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 pathological 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 shows high sensitivity, specificity, and accuracy [4] but requires complex and expensive technology. Here, the potential of fast field cycling 1H-NMR relaxometry as a new tool for intraoperative margin assessment was evaluated.

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

The acquisition of proton longitudinal relaxation time (T1) as a function of the magnetic field strength (NMRD profile) was performed on a Starlab SpinMaster M/IC-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 B0 = 0.24 mT-24 mT).

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 significantly 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 R1 value measured at 0.02 MHz and 1 MHz, i.e. $R1_{0.02MHz}/R1_{1MHz}$
• 2R1: the sum of the R1 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 2R1 were found to be 2.19 and 24.0 s⁻¹, respectively. Fig. 2B reports the Ratio value as a function of the 2R1 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 (2R1). 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 2R1 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 does not need highly specialised operators for the data analysis obtained as it can be highly automated.