

**Purpose:** Develop an objective methodology for deformable coregistration of digital-autoradiography (DAR) and microscopy images acquired from sequential tissue sections, in the context of histopathological PET tracer validation.

**Method and Materials:** Tumor-bearing mice were injected with  $^{18}\text{F}$ -FLT and other markers including Hoechst (blood flow surrogate). After sacrifice, tumors were excised, frozen and sectioned. Multiple stacks of sequential  $8\mu\text{m}$  sections were collected from each tumor. Selected sections (reference) were used for DAR to image  $^{18}\text{F}$ -FLT uptake distribution. Sections adjacent to references were used to acquire histopathological data. Hoechst images were acquired for each section. To correct for deformations induced by tissue processing and image acquisition, Hoechst image of each non-reference section was warped onto the reference Hoechst using elastic registration. This transformation was then applied to other images acquired from the same tissue section. This way, all microscopy images were coregistered to the reference Hoechst image. The Hoechst to DAR image registration was done using rigid point set registration based on external markers visible in both images.

**Results:** Registration error was evaluated using sets of independent landmarks. The mean error of Hoechst to DAR (same section) registration was  $30.8\pm 20.1\mu\text{m}$ . The error of Hoechst-based deformable registration of histopathological images was  $23.1\pm 17.9\mu\text{m}$ . Total registration error was  $44.86\mu\text{m}$ . This supersedes current rigid registration methods with reported errors of  $100\text{-}200\mu\text{m}$ .

**Conclusion:** Deformable registration of DAR and histopathology images acquired from sequential sections is feasible and accurate when performed using corresponding Hoechst images.

Continuing Education: PET tracer validation, image-guided radiotherapy, deformable registration, digital autoradiography, immunofluorescent microscopy