

Workshop I: Cytome Diagnostics: Characterization of DNA damage and cytome biomarkers in human buccal cells using quantitative imaging cytometry

Instructors: Wayne Leifert, Maxime François, Michael Fenech
Commonwealth Scientific and Industrial Research Organisation (CSIRO)
Food and Nutritional Sciences, Nutritional Genomics & Genome Health
Diagnostics, Adelaide, Australia

The buccal mucosa is a stratified squamous epithelium consisting of four distinct layers. Human buccal cells are easily accessible in a minimally invasive manner, and exhibit cytological and nuclear morphologies that may be indicative of individual health status. With increasing age, the cell renewal process becomes less efficient due to accumulated DNA damage and subsequent cell death, which may result in a decreased thickness of the epidermis. DNA damage events in buccal mucosa can be measured by scoring abnormal nuclear features such as micronuclei which arise from broken chromosomes, nuclear buds reflecting DNA amplification events or binucleated cells which may be indicative of cytokinesis-arrest due to aneuploidy status¹. These biomarkers may also be used as bio-dosimeters of exposure to genotoxins such as ionising radiation and are associated with a higher risk for cancer and accelerated aging syndromes such as Down's syndrome and Alzheimer's disease¹⁻³.

Laser scanning cytometry (LSC) combines the properties and advantages of flow cytometry, quantitative imaging and immunohistochemistry with high-content, multi-color fluorescence analysis, and can be used to identify specific cells in a heterogeneous population as well as to score unique molecular events within them. Additionally, LSC provides intracellular and within-tissue localization of specific protein targets, generating data that offers the advantages of high-throughput analysis without sample loss. LSC quantifies the fluorescence intensity of localized molecular targets within nuclear and cytoplasmic structures of cells while maintaining the morphologic features of the examined tissue. For these reasons, LSC can be used successfully to study cellular and nuclear anomalies and DNA damage events in buccal cells.

In this workshop we will demonstrate specific technical details involved in buccal cell sample collection, slide preparation, optimal protocol design for image acquisition and data analysis of events measured in these cells by LSC. Real-time scanning on the iCys[®] Imaging Cytometer will be performed as part of the workshop. Buccal cell slides from Alzheimer cases and age- and gender-matched controls will be analysed to identify and quantify buccal cell subtypes, nuclear DNA content, and DNA-damage events such as micronuclei.

1. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M. Buccal micronucleus cytome assay. *Nat Protoc.* 2009;4(6):825-37.
2. Thomas P, Hecker J, Faunt J, Fenech M. Buccal micronucleus cytome biomarkers may be associated with Alzheimer's disease. *Mutagenesis.* 2007 Nov;22(6):371-9.
3. Thomas P, Harvey S, Gruner T, Fenech M. The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls. *Mutat Res.* 2008 Feb 1;638(1-2):37-47.