Minimally Invasive Micro-Indentation

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MINIMALLY INVASIVE MICRO-INDENTATION

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Introduction to this thesis

This chapter is a preface to the thesis. It introduces the field of tissue mechanics and provides the reader with a brief overview of the relevance of tissue stiffness measurements and in situ tissue stiffness measurements in particular. This chapter also contains the scope and the outline of the thesis.

**Keywords:** Mechanical properties – *In situ* – Tissue stiffness – Scope and outline
Biological tissues can be characterized in many different ways. Whether one aims to differentiate between structure or function in a particular region in the human body or between normality and abnormality in case of trauma or disease, the main goal is always to find a proper form of contrast. This contrast can be provided intrinsically, for instance by means of attenuation of ultrasound or light, or by an external agent, such as radioactive tracers in positron emission tomography (PET) or single-photon emission computed tomography (SPECT). Tissue can be characterized based on qualitative contrast, which may rely on one or more characteristics, or quantitative contrast, which is based on a measured value and can be used to compare variables to other research. An example of a quantitative, intrinsic type of contrast is tissue mechanical contrast.

1.1 Tissue stiffness as a contrast

Biological tissues have been characterized in terms of their stiffness for hundreds of years. Stiffness, or rigidity, of a material is defined as the extent to which a material resists deformation in response to an applied force [1]. Stiffness is often reported as the elastic modulus and is the dominant component of the (bio)mechanical properties of a tissue. As illustrated by Figure 1.1, the mechanical properties vary markedly between organs and tissues and are inherently related to tissue function [2]. On the one hand, soft, compliant tissues such as brain or lung exhibit low stiffness, whereas, on the other hand, tissue which is exposed to high mechanical loading, such as bone, exhibit elastic moduli that are several orders of magnitude higher. It is thus evident that tissues can be characterized based on their mechanical properties alone [3, 4].

An elegant way to identify tissues based on their stiffness is by means of palpation [5]. The skill of palpation, i.e. the process of using one’s hand to examine the body, is practiced by physicians all over the world. In fact, as far back as in ancient Greece Hippocratic physicians recommended palpation of the patients abdomen to detect hardening or pain [4]. Nowadays, palpation is standard practice during a physical examination and is the main procedure for a wide variety of conditions, one example being early breast cancer diagnoses.

Although a very effective methods, palpation also has its drawbacks. First and foremost, palpation presents a qualitative result, meaning that the physician is unable to relate his/her observation to an absolute scale. The outcome may also rely greatly on the physician’s level of skill, increasing the method’s susceptibility for debate. In order to gain more scientific value, a quantitative result is required, allowing for comparison between different methods and enabling the generation of comprehensive images and maps [5]. Moreover, palpation measurements are limited to tissues accessible to the physician’s hand. This limitation restricts the method to superficial recordings and is characteristic for many state-of-the-art methods to characterize tissue mechanical properties (an overview of which can be found in Chapter 3).
1.2 The need for *in situ* stiffness measurements

The Latin phrase *in situ* translates literally to ‘on site’ or ‘in position’ and is used to describe where an event takes place. In an *in situ* experiment a phenomenon is examined exactly in the place where it occurs (e.g. observation of a single cell within a whole organ). An *in situ* stiffness measurement is performed on a sample while it is still within its original surroundings.

The mechanical properties of sensitive tissues are most likely dependent on their direct surroundings. When conditions such as confinement, osmolarity, or humidity are varied, for instance during extraction from their surroundings, the mechanical properties of the tissue may be altered [6]. This holds true for numerous tissues that, when inside the human body, are exposed to strain (such as skin, arteries, veins, cartilage), confinement (brain, eye, intervertebral disc), or swelling. Extraction of these highly sensitive tissues to perform stiffness measurements may cause structural changes which could generate unwanted artifacts. It is thus of paramount importance to develop instruments that could measure the viscoelastic response of a tissue without necessarily excising it [7].

Moreover, an *in situ* tool enables monitoring of tissue stiffness in its natural environment over time. One could, for example, follow the development of a tumor in terms of mechanical changes inside a 3D tissue volume or track the change of stiffness during induced degradation of cartilage inside a joint. Furthermore, by applying (experimental) treatments one may also follow their ability to reverse these traumas. Complex processes like tumor development are dependent on many variables and can often only be induced when the tissue can rely on its natural surroundings [6].

In situ stiffness characterization can also benefit the fabrication of biomaterials. Since tissue can be mechanically sensitive to their surroundings, it may be beneficial to mechanically characterize biomaterials designed for integration in the body in an environment that resembles their intended area, to avoid a possible mismatch between *in situ* and *ex situ* tissue mechanical properties.

![Figure 1.1: Variation of tissue stiffness in the human body][1]

[1]: image.png
1.3 Scope and relevance of the thesis

In this thesis the development of a new method to measure the mechanical properties of soft biological tissue \textit{in situ} is described. The method relies on the gentle probing of the sample material with a micro-machined force sensor – a procedure called micro-indentation – at the opening of a rigid needle. The force sensor is based on ferrule-top technology, earlier developed by our group. First, the principle of \textit{in situ} micro-indentation is investigated by developing and testing an upscaled prototype of the indenter. Afterwards, the dimensions of the device are significantly reduced and the method is improved to include a viscoelastic characterization of the target material. The new \textit{in situ} micro-indenter, combined with the versatility of the probe it is based on, has the potential to trigger an entire new generation of experiments that might enable a deeper understanding of the role of mechanics in physiology and tissue engineering.

1.4 Outline of the thesis

This thesis focuses on micro-indentation using ferrule-top technology. The main aim of the thesis is to develop a minimally invasive device that is able to characterize soft tissues on the basis of their mechanical properties. Several sensor miniaturization steps were required to successfully perform an \textit{in situ} measurement of tissue stiffness. The thesis is structured on the basis of these miniaturization steps:

- **Chapter 2** gives an introduction to fiber-top and ferrule-top technology. In this chapter the process that led to the development of the ferrule-top sensor is discussed. To give the reader an understanding of the probe, the current fabrication procedure of the sensor is described step by step. Afterwards, an overview of the interferometric readout scheme, including its linearization, is presented. Finally, several practical applications of ferrule-top devices are introduced.

- **Chapter 3** introduces the field of tissue mechanics. The different models that are used to estimate tissue elasticity and viscoelasticity in the literature are discussed, along with a presentation of conventional and state-of-the-art methods to measure tissue mechanics.

- **Chapter 4** presents an experimental calibration method for force transducers with interferometric readout. The method relies on the application of a constant pressure by the transducer on an analytical balance using a negative feedback loop. The loop allows one to keep the displacement of the transducer stable over time. The method requires only measurements of weights and laser wavelengths, both of which can be, in principle, referred to metrological standards.

- **Chapter 5** describes the first integration of a ferrule-top device in an \textit{in situ} tool, allowing the user to measure the Youngs Modulus of a material at the opening of a 5 mm diameter needle. The probe is actuated at the end of the needle by means of a
1.4 Outline of the thesis

steel cable that is controlled via a piezoelectric actuator located at the proximal end. The value of the device is demonstrated by measurements of samples with layers of varying stiffness.

Chapter 6 reports further miniaturization of the ferrule-top needle-based indenter to 1.3 mm diameter. This minimally invasive micro-indenter is used to map the viscoelastic properties of a complex, confined sample, namely, the nucleus pulposus of the intervertebral disc. After comparison with literature values, the findings show that the mechanical properties of a biological tissue in its local environment may be different than those that one would measure after excision.

Chapter 7 expands on the advantages of in situ measurements as introduced in chapter 6. Using the minimally invasive micro-indentation technique, an in situ rheological characterization is performed of the nucleus pulposus before, during and after axial loading of the intervertebral disc. An increase in storage modulus and a decrease of \( \tan(\phi) \) is found during the mechanical loading, suggesting an increase in stiffness due to a loss of liquid.

Chapter 8 presents a high throughput fabrication procedure for batch production of MEMS devices directly on top of an optical fiber. Using this new top-down approach, fiber-top sensors can be fabricated with a diameter of only 125 \( \mu \)m. We describe in details the 8 steps of the procedure and we show its application to the fabrication of several cantilever-based structures. Overall, we report a process yield of 80% functioning MEMS devices.

Chapter 9 is used to give an outlook to future research. Several new applications of minimally invasive micro-indentation are discussed. Additionally, some alterations to the design of the device are suggested. With the emphasis on miniaturization, an interesting alternative fabrication method for fiber-top probes is presented.

The main part of the thesis is followed by a general Summary of the work.

The last two chapters serve as an appendix to this thesis. In Appendix A, an alternative approach for interferometric force sensing on the tip of a needle is discussed. Several concepts for the design of a force sensor based on a fiber-optic Fabry-Pérot interferometer to measure needle-tissue interaction forces on the tip of a 18 G needle are investigated, where special attention is given to concepts for a sensor with (1) an intrinsic low cross-sensitivity to temperature and (2) elementary design and fabrication. In Appendix B a short summary of the batch production of a cantilever sensor on top of an optical fiber is presented.

A considerable part of the experiments described in this thesis were performed in collaboration with other research groups or industry partners. The work presented in this thesis was primarily funded by the Netherlands Organization for Scientific Research (NWO) under the banner of Applied and Engineering Sciences (TTW, former STW) and by the European Research Counsel (ERC).
Fiber-top and ferrule-top force sensors

In this chapter the process that led to the development of the ferrule-top sensor is discussed. To give the reader an understanding of the probe, the current fabrication procedure of the sensor is described step by step. Afterwards, an overview of the interferometric readout scheme, including its linearization, is presented. Finally, several practical applications of ferrule-top devices are introduced.

**Keywords:** Optical fiber sensor – Ferrule-top – Fabry-Pérot interferometer – Cantilever – Micro-indentation
2 Fiber-top and ferrule-top force sensors

Figure 2.1: Illustration of the difference in working principle between intrinsic and extrinsic OFS. A) Intrinsic OFS utilize the fiber itself for sensing purposes. B) Extrinsic OFS require an external transducer to record the parameter of interest.

2.1 Introduction

A sensor, in the broadest definition, is a component that transforms a physical signal, such as temperature or pressure, into an electronic signal [8]. Sensors can be seen as the interface between the physical world, in which we live, and the realm of electrical circuits, where everything relies on moving electrical charges. The digitalized signals can then be further processed, allowing us to interpret and quantify the physical phenomena around us. In other words, sensors can be seen as the eyes and ears of computers [8].

An example of a versatile, multi-purpose sensors are optical fiber sensor (OFS). Over the last few decades, optical sensors have spread rapidly in the scientific community. As with many scientific discoveries, the development of the optical sensor was tremendously pushed by industry [9]. Since the successful development of an optical fiber with an attenuation low enough for communication purposes in the early 1970s [10], in combination with the rise of compact GaAs semiconductor lasers at the same time [11, 12], the industrial demand for optical fiber components for telecommunication applications has resulted in superior performance and lower cost. Additionally, a second revolution emerged in the late 1970s as prices of optoelectronic components dropped, driven by the mass production of new commercial products such as compact disc players, personal copiers, and laser printers. These industrial efforts resulted in a tremendous scientific progress in development of new optical fiber sensors and techniques [13].

By measuring the various properties of light, including intensity, phase, polarization and wavelength, OFS can record a wide range of physical properties and they have, thus, become an interesting solution in many scientific and industrial applications [14]. Moreover, the multitude of advantages of OFS (e.g. high sensitivity, high resolution, high accuracy, mechanical stability, easy adaptation to harsh environments, small footprint, simple multiplexing) combined with the possibility of remote sensing in a very minimally invasive approach have made OFS a prime candidate for biomedical sensing applications.

Looking at their working principles, OFS can be distinguished in two main groups.
2.1 Introduction

Figure 2.2: Illustration of a simple FP cavity, consisting of two semi-transparent mirrors with reflectivity $R_1$ and $R_2$ separated by a cavity with length $d$, with $P_i$, $P_r$ and $P_t$ the incident, reflected, and transmitted optical power, respectively [24].

Intrinsic sensors use the optical fiber itself as sensing element, whereas extrinsic sensors use the fiber only to relay the signal of a remote (point) sensor to the electronics that process the signal (Figure 2.1). Intrinsic sensors can be divided in two subgroups: distributed and semi-distributed. In the former, the unmodified fiber is utilized as a whole by monitoring scattering (e.g. Rayleigh, Brillouin or Raman scattering). Using distributed sensing techniques a spatial resolution down to 8 mm can be achieved over more than 10 kilometers, with applications in, amongst others, monitoring of large structures such as bridges, dams or pipelines [15, 16]. Semi-distributed fiber sensing is mainly based on Fiber Bragg gratings (FBGs) and is typically employed over much shorter distances [9, 17]. Fiber sensing using FBGs is based on periodic longitudinal modulation of the refractive index in the fiber core, which can be tuned to reflect a specific wavelength [18]. The selectivity of the wavelength is dependent on the period of the modulation in the fiber core. This modulation pattern is sensitive to external disturbances, such as a change in stress or temperature, which will lead to a change in the characteristic wavelength, which, in turn, can be measured in reflection or transmission mode. FBGs have been primarily used for selective filtering applications in optical communication systems. Recently, however, FBGs have been increasingly employed as optical fiber sensors [19, 20]. Applications include needle shape sensing [21], seismologic sensing and pressure sensing in extremely harsh environments [22].

Extrinsic OFS are able to perform measurements only in a single point. The fiber is used solely to guide the light to a sensing region, which is generally located in close vicinity of the end of the fiber. Thanks to their many advantages, a large number of applications using extrinsic point sensors has been reported in the literature. Examples of applications reach from the medical field (e.g. optical coherence tomography), to chemistry (chemical sensing), industry (displacement monitoring in process lines) and the military (temperature sensing in engines) [9]. Extrinsic OFS are primarily based on Fabry-Pérot interferometers (FPIs). The core of an FPI consists of a cavity where light is reflected between two parallel surfaces, as illustrated in Figure 2.2. The smallest change in distance or refractive index of the cavity medium will cause a change in the sensors output signal. This change in distance or refractive index can be provoked by many different physical phenomena, making the FPI a very versatile tool for fiber sensing [23].
A decade ago, our group proposed a new kind of optical fiber point sensor, which was introduced as fiber-top technology [25]. The sensor is fabricated out of the material of the optical fiber itself and is focused around a Fabry-Pérot cavity. The device is based on a micro-machined cantilever suspended above the core of a single mode optical fiber, which is used to interrogate the position of the cantilever (Figure 2.3). In a series of publications, the versatility of the novel device was demonstrated by performing position and temperature sensing, hydrogen detection, and surface topology mapping [26–29]. Besides the versatility of the sensor, fiber-top devices offer several other distinct advantages. The all-optical readout offers high sensitivity and resolution in almost all optical transparent environments and is inherently immune to electromagnetic interference, unlike electronic readouts. Moreover, the micrometer dimensions of the sensor facilitate operation in very hard to reach locations and add to the biocompatibility of the device. Finally, the interferometric readout does not require any alignment, making the device truly a plug-and-play solution. This is particularly useful for applications where any manipulation of the sensor is unwanted.

The small size of the fiber-top sensor, however, is at the same time its greatest drawback. The precise fabrication of the cantilever requires the accuracy and precision of a laborious and expensive technique called focused ion beam (FIB) milling. This technique is time-consuming and unsuitable for series or batch production. Fiber-top sensors thus have to be produced one-by-one; an unpractical approach if one takes into account the fragile nature of the device.

To overcome this limitation, two alternative approaches have been proposed. Conventional micro-electro-mechanical systems (MEMS), such as the accelerometers in mobile phones or the micromirrors in projectors, are fabricated via a top down process of etching and photolithography [30]. A similar approach can be used to fabricate fiber-top MEMS devices directly on top of a cleaved optical fiber, as already showed by our group [31, 32]. By growing and patterning alternate layers of structural and sacrificial materials directly on top of an optical fiber, suspended cantilever transducers, with the same high quality as the FIB sensors, can be fabricated. The biggest advantage is that this process can now be performed in batches of up to 18 fibers at once. This method will be further discussed in Chapter 8.

Alternatively, the original approach of fiber-top probes can be upscaled with one
2.2 Fabrication of ferrule-top devices

The fabrication of ferrule-top devices can be divided in 5 important steps, as illustrated in Figure 2.4. In this section the fabrication of the probe is described in detail.

The building block of the ferrule-top probe is a 3x3x7 mm³ borosilicate glass ferrule (Vitrocom). The ferrule is equipped with a centered, cylindrical bore-hole with a diameter of 128µm, which can be used to house a stripped single mode optical fiber firmly underneath the cantilever. In step I, the ferrule is mounted in the wire cutter to carve a 3x0.4x0.4 mm³ ridge on the top facet of the ferrule using the diamond coated wire. The typical diameter of the wire during this operation is 250µm. Subsequently, a groove with a cross section of 0.2x0.4 mm² is machined along the side of the ferrule. The ferrule is then unmounted from the wire cutter and positioned under a microscope equipped with micrometer precision manipulators.
2 Fiber-top and ferrule-top force sensors

Figure 2.5: Schematic view of the interferometric readout. Light propagating through the fiber is reflected on mirrors $R_1$ and $R_2$, which causes the periodic readout signal on the detector.

In step II, a borosilicate ribbon (Vitrocom), previously coated with chromium (10 nm, for adhesion) and gold (100 nm), is lowered onto the ridge, precisely aligned with the groove (or bore-hole) and glued firmly on the ridge. The ribbons have a fixed thickness (i.e. 20 µm, 30 µm, or 40 µm), but can be adjusted in width and length in step III. Cyanoacrylate (CA) glue is used in all the gluing steps during fabrication. CA was found to be the most stable glue during drift and stress tests in air and liquid, performed with several epoxy and UV-curing glues.

In step III, the ferrule is mounted on a picosecond-laser ablation system (Optec System with Lumera Laser source) to cut the ribbon to a cantilever with the desired dimensions. On the backside of the ferrule the ribbon is cut over its full width just behind the ridge. Because of the high accuracy of the ablation process the length of the cantilever can be very precisely determined (5 µm resolution).

In step IV, a borosilicate sphere (radius equal to 75-150 µm) is glued at the tip of the cantilever.

Finally, in step V, a cleaved single mode optical fiber (SMF28, Corning) is slid and glued into the lateral groove (or the bore-hole). This fiber enables the interferometric readout of the cantilever position, as explained in the next section. The ferrule, the cantilever and the fiber are so well held together by the CA glue that the sensor can be treated as a single mechanical piece.

2.3 Interferometric readout and linearization

The readout principle of ferrule-top probes is based on Fabry-Pérot interferometry [36]. Interference occurs when two (or more) light waves of the same frequency interact. Interference can be constructive, when two waves form a new wave with a greater amplitude, or destructive, in which case the resultant wave has a lower amplitude. Interferometric readouts are used widely because of their stability, robustness and easy of use. Moreover, well-designed fiber optic interferometers are able to achieve readout resolution of well below 1 µm for bandwidths up to 100 kHz [37].

In case of the Fabry-Pérot interferometer, the interfering light waves are generated by two parallel semi-transparent mirrors [24,38]. The mirrors form an optical cavity, also called the Fabry-Pérot cavity or etalon, in which multiple light reflections occur. The light leaving the cavity forms an interference pattern that is characteristic for the
absolute distance between the two mirrors. In a ferrule-top device the Fabry-Pérot cavity is created between the cleaved end of the optical fiber and the reflecting bottom surface of the cantilever structure (Figure 2.5) [33]. An infrared laser (\(\lambda = 1550\) nm) is coupled to the fiber via an optical fiber coupler (see figure). A small part of the incoming light propagating through the optical fiber towards the cavity is reflected from the fiber-to-air interface via Fresnel reflection, forming the first interference beam. The remainder of the light travels through the cavity and reflects from the air-to-cantilever surface. This light travels through the cavity once more and is coupled back into the optical fiber, forming the second interference beam. The two beams propagating back through the fiber to the detector form an interference pattern, whose amplitude is measured by a detector aligned with the exit of the coupler. The interference pattern formed by the two beams in a lossless FPI can be described by [24]:

\[
I = I_0 \frac{R_1 + R_2 - 2\sqrt{R_1 R_2} \cos \phi}{1 + R_1 R_2 - 2\sqrt{R_1 R_2} \cos \phi},
\]

(2.1)

where \(I_0\) is the intensity of the light source, \(R_1\) and \(R_2\) are the reflectivity of both FPI mirrors, and \(\phi\) is the phase difference between the two beams, which is dependent on the cavity length \(d\) and is defined by:

\[
\phi = \frac{2\pi L}{\lambda} = \frac{4\pi nd}{\lambda},
\]

(2.2)

where \(L\) is the optical path length, \(n\) is the refractive index of the cavity medium and \(\lambda\) corresponds to the wavelength in vacuum.

The number of reflections inside the Fabry-Pérot cavity is characterized by the finesse of the FPI. The effect of the finesse on the interferometric signal is shown in Figure 2.6. In a high finesse FPI the light is able to reflect multiple times between the two mirrors, leading to an increased sensitivity for minute changes in the cavity length. However, the range over which the FPI is sensitive for cavity length changes decreases dramatically with increased finesse [24]. Therefore, ferrule-top devices are fabricated to work under low finesse conditions, where the reflectivity of the mirrors is tuned such that multiple reflections in the cavity can be neglected. For a low finesse cavity, assuming that \(R_1 \ll 1\) and \(R_2 = 1\), the intensity at the output of the interferometer is described by [24,25,36]:

\[
I(d) = I_0 \left[1 + V \cos \left(\frac{4\pi nd}{\lambda} + \varphi_0\right)\right],
\]

(2.3)

where \(\varphi_0\) is a constant phase shift that only depends on the geometry of the sensor.

The periodic nature of the output can be described to consecutive constructive and destructive interference. A single period of \(\phi = 4\pi nd/\lambda\) is referred to as a fringe. In equation 2.3, \(V\) represents the fringe visibility and is given by the maximum \((V_{\text{max}})\) and minimum \((V_{\text{min}})\) output of the fringe pattern [36]:

\[
V = \frac{V_{\text{max}} - V_{\text{min}}}{V_{\text{max}} + V_{\text{min}}},
\]

(2.4)
The fringe visibility largely determines the signal-to-noise ratio of FPI-based sensors and is dependent on the reflectivity of both FPI mirrors ($R_1$ and $R_2$) as well as the cavity length, cavity medium and the parallelism of the two mirrors [39].

FPI sensors can be interrogated using either high or low coherence light sources. Monochromatic light sources with a long coherence length, such as lasers, allow one to achieve high resolution measurements over long distances (as long as the optical path difference $L$ is less than the coherence length [24]), but the detection of cavity length changes is limited to half a fringe, due to the periodicity of the fringe pattern. In fact, to optimize sensitivity, one has to operate in close vicinity of the quadrature point, where a linear approximation of the periodic readout signal can be applied [24, 36]. On the other hand, low coherence light sources, such as superluminescent diodes, are not limited to single fringe operation and allow one to determine the absolute size of the cavity [40]. Unfortunately, low coherence methods lack the sensitivity that can be achieved with lasers.

The ferrule-top fabrication process, as described above, does not provide the accuracy needed to fabricate Fabry-Pérot cavities that inherently work in quadrature [41]. Therefore, our ferrule-top devices are interrogated by a laser with tunable wavelength. In this way, the FPI can be tuned to operate around quadrature at all times.

Although effective for small displacements around quadrature, the non-linearity of the output signal renders the method not ideal for the measurement of larger displacements. In applications such as micro-indentation, a linear readout is required over a large displacement ($d \gg \lambda$) [42]. In order to increase the dynamic range and linearize the amplitude response over the complete deflection range, the wavelength of the laser is modulated around the central wavelength ($\lambda_c$) according to:

$$\lambda(t) = \lambda_c + \delta \lambda \cos(\omega t),$$

where $\delta \lambda$ and $\omega$ represent the amplitude and frequency of the modulation, respectively. Assuming, for the sake of simplicity and without loss of generality, $\varphi_0 = 0$, the expected time dependent amplitude of the photodiode during wavelength modulation is given.
2.4 Applications of ferrule-top devices

by:

\[ W(t) = W_0 \left( 1 + V \cos \left[ \frac{4\pi d}{\lambda_c + \delta \lambda \cos(\omega t)} \right] \right). \] (2.6)

This time dependent response contains a DC component, encoding for the movement of the reflective surface, and a component that oscillates at frequency \( \omega \), originating from the modulation of the wavelength. The contribution of each component can be assessed in detail by making a first order Taylor expansion of \( W(t) \) around \( \frac{\delta \lambda \cos \omega t}{\lambda_c} = 0 \):

\[ W(t) \approx \cos \left( \frac{4\pi d}{\lambda_c} \right) + \left( \frac{4\pi d \delta \lambda \cos(\omega t)}{\lambda_c^2} \right) \sin \left( \frac{4\pi d}{\lambda_c} \right). \] (2.7)

The low frequency component (which we will denote with \( W_{dc} \)) and the high frequency component (which we will denote with \( W_\omega \)) are now described by the first and second term of the Taylor expansion, respectively. It can be readily observed from eq. 2.7 that \( W_{dc} \) and \( W_\omega \) are separated by a 90 deg phase shift. This particular relation allows one to linearize the output signal, as illustrated in Figure 2.7, and apply phase unwrapping to obtain a continuous linear response for the displacement of the mirror \( (D_m) \):

\[ D_m = \frac{\lambda_c}{4\pi} \arctan(W_{dc}/W_\omega). \] (2.8)

\( W_{dc} \) can then be recorded by means of a low-pass filter with a cut-off frequency below the modulation frequency. To record \( W_\omega \), the unfiltered amplitude response of the photodiode is sent to a lock-in amplifier, which is locked at frequency \( \omega \) via a square wave reference signal. We note that, thanks to the high bandwidth of our measurement, acquisition of \( W_{dc} \) and \( W_\omega \) and the following linearization of the signal is performed in real time. Using this method, a typical readout resolution of below 1 nm can be obtained for a 75 kHz bandwidth.

2.4 Applications of ferrule-top devices

Thanks to their versatility, ferrule-top devices have been used in a series of very diverse experiments. Primarily, ferrule-top sensors were design to measure very small forces, also known as Casimir forces, between a gold plated plate and a gold coated sphere under varying environmental conditions [43,44]. The ability to operate in harsh environments gives the ferrule-top sensors its advantage over traditional Casimir force equipment, which cannot easily adapt to different environments, ranging, for example, from low temperature vacuum to room temperature liquids. The robust operation of the ferrule-top probe was further employed to measure humidity, pressure, temperature and (acoustic) vibrations under various conditions [45–47].

Functionalization of the cantilever offers a new range of applications in chemical detection and biochemical recognition [48–50]. By coating the cantilever with a proper receptor that, upon contact with the target substance, gives rise to mechanical stress on the cantilever, ferrule-top sensors can be exploited as e.g. hydrogen sensors [27].
Figure 2.7: Linearization of an interferometric readout by wavelength modulation for increased dynamic range, demonstrated for small (A–C) and large (D–F) cavity length change. (A and D) Interferometer ($W_{dc}$, black) and wavelength modulation amplitude from the lock-in amplifier ($W_{\omega}$, red) show constant visibility with a 90 deg phase-shift. (B and E) Scaled $W_{dc}$ and $W_{\omega}$ signals form a circle whose phase directly corresponds to the change in cavity length (phase in rad). The unwrapped phase angle shows a linear relation to the deflection with high sensitivity over small (C) and large (F) deflections [42].

Surface topology, via atomic force microscopy, is another interesting application of ferrule-top technology. With a sharp tip mounted on the end of the cantilever, the probe can be scanned over the surface of a sample to obtain topological information [51, 52]. Additionally, spectral information of the sample can be obtained by coupling an optical fiber to the tip of the probe and performing scanning near-field optical microscopy [34, 53] or optical coherence tomography [54] directly through the tip.

Finally, ferrule-top cantilever can be used as all-optical photoacoustic spectrometers. In this application, the ferrule is equipped with two optical fibers, one for laser excitation of the gas and one for the interferometric readout of the transducer [55, 56]. By selecting the proper wavelength, selective molecules in the gas can be excited. The excited molecules create a pressure wave, the amplitude of which can be monitored by the highly sensitive Fabry-Pérot cavity. Using this approach, the concentration of various molecules in the gas can be recorded.

2.5 Ferrule-top micro-indentation

This thesis is focused on the application of ferrule-top micro-indentation. This application is directly related to atomic force microscopy [57]. Instead of scanning the probe over the surface, the tip of the probe is lightly pressed into the surface of a sample. Depending on the sample, the tip can be conical, cylindrical or spherical. The
2.6 Conclusions

Ferrule-top sensors are highly versatile devices at the tip of an optical fiber that rely on some of the distinct advantages of optical fiber sensors. Thanks to their all-optical nature, they are able to operate remotely, under harsh conditions, with a very small footprint and without laborious calibration procedures before each measurement. Using a linearized interferometric readout, centered around a Fabry-Pérot cavity, a position resolution below 1 nm can be obtained for 75 kHz bandwidth. Applications include temperature and pressure sensing, atomic force microscopy, photoacoustic spectroscopy and, the main topic of this thesis, micro-indentation.
Introduction to tissue mechanics

This chapter is meant as a brief introduction to the field of tissue mechanics. After a short discussion of the history and importance of biomechanics, an overview is given of the main methods to record mechanical properties of biological materials at different scales. The chapter continues with a description of the different models that are used to estimate tissue elasticity and viscoelasticity by means of indentation.

Keywords: Indentation models – Elastic modulus – Hertz – Adhesion – Stress – Creep – Dynamic Modulus
3.1 History and importance of mechanics

The mechanical properties of a material describe its behavior under pressure or force. These intrinsic properties can be related to function for many materials in both the plant and animal kingdoms. Structural skeletons in animals and plants provide strength and support (e.g., the spine or a tree trunk) and offer protection for delicate organisms or internal organs (e.g., ribcage, skull, shells).

The recent history of research on mechanical functioning of biological materials, specifically in the context of the human body, extends back to 1452, with Leonardo da Vinci. He pioneered many studies on the workings of the human skeleton, the vascular system, and other internal organs, studied and described whole-body motion, and designed experiments to observe the mechanical responses of organs and tissues.

The next major breakthrough in the field of biomechanics came in 1678 when Robert Hooke, who earlier coined the term cell for describing biological organisms, published his law of elasticity, which describes the linear relation between force and extension in an elastic spring. This discovery facilitated a quantification of the applied force, which enabled Leonhard Euler to develop the concept of Young’s modulus as a measure of the stiffness of a solid material in 1727. The method became widely accepted some 80 years later when Thomas Young described the characterization of elasticity in 1807.

Nowadays, the interest in mechanical properties of biological tissue is wide-spread and continues to grow [6,58], aided by the development of new tools and methods that are able to probe previously unaccessible characteristics of materials and tissues [59–64]. Earlier research has demonstrated that, besides their use as important tissue characterization parameter [4], mechanical properties can influence a wide range of physiological processes. Cell growth, cell signaling, tumor development and the related process of angiogenesis have been shown to be influenced by the stiffness of the surrounding extracellular matrix [65,66]. Furthermore, tumors are generally stiffer than the normal surrounding tissues, providing a possibility for early diagnosis [67]. Intrinsic cell stiffness as well as substrate flexibility play an important role in the migration of white blood cells. Moreover, gradients of substrate rigidity have shown to direct cell migration [68]. In wound healing, microdeformation of the wound bed can be exploited to maintain intense cell proliferation and angiogenesis in poorly healing wounds. On the contrary, shielding from mechanical forces in linear wounds can be used as a strategy to prevent of excessive scarring [69, 70]. Even a highly complicated process such as the differentiation of stem cells is sensitive to the stiffness of the extracellular environment [71–73].

In the field of tissue engineering, characterizing the mechanical properties of (bio)materials as well as mapping the local mechanics of the target area has proven invaluable for the development of artificial tissue constructs such as engineered cartilage or skin, as maintaining mechanical stability at the defect site of the host is of key importance [74,75].

Biomechanical experiments on brain tissue, one of the softest tissues of the human body, offer valuable insights in the mechanics behind the structural heterogeneity that forms the gray and white matter and understanding brain tissue mechanics will aid in unraveling tumor development, Alzheimer’s, and the brain’s response to traumatic
3.2 Methods to measure mechanical properties

The mechanical properties of biological tissues can be recorded over a broad range of length scales, as illustrated by Figure 3.1. The application of medical imaging techniques to map the mechanical properties of tissue, known as elastography, has been developed extensively over the past 25 years and is based mainly on ultrasound imaging (UI) and magnetic resonance imaging (MRI). Magnetic resonance elastography (MRE) [80] and ultrasound elastography (UE) are based on imaging the propagation of mechanically induced acoustic waves in tissue and have been applied for clinical use in cancer detection in vivo [81, 82]. However, the spatial resolution of UI and MRI, together with the macroscopic nature of the wave propagation in the tissue, limit the spatial scales of UE and MRE for elasticity imaging to a macroscopic level with organ-size field of view (∼100 µm and ∼1 mm for UE and MRI, respectively).

Using optics, imaging with a lower spatial resolution can be achieved at the...
expense of penetration depth. Optical coherence tomography (OCT) is a three-dimensional imaging modality with micrometer scale spatial resolution and millimeter scale penetration depth in scattering tissues [84]. Similarly to MRE, OCT elastography, also termed as optical coherence elastography (OCE), relies on the detection of localized tissue deformations induced by external stimulation [85]. Although the spatial resolution of OCE is promising, the technique is hampered by a lack of quantitative results for the elastic modulus [83]. Recently, significant improvements towards quantitative elasticity mapping with OCE have been obtained by mapping the induced stress in the tissue with the aid of a calibrated elastic element [86]. One can obtain a spatial resolution of 15-100 $\mu$m using OCE with a maximum imaging depth of 3 mm [7].

Several other optical elastographic methods have been developed based on optical imaging techniques and are compared with UE, MRE and OCE based on resolution and field of view in Figure 3.1. Laser speckle imaging [87], holohraphic imaging [88], confocal brillouin microscopy [64], optical tweezers [89] and multiphoton microscopy [90] all operate on their own length scale. Elastography measurements based on these optical techniques hold great potential for observation of elastic moduli on various spatial- and timescales.

Several other, more quantitative methods, rely on measurements of the (localized) stress inside a material under the influence of controlled displacement or force. These test can be performed in tensile, rotational or compressive motion and result in force-displacement curves, from which the elastic modulus of a material can be determined. In a tensile test a specimen is fixed between a force sensor and an actuator, which is used to elongate the sample to a fixed strain [91]. The (average) elastic modulus of the sample can then be determined from the resulting load-displacement curves. Using more sophisticated protocols, one can extract the frequency dependent elastic and viscous moduli of a sample by using shear rheology [92]. A rheometer controls the shear stress or shear strain, applied via rotary movement, and records the resulting strain or stress for each frequency. By recording the shear modulus over a broad range of frequencies, a quantitative viscoelastic analysis of soft tissue can be performed [93]. The main drawbacks of the rheometer are its spatial resolution (i.e. averaging of the elastic modulus over a large volume) and its inability to perform in situ or in vivo measurement of the elastic modulus.

Over the last decade, indentation has emerged as a leading technique for characterizing the mechanical behavior of materials. Its increased application for the study of biological materials can be ascribed to its adaptability, easy-of-use and its ability to combine localized, quantitative measurements with large-scale qualitative mapping of heterogeneous materials. During indentation a probe with a known shape is brought into contact with a flat surface of material, typically under load control [94]. The load and displacement are continuously monitored during the full loading and unloading contact cycle. Only the material in close proximity of the contact is mechanically tested, thus enabling examination of local variation of mechanical properties. Indentation testing can be performed over various length scales, from sub-nanometer level to several millimeters, with corresponding depth sampling [94]. Nano-indentation, using an atomic force microscope (AFM) [95], is able to mechanical characterize single cells [96,97] or even cellular components such as DNA [98] and proteins [99].
Using much larger probes, instrumented indentation has a footprint of at least 1 mm, thus operating at organ level, and can be more readily applied for in vivo measurements [100–102]. Situated between these two extremes, micro-indentation – the topic of this thesis – is generally based around tissue contact with tips with a diameter of 50-100 µm and therefore samples the mechanical behavior on the intermediate scale, between that of cells and that of organs (i.e. at the tissue level) [42]. The main drawback of indentation testing – its inability to reach below the surface – is also addressed in this thesis.

### 3.3 Indentation theory

Any well performed indentation experiment, independent on the probe size, results in force-displacement data of the sample. These results, however, are not absolute and depend on the test method. Therefore, to enable a more quantitative comparison, further data analysis is required.

For sufficiently small strains, a linear relation between stress and strain is given by Young’s Modulus $E$, for perpendicular, compressive force, and the Shear Modulus $G$ for force parallel to the contact area. Stress and strain are given by $\sigma = F/A$ and $\epsilon = (l - l_0)/l_0$, where $F$ is the applied force, $A$ is the area on which the force is applied, $l_0$ is the initial length of the material and $l$ is the stretched length. The range over which the relation between stress and strain is linear is referred to as the linear-elastic regime. Most indentation models are only valid in the linear-elastic regime, as they were originally designed for research on very hard materials such as metals and glass.

For the sake of simplicity this section is divided in static indentation models and dynamic indentation models. Static indentation models, such as the Oliver and Pharr model or the Hertz model, produce one elastic modulus, generally Young’s Modulus, per indentation. They are based on elastic-plastic deformation and do not incorporate viscoelasticity. Dynamic models can compute the frequency dependent elastic modulus – and in some cases the viscous modulus – and thus allow for a viscoelastic characterization of materials. Incorporation of a dynamic indentation model oftentimes requires adaptations to the indentation profile. In the next part of this section the most common indentation models are discussed.

#### 3.3.1 Static indentation models

This section treats models that assume only elastic deformation and potentially allow for plasticity. These models are so called static indentation models as they do not allow for viscoelasticity. They can be applied to any indentation with a fixed strain rate during loading and unloading. In particular, the Hertz and Oliver-Pharr models are discussed.

**The Oliver and Pharr model**

Warren Oliver and George Pharr published a landmark approach to derive the elastic modulus from indentation force-displacement data in 1992 [104]. Their straightforward mechanism soon became widely used and was later adapted to include indentation
Introduction to tissue mechanics

Figure 3.2: Schematic view of the elastic indentation of a flat plane surface with a small spherical indenter of radius $R$. Starting from zero load ($P = 0$), $P$ is increased until the maximum indentation depth ($h_{max}$) is reached. The contact radius ($a$) and the corresponding contact depth ($h_c$) depend on the depth of indentation. The inset shows a typical load-indentation curve along with the definition of the parameters used for the Oliver and Pharr analysis [103].

with a spherical tip [105]. It is important to recall that, to describe the indentation of an elastic material with a spherical indenter the contact area $A$ between the tip and the sample must be correctly modeled. A schematic of the contact between a spherical indenter and a flat planar surface is presented in Figure 3.2. The contact depth ($h_c$) of the sphere can be determined from the final and maximum indentation depths ($h_f$ and $h_{max}$, respectively), as postulated by Field and Swain [106]:

$$h_c = \frac{h_{max} + h_f}{2}. \quad (3.1)$$

The final indentation depth $h_f$ is the depth where, during unloading, the load equals zero. Now, from a geometrical point of view, the radius of the circle of contact $a$ can be calculated from:

$$a = \sqrt{(2Rh_c - h_c^2)}, \quad (3.2)$$

where $R$ is the radius of the spherical tip. For a contact depth significantly smaller than the radius of the indenter the quadratic term in the square root can be neglected. Assuming initial elastic unloading, the Young Modulus $E$ of the indented material can be estimated from the experimental data by Hertzian contact mechanics [107]:

$$E = \frac{S\sqrt{\pi}}{2\sqrt{A}}(1 - \nu^2). \quad (3.3)$$

Here, $S = dP/dh$ is the slope of the initial unloading curve (i.e., 85% and 65% of the load at maximum indentation), $A = \pi a^2$ is the area of the contact circle and $\nu$ is
the Poisson ratio of the indented material. For biological tissue, which is generally assumed to be incompressible, \( \nu = 0.5 \) \([108,109]\).

Although the Oliver-Pharr method is a widely accepted approach to compute Young’s moduli from indentation results, it is developed for elastic-plastic materials and therefore not appropriate for viscoelastic materials \([110]\). The derivation relies heavily on several assumptions that are valid for hard materials but may be violated in the case of soft matter such as biological tissue:

1. The surfaces in contact are smooth and continuous; the sample surface is flat.
2. The strains are small and within the linear-elastic regime.
3. Each solid can be considered as an elastic half space; the elasticity of the indenter is infinitely higher than that of the indented surface. The elasticity of both solids is homogeneous.
4. The surfaces in contact are frictionless and un-adhesive.
5. The initial loading and unloading is purely elastic.

It is important to realize that this model, respecting the above-mentioned assumptions, only produces sensible values for the elastic modulus \( E \) if elastic-plastic deformation of the sample occurs. Viscoelastic behavior can be easily mistaken for plastic deformation on short time scales. Moreover, further complications can arise when substantial adhesion between the sample and the indenter tip occurs, which may influence the initial unloading slope. Therefore, in the presence of strong viscoelastic and adhesive behavior, it may be more accurate to measure elasticity using the Hertz model.

**The Hertz model**

The Hertz model is based on the theory of contact mechanics developed by Heinrich Hertz in 1882 \([107]\). It relies on a fit of the model derived by Hertz to the initial, elastic loading part of the force-displacement curve and is, therefore, less sensitive to the influences of viscoelasticity and adhesion. Hertz describes the load \( P \) between two elastic-plastic solid bodies as:

\[
P = \frac{4}{3} E' R^{1/2} \delta^{3/2},
\]

where \( \delta \) represents the indentation depth and \( E' \) is the effective Young modulus. Where Oliver and Pharr fit a linear equation to the initial unloading part of the force-displacement curve to find \( dP/dh \), one can alternatively fit equation 3.4 directly to any elastic part of the force-displacement information and solve for \( E' \) (Figure 3.3). To avoid the effects of viscoelasticity and adhesion that occur during unloading, it is evident that a fit during the initial, elastic loading phase is more sensible. For this approach to succeed, a clear definition of the contact point is required to specify the fitted range. Moreover, a precise description of the contact area \( A \) is required. Hertz defined the relation between the indentation depth \( \delta \) and the contact radius \( a \) as:
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Figure 3.3: Illustration of a fit of the Hertz model to the initial elastic loading part of the load-indentation curve to obtain the elastic modulus of a sample. The fit starts at the statistically determined point of contact.

\[ \delta = \frac{a^2}{R}. \]  \hspace{1cm} (3.5)

Using this definition, together with equation 3.1 and 3.2, an optimized value for \( E' \) can be computed by optimizing the fit for \( P \). The effective Young modulus depends on the poission ratio, the YM of the indenter as well as the YM of the sample material:

\[ \frac{1}{E'} = \frac{1 - \nu_1^2}{E_1} + \frac{1 - \nu_2^2}{E_2}. \]  \hspace{1cm} (3.6)

Assuming that the indenter is much stiffer than the material under investigation, the Young modulus of the material can be approximated by:

\[ E = \frac{E'}{1 - \nu^2}. \]  \hspace{1cm} (3.7)

A note on adhesion

Extensive research has been performed to incorporate the effects of adhesive and capillary forces between the tip and the sample in Hertzian contact mechanics [111–115]. The effects of adhesion include an increase in contact area \( a \) during indentation and a positive contact area at negative loads after unloading (i.e. the indenter sticks to the sample). Analytical models such as the JKR model [111] and the DMT model [112] offer modifications to the Hertz equations to take the effect of adhesion into account in different situations. The JKR model is developed for low acuity (i.e. large tip radius \( R \)) indentations on soft materials, whereas the DMT model predicts the indentation behavior of a stiff material probed with small tip radius. Clarification about the use of
3.3 Indentation theory

the two model was given by Tabor [113], who introduced a coefficient \( \mu \) to determine which theory is applicable:

\[
\mu = \left( \frac{R\gamma^2}{E\gamma^2 z_0^3} \right)^{\frac{1}{3}}, \quad (3.8)
\]

where \( z_0 \) is the equilibrium separation between the atoms of the surfaces in contact (i.e., the distance between the atoms at which the force on each atom is zero) and \( \gamma \) is the energy per unit contact area, also termed the work of adhesion, which depends on the surface energies of the two contacting surfaces and an interaction term which is often neglected (\( \gamma = \gamma_1 + \gamma_2 - \gamma_{12} \)). If \( \mu \) is large, JKR theory applies and if it is small, the DMT model is more valid. The DMT model is not further discussed, as its applications lie beyond the scope of this thesis. Following the JKR model, a description of \( \gamma \) can be obtained based on the critical load \( P_{\text{adhesive}} \) at which the indenter separates from the surface (i.e., the maximum adhesive force, as illustrated in Figure 3.4):

\[
\gamma = \frac{3}{2} \frac{P_{\text{adhesive}}}{\pi R}. \quad (3.9)
\]

The radius of the circle of contact at a given load \( P \) is modified from the Hertz equation to include the contact surface energy \( \gamma \):

\[
a^3 = \frac{3R}{4E'} \left( P + 3\gamma \pi R + \sqrt{6\gamma \pi RP + (3\gamma \pi R)^2} \right). \quad (3.10)
\]

When \( \gamma = 0 \) equation 3.10 reverts to the simple Hertz equation without adhesion. However, in case of adhesion (\( \gamma \neq 0 \)), if \( P = 0 \), the contact area radius becomes
non-zero and is given by:

\[ a^3 = \frac{18\gamma \pi R^2}{4E'} \]  

(3.11)

Using this modified description of the contact radius an alternative value for the effective Young modulus \( E' \), accounting for surface adhesion, can be computed.

### 3.3.2 Dynamic indentation models

The previously described indentation models have only taken time-independent deformation into account. Soft biological materials consist of collagen and protein networks, cells (filled with liquid) and interstitial fluid which may move around freely. The mechanical behavior of the sum of these components is often dependent on how fast the deformation is applied (i.e. strain rate dependent). In this section the most-common indentation models that include viscoelasticity are discussed.

**Creep: Strain increase**

A frequently used method to investigate viscoelasticity with an indenter is to apply a constant stress and monitor the evolution of the strain over time. When subjected to constant stress, viscoelastic materials experience a time dependent increase in strain. Ideally, this constant stress is applied via a step-function. It is, however, important to realize that a step-function increase in the applied stress is in practice impossible. Thus, the response of the relaxation will always depend on the rate at which the stress is applied.

A simple model that is often used to simulate the creep behavior of polymers is the Kelvin-Voigt model. As shown in Figure 3.5, it consists of a spring and dashpot connected in parallel and may be expanded by adding more springs and dashpots in parallel. The strain response of this system over time \( \epsilon(t) \) to a certain applied stress \( \sigma \) is given by:

\[ \epsilon(t) = \frac{\sigma}{E}(1 - e^{-t/\tau}) \]  

(3.12)

where the relaxation time \( \tau = \eta/E \) and \( E \) is the shear modulus of the sample material. As with a viscoelastic material, when the stress is released, the strain gradually decreases to its undeformed value. Taking the recorded load and indentation as analogi for stress and strain, respectively, the model can be fit to the force-displacement data to obtain an estimate of \( E \) [110,116]. One needs to keep in mind that, with a change in indentation depth, the contact area changes, which in turn changes the stress on the sample. To maintain a constant stress, a load-controlled indentation is required. Moreover, the increase in indentation depth may exceed the linear-elastic regime, which may result in a non-linear tissue response. For non-linear elastic materials, it may be more practical to employ a stress relaxation method.
3.3 Indentation theory

Figure 3.5: A) Schematic representation of the Kevin-Voigt model with one spring ($E$) and one dashpot ($\eta$) in parallel. B) Applied stress in a theoretical creep experiment. C) The induced strain increase as a function of time upon constant stress.

Figure 3.6: A) Schematic representation of the Maxwell model with a dashpot ($\eta$) and a spring ($E$) in series. B) Applied strain in a theoretical stress relaxation experiment. C) The induced stress relaxation as a function of time upon constant strain.

Stress relaxation

Analogous to the creep experiment, one can also keep the strain (or, indentation depth) constant and monitor the relaxation of the stress (load) over time. Stress relaxation in a viscoelastic material is preferably modeled by a Maxwell model, which consists of a spring and dashpot placed in series, as illustrated in Figure 3.6. Again, for a theoretical simulation, a step function increase in the strain is assumed. The stress response of the material, according to a simple Maxwell model, is given by:

$$\sigma(t) = \sigma_0 e^{-t/\tau}. \tag{3.13}$$

An exponential fit with a given time constant can be applied to obtain an estimate of the elastic modulus $E$.

Dynamic mechanical analysis

In case of a linear viscoelastic material, one can perform a dynamic indentation experiment to resolve the frequency dependent storage and loss modulus of the material. This approach is based on oscillations of the load (or indentation depth) at different frequencies on top of a feedback-controlled constant load (or indentation depth). As shown in Figure 3.7, during the oscillations the response of the material
is carefully monitored. The storage modulus $E'$ represents the material’s capacity to store energy; it is the component in phase with the applied displacement or load. The loss modulus $E''$ represents the material’s capacity to dissipate energy; it is the component $90^\circ$ out of phase with the applied displacement or load. The ratio $E''/E' = \tan \phi$ is called the loss factor and is often used as a measure of damping in a linear viscoelastic material [117]. The application of this approach to (nano)indentation was developed by Herbert, Oliver and Pharr [117], who applied Sneddon’s stiffness equation to relate stress and strain to load and indentation [118]. Using the derivation for a spherical indenter, the dynamic moduli can be expressed in terms of the phase shift $\phi$ between load and indentation:

$$\frac{E'}{1 - \nu^2} = \frac{P_0}{h_0} \cos(\phi) \frac{1}{a}$$  \hspace{1cm} (3.14)

and

$$\frac{E''}{1 - \nu^2} = \frac{P_0}{h_0} \sin(\phi) \frac{1}{a},$$  \hspace{1cm} (3.15)

with $P_0$ the amplitude of the dynamic load, $h_0$ the amplitude of the oscillatory indentation depth and $a$ the radius of the contact area. Referring back to the previous section, creep or strain increase appears immediately after a constant load is applied to a viscoelastic material. To prevent creep effects from influencing the measurement of $E'$ and $E''$, dynamic indentation should be performed on a relaxed sample. Before starting the oscillations, enough time should be given at the static load (or depth) to allow for constant load and indentation depth over time. Moreover, one should ensure to remain in the linear viscoelastic regime to obtain a valid characterization of the material at different deformation rates.
Combining all the previous approaches, one could, theoretically, perform a Hertz fit, creep or stress relaxation analysis, dynamic mechanical analysis, the Oliver-Pharr method and even an adhesion measurement in one sample-tip contact. Practically, however, one should select the preferred method for the sample under investigation and optimize the indentation profile for the selected application.
A metrological approach for the calibration of force transducers with interferometric readout

We introduce an experimental calibration method for force transducers with interferometric readout. The head of the transducer is compressed on the pan of a weighing scale until the first maximum of interference is reached. An optomechanical feedback loop makes sure that the force applied remains constant during the integration time of the weighing scale. At the end of the integration time, the transducer is forced to move to the next maximum of interference, where it is again locked into position to allow the user to read the corresponding increase in weight on the scale. Repeating a similar procedure for a series of consecutive maximum-to-maximum steps, one can finally plot the weight indicated by the scale as a function of the displacement of the head of the transducer, and, from there, extract its spring constant. The method relies only on measurements of weights and laser wavelengths, both of which can be, in principle, referred to metrological standards.

Keywords: Force transducers – Calibration – Ferrule-top probe – Cantilevers – Atomic force microscopy

4.1 Introduction

Micro- and nanomachined linear force transducers often rely on two main components: a spring that bends in proportion to the force applied to the sensing head, and a readout unit that measures the extent of that bending. To translate the output of the readout unit into force, one needs to know the spring constant of the spring, which has thus to be accurately calibrated [119,120]. Unfortunately, calibration is not always a straightforward procedure. In the field of atomic force microscopy (AFM), for instance, despite the numerous calibration methods already developed to assess the spring constant of cantilevers (for an overview see [121]), there is still no accepted protocol that can provide accurate values in all circumstances. Dynamic calibration methods, in fact, make use of mechanical models and simulations that may hinge on heavy approximations [122–127] and that may require accurate knowledge of the geometrical features of the spring [121,128–133]. Static calibration procedures, on the contrary, can provide direct accurate measurements [126,134–136], but often necessitate tedious steps and extensive know-how to be correctly implemented.

To solve this problem, Doering et al. proposed an alternative static method that can be implemented via a series of straightforward steps [137]. The method relies on the idea of mounting the AFM cantilever on a calibrated piezoelectric translation stage, which is then used to push the free handing end of the cantilever against the pan of a weighing scale. The spring constant of the cantilever is inferred by measuring the weight indicated by the scale as a function of the extension of the piezoelectric device, which is assumed to be equal to the bending of the cantilever. Despite its simplicity, this approach seems to have the potential to outperform all the other static methods proposed so far in the literature [137]. However, the method suffers from a few limitations. In first place, one needs to rely on the calibration of the piezoelectric translator – an assumption that may affect the systematic error of the measurement. Furthermore, during the integration time that the weighing scale needs to measure the force applied, the bending of the cantilever must be kept constant – a technical detail that may become quite detrimental in the presence of vibrations, especially for a long integration time. Finally, because of the rather long mechanical loop of the setup (several centimeters), the system may suffer from long term drifts, which may again give rise to significant errors.

In this paper, we show that, for a force transducer equipped with interferometric readout (such as those proposed, for instance, in [36,138–140]), one can refine the weighing scale method by adding a high gain negative feedback loop designed to keep the bending of the cantilever equal to a multiple of the wavelength of the readout laser. The method relies only on measurements of weights and laser wavelengths, both of which can be referred, in principle, to metrological standards. To demonstrate the feasibility of our approach, we present the results obtained while testing it on a ferrule-top micromachined device [33,41,45], which has already been proven to be an interesting candidate for the development of a new generation of AFMs [34,51,54].
4.2 Experimental Details

4.2.1 Ferrule-top cantilevers: fabrication

The ferrule-top transducer used in our experiment is obtained by gluing a borosilicate cantilever on top of a borosilicate ferrule. The position of the free-hanging end of the cantilever can be monitored via a single mode optical fiber, anchored to the side of the ferrule. The most important steps of the fabrication process are shown in Figure 4.1 (see also [34]).

In step I, a $3 \times 3 \times 7 \text{mm}^3$ borosilicate glass ferrule is mounted on a wire cutter to carve a $3 \times 0.4 \times 0.4 \text{mm}^3$ ridge on the top facet of the ferrule and a groove with a cross section of $0.2 \times 0.4 \text{mm}^2$ on the side opposite to the ridge. The ferrule is then taken out of the wire cutter and positioned under a microscope equipped with micrometer precision manipulators. In step II, a borosilicate cantilever, previously coated with chromium (10 nm) and gold (100 nm), is aligned with the groove and glued with wax onto the ridge\(^1\). In step III, the ferrule is mounted on a ps-laser ablation system (Optec System with Lumera Laser source) to cut the cantilever on both ends of the ferrule. Because of the high accuracy of the ablation process the length of the cantilever can be very precisely determined (5\text{µm} resolution). In step IV, a glass sphere (radius equal to 100-150 \text{µm}) is glued at the tip of the cantilever. Finally, in step V, a cleaved single mode optical fiber (Corning SMF28) is slid and glued into the lateral groove. This fiber will be used to detect the deflection of the cantilever, as explained in section 2.2. The ferrule, the cantilever and the fiber are so well held together by the wax and the glue that the sensor can be treated as a single mechanical piece. Using this method we have prepared a macro cantilever with length ($L$) equal to 2.65 mm, width ($w$) equal to 0.16 mm, and thickness ($t$) equal to 0.02 mm\(^2\) (see Figure 4.1).

\(^1\)The wax dries to a very hard material that adheres well to both the ferrule and the cantilever. Drift and stress tests in ambient environment showed no observable difference between wax and various epoxy glues.

\(^2\)Although the size of this cantilever differs significantly from standard AFM cantilevers, the method illustrated in this paper holds for cantilevers of any size or stiffness, as long as there is no significant mechanical drift between the position of the end of the fiber and that of the cantilever.

Figure 4.1: Fabrication process for ferrule-top probes (not to scale) (see also [34]), along with a microscope image of a ferrule-top cantilever (scalebar = 1110 \text{µm}). We refer the reader to the main text for the details. Here we only add that the ferrule is delivered by the manufacturer with a central bore hole (not shown in the schematic drawing), which, however, is not used in this application.
Figure 4.2: Schematic view of the readout system used to detect the bending of the cantilever. No light reflects at the terminated fiber end.

4.2.2 Ferrule-top cantilevers: readout

The detection of cantilever bending relies on Fabry-Pérot interferometry and has been described in previous papers [33, 36, 140]. The distal end of the readout fiber is connected to a laser via an optical fiber coupler, as illustrated in Figure 4.2. At the cleaved end of the fiber, a small part of the incident laser light reflects back. Most of the light, however, passes through the end and reflects on the metal interface underneath the cantilever. The two signals (i.e., the one reflected at the cleaved end of the fiber and the one reflected by the cantilever bottom surface) create an interference pattern whose amplitude is measured via a photodiode aligned with the exit of the coupler. Following [33, 36], if multiple reflections are neglected, one can describe the amplitude of the ideal interference signal in the photodiode by 3:

\[ W(d) = W_0 \left[ 1 + V \cos \left( \frac{4\pi d}{\lambda} + \varphi_0 \right) \right], \]

where \( d \) is the gap size, \( \varphi_0 \) is a constant phase shift that only depends on the geometry of the probe, \( \lambda \) is the wavelength of the laser, and \( W_0 \) and \( V \) are the midpoint interference signal and the fringe visibility, respectively. Movement of the cantilever causes a change in the size of the gap between the cantilever and the fiber end. This, in turn, leads to a change in the interference signal. By monitoring this signal, one can measure cantilever displacements.

4.2.3 Calibration method: overview

The calibration method presented in this paper relies on the idea to push the free hanging end of the cantilever against a calibrated weighing scale, measure the weight registered by the balance as a function of the cantilever deflection, and use Hooke’s law [141] to extract the spring constant of the cantilever from the weight registered by the scale and the wavelength of the laser used in the readout.

To achieve this goal, the wavelength of the laser is modulated around a fixed value \( \lambda_0 \) according to:

\[ \lambda(t) = \lambda_0 + \delta \lambda \cos(\omega t), \]

\(^3\)Mode hopping in the laser is minimized by introducing an isolator in the optical path.
where $\delta \lambda$ and $\omega$ represent the amplitude and the angular frequency of the oscillation, respectively. Substituting equation 2 in equation 1, one obtains the expected time dependent function of the readout output:

$$W(t) \propto \cos \left[ \frac{4\pi d}{\lambda_0 + \delta \lambda \cos (\omega t)} + \varphi_0 \right] = \cos \left[ \frac{\alpha}{1 + x} + \varphi_0 \right],$$

(4.3)

where

$$\alpha = \frac{4\pi d}{\lambda_0}$$

(4.4)

and

$$x = \frac{\delta \lambda \cos (\omega t)}{\lambda_0}.$$  

(4.5)

For small values of $\delta \lambda/\lambda_0$, equation 4 can be approximated by the first order Taylor expansion around $x = 0$:

$$W(x) \propto \cos(\alpha + \varphi_0) + \alpha \sin (\alpha + \varphi_0) \times x + O(x^2).$$  

(4.6)

Let’s assume that the cantilever is compressed against the pan of the balance of an amount $d_1$ such that:

$$\alpha + \varphi_0 = \frac{4\pi d_1}{\lambda_0} + \varphi_0 = \frac{1}{2} \pi \times n, \quad n = 1, 3, 5, \ldots,$$

(4.7)

which implies that, for $\lambda = \lambda_0$, the output signal of the readout is at quadrature. Under these circumstances, as the wavelength of the laser oscillates around $\lambda_0$, the readout signal contains a component that oscillates at frequency $\omega$:

$$W(t) \propto 0 + \left[ \frac{4\pi d}{\lambda_0} \right] \times \frac{\delta \lambda}{\lambda_0} \cos(\omega t) + O(x^2).$$  

(4.8)

Now let’s assume that the cantilever is compressed against the pan of the balance of an amount $d_2$ such that:

$$\alpha + \varphi_0 = \frac{4\pi d_2}{\lambda_0} + \varphi_0 = 2\pi \times n, \quad n = 1, 2, 3, \ldots,$$

(4.9)

which implies that, for $\lambda = \lambda_0$, the output signal of the readout is at a maximum of interference. Under these circumstances, as the wavelength of the laser oscillates around $\lambda_0$, the readout signal does not contain any component oscillating at frequency $\omega$:

$$W(t) \propto 1 + 0 \times \frac{\delta \lambda}{\lambda_0} \cos(\omega t) + O(x^2).$$  

(4.10)

From this example, it is clear that, by modulating the wavelength of the readout at frequency $\omega$, one can distinguish maxima (or, equivalently, minima) of interference from quadrature points by measuring the component of the readout output signal that oscillates at frequency $\omega$. To maintain the deflection of the cantilever constant, one
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4.4 A metrological approach for the calibration of force transducers with interferometric readout

Figure 4.3: Schematic view of the calibration setup with the closed-loop feedback control system.

can thus mount the probe on a piezoelectric stage that, driven by a high-gain negative feedback loop fed with a signal proportional to the $\omega$ component of the readout system, keeps the latter equal to zero.

Interestingly, to move from one maximum (or minimum) of interference to the next one, one needs to deflect the cantilever of an exact amount $\delta d = \lambda_0/2$. Measuring the change of weight registered by the balance as the cantilever moves through a series of maxima (or minima) of interference, it is then possible to obtain a calibration of the spring constant of the cantilever on the basis of only two parameters: the weights measured by the balance and the wavelength of the laser ($\lambda_0$).

4.2.4 Calibration method: experimental setup

Figure 4.3 shows a schematic view of the experimental setup used to demonstrate our calibration method.

The ferrule-top probe is connected to a commercial interferometer (OP1550, Optics11), which is equipped with a tunable infrared laser. The wavelength of the laser is internally controlled with 10 pm accuracy, and can be swept by driving the injection current with a sinusoidal signal. For our experiment, we used a central wavelength of 1531 nm, modulated at 10 kHz with a modulation amplitude of 0.5 mA, corresponding to a modulation of approximately 50 pm to 100 pm.

The probe is mounted on a large stroke (500 $\mu$m) piezoelectric translator (Piezo I in Figure 4.3, P-603.5S1, PI GmbH) driven, in open loop, by a 5 nm resolution servo-controller (E-665.SR, PI GmbH). The holder contains a second piezoelectric translator (Piezo II in Figure 4.3, 10 $\mu$m stroke, Thorlabs GmbH), which is operated in closed loop via a strain gauge system. For a controlled force measurement we make use of an analytical self calibration balance (MSE125P-100-DU, Sartorius AG) that has a readability of 15 $\mu$g (or force resolution of 150 nN) with an integration time of $\approx 6$ s. To reduce vibrations and airflow, the setup is built within draft shields and is mounted on a passive anti-vibration stage (Nexus, Thorlabs GmbH).

The position of the cantilever is carefully controlled by means of a negative feedback
circuit (gain = 19.8 dB, τ = 90 ms). This circuit is driven by a lock-in amplifier (SR830, Stanford Research Systems), which is locked at frequency ω. The time constant of the lock-in amplifier is set to 30 ms. A proportional-integral-derivative (PID)-controller, driven by the lock-in amplifier, is connected to the 10 µm stroke piezoelectric translator to adjust the bending of the cantilever such that the output of the lock-in amplifier, at frequency ω, is set to zero, corresponding to a maximum (or minimum) of interference.

4.2.5 Experimental procedure

To demonstrate the working principle, we have calibrated the ferrule-top cantilever described in the previous section according to the following procedure.

The ferrule-top probe is positioned, within 2 µm above the weighing pan of the balance, by means of the large-stroke z-translator (Piezo I). The loop is then closed, causing the second piezoelectric translator to scan down, bring the probe in contact with the pan of the weighing scale, and compress the cantilever until it encounters the first maximum of interference. The weight measured while the applied force is locked to this set value is the first measurement point of our experiment.

Starting from this maximum of interference, then, we apply, in closed loop, a step of approximately $\frac{1}{2} \lambda_0$ to Piezo I with a stroke significantly faster than the reaction time of the feedback ($\tau = 90$ ms). This procedure allows us to move to the next maximum of interference, thereby increasing the cantilever deflection. The step size of the z-translator does not have to be exactly $\frac{1}{2} \lambda_0$: since the loop is still closed, the small-stroke piezoelectric translator adjusts the bending of the cantilever in such a way that the signal is locked to the next maximum, corresponding to an additional deflection of exactly $\frac{1}{2} \lambda_0$. Using this method discrete indentation steps of $n \times \frac{1}{2} \lambda_0$ are possible (with $n = 1, 2, 3, \ldots$), with a resolution corresponding to that of the laser wavelength (10 pm).

To calibrate the cantilever described in section 2.1, we repeated the calibration procedure explained above for 9 times. Before each run, we calibrated the weighing scale using its internal calibration procedure. In each run, we measured the weight indicated by the scale for 20 steps of $\frac{1}{2} \lambda_0$ ($\lambda_0 = 1551$ nm), resulting in a maximum cantilever deflection of around 16 µm, and then calculated the spring constant of the cantilever from the linear fit of the force-bending curve.

To illustrate the effect of the feedback loop on noise reduction, we also repeated a set of 4 runs with the feedback loop disabled.

4.3 Results and Discussion

Figure 4.4A reports a typical force-bending curve obtained with the feedback loop method. A linear response is observed over the entire deflection range. Fitting each of the 9 force-bending curve with a first order linear regression, and calculating the weighted average of the slope, one obtains a value for the spring constant of the cantilever equal to $1.1627 \pm 0.0047$ N/m. Figure 4.4B further shows the distribution of the residuals of the fits, which are indeed spread according to a Gaussian distribution. Performing a t-Test on these data, one can show that the mean of the residuals does
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Figure 4.4: A) Typical force-bending curve together with a first order regression; B) Histogram of the residuals of the force-bending curves.

Figure 4.5: A) Residuals of the force-bending curves with (squares) and without (triangles) feedback; B) Force readout when a stable pressure is applied to the pan of the balance. The feedback loop was disabled after 14 seconds and re-enabled after 124 seconds.

not significantly differ from zero ($p << 0.001$). Therefore it can be concluded that the linear regressions correlate very well with the obtained datasets.

To demonstrate the added value of the high-gain feedback loop, in Figure 4.5A we compare the residuals of the force-bending curves obtained with our method with those obtained from the data collected in the 4 runs without feedback. It is clear that our method is indeed capable of reducing the error significantly. Figure 4.5B further shows the effect of the feedback loop when a constant pressure is applied to the pan of the balance. One can observe that, as soon as the feedback loop is disabled, the readout signal becomes much more noisy.

Concerning the systematic error, it is important to stress once again that our method completely eliminates the calibration of the piezoelectric transducer and significantly reduces the effects of mechanical drifts. The changes in the bending of the cantilever, in fact, are multiples of the wavelength of the laser used in the
interferometer, which, in our case, is known with an accuracy of 10 parts per million, and is thus completely negligible. The main source of systematic error is the one introduced by the weighing scale, which is certified for 15µg. A systematic error of 15µg on the first and the last point of Figure 4.4A would give rise to a systematic error on the spring constant of 0.0088 N/m ($\Delta k = \frac{\text{readability}}{(\lambda/2)}$). Therefore, assuming that, after internal calibration, the systematic error of the balance is equal to its readability, we can conclude that the spring constant of the cantilever is equal to:

$$k = 1.1627 \pm 0.0047 \, \text{(stat)} \pm 0.0088 \, \text{(syst)} \, \text{N/m.} \quad (4.11)$$

Substituting the weighting scale with one with 100 ng readability (e.g., MSA2.7S-000-DF, Sartorius AG), one could reduce the systematic error even further down to $\approx 100\mu \text{N/m}$.

It is important to stress that the calibration method presented here already accounts for the fact that the fiber is not aligned with the end of the cantilever, where the sphere enters in contact with the pan. When, after calibration, the transducer will be used in practical applications, in fact, the only information that the user will be able to obtain is the displacement of the cantilever at the point where the fiber is. Multiplying that number times the number indicated in equation 11, the user will now know exactly the magnitude of the force applied on the sphere at the end of the cantilever, which is the information that the force transducer is indeed supposed to give.

4.4 Conclusions

We have introduced an improved method for the calibration of force transducers with interferometric readout. This method relies on the application of a constant pressure by the sensor on an analytical balance using a negative feedback loop. The loop allows one to keep the displacement of the transducer stable over time and to simultaneously measure the displacement of the transducer as a multiple of the wavelength of the laser in the readout. By using this feedback loop, our calibration method is able to offer calibrations according to metrical standards. The key parameters, displacement and weight, are measured with an accuracy of 10 pm and 15µg, respectively. Other advantages over the well-known calibration methods are high throughput and ease of use. The method presented is non-destructive (as long as the contact point of the transducer does not damage when a force is applied to it), reproducible, and universal, and can therefore pave the way for the use of more complex cantilever devices in the future.

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Disclosures

D. Iannuzzi is co-founder and shareholder of Optics11.
Characterizing tissue stiffness at the tip of a rigid needle using an opto-mechanical force sensor

We present a novel device that allows the user to measure the Young Modulus of a material at the opening of a 5 mm diameter needle. The device relies on a miniaturized cantilever spring mounted at the end of the needle and interrogated via Fabry-Pérot optical fiber interferometry. The probe is repetitively brought in and out of contact with the sample at the end of the needle by means of a steel cable that is controlled via a piezoelectric actuator located at the proximal end. We demonstrate the ability of our device to detect and quantify layers of varying stiffness during needle insertion in a gelatin phantom and to successfully locate tissue boundaries in bovine liver tissue embedded in gelatin.

**Keywords:** Ferrule-top technology – In situ indentation – Remote actuation – Interferometry – Tissue Stiffness – Micromechanics – Minimally invasive instrument

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5 Characterizing tissue stiffness at the tip of a rigid needle using an opto-mechanical force sensor

5.1 Introduction

The mechanical properties of biological networks are often overseen in functional research of healthy tissue as well as in the diagnosis of potentially diseased tissue and in treatment monitoring. For example, in the classification of skin conditions (such as scars or burn wounds) and the following control of disease progression or healing, physicians in most cases prefer visual assessment and subjective scaling above quantitative mechanical information [142–145]. Moreover, tissue mechanics can be linked to a wide array of physiological processes [146–148]. Cells have very sophisticated methods to sense and adapt to their mechanical environment. Stem cells, for instance, have been proven to adapt their differentiation to the stiffness of their extracellular matrix [71, 72] and white blood cells, as well as tumor cells, are able to manipulate their own stiffness and shape to migrate in and out of blood vessels [149]. At the tissue level, the interplay between the cellular mechanics and the extracellular network determines the stiffness at the micron scale, changes of which have been associated with Alzheimer’s disease [150] and multiple sclerosis [151, 152] (in the brain), cancerous growth [153, 154] (breast) and osteoarthritis [155, 156] (cartilage). Hence, a method to quantify local mechanical properties of tissue, preferably \textit{in situ}, is of high interest.

Classically, the biomechanical response of complex networks is assessed by means of Atomic Force Microscope (AFM) nanoindentation [157–162]. The utilization of an AFM for classification of biological tissues has, however, some principle limitations that cannot be easily overcome, such as size, stability and flexibility. To mitigate those limitations, we have recently introduced a new probe, called \textit{ferrule-top cantilever}, that provides a good alternative for indentation of biological samples in harsh environments [41, 163, 164].

Both AFM and ferrule-top indentation are restricted to probing the surface of a sample, while the advantages of probing in depth (i.e., underneath the surface) would clearly be multiple. By integrating an indenter at the tip of a needle one could not only quantify between layers of different stiffness, but also navigate to the target location and perform a minimally invasive measurement based on the tissue mechanical properties. An example of an \textit{in situ} AFM indenter for arthroscopic knee cartilage inspection was presented by Imer \textit{et al.} [165]. The indenter consists of an extensive stabilization stage connected to a piezoelectric scanning module, both of which are inserted into the sample, resulting in a large footprint. Moreover, the lack of calibration of the piezoelectric tube hampered a quantitative analysis.

Here, we demonstrate a ferrule-top indenter on the distal end of a rigid needle and we show its ability to quantify local mechanical properties of tissue \textit{in situ}. Thanks to the remote actuation of the sensor by a piezoelectric translator the size of the needle is limited to the dimensions of the indentation probe at the tip. The performance of our indenter is tested on an engineered layered sample as well as on biological tissue.
5.2 Experimental section

5.2.1 The ferrule-top force transducer

The indenter relies on the working principle of bulkier ferrule-top instruments, which is here briefly discussed (for more details, see [35,41,42]). A ferrule-top force transducer consists of a borosilicate cantilever, equipped with a small sphere on its free hanging end (see Figure 5.1A), and assembled over a 3 mm x 3 mm x 7 mm borosilicate ferrule (see Figure 5.1B). A single mode optical fiber is glued at the side of the ferrule and aligned with the free hanging end of the cantilever. The cleaved end of the fiber and the bottom surface of the cantilever form a Fabry-Pérot cavity (more details in section 5.2.4), which allows one to detect the bending of the cantilever with nanometer precision. Pushing the sphere of the cantilever against a sample, and measuring the indentation depth as a function of the deflection of the cantilever (and, hence, of the force applied), one can infer the Young Modulus of the sample.

5.2.2 Indentation module

To reduce the dimensions of the indenter, we have developed an indentation module that enables remote actuation of the force transducer. A schematic view of the indentation module is shown in Figure 5.1B. The optical force transducer is housed in a square borosilicate capillary with an inner lumen of 3.05 mm x 3.05 mm, which restricts the movement of the probe to the axial direction. The probe is mechanically connected to a calibrated piezoelectric translator via a steel cable (diameter = 120µm) similar to those that are commonly used to actuate the tip of surgical steerable needles [166,167]. A small compression spring is used to load the probe against a backplane in the capillary. If the spring is initially compressed with pretension, in fact, the movement of the piezoelectric translator can be smoothly transferred, from remote position, to the probe. The translator (P-602.5L8, Physike Instrumente GmbH) has a 500µm stroke and a 325 N blocking force. It is important to note that, due to hysteresis in the steel cable and friction between the capillary and the probe, in our case, it is not possible to assume that the movement of the probe is exactly equal to that indicated by the strain gauge feedback system of the driving piezoelectric device. To solve this issue, the movement of the probe is monitored by a second single mode optical fiber, anchored to the backplane of the square capillary and aligned with the back of the ferrule (see Figure 5.1B).

5.2.3 Experimental setup

The indentation module is housed at the distal end of a 20 cm long custom designed needle, that is used to insert the sensor into the specimen. Figure 5.1C shows a schematic view of the ferrule-top indenter at the tip of the rigid needle. The needle (diameter = 5 mm) is fixed to a motorized linear translation stage (LTS300, Thorlabs GmbH) that is used for insertion in the sample. At the proximal end of the needle the steel cable is fixed to the piezoelectric translator, which in turn is mounted on a coarse position stage, allowing for adjustment of the pretension in the cable-spring system.
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Figure 5.1: Schematic view of the experimental setup, showing a microscope image of the indenter (A), a closeup of the indentation module (B), and a sketch of the complete needle insertion setup (C) (not to scale).

The two optical fibers (i.e., the cantilever deflection readout and the sensor movement readout), are fed through the lumen of the needle and connected to two interferometric readout systems (OP1550, Optics11). Both interferometers are equipped with a tunable infrared laser (35 nm tunability), the wavelength of which is internally locked with 10 pm accuracy by a feedback system, and can be swept by driving the injection current sinusoidally.

The sample is placed on an xy-translation stage (MAX312D, Thorlabs GmbH), which is used to select a position for needle insertion. To reduce vibrations, the setup is built on a passive anti-vibration stage.

5.2.4 Working principle

As previously stated, both the detection of cantilever bending and the monitoring of the position of the sensor rely on Fabry-Pérot interferometry. Figure 5.2 illustrates the basic scheme of the detection mechanism that is employed. The laser is coupled into the Fabry-Pérot cavity via the 10% arm of a 90/10 fiber coupler. Before entering the cavity, a small fraction of the incident light is coupled back into the fiber due to the refractive index mismatch between the fiber core and the medium inside the cavity. The remainder of the light passes through the cleaved fiber end, reflects on the other surface of the cavity (in our case, either the bottom of the cantilever or the bottom of the ferrule) and is collected back into the fiber. The amplitude of the

Declaration of interest: D. Iannuzzi is shareholder of Optics11.
interference pattern created by the two backpropagating signals is measured by a
photodiode aligned with the exit of the coupler and can be described by\(^1\):

\[
W(d) = W_0 \left[ 1 + V \cos \left( \frac{4\pi d}{\lambda} + \varphi_0 \right) \right], \quad (5.1)
\]

where \(d\) is the length of the cavity, \(\lambda\) is the central wavelength of the laser, \(\varphi_0\) is a
constant phase shift that only depends on the geometry of the probe, and \(W_0\) and \(V\) are the midpoint interference signal \(((W_{\text{max}} + W_{\text{min}})/2)\) and the fringe visibility
\(((W_{\text{max}} - W_{\text{min}})/(W_{\text{max}} + W_{\text{min}}))\), respectively. One can recognize that a displacement
of the mirror can be immediately identified from the output signal of the photodiode.
In our setup, this principle is employed to assess the position of both the cantilever
and the sensor (see Figure 5.1). One single mode fiber is positioned perpendicular to
the gold coated bottom facet of the cantilever and a second fiber is anchored at the
backplane of the square capillary, continuously monitoring the displacement of the
sensor. Multiple reflections in the cavity can be prevented by a slight rounding of the
cleaved facet of the fiber, obtained by exposing the tip to a brief plasma arc before
mounting it in the setup.

The detection method described is highly sensitive for relative mirror movements
and is straightforward to implement for small displacements. One can simply tune the
wavelength to quadrature, where the sensitivity to mirror movement is maximum and
the output of the readout is linear. Although effective for small displacements around
quadrature, the non-linearity of the output signal renders the method not ideal for the
larger displacements required for indentation measurements, where a linear readout
is required over a large displacement \((d \gg \lambda)\). In order to linearize the amplitude
response over the complete deflection range we modulated the wavelength of the laser
around the central wavelength \((\lambda_c)\) according to [42]:

\[
\lambda(t) = \lambda_c + \delta \lambda \cos(\omega t), \quad (5.2)
\]

where \(\delta \lambda\) and \(\omega\) represent the amplitude and frequency of the modulation, respectively.
Assuming, for the sake of simplicity and without loss of generality, \(\varphi_0 = 0\), the expected
time dependent amplitude of the photodiode during wavelength modulation is given
by:

\[
W(t) = W_0 \left( 1 + V \cos \left( \frac{4\pi d}{\lambda_c + \delta \lambda \cos(\omega t)} \right) \right). \quad (5.3)
\]

This time dependent response contains a DC component, encoding for the movement of the
reflective surface, and a component that oscillates at frequency \(\omega\), originating from
the modulation of the wavelength. The contribution of each component can be assessed
in detail by making a first order Taylor expansion of \(W(t)\) around \(\frac{\delta \lambda \cos(\omega t)}{\lambda} = 0\):

\[
W(t) \approx \cos \left( \frac{4\pi d}{\lambda_c} \right) + \left( \frac{4\pi d \delta \lambda \cos(\omega t)}{\lambda_c^2} \right) \sin \left( \frac{4\pi d}{\lambda_c} \right). \quad (5.4)
\]

The low frequency component (which we will denote with \(W_{\text{dc}}\)) and the high frequency
component (which we will denote with \(W_{\omega}\)) are now described by the first and second

\(^1\)Mode hopping in the laser is minimized by introducing an isolator in the optical path.
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Figure 5.2: Sketch of the interferometric scheme used to measure the deflection of the cantilever and the movement of the ferrule. The cavity formed by the cleaved end of the fiber and the surface in front is interrogated by a monochromatic tunable laser. The difference in intensity due to a change in interference (caused by movement of the mirror) is detected in the photodiode.

term of the Taylor expansion, respectively. It can be readily observed from eq. 5.4 that \( W_{dc} \) and \( W_\omega \) are separated by a 90 deg phase shift. This particular relation allows one to linearize the output signal and apply phase unwrapping to obtain a continuous linear response for the displacement of the mirror (\( D_m \)):

\[
D_m = \frac{\lambda_c}{4\pi} \arctan\left(\frac{W_{dc}}{W_\omega}\right).
\]  

(5.5)

\( W_{dc} \) can then be recorded by means of a low-pass filter with a cut-off frequency below the modulation frequency. To record \( W_\omega \), the unfiltered amplitude response of the photodiode is sent to a lock-in amplifier, which is locked at frequency \( \omega \) via a square wave reference signal. We note that, thanks to the high bandwidth of our measurement, acquisition of \( W_{dc} \) and \( W_\omega \) and the following linearization of the signal is performed in real time.

5.2.5 Specimen preparation

To demonstrate the working principle of our device, we performed a series of insertions consisting of multiple indentation measurements at varying depth on two gelatin-based phantoms (Gelatin from bovine skin, Sigma-Aldrich). In the first specimen a stiffness gradient was created by compiling layers (10 mm in height) with decreasing mass gelatin to water ratio, hence creating a sample with layers of decreasing stiffness from bottom to top. Starting from a layer of 15% mass gelatin at the bottom, each following layer contained 2.5% less mass gelatin, ultimately creating a specimen with 6 layers of decreasing stiffness: 15%, 12.5%, 10%, 7.5%, 5% and 2.5% mass gelatin. For each layer, the gelatin was dissolved in demineralised water at 60 °C, poured in the container and kept at 4 °C for 30 minutes to allow the layer to stiffen. After the final layer was poured, the sample was stored 4 °C overnight and measured the next day.

The second specimen consisted of a square piece of bovine liver (6x6x3 mm³) fixated in a gelatin solution. To create a base layer (20 mm in height), gelatin was dissolved at a 12% mass to water ratio at 60 °C, poured in a container and stored overnight at 4 °C. Subsequently, the liver sample was placed on top of the first gelatin layer and a second solution of gelatin (12%) was poured over the sample until it was fully submerged. The gelatin solution was cooled down to 40 °C prior to pouring to prevent thermal damage of the tissue. After the container was filled with gelatin (±
5.2 Experimental section

Figure 5.3: Schematic view of the elastic indentation of a flat plane surface with a small spherical indenter of radius $R$. Starting from zero load ($P = 0$), $P$ is increased until the maximum indentation depth ($h_{max}$) is reached. The contact radius ($a$) and the corresponding contact depth ($h_c$) depend on the depth of indentation.

20 mm above the sample), it was again stored overnight at 4 °C to ensure proper stiffening.

5.2.6 Experimental details and indentation protocol

For this experiment, the cantilever was equipped with a spherical borosilicate bead of radius equal to 85 µm. For optimal sensitivity, the cleaved end of the single mode fiber was positioned directly underneath the center of the microbead, positioning the Fabry-Pérot cavity directly underneath the point of contact (Figure 5.1A). A central wavelength of 1551 nm was used in both interferometers in combination with a 90 kHz sinusoidal wavelength modulation of approximately 50-200 pm modulation depth. The spring constant of the cantilever was measured to be $12.97 \pm 0.06$ N/m via the method reported in [35].

Four needle insertions were placed in the layered specimen and six in the animal liver specimen. Stiffness data were recorded at seven depth: one at the surface and six at increasing depth, each position separated by 10 mm. At each point in depth 5 indentation curves were recorded at 3 different locations, for a total of 15 indentations per depth position. All the measurements were performed in air in order to best preserve the gelatin structure. For each depth level, the probe at the end of the needle was carefully lowered with the motorized linear stage until contact was found between the indenting tip of the ferrule-top probe and the sample, identified by backwards bending of the cantilever of a few tens of nanometers. Starting from this position, the indentation movement was generated by applying a ramp-shaped voltage profile to the piezoelectric translator. This movement was in turn translated through the needle shaft and resulted in an indentation stroke of the sensor. Prior to each measurement, the system was adjusted such that the stroke of the sensor ($d_f$) was maximum 30 µm. The indentation depth ($d_i$) inside the sample, however, depends not solely on $d_f$ but also on the displacement cantilever ($d_c$). The indentation depth was continuously recorded during the measurement and is given by:

$$d_i = d_f - d_c. \tag{5.6}$$

If the spring constant of the cantilever ($k$) is known, the load ($P$) applied during the
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**Figure 5.4:** A) Relative movement of the piezoelectric translator (red), of the ferrule, and of the cantilever as a function of time in one of the indentation stroke. The micro-movement of the sensor (green) and the cantilever (blue) at the tip of the needle is compared with the movement of the piezoelectric transducer at the proximal end. The remote actuation of the ferrule results in a smooth, reliable indentation movement. The pretension of the spring is adjusted such that the hold time at maximum load is 250 ms. B) Load-indentation curve obtained from the data reported in A, along with the definition of the parameters used for the analysis of the data.

indentation is:

\[ P = k \cdot d_c. \] (5.7)

To validate our measurements, before the first insertion of the needle, we cut a cross-sectional slice of the specimen, in which the different layers were clearly identifiable. The slice was then indented on the surface at locations corresponding to the different layers. Each reference measurement was obtained as an average of 15 indentations spread over 3 locations.

### 5.2.7 Analysis

In order to derive the elastic modulus from the raw indentation data, we used the method for spherical indentation described by Oliver and Pharr [104, 105]. It is important to recall that, to describe the indentation of an elastic material with a spherical indenter the contact area \( A \) between the tip and the sample must be correctly modeled. The contact depth \( (h_c) \) of the sphere can be determined from the final and maximum indentation depths \( (h_f \text{ and } h_{\text{max}}, \text{ respectively}) [106] \) (Figure 5.3):

\[ h_c = \frac{h_{\text{max}} + h_f}{2}. \] (5.8)

Now, from a geometrical point of view, the radius of the circle of contact can be
5.3 Results and discussions

5.3.1 Calculating Young's Modulus

The Young Modulus of the indented material can be estimated from the experimental data by Hertzian mechanics:

\[ E = \frac{S\sqrt{\pi}}{2\sqrt{A}}(1 - \nu^2). \]  

Here, \( S = dP/dh \) is the slope of the initial unloading curve (i.e., 85% and 65% of the load at maximum indentation), \( A = \pi a^2 \) is the area of the contact circle and \( \nu \) is the Poisson ratio of the indented material. In our case \( \nu = 0.5 \), as both gelatin and liver tissue were assumed to be incompressible [108, 109].

5.3 Results and discussions

The response of the ferrule-top probe to a ramp-like movement of the piezoelectric transducer as observed in one of our indentation stroke is reported in Figure 5.4A. One can note that, at the start of the ramp, the friction of the probe inside the indentation module prevents the probe from moving. However, as the force builds up the friction is overcome and a smooth, reliable indentation movement is initiated. We observed that the amount of friction (and the hereby related hold time) is adjustable by varying the initial loading of the compression spring. Using our method, we were able to remotely actuate the indenter with the precision required for our indentation measurement. Figure 5.4B reports a typical load versus indentation depth curve obtained with our system. Note that indentation in our case describes the deformation of the measured sample, not the deflection of the cantilever.

In Figure 5.5, we compare the Young Moduli measured during four separate needle insertions in the gelatin gradient specimen with the results obtained from the reference slice. One can conclude that there is a good agreement between the two measurements, although some outliers are, at times, observed. Some of those outlying data may be due to the presence of contaminations on the measured surface. The results obtained at 60 mm depth, on the contrary, can be probably ascribed to the fact that the bottom layer was too close to the bottom of the glass container.

Figure 5.6 shows the Young Modulus at increasing depth for six insertions in the fixed liver specimen and the corresponding cross-sectional reference. As one can see from the reference, the specimen consisted of the different stiffness levels; 1) the surface of the specimen, 2) the encapsulating gelatin and 3) the liver. The surface of the specimen was found to be noticeably stiffer than the gelatin underneath, most likely due to the evaporation of water that occurs when the specimen is in contact with air. The liver, one order of magnitude softer than the surrounding gelatin, was positioned between 25 and 45 mm from the surface. The difference in stiffness between gelatin and the liver tissue is clearly visible in the reference and for each insertion. The homogeneous stiffness distribution of the gelatin over the
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Figure 5.5: Young Modulus versus depth in the gelatin gradient specimen for 4 insertions (closed circles, squares, diamonds, triangles) compared to surface measurements on a cross-sectional reference slice (crosses). The dashed line was added as a guide for the eye. Still, it is to note that that line scales like the square power of the depth, which corresponds to the trend expected from theory [168].

entire specimen (with exception of the surface), as demonstrated by the reference measurement, is less predominant when each insertion is inspected individually. This variability can be attributed to the inhomogeneity of the measured surface during the insertion; surface roughness can cause the contact to be ill-defined and can lead to an under- or overestimation of the sample stiffness. However, when all the insertions are grouped together (Figure 5.7), a good agreement is observed between the gelatin measurements at different depths, as well as between the liver indentations at 30 and 40 mm.

5.4 Limitations

In the following section we discuss some of the limitations of our indenter in its current form.

The design of the cantilever for this experiment was optimized to measure the stiffness of materials with Young Modulus between 1 kPa and 100 kPa. To cover the wide range of biological tissue stiffness, which varies over at least three orders of magnitude [169], one may need to use cantilevers with different spring constants – a major complication for future applications. Furthermore, throughout the entire analysis, we have implicitly neglected the viscous and plastic components of the sample mechanics. Both these issues have been recently addressed by another paper of our group [42], where we showed that, applying a dynamic modulation analysis, it is possible to measure both the loss and storage modulus of a largely heterogenous material. Still, the method adds some limitations, as it is not as fast and as straightforward as the one presented here.

A further obvious limitation of our needle indenter here is its large diameter. Work is under way to reduce the dimensions of all its components to embed the device in a 3 mm diameter needle.
5.4 Limitations

Figure 5.6: Boxplot of Young Modulus versus depth in the fixed liver specimen for six needle insertions, compared with a cross-sectional reference (dashed line). The lever slide was placed 25 mm underneath the surface.

Figure 5.7: Boxplot of the grouped Young Moduli versus depth in the liver specimen for all six indentations, compared with the cross-sectional reference (dashed line).
Another possible drawback is the risk that, during perforation, some debris of the sample enters the Fabry-Pérot cavity between the fiber and the cantilever. Although we have not observed any nuisance when indenting inside the liver specimen, one could circumvent this problem altogether by designing a membrane based sensor.

Finally, for further research on biological samples, sterilization of the device may become necessary. We designed the device such that the sensitive part, which will not survive a repetitive sterilization procedure, can be disposed without discarding the main working elements of the indenter.

5.5 Conclusions

We have successfully developed a cantilever based, all-optical indenter at the tip of a rigid needle. The indentation measurement is enabled by a sensor that probes the mechanical properties of the underlying specimen by indentation using a microsphere. The sensor is remotely actuated by a strain gauge controlled piezoelectric translator driving a microscopic cable and spring system. The movement of the sensor as well as the movement of the cantilever is interrogated by Fabry-Pérot interferometry. We have performed stiffness measurements at fixed depth positions during needle insertion in gelatin phantoms and animal liver specimens. The measurements showed that we are able to quantify a stiffness gradient in depth and that we can successfully identify stiffer layers in a uniform sample. Measurements in a gelatin embedded animal liver confirmed that we can localize the liver based on mechanical contrast. Moreover, as the needle protrudes further inside the liver tissue, a quantitative analysis of the liver can be made based on the mechanical properties alone. Despite some limitations, our needle may, on the long term, ultimately evolve into a minimally invasive tool for the analysis of the mechanical properties of tissues, with potential applications in needle navigation or tissue diagnostics.

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Experiments regarding the mechanical properties of soft tissues mostly rely on data collected on specimens that are extracted from their native environment. During the extraction and in the time period between the extraction and the completion of the measurements, however, the specimen may undergo structural changes which could generate unwanted artifacts. To further investigate the role of mechanics in physiology and possibly use it in clinical practices, it is thus of paramount importance to develop instruments that could measure the viscoelastic response of a tissue without necessarily excising it. Tantalized by this opportunity, we have designed a minimally invasive micro-indenter that is able to probe the mechanical response of soft tissues, in situ, via an 18G needle. Here, we discuss its working principle and validate its usability by mapping the viscoelastic properties of a complex, confined sample, namely, the nucleus pulposus of the intervertebral disc. Our findings show that the mechanical properties of a biological tissue in its local environment may be indeed different than those that one would measure after excision, and thus confirm that, to better understand the role of mechanics in life sciences, one should always perform minimally invasive measurements like those that we have here introduced.

**Keywords:** Mechanical properties – Minimally invasive indentation – Dynamic mechanical analysis – Intervertebral disc – *In situ* indenter

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6.1 Introduction

It is widely recognized that the micromechanical environment that surrounds cells and biological tissues can influence a large number of fundamental physiological processes, including cell growth, cell signaling, cell migration, tumor development, angiogenesis, wound healing, scar formation, and even a highly complex process such as stem cell differentiation [65,66,68 –73]. In the field of tissue engineering, it has been further demonstrated that the mechanical stability at the defect site of the host is of key importance for the growth of successful biocompatible materials [74, 75]. It is thus not surprising that, over the last decade, there has been increasing attention to the development of experimental tools and methods that could locally map the mechanical properties of cells and tissues for both fundamental research and clinical applications [59–64].

At present, the characterization of the mechanical properties of biological samples is still mostly carried out by means of (micro-)indentation [100]. For instance, recent indentation experiments on brain tissue – one of the softest tissues of the human body – have proven to offer valuable insights in the mechanics behind the structural heterogeneity that forms the gray and white matter. These studies hold promise for a better understanding of the response of brain tissue to life threatening conditions as severe as cancerous tumors, Alzheimer’s, and traumatic injury [76 –78]. On the other side of the scale, in the field of orthopedics, indentation is known to provide previously neglected mechanical information on a tissue as stiff as cartilage [79], proving the great versatility of the approach.

(Micro-)indentation experiments, however, suffer from one major limitation. To perform the measurement of the mechanical properties of a sample, the head of the (micro-)indenter, which is typically rather bulky, needs to move into contact with the top surface. Experiments can thus be performed only on excised specimens. Yet, the extraction and preservation protocol can introduce biases that may lead to wrong conclusions. The mechanical properties of most sensitive tissues, in fact, can be altered by drying, swelling and loss of confinement, or external stress [170 –172]. One can conclude that an unbiased recording of tissue mechanical properties can only be obtained when the target tissue is still within the original surroundings. There is thus a strong demand for a device that could measure the mechanics of a sample below its surface via non- or minimally invasive means, and, in that way, enable true in situ characterization of tissue viscoelasticity [7, 103, 155]. In 2006, for example, Imer and colleagues introduced the so-called scanning force arthroscope, which is able to perform in vivo nano-indentation measurements during a standard arthroscopic procedure. Unfortunately, this instrument relies on a bulky stabilization module that prevents minimally invasive experiments [165]. More recently, our group has developed a needle-based micro-indenter, which, however, can only operate via apertures as large as 5 millimeters [103]. Alternative non-invasive techniques, such as optical coherence elastography, magnetic resonance elastography, and ultrasound elastography, certainly circumvent the excision issue, but are limited to provide the mechanical properties of tissues only at a relatively macroscopic scale and generally lack the ability to provide quantitative results [7,173,174].

To solve this impasse, in this paper, we present a new micro-indenter that is able to...
Figure 6.1: Sketches and microscope images of the optical force transducer used at the tip of the MIMI indenter. (A) Schematic model of the probe, which consists of a cantilever indentation spring (gold), an optical fiber for the interferometric readout of the displacement of the cantilever (red), and a borosilicate sphere to create a spherical contact with the indented surface (blue). The inset shows a schematic of the indentation procedure, where emphasis is put on the interferometric readout and the movement of the piezoelectric transducer (not-to-scale); (B) Microscope image of the probe, showing the interferometric cavity; (C) Top view of the sensor.

The main purpose of this study was to design a minimally invasive in situ indenter for viscoelastic characterization of tissues below the top surface. The indenter is based on a micro-machined cantilever spring operating as force transducer, the displacement of which is monitored by a Fabry-Pérot interferometer. The free hanging end of the cantilever is equipped with a spherical tip, which is used to indent the tissue (Figure 6.1). After needle insertion, a piezoelectric manipulator, fixed at the proximal end of the needle, advances the probe inside the needle until a predefined load on the sample is achieved. After contact has been reached, a sinusoidal frequency sweep is imposed on the cantilever and the indentation response of the sample is recorded to determine the frequency dependent storage- and loss moduli of the sample. The fabrication details of the probe and the measurement protocol are further discussed in the Methods section (see also [42]).

To validate our instrument against an established technique, we measured the storage and loss moduli of a polymeric reference sample (Poly(DiMethyl) Siloxane (PDMS) with a crosslinker:prepolymer weight ratio of 1:20) in three different experi-
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Figure 6.2: Schematic overview of the measurements performed to validate the MIMI indenter. The areas shaded with diagonal lines represent the PDMS sample. A) First experiment: indentation of the top surface of a PDMS sample performed with a validated table top indenter as presented in [42]. B) Second experiment: indentation inside a PDMS sample performed with our MIMI indenter. C) Third experiment: indentation inside a PDMS sample inside the NP of an IVD performed with our MIMI indenter. Not to scale.

Figure 6.3: Quantitative comparison of the storage and loss moduli obtained in the three experiments sketched in Figure 6.2, plotted as a function of the probed frequency.

ments. The first experiment (Figure 6.2A) was performed on the top surface of the sample by means of a table top indenter described in previous research, which was thoroughly validated against traditional macroscopic shear rheology [42]. In the second experiment (Figure 6.2B), we used our MIMI indenter to pierce the same sample and measure the mechanical properties of the material in the bulk. For the third experiment (Figure 6.2C), which is designed to test for any systematic errors of our instrument after insertion through a rigid material, we prepared a dummy sample obtained by replacing the NP of a goat’s IVD with a polymeric sample identical to the one used in the previous two experiments. Measurements of the mechanical properties of the dummy NP were then carried out by inserting our MIMI indenter through the AF. For more details, we refer the reader to the Methods section.

Figure 6.3 shows the dynamic response of the PDMS for a frequency range of 0.05-10 Hz as measured in the three experiments. Each frequency scan (containing 15 frequencies) was fully independent and no fitting was applied. Storage and loss moduli
6.2 Results

Figure 6.4: Dynamic response of the NP of disc 1, for a frequency range of 0.5-10 Hz, measured in situ using the needle-based MIMI probe. We performed indentations on three independent locations. Each location represents an average of 5 frequency sweeps. Variability of $G'$ (closed symbols) and $G''$ (open symbols) is found to be marginal within one disc. ($G'$ and $G''$, respectively) are presented for 5 frequency sweeps per experiment. All five sweeps were performed in the same location. It can be observed from Figure 6.3 that the frequency dependent storage and loss moduli are in quantitative agreement with each other.

To demonstrate the capabilities of the MIMI indenter in full, we have further measured the storage and loss moduli of the NP without extracting it from the IVD. The NP is a proteoglycan-rich type of connective tissue confined between the two endplates of the disc and the AF. It is known to play a crucial role in the mechanical function of the IVD and its degeneration is considered one of the underlying factors of low back pain [175,176]. An accurate assessment of the mechanical properties of this tissue is thus extremely relevant to a better understanding of the causes of certain conditions and on the definition of protocols for engineered replacement materials. The mechanical properties of the NP, however, may be drastically altered when the original confinement of the NP is released or when brought in contact with air or a liquid with unphysiological osmolarity. Not surprisingly, different rheology measurements on extracted NPs, performed according to various protocols, do provide different values for the elastic and viscous moduli of the material [177–181]. Our needle-based indenter, however, gives us the opportunity to record the in situ values of $G'$ and $G''$ of the NP while maintaining the original confinement and environment inside the disc. In our measurements, we decided to limit the frequency of the sweep to 0.5-10 Hz, as measuring at faster timescales reduces the influence of time dependent changes that occur in soft, hydrated tissues. Moreover, for the sake of measurement time, we reduced the number of frequencies in the sweep to 5. A longer measurement time would lead to increased deterioration in the sample, which may cause a change in viscoelastic properties over time. In Figure 6.4 we report the results obtained by
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Figure 6.5: Boxplot of the in situ storage- (A) and loss (B) modulus of the NP at 1.0 Hz. Entries are separated by disc. Locations within a disc are indicated by letters. Even though a large variety in \(G'\) and \(G''\) is observed between discs, the variation between different locations within one disc is minor.

6.3 Discussion

The agreement between the results of the first and second experiment on the PDMS sample demonstrates that our MIMI approach is able to accurately capture the viscoelastic properties of an homogeneous material below its top surface. The agreement of these two sets of data with those obtained with the dummy IVD further shows that the insertion of the needle through a rigid material (i.e., the AF) does not introduce any systematic error in the measurements. Moreover, the decreasing trend of the loss modulus of PDMS with decreasing frequency is well-known and confirmed by literature [182].

As for the experiments performed on the IVD, data collected on the same disc were found to be consistent. Measurements performed on different discs, however, seem to suggest that the mechanical properties of the NP vary significantly from sample to sample, as already reported in the literature [177–179]. The storage modulus of the NP varied between 1.7 and 7.2 kPa over all the recorded discs. These quantitative differences in \(G'\) may be described to the position of the disc in the spine of the animal [180,183].

Most importantly, we have observed a sharp contrast between the NP moduli for goat IVD measured in situ and those reported in the literature after extraction, which were presented in the order of 10-50 kPa [179]. This order of magnitude difference may be caused by the extensive procedure that is required to extract the NP from the IVD. Prior to rheological testing, the IVD is cut open and the nucleus is surgically
removed, both of which are procedures that could damage NP structure. Moreover, during sample preparation the NP is exposed to air and the original confinement is lost, resulting in drying and swelling. The loss of confinement and the resulting swelling of the nucleus tissue may lead to the higher storage modulus reported in the literature. The NP, in fact, consists of large amounts water-binding proteoglycans, embedded in loosely structured collagen fibrils, which are not fully stretched in their native state due to the confinement conditions [176]. Upon release of these constraints, we hypothesize that the swelling enables the collagen fibers to fully stretch, thereby increasing the stiffness of the tissue.

From the results obtained in the IVD experiments, we conclude that the mechanical properties of the NP can be accurately observed only when the NP is probed in its native environment, where the tissue is properly confined and exposed to its natural hydration conditions. One can further extrapolate this result to the numerous other tissues that, when inside the human body, are exposed to strain (such as skin, arteries, veins), confinement (brain, eye), or swelling. These highly sensitive tissues may all have a strong mechanical reaction to extraction from their surroundings. Measurements in situ are therefore mandatory for a quantitative assessment of the role of mechanics in their development or degradation. Similarly, we propose that biomaterials designed for integration in the body should be mechanically characterized in an environment that resembles their intended area – such as a loaded disc culture system [184] – to avoid a possible mismatch between in situ and ex situ tissue mechanical properties.

In conclusion, we have introduced an indenter that can perform accurate measurements of the elastic and viscous properties of a material at the end of an 18G needle. We believe that the localized and minimally invasive character of MIMI measurements, combined with the versatility of the probe it is based on, may soon trigger an entire new generation of experiments that will enable a deeper understanding of the role of mechanics in physiology and tissue engineering.

6.4 Outlook and Limitations

We have chosen to limit the band of the frequency sweep to 0.05-10 Hz, corresponding to timescales in which most natural processes occur. Oscillations with very low frequencies ($F < 0.05$ Hz) may be biologically significant but were not attainable for in situ application. The analysis of $G''$ is highly sensitive to perturbations in the low frequency range, as the energy stored in the sample (and therefore the obtained phase shift) at those frequencies is minimal. Friction in the needle shaft caused by piercing the AF tissue hampered the movement of the probe in the lumen of the needle when the speed was very low, resulting in an erroneous phase estimation of the oscillating indentation signal. The upper limit of the frequency band is dependent on the resonance frequency of the piezoelectric translator and can be increased when a translator with a smaller maximum displacement is selected.

One of the main limitations of our force sensor is the fragility of the cantilever. During the course of this study we had to replace the sensor several times due to failure as a result of overloading or obstruction of the cantilever. Future experiments may benefit from force sensors with innovative designs such as membranes or MEMS
based structures. Miniaturization of the sensor to sub-millimeter size would reduce the impact on the sample even more.

6.5 Methods

6.5.1 Force transducer and readout

The optical force transducer was built in-house out of borosilicate parts and consisted of a cantilever mounted on top of a cleaved single mode optical fiber. An extensive description of probe fabrication can be found elsewhere, although probes have been slightly adapted for this study [42]. Instead of using a ferrule, we mounted the cantilever on an 8 cm long borosilicate capillary (diameter: 1 mm, wall thickness: 0.21 mm, Science Products GmbH), as illustrated in Figure 6.1. The optical fiber (Corning SMF-28) is supported by a second borosilicate capillary (diameter: 0.55 mm, wall thickness: 0.075 mm, Vitrocom), rigidly mounted inside the first capillary. A Fabry-Pérot cavity was created between the cleaved facet of the fiber and the cantilever by coupling the distal end of the fiber to an interferometer (OP1550 V2, Optics11). The recorded intensity signal on the detector encodes for the deflection of the cantilever, which can be obtained by lock-in detection, as described in previous work [35]. A schematic of the experimental setup is presented in Figure 6.6. The probe was mounted on a long-range piezoelectric transducer (P-602.5L8, Physik Instrumente GmbH), which in turn was mounted on a coarse positioning stage. To enable a minimally invasive measurement, the probe was inserted into an 18G needle (in-house fabricated from a stainless steel capillary with diameter: 1.3 mm, wall thickness: 0.1 mm, Salomon’s metalen b.v.) and was able to move freely in axial direction with respect to the needle thanks to the piezoelectric transducer and a manipulator. The needle was fixed on a motorized linear stage (LTS300, Thorlabs GmbH), which was used for needle insertion.

Before installation in the needle, the spring constant of newly fabricated probes was calibrated using an in-house developed calibration method [35]. Cantilever spring constants varied between 60-70 N/m, slightly depending on the exact position of the spherical indentation tip at the far end of the cantilever. Prior to each experiment, adequate probe performance was confirmed by a calibration procedure on glass. Whenever a probe did not perform satisfactory due to drifts or bad interference fringe visibility, it was discarded and replaced. A possible geometrical offset between the position of the cleaved optical fiber and that of the spherical tip was accounted for during the calibration procedure on glass.

6.5.2 Dynamic mechanical analysis

DMA can be used to obtain information about the dynamic mechanical moduli of a sample. Its application to indentation has been described in previous research [42,117]. By means of a feedback control loop on the bending of the cantilever a predefined oscillatory load-sweep was applied on top of a fixed static load. Control of the applied load (i.e. cantilever deflection, instead of only probe movement) was essential to ensure that a consistent stress was applied to the sample at each indentation. Upon contact with the tissue, the feedback-controlled piezoelectric transducer moved the probe
6.5 Methods

Figure 6.6: Schematic of the (A) experimental setup and (B) readout and feedback control. (A) The probe is mounted on a long-range piezoelectric translator (orange), which is attached to a manual translation stage (green). The probe is housed in an 18G needle, fixed on a motorized linear stage. The sample is clamped in front of the needle and can be positioned in three dimensions (XYZ). (B) Real-time control of cantilever displacement (i.e. load control) by means of the piezoelectric translator is enabled by high-frequency wavelength modulation.

forward (thus bending the cantilever) until a predefined value for the applied load (∼300µN) by the cantilever was reached. This load was kept stable for at least 60 s to allow for dissipation of the tissue. Afterwards, the load was oscillated sequentially (amplitude ∼ 10µN) for a finite number of increasing frequencies (5 periods each), logarithmically spaced between 0.05 Hz (0.5 Hz for the NP measurements) and 10 Hz. During all indentations it was ensured that the indentation depth stayed within the linear viscoelastic regime and that indentation depth was much smaller than the bead radius (the maximum static indentation depth was 40µm). Shear storage and loss moduli were obtained by employing an analytical solution for oscillatory indentation using a spherical indenter obtained in previous research [42,117].

Although our indenter is limited to a compressive motion, moduli in this study are presented as shear moduli (i.e. the ratio of shear stress to shear strain). The modulus of compression ($E$) was converted into the shear modulus ($G$) by means of Poission's ratio, which was assumed to be 0.5 for roughly incompressible soft biological tissue [185,186]. We have explicitly made the conversion to shear modulus to facilitate a quantitative comparison of our in situ results with classical rheology as well as other shear-based techniques.

6.5.3 Measurement protocol

During the in situ measurements discs were placed in a clamp mounted on top of a three-axis micro manipulation stage (MAX312D, Thorlabs GmbH) and positioned in front of the needle (Figure 6.6). Prior to the first insertion, discs were probed with a 21G hypodermic needle (Neolus NN-2138R, Terumo) to locate a suitable insertion trajectory. Subsequently, the 18G needle, housing the micro-indenter, was inserted through the annulus. All measurements were performed 1-3 mm inside the NP. After reaching the target location, insertion of the needle was stopped and the
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The probe was carefully advanced until contact with the tissue was observed. In case of contamination of the lumen of the needle, which was indicated by premature contact with the tissue, the probe and the needle were retracted and the lumen was cleaned. After finding contact with the tissue, the probe was retracted for 100µm and the dynamic mechanical analysis procedure was started (see section Dynamic mechanical analysis). Five frequency sweeps were performed for each needle insertion. After indentation, the probe and needle were retracted to their respective starting positions.

For the ex situ reference measurements the probe was positioned such that it slightly protruded the tip of the needle. The sample was placed vertically in front of the device and the probe was moved forward until contact was found. Afterwards, the aforementioned measurement procedure was followed.

6.5.4 Intervertebral disc preparation

Isolated intact spines of skeletally mature female milk goats (age 3-4 years) were obtained from a local butcher and processed immediately after isolation. To prepare specimens for testing, individual discs were separated from the spine with an oscillating saw, maintaining 2-5 mm of endplate on both sides. Subsequently, discs were brushed clean, rinsed and stored in physiological saline soaked gauzes at -20 °C. Before testing, discs were thawed in lukewarm saline water for 30 min.

6.5.5 Reference disc- and sample preparation

To verify proper functioning of our needle based indenter during in situ measurements, we tested a dummy IVD sample in which the NP was replaced with PDMS (Sylgard 184, Dow Corning) with a crosslinker:prepolymer weight ratio of 1:20. The PDMS was mixed, degassed for 30 min, poured into a glass petridish and allowed to cure at room temperature in a flow chamber for 96 hrs. Afterwards, the NP of an IVD was surgically removed by tweezers and a scalpel after drilling a small hole through the bone of the endplate. After NP removal, a cylinder of PDMS was cut from the cured sample and placed firmly into the disc. To fixate the PDMS in all directions the disc was resealed. The remainder of the PDMS in the petridish served as the polymeric reference sample.

Code availability

The computer code used to generate the results of this study is available on reasonable request from the corresponding author.

Data availability

All raw and processed data that support the findings of this study are available from the corresponding author upon reasonable request.
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Author contributions

SB performed the experiments, data-analysis and wrote the report. KE was responsible for IVD preparation and handling and literature research. SB and KE contributed to the design of the experiments. TS and DI conceived the experiments and offered guidance and supervision. All authors contributed to the editing of the report.

Competing interests

DI is co-founder and shareholder of Optics11.
Stiffening of the nucleus pulposus upon axial loading of the intervertebral disc: an experimental in situ study

Mechanical loading is inherently related to the function and degeneration of the intervertebral disc. We present a series of experiments aimed at measuring the effect of a loading/unloading cycle of the intervertebral disc on the mechanical properties of the nucleus pulposus. The study relies on our new minimally invasive micro-indenter (Beekmans et al 2017 Scientific Reports 7 11364), which allows us to quantify the storage and loss moduli of the nucleus pulposus by inserting an optomechanical probe in an intact (resected) intervertebral disk through the annulus fibrosis via a small needle. Our results indicate that, under the influence of compressive loading, the nucleus pulposus exhibits a more solid-like behavior.

Keywords: intervertebral disc – nucleus pulposus – mechanical loading – minimally invasive micro-indentation – in situ viscoelastic properties

7 Stiffening of the nucleus pulposus upon axial loading of the intervertebral disc: an experimental in situ study

7.1 Introduction

The nucleus pulposus (NP), the water-rich gelatinous center of the intervertebral disc (IVD), primarily bears the pressure on the spine and plays a major role in the degeneration of the IVD. The NP is strongly confined between the annulus fibrosis (AF) and the two vertebral end-plates (VEP) of the IVD [187]. The NP contains large concentrations of negatively charged proteoglycans (PGs), which cause it to retain water and maintain its swelling pressure [188]. Surrounding the PGs, sparsely arranged collagen fibrils serve as a supporting matrix. Due to the limited amount of structural components inside the NP, its mechanical properties are dependent on the amount of PGs and water in the NP [189]. As the amount of water in the IVD slowly changes with loading [190], the mechanical properties likely depend on the loading history.

During a 24 hr day and night period, the spine is exposed to a cycle of mechanical axial loading and unloading. Mechanical loading can have various direct and indirect effects on the IVD, including, in severe cases, disc degeneration [176]. Although the metabolic effects of the NP of disc loading have been described extensively [191], little research effort has been spent investigating the effect of loading on the mechanical properties of the NP. This may be ascribed to the complex confinement of the NP within the IVD. Conventional mechanical characterization instruments, such as rheometers, require extraction of the NP from the disc, which may result in damage caused by (A) the surgical procedure, (B) exposure to air or water, resulting drying or swelling, and (C) bulging due to the loss of confinement, all of which are known to alter the mechanical properties of the NP. The mechanical properties of the unconfined NP are thus dependent on the measurement protocol, as indicated by a large spread in elastic moduli in the literature values [177–180,183].

Recently, our group presented a novel minimally invasive device that allows the user to measure the mechanical properties of biological tissue, such as its stiffness, at the tip of a rigid needle by means of micro-indentation [103]. This method has been further improved to incorporate a full rheological analysis using an 18G needle – an approach dubbed as minimally invasive micro-indentation (MIMI) [192]. The small footprint of this device allows for minimally invasive measurements of the localized mechanical properties of a material beneath its surface. The ability to perform sub-surface stiffness measurements is in sharp contrast with conventional mechanical testing instruments, the range of which is limited to the surface of a sample. Hence, this approach enables us to record the mechanical properties of the NP, in terms of the elastic and viscous modulus, while maintaining NP confinement, as presented in [192]. Using our minimally invasive indenter, here, we present a full, in situ characterization of the mechanical properties of the confined NP, thus bearing the native confinement by the AF and VEP, during a cycle of mechanical loading and unloading of the IVD.
7.2 Methods

7.2.1 Intervertebral disc preparation

Isolated intact spines of skeletally mature female milk goats (age 3-4 years) were obtained from a local butcher and processed immediately after isolation. To prepare specimens for testing, individual discs were separated from the spine with an oscillating saw, maintaining 2-5 mm of flat endplate on both sides. Subsequently, discs were brushed clean, rinsed and stored in physiological saline soaked gauzes at -20° C. Before testing, discs were thawed in lukewarm saline water for 30 min. The typical cross-sectional area of the discs was 900 mm$^2$.

7.2.2 Minimally invasive micro-indentation

MIMI is based on an optical force transducer at the tip of a thin needle and employs the bending of a micro-machined cantilever to infer the viscoelastic properties of a sample by means of dynamic mechanical analysis (DMA) [42, 103, 117]. The details of the device are described in previous work [192]. The cantilever, the bending of

Figure 7.1: Schematic view of the experimental setup. See [192] for details. A) Close-up image of the MIMI probe at the tip of an 18G needle. B) Schematic of the probe, mounted on a long-range piezoelectric translator (orange) which is attached to a manual translation stage (green). The probe is housed in a thin needle, fixed on a motorized linear stage. The IVD (pink) is clamped in front of the needle between a pivoting and a flat plate and can be positioned in three dimensions. C) Schematic of the needle inside the IVD. Load can be applied on the IVD by increasing the compression force. The NP is highlighted by diagonal lines. Not to scale.
which is monitored via a Fabry-Pérot cavity using a cleaved single mode optical fiber, is equipped with a spherical tip with a diameter of around 200µm. The probe is mounted in the lumen of an 18G needle and can be retracted to a safe distance from the needle tip (figure 7.1A). By means of a long-range piezoelectric transducer (P-602.5L8, Physike Instrumente GmbH), mounted at the proximal part of the needle, fine positioning of the probe can be achieved. The needle is fixed on a motorized linear stage (LTS300, Thorlabs GmbH), which is used for needle insertion (figure 7.1B).

Before installation in the needle, the spring constant of newly fabricated probes is calibrated using a customized calibration method for cantilevers with interferometric readouts [35]. Effective cantilever spring constants varied between 60-70 N/m.

To obtain a mechanical characterization of the NP with the micro-indenter, we have made use of dynamic indentation [42]. DMA is based on load- or indentation controlled oscillations applied on top of a fixed static load. Precise control of load (i.e. cantilever bending) or indentation depth is crucial to ensure consistent stress or strain, respectively, in the sample with each indentation. We have performed our dynamic indentations in load-control mode. After contact with the NP is confirmed, the probe is moved forward (thus bending the cantilever) until the static load applied on the tissue matches the preset value (∼300µN). To allow the tissue to equilibrate, we maintain the fixed static load for 60 s. Afterwards, the load is swept over 5 frequencies (oscillations with 5 periods each), logarithmically spaced and in increasing order between 0.5 Hz and 10 Hz. The amplitude of the oscillations is set to 10µN in order to 1) keep the indentation depth in the viscoelastic regime and 2) ensure that indentation depth is much smaller than the bead radius (max indentation depth ∼40µm). We obtain shear storage and loss moduli (\(G'\) and \(G''\)) from the dynamic indentation results by applying an analytical solution for DMA [117].

### 7.2.3 Measurement protocol

Prior to each experiment, an indentation procedure on glass is performed to account for a possible geometrical offset between the position of the cleaved optical fiber and that of the spherical tip. IVDs are clamped on top of a three-axis micro manipulation stage (MAX312D, Thorlabs GmbH) and are positioned in front of the needle (Fig. 7.1). The clamping force is minimized to prevent initial axial stress on the disc. Before the first insertion, unloaded discs are probed with a 21G hypodermic needle (Neolus NN-2138R, Terumo) to locate a suitable insertion trajectory. Subsequently, the 18G needle (manufactured in-house from a stainless-steel capillary with diameter: 1.3 mm, wall thickness: 0.1 mm, Salomon’s metalen b.v.), housing the micro-indenter, is inserted through the AF until 1-3 mm inside the NP (figure 7.1C). After reaching the target location, insertion of the needle is stopped and the probe is carefully advanced using a coarse long-range transducer until contact with the tissue is observed. Upon contact with the NP tissue, the probe is retracted for 100µm and dynamic indentation is started. In case of contamination of the lumen of the needle, indicated by premature contact with the tissue, the probe and the needle are retracted and the lumen is cleaned. Five frequency scans are obtained for each insertion.

To study the effect of IVD loading on the mechanical properties of the NP, the whole IVD is compressed rapidly (∼2 s) in the clamp until a strain on its total height
7.3 Results and discussion

Figure 7.2: Quantitative comparison of the viscoelastic behavior of the NP before, during and after loading of disc 1, as measured in situ for 2 central sites in the NP (different insertion angle, same penetration depth) in the same disc (A and B). Frequency dependent storage (closed symbols) and loss (open symbols) moduli are averaged over 5 frequency sweeps at each location. An increase in $G'$ and $G''$ during the loaded state can be observed for both sites. For site (A), $G'$ and $G''$ present a low degree of recovery, contrary to site (B), where a high degree of recovery can be observed.

of 10% is reached, corresponding to approximately 1000 N. The top plate of the clamp allows for sufficient pivot to ensure axial loading. After 30 min, the compressed disc is re-inserted with the needle (i.e. in the same location) and NP mechanical properties are recorded according to the same protocol. Afterwards, the disc is unloaded and, after 30 min, the same location in the NP is tested once more. Cycles of 30 min have been chosen to allow for observable changes in the sample while avoiding significant drift in the measurement system. The compressive load is not continuously monitored and, therefore, a small amount of stress relaxation could not be prevented. This is neglected in the further analysis, as the aim of this study is to observe the effect of a significant load on the IVD to the dynamic elastic moduli of the NP. The data for $G'$ and $G''$ were assumed to be distributed normally. Statistical differences between loading states were investigated by means of a paired Student’s t-test.

7.3 Results and discussion

In this study, 6 sites (2 per disc), all in the center of the NP, were successfully characterized in terms of storage and loss modulus during a cycle of loading and unloading. Each site was approached from a different angle but it was ensured that the penetration depth in the IVD was always the same. Needle insertions were performed before, during and after loading on the IVD. Five dynamic indentations were performed for each needle insertion. The time between states of loading/unloading was set to 30 min for each cycle.

In figure 7.2 we report the in situ frequency dependent storage- and loss moduli of the NP measured before, during and after loading of disc 1. We observed a systematic increase in both $G'$ and $G''$ in the NP after loading of the IVD. After releasing the load, the NP showed recovery in all the measured sites, albeit to a variable extent, as
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Figure 7.3: Storage modulus \( (G') \) and \( \tan(\phi) \) at 2.2 Hz for six cycles of loading and unloading on six central sites in the NP. Each bar or point represents an average of 5 measurements at 2.2 Hz, corresponding to those depicted in figure 7.2 for disc 1A and 1B. Statistical differences in \( G' \) between the different loading state are indicated directly above the bars (* indicates \( \alpha < 0.05 \), ** indicates \( \alpha < 0.01 \)). Statistics for \( \tan(\phi) \) are shown at the top of the chart. All error bars are standard deviations.

illustrated by the example in figure 7.2. Figure 7.2A shows a low degree of recovery, whereas in figure 7.2B an example of a high degree of recovery can be seen. We report a minor upwards trend for both \( G' \) and \( G'' \) with increasing frequency. This weak-gel like rheological behavior has been reported earlier for porcine NP and can be related to the NP’s physiological role as a shock absorber [180].

To illustrate the effect of IVD loading on the mechanical properties of the NP, in figure 7.3 we show the average storage modulus at 2.2 Hz for each tested site in the NP before, during and after loading, as well as the total average for \( G' \) at 2.2 Hz. The storage and loss moduli of the unloaded NP varied slightly between the measured sites, as was expected with varying locations and discs. The total average \( G' \) at 2.2 Hz of the unloaded NP was \( 4800 \pm 800 \) Pa, in agreement with previous work – a comparison of \( G' \) and \( G'' \) of the unloaded NP with literature values can be found in [192]. A systematic increase of \( G' \) during loading can be clearly observed in figure 7.3 for each of the sites, and is confirmed by the total average \( (19100 \pm 4100 \) Pa). An increase of \( G' \), corresponding to a stiffening of the material, can be correlated to one of the main biological functions of the NP, namely maintaining the height of the IVD under compressive loading [193]. An increase in whole disc compressive stiffness during axial loading has been reported repeatedly in the literature before [194,195].

The value of \( G' \) of the NP did, in most cases, not recover to its unloaded level after releasing the load. Two possible explanations for the absence of full recovery are: 1) the recovery time of 30 min was not sufficient or 2) the induced mechanical load was too high, causing irreversible damage to the NP. Mechanical overloading is associated with accelerated disc degeneration and it has been reported that disc cells,
including the NP, respond to mechanical loading in a manner that depends on the magnitude, duration, and frequency of the loading [196]. A recovery time of 30 min may be relatively short with respect to a physiological loading/unloading cycle of 24 hours [194]. Performing measurements with a longer waiting time is, however, not ideal either. Using short cycles, the sample remains in similar conditions throughout the measurement. Moreover, by doing so, significant drift in the measurement system is avoided while, at the same time, a recovery time of 30 min enables observable differences in fluid content. For the same reason we reduced the frequency sweep range to 0.5-10 Hz and decided to limit the amount of frequencies in the sweep to five, as measuring at longer timescales increases the influence of time dependent changes that occur in soft, hydrated tissues. The varying amount of recovery of the sites may also be attributed to the position of the disc in the spine [180], or to the location that was probed within the NP, as the strain is not uniform inside the NP during loading [197].

Additionally, in figure 7.3 we report the value of tan(φ) (\(= G''/G'\)) during the loading cycle. Tan(φ), sometimes called the loss factor, quantifies the balance between the loss and storage modulus and can be seen as a weighted indicator of the viscosity of a material. A value for tan(φ) above unity indicates a more liquid-like behavior, whereas a tan(φ) closer to zero suggests more solid-like properties. The average tan(φ) value before and after compression of the disc was 0.21 ± 0.03 and 0.20 ± 0.03, respectively, indicating gel-like behavior of the NP. During compression, the average tan(φ) dropped to 0.14 ± 0.03, indicating that the NP has lost a significant amount of liquid to the AF or VEP – no fluid outflow was observed during the loading of the disc – and thus demonstrates stiffening under compression. This is in line with the suggestions that the NP is a material with biphasic/poroelastic properties [198,199], as the compression will induce a loss of water, increasing the solid to fluid ratio, and therefore shifting towards a solid-like behavior. Furthermore, after the fluid is expelled, the solid matrix will stiffen to withstand the compressive forces [200]. This observation is in sharp contrast with the traditional description of the NP as an incompressible rubber often used in computational modeling [201,202].

In this study, \(G'\) and \(G''\) are calculated using an indentation model, which is set in a viscoelastic framework. This analysis provides information on the mechanical behavior of the NP under compression in situ. However, the lack of a direct measurement of poroelastic properties is a limitation of this study. Poroelasticity is often described by means of finite element models which require a-priori information [203,204]. Following [203], one can find evidence of poroelasticity by observing a shift in the characteristic frequency of tan(φ) with varying indentation depth. This frequency shift can be best observed when a continuous frequency sweep is applied to the sample. Due to the discrete amount of frequencies probed during DMA, the current study is limited to an analysis in a viscoelastic framework. Therefore, to assess the poroelastic behavior of the NP in more detail, future experiments could include denser frequency sweeps or employ a continuous sweep model, such as in [203].

Loading of the IVD by means of compression results in a substantial amount of stress in the tissue, in particular in the AF. Once the needle has penetrated the AF, part of this stress may be transferred to the needle shaft. In order to assess whether the stress on the needle has an influence on the dynamic analysis we have designed a reference experiment, in which the needle is subjected to stresses comparable to those
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Figure 7.4: Reference experiment on a polymeric sample to test the influence of stress on the needle shaft on the dynamic analysis. Measurements of storage- and loss moduli were performed according to the same protocol as the IVD measurements. Each bar represents the average $G'$ of 5 measurements at 2.2 Hz. No significant deviation of $G'$ or $\tan(\phi)$ was found during loading of the disc. All error bars are standard deviations.

during a loading experiment, while the reference sample is insensitive to mechanical loading (we used a polymeric sample, prepared as in [42]). As the mechanical properties of the reference sample are insensitive to stress, the same results for $G'$ and $G''$ were expected before, during and after loading. Figure 7.4 reports the average for $G'$ at 2.2 Hz of 5 frequency sweeps for 5 cycles of loading and unloading. From the absence of an increase in $G'$ during loading it can be concluded that stress on the needle shaft due to loading of the disc does not influence the dynamic mechanical analysis. Therefore, the findings of this study are not influenced by a systematic error induced by the measurement device.

7.4 Conclusions

Concluding, in this study we have recorded the in situ mechanical properties of the NP during a cycle of loading and unloading using our in-house developed minimally invasive micro-indentation technique. A full, in situ, mechanical characterization of the NP before, during, and after mechanical loading of the IVD showed an increase of the storage and loss modulus, and, more importantly, a decrease of $\tan(\phi)$ after 30 min of axial loading. Recovery of the moduli was observed in all cycles 30 min after releasing the load, although only 50% of the sites recovered to the same level as before testing. Our results indicate stiffening of the NP during axial IVD compression, which is in line with the suggestion that the NP can also be described as a poroelastic material.
Acknowledgments

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Author contributions

SB performed the experiments, data-analysis and wrote the report. KE was responsible for IVD preparation and handling and literature research. SB and KE contributed to the design of the experiments. TS and DI conceived the experiments and offered guidance and supervision. All authors contributed to the editing of the report.

Competing interests

DI is co-founder and shareholder of Optics11.
Optimization of the batch production of silicon fiber-top MEMS devices

We present a fabrication procedure for batch production of MEMS devices directly on top of an optical fiber. The procedure relies on the approach introduced earlier by our group (K. B. Gavan et al, Top-down approach to fiber-top cantilevers, Opt. Lett., 36, 2898-2900 (2011)), which has been here optimized to obtain higher yield and increased reliability. We describe in details the 8 steps of the procedure and we show its application to the fabrication of several cantilever-based structures. Overall, we report a process yield of 80% functioning MEMS devices in our final batch.

Keywords: Batch production – Fiber-top device – Fabrication – Silicon – Photolithography

8.1 Introduction

Micro-electro-mechanical systems (MEMS) generally consist of miniaturized (electro) mechanical elements that are usually fabricated by growing and patterning alternate layers of sacrificial and structural materials on a flat surface. MEMS devices can vary from relatively simple structures, having no moving elements, to extremely complex electromechanical systems with multiple functional elements under the control of integrated microelectronics [205,206]. The quality of MEMS has seen a remarkable development over the last decades, resulting in a wide variety of applications such as accelerators, gyroscopes, inkjet printers, pressure sensors and optical switches [207–209], to name a few. Functionalized MEMS devices further enable physical, chemical and bio-sensing, and can even be embedded into biomedical instrumentation [210–212].

For utilization in liquids and harsh environments, where electronics is prone to failure, the movement of the mechanical parts of a MEMS device has to be monitored optically. For this kind of applications, one may resort to the use of optical fiber interfaces, which adapt well to the development of all-optical remote sensing readouts [213]. For instance, MEMS devices can be produced with traditional semiconductor technology in flat wafers and then glued onto the end of an optical fiber [214,215]. Alternatively, one can use the cleaved end of the optical fiber as the very same building block from which a MEMS device can be obtained [25,216–222] – an approach that has been dubbed as fiber-top technology. Unfortunately, both approaches have considerable disadvantages, such as cumbersome manufacturing, high cost of production, and lack of versatility.

As a solution to this problem, a few years ago our group has proposed to fabricate MEMS devices on the cleaved end of a fiber via a top-down process similar to that used in semiconductor technology [31,32]. This approach does not require any gluing procedure, making the fabrication process simpler, more cost effective, and more reliable. Furthermore, it relies on a series of steps that, in principle, can be used to parallelize production. Due to a series of technical obstacles, however, the method was never tuned to a point where series, low cost production could be achieved.

To overcome this impasse, we have spent a dedicated engineering effort to develop and fine-tune all the equipment needed to optimize our top-down fabrication process. In this paper, we present the results of this effort, demonstrating that we can indeed fabricate fiber-top sensors via a parallel process with an 80% effective fabrication yield in our final batch.

8.2 Fabrication procedure

The direct fabrication of a MEMS device on top of an optical fiber consists of 4 major processes (sputter deposition, align-and-shine (AS) photolithography, reactive ion etching (RIE), and chemical wet etching) divided into the 8 steps illustrated in Figure 8.1 and discussed here below.
8.2 Fabrication procedure

Figure 8.1: Schematic overview of the 8 steps required to fabricate a MEMS device directly on top of an optical fiber (not to scale). Note that, for a better reading of the figure, here we show a cross section of the fiber. A) Cleaved and rounded single mode optical fiber. B) Sputter deposition of 3µm gold followed by 1µm silicon. C) Application of the photoresist. D) Align-and-shine photolithography. E) Development of the photoresist. F) Reactive ion etching of the structural silicon layer. G) Removal of the photoresist. H) Chemical (wet) etching of the sacrificial gold layer.

8.2.1 Step 1: Fiber preparation

The MEMS device is built on the cleaved end of a standard single-mode optical fiber (SMF 28e+, Corning Inc). This fiber has a cladding diameter of 125µm and a core diameter of 8µm. The fiber is protected on the outside by a dual acrylate coating with a diameter of 250µm to prevent fractures of the glass fiber. Before cleaving the fiber, this coating is stripped away such that only 3 mm of cladding is exposed after cleaving.

In our top-down fabrication method the cleaved facet of the optical fiber is the substrate on which the consecutive layers are deposited. The quality of the cleave defines the growth conditions and surface morphology of the deposited layers and, ultimately, the quality of the MEMS device. To produce our devices in a reliable manner, it is thus imperative to start with a flat and well defined smooth surface morphology. Upon cleaving, hand-held cleaving machines, like those developed for the telecom industry, leave a fish-shell surface morphology behind (see Figure 8.2A), which is not suitable for our purpose. To obtain a flat and perpendicular end plane of the fiber, one must therefore rely on laboratory cleaving machines that offer a better control on the cleaving process (see Figure 8.2B).

The sharp edge of the cleaved fiber can, however, introduce weak spots in the deposited layers, eventually resulting in severe delamination of the deposited material around the edge (Figure 8.2C). To avoid this problem, immediately after cleaving, the fibers are exposed to an isothermic plasma field (Large Diameter Splicing System (LDS2), 3 SAE Technologies) that rounds off the sharp edges. We obtained a bending radius of approximately 15µm, resulting in a central flat area with a diameter of 80µm (Figure 8.3A and 8.3B).

For further processing the fibers are mounted in a batch holder (Figure 8.3C) which is compatible with all process steps, excluding AS photolithography, where the fibers have to be processed one-by-one. The batch, consisting of 18 fibers, is mounted inside the batch fiber holder unless otherwise stated.
Figure 8.2: Light microscope images of the cleaved fiber facet. A) Fish-shell morphology after using a standard, hand-held fiber cleaver (Fitel S325A One-Step Fiber Cleaver). B) Flat surface morphology obtained by means of a laboratory cleaver (Vytran LDC 200). Note the local tension area caused by the impact of the diamond axe in the lower righthand corner. C) Delamination of a chromium layer starting at the sharp edge of the fiber (i.e. when the fiber was not rounded).

Figure 8.3: A) Fiber after cleaving. B) Fiber after exposure to plasma (note the rounded edges). C) The batch fiber holder containing 18 fibers.
8.2.2 Step 2: layer deposition

Deposition equipment.

The fiber-top MEMS devices fabricated in our facility are machined on a 1µm thick structural silicon layer anchored on a 3µm thick sacrificial gold layer. Both layers are deposited on the fibers by means of sputtering deposition.

Sputtering deposition can produce pure and uniform films even when the substrate is not heated up at high temperatures – a major advantage for our fabrication process, in which we aim to prevent damage to the dual acrylate coating of the fibers. Unfortunately, the rate of deposition that one can obtain with our method is notoriously low, forcing us to rely on long sputtering sessions. Long periods of sputtering, however, may lead to cross-contamination of the source materials. This can be prevented by mounting long tubular chimneys on the sputter sources. Ductile metals like gold form a thick, stable layer on the inside of the chimneys and, thus, enable use of the source for many (long) runs without any further maintenance on the machine. For the silicon source, however, the same solution is actually counterproductive. Due to the high intensity of the plasma and the high deposition rate inside the chimney, the compressive stress of the deposited layer of silicon in the chimney increases rapidly, leading to delamination of the silicon layer into a fine silicon powder. This powder becomes ionized in the sputter plasma and gets drawn into the gap between the grounding ring and the target material, causing electrical short-cuts that prematurely stop the deposition process. To limit cross-contamination and reduce the probability of source shortcuts, we have equipped the system with a series of shutters mounted in close proximity of the target material. Furthermore, the silicon source is unmounted and cleaned after every deposition.

Our machine is equipped with four sputter sources (A320 UHV, AJA Company), containing 2 inch diameter high purity targets of titanium, chromium, gold and silicon, mounted in a custom built ultra-high vacuum (UHV) system (Demaco Vacuum N.V.). The vacuum system is evacuated using a turbo molecular drag pump (TMU 450 UHV, Balzer), resulting in a base pressure of the UHV system of $2 \times 10^{-9}$ mbar after a 48 hrs bake out (100° C). The four sputter sources are connected to an RF power supply of 300 W (R301, Seren) in combination with a Seren MC automatic matching box and a DC power supply of 500 W (MDX 500, Advanced Energy). We use an RF power supply (RFG-300-A, Yima Co.), electrically connected to the fiber holder, for plasma cleaning of the fibers. During deposition, argon gas of 6N purity is used as sputter gas. The flow of argon gas is controlled by a MKS flow controller type 247. A rest gas analyzer (LM502, Spectra Vacsan) is included to measure the quality of the rest gas in the system. A load-lock system (CF64, MKS) is connected to the deposition chamber to facilitate the introduction of the fibers. The load-lock system is pumped down to $2 \times 10^{-9}$ mbar (HiCube 80, Pfeiffer) before the fibers are introduced into the deposition chamber.

Titanium sublimation.

Before introducing the fibers into the deposition system, the titanium source is run for 15 min using 150 W RF power and a flow of 50 ml/min argon gas at $7 \times 10^{-3}$
mbar to cover the inside of the vacuum system with a 100 nm titanium layer. This layer acts as a titanium sublimation pump that captures oxygen, hydrogen, and water of the rest gas, thus enabling the production of an oxygen- and hydrogen-free silicon film. The argon gas settings are the same for all the successive steps and depositions unless stated otherwise.

**Plasma cleaning.**

Next, the fibers are introduced into the chamber via the load-lock system, where carbon hydrides and water on the fiber surface are removed by means of an RF plasma cleaning procedure (3 min, 30 W). While mounted in the deposition machine, the back ends of the fibers are protected by a ceramic tube mounted on the holder.

**Chromium adhesion layer.**

A 10 nm chromium adhesion layer is deposited at 80 W RF sputter source power, resulting in a deposition rate of 0.1 nm/s. To prevent contamination of the cleaved fiber surface the chromium deposition is started during the last 30 s of the plasma cleaning. The total chromium deposition time is set to 100 s. This adhesion layer is of paramount importance since noble metals do not adhere well on glass.

**Gold sacrificial layer.**

To facilitate a gradual transition towards gold deposition, the gold sputter source is started during the last 30 s of the chromium deposition. In this way an intermixed layer between the chromium and the gold is created (of about 20 nm) to further enhance the gold adhesion. After 30 s of mixed deposition, the chromium source is turned off and a layer of 3 μm gold is deposited using 20 W DC power and an effective deposition rate of 0.55 nm/s. The total gold deposition time is set to 91 min.

**Silicon structural layer.**

After gold deposition, an intermixed layer of gold and silicon (of about 20 nm) is created by simultaneous sputtering of gold and silicon for 30 s, after which 1 μm silicon is produced using 150 W RF power and an effective deposition rate of 0.19 nm/s (Figure 8.1B). The total silicon deposition time is set to 88 min.

### 8.2.3 Step 3: Application of the photoresist

In our process, the silicon layer is patterned via a selective RIE etching step, which is preceded by a photolithography step that defines which parts of the layer have to be removed and which parts have to remain. The photolithography step is achieved via AS photolithography, which is described in [31,32] and later in the text. To achieve good reproducibility in the fabrication process, it is important that the photoresist (PR) layer is uniform and flat. In our previous experiments [31,32], this detail was somewhat neglected. In earlier AS photolithographic experiments, in fact, the PR layer was applied by dipping the fibers in a solution of PR and acetone [31].
8.2 Fabrication procedure

Figure 8.4: Light microscope images of silicon wafers (A-C) and an optical fiber (D) after photoresist spray coating. A) Open photoresist coverage after 1 spray cycle of the spray coater (layer thickness = 0.9 µm). B) Closed coverage after two spray cycles (layer thickness = 1.6 µm). C) Coverage after three spray cycles (layer thickness = 2.3 µm). D) Spray coated photoresist applied on an optical fiber (with deposited layers) using two spray cycles.

approach resulted in a highly curved PR layer that would be too thin at the edges to withstand the RIE etching step.

To avoid this problem and obtain a more evenly distributed PR layer on top of the fibers, we have thus decided to switch to a spray coating machine (Altaspray AS8, Suss Tec GmbH). The spray coating is based on micro gear pumps that, in combination with a nitrogen gas jet, are able to produce sub-micrometer PR droplets. For optimal coating, we use low-viscosity PR (AZ4999, Mircochemicals GmbH), which is designed for micro-droplet formation [223], diluted with methyl ethyl ketone (MEK) as fast evaporating thinner, and 1-methoxy-2-propyl-acetate (PGMEA) as a slower evaporating solvent. During the flight between the spray nozzle and the substrate the MEK evaporates quickly, increasing the viscosity of the droplets to an intermediate value. When the PR has reached the substrate, the viscosity has increased to such an extent that flowing on the substrate is minimal. The diffusion of the PR on the sample surface is further reduced by maintaining a substrate temperature of 75°C during spray coating, resulting in further evaporation of the solvent and an increase of the PR viscosity. A viscosity which is too low causes macroscopic PR flowing, thereby reducing the coverage of the PR film on the rounded edge of the fiber [224].

A homogeneous PR film requires, nevertheless, flowing of the PR on the substrate for at least 1-2 µm, thus defining an upper limit for the resist viscosity or, in other words, a certain minimum for the remaining solvent concentration. We have performed several experiments with the Altaspray AS8 manual system to optimize PR film coverage and layer thickness. The standard process for spray coating on a 10 cm diameter substrate consists of four cycles of the nozzle in a zig-zag movement scanning over the whole substrate surface area. After each cycle the substrate is rotated 90°. Tests have been done on standard silicon wafers analyzing the PR coverage, thickness and morphology after each consecutive cycle using a profilometer (Dektak 8, Veeco).

Figure 8.4A shows the film produced after one spray cycle. Although the film thickness is found to be approximately 1 µm, the film shows open spots with a diameter of 20 µm. After two spray cycles a film thickness of 1.6 µm is obtained (Figure 8.4B). This film is completely closed with the exception of a few minor irregularities. After applying three spray cycles a film thickness of 2.3 µm is realized and no open spots can be discerned (Figure 8.4C).
To achieve the thinnest closed layer, PR is spray coated on the fibers in 2 spray cycles. To apply the PR on the optical fibers, the batch fiber holder (Figure 8.3C) – holding the cleaved fibers with deposited layers of gold and silicon – is mounted vertically on an aluminum disk of 10 cm diameter that served as a dummy wafer to run through the spray coating process as described above. The PR coverage on the fiber top, the sidewalls, and the rounded edge was found to be uniformly 1.5 \( \mu \)m thick and, therefore, sufficient to withstand the RIE process (Figure 8.4D).

8.2.4 Step 4: Align-and-shine photolithography

Upon completion of the PR prebake (95\(^\circ\) C, 1 min), the fibers are serially patterned via our in-house developed AS photolithography technique (Figure 8.1D) [31, 32]. The AS photolithography approach relies on standard contact-mask lithography, where, however, the mask is written on the facet of a multimode fiber (Figure 8.5). The facet of the mask fiber is then brought to perfect contact with the facet of the PR coated fiber (the target fiber). Shining light from the other end of the mask fiber, one can then transfer the pattern to the PR layer. We refer the reader to our earlier work for further details [31,32].

To align the two facets, the mask fiber is mounted on a manual stage (nanomax TS XYZ, Thorlabs GmbH), while the target fiber is mounted on a motorized platform (Nanomax 343/m XYZ, Thorlabs GmbH) with stepper motor controller (SSC103, Thorlabs GmbH) with a step resolution of 0.1 \( \mu \)m. Automated fine positioning of the target fiber is performed by a LabView program that, via a shape recognition algorithm, recognizes the position of both fibers and moves the target fiber into contact with the mask fiber. The input for the feedback on the controller is the image of two webcams, each in front of a 40x microscope objective and operating in orthogonal directions. Illumination is achieved by low intensity red LEDs, which were found to have no influence on the quality of the lithographic process. After alignment, light (420 nm, 30 \( \mu \)W) is coupled through the mask fiber for 5 s. During this step the fibers are processed one-by-one to guaranty a good alignment for each fiber.

8.2.5 Step 5: Development of the photoresist

After exposure of the target fiber, the PR is developed in a standard TMHA developer for 1 min (Figure 8.1E). No hard bake is applied to prevent deterioration of the pattern.

8.2.6 Step 6: Reactive ion etching

RIE (Figure 8.1F) has become the standard dry etching technique in MEMS production and is often used to remove materials that are laborious to etch chemically such as silicon, silicon nitride and silicon carbide [225–229]. Our RIE process is based on a two-step approach. First, an SF\(_6\) plasma is created, enabling fluoride species to dissociate according to the following reaction: \( \text{SF}_x + e^- \rightarrow \text{SF}_{(x-1)^-} + F^* \) \((3 \leq x \leq 6)\). Afterwards, the dissociated fluoride can react chemically with silicon
8.2 Fabrication procedure

Figure 8.5: Schematic view of the masks used for AS photolithography. A) Single cantilever arm. B) Double cantilever arm (80 degrees V-shape). C) Double cantilever arm (180 degrees bridge structure). D) Quadruple cantilever arm (90 degrees cross structure).

Figure 8.6: Images of the RIE equipment. A) View of the chamber during the RIE process. The SF₆ plasma is visible as a green glow. The RIE stub (b) is mounted on top of the RF electrode (a) and directly underneath the circular counter-electrode (c). B) Top view of the RIE stub containing the batch fiber holder. C) Bottom view of the RIE stub showing the circular slit for secure storage of the fiber ends.

(Si + 4 F• → SiF₄). The volatile product of this reaction is sputtered in a strongly anisotropic etch process [230, 231].

Since all the equipment in the semiconductor industry is designed for utilization in flat wafers, we implemented our own RIE chamber, in which we increased the distance between the two capacitor plates to 20 cm (Figure 8.6A). Furthermore, we designed a customized RIE stub, taking into account the maximum permitted bending radius of SMF28 optical fibers (Figure 8.6B and 8.6C). This RIE stub contains the batch fiber holder and can be transported into the main reaction chamber via a loadlock system. After transfer to the reaction chamber, the RIE stub is clamped onto the RF electrode by a horseshoe like clamp to insure good thermal contact with the water-cooled RF electrode.

The reaction chamber (custom built by Demaco Vacuum Systems) is pumped down to $1 \times 10^{-7}$ mbar by a Pfeiffer HiPace 300C molecular drag chemical resistant turbo molecular pump. To prevent degradation of the TM pump by the aggressive reaction products of the RIE process, the pump is purged with N₂ gas to dilute the reaction gas. The TM pump is backed by an oil free pre-vacuum pump (Adixen ACP 15G, Pfeiffer). During RIE processing the pump outlet gas of the reaction chamber is diluted with a high flow of compressed air and lead through a water container to capture hazardous byproducts of the RIE reaction such as HF. A pH indicator is added to the container.
8 Optimization of the batch production of silicon fiber-top MEMS devices

Table 8.1: Effective RIE rates of various structural layers and standard photoresist. Values are obtained after 10 min of etching using the optimal settings for the RIE parameters.

<table>
<thead>
<tr>
<th>Material</th>
<th>Etch depth (nm)</th>
<th>Etch rate (nm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon</td>
<td>4000</td>
<td>6.7</td>
</tr>
<tr>
<td>Silicon oxide</td>
<td>160</td>
<td>0.27</td>
</tr>
<tr>
<td>Silicon nitride</td>
<td>100</td>
<td>0.17</td>
</tr>
<tr>
<td>PR (maP1205)</td>
<td>200</td>
<td>0.33</td>
</tr>
</tbody>
</table>

to monitor the acidity of the water filter. The SF$_6$ gas is flow controlled (Model 5878, Brooks) and the pressure is maintained by an adaptive pressure controller (PM-3, VAT). An AJA 100/300 power supply of 300 W in combination with an AJA MC2 automatic matching box is used for the creation of the RIE plasma.

In order to optimize our RIE process, experiments have been conducted on flat silicon wafers glued to the surface of the RIE stub. Either silicon nitride, silicon oxide or silicon was deposited on the wafer to compare the etching rate of various structural layers. Standard photolithographic processing was used to pattern a grid of squares of 50 x 50 µm$^2$ in PR (maP1205, Micro Resist Technology GmbH) spun on the wavers. After RIE, the depth of the etched patterns was measured using an optical profiler (Wyko NT 9100, Veeco). The highest etching rates were obtained with 30 W RF power, 10 ml/min SF$_6$ flow, and $5 \times 10^{-2}$ mbar SF$_6$ pressure. Table 8.1 lists the etching rates for the various structural materials using the optimal RIE settings. Clearly, our etching process is very favorable for selective removal of silicon.

Inside the batch fiber holder, the fibers are clamped around the acrylate coating and slightly protruded from the holder, resulting in very little thermal contact between the fiber tip and the holder. To prevent overheating of the PR during the RIE process, we rely on a pulsed RIE process instead of continuous processing. Optimal results were found using a duty cycle of 30% (2 min of etching alternated with 5 min cooling), during which the RF power supply and the SF$_6$ flow were switched on and shut off. In this way, the etching of a 1 µm silicon layer can be completed after 3 duty cycles, (i.e. a total etching time of 6 min). All silicon fiber-top MEMS devices described in this work were produced using the optimal settings described above.

8.2.7 Step 7: Photoresist removal

After the RIE process, the PR is removed using a PR dissolving solution (REM400, Micro Resist Technology GmbH) (Figure 8.1G). Nevertheless, in several cases we observed the presence of PR residues around the sharp edges of the silicon structure. Because this thin PR veil may obstruct the chemical etching of the sacrificial layer, we routinely perform an oxygen plasma etch in the RIE chamber after chemical PR removal to ensure no PR remains on the MEMS device (30 W RF power, 10 ml/min O$_2$ flow, $5 \times 10^{-2}$ mbar O$_2$ pressure, 4 min).

8.2.8 Step 8: Chemical etching

To free the MEMS from the substrate, one has to remove the sacrificial layer (Figure 8.1H). This step is performed via chemical etching by immersing the fibers in a
8.3 Results and discussion

Figure 8.7: Images of a suspended V-shape cantilever on top of an optical fiber after chemical etching. A) Light microscope image of the MEMS device after the etching of the gold sacrificial layer. Note the strong reflection of the thin chromium layer underneath the cantilever, as well as the sharp edges of the pattern and the precise alignment of the paddle above the core of the fiber. B) Light microscope image of the device after etching of the chromium layer. Note the lack of reflection in the central circle. C) SEM image of the MEMS device, demonstrating the cleaved surface of the fiber (a), the suspended central paddle (b) and the sacrificial gold layer (c). Note the typical columnar growth mode of the gold layer.

potassium iodine (KI) solution, where we add 0.3% molecular iodine (I₂) to speed up the etching process. The etching of the 3µm layer of gold is performed by stirring the fiber continuously in the etchant and is stopped as soon as the silicon structure is completely suspended (i.e., 4 min after immersion).

Although the chromium adhesive layer has a thickness of only 10 nm, it may severely hamper the performance of the MEMS device by disturbing the coupling efficiency between the fiber core and the paddle of the cantilever. The chromium layer acts as a mirror, reflecting incoming light away from the fiber core, as illustrated by the strong reflection in the central circle in Figure 8.7A. Therefore, to prevent attenuation of the optical signal by the chromium layer, an additional chemical etch is performed for 1 min (Chromium etchant, Sigma-Aldrich), after which there is no reflection from the area below the cantilever (Figure 8.7B). Figure 8.7C demonstrates a scanning electron microscope (SEM) image of the suspended V-shape cantilever after all the processing steps.

8.3 Results and discussion

Four different suspended cantilever structures have been successfully fabricated out of silicon directly on top of an optical fiber in a batch process. We report an overall manufacturing yield of around 80% during the processing of the last batch of 18 fibers. The remaining 20% did not present a functional sensor, mostly due to contamination of the deposited layer when the fiber was positioned at the edge of the batch holder, or, due to mishandling during the manual processing steps (see table 8.2). Figure 8.8 reports an overview of the fabricated structures on top an optical fiber. The single arm cantilever is 20µm long and 5µm wide, matching very well the dimensions of the mask fiber. The suspended paddle has a diameter of 10µm. From the SEM images
Optimization of the batch production of silicon fiber-top MEMS devices

Figure 8.8: SEM images of four different suspended MEMS structures in silicon on top of an optical fiber. A) Single cantilever arm. B) Double cantilever arm (80 degrees V-shape). C) Double cantilever arm (180 degrees bridge structure). D) Quadruple cantilever arm (90 degrees cross structure).

The thickness of the cantilever is estimated to be around 1 µm, which corresponds well with the target value for the silicon layer during the sputter deposition.

To demonstrate that our MEMS devices are working according to design, we have coupled the other end of the fiber to a commercial interferometer (OP1550, Optics11) (for a description of the readout method, see [25]). Figure 8.9 shows the interferometric output signal of the cantilever structure upon contact with a sharp tip, periodically driven by a piezoelectric translator. One can observe that, when in contact, the interferometric signal follows the movement of the piezoelectric translator. The optical signal can thus be used as a direct readout for cantilever displacement.

The optical signal may be distorted by deviations in the alignment of the pattern during the AS procedure, in particular when the applied photoresist is not evenly distributed over the circumference of the target fiber. In our experiments we have experienced a maximum deviation of around 2 µm. All cantilever designs are equipped with a central circular paddle of 10 µm diameter for the reflection of the light coming from the core of the fiber. Since the core of the single-mode fiber is 8 µm in diameter and the light bundle is divergent, we never experienced any problems in the optical readout of our devices.

In Figure 8.10 we report a characterization of the spring constant of our MEMS devices. Force and displacement data of the sensors have been obtained by pushing the paddle of the MEMS devices against a macroscopic cantilever, which has been calibrated according to a protocol developed by our group [35]. To ensure that the contact is made at the center of the paddle, a 10 µm borosilicate sphere is glued onto the paddle. Force data is recorded for bending of the MEMS cantilever from 10-1500 nm. The average spring constant of the single cantilever arm, recorded on two sensors, was 40 N/m and was independent on cantilever bending. This value is consistent with simulations performed for the single arm cantilever and indicates that the silicon layer was slightly thicker than 1 µm (Figure 8.10A). The spring constants of the more complex MEMS devices are found to be dependent on the bending of the lever as illustrated in Figure 8.10C and 8.10D. For the first 200 nm of bending
Note on residual stress

Residual stress in thin films is a major concern for the operation and reliability of MEMS, especially in the fabrication of suspended membranes. Residual stress can be compressive, which makes the film expand parallel to the surface, or tensile, causing the film to shrink. A compressive membrane may buckle, whereas a tensile membrane can break in the presence of pressure or high temperature gradients. During our initial fabrication process our MEMS sensors demonstrated a high amount of compressive stress, most evident in the cross structure, as illustrated in Figure 8.11. The silicon...
layer was, in this batch, 0.3 μm thick. When the chemical etch process was stopped before the central paddle was completely separated, the legs of the cross appeared to be highly bent (Figure 8.11A). The legs, however, did not break, demonstrating the high flexibility of the silicon. After the completion of the etch process one can observe a high amount of buckling in the structure (Figure 8.11B).

In order to reduce the amount of stress in our silicon films we have optimized our deposition process. Because our deposition rate, argon flow and argon pressure were already within reasonable boundaries, we have mainly focused on optimizing the substrate temperature during deposition. Since silicon has a melting temperature of 1961 K, a room temperature deposition results in a homologous temperature \( T/T_{\text{melting}} \) of 0.15. Films deposited at homologous temperatures between 0.1 and 0.5 are typically in the transition zone of the Thornton zone diagram for sputtered films [226]. Films in this zone can have either tensile or compressive stresses depending on the remaining deposition parameters [232]. Other reports, however, suggest a temperature of 250° C to be the transition temperature from tensile to compressive stress in sputter deposited silicon films [233]. Each deposition system has its own peculiarities so definitive parameters can only be obtained experimentally. Moreover, as previously discussed, there is a poor thermal contact between the fibers and the batch holder, so the effective temperature of the fiber tip is not known.

During the layer deposition, heat is generated by the sputter source. Initially, the fiber holder was placed in close proximity (15 cm) from the sputter target to obtain the high deposition rate necessary to produce a thick film. A thermocouple, connected to the fiber holder, indicated an increase of 20° C above room temperature during silicon sputtering. However, at this distance the top of the plasma was just hitting the tips of the fibers. The impact of highly energetic argon and silicon ions from the plasma onto the substrates increases the surface mobility of the atoms on the substrate, leading to a higher effective substrate temperature during film growth. As a second heat source we applied RF plasma heating around the batch holder. Using 30 W RF power the temperature of the fiber holder increased to 150° C. Combined with the additional heat input of the plasma sputter source and the increased surface mobility on the substrate during deposition, we estimate the effective substrate temperature to be around 250° C.

In several experiments we stepwise decreased the RF power of the plasma heating...
and increased the distance between the target and the fiber holder, thus reducing the heat input and the added surface mobility. Consequently, increasing the distance to the substrate reduced the deposition rate. The best layer quality was achieved at a substrate distance of 30 cm with discontinuation of the plasma heating. At this distance the deposited silicon layer may still be subjected to a minor amount of stress. Therefore, we increased the thickness of the silicon layer to 1.0 µm, as suggested in [226]. Although not evidently visible in the SEM images (Figure 8.8), the minimal residual stress in the layer is revealed by the behavior of the spring constant of the sensors. From these results we estimate the buckling of the sensors to be around 600 nm.

8.5 Conclusions

We have demonstrated that, using our AS process coupled to sputter deposition, PR spraycoating, RIE, and chemical etching, all optimized towards optical fiber processing, we are able to reliably produce silicon MEMS devices directly on top of an optical fiber via a batch process. Four different MEMS devices have been realized on the top of a standard optical fiber based on a suspended cantilever supporting a round paddle above the fiber core. We tested the performance of the fabricated devices by mechanically actuating the silicon cantilever while looking with an interferometric readout at the displacement thereby induced. Furthermore, we characterized the spring constant of the various cantilever structures by pressing against a pre-calibrated cantilever. The process yield during the processing of the last batch consisting of 18 fibers was around 80%, making our technique feasible for series production of miscellaneous fiber-top MEMS devices. The yield may be improved by further optimization of the deposition process and the photoresist application and removal. The throughput time may be reduced by automating the AS photolithography process and the wet etching steps further. We believe that our work paves the way for a large variety of remote sensing applications that make use of MEMS based devices fabricated directly on top of an
optical fiber.

**Acknowledgments**

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**Competing interests**

DI is co-founder of Optics11. DI and MS are shareholders of Optics11.
Looking ahead

In this chapter a view on future applications and modifications of the approach presented in the thesis is provided. First, several promising applications of the current device are explored. Afterwards, some alterations to the setup are suggested, such as a steerable device and an alternative way to mount the indenter. Finally, with examples such as the micro-scale steerable needle and an alternative fabrication method for cantilever-based fiber-top probes, the topic of further miniaturization is discussed.

**Keywords:** Alternative applications – Miniaturization – Micro-scale steerable needle – Lateral indenter – Membrane based sensors

In this work a novel approach to characterize the viscoelastic properties of soft biological tissue, dubbed as minimally invasive micro-indentation, is presented. By integrating an indenter based on ferrule-top technology into a thin needle, sub-surface measurements of tissue mechanical properties can be performed. Thanks to the all-optical nature of the probe the measurements can be performed in harsh environment such as in liquid and under high temperature. Various designs of the device are presented in the thesis, with emphasis on the continued miniaturization of the indenter. The approach is applied on artificial samples with varying degrees of stiffness as well as bovine liver submerged in gelatin. To illustrate the potential of in situ stiffness measurements, the stiffness of the nucleus pulposus of the goat’s intervertabral disc is recorded without extracting the nucleus.

A thin, rigid needle with a sensor able to measure a material’s stiffness anywhere underneath its surface opens up a field of interesting applications. One could, for instance, record the viscoelastic properties of brain tissue, while the brain remains within its natural surroundings in the skull, in a minimally invasive way. On a more fundamental level, the influence of extraction of tissue from its surroundings on the mechanical properties can be studied. Simultaneously, one could examine the effect of extraction on the mechanics of the host tissue. A characterization of the mechanical properties of the host tissue can be of great importance in the field of tissue engineering, where matching the local stiffness of the implant with that of the host is one of the main challenges. In cancer studies, it may be valuable to compare the stiffness of the outside of a tumor to that of the tumor’s inner tissue.

The integration of an indenter in a steerable needle remains a big challenge. An indentation measurement relies on accurate translation of the indentation movement from the actuator to the indenter. This is hampered as soon as a bend is introduced along the translation path. In Chapter 5, we have made an attempt to work around this problem by monitoring the displacement of the probe as well as that of the cantilever. In this way, the accuracy of the indentation is not dependent on the translation path. By using a thin steel cable to translate the actuation, bending of the needle should be possible. The probe, as presented in Figure 5.1B, was tested at maximum needle bending (i.e. 90 degrees, see Figure 9.1) and good load-indentation results were obtained. Inspired by these results, several designs for the indentation module were developed and tested, as illustrated in Figure 9.2. Unfortunately the dimensions of the sensor were not sufficient for potentially interesting applications and it was decided to focus on a rigid needle. Looking ahead, the development of a steerable needle with a diameter of ~ 1.5 mm should be investigated in more detail, but remains an engineering challenge.

To date, the smallest steerable steerable needle available measures just under 1 mm in outer diameter [234]. The miniaturization of steerable needles is an ongoing challenge. Smaller steerable needles enable procedures at hard-to-reach locations in valuable organs. We have explored the possibility of manufacturing a micro-scale steerable needle out of a 200 x 200µm square borosilicate capillary. The bendable segment, normally fabricated by laser incisions in the stainless-steel shaft, is produced by carving a puzzle structure in the capillary wall by ps-laser ablation. In Figure 9.3A, one puzzle shackle in the capillary wall is illustrated. By performing these carvings on each of the four sides of the capillary (Figure 9.3B) and linking at least three
Figure 9.1: Indentation test at maximum bending.

Figure 9.2: Examples of alternative designs for the indentation module. A) Square ferrule holder with small ridges to minimize the friction and feed-throughs for the steel cable and the optical fibers. B) Holder to mount a square ferrule on a round needle, including a slit and feed-through for fibers and cables.
Looking ahead

Figure 9.3: Light microscope images of the micro-scale steerable needle. A) Puzzle structure carved in the micro capillary. B) By carving puzzle structures on each of the four sides, a 3D shackle can be fabricated. C) Vertical bending of the capillary due to airflow. D) Horizontal bending due to gravity. The scale bar equals 200 µm.

shackles into a chain (Figure 9.3C), the capillary is able to bend for at least 20 degrees (Figure 9.3D). By attaching two micro-cables to the tip, the device could be made steerable.

Adapting the device presented in this thesis to a lateral indenter (i.e. indentation along the shaft of the needle), as illustrated in Figure 9.4, is a logical next step in this project. The lateral indenter would be able to continuously monitor tissue stiffness while the needle penetrates deeper into the tissue. Moreover, the integration of a cantilever in the needle shaft instead of the needle tip would greatly reduce the fragility of the device. For a successful optical readout, one has to modify the optical fiber as shown in the magnification of Figure 9.4. The challenge of this approach is to translate the indentation movement orthogonally in a very narrow volume (i.e. in the lumen of the needle).

Ultimately, one would like miniaturize the sensor to the size of a single mode optical fiber (i.e. 250 µm). Such a device can be integrated in a very thin needle (diameter < 1 mm) to perform truly minimally invasive measurements. In Chapter 8, we present a top-down fabrication method for cantilever-based sensors produced directly on top of a single mode optical fiber. Alternatively, a less fragile sensor could be produced when the cantilever is replaced by a membrane. An additional advantage of the latter is the closed cavity, which would be less prone to contaminations.

In collaboration with Philips Research we have designed a novel concept, based on devices with flexible membranes, which can be mass manufactured and easily assembled at the tip of an optical fiber. The resulting device is compatible with a
Figure 9.4: Sketch of the lateral indenter inside a needle. The magnification shows the readout scheme. By polishing the fiber at a 45° angle, the incoming light (orange arrows) is directed towards the cantilever and the reflected light (green arrows) is collected.

Figure 9.5: Concept for a flexible membrane sensor, mountable on a 250 µm diameter optical fiber, and compatible with Fabry-Pérot interferometry method [237].

Fabry-Pérot interferometry readout for the membrane displacement. The conceptual design for the new device can be seen in Figure 9.5. In order to fabricate the arbitrary silicon shape required, we propose to use a previously presented technology platform by Philips Research called Flex-to-Rigid (F2R) [235]. The feasibility to insert an optical fiber on an F2R device was demonstrated before [236]. Furthermore, this new concept makes use of a new process flow developed by Philips Research, called vacuum trenches, which allows flexible SiO₂ membranes with an in-silicon cavity underneath (Figure 9.6). The membrane sensitivity is easily tunable by controlling its thickness during the processing. An overview of the process steps is given in Figure 9.7. For more details the reader is referred to [237].
**Figure 9.6:** First test to fabricate membranes on vacuumtrenches, in this case made with 1μm circular holes. A) Mesh after deep etching through the holes. The scalebar equals 24μm. B) After closing the membrane with SiO$_2$ [237].

**Figure 9.7:** Overview of the process steps. A) Fabrication of the two-step mask on the back side and vacuumtrenches on the front side. B) Deposition of an aluminum pad in the center and etching of the circumference of the device. C) Spinning of polyimide and application of hard-etch aluminum mask on front side. First deep etch from the back side. D) Second deep etch from the back side. Etching of polyimide and removal of hard-etch mask on the front side [237].
Summary of the thesis

Minimally Invasive Micro-Indentation

The mechanical properties of biological tissue describe its behavior under pressure or force. These intrinsic tissue properties can often be related to function. For instance, the spine provides strength and structural support and the ribcage and skull offer protection for delicate internal organs such as the lungs and the brain. On a smaller scale, it has been shown that cells and tissues feel and respond to the mechanical properties of their surroundings. The micro-mechanical environment of a tissue thus has a direct influence on its functioning and vice versa.

Despite the diagnostic opportunities as well as a clear relation to function, very few methods are available to accurately assess tissue mechanical properties on the micro-scale. Most clinical methods to measure tissue stiffness rely on large scale imaging techniques that lack the resolution necessary to differentiate on the micro-level, while material science approaches such as the atomic force microscope are focused on the nano-level and fail to capture the broader view. In this thesis a novel approach for micro-indentation is presented based on ferrule-top technology.

A ferrule-top sensor consists of a cantilever accurately positioned above a single mode optical fiber. By coupling monochromatic laser light through the fiber, the displacement of the cantilever with respect to its neutral position can be monitored very accurately. Traditionally, ferrule-top probes are fabricated out of borosilicate parts and their diameter is around 3 mm. The cantilever can be equipped with various tips or coatings depending on the application.

In order to perform accurate force measurements with a ferrule-top device, the spring constant of the cantilever has to be determined. As part of this thesis an experimental calibration method for force transducers with interferometric readout has been developed. The method relies on the idea of mounting the sensor on a calibrated piezoelectric translation stage, which is then used to push the free handing end of the cantilever against the pan of a weighing scale. The displacement of the translation stage is regulated by a high gain negative feedback loop designed to keep the bending of the cantilever equal to a multiple of the wavelength of the readout laser (i.e. in the maximum of the interference pattern). At the end of the integration time, the transducer is forced to move to the next maximum of interference, where it is again locked into position. Repeating a similar procedure for a series of consecutive maximum-to-maximum steps, one can finally plot the weight indicated by the scale as a function of the displacement of the cantilever, and, from there, extract its spring constant.

Using a calibrated ferrule-top sensor, the stiffness of soft biological tissue can be determined by means of micro-indentation. This technique is based on measurements of displacement of the tissue (i.e. indentation) as a response of a force applied by
the sensor. Conventional table-top indenters are limited to the surface of a sample. The main aim of this thesis is to develop an in situ indenter that enables sub-surface measurements. Therefore, a novel device has been designed that allows the user to measure the Young Modulus of a material at the opening of a 5 mm diameter needle. The device is equipped with a ferrule-top cantilever with a spherical tip, which is repetitively brought in and out of contact with the sample at the end of the needle by means of a steel cable that is controlled via a piezoelectric actuator located at the proximal end. The ability of the device to detect and quantify layers of varying stiffness is demonstrated during needle insertion in a gelatin phantom. Moreover, it is shown that, using this approach, tissue boundaries in bovine liver tissue embedded in gelatin can be successfully located.

A miniaturization step is required to apply the in situ indenter for stiffness measurements in relevant pre-clinical research. The outer diameter of the device is reduced to 1.3 mm to enable measurements of the mechanical properties of intervertebral discs of goats. Moreover, the indentation procedure is adapted to record the localized dynamic storage and loss moduli of a sample. Benefiting from the smaller dimensions, the device is applied to map the viscoelastic properties of a complex, confined sample, namely, the nucleus pulposus of the intervertebral disc. The findings in this project show that the mechanical properties of a biological tissue in its local environment may be different than those that one would measure after excision of the tissue and, thus, depend on the local surroundings.

The smaller footprint of the device opens up a wide range of applications in which the response to a varying parameter can be monitored. For instance, by recording the viscoelastic properties of the nucleus pulpusus before, during and after axial loading of the intervertebral disc, it is found that, by losing liquid, the nucleus becomes more elastic during axial loading.

A continued strive towards further miniaturization is an important theme in this thesis. In the final chapters two alternative approaches to produce MEMS structures (such as a cantilever) on an optical fiber are presented. The first approach consist of a method to fabricate MEMS devices directly on the cleaved end of an optical fiber via a top-down process similar to that used in semiconductor technology. By growing and patterning alternate layers of structural and sacrificial material, fiber-top sensors with a diameter of 125µm can be produced. In collaboration with Philips Research a concept for a membrane based sensor has been developed that can be fabricated out of a silicon waver in a batch process.

Overall, a dedicated effort has been made to develop and test an in situ minimally invasive micro-indenter. The continued emphasis on miniaturization has lead to a small device that is suitable for pre-clinical research as well as several valuable pathways to continue the research project.
Appendix A: Fiber-Optic Fabry-Pérot Interferometers for Axial Force Sensing on the Tip of a Needle

A range of complex percutaneous procedures, such as biopsy or regional anesthesia, rely heavily on accurate needle insertion. Small variations in the mechanical properties of the pierced tissue can however cause deviations from the projected needle path and can thus result in inaccurate placement of the needle. Navigation of a rigid needle towards the target tissue is traditionally based on the surgeon’s capacity to interpret small variations in the needle insertion force. A more accurate measurement of these small force variations enables improvement in needle targeting, can potentially aid in enhancing force feedback in robotic needle placement and can provide valuable information on tissue-tool interaction. In this study we investigated several concepts for the design of a force sensor based on a fiber-optic Fabry-Pérot interferometer to measure needle-tissue interaction forces on the tip of a 18 G needle, where special attention was given to concepts for a sensor with (1), an intrinsic low cross-sensitivity to temperature and (2), elementary design and fabrication. Three concepts, using either a quartz capillary, an Invar capillary or a thin polyimide film as the force sensitive element were prototyped and subjected to both static and dynamic testing. The force transducer based on a quartz capillary presented the lowest cross-sensitivity to temperature (12 mN/°C) and good accuracy (maximum measurement error of 65 mN/10 N) in a measurement of static forces. However, limited strength of the sensor is expected to prevent usage of the quartz capillary in small diameter needles. The concepts for a sensor based on an Invar capillary or a thin polyimide film proved a higher cross-sensitivity to temperature (50 mN/°C and 220 mN/°C, respectively) and higher maximum measurement error (350 mN/10 N, 800 mN/10 N), comparable to those of FBG-based sensors reported in literature, but are likely to be more suitable for integration in very small biopsy needles.

Keywords: Fabry-Pérot interferometer – needle-tissue interaction force – low temperature cross-sensitivity – fiber optic force sensor – tip force sensing

Appendix A: Fiber-Optic Fabry-Pérot Interferometers for Axial Force Sensing on the Tip of a Needle

Introduction

Needles are nowadays used for a wide variety of therapeutic as well as diagnostic medical procedures, as percutaneous (through the skin) needle insertion has proven to be an ideal method to gain invasive access to regions deep into the human body with minimal tissue damage [238,239]. As a result, minimally invasive needle insertion has become one of the most common procedures in medicine [239,240]. Regional anesthesia, biopsy, neurosurgery, deep brain stimulation, catheterization, ablation and brachytherapy are a few examples of complex procedures that rely heavily on the use of medical needles and accurate needle insertion [238,241,242]. New developments to improve needle insertion are of great interest due to the frequent occurrence of percutaneous needle insertions in healthcare [239]. One of the main developments of the last decennia in this growing field is technology enhanced needle insertion, such as image guidance and force feedback during perforation of the tissue [243]. Many recent academic studies related to needle insertion focus on tissue and tissue-tool-interaction models to model insertion, force feedback, robot assisted needle insertion devices, steerable needles and instrumented needles [239,244–249].

Accurate measurement of forces acting on a needle tip can be useful for multiple applications related to needle insertion, including research on tissue-tool interaction and (enhancing) force feedback. In present times a preference can be observed towards (fiber-)optical sensors for the measurement of forces on the tip of a needle. One of the main advantages of fiber-optic sensors is the potentially small size of the sensor as well as the required cabling [9]. Furthermore, fiber-optic sensors (to be precise, their optical sensing part) are inherently immune to electromagnetic interference, while electronic sensors are susceptible to electromagnetic interference to at least some degree. This is an important advantage in terms of medical applications, as it allows usage of the sensor in combination with RF-ablation and MRI scanners; environments with a need for force sensing at the tip of a needle (e.g., force feedback in robotic needle insertion devices [250,251]) but which are not optimal for electronic sensing systems. Several other advantages of fiber-optics sensors include (a) a fully dielectric working mechanism and hence no possibility of flow of electric current to the patient [9,252,253]; (b) biocompatibility and thus no predicament with invasive measurements and, finally; (c) the potential for multiplexing several fiber-optic sensors in a single optical fiber, allowing for a multidisciplinary diagnostic tool at the tip of a needle [9,253].

Despite the recent research activity, most force sensors for the measurement of axial forces on the tip of a needle discussed in literature, including optical sensors such as Fiber Bragg Gratings (FBGs), are unsuited for in vivo applications due to their large cross-sensitivity to temperature [249,254]. Therefore, the primary requirement for the design of our force sensor is a low cross-sensitivity to the temperature of the needle shaft. Secondly, the sensor should fit inside an 18 G needle and, more importantly, should be relatively easy to manufacture and assemble. Moreover, the sensor should be able to measure axial forces up to 10 N with a maximum measurement uncertainty of 500 mN/10 N [254,255], while the sensor is exposed to temperature variations between 17 °C and 41 °C.

To comply with the requirements of low cross-sensitivity to temperature and
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elementary fabrication it was chosen to design a sensor based on Fabry-Pérot interferometry [36,140]. The sensitive part of the Fabry-Pérot interferometer (FPI) can be fabricated on top of a cleaved optical fiber and can potentially be extremely short, thus making the FPI particularly suited to measure forces on a needle tip. Moreover, the cross-sensitivity for temperature of an FPI-based strain gage has been reported as low as 0.95 pm/°C, which is roughly a factor 90 lower than that of an FBG-based sensor [256], indicating promising opportunities for FPI-based force sensors. It is estimated that the dimensions of the elastic element required to obtain a workable force range and sensitivity can be kept small, ensuring that the FPI sensor will fit in a very thin needle. Finally, FPI sensors are low cost, disposable and straightforward to fabricate and therefore seem the logical choice as a force sensor for harsh environments.

The goal of this study was to design a force sensor based on an FPI, incorporated in a needle tip, for the measurement of forces acting on the needle tip in axial direction of the needle. To realize this goal eight concepts for the force sensor were developed. Out of these eight concepts, the three most promising designs were simulated using finite element analysis. After selection of the most promising materials and production method, the three concepts were prototyped and subjected to both static and dynamic testing. On the basis of these tests a recommendation for further research will be given.

**Working Principle and Readout of the Force Sensor**

Figure A.1 presents a simplified model of the heart of the force sensor, the Fabry-Pérot interferometer. The interferometer consists of an elastic element which is centred between two partially reflective mirrors and is fabricated on top of the cleaved end of an optical fiber. Light propagating through the fiber partially reflects on the first mirror of the FPI and travels back into the fiber. The remainder of the light propagates through the elastic element, reflects on the second mirror and is ultimately collected in the optical fiber. The light waves propagating back to the light source interfere with each other and encode for the length of the elastic element. The back-propagating light is collected by a detector via a 90/10 fiber coupler (Mode hopping in the laser is minimized by introducing an isolator in the optical path), as demonstrated in Figure A.2. When we neglect multiple reflections in the elastic element, we can describe the amplitude of the ideal interference signal in the photodiode by:

\[
W(d) = W_0 \left[ 1 + V \cos \left( \frac{4\pi L}{\lambda} + \varphi_0 \right) \right]
\]

where \(L\) is the length of the elastic element, \(\varphi_0\) is a constant phase shift that only depends on the geometry of the probe, \(\lambda\) is the wavelength of the laser, and \(W_0\) and \(V\) are the midpoint interference signal and the fringe visibility, respectively. The interference signal on the detector can be transformed to a linear readout of \(L\) following the mathematical derivation in [103].

A major requirement of the to-be-designed force sensor was a low intrinsic cross-sensitivity to temperature. FPIs are reported to have a particularly low sensitivity to temperature when the cavity is filled with air [257,258]. The elastic element,
Appendix A: Fiber-Optic Fabry-Pérot Interferometers for Axial Force Sensing on the Tip of a Needle

Figure A.1: Simplified model of the Fabry-Pérot interferometer inside the force sensor, showing the mirrors of the FPI in purple and light blue with an FPI cavity medium in between with a refractive index $n$ that depends both on temperature and stress inside the medium resulting from the force applied on the sensor. The mirrors are separated by an elastic element with distance $L$ that depends on the temperature of the sensor and the force exerted on the elastic element.

Figure A.2: Schematic view of the readout system used to detect changes in the length of the elastic element in the FPI sensor. No light reflects at the terminated fiber end.

Connecting the two mirrors, can be seen as a spring with compliance $c$, which carries the entire load on the force sensor and is assumed to compress according to Hooke’s law. The compliance of the spring is linearly dependent on the temperature of the sensor and independent of the exerted force:

$$c = \frac{\partial L}{\partial F} = c_0 + \frac{\partial c}{\partial T} T. \tag{9.2}$$

The change in length of the FPI cavity upon exertion of force is assumed to be equal to the change in length of the spring plus the change in geometrical length of the cavity due to expansion of the material connecting both mirrors. This geometrical change of cavity length is assumed to depend linearly on the temperature of the sensor and to be proportional to the coefficient of thermal expansion (CTE) of the spring’s material and the length of the FPI cavity (this definition implies that the length of the spring does not have to match the length of the FPI cavity). The length of the cavity is $L_0$ when no force is exerted on the sensor for a given temperature. The cavity length is thus:

$$L = \left( L_0 + \frac{\partial L}{\partial T} T + \left( c_0 + \frac{\partial c}{\partial T} T \right) F \right). \tag{9.3}$$

The refractive index of the medium between the FPI mirrors is assumed to depend linearly on the temperature and the force exerted on the sensor (the latter will only be the case when the FPI cavity medium is a solid material). The refractive index is $n_0$ when no force is exerted on the sensor and the temperature of the sensor is ‘zero’.
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The refractive index is thus:

\[ n = \left( n_0 + \frac{\partial n}{\partial T} T + \frac{\partial n}{\partial F} F \right). \]  
(9.4)

The equation for the optical path difference (\( \text{OPD} \)) of an FPI is a function of the FPI cavity length \( L \) and the refractive index of the FPI cavity medium:

\[ \text{OPD} = 2 \times n \times L. \]  
(9.5)

Summarizing all of the above, the \( \text{OPD} \) of the FPI of the force sensor as function of the force exerted on the force sensor and the temperature of the force sensor is thus:

\[ \text{OPD}(T,F) = 2 \times \left( n_0 + \frac{\partial n}{\partial T} T + \frac{\partial n}{\partial F} F \right) \left( L_0 + \frac{\partial L}{\partial T} T + \left( c_0 + \frac{\partial c}{\partial T} T \right) F \right). \]  
(9.6)

A change in \( \text{OPD} \) is only dependent on force or temperature. Cross-Sensitivity of the force sensor to temperature can occur in the form of a constant error and a modifying error. Change of the \( \text{OPD} \) as result of a change in temperature due to thermal expansion of the spring and a change in refractive index of the FPI cavity medium will result in a constant measurement error throughout the measurement range. The dependency of the Young modulus of the material of the spring on the temperature will on the other hand cause a modifying error on the force exerted on the sensor that will increase with increasing force. Assuming that the compliance of the spring does not significantly depend on temperature, the change in \( \text{OPD} \) can be described by:

\[ \Delta \text{OPD}_{\text{linearized}} = 2 \times \left( \frac{\partial n}{\partial T} \times L + 2 \times n \times \text{CTE} \times L \right) \Delta T + 2 \times \left( \frac{\partial n}{\partial F} \times L + n \times c \right) \Delta F. \]  
(9.7)

This is a reasonable assumption, as long as materials with a high glass transition temperature are selected for the spring (see Section 9). Equation (9.7) shows that the cross-sensitivity to temperature of an FPI used to measure force can be minimized by balancing the thermo-optic coefficient of the medium inside the FPI cavity and the CTE of the spring of the FPI. Both the thermo-optic coefficient and CTE should nonetheless be preferably as small as possible to minimize cross-sensitivity to temperature, as both material properties will likely never be sufficiently perfect in balance. Minimizing the FPI cavity length will also minimize the cross-sensitivity to temperature, as long as other parameters like the compliance of the spring remain constant. A reduction of the cavity length is limited by the manufacturing method and the minimum required length of the FPI cavity for good functionality. The cross-sensitivity to temperature can furthermore be minimized by maximizing the compliance of the elastic element of the FPI or maximizing the elasto-optic coefficient of the FPI cavity medium. This method of reducing the measurement error due to temperature disturbances is strongly limited by the range in which changes of \( \text{OPD} \) can be measured. The compliance of the elastic element can be increased by selecting an appropriate material with a low Young modulus for the elastic element and optimizing the dimensions and geometry of the elastic element of the FPI.
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Concept Design and Fabrication

Several concepts for a FPI-based system to measure forces on the tip of the stylet of a needle, with a low cross-sensitivity to temperature, were generated based on an analysis of the requirements and suitable materials. In particular many combinations of different types of force-sensitive elements and placements of FPI sensors for force and temperature measurement were identified. The three most promising concepts, namely the quartz capillary concept, the Invar capillary concept and the polyimide film concept, are presented in Figure A.3 and will be described in the next sections. We have selected two concepts with an air-filled cavity to minimize sensitivity to temperature and a column based spring of either quartz or Invar, based on yield strength, Young modulus and CTE. Furthermore, for comparison, one concept based on a polymer thin film that functions both as FP cavity medium and force sensitive element was selected. The concepts are demonstrated as prototypes, meaning that their design might not be ideal for the final application.

Figure A.3: The three most promising concepts for a fiber-optic force sensor, based on Fabry-Pérot interferometry, in the tip of the stylet of a trocar needle to measure forces in axial direction on the needle tip, showing A) the quartz capillary concept; B) the Invar capillary concept; and C) the thin film concept. The illustrations of each concept show an axial cross-section of the stylet tip and a transverse cross-section of the stylet tip at locations containing an FPI cavity.

Quartz Capillary Concept

The sensing element of the quartz capillary concept (Figure A.4) consists of a 6 mm long and 0.7 mm outer diameter clear fused quartz ferrule and two cleaved single mode optical fibers (SMF-28). The ferrule has a centred bore hole of $127 \pm 1 \mu m$ diameter with a 2 mm long tapered lead-in on one side of the ferrule. The prototype sensor was fabricated by glueing two fibers into the central bore hole of the quartz ferrule with a heat curable epoxy (EPO-TEK 353ND) (For higher mechanical and thermal stability the stripped fibers can be fused in the ferrule. For practical reasons this is neglected in the prototype). One of the fibers is connected to the FPI interrogation device, creating a Fabry-Pérot (FP) cavity between the facets of the cleaved fibers, while the other is sputter coated with a gold film ($\pm 50$ nm) to prevent a second cavity. The force sensitive element of the quartz capillary concept thus consists of a hollow quartz column around the FP cavity, which is closed off from the environment by the
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ferrule and glue. Using a two component epoxy, the ferrule was subsequently glued into an stainless steel (AISI 304) stylet tube such that the sensitive part of the sensor still protrudes from the stylet. Finally, the needle tip was glued on top of the ferrule.

The cross-sensitivity to temperature of the quartz capillary concept is mainly dependent on the geometry of the column. However, a trade-off exists between the cross-sensitivity to temperature and the strength of the sensor, as a thin column will be much more susceptible to transverse stresses. During temperature fluctuations the expansion of the entire length of the column of the force sensitive element will result in a change of geometrical length of the FP cavity. However, the CTE of quartz nearly compensates the thermo-optic coefficient of air and matches the CTE of the silica fibers, hence the measurement error due to temperature disturbances, resulting from the thermal expansion of the quartz column and the thermo-optic coefficient of air (see Equation (9.7)), is expected to be lower than the maximum measurement uncertainty.

The brittle nature of the quartz glass column is the main weakness of the concept, but is not expected to hamper the performance in this study (see Section 9). Although the column structure was chosen to minimise the influence of transverse forces, a certain sensitivity towards the lateral bending force will remain imminent, unless the centre of the FPI cavity is located perfectly on the neutral axis of the needle. The cross-sensitivity can be approximated as the ratio of the stress in axial direction in the ferrule at the center of the FPI cavity per Newton transverse force and the stress per Newton axial force on the needle tip. The most important assumption of this approximation is that optical path of the light in the Fabry-Pérot cavity bends with the bending of the ferrule, which is not the case in practice. The calculated measurement error is therefore an underestimation, as the change in geometric length of the optical path through the FPI cavity due to bending will be larger than approximated. Furthermore, bending of the ferrule causes a wedge angle of the FPI mirrors which reflects light out of the FPI cavity, reducing visibility of the sensor particularly when the FPI cavity is long.

The ratio of the stress inside the ferrule per Newton transverse force and the stress

![Figure A.4: Cross-section of the geometry of the quartz capillary concept. The metal stylet tube and needle tip are shown in grey, the quartz ferrule in green, the glue layers in orange and the silica optical fiber in blue. The thickness of the glue layer used to fixate the optical fibers in the borehole of the quartz ferrule is enlarged for illustrative purposes. The FPI-cavity length was varied between 100 µm and 500 µm.](image)
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per Newton axial force on the needle tip at the center of the FPI cavity can be described by:

\[ \sigma_{\text{trans}} \sigma_{\text{ax}} = \frac{4 \times L_{\text{tip}} \times x}{r^2} \]  

(9.8)

where \( x \) is the eccentricity of the FPI from the neutral axis of the ferrule along the axis of the transverse force, \( r \) the radius of the ferrule and \( L_{\text{tip}} \) the moment arm of the transverse force with the respect to the location of the center of the FPI. The cross-sensitivity to transverse force on the needle tip is according to Equation (9.8), approximately 42 mN per Newton transverse force per micron eccentricity of the FPI cavity. As a result of the low tolerance on the eccentricity of the boreholes of the ferrules used in our sensor, we expect the eccentricity in the sensor not to exceed 10\( \mu \)m. The measurement error due to a 1 N transverse force on the needle tip is, therefore, estimated to be in the order of a few hundred milli-Newtons. Significantly reducing the sensitivity of the force sensor in the needle tip to transverse force on the needle tip is not straightforward, as it depends heavily on the precision of the manufacturing method.

**Invar Capillary Concept**

Similarly to the fabrication of a sensor according the quartz capillary concept, the Invar capillary concept (Figure A.5) consists of a cleaved single mode optical fiber, glued into the central bore hole of a quartz ferrule using a UV curable adhesive. However, this time the cleaved facet of the fiber is aligned with the facet of the ferrule. Next, the quartz ferrule with the integrated fiber is carefully inserted into an Invar capillary stylet tube until a calibrated depth and fixed with slowly curing glue. The insertion depth will determine the length of the FPI cavity. The end of a needle tip, also fabricated out of Invar, is polished to create a mirroring surface. The needle tip is then attached to the stylet tube by means of a press fit. An air filled FPI cavity will thus be created between the fiber-air transition at the end of the optical fiber and the polished surface of the Invar needle tip. The stylet tube will thus be used as an elastic column, creating the force sensitive element.

![Figure A.5: Geometry and dimensions of the Invar capillary concept, showing Invar parts in grey, a fused quartz ferrule in green, an optical fiber in blue and glue layers in orange. The press-fit connection between the needle tip and the stylet tube is shown as a dashed line. The aim for the FPI-cavity length was 100\( \mu \)m.](image)

An advantage of the Invar capillary concept compared to the quartz capillary concept is the strength of the sensor. The metal stylet tube will carry practically all the tensile stress due to transverse forces on the needle tip and the brittle quartz ferrule
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will hardly be exposed to large tensile stresses. The cross-sensitivity to lateral forces is
not expected to differ significantly from the quartz capillary concept, as the eccentricity
still depends on the accuracy of the placement of the borehole (see Equation (9.8)).
The cross-sensitivity to temperature of the FPI is expected to be higher than the
quartz capillary concept, due to the slight mismatch in CTE of the quartz ferrule and
the Invar stylet tube acting as the force sensitive element. Nonetheless, the required
accuracy can be achieved when other sources of measurement errors do not contribute
much to the total measurement error. Moreover, the relatively high cross-sensitivity
to temperature could be compensated by introducing a second, temperature sensitive
FPI, by filtering or by calibrating the sensor at body temperature.

There is unfortunately quite some chance that slip of the press fit connection of
the needle tip, which also acts as second mirror of the force sensing FPI, will introduce
an hysteresis error in the force sensor. Using glue instead of a press fit to attach the
needle tip to the stylet tube could be a solution for this problem. Usage of glue is
however not preferred, as there is a great risk that glue will flow into the FPI cavity
that cannot be removed before the glue is cured. Furthermore, the usage of Invar,
a ferromagnetic material, is a disadvantage of this concept, as it makes the needle
unsuited for MRI-related applications.

Polyimide Film Concept

The two previously proposed concepts have FP cavities that can be strenuous to
manufacture; during fabrication the challenge lies in precisely determining the length
of the cavity, reproducing this cavity length for multiple sensors, and preventing that
the air filled cavities are accidentally flooded with the glue that is used to assemble the
sensor and the needle. The polyimide film concept therefore uses a polymer thin film
as both FP cavity medium and force sensitive element, as illustrated in Figure A.6.
The length of such a cavity can be precisely controlled and reproduced with a thin
film deposition method.

A sensor based on the parallel thin-film concept is fabricated fixing a cleaved single
mode optical fiber in the bore hole of the quartz ferrule, analogous to the previously
described concepts. A chromium film, which will act as the reference mirror of the
force sensing FPI, is afterwards deposited by means of sputtering on the top facet of
the quartz ferrule. A spin coated polyimide layer is subsequently deposited on top of
the titanium-dioxide layer after treatment of the surface with an adhesion promoter.
The elastic modulus of polyimide is expected to be hardly dependent on temperature
thanks to its high glass transition temperature [259]. A thick chromium layer is finally
deposited on top of the polymer layer by means of sputtering to serve as the second
mirror of the FPI and prevent a double FP cavity. The needle tip can be glued on top
of the ferrule and the ferrule can be glued into the stylet tube, identical to the quartz
capillary concept. To increase strength and stability of the sensor, a polymer needle
tip (or a metal needle tip with a polymer transition piece) can be attached on top of
the stylet tube, a procedure that is unnecessarily complicated for the prototype phase.
The sensor can be interrogated with interferometry by coupling the distal end of the
fiber to the readout system.

Of the three presented concepts the polyimide film concept was expected to have
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Figure A.6: A) Sketch of the cross-section of the thin-film concept. B) Close up image of the proof-of-concept of the thin-film device, after deposition of the last 100 nm thick chromium layer. A small particle seems to be partially covering the optical fiber located in the center of the ferrule.

the highest cross-sensitivity to temperature due to the large mismatch between the CTE of the polymer and that of the quartz substrate and due to the high thermo-optic coefficient of the polymer used for the combined FPI cavity medium and force sensitive element. The thermo-optic coefficient of polymers can be estimated with following equation, that uses the volumetric thermal expansion coefficient of the polymer to predict its thermo-optic coefficient [260]:

\[ \frac{dn}{dT} = -\left( \frac{dn}{d\rho} \right)_T \times CTE_{vol} - \left( \frac{dn}{dT} \right)_\rho. \]  

(9.9)

Disregarding the elasto-optic effect, the change in \( \Delta OPD \) of the FPI as function of force applied on the needle tip can be estimated with the following equation:

\[ \Delta OPD(\Delta F) = \frac{2 \times n_{polyimide} \times L_{cavity}}{E_{polyimide} \times A_{ferrule}} \times \Delta F. \]  

(9.10)

This equation is only valid for a thin film of which the substrate has identical mechanical properties as the polymer of the film itself and the stress distribution in the thin film due to the applied force is uniform. The change in \( OPD \) due to a change in temperature can be estimated by:

\[ \Delta OPD(\Delta T) = 2 \times \left( \frac{dn}{dT}_{polyimide} + n_{polyimide} \times CTE_{polyimide} \right) \times L_{cavity} \times \Delta T, \]  

(9.11)

when the thin film is deposited on a substrate with the same thermal and mechanical properties. The constant measurement error due to a change of temperature of 24 °C for a FPI made on a 0.7 mm diameter ferrule with a polyimide layer with a stiffness of 3.3 GPa, CTE of 32 µstrain/K and a layer thickness of 10µm was estimated to be a 8 mN (marginally worse when the elasto-optic effect would have been taken into account). The low cross-sensitivity to temperature of the thin-film concept is the result of the influence of the CTE and the thermo-optic coefficient on the change in \( OPD \) of the FPI due to a change in temperature that nearly cancel each other out. This is however not the case when the polyimide film is deposited on a substrate with different mechanical and thermal properties than the polymer film.
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It is therefore expected that the introduction of a second FPI sensor, solely dedicated to measuring temperature variations, might be beneficial to this concept. This temperature sensitive FP cavity can be positioned in a groove machined on the long edge of the ferrule. The polyimide film sensor is expected to be robust when a needle tip is moulded onto the stylet tube, but more fragile when the needle tip is glued. The FPI sensor is expected to be hardly sensitive to transverse forces, which can only result in a minimal shear of the polymer layer, thanks to the small cavity length.

Finite Element Analysis

Before prototyping of the three concepts a finite element analysis (FEA) of each concept was performed in order to assess the strength of the design and the sensitivity of the sensor to force. In addition, the influence of disturbances on the measurement uncertainty of the three designs were investigated. The FE simulations were performed with Solidworks and COMSOL Multiphysics. For all concepts, the bond line thickness of the glue, used to bond the ferrule to the needle tip and to the stylet tube, was assumed to be 25 µm. The Young modulus and the Poisson ratio of the glue, unless otherwise indicated, were assumed to be 0.5 GPa and 0.3, respectively.

Quartz Capillary Concept

A cross-section of the geometry of the axisymmetric FE-model of the quartz capillary concept is presented in Figure A.4. FE-simulations (based on the modified Mohr-Coulomb criterion [261]) showed that the maximum equivalent stress due to a transverse load of 1 N on the needle tip was 47 MPa in the protruding quartz element. This is marginally lower than the reported maximum tensile stress of quartz glass (50 MPa). However, the maximum tensile stress of the quartz glass of the ferrule is expected to be at least a 2 fold higher as a result of very high surface quality, as was further supported by experiments in our lab. It was therefore decided that the limited strength of the quartz column was not a reason to discard the concept, particularly since transverse forces over 1 N are not expected. Further results of the FE-analysis are presented in Table 9.1.

Simulations were performed for sensors manufactured with NOA 68 UV curable adhesive or EPO-TEK 353ND epoxy to fixate the optical fiber in the ferrule, for Invar and metal stylet tubes and for a cavity length of 100 or 500 µm. For comparison, a glass fuse between the fiber and the ferrule is also taken into account. It can be observed, as expected, that the glue significantly determines the compliance of the force sensitive element, particularly when the compliant UV curable adhesive is used. A change in cavity length due to a 10 N load on the tip of at least 100 nm is preferred for a good visibility of the sensor. A relatively strong influence of the material of the stylet tube can be observed on the change in cavity length caused by temperature. The advantage of the Invar stylet tube was, however, not regarded as significant for this concept. The disadvantage on the measurement error of gluing the fiber in the ferrule with respect to fusing was non-existent according to our FE-simulations. The
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Table 9.1: Results of the FE-analyse of the sensitivity of the FPI of the quartz capillary concept to force and temperature. The first column shows the type bond used to fix the optical fiber in the ferrule (UV curable NOA 68, epoxy EPO-TEK 353ND or fused glass), the material used for the stylet tube and needle tip and the cavity length. The second column shows the change in geometrical cavity length ($OPD$) due to application of a 10 N load on the needle tip. The third column shows the change in cavity length ($OPD$) as a response to an increase in temperature of 24 °C, accounting for the CTE of materials used in the needle tip and the thermo-optic coefficient of air. The last column shows the expected measurement error due to cross-sensitivity to temperature in mN/°C.

<table>
<thead>
<tr>
<th>Glue Type, Cavity Length (µm)</th>
<th>Change in $OPD$ due to 10 N Axial Force (nm)</th>
<th>Change in $OPD$ due to 24 °C Temp. Incr. (nm)</th>
<th>Error due to Cross-Sensitivity to Temp. (mN/°C)</th>
</tr>
</thead>
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High compliance and high CTE of the UV curable glue, resulting in the highest change in $OPD$ due to force and temperature, made the use of this adhesive unfavourable.

Invar Capillary Concept

A cross-section of the geometry of the Invar capillary FE-model is presented in Figure A.5. The Invar capillary was not tested for the maximum equivalent stress due to a transverse load of 1 N. A collapse of the Invar capillary was not expected thanks to its high maximum tensile stress. The results of an extensive FE-analysis, taking into account the modulus, CTE and thickness of the epoxy used to bond the ferrule in the capillary, the CTE of the Invar capillary and the type of adhesive used to fix the fiber into the ferrule, is presented in Table 9.2. It was concluded that usage of a stiff epoxy with a medium CTE was preferred to bond the ferrule inside the Invar capillary. It can further be observed that the error due to cross-sensitivity to temperature does not significantly depend on the used type of adhesive. A change in FPI cavity length due to a 10 N axial force on the needle tip of 200–300 nm and a constant measurement error up to 1.5 N due to a temperature change of 24 °C were expected based on the FE-analysis.

Polyimide Film Concept

Modeling the Polyimide film concept with a FE-model showed a severe impact of the quartz glass substrate, with a high stiffness and a low CTE, on the cross-sensitivity of the concept to temperature. The Polyimide film (10 µm, 3.3 GPa, CTE 32 µstrain/K) was modelled to be deposited on a quartz ferrule housing an optical fiber. The change in geometrical FPI cavity length was defined as the change in thickness of the polyimide film along the axis of symmetry of the model. The elasto-optic effect was not taken into account. The change in cavity length due to a change in temperature was calculated
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based on the thermal expansion of the thin film along the axis of symmetry of the model and the change in refractive index of the polymer film. The force on the needle tip was modelled as a distributed load over the entire polymer film. The change in FPI cavity length due to a 10 N load is expected to be approximately 52 nm based on the FE-model (about equivalent to a change in length of 100 nm of an air-filled FPI cavity, due to the difference in refractive index). A change in FPI cavity length due to a 10 N force in the order of 79 nm would be anticipated had the thin polymer layer been deposited on a polyimide ferrule with identical mechanical properties as the polyimide film, which could be realized using plastic fibers.

The constant measurement error due to a 24 °C temperature change was expected to be approximately 2 N based on the FE-model. This high sensitivity to temperature is due to the low expansion coefficient of the quartz substrate that prevents the polyimide layer from expanding in lateral direction. The polyimide expands more in axial direction instead, which is not compensated by variation in the refractive index of the polyimide. The presence of the adhesive used to fix the fiber in the ferrule hardly effected the sensitivity of the FPI to either temperature or force. Finally, literature indicated quite some dependence of the Young’s modulus of polyimides on temperature (about 5% change in modulus for a temperature change from 20 °C to 40 °C, corresponding to a modifying error due to cross-sensitivity to temperature of about 0.5 N for a force of 10 N on the needle tip), despite a high glass transition temperature [262].

Experimental Methods

The prototypes of the needle tips containing an FPI-based force sensor were tested using a custom made calibration setup shown in Figure A.7. The calibration setup is placed on an active, air-stabilized optical table to minimize the effect of vibrations of the environment on the assessment of the resolution of the force sensors. The

![Figure A.7: Calibration setup used to test prototypes of the needle tip containing an FPI-based force sensor.](image)

calibration setup contains an ATI Nano 17 (ATI Industrial Automation, Apex, NC,
Appendix A: Fiber-Optic Fabry-Pérot Interferometers for Axial Force Sensing on the Tip of a Needle

USA) force sensor that serves as the reference sensor to determine the calibration force on the needle tip. Prototypes of the needle tips were mounted on the ATI Nano 17 sensor using a custom built needle tip mount. The ATI Nano 17 sensor was used to measure forces exerted in axial and transverse direction on the needle tip, as the calibration setup does not prevent accidental exertion of transverse forces on the needle tip.

During the calibration procedure axial forces were exerted on the needle tip both statically and dynamically. The force sensors were preloaded with the weight of the calibration setup, which was defined as a calibration force of 0 N. The needle tip was pressed onto the rigid bottom of a plastic water bath that could be filled with cold and hot water to test the cross-sensitivity of the prototype to temperature. A thermocouple was used to measure the water temperature. The accuracy of the thermocouple was in the order of 1 °C, which was determined by comparing readings of the thermocouple with readings of the precise thermometer of the IKA C-MAG HS 7 (IKA Werke GMBH, Staufen im Breisgau, Germany) heating plate used to heat water up to body temperature.

The force sensor of each prototype was tested for visibility prior to calibration and was interrogated using a commercial interferometer (OP1550 V2, Optics11, Amsterdam, The Netherlands). The wavelength was modulated around 1550 nm at 3 kHz with a modulation depth of approx. 50 pm. Data acquisition during the calibration procedures was performed using a custom application written in MATLAB.

Static Calibration

Axial static loads were exerted on the needle tip by placing well-determined weights on the platform of the calibration setup. Each prototype was first loaded 3 times for 60 s with the maximum calibration force (10 N). The calibration procedure was then performed by loading-and-unloading the prototype at least three times in small steps up to 10 N, during which readings of the reference force sensor and the prototype were obtained. A reading of the force sensors was obtained at least 5 s after changing the calibration force and was averaged over 1 s. Three times the standard deviation of the acquired 1 s long signal was defined as the resolution of the force sensor. Static calibration of prototypes was always performed at room temperature without submerging the prototype in water, as the water temperature could not be kept constant for a sufficiently long time. Static calibration took in practice at least 10 min per prototype. Due to this long measurement time significant drift of readings of the sensor, for example due to creep of the force sensitive element as a result of varying room temperature, may occur in the static calibration results. An average of at least three calibration cycles was taken for each concept to neutralise the drift.

Dynamic Calibration

Dynamic calibration of the force sensors was performed with the needle tip submerged in water and consisted of several 15 s long measurements. Arbitrary calibration forces were exerted on the prototype during these measurements by manually exerting force on the weight platform. The water was changed in between the measurements to
vary the temperature of the environment. During the measurement the temperature of the water slowly changed to room temperature. Moreover, the transverse force on the needle tip varied strongly during dynamic calibration. This procedure can therefore give an impression of the performance of the needle tip force sensors under practical circumstances, where they can also be exposed to transverse forces and varying temperatures. The sensors were afterwards submerged in cold and hot water baths to evaluate the drift due to the change in temperature. The IKA heating plate was used to keep the hot water bath at constant temperature during these drift experiments.

Data Analysis

Interpolation functions, describing the readings of the optical detector of the sensor as function of the calibration force, were made by fitting an sinusoidal (in the case of small compression) or third order polynomial function (for larger compressions) to the data obtained during the static or dynamic calibration of the force sensor. The measurement error of prototypes was assessed as the difference between the calibration force according to the reference sensor and the force measured with the prototype, calculated from the reading of the FPI with the determined interpolation function. Errors can be made in the assessment of the measurement error of purely axial forces during static calibration when the transverse force on the needle tip varies during the calibration procedure. The estimated response of the FPI to transverse forces on the needle tip, derived from the ATI Nano 17, was therefore subtracted from the measured response of the FPI.

Results

In this section the results for static and dynamic calibration will be discussed for prototypes of all three concepts.

Quartz Capillary

The tested prototype had an FPI cavity length of roughly 500 µm and the ferrule protruded approximately 1.5 mm from the stylet tube (the FPI cavity was located as close as possible to the edge of the stylet tube). The change in FPI cavity length due to application of a load of 10 N on the needle tip was about 310 nm, as was predicted by the FE-simulations (Table 9.1).

Results of static calibration of the prototype are shown in Figure A.8. The maximum observed measurement error during static calibration remains below 65 mN (without accounting for the resolution of the sensor). The largest measurement errors are observed at the limits of the measurement range. The resolution, as well as the distribution of noise, of the FPI-based sensor and the ATI Nano 17 sensor are found to be very similar.

In Figures A.9 and A.10 we present the results of the dynamic calibration of the prototype. To test the temperature sensitivity of the sensor, the water temperature was increased stepwise from room temperature (22 °C) to the body temperature...
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![Graphs showing the response of the FPI sensor, force measured with the FPI sensor, and measurement error of the FPI sensor as functions of calibration force.]

Figure A.8: Results of static calibration of a prototype of the quartz capillary concept. The response of the FPI as function of the calibration force, together with a fitted sinusoidal interpolation function, is shown on the left. The center figure shows the force measured with the FPI, calculated using the interpolation function, as function of the calibration force and the identity line \((y = x)\) as a reference. The measurement error of the FPI sensor as function of the calibration force is shown on the right.

of a feverish patient (40 °C) during dynamic calibration. The maximum observed measurement error was 534 mN for a measurement range up to 20 N. The resolution of the prototype was estimated to be 9.5 mN, by analyzing a 1 s long measurement of the response of the prototype for a calibration force of 0 N. The resolution of the reference force sensor, the ATI Nano 17, was determined to be 2.7 mN. The maximum observed measurement error at room temperature, during dynamic calibration, was 388 mN.

Cross-sensitivity of the prototype to temperature was investigated by fitting separate linear interpolation functions to data obtained during dynamic calibration of the sensor at temperatures below 22.5 °C and data obtained at temperatures above 40 °C. Usage of the interpolation function determined for the response of the prototype at 40 °C while the needle tip is at room temperature (22.5 °C), would result in an error of varying between 190 mN and 240 mN within the measurement range (on average 215 mN) according to the fitted interpolation functions. This corresponds to a cross-sensitivity to temperature of 12 mN/°C. The maximum resultant transverse force on the tip reached up to 1.1 N during dynamic calibration. The cross-sensitivity of the FPI to transverse forces was estimated to be 0.3–0.5 N/N axial force based on the regression model.

**Invar Capillary**

The Invar capillary prototype had an FPI cavity length of approximately 160 μm. The change in FPI cavity length due to a force of 10 N on the needle tip was approximately 700 nm.

The results of static calibration of the prototype are presented in Figure A.11. Five instead of three calibration cycles were performed as the sensor seemed to suffer from significant drift, possibly due to viscoelastic creep of the glue. The maximum observed measurement error was 445 mN. The maximum observed error of the FPI
for the measurement of axial forces, after correction for variation in the resultant transverse force, is about 350 mN. The resolution of the prototype is estimated to be in the worst case, disregarding outliers, about 8 mN, which is about 2.5 times worse than the resolution of the ATI Nano 17 sensor. The resolution of the prototype is in the best case about equal to the resolution of the reference sensor.

In Figures A.12 and A.13 we present the results of dynamic calibration of the prototype of the Invar capillary concept at constant temperature. The Invar prototype demonstrated significant drift as a result of temperature variations and could therefore not be calibrated at fluctuating temperature.

The maximum observed measurement error during dynamic calibration using a 3rd order polynomial interpolation function was about 800 mN. The maximum resultant transverse force on the tip was 0.5 N during dynamic calibration. The cross-sensitivity of the FPI to transverse forces was estimated to be 0.6–0.9 N/N axial force based on the regression model.

After submerging the sensor in water of 40 °C, the prototype showed a continuous drift of 25 mN/s. An estimate for the cross-sensitivity to temperature could not be given as it was not possible to calibrate for the drift.

**Thin Film**

The FPI cavity length of the proof-of-concept was estimated to be in the order of 8.5 µm to 9.5 µm. The reflection of the FPI was less than expected, most likely caused by a dirt particle on the top of the fiber, which was observed under the microscope (Figure A.6b). The sensitivity of the FPI to force was found to be around 30% of the predicted sensitivity in the FE-model. The change in FPI cavity length due to a force of 10 N on the sensor was approximately 16 nm.

Results of static calibration of the proof-of-concept, using a 3rd order polynomial
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Figure A.10: Results of dynamic calibration with temperatures varying between 22 °C and 40° of the prototype of the quartz capillary concept, shown as function of time. The graphs show, from top to bottom: (1) the calibration force and the force measured with the prototype, calculated using the interpolation function shown in Figure A.9; (2) the measurement error of the prototype; (3) the temperature of the water bath in which the needle tip is submerged; (4) the transverse force on the needle tip, shown as the resultant transverse force (F\text{tan}) and its two orthogonal components (F_x and F_y). The dotted lines in the top graph (1) indicate the 15 s long fragments of which the dynamic calibration dataset is composed.

The interpolation function, are shown in Figure A.14. The maximum observed measurement error within the measurement range is about 800 mN. The resolution of the proof-of-concept remains to be improved and slowly deteriorated during static calibration from 25 mN to 40 mN. This is significantly lower than the resolution of the reference sensor used during the calibration procedure (2.7 mN). Results of dynamic calibration at constant temperature of the thin film proof-of-concept, using a 3rd order polynomial interpolation function, are shown in Figures A.15 and A.16. The maximum observed measurement error within the shown dataset, consisting out of two separate 15 s long measurements, is on average 800 mN.

Calibration of the thin film sensor at fluctuating temperatures proved laborious due to similar drifts as found with the Invar capillary prototype. The measurement error due to cross-sensitivity to temperature is for a temperature change from about 23 °C to 39 °C estimated to be on average 3.5 N when linear interpolation functions are used to describe the response of the FPI. This results in a cross-sensitivity to temperature of 220 mN/°C. The thin film sensor was rather insensitive to transverse forces in its current form; the maximum resultant transverse force did not exceed 0.5 N during dynamic calibration. The cross-sensitivity of the FPI to transverse forces was estimated to be around 0.03 N/N axial force.
Figure A.11: Results of static calibration of a prototype of the Invar capillary concept, not accounting for transverse force on the needle tip. The response of the FPI as function of the calibration force, together with a polynomial fit, is shown on the left. The graph in the center shows the force measured with the FPI, calculated using the interpolation function, as function of the calibration force and the identity line ($y = x$) as a reference. The measurement error of the FPI-based sensor as function of the calibration force is shown on the right.

Discussion

Three concepts were presented for integrating an FPI sensor into the tip of a needle in order to measure axial forces. Prototypes of these concepts were analysed in an FE-environment, built and calibrated, both statically and dynamically, and their performance was evaluated in terms of accuracy for axial forces, sensitivity for lateral forces and temperature cross-sensitivity.

Although the calibration setup as described in Section 9 was not designed to separate axial from transverse loads on the needle tip, the ATI Nano 17 reference sensor was able to detect the transverse loads applied on the needle tip. Where possible, the measured load by the FPI sensor was corrected for the transverse load given by the ATI Nano 17. Small differences in alignment between the reference sensor and the FPI sensor might have led to an over- or under correction and thereby influenced the calculated sensitivity of the FPI sensor to axial forces. The variation of transverse forces on the needle tip was minimized as much as possible by using masses on the weight platform during static calibration. However, small unintentional variations of the transverse force, especially during dynamic calibration, were unavoidable.

The calibration results of the quartz capillary concept agreed very well with the FE-simulations. Both the expected force sensitivity and temperature sensitivity were demonstrated by the prototype. The measured cross-sensitivity to transverse forces matched the theoretical calculation discussed in Section 9. The quartz column did not show any sign of damage after calibration, despite transverse forces up to 1.1 N during the procedure.

The force response of the Invar capillary concept agreed with FE-analysis. Regrettably, due to heavy drift of the FPI sensor after a temperature change, we were not able to report on the temperature cross-sensitivity of the concept. It is, however, safe
Figure A.12: Results of dynamic calibration of a prototype of the Invar capillary concept. The response of the FPI as function of the calibration force, together with a fitted third order polynomial interpolation function, is shown on the left. The graph in the center shows the force measured with the FPI-based sensor, calculated using the interpolation function, as function of the calibration force and the identity line ($y = x$) as a reference. The measurement error of the FPI-based sensor as function of the calibration force is shown on the right.

...to assume that the concept as such is unfit for use in an environment with fluctuating temperature. The observed drift is most likely caused by the mismatch in CTE between quartz and Invar the multitude of glue joints used in the current prototype. Unlike the quartz based prototype, the FPI cavity of the Invar concept exists of two different materials and entails a large mechanical loop including several glue joints. Replacing these joints with fuses in a future prototype may improve the temperature sensitivity of the design drastically. Out of the two capillary designs, the Invar concept was expected to be the most resistant to lateral forces. However, dynamic calibration resulted in a larger cross-sensitivity to lateral forces for the Invar prototype. This decreased performance can be described to an underestimation of the effect of the several glue joints in the Invar concept. An additional downside of the Invar concept is the loss of potential MRI compatibility.

The force sensitivity of the thin film concept was only 30% of the sensitivity expected from the FE-analysis. This can be partly explained by a slightly thinner polyimide layer as well as a possible underestimation of the Young modulus due to neglect of the elasto-optic effect. Fabrication of the FPI by means of thin film deposition proved to be an advantage. The controllability of the sensitivity of the sensor during manufacturing makes the thin film concept better suited for usage in combination with interferometry compared to the other two concepts. Despite this fabrication advantage, the sensitivity of the thin film prototype tested in this study was high, as predicted by the FE-analysis. The simulations show that when the polyimide layer is deposited on a substrate with a similar CTE, the temperature sensitivity can be reduced to as low as 330 µN/°C. The main drawback of a polyimide elastic element is the sensitivity of its compliance to the temperature of the sensor [262]. In this case, incorporation of an additional temperature sensing FPI inside the needle tip might
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Figure A.13: Results of dynamic calibration of a prototype of the Invar capillary concept at room temperature shown as function of time. The graphs present, from top to bottom: (1) the calibration force and the force measured with the prototype, calculated using the interpolation function shown in Figure A.12; (2) the measurement error of the prototype; (3) the transverse force on the needle tip, shown as the resultant transverse force (\(F_{\text{tan}}\)) and its two orthogonal components (\(F_x\) and \(F_y\)). The dotted lines in the top graph (1) indicate the 15 s long fragments of which the dataset obtained during dynamic calibration is composed.

still be required when no polymer for the thin film can be found with a constant elastic modulus within working temperature range. The sensitivity to transverse stresses was low during static and dynamic calibration. However, addition of a needle tip to the prototype might increase the transverse force per Newton axial force. Therefore, the cross-sensitivity to transverse forces may be underestimated in this study.

The FEA did not account for undefined properties of the glue that could result in measurement errors, such as creep or viscoelastic behaviour of the glue or a temperature dependent compliance. These properties made the use of a compliant glue less attractive, as they would be more evident. To avoid creep and drift as much as possible, non compliant, glass-like glues were selected for FEA. Ultimately the FE-model was able to predict the behaviour of the various prototypes adequately.

Calibration of the quartz capillary concept yielded an order of magnitude improvement in measurement uncertainty of static axial forces compared to FBG-based sensors designed earlier at the TU Delft, approaching the accuracy and resolution of the ATI NANO 17. The intrinsic low cross-sensitivity to temperature of the prototype was nearly two orders of magnitude better than FBG-based needle tip force sensors shown in literature [249]. The exceptional low cross-sensitivity of the prototype comes at the cost of a quite fragile needle tip. More reliable strength tests are required to determine the safety margin of the design, although FE-simulations showed that the current design could be sufficiently strong for integration in 18 G needles.

Marginally visible in the response of the quartz capillary prototype, but more evident with the Invar capillary sensor, was the effect of the glue joints in the sensor and between the sensor and the needle shaft. Measurements of the FPI sensor seemed to lag slightly behind those of the reference sensor during dynamic calibration, resulting in a
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Figure A.14: Results of static calibration of a proof-of-concept of the thin film concept. The response of the FPI as function of the calibration force, together with a fitted 3rd order polynomial interpolation function is shown on the left. The center figure shows the force measured with the FPI, calculated using the interpolation function, as function of the calibration force and the identity line ($y = x$) as a reference. The measurement error of the FPI sensor as function of the calibration force is shown on the right.

A significant sensitivity of all prototypes to transverse force could be observed, predominantly during the dynamic calibration procedure. Using the current manufacturing method and materials it proved impossible to fabricate a sensor that is perfectly located on the neutral axis of the needle. Therefore, for future prototypes, it is recommended to implement a biaxial or, preferably, a triaxial FPI sensor. The implementation of a triaxial FPI sensor could, specifically in the case of the Invar-based prototype, increase the accuracy by separating the transverse forces from the axial forces.

The proposed prototypes proved to be able to detect small force variations while maintaining a low cross sensitivity to temperature. While the current prototypes were optimized for an application with a 18 G needle, all optical FPI force sensors are likely to be suitable for integration in very small biopsy needles. Their ability to measure small variations in the needle insertion force can be employed for needle targeting in future research.
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Figure A.15: Results of dynamic calibration of a proof-of-concept of the thin-film concept at room temperature. The response of the FPI as function of the calibration force, together with third order polynomial interpolation function, is shown in the figure on the left. The figure in the middle shows the force measured with the proof-of-concept, calculated using the interpolation function, as function of the calibration force and the identity line \( y = x \) as a reference. The measurement error of the proof-of-concept as function of the calibration force is shown on the right.

Conclusions

A study of different concepts for a small fiber-optic force sensor based on Fabry-Pérot interferometry to measure forces in axial direction on the tip of a needle was performed. The goal was to design a sensor with a low cross-sensitivity to temperature of the needle tip, an adequate resolution to measure axial loads in the desired range and the sensor was furthermore preferred to be easily manufacturable and, possibly, MRI compatible. Three different concepts for a force sensor in the needle tip with distinct advantages were investigated in more detail. The force sensor based on a quartz capillary resulted in a very low cross-sensitivity to temperature and high axial force resolution, but might be hampered by the fragility of the quartz element. The Invar-based force sensor demonstrated good mechanical strength and decent force resolution but suffered from a higher cross-sensitivity to temperature and transverse forces. The thin film sensor showed great promise for future research, although the current prototype was not able to reach the desired resolution and temperature sensitivity. The small size of this sensor, enabling a tri-axial readout of the force, and the possible integration with plastic fibers, however, makes this concept the most promising for continued research.

Acknowledgments

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Figure A.16: Calibration results of dynamic calibration of a proof-of-concept of the thin-film concept at room temperature, shown as function of time. The graphs show, from top to bottom: (1) the calibration force and the force measured with the proof-of-concept, calculated using the interpolation function shown in Figure A.15; (2) the measurement error of the proof-of-concept; (3) the transverse force on the needle tip, shown as the resultant transverse force ($F_{tan}$) and its two orthogonal components ($F_x$ and $F_y$). The dotted lines in the top graph (1) indicate the 15 s long fragments of which the dataset obtained during dynamic calibration is composed.

Author contributions

T.L., S.B. and D.v.G. conceived and designed the experiments; T.L. performed the experiments and analyzed the data; S.B. contributed materials/tools; S.B. wrote the paper; D.v.G. and J.v.d.D. offered guidance and supervision.

Conflict of interests statement

The authors declare no conflict of interest.
Table 9.2: Results of FE-analysis of the Invar capillary concept, investigating the sensitivity of the force sensing FPI in the needle tip to force and temperature, as function of the CTE of Invar, the thickness of the epoxy layer between the ferrule and Invar capillary, the modulus of elasticity and CTE of the epoxy and the type of glue used to fix the optical fiber into the ferrule. The needle tip was assumed to be attached rigidly, without a glue layer, to the stylet tube. The FE-model accounts for the elastic modulus and CTE of used materials and the thermo-optic coefficient of air. The sensitivity to force is shown as the change in geometrical cavity length due to an applied axial force of 10 N on the needle tip. The sensitivity to temperature is shown as change in cavity length due to a temperature change of 24 °C. The last three columns show the expected measurement error due to cross-sensitivity to temperature in mN/°C.

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<td>-239</td>
<td>19.6</td>
<td>3.80</td>
</tr>
</tbody>
</table>
Appendix B: Batch production of silicon fiber-top cantilever devices

We present a fabrication procedure for batch production of MEMS devices directly on top of an optical fiber. Here, the stepwise fabrication procedure is described and the performance of the final device is demonstrated.

**Keywords:** batch production – fiber-top device – cantilever – MEMS – Photolithography

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Appendix B: Batch production of silicon fiber-top cantilever devices

Figure B.1: Schematic overview of the 8 steps required to fabricate a MEMS device directly on top of an optical fiber (not to scale). Note that, for a better reading of the figure, here we show a cross section of the fiber. A) Cleaved and rounded single mode optical fiber. B) Sputter deposition of 3 μm gold followed by 1 μm silicon. C) Application of the photoresist. D) Align-and-shine photolithography. E) Development of the photoresist. F) Reactive ion etching of the structural silicon layer. G) Removal of the photoresist. H) Chemical (wet) etching of the sacrificial gold layer.

Introduction

The quality of Micro-Electro-Mechanical Systems (MEMS) has seen a remarkable development over the last decades, resulting in a wide variety of applications such as accelerators, gyroscopes, inkjet printers, pressure sensors and optical switches [207]. Furthermore, functionalized MEMS devices enable physical, chemical and bio-sensing, and can be embedded into biomedical instrumentation [210]. For utilization in liquids and harsh environments, however, most biomedical MEMS require an optical readout and, thus, an optical interface. To solve this issue, MEMS devices can be produced with semiconductor technology and glued onto the end of an optical fiber [214] or fabricated directly out of the fiber itself [263] – an approach that has been dubbed as fiber-top technology. Both approaches have considerable disadvantages such as cumbersome manufacturing, high cost of production, and lack of versatility. As a solution to this problem, a few years ago our group has proposed to fabricate MEMS devices by growing and patterning alternate layers of structural and sacrificial material directly on the cleaved end of the fiber [31,32]. The method, however, was never tuned to a point where series, low cost production could be achieved. Here, we present an update on that topic and show that we can indeed fabricate fiber-top sensors via a parallel, cost effective process.

Fabrication procedure

The fabrication process consists of eight major steps, as illustrated in Figure B.1.
Appendix B: Batch production of silicon fiber-top cantilever devices

Figure B.2: A) Fiber after cleaving. B) Fiber after exposure to plasma (note the rounded edges). C) 18 Fibers in the batch holder.

Optical fiber preparation

Our MEMS devices are fabricated on top of a standard single-mode optical fiber (SMF28, Corning). The fibers are cleaved (LDC 200, Vytran), exposed to oxygen plasma (Figure B.2), and placed in the batch holder (Figure B.2C).

Layer deposition

The batch holder is mounted inside a custom built UHV sputtering system (Demaco Vacuum N.V.), where the fibers are coated with a thin layer of chromium (adhesion layer, 10 nm), gold (sacrificial layer, 3 µm, and silicon (structural layer, 1 µm).

Photorestist application

The fibers, still mounted in the batch holder, are then coated with an evenly flat, 1.5 µm thick photoresist film by means of photoresist spray coating (AS8, Altaspray).

Align-and-shine photolithography

Upon completion of the photoresists baking process, the fibers are serially patterned via our in-house developed Align-and-Shine (AS) photolithography technique [31]. The AS method is based on the precise alignment of a wide-core multimode mask fiber and the target fiber inside a modified optical fiber fusion splicing machine (FSU 905, Ericsson). The mask fiber is coated with a chromium and chromium oxide layer, which had been previously inscribed with the photolithographic pattern using focused ion beam milling (Figure B.3A). After aligning, the fibers are brought to contact and UV light is coupled through the mask fiber for 5 s. After exposure, the photoresist is developed according to standard lithography recipes.

Reactive Ion Etching

At the end of the AS process, the fiber holder is mounted in a custom-built reactive ion etch (RIE) chamber. Using an SF6 plasma, the pattern is etched in the silicon layer.
Appendix B: Batch production of silicon fiber-top cantilever devices

Figure B.3: A) The mask used for Align-and-Shine Photolithography. B) SEM image of the suspended cantilever structure on top of an optical fiber.

Figure B.4: Readout output signal in correspondence with a mechanical deformation of the cantilever caused by a sharp tip driven by a piezoelectric translator.

Chemical Etching

A chemical etching step is required to create a suspended structure in silicon on top of the optical fiber. The chemical etching of the sacrificial gold layer is carried out using potassium iodine (KI) under continuous stirring. An additional short chromium etch is performed to remove the adhesive chromium layer from the core.

Results

A suspended cantilever structure has been successfully fabricated out of silicon directly on top of an optical fiber in a batch process (Figure B.3B). We report an overall process yield of around 80% during the batch processing. Figure B.4 demonstrates the interferometric output signal of the cantilever structure upon contact with a sharp tip, as recorded by a commercial interferometer (OP1550, Optics11) (for a description of the readout method, see [263]). The optical signal is a direct readout for cantilever displacement and a proof that the device works according to design.
Appendix B: Batch production of silicon fiber-top cantilever devices

Conclusions

We have demonstrated that, using our AS process coupled to other custom-built optical fiber microfabrication tools, we are able to reliably produce silicon MEMS devices directly on top of an optical fiber via a batch process. We tested the performance of the obtained devices by mechanically actuating the cantilever while looking with an interferometric readout at the mechanical displacement thereby induced. Our work paves the way for a large variety of remote sensing applications that make use of MEMS based devices fabricated directly on top of an optical fiber.

Acknowledgments

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Conflict of interest statement

DI is co-founder of Optics11. DI and MS are shareholders of Optics11.
List of publications and achievements

Publications


Awards and achievements

1. High Five Award 2016 of the Iannuzzi group for the support provided to other members of the group.

2. Young Investigators Award for ‘excellence in research’ and recognizing ‘outstanding, interdisciplinary scientific work’ at iSMIT/DMD ’16 (out of > 200 participants)
3. **2nd best poster** at Dutch Biophysics ’16 (out of > 200 candidates)

4. Selected for the ‘**Highlights of 2015**’ special edition of Surface Topography: Metrology and Properties

5. **4th best poster** at BIGGS ’14 (out of 50 candidates)

6. **3rd best poster** at DOC ’14 (out of 45 candidates)

**Contributions to conferences: oral presentations**

1. ”**High Throughput Batch Production of Fiber-top MEMS Devices**”, OMN, Santa Fe, USA, 2017.

2. ”**In situ dynamic mechanical analysis of heterogeneous soft tissue using a 1.3 mm probe**”, SPIE PW BIOS, San Francisco, USA, 2017.

3. ”**Mechanical properties of biological tissues at the end of a 1.2 millimeter needle: development of the instrument and applications to the physics of the intervertebral disk**”, Annual LaserLaB Symposium, Amsterdam, The Netherlands, 2016.


7. ”**Sensing at the tip of a fiber**”, LAMELIS, Szeged, Hungary, 2014.

**Contributions to conferences: poster presentations**

1. ”**In situ dynamic mechanical analysis of soft tissue using a 1 mm probe**”, iSMIT/DMD, Delft, The Netherlands, 2016.

2. ”**In situ dynamic mechanical analysis of soft tissue using a 1 mm probe**”, Dutch Biophysics, Veldhoven, The Netherlands, 2016.


5. ”**Real-time Identification of Tissue at the Tip of a Needle**”, Biophotonics, Florence, Italy, 2015.


De mechanische eigenschappen van biologisch weefsel beschrijven diens vervorming onder invloed van druk of kracht. Deze intrinsieke eigenschap van weefsel kan vaak worden gerelateerd aan de functie van desbetreffend weefsel in het lichaam. De ruggegraat zorgt bijvoorbeeld voor kracht en structurele ondersteuning in het menselijk lichaam en de schedel en ribbenkast bieden bescherming voor kwetsbare inwendige organen zoals de hersenen en de longen. De literatuur toont aan dat, ook op microscopisch niveau, cellen en weefsels gevoelig zijn voor en reageren op de mechanische eigenschappen van hun omgeving. Het micro-mechanisch milieu van een weefsel heeft dus een directe invloed op cel en weefsel functie.

Ondanks de bovengenoemde evidente relatie met weefselsfunctie en de grote diagnostische waarde van mechanistische eigenschappen van weefsels zijn er slechts zeer weinig procedures beschikbaar voor het meten van deze eigenschappen op microscopisch niveau. De meeste klinische methodes geschikt voor het meten van weefsel stijfheid maken gebruik van macroscopische beeldvormende technieken. Deze technieken beschikken echter niet over de benodigde resolutie om onderscheid te maken op microscopisch niveau. Daartegenover staan de meetmethoden voor materiaaleigenschappen uit de vaste stoffysica, die vaak gericht zijn op nano-niveau en daardoor het microscopische overzicht missen. In deze thesis is een nieuwe aanpak voor micro-indentatie geïntroduceerd, gebaseerd op ferrule-top technologie.

Een ferrule-top sensor bestaat uit een microscopische uitkragende ligger of cantilever die nauwkeurig is gepositioneerd boven een optische glasvezel. De verplaatsing van de cantilever ten opzichte van zijn neutrale positie kan uiterst accuraat worden uitgelezen door een monochromatische laser door de glasvezel te schijnen. De sensor is gefabriceerd uit boriumsilicaat (glas) en is 3 mm in diameter in zijn standaard uitvoering. Afhankelijk van de toepassing kan de cantilever worden uitgerust met verscheidene tips en coatings.

Voordat een ferrule-top sensor kan worden gebruikt voor nauwkeurige krachtmetingen dient de verconstante van de cantilever vastgesteld te worden. Als een onderdeel van deze thesis is een experimentele kalibratie methode ontwikkeld speciaal voor krachtsensoren met een positie uitlezing gebaseerd op interferentie van laserlicht. De methode berust op een aanpak waarbij het vrij hangende uiteinde van de cantilever voorzichtig tegen de pan van een weegschaal wordt gedrukt. De verplaatsing van de sensor wordt geleverd door een nauwkeurig gekalibreerd piezo-elektrisch motortje, welke wordt angestuurd door een gevoelig reguleringsysteem dat gebruik maakt van negatieve terugkoppeling. Dit systeem zorgt ervoor dat de buiging van de cantilever wordt gefixeerd op een waarde overeenkomstig met een maximum van het interferentiepatroon. Nadat de bijbehorende waarde voor de uitgeoefende kracht is opgeslagen.
krijgt de motor de opdracht om de cantilever te buigen tot het volgende maximum in het interferentie patroon. Door deze stappen een aantal keer te herhalen kan de gemeten kracht worden uitgezet tegen de buiging van de cantilever. Uit deze grafiek kan vervolgens de veerconstante van de cantilever worden afgeleid.

Wanneer de ferrule-top sensor eenmaal gekalibreerd is kan de stijfheid van biologisch weefsel worden bepaald door middel van micro-indentatie. Deze techniek meet zorgvuldig de verplaatsing van het weefsel (m.a.w. de indentatie) naar aanleiding van de druk die geleverd wordt door de sensor. Traditionele indentatie apparatuur is gelimiteerd tot de oppervlakte van een weefsel monster. De doelstelling van deze thesis is het ontwikkelen van een \textit{in situ} indentatie instrument dat lokale metingen van weefsel stijfheid onder het oppervlakte mogelijk maakt. Om dit te bereiken is een nieuw instrument ontworpen dat de gebruiker de mogelijkheid geeft de Young Modulus van een materiaal te meten aan het uiteinde van een 5 mm brede naald. Het instrument is uitgerust met een ferrule-top cantilever met een microscopische balvormige tip. Met behulp van een piezo-elektrische actuator aan de achterkant van de naald, verbonden door de sensor door middel van een stalen kabeltje, wordt de tip van de sensor herhaaldelijk in en uit contact gebracht met het specimen. De capaciteit van het instrument om lagen van verschillende stijfheid te herkennen en te kwantificeren is aangetoond in een gelaagd gelatine monster. Daarnaast is het aangetoond dat met deze nieuwe methode de verschillende overgangen in stijfheid in leverweefsel succesvol kunnen worden geïdentificeerd.

Voordat het ontworpen \textit{in situ} indentatie instrument toegepast kan worden in relevant preklinisch onderzoek was een miniaturisatie stap noodzakelijk. Om metingen te van de mechanische eigenschappen van tussenwervelschijven van geiten uit te voeren is de buitendiameter van het instrument verkleind tot ongeveer 1.3 mm. Daarnaast is het indentatie protocol aangepast om de lokale dynamische moduli van een specimen vast te leggen. Door middel van de dynamische moduli kan onderscheid gemaakt worden tussen de hoeveelheid opgeslagen energie (storage modulus) en verloren energie (loss modulus) in een specimen tijdens een indentatie. Het aangepaste instrument is toegepast om de viscoelastische eigenschappen van een volledig ingesloten specimen te meten, namelijk de nucleus pulposus van de tussenwervelschijf. De resultaten van de metingen in dit project tonen aan dat wanneer de mechanische eigenschappen van biologisch weefsel worden gemeten terwijl het weefsel zich in zijn natuurlijke omgeving bevindt, deze daadwerkelijk kunnen verschillen van de waarden die worden gemeten wanneer het weefsel uit zijn omgeving wordt verwijderd. Dit toont aan dat de mechanische eigenschappen van biologische weefsels afhankelijk zijn van het lokale milieu.

De kleine afmetingen van het instrument maken een groot aantal toepassingen mogelijk waarbij de lokale reactie van een systeem ten gevolge van een variërende parameter kan worden gemeten. Als voorbeeld is er gekeken naar de verandering in viscoelastische eigenschappen van de nucleus pulposus ten gevolge van verticale druk op de wervelschijf. Tijdens deze experimenten is er gevonden dat de nucleus door middel van vochtverlies een elastische reactie vertoont gedurende verticale druk op de wervelschijf.

Een belangrijk thema in deze thesis is een continu streven naar verdergaande miniaturisatie. In de laatste hoofdstukken worden twee alternatieve aanpakken om
zogenaamde MEMS structuren (zoals bijvoorbeeld de cantilever) te fabriceren op een optische glasvezel. De eerste methode beschrijft een procedure om MEMS structuren direct op het uiteinde van een optische glasvezel te fabriceren met behulp van een omgekeerd fabricage proces zoals vaak wordt gebruikt in de semiconductor industrie. Fiber-top sensoren met een diameter van 125\(\mu\)m kunnen worden geproduceerd door middel van het aangroeien en selectief verwijderen van materiaal direct op het uiteinde van de glasvezel. De tweede aanpak komt voort uit een samenwerking met Philips Research, waarin een concept is ontwikkeld voor een fiber-top sensor gebaseerd op een bewegend membraan. Deze microscopische membranen kunnen met duizenden tegelijk worden gefabriceerd uit een silicium wafer met behulp van een batch proces.

Samenvattend is er voor deze thesis een gespecialiseerde in situ minimaal invasief micro-indentatie instrument voor het meten van weefsel stijfheid onder het oppervlak ontworpen, ontwikkeld en getest. De constante nadruk die gelegd is op miniaturisatie heeft ertoe geleid dat het ontwikkelde instrument geschikt is voor preklinisch onderzoek en dat er daarnaast nog verschillende mogelijkheden zijn om het onderzoeksproject te vervolgen.
Acknowledgments

This PhD project has been a journey for me. Not only literally, as I have been able to visit Ireland, Sweden, Denmark, Latvia, Hungary, Italy, The United States and countless places in The Netherlands, but also psychologically. As with any challenging project, it has seen both low points and highlights. Destroying all my newly fabricated force sensors in one thoughtless movement, basically undoing the work of the past week and, as I learned, inherently related to working with glass fibers, has left me seriously reconsidering my commitment to the project on several occasions. My motivation definitely dropped to a minimum in the summer of 2016, when my setup mysteriously stopped working and everything I tried to fix it only seemed to make it worse. These moments have been, however, always insignificant compared to the feeling of pride when the next paper was published. Moreover, I would argue that these low points have made me stronger, as I have learned that, oftentimes, its is necessary to fail and start over in order to succeed. I have, more than anything, learned how to plan, set up and execute experiments independently. But I have also learned how to collaborate, how to present research and how to write in scientific English.

I would not have been able to achieve the results presented in this thesis without the help and support of a great deal of people. First and foremost, I need to thank my daily supervisor, advisor, co-author and promoter, Davide. You recognized my potential when I joined the group at the end of 2012 and put your trust in me ever since. Thank you for offering this position to me and enabling me to grow in it as well. I truly admire your enthusiasm for scientific research and your ability to transfer this to others. Your ingenuity and creativity always led to new ideas that, in turn, inspired me to continue. I am thankful for your persistence in correcting my writing and encouraging me to perform ‘one more series of measurements’ when, in my opinion, the work was done. The end result was always that much better because of it, which was, in turn, appreciated by our peer reviewers. Although it might not have shown on a daily basis, I have very much enjoyed our collaboration.

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Morris, Ata and later Hedde, thank you for your company and introducing me to the world of ferrule-top probes. I also would like to thank the guys from Fijnmechanische Instrumentatie.

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Nelda, you have been my biggest support throughout almost my entire PhD period. Combining the role of girlfriend and colleague, you have always been by my side when I needed it. Thank you for being with me and making everything else seem irrelevant.
Curriculum Vitae

Steven Vincent Beekmans was born in Heemstede, The Netherlands on June 17, 1989. In 2007, at 18 years old he graduated from the secondary school 'Coornhert Lyceum' in Haarlem, combining natural sciences with biology, French and computer science. Aiming to combine his interests in natural sciences and medicine, he received his Bachelor degree in Medical Natural Sciences in 2010 at the VU University Amsterdam. Afterwards, he interrupted his studies for a six month long trip through South America, Australia and Africa. In 2013, he obtained his Master degree in Medical Natural Sciences at the VU University Amsterdam with a weighted average grade of 8.3/10. Specializing in Medical Physics, he performed research internships at the VU Medical Center, working on reconstruction methods for SPECT/CT imaging, and in the VU Biophotonics and Medical Imaging group, studying reflectance spectroscopy of human skin. During his research internships he developed an interest in combining experimental physics with medical imaging, which has driven him to pursue his doctorate degree on the boundary of these disciplines.

From September 2013 to September 2017 he has been a PhD candidate in the group of Prof. Davide Iannuzzi, part of the Biophotonics and Medical Imaging group at the VU University. During this period he worked on a project to implement ferrule-top force sensors as a versatile tool for micro-indentation of soft tissues, which ultimately led to the development of the minimally invasive micro-indentation approach described in this thesis. Initiating and preserving successful collaborations with several other research groups has been paramount for the success of this project.
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