Fiscal Year:	FY 2010	**Task Last Updated:**	FY 12/09/2009
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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Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2007 NSBRI-RFA-07-01 Human Health in Space
Start Date:	10/01/2007	**End Date:**	09/30/2011
No. of Post Docs:	1	**No. of PhD Degrees:**	2
No. of PhD Candidates:	3	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NSBRI
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Ho, Chih-Ming (University of California, Los Angeles) Kasdan, Harvey (IRIS International) Adams, Thomas (IRIS International)		
Grant/Contract No.:	NCC 9-58-TD01301		
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Task Description:	The specific aims of the project include (a) 5-part WBC differential, (b) analysis of WBC subtypes (e.g., CD4+ T helper and natural killer cells), and (c) serum/plasma protein biomarker analysis (e.g., for infection, radiation and bone loss monitoring). This project is a continuation of a related project entitled "Handheld Body-Fluid Analysis System for Astronaut Health Monitoring," in which we explored electrical impedance sensing, fluorescence optical sensing, and flow separation of blood cells in microfluidic devices and portable platforms. We successfully demonstrated fluorescent sensing and counting for WBC count and 2-part differential with a portable prototype micro flowcytometer. For the current project, a major effort is proposed to extend the 2-part WBC differential to a 5-part WBC differential, add cell surface marker detection and analysis capability to the platform repertoire, and add plasma protein detection and analysis capability to the platform repertoire. Our approach to achieve the objectives is to extend the capability of the micro flowcytometer to enable a more comprehensive WBC differential, and allow detection of fluorescent labels attached to ligands used for cell surface marker and plasma protein detection. The second component necessary for extending the platform capability is the offline data analysis software. This software is being developed in Matlab to facilitate both quantitative assessment of fluorescence detection and cell and analyte recognition and quantitation. In the last funded year, we successfully tested the proposed micro flowcytometer in a zero-G parabolic flight test in collaboration with research scientists from Wyle cooperation. The test demonstrated the facility of doing WBC differential count in zero/micro gravity environment with the proposed prototype. Results similar to on-ground test are obtained. The prototype was shown to be convenient for operation. One flight crew learned to operate the prototype and carried out the test after a brief training. For improving the differential capability of the prototype, we worked on searching for new staining method and optimizing the previously proposed Acridine Orange staining. We successfully demonstrated 4-part WBC differential count (i.e. Lymphocyte, Monocyte, Neutrophil and Eosinophil) with a two color laser-induced fluorescence (LIF) detection scheme. The dye combination FITC and PI stains WBC in blood with selective affinity and shows different fluorescence signatures for each of the 4 types. The differential capability of the platform is largely improved from 2-part differential (i.e. Lymphocyte, Non-Lymphocyte) to 4-part differential (i.e. Lymphocyte, Monocyte, Neutrophil and Eosinophil). The previously proposed fast staining with Acridine Orange was also optimized so that the differential capability was expanded from 2-part to 3-part (Lymphocyte, Monocyte and Neutrophil). We also worked on improving the proposed platform on its excitation source. By replacing the LED with a laser excitation, the induced fluorescence intensity is largely enhanced. With the improved prototype, WBC subtype counting such as CD4+ T cells with fluorophore conjugated antibody staining whole blood was also demonstrated. The sensitivity of the improved prototype was capable to detect fluorescence signals from the fluorophore after the conjugated antibody adhering to the cell surface, which provided a useful method of approaching out specific aim for WBC subtype analysis. To analyze WBC subtypes, we proposed to use continuous flow separation at upstream and dielectric properties characterization at downstream. Our previous design had achieved a very compact design capable of continuous cell separation. The geometry of the sorting region has been further optimized to improve sorting efficiency and enhance continuous operation. Experiments have been performed to successfully separate particles and embryoid bodies into size-dependant groups. Electrical Impedance Spectroscopy (EIS) is explored for dielectric properties characterization which employed a microelectrode array in combination of a novel cell patterning method for cell impedance measurements on the single-cell basis. Utilizing photolithographically patterned SAMs and stepwise protein immobilization enable the precise formation of single-cell arrays. Target cells are immobilized onto detection electrodes and their impedance spectra are measured to discriminate different WBC subtypes. For the coming year, we plan to work on improving the platform on its detection part. To expand the detecting spectrum range, we plan to explore of using mini spectrometer on the micro flowcytometer platform instead of the PMT detectors. Spectrum analysis could provide the potential of analyzing multi-color fluorescence in a compact size prototype. Further, we will explore WBC counting and differential with fluorophore conjugated antibodies. A cocktail of fluorescent dyes including acridine orange will be investigated to stain blood for five part WBC differential, also for WBC subtype counting. For WBC subtype separation and counting, with the successful optimization of the continuous flow separation device, integration of micromixer and deionier as well as DEP focusing devices and Coulter counters will be investigated. The fluorescent particle immunoassay (FPIA) will be investigated for on-chip plasma protein detection.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The devices under development can be used for earth-based applications. The proposed device use cartridge and hand held system. The cartridge will be cheap and disposable. The results will be available almost immediately to the patients without going through central lab facilities. The device can be used in emergency room, on ambulance as well as at home. As the senior population continuous to grow, this kind of device will find more and more appealing in point-of-care applications.
	For the first funding year, we proposed to optimize the blood staining and testing procedure for 5-part WBC differential, optimize hydrodynamic separator for WBC subtype separation, and plasma preparation for plasma biomarker detection. We are on the schedule. 1. Blood staining and testing procedure optimization: We demonstrated 4-part WBC differential count including Lymphocyte, Monocyte, Neutrophil and Eosinophil, with a combination staining of FTIC and PI. We also demonstrated 3-part WBC differential count by an optimized Acridine Orange staining. 2. Fluorophore conjugated antibody staining of whole blood: As an alternative to chemical staining, we demonstrated WBC differential and WBC subtype counting with fluorophore conjugated antibody. By using a laser module for excitation, the sensitivity of the prototype is largely improved to detect signals from fluorophore conjugated antibody. Monocyte count and WBC subtype count such as CD4+ have been demonstrated on the proposed micro flowcytometer. 3. Zero-G parabolic flight test: We tested the portable micro flowcytometer prototype on a zero-G parabolic flight test, which is important to validate using the prototype in a zero/micro gravity environment. WBC differential similar to on ground test has been achieved. Experiences from the flight test are being used for improvement of the prototype, such as

Task Progress:	reinforcing the mechanical structure and increasing the pumping efficiency under low environmental pressure. 4. Hardware improvement: The excitation source of the prototype has been upgraded to a blue laser from a LED. The laser excitation provides stronger fluorescence intensity for detection. Now we are working on improving the detection component. Analysis of fluorescence spectrum would be explored with a mini-spectrometer detector. 5. Optimization of hydrodynamic separator for WBC subtype separation: The geometry of the sorting region has been further optimized to improve sorting efficiency and enhance continuous operation. Computational simulations were utilized to improve the design of various components including the hydrodynamic focusing region, the sorting region and the collection bins. Experiments have been performed to successfully separate particles and embryoid bodies into size-dependant groups. 6. WBC subtype analysis with EIS: Characterization of single-cell dielectric properties using EIS requires immobilization of the target cells onto detection electrodes with accurate position control at the single-cell level. We have refined our previously developed cell patterning technique through optimizing the fabrication process to achieve high selectivity protein patterns enabling for the precise formation of single-cell arrays. We have also improved the detection sensitivity by increasing the effective electrode surface area through a polypyrrole (PPy)-electrode coating and by using a low conductivity cell suspension buffer.
Bibliography Type:	Description: (Last Updated: 08/30/2018)
Articles in Peer-reviewed Journals	Li N, Ho CM. "Patterning functional proteins with high selectivity for biosensor applications." Journal of Laboratory Automation. 2008 Aug;13(4):237-42. http://dx.doi.org/10.1016/j.jala.2008.04.001 , Aug-2008
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