

Purpose: To propose a new objective method for deformable coregistration of multimodality images acquired with digital autoradiography (DAR) and microscopy in the context of PET tracer histopathological validation. To analyze the spatial concordance between the uptake pattern of 18F-fluorothymidine (FLT) as imaged with DAR and the distribution of cell proliferation as revealed by immunofluorescence microscopy imaging.

Methods: Tumor-bearing mice were injected with FLT and other markers including bromodeoxyuridine (cell proliferation). After sacrifice, tumors were excised, frozen and sectioned. Multiple stacks of sequential 8 $\mu$ m sections were collected from each tumor. Selected sections were used for DAR to image FLT uptake distribution. Adjacent sections were used to acquire histopathological data. To correct for imperfections of the tissue cutting and collection, all images were deformably coregistered to the FLT DAR image based on biological images of tumor blood flow (Hoechst) that was acquired from each tissue section used. For each FLT DAR – cell proliferation microscopy image pair, object-based analysis was conducted, including overlap and relative operating characteristics (ROC) analysis.

Results: Total registration error of proposed coregistration method was 44.86 $\mu$ m. This supersedes current rigid registration methods with reported errors of 100-200 $\mu$ m. In tumors with well-compartmentalized functional aspects, area under the ROC curve (AUCroc= 0.7) indicated FLT DAR image thresholding as an accurate method of detecting cell proliferation. For these tumors, Dice overlap index indicated maximum detection rates at thresholds between 20% and 40% of the maximum DAR intensity. For the tumors characterized by more heterogeneous distribution of cell proliferation across the tumor section, FLT DAR image thresholding could not predict cell proliferation beyond random chance.

Conclusions: We developed a comprehensive method of obtaining and analyzing coregistered images of cell proliferation markers and intratumoral uptake of FLT. Tumor microenvironment heterogeneity is a significant factor affecting the utility of FLT for imaging cell proliferation.