

Workshop IV: Immunophenotyping in Fine Needle Aspirates Using Laser Scanning Cytometry

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Characterization of cell surface antigens of hematolymphoid cells is central to the diagnosis of lymphoma and leukemia and constitutes a large component of the work of most flow cytometry laboratories. Immunophenotyping by laser scanning cytometry (LSC) offers the following advantages:

- Requires fewer cells
- Has a lower cost per reportable result
- Is easily instituted in laboratories that only need to immunophenotype samples on an intermittent basis (i.e., instrument is extremely stable without daily maintenance or use)
- Can be accomplished by a simple standardized assay (once set up) requiring few decisions on the part of the technologist, applicable to laboratories where there is limited expertise in immunophenotyping.
- Leaves cells *in situ* after data acquisition so that they can be re-analyzed to clarify results or re-stained with other antibodies with or without permeabilization.
- Allows recovery of DNA from the sample after analysis (without cell sorting) and permits use of cells for FISH
- Easily permits cell immunophenotype to be linked to cell morphology.

In this session we will consider each of the components of the process of immunophenotyping by LSC. All participants will perform some of the components of the process using non-biological materials and will analyze a pre-prepared sample on the iCys. We will demonstrate the following:

- Acquisition of “cytologic” samples by needle aspiration and use of the material so obtained to make either a cell suspension (surface marker immunophenotyping) or cell (paraffin) block for immunohistochemistry.
- Cell surface marker immunophenotyping by LSC, using the Clatch Slide and method as the prototype.
- Immunophenotyping on paraffin sections or cytospin preparations stained with chromogenic dyes, illustrating the process of spectral deconvolution.

Participants will learn to:

- 1) Define an analysis protocol based on appropriate choices of antibodies and fluorochromes.
- 2) Prepare the sample to satisfy the requirements or constraints of the analytical system.
- 3) Set up and execute the defined analysis protocol.
- 4) Assess the quality of the sample preparation and the acquired data.