-dimensional tissue in a sample of living or fixed tissue. The figure shows the three-dimensional model assembled and joined to a vibratome.

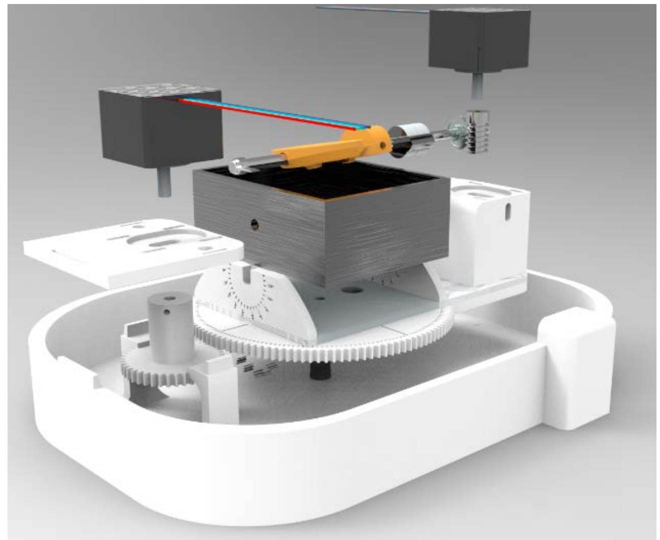

Figure 3. Automatic positioning device for cutting three-dimensional tissue in a sample of living or fixed tissue. The figure shows the three-dimensional model disassembled.

The *Ice container* (Fig. 4, Element 10) was made to contain the rotational mechanical system and dry ice. It has a central axis which was designed to be concentric with the *Structural support* in its center.

The *Inner container* was made to be filled with buffered liquid. However, it was necessary to drill two holes at either end in order to allow the *Shaft* to pass through. Moreover, in order to prevent the liquid from leaking out through these holes, a pair of O-shaped rings were used. To ensure that the inclination mechanical system (a worm gear and a toothed gear) was protected from the dry ice, it was placed inside the *Gearbox*.

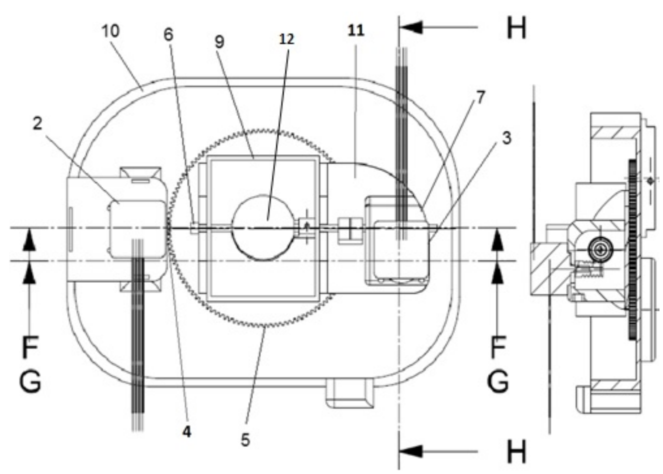

Figure 4. Schematic illustration of the 3D positioning system. The figure on the left shows the top view of the plant and on the right a side view showing the cutting G is depicted. 1-Sample platform, 2-Rotation engine, 3-Angular engine, 4-Rotation power gear, 5-Rotation gear, 6-Shaft, 7-Gearbox, 9-Inner container, 10-Ice container, 11-Structural support, 12-Samples platform.[16]

To enable a rotational movement of the platform, an electromechanical system which consists of the *Rotation engine*, the *Rotation power gear* and the *Rotation Gear* was developed (Fig. 4, Element 2, 4 and 5). To keep a simultaneous movement between the *Rotation gear* and the *Structural support*, both elements were fixed in a solid structure.

The *Rotation engine* and the *Angular engine* have a minimum step angle of 1.8 degrees (200 steps / revolution). Each phase needs 280 mA to 7.4 V, allowing a torque of 650 g-cm (9 oz-in). To drive both engines a pair of stepper controllers A4988 (Pololu Robotics and Electronics, Las Vegas, NV, USA) was used. In order to calculate the exact position of zero at inclination and rotation, a pair Hall effect sensors was installed.

C. Manufacturing materials

The *Inner container* where the living tissue sample is deposited was built in Alumide®; this to achieve maximum thermal transfer. Alumide® is a material of a metallic grey color, composed of aluminum-filled polyamide powder, which is characterized by its rigidity and metallic appearance [15].

The *Ice container* and the *Structural support,* described above, were composed of white plastic materials. It was finished with matte plastic (PA 2200). The *Shaft* that supports the platform was made with stainless steel 420 SS+.

D. Acquisition system

To validate the system, a motion-capturing sensor was used. The more adaptable sensor was the MPU 9150 (InvenSense, Sunnyvale, CA, USA), which is a microelectromechanical system (MEMS) embedded in a microchip. This contains an accelerometer, a gyroscope and

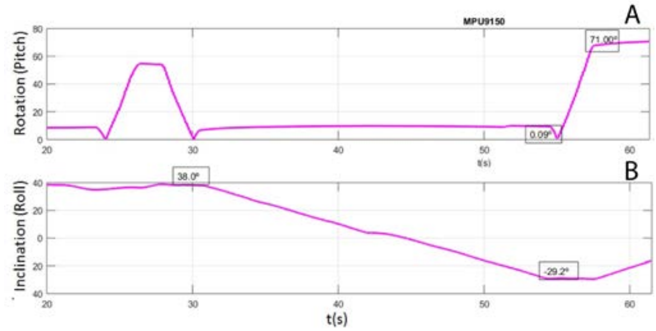

Figure 5. System specifications. (A) Rotation system from zero to the maximum value acquired with the MPU-9150. (B) Maximum inclination difference that was able to do, starting from the most negative inclination to the most positive inclination.

magnetometer. It also includes a Digital Motion Processor (DMP), which enables the calculation process in the main microcontroller. This works at 200 Hz and the results are delivered to the main microcontroller.

Furtehrmore, the system was connected to an Arduino Mega to read the digital signals provided by MPU 9150, and it was also connected by USB to Matlab (The Mathworks, Inc., Natick, MA, USA).

III. Results and Discussion

A. Systems specifications

To know mechanical limits of the automatic positioning device, movements from zero to the end of the path were programmed in a depuration script. The orientation changes were quantified with MPU-9150 as it is shown in Fig. 5.

The mechanical limits were defined as the maximum rotation or inclination angle that is possible to obtain. The

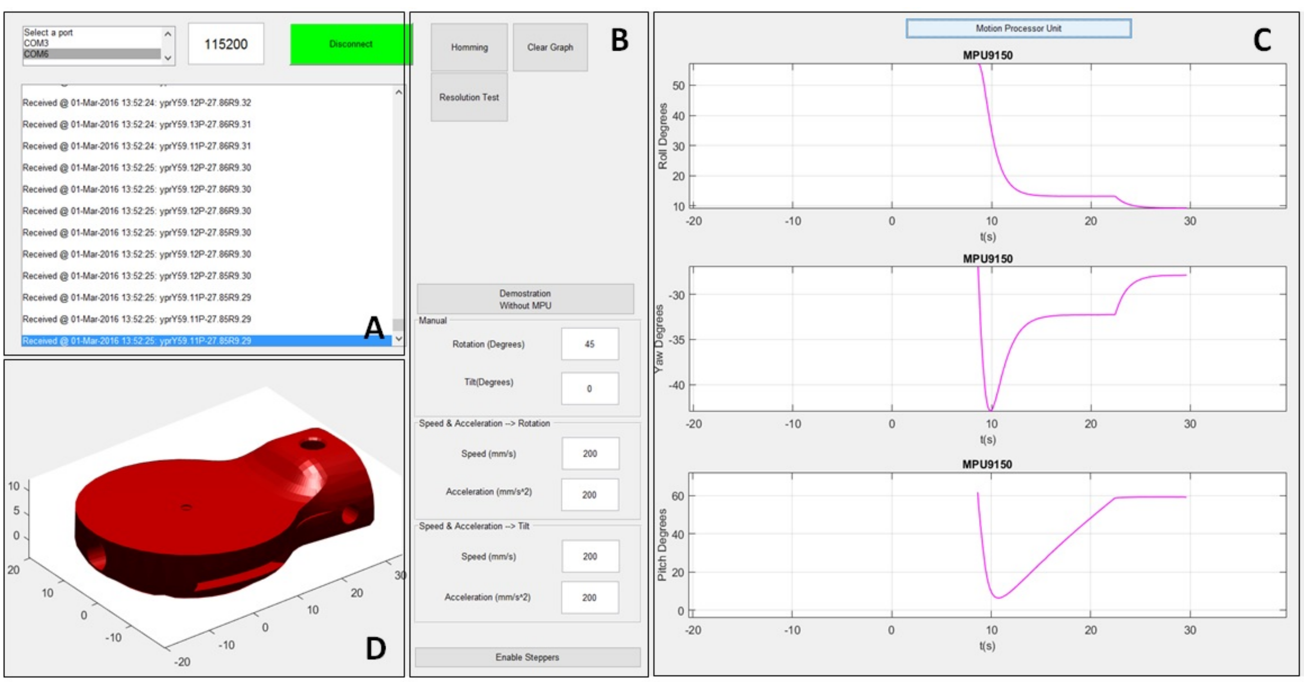

Figure 6. Graphical user interface. This Graphical User Interface (GUI) was made to control each movement of the system and to visualize all of orientation data. At zone A communications settings can be changed to communicate with the system properly. The device setting is provided in zone B. The sensor measurements can be shown in the C zone. Inside of D zone, a real time representation of the Sample's platform is shown while the system is working.

maximum rotation range (pitch) was 70.01 degrees as shown in Fig. 5A. The maximum inclination range (roll) was 67.20 degrees, between -33.6 and +33.6 degrees, as shown in Fig. 5B.

The resolution of the system was defined as the minimum movement that is possible to do with the device. It was obtained by making a test with Arduino firmware and Matlab. The test was started moving the engines gradually. When movement was detected by the MPU, the stop order was send. Then, the number of engine steps divided the movement increment. As a result, the minimum rotation movement obtained was 0.66 degrees and minimum inclination movement was 0.08 degrees.

B. Graphical user interface

To control the system a Graphical User Interface (GUI) was created in Matlab (Fig. 6). It permits to setup USB communications with Arduino and read the I2C protocol provided by the MPU. Arduino firmware interpreted the commands from Matlab to control the movement accurately. Simultaneously MPU data were read by I2C bus and was shown in the GUI. In right side of the GUI the orientation data can be viewed in real time. The 3D object on the lower left side of the GUI, mimics the movement of the platform, to make it easier to interpret.

IV. Conclusions

The concept of an automatic positioning device for cutting three-dimensional tissue in living or fixed samples was proposed, developed and tested. It was designed to give researchers the ability to use it as an enhanced vibratome accessory. The device was designed and developed with professional 3D CAD software. The prototype was manufactured with selective laser sintering technologies. In addition, the device is easy to assemble and disassemble without any tools to make it easier to clean.

The device means the user is able to change sample orientation when the slicing process has started. This makes the device a highly competitive product because it improves tissue research possibilities in almost all biological fields. This is especially true in neurological research where it is important to obtain the whole path or structures of the animal's brain.

The presented automatic positioning device achieves very precise and smooth movements, as reflected in the tests performed with orientation sensors. The device makes it possible to slice samples along different paths or guidelines. The device is currently undergoing tests with a commercial vibratome in order to obtain slices of whole tracts.

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