1 Introduction.

A large amount of research is devoted to the study of muscles, including muscle diseases, fundamental mechanisms, etc. One aspect, muscle contraction, has received a lot of attention, although as yet understanding of the fundamental process is incomplete.

This project was to design and construct a positive feedback device to allow Biophysicists to investigate the isometric response of muscle tissue. In an isometric contraction, the muscle tension is allowed to vary, whilst keeping the length fixed. This requires a circuit to detect changes in length and supply a signal that is suitable for driving a servo-motor, that subsequently corrects for the movement, returning the muscle to its original length.

In this report, I will briefly outline the biological background, before moving on to concentrate on the design aspects and give detailed information on the operation and testing of the circuit. It is hoped that this report will in future serve as a kind of user's manual, such that the device can be adjusted or modified if necessary.

2 Background information.

2.1 Muscle structure and contraction.

Muscles comprise bundles of fibres a few centimetres in length and 20-100 μ m in diameter, surrounded by connective tissue (See figure 1). Every fibre is filled with approximately a thousand rod-like structures called myofibrils, each about 1 μ m in diameter. Each myofibril is divided up into cylindrical sections by thin partitions called Z-lines or Z-discs. These also cross from myofibril to myofibril throughout the fibre, dividing it into repeating units called sarcomeres.

Thin filaments of actin emerge from the Z-line, making up the I-band, while thick myosin filaments make up the A-band. The actin elements of the I-band extend into the A-band, leaving a less dense region in the centre, where there is no overlap. This is the H-zone. This variation in density is the cause of diffraction of a monochromatic light beam, allowing changes in sarcomere length to be observed. The cross section (figure 1) shows how the filaments from the I- and A-bands interleave in a hexagonal structure.

On stretching or shortening of the muscle fibre, the A-bands remain at constant length, while the I-bands expand and contract. This was shown by Dr. H. Huxley, and leads to the sliding filament theory of muscle contraction: contraction occurs by the interdigitation of the I-band

Figure 1: Structure of vertebrate skeletal muscle.



(actin) and A-band (myosin) filaments. The process by which the filaments pull themselves past each other to interleave in this way is complex, and is still not fully understood; it is the subject of considerable investigation in this field.

2.2 Monochromatic light diffraction experiments.



Figure 2 shows a typical experimental set-up that is used for obtaining diffraction patterns from plaice fin muscle. The muscle is mounted inside a chamber and immersed in a cool solution known as ringer's solution; this closely matches conditions in the fish plasma, which helps to preserve the muscle for a long time. Monochromatic light from a laser passes through the muscle, and the resulting diffracted beams are observed using an array of photodiodes. The central part of the array is usually masked to avoid overloading due to the high intensity of the central, undiffracted beam.

Figure 4: Typical optical diffraction pattern.



The array is scanned by a computer, to produce a chart similar to that shown in figure 4. The first order peaks can be easily seen. Statistical analysis can be performed, using the computer, to filter the noise and obtain two best-fit Gaussian curves to fit peaks. From this, the sarcomere length can be calculated. The tension in the muscle can also be measured, using a force transducer connected to the wire holding the muscle in place inside the muscle chamber. The muscle is stimulated using a number of short (2 mS) voltage pulses occurring every 20 mS, for a period of say 200 mS. During the resulting contraction, the sarcomere length and tension in the muscle change and are recorded by the computer for later analysis. Even when the muscle is fixed at both ends, the sarcomere length is still found to change by up to 8%. In addition, the intensity of one of the diffracted beams can be up to 7 times that of the other.

2.3 X-ray diffraction experiments.

Figure 3: Typical X-ray diffraction pattern.



In a similar way to that decribed above, X-rays may be used instead of visible light, to obtain higher resolution diffraction patterns, due to their shorter wavelength. The differences are that the machine is a lot larger: a typical distance of the screen or photographic plate to observe the pattern, from the muscle, is six metres. The images which are produced are highly complex and must undergo mathematical transform analysis using a computer, before any useful information can be extracted. A typical pattern is shown in figure 3. Again, the observations are performed during muscle stimulations, so that the changing muscle structure during contraction can be accurately observed. In this way Biophysicists hope to gain a greater understanding of the processes behind muscle contraction.

2.4 The need for a positive feedback circuit.

Biophysicists would like to be able to see changes in muscle structure during contractions at constant tension, or constant sarcomere length (Isometric contractions) Unfortunately merely clamping the muscle at its two ends is no guarantee that the intervening tissue will remain at a constant length: the length seems able to re-distribute itself so to speak, parts of it contracting and parts lengthening. In fact light diffraction studies have shown that the sarcomere length changes by 7-8% during some contractions (previous section). Therefore some means of keeping sarcomere length constant is required.

One way of doing this is to shine a laser beam through the muscle in addition to the X-rays; the passage of this beam will not affect the characteristics of the muscle, nor will it alter the X-ray diffraction pattern. The position of the two first order diffraction peaks could be measured in some way and used to provide a positive feedback signal, in such a way that a decrease in sarcomere length would cause a servo-motor to lengthen the muscle as a whole, while an increase would have the opposite effect. Thus while the whole muscle's length is not kept constant, the sarcomere length at the position of X-ray and optical measurement is. This was the subject of this project.

3 Design Solution.

Figure 6: Requirements of processing circuit:



tig 6c: Beams are closer together, indicating sarcomere length has increased; the circuit's output should become positive to indicate this to the servo-motor controller.

> SEPARATION LESS THAN IN A. SEPARATION REATER THAN

Lateral effect transistors (figure 8) are devices having a large photosensitive area (~20x20mm), which when illuminated by a strong light beam such as a laser, will give positional information (See later, section 4). In this application, only a horizontal position is required; taking the difference between the outputs from the two side connections of the transistor, (subtracting them) will give a voltage that is linearly dependent on the horizontal position of the beam on the detector surface. However, this alone would not be sufficient as the condition specified in figure 6b would not be met; ie when both diffracted beams shift by the same amount, the output would not be zero. Therefore two lateral effect transistors are necessary; the difference between their position voltages will not alter if the diffracted beams merely shift, but will change as required if the beams move closer together or further apart.

Figure 5 shows the experimental arrangement, and figure 7 shows a circuit block diagram that will achieve the required positive feedback. The output of this circuit can then be used to control a servo-motor, that can change the length of the muscle. In this way, any deviation in the sarcomere length during muscle stimulation will immediately be detected and corrected by the motor. Note also, with reference to the block diagram, figure 7, that some sort of Automatic Gain Control (AGC) circuit is needed, so that the changes in intensity between the diffracted beams (section 2.2 and figure 4) do not distort the output. This will be discussed in more detail in the next section. Not shown on the block diagram is the power supply: this must convert the 240 V AC mains voltage into positive and negative 15 volt supplies for the op-amps.

SAMO SEPARATION AS IN A.

tig 6d: Beams move aparts;

should be negative now.

sarc. length decreased; output



Figure 7 Circuit Block Diagram.



4 Detailed Circuit Description.

The individual parts of the block diagram of figure 7 will now be described in more detail, paying particular attention to the Automatic Gain Control (AGC) circuits, which are complex in comparison to the other circuit elements, and require sensitive adjustment.

4.1 Detectors



The two position sensing detectors are the type PIN SC/1OD lateral effect transistors. These transistors have a common connection and four side connection pins to the sides of the active area, as illustrated in figure 8. If a narrow beam illuminates the transistor, then the output from each connection is proportional to the distance of the light spot from that side of the detector area. Thus the difference between outputs from two opposite side connections is linearly dependent on the horizontal position of the light beam on the detector, ignoring changes in beam intensity.

Because the beam intensity is liable to change during the muscle contraction, this must be compensated for.

Movement of the beam in the vertical direction was found to alter the output at the horizontal connections. Therefore it is recommended that in use, the light beam is either focused onto the central horizontal area of the detector surface using a Fresnel lens, or the detector covered except for a narrow horizontal slit (See fig 9).



4.2 Pre-amplifiers

[Diagram 1 shows the main circuit diagram]



The circuit around IC1 is a simple operational amplifier circuit in negative feedback configuration. This amplifier biases the lateral effect transistor and increases its output to a level which is suitable for further processing. IC2-4 are similar circuits, to amplify the other inputs from the detectors.

In these high gain amplifiers it is important to use the offset-null facility of the type 741 op-amp, in order to get an accurate representation of the input signal, with no d.c offset. VR1-4 are multi-turn presets used for this purpose. To adjust the offset null of a particular op-amp, the input should be connected to ground through a low resistance such as 100 ohms, and the output of the op-amp (pin 6) monitored with an accurate

4.3 First difference amplifiers

The amplified signals from the two opposite sides of each detector are fed to the circuit around IC5 and R5, 6, 11 & 14. This op-amp is configured as a unity-gain difference amplifier, and subtracts the amplified signal from input 2 from that of input 1. A similar circuit, consisting of IC6 and R15, 16, 20, 24 takes the difference between inputs 3 and 4.

4.4 Automatic gain control (AGC)

As mentioned earlier the intensity of the two beams incident on the detector is likely to vary during muscle stimulation. Therefore it is necessary to design a circuit to compensate for this variation, on a time scale so fast that the final output will give a true indication of the beam separation, undistorted by intensity variations that can occur on a time scale of the order of 5mS.

Considerable detail will be presented here, as the design of this crucial part of the circuit occupied by far the most time during this project. Also, it is an interesting and innovative circuit, in comparison to the fairly standard op-amp configurations used elsewhere in the design.

At first sight this may seem a formidable task; however a reasonably simple solution was designed using a transconductance operational amplifier. The type LM13700 chip is a dual transconductance op-amp; the difference between these amplifiers and the usual, straight-forward op-amps is as follows. In a normal op-amp the amplifier gain is determined by the combination of resistors used in the negative feedback loop, whereas in a transconductance op-amp, the gain is governed by the current through an extra input connection (pins 1 and 16)

The original idea (illustrated in diagram 3) was to sum the signals from the two sides of the detector: this sum is independent of beam position and varies solely as intensity. This signal is passed through a transconductance op-amp, and the result compared with a preset value using an ordinary 741 op-amp configured as a comparator. The output of this comparator charges up a capacitor when the incoming sum signal falls below the preset value. The voltage across this capacitor, when fed back to the gain control input of the transconductance op-amp acts to increase the gain, thus compensating for the fall in intensity.

After a small interval (determined by the time constant of the R,C combination), the gain will have increased too much, and the amplified sum will now be above the preset value, causing the comparator to change state and start discharging the capacitor again. This reduces the gain, until such a level that the whole process starts again. Thus whatever the input sum signal, the gain of the amplifier always adjusts itself to bring its output up (or down) to the preset value. The same signal that alters the gain of this transconductance op-amp can also be used to control another, this one amplifying the difference signal. In such a way, the difference output is amplified if the intensity is low, attenuated if the intensity is high (relative to the preset intensity) This signal is now a 'true' difference, independent of the intensity of the incident beam.

Diagram 3: Original AGC Idea



In order to ensure that the gain fluctuations are small enough not to cause a significant oscillating signal to modulate the amplified difference, thus distorting it, the time constant of the R/C combination must be relatively large. Unfortunately, when the time constant is increased, this increases the response time of the circuit. Since the intensity can change on a time scale of only milliseconds, the response must be faster than this. It was found to be impossible to have a low enough time constant to allow fast response, and yet at the same to time have a large enough time constant to keep distortion of the amplified signal below a reasonable level.

The solution is in a deficiency of practical op-amps. The ideal, perfect op-amp would have the following characteristics:

It would:

i) Draw no input current,

ii) Have a closed loop gain dependent only on the feedback elements of its host circuit,

iii) Drive a low resistance load,

iv) Have constant gain with respect to frequency,

v) Be able to follow indefinitely sharp pulses independent of amplitude.

This last characteristic is particularly important here. A practical, real-world op-amp has a 'slew rate' usually measured in V/ μ S, which is the maximum rate at which its output voltage can change, due to its internal circuit. A typical value is 1V/ μ S, which is close enough to ideal in most situations, as to be neglected.

However, in this design, it was found that this deficiency of the practical op-amp can actually be used to advantage here. Removing the capacitance altogether and coupling the comparator output directly through a resistor to the gain control of the transconductance op-amp, the time constant is now so small (only determined by the op-amps internal capacitance) that the comparator op-amp cannot change its output fast enough to keep up with the changes at its inputs. Hence its comparator function is no longer a simple fully-positive-if-greater-than, fully-negative-if-less-than output, but rather a voltage somewhere in between, that depends on how much is the difference between its inputs. It should be noted that this is not just another form of difference amplifier: here there is no negative feedback etc, the op-amp is configured as a comparator, but due to the finite slew rate, its output cannot change fast enough. A normal difference amplifier cannot be used here.

This novel usage of a component's deficiencies, rather than the usual constant attempts to minimise them, solves both problems with the automatic gain control circuit: firstly, since there is no actual switching between fully positive and fully negative, the gain of the transconductance op-amp does not oscillate strongly as before; secondly, because there are no external time-constant components to slow down the reaction time, the reaction time is extremely fast, in fact limited only by the fourth op-amp deficiency listed above: the finite frequency

response of practical op-amps. In this application, the response time is far less than that required by the time scale of intensity variations. This was demonstrated during later testing.

With reference to the main circuit, diagram 1, the automatic gain control is implemented in the circuit around IC7a, and IC9, with the automatic gain control signal routed to IC7b which amplifies the primary difference signal. The output of this amplifier is the intensity independent difference between the inputs 1 & 2.

IC6, IC8a, and IC8b form a duplicate circuit providing AGC for the difference between inputs 3 & 4.

It should be noted that as the beam intensity changes, the AGC circuit will compensate for changes in the total light incident on the detector, hence any ambient light in the lab will, if unshielded, cause the device to operate inaccurately. Therefore the detectors must be well shielded from ambient lighting, or the experiment conducted in a darkened room. If this is likely to be a problem, op-amp subtracter circuits could be added directly before the inputs to the AGC circuits, i.e at pin 4 of IC7a and IC8a, to remove the ambient light contribution to the signal by subtracting a predetermined amount representing the light. However, if the ambient light is not constant, for instance if it is altered slightly by people moving around in the room, or if it is directional so that it has more effect at one side of the detector than the other, the output will still be affected. Hence it is recommended that the ambient light is prevented from reaching the detectors, either by shielding or by use in a darkened laboratory.

The AGC circuits are adjusted for the required range of intensity conditions using the presets VR5 & VR6. To make the adjustment, the detectors should be set up as in fig 10, using a laser and diffraction grating. A filter should be inserted in the beam path immediately after the laser, chosen so as to reduce the intensity of the beam to roughly the same as that expected from the muscle under investigation. The voltage at pin 5 of IC7 should be monitored with a high impedance (>20 M-ohm voltmeter or oscilloscope, and VR5 adjusted until this voltage remains constant whatever light level is incident on the detector. The light level can be varied by holding a polaroid filter in front of the detector: at a certain angle the filter will allow most of the light through, then as it is rotated the light will be reduced until after 90 degrees, it is stopped entirely. The circuit obviously cannot compensate indefinitely to cope with this situation, however in tests it was found that the light level can be reduced by a factor of at least 20 before the AGC circuit starts to fail to compensate. This is easily sufficient in this application. Once VR5 has been adjusted to give a constant voltage over a range of light intensities, the voltage at pin 5 of IC8 must be monitored in a similar way, and VR6 adjusted until this voltage equals that previously measured, at pin 5 of IC7. Now both AGC circuits will be correctly calibrated to compensate for changes in intensity over a wide range, and in addition any possible differences between the gain of the two lateral effect transistors is compensated for.

Note that this adjustment of the AGC circuits also has an effect on the magnitude of the final output of the device. In this application this should not cause any difficulties, however if problems do arise, another non-inverting op-amp amplifier could be connected, following the final difference amplifier.



4.5 Final difference amplifier

The relative difference between the beam position on the two detectors is found by IC11, configured as a difference amplifier. The values of resistors R28,40-42 are necessarily large, as the output of the LM13700 transconductance amplifier is unbuffered, and hence cannot source more than a tiny current without introducing severe distortion. The capacitor C1 is included to filter out any small high frequency oscillations that could be produced in the AGC circuits, and to eliminate high frequency noise.

4.6 Power supply



Diagram 2: Power Supply Circuit.

The circuit of the power supply is shown in diagram 2. This is a standard transformer, rectifier and regulator arrangement; the two secondary windings of the transformer are both utilised, to provide + and -15 volts supply to the op-amps. Rectification is accomplished by bridge rectifiers, and the output heavily smoothed (C4-7); this is followed by voltage regulators, type 7815 (IC12) for the positive supply, and type 7915 (IC13) for the negative supply. Further smoothing is effected by capacitors C2 and C3.

5 Testing of the Device.

The testing of this device was carried out in two phases: static testing, to monitor the output of the position sensing detectors in order to check that the output is linearly dependent on position; and dynamic testing, to make sure that the circuit can respond fast enough and accurately enough to the changes at its inputs. Recall that in this application, the muscle sarcomere length will be changing on a time scale of 10 mS or less. In addition, the AGC circuit's response was determined.

5.1 Static Testing: longitudinal.



A laser and diffraction grating were used to obtain two beams, which were directed onto the detectors, mounted perpendicularly to the optical axis as in figure 11. The detectors were placed on a piece of veroboard, and held in an adjustable clamp. The position of the detector board could be adjusted accurately using a knob on the clamp; 20 turns of the knob corresponded to about 40mm of linear movement. The voltage output of the device was monitored with a digital voltmeter, while the detector board was moved longitudinally along the optical axis, in half-turn steps (corresponding to approximately 1 mm movements) This caused the two diffracted beams to move apart on the detectors. In the real experiment, the varying separation of the beams would indicate the changing sarcomere length. Simulating the sarcomere movements in this way

is a good test of the linearity of the circuit and detectors. The voltage at each point was recorded, and the entire set of results repeated five times. Statistical calculations were performed on the five voltage readings at each point, to determine mean and standard deviation. The results are shown plotted against distance (turns of the clamp knob) in figure 13. The exact units of distance are unimportant here.

Notice that the graph tails off at either end: this is where the diffracted laser beams fall off the edge of the detectors.

The graph shows excellent linearity: it is almost an exact straight line. Note that in this testing, no special precautions were taken to shield the detector from ambient light, although fluorescent lighting in the laboratory was found to cause a large 100 Hz disturbance at the output, and so the measurements were conducted in daylight, with artificial lighting switched off. In a darkened laboratory, or with elaborate detector shielding, the already excellent output can only improve further.



5.2 Static testing: Transverse.



Recall that as discussed previously, the output must be unaffected by both diffracted beams shifting simultaneously, that is, the light spots on the detectors move relative to the detectors, but not relative to each other. This condition may be simulated by keeping the detector board at a fixed distance from the diffraction grating, but moving it transverse to the optical axis, see fig 11.

Once again five sets of results were recorded across the full area of the detectors, and subjected to statistical analysis to determine the standard deviation and mean. The

graph in fig 14 is a plot of the output voltage against transverse distance moved, again in arbitrary distance units, 1 turn corresponding to a movement of about 2 mm.

For relative comparison with the longitudinal graph (fig 13) this graph is plotted using the same vertical scale. The output is nearly zero, indicating again excellent linearity of the detectors, not only individually, but also comparative to each other. If the output of the detectors was



slightly different at a given beam position on their surface, this difference would be noticeable on this transverse graph. The graph in fig 15 shows the same results, but on a larger scale for closer inspection.

Notice that at the ends, the output suddenly increases: this is due to non-linearity at the very edges of the detectors, and is of no concern.

Even in these uncontrolled lighting conditions, the magnitude of the output for transverse movements across the whole central portion of the detector is less than 0.5% of the magnitude of the voltage change for the longitudinal direction. This is excellent; with improved light shielding etc, this could even improve further.

5.3 Dynamic Testing.

In order to test the reaction times of the circuit, it is necessary to cause the beam positions to vary quickly across the detector surface. It would be difficult to rapidly change the spacing or position of the diffraction grating, or to insert some optical shifting mechanism in the light path to move the diffracted beams; therefore I decided to attempt to rapidly move the detectors. The easiest way of doing this is to mount the detector board onto the cone of a loudspeaker, then connect this to a signal generator in order to vibrate the cone back and forth.

The apparatus was set up as in figure 12; the loudspeaker was mounted perpendicular to the optical axis, so that the vibrations of its cone would cause the detectors to be moved rapidly in the longitudinal direction. This simulates rapid movements of the muscle sarcomere. The detector was taped securely onto the cone of a 10 inch loudspeaker, connected to a signal generator at maximum output setting. The connections to the detectors were made with very thin enamelled wire, so that they would impede the movement as little as possible. The frequency was varied across a wide range, to test the response of the circuit.

Quantitative measurements of this kind of testing are difficult to make: there is no alternative way of measuring the displacement of the loudspeaker cone, hence nothing to compare the circuit's output against. However, good qualitative observations were made.

The movement of the loudspeaker cone was tiny: at the most, that is, at the loudspeaker's resonance frequency (~35-45 Hz), the movement of the cone was less than 2mm, and quickly became very small for frequencies over 100 Hz. Despite this the output from the circuit, viewed on an oscilloscope along with the signal generator output (sine wave), remained strong, easily distinguishable from background noise, for frequencies in excess of 2 KHz. This corresponds to a period of only 0.5 mS, far shorter than the response time required in order to be able to



react quickly to the changes in sarcomere length.

In order to further check the response of the circuit, one of the inputs was connected to the signal generator instead of the detector, and the other inputs grounded. The output of the device accurately resembled the input at frequencies well above 5 KHz; at frequencies higher than this the signal began to be attenuated. This is due to the finite bandwidth of the 741 and LM13700 op-amps.

5.4 AGC circuit response.

So that the reaction time of the AGC circuit could be checked, the sum input to one AGC circuit was temporarily disconnected and replaced by a connection to the signal generator, with its output set

for a fairly low amplitude. The preset was adjusted to give a constant voltage at the output (pin 5) of the LM13700a, as required. (Refer to main circuit, diagram 1). This indicated that the oscillations were being treated as intensity variations and compensated for; as further confirmation, the gain input (pin 1) and also the output of the second transconductance op-amp LM13700b showed sine wave oscillations 180 degrees out of phase to the simulated 'sum' input, as expected. The AGC circuits continued to compensate for the perceived variations in intensity, for frequencies exceeding 5 KHz; at these frequencies the gain of the op-amps has started to decrease anyway, due to their bandwidth (as found in section 4.3)

6 Conclusion.

The design and construction of this position sensing and positive feedback device should easily suffice for the purposes of isometric muscle experiments. The only adjustment which needs to be made to the circuit is that of the AGC thresholds, VR5 & 6. The output of the circuit is suitable for direct connection to servcontroller equipment, to alter the length of the muscle as required. Extensive testing (section 4) showed that the characteristics of the device, including linearity, immunity to intensity variations, and response times, were all excellent. In most cases the performance of the device exceeds what is required of it by an order of magnitude, such that it could also find many other applications. For example, it could be used to measure the sarcomere length, while muscle tension is kept constant.

Appendix: Connections and Layouts.

Fig 16: Front panel layout:





	OFFSET NULLS		AGC ADJUST		\bigcirc	
Ø	Ø	Ø	Ø	0	G	MAINS
VRI	NR2	VR3	VR4	VRS	VRG	IN.