# LOW FIELD NMR RELAXOMETRY FOR INTRAOPERATIVE TUMOUR MARGIN ASSESSMENT IN BREAST-CONSERVING SURGERY

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**INTRO** As breast-conserving surgery is routinely applied for treatment of **breast cancer** (the most common form of cancer for women), the need for new technology to improve intraoperative **margin assessment** has become increasingly important [1]. The current gold standard for margin classification is microscopic pathologic evaluation of the excised tissue following formalin fixation, paraffin embedding, and Hematoxylin & Eosin staining. The method is robust and accurate, but it is time-consuming. Several intraoperative margin assessment strategies (flow cytometry, fluorescent dye-based method, mass spectroscopy, micro-elastography, UltraSound,…) have been proposed to reduce the need for re-excision, but they all have significant clinical and technical limitations that have hampered widespread adoption [2,3]. Recently, a mobile MRI scanner (ClearSight™ system), that exploits diffusion-weighted (DWI)-MRI, has been proposed for tumour margin. The method show high sensitivity, specificity, and accuracy [4] but requires complex and expensive technology. **Here, the potential of fast field cycling $^1$H-NMR relaxometry as a new tool for intraoperative margin assessment was evaluated.**

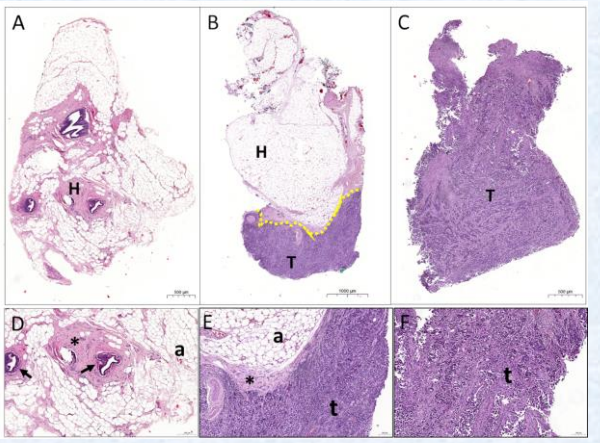
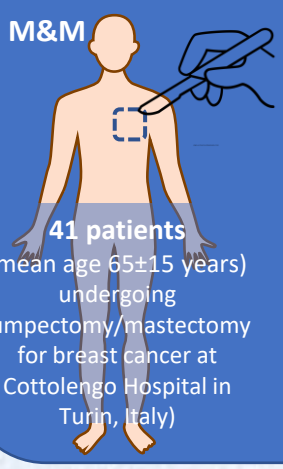



**Figure 1.** Representative images of healthy (A), mix (B) and tumour (C) breast tissue samples stained by H&E. (D, E, F) magnification of (A, B, C) respectively. Arrows indicate normal mammary ducts, asterisks indicate fibrous tissue, "a" indicate adipocytes and "t" tumour cells

**M&M**



**41 patients** (mean age 65±15 years) undergoing lumpectomy/mastectomy for breast cancer at Cottolengo Hospital in Turin, Italy)

**104** freshly excised **breast tissue samples** (weight between 16-114 mg)
Each sample was taken from different areas of the surgical specimen by the pathologist, following an on-site, real-time, macropathology gross examination, to obtain a variety of tissue types.

104 samples
H Healthy (n=40) - margin
M Mix (n=43)
T Tumour (n=21) + margin

The individual tissue samples were analyzed by
- ✓ **relaxometric method**
- ✓ **histopathology evaluation (Fig. 1)**

The acquisition of proton longitudinal relaxation time ($T_1$) as a function of the magnetic field strength (NMRD profile) was performed on a Stelar SpinMaster FFC-NMR relaxometer, equipped with a signal-detection microcoil of 10 mm diameter, at 10 °C in the 0.02–1 MHz Proton Larmor Frequencies range (corresponding to $B_0$ = 0.24 mT-24 mT).

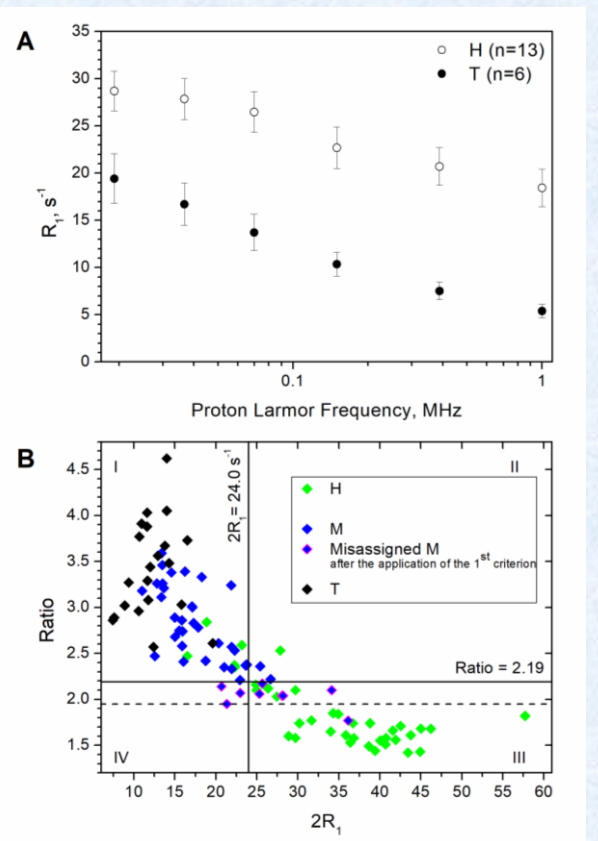



**Figure 2. A**) Comparison between typical $R_1$ NMRD profiles of healthy specimens (H, n=13) and tumour samples (T, n=6). Error bars represent the SD. **B**) The Ratio value as a function of the $2R_1$ value (see text).

**R&D** As shown in **Fig. 2A**, both H and T tissue relaxation rates increase when the magnetic field strengths decrease but the relative values and slopes of the two curves are significantly different. This finding appears to be associated, first of all, with the different water content and water mobility characteristics of the tissues [5]: tumour tissue has a protein/fat/water content that is highly altered with respect to healthy breast tissue, in which adipocytes are dominant and lipids account for up to 70-80% of tissue content. As previously reported [6,7], lipid protons relaxation rates show significatively less dispersion with the magnetic field strength in the range 0.02-10 MHz than water/protein protons. Therefore, relaxation rates of H tissues show higher values and a less pronounced dispersion with the magnetic field with respect to T tissues.
Accordingly, we defined **2 relaxometric quantifiers** that captured this peculiar behavior and allowed assessing the presence of tumour cells in a breast tissue specimen:

- **Ratio** : the ratio between the $R_1$ value measured at 0.02 MHz and 1 MHz, i.e. $R_1^{0.02MHz}/R_1^{1MHz}$
- **$2R_1$** : the sum of the $R_1$ values measured at 0.39 MHz and 1 MHz

According to the Receiver Operating Characteristic (ROC) curve analysis, the best **cutoff values** for Ratio and $2R_1$ were found to be 2.19 and 24.0 $s^{-1}$, respectively. **Fig. 2B** reports the Ratio value as a function of the $2R_1$ value for all the 104 samples. The two identified cutoff values correspond to the horizontal and vertical thick lines.

With the aim of developing a method that is at the same time rapid and accurate for the intraoperative margin assessment, we considered a **2 criteria protocol** in which the verification with the second criterion (and therefore the execution of an additional measure) is necessary only for a small number of borderline samples (**Fig. 3**). Then, only the samples having a Ratio between 1.95 ≤ Ratio ≤ 2.19 (6 H and 7 M) were considered for the second criterion ($2R_1$). The rationale relies on the observation that in this area fell misassigned M specimens containing a significant amount of adipose tissue (58.4 %+- 8.6 % (SE)) and this type of composition affects the Ratio more than the $2R_1$ parameter. In this way, the H sample assignment was confirmed and 3 of the False Negative (the samples in the quadrant IV) could now be correctly assigned.

A **sensitivity, specificity, and accuracy of 92%, 85%, and 89%,** respectively, were achieved. The relaxometric method is low cost, fast and it does not need highly specialised operators for the interpretation of the data obtained as it can be highly automatized.

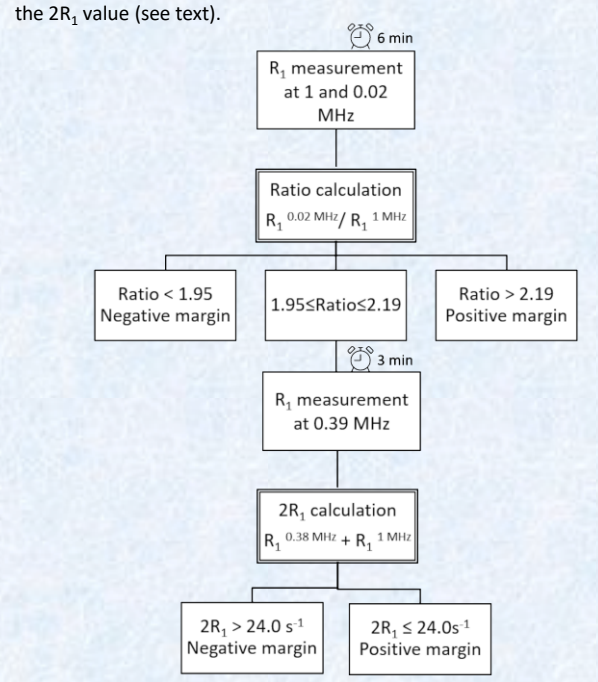



**Figure 3** The proposed protocol

**References**: [1] J. Fajdic *et al. Acta Inform. Medica* **2013**, *21*, 16; [2] B.W. Maloney *et al. J. Biomed. Opt.* **2018**, *23*, 1; [3] A.R. Pradipta *et al. Adv. Sci.* **2020**, *7*, 1901519; [4] M. Papa *et al. J. Surg. Oncol.* **2016**, *114*, 22; [5] M. R. Ruggiero *et al. Angew. Chemie Int. Ed.* **2018**, 57, 7468;[6] E. Di Gregorio *et al. Sci. Rep.* **2019**, *9;* [7] L.M. Broche *et al. Sci. Rep.* **2019**, *9*.