



# User Manual



Saturn™ Laser System



RI IMSI™



RI Viewer™

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## PREFACE

This manual is the sole property of Research Instruments, and along with the Research Instruments products to which it applies, is provided for the exclusive use of Research Instruments clients.

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Saturn™, RI Viewer™ and RI IMSI™ are trademarks of Research instruments Ltd. All other brand names are trademarks of their respective owners.

The information in this manual is current at the time of publication. Our commitment to product improvement requires that we reserve the right to change equipment, procedures and specifications at any time.

**Note** Assisted Hatching is not recommended for routine use in all ART patients.



The system should be operated by qualified and trained personnel only.

## Section 2

### Introduction to Saturn™ Laser Systems

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## INTRODUCTION TO SATURN™ LASER SYSTEMS

### Intended Use

The Saturn™ Laser System is intended 'to ablate a User defined section of a gamete or embryo within ART'. Applicable indications for use are subject to the regulations of the country into which the device is sold. Availability of a Saturn™ Laser System for clinical use is dependent on the regulatory approval status of the Saturn™ Laser System within the country the device is intended to be sold into.

**CE**  
0120

**USA ONLY** Ref: FDA 510(k) K060764 and K083208.

The Saturn™ Laser System is to be used to drill a small tangential hole in, or to thin, the zona pellucida of the embryo in selected in vitro fertilization (IVF) patients with otherwise poor prognosis for successful pregnancy outcome such as:

Advanced maternal age

Prior failed IVF

Cryopreserved embryos

Abnormal zona pellucida morphology

**R<sub>X</sub>Only**

**Caution:** Federal law restricts this device to sale by or on the order of a physician or a practitioner trained and certified in its use

### Applicable Part Numbers

6-47-500 - Saturn 5 Active™ Laser

6-47-501 - Saturn 5™ Fixed Laser

6-47-510 - Saturn 5 Active™ Laser competitor replacement

6-47-511 - Saturn 5™ Fixed Laser competitor replacement

6-47-512 - Upgrade from Saturn™ to Saturn 5 Active™

6-47-513 - Upgrade from Saturn™ to Saturn 5™ Fixed

6-47-514 - Upgrade from Saturn 3 Active™ to Saturn 5 Active™

6-47-515 - Upgrade from Saturn 5™ Fixed to Saturn 5 Active™

### Microscope Compatibility

Zeiss Axiovert 40/100/200/Observer

Nikon TMD, D200/300, TE200/300, TE2000, Ti

Leica DMIRB, DMI3000B/4000B/6000B, DMIL

Olympus IMT2, IX50/70, IX51/71/81, IX53/73/83

## **Installation**

Installation of the Saturn™ Laser System should be carried out by a Research Instruments technician or other RI-authorized personnel. Incorrect installation could result in reduced power output, laser misalignment or laser malfunction.

All relevant sections of this manual should be read and understood fully before any operation of the Saturn™ Laser System takes place. If the operator is unsure of any of the information contained in this manual, they should contact Research Instruments or an appointed representative before attempting to use this equipment.

A Patients' Guide to Laser Assisted Hatching (LAH) is provided on the RI Viewer™ installation CD (LAH Patients Guide.pdf). This can be opened using Adobe Reader or other PDF viewers. We recommend that copies are printed and made available to patients.

For outside of the USA an installation manual will be supplied (6-47-500IM)

No user serviceable parts.

## Section 3

### Saturn™ Laser Systems Safety Precautions

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#### SATURN™ LASER SYSTEMS SAFETY PRECAUTIONS

##### Safety



This indicates cautionary text which should be followed to avoid injury to users or damage to samples

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Laser radiation is present within the control unit, the fibre optic patch lead and through the microscope to output at the objective. The Saturn™ laser system has been designed to ensure that emission of laser radiation at any point is within the limits stipulated by international safety standards, IEC60825-1:2008, and US 21CFR1040.10 for unprotected viewing (Class 1). As an extra precaution, an infrared blocking filter has been incorporated into the optical path leading to the eyepieces to reduce laser emission by a further 99%.



**CAUTION** hazardous radiation exposure may occur through the use of controls, executing adjustments or the performance of procedures other than those specified herein. Servicing and adjustment other than that specified in this manual should only be carried out by RI or RI authorised agents.



**DO NOT** disassemble or modify any part of the Saturn™ Laser system, or substitute any component for any other. Doing so may result in exposure to dangerous levels of laser radiation. This voids the warranty and/or service contract.



**DO NOT** use a magnifying glass or eye loupe above the objective lens.



**DO NOT** operate the laser unless it is properly mounted to a microscope.



**ONLY** use the power cable and power supply adaptor supplied with the system.

The cable to the power supply is the Disconnect Device for this equipment. To remove all electrical power from this product, disconnect the power cable from the electrical outlet. Equipment should be positioned so as to allow easy access to the power cable. The appliance coupler or mains plug is used as the disconnect and must remain readily operable.



**WARNING** To avoid the risk of electric shock, this equipment must only be connected to a supply mains with protective earth.



**WARNING** Not to be used in a patient environment



**WARNING** Before performing any procedure, the pilot laser must be used to check the alignment of the Active positioning system. The system must be re-aligned when necessary. See Section 6 - How to Check Laser Target Alignment.



**WARNING** Thermal lensing is a defocusing of the laser beam caused by changes to the refractive index of the medium as it is heated. The degree of defocus depends on the total beam energy deposited in the medium. While slight defocus has no effect on the drilling properties, this effect may become a problem for higher beam energies. To eliminate any significant effects from thermal lensing, we recommend that the pulse length be kept below 1000µs for clinical Laser Assisted Hatching (LAH) zona drilling.



**WARNING** Only a single opening should be made in the zona pellucida. Multiple openings or those that are too small may prevent embryo hatching or lead to abnormal development.



**WARNING** Do not operate the pilot laser with an embryo in the field of view. Exposure to the pilot laser may damage the embryo.



**WARNING** The microscope itself must be maintained to a high standard. Problems such as worn focus mechanisms or an insecure video camera may lead to unreliable focus and image stability, and could lead to embryo damage.



**WARNING** Do not operate the laser without the RI Viewer™ software running, as targeting and hole size indication will not be active, and the embryo may be damaged.



**WARNING** The embryo may be damaged if objectives other than the red Saturn™ laser objective is used.



**WARNING** Do not modify this equipment without authorization of the manufacturer.



**WARNING** Where an ITO glass heated stage is fitted, do not fire the laser with the microscope focussed into the ITO glass surface as this may damage the ITO coating.

### Precautions

- To minimise the risk of damage to blastomeres, administer as few laser pulses as possible at the lowest energy levels possible to achieve the prescribed zona drilling or thinning effect.
- Direct the laser beam toward a section of the zona pellucida where the adjacent perivitelline space is widest or next to an area of fragmentation.
- A holding pipette should be used during laser treatment to minimize the risk of embryo movement.
- Small openings in the zona pellucida may lead to embryo constriction and abnormal development.
- To date there are no known reports showing a greater occurrence rate of major or minor defects in children derived from laser-hatched embryos. Long-term follow-up data on children born from laser-hatched embryos does not yet exist. A study of 134 such babies\* found no increase in the major congenital malformations, chromosomal aberrations or minor congenital malformations between the LAH treated group and all deliveries at their hospital.
- The device is not affected by and does not present any reciprocal interference to the microscope.
- During normal use there is no contact with patient/sample. Standard lab cleaning procedures can be applied.

\*Kanyo, K., Konc, J. "A follow-up study of children born after diode laser assisted hatching." *European Journal of Obstetrics and Gynaecology*. 110: 176-180 (2003).

# Section 3

## Saturn™ Laser Systems Safety Precautions

### Guidance and Manufacturer’s Declaration – Electromagnetic Emissions (IEC 6060 1-1-2)

The Saturn™ Laser System is intended for use in the electromagnetic environment specified below.

The customer or the user of the Saturn™ Laser System should assure that it is used in such an environment.

Emissions test	Compliance	Electromagnetic environment - guidance
RF emissions CISPR 11	Group 1	The Saturn™ Laser System uses RF energy only for its internal function. Therefore, its RF emissions are very low and are not likely to cause any interference in nearby electronic equipment.
RF emissions CISPR 11	Class B	The Saturn™ Laser System is suitable for use in all establishments, including domestic establishments and those directly connected to the public low-voltage power supply network that supplies buildings used for domestic purposes.
Harmonic emissions IEC 61000-3-2	Class A	
Voltage fluctuations/flicker emissions	Complies	

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### Guidance and Manufacturer's Declaration — Electromagnetic Immunity

The Saturn™ Laser System is intended for use in the electromagnetic environment specified below. The customer or the user of the Saturn™ Laser System should ensure that it is used in such an environment.

IMMUNITY test	IEC 60601 Test level	Compliance level	Electro magnetic environment - guidance
Electrostatic discharge (ESD) IEC 61000-4-2	± 6 kV contact ± 8 kV air	± 6 kV contact ± 8 kV air	Floors should be wood, concrete or ceramic tile. If floors are covered with synthetic material, the relative humidity should be at least 30 %.
Electrical fast transient/burst IEC 61000-4-4	± 2 kV for power supply lines ± 1 kV for input/ output lines	± 2 kV for power supply lines ± 1 kV for input/ output Lines	Mains power quality should be that of a typical commercial or hospital environment.
Surge IEC 61000-4-5	± 1 kV line(s) to line(s) ± 2 kV line(s) to earth	± 1 kV differential Mode ± 2 kV common mode	Mains power quality should be that of a typical commercial or hospital environment.
Voltage dips, short interruptions and voltage variations on power supply input lines IEC 61000-4-11	<5 % $U_T$ (>95 % dip in $U_T$ ) for 0.5 cycle 40 % $U_T$ (60 % dip in $U_T$ ) for 5 cycles 70 % $U_T$ (30 % dip in $U_T$ ) for 25 cycles <5 % $U_T$ (>95 % dip in $U_T$ ) for 5s	<5 % $U_T$ (>95 % dip in $U_T$ ) for 0.5 cycle 40 % $U_T$ (60 % dip in $U_T$ ) for 5 cycles 70 % $U_T$ (30 % dip in $U_T$ ) for 25 cycles <5 % $U_T$ (>95 % dip in $U_T$ ) for 5s	Mains power quality should be that of a typical commercial or hospital environment. If the user of the Model 004 image intensifier requires continued operation during power mains interruptions, it is recommended that the Model 004 Image Intensifier be powered from an uninterruptible power supply or a battery.
Power frequency (50/60 Hz) magnetic field  IEC 61000-4-8	3 A/m	0.3 A/m	If image distortion occurs, it may be necessary to position the Model 004 image intensifier further from sources of power frequency magnetic fields or to install magnetic shielding. The power frequency magnetic field should be measured in the intended installation location to assure that it is sufficiently low.

**Note:**  $U_T$  is the a.c. mains voltage prior to application of the test level.

# Section 3

## Saturn™ Laser Systems Safety Precautions

### Guidance and Manufacturer’s Declaration — Electromagnetic Immunity

The Saturn™ Laser System is intended for use in the electromagnetic environment specified below. The customer or the user of the Saturn™ Laser System should assure that it is used in such an environment.

Conducted RF	3 Vrms	3 Vrms	Portable and mobile RF communications equipment should be used no closer to any part of the Model 006, including cables, than the recommended separation distance calculated from the equation applicable to the frequency of the transmitter.
IEC 61000-4-6	150 kHz to 80 MHz		
Radiated RF	3 V/m	3 V/m	<p><b>Recommended separation distance</b></p> $d = 1,2\sqrt{P}$ $d = 1,2\sqrt{P}$ 80 MHz to 800 MHz $d = 2,3\sqrt{P}$ 800 MHz to 2,5 GHz where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer and d is the recommended separation distance in metres (m). <p>Field strengths from fixed RF transmitters, as determined by an electromagnetic site survey, <sup>a</sup> should be less than the compliance level in each frequency range. <sup>b</sup></p> <p>Interference may occur in the vicinity of equipment marked with the following symbol:</p> 
IEC 61000-4-3	80 MHz to 2.5 GHz		

**Note 1:** At 80 MHz and 800 MHz, the higher frequency range applies.

**Note 2:** These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.

<sup>a</sup> Field strengths from fixed transmitters, such as base stations for radio (cellular/cordless) telephone and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast cannot be predicted theoretically with accuracy. To assess the electromagnetic environment due to fixed RF transmitters, an electromagnetic site survey should be considered. If the measured field strength in the location in which the Model 006 is used exceeds the applicable RF compliance level above, the Model 006 should be observed to verify normal operation. If abnormal performance is observed, additional measures may be necessary, such as re-orienting or relocating the Model 006.

<sup>b</sup> Over the frequency range 150 kHz to 80 MHz, field strengths should be less than 3 V/m.

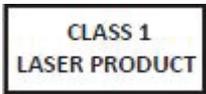
**User Interface Icons**

Symbol	Meaning	Symbol	Meaning
	Take a picture		Laser control
	Take a video		General settings
	Stop video recording		Additional camera settings
	View images		Take measurements
	Choose objective magnification		Display rulers tool
	Increase/Decrease zoom level		Instructions For Use

## Section 3

### Saturn™ Laser Systems Safety Precautions

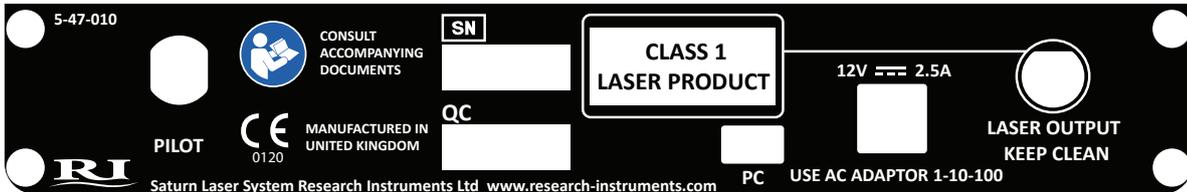
#### Symbols

Symbol	Meaning
	Caution: Federal law restricts this device to sale by or on the order of a (licensed healthcare practitioner).
	Indicates disposal of goods instruction
	Class 1 laser product
	In accordance with European Medical Device Directive 93/42/EEC
	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that cannot, for a variety of reasons, be presented on the medical device itself.
	Important safety instructions
	The first four digits are a unique identifier assigned to the control unit and the last 2 digits signify the year of manufacture, e.g. 5001/13 (this denotes a unique serial number of 5001 and a year of manufacture 2013).

## SATURN™ LASER SYSTEMS PRODUCT OVERVIEW

### Hardware Overview

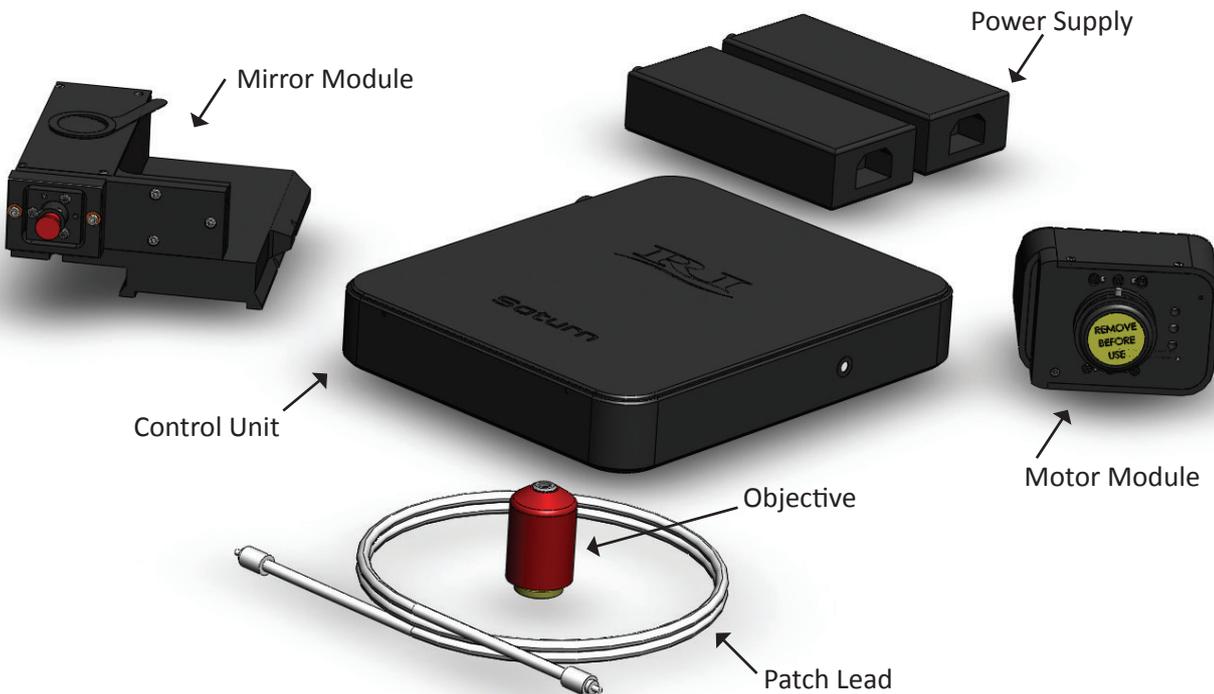
#### Rear Panel



**Pilot laser brightness:** The brightness of the pilot laser can be adjusted by turning the control to obtain the clearest image in the target alignment procedure.

The light on the front of the control unit indicates that the control unit is operational. If the unit is plugged in and the light is not on, contact Research Instruments for assistance.

### System Components



**Note** Actual components may differ from those shown.

The Saturn™ Laser System comes supplied with the following parts a control unit containing the lasers, mirror module, armoured patch lead and a special objective. If you have a Saturn Active™ system then an additional motor module will be supplied. The laser beams are delivered to the specimen through a fibre optic patch lead, collimator module (that is part of the mirror module for a fixed system or the motor module for an active system) and the special objective.

A foot pedal for firing the laser is available as an optional extra, this is attached to the PC by a mini USB cable.

The motor module receives coordinates from USB which moves the motors to those coordinates, the control unit then receives commands from USB to fire for a specifies length of time.

## Section 4

### Saturn™ Laser Systems Product Overview

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#### Packaging and Handling Requirements

No special packaging or handling requirements

#### Saturn™ Laser System Component Part Numbers

Part Number	Description
5-47-010	Control Unit with 1.5m Patch Lead
5-47-100	Motor Module (Saturn™ Active)
1-10-100	12V 2.5A Medical PSU (x2 for Saturn Active™)
2-45-801	Laser Objective (red)

#### Saturn™ Laser System Mirror Module Part Number and Descriptions

##### Saturn Active™ Mirror Modules

Part Number	Description
5-46-163	Mirror module for IX53/73/83 left
5-46-150	Mirror module for IX70 left
5-46-160	Mirror module for IX70 right
5-46-151	Mirror module for IX71 left
5-46-152	Mirror module for IX71 right
5-46-159	Mirror module for IMT2
5-46-135	Mirror module for TMD
5-46-132	Mirror module for D300
5-46-130	Mirror module for TE300
5-46-140	Mirror module for TE2000 with Integra Ti™
5-46-141	Mirror module for TE2000 without Integra Ti™
5-46-142	Mirror module for Nikon Ti with Integra Ti™
5-46-144	Mirror module for Nikon Ti with X-Y
5-46-158	Mirror module for Ax40
5-46-110	Mirror module for Ax200/Observer
5-46-120	Mirror module for DMIRB
5-46-122	Mirror module for DMI3000B
5-46-121	Mirror module for DMI4000B/6000B

**Saturn™ Mirror Modules**

5-45-163	Collimator module for IX53/73/83
5-45-383	Collimator module for IX70
5-45-387	Collimator module for IX71
5-45-388	Collimator module for IX71 fluor
5-45-580	Collimator module for IMT2
5-45-411	Collimator module for TMD
5-45-301	Collimator module for D300/TE300
5-45-545	Collimator module for TE2000/Nikon Ti
5-45-546	Collimator module for TE2000/Nikon Ti fluor
5-45-575	Collimator module for Ax40
5-45-009	Collimator module for Ax100
5-45-011	Collimator module for Ax200/Observer
5-45-450	Collimator module for DMIRB
5-45-470	Collimator module for DMIL
5-45-461	Collimator module for DMI3000B
5-45-460	Collimator module for DMI4000B/6000B

## Section 4

### Saturn™ Laser Systems Product Overview

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#### Laser Specifications

Laser Specifications	Ablation Laser	Pilot Laser
Output wavelength	1480nm	650nm
Power output from patch lead	400mW	130µW (max)
Maximum pulse length	2.0ms	None
*Laser Safety Classification	Class 1	Class 1

\* IEC60825-1: 2008, US 21CFR 1040.10

#### Objective Specifications

Magnification	40x
N.A.	0.49
Working distance	2.5mm
Parfocal distance	45mm (adaptor supplied for Nikon CFI60 systems)

#### Electrical Specifications

##### Mains Adaptor for Control Unit and Motor Module

Input	100-240VAC, 50-60Hz, 0.8-0.4A
Output Power (maximum)	30W
Voltage	12VDC
Current (maximum)	2.50A
Earth Connection	Mandatory 3-pin plug for earthing (grounding)

#### Dimensions

Control unit	220mmx180mmx34mm (8.6"x7.1"x1.3")
Weight	1.2Kg

#### Operating Range

Temperature	10°C (50°F) to 42°C (108°F)
Humidity	15% to 85% RH (Non Condensing)

##### USA Only

Compliance with the emissions requirements of CISPR 22 Class A requires the following warning: "This is a class A product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate measures."

## RI VIEWER™

### Introduction

RI Viewer™ is the software that interacts with the Saturn™ and RI IMSI™ range of products from Research Instruments for use within an ART laboratory. Connected to a camera attached to a microscope, it provides on screen images of samples under the microscope. It can record video and store still images from the microscope in a PC's file system. Supplied PC's are IEC 60950-1 approved.

The operator should be positioned in such a place to easily access the microscope and also view the image on the PC monitor.

### How to View Live Images

A camera will need to be connected to the PC running RI Viewer™ and the drivers installed correctly to view live images within RI Viewer™. The dongle must also be fitted to a USB port.

RI Viewer™ software will automatically recognise both analogue and compatible digital camera devices connected to the PC. If there is only one camera device detected, it will show the live image for that device. If multiple camera devices are connected it will use the camera that was last selected from the Video Source drop down box.

If there are multiple cameras attached to the PC, you may select the device you want the live image to be viewed from. To do this select the camera from the dropdown list on the Settings panel. See Section 11 - How to Select Cameras.

### How to Zoom & Pan the Image

There are three ways to zoom into an area on the screen.

1. Place the mouse at a point on the screen and click and hold the right mouse button then release. This will zoom into the spot where the mouse is placed. To zoom out, click and hold the right mouse button.
2. Place the mouse at a point on the screen and use the scroll wheel on the mouse (if available). This will give the user control over how much digital zoom is given.
3. Using the tool bar at the bottom of the screen, click the + icon to zoom in incrementally. To zoom out by the same amount, click on the - icon on the tool bar. The magnifying glass icon will zoom in and out by the preset zoom.

Whilst using digital zoom, a thumbnail image of the screen will appear in the top left of the screen. At the top of this panel is the amount of magnification you are using. See Section 11 - How to Set the Preset Zoom. When zoomed out fully, this panel will not appear on the screen.

Zooming in and zooming out can also be achieved using the foot pedal. See Section 11 - How to Configure Foot Pedal/Keyboard.

### Changing Camera Settings

Clicking  on the tool bar will show a panel indicating the camera name and frames per second (fps). Clicking on **Additional Settings** will allow the user to adjust settings on the video source. Only settings available to the video source chosen will appear.

## Section 5

### How to Take a Picture

1. Click  on the tool bar.
2. A camera icon will flash briefly in the centre of the screen to let the user know that an image has been taken.

Images are stored in a .bmp file format.

Images are named by default (date and time) yyyyymmddhhmmss.bmp. For example, a picture taken on the 3<sup>rd</sup> of January 2012 at 10:35:02 would be named 20120103103502.bmp.

### How to Record Video

1. Click  on the tool bar.
2. Whilst video is being captured, a recording notification will flash in the top left corner of the screen.
3. Click  on the tool bar and the recording notification will stop.
4. Each video recording can last for a maximum of one hour. This eliminates the problem of accidentally leaving the software recording with the possibility of rendering the computer unusable.

Recorded videos are stored in a .wmv file format

Recorded videos are named by default (date and time) yyyyymmddhhmmss.wmv. For example, a video taken on the 3<sup>rd</sup> of January 2012 at 10:35:02 would be named 20120103103502.wmv.

### How to Perform Measurements

1. Click  on the tool bar.
2. Click on the screen to select a start point for the measurement.
3. Click on the screen a second time to select an end point for the measurement.

The ends of the line can be dragged to change the measurement.

Lines can be removed by clicking the cross in the context window.

Lines are shown during laser operation but cannot be dragged/created and no measurements will be displayed.

Lines and measurements are shown on an image when images are taken in line mode.

## SATURN™ LASER SYSTEMS OPERATION

### How to Configure the Laser for a New Procedure

1. Check objective calibration.
2. Check laser target alignment.
3. Check hole size calibration.

### How to Check Objective Calibration

1. Click  on the tool bar.
2. Click the **Objective Calibration** button to open the Objective Calibration panel.
3. Place an object of known dimensions (a stage micrometer is supplied with each system for this purpose) in the field of view.
4. Ensure that the objective selected in RI Viewer™ matches that being used on the microscope. See Section 11 – How to Select Objectives.
5. The stage micrometer supplied measures 100µm between the longer lines. For best accuracy, position each end point of the line at exactly the same relative position on the scale, for example at the right hand edge of each vertical line.
6. Drag the rulers to the point where the stage micrometer is going to be measured from and use the fine adjustment up and down until the ruler scale matches the stage micrometer.

### How to Check Laser Target Alignment

1. Click  on the toolbar. If the laser has not been aligned the Laser Target Alignment will start automatically.
2. Follow the on screen instructions.

Once Target Alignment has been completed, it can be accessed again by clicking **Laser Settings**, then **Align Laser** and finally clicking the **Target Alignment** button. This process is the same as the initial Target Alignment.

# Section 6

## Saturn™ Laser Systems Operation

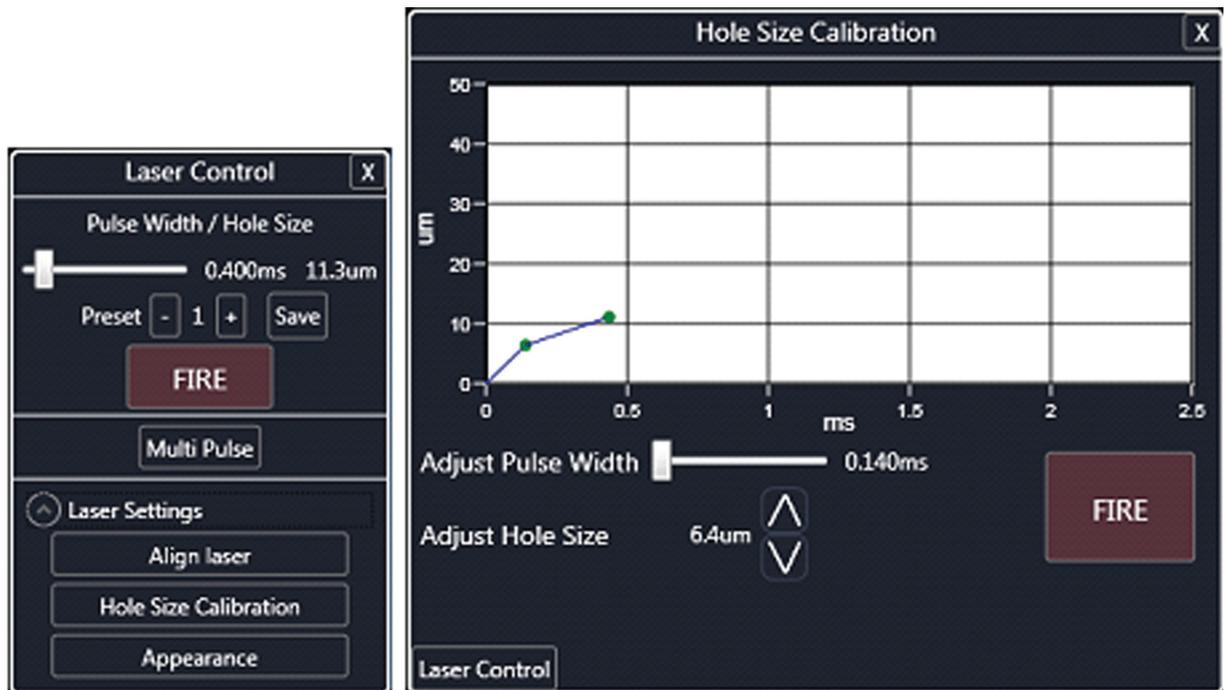
### How to Check Hole Size Calibration

The laser objective **must** be calibrated correctly for the Hole Size Indicator to work correctly.

The actual size of hole a laser produces may vary depending on the type of Petri dish and media, the characteristics of the embryo and other factors. For this reason, the Hole Size Indicator may be calibrated.

To calibrate the Hole Size Indicator, you must be using the red laser objective and select the 40X objective in RI Viewer™. A suitable test specimen (which will be drilled) should be in the laser target area.

1. Click the **Laser Settings** button on the Laser Control panel.



2. Click the **Hole Size Calibration** button.
3. Put the laser target on top of the specimen.
4. Fire the laser and ablate an area of the sample specimen.
5. Click the up and down arrows next to Adjust Hole Size to match to the size of the drilled hole.
6. Click the **Save Point** button when you are happy that the sizes match.
7. Repeat for a number of pulse widths to create a curve on the graph



Repeated hole size calibration can sometimes introduce incorrect points on the graph. The graph should normally be a straight line or smooth curve with a positive (increasing) gradient. If a point leads to a negative gradient then a warning will be displayed on the chart. This should be corrected as it will imply two different pulse lengths for a given hole size, and may lead to unexpected hole sizes.

### How to Remove a Point from the Hole Size Calibration Graph

1. Click the point you wish to remove.
2. Click the **Remove** button.

The warning will disappear as soon as the negative gradient has been resolved.

### How to Fire the Laser (Single Pulse)

1. Find the sample under the microscope using an objective with wide field of view (e.g. 4x objective).
2. Hold the sample with a holding pipette at the bottom of the dish.
3. Switch to the Laser Objective and focus on the sample.
4. Click  on the tool bar.
- 5.a Fixed System - position the sample such that the desired hole location is under the target.
- 5.b Active System - position the sample in the field of view. Click on the desired position of the hole.
6. Click the **Fire** button. The control unit will beep to confirm that the laser has fired.

In some installations, the field of view is larger than the range of movement of the laser. If the cursor is moved to a position on the screen where the laser cannot reach, the unreachable area will be highlighted. The target cannot be placed in this area.

### How to Adjust Hole Size/Pulse Width

1. Hold the left mouse button down and drag until the desired size is reached.
2. Click and drag the slider on the Laser Control panel.
3. Fine adjustment can be made using the up down keys.

### Laser Target

RI Viewer™ displays a laser target on the image. This indicates where the laser beam is focused, and thus where the hole will appear.

**Note** to use the laser system, the specimen must be viewed with the red laser objective.

The target will only be shown when the system is in laser mode.

The appearance and size of the laser target can be modified. The Target Appearance is displayed in the Laser Settings panel. You can change its appearance by adjusting the sliders. This is also where the size of the laser Exclusion Zone can be set.

### Exclusion Zone

The Exclusion Zone is used to show whether critical cells are close to the laser ablation area and may be affected by the heat. The default setting of 8µm is based on previous laboratory data\*. This can be adjusted via the Target Appearance panel using the slider or input box. However, it is the responsibility of users to carry out appropriate trials to satisfy themselves of the safety of the chosen setting if the size is reduced from the default.

*\*K. Chatzimeletiou, K., Picton, H.M. & Handyside, A.H., 2001. Use of a non-contact, infrared laser for zone drilling of mouse embryos: assessment of immediate effects on blastomere viability. Reproductive Biomedicine Online, 2(3), p.178. Available at: <http://www.ncbi.nih.gov/pubmed/12537793>*

### Hole Size Indicator

The circle that is displayed indicates the estimated size of the hole that will be created when the laser is fired. Actual hole sizes may vary due to natural differences between specimens.

The Hole Size Indicator can be turned on or off from the Target Appearance panel on the Laser Settings panel.

## Section 6

### Saturn™ Laser Systems Operation

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#### Preset Pulse Lengths

The preset values can be changed using RI Viewer™. Pulse lengths are limited to stay within Class 1 laser safety standards.



Choose the preset by clicking the + or - buttons. To change a preset, select the preset, set the desired value using the slider, and click the **Save** button.

Hole sizes can also be chosen by clicking on the screen and dragging a hole of the size you need. The pulse width will be calculated automatically to create the size of hole required.

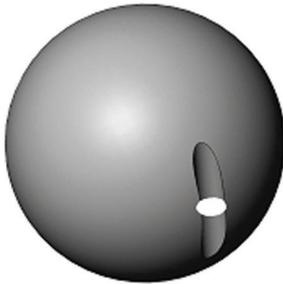
#### How to Shut Down

1. Close RI Viewer™ software.
2. Unplug the device from the power outlet.

## ASSISTED HATCHING PROCEDURE

### Introduction

The aim of Laser Assisted Hatching (LAH) is to locally weaken the zona pellucida. The specimen is viewed along the optical axis and the laser creates a trench in the zona pellucida which extends above and below the apparent hole.



Circle shows hole as viewed through microscope

To minimize the risk of damage to blastomeres, users should administer as few laser pulses as possible at the shortest pulse lengths possible to achieve prescribed zona drilling or thinning effects.

The optimum diameter of the hole is determined by the thickness and/or hardness of the zona. Larger holes are necessary in thicker zona. Smaller holes are preferable for thin zona. The hole may fully breach the zona, whilst some users prefer to ablate only a fraction of the zona thickness, but across a wider area. This process is commonly referred to as zona thinning.

Only a single opening should be made in the zona pellucida. Multiple openings or those that are too small may prevent embryo hatching or lead to abnormal development.

The site chosen for the hole should be a section of the zona pellucida where the adjacent perivitelline space is widest or next to an area of fragmentation. This will minimise possible damage due to heating of adjacent blastomeres. The Exclusion Zone display can help with this.

Note: Assisted Hatching is not recommended for routine use in all ART patients.

### Summary

1. Select the red laser objective on the microscope and check the Objective Calibration settings.



**WARNING** -The embryo may be damaged if objectives other than the red Saturn™ laser objective is used.

2. Check the target alignment before each LAH procedure. See Section 6 - How to Check Laser Target Alignment.
3. Check the temperature of the specimen (37°C).
4. Focus at the mid-plane of the embryo.
5. Position the embryo against the surface of the Petri dish using a micromanipulator and holding pipette to minimise embryo movement.
6. Choose a suitable location for the hole.
7. The first attempt to make a hole should be made with a short pulse. If the hole is too small, then use progressively longer pulses until the desired hole size is achieved.

## Section 7

### Assisted Hatching Procedure

#### Hole Size Selection

For safe and effective treatment we recommend that pulses used are in the range 15 to 1000ms.

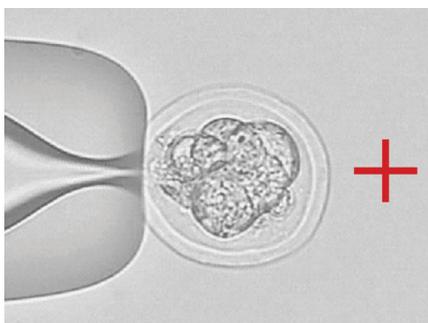
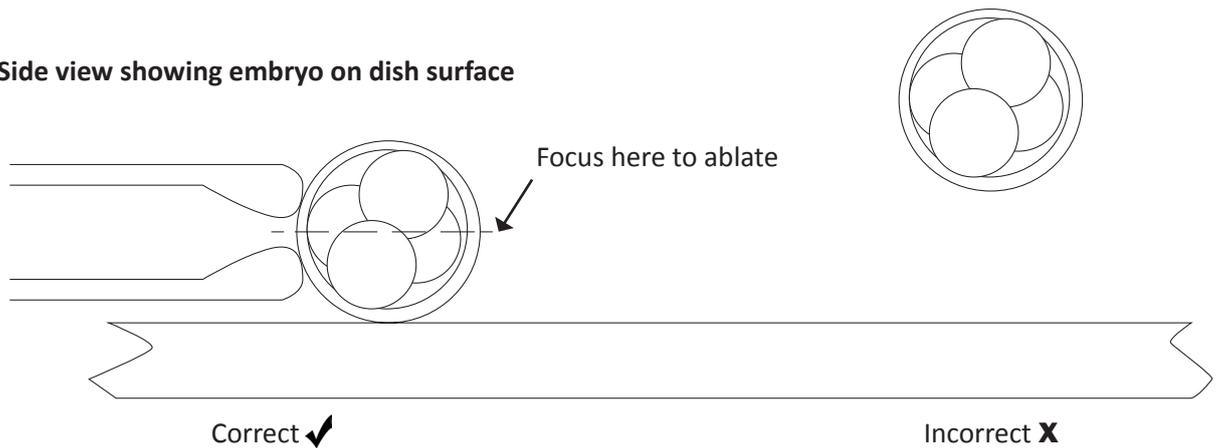
Pulses that are very short may be ineffective and pulses that are too long may damage the embryo. For this reason, a warning will appear on the **Fire** button when pulse width is 15ms or below and when the pulse width is greater or equal to 1000ms.

When the warnings are shown, the laser can still be fired, but an initial click on the **Fire** button is required to acknowledge the warning. If the pulse width is still in the warning range, then the button will go back to the warning state after the laser has fired.

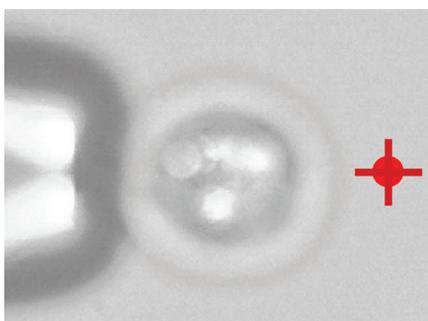
#### Ablation Procedure

Focus on the surface of a Petri dish and ensure that the target is correctly aligned to the pilot laser spot. Place the dish with the specimen on the microscope stage and hold the specimen on the bottom of the dish with a holding pipette. Check you are using the red Saturn™ laser objective. To ablate the zona, the focal plane must be at the mid section of the embryo. Refocus the microscope until the image of the zona is sharp.

#### Side view showing embryo on dish surface



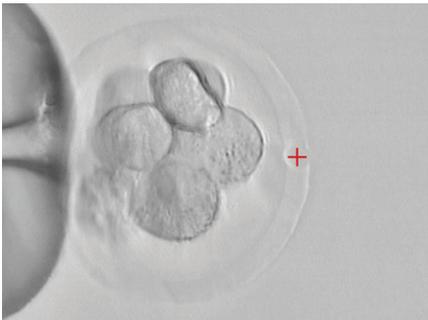
Correct focus - specimen is sharply focussed.



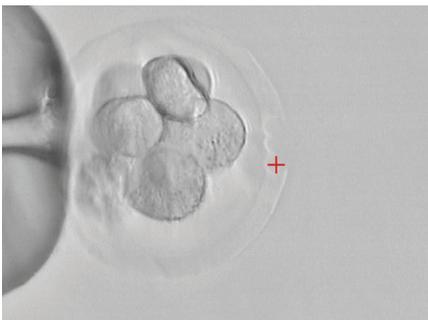
Incorrect focus for drilling.



Move the specimen into the field of view. Select a short pulse length using the handset or the RI Viewer™ controls. The different settings do not vary the power of the laser; they control the length of time that the laser is fired.



Click on the zona pellucida where you want the hole to appear. **Fire** the laser by clicking the **Fire** button in RI Viewer™. A small hole should appear in the zona. If a larger hole is required, increase the laser pulse time and fire again.



To create larger holes and for zona thinning the area should be ablated using a series of small diameter holes that overlap slightly, joining to form one larger hole.

7



Do not attempt to make a large hole with just one firing of the laser. Although this will not cause damage to the instruments, it could potentially damage the embryo due to the greater heating effect of the long pulse length.

For further information on the methodology of laser ablation, refer to the many published scientific papers on the subject, or seek advice from a practitioner who is experienced in the technique.

## Section 8

### Saturn™ Laser Systems Additional Modes

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#### SATURN™ LASER SYSTEMS ADDITIONAL MODES



In the USA, Saturn™ Laser Systems are 510(k) cleared for Assisted Hatching only. The operations in this section are for investigational purposes only

##### How to Enable Biopsy Mode

1. Click  on the tool bar.
2. Tick **Enable Biopsy Mode** (this option is available only with Saturn™ Active).

##### How to Enable Multi-Pulse Mode

1. Click  on the tool bar.
2. Tick **Enable Multi-Pulse Mode**.

##### How to Fire the Laser (Biopsy Mode)

Biopsy Mode allows you to create a line of holes that will be drilled automatically. This mode will only be accessible if the Active component is installed.

1. Switch to the Laser Objective.
2. Focus on the sample.
3. Click  on the tool bar.
4. Check the sample is just on the bottom of the dish.
5. From the Laser Control Panel, click **Biopsy**. You will be taken to the Laser Biopsy panel.
6. Click where the desired start point of the line. The line will follow your cursor.
7. Click the desired end point of the line. The holes to be drilled are overlain on the image.
8. The size and number of holes can be changed by moving the two sliders on the panel.
9. The ends of the line can be adjusted by dragging them.
10. A point in the middle can be dragged to create a curved line.

Press **Start Firing** to begin the firing procedure. The laser will then drill each hole in sequence. To interrupt this procedure press **Stop**.

### How to Fire the Laser (Multi-Pulse Mode)

The laser target cannot be moved during Multi-Pulse Mode.

1. Switch to the Laser Objective.
2. Focus on the sample.
3. Click  on the tool bar.
4. Check the sample is just on the bottom of the dish.
5. From the Laser Control panel, click **Multi-Pulse**. You will be taken to the Multi-Pulse Laser panel.
6. Click on the screen to select the location to fire the laser (Active only).
7. Select the number of pulses per second.
8. Click **Fire Multi-Pulse**.
9. Move the sample with the holding pipette to ablate the required region. The control unit will beep continuously while the laser is firing.
10. Click **Stop** to complete the procedure. The laser will also stop firing automatically after 20 seconds.

# Section 9

## SATURN™ LASER SYSTEMS TROUBLESHOOTING

Problem	Possible Cause	Solution
No lights on control unit	No power input	Check the power supply is plugged in to the mains and switched on
	Incorrect power supply	Use only the power supply provided
No video image	Camera not connected	Make sure camera is connected to PC and to power supply if appropriate
	Incorrect camera settings	Check brightness/contrast settings (section How to view live image)
	Camera port not selected	Select camera port
Laser having no effect	Wrong objective used	Only use the red Saturn™ objective
	Target misaligned	Check alignment
	Port selector misaligned	Check port selector is in correct position*
	Dirt on fibre optic	Service required
	Embryo above dish surface	Check the embryo is touching the dish surface
	Dirt on fibre optic	Service required
Hole size smaller than expected	Kinked fibre optic	Straighten fibre optic patch lead - minimum bend radius 50mm
	Embryo too cold	Check temperature should be 37°C
	Dirt/oil on objective	Clean objective
Hole size larger than expected	Excessive pulse length	Choose smaller hole size
	Hole Size Indicator not calibrated	Check calibration. Check for negative gradient
Laser position inaccurate	Port selector misaligned	Check port selector is in correct position*
	Calibration not done	Calibrate each time unit is switched on

*\*Some port selectors do not return to exactly the same position when moved. Check the target alignment each time the port selector is moved.*

## RI IMSI™

### Introduction

Please read this manual carefully before using the system, and follow the instructions to ensure that your RI IMSI™ system will work reliably. Obtaining high quality images requires careful set-up and regular cleaning.

This manual does not contain detailed instructions for using micromanipulators, the microscope or additional optical components. For detailed information, refer to the individual manuals supplied with each system component.

### Specifications

Objective	RI - HMC® 60x/0.7 or 63x/0.7 (Depending on microscope)
Condenser	RI - HMC® WD45mm, NA-0.6 (Depending on microscope)
Contrast	RI - HMC® High Sensitivity Modulation Contrast System
DC2 Digital Camera	High sensitivity, 1.4 MP, 2/3" colour CCD
Monitor	21" 1600 x 1200, DICOM compliant medical imaging monitor
Software	RI Viewer™ software included
Magnification	Up to 7000x without loss of resolution

### Hardware Overview

#### HMC System

Most systems will be supplied with a purpose made HMC system for IMSI from Modulation Optics. However, other configurations may be supplied. Consult the instructions supplied with the optical system.

For the image quality to be suitable for IMSI, the optical components must be set up exactly according to the instructions. At high magnification, small misalignments can have a significant effect on the image quality.

#### Condenser

Follow the instructions supplied with the condenser system. The illumination field apertures should be adjusted such that it is just outside the field of view of the 4x objective. Closing the aperture will reduce NA and resolution.

#### Objectives

The 40x and 60x objectives are fitted with coverslip correction collars. These **must** be in the correct position to obtain the best image quality.

1. Start by turning the collar to align the scale to the thickness of the Petri dish (typically 1.0mm). **Note** that some objectives have a fixed cap which provides some correction, so the correction collar should be set such that the sum of the two is 1.0mm. For example, the cap shows a correction of 1.1mm, so the collar scale should be set to -0.1.
2. Focusing on a sperm sample, turn the correction collar slightly and refocus. If the resolution appears better then try a further slight adjustment. If the image is worse then try a small adjustment in the other direction.

60x objectives have a shallow depth of field, so the focus may have to be frequently adjusted when observing moving sperm.

# Section 10

RI IMSI™

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## IMSI Monitor

We recommend a monitor designed for medical imaging, and that the PC is fitted with a separate PCI-E graphics card with DVI (Digital Video Interface) output and at least 256MB memory.

Place the monitor in a convenient position close to the microscope.

Adjust the height and angle of the monitor such that the operator can comfortably look towards the centre of the screen.

## Monitor Settings (for Medical Monitor Supplied by RI)

The M (Mode) button on the bottom edge of the monitor switches between display modes for different purposes. Confirm the chosen mode by pressing the I button. We suggest using DICOM mode for IMSI for the best image contrast and brightness. If the PC is used for other purposes (for example, office applications), the high brightness and colour temperature of the DICOM mode may be tiring. Change to Text mode for general computer use.

In Text mode the brightness and colour controls can be adjusted for comfortable viewing. We suggest setting Text mode to a brightness of 30% with a colour temperature of 6500K.

Brightness and colour cannot be manually adjusted in DICOM mode, as these settings are fixed to conform to the DICOM standard. See the monitor manual, supplied separately, for detailed instructions.

## Sample Preparation

A solution of PVP is normally used to prepare a sperm drop in order to increase the viscosity of the medium and thus to slow the movement of the sperm. 60x objectives have a shallow depth of field. If sperm are allowed to move vertically within the drop, they will tend to move out of focus and may be hard to observe. It may be helpful to use a pipette to drag the edges of the drop outwards in a number of places to create shallow areas which will confine the sperm.

## Observation

1. Place the dish on the microscope stage.
2. Focus on the top surface of the dish using a low magnification objective. It may help to focus on the edge of the media droplet to begin with in order to focus on the surface.
3. Use the micromanipulator controls to place the tip of an injection pipette in the field of view.
4. Use progressively higher magnification and the x-y stage to locate an area of active sperm.
5. For examination of individual sperm morphology, select the 60x/63x objective and matching HMC position in the condenser turret.
6. Using the blue filter supplied with the HMC system will give colour images a blue cast, but will improve image resolution.
7. The software zoom feature can be used to increase the magnification. See Section 5 - RI Viewer™. This feature should be used sparingly, as high zoom levels will exceed the resolution available from the microscope optics. Use the micromanipulation system and a sperm injection micropipette to pick up the selected sperm and transfer them to the dish/droplet containing the oocytes to be fertilised.

## Maintenance

### General

The microscope must be maintained to a high standard. Problems such as worn focus mechanisms, dirt on optics, etc. may result in reduced image quality.

It is essential that optical components are correctly adjusted to achieve the best image quality.

### Cleanliness

All exposed optical surfaces must be kept clean. Use an air blower followed by a dry lens cloth to remove dust particles. Oils from the skin, if a lens has been touched, and other marks can usually be removed with isopropyl alcohol wipes, followed by wiping with a dry lens cloth.

### Monitor Calibration

The monitors we supply will provide a high quality image with minimal set up. However, performance of any monitor will change slowly over time, and periodic calibration will ensure a high quality image for as long as possible.

Larger hospitals may have calibration facilities already in place to cover monitors used for X-ray imaging. Contact your IT/maintenance department for information.

Calibration involves measuring the screen output using a colorimeter. The measurements are stored by the graphics card and used to compensate for any slight errors. **Note** the colorimeter is specific for DICOM calibration on these monitors. General purpose colorimeters (e.g. Datacolor Spyder3) are not compatible.

Colorimeters can be supplied by RI, or a calibration service may be offered by your RI distributor or local representative of the monitor manufacturer. We suggest calibrating your monitor every twelve months.

# Section 10

RI IMSI™

## Troubleshooting

<b>Problem</b>	<b>Possible Cause</b>	<b>Solution</b>
No video image	Camera not connected	Ensure USB cable is properly connected and undamaged
	Port selector closed	Ensure the microscope's port selector is set to the appropriate position
Poor quality image	Correction collar out of adjustment	Turn the correction collar on the objective to the correct position
	Dirt on optics	Make sure all accessible optical surfaces are perfectly clean
	HMC system out of alignment	Check condenser height and slit aperture alignment
	Monitor in Text mode	Set monitor to DICOM mode
	Unsuitable graphics card	Fit the graphics card supplied with the monitor
Blurred image	Too much digital zoom	Excessive zoom cannot resolve more detail. Reduce zoom level

## APPENDICES

### How to Select Cameras

1. Click  on the tool bar. The Video Source drop down box will have a list of camera names referring to the cameras connected to the PC.
2. Click on the **drop down box** to allow the selection of cameras. Clicking on the required camera in the list will change the live image to that of the selected camera.

### Camera Flipping

1. Click  on the tool bar.
2. Tick **Flip Video Horizontal** (for camera devices that support these options).
3. Tick **Flip Video Vertical** (for camera devices that support these options).

### How to Set the Preset Zoom

1. Click  on the tool bar.
2. Drag the **Preset Zoom** slider to the required zoom.

### How to Configure the Foot Pedal/Keyboard

1. Click  on the tool bar.
2. From the Settings panel click the **Keyboard/Foot pedals Shortcuts**.
3. Click the corresponding edit button to assign a shortcut.
4. Hold down the key or press the foot pedal to assign.
5. Click **Save and Quit**.

Once the shortcuts are set up, press the shortcut keys or the corresponding foot pedal to activate the command.

### How to View Still and Recorded Images

1. Click  on the tool bar.

From this location, the user can use their preferred image viewer to review, edit, email, rename and move the image file to a different location for further observations or the manipulation of any images that have been captured.

# Section 11

## Appendices

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### How to Select Objectives

1. Click the magnification on the tool bar .
2. Click the required objective magnification.

### How to Add Objectives

1. Click  on the tool bar.
2. Click the **Objective Calibration** button.
3. Click **New Objective**. The magnification will be the same as the current magnification.
4. Click the required objective to edit **Objective magnification** and **Fine Adjustment** values.

### How to Remove Objectives

1. Click  on the tool bar.
2. Click the **Objective Calibration** button.
3. Click **X** next to an objective.

## **SATURN™ LASER SYSTEMS CARE AND MAINTENANCE**

We recommend that a non PVC dust cover is placed over the microscope when not in use. Plasticisers commonly used in PVC are toxic to embryos.

The control unit case should be cleaned with a cloth moistened with isopropyl alcohol only. If any liquids are spilt over the control unit switch off immediately and remove the power connector. Clean the spill and ensure that the unit is completely dry before switching on. If it is suspected that any liquids have gone inside the case contact RI for advice before switching on.

Routine maintenance simply involves ensuring that all optical components are kept clean. The lens on the end of the Saturn™ objective should be cleaned occasionally by wiping gently with a dry lens cloth.

Every six months the collimator module should be removed and the dichroic mirror cleaned with a dry lens cloth. This should be done by RI or an appointed representative as the laser alignment must be checked and readjusted after removing the collimator module.



We recommend that the system undergoes a routine service at least every twelve months. This can normally be carried out by your distributor.

For further advice on microscope cleaning, please contact RI or your microscope supplier.

In the event that you have a problem with RI instruments, first look at the Troubleshooting section. If you require any further help, contact your distributor or RI's service team direct. We will try to resolve the problem as quickly as possible.



### **Disposal of Goods**

If any electronic component is no longer serviceable, it must be sent back to RI to be destroyed in an environmentally safe way. Do not dispose of with 'normal' waste.

## Section 13

### Returns Procedure

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#### RETURNS PROCEDURE

1. Contact RI to obtain a Returned Materials Authorisation (RMA) number. **Note** goods will not be replaced or refunded without prior agreement and clearly stating the RMA number.
2. Pack the item carefully in its original packaging. RI will not accept responsibility for damage due to incorrect packaging. Replacement items or additional repairs will be invoiced.
3. Clearly label the consignment with the RMA number, mark the package "Urgent - Returned Items For Repair", and ship to the address below. Goods should be insured for their full value during shipping.

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#### Customer Feedback

Thank you for purchasing an RI product. To help RI develop the best tools for ART, we rely on customer feedback. If you have any suggestions of how we can improve our products or the information we provide with it, please send them to [feedback@research-instruments.com](mailto:feedback@research-instruments.com). Your feedback will help us develop the product and supporting materials to meet your future needs.

Thank you





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