Protein Analysis Using Mini-Flow Cytometer

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The Vision for Exploration calls for research into human adaptation to long-term spaceflight. For crew monitoring, there is a lack of advanced clinical analysis instrumentation. Therefore, we have considerable interest in analyzing cells, cell surface markers, and proteins expressed in biological fluids (saliva, blood, urine) on orbit. Information obtained on orbit provides researchers with instant feedback, allowing for optimization of medical intervention in real time. In addition, analyzing a sample on orbit eliminates the need to store the sample or return it to Earth. On-orbit instrumentation should yield as much information as possible from a sample, and it should have minimal space, power, and crew requirements.

Flow cytometers can measure white blood cells, fluorescently labeled cells, allowing genomic and proteomic studies, viability, apoptosis, cell cycle, and a variety of other assays to be performed. In the Guava flow cytometer, cells are sorted into a column without sheath fluid by the flow cell, and a laser (488 nm and/or 532 nm) interrogates the cells as they pass through the illumination volume. Two photomultiplier detectors with fixed-wavelength filters detect fluorescence from the cell.

While detection and measurement of live cell fluorescence is possible, the instrument's capabilities were broadened by measuring fluorescent-labeled microspheres bound to proteins of interest. In microbead assays, a set of beads is encoded using a fluorescent dye with known concentration, and functionalized with an antibody tag for the protein of interest. By varying the concentration of the fluorescent dyes in the beads, multiple analyte beads can be used simultaneously, allowing several analytes to be detected within one sample. Each well is incubated with a mixture of microbeads corresponding to a range of analytes. Each analyte measured is bound to a microbead impregnated with a specific concentration of fluorescent dyes to uniquely identify the analyte bound to the bead. In this way, each bead can use the same reporter molecule for the analytical signal, while the identity of the bead (given by the concentration of the fluorescent dye of the bead itself) gives the analyte it captures. We evaluated up to six analytes simultaneously. Readout of the microbeads takes place in the flow cytometry channel, where the beads are focused and interrogated with a laser. One of the fluorescent channels (PM1) identifies the bead (analyte) and the second channel (PM2) quantifies the concentration of the analyte.

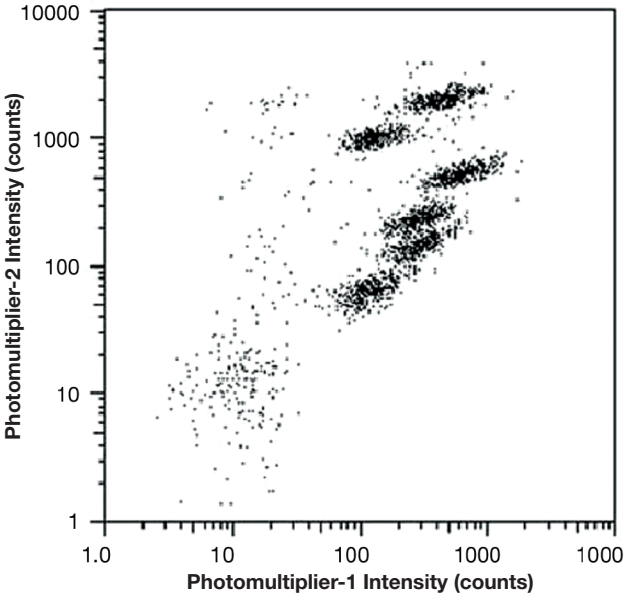

Fig. 1. *Fluorescent intensity obtained in Photomuliplier-2 (Y-axis) Red Fluorescence and Photomultiplier-1.*

Since several proteins can be measured simultaneously, the flow cytometer system can measure a panel of cytokines or other indicators of stress, disease, or injury simultaneously. Cytokines are of interest for crew health since many of these proteins are associated with disease, injury, and stress. Cytokines have been associated with cardiovascular stress and aging, and can serve as markers for the onset of many space-related medical problems. Figure 1 indicates the data simultaneously obtained from six proteins, namely: Interleukin (IL)-2; IL-4; IL-6; IL-10; Tumour necrosis factor-alpha; and Interferon. The Y-axis represents the bead fluorescence data and identifies the bead (analyte), and the X-axis represents the concentration of the analyte. Figure 2 shows that large concentration ranges of the analytes can be monitored using this technology. Using a single sample (serum, saliva, or urine) from the crew member, multiple analytes can simultaneously be measured.

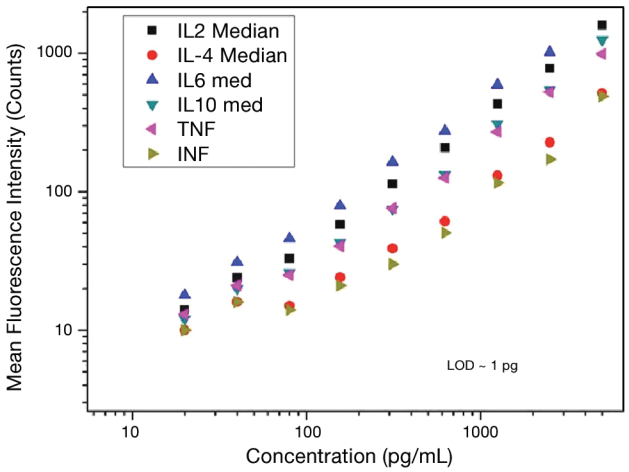

Fig. 2. *Fluorescent intensities of six proteins at the concentrations range between 20 picogram/ml and 40 nanogram/ml.*