Bimodal and Multiscale ^{19}F-MRI-Raman Detection of Fluorinated Nanoparticles for the *in-situ* Detection of Tumoral Lesions

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The failure to accurately detect tumoral margins during surgery is responsible for the need for a second surgical intervention in 3-40% of cases, associated with worse prognosis, patient's discomfort and increased costs for the healthcare systems. The possibility of combining contrast agent-based tumor-targeting MRI imaging strategies with techniques enabling the intraoperative *in-situ* detection of the very same contrast agent would allow the accurate identification of the tumoral lesion, thereby improving surgical timing and reducing recurrence.

Here we present a bimodal and multiscale approach for the detection of fluorinated nanoparticles (FNPs) *in-vivo* by ^{19}F-MRI imaging at whole body scale, and for the subsequent *in situ* detection of the same nanoparticles at their site of accumulation (e.g., after tumor targeting) using Raman spectroscopy, with sub-millimeter accuracy.

FNPs, made starting from PLGA nanocarriers loaded with PERFECTA, a perfluorinated molecular probe characterized by exceptional ^{19}F MRI sensitivity and distinct Raman signatures [1], were first stabilized with either polyvinyl alcohol (PVA) or sodium cholate (NaC). *In vitro* Raman imaging was used to monitor FNPs uptake in NIH/3T3 murine fibroblasts. Measurements were carried out with a home-built Raman micro-spectroscopy system (660 nm excitation wavelength), enabling hyperspectral imaging throughout the cell volume (step size = 0.7 µm; pixel dwell time = 750 ms). Raman imaging revealed the time-dependent internalization of FNPs, allowing the separate tracking of their fate, and, furthermore, the eventual degradation of both the fluorinated core and the PLGA-based carrier, independently, thanks to specific Raman signals of these two molecules.

Subsequently, FNPs were administered, intratumorally or intravenously, in BALB/c mice bearing orthotopic mammary tumors and detected by *in-vivo* ^{19}F-MRI. After MRI experiments, Raman detection of FNPs was confirmed on intact tumors both *in situ* (within the euthanized animal) and *ex vivo* after surgical excision, on high-MRI signal sites. Scattered photons coming from depths of up to 2.5 mm were collected using both SORS and micro-SORS strategies, based on 785 nm excitation. Further analysis will be conducted on tissue sections obtained from intravenously injected tumor-bearing mice to investigate the depth-dependent distribution and internalization of PERFECTA within the tumor microenvironment.

Our bimodal and multiscale approach opens the door to future *in vivo* investigations: PERFECTA-based FNPs could be used to identify tumor localization via ^{19}F-MRI, followed by *in situ* and *in vivo* characterization of tumor margins using portable SORS devices equipped with fiber-optic probes, supporting intraoperative detection of residual cancer cells.

References

*[1] Chirizzi, Cristina, et al. "A bioorthogonal probe for multiscale imaging by 19F-MRI and Raman Microscopy: from whole body to single cells". (**2021**)*