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| **Fiscal Year:** | FY 2012 | **Task Last Updated:** | FY 02/14/2012 |
| **PI Name:** | Tai, Yu-Chong Ph.D. | | |
| **Project Title:** | In-flight Blood Analysis Technology for Astronaut Health Monitoring | | |
| **Division Name:** | Human Research | | |
| **Program/Discipline:** | NSBRI | | |
| **Program/Discipline--Element/Subdiscipline:** | NSBRI--Smart Medical Systems and Technology Team | | |
| **Joint Agency Name:** | | **TechPort:** | Yes |
| **Human Research Program Elements:** | (1) **ExMC**:Exploration Medical Capabilities | | |
| **Human Research Program Risks:** | (1) **Medical Conditions**:Risk of Adverse Health Outcomes and Decrements in Performance Due to Medical Conditions that occur in Mission, as well as Long Term Health Outcomes Due to Mission Exposures | | |
| **Space Biology Element:** | None | | |
| **Space Biology Cross-Element Discipline:** | None | | |
| **Space Biology Special Category:** | None | | |
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| **Zip Code:** | 91125 | **Congressional District:** | 29 |
| **Comments:** | | | |
| **Project Type:** | Ground | **Solicitation / Funding Source:** | 2007 NSBRI-RFA-07-01 Human Health in Space |
| **Start Date:** | 10/01/2007 | **End Date:** | 10/01/2011 |
| **No. of Post Docs:** | 0 | **No. of PhD Degrees:** | 3 |
| **No. of PhD Candidates:** | 2 | **No. of Master' Degrees:** | 0 |
| **No. of Master's Candidates:** | 0 | **No. of Bachelor's Degrees:** | 1 |
| **No. of Bachelor's Candidates:** | 2 | **Monitoring Center:** | NSBRI |
| **Contact Monitor:** | | **Contact Phone:** | |
| **Contact Email:** | | | |
| **Flight Program:** | | | |
| **Flight Assignment:** | NOTE: end date changed to 10/1/2011 to accommodate final reporting (Ed., 2/21/2012) | | |
| **Key Personnel Changes/Previous PI:** | | | |
| **COI Name (Institution):** | Ho, Chih-Ming ( University of California, Los Angeles )<br>Kasdan, Harvey ( IRIS International )<br>Adams, Thomas ( IRIS International ) | | |
| **Grant/Contract No.:** | NCC 9-58-TD01301 | | |
| **Performance Goal No.:** | | | |
| **Performance Goal Text:** | | | |

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| **Task Description:** | Medical events happened frequently to astronauts in space. For example, even the Space Shuttle Program alone reported 1867 incidences 1981-1998. Moreover, some events were serious viral/bacterial diseases such as urinary tract, conjunctivitis, acute respiratory, dental, and Varicella Zoster virus infections. Ideally, treatment on astronauts should be based on precise medical information. Meanwhile, blood is one of the most important body fluids related to health and there's tremendous information in blood. Blood analysis, if possible, should be the first important step of health monitoring for sick and healthy astronauts. Blood analysis can also be a powerful technique to monitor bone loss and radiation effects. Therefore, NASA should have an in-space, real-time blood analysis capability. However, NASA still doesn't have blood analysis capability other than blood gas and electrolyte analysis. This proposal is specifically to develop an in-box blood analysis technology for NASA. As a whole, we believe that the lab-on-a-chip technology is the best choice for multiple blood analysis in space. Therefore, our long-term objective is to develop blood analysis in-a-box using lab-on-a-chip technology specifically for space applications, emphasizing small form factor, lightweight, and autonomous operation to accommodate Crew Exploration Vehicle (CEV) and International Space Station (ISS) size requirement for medical kits.<br>The specific aims for this project period are to develop space technologies for (a) 5-part WBC (white blood cell) differential, (b) analysis of WBC subtypes (e.g., CD4+ T helper and natural killer cells). Our approach to achieve the goal is to develop the capability of minimally diluted micro flow cytometer to enable a comprehensive WBC differential, and allow detection of fluorescent labels attached to ligands used for cell surface marker for WBC subtype analysis. Embedded in the two specific aims is a research component on the data analysis software. This software has been developed in Matlab to facilitate both quantitative assessment of fluorescence detection and cell and analyte recognition and quantitation.<br><br>For the last funding years, we worked extensively on searching for a new staining method and optimizing the previously proposed Acridine Orange staining. We successfully developed a series of assays including a 4-part differential assay (i.e., Lymphocyte, Monocyte, Neutrophil, and Eosinophil) with a cocktail staining of fluorescent dyes fluorescein isothiocyanate (FITC) and propidium iodide (PI), a 5-part differential assay (i.e., Lymphocyte, Monocyte, Neutrophil, Eosinophil, and Basophil) with a cocktail staining of fluorescent dyes fluorescein isothiocyanate (FITC), propidium iodide (PI), and Basic Orange 21, and a specific assay for the rare cell type basophil differential using fluorescent dye Basic Orange 21. The differential assays were investigated in a correlation study with the commercial hematology analyzer, and further verified with the purified WBC types. For the Acridine Orange assay, the differential capability is also extended from 2-part (Lymphocyte and Neutrophil) into 4-part (Lymphocyte, Monocyte, Neutrophil, and Eosinophil). The time and temperature dependence of the Acridine Orange staining are also investigated.<br><br>Within the project period, we have also explored the possibility of improving the (box) platform in terms of spectroscopic detection. Two different approaches have been implemented; one uses a commercial mini-spectrometer and the other approach uses a 8-channel PMT (photomultiplier) module. Measurement of fluorescent emission spectrum from blood cells stained with the dye assay has been successfully demonstrated on the spectroscopic approach. Single cell fluorescence emission spectrum has been measured on the mini-spectrometer prototype. Distinct spectrums were measured from lymphocyte, neutrophil, and eosinophil cells. Besides, multicolor fluorescent beads have been successfully measured on the 8-color reader. Those two approaches enable detection of multiple fluorophore simultaneously from WBC subtype immune-staining. The additional spectral information should provide better discrimination between multiple fluorophores used simultaneously. It may also provide additional information about the intracellular environment in which Acridine Orange fluorescence occurs, leading to efficient WBC subtype discrimination. For WBC subtypes analysis, we have also successfully demonstrated assays that identified and counted CD4 and CD8 WBCs. In addition, we also developed synthesized peptides specifically targeted for leukocyte cells. The binding peptides were custom-synthesized with a fluorescein fluorophore attached to their n-terminus for binding quantification. A library of 72 potential peptide candidates has been tested using a modified protocol for leukocytes utilized in our studies. Among the results, 4 peptides from our initial library exhibited a 2-3x higher binding strength to the B-cells compared to the other peptides, which confirmed the capability of this approach for WBC subtype analysis. |
| **Rationale for HRP Directed Research:** | |
| **Research Impact/Earth Benefits:** | This project developed assays and instrumentation (i.e., hardwares, and softwares) that provide new ways of WBC count and subtype analysis. This project also proved that these new methods are as good as, if not better, as currently available commercial methods on Earth. Therefore, for the first time, this project provided the capability for NASA to do blood cell analysis in space, although further improvement needs to be done over our prototype for space qualification. In addition, both the developed assays and instrument can be used on Earth, too, and the technology has been licensed to a company, i.e., LeukoDx Inc., for the development of a point of care sepsis monitoring system initially targeted for the detection and monitoring of neonatal sepsis. |
| **Task Progress:** | 1. Blood staining and testing procedure optimization: A 5-part WBC differential (Lymphocyte, Monocyte, Neutrophil, Eosinophil, and Basophil) assay using a staining cocktail of FTIC, PI, and Basic Orange 21 has been developed. The differential capability has been investigated with a correlation study with a commercial hematology analyzer and further verified with purified individual WBC types. In addition, a specific assay was developed for the differential count of the rare population, Basophil, using the fluorescent dye BO21.<br>2. Verification of the differential assays with purified WBC types. A procedure of preparing purified WBC individual types (Lymphocyte, Monocyte, Neutrophil, Eosinophil, or Basophil) has been developed. The differential capability of the 5-part assay (PI, FITC, BO21) and the Basophil specific assay (BO21) was verified with the purified WBC types. The staining pattern observed from the purified WBC types also provided a useful tool to study new assays.<br><br>3. Spectrum analysis capability. One unit of the prototype has been upgraded from two-color detection to spectrum analysis with a commercial mini-spectrometer. Fluorescence spectrum measurment of dye (Acridine Orange) stained white blood cells were successfully demonstrated on the microfluidic chip. Distinct spectrums were measured from the Lymphocyte, Neutrophil, and Eosinophil cells. In addition, the detection of lymphocyte subtype cells were also demonstrated with the spectrum measurement system, which paved the way for simultaneous measurement of multiple subtype cells.<br><br>4. Planning for the new generation cartridge. Components of the next generation cartridge were successfully demonstrated. In the current cartridge, manual handling was involved to process the blood sample before test, and an external pump and waste collection tube were need for the fluidic operation. In the next generation cartridge, the whole |

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| | test will be integrated into a 1cm x 1cm x 3mm chip without external fluidic connection. We successfully demonstrated the on-chip staining of blood sample with fluorescent dyes on the microchip. Besides, basic components of on-chip pump, on-chip valve, and long term reagent storage capability were also demonstrated. |
| **Bibliography Type:** | Description: (Last Updated: 08/30/2018) |
| **Articles in Peer-reviewed Journals** | Shi W, Guo L, Kasdan H, Tai YC. "Four-part leukocyte differential count based on sheathless microflow cytometer and fluorescent dye assay." Lab Chip. 2013 Apr 7;13(7):1257-65. https://doi.org/10.1039/c3lc41059e ; PubMed PMID: 23389050 , Apr-2013 |
| **Awards** | Shi W. "2011 Lemelson-MIT Caltech Student Prize, 2nd Place, March 2011." Mar-2011 |
| **Papers from Meeting Proceedings** | Shi W, Guo LW, Tai YC. "A Microfluidic Blood-Clogging Valve for On-Chip Blood Analysis." 16th International Conference on Solid-State Sensors, Actuators and Microsystems (Transducers' 11), Beijing, China, June 5-9, 2011. 2011 16th International Solid-State Sensors, Actuators and Microsystems Conference (TRANSDUCERS 2011). Piscataway, NJ : Institute of Electrical and Electronic Engineers, Inc., 2011. p. 1923-1926. http://dx.doi.org/10.1109/TRANSDUCERS.2011.5969375 , Jun-2011 |
| **Papers from Meeting Proceedings** | Shi W, Guo LW, Kasdan H, Fridge A, Tai YC. "Leukocyte 5-part differential count using a microfluidic cytometer." 16th International Conference on Solid-State Sensors, Actuators and Microsystems (Transducers' 11), Beijing, China, June 5-9, 2011.<br>2011 16th International Solid-State Sensors, Actuators and Microsystems Conference (TRANSDUCERS 2011). Piscataway, NJ : Institute of Electrical and Electronic Engineers, Inc., 2011. p. 2956-2959. http://dx.doi.org/10.1109/TRANSDUCERS.2011.5969374 , Jun-2011 |