Naval Research Laboratory
Washington, DC 20375-5320

NRL/6930/MR—2023/4

Design and Application of a Microscope Mounted Manual Micromanipulation Device

DR. ERIC O. MCGHEE

Laboratory for Molecular Interfaces Branch
Center for Biomolecular Science and Engineering Division

DR. QIN LU

Molecular Interfaces and Tribology Section
Chemistry Division

DR. CHRISTOPHER SPILLMANN

Laboratory for Bio/Nano Science and Technology Branch
Center for Biomolecular Science and Engineering Division

September 11, 2023

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REPORT DOCUMENTATION PAGE

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1. REPORT DATE *(DD-MM-YYYY)*
11-09-2023

2. REPORT TYPE
NRL Memorandum Report

3. DATES COVERED *(From - To)*

4. TITLE AND SUBTITLE
Design and Application of a Microscope Mounted Manual Micromanipulation Device

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Eric O. McGhee, Qin Lu, and Christopher Spillmann

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
1Y78

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Research Laboratory
4555 Overlook Avenue, SW
Washington, DC 20375-5320

8. PERFORMING ORGANIZATION REPORT NUMBER
NRL/6930/MR--2023/4

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Naval Research Laboratory
4555 Overlook Avenue, SW
Washington, DC 20375-5320

10. SPONSOR / MONITOR'S ACRONYM(S)
NRL

11. SPONSOR / MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This report describes the design and application of a custom designed and fabricated 3-axis micromanipulation device capable of being mounted on a Nikon Ti2 microscope base, standard to contemporary Nikon confocal systems. This device enables micromanipulation of a variety of probe units for an assortment of unspecified tasks with a rapidly achievable manual spatial resolution near 10 µm. A demonstration of the device is included in the report describing micromanipulation of a working electrode in context of a barnacle epicuticle electrochemical observation.

15. SUBJECT TERMS
Microscope
Micromanipulator
Patch clamp
Probe

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U	U	U	U	14	Eric McGhee
					19b. TELEPHONE NUMBER *(include area code)* (202) 309-8688

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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CONTENTS

FIGURES

EXECUTIVE SUMMARY

This report describes the design and application of a custom designed and fabricated 3-axis micromanipulation device capable of being mounted on a Nikon Ti2 microscope base, standard to contemporary Nikon confocal systems. This device enables micromanipulation of a variety of probe units for an assortment of unspecified tasks with a rapidly achievable manual spatial resolution near 10 µm. A demonstration of the device is included in the report describing micromanipulation of a working electrode in context of a barnacle epicuticle electrochemical observation.

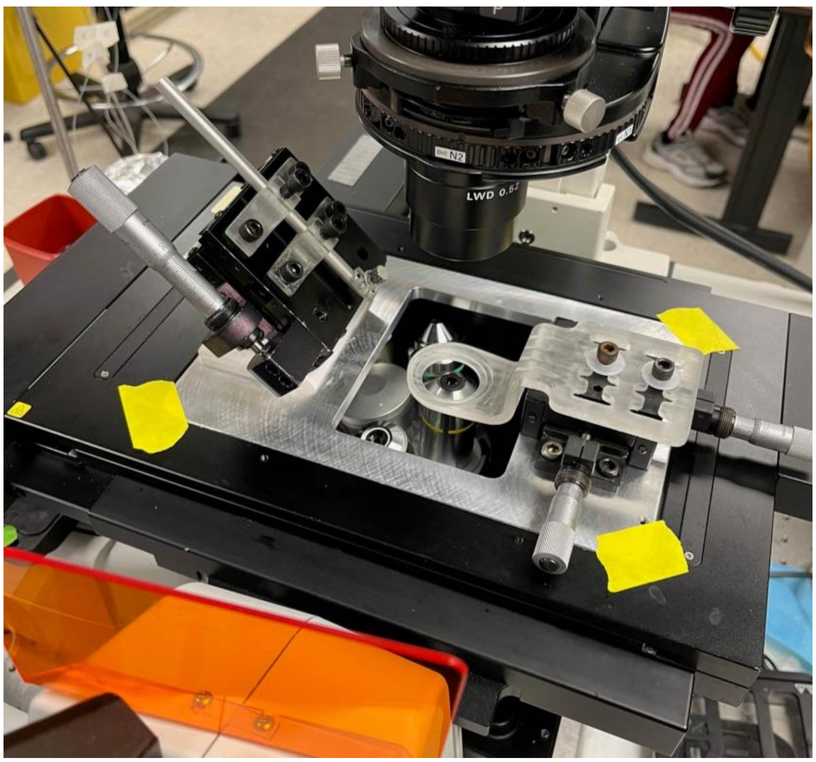

Fig. ES1 — image of device on microscope

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DESIGN AND APPLICATION OF A MICROSCOPE MOUNTED MANUAL MICROMANIPULATION DEVICE

1. INTRODUCTION

The combination of microscopic observation and micromanipulation of an object is conceptually traditional, with manipulation devices ranging from manual stages, motorized stages, pneumatic control devices, optical tweezers, and piezo manipulators. The specific choice of the micromanipulator style is highly application dependent with ranges of cost and complexity across device styles and includes considerations related to systematic forces, manipulation precision/range/axes, and probe context with characteristic tradeoffs existing across these considerations - i.e., a high precision piezo system generally does not have high spatial range. Once experimental considerations are bounded, choices of commercial systems targeting specific constraints are generally limited outside of standard applications without expensive customization and combination of various manipulation types – e.g. a piezo stage (fine control) stacked onto a motorized stage (coarse control). The nature of the current research program to access, sample, and manipulate an acorn barnacle secretion at the basal leading edge of the substrate interface is dynamic and semi-bounded, limiting the contextual device design choice. Thus, the decision was made to develop and design a relatively simple, manual, moderate precision device system with a high spatial manipulation range to accommodate a large range of potential activities onto a single microscope mounted device. As it applies to acorn barnacle research, the design described here allows physical access to a notoriously difficult region to study to enable a combination of spatial manipulation (probe placement, material collection) and spectroscopic/microscopic observation (confocal microscopy).

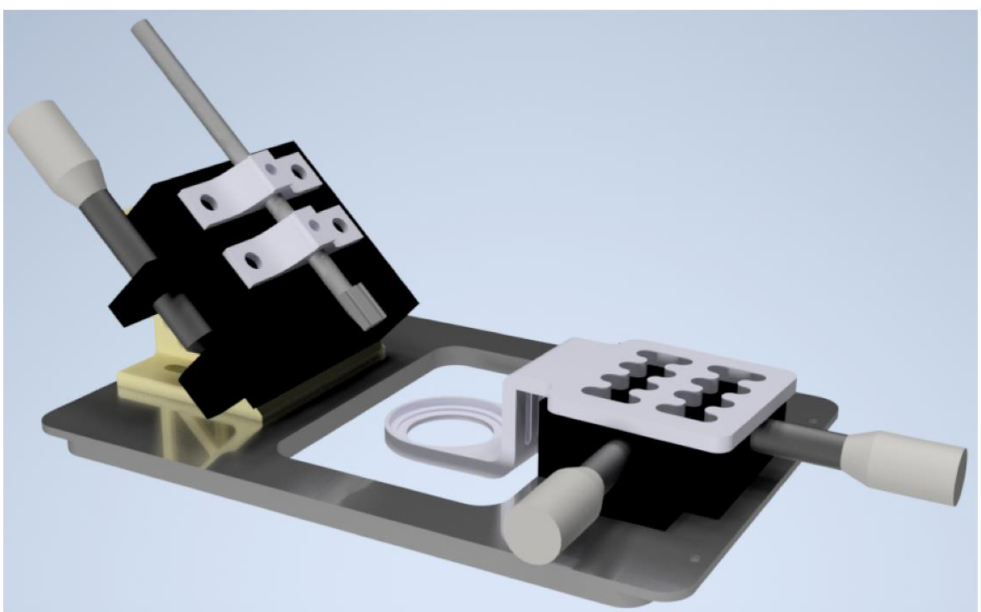

Fig. 1 — CAD rendering of microscope-mounted micromanipulation device. Fasteners not shown.

Manuscript approved September 8, 2023.

1.1 Device design

1.1.1 Necessary axes and design choices

For our desired applications, the manipulation system requires three-axis motion with a highly modifiable axis basis while allowing rotation of the sample inside a probe transitory motion axis. The device is detailed to allow fine control of a probe as it approaches a desired location within the sample location. Therefore, at least one component from the three-axes must be isolated – the **z**-axis was chosen to be separated from the **x-y** axis due to inherent usability. Optionally, and included in this design report, a secondary axis basis was created named **u**, the probe axis, by mounting the z-stage on an angled bracket, offsetting the **z**-axis translation 45-degrees from its primary axis (see Figure 1 for detail). This offset was chosen to allow an approach angle for the probe while keeping manipulation equipment out of the way of the microscope condenser turret. The acorn barnacles that are the focus of this application have regions of interest near their peripheral base and an opaque bottom, rendering observation during an overhead probe approach impossible. A vertical approach could be added by modification to the **z-**stage holder by combining a vertical **z-**stage holder in conjunction with an orthogonal probe extension arm, allowing condenser turret operation, and optical axis approach.

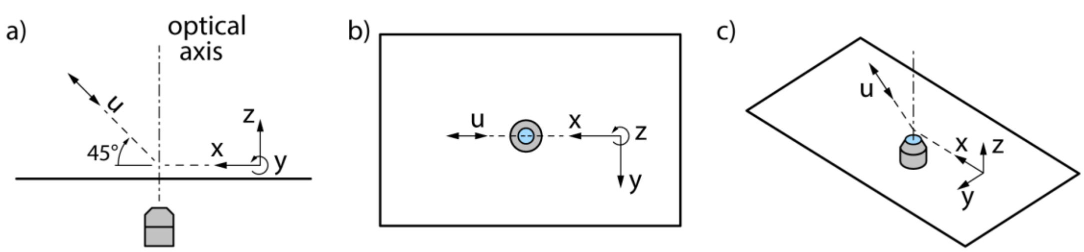

Fig. 2— a) side view of translation axes, b) top view of translation axes, c) isometric view showing all axes

1.1.2 Microscope attachment and optical path

A chassis capable of fitting into the microscope stage with optical path access is necessary for *in situ* use of the micromanipulation system. To accomplish this, an aluminum chassis was fabricated with a large orifice for coarse sample translation and optical path freedom. A bolt pattern was established to carry the translation bases. Drawings were utilized from the Nikon Ti2 microscope base to generate necessary chassis dimensions in addition to caliper measurements to fill in omitted dimensions from the microscope drawings. When mounted to the microscope, simply securing the device using standard adhesive tape prevents translation of the device during micromanipulation.

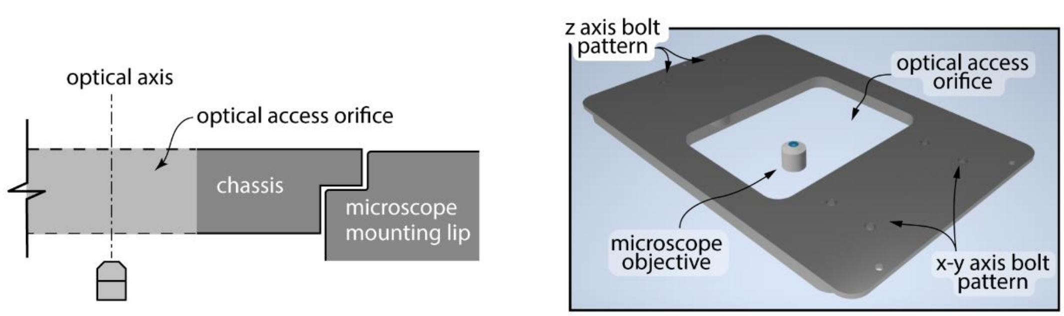

Fig. 3 — side view cutaway illustration of optical axis through access orifice with isometric rendering of chassis with microscope objective seen through optical access orifice

1.1.3 Adjustment and control

A sample holder with an open bolt pattern was developed specifically to accommodate 36 mm culture dishes and is fixed to the **x-y** axis manipulator providing coarse adjustment of the sample location, while also providing free rotation of the culture dish. Both the **z-**axis, through channel bolt patterns on the z-stage bracket, and **x-y** axis, through the sample holder's open bolt pattern, are highly adjustable axis bases allowing for coarse adjustment of the sample before fine adjustment. Additionally, the probe can be coarse adjusted along the probe axis to approach the sample before fine translation by modifying its position in the probe holders. Coarse adjustment of the system is completed first, aligning the axes with the position of interest on the sample and approaching the probe towards the sample. Once coarse adjustment is completed, fine manipulation of the probe along the **u-**axis and sample manipulation across the **x-y** axes can be accomplished by maneuvering the micromanipulators during simultaneous observation of probe position relative to sample position using the microscope. Together, the adjustability of the sample and probe allow high dynamic range of axis basis and fine probe control, providing dynamic positional capability for a variety of tasks.

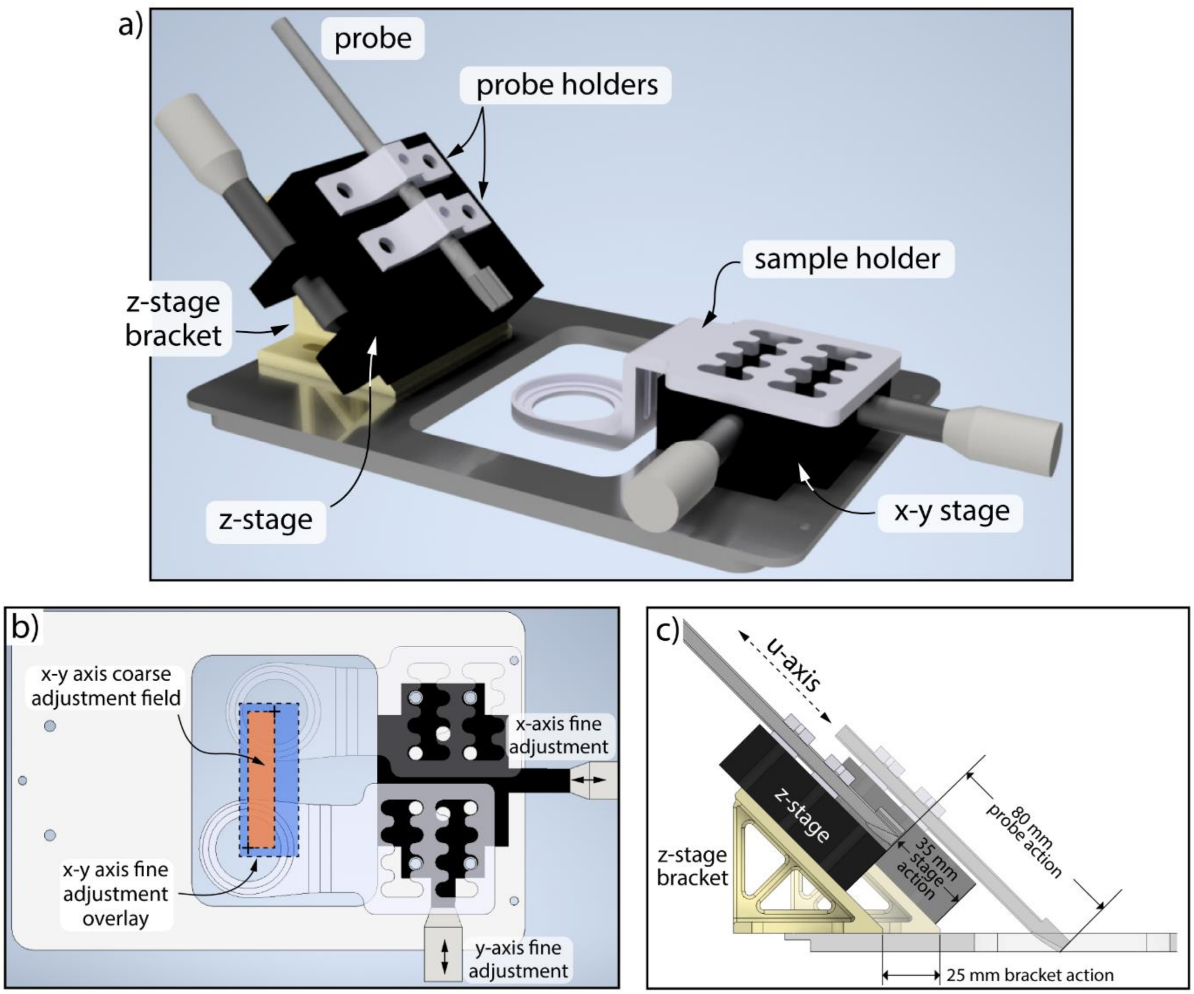

Fig. 4 — component naming scheme and translation explanation. a) isometric view of device with component naming scheme shown. b) illustrative description of motion of the sample using the open bolt pattern design on the sample holder; the coarse adjustment field (orange dashed region) shows the maximum range the sample holder can be coarse translated using the bolt pattern, and the fine adjustment overlay (blue dashed region) shows the additional maximum extent of translation using the x-y stages. c) maximum coarse translation extent for the probe, bracket, and z-stage.

1.1.4 Component construction

Components were built using a combination of 3D printing and machining depending on part utilization and purpose. A FormLabs 3+ sterolithographic 3D printer was used to develop parts for this device due to its material adaptability, ease of use, and moderate precision. The microscope chassis was prototyped initially using 3D printed general-purpose resin but high compliance was observed under micromanipulation and, therefore, outside of an acceptable tolerance. The working version of the microscope chassis was formed by machining aluminum to counteract this compliance, providing a rigid base for manipulators. The sample holder was also first prototyped using general-purpose resin, but was later built using a rigidized resin (Rigid 10K, FormLabs) since curing the form-factor with general purpose resin produced warping resulting in an out-of-optical-plane sample base. The **z**-axis manipulator holder was formed using rigidized resin (Rigid 10K) to prevent compliance during manual operation of the micromanipulator. The **x-y** stage was bolted to the microscope chassis using steel fasteners. The sample holder was secured to the **x-**y stage using steel fasteners and a large polycarbonate washer. The **z-**stage holder was secured to the microscope chassis using polycarbonate bolts in order to prevent damaging the component as secure fastening could crack the rigid resin. The **z-**stage was secured to its holder again using polycarbonate fasteners. Probe holders were formed using general-purpose resin on the 3D printer and fastened to the **z-**stage using steel fasteners. All fasteners used in this device are ¼-20 fasteners of variable length depending on position and component.

2. DEMONSTRATION OF DEVICE

2.1 Simultaneous *in situ* electrochemical and confocal observation of *A. amphitrite* epicuticle region

The main driver of the micromanipulation system was to enable a micro-sized physical probe to be brought into close ($\pm 10\ \mu m$) proximity with a research sample, specifically to the basal region of the acorn barnacle *A. amphitrite.* Generation of reactive oxygen species has been hypothesized to occur at the peripheral epicuticle region of this barnacle, coinciding with presence of a surface clearing material (SCM) produced by *A. amphitrite* (Fears, *et al.*, Adv. Sci., 5(6):1700762). The SCM has been observed by Fears *et al.* to effectively remove microbial biofilm from the substrate, presumably in preparation for the barnacle base to expand during growth through its molt cycle. The production of reactive species may provide the crucial component to clear biofilm by providing a clean surface for follow-on adhesive chemistries. To confirm the coincidence of reactive species with presence of SCM production, *in situ* observation, both optically and electrochemically, is required. Previous efforts to catalog the hypothesized reactive species by optical observation alone using targeted fluorescent staining has proven inadequate. To overcome this roadblock, the micromanipulation device has been designed to provide configurable and precise access of an electrode tip to areas of interest along a barnacle's periphery at the substrate interface, demonstrating the adaptability of the device described herein.

Configuration of the device to provide electrochemical observation was rapidly and easily achieved (Fig. 5). Electrodes were attached to the probe simply using a paper clip near the end of the probe, and the bolt and washer at the probe tip to securely fasten the electrode tips during observation. An additional part was 3D printed to hold the reference electrode in a vertical position above the sample holder. This part was fastened to the sample holder using an extended bolt and pliable washer. An identical open bolt pattern was generated on this part to enable coarse adjustment access during use.

Electrochemical and microscopic observation was collected simultaneously using a variety of open circuit potential, and voltage sweeps (200 mv/s, 50 mv/s, and 10 mv/s) (Fig. 6). The purpose of this report is a description of the micromanipulation setup and, therefore, conclusions on data collected using this system are not described here. Instead, we demonstrate the utility of the system to allow for placement of electrochemical electrodes near an acorn barnacle base while also imaging in a brightfield and/or confocal mode.

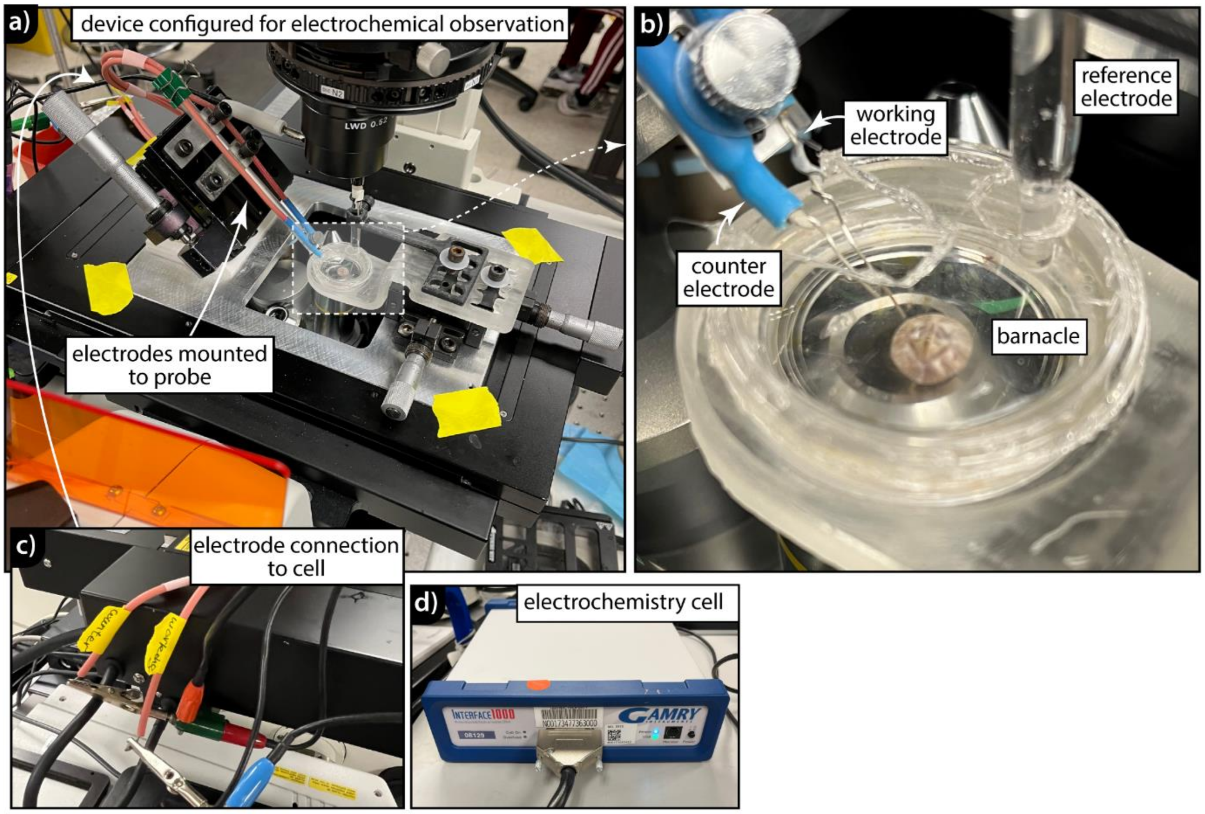

Fig. 5 — a) image of device configuration setup on a Nikon Ti2 base A1R confocal microscope. b) zoom in of sample region showing electrode placement and barnacle under observation above microscope objective,.c) simple connection of electrode cables and adherence of cables to microscope base by adhesive tape. d) image of electrochemical cell used in experimentation

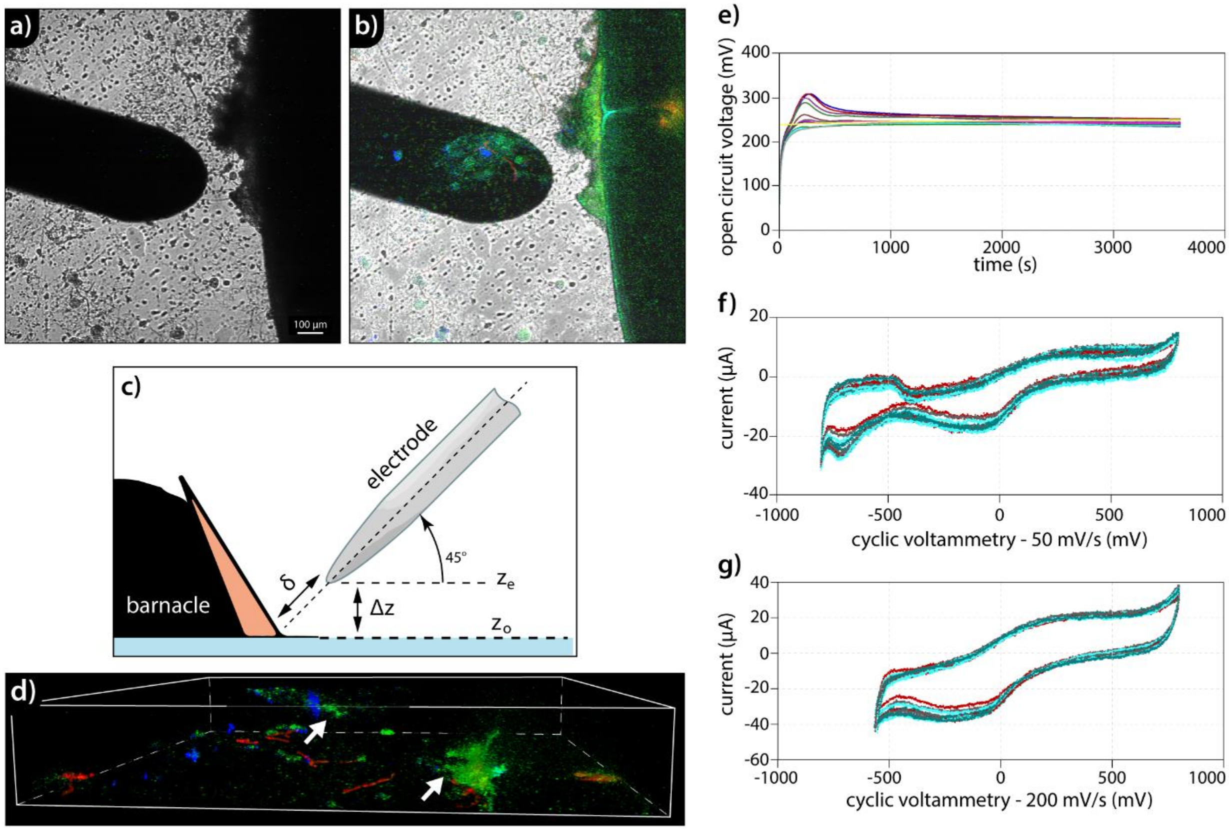

Fig. 6 – a) transmission image showing electrode (left) near barnacle (right). b) max intensity projection of probe and barnacle epicuticle showing autofluorescent region of interest and detritus on probe. c) geometric illustration of probe approach to barnacle epicuticle region. Using focal plane determination of z-positions of the glass (z_o), the electrode tip (z_e), and finding their differential distance (Δz), combined with known approach angle, a user can rapidly determine the distance of the probe from the area of interest (δ). d) Reconstructed 3D fluorescent image of probe near epicuticle region of interest. Arrows show probe surface (left arrow) and epicuticle region (right arrow). e) plot of open circuit potential as a function of time using the setup in Fig. 5. f-g) Cyclic voltammogram data collected during overnight observation.

3. CONCLUSIONS

A microscope-mounted micromanipulation device is described for coarse and fine adjustment of a probe to approach a sample under microscopic observation. Simultaneous operation of the microscope and the manipulation device enables *in situ* observation of a sample while a probe carrying an unspecified device either manipulates or observes processes of a sample. The device was designed to be simple, easily configurable, and readily adaptable to new experimental needs. Use of consistent fasteners, loose tolerances, and 3D printing enables ready customization. Despite the low tier manufacturing, device precision can exceed 10 μm. Practical use of the device is demonstrated in an electrochemical experiment and provides successful probe placement over the course of a >12 hr experiment.

4. APPENDIX

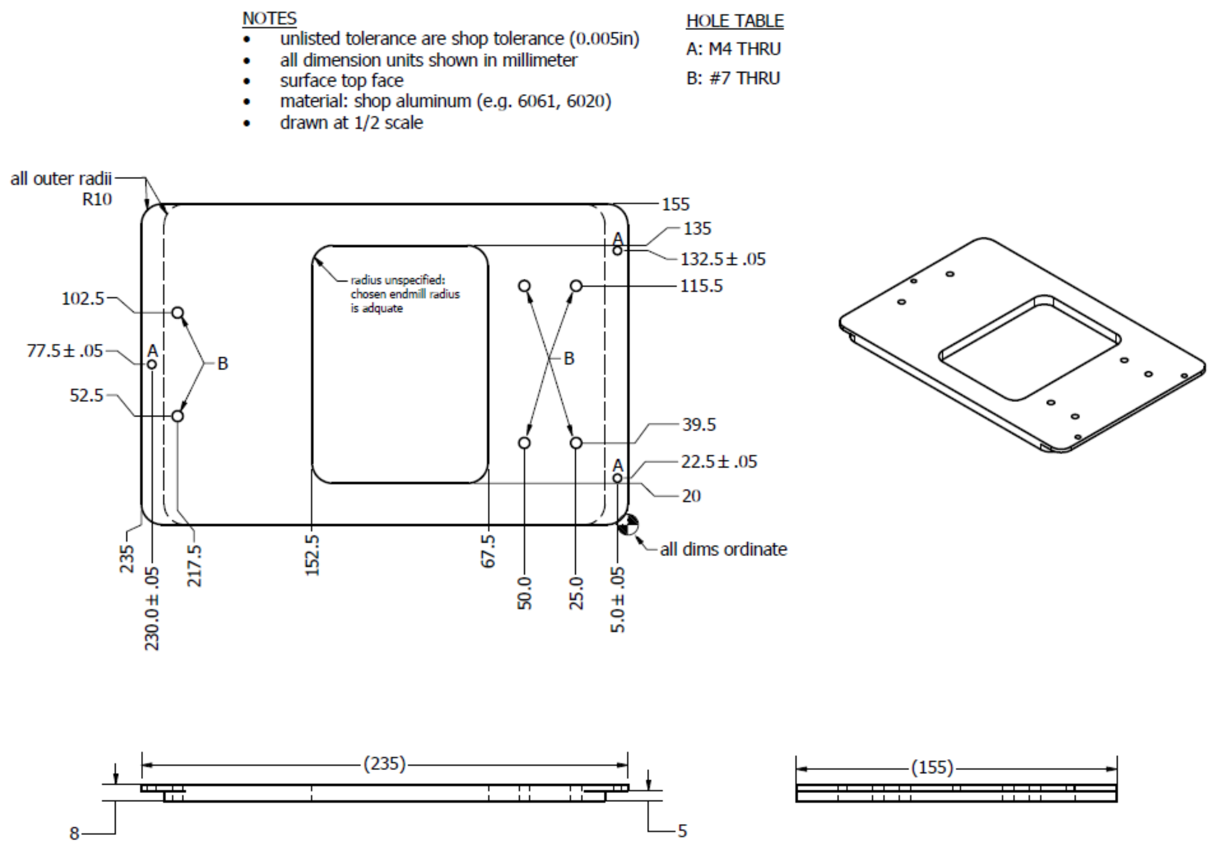

Fig A1 – microscope chassis part drawing.

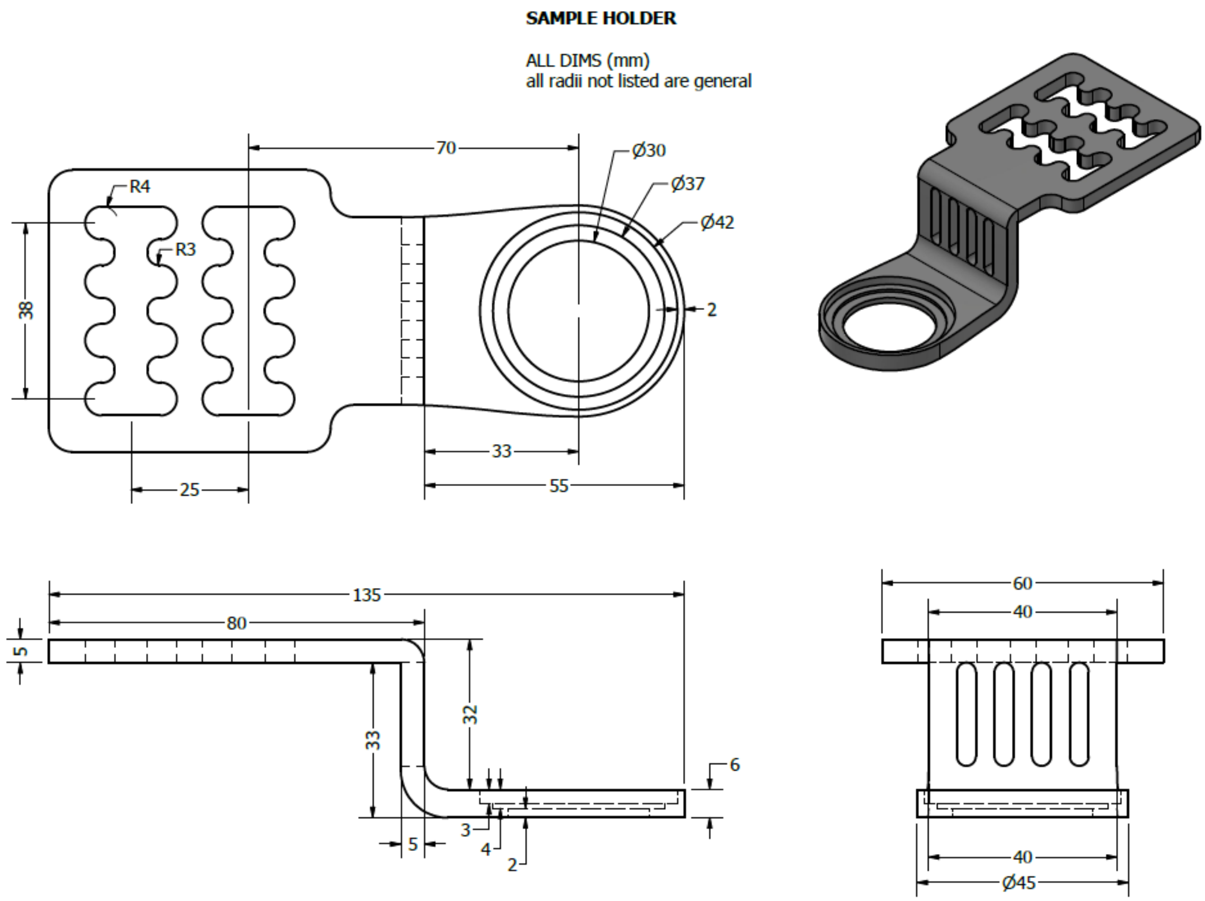

Fig A2 – sample holder part drawings. Unlisted dimensions are open for customization

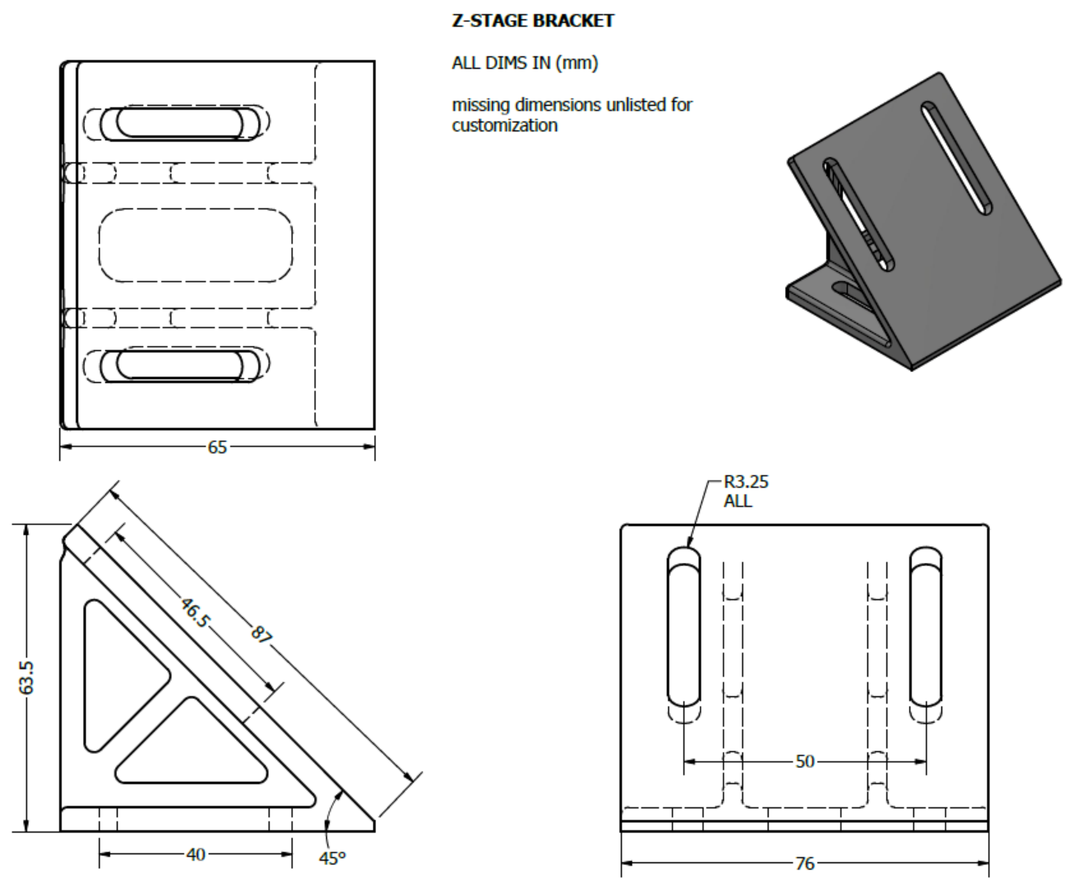

Fig A3 – z-bracket part drawings. Unlisted dimensions are open for customization