UNIVERSITY OF LOUISIANA AT LAFAYETTE

STEP Committee

Technology Fee Application

Purchase of Micropipettes for Chemistry Laboratory Courses

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Name of Submitter

(Faculty or Staff Only)

Department of Chemistry

Organization

Title: Purchase of Micropipettes for Chemistry Laboratory Courses
Date: 07/12/2017
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ABSTRACT:

Over the past several decades, advances in chemistry have influenced many changes in experimental techniques and methods, including the volume of solutions and samples. Depending upon the procedure being performed, chemistry experiments can utilize a variety of volumes ranging from several hundreds of liters to very small microliter volumes. Pipetting is a critically important technique in chemistry experiments to ensure accurate experimental results and is used in all of the chemistry lab courses. CHEM 115 (General Chemistry Lab), CHEM 233 and CHEM 234 (Organic Chemistry Labs I and II), CHEM CHEM 312 (Physical Chemistry Lab), CHEM 319 (Biochemistry Lab), CHEM 362/462 (Undergraduate Research Labs), and CHEM 452 (Inorganic Chemistry Lab) are foundational courses for numerous students in the colleges of science, engineering and education. The Department of Chemistry offers ~15 sections of CHEM 115, ~6 sections of CHEM 233 and CHEM 234, 1 section of CHEM 312, 1 section of CHEM 319, and ~20 sections of CHEM 362/462 and 2 sections of CHEM 452 throughout the year, and all sections together have more than 500 students. All these labs require micropipetting on either daily or specific experiment basis. In addition, the chemistry department has a variety types of spectroscopic instruments including UV-Vis, fluorescence, FTIR, Roman, GC-MS and NMR, and all these spectroscopic techniques require pipetting skills. Our chemistry faculty published ~20 papers every year and many publications contain the results from these instruments. Many undergraduates are the co-authors of those publications. While the chemistry department currently has 20 micropipettes, 15 of them are more than 10 years old and did not work correctly or did not work at all during the past semester. As a result, a significant fraction of students were not able to complete the experiments in the intended manner. The main purpose of this proposal is to request purchase of 40 new micropipettes to replace instruments that are known to be defective. Our students will obtain more accurate data, and be more efficient in lab. We expect that the new instruments will have a positive impact on hundreds of students each year. Our department has discussed the possibility of writing an enhancement proposal for the purchase of micropipettes for chemistry labs through board of regents support funds. However, because micropipettes are small commonplace instruments, we feel that a STEP technology fee application is the most sensible way to attempt to address our instrumental deficiencies. Chemistry labs need new micropipettes. This situation compels us to submit this proposal for university support.

Purchase of Micropipettes for Chemistry Laboratory Courses

A. Purpose of grant and impact to student body as a whole

Because there is no graduate program in the Department of Chemistry, the department's mission is focused on undergraduate teaching and training. Currently, the Department of Chemistry offers ten laboratory courses: CHEM 112 (General Chemistry for Education Majors), CHEM 115 (General Chemistry), CHEM 222 (Analytical), CHEM 252 and 452 (Inorganic), CHEM 233 (Organic I), CHEM 234 (Organic II), CHEM 311 (Physical I), CHEM 312 (Physical II), CHEM 319 (Biochemistry) and CHEM 362/462 (Undergraduate Research I/II) for the students of the Colleges of Science, Engineering, and Education, as well as courses designed for those interested in non-technical fields.

CHEM 115 (General Chemistry Lab) is designed to reinforce concepts learned in lectures of general chemistry (CHEM 107 and CHEM 108) and provides an introduction into basic laboratory techniques. This lab is a required course for undergraduates majoring in biology, chemistry, physics, chemical engineering, civil engineering, petroleum engineering, and kinesiology. The department typically offers eight sections of CHEM 115 in fall semesters and nine sections in spring semesters. There are also four to five summer sections of CHEM 115, although the number of sections is somewhat variable. Each section has ~25 students (American Chemical Society (ACS) limits number of students in any chemistry lab to 25), and every section is typically full, and has a substantial wait list. It is estimated that approximately 500 students take this lab every year.

Table 1. List of undergraduate majors which require CHEM 115 for graduation

	CHEM 115 General Chemistry
Biology	X
Microbiology	X
Biodiversity	X
Chemistry	X
Chem Eng	X
Civil Eng	X
Petroleum Eng	X
Physics	X
Kinesiology	X

Chemistry is a traditional branch of science and a fundamental field. Its applications are to address the challenges facing the world by combating diseases, providing clean water and safe food, developing new sources of energy, developing new materials, and confronting climate changes through interdisciplinary nature of chemistry. To obtain skills for solving world challenges, requires an appropriate suite of modern chemical instrumentation and specialized lab apparatus to analyze chemical properties of small and large molecules. Providing student access to this instrumentation supports the undergraduate instructional and research missions of the chemistry department and the university. Over the past several decades, advances in chemistry have influenced many changes in experimental techniques and methods, including the volume of solutions and chemical or biological samples used. Depending upon the procedure being performed, chemistry experiments can utilize a variety of volumes of samples and buffers, ranging from several hundreds of liters to very small microliter (μl) volumes. Pipetting is a critically important technique in chemistry experiments to ensure accurate experimental results. For example, in typical biochemistry experiments, biopolymers and reagents such as DNA, enzymes and buffers are transferred by pipetting into small microcentrifuge tubes which serve as reaction vessels. For these type of reactions, microliter volumes are typically used. There are 1,000 microliters in 1.0 milliliter of a solution. To put it in perspective, a 50 µl sample is approximately equal in size to a single raindrop. A raindrop-sized sample is relatively large when compared to experimental samples which often are 1.00 to 50.0 µl in volume. To measure µl volumes, a special instrument called a micropipette is used. The variable automatic micropipette is the preferred instrument for delivering accurate, reproducible volumes of sample. These instruments are manufactured to deliver samples in various ranges (e.g., 0.5-10 µl, 5-50 µl, 200-1000 µl, *etc*.) and usually can be adjusted in one-hundred microliter increments depending on the size. Typically, these instruments have an ejector button for releasing the tip after sample delivery. Variable automatic micropipettes can also be multi-channeled, designed to uniformly deliver several samples at the sample time. However, for this experiment, only one sample will be delivered at a time.

Almost all chemistry labs require pipettes or micropipettes. Some labs need them on daily basis while other labs require this instrument dependent on the specific experiments being performed. Currently, our department has 20 micropipettes including size of P10, P20, P100, and P1000. Fifteen of them were purchased more than ten years ago and are not functioning correctly. Our department spends money every year for checking accuracy of ~25 scales in all chemistry labs. This service is done annually in August by a service company in Texas and a technician from the company come to our department to check the scales. Two years ago, we requested the technician to repair micropipettes which were not functioning. However, the technician could not repair them. The micropipettes were getting worse over the time. In the chemistry labs, especially in CHEM 319 and CHEM 362/CHEM 462, 8 to 10 students have to share one micropipette which can give accurate volumes. Students got very frustrated since they often have to wait for more than 20 minutes. Students do not want to use the ones that give inaccurate volumes. They often see errors as high as ~80% when switching between good and bad ones in the same lab. New micropipettes would allow students to more easily collect their data, and it reduces student frustration and facilitates conceptual understanding. It reaches the point that our faculty members need to write this STEP to purchase new micropipettes. The new micropipettes would allow faculty to teach students to meet the ACS standards.

The main purpose of the proposal is to purchase micropipettes for CHEM 115, CHEM 233, CHEM 234, CHEM 252, CHEM 312, CHEM 319, CHEM 362, CHEM 452 and CHEM 462 labs. Drs. Xu, Gallo, Knierim, and Srivastava along with other faculty members teach those courses. This situation requires us to initiate this STEP proposal, seeking support from our university. Students will benefit in the following ways:
(i) Spectroscopy, widely used in research and diagnosis, is a fundamental technology in all chemistry labs. The Department of Chemistry has an Agilent fluorescence, an Agilent UV-Vis, an Agilent FTIR, Ultimate 3000 HPLC-Dionex, an Agilent GC-MS, a StellaNet HR-TEC Raman spectrometer, and a Varian NMR 400 MHz spectrometer. Students trained in spectroscopy will also help economic development by providing a highly-trained scientific workforce to industries, and academic institutes. Students with practical experience with spectroscopy are valued by employers. All these types of spectrometers in chemistry labs discussed here require pipetting or micropipetting skills.

(i) CHEM 319-Biochemistry Lab: Micropipettes have been used on daily basis in both biochemistry teaching and in research labs for last ten years. There are 14 experiments designed in this lab for students to learn lab skills and to understand CHEM317 lectures deeper. The objective of Experiments 3-5 in Biochemistry Lab is to purify lysozyme from egg white and to characterize the enzyme by electrophoresis and enzymatic reaction. Experiments 3-5 involve (1) isolation of proteins from egg white by size exclusion and ion exchange chromatography; (2) protein content analysis by Bradford, Folin-Lowry and UV-Vis methods; (3) separation of the purified proteins by SDS-PAGE. Electrophoresis was used in protein SDS-PAGE lab. In addition to protein purification from egg white, students will isolate cytoplasmic proteins, nuclear proteins and whole cell proteins from mammalian cell cultures, and resolve proteins in SDS-PAGE based on published methods to characterize proteins. Students need to pipet 10.0 µL to 3.00 mL. An example of the volumes used in the experiments is shown in the following **Table 1**.

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Tube Number	Water mL	1mg/mL Lysozyme mL in assay	Reagent A mL	Reagent B mL	Reagent C mL	mg Protein (lysozyme)	ABS at 650 nm
1A	1.0	0	0.9	0.1	3.00		Use to zero
2A	0.99	0.01	0.9	0.1	3.00		
3A	0.98	0.02	0.9	0.1	3.00		
4A	0.96	0.04	0.9	0.1	3.00		
5A	0.92	0.08	0.9	0.1	3.00		
6A	0.84	0.16	0.9	0.1	3.00		

Experiment 6 allows students to perform enzyme kinetics. The main objectives are (1) to gain hand-on experience on enzyme assay; (2) to gain deeper understanding of enzyme kinetics discussed in CHEM 317 lecture. This is an essential skill for pharmaceutic company. In this experiment, assays will be done to determine the effect of enzyme concentration and substrate concentration on the phosphatase catalyzed hydrolysis of p-nitrophenylphosphate (p-NPP). To set-up this assay, pipetting the water, buffer, p-NPP and enzyme at microliter and milliliter scale in the following **Table 2** is required.

Tube#	H_2O (mL)	Tris-ascorbate Buffer (mL)	11mM pNPP (mL)	Phosphatase Solution (mL)	*Enzyme Conc. mg/mL	0	0.5 min	1.0 min	1.5 min	2.0 min	2.5 min	5.0 min
Blank	*1.2*	*1.2*	*3.6*	*0.0*								
Assay1	1.2	0.9	3.6	0.3								
Assay2	1.2	0.6	3.6	0.6								
Assay3	1.2	0	3.6	1.2								

Experiments 9-11 were designed based on Dr. Xu's EMBO J paper. Four endogenous genes were dependent on the physical interactions between CREB and CBP in response to cAMP signaling. The endogenous cAMP responsible genes were verified by luciferase reporter assay. Two of the constructed reporter genes: *cfos* and *Areg*, were used in experiments 9-11. The objective of these three labs is to identify plasmids using two independent methods: enzyme digestion and polymerase chain reaction (PCR). These three labs include (1) isolation of plasmid DNAs: *cfos* and *Areg* luciferase reporter constructs; (2) enzyme digestion of the plasmids and gel electrophoresis; (3) PCR and gel electrophoresis. An example of micropipetting is shown in the following procedure:

Procedure: Set-up a 50 µl of PCR reaction. Label the thin-walled 0.5 ml PCR tubes.

DNA template (the plasmid A you isolated before): 5.0-10 ng
10X PCR buffer: 5.0 µl

5 X Q solution	10.0 µl
10 mM dNTP:	1.0 µl
20 pmol/µl Areg sense primer:	0.5 µl
20 pmol/ µl Areg anti-sense primer:	0.5 µl
2.5U/100µl Taq polymerase:	0.5 µl
mQ H_2O:	to 50 µl

The objectives of Experiments 13-14 are to learn mammalian cell culture, to isolate and to characterize signaling-dependent mRNAs. Experiments 13-14 include (1) mammalian cell culture and cell treatment by anti-cancer drugs and cAMP activator; (2) RNA isolation and reverse transcriptase reaction; (3) semi-quantification of RNA by reverse transcriptase (RT)-PCR. A thermal cycler is used in semi-quantifying specific genes Mammalian cell cultures designed in these two experiments need micropipettes. The following example is to show scale of volumes used in the experiments:

1) Set up your cDNA reactions

RNA sample (500 ng/µL)	1.0 µl
500 µg/ml of Oligo d(T)	1.0 µl
10 mM dNTP	1.0 µl
dH_2O	10.0 µl

2) Heat your mixture at 65ºC for 5 min
3) Cool the mixture on ice for 5 min
4) Add

5X First strand buffer	4.0 µl
0.1 M DTT	2.0 µl
Reverse transcriptase	1.0 µl

5) Perform cDNA synthesis at 42ºC for 50 min

(iii) Pipetting is a fundamental technique in general chemistry lab. Purchase of micropipettes would allow students to learn new skills. CHEM 115 lab is an introductory lab required for higher level chemistry labs (inorganic, organic, analytical, physical and biochemistry labs). Micropipetting is a fundamental skill for upper level chemistry labs and in future careers, so improving student confidence with the instrument should help both inside and outside the classroom;

(iv) CHEM 233 and CHEM 234-Organic Chemistry I and II Lab: Drs. Gallo, Simon and Junk teach this lab. Measurement in chemistry labs is a fundamental technique used by all students in the sciences. The use of micropipettes is common in the biochemistry labs but it can also be used in the organic lab. Several in organic labs require the measurement of small volumes and micropipettes will be used in these experiments. In CHEM 233, the synthesis of 1-bromobutane and 2-chloro-2-methylpropane by SN2 and SN1 pathways respectively are carried out. The kinetics of these substitution reactions can be determined and micropipettes will be used to make dilutions of the reactants in determining the rate law. In CHEM 234, a series of triarylmethane dyes from 4-bromo-N,N-dimethylaniline will be prepared and the quantitation of these dyes is determined by UV-Vis spectroscopy. Serial dilution of the synthesized dyes using micropipettes will assist in calculating the concentration of the dyes, malachite green and crystal violet.

(v) CHEM 311 and CHEM 312-Physical Chemistry I and II Labs: This course, interdisciplinary courses between physics and chemistry, are taught by Drs. Perkins and Knierim. The availability of micropipettes capable of dispensing 100 to 1000 microliters will benefit students in the physical chemistry lab courses. In particular, the ability to precisely dispense small amounts of solution is required for an enzyme kinetics experiment. This experiment is of particular interest because it gives students the opportunity to combine information from two different lecture courses, biochemistry and physical chemistry, while carrying out the experiment and interpreting the results;

(vi) CHEM 252 and CHEM 452-Inorganic Chemistry Labs: Dr. Srivastava teaches the courses. Accurate and precise measurement in chemistry is a fundamental prerequisite. In the chemistry lab it becomes even more essential to deliver reagents as accurately as possible. Some of experiments in teaching chemistry labs require microliter amount of samples. To deliver microliter aliquots we need more precise and accurate delivery system. The micropipettes are one of them. It delivers samples in the fraction of a microliter. Therefore to train students of those classes described above we need pipets of 5, 10, 25, and 50 microliters sizes.

(vii) CHEM 362 and CHEM 462: Research quality is an important indicator of success of an academic program since high quality research will better prepare students to pursue intellectual inquiry. The faculty members have been able to obtain funding to enhance their research activities. Dr. Srivastava received grants from ITRS program and NSF. Dr. Xu has obtained support from ITRS program, an NSF EPSCoR award, and a RCS award. A number of independent and collaborative projects have been developed by our faculty members. For example, undergraduate students in Dr. Srivastava's inorganic chemistry lab synthesized small metal compounds, which were tested in mammalian cell system by undergraduate students in Xu's biochemistry lab. The results from this collaborative effort were published in *Int. J of Oncology* in 2010. Undergraduate students in CHEM 319, CHEM451, and CHEM 362 continued this work last semester and have obtained very interesting results (**Figure 1**, unpublished data). In Dr. Gallo's lab, undergraduate research involving the use of vegetables and fruits as biocatalysts in the asymmetric reduction of aldehydes and ketones via green chemistry will use the micropipettes. Small concentrations of the substrate (e.g. acetophenone) will be measured using the micropipettes followed by addition of the biocatalyst and the reaction followed by GC to determine the percent conversion of reduced product. We are very proud of our undergraduates. The examples demonstrated that the proposed instrument is essential for undergraduate research. All the papers published from Dr. Xu's lab contain the data from this instrument. Dr. Xu has been conducting and will continue to conduct surveys on undergraduate research with the goal of integration of research with teaching. Students are interested in learning lab techniques (**Figure 2,** unpublished data). The research projects introduce students to real scientific investigations through project-based experiments. Project-based learning uses complex real world problems to captivate student curiosity, motivating them to recognize and research the abstract concepts and principles

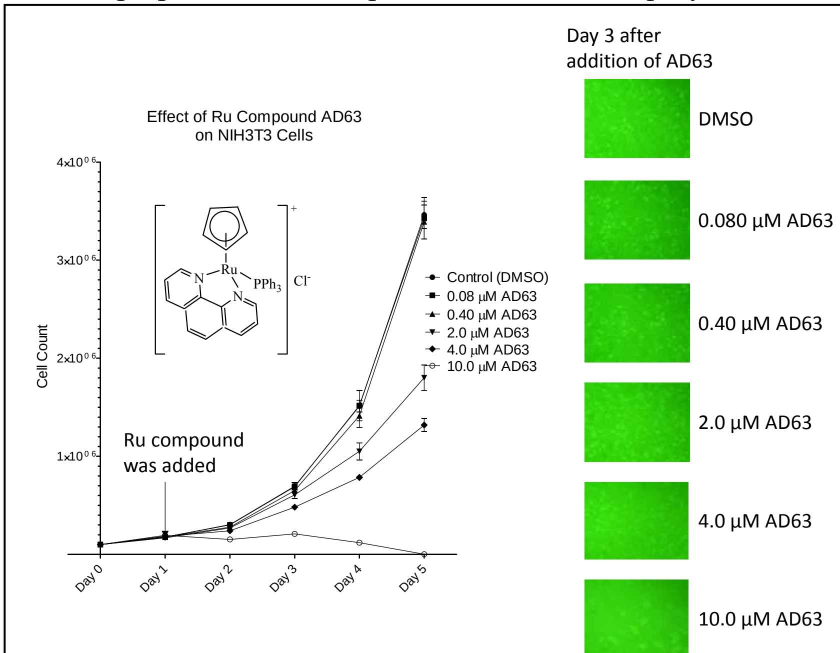

Figure 1. Ru compounds synthesized in inorganic lab were tested in biochemistry lab. This compound has an anti-cancer activity in dose-dependent manner.

they need to know. Collaborative research between faculty on or off campus will provide students with a group-based work environment that allows the growth of interpersonal, organizational, and teamwork skills that will better prepare students for their future scientific workforce. Prospective employers in the scientific and engineering fields have suggested that building skills for interdisciplinary problem solving are important, and this can be exercised in new and more interesting ways after employment.

B. Projected lifetime of enhancement

Micropipettes should be in a good working condition in the lab for five to ten years, only requiring regular basic maintenance.

C. Person(s) responsible for
Implementation: Wu Xu
Installation: Wu Xu
Maintenance: Departmental committee made of up faculty member: Wu Xu
Operation: Faculty of Chemistry Department
Training: Wu Xu will conduct training for faculty members, following their training by the manufacturer

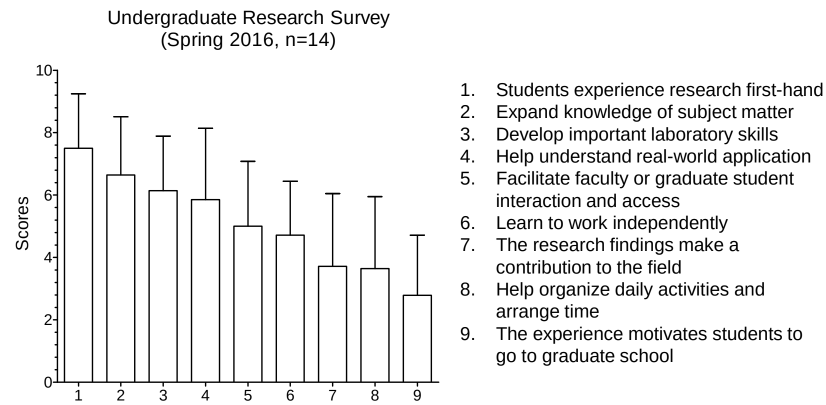

Figure 2. The survey result on undergraduate research. Higher score means higher priority for students.

Budget Proposal

1. Equipment $15,540.00

Catalog No.	Qty	Description	Price
F144802G	10	Gilson™ PIPETMAN Classic™ Pipets, P10	$388.50
F123600G	10	Gilson™ PIPETMAN Classic™ Pipets, P20	$388.50
F123615G	10	Gilson™ PIPETMAN Classic™ Pipets, P100	$388.50
F123602G	10	Gilson™ PIPETMAN Classic™ Pipets, P1000	$388.50
FREIGHT CHARGE			$0.00
		Total:	$15,540

2. Software $ 0.00

No software is required.

3. Supplies $0.00

4. Maintenance $ 0.00

Routine maintenance will be covered by department.

5. Personnel $ 0.00

No personnel required

6. Other $ 0.00

None

TOTAL:	**$15,540.00**

D. Other relevant information

None

E. Previous STEP projects

Drs. Xu, Gallo and Knierim successfully authored a STEP proposals “Smart Classrooms in Chemistry”, funded in 2014 or 2015.