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“In vivo” Field-Cycling relaxometry of tumours. Evidence for the role of the intracellular water lifetime as tumour biomarker.

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Synopsis
This work aims at developing an innovative diagnostic strategy, based on the “in vivo” measurements of longitudinal relaxation times at low and ultralow magnetic fields with Fast Field Cycling PFG-NMR to obtain quantitative information on tumour microstructural potential, due to different water content and mobility, that is invisible to standard MRI. Preliminary results show that the endogenous contrast between normal and diseased tissue, due to differences in T1, is much greater at low field and the shape of the relaxation dispersion profiles may be used as a reporter of the molecular dynamical processes, biomarkers of the disease grade.

Introduction
Magnetic resonance imaging (MRI) has had a wide role in the field of oncology over the last several decades. The MRI diagnostic power arises basically from the differences in the longitudinal and transverse proton relaxation times between healthy and pathological tissues. However, at the high field strength of the currently available scanners (1.5T), changes in T1 do not appear sensitive enough to report on peculiar aspects of the tumour stage. However, there is an offsite opinion that, at low magnetic field strength, the marked increase of T1 observed in biological tissues might be beneficial to improve the MRI diagnostic potential in tumour phenotyping (1-8). Herein it is shown that the in vivo acquisition of 1H nuclear magnetic resonance dispersion (NMRD) profiles from 0.01 to 10 MHz, proton Larmor frequency fully supports this expectation as the observed T1 at low magnetic fields (0.2 T) “in vivo”, an experimental animal model, allow a clear discrimination between tumours endowed with different metastatic potential.

Methods
1H NMRD profiles are acquired on Fast Field Cycling relaxometers able to switch the magnetic field between different field-strengths, during the measurement procedure. A field cycle overcomes the problem of the low sensitivity at low magnetic fields and allows rapid acquisition. The currently available relaxometers are designed for liquid samples measurements. Therefore, in order to host a mouse, a commercially available relaxometer had to be modified with the implementation of a 40 mm 0.3T Field Cycling magnet and a dedicated 11 mm external detection coil placed around the mouse’s leg (Fig. 1), where is located the tumour xenograft prepared with mouse mammary adenocarcinoma cells, namely TAK 0.1T, 168Fam injected in the leg muscle. These cells display different characteristics in terms of aggressiveness and metastatic potential (i.e. 168Fam vs 1A4).4

Results
A simple inspection on the obtained NMRD profiles, allow us to clearly distinguish healthy from tumour tissues at the tumour tissues invariably show lower T1 values in particular at low magnetic field strengths. The T1 elongation was different for the three tumour models (Fig. 2) essentially reflecting their different aggressiveness. To better understand this behavior one needs to recall that each T1 in the profile represents an average of the T1 values of water molecules in different tissue microenvironments; the extra cellular spaces (ECS), the extracellular compartment characterized by a more restricted water molecules mobility (ECS-T), being ECS and ECS the respective volume fractions. Water molecules can cross the barriers between the two compartments thus contributing to mixing, at some extent, the relaxation rates of the intracellular and extracellular compartments. Therefore, the water exchange rate from the ECS to the extracellular space have to be introduced in the model used for the fitting of the T1 decay. According to this incompartmental model, the time evolution of T1 is dependent on the relationship between the absolute values of the relaxation terms (k1T1-ECS, k1T1-ECS), and an “exchange” term (k1T1-ECS), also defined as the NMR “exchanger” speed. (1-11) The most striking result from the fitting procedure is the large variation of k1T1-ECS to indicate that the water exchange rate across the plasma membrane is a distinctive hallmark that differentiates most representative of healthy cells and tumour cells. This finding clearly reports on the peculiar characteristics of the given tumour cell type. In fact, the intracellular water lifetime (n=10) values obtained for three breast cancer cell lines are inversely proportional to their metastatic potential (4T1A vs 1A4).4

Discussion
The herein reported results open new horizons for the non-invasive evaluation of tumour microstructural phenotypes, by providing useful information related to the tumour microstructural propperty. The simultaneous fitting of T1 over an extended range of magnetic field strengths allows attaining a good estimation of k1T1-ECS crucial to set function. Cell water content and volume are related to the concentration of intracellular osmotic active compounds as well as to the extracellular toxicity. Ion pumps or active transporters (sodium-potassium-transporters) located in the presence of a pathological state, can be exploited as a specific reporter of the cellular state. k1T1-ECS on the activities of a number of transporters (Na+/K+ ATPase and collectively it may represent an hallmark of tumour cells aggressiveness.

Conclusion
We may conclude that the measurement of biofilm permeability provides insights for more specific assessment of the pathophysiological status of tumours as well as other biological tissues. Despite the ICT-CNMM instrumentation is not embedded with spatial resolution, fundamental knowledge obtained in this study can open the route to new diagnostic horizons in oncology until now uncharted.

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References

Figures
Photographs of the Fast Field Cycling NMR relaxometer with the introduced modifications for the acquisition of in vivo NMRD profile of tissue portions of the mouse leg.

Observed relaxation rates as a function of the magnetic field strength (NMRD profile of mouse leg tissue; 10-15 days after the intramuscular inoculation of 4T1 cells (green symbols). TSA (red symbols) and 168Fam (black symbols))

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