Hyperspectral Cytometry at the Single-Cell Level Using a 32-Channel Photodetector : preliminary results on bacteria

Gérald Grégori*†[1], Bartek Rajwa[2], Valery Patsekin[3], Kathy Ragheb[4], Cheryl Holdman[5], James Jones[6], and Paul Robinson[7]

[1]Laboratoire de Microbiologie, Géochimie et Ecologie Marines (LMGEM) – Université de la Méditerranée – 163 avenue de Luminy, Case 901, Batiment TPR1, France

[2]Purdue University Cytometry Laboratories (PUCL) – Bindley Bioscience Center 1203 West State Street Discovery Park, Purdue University West Lafayette, IN 47907-2057, United States

[3]Purdue University Cytometry Laboratories (PUCL) – Bindley Bioscience Center 1203 West State Street Discovery Park, Purdue University West Lafayette, IN 47907-2057, United States

[4]Purdue University Cytometry Laboratories (PUCL) – Bindley Bioscience Center 1203 West State Street Discovery Park, Purdue University West Lafayette, IN 47907-2057, United States

[5]Purdue University Cytometry Laboratories (PUCL) – Bindley Bioscience Center 1203 West State Street Discovery Park, Purdue University West Lafayette, IN 47907-2057, United States

[6]Weldon School of Biomedical Engineering – Purdue University, West Lafayette, Indiana 47907, United States

[7]Purdue University Cytometry Laboratories (PUCL) – Bindley Bioscience Center 1203 West State Street Discovery Park, Purdue University West Lafayette, IN 47907-2057, United States

Abstract

Despite recent progress in cell-analysis technology, rapid classification of cells remains a very difficult task. Among the techniques available, flow cytometry (FCM) is considered especially powerful, because it is able to perform multiparametric analyses of single biological particles at a high flow rate (up to several thousand particles per second). Moreover, FCM is nondestructive, and flow cytometric analysis can be performed on live cells. The current limit for simultaneously detectable fluorescence signals in FCM is around 8–15 depending upon the instrument. Obtaining multiparametric measurements is a very complex task, and the necessity for fluorescence spectral overlap compensation creates a number of additional difficulties to solve. Further, to obtain well separated single spectral bands a very complex set of optical filters is required. This study describes the key components and principles involved in building a next-generation flow cytometer based on a 32-channel PMT array detector, a phase-volume holographic grating, and a fast electronic board. The system is capable of full-spectral data collection and spectral analysis at the single-cell level. First attempts on microbes (bacteria) have been performed and have clearly shown that sensitivity is high enough to extend application of this new device to microbes. Preliminary results will be presented.

*Speaker

†Corresponding author: gerald.gregori@univmed.fr

Keywords: Hyperspectral cytometry, 32 channel, PMT, single cell