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.