

Contrast-enhanced magnetomotive ultrasound imaging (CE-MMUS) for colorectal cancer staging: Assessment of sensitivity and resolution to detect alterations in tissue stiffness

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Abstract— A key challenge in the treatment of colorectal cancer is identification of the sentinel draining lymph node. Magnetomotive ultrasound, MMUS, has identified lymph nodes in rat models: superparamagnetic iron oxide nanoparticles (SPIONs) accumulated in the lymph are forced to oscillate by an external magnetic field; the resulting axial displacement is recovered allowing structure delineation with potential to indicate alterations in tissue stiffness, but it is limited by small vibration amplitudes. We propose CE-MMUS using SPION loaded microbubbles (SPION-MBs) to enhance sensitivity, reduce toxicity, and offer additional diagnostic or perfusion information. Laser doppler vibrometry measurements were performed on SPION containing tissue mimicking material during magnetic excitation. These measurements show a vibration amplitude of $279 \pm 113 \mu\text{m}$ in a material with Young's modulus of $24.3 \pm 2.8 \text{ kPa}$, while the displacements were substantially larger, $426 \pm 9 \mu\text{m}$, in the softer material, with a Young's modulus of $9.6 \pm 0.8 \text{ kPa}$. Magnetic field measurement data was used to calibrate finite element modelling of both MMUS and CE-MMUS. SPION-MBs were shown to be capable of inducing larger tissue displacements under a given magnetic field than SPIONs alone, leading to axial displacements of up to 2.3x larger. A doubling in tissue stiffness (as may occur in cancer) reduces the vibration amplitude. Thus, there is potential for CE-MMUS to achieve improved stiffness sensitivity. Our aim is to define the potential contribution of CE-MMUS in colorectal cancer diagnosis and surgical guidance.

Keywords—magnetic microbubbles, contrast-enhanced magneto-motive ultrasound, elastography, colorectal cancer, SPION

I. INTRODUCTION

Colorectal cancer is the 2nd most common cause of cancer death in the UK, accounting for 10% of all cancer deaths (2016) [1] (2015) [2]. Treatment for rectal cancer often involves major resectional surgery that, in addition to carrying a significant risk of complications, may result in stoma; Both with the potential to substantially reduce a patient's quality of life. All patients undergo contrast CT and MRI imaging during pre-treatment staging, which remains limited in the detection and characterisation of lymph tissue. Furthermore, identification of the sentinel draining lymph, a key indicator of lymphatic spread is not possible leaving the surgeon to perform major surgery to ensure complete oncological treatment. Development of a sentinel node detection technique could potentially lead to targeted surgical resection, and improve quality of life, yet ensure effective oncological treatment.

Magneto-motive ultrasound (MMUS) offers a multimodal imaging alternative for sentinel lymph node detection [3]. Superparamagnetic iron oxide nanoparticles (SPIONs) drain through the lymph after intra-dermal injection and can be imaged using MRI. Excitation with a low frequency (4-16 Hz) electromagnet causes the SPIONs to oscillate, creating a low frequency tissue vibration that can be imaged using ultrasound (centre frequency 20-30 MHz). Despite the small amplitude of displacement involved (1-2 μm [4]), filtering for the known frequency of vibration provides a means of overcoming the diffraction limit of conventional ultrasound imaging such that tissues that have taken up SPIONs can be distinguished from their surroundings. Since the amplitude of tissue displacement will be influenced by stiffness, MMUS also has the potential to present diagnostic information. MMUS therefore has the

potential to replace dye and radioactive tracers for lymph node identification during surgery. This work proposes the use of microbubbles (MB) labelled with SPIONS (SPION-MBs) in conjunction with MMUS to generate larger tissue displacements capable of improving sensitivity of the technique which we refer to as contrast enhanced magneto-motive ultrasound (CE-MMUS). SPION-MBs have previously been developed and demonstrated for use in targeted drug delivery [5] and targeted imaging, as they can be guided to a desired location with the use of a permanent magnet [6], and this background magnetic field has been found to enhance MB cavitation amplitudes [7, 8]. MB have already been demonstrated as an effective contrast agent for the lymph system via intra-dermal injection [9]. However, their behaviour under an oscillating magnetic field is yet to be studied. We investigate how SPION-MBs can be used to generate larger tissue displacements as compared to SPIONS alone.

II. METHODS

A. Tissue Mimicking Material

Tissue mimicking material (TMM) was prepared by mixing polyvinyl alcohol, PVA (molecular weight 89,000-98,000, 99% hydrolyzed, Sigma-Aldrich UK company Ltd. Dorset, England), and 0.01 % benzalkonium (Sigma-Aldrich UK company Ltd. Dorset, England) in deionized water kept below the boiling point until completely dissolved, and then left to cool. Two concentrations were prepared, five and ten weight percent of PVA. Samples were prepared by transferring one millilitre of PVA solution into cylindrical moulds, with internal diameter of 17 mm. Three replicas were made of each concentration PVA, and 0.5 mg/mL of 10 nm diameter SPIONS (WHKS1S12, Liquid Research Ltd, Bangor, UK) was added and mixed to form a homogeneous distribution. The samples were then subjected to three 24 h freeze thaw cycles to form a cryogel (PVA-c) by crosslinking the polymer. The finished samples were stored in sealed plastic containers.

B. Compression testing

In order to evaluate the final stiffness, each sample was subjected to compression testing (Bose 3100, Framingham, Massachusetts, U.S.). The height was individually measured using a digital caliper, and the diameter assumed equal to the internal diameter of the mould. The samples were placed in contact with the load cell, and compressed by 0.01 mm. After ten minutes, a linear ramp was applied, compressing the sample to 5 % strain at a rate of 0.02 mm per second. Force as a function of displacement was exported and scaled by surface area and initial height to obtain stress and strain, from which Young's Modulus could be estimated using the slope of a linear regression.

C. Magnetic Excitation

The TMM samples containing SPIONS were subject to magnetic excitation from an in-house built electromagnetic solenoid, see Fig. 1. The predominating, axial force on a single SPION can be approximated by

$$F_n = \frac{V_m \chi_n}{\mu_0} B_z \frac{\partial B_z}{\partial z} \quad (1)$$

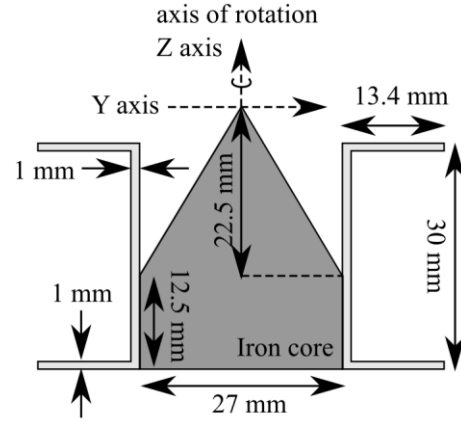


Figure 1. Cross-sectional schematic of the solenoid core (annealed soft iron, SEKELS GmbH) and spool. Spool fits 480 turns of 0.75 mm diameter enameled copper wire.

where V_m is the magnetic volume of the SPION, χ_n is the magnetic susceptibility of the SPION, μ_0 is the permeability of free space, and B_z is the magnitude of the magnetic flux density in the Z axis direction.

A 20 Hz sinusoidal signal was amplified (Behringer EP4000) and used to drive the solenoid. The vertical component of the output magnetic field was mapped using a Hall probe with active area 0.2x0.2 mm connected to a gaussmeter (GM08, Hirst Magnetic Instruments Ltd, Cornwall, UK). A 2D scan over a 4x4 mm area, covering the core tip was conducted in 0.25 mm increments, as well as a 6 mm vertical scan above the centre point.

D. Laser Doppler Vibrometry

Driving the solenoid at 20 Hz will generate a 40 Hz magnetic force acting on the magnetic nanoparticles, see equation 1, but it can also create a mechanical vibration. In order to mechanically decouple the sample, it was placed on a holder just above the surface of the solenoid, underneath the laser Doppler vibrometer, LDV (Polytec, Waldbronn, Germany), scanning head. The laser spot was aligned with the core tip and the center of the sample before starting the measurement. A 3 V peak to peak signal, by 30 dB, was used to drive the solenoid, a typical measurement ran for 7 s. LDV measures velocity which was converted to displacement by integration.

E. Finite Element Modelling

A finite element modelling (FEM) software (COMSOL Multiphysics, Stockholm, Sweden) was used to simulate the effect of SPIONS organized in a bubble shell formation, as compared to homogeneously distributed. First, a model of the solenoid was constructed to simulate the field. Second, the computed field was used as an input to a second model containing either a single MB (diameter 1.2 μm , shell thickness 2 nm) with magnetic properties confined to the shell, or a sphere of the same radius with the same amount of magnetic material distributed throughout the volume. Poisson's ratio was set to 0.499, Young's modulus was 100 MPa for air and 60 kPa elsewhere, density of the surrounding material was 997 kg m^{-3} ,

1100 kg m⁻³ for the shell/sphere, and 11.2 kg m⁻³ for air. The electromagnetic properties used in the simulations are summarized in table 1.

TABLE I. ELECTROMAGNETIC PARAMETERS

Material	Material Property		
	Permeability (relative)	Permittivity (relative)	Conductivity (S/m)
Air	1	1	0
Copper	1	1	5.998x10 ⁷
Iron	2 x10 ⁶	1	1.12x10 ⁷

III. RESULTS

A. Magnetic Field Mapping

Measurements confirmed that the field was rotationally symmetrical, Fig. 2, with the peak value above the core tip of 1.38 T at a frequency of 20 Hz and 30 dB amplification. Furthermore, the field dropped off with vertical distance to the core tip, according to Fig. 4.

B. Compression Testing

The material response was predominantly linear at the rate and level of strain. Linear regression estimates of Young's Modulus, presented in Fig. 3, were 9.6 ± 0.8 for 5 % PVA-c and 24.3 ± 2.8 for 10 % PVA-c.

C. Laser Doppler Vibrometry

The largest vibration velocities and amplitudes were found at 40 Hz, double the excitation frequency. Out of the 6 samples containing SPIONs, the signal to noise ratio in 1 of each PVA concentration was inadequate. The softer 5 % PVAc samples display notably larger displacements, at 40 Hz the displacement of the 5 % PVA-c samples were $426.9 \pm 9 \mu\text{m}$ while the 10 % PVA-c samples displaced on average $278 \pm 113 \mu\text{m}$ at the same frequency.

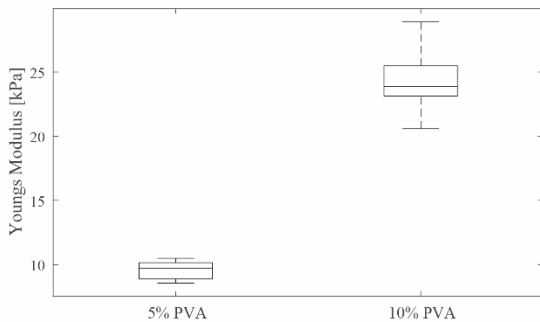


Figure 3. Box plot of estimated Young's modulus from the slope of regression lines. Compression testing was carried out directly after the samples had completely defrosted, and repeated 6 hours later.

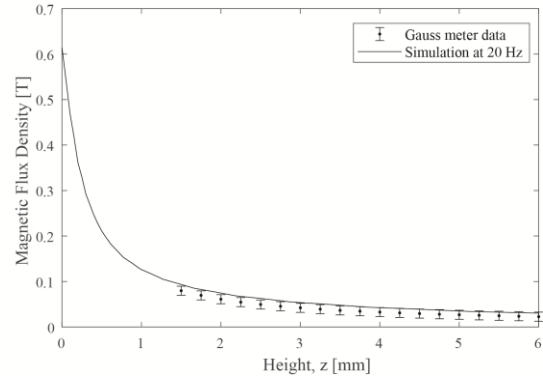


Figure 4. Magnetic flux density measured along the centre line of the solenoid and simulated. A 10 dB amplification was used.

D. Finite Element Results

The simulated field at 20 Hz, Fig. 4, was similar to the measurement and 0.01 T higher throughout along the centerline.

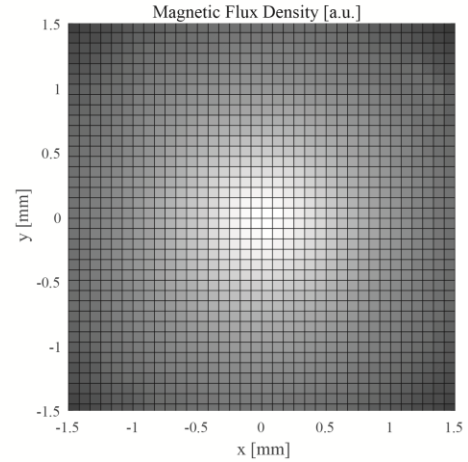


Figure 2. Normalized magnetic flux density measured above the core tip.

The second part of the simulation, comparing the displacement of a single solid sphere with that of a MB, indicate that the geometric configuration of the magnetic material has an effect on the resulting displacement. The simulated displacement was 2.3x larger when the magnetic material was located in a shell as compared to homogeneously distributed.

IV. DISCUSSION AND CONCLUSION

The results indicate that the development of MBs for MMUS could increase the vibration amplitude, and thereby also the sensitivity and range of tissue stiffness sensing.

The amplitude of vibration depends on the viscoelastic properties of the tissue, or in this case TMM. The displacement amplitude was found to be significantly smaller in the stiffer samples. The increase in Young's modulus from 9.6 to 24.3 kPa (153%), caused the displacement amplitude, as measured by LDV, to decrease by 35%.

Our simulation results show that the organization of magnetic material in a MB shell affects the induced vibration.

Furthermore, it suggests that the amplitude would be 2.3x larger in the MB case, as compared to a homogeneous distribution of magnetic material, which would directly affect the sensitivity. This implies that CE-MMUS would be more sensitive and would be capable of inducing a detectable movement in stiffer tissues, than MMUS using free SPIONs.

The different concentration of PVA in the TMMs were used to illustrate the relation between stiffness and vibration amplitude, but with only two concentrations, the results cannot be generalized. Furthermore, the analysis of the stress-strain data was done assuming a linear relation, and only extracting a single parameter, Young's modulus. While the linear assumption was valid in this regime, it is likely that mechanical non-linearity would influence the response at other frequencies. Therefore, characterizing the viscoelasticity would be preferable.

The excitation frequency of the solenoid was largely based on the sensitivity of the LDV system. In order to optimize the choice for future ultrasound applications, it is important to take the frequency response of the amplifier and solenoid into account, as well as heating of the solenoid. While the simulated field agreed well with the measurements at the settings used in this study, there are some discrepancies between the frequency response and linearity of the physical and the simulated system that would need to be accounted for, in order for the model to be valid at other driving currents.

Despite the driving frequency being selected with the LDV sensitivity in mind, the signal to noise ratio was only sufficient in four out of the six measurements. This could be due to the surface structure of the samples, and the ability to scatter light, causing the intensity of the returning laser reflection to be low. Using reflective paint on the top surface could alleviate this issue, and more repeats are necessary, in order to get more reliable measurements.

There are assumptions and simplifications in the model comparing MB to a solid sphere response. The mechanical parameters of the components are difficult to characterize, and depend on strain level, rate, temperature, ambient pressure, etc. The choice of these parameters influences the outcome in terms of vibration amplitude. Moreover, the susceptibility in equation 1, χ_n , is constant in the simulation, which is a valid assumption only when the magnetization of the particles is far from saturation.

The results are promising for the potential of CE-MMUS, indicating that smaller, stiffer inclusions can be made to oscillate, at an amplitude that is detectable by ultrasound.

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