Robotic Stereotaxic System based on 3D skull reconstruction to improve surgical accuracy and speed

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Abstract

Neuroscience in-vivo animal studies often require injecting DNA material or fluorescent dyes into specific brain regions within the animal's skull. Currently, these types of injections or surgical procedures are done manually by skilled researchers using mechanically based stereotaxic platforms. However, alignment can be very time-consuming and prone to error due to the small size of brain targets.

We developed a next generation robotic stereotaxic platform for small animals by combining a three-dimensional (3D) skull profiler sub-system and a full six degree-of-freedom (6DOF) robotic platform to improve spatial accuracy and surgical speed. The 3D skull profiler is based on structure illumination in which a video projector projects a series of horizontal and vertical line patterns onto an animal skull and capture by two two-dimensional (2D) regular CCD cameras to reconstruct an accurate 3D skull surface based on geometrical triangulation. Using the reconstructed 3D skull profile, the skull can be guided and repositioned using a 6DOF robotic platform which is based on the Stewart design to precisely and accurately align a surgical tool with the intention of reaching a specific brain target. The system was evaluated using mechanical measurement techniques and the precision targeting of the platform was demonstrated using an agar brain phantom. An anesthetized Monogonal gerbil was also used for the system to target the medial nuclear of the trapezoid body (MNTB) by dye injection using a glass pipette. Fluorescence imaging of the excised brain slices confirmed the accuracy of targeting the brain nucleus. The results indicate that this new stereotaxic system can improve accuracy and speed of small-scale brain surgeries for neuroscience studies which may accelerate neuroscience discoveries and reduce the attrition rate of experimental animals.

Introduction

Computer vision and robotics have made significant impact on modern surgical practice. Several computer vision and robotic systems have already been used in operating rooms to improve treatment outcomes and reduce the risks of surgical procedures. In order to increase dexterity and accuracy, some surgical robots were designed to perform motion tasks with multiple degrees of freedom for minimally invasive robotic surgeries (MIRS) [1][2], ranging from medium-sized laparoscopy and endoscopy [3] to small-sized tissue grafting [4].

On the research side, stereotaxic (or stereotactic) surgery for small animals is an indispensable tool for systemic neuroscience studies. Stereotaxic surgeries are routinely performed in neuroscience laboratories on a daily basis for a variety of surgical procedures, including the creation of site-targeted lesions, injection of anatomical tracers, implantation of electrophysiology electrodes, and insertion of optical fibers or micro-dialysis probes [5]–[7]. However, these stereotaxic surgical procedures are often time-consuming and prone to error due to the small size of the brain nuclei.

Current stereotaxic systems routinely used in the laboratory for small animals are largely hand-driven and do not take advantage of modern electronic, mechanical and computer technologies. However, such mechanical systems have the potential to reduce surgical time and increase surgical accuracy. In addition, stereotaxic atlases, which are compiled meticulously by a few anatomy experts and are only available for very limited number of species and age groups, are used to estimate the 3D coordinates of the brain area with respect to anatomical landmarks on the skull. The current fully mechanical based injections or surgical procedures have not been automatically conducted and are prone to error with a success rate of only about 30% in correctly positioning an electrode or a surgical tool to a nucleus within a rodent brain.

In recent years, there have been efforts to develop robotic stereotaxic systems that can automate the stereotaxic surgeries to reduce human operations to improve surgical accuracy and time. Pak et al developed an automated craniotomy system which controls a motorized drill based on impedance measurement to open a cranial window as small as several millimeters with high repeatability. Although the system is robotically and electronically controlled, the system is not a full stereotaxic system lacking the capabilities to perform stereotaxic positioning and automatic insertion of the surgical tools. The system was designed to retrofit to existing manual stereotaxic systems and to open holes on the skull automatically with a high spatial precision. Neurostar, on the other hand, developed a robotic stereotaxic system in which the three translational actuators were motorized, and an electronic brain atlas was added to the control computer to guide the actuators for stereotaxic surgeries. The system can also optionally equip with electronic drill and microinjector to provide automatic skull drilling and dye injections. However, the Neurostar system only provide three motorized translational actuators and rotational positioning remains to be manually controlled. This makes the system impossible to automatically align the animal to the “skull flat” position, which is critical for precise nucleus targeting to avoid blood vessels or other important brain regions. The system also uses a pair of 2D CCD cameras to identify the single Bregma landmark on the skull to guide the surgical tool, but the design lacks the needed hardware, such as structure illumination light sources, for full 3D skull profile reconstruction. Another robotic stereotaxic surgical system was developed by Brainsight. The Vet robot of Brainsight uses a full 6 degree-of-freedom robotic arm to guide a surgical tool to a brain region. The system also integrated with MRI or CT images for automatic stereotaxic guidance. The Vet robot also equipped with two CCD camera for 3D positioning registration and a 200 um positioning accuracy were demonstrated on their system. Comparing to the Neurostar system, the Brainsight system has a full 6 degree-of-freedom robotic arm which allows surgical insertions in all angles. However, the stereo camera system still lacks the capability to reconstruct the skull profile in 3D and only a single target point can be identified. Another disadvantages of using a full 6 degree-of-freedom robotic arm is the high cost of ownership and may makes the system difficult to be adapted by a larger number of neuroscience laboratories.

The objective of this study was to develop a fully automated stereotaxic system for small animals. The system may be able to significantly improve the surgical speed and the success rate for brain surgery due to improved spatial resolution and accuracy for both measurement and the manipulation systems. The system is equipped with a 3D skull profiler which is based on structure illumination and geometrical triangulation to map the skull surface of a small rodent with high spatial (sub-millimeter) precision, and a full 6DOF robotic platform to provide a large translational and rotational range of motions to position the animal for precise stereotaxic procedures. In this paper, the design and construction of the system is discussed in details and the accuracy evaluation of the system using both mechanical measurement and actual stereotaxic injection on brain phantoms and anesthetized rodents are also demonstrated.

Results

The main goal of this project was to design and build a stereotaxic device that can perform the surgery automatically without human intervention for small animals which can lead to increased targeting accuracy especially for small and deep brain nuclei. More specifically, we intended to address two areas of inaccuracy that many existing devices have: 1) The acquisition of measurements needed to obtain skull-flat and 2) inaccuracies with the movement of the various axes to adjust, rotate, and tilt the animal's skull into the correct position.

To address the first area of concern (measurements of skull-flat), we developed a computer vision system that performs 3D scans of an animal's skull. The computer vision system is a 3D skull profiler that can scan the rodent's skull using a video projector to project line patterns and imaged by two regular CCD cameras on the two ends. The acquired images can be used to calculate a 3D skull profile with sub-millimeter spatial resolution based on the techniques of structure illumination and geometrical triangulation.

To address the second area of concern (precision of movement), we developed a stereotaxic platform that is based on a hexpod (Steward platform) design. A Steward platform consists of two plates that are connected to each other via six motorized axes. Lengthening or shorting these 6 axes in particular synergistic ways will allow the top plate to rotate, tilt, or move against the bottom plate with six DOF (X, Y, Z, roll, yaw, pitch). Based on the spatial information reconstructed from the 3D skull profile, the animal skull can be moved to a desired location through estimating the movement required to move the robotic platform which is securely holding the animal.

Figure 1 shows both the schematic diagram and the actual photographic image of the automated robotic stereotaxic system illustrating the major components required for the system.

3D skull profile reconstruction using structure illumination

The skull of a rodent can be reconstructed in the 3D space through the technique of structure illumination. Figure 2A shows example images taken by the left and right 2D CCD cameras illuminated with vertical and horizontal line patterns. A total of 42 photos were taken by each camera with the spatial frequencies varying from 0.025 to 12.8 lines/millimeter for 3D reconstruction. Due to optical projection, vertical displacement on the rodent's skull in turn creates lateral displacements in the acquired 2D images, and these lateral displacements can be combined to estimate the vertical displacement of each point on the surface skull using geometrical triangulation. In order to correctly calibrate the camera systems to obtain sub-millimeter spatial reconstruction, 3D printed calibration targets and optical calibration targets with known dimensions were first imaged to estimate the correct parameters for the focal lengths of the CCD cameras and the relatively positional parameters for the cameras and the projectors. Through this calibration process, it was determined that the 3D reconstructed skull profile can achieve a spatial resolution of 0.0985±0.045 mm.

The 3D profiler was then used to reconstruct the skull profile of a bare Mongolian gerbil skull. The 2D normal CCD image and the reconstructed 3D profile of the gerbil skull were shown in Fig. 2C. The reconstructed 3D skull profile was also superimposed with black-and-white intensities obtained from a normal 2D skull image for better visualization. The stereotaxic landmarks and bone sutures can be clearly identified from the reconstructed 3D skull profile. The reconstructed skull profile has very few missing 3D points on the skull surface but the structures on the two sides of the skull were not reconstructed. This is due to the fact that only one camera can see one side of the skull, making the geometrical reconstruction impossible with the missing side information. However, the inability of reconstructing the side surfaces of the skull were not important for our purpose since the normal plane for the rodent's skull can be accurately determined based on the top skull surface alone.

After confirming the functions and accuracies of the computer vision 3D profiler with a bare skull, an anesthetized Mongolian gerbil was then used for system evaluation. The scalp of the gerbil was surgical opened exposing the top surface of the skull and the gerbil was secured to the robotic platform by gluing

the gerbil's skull to a metal rod attached to the top surface of the robotic platform (see below). The surface of the skull was then scanned by the 3D profiler without the use of image enhancer, fiducial markings or other treatment methods. The reconstructed 3D surface profile is shown in Fig. 2D in two different viewing angles (top figure). The skull surface was well-reconstructed showing details of the skull surface including the stereotaxic landmarks and part of exposed scalp. Using our custom control software, the reconstructed 3D skull profile can be manipulated on the computer screen in real-time using a computer mouse, and the Bregma and Lambda points can be readily identified by the users. (bottom figure) Based on the two selected stereotaxic landmark points, the mid-point and two additional points 4 mm away from the mid-points perpendicular to the center line on both sides were estimated. Based on these points, two 3D perpendicular lines can be determined defining a normal surface plane for the gerbil skull, and this normal plane can then be used to move the robotic platform to the "skull-flat" position for subsequently alignment and stereotaxic procedures.

Full six degree-of-freedom robotic stereotaxic platform developed using 3D prototyping technology

The full six degree-of-freedom robotic stereotaxic platform were manufactured using rapid prototyping technology. The majority components of the robotic stereotaxic platform were manufactured using 3D printing technology and were designed using Solidworks 3D CAD design software (Dassault Systemes, France). The components were then printed using the PolyJet 3D printing system with a spatial resolution of 28 µm. Rapid prototyping manufacturing technology allows the platform to be easily configured to hold different animal species and sizes. In addition, for stereotaxic work to succeed with live animals, a number of accessories are required. For example, many animal species and/or surgical procedures require that the animal maintain physiological temperature despite the fact that many species cool down during anesthesia. Also, the animal's head needs to be immobilized in some ways so it remains stable and in position despite the surgeon's handling and manipulation. Depending on the brain area and investigator's preference, this fixation may occur via ear bars, bite bars, head posts, molds to rest the head, or a combination of these methods. Figure 3 shows some example custom add-ons that was also manufactured with 3D printing prototyping technology. Figure 3A is a custom head rest that is specially designed for Mongolian gerbils. In addition, traditional stereotaxic devices typically use ear bars to secure the rodent on the stereotaxic platform; however, some neuroscience experiments especially for studying the auditory pathway requires the animal's ears are clear of obstruction. For that reason, a rotatable and extendable manipulator was designed to hold a head post which can be glued to the skull of the rodent for secure placement. Figure 3B to D are the sub-components of the manipulator and can be assembled to attach to the top plate of the robotic platform to secure a Mongolian gerbil, as shown in Figure 3H. Temperature control is essential to the survivability of the rodent and the rapid prototyping technology allows easily integration of temperature control to the robotic stereotaxic platform. Figure 3E shows the heating pad controlled by a PID controller with a thermostat placed under the rodent to provide accuracy temperature of 37ºC throughout the entire stereotaxic surgery. The heating pad can be embedded onto the top plate of the platform seamlessly to provide maximum comfort for the small animal. Figure 3G shows the top and bottom plates of the robotic stereotaxic platform rapidly prototyped using 3D printing technology. The technology allows custom mounting holes and custom placement grooves for the top plate to easily mount custom components for securing the animals or other life-support related installments.

Considering that the radius of a small rodent's skull is in the order of 20 mm and the typical dimension of a brain region inside a small rodent's brain is 0.2 mm, a translational and rotational accuracies of 200 µm and 0.5 degree is required to accurately target the brain region. In addition, the translational and rotational motions of the robotic platform have to be linear to ensure smooth positioning for stereotaxic surgeries. Therefore, the robotic platform was first characterized using mechanical calibration techniques. Three mechanical dial gauges (Model 25-611, L. S. Starrett, USA) and a digital 9 degree-of-freedom inertial measurement unit (IMU) chip (MPU9150A, InvenSense, USA) were used to measure the both the

translational displacement and the rotational angles of the robotic platform against the desired translational distances or a rotational angles issued by the control computer. The three translational gauges were mounted against the platform perpendicular to one another for all the three axes and the IMU chip is directly mounted at the center of the top platform to measure the rotational angles. Figure 4 shows some selected calibration curves (the z linear axis and the pitch angle) to demonstration the linearity in both translational and rotational motions. Figure 4C compares the desired and actual movements in all three translational and rotational axes, and the maximum error is less than 8.7 %. Using the calibration tools, the robotic platform was determined to have a full translational distance of ± 15 mm with an accuracy of ± 0.25 mm, and a full rotational angle of ± 20° with an angular accuracy of better than ±0.1^{o}, as summarized in Table 1.

The robotic platform was also evaluated using a brain phantom made from agar to demonstrate its positioning accuracy. The robotic platform was first programmed to print a "cross" pattern with an interspacing of 1mm on the brain phantom, as shown in Fig. 4D. The diameter of the glass micropipette used in the injection was estimated to be ~5 µm and there is no observable derivation of the printed pattern on the brain phantom. Injection depth accuracy was also demonstrated using the agar phantom brain. A vertical injection of 1 mm distance difference was imprinted as shown in Fig. 4E, and the injection printing again have no observation derivation.

Stereotaxic brain injection to a deep nucleus of an anesthetized gerbil

In order to test the actual stereotaxic surgical performance of the system in a typical neuroscience experimental setting, an anesthetized gerbil was placed on the top plate of the robotic stereotaxic platform secured by a head post attached to the top plate, as shown in Fig. 5A. The scalp of the gerbil was first opened exposing the skull, and structure illumination scanning was followed to construct the 3D skull profile on the computer. The Bregma and Lambda landmarks were then identified through clicking on the computer to determine the skull normal surface. A "skull flat" position was then achieved through estimating the required translational and rotational displacements using the skull normal surface and the robotic platform were commanded to move the gerbil to the determined positions. The gerbil was also positioned with translation and rotational displacements of 4 mm and 20 degree away from the Lambda in which the displacements were estimated based on a gerbil brain atlas to target the MNTB brain region. After the drill position was marked and a small hole was drilled manually, the skull was rescanned by the 3D computer vision skull profiler and the robotic platform was moved to compensate for any positional errors induced by the drilling process. A glass pipette filled with either cascade-blue or micro-ruby as fluorescent coloring agents was inserted to the gerbil head at a depth of 7.5 mm by raising the robotic platform, as shown in Fig. 5B. After the injection, the gerbil was euthanized, and the brain was extracted and sectioned for fluorescence imaging. Figure 5C shows the fluorescent image of the brain slice with the fluorescence dyes reaching the intended MNTB target, as compared to a reference brain atlas as shown as an insert. This simulated neuroscience animal stereotaxic surgery confirms that the stereotaxic system can inject to the intended brain region precisely with very minimal user inputs.

Discussion (1 page)

In this paper, we have demonstrated that an automatic robotic stereotaxic system was realized through combining a 3D computer vision optical skull profiler based on the techniques of structure illumination and geometrical triangulation, and a 6DOF robotic platform based on the Stewart design. Through this combination, the skull profile of the small rodent can be reconstructed in the computer space with high degree of accuracies to guide the skull repositioning. The 6DOF robotic platform can then be instructed by the positioning estimation to move the rodent's skull to the correct position for stereotaxic surgeries. To the best of our knowledge, a 3D optical profile is the first time being used to capture the 3D point cloud of the rodent's skull for stereotaxic purposes. Despite other existing robotic stereotaxic systems use single or multiple CCD cameras, these cameras are mostly used to determine the coordinate of a single or multiple fiducial spheres or landmarks. The advantage of using our approach is that no preparation or device mounting is required for the stereotaxic surgery to provide a full 3D reconstruction of the skull profile for very accuracy stereotaxic positional identification. In addition, our robotic stereotaxic platform based on the Stewart design provides full 6DOF motions in all translational and rotational axes with a good range of movements (± 15 mm and ± 20º) and high precisions (± 0.25 mm and ± 0.1º), which is adequate to cover all translational and rotational needs of small rodent stereotaxics. In addition, the range of movements of the robotic platform is simply related to the variation lengths of the six arms, and the platform can be designed in terms of platform size and arm lengths to accommodate for other animal dimensions.

Another advantage of the design is that the system is constructed with relatively low-cost components, making the system highly affordable to many neuroscience laboratories. The 3D optical skull profiler is constructed based on two low-cost regular CCD cameras with two 10x zoom lens and a commercial computer projector with a low-cost 100 mm convex lens. In this design, there are no needs to use special time-of-flight 3D cameras or other special optical components for the 3D reconstruction. In addition, the robotic platform were built using 6 low-cost digital servos and the other components were 3D printed using rapid prototyping technologies. Traditionally, six linear translational actuators were used to realize the Stewart platform design but these linear translational shafts are required to have good spatial resolution, making such a platform to be relatively high cost to build. In contrast, digital rotational servos are much more economical. Mathematically, based on simple trigonometric relation as illustrated in the supplementary information, linear translational distances can be expressed in rotation angles for the digital servos to achieve the same movement outcomes for the stereotaxic platform. Therefore, in this work, digital servos are chosen to be used to build the rotational platform to make the device for assessable to the neuroscience community to accelerate discoveries. In addition, compared to other existing robotic stereotaxic systems which uses a 6DOF robotic arm to guide the surgical tools, the fundamental difference between the two designs is that our design moves the animal together with the robotic platform while the other design keeps the animal stationary. However, as long as the animal is secured with proper support, as demonstrated by using various positional add-ons, our robotic platform can achieve the same result but with a significant cost reduction.

An increasing number of experimental approaches in neuroscience require the precise placement of a recording electrode, injection pipette or some other tool into a specific brain area that can be quite small and/or located deep beneath the surface. With traditional methods and devices, this can be challenging for a number of reasons. First, some brain nuclei are less than 0.5 mm in diameter and may be as deep as 7-10 millimeters in typical rodent species, requiring a target accuracy of better than 1 degree. Second, investigators try to minimize the size of the opening in the animal's skull through which the tool is advanced in order to minimize surgical trauma from the intervention. Third, jaws, face, or ears additionally limit the locations on the head where craniotomies can be performed, such that for practical reasons, the majority of craniotomies use a dorsal approach. This lack of sophisticated technology is especially unacceptable for many other types of experiments that target small and deep brain areas (midbrain, brain stem, thalamus, subregions of hippocampus, and many other types of non-surface structures). As a result of this lack of sophisticated technology, many in-vivo manipulations either require a significant amount of experience by

the experimenter, and/or have a significant failure rate. Failed experiments are costly in terms of wasted investigator time, research animals and materials. However, even in cases where a lab has the expertise to target the desired brain area with a relatively high success rate (for example, because they employ a very skilled student/postdoc/lab tech), this is problematic since the success depends on that person and their "magic touch" – effectively reducing the reproducibility of these experiments for all other labs that don't have access to that person. Even the same lab may have trouble reproducing their own experiments once that person with the "magic touch" leaves.

Further improvement of the system can be enhanced in the following areas. The current software was written with the Python programming language, which its mathematic calculation can be slowed due to the interpretative nature of the programming language. In addition, single core computing was only used in the 3D reconstruction algorithm and improvements in calculation speed can be achieved using multiple CPU cores or GPU acceleration to reduce the time needed for the reconstruction. In addition, the current software requires users to visually identify the Bregma and Lambda points on the 3D reconstructed skull profile to calculate the normal surface of the skull, and this identification process can further be automated using image processing techniques to identify the two landmarks in the future. Currently skull drilling was still performed manually and additional drilling add-on can be installed next to the nanoinjector to allow automatic drilling. An impedance based sensor similar to Ref. 2 can also be installed on the drill bit and the robotic platform to allow precise drilling of the skull without damaging brain tissue.

Conclusion (1 page)

A new type of stereotaxic system for small animal brain surgeries has been developed through combining a 3D computer vision sub-system and a 6DOF robotic platform. High resolution 3D reconstruction of an animal skull has been demonstrated. No special 3D camera or hardware was required, but a series of images were captured by two regular computer cameras mounted on both sides of the skull. A series of structured patterns were projected onto the animal skull using a video projector to increase optical contrast to allow high resolution 3D capture on a relatively featureless skull structure. Six low-cost digital servos were used to control the 6 extendable arms to allow placement of the top platform in all 3 translational and 3 rotational positions with few limitations. Both the 3D camera sub-system and the robotic platform have been characterized to have sub-millimeter and sub-degree spatial resolution, adequate to precisely target a small neural target within the animal's head. A simulated stereotaxic surgery in a typical neuroscience experimental setting using an anesthetized Monogonal gerbil has confirmed that the medial nuclear of the trapezoid body (MNTB) can be accurately targeted with the system. We anticipate that this new development can help the advancement of neuroscience research through an increased success rate for stereotaxic surgeries and reduced surgical time to increase animal survivability.

Methods and System Design

Optical structure illumination for precision 3D skull mapping

A video projector (S1, ASUS) was mounted slightly offset from the middle longitudinal axis of the entire setup due to the fact that commercial projectors were designed to project videos in a tilted upward position. Since the video projector is designed to project videos meters away, a 75 mm bi-convex lens (LB1901-A, Thorlabs, USA) was placed in front of the project to focus the projecting images to a much shorter distance onto the rodent skull. Two 2D CCD cameras (BCE-B013-U, Mightex) were mounted on two sides of the projector to capture skull images illuminated with line patterns from the video projector. Two 10x zoom lenses (MLH Macro 10X, Computar) were also mounted onto the two CCD cameras to reduce the field of view onto the rodent skull to maximize the image resolution for the 3D reconstruction. The two CCD cameras used have a pixel density of 1280x1024 covering a field-of-view in the size of about 50x40mm, this translate to a lateral spatial resolution of 30x35 µm for the 3D reconstructed profile.

A series of horizontal and vertical back-and-white lines with increasing spatial frequencies (structured illumination) were projected onto the rodent's skull and the two CCD cameras were used to capture 2D images of the projected lines from both sides. Using these captured 2D images, the rodent skull can be reconstructed in 3D based on the idea that the projected lines are laterally displaced when projected onto a non-flat surface, and the degree of lateral displacement is linear proportional to the vertical corrugation of the rodent's skull. The surface point $\vec{P}$ in the 3D space can be estimated based on geometrical triangulation using the 2D images from the two cameras in which

$$P = \frac{1}{2}(C^L + C^R + \lambda^L \cdot v^L + \lambda^R \cdot v^R)$$

where v^L and $v^R$are the viewing vector pointing towards the point P for the left and right cameras; C^L and C^R are the 3D coordinates of the center points of the left and right cameras; and λ^L and λ^R are parameters can be calculated based on geometric triangulation. Detail discussion for the equations to calculate λ^L and λ^R, and the 3D structure illumination methods can be found in the supplementary information. A custom Python program was written to process the captured 2D images to reconstruct the 3D skull profile. There are a total of 42 vertical and horizontal line patterns projected onto the rodent's skull and captured by each of the CCD cameras with a frame rate of 25 fps. All the images were captured in a standard laboratory lighting conditions with no light shielding employed. The exposure time for each image was 20 ms with an average of 8 frames to improve image contrast and to remove optical noise induced by the projector and room lighting. The overall image capture time was about 86 second. An Intel Core i5-4430 CPU @ 3.00GHz with 16.0 GB RAM desktop computer was used for the reconstruction process using a custom Python program and the process time to reconstruct the 3D profile was approximately 9 seconds.

Full 6 degree-of-freedom robotic platform

The robotic platform provides a full 6 degree-of-freedom - 3 translational and 3 rotational - movements to allow precise positioning of the rodent's skull. The platform is based on the Stewart design in which six motorized arms attached to a moveable top plate and a stationary bottom plate. The 6 arms are coordinated in their movements to give the top plate all 6 degrees of motions. Differs from other conventional Stewart designs in which 6 linear actuators are used, the robotic platform used in this project was built with 6 digital rotational servos (Dynamixel, AX-12A) and each connected to a short and a long arm forming a semi-triangle with a rotational pivot. The short and long arms then act as the two short edges of the semi-triangle and through rotating the servo angle, the long arm of the semi-triangle can be shortened and lengthened to provide the needed linear extensions for the coordinated platform motion. Details of the control algorithms of the platform can be found in the supplementary information. The design allows the platform to have precise sub-millimeter translational and sub-degree rotational positioning accuracies and at the same time reduces the cost of ownership using low cost rotational servos. In the current design, the top and bottom plates were designed with Solidworks mechanical design software and were 3D printed by a 3D plastic

printer (Objet30, Stratasys) with a printing spatial resolution of 28 µm. The top plate was also embedded with a heat plate which was controlled by a proportional-integral-derivative (PID) controller to maintain 37ºC body temperature for the rodent. A number of accessories, such as ear and bite bars, can also be mounted on the top plate for securing the animal position on the platform.

Fully automatic stereotaxic skull alignment and positioning

Fully automatic stereotaxic surgery can be achieved through coordinating the 3D reconstructed skull profile obtained from the computer vision system with the movements of the 6 DOF stereotaxic platform through a custom Python program. A small rodent can be securely supported on the top plate of the robotic platform either using a pair of ear bars or a metal head post glued to the rodent's skull, and the temperature of the rodent can be maintained through a computer-controlled heat pad. After the head fur was removed and the skull was exposed, a series of structured illuminated images can then be taken by the two CCD cameras and the 3D skull profile can be reconstructed by the software routine. Users were then requested to identify the Bregma and Lambda landmark positions through clicking on the reconstructed skull profile. Based on the 3D coordinates of the Bregma and Lambda, the mid-point between the two landmark points can be automatically estimated. In addition, two points that is 4 centimeters away from this mid-point and are perpendicular to the Bregma and Lambda points will be estimated. Thus, these five points (Lamdba, Bregma, mid-point, two side points) form two perpendicular lines to define a 3D surface normal plane for the rodent's skull. Using this surface normal plane, the robotic plane can be translated and rotated to achieve the "skull flat" position. At this point, the users can enter the desired translational and rotational displacements referenced to the lambda landmark of the skull for the robotic platform to move to the desired location.

Animal protocol and procedures for stereotaxic evaluation

All experimental procedures complied with all applicable laws and NIH guidelines and were approved by the University of Colorado IACUC. All experiments were conducted in Mongolian gerbils (*Meriones unguiculatus)*. Animals were first anesthetized with a mixture of ketamine-xylazine (60mg/kg-5mg/kg), and a maintenance dose (25mg/kg-5mg/kg) was given following complete anesthesia to maintain the anesthetized state. Once the animal was properly anesthetized, the fur over the skull was shaved off and the underlying skin sanitized with a disinfectant. Skin and muscle overlying the skull were removed and a craniotomy was made in the skull at 1.2 mm lateral and 1.2 mm posterior of lambda using a dental drill. The coordinates used here allowed us access the auditory brainstem for dye injections into the medial nucleus of the trapezoid body (MNTB). Dye injections were made using a Nanoliter injector (World Precisions Instruments, Sarasota, FL). After all injections were concluded, the animal was given an overdose of pentobarbital (0.03 mL/g) and perfused transcardially with phosphate buffer solution (PBS) and 4% Paraformaldehyde (PFA). Once perfusion was complete, the brain was removed and placed into 4% agar. The brainstem and cerebellum were then sliced coronally in 100 µm sections using a vibratome (Leica VT 1000s, Nussloch, Germany). The sections were then stained with a 1:100 concentration of Neurotrace Nissl stain (ThermoFisher, Waltham MA, 435/455 blue fluorescent Nissl stain) diluted in antibody solution (ABS) [61]. The brain slices were then mounted on slides with Fluoromount-G (Diagnostic BioSystems, Pleasanton CA) and imaged with an Olympus FV1000 (Tokyo, Japan) confocal microscope using the laser lines of 405nm and 488nm to image the Nissl (cell body indicator).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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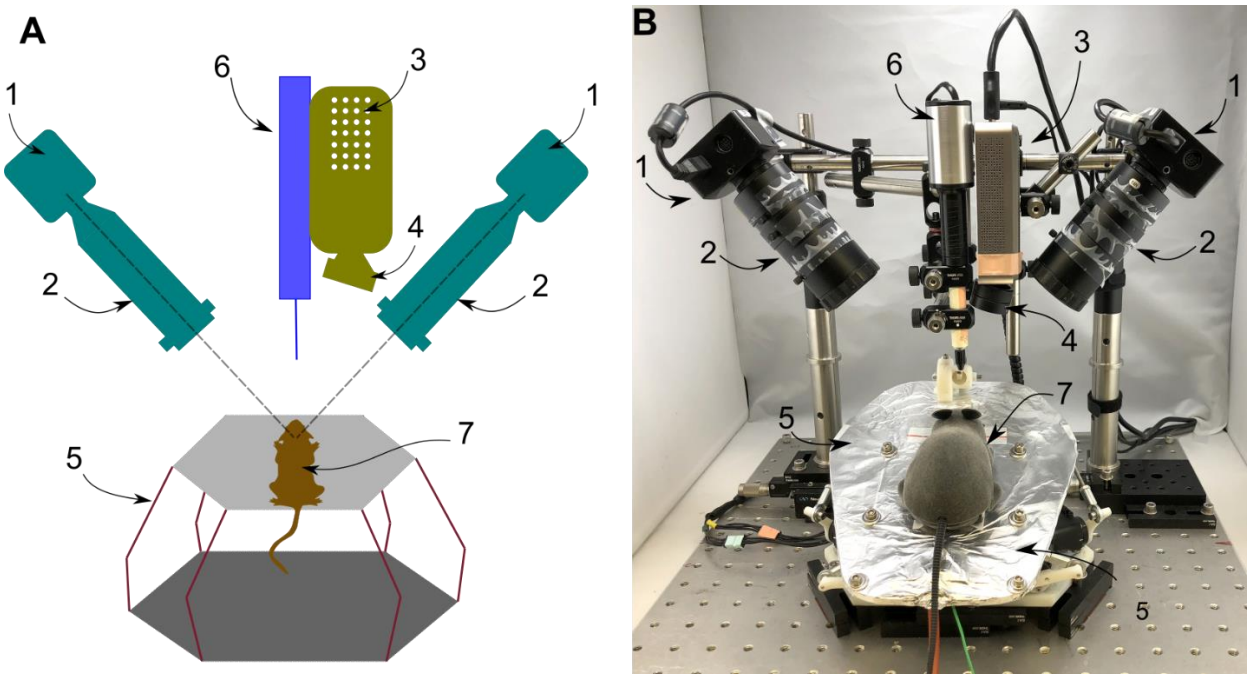

Figure 1 Fully automated robotic stereotaxic system based on 3D structure illumination. **A.** Schematic diagram of the stereotaxic system illustrating the crucial components of the system. Two cameras (1) mounted on the two sides of the center axis were equipped with zoom lens to focus on the skull of the rodent (7). A video projector (3) was used to project structure light patterns on the skull for 3D skull profile reconstruction. A 100 mm convex lens with a small slanted angle was used to focus the projection plane of the structured image onto the skull. A 6 degree-of-freedom (3 translation and 3 rotation) robotic platform (5) was used to secure the rodent and allowed positioning the animal's head with sub-millimeter spatial resolution. A surgical device (6) such as a nanoliter injector can be placed along the center axis to perform stereotaxic surgical procedures. **B.** Photographic image of the actual automated stereotaxic system.

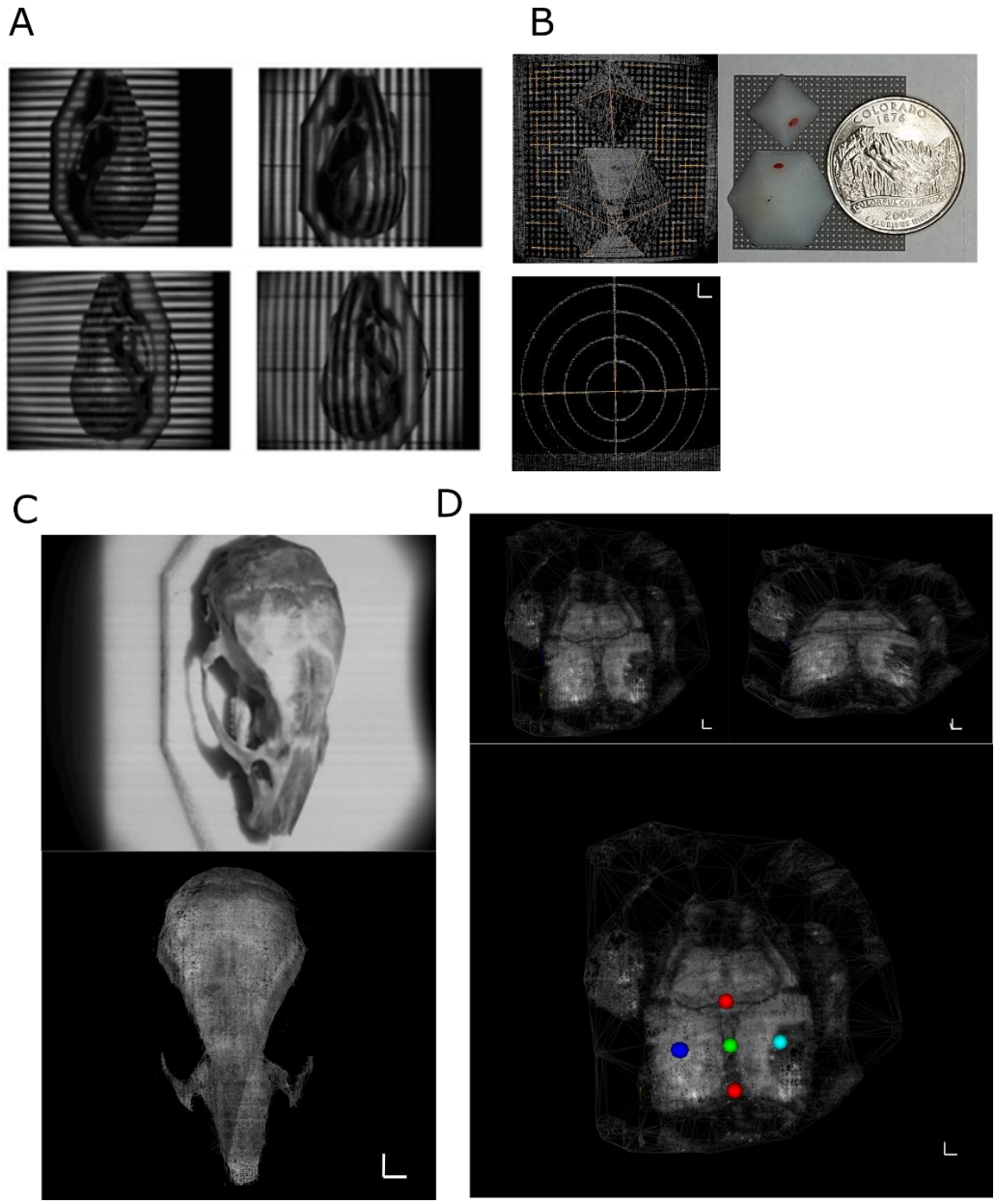

Figure 2: 3D skull profile reconstruction using structure illumination. **A.** A Mongolian gerbil skull illuminated by example vertical and horizontal lines observed by the left (top) and the right (bottom) cameras. **B.** Reconstructed calibration standards by structure illumination (top left) and the 3D printed calibration standard in pyramided shapes (top right). Reconstructed optical target (bottom left) and the calibrated optical target (bottom right) for calibrating the reconstruction routine to obtain sub-millimeter spatial resolution. **C.** A regular 2D image taken by the left black and white CCD camera (top) and the reconstructed 3D skull profile (bottom) of a Mongolian gerbil skull. The reconstructed 3D points of the skull profile were colored with the greyscale intensity to visualize stereotaxic landmarks (Bregma and Lambda). **D.** Reconstructed 3D skull profile of an anesthetized Mongolian gerbil in two different view angles clearly showing the exposed skull and skin (top). The Bregma (red dot) and Lambda (green dot) was used to identify and to estimate the center intersection point (blue dot) and two points 4 millimeters perpendicular to the center line (bottom). Note: all scalebars are 1 mm in length.

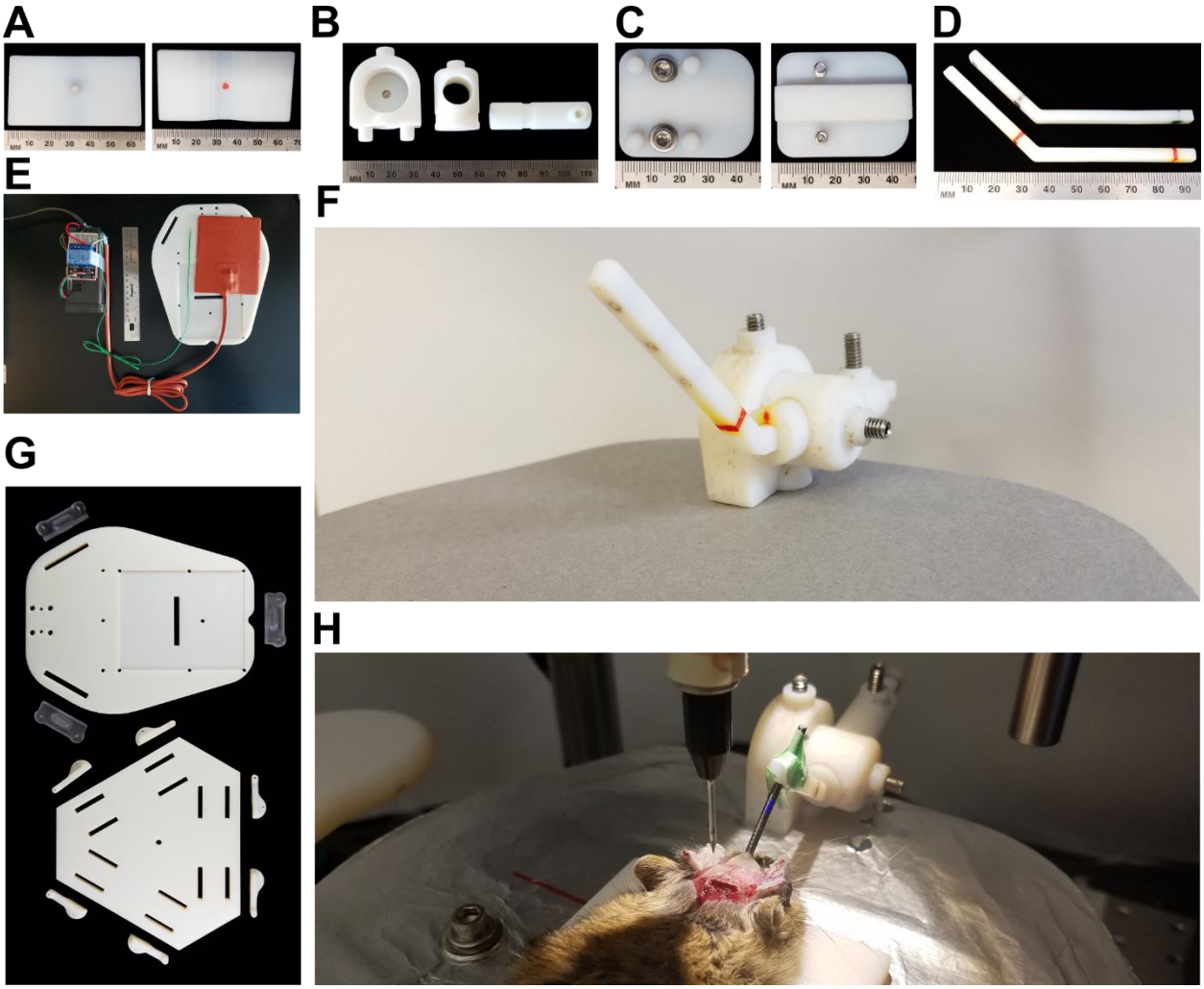

Figure 3: Components and add-ons of the six degree-of-freedom robotic platform. **A.** A 3D printed heat rest that can be optionally attached to the platform to better position the animal's head for the stereotaxic surgery. **B to D.** 3D printed sub-components of a rotatable and extendable manipulator to hold a metal bar that can glue to the animal's skull for holding the animal securely for positioning. **E.** A temperature heat pad controlled by a PID controller can be embedded into the top plate of the robotic platform to maintain the body temperature of the rodent. G. 3D printed top and bottom plates of the robotic platform in which the top plate has several mounted holes to allow add-ons to be installed optionally.

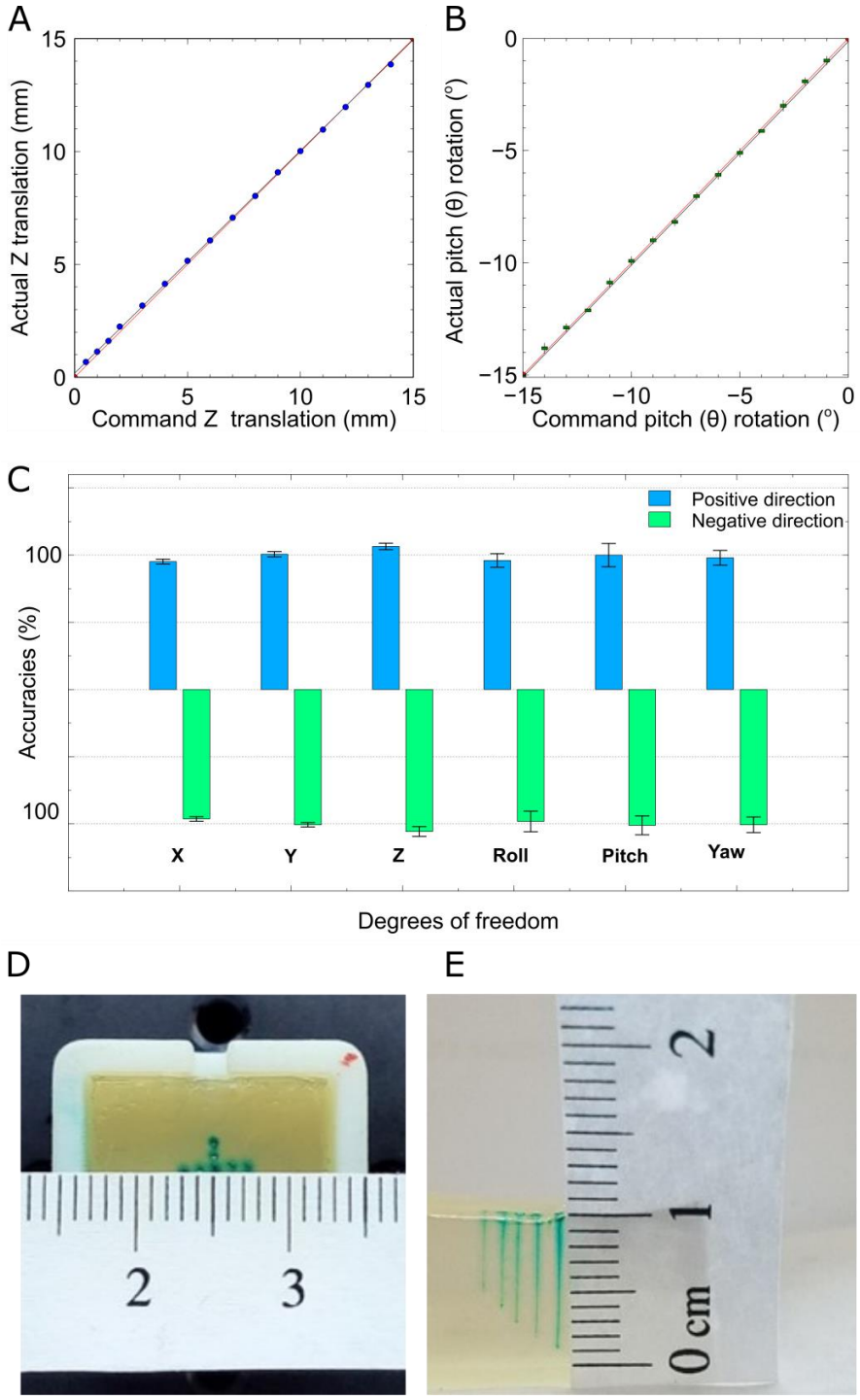

Figure 4: Positioning accuracy of the robotic stereotaxic platform **A.** Desired Z translational positions against the actual Z translational positions by the platform. **B.** Desired pitch rotational angles against the actual pitch rotational angle. **C.** The estimated accuracies in all six degree-of-freedom motions for the robotic platform. **D.** A cross pattern with an inter-spacing of 1 mm was injected on an agar brain phantom using the stereotaxic platform. **E.** Longitudinal injections with a depth difference of 1 mm were imprinted into a agar brain phantom using the platform.

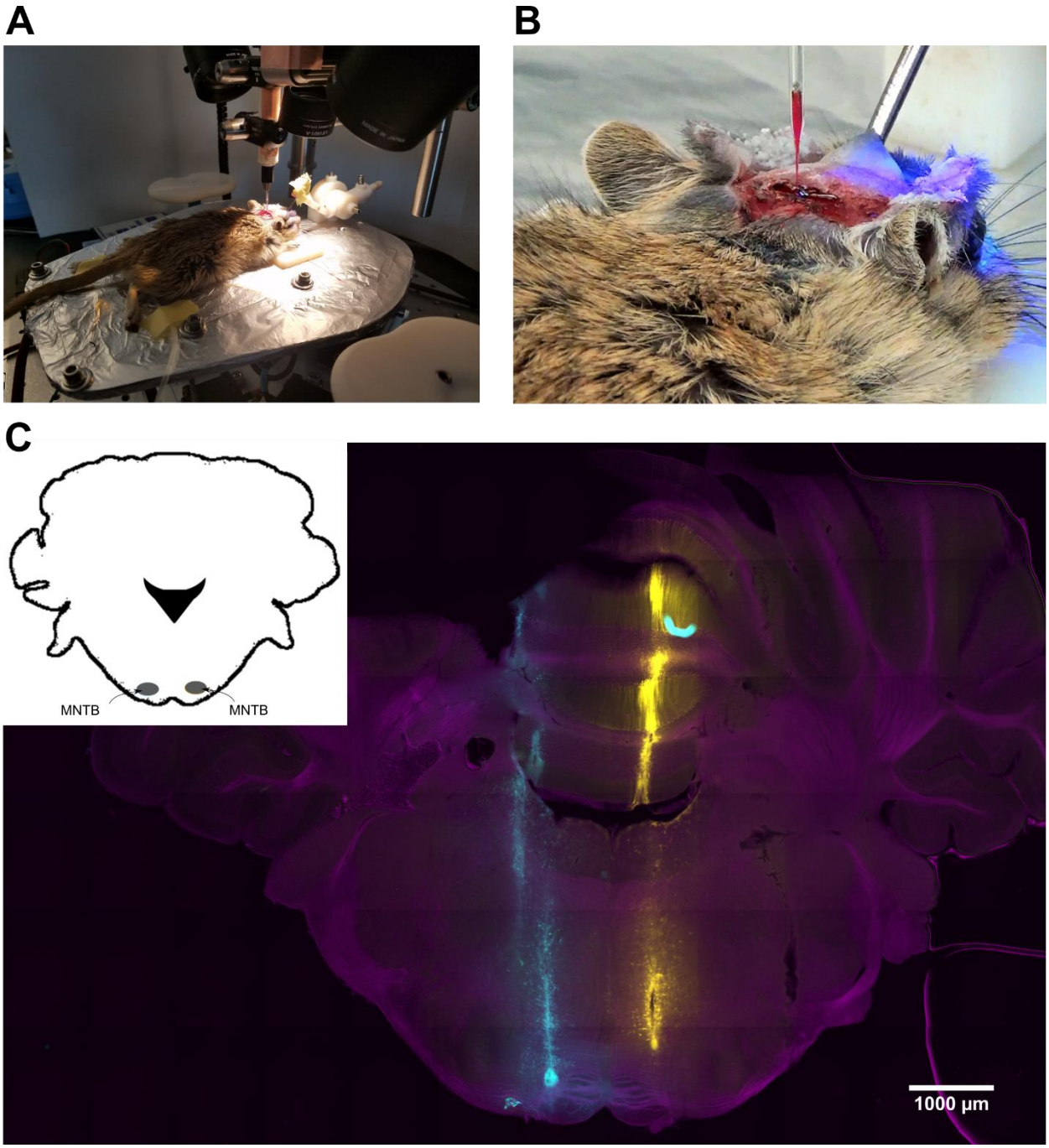

Figure 5: Fully automatic stereotaxic brain surgeries on a Mongolian gerbil. **A.** The exposed skull of an anesthetized Mongolian gerbil was first 3D scanned and automatically positioned for stereotaxic injection to the medial nucleus of the trapezoid body (MNTB) deep within the brain. **B.** Close-up view of the stereotaxic injection with the glass micropipette needle was inserted into the animal's brain. **C.** Fluorescence imaging of an excised brain slice showing the successful targeting of the MNTB region. Upper left is the schematic brain atlas showing the locations of the two MNTBs.

Top platform	**Measured ranges and accuracies**	
	Translational	***Rotational***
Maximum range	± 15 mm	± 20°
Resolution	± 0.25 mm	± 0.1°

Table 1: Maximum translational and rotational ranges and resolutions of 6 DOF robotic platform