

Workshop: Tissue Applications

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David Krull is Principal Scientist at GlaxoSmithKline where he manages a molecular pathology lab within Safety Assessment, developing automated immunohistochemical and immunofluorescent methods for evaluation of tissues from toxicology studies and performing a variety of chromagenic and fluorescent analyses. Prior to joining GSK in 2004, he spent 15 years at Pfizer in Groton, CT.

Abstract

This workshop is designed to introduce the audience to whole tissue analysis using laser scanning cytometry (LSC). Topics covered will include experimental design, tissue sample preparation, multiplexing of both chromagenic and fluorescent dyes, building an LSC protocol for tissue analysis, and different strategies for LSC data analysis in histocytometry.

We will use heart and kidney sections from rats treated with doxorubicin to illustrate these principles. Doxorubicin is a chemotherapeutic agent that has been shown to cause cardiotoxicity and can potentially damage other vital organs. To study the toxic effects of doxorubicin, heart and kidney tissues were collected from doxorubicin-dosed and control rats and embedded in paraffin. Five- μ m tissue sections were cut, and antigens of interest were immuno-labeled either chromatically or fluorescently. In the chromatically-labeled samples, aquaporin 3 was stained with Warp Red, Kim1 with DAB, SMA with Vina Green, and the nuclei were counterstained with hematoxylin. In the fluorescently-labeled samples, aquaporin 3 was stained with Alexa 647, Kim1 with Alexa 555, SMA with Alexa 488, and the nuclei were counterstained with DAPI. Different LSC data analysis strategies will be applied to quantify the expression and localization of the antigens in order to understand the toxicity of doxorubicin.