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**14. ABSTRACT**
We propose to purchase a spinning disk confocal unit, to enable high spatial and temporal resolution imaging of dynamic artificial cells. This instrument will support Department of Defense (DoD) sponsored research efforts by several labs at UCSD, will accelerate progress on a DoD Multidisciplinary Research Initiative (MURI) related to dynamic artificial cells, and greatly strengthen the education of students with respect to the research of dynamic artificial cells. The spinning disk confocal unit will enable 4 color imaging of synthetic cells, and will allow rapid capture of artificial cell dynamics to capture shape changes, growth, and division events. The device will also

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| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 15. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | Neal Devaraj |
| UU | UU | UU | UU | | 19b. TELEPHONE NUMBER 858-534-9539 |

## Report Title

Final Report: Spinning Disk Confocal Unit for Observing and Controlling Dynamics in Artificial Cells

## ABSTRACT

We propose to purchase a spinning disk confocal unit, to enable high spatial and temporal resolution imaging of dynamic artificial cells. This instrument will support Department of Defense (DoD) sponsored research efforts by several labs at UCSD, will accelerate progress on a DoD Multidisciplinary Research Initiative (MURI) related to dynamic artificial cells, and greatly strengthen the education of students with respect to the research of dynamic artificial cells. The spinning disk confocal unit will enable 4 color imaging of synthetic cells, and will allow rapid capture of artificial cell dynamics to capture shape changes, growth, and division events. The device will also enable long-term imaging of dynamics without significant phototoxic side effects. The laser excitation system will also provide rapid photoactivation capabilities with excellent spatial and temporal resolution. Coupled with photoactivated membrane synthesis and protein modification, this system will accelerate progress in achieving 4D control over vesicle dynamics and understanding how localization of proteins and lipids can affect phenomenon such as vesicle shape, size, and protein synthesis. The system will upgrade and interface with an existing Zeiss Axiobserver Z1 microscope equipped with a Hamamatsu scientific CMOS camera. This microscope is housed in a dedicated microscopy room provided by the UCSD Department of Chemistry and Biochemistry. This microscopy resource will provide opportunities to educate and train several graduate students and postdoctoral fellows on a state-of-the-art confocal microscopy system. Students will gain immediate and long-term access to a dedicated confocal system, facilitating novel and challenging and long time duration experiments to be setup readily. The proposed spinning disk confocal system would accelerate current DoD support research programs, provide support for research objectives that are of interest to DoD, and will allow students and postdoctoral fellows to obtain valuable research related education related to working with high performance optical microscopy systems.

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## Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

### (a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

**TOTAL:**

**Number of Papers published in peer-reviewed journals:**

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### (b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

**TOTAL:**

**Number of Papers published in non peer-reviewed journals:**

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### (c) Presentations

**Number of Presentations:**

## Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

**TOTAL:**

**Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

**TOTAL:**

**Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):**

## (d) Manuscripts

Received Paper

**TOTAL:**

**Number of Manuscripts:**

## Books

Received Book

**TOTAL:**

| Received | Book Chapter |
|---|---|

**TOTAL:**

## Patents Submitted

## Patents Awarded

## Awards

### Graduate Students

| NAME | PERCENT_SUPPORTED |
|---|---|

**FTE Equivalent:**
**Total Number:**

### Names of Post Doctorates

| NAME | PERCENT_SUPPORTED |
|---|---|

**FTE Equivalent:**
**Total Number:**

### Names of Faculty Supported

| NAME | PERCENT_SUPPORTED |
|---|---|

**FTE Equivalent:**
**Total Number:**

### Names of Under Graduate students supported

| NAME | PERCENT_SUPPORTED |
|---|---|

**FTE Equivalent:**
**Total Number:**

## Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ..... 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: ..... 0.00

## Names of Personnel receiving masters degrees

NAME

**Total Number:**

## Names of personnel receiving PHDs

NAME

**Total Number:**

## Names of other research staff

| NAME | PERCENT_SUPPORTED |
|---|---|

**FTE Equivalent:**

**Total Number:**

## Sub Contractors (DD882)

## Inventions (DD882)

## Scientific Progress

## Technology Transfer

**Final Report: Proposal No. 66891-LS-RIP Spinning Disk Confocal Unit for Observing and Controlling Dynamics in Artificial Cells**

**Acquired equipment and cost**

Please see attached purchase order from UCSD (page 4) to the vendor Carl Zeiss Microimaging Inc. In summary, $200,000 was spent to acquire a 4-laser spinning disc confocal unit with a Direct FRAP (fluorescence recovery after photobleaching) add-on for photoactivation studies. The instrument was purchased in September 2015 and was installed in January 2016. There was a delay due to the requirement that equipment required shipment to Germany and back to our lab.

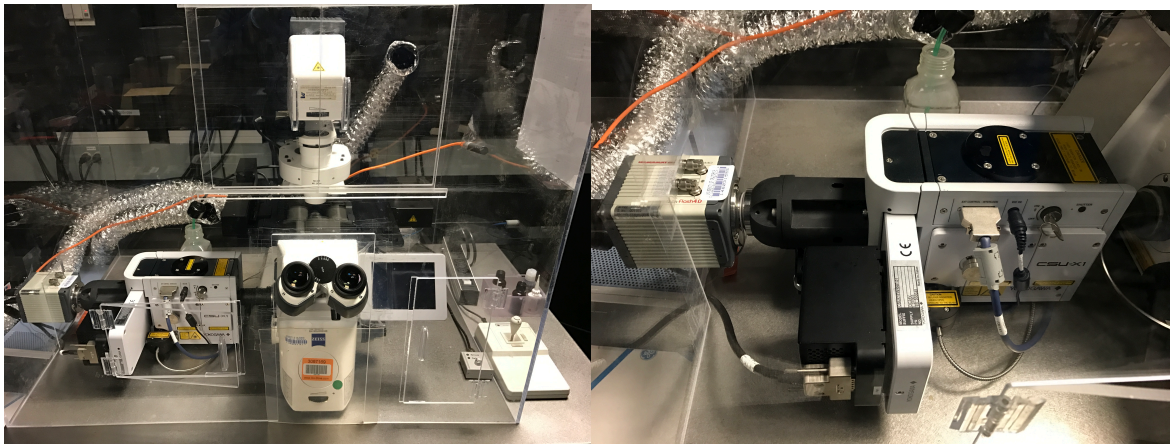

**Figure 1. Left: Photograph of the spinning disc confocal microscope. Right: closeup of the spinning disc confocal unit**

**Summary of the research projects on which equipment has been or will be used**

The instrument supports Department of Defense sponsored research efforts by several labs at UCSD, and was primarily used on a Department of Defense Multidisciplinary Research Initiative (MURI) related to dynamic artificial cells. The instrument has supported two DoD funded projects at UCSD. The first is a Multidisciplinary University Research Initiative (MURI) funded by the DoD Army Research Office (ARO) entitled "Dynamic Artificial Cells Composed of Synthetic Bioorthogonal Membranes" (W911NF-13-1-0383). The second project is a DoD U.S. Army Construction Engineering Research Lab (CERL) project entitled "Controlling Functional Group Architecture in Artificial Cells" (W9132T-14-2-0002).

**Exemplary DoD relevant research projects for which the microscope has been used**

Imaging *De novo* phospholipid vesicle formation using a single soluble enzyme:

Linking gene expression with phospholipid formation with high efficiency and selectivity is an important goal in the pursuit of synthesizing artificial cells. We designed a non-natural enzyme based method for de novo formation of phospholipid vesicles from simple water soluble precursors. We utilized a soluble mycobacterial enzyme FadD10 to generate fatty acyl

adenylates from corresponding saturated fatty acids in presence of ATP and $Mg^{2+}$. Upon formation, the adenylate spontaneously reacts with an amine-functionalized lysolipid in a highly chemoselective manner to form phospholipid, which thereby self-assemble to form vesicles. Interestingly, during self-assembly of the phospholipids, the enzyme gets encapsulated with high efficiency.

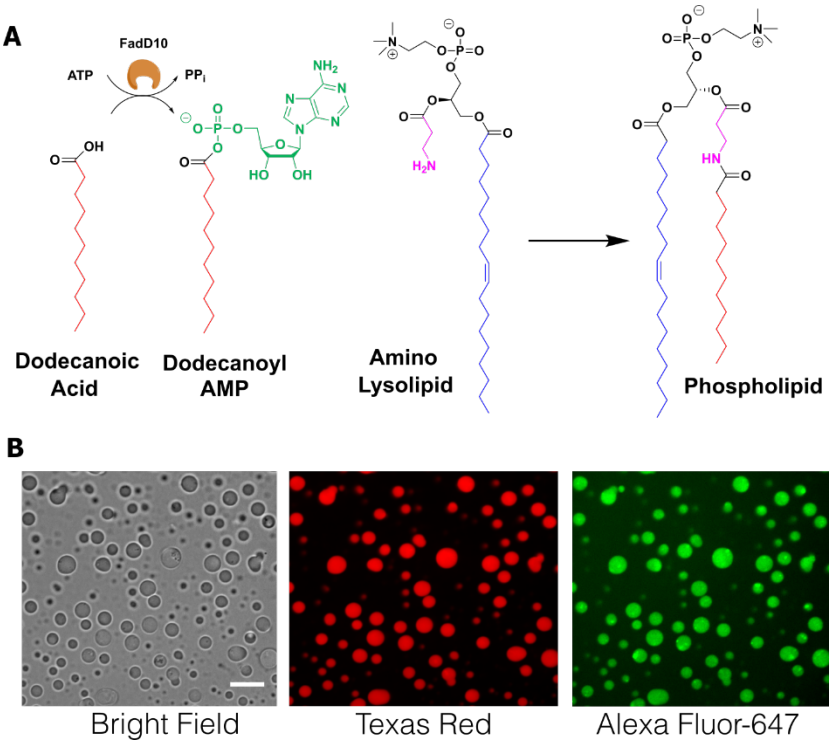


**Figure 2. *De novo* vesicle formation using a soluble enzyme FadD10: (A)** FadD10 activates a saturated fatty acid (dodecanoic acid) to the corresponding adenylate in presence of ATP and $Mg^{2+}$. An amino-lysolipid reacts with this intermediate spontaneously to form phospholipid via amide bond formation. **(B)** Copious formation of highly multilamellar vesicles along with efficient encapsulation of fluorescently (Alexa Fluor® 647) labeled FadD10 observed by spinning disc confocal microscopy (scale bar: 10 µm)

We further found that, when a DNA encoding FadD10 is added to a cell-free transcription-translation system (PURExpress®), the enzyme can be expressed and phospholipid vesicles can be formed de novo when suitable precursors are added. Our current efforts are focused towards achieving growth and division of vesicles expressing FadD10 using microfluidics based methods. The purchased microscope was used for imaging and monitoring the de novo formation and growth of these synthetic vesicles (Figure 2B).

Imaging Gene expression in porous artificial cells with nuclei

All living cells exchange matter with their environment when they take up nutrients, excrete waste products or communicate with neighboring cells. We are interested in engineering artificial cells that can exchange molecules with their environment. We use a microfluidic

method to produce porous polyacrylate capsules with a "nucleus" that contains their genome. The artificial cells' "nuclei" are formed by a clay hydrogel that tightly binds DNA. When we add transcription and translation ("TX-TL") reagents to these capsules they synthesize proteins that are encoded in the DNA in their nuclei. The capsules are very porous, so newly synthesized proteins quickly leave the capsule they originate from. In order to localize protein products to capsules we add a plasmid that contains an array of binding sites for the TetR repressor. When capsules produce a fusion protein of TetR repressor and GFP, nuclei increase in fluorescence over time as shown in Figure 3, taken using our purchased instrument.

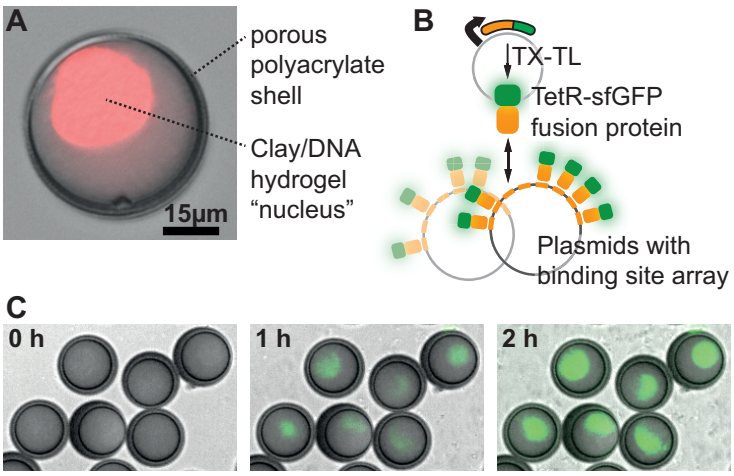


**Figure 3. Polyacrylate capsules with a "nucleus" are capable of gene expression.**
**(A)** Image of a capsule containing a clay/DNA nucleus stained with gel red. **(B)** Schematic of plasmids contained in the nucleus. **(C)** When TX-TL reagents are added, TetR-sfGFP protein accumulates inside the capsules.

We found that capsules with different genomes can communicate with each other because they can take up proteins synthesized by neighboring capsules. In a mixture of producer capsules that only carry the plasmid to synthesize TetR-sfGFP and collector capsules that only carry the plasmid with TetR binding sites, nuclei in collector capsules light up. We are now exploring ways to form spatial patterns in a "tissue" of artificial cells. This work will require confocal imaging using the purchased instrument and is part of our DoD MURI.

**Future enhanced research capabilities for performing research and research-related education of interest to DoD**

The purchased instrument will support several areas of technical interest to the DOD, including enhancing our ability to understand and control the function, structure, and organization of biomolecules. We believe the instrument will support future high-risk research in biomolecular self-assembly.

**UCSD Purchase Order:** 91131729
**Vendor:** CARL ZEISS MICROIMAGING, LLC

| Quantity | Catalog No. | Description | Amount |
|---|---|---|---|
| | | CARL ZEISS MICROSCOPY, LLC OBSERVER SD | |
| 1 | 410901368099900 | CSU-X1M & laser bench 3F f/Observer | $113,481.12 |
| 1 | 4261189000000000 | Camera adapter 60N 1.0x long with TL 1x | $1,244.00 |
| 1 | 4101361045110000 | ZEN SW Driver Hamamatsu Cam. HW Lic. Key | $691.00 |
| | | Lasers | $12,927.00 |
| 1 | 4006009000000000 | Laser 405nm 20mW | $13,281.00 |
| 1 | 4006009040000000 | Laser 488nm 30mW | $12,927.00 |
| 1 | 4006009090000000 | Laser 561nm 20mW | $10,774.00 |
| 1 | 4006009121000000 | Laser 638nm 75mW | $379.00 |
| 1 | 0000001031343000 | Emission Filter BP 450/50 d=25x4 ( E ) | $484.00 |
| 1 | 4890388002000000 | Emission filter BP 525/50 DMR 25 | $484.00 |
| 1 | 4890638002000000 | Emission filter BP 629/62 | $447.00 |
| 1 | 4880508003000000 | Emission filter BP 690/50 | $484.00 |
| 1 | 4890618003000000 | Emission filter DBP 527/54+645/60 | |
| | | DirectFRAP module | |
| 1 | 4109009199000000 | DirectFRAP Kit with Fiber | $11,808.00 |
| 1 | 4236359010000000 | Fiber oscillator | $768.00 |
| 1 | 4236399061000000 | Beampath Switch, fast f. Axio Observer | $3,349.00 |
| | | Laser safety upgrade for AxioObserver S/N 3834000449 | |
| | | Retrofit specific for spinning disc | |
| | | 4329279011000 basic Laser safety | |
| | | 425155 Sideport 60N L/R 100 3 position f/ Observer | |
| | 000000171441 | Upgrade cost incl parts, labor & shipping | $10,000.00 |
| | | Shipping Charges | $385.00 |
| | | Subtotal: | $193,913.12 |
| | | CA Sales Tax: | $7,378.26 |
| | | Total: | $201,291.38 |
| | | Amount transferred to PI's second fund source: | $1,291.38 |
| | | TOTAL charged to PI's DURIP award: | $200,000.00 |