INSTRUCTION MANUAL

Model
902A

Laser Diode Sarcomere Length

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Aurora Scientific Inc.
360 Industrial Parkway South, Unit 4
Aurora, Ontario, Canada L4G 3V7

Toll Free: 1-877-878-4784
Telephone: 1-905-727-5161
Facsimile: 1-905-713-6882
Email: info@AuroraScientific.com
Web site: www.AuroraScientific.com

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1.0 Introduction

The 902A Laser Diode Sarcomere Length system is intended for manual measurement and setting of sarcomere length before or during an experiment. The system includes: target holder, target, laser diode, XY stage mount for the laser diode, C-mount, laser diode on/off switch and laser diode power supply.

The system can be used with either the 801C Small Intact Muscle Test Apparatus or the 802D Permeabilized Fibre Test Apparatus available from Aurora Scientific Inc. However there are differences between the target holders used for each model.

In operation a laser beam is projected vertically upwards out of an unused objective port on an inverted microscope and through the tissue suspended in the apparatus tissue bath. The laser beam is diffracted by the sarcomeres in the tissue and this diffraction pattern is projected on the target. The laser beam separation is compared with lines printed on the target to determine and set the sarcomere length.

CAUTION

Laser radiation is present in and above the inverted microscope. Wear proper eye protection or ensure you are protected from direct exposure to the laser beam.

Do not look through microscope eyepieces when laser is on.

The model 902A includes a Class II laser. Class II lasers are low power (< 1mW), visible light lasers that could possibly cause damage to a person's eyes. If class II laser beams are directly viewed for long periods of time (i.e. > 15 minutes) damage to the eyes could result. Avoid looking into a Class II laser beam or pointing a Class II laser beam into another person's eyes. Avoid viewing Class II laser beams with telescopic devices such as the eyepieces of a microscope. Realize that the bright light of a Class II laser beam into your eyes will cause a normal reaction to look away or close your eyes. This response is expected to protect you from Class II laser damage to the eyes.

2.0 Specifications

Model: 902A Laser Diode Sarcomere Length

Laser Diode

Model:	Edmund Optics Model: NT59-080
Type:	CW Spot
Laser Class	II
Output Power:	1.00 mW
Wavelength:	635 nm
Focus Beam Diameter:	0.15 mm (at 300mm)
Collimated Beam Diameter:	3x2 mm (at 10m)
Operating Current:	60 mA maximum
Diameter:	12.0 mm
Length:	41.5 mm
Power Supply:	Input: 100-240VAC, 50/60Hz. Output: 5VDC @ 2.0A

SL Target

Base:	3” x 1” Microscope Slide
Target Overlay:	Translucent Film with pre-printed SL lines
Target SL:	Option 1: 2.0, 2.1, 2.2, 2.3, 2.54, 10.0 µm Option 2: 1.8, 2.0, 2.2, 2.4, 2.6, 10.0 µm Contact ASI for other SL lines in the range 1.8 to 10 µm

3.0 Setup and Use

3.1 Instrument Setup

The model 902A consists of two main subassemblies: laser diode and its mount and the target holder with the target. The laser diode and its mount are pre-assembled at the factory and all you need to do is attach the assembly to a microscope C mount port adapter. (This adapter is not included with the 902A, most microscopes include a C mount adapter but if yours doesn't then the adapter can be purchased from your microscope manufacturer. If you need to purchase an adapter then ensure that you order a 1x C mount port adapter, don't order an adapter with a built in lens.) The port adapter should have fine external threads on it (1" diameter by 25 threads per inch). The laser diode mount assembly has mating internal threads; carefully screw the laser diode mount assembly onto the port adapter. Note: it may be easier to remove the port adapter from the microscope, attach it to the laser diode mount and then insert the port adapter into the microscope port.

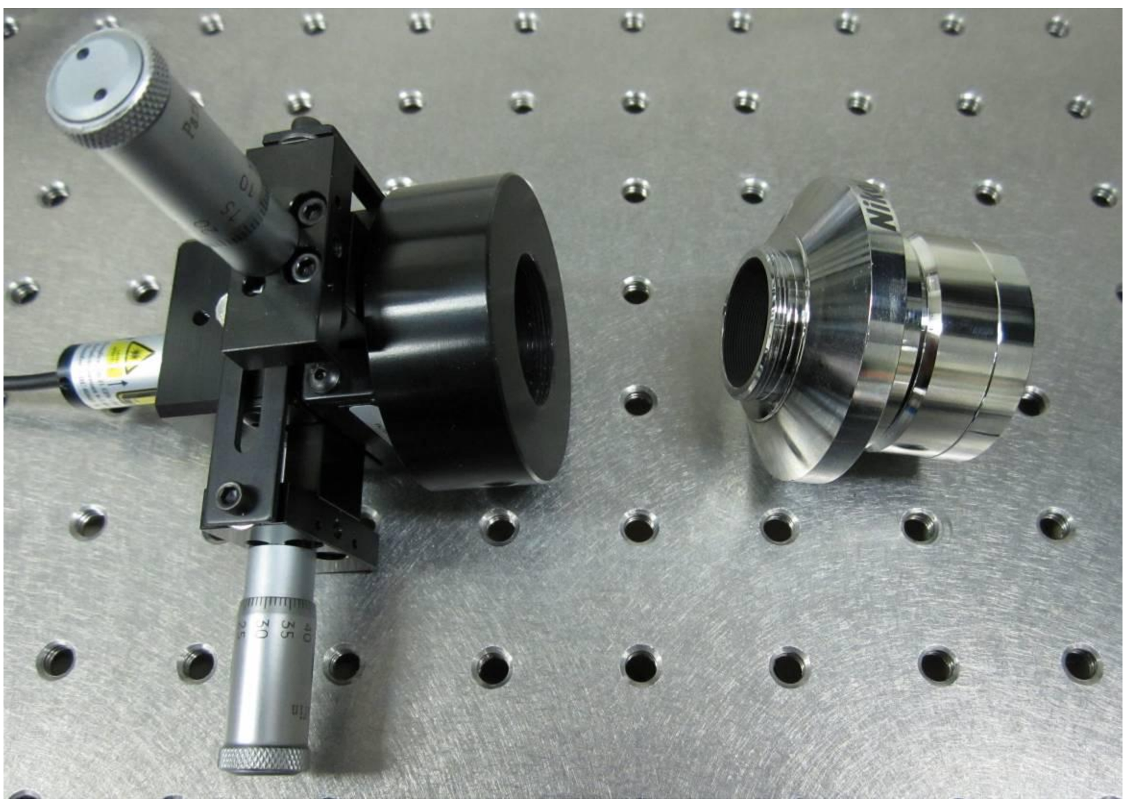

Figure 1 902A Laser Diode Assembly and C Mount Adapter (not included)

For safety reasons it is best to choose a microscope port that shuts off the eyepieces when the laser is in use. If this type of port is available on your microscope then please attach the port adapter to it. When attaching the laser diode mount to the port orient the XY stages so that one stage is horizontal and other is vertical.

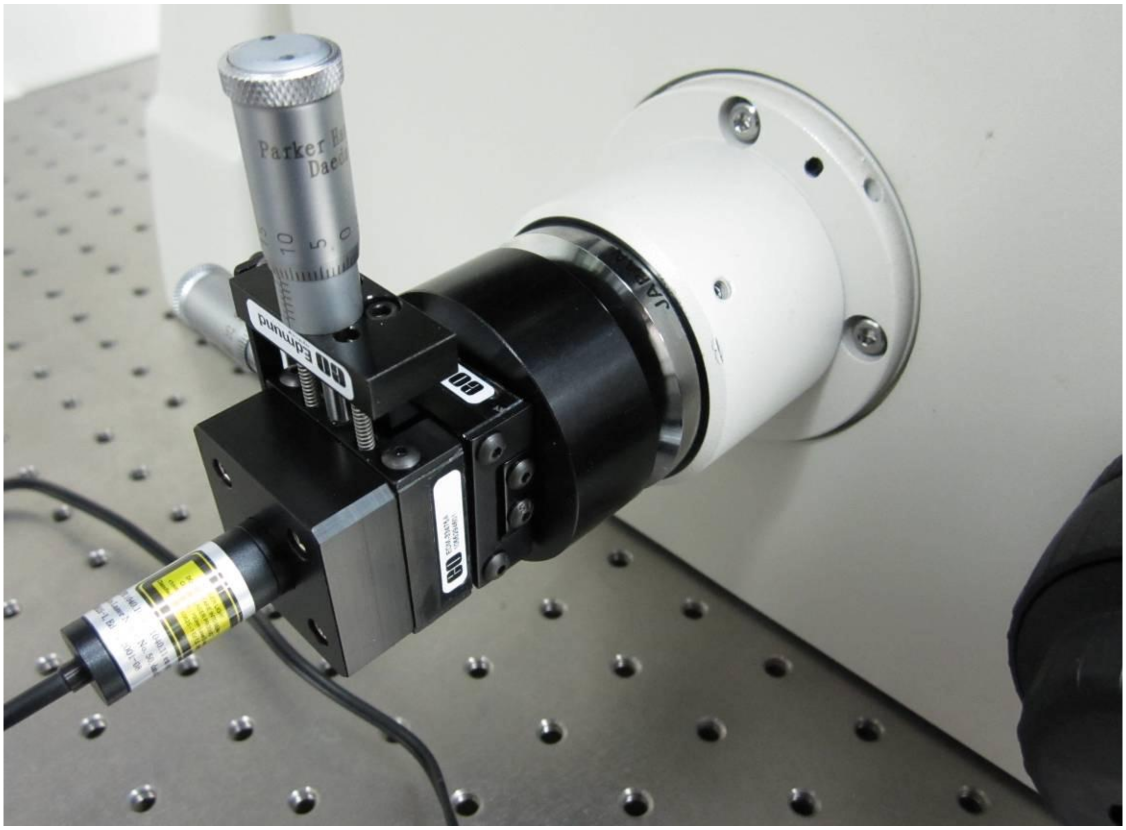

Figure 2 Laser Diode Assembly Mounted to Microscope

There are two variants of the target holder dependent on whether the target holder is to be used on an 801C or an 802D. See figures 3 and 4 for pictures of what the target holder looks like when attached to an 801C and an 802D. You may need to tilt the microscope's condenser mount backwards to obtain sufficient room above the apparatus for the 902A target holder. The centre line of the target should line up with the centre of the tissue bath and the long axis of the target should be in the same direction as the long axes of the muscle tissue.

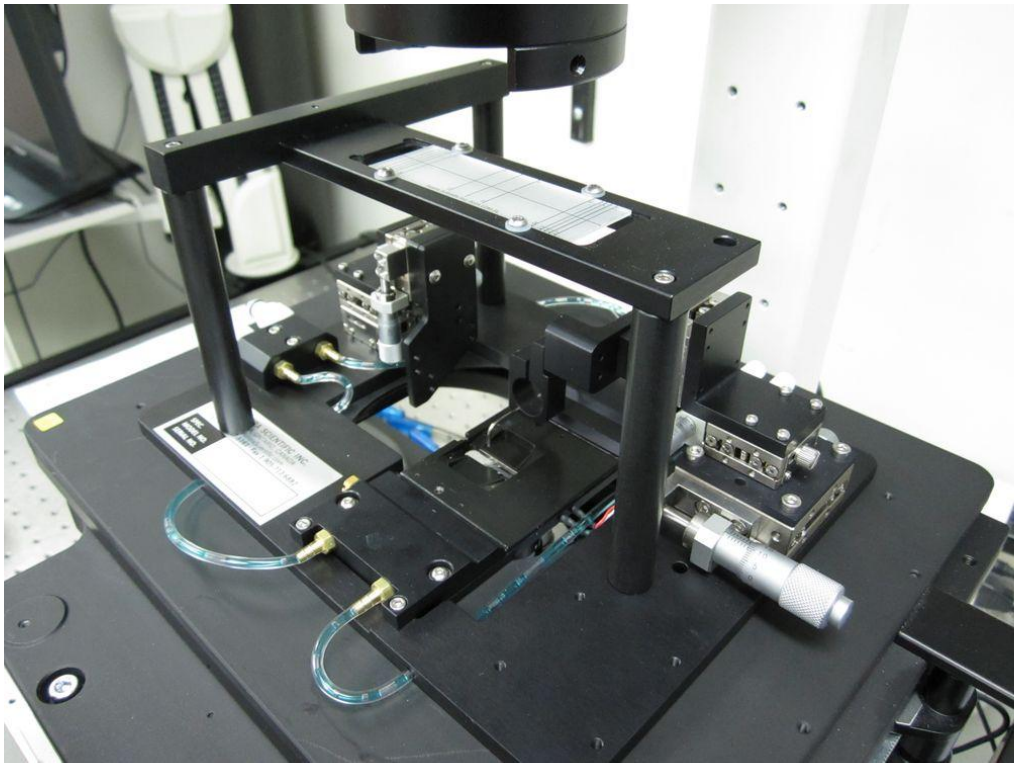

Figure 3 902A mounted on 801C Apparatus

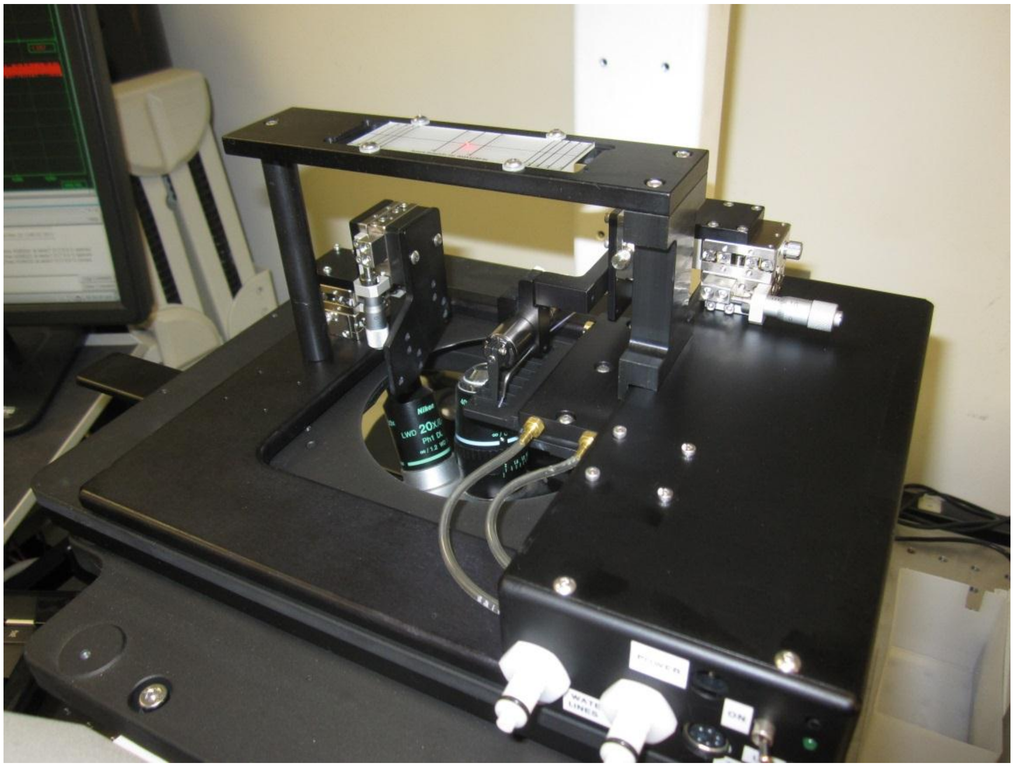

Figure 4 902A mounted on 802D Apparatus

3.2 Using the 902A

Check for Correct Laser Operation

Rotate the microscope turret to an unused location and remove any dust covers that may be at that location. Plug the 5 volt cord from the laser power supply/switch box into the connector attached to the laser diode. Ensure the switch is set to the off position and then plug the laser power supply into an appropriate AC outlet. Ensure that you are not looking into the unused objective port and switch on the laser. Look for the laser beam exiting the objective port; use a piece of paper to image the beam. If the laser beam is not present then check the power connections and the port settings on the microscope. If necessary remove the port adapter from the microscope and check for the beam exiting the port adapter, you may need to adjust the XY stages to ensure the beam exits through the holes in the stages. Once the beam can be seen exiting the port adapter then re-attach the adapter to the microscope and check for the beam exiting the objective port.

Figure 5 Laser Exiting an Unused Objective Port

Mount the Apparatus

Place the apparatus on the microscope stage and use the controls built into the microscope stage to move the apparatus and line up the tissue with the laser beam. Fine adjust the position of the 801C or 802D apparatus such that the beam enters the tissue bath and shines onto the tissue. Note: If you turn off the laser, rotate the turret to an objective and then adjust the apparatus so that you can see the tissue in the eyepiece then when you rotate the turret to the laser position and turn the laser back on the beam should shine on the tissue.

Never look through the eyepiece when the laser is on.

Fine Adjust the Laser Position

Use the X and Y stages attached to the laser diode for even finer control of the position of the laser beam with respect to the tissue. Due to the size of the holes drilled in the XY stages there is limited XY travel before the laser beam will be obstructed by the stage, this movement is less than the movement provided with the stage micrometer controls.

Attach the Target Holder and the Target

Place the target holder on the apparatus as shown in figures 3 and 4. You will notice that the target can slide in a direction parallel to the long axis of the tissue. Align the target holder and the target so that the un-diffracted laser beam strikes the centre of the target. If muscle tissue is present in the bath and if the laser is aligned correctly with the tissue you should see a first order diffracted beam on either side of the zero order beam. Figure 6 shows the diffraction pattern resulting from a 10 µm calibration slide. In this photo you can clearly see the very bright 0 order beam located in the centre of the target. The photo also shows 1st, 2nd, 3rd, 4th and 5th order beams on either side of the 0 order beam. You can also observe that the 1st order beams do line up with the 10 µm lines on the target. When measuring muscle it is unlikely that you will be able to see the beams above the 1st order.

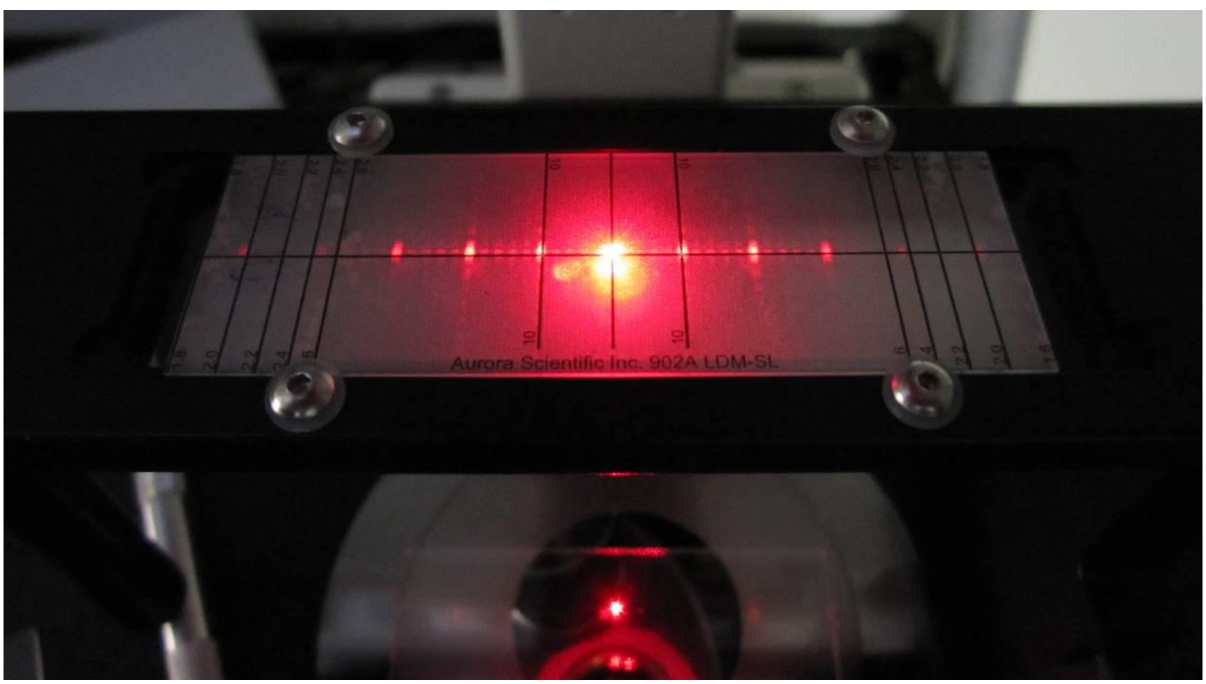

Figure 6 Diffraction Pattern resulting from 10µm Calibration Slide

Measuring Sarcomere Length

Align the zero order laser beam with the centre of the target (see figures 4 and 6 for a photo showing the zero order beam aligned correctly on the target). Observe the location of the first order beams with respect to the printed lines on the target. Depending on the option you purchased you will have lines labelled 10.0, 2.54, 2.3, 2.2, 2.1, 2.0 µm or 10.0, 2.6, 2.4, 2.2, 2.0, 1.8 µm. Compare the position of the first order diffracted beam with these lines to determine the current SL.

If a specific SL is required then adjust the X stage of either the force transducer or the motor to stretch or relax the tissue until the first order beam is aligned with the desired target line. Check that the zero order beam is still centred on the target and readjust the target if necessary. Once the desired SL is set turn off the laser, remove the target holder from the apparatus and return the condenser to its operating position. Proceed with your experiment. If at any point you need to check the SL simply place the target holder back on the apparatus, choose the empty turret position, select the laser port, turn on the laser, line up the zero order beam with the center of the target and read off the current SL from the location of the first order beams.

The liquid surface at the top of the bath can adversely affect the diffraction pattern. In some cases you may need to place a small piece of glass cover slip over the top of the muscle bath to eliminate any lens effect caused by the liquid surface.

3.3 Diffraction Calculations

The design of the 902A is based on the knowledge that sarcomeres in muscle tissue can act as a diffraction grating. When a plane wave of wavelength λ is incident normally on the tissue, each sarcomere in the tissue will act as a point source propagating in all directions. The light in a particular direction, θ, is made up of the interfering components from each sarcomere. Generally, the phases of the waves from different sarcomeres will vary from one another, and will cancel one another out partially or wholly. However, when the path difference between the light from adjacent sarcomeres is equal to the wavelength, λ, the waves will all be in phase. This occurs at angles θ_m which satisfy the relationship

$$d \sin\theta_m / \lambda = |m|$$

where d is the separation of the sarcomeres and m is an integer. Thus, the diffracted light will have maxima at angles θ_m given by

$$d \sin\theta_m = m \lambda$$

where d is the sarcomere spacing, m is the order (0, 1, 2, …), and λ is the wavelength of the laser (633 nm).

The light that corresponds to direct transmission is called the zero order and it is denoted $m = 0$. The other maxima occur at angles which are represented by non-zero integers of m. Of all the non-zero m values we are only interested in the first order light, $m = 1$.

For any given SL, the separation (s) between the zero order and the first order beam can be determined for a given distance between the tissue and the target. To do this the above equation is used to calculate the angle θ_m for a given SL (d) and then the distance between the tissue and the target is used to determine the separation at the target. For the 902A the distance between the target and the tissue is 100mm. Therefore the line separation (s) on the target, between the zero order and first order beams, can be determined as

$$s = 100 \tan\theta_m$$

The above calculations are valid regardless of other mediums between the tissue and the target (glass, water, air as is normally in the path when a fibre is immersed in a bath).

In many cases the presence of a free water surface will cause a lot of scatter and movement of the zero order and 1st order beams. To obtain better results it is suggested that a small piece of cover slip be placed over the top of the bath to present a fixed surface for the light to exit through.

Tables 1 and 2 present SL, θ_m and spot Separation (s) for the two target patterns that are available with the 902A. Note: the 10µm spacing is included so that you can check the calibration of the system using a standard 10µm Stage Micrometer (available from Aurora Scientific Inc.).

Table 1 Spot Separation vs SL – Option 1 Pattern

SL [um]	θ_m [rad]	Spot Separation (s) [mm]
2	0.323	33.48
2.1	0.307	31.72
2.2	0.293	30.15
2.3	0.280	28.73
2.54	0.253	25.82
10.0	0.064	6.36

Table 2 Spot Separation vs SL – Option 2 Pattern

SL [um]	θ_m [rad]	Spot Separation (s) [mm]
1.8	0.361	37.70
2	0.323	33.48
2.2	0.293	30.15
2.4	0.268	27.44
2.6	0.247	25.19
10.0	0.064	6.36

4.0 Warranty

The 902A Laser Diode Sarcomere Length system is warranted to be free of defects in materials and workmanship for three years from the date of shipment. Aurora Scientific Inc. will repair or replace, at our option, any part of the 902A system that upon our examination is found to be defective while under warranty. Obligations under this warranty are limited to repair or replacement of the instrument. Aurora Scientific Inc. shall not be liable for any other damages of any kind, including consequential damages, personal injury, or the like. Damage to the system through misuse will void this warranty. Aurora Scientific Inc. pursues a policy of continual product development and improvement therefore we reserve the right to change published specifications without prior notice.

4.1 Contacting Aurora Scientific Inc.

Technical assistance is available by regular mail, email, phone, or fax. Use the information below to contact Aurora Scientific Inc.

Address:	Aurora Scientific Inc. 902A Technical Assistance P.O. Box 2724 Richmond Hill, Ontario, CANADA L4E 1A7
Phone:	1 905 727-5161
Toll Free:	1 877 878-4784 (North America)
FAX:	1 905 713-6882
E-mail:	info@AuroraScientific.com
Web site:	www.AuroraScientific.com

5. 0 Terms and Conditions for Returning Equipment

1. Aurora Scientific Inc. **will not** accept any equipment returned without prior authorization in the form of a return material authorization (RMA) number.

2. **Please call Customer Service at (905) 727-5161 or toll free at 1-877-878-4784 to obtain an RMA number. Please be prepared to supply the model and serial numbers, these can be found on the ASI tag affixed to the product.**

3. Please package equipment properly. Goods that are damaged in shipment are the responsibility of the shipper.

4. **Aurora Scientific, Inc. withholds the right to assess charges for the repair or replacement of such damaged goods, regardless of warranty status.**

5. Warranty repairs will be shipped back to the customer via FedEx. If you require or request another form of shipment, the cost of such service is your full responsibility.

6. Aurora Scientific, Inc. **will not** be responsible for any return or replacement **shipping charges** incurred due to an incorrect order placed by the customer.

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Aurora Scientific Inc.
360 Industrial Pkwy. S., Unit 4
Aurora, ON, Canada
L4G 3V7
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