Validation of fluorescence-mediated tomography combined with micro-CT: a longitudinal *in vivo* comparison to PET-imaging

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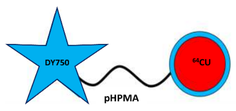
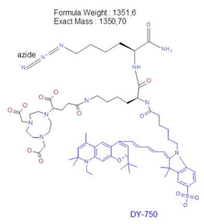
Introduction

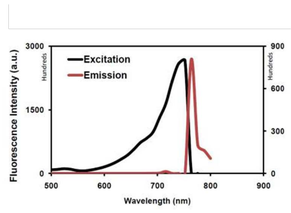
Fluorescence tomography (FLT), also called FMT (Fluorescence Mediated Tomography), is a fast-developing imaging technology that bears a high potential for routine preclinical organ biodistribution data assessment. However, these information are instead acquired by examination of excised organs different time points or using radiolabeling and nuclear imaging modalities (PET/SPECT). Although, FLT-imaging offers considerable advantages such as longitudinal imaging within the same animal at several time points without the burden of radioactivity, the accuracy of CT-guided FLT (Hybrid FLT/CT) quantification has not been determined before. Hence, in our study we would like to evidence the accurate detection and quantification of fluorescence signals in tumors and organs in a comparable range to PET.

Material and Methods

Probe synthesis

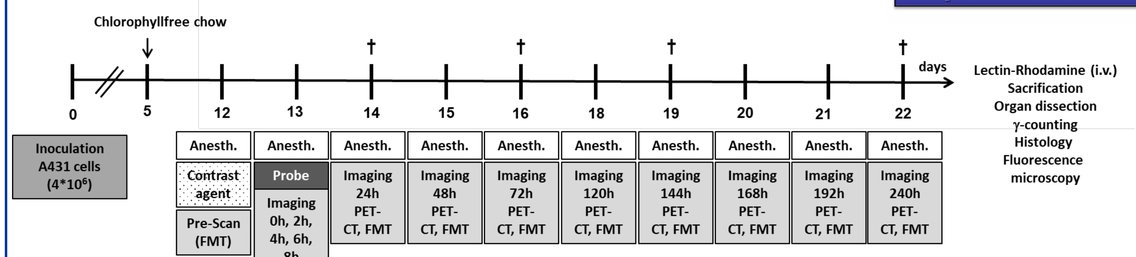






The dual-modality probe **DY750-HPMA-^{64}Cu** is based on a pHPMA and labelled with NIRF-dye DY750 for FLT imaging and a functional azide group for coupling a chelator (NODA-GA) and binding the radioactive tracer ^{64}Cu for PET imaging. Chemical structure and spectrophotometric analysis of DY750-HPMA-^{64}Cu are illustrated.

Experimental setup (*in vivo* and *ex vivo*)



After s.c. A431-tumor inoculation and growth, a contrast agent to enhance spleen and liver CT contrast was injected and pre-scans were acquired. Directly after synthesis, DY750-HPMA-^{64}Cu, 100 pmol dye/ 20 MBq, was i.v. injected. Imaging in PET-CT and FLT was performed directly after injection of the probe until 240h post-injection at the illustrated time points. *Ex vivo* 2D fluorescence reflectance and γ-counting analysis of excised organs were acquired 24h,72h,144h, and 240h post-injection.

Results

Detection of DY750-HPMA-^{64}Cu by FLT and PET

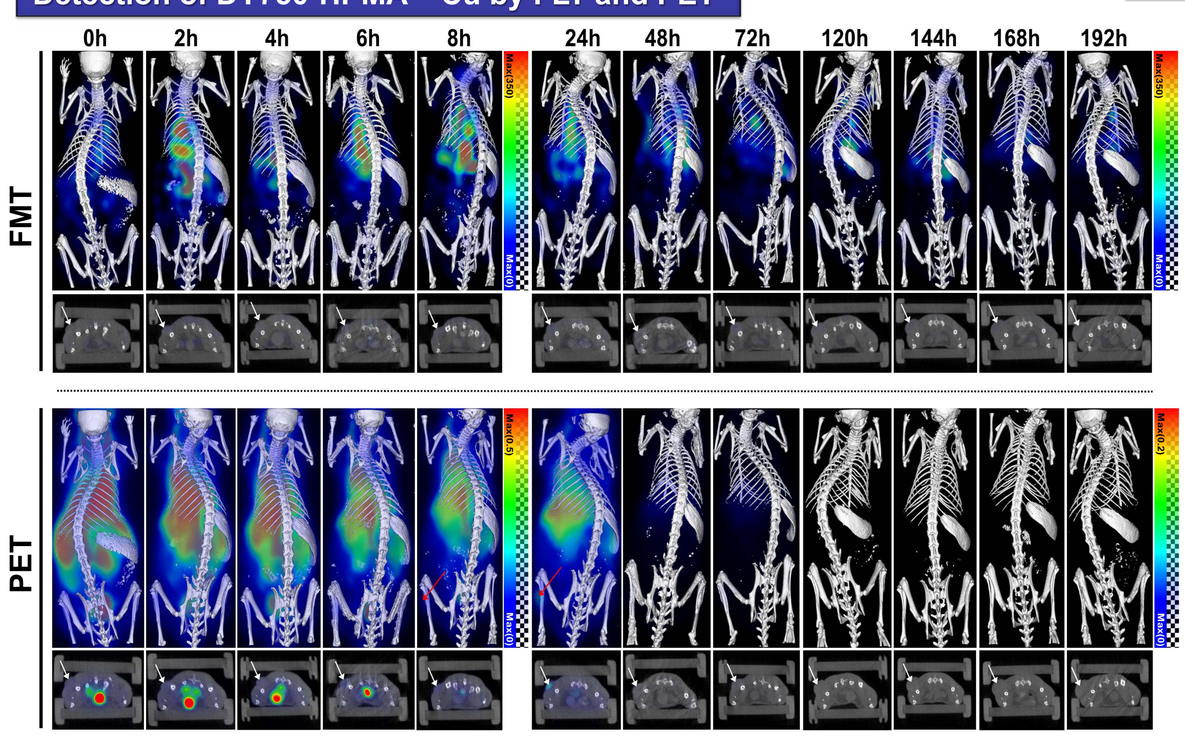
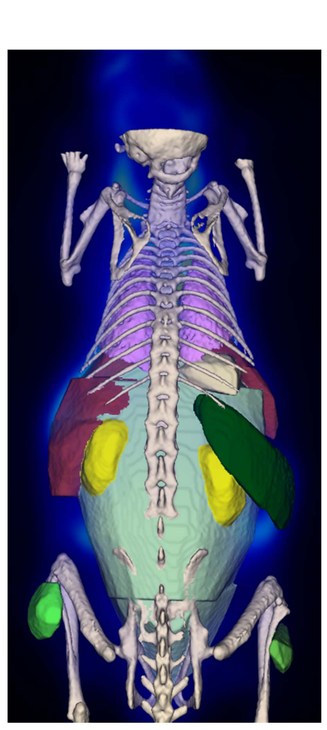


Figure 3: Longitudinal comparison of fluorescence and PET intensities (3D whole-body images and axial plane) evidence the detection of DY750-HPMA-^{64}Cu signals by both FLT and PET. Fluorescence signals are depicted in the upper panel, PET signals in the lower panel; both with CT-underlay and colour-coded. Tumors are highlighted by arrows (axial plane). High PET intensities in tumors are emphasised by red arrows (3D images).

Quantification of fluorescence and PET intensities



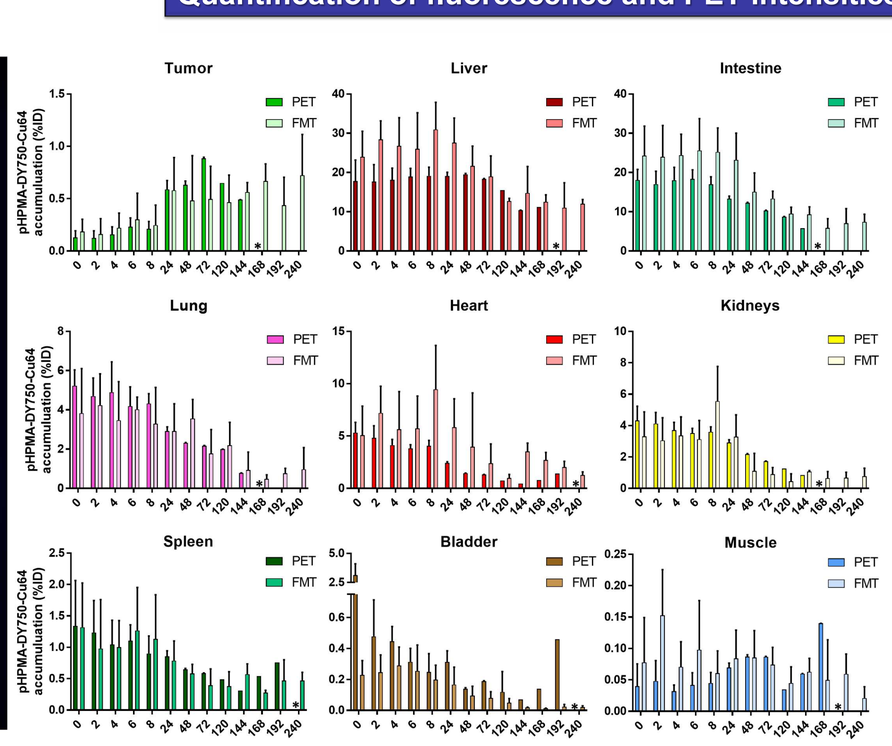


Figure 4: Quantification of fluorescence and PET intensities (half-life time corrected) exhibited similar percentage injected dose (%ID) in organs and tumor. At later time points, the PET signals decreased stronger due to the decay of the radioactive tracer and were not detectable in most organs after 144h, marked by *, whereas the fluorescence signals were still detectable. Along all time points, the variations in the FLT values were higher in comparison to PET.

2D Fluorescence reflectance images

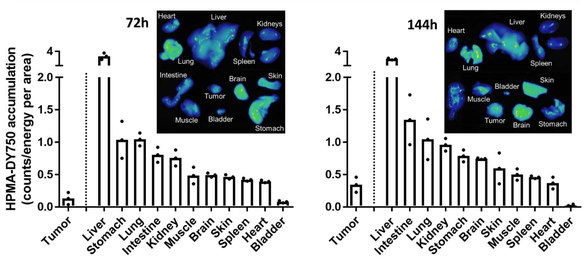
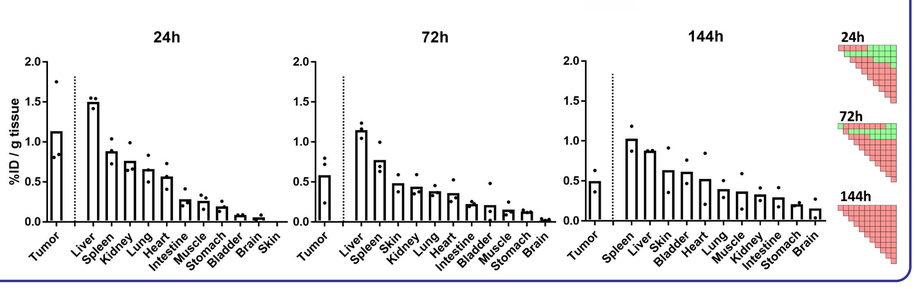


Figure 5: *Ex vivo* organ biodistribution.
Left: 2D fluorescence reflectance images and quantification of the fluorescence intensities (un-normalized) 72h, 144h, and 240h post-injection.
Right: γ-counting analysis of the ^{64}Cu-signal of selected organs 24h, 72h, and 144h post-injection. Significances ($p \leq 0.05$) are indicated by green boxes of the pair-wise significance matrices.

γ-counting analysis



Discussion

The dual-modality probe DY750-HPMA-^{64}Cu was detectable by both FLT and PET imaging. By quantification of the fluorescence signals and direct comparison to PET, a gold standard in non-invasive biodistribution assessment, we were able to prove the robustness of FLT quantification. Thus, we demonstrated the potential applicability of hybrid FLT-CT-imaging (MILabs BV, Utrecht, the Netherlands) for accurate biodistribution assessment of intravenous injected tracer with considerable advantages.

References

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Acknowledgements

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