# Unifying image analysis and flow cytometry software to analyse and present multi-parameter image data for quantitative pathology.

**Tam Nguyen[1], Clay Winterford[2], Amanda Stanley[1], Crystal Chang[2], Andrew Masel[2], Grace Chojnowski[1], and Nigel Waterhouse[1].**

**Flow Cytometry and Imaging, and Histology QIMR Berghofer Medical Research Institute, 300 Herston Road, Herston, Brisbane, QLD 4006. Australia.**

A recent surge in immunotherapy has raised interest in multi-parameter imaging to quantitate immune cells, tumour cells, and other cells in distinct areas of tissues (e.g. inside and outside the tumour margins). Multi-parameter flow cytometry is commonly used for immunophenotyping and quantitation of cells in peripheral blood, and is well understood in clinical settings. However flow cytometry cannot be used to quantitate cells in solid tissues, and analysis of tissue pathology in clinical settings remains largely restricted to measuring histological parameters (size of pathology) or single colours (e.g Dab staining). Immunophenotyping and quantitation of immune cells using multi-parameter imaging of solid tissue, similar to that obtained in liquid biopsies by flow cytometry, is likely to be essential for evaluating the effects and effectiveness of immune therapies.

Approaches for analysing multi-parameter flow cytometry and fluorescence imaging remain strikingly distinct despite their use of similar technologies for data acquisition (e.g. fluorescent dyes, lasers, filters, and PMTs/detectors etc). This may be due to the different size of data sets (100s vs 1000s of events) or different parameters being measured (such as counting overall fluorescence of cells vs analysing location of a fluorophore in the cell or tissue). We have therefore been evaluating the use of InForm, which is proprietary software for analysis of images obtained using the Perkin Elmer Vectra Quantitative Imaging System, together with FCS Express which is commonly used for flow cytometry. The data generated can now be easily merged from multiple images to generate large numbers of events for analysis. The data can be analysed using standard flow cytometry strategies or mined using more complex flow cytometry algorithms such as tSNE, and then back gated to the image to locate any cells of interest. The images can also be used as part of the gating strategy to segment the tissue for analysis.

The benefits of this strategy are that spectral un-mixing can be used to accurately generate and analyse complex multi-parameter data in different tissue areas. The data can then be analysed in a way that is easily understood by immunology researchers and clinicians who are familiar with results produced from flow cytometry software.