

Paul Wallace, Professor Oncology and Director Department of Flow & Image Cytometry at Roswell Park Comprehensive Cancer Center, is recognized for his expertise in clinical flow cytometry with a strong background in immunology and research interests in antigen processing and presentation. He is Past President of the International Society for the Advancement of Cytometry (ISAC) a past Councilor of the International Clinical Cytometry Society (ICCS) and this year's recipient of their Wallace H. Coulter award for lifetime achievement in clinical cytometry. Flow and Image Cytometry at Roswell Park offers a strong combination of clinical and research missions and under Dr. Wallace's direction actively works to build translational synergies between them. The clinical laboratory is focused on the diagnosis and monitoring of patients with leukemia and lymphoma with a recent emphasis on minimal residual disease. Before joining Roswell Park, Dr. Wallace was an Assistant Professor of Immunology at Dartmouth Medical School, Lebanon, NH (1993-2003); a cofounder of Zynaxis Cell Science, Inc., Malvern PA (1988-1991) the company that developed the PKH tracking dyes, and supervisor of Microbiology, Immunology, Serology, and Flow Cytometry at SmithKline Clinical Laboratories. He obtained his PhD from the Medical College of Pennsylvania in 1993 and his Masters from Idaho State University in 1979.



Tracking cell proliferation

The number of fluorescent dyes commercially available for cell tracking, and the subset useful for proliferation monitoring continues to expand rapidly. Dyes of one class, referred to as "protein dyes", react with proteins to form a covalent bond. Dyes of the other class, referred to as "membrane dyes", stably intercalate into the lipid bilayer of cell membranes via strong hydrophobic associations. They can be used to label almost any cell and have enabled biologists monitor a wide variety of tumor and immune cell functions including: migration and adhesion; proliferation of stem and progenitor cells; differentiation and growth control; mechanisms of antigen presentation; and interactions of effector and regulatory cells with each other and with tumor cells. Participants of this lab will learn how to stain cells with both protein and lipophilic dyes and how to correctly analyze and interpret the resulting data.

mRNA Cytometry. Until recently our ability to detect mRNA gene transcripts by flow cytometry has been limited. The relatively new branched DNA technique amplifies signal from a single mRNA species several thousand fold permitting the detection of as few as 5 copies of mRNA within a cell. The technique is compatible with antibody-based targeting allowing mRNA detection within specific subpopulations within a mixed population of cells. Examples in this lab will emphasize monocyte activation with LPS and the kinetics of cytokine mRNA and protein expression.

Relevant Literature:

1. Tario JD, Jr., Conway AN, Muirhead KA, Wallace PK. Monitoring Cell Proliferation by Dye Dilution: Considerations for Probe Selection. *Methods Mol Biol* 2018;1678:249-299.
2. Soh KT, Wallace PK. RNA Flow Cytometry Using the Branched DNA Technique. *Methods Mol Biol* 2018;1678:49-77.
3. Soh KT, Tario JD, Jr., Colligan S, Maguire O, Pan D, Minderman H, Wallace PK. Simultaneous, Single-Cell Measurement of Messenger RNA, Cell Surface Proteins, and Intracellular Proteins. *Curr Protoc Cytom* 2016;75:7 45 1-7 45 33.
4. Tario JD, Jr., Humphrey K, Bantly AD, Muirhead KA, Moore JS, Wallace PK. Optimized Staining and Proliferation Modeling Methods for Cell Division Monitoring using Cell Tracking Dyes. *J Vis Exp* 2012.
5. Tario JD, Jr., Muirhead KA, Pan D, Munson ME, Wallace PK. Tracking immune cell proliferation and cytotoxic potential using flow cytometry. In: Hawley TS, Hawley RG, editors. *Methods Mol Biol*. Volume 699. New York: Humana Press; 2011. p 119-164.

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