Dynamic instrumented palpation (DIP) - a new method for soft tissue quality assessment: application to prostate disease diagnosis

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Prostate samples were obtained from a total of 36 patients who had undergone transurethral resection of the prostate (TURP) to relieve prostatic obstruction and 4 patients who had undergone radical cystoprostatectomy for bladder cancer. Specimens (chips) recovered from TURP were of nominal size of 5mm × 8mm and thicknesses between 2-4mm, whereas those from the cystoprostatectomy were in the form of transverse slices of thickness approximately 6 mm.

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Overall, the study has demonstrated that dynamic mechanical properties

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Fig16.docx
Fig17.docx
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Abstract
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Overall, the study has demonstrated that dynamic mechanical properties can potentially be used for diagnosis of prostate condition using in vivo measurements.
Background

Benign prostatic hyperplasia (BPH) is a common prostate disease affecting older men, with 40% presenting with benign prostatic obstruction (BPO) which may affect their quality of life [1]. Histologically, BPH is characterised by the proliferation of the glandular and stromal components of the prostate. BPO can cause troublesome symptoms such as poor urinary flow, hesitancy, urinary frequency during the day or night, or, in the extreme, complete urinary retention. Prostate cancer (PCa) accounts for about 10% for all cancer cases in the world [2]. Before the widespread use of prostate specific antigen (PSA) as a screening test, the incidence of PCa was increasing, but now the focus is on assessing the grade of cancer, both for monitoring purposes and to optimise treatment strategies [3]. Histopathologically, 70% of PCa cases arise in the peripheral zone (following McNeal’s zonal anatomy of the prostate [4]) and 20% occur in the transition zone that is also common to benign prostatic disease (BPH).

Digital rectal examination (DRE) allows clinicians to assess the prostate by palpating the posterior surface of the prostate via the rectum. Symmetrical enlargement with a smooth and elastic consistency is consistent with benign enlargement of the gland. However, prostate cancer may result in a gland which can be nodular, stony, hard and asymmetrical. Further clinical investigations such as urinalysis, blood tests, uroflowmetry, transrectal ultrasound (TRUS) or prostate biopsy may be warranted depending upon the initial clinical history and physical examination.

The challenge remains to detect and differentiate BPH and PCa at the earliest possible stage, and techniques which can be applied relatively regularly without excessive discomfort are useful. The increase in size of the gland in BPH is reflected in an increase in the stromal component and also a greater stromal/epithelial ratio [2]. It is thought that BPO occurs as a consequence of static and dynamic components resulting from the bulk of adenoma (mass effect of the enlarged prostate upon the urethra) and the tone within the prostatic smooth muscle, respectively [5, 6]. Also BPO can occur in men with large or small prostates [7]. Early symptoms of PCa are rarely seen, as the lesion is likely to have arisen from the peripheral zone, and its enlargement is less likely to cause urinary obstruction as seen in BPH. If PCa is suspected from DRE or a raised PSA, histological analysis using needle biopsy guided by transrectal ultrasound (TRUS) is recommended. If the histological testing confirms PCa, then the patient may require a bone scan or magnetic resonance imaging (MRI) of the pelvis as staging investigations. PCa is graded according to the system described by Gleason which allows classification in 5 different grades which reflect the prognosis of the disease. An important aspect of staging is to distinguish organ confined disease, potentially curable, from those cases where the disease has spread outwith the prostate capsule. Potentially curative treatments such as radical prostatectomy or radical radiotherapy are offered to patients with confined disease. Although TRUS and computed tomography (CT) can be used to visualise malignant lesions within the prostate, magnetic resonance imaging (MRI) is the most widely used modality for PCa staging although MRI has its limitations in its ability to detect extra-prostatic disease [8]. Therefore, the accurate staging of PCa remains one of the most difficult aspects of the management of PCa encountered by clinicians.

All of the foregoing indicates that palpation (and, by implication, instrumented palpation) can aid prostate diagnosis, although it would be naïve to expect that a single value of elastic modulus could be used to distinguish between normal, cancerous and hyperplastic
conditions even within an individual. The technique advocated here is dynamic instrumented palpation \cite{9}, which involves the measurement of the dynamic elastic modulus at a range of frequencies, the value and variation of the component of this modulus with frequency yielding a characteristic of the tissue. Since biological tissue is essentially made up of relatively watery and relatively elastic components, it is expected that the characteristic will be related to the histological structure of the tissue at a range of scales. The principle is illustrated on the LHS of Figure 1, where the phase difference and amplitude ratio between a controlled sinusoidal displacement and the resulting reaction force, yield a measure of dynamic stiffness. The make-up of the tissue can be probed at a variety of scales, where it would generally be expected that finer scale features would require higher frequencies to be revealed.

There are limited published findings on diagnostic uses of prostate tissue mechanical properties at slow strain rates, as opposed to those involving elastic wave propagation. The earliest are due to Krouskop \textit{et al.} \cite{10} who tested small pieces of prostate in compression using a sinusoidally varying strain at 0.1, 1 and 4 Hz with two different pre-strains, 2\% and 4\%, measuring the load in order to obtain a dynamic modulus. They did not observe significant phase differences between strain and load, so used the elastic modulus (as opposed to dynamic modulus) as a measure of tissue stiffness, and did not observe any systematic variations with frequency. “Normal” prostate samples had moduli of 40-85 kPa, whereas BPH samples had moduli of 30-55 kPa. Samples with PCa had higher moduli than either BPH or normal tissue, but there was a significant effect of the amount of pre-compression on the modulus; between 75 and 120 kPa for 2\% pre-compression and between 190-270 kPa for 4\% pre-compression. Phipps \textit{et al.} \cite{11} reported values of dynamic elastic modulus, \(|E'|\), between 20 kPa and 160 kPa measured on chips removed during trans-urethral resection of prostates of 17 patients with BPH. They used a specially-developed indentation test machine using a 2 mm diameter ball-end probe which oscillated the strain around a mean of around 20\% at frequencies between 1 and 50 Hz. They found a correlation (albeit a weak one) between the percentage of smooth muscle in the individual samples and \(|E'|\), although this correlation was considerably stronger in the samples which contained predominantly stromal tissue. In later work, Phipps \textit{et al.} \cite{12} observed statistically significant differences between prostate chips affected by cancer and those affected by BPH, with the mean \(|E'|\) rising from around 100 kPa for BPH to around 110 kPa for PCa. Within the same sample set the mean tangent of the phase angle between strain and stress fell from around 0.22 in BPH to around 0.15 in PCa. The same group \cite{Yang \textit{et al.}, 13} carried out point measurements on 6 mm thick transverse slices of human prostate recovered from cystectomy and radical prostatectomy using a similar method to \cite{11} and \cite{12}. They found \(|E'|\) to increase with frequency between 5 Hz and 30 Hz, whereas the tangent of the phase angle showed a more complex variation with frequency, decreasing from about 0.3 at 5 Hz to almost zero at 20 Hz before increasing sharply between 25 Hz and 30 Hz. The values of \(|E'|\) were significantly higher for the slices than for the chips, being in the region of 100-200 kPa for stromal areas and even higher for areas designated “epithelial”. The other major systematic study is by Zhang \textit{et al.} \cite{14} who tested 17 cores of diameter 8 mm and length 7 mm obtained from 8 patients. They measured stress relaxation at 5\% compressive strain and followed the curve over 700 s. Although they fitted the results to a Kelvin-Voight visco-elastic model, they calculated values of \(|E'|\) using these parameters for 150 Hz vibration frequency (for sonoelastography applications) citing values of 15.9±5.9 kPa for “normal” tissue and 40.4±15.7 for cancerous tissue. They also indicated that the ratio of storage modulus to loss modules (effectively the inverse of the tangent of the phase angle) was 2.8±0.3 for “normal” tissue and 2.7±0.3.
for cancerous tissue. Although they do not specifically cite elastic properties, Jalkanen et al. [15] have shown that PCa leads to changes in dynamic stiffness in transverse sections (10-15mm thick) taken from ten patients. More recently, the same group [16] have carried out preliminary studies on a whole excised prostate gland showing a statistically significant difference in stiffness between areas considered to contain cancer and other areas of the prostate. Finally, although it is not applied to prostate materials, some very recent work by Barnes et al. is worth noting here. These authors have obtained 10 specimens of human bladder tumours from 8 patients. The specimens were of various sizes and shapes but were roughly spherical to ovoid with a major diameter of around 10mm, and were tested in compression using a circular cylindrical plate of diameter 20mm and subjected to a sinusoidally varying displacement at frequencies between 0.01 and 30Hz. The mean storage modulus was found to vary between 50kPa and 80kPa over the frequency range, whilst the loss modulus was found to vary little from 20kPa in the range from 0.01 to 1Hz, and to increase to about 50kPa between 1 and 30Hz.

It is clear from the foregoing that dynamic modulus or dynamic stiffness offers a relatively non-invasive way of diagnosing soft tissue cancer in vivo, provided that a way of making the necessary measurements can be devised. However, it is also clear that a variety of parameters, such as specimen size, pre-strain and testing frequency can all affect results to an extent that outweighs the differences expressed by patient condition. Also, all researchers report considerable inter-patient variations making it valueless to cite a single value for the stiffness of cancerous (or non-cancerous) prostate. The purpose of the present paper, therefore, is to attempt to isolate the effects of test conditions from the patient-specific and condition-specific effects. To do this, reference will be made to tests on tissues obtained from 32 different patients using chips from trans-urethral resection of the prostate and slices of prostate obtained from radical cysto-prostatectomy procedures, and use will be made of histopathological measurements as well as diagnostic measures.

Prostate Samples
Specimens from a total of 32 patients, recruited specifically for this study were used, ethical approval having been obtained with the patients giving written informed consent. Two distinct sets of samples were obtained; chips recovered from trans-urethral resection of the prostate (TURP) and slices of whole prostate obtained from patients who had undergone radical cysto-prostatectomy (removal of bladder and prostate).

The TURP chips were of nominal size 5mm × 8mm and thicknesses from 3-4mm, the number obtained from each patient depending on the size of the resection tool and the total prostate volume to be removed. Immediately after removal, the tissue specimens were immersed in physiological buffer solution (PBS) at room temperature and transported to the laboratory for mechanical testing within 20 minutes of collection. 17 patients diagnosed with symptomatic BPH underwent TURP yielding a total of 84 samples. The 17 patients had a mean age of 71 years (range 58 to 84) and had a mean prostate-specific antigen (PSA) level of 6.8ng/mL (range 0.8 to 28 ng/mL). A total of 32 fresh tissue specimens were collected from 11 PCa patients undergoing TURP to relieve prostatic obstruction. These patients had a mean age of 75 years (range 67-84) and had a mean PSA level of 23ng/ml (range 0.5 – 75ng/ml). The PCa specimens contained at least 30% malignancy and were of Gleason sum 3+3 or greater, as assessed by a histopathologist.
A total 4 transverse slices were obtained from 4 patients undergoing radical
cystoprostatectomy for bladder cancer. Immediately after excision, the prostate and
bladder was transported to the pathology department where a transverse slice of
approximate thickness 6mm was taken from close to the bladder neck in order to include
the transition zone. The specimens were tested immediately. Although prostate cancer was
later detected in some of the patients, the tested sections were subsequently examined by a
histopathologist to confirm that no cancer was present in the areas tested. The patients had
a mean age of 72 years (range 62-80).

Experimental Procedures
The prostate specimens underwent cyclic compression at actuation frequencies between
5Hz and 30Hz, using a set-up “calibrated” on engineered phantom materials [9]. A ball-
nosed probe of diameter 2mm was used as the indenter and this was advanced to a pre-
strain of around 5% before oscillating the probe, whilst measuring the reaction force as a
function of time. For chips, the probe was centred on the specimen and a single-point
measurement taken, whereas, for slices, between 10 and 18 points were probed and
marked with ink so that they could be found later in the histological slides. The test set-up
is shown in Figure 1 alongside the diagrammatic representation of the DIP method.

The indenter position was converted to a nominal strain (as a percentage of the specimen
thickness) and the force converted to a nominal stress using a simple Hertzian contact
assumption. The raw stress and strain waveforms were then signal-averaged to obtain an
amplitude and a phase and the dynamic modulus expressed as amplitude ratio (|E*|) and
phase difference (tan δ). The mean effective modulus (E*) was also determined (mean
stress / mean strain) although this was not expected to vary significantly with frequency.

After mechanical testing, the specimens were fixed in 10% buffered formalin for at least
48 hours, embedded in paraffin and sectioned using a rotary microtome. Consecutive 4µm
thick sections from each sample were prepared and subjected to specialist staining to
reveal smooth muscle and epithelial cells (around the glandular acini), respectively, using
a protocol described elsewhere [11]. For chips, the entire slice fitted onto a single
microscope slide and yielded 60-100 frames at a magnification of ×200, deemed to be the
minimum magnification which would offer sufficient resolution for image analysis. For
the whole prostate slices, the process was a bit more complex, involving 3000-4000 frames
per slice, which also had to be stitched together to provide an image of the slice which had
been mechanically probed. Figure 2 shows sections from part of a slice stained to reveal
smooth muscle (left) and epithelial cells (right). The top half of the figure shows the entire
section, consisting of 759 frames, and the bottom half shows two different sample frames
after treatment by the image analyser to identify smooth muscle (left) and epithelial
regions (right). The image analyser assigned a percentage of smooth muscle (actually, the
myosin component, %MY) by calculating the area fraction stained brown. Because the
epithelial cells simply outline the gland lumen, this component (essentially a fluid-filled
bag including its envelope) was determined by filling in the area outlined by the epithelial
cells and a percentage of epithelial-lined gland (%EP) was assigned to each frame. As well
as each of these histological components, probe points on the slices were each given a
tissue condition classification; stromal (S) or nodular (N), both outlined in Figure 2, with
two classifications between these (mostly stromal, S?, or mostly nodular, N?). For chips,
the tissue classification was the condition for which the patient was being treated (i.e BPH
or PCa).
Results

Figures 3 to 7 show the results of mechanical testing of the prostate chips. The mean amplitude ratio of BPH chips was found to be between 83kPa and 92kPa with a standard deviation between 39kPa and 41kPa at actuation frequencies between 5Hz and 30Hz. From a perusal of Figure 3, it is clear that any systematic variation of amplitude ratio with frequency is small in the face of the inter-patient and inter-sample variations. By contrast, the mean phase difference (Figure 4) varied considerably across the range of actuation frequency, from around 0.2 at 5Hz, gradually falling almost to zero at 20Hz and then increasing to around 0.4 at 30Hz. The standard deviation was around 0.05 for actuation frequencies between 5Hz and 25Hz, and, for 30Hz, was 0.2. For PCa chips, the mean amplitude ratio was between 109kPa and 116kPa with a standard deviation between 53kPa and 58kPa, and, although the magnitude was higher than for BPH chips, again no change was evident with actuation frequency (Figure 5). The mean phase difference (Figure 5) exhibited a similar profile to BPH chips, but with generally lower values and with a larger scatter in the 5-25Hz range. Finally, the mean (over all frequencies) of the mean effective moduli for the BPH and PCa chips were 21.2kPa and 39.7kPa with standard deviations of 16.6kPa and 44.0kPa, respectively (Figure 7). ANOVA analyses showed that there was not only a statistically significant difference between the mean effective modulus of BPH and PCa chips, but also that there was a statistically significant difference between patients within a given class (BPH or PCa).

Figure 8 shows the most heterogeneous slice of the four examined, identifying the probe points and the values of $|E^*|$ and tan $\delta$ at 5Hz actuation frequency. The sections are stained for smooth muscle but one very large nodule is evident, along with a number of smaller ones.

Figures 9 and 10 show how the measured values of amplitude ratio and phase lag vary with frequency and classification for the points shown in Figure 8 and also for all measured points in all four slices (a total of 57 points). As can be seen, $|E^*|$ varies substantially with frequency and the variability both within groups (e.g. stromal and nodular) and between groups also increases with frequency. Generally, the stromal tissue has lower amplitude ratio than the nodular and the foregoing observations are evident (although less clear) when data from all four patients are considered. The variation of tan $\delta$ with frequency is rather similar to that for the chips, again with the scatter being rather higher at 30Hz. There is no clear stratification of nodular and stromal tissue in the phase lag. Again, the same effects can be seen when all the patient data are included. As expected, $\bar{E}$ did not vary significantly with frequency and the mean values (over all frequencies and all points) did not separate nodular from stromal tissue; $\bar{N} = 36kPa$, $\bar{N}^? = 48kPa$, $\bar{S}^? = 37kPa$ and $\bar{S} = 47kPa$.

Table 1 summarises the mean and range of the two histological indicators (epithelial and smooth muscle) per patient classification (PCa or BPH). Clearly, the small variations between the two conditions are dwarfed by the very large range in the means per patient.

<table>
<thead>
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<th>Component/condition</th>
<th>Mean (%)</th>
<th>Min (%)</th>
<th>Max (%)</th>
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<tr>
<td>%EP / PCa</td>
<td>16</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>%MY / PCa</td>
<td>28</td>
<td>16</td>
<td>37</td>
</tr>
<tr>
<td>%EP / BPH</td>
<td>19</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>%MY / BPH</td>
<td>26</td>
<td>11</td>
<td>45</td>
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</table>
Table 1: Summary histological indicators for prostate chips

Figure 11 shows the data as histograms over equal intervals in the ranges given in Table 1, which should be read in the context that the BPH histograms were generated from 84 samples involving 17 patients and the PCa histograms from 32 samples involving 11 patients.

The histological data for the prostate slices have two dimensions of variability, within patients (heterogeneity) and between patients (patient specificity). To assess the first of these, the largest of the prostate slices, which required four sections (for each stain) to cover its area, was analysed in detail. First, one representative S-area and one N-area were selected for each of the sections (between 252 and 437 frames) for each of the indicators. The %EP and %MY was determined for the S- and N-designated areas and for the whole section, with the results shown in Table 2. Although the expected result (higher %EP in N and higher %SM in S) is statistically significant, there is clearly quite a variation, particularly in the %EP, which is probably associated with the size of the features (of the order of the frame size, see Figure 2.)

<table>
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<th>Designation</th>
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<th>Section 3 (Mean ± SD)</th>
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<tr>
<td>%EP / N</td>
<td>58 ± 23</td>
<td>51 ± 27</td>
<td>36 ± 22</td>
<td>49 ± 22</td>
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<tr>
<td>%EP / S</td>
<td>24 ± 21</td>
<td>19 ± 16</td>
<td>24 ± 20</td>
<td>18 ± 15</td>
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<tr>
<td>%EP / Section</td>
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<td>31 ± 29</td>
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<tr>
<td>%MY / N</td>
<td>18 ± 11</td>
<td>15 ± 7</td>
<td>20 ± 11</td>
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<tr>
<td>%MY / S</td>
<td>42 ± 8</td>
<td>30 ± 5</td>
<td>37 ± 5</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>%MY / Section</td>
<td>34 ± 16</td>
<td>19 ± 9</td>
<td>27 ± 11</td>
<td>30 ± 13</td>
</tr>
</tbody>
</table>

Table 2: Summary histological indicators for one prostate slice

In order to assess the local histological features, an area of $7 \times 7$ frames ($4.9 \text{ mm} \times 3.5 \text{ mm}$) around each probe point was used, this being a compromise between precision of location (the probe is encompassed by about $7 \times 7$ frames) and the possibility that the testing could distort the surface histology. Figure 12 summarises the histological indicators for all of the probe points classified into two groups, predominantly nodular (N and N?) and predominantly stromal (S and S?). Clearly, the relationship observed in Table 2 is preserved across all of the patients for each of the probe points, although with less precision reflecting the local heterogeneity and patient specificity of the histological structure.

Structure-property-condition relationship
The results have used six different “condition indicators”, BPH and PCa for chips and N, N?, S and S? for slices, and two histological indicators %EP and %MY. In fact, the situation is more complicated than this, not only because the prostate is rather a heterogeneous organ but also because none of the indicators is, in itself, a complete description of the tissue even at a local level. Most importantly, prostate cancer can be more or less diffuse and is characterised by a lack of differentiation of cell types, whereas benign hyperplasia is characterised by a proliferation of the glandular component. Also, the conditions of BPH and PCa may coexist in a given individual and, in a sense, PCa is a
histological as well as a condition indicator, although the continuous histological indicator of PCa (Gleason Grade) cannot be determined at chip level.

Nevertheless, it is possible to discern structure-condition-property relationships for prostate tissue, which will help to establish the diagnostic power of DIP. Because the histological indicators are continuous (as opposed to the discrete nature of the condition indicators), it is possible to use correlation analysis for the structure-property relationship, although this needs to be seen through the filter of the statistical relationship between the structure and the condition established in the previous section.

For the prostate chips, the ability to distinguish between cancer and BPH was quantified using single-factor analysis of variance (ANOVA) where a statistical probability ($p$) is assigned to the likelihood that two sample sets of data are drawn from the same population. It is usual to consider $p$-values of 0.05 and lower to indicate that the samples are different.

Tables 3 to 5 show the test applied to data for each frequency for each of the mechanical indicators $|E^*|$ and $\tan \delta$. As can be seen from Tables 3 and 4 the mechanical properties of both BPH and PCa chips were significantly different between patients at all frequencies. Despite this (Table 5) the amplitude ratio was able to distinguish between PCa and BPH for all patients at all frequencies except 30 Hz. The phase difference was less good at making the distinction, being best at the extremes of frequency (5Hz and 30 Hz). Table 6 summarises the elastic properties for BPH and PCa as determined here (Figures 3 to 7) and, to facilitate comparison with the literature, the storage modulus $E' = \overline{E}\cos\delta$ and the loss modulus $E'' = \overline{E}\sin\delta$ have been calculated from the other measured constants.

| Actuation Frequency (Hz) | $|E^*|$ between prostates | $\tan \delta$ between prostates |
|--------------------------|---------------------------|---------------------------------|
| 5                        | 0.011                      | 0.023                           |
| 10                       | 0.007                      | 0.002                           |
| 15                       | 0.005                      | 0.002                           |
| 20                       | 0.002                      | 0.006                           |
| 25                       | 0.001                      | 0.002                           |
| 30                       | 0.001                      | $1.15 \times 10^{-7}$           |

Table 3: ANOVA results for amplitude ratio and phase difference between patients being treated for BPH

| Actuation Frequency (Hz) | $|E^*|$ between prostates | $\tan \delta$ between prostates |
|--------------------------|---------------------------|---------------------------------|
| 5                        | 0.016                      | 0.004                           |
| 10                       | 0.019                      | $2.64 \times 10^{-8}$           |
| 15                       | 0.013                      | $4.71 \times 10^{-10}$          |
| 20                       | 0.007                      | $3.18 \times 10^{-11}$          |
| 25                       | 0.005                      | $1.23 \times 10^{-10}$          |
| 30                       | 0.006                      | $4.59 \times 10^{-15}$          |

Table 4: ANOVA results for amplitude ratio and phase difference between patients being treated for PCa
Table 5: ANOVA results for amplitude ratio and phase difference between BPH and PCa for all patients

<table>
<thead>
<tr>
<th></th>
<th>BPH (5Hz)</th>
<th>PCa (5Hz)</th>
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<tr>
<td>$</td>
<td>E'</td>
<td>$ (kPa)</td>
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<tr>
<td>$\tan \delta$ (5Hz)</td>
<td>0.22</td>
<td>0.19</td>
</tr>
<tr>
<td>$E'$ (Pa) (5Hz)</td>
<td>20.8</td>
<td>39.0</td>
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<tr>
<td>$E''$ (Pa) (5Hz)</td>
<td>4.58</td>
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<td>$E$ (Pa)</td>
<td>21.3</td>
<td>39.7</td>
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</table>

Table 6: Mean values of elastic properties determined from chips (Figures 2 to 6)

Based on the ANOVA results, the frequency of 5Hz was chosen for the morphological correlation analysis of the BPH chips and, to avoid distorting the data too much with the heterogeneity of the organ, specimens from the bladder neck were excluded from the analysis. Table 7 summarises the various correlations and none of these is particularly strong, unsurprising since these data inherently contain in-patient and between-patient variations. Rather more success in identifying the relationship between morphology and properties was obtained when mean values for the mechanical and morphological data were used. Figures 13 and 14 show the two best correlations, between %MY and amplitude and phase lag, respectively, although it should be emphasised that each point in these figures has a variability in both the x- and y- dimensions. The relationship between smooth muscle percentage and amplitude ratio has been confined to stromal chips (i.e. those containing less than 15% glandular material) and is of the expected positive sense amounting to about 2kPa/%MY. For phase lag, there was an expected negative correlation with smooth muscle tending towards zero at zero glandular component (100%SM), which is as would be expected. The fact that this relationship is not seen as strongly between phase lag and %EP is attributed to the small size of the samples and the likelihood that water is expressed permanently from the whole sample under testing. All of this should be seen in the context that only part of the mechanical response (a particular frequency) has been selected and the probed volume includes material under the surface, which is not revealed in a histological section.

Table 7: Structure-property summary for chips from patients being treated for BPH (all properties measured at 5Hz)
When comparing the mechanical properties of the prostate slices to those obtained from TURP chips, it has to be acknowledged that the slices were taken close to the bladder neck to include the transition zone, where BPH first develops. In view of the evolutions shown in Figures 9 and 10, summary data of the elastic properties have been calculated at both 5 Hz and 30 Hz, shown in Table 8. The first thing to notice about these data is that all modulus values are considerably higher than either the PCa or BPH values measured on chips, and this gross difference is attributed mainly to the size effect where the larger volume of specimen leads to less water being expressed from the sample during testing. Somewhat counter-intuitively, the points classified as nodular have higher amplitude ratios and (marginally) smaller phase differences on average, although the mean effective modulus is marginally smaller for the nodular points.

<table>
<thead>
<tr>
<th>Mean property</th>
<th>Nodular (n = 37)</th>
<th>Stromal (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E'$ (kPa) (5Hz)</td>
<td>141</td>
<td>115</td>
</tr>
<tr>
<td>$\tan \delta$ (5Hz)</td>
<td>0.28</td>
<td>0.31</td>
</tr>
<tr>
<td>$E'$ (Pa) (5Hz)</td>
<td>281</td>
<td>310</td>
</tr>
<tr>
<td>$E''$ (Pa) (5Hz)</td>
<td>78.7</td>
<td>96.2</td>
</tr>
<tr>
<td>$</td>
<td>E'</td>
<td>$ (kPa) (30Hz)</td>
</tr>
<tr>
<td>$\tan \delta$ (30Hz)</td>
<td>0.41</td>
<td>0.45</td>
</tr>
<tr>
<td>$E'$ (Pa) (30Hz)</td>
<td>270</td>
<td>296</td>
</tr>
<tr>
<td>$E''$ (Pa) (30Hz)</td>
<td>111</td>
<td>133</td>
</tr>
<tr>
<td>$E$ (Pa)</td>
<td>292</td>
<td>325</td>
</tr>
</tbody>
</table>

Table 8: Mean values of elastic properties determined from slices (Figures 9 and 10)

Attempts to correlate mechanical properties individually with the histological measures proved ineffective with none of the mechanical variables at 5Hz or 30Hz showing a strong correlation with either %EP or %MY and Figures 15 to 17 explain why this might be as well as suggesting the way forward. First of all, the scatter plot of average %MY vs %EP for each measurement point (Figure 15) shows that, although the points classified as nodular have higher %EP, there is considerable overlap, especially in the classification N? Figure 14 gives one example of modulus classification, confirming the relatively low modulus of stromal tissue. This figure also shows that the higher modulus of nodular tissue (and the associated increase with percentage glandular component) is confined to a subset of the points probed, and excludes the points on very large nodules. It is possible that the acinar area could also have an influence since, for the same proportion of glandular material, a finer distribution might be expected to make the tissue stiffer due to the greater proportion of epithelium to lumen. Figure 17 confirms the (counter-intuitive) observation made from Table 8 that it is the stromal tissue which exhibits the greater phase lag, and this is especially evident at low volume fractions of glandular material at the probe point. Within material that is classified as nodular, however, there is a weak tendency for the expected increase in phase lag with percentage of glandular material at the probe point.

The foregoing discussion on the structure-property relationship as revealed by the prostate slices shows the inter-relationship between classification, constitution and mechanical properties to be complex and not (at least with the amount of data available) amenable to simple statistical analysis. In order to assess if a relationship exists at all, the data were subjected to a pattern recognition approach using Artificial Neural Networks (ANNs).
First, an ANN curve-fit was attempted. For a given point, the histological indicators (%SM and %EP) and the mechanical indicators ($|E^*|$ and tan $\delta$) were presented in various configurations to a proprietary Levenberg-Marquardt neural network curve fitting algorithm. Not too surprisingly, the single variable pattern recognition yielded little more than Figures 16 and 17 by way of identifying correlation. However, when both histological variables were used against $|E^*|$ and tan $\delta$ at 5Hz and 30Hz along with the mean of $E$ (i.e. 5 mechanical features) the correlations improved considerably, Figure 18, although the total number of sample points is rather small for the number of variables, and the use of individual patient sets gives unstable results.

An ANN was also used to investigate the degree to which the mechanical indicators could be used to predict the classification of the local areas probed. Again due to the limited number of probed points (even across all patients) it was necessary to reduce the number of targets to two (predominantly nodular, N and N?, and predominantly stromal, S and S?) to avoid instability in training of the network. For demonstration purposes, a simple feed-forward network with one hidden layer was used to train the entire pattern of mechanical indicators ($E$, $E^*$ and tan $\delta$ as a function of frequency, i.e. 15 indicators) to the targets. The results, shown in Figure 19, indicate good discrimination of the classification, even with such a limited data set.

**Discussion**

As indicated in the background, a wide range of values of dynamic mechanical properties of human prostate have been reported using different measurement conditions and sample configurations. Despite this, some clear patterns can be seen in the figures summarised in Table 9. The most obvious of these is the effect of time, with low frequency and/or quasi-static tests giving rise to substantially lower values of apparent elastic modulus. Somewhat surprisingly, though, this change is not reflected to a great degree in phase measurements. This is possibly due to the fact that measurement made on “open cell” tissue can express water thus masking the recovery effect that would occur in vivo.

The effect of disease on dynamic mechanical properties is a bit more elusive, with some authors reporting a substantial increase in modulus with cancer and a decrease with BPH, as one might intuitively expect. The current work has shown that actually there is a more robust and explicable effect of tissue microstructure, when classified along the lines of “nodular” and “stromal”, the stromal tissue having generally lower values of dynamic modulus and higher values of phase difference than nodular issue. With the current data set, it was not possible to confirm with any degree of certainty whether this relationship could be pursued down to histological component level, nor was there sufficient data to establish the effect of cancer load.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Modulus value(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krouskop et al.</td>
<td>$E$ 0.1-4Hz, 40-85kPa (normal), 30-55 (BPH), 75-270 (PCa)</td>
</tr>
<tr>
<td>Phipps et al.</td>
<td>$</td>
</tr>
<tr>
<td>Phipps et al.</td>
<td>$</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>$E^*$, $\tan(\delta)$ Stress relax, 10-22kPa, 0.3-0.4 (normal), 24-56, 0.3-0.4 (PCa)</td>
</tr>
</tbody>
</table>
In summary, given the differences in measurement conditions and sample configuration, the results here are consistent with other published data, although a more quantitative structure-property relationship must await measurements where distribution and grade of PCa are determined as well as mechanical properties.

The current work has several limitations, which need to be resolved before a reliable diagnostic device based on palpation can be developed:

- The palpated samples need to be closer to that experienced in vivo, either whole prostates or in vivo measurements
- A method needs to be devised for making the necessary force and displacement measurements and control in vivo
- The effect of probe size relative to the structural unit needs to be investigated
- The effect of probing frequency relative to the scale of fluid movements within the tissue needs to be established
- Much more data with quantifiably different disease states needs to be gathered, preferably under in vivo conditions
- The structure within the palpated volume (typically a hemisphere of radius around twice the indenter) needs to be established with greater precision than is possible with a single histological section at the surface

Conclusions
The paper has demonstrated that a new method, dynamic instrumented palpation, has the potential to be used as an in vivo assessment of the condition of human prostate. Detailed findings supporting this general conclusion follow:

- The dynamic elastic modulus can distinguish between morphologies (as quantified by the percentages of smooth muscle and glandular components in the tissue being tested) and between expert assessments of condition of the prostate globally (cancer or BPH) and locally (nodular or stromal).

- Within a given classification, measured mechanical properties vary significantly within and between patients. The within-patient variation can be largely attributed to regional morphology using simple correlations, but this is more difficult to assess with the current data set between patients.

- Measured mechanical properties depend critically on the size of the specimen relative to the volume of material being deformed by the indenter. This is attributed to the movement and confinement of fluid within the specimens and has considerable implications for in vivo applications.
The relationship between structure, condition and properties is a complex multivariate problem amenable to pattern recognition approaches, demonstrated here for multi-point probing of prostate slices across four different patients. A good correlation has been found between mechanical indicators and histological constitution and the pattern of mechanical measurements at a point can be used for classification of prostate condition. Thus, it is expected that multi-point measurements on the surface of whole prostates will require such analysis to establish the relationship and identify the significant structural and mechanical indicators.

Overall, the work provides a strong basis for the development of in vivo measurement devices, for which there is the added complexity of perfused tissue, support conditions of the prostate and the three-dimensional heterogeneity of the prostate.

Acknowledgements
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References


Figure 1: Measurement principle for dynamic instrumented palpation (DIP): (left) schematic of loading and structure of solid involving a modulated displacement and measurement of the reaction force, (right) realisation of the measurement and control for excised tissue specimens.
Figure 2: Histology and classification of prostate tissue. Top: 759-frame composite of consecutive sections from a prostate slice, left stained with anti-smooth muscle antibody and right stained with anti-PSA. Solid outline shows area classified as nodular and dotted outline shows area classified as stromal. Bottom: Image analyser interpretation of smooth muscle in a single 0.7mm×0.5mm frame (left) and of epithelial cells outlining glands (right).
Figure 3: Amplitude ratio for prostate chips from patients treated for BPH (17 patients, 84 samples)
Figure 4: Phase difference for prostate chips from patients treated for BPH (17 patients, 84 samples)
Figure 5: Amplitude ratio for prostate chips from patients treated for PCa (11 patients, 32 samples)
Figure 6: Phase difference for prostate chips from patients treated for PCa (11 patients, 32 samples)
Figure 7: Mean effective modulus vs patient condition for prostate chips
Figure 8: Whole prostate slice with anti-smooth muscle stain showing probe points
Figure 9: Variation of $|E'|$ with frequency: top for each of the points shown in Figure 7 and bottom for all on points on all sections. Key: $N =$ nodular, $N?$ = predominantly nodular, $S =$ stromal, $S?$ = predominantly stromal.
Figure 10: Variation of tan δ with frequency: top for each of the points shown in Figure 7 and bottom for all on points on all sections. Key: N = nodular, N? = predominantly nodular, S = stromal, S? = predominantly stromal.
Figure 11: Histograms of histological components: (a) epithelial / cancer, (b) epithelial / benign hyperplastic, (c) smooth muscle / cancer, (d) smooth muscle / benign hyperplastic.
Figure 12: Histological indicators for all prostate slice probe points. Nodules = N and N? and Stroma = S and S?
Figure 13: Correlation between mean amplitude ratio and mean percentage area of myosin for BPH chips with less than 15% of epithelial lumen (predominantly stromal tissue)

R² = 0.709
Figure 14: Correlation between mean phase difference and mean percentage area of myosin for BPH chips

R² = 0.578
Figure 15: Scatter plot of histological measures for each of the tissue condition classifications
Figure 18: Neural network curve fit of mean histological indicators (%SM and %EP) against mechanical indicators ($E'$ and $\tan \delta$ along with $E'$). Training on 31 samples, validation on 7 samples and testing on 7 samples, with $R$ value of 0.7.
Figure 19: Neural network analysis of condition classification (nodular or stromal) against mechanical indicators ($\varepsilon'$ and $\tan\delta$ and $\tilde{\varepsilon}$ as a function of frequency). Training on 39 samples, validation on 8 samples and testing on 8 samples.