A dynamometer for nature's engines

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Muscle is an amazing natural thermodynamic engine that converts chemical energy into mechanical work. Each muscle twitch is 'sparked' by an electrically-released pulse of calcium ions, which trigger force-development and cell shortening, at the cost of energy and oxygen, and the dissipation of heat. Like other engines, muscle is inefficient at converting chemical energy to mechanical energy; at least 80 % of the energy consumed by the muscle is released as heat. When we want to measure or enhance the performance of a human-made engine, such as an internal combustion engine, we typically attach it to a *dynamometer* – a device that can apply a controlled load to the engine, and measure its fuel consumption and work output. Imagine that we had a similar tool that allowed us to inspect nature's engine – muscle tissue – and thereby measure its energetic performance. Such a tool would allow us to better understand the muscle's work output and energy consumption, diagnose problems with the engine, tune its performance, and observe its response to changes in load, or to additives in its fuel. In this article we report on our construction of a dynamometer for heart muscle.

Over several years, our bioinstrumentation team at the Auckland Bioengineering Institute has developed unique systems [1-4] to measure, simultaneously, the energetic processes underlying heart muscle contraction, while subjecting isolated samples of heart tissue to realistic contraction patterns that mimic the pressure-volume-time loops experienced by the heart with each beat. These devices are thus effectively 'dynamometers for heart muscle'. Their development has required us to create our own actuators, force transducers, heat sensors, and optical measurement systems and methods. Our instruments make use of several different measurement modalities which are integrated in a hardware-based real-time acquisition and control environment and interpreted with the aid of computational models. In this article, we provide a brief overview of the instrumentation and measurement technologies that we have developed and integrated into these unique devices.

Fuel supply

Cardiac tissue contraction is a cyclic process that imposes a high energy demand. However, once tissues are disconnected from the vasculature of the heart, energy supply to the muscle

cells is limited primarily by the rate at which oxygen can diffuse into the tissue. For this reason, researchers prefer to study thin (~100 µm diameter, ~1 mm length) cylinders of axially-arranged heart muscle cells known as 'cardiac trabeculae'. These can be thought of as approximately one-dimensional actuators, and are slender enough that oxygen can diffuse into the tissue while it is superfused in an oxygenated saline solution. However, their tiny size and delicate form places extreme constraints on systems for maintaining their viability, measuring or controlling their length and force production, and measuring their shape and heat production.

Our approach to this challenge has been to suspend these tiny samples (usually obtained from the hearts of rats) between two pairs of platinum hooks, which are attached to quartz tubes (Figure 1). A steady stream of oxygenated and nutrient-rich solution flows over the length of the muscle, providing fuel and removing waste products. The muscle is typically inserted length-wise into a glass bath of 1 mm square cross section, which allows optical access for imaging systems. Access is provided for optical probes to measure the partial pressure of oxygen in the fluid upstream and downstream of the muscle. Platinum electrodes are placed near the muscle, and provide a means for imposing a pulsed electric-field (~1 kV/m, 5 ms) to initiate muscle contraction. At body temperature these samples can be electrically stimulated to contract at rates of up to 10 Hz.

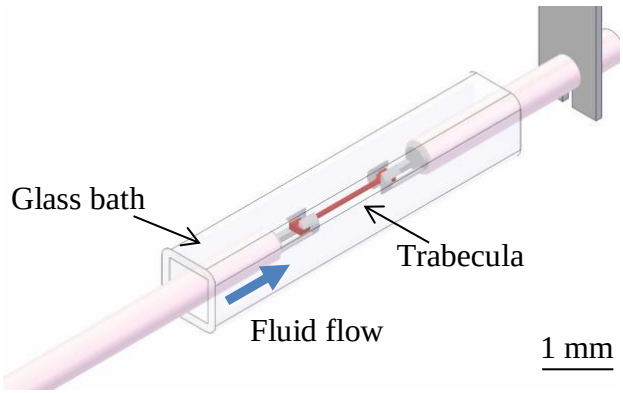

Fig. 1. Close-up view of a trabecula mounted onto hooks, and glass bath with fluid flow indicated by the blue arrow.

Firing the engine

Following each electric field stimulation event, cardiac muscle cells release a 'spark' of calcium ions from their internal stores into the interior of the cell. Calcium ions allow protein-based molecular motors to cyclically interact by creating 'cross-bridges', which result in force development. After ~100 ms, the calcium is pumped from the cellular environment back into its internal storehouse in readiness for the next stimulated contraction. The intracellular

calcium concentration can be measured by the use of a fluorescent probe (Fura2) which binds to the free calcium within the cell. The fluorophore is excited by alternately illuminating the tissue with UV light of 340 nm and 380 nm wavelength, through a 20× microscope objective (Figure 2A). The fluorescent emission excited by these wavelengths (at 580 nm) is collected from the tissue through the same objective, and redirected to a photomultiplier tube. The magnitude and ratio of the fluorescence signals is an index of the concentration of free calcium within the cell, and thus of the key process that initiates contraction.

Force and length measurement and control

During contraction, cardiac muscle can develop a stress of up to 100 kPa. In a sample of this size, this corresponds to a force of approximately 1 mN. A common technique for measuring forces of this magnitude is to use a silicon beam implanted with piezoelectric strain gauges. However, these are very delicate and prone to thermal drift and accidental breakage. Our solution to this problem has been to develop our own stainless-steel cantilever force sensor (Fig 2B) and to measure its deflection using a focused heterodyne laser interferometer (Fig 2C). The stainless-steel surface provides sufficient diffuse reflection to allow cantilever deflection to be measured to 100 μm or more, to the interferometer resolution of 0.309 nm. The corresponding force production can thus be resolved to approximately 1 μN resolution over a 20 kHz bandwidth (Figure 3A).

If muscle contraction is unimpeded, trabecula shortening of several hundred micrometers can occur. Thus, a second interferometer beam (Fig 2D) is used to monitor the position of a custom voice-coil linear motor (Fig 2E) that controls the length of the muscle, via a tube and hooks. Both interferometer signals update internal 36-bit position registers in a laser axis card (Keysight N1231B), and are read into LabVIEW RealTime via a field programmable gate array (FPGA). Muscle length control is achieved using a PID algorithm implemented in the FPGA, updated at a rate of 20 kHz.

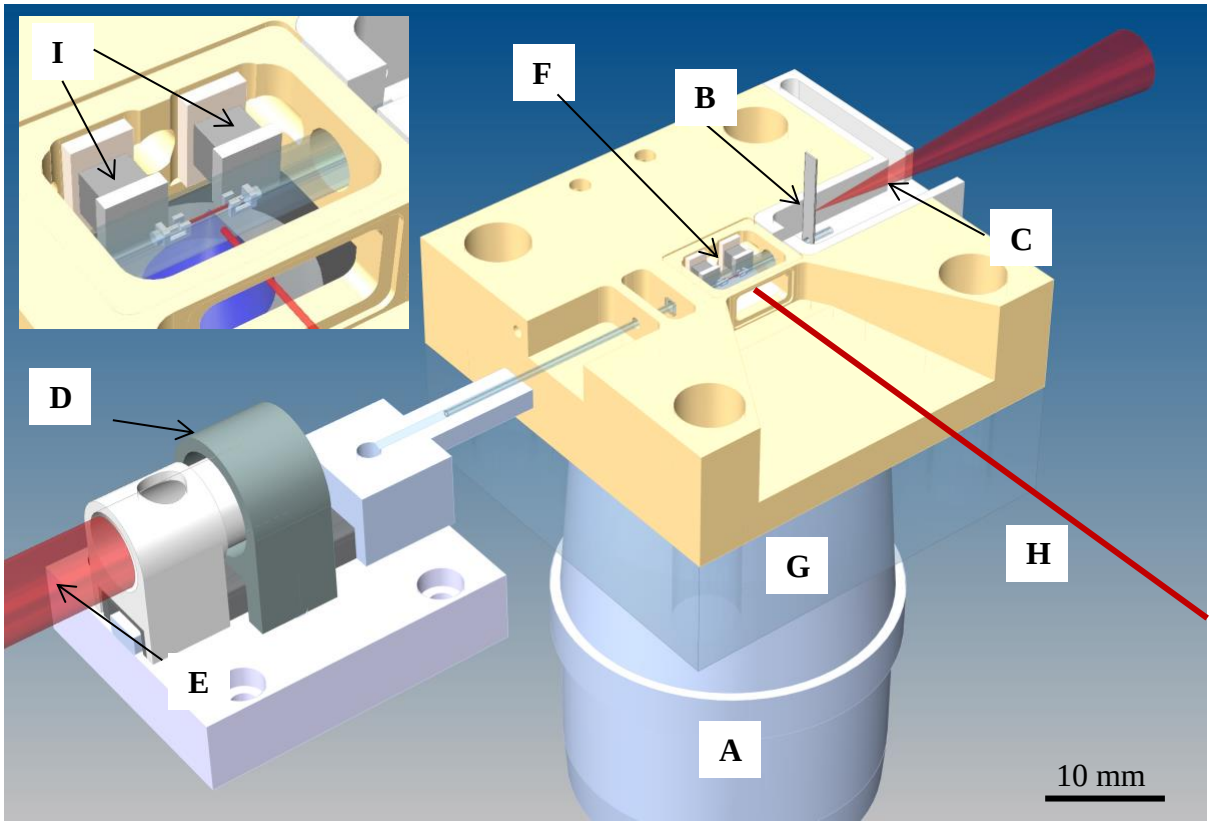

Fig. 2. Components of our measurement system: **A.** Calcium and brightfield imaging microscoope objective. **B.** Cantilever force sensor. **C.** Force sensor laser intereferometer beam. **D.** Linear voice-coil motor. **E** Linear motor interferometer laser beam. **F.** Muscle measurement chamber. **G.** Temperature controlled copper base (top not shown). **H**. Scanning optical coherence tomography laser. Inset: Close up view of measurement chamber (as in Figure 1) showing **I.** Heat sensors.

An important feature of any dynamometer is its ability to apply a precisely controlled mechanical impedance to the motor under test. With real-time estimates of force and length, we are able to exploit the determinism of the FPGA environment to dynamically modulate the mechanical impedance presented to the muscle by the voice coil motor. This capability allows us to measure and control the work done by the muscle during contraction. In our most recent devices [5], we dynamically compute in the FPGA an equivalent lumped-parameter model of the circulatory system load that the muscle would experience *in vivo*, and use this to dictate the length of the muscle as it cyclically develops force, shortens, relaxes and is restretched to its original length.

Estimates of muscle length are computed by our model at a rate of 20 kHz, and provide the set-point for the voice-coil motor. The result is that the muscle expresses a series of force-length loops ('work-loops'), where the area enclosed by each loop is the work done by the muscle on the voice coil motor (Figure 3C). The work-loops described by the muscle samples thus replicate the pressure-volume dynamics of the heart. By varying model parameters, we can mimic a variety of medical conditions such as high blood-pressure, or under acute vigorous exercise interventions, to discover how these affect the work output.

Heat rate measurement

For more than a century, measurements of heat production have provided useful insight into the energetic characteristics of muscle tissue, in health and disease. A variety of calorimeters has been constructed specifically for this purpose. In these designs, muscle heat-rate (i.e. thermal power output, typically ~ 10 μW) is inferred from the slight temperature increase (~1 mK) imparted by the muscle to the surrounding flowing fluid. Usually, temperature is measured up-stream and down-stream of the muscle.

Our first muscle calorimeters measured the temperature increase using an array of thin-film infrared thermopile sensors closely arranged around the square-cross section glass tube housing the muscle. In this approach, heat conducted from the glass tube through a 100 μm layer of air, raising the temperature of a thin membrane upon which the hot-junctions of a thermopile had been deposited. The thermopile produced a microvolt-level signal in proportion to the temperature increase of the fluid. While this approach was effective, the high resistance of the thin-film thermopile resulted in a high level of Johnson noise, and thus a modest signal:noise ratio.

In subsequent work, we explored a variety of other temperature measurement methods [6] including the use of a laser interferometer to read the deflection of a vapour-pressure thermometer inspired by a 'hand bubbler' [7]. However, careful modelling, analysis, and experimentation has led us to our current design, in which we use two bismuth telluride thermopile arrays (thermoelectric modules, used as sensors, rather than as heat pumps), upstream and downstream of the muscle (Fig 2I). The nanovolt-level signals arising from these sensors are amplified by precision amplifiers (EM Electronics) and digitized by 24 bit delta-sigma A/D converters (National Instruments). Although the signal level arising from these thermopiles is much lower than that of the thin film thermopiles we used previously, their very low electrical resistance effectively removes Johnson noise as a limiting factor. With this approach, we can measure the temperature increase of the superfusing fluid to a resolution of 0.4 μK over a 1 Hz bandwidth [1-3]. This corresponds to a muscle power output resolution of 2.6 nW at room temperature, and a signal:noise ratio of 1700 (see Figure 3B for an example measurement).

The achievement of this noise-limited heat resolution requires the muscle experiment to be enclosed by a pair of insulated copper blocks (Fig 2G), which are temperature controlled to sub milliKelvin resolution. We use thermistors as temperature sensors on these blocks, and measure

their resistance using a data acquisition device (Keysight 34970A). Heat is pumped in and out of the blocks using thermoelectric coolers driven by linear amplifiers.

Having the ability to precisely control the work-done (W) by the muscle, and to simultaneously measure its heat production (Q), we can compute the efficiency (ϵ) of the muscle from

$$\epsilon = \frac{W}{W+Q} \tag{1}$$

as the parameters describing the load faced by the muscle are varied. Typically, we find that the efficiency of cardiac muscle samples varies with load up to a maximum of ~ 15 % (Figure 3D).

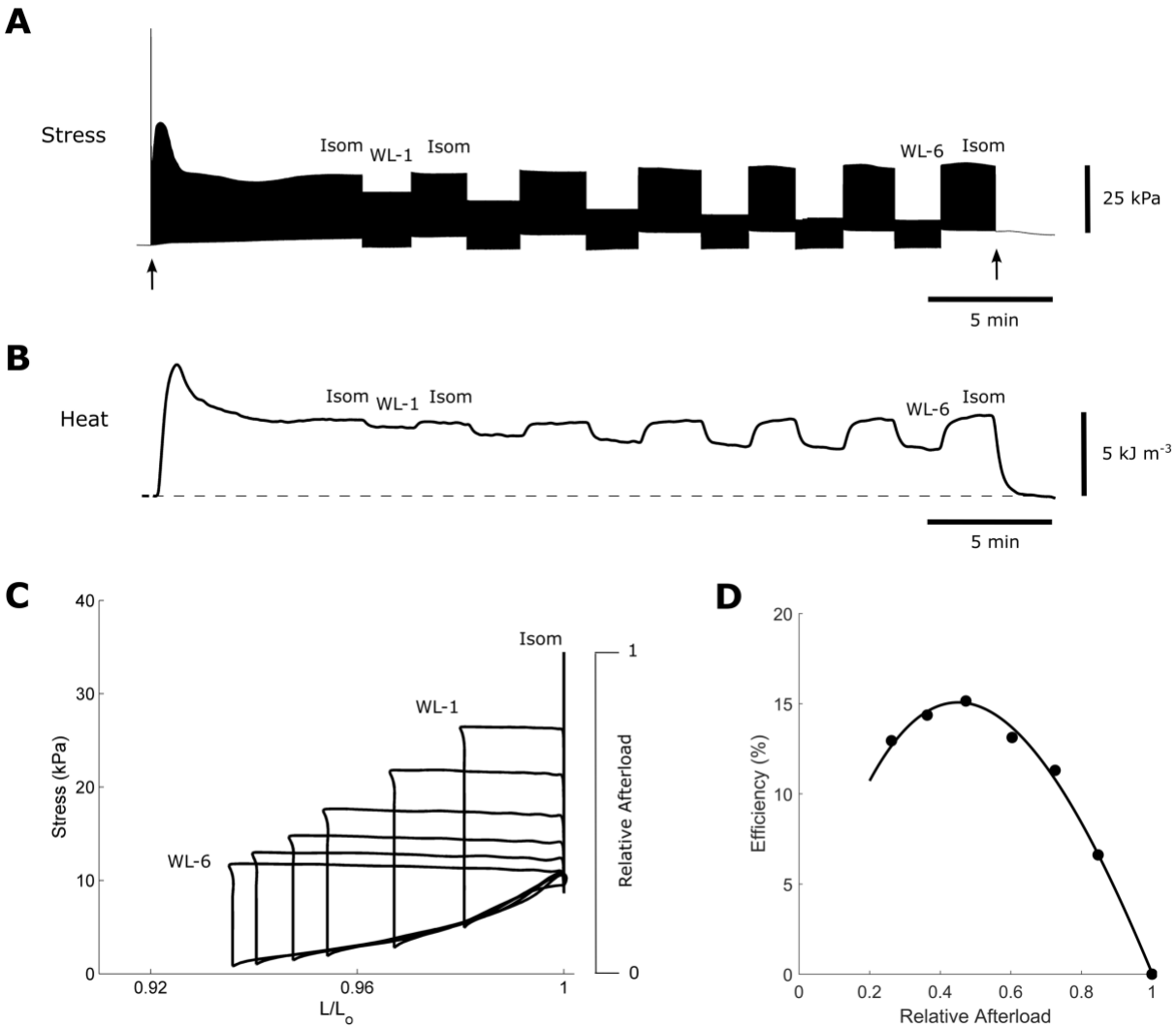

Fig. 3. A representative experimental record from a single trabecula undergoing isometric and work loop contractions. Stimulation started at the time indicated by the first arrow and halted at the second arrow. The trabecula underwent work loop contractions at 6 different afterloads (labelled 'WL 1' and 'WL 6') until steady state (~2 min) before and after an isometric contraction (labelled 'Isom'), where its stress production (**A**), suprabasal heat output (**B**), and length change (not shown) were simultaneously recorded. Steady-state work-loops revealed from plotting stress as a function of length revealed from plotting stress as a function of length, where L_o denotes length that yield optimal stress output (**C**). Steady-state efficiency as a function of load where peak efficiency occurrred at around 0.45 relative afterload (**D**). Reproduced from [8].

Optical imaging systems

The optical access provided by the use of a square cross-section glass bath enables us to gather images of the muscle in two different directions. Thus, we have incorporated two different imaging systems into our devices: an optical coherence tomography (OCT) system for measuring the external 3D geometry of the tissue, and a brightfield microscope that captures transmission images of the internal muscle structures.

Muscle shape by Optical Coherence Tomography imaging

The geometry (diameter, length, and shape) of trabeculae can vary considerably among samples. Hence, it is desirable that we can accurately image each trabecula being investigated during an experiment. To this end, we constructed an OCT system and developed a gated imaging procedure to image actively contracting trabeculae and to reconstruct their time-varying geometries. OCT is based on low coherence interferometry, and provides three-dimensional images of tissue reflectance. It is ideal for imaging translucent media such as biological tissue, and has been frequently used to image the retina, gastrointestinal tissue, arteries, and embryonic heart. No special tissue preparation is required, the resolution and depth are of the required scale, and the muscle can be imaged while contracting inside the glass bath (Figure 4A).

By imaging a single cross section while monitoring developed force, we have found that gated stimulation of the muscle is sufficiently repeatable to allow us to coherently stitch together geometric data from multiple contractions to form a 3D representation of a single muscle contraction over its entire cycle [9]. These data allow us to properly compute the engineering stress along the length of the muscle, and to follow dynamic changes in muscle shape during contraction. They also allow us to accurately normalise the heat production of the tissue to its total volume, allowing us to make comparisons of energy output between different samples of tissue.

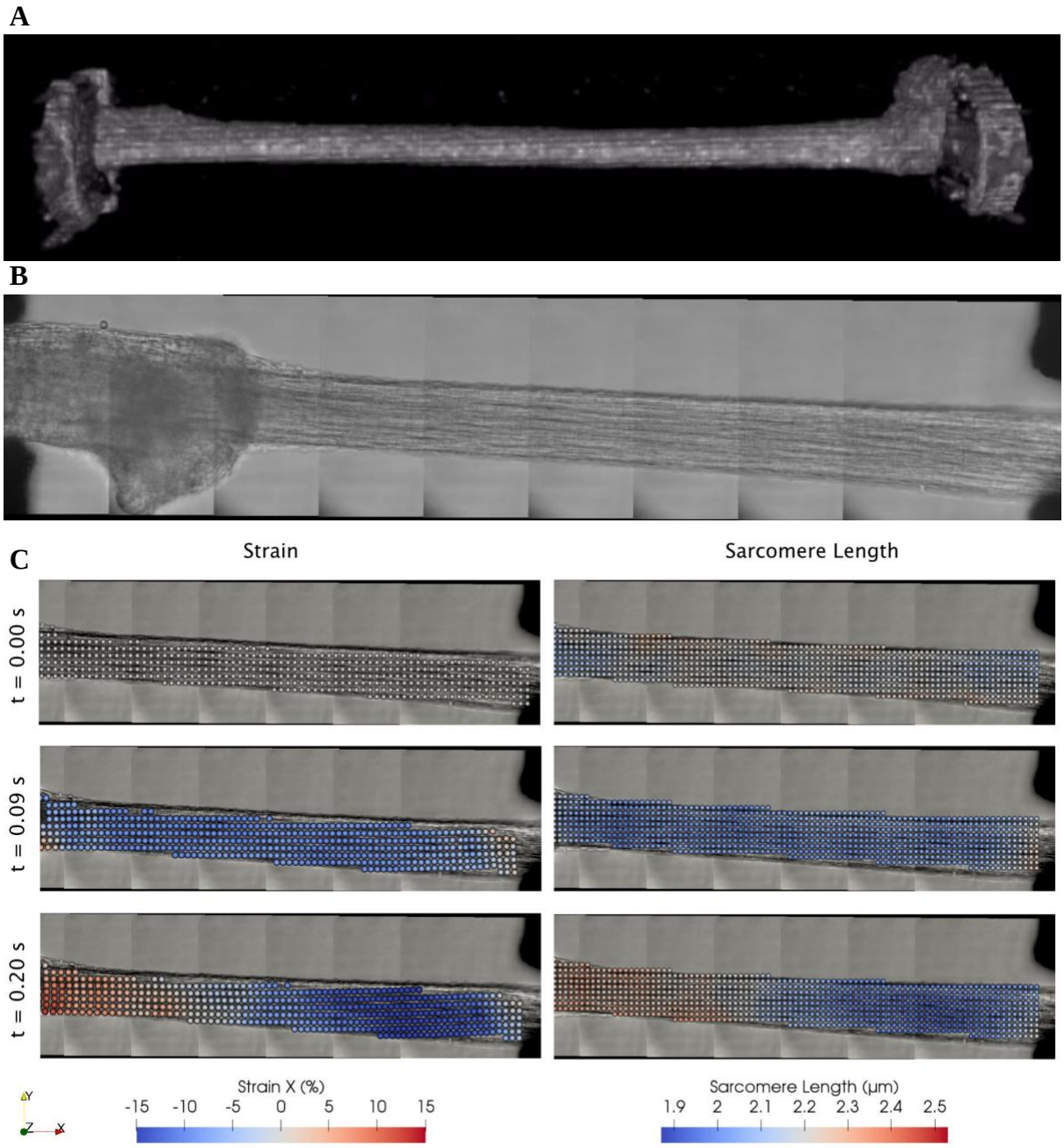

Fig. 4. Typical optical imaging data: **A.** 3D reconstruction of a trabecula under tension. **B.** Composite of brightfield images through a trabecula. **C**. Processed brightfield images, showing the horizontal strain (left) and sarcomere length (right) of tracked material points, at rest (top), peak force (middle), and during relaxation (bottom). This trabecula is approximately 1.4 mm long.

Internal shortening and sarcomere length by brightfield image registration

It is not just the external geometry of the muscle that changes during its contraction; the force production of the contractile proteins internal to each cell can give rise to quite complicated motion within each trabecula. Fortunately, transmission images of the trabecula can reveal a rich set of useful information about the motion of internal structures during contraction. Such images also reveal information about the repetitive spacing between the contractile proteins (i.e. the 'sarcomere length'), and how this varies throughout the trabecula during a muscle

twitch. Both of these parameters of interest can be computed using image processing techniques.

Digital image correlation (DIC) is a technique wherein a displacement field can be computed from two images that contain translations in position. The technique involves dividing each image into subimages, and correlating visible features between the two images to determine the relative displacement of each region. Cardiac trabeculae have natural features that arise from their contractile proteins and the connective tissue surrounding them, making them suitable candidates for DIC analysis.

We have thus developed a highly accurate, robust and efficient image registration technique [10] to track the motion of features internal to the trabecula, and to reconstruct this motion throughout the time-course of a twitch. We first scan along the length of the muscle in order to gather 2D brightfield images through its thickness, and then stitch these together to create a composite image of the muscle (Figure 4B). We then divide this composite image into regularly spaced subimages, and consider the centre of each subimage to comprise a 'material point' for further tracking. By applying our image registration technique to adjacent subimages in time we can track the average displacement of each material point during muscle contraction.

Our image registration technique allows us to resolve material point motion to millipixel resolution. By dividing the displacement of adjacent material points by their original spacing, we can compute an estimate of local strain. Having tracked the displacement of each material point, we can also follow the spacing of the contractile proteins that surround the material point. The repeated protein spacing in cardiac muscle gives rise to striped features known as sarcomeres. Their spatial frequency can be estimated by selecting a window around each material point, and applying the 2D Fourier transform.

These techniques yield 2D maps of local deformation and strain (Figure 4C) throughout the length of the trabecula, and over the time-course of a twitch, at a spatial resolution far exceeding that of previous techniques. Our results have revealed that internal strain within a muscle can be highly non-uniform even during fixed-length contractions.

System Integration

All of these data acquisition, imaging, measurement and control techniques are synchronised in a single hardware/software system developed in National Instruments LabVIEW RealTime and LabVIEW FPGA environments, running in an 8-core Intel Core i7 workstation. This

approach has provided us with a great deal of flexibility, and control over the system architecture, with tight synchrony between each of the measurement modalities (Figure 5).

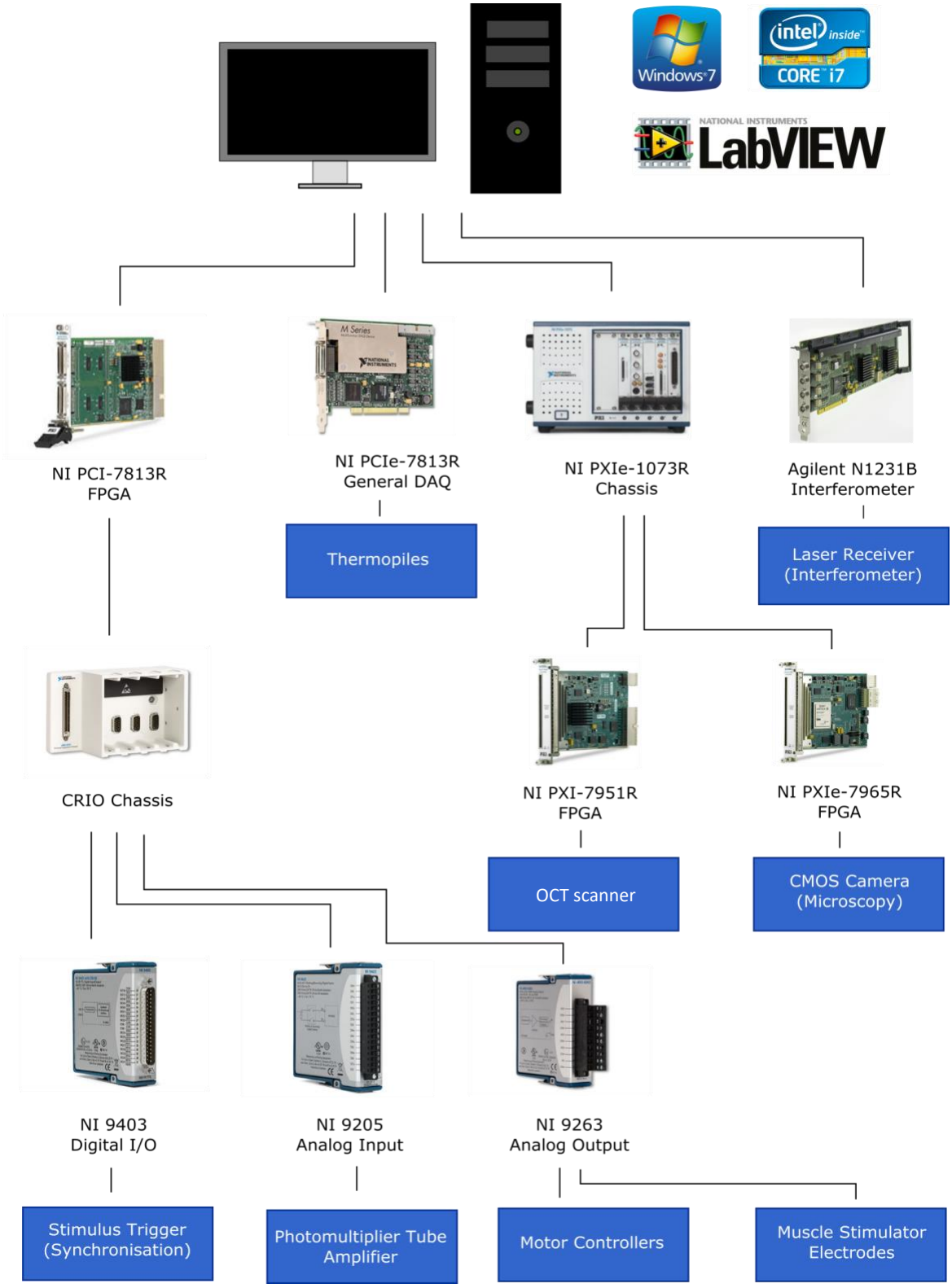

Fig. 5. Hardware components and architecture of our measurement system.

Insights

Equipped with these unique capabilities, we have probed the mechano-energetics of failing hearts from rats with diabetes. We have found that the peak stress and peak mechanical efficiency of diabetic tissues are normal, despite them suffering prolonged twitch duration. We have also shown that pulmonary hypertension reduces the peak efficiency of the right-heart tissues, but not the left-heart tissues. In another program of research, we have demonstrated that despite claims to the contrary, dietary supplementation of fish-oils has no effect on heart muscle efficiency. None of these insights was fully revealed until the development of this instrument.

Future work

In the near future we plan to supplement these technologies with:

- a device for measuring the dynamic stiffness of permeabilized tissue samples,
- devices for high-throughput cell testing, and 3D myocyte bio-printing.

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Biographies

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Poul Michael Fønss Nielsen is a professor in the Auckland Bioengineering Institute and Department of Engineering Science at the University of Auckland, New Zealand. His research focuses on using novel instrumentation, detailed computational models, and quantitative descriptions of physical processes to gain a better understanding of human physiology. Many of his projects couple mathematical modelling with innovative instrumentation to improve our ability to understand and interpret measurements of complex biological systems, subject to the constraints of well-understood physical conservation and balance laws. He is a member of IEEE, and the secretary of the New Zealand chapter of the IEEE Instrumentation and Measurement Society.

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