Review

The Role of pH in Breast Cancer Screening

Dyutika Kantamneni *, Saumya Gurbani, Mary Salvatore

Posted Date: 7 November 2025

doi: 10.20944/preprints202511.0512.v1

Keywords: breast neoplasms; early detection of cancer; diagnostic imaging; mammography; tumor microenvironment; proton magnetic resonance spectroscopy; carbon-13 magnetic resonance spectroscopy

 doi:10.20944/preprints202511.0512.v1

Review

The Role of pH in Breast Cancer Screening

Dyutika Kantamneni [1,*], Saumya Gurbani [2] and Mary Salvatore [1]

[1] Jacobi Medical Center Radiology, USA

[2] Emory University Radiology, USA

* Correspondence: dyu_121@yahoo.co.in

Abstract

Breast cancer screening, while vital for reducing mortality, faces significant limitations in sensitivity and specificity, particularly in dense breasts. Current modalities primarily detect anatomical changes, often missing biologically aggressive tumors at their earliest stages. The altered metabolism of cancer cells establishes a characteristic inverted pH gradient that drives tumor invasion, metastasis, and treatment resistance. This makes tumor acidity a compelling, functional biomarker for early detection. This review synthesizes the emerging role of pH as a diagnostic biomarker and provides a critical evaluation of advanced imaging techniques for its non-invasive measurement. We detail the biological underpinnings of tumor acidosis, emphasizing its regulation through glycolytic reprogramming and dysregulated proton transport. Our analysis encompasses a broad spectrum of pH-sensitive imaging modalities, including magnetic resonance methods such as Chemical Exchange Saturation Transfer (CEST) MRI for extracellular pH mapping and multi-nuclear Magnetic Resonance Spectroscopy (MRS) using ^{1}H, ^{31}P, and ^{19}F nuclei to probe various cellular compartments. Furthermore, we examine hyperpolarized ^{13}C MRI for real-time metabolic flux imaging, where metrics like the lactate-to-pyruvate ratio show significant predictive value for treatment response. The review also assesses optical and photoacoustic imaging techniques, which offer high sensitivity but are often constrained to superficial tumors. Imaging tumor pH provides a powerful functional window into the earliest metabolic shifts in breast cancer, far preceding macroscopic anatomical changes. The ongoing development and clinical validation of these pH-sensitive imaging techniques hold immense promise for revolutionizing breast cancer screening by enabling earlier, more specific detection and personalized risk stratification, ultimately aiming to improve patient outcomes.

Keywords: breast neoplasms; early detection of cancer; diagnostic imaging; mammography; tumor microenvironment; proton magnetic resonance spectroscopy; carbon-13 magnetic resonance spectroscopy

1. Introduction

Despite widespread adoption of breast cancer screening, tumours can still remain underdetected, contributing to continued morbidity and mortality [1]. The most common cancer among women worldwide is breast cancer with 2.3 million new cases annually accounting for 30% of cancers in women with rising incidence rates [2,3]. To reduce mortality by early detection of cancer, US Preventive Services Task Force (USPSTF) recommends biennial screening digital mammography (DM) and digital breast tomosynthesis (DBT, also known as 3D-mammography) for women aged 40 to 74. Supplemental modalities such as breast ultrasonography, magnetic resonance imaging (MRI) and contrast enhanced mammography are advised for women with high lifetime risk based on familial and personal factors [4]. While patients with dense breasts are challenging to screen with DM and DBT, there is currently insufficient evidence for the use of supplemental screening modalities in these populations.

 doi:10.20944/preprints202511.0512.v1

While these imaging tools can identify structural changes, they have notable limitations including reduced sensitivity and specificity in dense breasts, risk of false positives and false negatives, and potential for overdiagnosis, all of which can lead to increased testing, invasive follow-up procedures, and psychological distress [4–8]. Moreover, by the time morphological abnormalities are visible, some tumours may already exhibit aggressive behaviour [9]. This underscores the need for screening approaches capable of detecting cancer earlier and with greater biological specificity, particularly in patients with dense breast tissue. One promising avenue is functional and metabolic imaging, such as targeting tumour pH. Cancer cells, unlike normal cells, exhibit altered metabolism to support intensive growth and turnover characterized by a more basic intracellular pH and more acidic extracellular pH due to the *Warberg effect* [10–14]. The *Warburg effect* is a well known phenomenon where tumours even in presence of sufficient oxygen produce lactate using the glycolytic pathways instead of oxidative phosphorylation [14]. An acidic pH in breast cancer cells has been seen to contribute to increased invasive growth, migration, aggressiveness, metastasis, and poor prognosis, making pH a compelling biomarker for early breast cancer screening [15–21].

Recent improvements in advanced imaging modalities can help improve our ability to detect breast cancer earlier by overcoming the limitations of traditional techniques, and may provide evidence for future improvements in screening and diagnostic guidelines, particularly for patients with dense breast tissue. This review synthesizes current evidence and emerging advances in pH-sensitive breast imaging, comparing these innovations with established modalities to evaluate their feasibility for earlier cancer detection.

2. Tumor pH Biology and and Its Role in Breast Cancer

2.1. Why pH Is a Hallmark of Cancer?

Tumor pH can be used as a diagnostic marker for tumors, where the central theme is an inversion of the pH gradient due to an altered metabolism, a fundamental reversal from physiology that Koltai T termed "*the cancer pH paradigm*" [22–26]. Typically, extracellular pH (pHe) ranges from 7.3 and 7.4 while intracellular pH (pHi) ranges from 7.0 to 7.3 [27,28]. The reversal of this pHi/pHe gradient in cancer is a result of interplay between glycolytic reprogramming and dysregulated proton dynamics [14,29,30]. The Warburg effect, describing tumor cells' increased oxidative phosphorylation, drives lactate and proton(H+) overproduction; these acids are extruded via a Na+/H+ exchanger (NHE), the H+-lactate co-transporter, monocarboxylate transporters (MCTs), and the H+-ATPase (H+ pump) which lead to secretion of H+ creating an acidic tumor microenvironment and an alkaline cytosolic environment [29,30]. The change to an alkaline pHi promotes cell proliferation and cell survival by limiting apoptosis, associated with intracellular acidification [31–36]. Extracellular acidosis actively drives malignancy by promoting invasion (via MMP-9 activation), evasion of immune surveillance, and enhancing chemoresistance among other effects [24,37–44]. In breast cancer, this pH inversion critically influences tumor aggressiveness, metastatic potential and treatment response, while also offering opportunities for early detection strategies.

2.2. Acidic Shift in Breast cancer

In cancer there are multiple pathways that contribute to the regulation of pHe/pHi [45,46]. Cancer cells tend to have a higher alkaline pHi (>7.4) and lower acidic pHe (6.7-7.1) compared to normal cells [11]. This shift is not a passive occurrence but an active, orchestrated process driven by metabolic reprogramming and molecular regulation. In breast cancer cells, the acidity of the extracellular environment is attributed to multiple causes. The Warburg effect (aerobic glycolysis) is initiated by oncogenic signaling and a failure of energy-sensing checkpoints, particularly the loss of the LKB1-AMPK pathway, which allows cells with inefficient, acid-producing metabolism to evade growth arrest and continue proliferating [47,48]. Malignant tumors have increased tumor pHi than lower grade tumors [49]. Acid extrusion is achieved through the coordinated upregulation of specific transporters on the plasma membrane or through phagosomes [50,51]. Monocarboxylate transporters

 doi:10.20944/preprints202511.0512.v1

MCT1 and MCT4 (SLC16A1, SLC16A3) are pivotal, co-exporting lactate alongside protons (H+) into the extracellular space, directly acidifying the tumour microenvironment [52–54]. Their expression and membrane localization are dependent on their association with the chaperone protein CD147, which is itself linked to poor prognosis [55–57]. Simultaneously, proton pumps, specifically the plasma membrane H+-ATPase, operate aggressively and uniformly across the cell surface to pump protons out, further contributing to the severe extracellular acidification [50,58]. Additionally phagosomes can also contribute as they lead to increased leakage of protons [51,59,60].

The Na+/H+ exchanger (NHE1) extrudes intracellular H+ in exchange for extracellular Na+, while the Na+,HCO3– cotransporter NBCn1 (SCL4A7)imports bicarbonate ions, which buffer the cytosol [61–63]. Lowered cancer cell proliferation and reduced breast tumor growth is seen when the expression of NBCn1 is disrupted, but elevated levels are associated with shorter survival and increased metastasis [64,65]. In contrast NHE1 is linked to promoting motility in ErbB2-positive breast cancer cells, stimulating epithelial to mesenchymal transition (EMT) and contributing to chemotherapy resistance [61,62,66–68]. A loss of extracellular HCO3- sensing protein receptor protein tyrosine phosphatase RPTPγ is seen in premalignant lesions of the breast. The upregulation or downregulation of these transporters is not random; it is directly driven by oncogenic pathways. The tyrosine kinase ErbB2 (HER2), a key driver in certain breast cancer subtypes, enhances the expression and activity of both NHE1 and NBCn1 via the ERK-RSK signaling pathway. This direct link ensures that the pH regulatory machinery is integrated with the core proliferative signals of the cancer cell [69–71].

The resulting inverted pH gradient promotes invasion and metastasis. Intracellular alkalinization protects the cancer cell from acid-induced damage and promotes proliferation [50,58]. Conversely, extracellular acidification remodels the tumour microenvironment by activating secreted enzymes like cathepsins that degrade the extracellular matrix (ECM), clearing a path for invasion. Thus, the coordinated activity of MCTs, H+-ATPases, NHE1, and NBCn1, all regulated by oncogenic drivers, creates a permissive environment for breast tumour growth, invasion, and ultimately, poor patient outcomes.

3. Imaging Techniques for pH Detection in Breast Cancer

Because of changes in pH in tumor, imaging techniques are being evaluated to measure in vivo acidosis, including magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), nuclear medicine (positron emission tomography, PET), electron paramagnetic resonance (EPR), optical imaging (OI), and photoacoustic imaging (PAI). In this section, we review these imaging techniques and their application in breast cancer.

3.1. MRS Methods

MRS techniques offer the ability to derive metabolic information without use of ionizing radiation with limited radiation exposure [72,73]. While MRI depends on the signal of all the protons, in contrast MRS focusses on how different proton groups in molecules resonate at slightly different frequencies in a magnetic field [74]. MRS techniques seek to identify and quantify different cellular chemicals based on the relative signals detected at these resonance offsets, providing quantitative insight into tissue metabolism in vivo [73,75]. MRS operates on the same scanner as an MRI but is uniquely designed to detect and quantify the concentrations of specific endogenous metabolites within living tissue [74,75]. By revealing these metabolic alterations, MRS can identify pathological processes even before any structural damage becomes visible [73]. However, the technique presents significant technical challenges, as it is highly sensitive to magnetic field imperfections, must detect molecules present in very low concentrations, and requires complex analysis to interpret the resulting spectral data [72,73].

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

^{1}H-MRS represents a valuable approach for assessing tumor microenvironment parameters through detection of both endogenous metabolites and exogenous probes, leveraging the high

 doi:10.20944/preprints202511.0512.v1

natural abundance (99.98%) and superior sensitivity of the proton nucleus in human tissue [73,76]. This technique has demonstrated clinical utility in breast cancer characterization through detection of elevated choline compounds (tCho), which serve as a biomarker of increased membrane turnover in malignant tissues, with diagnostic sensitivity and specificity ranging from 71-80% [77–79]. More recently, attention has turned to lactate as a hypoxia-associated metabolite, with studies demonstrating that lactate can be detected in the human breast noninvasively using ^{1}H MRS [80].

However, conventional ^{1}H-MRS faces challenges in breast tissue due to the high concentration of lipids in breast tissue. Lipid resonances can overlap with many of the other chemical compounds relevant, including choline. Techniques used elsewhere in the body, such as placement of saturation bands or masking of lipid-containing tissues, can be difficult to implement in the breast [81,82]. Double-quantum filtered (DQF) MRS methods have been developed to suppress these interfering lipid signals, enabling specific lactate detection that reveals significantly higher levels in grade III compared to grade II breast lesions, supporting its potential as a biomarker of tumor aggression [82–88].

For direct measurement of extracellular pH (pHe), exogenous imidazole-based probes such as (±)2-imidazole-1-yl-3-ethoxycarbonylpropionic acid (IEPA) and (±)2-(imidazol-1-yl)succinic acid (ISUCA) have been developed, featuring pH-sensitive ^{1}H resonances in the 7-9 ppm range, a spectral region relatively free from endogenous interferences [89–94]. These compounds enable ratiometric measurements through pH-dependent chemical shifts of their H-2 proton relative to internal reference protons, allowing concentration-independent pH assessment. Preclinical studies in orthotopic breast cancer models have successfully generated pHe maps using these approaches, revealing acidic regions within tumors that correlate with aggressive features [90,95]. While ^{31}P MRS offers a more direct way to monitor phospholipid metabolites, energy metabolites, and intracellular pH levels, the increased spectral resolution at higher field strengths (7T versus 1.5T) has enabled distinct detection of phosphoethanolamine (PE) metabolites from phosphocholine (PC) metabolites in patient studies [96–100]. Clinical observations show altered metabolite ratios in breast cancer patients, with elevated PE resonances compared to PC resonances, which may partially explain the tCho levels detected. Furthermore, alterations in pH levels have been observed clinically through split Pi resonances, with calculations yielding pH values of 7.5 and 6.9 [101,102].

However, the technique faces challenges including relatively small pH-dependent chemical shifts (approximately 0.7 ppm over the physiological range) that require high magnetic field strengths (e.g., 3T) for adequate resolution, and spatial resolution on the order of multiple millimeters that may be insufficient for characterizing heterogeneous tumor microenvironments [95]. Additionally, some imidazole-based probes exhibit buffering capacity that could potentially alter the pHe they are intended to measure, although recent developments in diimidazole compounds aim to address these limitations with enhanced sensitivity and reduced buffering capacity [89,103]. Despite these challenges, the technique's compatibility with standard clinical MRI systems and its ability to simultaneously assess multiple metabolic parameters position it as a promising multiparametric approach for breast cancer evaluation.

Chemical Exchange Saturation Transfer (CEST) MRI:

Chemical exchange saturation transfer (CEST) imaging refers to a method to measure low-concentration solutes by the phenomenon of magnetization transfer (MT). In MT, a specific proton group (e.g., amides) are stimulated at their resonance frequency using radiofrequency (RF) pulses, saturating their magnetization. This magnetization is transferred to surrounding water due to the exchange of the excited protons with protons in bulk water. Repeated cycles of stimulation and transfer will lead to a build-up of signal in bulk water, which cannot be saturated given its concentration is orders of magnitude higher than the stimulated proton groups. This increased signal can then be more easily detected [104–108]. The chemical exchange of saturated protons with protons of water is pH dependent, and therefore measurement of the rate of transfer can be used to estimate tissue pH [109,110]. CEST imaging has proven effective in detecting acidic pH of breast tumors and revealing their metastatic potential, as the CEST effect diminishes at higher, more basic pH levels due

 doi:10.20944/preprints202511.0512.v1

to an excessively rapid exchange rate. CEST is broadly categorised into two types: endogenous and exogenous CEST [111–114].

a. Endogenous CEST: This approach focuses on stimulation of chemical groups endogenously found in tissues. The most established method is amide proton Transfer(APT) imaging, which focuses on amide protons (proteins and peptides) [114]. The exchange rate between the amide protons and water decreases with decreasing pH [110,115–119]. Since cancer cells exhibit higher intracellular protein concentrations due to rapid turnover, the APT signal is often increased [120–122]. Clinically, APT has demonstrated high diagnostic performance, distinguishing between benign and malignant breast lesions with an AUC as high as 0.959 [123,124]. Its signal shows a positive correlation with tumor stage and Ki-67 proliferation index and decreases following effective neoadjuvant therapy [125]. A current limitation is it has mostly been studied for characterizing larger lesions compared to smaller ones.

Other endogenous CEST methods target different protons: hydroxyl groups (e.g., glycCEST,gagCEST used in humans to distinguish tumor from surrounding tissue) and amine groups of glutamate (gluCEST) [103,126–128]. While GluCEST has shown promise in mouse models for monitoring early response to glutaminase inhibitors in TNBC, its clinical translation is limited by high magnetic field requirements and a focus on specific subtypes [129]. Although amide and guanidyl CEST can map intracellular pH (pHi), these studies require high magnetic strengths and lack simultaneous compensation for confounding tumor effects, limiting their current clinical understanding.

b. Exogenous CEST:

This method utilizes administered CEST contrast agents to specifically map the extracellular pH (pHe) of the tumor microenvironment [130]. It employs FDA-approved, non-ionic iodinated X-ray contrast agents known for high water solubility, low toxicity and is pH dependent, such as Iopromide and Ioversol [131–133].

A key advance is the development of acidoCEST MRI, a technique that takes advantage of differences in CEST effects of its two amide groups at different pH levels; a ratio of the magnitude of these two CEST effects was found to be highly linearly correlated with pH [133]. This allows for a ratiometric analysis method that calculates pHe independent of the agent's concentration, a significant advantage for use in human subjects [131,134,135].

However, exogenous CEST faces challenges. Many pioneering studies were conducted at 7T, which is not widely available even at academic institutions, and validation on clinical 3T systems is needed. The accurate measurement range is typically limited to pH < 7.2, and while many tumor microenvironments are at pH < 7.4, the technique remains sensitive to confounding variables like temperature, pH, contrast agent and magnetic field (B0/B1) inhomogeneity, necessitating careful experimental control [136,137].

31P - MRS (phosphorous)

Phosphate compounds play a central role in cellular energy metabolism; all energetic processes in human tissue cells involve phosphate-containing metabolites such as phosphocreatine (PCr),inorganic phosphate (Pi) and adenosine triphosphate (α-β-γ-ATP) [138]. Phosphorous MR Spectroscopy (31P-MRS) relies on the same phenomenon of difference in resonance frequencies of phosphorous when it is bound in different chemicals. 31P-MRS can measure intracellular pH by assessing the chemical shift difference between different phosphorus chemical compounds, with this difference changing based on pH; this technique has been used to assess pH changes in multiple tissues and tumors [139]. Inorganic Pi is most affected by changes in pH particularly in tumors, whereas the PCr is used as a reference since it has a relatively stable resonance position across physiologic pH [140–142]. Most studies have focussed on the quantification of phosphorus metabolites in breast cancers as compared to normal tissue with focus on changes of these metabolites under chemotherapy, with increase in pHi following treatment.Studies on 3TMRI show improved signal to noise ratio and spectral resolution in the human breast in vivo [143–148].While MRS can be used to study the intracellular pH changes which are the very initial changes that take place in cancer

 doi:10.20944/preprints202511.0512.v1

cells further studies have been done to assess the extracellular pH [149]. 31P MRS of 3-aminopropylphosphate (3-APP), a non-toxic membrane impermeant, is used to measure extracellular pH of tumor. It has been demonstrated to show changes in breast cancer cell lines [23]. A major benefit of 31P-MRS is the lack of a need for water suppression in order to detect phosphorous metabolites, unlike detecting in vivo metabolites with 1H-MRS [148]. Limitations of a 31P-MRS include lower sensitivity as Pi concentrations are generally lower than proton concentrations, and thus it is essential to use techniques to acquire sufficiently high SNR for pH quantification [139].

19F - MRS (Fluoride)

^{19}F Magnetic Resonance Spectroscopy (MRS) has emerged as a powerful technique for probing the tumor microenvironment, particularly extracellular pH (pHe), due to the near absence of endogenous fluorine background in biological tissues [150–152]. This lack of background signal allows for unambiguous detection of exogenous fluorinated probes, while the nucleus's high gyromagnetic ratio and large chemical shift dispersion (~300 ppm) provide excellent spectral resolution [150]. The development of effective 19F pH indicators requires careful optimization of pharmacokinetic properties, including a pK_a within the physiological range, high sensitivity and specificity, low toxicity, efficient cellular delivery, and appropriate membrane permeability characteristics [149,151–154].

Several classes of fluorinated probes have been developed for pH sensing. Early approaches utilized fluorinated alanines, which demonstrated pH-dependent chemical shifts but were limited to perfused cell systems and were not applied in vivo [155–158]. More advanced probes include fluorinated vitamin B6 analogues such as 6-fluoropyridoxol and its trifluoromethylated derivative, which exhibit improved pK_a values and membrane impermeability, making them suitable for extracellular pH measurement in tumor xenograft models [159]. The fluoroaniline sulfonamide ZK-150471 has shown particular promise, demonstrating superior signal-to-noise ratio and pH sensitivity compared to ^{31}P MRS probes like 3-APP [149,160].

Recent innovations focus on "smart" contrast agents and nanoscale delivery systems [161–163]. PEGylated nanogels containing perfluorocarbons exhibit pH-dependent size changes, enabling indirect pH estimation through ^{19}F signal variations [164–169]. Molecular switches acting as ratiometric probes eliminate the need for external references, paramagnetic relaxation enhancement strategies using Mn^{2+} ions have improved signal intensity by over five-fold [165,167,170,171]. Liposil-encapsulated fluorinated ionic liquids represent another advancement, enabling multiplexed ^{19}F MRI with enhanced relaxation properties [171].

Despite these advantages, ^{19}F MRS faces challenges including probe instability, nonspecific accumulation due to hydrophobicity, and limited spatial resolution despite high field strengths [160,164] While extracellular pH measurement is well-established, simultaneous intracellular pH assessment remains challenging. Nevertheless, the continuous development of novel fluorinated compounds and encapsulation strategies positions ^{19}F MRS as a valuable complementary approach to proton-based methods for characterizing tumor acidosis in breast cancer research and potential clinical applications [164,167].

3.2. Hyperpolarized ^{13}C MRS:

Hyperpolarized carbon-13 (^{13}C) magnetic resonance imaging (MRI) is a real-time, non-invasive method that enhances signal of ^{13}C-labeled compounds through cooling a 13C-enriched sample with a polarizing agent to near absolute zero in presence of strong magnetic fields (3.35 - 7T) for 2 hours and transferring polarization via microwave irradiation to improve visualization of metabolic activity. However, this technique requires specialized equipment for polarization and rapid quality control, necessitating dedicated multinuclear MRI scanners with custom ^{13}C coils. These technical and economic constraints have currently limited its widespread clinical implementation [172].

Two primary approaches facilitate pH quantification using hyperpolarized ^{13}C MRI. The first method utilizes ^{13}C-bicarbonate, which undergoes pH-dependent equilibrium with $^{13}CO_2$ via carbonic anhydrase catalysis, enabling quantitative mapping of extracellular pH through signal

 doi:10.20944/preprints202511.0512.v1

intensity ratios. The second approach employs [1-^{13}C]pyruvate, whose metabolic conversion to $^{13}CO_2$ in highly aerobic tissues permits similar ratio-based calculations primarily reflecting intracellular pH. Since both compounds are endogenous metabolites, these techniques show significant potential for clinical translation in mapping pH distributions in human cancers [173].

In breast cancer applications, the hyperpolarized ^{13}C-lactate signal has demonstrated utility as a potential biomarker for tumor grading, with detectable signals in triple-negative and high-grade tumors but absence in lower-grade malignancies [174,175]. The lactate-to-pyruvate ratio (Lac/Pyr) correlates strongly with HIF1α expression, linking metabolic activity to hypoxia and tumor volume, while also showing associations with lactate dehydrogenase A (LDHA) and monocarboxylate transporter 1 (SLC16A1/MCT1) expressions [176].

The technology shows particular promise for treatment response assessment, detecting metabolic alterations within 7-11 days post-treatment. Increases in Lac/Pyr ratio ≥20% following therapy have demonstrated predictive value for pathological complete response (pCR) [176,177]. However, data interpretation requires careful consideration since the ^{13}C-lactate signal reflects complex interactions involving substrate delivery, transporter expression, and enzymatic activity rather than only lactate production. This complexity is evidenced by paradoxical observations such as increased lactate conversion following pazopanib treatment [178].

Recent technological innovations address limitations of conventional polarization methods. Novel approaches like Spin-Lock Induced Crossing with Signal Amplification By Reversible Exchange (SLIC-SABRE) enable rapid generation of hyperpolarized [1-^{13}C]pyruvate within minutes at reduced cost, facilitating high-throughput studies. Applications in transgenic breast cancer models have successfully identified elevated lactate metabolism and metabolic subcompartments corresponding to histological profiles, demonstrating potential for overcoming current implementation barriers [179].

While challenges remain regarding technical complexity, cost, and data interpretation, ongoing advancements in hyperpolarized ^{13}C MRI position it as a transformative approach for non-invasive pH monitoring in breast cancer management. The capability to track spatial and temporal pH dynamics provides unique insights into tumor heterogeneity and treatment response, offering significant potential for advancing personalized cancer care [174].

3.3. Optical and Fluorescent Probes for tumor pH detection

Optical imaging represents a powerful approach for detecting the acidic tumor microenvironment, leveraging light-based technologies to provide accurate, real-time data on biochemical properties including extracellular pH (pHe) [180,181]. These methods are particularly valuable for their high sensitivity and versatility, though their application is primarily limited to superficial or accessible tumors [101,182–187]. A key advantage of these approaches is their capacity for ratiometric measurements, where the ratio of signals at different emission or excitation wavelengths enables pH quantification independent of probe concentration, photobleaching, or tissue depth variations. This capability for concentration-independent measurement significantly enhances accuracy and reliability in pH detection [188].

For in vivo applications, the near-infrared (NIR) range (650-900 nm) is particularly valuable due to reduced light absorption and scattering by biological tissues, allowing penetration to deeper depths of the tissue of up to 1 cm. Several optical techniques have been developed for pH sensing, each with distinct characteristics [103,133].

Optical sensors such as Optical coherence tomography (OCT) and photoacoustic imaging (PAI) are a non-invasive technique to detect tumors [189]. OCT employs non-invasive light-based imaging to generate high-resolution, micrometer-scale images of tissue architecture. OCT takes advantage of the high spatial resolution of near-infrared light to detect micrometer-level reflectances in tissue, an optical analogue of ultrasound [190]. OCT has demonstrated particular utility in identifying early-stage breast malignancies that evade detection by conventional imaging modalities. Clinical investigations have validated OCT's diagnostic capability, reporting sensitivity of 93% and specificity

 doi:10.20944/preprints202511.0512.v1

of 85% in distinguishing cancerous breast tissue, establishing its potential as an effective tool for early cancer detection [191]. Fluorescence imaging employs pH-sensitive dyes that change their emission properties in response to acidity. Optical coherence tomography (OCT), though not a direct pH sensor, provides high-resolution structural imaging that can complement pH measurements. The development of various probes has been crucial for advancing optical pH detection, ranging from visible-light probes like seminaphthorhodafluor-1 (SNARF-1) for preclinical window chamber models to NIR fluorescent probes such as modified rhodamine bound to dextran (Dex-Me-pEPPR) and activatable nanoparticles that turn on fluorescence in acidic conditions [184,192,193]. The pH can be measured independent of concentration by assessing the ratio of fluorescence signals at different emission wavelengths or at different fluorescence lifetimes [17,182,185,186,192].

PAI has emerged as a particularly promising modality for deeper tissue pH sensing, utilizing probes designed with pH-dependent and pH-independent absorption peaks [180]. To overcome the depth limitations of optical methods like photoacoustic imaging (PAI) has emerged as a modality for deeper tissue pH sensing. PAI is a hybrid modality that combines optical imaging with ultrasound detection, providing high spatial-temporal resolution for imaging structures several centimeters deep with exceptional capital and temporal resolution [194,195]. This technique can identify tumors as small as 1-2 mm with a sensitivity of 91%. Systems including albumin-based nanoparticles with benzo-α-phenoxazine and IR825, or polymer nanoparticles containing SNARF-5F, enable pH quantification through ratioing of optoacoustic signals at different excitation wavelengths [196]. Despite their considerable promise, the clinical translation of optical and photoacoustic pH-sensing for broad breast cancer screening faces hurdles. The primary limitation remains depth penetration, which restricts these methods to superficial or accessible tumors [101,196]. Furthermore, challenges related to the biocompatibility, stability of probes, along with addressing the complexities of absolute pH quantification due to nonlinear response curves [103]. Consequently, these technologies are currently best suited for specialized applications such as intraoperative guidance or endoscopic procedures, where their high sensitivity and capacity for spatial pH mapping can be fully leveraged [103,194–197].

Table 1. Diagnostic Imaging techniques for pH detection for breast cancer screening.

Technique	Primary Measure	Key Strength	Key Finding	Main Limitation
^{1}H MRS (Conventional)	Total Choline (tCho) concentration	High endogenous concentration; widely available on standard clinical MRI systems	71-80% sensitivity/specificity for malignant lesion detection	Cannot directly measure pH; lipid signal contamination
^{1}H MRS (DQF)	Lactate concentration	Specific lactate detection by suppressing lipid signals	Higher lactate in grade III vs. grade II lesions; links to hypoxia	50% inherent signal loss; challenging for small lesions

 doi:10.20944/preprints202511.0512.v1

^{1}H MRS (Exogenous Probes)	Chemical shift of probe's H-2 proton	Ratiometric, concentration-independent pHe measurement	Successful pHe mapping in preclinical models reveals acidic regions	Small chemical shift range (~0.7 ppm); may alter native pHe
^{31}P MRS	Chemical shift difference of inorganic phosphate compared to pH-independent phosphates I	Direct measurement of intracellular pH; monitors energy metabolism	Resolves multiple pH compartments via Pi splitting	Low endogenous concentration; poor spatial/temporal resolution
^{19}F MRS	Chemical shift of exogenous ^{19}F probe	Negligible biological background; large chemical shift dispersion	Superior SNR vs. ^{31}P MRS; enables specific pHe mapping	Requires exogenous probes; limited clinical translation
Hyperpolarized ^{13}C MRS	Lac/Pyr or $H^{13}CO_3^-/^{13}CO_2$ ratio	>10,000x signal enhancement for real-time metabolic flux	Lac/Pyr increase ≥20% predicts pCR post-treatment	Extreme cost/technical complexity; short signal lifetime
CEST MRI (Endogenous)	Amide proton transfer (APT) effect	No contrast agent needed; correlates with tumor aggression	High AUC (~0.96) for malignancy; tracks therapy response	Confounded by multiple factors; less sensitive in small lesions
CEST MRI (Exogenous)	Chemical exchange of iodinated agents	Ratiometric, concentration-independent pHe measurement	Revealed "pH-neutral" tumors; quantitative pHe mapping	Limited to acidic range (pH <7.2); requires high field strength (e.g., 7T)

 doi:10.20944/preprints202511.0512.v1

Fluorescence Imaging	Fluorescence intensity ratio	High sensitivity; real-time ratiometric quantification	Direct correlation between low pHe and high tumor invasion	Limited to superficial tumors (<1 cm depth)
Optical Coherence Tomography	Tissue microstructure changes	High spatial resolution (~micrometers); non-invasive	93% sensitivity/85% specificity for cancerous tissue	Does not directly measure pH; structural context only
Photoacoustic Imaging	Optoacoustic signal ratio	Deeper penetration than pure optical; combines optical/ultrasound	91% sensitivity for lesions 1-2 mm; quantifies tumor pH	Nonlinear response curves; probe biocompatibility issues

4. Discussion

This comprehensive review establishes tumor acidosis as a fundamental, targetable hallmark of breast cancer with profound implications for early detection. The inverted pH gradient, characterized by alkaline intracellular and acidic extracellular environments, is not merely a metabolic byproduct but an active driver of tumor progression, invasion, and treatment resistance. The central conclusion of this analysis is that pH-sensitive imaging represents a paradigm shift in breast cancer screening, moving beyond anatomical assessment to functional, metabolic characterization.

The primary conclusion is that multiple imaging modalities have now matured to the point of providing reliable pH measurement. Techniques such as CEST MRI, particularly the exogenous acidoCEST approach, and advanced MRS methods (^{19}F, ^{31}P) offer clinically feasible pathways to quantify extracellular and intracellular pH with increasing precision. Hyperpolarized ^{13}C MRI, despite its current technical complexity, provides unprecedented insight into real-time metabolic flux, with the lactate-to-pyruvate ratio emerging as a powerful predictive biomarker for treatment response.

The relevance of these findings to the field is substantial and multi-faceted. First, pH imaging addresses critical limitations of current screening, particularly in dense breasts where structural modalities underperform. By detecting metabolic alterations that precede macroscopic tumor formation, these techniques enable truly early cancer detection. Second, the ability to stratify tumors based on their pH profile and metabolic activity offers new avenues for personalized risk assessment and treatment selection, potentially identifying aggressive tumors that warrant more intensive management while sparing patients with indolent disease from overtreatment.

The translation of these technologies into routine clinical practice represents the next frontier. While challenges remain in standardization, cost reduction, and validation across diverse populations, the convergence of biological understanding and imaging innovation positions pH-based screening as a transformative approach. Future efforts should focus on integrating these functional techniques with existing structural modalities, developing standardized protocols, and validating pH thresholds that reliably predict clinical outcomes. The implementation of pH-sensitive imaging holds the potential to revolutionize breast cancer management by enabling detection at its

 doi:10.20944/preprints202511.0512.v1

earliest metabolic origins, ultimately reducing mortality through timely intervention and personalized therapeutic strategies.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, M.S and D.K.; methodology, D.K.; writing—original draft preparation, D.K. and M.S; writing—review and editing, S.G and D.K.; supervision, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
^{1}H-MRS	Proton Magnetic Resonance Spectroscopy
^{31}P-MRS	Phosphorus-31 Magnetic Resonance Spectroscopy
^{19}F-MRS	Fluorine-19 Magnetic Resonance Spectroscopy
^{13}C-MRS	Carbon-13 Magnetic Resonance Spectroscopy
CEST	Chemical Exchange Saturation Transfer
APT	Amide Proton Transfer
BOLD	Blood Oxygen Level–Dependent
PET	Positron Emission Tomography
CT	Computed Tomography
US	Ultrasound
pHe	Extracellular pH
pHi	Intracellular pH
Na^+/H^+ exchanger (NHE1)	Sodium/Hydrogen Exchanger Isoform 1
MCT	Monocarboxylate Transporter
CAIX	Carbonic Anhydrase IX
NMR	Nuclear Magnetic Resonance
RF	Radiofrequency
ppm	Parts per Million
TR/TE	Repetition Time / Echo Time
FID	Free Induction Decay
PCr	Phosphocreatine
Pi	Inorganic Phosphate
CO_2	Carbon Dioxide
^{19}F	Fluorine-19 (nucleus used in MRS)
^{13}C	Carbon-13 (nucleus used in MRS)

 doi:10.20944/preprints202511.0512.v1

DNP	Dynamic Nuclear Polarization
PA	Photoacoustic
PAI	Photoacoustic Imaging
pKa	Acid Dissociation Constant
B_0	Main Magnetic Field Strength
B_1	Radiofrequency Magnetic Field
T_1/T_2	Longitudinal / Transverse Relaxation Time
SPIO	Superparamagnetic Iron Oxide
pH-sensitive MRI	Magnetic Resonance Imaging sensitive to pH contrast mechanisms
FDA	U.S. Food and Drug Administration

References

1. Mahfuz Al Hasan S, Bennett D, Toriola A. Screening programmes and breast cancer mortality: an observational study of 194 countries. *Bull World Health Organ*. 2025;103(8):470-483. doi:10.2471/BLT.24.292529
2. Breast Cancer Statistics | How Common Is Breast Cancer? Accessed October 29, 2025. https://www.cancer.org/cancer/types/breast-cancer/about/how-common-is-breast-cancer.html
3. Kim J, Harper A, McCormack V, et al. Global patterns and trends in breast cancer incidence and mortality across 185 countries. *Nat Med*. 2025;31(4):1154-1162. doi:10.1038/s41591-025-03502-3
4. US Preventive Services Task Force, Nicholson WK, Silverstein M, et al. Screening for Breast Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA*. 2024;331(22):1918. doi:10.1001/jama.2024.5534
5. Trentham-Dietz A, Chapman CH, Jayasekera J, et al. *Breast Cancer Screening With Mammography: An Updated Decision Analysis for the U.S. Preventive Services Task Force*. Agency for Healthcare Research and Quality (US); 2024. Accessed October 29, 2025. http://www.ncbi.nlm.nih.gov/books/NBK603560/
6. Trentham-Dietz A, Chapman CH, Jayasekera J, et al. Collaborative Modeling to Compare Different Breast Cancer Screening Strategies: A Decision Analysis for the US Preventive Services Task Force. *JAMA*. 2024;331(22):1947. doi:10.1001/jama.2023.24766
7. Nelson HD, Cantor A, Humphrey L, et al. *Screening for Breast Cancer: A Systematic Review to Update the 2009 U.S. Preventive Services Task Force Recommendation*. Agency for Healthcare Research and Quality (US); 2016. Accessed October 29, 2025. http://www.ncbi.nlm.nih.gov/books/NBK343819/
8. Henderson JT, Webber EM, Weyrich M, Miller M, Melnikow J. *Screening for Breast Cancer: A Comparative Effectiveness Review for the U.S. Preventive Services Task Force*. Agency for Healthcare Research and Quality (US); 2024. Accessed October 29, 2025. http://www.ncbi.nlm.nih.gov/books/NBK603789/
9. Irvin VL, Zhang Z, Simon MS, et al. Comparison of Mortality Among Participants of Women's Health Initiative Trials With Screening-Detected Breast Cancers vs Interval Breast Cancers. *JAMA Netw Open*. 2020;3(6):e207227. doi:10.1001/jamanetworkopen.2020.7227
10. Persi E, Duran-Frigola M, Damaghi M, et al. Systems analysis of intracellular pH vulnerabilities for cancer therapy. *Nat Commun*. 2018;9(1):2997. doi:10.1038/s41467-018-05261-x
11. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. *Nat Rev Cancer*. 2011;11(9):671-677. doi:10.1038/nrc3110
12. Kato Y, Ozawa S, Miyamoto C, et al. Acidic extracellular microenvironment and cancer. *Cancer Cell Int*. 2013;13(1):89. doi:10.1186/1475-2867-13-89
13. Kallinowski F, Schlenger KH, Runkel S, et al. Blood flow, metabolism, cellular microenvironment, and growth rate of human tumor xenografts. *Cancer Res*. 1989;49(14):3759-3764.
14. Warburg O, Wind F, Negelein E. THE METABOLISM OF TUMORS IN THE BODY. *J Gen Physiol*. 1927;8(6):519-530. doi:10.1085/jgp.8.6.519
15. Lee S, Shanti A. Effect of Exogenous pH on Cell Growth of Breast Cancer Cells. *Int J Mol Sci*. 2021;22(18):9910. doi:10.3390/ijms22189910

 doi:10.20944/preprints202511.0512.v1

16. Ward C, Meehan J, Gray ME, et al. The impact of tumour pH on cancer progression: strategies for clinical intervention. *Explor Target Anti-Tumor Ther*. 2020;1(2):71-100. doi:10.37349/etat.2020.00005
17. Estrella V, Chen T, Lloyd M, et al. Acidity Generated by the Tumor Microenvironment Drives Local Invasion. *Cancer Res*. 2013;73(5):1524-1535. doi:10.1158/0008-5472.CAN-12-2796
18. Gillies RJ, Gatenby RA. Hypoxia and adaptive landscapes in the evolution of carcinogenesis. *Cancer Metastasis Rev*. 2007;26(2):311-317. doi:10.1007/s10555-007-9065-z
19. Morita T, Nagaki T, Fukuda I, Okumura K. Clastogenicity of low pH to various cultured mammalian cells. *Mutat Res*. 1992;268(2):297-305. doi:10.1016/0027-5107(92)90235-t
20. Shi Q, Le X, Wang B, et al. Regulation of vascular endothelial growth factor expression by acidosis in human cancer cells. *Oncogene*. 2001;20(28):3751-3756. doi:10.1038/sj.onc.1204500
21. Bix G, Castello R, Burrows M, et al. Endorepellin In Vivo: Targeting the Tumor Vasculature and Retarding Cancer Growth and Metabolism. *JNCI J Natl Cancer Inst*. 2006;98(22):1634-1646. doi:10.1093/jnci/djj441
22. Wike-Hooley JL, Haveman J, Reinhold HS. The relevance of tumour pH to the treatment of malignant disease. *Radiother Oncol*. 1984;2(4):343-366. doi:10.1016/S0167-8140(84)80077-8
23. Gillies RJ, Liu Z, Bhujwalla Z. 31P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate. *Am J Physiol*. 1994;267(1 Pt 1):C195-203. doi:10.1152/ajpcell.1994.267.1.C195
24. Tannock IF, Rotin D. Acid pH in tumors and its potential for therapeutic exploitation. *Cancer Res*. 1989;49(16):4373-4384.
25. Swietach P, Boedtkjer E, Pedersen SF. How protons pave the way to aggressive cancers. *Nat Rev Cancer*. 2023;23(12):825-841. doi:10.1038/s41568-023-00628-9
26. Koltai T. The Ph paradigm in cancer. *Eur J Clin Nutr*. 2020;74(S1):14-19. doi:10.1038/s41430-020-0684-6
27. Michl J, Monterisi S, White B, et al. Acid-adapted cancer cells alkalinize their cytoplasm by degrading the acid-loading membrane transporter anion exchanger 2, SLC4A2. *Cell Rep*. 2023;42(6):112601. doi:10.1016/j.celrep.2023.112601
28. Boron WF. Regulation of intracellular pH. *Adv Physiol Educ*. 2004;28(1-4):160-179. doi:10.1152/advan.00045.2004
29. Chesler M, Nicholson C. Regulation of intracellular pH in vertebrate central neurons. *Brain Res*. 1985;325(1-2):313-316. doi:10.1016/0006-8993(85)90330-0
30. Nishisho T, Hata K, Nakanishi M, et al. The a3 isoform vacuolar type H^+-ATPase promotes distant metastasis in the mouse B16 melanoma cells. *Mol Cancer Res MCR*. 2011;9(7):845-855. doi:10.1158/1541-7786.MCR-10-0449
31. Pouysségur J, Franchi A, L'Allemain G, Paris S. Cytoplasmic pH, a key determinant of growth factor-induced DNA synthesis in quiescent fibroblasts. *FEBS Lett*. 1985;190(1):115-119. doi:10.1016/0014-5793(85)80439-7
32. Moolenaar WH. Effects of growth factors on intracellular pH regulation. *Annu Rev Physiol*. 1986;48:363-376. doi:10.1146/annurev.ph.48.030186.002051
33. Kapus A, Romanek R, Qu AY, Rotstein OD, Grinstein S. A pH-sensitive and voltage-dependent proton conductance in the plasma membrane of macrophages. *J Gen Physiol*. 1993;102(4):729-760. doi:10.1085/jgp.102.4.729
34. Denker SP, Huang DC, Orlowski J, Furthmayr H, Barber DL. Direct Binding of the Na–H Exchanger NHE1 to ERM Proteins Regulates the Cortical Cytoskeleton and Cell Shape Independently of H+ Translocation. *Mol Cell*. 2000;6(6):1425-1436. doi:10.1016/S1097-2765(00)00139-8
35. Alterations of intracellular pH homeostasis in apoptosis: origins and roles | Cell Death & Differentiation. Accessed October 29, 2025. https://www.nature.com/articles/4401466
36. Matsuyama S, Llopis J, Deveraux QL, Tsien RY, Reed JC. Changes in intramitochondrial and cytosolic pH: early events that modulate caspase activation during apoptosis. *Nat Cell Biol*. 2000;2(6):318-325. doi:10.1038/35014006
37. Kato Y, Ozawa S, Tsukuda M, et al. Acidic extracellular pH increases calcium influx-triggered phospholipase D activity along with acidic sphingomyelinase activation to induce matrix metalloproteinase-9 expression in mouse metastatic melanoma. *FEBS J*. 2007;274(12):3171-3183. doi:10.1111/j.1742-4658.2007.05848.x

 doi:10.20944/preprints202511.0512.v1

38. Kato Y, Nakayama Y, Umeda M, Miyazaki K. Induction of 103-kDa gelatinase/type IV collagenase by acidic culture conditions in mouse metastatic melanoma cell lines. *J Biol Chem*. 1992;267(16):11424-11430.
39. Comito G, Iscaro A, Bacci M, et al. Lactate modulates CD4+ T-cell polarization and induces an immunosuppressive environment, which sustains prostate carcinoma progression via TLR8/miR21 axis. *Oncogene*. 2019;38(19):3681-3695. doi:10.1038/s41388-019-0688-7
40. Lactate modulation of immune responses in inflammatory versus tumour microenvironments | Nature Reviews Immunology. Accessed October 29, 2025. https://www.nature.com/articles/s41577-020-0406-2
41. Calcinotto A, Filipazzi P, Grioni M, et al. Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. *Cancer Res*. 2012;72(11):2746-2756. doi:10.1158/0008-5472.CAN-11-1272
42. Brand A, Singer K, Koehl GE, et al. LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. *Cell Metab*. 2016;24(5):657-671. doi:10.1016/j.cmet.2016.08.011
43. Rolver MG, Holland LKK, Ponniah M, et al. Chronic acidosis rewires cancer cell metabolism through PPARα signaling. *Int J Cancer*. 2023;152(8):1668-1684. doi:10.1002/ijc.34404
44. Raghunand N, Gillies RJ. pH and drug resistance in tumors. *Drug Resist Updat Rev Comment Antimicrob Anticancer Chemother*. 2000;3(1):39-47. doi:10.1054/drup.2000.0119
45. Gupta S, Farooque A, Adhikari JS, Singh S, Dwarakanath BS. Enhancement of radiation and chemotherapeutic drug responses by 2-deoxy-D-glucose in animal tumors. *J Cancer Res Ther*. 2009;5 Suppl 1:S16-20. doi:10.4103/0973-1482.55135
46. Pelicano H, Martin DS, Xu RH, Huang P. Glycolysis inhibition for anticancer treatment. *Oncogene*. 2006;25(34):4633-4646. doi:10.1038/sj.onc.1209597
47. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol*. 2007;8(10):774-785. doi:10.1038/nrm2249
48. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science*. 2009;324(5930):1029-1033. doi:10.1126/science.1160809
49. Toft NJ, Axelsen TV, Pedersen HL, et al. Acid-base transporters and pH dynamics in human breast carcinomas predict proliferative activity, metastasis, and survival. *eLife*. 2021;10:e68447. doi:10.7554/eLife.68447
50. Swallow CJ, Grinstein S, Rotstein OD. A vacuolar type H(+)-ATPase regulates cytoplasmic pH in murine macrophages. *J Biol Chem*. 1990;265(13):7645-7654. doi:10.1016/S0021-9258(19)39163-X
51. Young PR, Zygas AP. Secretion of lactic acid by peritoneal macrophages during extracellular phagocytosis. The possible role of local hyperacidity in inflammatory demyelination. *J Neuroimmunol*. 1987;15(3):295-308. doi:10.1016/0165-5728(87)90123-8
52. Halestrap AP, Wilson MC. The monocarboxylate transporter family--role and regulation. *IUBMB Life*. 2012;64(2):109-119. doi:10.1002/iub.572
53. Doherty JR, Yang C, Scott KEN, et al. Blocking lactate export by inhibiting the Myc target MCT1 Disables glycolysis and glutathione synthesis. *Cancer Res*. 2014;74(3):908-920. doi:10.1158/0008-5472.CAN-13-2034
54. Kumar A, Kant S, Singh SM. Targeting monocarboxylate transporter by α-cyano-4-hydroxycinnamate modulates apoptosis and cisplatin resistance of Colo205 cells: implication of altered cell survival regulation. *Apoptosis*. 2013;18(12):1574-1585. doi:10.1007/s10495-013-0894-7
55. Ullah MS, Davies AJ, Halestrap AP. The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. *J Biol Chem*. 2006;281(14):9030-9037. doi:10.1074/jbc.M511397200
56. Xu D, Hemler ME. Metabolic activation-related CD147-CD98 complex. *Mol Cell Proteomics MCP*. 2005;4(8):1061-1071. doi:10.1074/mcp.M400207-MCP200
57. Guo H, Li R, Zucker S, Toole BP. EMMPRIN (CD147), an inducer of matrix metalloproteinase synthesis, also binds interstitial collagenase to the tumor cell surface. *Cancer Res*. 2000;60(4):888-891.
58. Martinez-Zaguilan R, Lynch RM, Martinez GM, Gillies RJ. Vacuolar-type H(+)-ATPases are functionally expressed in plasma membranes of human tumor cells. *Am J Physiol*. 1993;265(4 Pt 1):C1015-1029. doi:10.1152/ajpcell.1993.265.4.C1015

 doi:10.20944/preprints202511.0512.v1

59. Rochefort H, Liaudet E, Garcia M. Alterations and role of human cathepsin D in cancer metastasis. *Enzyme Protein*. 1996;49(1-3):106-116. doi:10.1159/000468620
60. Montcourrier P, Mangeat PH, Valembois C, et al. Characterization of very acidic phagosomes in breast cancer cells and their association with invasion. *J Cell Sci*. 1994;107 (Pt 9):2381-2391. doi:10.1242/jcs.107.9.2381
61. Lauritzen G, Stock CM, Lemaire J, et al. The Na+/H+ exchanger NHE1, but not the Na+, HCO3(-) cotransporter NBCn1, regulates motility of MCF7 breast cancer cells expressing constitutively active ErbB2. *Cancer Lett*. 2012;317(2):172-183. doi:10.1016/j.canlet.2011.11.023
62. Boedtkjer E, Moreira JMA, Mele M, et al. Contribution of Na+,HCO3(-)-cotransport to cellular pH control in human breast cancer: a role for the breast cancer susceptibility locus NBCn1 (SLC4A7). *Int J Cancer*. 2013;132(6):1288-1299. doi:10.1002/ijc.27782
63. Lee S, Mele M, Vahl P, Christiansen PM, Jensen VED, Boedtkjer E. Na+,HCO3- -cotransport is functionally upregulated during human breast carcinogenesis and required for the inverted pH gradient across the plasma membrane. *Pflugers Arch*. 2015;467(2):367-377. doi:10.1007/s00424-014-1524-0
64. Lee S, Axelsen TV, Andersen AP, Vahl P, Pedersen SF, Boedtkjer E. Disrupting Na^+, HCO_3^--cotransporter NBCn1 (Slc4a7) delays murine breast cancer development. *Oncogene*. 2016;35(16):2112-2122. doi:10.1038/onc.2015.273
65. Lee S, Axelsen TV, Jessen N, Pedersen SF, Vahl P, Boedtkjer E. Na+,HCO3--cotransporter NBCn1 (Slc4a7) accelerates ErbB2-induced breast cancer development and tumor growth in mice. *Oncogene*. 2018;37(41):5569-5584. doi:10.1038/s41388-018-0353-6
66. Intracellular pH modulation of ADF/cofilin proteins - Bernstein - 2000 - Cell Motility - Wiley Online Library. Accessed October 29, 2025. https://onlinelibrary.wiley.com/doi/abs/10.1002/1097-0169%28200012%2947%3A4%3C319%3A%3AAID-CM6%3E3.0.CO%3B2-I
67. Kumar AP, Quake AL, Chang MKX, et al. Repression of NHE1 Expression by PPARγ Activation Is a Potential New Approach for Specific Inhibition of the Growth of Tumor Cells In vitro and In vivo. *Cancer Res*. 2009;69(22):8636-8644. doi:10.1158/0008-5472.CAN-09-0219
68. Miraglia E, Viarisio D, Riganti C, Costamagna C, Ghigo D, Bosia A. Na^+ /H^+ exchanger activity is increased in doxorubicin-resistant human colon cancer cells and its modulation modifies the sensitivity of the cells to doxorubicin. *Int J Cancer*. 2005;115(6):924-929. doi:10.1002/ijc.20959
69. Sloth RA, Axelsen TV, Espejo MS, et al. Loss of RPTPγ primes breast tissue for acid extrusion, promotes malignant transformation and results in early tumour recurrence and shortened survival. *Br J Cancer*. 2022;127(7):1226-1238. doi:10.1038/s41416-022-01911-6
70. Zhou Y, Skelton LA, Xu L, Chandler MP, Berthiaume JM, Boron WF. Role of Receptor Protein Tyrosine Phosphatase γ in Sensing Extracellular CO2 and HCO3. *J Am Soc Nephrol JASN*. 2016;27(9):2616-2621. doi:10.1681/ASN.2015040439
71. Boedtkjer E, Hansen KB, Boedtkjer DMB, Aalkjaer C, Boron WF. Extracellular HCO3- is sensed by mouse cerebral arteries: Regulation of tone by receptor protein tyrosine phosphatase γ. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab*. 2016;36(5):965-980. doi:10.1177/0271678X15610787
72. Khramtsov V, Grigor'ev I, Foster M, Lurie DJ, Nicholson I. Biological applications of spin pH probes. *Cell Mol Biol*. Published online December 1, 2000. Accessed October 29, 2025. https://www.semanticscholar.org/paper/Biological-applications-of-spin-pH-probes.-Khramtsov-Grigor%E2%80%99ev/7ec48d8b31b72163cb7f467d793a8c989f6dac88
73. Gillies RJ, Morse DL. In vivo magnetic resonance spectroscopy in cancer. *Annu Rev Biomed Eng*. 2005;7:287-326. doi:10.1146/annurev.bioeng.7.060804.100411
74. Tognarelli JM, Dawood M, Shariff MIF, et al. Magnetic Resonance Spectroscopy: Principles and Techniques: Lessons for Clinicians. *J Clin Exp Hepatol*. 2015;5(4):320-328. doi:10.1016/j.jceh.2015.10.006
75. Rhodes CJ. Magnetic Resonance Spectroscopy. *Sci Prog*. 2017;100(3):241-292. doi:10.3184/003685017X14993478654307
76. Aime S, Botta M, Milone L, Terreno E. Paramagnetic complexes as novel NMR pH indicators. *Chem Commun*. 1996;(11):1265-1266. doi:10.1039/CC9960001265

 doi:10.20944/preprints202511.0512.v1

77. Bitencourt AGV, Goldberg J, Pinker K, Thakur SB. Clinical applications of breast cancer metabolomics using high-resolution magic angle spinning proton magnetic resonance spectroscopy (HRMAS 1H MRS): systematic scoping review. *Metabolomics Off J Metabolomic Soc*. 2019;15(11):148. doi:10.1007/s11306-019-1611-5
78. Choline metabolism in malignant transformation | Nature Reviews Cancer. Accessed October 29, 2025. https://www.nature.com/articles/nrc3162
79. Sharma U, Jagannathan NR. In vivo MR spectroscopy for breast cancer diagnosis. *BJR Open*. 2019;1(1):20180040. doi:10.1259/bjro.20180040
80. Mellon EA, Lee SC, Pickup S, et al. Detection of Lactate with a Hadamard Slice Selected, Selective Multiple Quantum Coherence, Chemical Shift Imaging Sequence (HDMD-SelMQC-CSI) on a clinical MRI scanner: Application to Tumors and Muscle Ischemia. *Magn Reson Med Off J Soc Magn Reson Med Soc Magn Reson Med*. 2009;62(6):1404-1413. doi:10.1002/mrm.22141
81. Maudsley AA, Darkazanli A, Alger JR, et al. Comprehensive processing, display and analysis for in vivo MR spectroscopic imaging. *NMR Biomed*. 2006;19(4):492-503. doi:10.1002/nbm.1025
82. He Q, Shungu DC, van Zijl PC, Bhujwalla ZM, Glickson JD. Single-scan in vivo lactate editing with complete lipid and water suppression by selective multiple-quantum-coherence transfer (Sel-MQC) with application to tumors. *J Magn Reson B*. 1995;106(3):203-211. doi:10.1006/jmrb.1995.1035
83. Thakur SB, Yaligar J, Koutcher JA. In vivo lactate signal enhancement using binomial spectral-selective pulses in selective MQ coherence (SS-SelMQC) spectroscopy. *Magn Reson Med*. 2009;62(3):591-598. doi:10.1002/mrm.22065
84. Payne GS, Harris LM, Cairns GS, et al. Validating a robust double-quantum-filtered (1) H MRS lactate measurement method in high-grade brain tumours. *NMR Biomed*. 2016;29(10):1420-1426. doi:10.1002/nbm.3587
85. Thakur S, Annarao S, Bokacheva L, Oh JH. Measurement of lactate concentrations in the breast mammary tumors using selective multiple quantum coherence editing sequence at 4.7 T. In: *Joint Annual Meeting ISMRM-ESMRMB. Milano, Italy: International Society of Magnetic Resonance in Medicine*. 2014. Accessed October 29, 2025. https://archive.ismrm.org/2014/3774.html
86. Rizwan A, Serganova I, Khanin R, et al. Relationships between LDH-A, Lactate and Metastases in 4T1 Breast Tumors. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2013;19(18):10.1158/1078-0432.CCR-12-3300. doi:10.1158/1078-0432.CCR-12-3300
87. Serganova I, Rizwan A, Ni X, et al. Metabolic imaging: a link between lactate dehydrogenase A, lactate, and tumor phenotype. *Clin Cancer Res Off J Am Assoc Cancer Res*. 2011;17(19):6250-6261. doi:10.1158/1078-0432.CCR-11-0397
88. Cheung SM, Husain E, Masannat Y, et al. Lactate concentration in breast cancer using advanced magnetic resonance spectroscopy. *Br J Cancer*. 2020;123(2):261-267. doi:10.1038/s41416-020-0886-7
89. García-Martín ML, Hérigault G, Rémy C, et al. Mapping extracellular pH in rat brain gliomas in vivo by 1H magnetic resonance spectroscopic imaging: comparison with maps of metabolites. *Cancer Res*. 2001;61(17):6524-6531.
90. van Sluis R, Bhujwalla ZM, Raghunand N, et al. In vivo imaging of extracellular pH using 1H MRSI. *Magn Reson Med*. 1999;41(4):743-750. doi:10.1002/(sici)1522-2594(199904)41:4%3C743::aid-mrm13%3E3.0.co;2-z
91. Gil MS, Cruz F, Cerdán S, Ballesteros P. Imidazol-1-ylalkanoate esters and their corresponding acids. A novel series of extrinsic 1H NMR probes for intracellular pH. *Bioorg Med Chem Lett*. 1992;2(12):1717-1722. doi:10.1016/S0960-894X(00)80463-1
92. Gil S, Zaderenzo P, Cruz F, Cerdán S, Ballesteros P. Imidazol-1-ylalkanoic acids as extrinsic 1H NMR probes for the determination of intracellular pH, extracellular pH and cell volume. *Bioorg Med Chem*. 1994;2(5):305-314. doi:10.1016/s0968-0896(00)82186-0
93. Provent P, Benito M, Hiba B, et al. Serial In vivo Spectroscopic Nuclear Magnetic Resonance Imaging of Lactate and Extracellular pH in Rat Gliomas Shows Redistribution of Protons Away from Sites of Glycolysis. *Cancer Res*. 2007;67(16):7638-7645. doi:10.1158/0008-5472.CAN-06-3459
94. Carbonic anhydrase IX is a pH-stat that sets an acidic tumour extracellular pH in vivo | British Journal of Cancer. Accessed October 29, 2025. https://www.nature.com/articles/s41416-018-0216-5

 doi:10.20944/preprints202511.0512.v1

95. Bhujwalla ZM, Artemov D, Ballesteros P, Cerdan S, Gillies RJ, Solaiyappan M. Combined vascular and extracellular pH imaging of solid tumors. *NMR Biomed*. 2002;15(2):114-119. doi:10.1002/nbm.743
96. Haselgrove JC, Subramanian VH, Leigh JS, Gyulai L, Chance B. In vivo one-dimensional imaging of phosphorus metabolites by phosphorus-31 nuclear magnetic resonance. *Science*. 1983;220(4602):1170-1173. doi:10.1126/science.6857240
97. Podo F. Tumour phospholipid metabolism. *NMR Biomed*. 1999;12(7):413-439. doi:10.1002/(sici)1099-1492(199911)12:7%3C413::aid-nbm587%3E3.0.co;2-u
98. Glaholm J, Leach MO, Collins DJ, et al. In-vivo 31P magnetic resonance spectroscopy for monitoring treatment response in breast cancer. *Lancet Lond Engl*. 1989;1(8650):1326-1327. doi:10.1016/s0140-6736(89)92717-7
99. Leach MO, Verrill M, Glaholm J, et al. Measurements of human breast cancer using magnetic resonance spectroscopy: a review of clinical measurements and a report of localized 31P measurements of response to treatment. *NMR Biomed*. 1998;11(7):314-340. doi:10.1002/(sici)1099-1492(1998110)11:7%3C314::aid-nbm522%3E3.0.co;2-z
100. Arias-Mendoza F, Zakian K, Schwartz A, et al. Methodological standardization for a multi-institutional in vivo trial of localized 31P MR spectroscopy in human cancer research. In vitro and normal volunteer studies. *NMR Biomed*. 2004;17(6):382-391. doi:10.1002/nbm.915
101. Robey IF, Baggett BK, Kirkpatrick ND, et al. Bicarbonate Increases Tumor pH and Inhibits Spontaneous Metastases. *Cancer Res*. 2009;69(6):2260-2268. doi:10.1158/0008-5472.CAN-07-5575
102. Negendank W. Studies of human tumors by MRS: a review. *NMR Biomed*. 1992;5(5):303-324. doi:10.1002/nbm.1940050518
103. Chen LQ, Pagel MD. Evaluating pH in the Extracellular Tumor Microenvironment Using CEST MRI and Other Imaging Methods. *Adv Radiol*. 2015;2015:1-25. doi:10.1155/2015/206405
104. Van Zijl PCM, Yadav NN. Chemical exchange saturation transfer (CEST): What is in a name and what isn't? *Magn Reson Med*. 2011;65(4):927-948. doi:10.1002/mrm.22761
105. Cai K, Haris M, Singh A, et al. Magnetic resonance imaging of glutamate. *Nat Med*. 2012;18(2):302-306. doi:10.1038/nm.2615
106. Sherry AD, Woods M. Chemical exchange saturation transfer contrast agents for magnetic resonance imaging. *Annu Rev Biomed Eng*. 2008;10:391-411. doi:10.1146/annurev.bioeng.9.060906.151929
107. Song X, Airan RD, Arifin DR, et al. Label-free in vivo molecular imaging of underglycosylated mucin-1 expression in tumour cells. *Nat Commun*. 2015;6(1):6719. doi:10.1038/ncomms7719
108. Sun PZ, Lu J, Wu Y, Xiao G, Wu R. Evaluation of the dependence of CEST-EPI measurement on repetition time, RF irradiation duty cycle and imaging flip angle for enhanced pH sensitivity. *Phys Med Biol*. 2013;58(17):N229-N240. doi:10.1088/0031-9155/58/17/N229
109. Englander SW, Downer NW, Teitelbaum H. Hydrogen Exchange. *Annu Rev Biochem*. 1972;41(1):903-924. doi:10.1146/annurev.bi.41.070172.004351
110. Igarashi T, Kim H, Sun PZ. Detection of tissue pH with quantitative chemical exchange saturation transfer magnetic resonance imaging. *NMR Biomed*. 2023;36(6):e4711. doi:10.1002/nbm.4711
111. Anemone A, Consolino L, Conti L, et al. Tumour acidosis evaluated in vivo by MRI-CEST pH imaging reveals breast cancer metastatic potential. *Br J Cancer*. 2021;124(1):207-216. doi:10.1038/s41416-020-01173-0
112. Corrado A, Lorito N, Anemone A, et al. In vivo imaging of the spatial heterogeneity of intratumoral acidosis (pH) as a marker of the metastatic phenotype in breast cancer. *Breast Cancer Res*. 2025;27(1):112. doi:10.1186/s13058-025-02065-y
113. Chan KWY, Jiang L, Cheng M, et al. CEST-MRI detects metabolite levels altered by breast cancer cell aggressiveness and chemotherapy response. *NMR Biomed*. 2016;29(6):806-816. doi:10.1002/nbm.3526
114. Zhou J, Lal B, Wilson DA, Laterra J, Van Zijl PCM. Amide proton transfer (APT) contrast for imaging of brain tumors. *Magn Reson Med*. 2003;50(6):1120-1126. doi:10.1002/mrm.10651
115. Sun PZ, Wang E, Cheung JS, Zhang X, Benner T, Sorensen AG. Simulation and optimization of pulsed radio frequency irradiation scheme for chemical exchange saturation transfer (CEST) MRI—demonstration of pH-weighted pulsed-amide proton CEST MRI in an animal model of acute cerebral ischemia. *Magn Reson Med*. 2011;66(4):1042-1048. doi:10.1002/mrm.22894

 doi:10.20944/preprints202511.0512.v1

116. Sun PZ, Wang E, Cheung JS. Imaging acute ischemic tissue acidosis with pH-sensitive endogenous amide proton transfer (APT) MRI—Correction of tissue relaxation and concomitant RF irradiation effects toward mapping quantitative cerebral tissue pH. *NeuroImage*. 2012;60(1):1-6. doi:10.1016/j.neuroimage.2011.11.091
117. Zhou J, Payen JF, Wilson DA, Traystman RJ, van Zijl PCM. Using the amide proton signals of intracellular proteins and peptides to detect pH effects in MRI. *Nat Med*. 2003;9(8):1085-1090. doi:10.1038/nm907
118. Mori S, Eleff SM, Pilatus U, Mori N, van Zijl PC. Proton NMR spectroscopy of solvent-saturable resonances: a new approach to study pH effects in situ. *Magn Reson Med*. 1998;40(1):36-42. doi:10.1002/mrm.1910400105
119. Zhou J, Lal B, Wilson DA, Laterra J, van Zijl PCM. Amide proton transfer (APT) contrast for imaging of brain tumors. *Magn Reson Med*. 2003;50(6):1120-1126. doi:10.1002/mrm.10651
120. Zhou J, Heo HY, Knutsson L, van Zijl PCM, Jiang S. APT-weighted MRI: Techniques, current neuro applications, and challenging issues. *J Magn Reson Imaging JMRI*. 2019;50(2):347-364. doi:10.1002/jmri.26645
121. Yan K, Fu Z, Yang C, et al. Assessing Amide Proton Transfer (APT) MRI Contrast Origins in 9 L Gliosarcoma in the Rat Brain Using Proteomic Analysis. *Mol Imaging Biol*. 2015;17(4):479-487. doi:10.1007/s11307-015-0828-6
122. Lee DH, Heo HY, Zhang K, et al. Quantitative assessment of the effects of water proton concentration and water T1 changes on amide proton transfer (APT) and nuclear overhauser enhancement (NOE) MRI: The origin of the APT imaging signal in brain tumor. *Magn Reson Med*. 2017;77(2):855-863. doi:10.1002/mrm.26131
123. Xu M, Shan D, Zhang R, et al. Differentiation of breast cancer subtypes and correlation with biological status using functional magnetic resonance imaging: comparison with amide proton transfer-weighted imaging and diffusion-weighted imaging. *Quant Imaging Med Surg*. 2025;15(7):6102-6117. doi:10.21037/qims-24-2174
124. Lee RC, Boparai MS, Duong TQ. Detection of breast cancer lesions using APT weighted MRI: a systematic review. *J Transl Med*. 2025;23(1):141. doi:10.1186/s12967-025-06153-7
125. Liu Z, Wen J, Wang M, et al. Breast Amide Proton Transfer Imaging at 3 T: Diagnostic Performance and Association With Pathologic Characteristics. *J Magn Reson Imaging*. 2023;57(3):824-833. doi:10.1002/jmri.28335
126. Van Zijl PCM, Jones CK, Ren J, Malloy CR, Sherry AD. MRI detection of glycogen in vivo by using chemical exchange saturation transfer imaging (glycoCEST). *Proc Natl Acad Sci*. 2007;104(11):4359-4364. doi:10.1073/pnas.0700281104
127. Ling W, Regatte RR, Navon G, Jerschow A. Assessment of glycosaminoglycan concentration in vivo by chemical exchange-dependent saturation transfer (gagCEST). *Proc Natl Acad Sci*. 2008;105(7):2266-2270. doi:10.1073/pnas.0707666105
128. Cai K, Haris M, Singh A, et al. Magnetic resonance imaging of glutamate. *Nat Med*. 2012;18(2):302-306. doi:10.1038/nm.2615
129. Zhou R, Bagga P, Nath K, Hariharan H, Mankoff DA, Reddy R. Glutamate-Weighted Chemical Exchange Saturation Transfer Magnetic Resonance Imaging Detects Glutaminase Inhibition in a Mouse Model of Triple-Negative Breast Cancer. *Cancer Res*. 2018;78(19):5521-5526. doi:10.1158/0008-5472.CAN-17-3988
130. Ward KM, Balaban RS. Determination of pH using water protons and chemical exchange dependent saturation transfer (CEST). *Magn Reson Med*. 2000;44(5):799-802. doi:10.1002/1522-2594(200011)44:5%3C799::aid-mrm18%3E3.0.co;2-s
131. Longo DL, Dastrù W, Digilio G, et al. Iopamidol as a responsive MRI-chemical exchange saturation transfer contrast agent for pH mapping of kidneys: In vivo studies in mice at 7 T. *Magn Reson Med*. 2011;65(1):202-211. doi:10.1002/mrm.22608
132. Longo DL, Michelotti F, Consolino L, et al. In Vitro and In Vivo Assessment of Nonionic Iodinated Radiographic Molecules as Chemical Exchange Saturation Transfer Magnetic Resonance Imaging Tumor Perfusion Agents: *Invest Radiol*. 2016;51(3):155-162. doi:10.1097/RLI.0000000000000217
133. Chen LQ, Howison CM, Jeffery JJ, Robey IF, Kuo PH, Pagel MD. Evaluations of extracellular pH within in vivo tumors using acidoCEST MRI. *Magn Reson Med*. 2014;72(5):1408-1417. doi:10.1002/mrm.25053
134. Chen M, Chen C, Shen Z, et al. Extracellular pH is a biomarker enabling detection of breast cancer and liver cancer using CEST MRI. *Oncotarget*. 2017;8(28):45759-45767. doi:10.18632/oncotarget.17404

 doi:10.20944/preprints202511.0512.v1

135. Moon BF, Jones KM, Chen LQ, et al. A comparison of iopromide and iopamidol, two acidoCEST MRI contrast media that measure tumor extracellular pH. *Contrast Media Mol Imaging*. 2015;10(6):446-455. doi:10.1002/cmmi.1647
136. Wu R, Longo DL, Aime S, Sun PZ. Quantitative description of radiofrequency (RF) power-based ratiometric chemical exchange saturation transfer (CEST) pH imaging. *NMR Biomed*. 2015;28(5):555-565. doi:10.1002/nbm.3284
137. Wu R, Liu C, Liu PK, Sun PZ. Improved measurement of labile proton concentration-weighted chemical exchange rate (k_{ws}) with experimental factor-compensated and T_1 -normalized quantitative chemical exchange saturation transfer (CEST) MRI. *Contrast Media Mol Imaging*. 2012;7(4):384-389. doi:10.1002/cmmi.505
138. Sharma U, Jagannathan NR. Magnetic Resonance Imaging (MRI) and MR Spectroscopic Methods in Understanding Breast Cancer Biology and Metabolism. *Metabolites*. 2022;12(4):295. doi:10.3390/metabo12040295
139. Rata M, Giles SL, deSouza NM, Leach MO, Payne GS. Comparison of three reference methods for the measurement of intracellular pH using^{31} P MRS in healthy volunteers and patients with lymphoma. *NMR Biomed*. 2014;27(2):158-162. doi:10.1002/nbm.3047
140. Moon RB, Richards JH. Determination of intracellular pH by 31P magnetic resonance. *J Biol Chem*. 1973;248(20):7276-7278.
141. Stubbs M, Bhujwalla ZM, Tozer GM, et al. An assessment of 31P MRS as a method of measuring pH in rat tumours. *NMR Biomed*. 1992;5(6):351-359. doi:10.1002/nbm.1940050606
142. Ackerman JJ, Soto GE, Spees WM, Zhu Z, Evelhoch JL. The NMR chemical shift pH measurement revisited: analysis of error and modeling of a pH dependent reference. *Magn Reson Med*. 1996;36(5):674-683. doi:10.1002/mrm.1910360505
143. Oberhaensli RD, Hilton-Jones D, Bore PJ, Hands LJ, Rampling RP, Radda GK. Biochemical investigation of human tumours in vivo with phosphorus-31 magnetic resonance spectroscopy. *Lancet Lond Engl*. 1986;2(8497):8-11. doi:10.1016/s0140-6736(86)92558-4
144. Ng TC, Grundfest S, Vijayakumar S, et al. Therapeutic response of breast carcinoma monitored by 31P MRS in situ. *Magn Reson Med*. 1989;10(1):125-134. doi:10.1002/mrm.1910100112
145. Merchant TE, Thelissen GR, de Graaf PW, Den Otter W, Glonek T. Clinical magnetic resonance spectroscopy of human breast disease. *Invest Radiol*. 1991;26(12):1053-1059. doi:10.1097/00004424-199112000-00003
146. Twelves CJ, Porter DA, Lowry M, et al. Phosphorus-31 metabolism of post-menopausal breast cancer studied in vivo by magnetic resonance spectroscopy. *Br J Cancer*. 1994;69(6):1151-1156. doi:10.1038/bjc.1994.226
147. van der Kemp WJ, Stehouwer BL, Luijten PR, van den Bosch MA, Klomp DW. Detection of alterations in membrane metabolism during neoadjuvant chemotherapy in patients with breast cancer using phosphorus magnetic resonance spectroscopy at 7 Tesla. *SpringerPlus*. 2014;3:634. doi:10.1186/2193-1801-3-634
148. Li H. A Non-invasive way to Detect Phospholipid Metabolism of Cancer: In vivo31P-MRS. *J Radiol Radiat Ther*. 2024;12(1):1-7. doi:10.47739/2333-7095/1103
149. Ojugo AS, McSheehy PM, McIntyre DJ, et al. Measurement of the extracellular pH of solid tumours in mice by magnetic resonance spectroscopy: a comparison of exogenous (19)F and (31)P probes. *NMR Biomed*. 1999;12(8):495-504. doi:10.1002/(sici)1099-1492(199912)12:8%3C495::aid-nbm594%3E3.0.co;2-k
150. Gillies RJ, Raghunand N, Garcia-Martin ML, Gatenby RA. pH imaging. A review of pH measurement methods and applications in cancers. *IEEE Eng Med Biol Mag Q Mag Eng Med Biol Soc*. 2004;23(5):57-64. doi:10.1109/memb.2004.1360409
151. Deutsch CJ, Taylor JS. Intracellular pH as Measured by^{19} F NMRa. *Ann N Y Acad Sci*. 1987;508(1):33-47. doi:10.1111/j.1749-6632.1987.tb32892.x
152. Deutsch CJ, Taylor JS. New class of 19F pH indicators: fluoroanilines. *Biophys J*. 1989;55(4):799-804. doi:10.1016/S0006-3495(89)82879-6

153. Prior MJW, Maxwell RJ, Griffiths JR. Fluorine-19F NMR Spectroscopy and Imaging In-Vivo. In: Rudin M, ed. In-Vivo *Magnetic Resonance Spectroscopy III:* In-Vivo *MR Spectroscopy: Potential and Limitations*. Springer; 1992:101-130. doi:10.1007/978-3-642-77218-4_4
154. Mehta VD, Kulkarni PV, Mason RP, et al. 6-Fluoropyridoxol: A novel probe of cellular pH using^{19} F NMR spectroscopy. *FEBS Lett*. 1994;349(2):234-238. doi:10.1016/0014-5793(94)00675-X
155. Deutsch C, Taylor JS, Wilson DF. Regulation of intracellular pH by human peripheral blood lymphocytes as measured by 19F NMR. *Proc Natl Acad Sci U S A*. 1982;79(24):7944-7948. doi:10.1073/pnas.79.24.7944
156. Deutsch C, Taylor JS, Price M. pH homeostasis in human lymphocytes: modulation by ions and mitogen. *J Cell Biol*. 1984;98(3):885-893. doi:10.1083/jcb.98.3.885
157. Taylor JS, Deutsch C, McDonald GG, Wilson DF. Measurement of transmembrane pH gradients in human erythrocytes using 19F NMR. *Anal Biochem*. 1981;114(2):415-418. doi:10.1016/0003-2697(81)90504-2
158. Taylor JS, Deutsch C. Fluorinated alpha-methylamino acids as 19F NMR indicators of intracellular pH. *Biophys J*. 1983;43(3):261-267. doi:10.1016/S0006-3495(83)84349-5
159. Mason RP. Transmembrane pH gradients in vivo: measurements using fluorinated vitamin B6 derivatives. *Curr Med Chem*. 1999;6(6):481-499.
160. McSheehy PM, Seymour MT, Ojugo AS, et al. A pharmacokinetic and pharmacodynamic study in vivo of human HT29 tumours using 19F and 31P magnetic resonance spectroscopy. *Eur J Cancer Oxf Engl 1990*. 1997;33(14):2418-2427. doi:10.1016/s0959-8049(97)00336-5
161. Peterson KL, Srivastava K, Pierre VC. Fluorinated Paramagnetic Complexes: Sensitive and Responsive Probes for Magnetic Resonance Spectroscopy and Imaging. *Front Chem*. 2018;6:160. doi:10.3389/fchem.2018.00160
162. Mizukami S. Development of Molecular Imaging Tools to Investigate Protein Functions by Chemical Probe Design. *Chem Pharm Bull (Tokyo)*. 2011;59(12):1435-1446. doi:10.1248/cpb.59.1435
163. Carril M. Activatable probes for diagnosis and biomarker detection by MRI. *J Mater Chem B*. 2017;5(23):4332-4347. doi:10.1039/C7TB00093F
164. Oishi M, Sumitani S, Nagasaki Y. On−Off Regulation of^{19} F Magnetic Resonance Signals Based on pH-Sensitive PEGylated Nanogels for Potential Tumor-Specific Smart^{19} F MRI Probes. *Bioconjug Chem*. 2007;18(5):1379-1382. doi:10.1021/bc7002154
165. Yu JX, Cui W, Bourke VA, Mason RP. 6-Trifluoromethylpyridoxine: Novel^{19} F NMR pH Indicator for in Vivo Detection. *J Med Chem*. 2012;55(15):6814-6821. doi:10.1021/jm300520q
166. Chen S, Yang Y, Li H, Zhou X, Liu M. pH-Triggered Au-fluorescent mesoporous silica nanoparticles for^{19} F MR/fluorescent multimodal cancer cellular imaging. *Chem Commun*. 2014;50(3):283-285. doi:10.1039/C3CC47324D
167. Huang X, Huang G, Zhang S, et al. Multi-Chromatic pH-Activatable^{19} F-MRI Nanoprobes with Binary ON/OFF pH Transitions and Chemical-Shift Barcodes. *Angew Chem Int Ed*. 2013;52(31):8074-8078. doi:10.1002/anie.201301135
168. Li Y, Zhang H, Guo C, Hu G, Wang L. Multiresponsive Nanoprobes for Turn-On Fluorescence/^{19} F MRI Dual-Modal Imaging. *Anal Chem*. 2020;92(17):11739-11746. doi:10.1021/acs.analchem.0c01786
169. Zalewski M, Janasik D, Kapała A, et al. pH-Sensitive Polymethacrylates as Potential Contrast Agents in^{19} F MRI. *Macromol Chem Phys*. 2022;223(14):2200027. doi:10.1002/macp.202200027
170. Janasik D, Jasiński K, Węglarz WP, Nemec I, Jewula P, Krawczyk T. Ratiometric pH-Responsive^{19} F Magnetic Resonance Imaging Contrast Agents Based on Hydrazone Switches. *Anal Chem*. 2022;94(8):3427-3431. doi:10.1021/acs.analchem.1c04978
171. Chen L, Jiang Y, Xiong N, Fan Y, Lin H, Gao J. Sensitive Multichannel^{19} F Magnetic Resonance Imaging Enabled by Paramagnetic Fluorinated Ionic Liquid-Based Probes. *ACS Nano*. 2025;19(9):9061-9069. doi:10.1021/acsnano.4c17959
172. Deen SS, Rooney C, Shinozaki A, et al. Hyperpolarized Carbon 13 MRI: Clinical Applications and Future Directions in Oncology. *Radiol Imaging Cancer*. 2023;5(5):e230005. doi:10.1148/rycan.230005
173. Gallagher FA, Kettunen MI, Brindle KM. Imaging pH with hyperpolarized^{13} C. *NMR Biomed*. 2011;24(8):1006-1015. doi:10.1002/nbm.1742

 doi:10.20944/preprints202511.0512.v1

174. Woitek R, Brindle KM. Hyperpolarized Carbon-13 MRI in Breast Cancer. *Diagnostics*. 2023;13(13):2311. doi:10.3390/diagnostics13132311
175. Gallagher FA, Woitek R, McLean MA, et al. Imaging breast cancer using hyperpolarized carbon-13 MRI. *Proc Natl Acad Sci*. 2020;117(4):2092-2098. doi:10.1073/pnas.1913841117
176. Woitek R, McLean MA, Ursprung S, et al. Hyperpolarized Carbon-13 MRI for Early Response Assessment of Neoadjuvant Chemotherapy in Breast Cancer Patients. *Cancer Res*. 2021;81(23):6004-6017. doi:10.1158/0008-5472.CAN-21-1499
177. Comment A, Merritt ME. Hyperpolarized magnetic resonance as a sensitive detector of metabolic function. *Biochemistry*. 2014;53(47):7333-7357. doi:10.1021/bi501225t
178. Ravoori MK, Singh SP, Lee J, Bankson JA, Kundra V. In Vivo Assessment of Ovarian Tumor Response to Tyrosine Kinase Inhibitor Pazopanib by Using Hyperpolarized^{13} C-Pyruvate MR Spectroscopy and^{18} F-FDG PET/CT Imaging in a Mouse Model. *Radiology*. 2017;285(3):830-838. doi:10.1148/radiol.2017161772
179. Petersen S, Nagel L, Groß P, et al. In Vivo Molecular Imaging of Breast Cancer Metabolic Heterogeneity Using [1-13C]Pyruvate-d3 Hyperpolarized By Reversible Exchange With Parahydrogen. *Chemistry*. Preprint posted online August 1, 2024. doi:10.26434/chemrxiv-2024-qk0fh
180. Bakshi SF, Guz N, Zakharchenko A, et al. Nanoreactors based on DNAzyme-functionalized magnetic nanoparticles activated by magnetic field. *Nanoscale*. 2018;10(3):1356-1365. doi:10.1039/C7NR08581H
181. Ivanov YuD, Pleshakova TO, Malsagova KA, et al. Detection of marker miRNAs in plasma using SOI-NW biosensor. *Sens Actuators B Chem*. 2018;261:566-571. doi:10.1016/j.snb.2018.01.153
182. Dellian M, Helmlinger G, Yuan F, Jain R. Fluorescence ratio imaging of interstitial pH in solid tumours: effect of glucose on spatial and temporal gradients. *Br J Cancer*. 1996;74(8):1206-1215. doi:10.1038/bjc.1996.518
183. Van Geel I, Oppelaar H, Rijken P, et al. Vascular perfusion and hypoxic areas in RIF-1 tumours after photodynamic therapy. *Br J Cancer*. 1996;73(3):288-293. doi:10.1038/bjc.1996.51
184. Martin GR, Jain RK. Noninvasive measurement of interstitial pH profiles in normal and neoplastic tissue using fluorescence ratio imaging microscopy. *Cancer Res*. 1994;54(21):5670-5674.
185. Gatenby RA, Gawlinski ET, Gmitro AF, Kaylor B, Gillies RJ. Acid-Mediated Tumor Invasion: a Multidisciplinary Study. *Cancer Res*. 2006;66(10):5216-5223. doi:10.1158/0008-5472.CAN-05-4193
186. Hassan M, Riley J, Chernomordik V, et al. Fluorescence Lifetime Imaging System for In Vivo Studies. *Mol Imaging*. 2007;6(4):229-236.
187. Li C, Xia J, Wei X, Yan H, Si Z, Ju S. pH-Activated Near-Infrared Fluorescence Nanoprobe Imaging Tumors by Sensing the Acidic Microenvironment. *Adv Funct Mater*. 2010;20(14):2222-2230. doi:10.1002/adfm.201000038
188. Anemone A, Consolino L, Arena F, Capozza M, Longo DL. Imaging tumor acidosis: a survey of the available techniques for mapping in vivo tumor pH. *Cancer Metastasis Rev*. 2019;38(1-2):25-49. doi:10.1007/s10555-019-09782-9
189. Majd SM, Salimi A, Ghasemi F. An ultrasensitive detection of miRNA-155 in breast cancer via direct hybridization assay using two-dimensional molybdenum disulfide field-effect transistor biosensor. *Biosens Bioelectron*. 2018;105:6-13. doi:10.1016/j.bios.2018.01.009
190. Huang D, Swanson EA, Lin CP, et al. Optical Coherence Tomography. *Science*. 1991;254(5035):1178-1181. doi:10.1126/science.1957169
191. Veselinovic J, Li Z, Daggumati P, Seker E. Electrically Guided DNA Immobilization and Multiplexed DNA Detection with Nanoporous Gold Electrodes. *Nanomaterials*. 2018;8(5):351. doi:10.3390/nano8050351
192. Lin Y, Wu TY, Gmitro AF. Error analysis of ratiometric imaging of extracellular pH in a window chamber model. *J Biomed Opt*. 2012;17(4):046004. doi:10.1117/1.JBO.17.4.046004
193. Wang Y, Zhou K, Huang G, et al. A nanoparticle-based strategy for the imaging of a broad range of tumours by nonlinear amplification of microenvironment signals. *Nat Mater*. 2014;13(2):204-212. doi:10.1038/nmat3819
194. Molecular Imaging by Means of Multispectral Optoacoustic Tomography (MSOT) | Chemical Reviews. Accessed October 30, 2025. https://pubs.acs.org/doi/10.1021/cr9002566

 doi:10.20944/preprints202511.0512.v1

195. Su R, Ermilov S, Liopo A, Oraevsky A. Laser optoacoustic tomography: Towards new technology for biomedical diagnostics. *Nucl Instrum Methods Phys Res Sect Accel Spectrometers Detect Assoc Equip*. 2013;720:58-61. doi:10.1016/j.nima.2012.12.035
196. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol*. 2006;7(5):392-401. doi:10.1016/S1470-2045(06)70665-9
197. Chen Q, Liu X, Chen J, Zeng J, Cheng Z, Liu Z. A Self-Assembled Albumin-Based Nanoprobe for In Vivo Ratiometric Photoacoustic pH Imaging. *Adv Mater*. 2015;27(43):6820-6827. doi:10.1002/adma.201503194