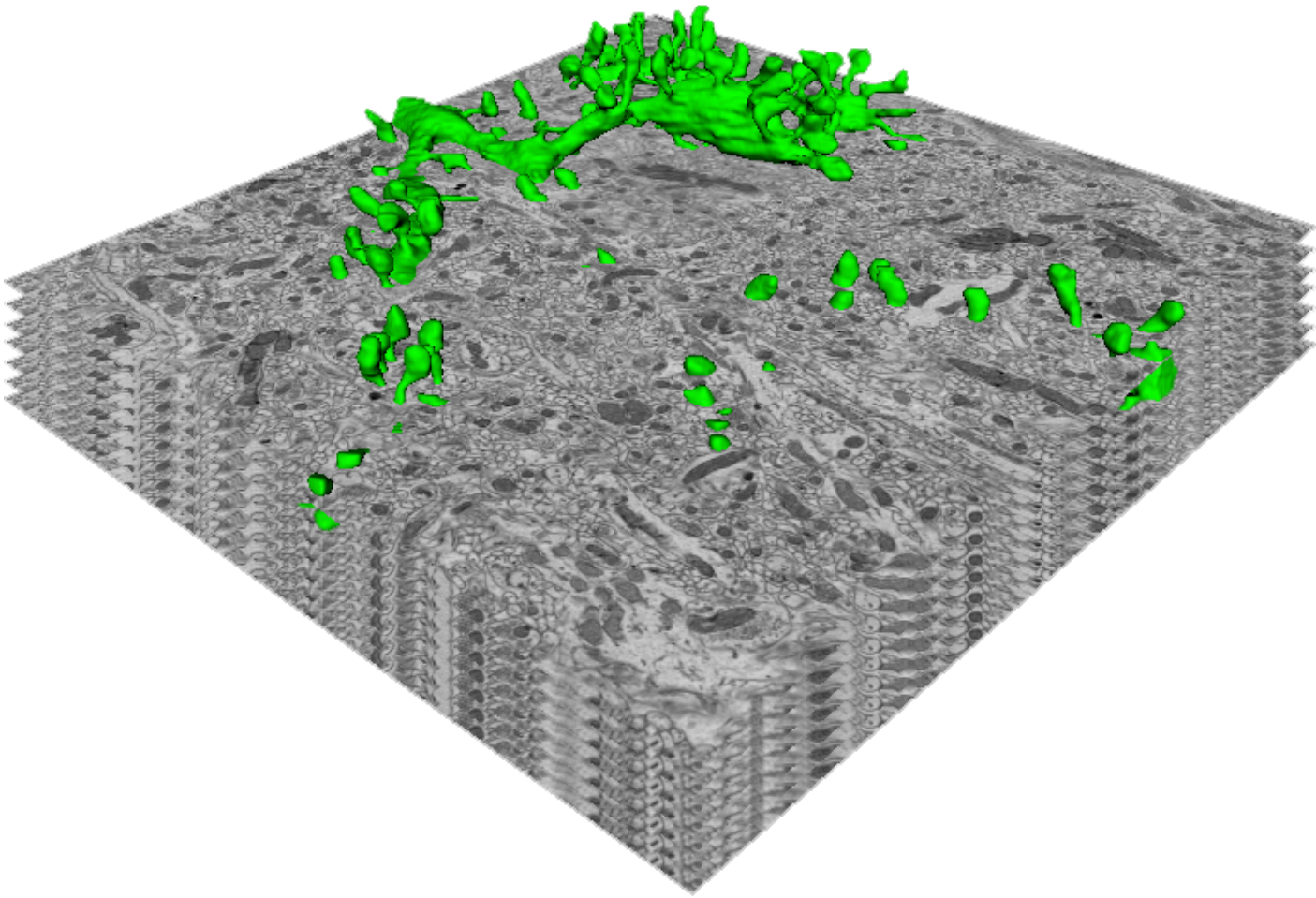
3View2XP

Users Guide



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Issue 14
Number of pages in document 102

Unpacking

All the boxes comprising the shipment should be inspected for any signs of damage before unpacking. If any severe damage is visible, the following procedures should be carried out immediately:

- Photograph the extent of the damage. Digital photographs sent by email are often the best method.
- Describe the extent of the damage to *Gatan UK* and / or their appointed agent.

- This will allow *Gatan UK* to estimate possible damage to the enclosed equipment and decide whether an insurance assessor and engineer needs to be present before further unpacking.

- Assuming there is no damage, all cartons should be opened and the contents removed and checked against the packing sheet.

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Support

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1. Safety of the 3View2XP System.

It is your responsibility to ensure your own safety, and the safety of the people working around you.

If the equipment is not used in the manner specified by Gatan UK, the protection provided by the equipment may be impaired.

Before you attempt to operate this equipment for the first time, please read the safety information and make sure that you are aware of the precautions that you must take to ensure your own safety.

1.1. Approvals and Safety Information.

This section presents approval and certification information for the 3View2XP system. It also provides a summary of the safety recommendations. Gatan recommends following all safety precautions to prevent harm to yourself or the equipment. Please follow all warnings marked on the equipment as well.

1.1.1. CE Certification.

The product described in this manual meets the intent of:

- **CE Directive, Decision No 768/2008/EC**
- **Low Voltage Directive, 2006/95/EC**
- **EMC Directive, 2004/108/EC**
- **Safety Standard, IEC 61010-1**
- **RoHS2.**

The product described in this manual has been developed, designed, and marketed for Research & Development, and sold on a business-to-business basis. As such, this product is exempt from the requirements of Directive 2011/65/EU of the European Parliament and of the Council of June 8th 2011 on the Restriction of the use of certain Hazardous Substances in electrical and electronic equipment (RoHS), per Article II, Clause 4(j).

A CE Declaration of Conformity is available from Gatan UK.

1.1.2. WEEE Directives

As a company that has always been firmly committed to environmentally responsible practices, Gatan UK is in accord with the content and spirit of Directive 2002/96/EC of the European Parliament and of the Council of 27 January 2003 on "Waste Electrical and Electronic Equipment" (commonly referred to as the WEEE Directive). The WEEE Directive applies to all products placed on the controlled market after 13 August 2005.

At present, Gatan UK's policy and instructions for customers wishing to dispose of equipment at the end of its life is presented on the Gatan web site at www.gatan.com.

1.1.3. **Safety certification.**

EMC amd LVD directives have been met through testing to the following harmonised standards:
EMC Emissions & Immunity:

EN61326 -1:2006 (IEC 61326-1:2005)
EN61000-3-2:2006
EN61000-3-3:2008

The Declaration of Conformity is available from Gatan UK., or from your authorised distributor.

X-ray safety directive; 96/29/EURATOM Ionizing Radiation has been met through testing at Gatan or at partners laboratories.

1.2. Symbols and Conventions in this Manual.

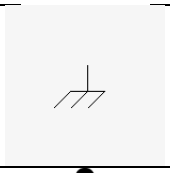
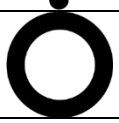
The following symbols appear in this document.

Danger: Indicates an immediately hazardous situation which, if not avoided, will result in death or serious injury. Danger is limited to the most extreme situations.

Warning: Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

Caution: Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Caution may also be used to alert against potentially unsafe practices.

1.3. Symbols and Warning Hazards Displayed on the Equipment.

SYMBOL	REFERENCE	DESCRIPTION
	IEC 60417-5031 (2002-10)	Direct current
	IEC 60417-5032 (2002-10)	Alternating current
	IEC 60417-5017 (2006-08)	Earth (ground) TERMINAL
	IEC 60417-5019 (2006-08)	Protective Conductor Terminal
	IEC 60417-5020 (2002-10)	Frame or chassis TERMINAL
	IEC 60417-5007 (2009-02)	On (Power)
	IEC 60417-5008 (2009-02)	Off (Power)

		Caution, possibility of electric shock
	ISO 7000-0434B (2004-01)	Caution - documentation must be consulted in all cases where this symbol is marked

The following table provides a list of warning hazards displayed and an overview of what these hazards mean.

Symbol	Meaning.	Location.
	Heavy Weight Hazard. Do not attempt to handle the 3View door as an individual unless a specialist stage exchange mechanism has been provided.	On either side of the 3View replacement door.
	High DC voltage is fed into these connectors via the cables which originate from the rear of the 3View controller. Upto -150V at up to 40mA. There are no additional warning signs on the inside of the door. Do not detach the HT coaxial cables without the power to the controller being turned off.	On the 3View door next to the STROKE and CUT SHV feed throughs. Once installed, the STROKE and CUT cabling are normally attached to these connectors.
	Do not use these covers as weight bearing handles.	Motor covers on the door.
	High DC voltage (upto -150V at upto 40mA) originates at the core of these SHV connectors. Do not detach the HT coaxial cables without power to the controller being turned off.	On the rear of the 3View Microtome stage controller.

	No user serviceable parts inside the controller.	On the rear of the 3VBSED controller.

1.4. Sevice Engineer and User Tasks.

The Gatan 3View2XP system is intended to be installed by a trained Gatan engineer. This installation is performed on an SEM which is already installed and functioning to the microscope manufacturer's specification.

This User's Guide describes the functionality of the installed product. It does not describe installation, service or fault finding tasks. Users are encouraged to read the User's Guide as it contains important information to help the user understand the product, to gain optimum results, and to limit any potential damage to the equipment.

Tasks which are appropriate for the user are described in the Users Guide. Tasks which may pose some hazard are highlighted as such. Users are minded to take circumvent risks by understanding them.

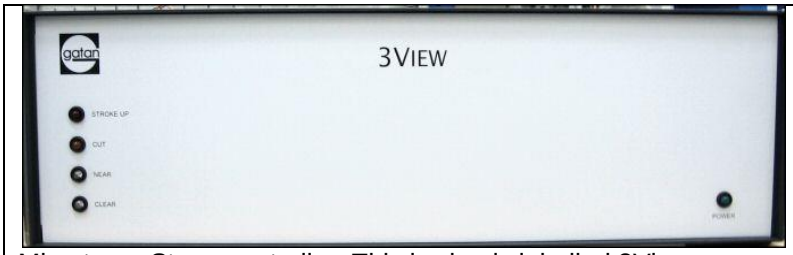
Some installation or repair tasks have risks associated with them. These tasks and risks are identified in the 3View2XP Service Manual. Tasks appropriate for a Gatan trained service engineer (subsequently referred to as service engineer) may be referenced in this User's Guide, but are described in more detail in the Service Manual.

Warning: ***For safety reasons, users should not attempt tasks described as being appropriate for service engineers.***

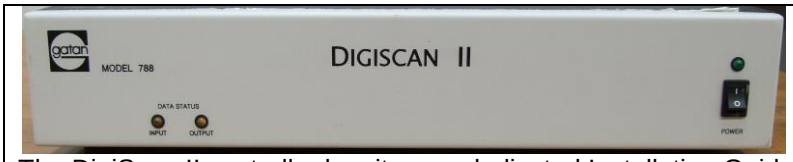
Warning: ***Do not attempt to use the machinery until it has been installed by a trained field service engineer. If the system is locked for shipping, then the field service engineer will unlock the system. If the user attempts to use it in a locked state, then damage will be caused to the motors and actuators.***

1.5. Electronic Controllers.

The 3View2XP system includes 3 electronics controllers. These are the Microtome Stage Controller, the DigiScan II Controller and the 3VBSED Controller. The following table helps identify these controllers.



Microtome Stage controller. This is simply labelled 3View



The DigiScan II controller has its own dedicated Installation Guide and is not described in detail in this 3View2XP Users Guide.

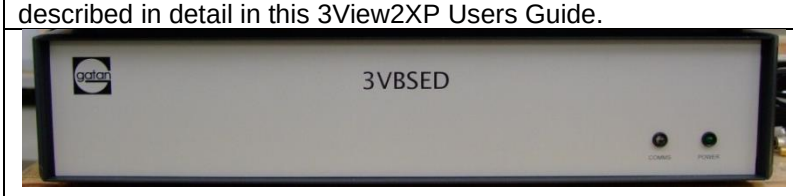


Figure 1 Identification of the 3 controllers.

It is common practice for the controllers to be powered from a main supply associated with the SEM. If this is the case, it is good practice to turn each unit on individually if the power to the microscope has been interrupted. This avoids potential problems with fuses in the SEM which may trip due to the large in-rush of current when all controllers are powered simultaneously.

Warning: ***There are NO user serviceable components inside any of these controllers. Users should NOT open these controllers to gain access inside them. Access is intended for service engineers only.***

1.5.1. Power Specifications of Controllers.

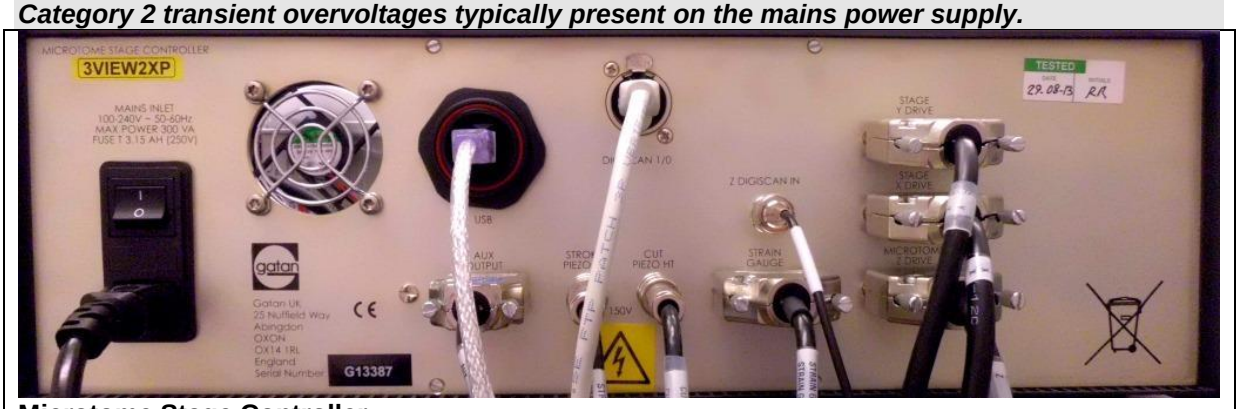
Lethal voltages are accessible inside each of these controllers. Disconnect the AC power supply before fuses or covers are removed. It is not sufficient to switch off the main power switch. Do not take risks with lethal voltages.

All 3 controllers have a single phase mains supply and are universal input.

Each mains supply must include an earth connection.

Each controller is compatible with mains supply voltage fluctuations of up to +-10% of the nominal voltage.

Category 2 transient overvoltages typically present on the mains power supply.



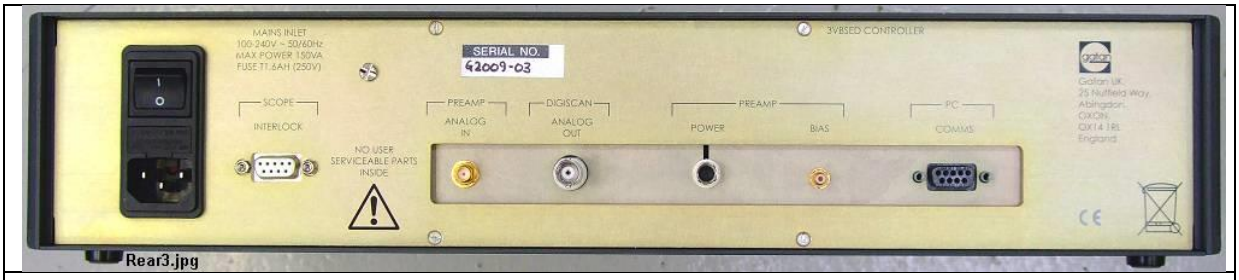
Microtome Stage Controller.

Mains Rating: *100-240V ~AC, 50-60Hz.*
Max Rated Power:300VA
Fuse Rating: *T 3.15 AH (250V) 20mm ceramic.*

Figure 2 Electrical Ratings of the Microtome Stage Controller.

DigiScan II Controller.
Mains Rating: *100-240V AC, 50-60Hz.*
Max Rated Power: 30W
Fuse Rating: *T 2AH (250V)*

Figure 3. Electrical Ratings of the DigiScan II controller.



3VBSED Controller.
Mains Rating: *100-240V AC, 50/60Hz.*
Max Rated Power: 150VA
Fuse Rating: *T 3.15AH (250V)*
These ratings are described on the rear of the unit

Figure 4. Electrical Ratings of the BSED controller.

The other connections on the rear of the Microtome Stage Controller, the DigiScan II controller and the BSED Controller pose no hazard, are low voltage and are described in more detail in Chapter 2.

1.5.2. Power Specification of PC and Monitor.

The PC and monitor is a standard product purchased from Dell in the UK. Please refer to the documentation supplied with the PC and monitor regarding safety and power specifications.

It is Gatan's policy to test and ship the equipment with correct fuses for the voltage specification at the destination country. However, it is the job of the installation engineer to ensure that no errors are made.

1.5.3. Environmental Specifications of Controllers.

The 3View2XP system is intended for laboratory use.

Temperature Specification.
It is expected that the system including all controller units and the PC is operated in a closely temperature controlled environmental compatible with the stable operation of Scanning Electron Microscopes. Low temperature variations (including avoiding direct exposure to sunlight) will improve the drift performance of the equipment.

Warning. ***The equipment has been designed using components which have been tested for safe operation in the temperature range 5 to 40 C. Do not use the equipment outside of this temperature range.***

Humidity Specification.
5-95% RH non condensing.

Altitude Specification.
Less than 2000m above sea level.

The equipment should not have been sold for installation at a laboratory above this altitude. If wishing to relocate, please contact Gatan UK for advice.

1.5.4. Ventilation of Controllers.

The Microtome Stage Controller and DigiScan units each contain a small fan in the rear of the controller units. The PC also contains a fan. The 3VBSED controller does not contain a fan.

The PC and controllers should be placed in a position to allow ventilation. The controllers should rest on their feet to allow air to be drawn through their bases and the rear of the units should not be blocked. The fans in the 2 units should turn freely with low audible noise. Do not block the fans. Turn the units off if their fans appear to be dysfunctional and seek help from an engineer to correct the problem.

1.5.5. Positioning of Controllers.

All of the mains powered controllers and the PC must be positioned so that access to the mains inputs on the rear of each unit is clear. They must not be positioned such that access is considered difficult or awkward. This is for safety purposes.

1.5.6. **Protective Ground (Earth).**

The following units must be connected to an electrical ground when they are installed and at all times during use.

- Microtome Stage Controller
- DigiScan II Controller
- 3VBSED controller

All three controllers are individually earthed through their IEC mains cables.

Warning: ***For the controllers, the ground wire (green / yellow), in the instrument power cable, must be connected to the laboratory electrical ground. Only use extension cables if they have an earth conductor. Do not disconnect the protective ground inside or outside the instrument and do not have external circuits connected to the instrument when its protective ground is disconnected.***

Warning: ***The instrument will not stop working if the earth wire is not connected, and there is no indication that you might be in danger. Make sure that it is checked at least annually.***

The replacement door is normally earthed by being attached to the earthed SEM chamber through the stage rails. This earth connection is checked at installation. If the unit is to be used for cutting when not attached to the SEM door, then the user should connect the earth cable as supplied with the system to the dedicated primary earth connection on the side of the door and connector this to an earth in the laboratory, e.g. from the SEM.

Figure 5. 3View door earth connection.

Photograph showing the configuration when the 3View door is not earthed ot the SEM chamber by means of the stage rails. A yellow / green earthing cable connects from the 3View2XP door to a primary earth in the laboratory.

1.6. Electrical Connections between Controllers.

A schematic of electrical connections of a fully installed 3View2XP system is given in Figure 34. The following detailed breakdown of connections is provided in this section as a reference and to illustrate the functionality and voltage levels employed.

1.6.1. **Microtome Stage Controller to 3View2XP Door.**

The cabling is designed so that it is NOT possible to make an incorrect connection which is unsafe to the user. The 3View2XP stage door includes seven vacuum tight electrical feed throughs. The following table provides details of the functionality of each connector, cable and feed through combination from the controller to the stage door.

Table 2 Microtome Stage Controller to 3View2XP Door Connections.

Connector on Microtome Stage Controller	***Cable***	***Vacuum feed through on Microtome Door***	***Description of Function***	***Maximum Voltage in connector / cable.***
Stage X Drive, 15way D(m) type	3m labelled **X**	12way circular labelled **X**	Drives X axis DC motor and senses whether min or max limit switch is reached.	15V
Stage Y Drive, 15way D(m) type	3m labelled **Y**	12way circular labelled **Y**	Drives Y axis DC motor and senses whether min or max limit switch is reached.	15V
Stage Z Drive, 15way D(m) type	3m labelled **Z**	12way circular labelled **Z**	Drives Z axis DC motor and senses whether min or max limit switch is reached. Z motor controls the specimen height and therefore the cut thickness.	15V
AUX 9way D(f) type.	3m labelled **AUX**	9way circular labelled **AUX**	Power and status position for the Near / Clear motor, LED illumination. If knife piezo vibration is offered it is included on this connector.	15V
STRAIN GAUGE 15 way D(f) type	3m labelled **SG**	6way circular labelled **SG**	Closed loop feedback controlling the accurate positioning of the Stroke piezo.	5V
STROKE PIEZO (HT) SHV	3m co-axial labelled **STROKE**	Female SHV labelled **STROKE**	High Tension DC voltage (150V) for controlling the Stroke Piezo actuator.	150V
CUT PIEZO (HT) SHV	3m co-axial labelled **CUT**	Female SHV labelled **CUT**	High Tension DC voltage (150V) for controlling the Cut Piezo actuator.	150V

Caution. ***NEVER connect or disconnect cabling from the controller to the door when the Microtome Stage Controller is powered. This can damage electrical components and such damage will not be covered under warranty.***

There are 3 similar DC motor cables for the X and Y stage movement and the Z specimen advance motor. There are 2 identical co-axial cables for the STROKE and CUT. The cables are identical except for the labelling. No electronic damage will be caused if the cables are connected wrongly, other than the software will not function correctly.

Caution. ***As some cables are identical it is possible to make incorrect connections between the Microtome Stage Controller and the stage door which is unsafe to the equipment, but this poses no hazard to the user. The cabling is labelled to avoid such errors. If any cable label is not visible, please refer to the cabling diagram or seek help.***

If the software is started when the X,Y,Z, or AUX cables are disconnected, the software does not report that the relevant motors (according to the cabling to the controller) are missing. Rather the controls will be missing from the software. The software should be exited and restarted with the cabling connected correctly. If the software is started when the STROKE, CUT, or SG cables disconnected, the software will not be aware of this fact.

1.6.2. **Additional Electrical Connections to the Controller.**

The remaining electrical connections to the Microtome Stage controller are detailed below.

Table 3. Additional Connections to Microtome Stage Controller.

Connector on Microtome Stage Controller	***Cable***	***Function***	***Max Voltage in connector / cable.***
USB II (B)	3m	Connects to internal USB hub for communication with the X,Y and Z DC motor controllers.	±5V
BNC **Z DigiScan IN**	BNC (F) co-axial	Analogue voltage output ramps to drive the CUT piezo actuator amplifier	±5V
DigiScan I/O RJ45 jack	0.5m. RJ45 to D-type	DigiScan controls the Stroke Up / Down logic status.	±5V

1.6.3. **DigiScan II Connections.**

Table 4 DigiScan connections.

Connector on DigiScan	***Cable***	***Connection to***	***Function***	***Max Voltage in connector / cable.***
DigiScan high density D type	DigiScan loom	DigiScan DDC	External scan control, microtome cutting control, and analog inputs	±10V
Digital I/O D type. **As described in above table.**	0.5m	Microtome stage controller	As above.	±5V
Firewire.	Firewire cable	PC	DigiScan communication	±5V

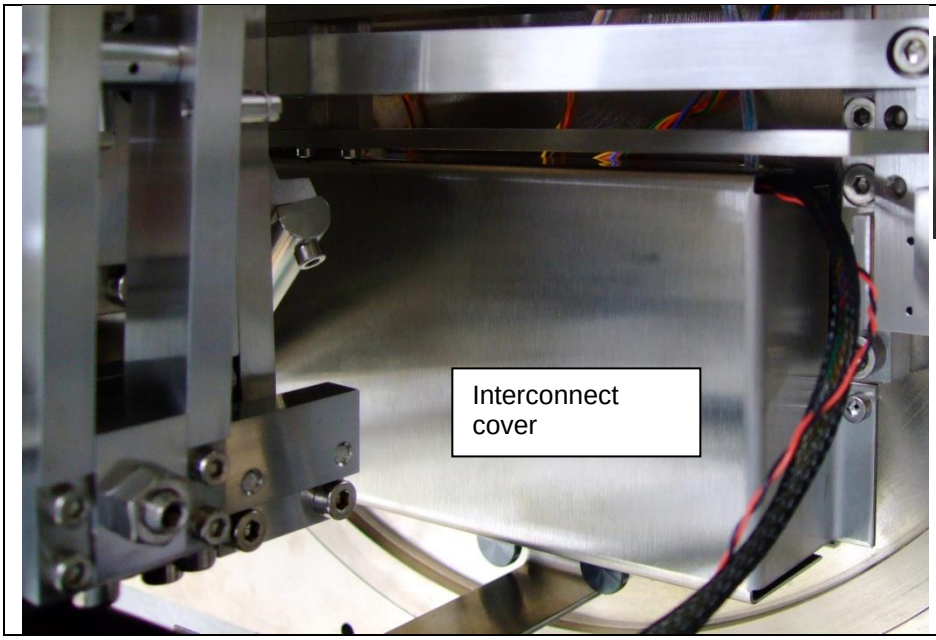
1.6.4. **3VBSED Controller Connections.**

Table 5 3VBSED controller connections.

Connector on 3VBSED controller	***Cable***	***Connection to***	***Function***	***Max Voltage in connector / cable.***
Pre-amp	Part of loom between BSED controller and pre-amp.	Pre-amp on chamber	Provides power to pre-amp	15V
Analog in.	"	"	Senses amplified signal from pre-amplifier.	500mV
V bias.	"	"	Sets bias on diode.	16V
Comms	RS232	Port 1 on PC	Provides Serial PC control of BSED controller.	5V

1.7. Protective Covers.

The 3View2XP system is manufactured with protective covers to all electronics components which are not inside a controller box. These remain in place during operation. These are located on the vacuum side (inside) of the 3View2XP door.



Near / Clear motor cover.

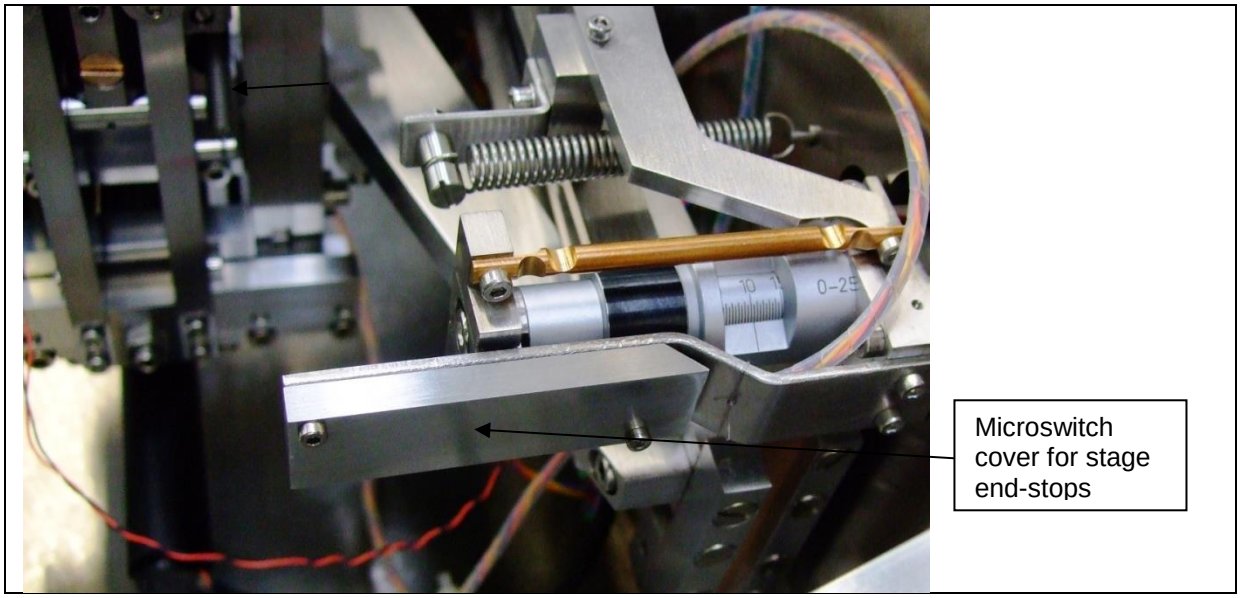


Figure 6. Protective covers on Y motor end stop microswitch.

Caution. ***The 3View2XP system is shipped in a state where the movements are locked for safety.***

Some of these locking mechanisms will be under the covers. A service engineer will unlock such components as part of the installation procedure. The system should not be moved from the laboratory from where it was installed. In case this is required, please contact Gatan for advice on installing appropriate locking mechanisms for protection from vibration.

1.8. Protection against Electric Shock.

2 co-axial cables extend from two SHV connectors on the rear of the Microtome Stage Controller, labelled “STROKE” and “CUT”, and are connected to two more hermetically sealed SHV connectors labelled “STROKE” and “CUT” on the stage door.

The co-axial cables and SHV connectors each contain a high tension voltage of up to 150V DC, with a maximum current of 40mA. The SHV connectors on the controller, cable and stage door, plus the internal interconnect as shown in figure 7, do not allow access to the high tension with a finger. The external electric hazard warning is not duplicated on the interior of the door but is also applicable to the internal high tension cabling and connectors.

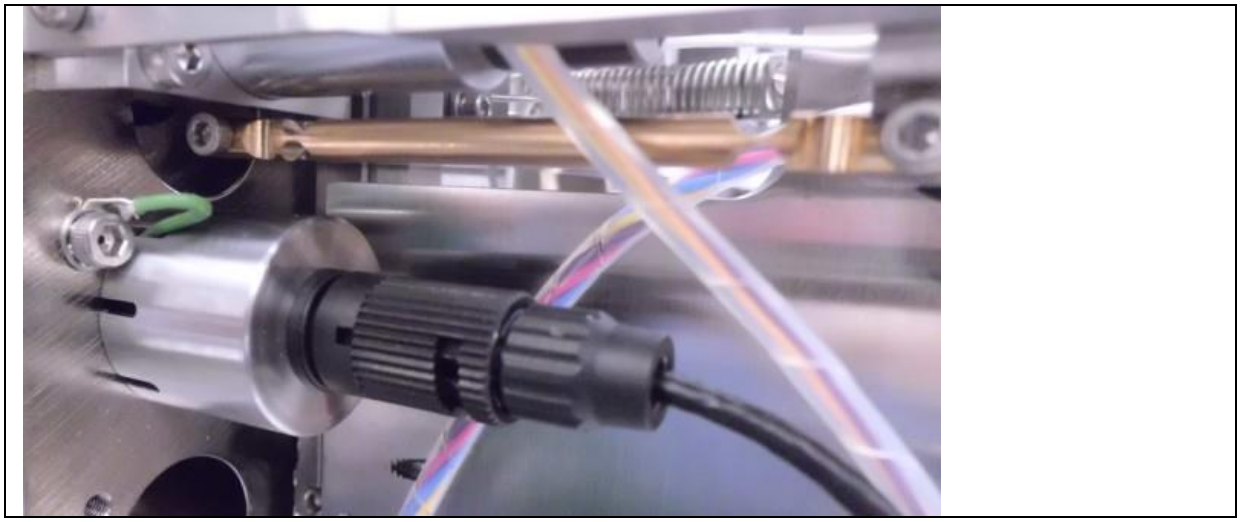


Figure 7. Internal cover and interconnects of the STROKE and CUT cables.

On the inside of the stage door, the user is protected from the high tension by a protective metal cover over each “STROKE” and “CUT” feed through. Users should not remove the interconnect, or the metal cover which protects the feedthrough.

The High Tension forms a Class 2 protection hazard as they are not earthed.

Warning: ***For safety reasons, do not interfere or remove this sheath over the HT wires which extend from the SHV feed throughs to the piezo elements on the microtome.***

None of the other signals in the cables or connectors of the whole 3View2XP system including controllers and stage door contain any electrical hazard. The maximum voltage in any of the other 5 cables from the Microtome stage controller to the stage door is 15V.

1.9. Working Environment.

The 3View2XP system is intended to be operated in a laboratory type environment suitable for safe operation of scanning electron microscopes.

Warning*. Do NOT operate the equipment in rain or excessive moisture environments. The equipment is not designed to be water or splash proof. Take care not to spill any liquids near the system*

1.10. Cleaning Protocol.

There is no strong cleaning requirement for the 3View2XP door and microtome assembly, the 3View2XP Microtome Stage controller, the DigiScan or the BSED controller unit. In the event that they need cleaning, wiping the exterior, but not the internals or back panels, with a lightly damp cloth will suffice.

As with all equipment intended for an electron microscope chamber, it is recommended to wear protective gloves when handling or touching components internal to the chamber vacuum.

The 3View2XP microtome creates very fine slices of material which are typically resin for the many 3View2XP applications. Standard Microtomy guidelines identify that caution is advised when handling powder from cut resin. The 3View2XP microtome does not create powder.

Sample waste accumulation

Hazardous Material Handling Precautions Associated with Sample Residue.
Each sample slice generated by 3View2XP may be expected to contain significantly less than 0.25nanograms of staining chemicals. The residue from sample slicing that may accumulate within the equipment will present no greater exposure risk to the user than the sample preparation process. During installation and removal of the sample, and the removal of any visible residue, follow the same safety precautions as during sample preparation.

- Refer to local safety procedures for handling and disposal of staining chemicals
- Ensure the correct Personal Protective Equipment (PPE) is worn
- Follow local requirements for disposal

1.11. Safety Precautions due to Weight.

Caution: ***The 3View2XP and SEM stage doors are heavy, (up to approximately 50Kg). Seek help before attempting to remove or re-install a SEM or 3View2XP door. This operation is NOT recommended for individuals.***

The 3View2XP door is normally attached to the SEM in an identical manner to the design of the SEM door that it replaces. This includes a safety withdrawal stop device. When attached it stops the stage withdrawing too far from the microscope chamber. This withdrawal stop may be fitted to the rear of the SEM where the rails poke through the bottom of the metalwork. Replacing the SEM stage with the 3View2XP stage requires removing and re-attaching this withdrawal stop device.

Caution: ***Do not leave a 3View2XP door or SEM door unattended without the withdrawal stop device fitted.***

A system that seems apparently stable may tend to fall forwards in case the gas levelling platform reaches the extent of its correction as the door opens fully. The SEM or 3View2XP replacement door should also be held manually in case the withdrawal stop device is temporarily removed.

Caution: ***The 3View2XP stage is supplied with 4 side handles to aid lifting. These can be detached. The motor test covers are not intended as handles for weight bearing. Using these to bear the weight may damage the vacuum integrity of the stage door.***

Caution: ***The SEM stage which is being swapped out will be a similar weight to the 3View2XP system. This may or may not have handles aiding movement, and may or may not have cautionary symbols. Users should take responsibility for their own safety when handling heavy components associated with the SEM.***

Handles screwed into the side are intended for lifting tasks. These can be unscrewed after the door has been successfully attached to the SEM. However, this is at the user's discretion.

The motor covers are not weight bearing handles. Always use the door handles on the side.

Figure 8. Identification of motor covers.

Depending on the stage door design, one of the 4 side handles may need to be be removed when the optical microscope mount is attached to the side of the door. This should be stored and re-attached when moving the stage. Washers should be present between the handles and the door to protect the paintwork of the door.

Depending on the stage door design, one or two rollers may be attached to the door. This can relieve bending pressure on stage rails when the door is fully open. They are not intended to touch the table plinth when the door is closed as this could affect the vibration isolation of the system.

1.12. X-ray safety.

The 3View2XP system is designed for electron microscopes operating at a maximum beam voltage of 30kV. X-rays are only generated when the 3View2XP door is fully closed and held secure by the presence of the vacuum in the SEM chamber. When the door is fully closed, the steel door and steel motor covers block X-rays generated at the specimen area to a level below that which is deemed safe by Gatan and below prescribed international standards.

X-ray protection through the BSED pre-amp is integral to the design. If the unit contains tamper proof screws and an X ray warning label on the inside of the lid, then the SEM should not be employed with this lid removed.

2. System Overview.

The Gatan 3View2XP is a second generation serial block face imaging system. The Gatan 3View2XP allows automated acquisition of 3D ultra-structure by sequentially imaging a freshly cut, block face. Furthermore the automated acquisition can take place over multiple regions of interest of different dimensions or over a montaged area, thereby providing a powerful tool for collecting data over considerable volumes. Using this technique the spatial resolution provided in the Z does not degrade with depth. Unlike other 3D imaging techniques, these systems offer electron microscopy resolution in X,Y and Z while allowing a field of view comparable to light microscopy.

The User's Guide covers the basic functionality of the 3View2XP system, with additional details covering the optional 3VBSED, Gatan's Back Scattered Detector. The overview chapter covers individual components of the 3View2XP hardware, and some background to their design and usage. Following this there are chapters covering software, the approach and cutting protocol, advice on specimen preparation and a final trouble shooting reference.

2.1. The SEM and Imaging Conditions.

Most users will be familiar with the traditional operation of an SEM where for high resolution work, short working distances are required. The 3View2XP system is designed to facilitate short working distances whilst still allowing the presence of a solid state back scattered detector, and the diamond knife to pass between the specimen and the bottom of the pole piece. For a 3View2XP aquisition the Gatan system takes complete control of the SEM, including scanning the electron beam, as well as the Gatan hardware. Users may still wish to use the SEM user interface during the setup process.

For Gatan Microscopy Suite 2.3 and later the Gatan software offers the user the choice of automated fine tuning of the focusing and stigmatism correction. Although the working distance and imaging conditions should not alter throughout and extended experiment, this facility is a powerful tool at ensuring that the highest resolution is not lost during extended automated acquisitions.

For electrically conductive specimens, then high vacuum conditions are normally favoured. For partially conducting or insulated specimens then 3View can be operated in low vacuum conditions as a way of coping with charge build up is obviously SEM dependent. For stained resin embedded biological specimens where the highest resolution imaging is sought in 3D, it is more common practice to optimize back scattered electron contrast through ideal specimen preparation, and then apply low injection conditions in high vacuum mode. With care a charge balance condition can be achieved whereby adequately stained areas show high resolution without charging artefacts. With suitably stained specimens this approach can provide higher resolution than applying low vacuum techniques to cancel the charge.

2.2. The Microtome

The 3View2XP microtome is attached to a replacement SEM door and X,Y stage. The 3View2XP microtome is different to traditional microtomes in that the specimen stays fixed in the horizontal plane and to perform a cut, the diamond knife moves across a stationary specimen. Between cuts the specimen increments higher towards the pole piece of the SEM and this increment defines the cut thickness. The cutting plane is the SEM working distance and this does not vary during an experiment. The Microtome design has inherent stability as required by the automated acquisition of sequential slices. The specially designed X,Y stage moves the microtome with precision as required when the automated acquisition is extended to multiple areas.

The Microtome is attached to the SEM replacement door and X,Y movement assembly. The mechanical method of attachment is via 4 screws in slotted holes. The Z height of the microtome and the cutting plane is defined by the diamond knife cutting plane and not pre-determinted by the height of the adjustable specimen holder block.

Caution: ***It is recommended that only service engineers adjust the Z height of the microtome and cutting plane.***

Caution: ***It is recommended that only service engineers remove the microtome from the 3View2XP stage door. Electrical disconnection requires access to the metal enclosure which covers the electrical feed throughs. Removal of this structure requires partial disassembly of the supporting arms which hold the microtome to the translation arms.***

It is recommended that users understand the mechanism of the Microtome as this will facilitate safe practice. The mechanism of the microtome is best understood with reference to the actuators which perform specific actions as explained below.

2.3. Cutting and Retracting of Diamond Knife.

The diamond knife is positioned above the specimen, whilst the knife arm pivot leaf spring is close to the base of the Microtome unit. The cutting operation is performed by the piezo pushing against the force of the retraction springs.

Note, great care is required to ensure that the diamond knife never cuts more than 250nm of specimen. Although diamond is strong, the very sharp diamond knife is relatively fragile.

Caution: ***The force of the retraction springs on the knife arm can be sufficient to squeeze, but not harm a finger placed in the gap. There is no reason to place a finger or other implement in the gap between the knife arm and the microtome body.***

In this manual and in the software, the term “CUT” and “CUTTING” are used to denote that the diamond knife moves in a cutting direction. Of course if the specimen is too low, then no actual cutting of the specimen will take place, and if the specimen is too high, then there is a danger of breakage. The 3View2XP software presents the user with warnings, but there is no interlocking process to ensure that the knife cannot perform either a cut or a retract operation in this unsafe condition.

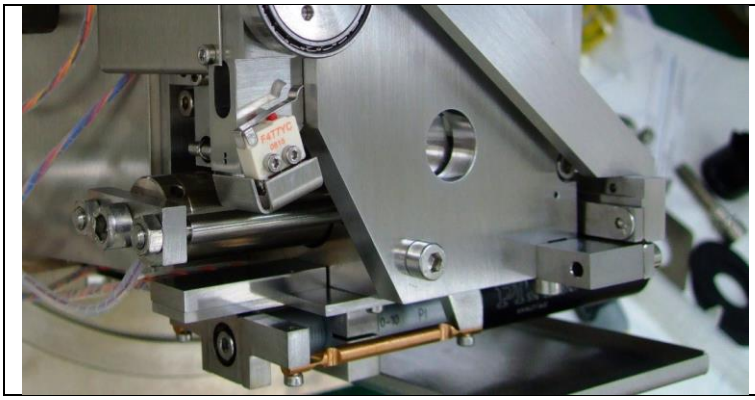


Figure 9. The knife piezo actuator.

The knife piezo actuator is horizontal and central in this figure.

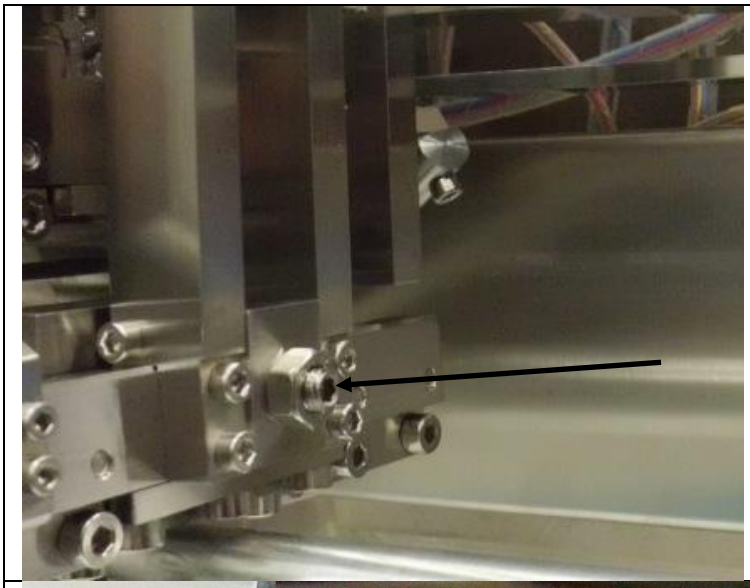
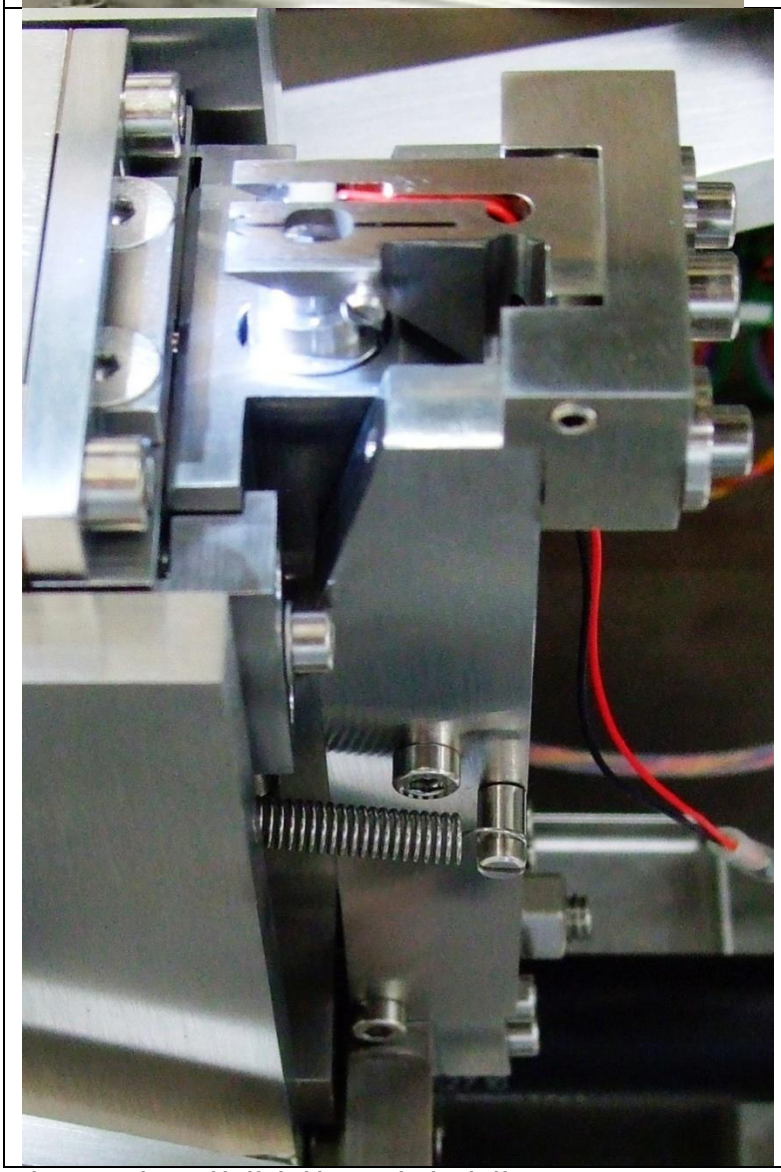


Figure 10. The leaf spring pivot of the diamond knife arm.

The knife piezo actuator pushes against the larger locked screw central on the pivot joint.

The screw insertion position of this locked screw provides coarse adjustment of the knife arm pivot with respect to the stationary specimen block holder.



In this photograph the knife arm is in the retracted position and the 2 return springs are least extended.

The red and black wires are connected to a miniature piezo element which is glued and clamped into the diamond knife holder.

The high frequency vibration of this element can be heard when the stroke is high and aids the cutting process.

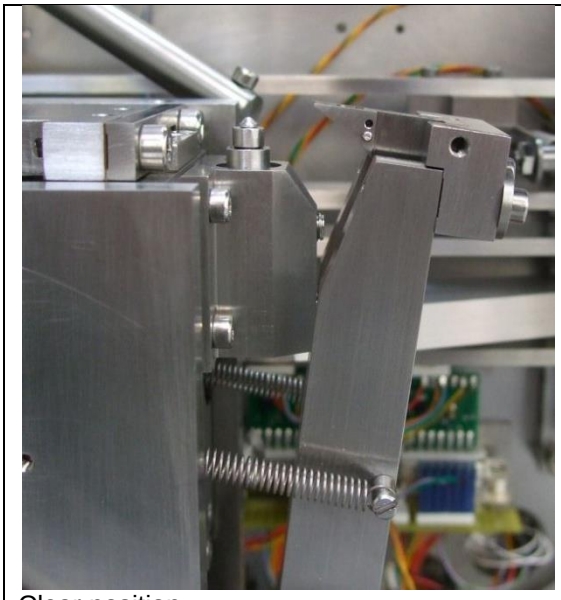
Figure 11. Diamond knife holder attached to knife arm.

Software controls the cutting speed and retracting speed.The knife piezo actuator pushes against a central, lockable screw head which, when unlocked, has coarse positional adjustment. This is shown in the above figure. This adjustment is used to manually set the knife arm position. This can be adjusted whatever the position of the knife piezo actuator. However, adjustment of the back position should allow the full actuator movement of the knife to cover the specimen width.

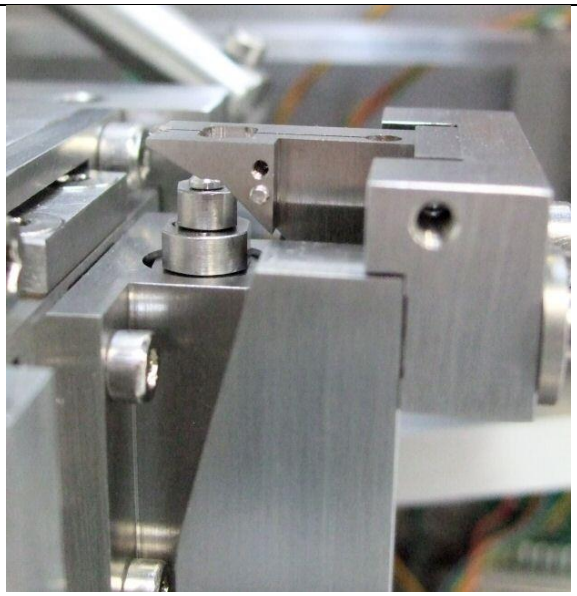
Use great care when making this adjustment to ensure that the specimen is lower than the diamond knife. The maximum movement of the diamond knife by the knife piezo actuator is 1.2mm. However, this movement is only over the specimen position when the Near / Clear motor places the knife arm in the “Near” position. When the motor is in the Clear position, the piezo actuator has no additional movement on the knife.

The Yellow LED on the front of the controller shows the Cut actuator status. It is illuminated when the knife is in the cut position, and off when retracted. The intensity ramps in between accordingly.

2.4. Nearing and Clearing of the Diamond Knife.



Clear position.
Default position for imaging.
Knife is not visible to electron beam.



Near Position.
Imaging is possible when retracted, (not CUT) but with some compromise, e.g. alteration to astigmatism.
Knife and shavings may be visible at low magnification.

Figure 12. The Clear and Near Positions.

The terms Nearing and Clearing denote movement of the diamond knife arm into the Near and Clear positions respectively. In the software this is shown as a ticked, or unticked CLEAR box. This is a manual request, but also takes place automatically during an acquisition. It is also shown as BLUE LED indicators on the front of the Microtome Stage Controller. The diamond knife holder arm pivot spring allows movement of the arm over a considerable span. This allows the Near / Clear motor to move the arm into one of 2 set positions.

When in the Clear position, the diamond knife position is completely cleared away from the specimen, imaging area and pole piece. This is the default position for imaging during a 3View2XP acquisition. This geometry allows the imaging process to take place after the cutting / clearing operation as this reduces the probability of cutting debris seen on the specimen surface during imaging.

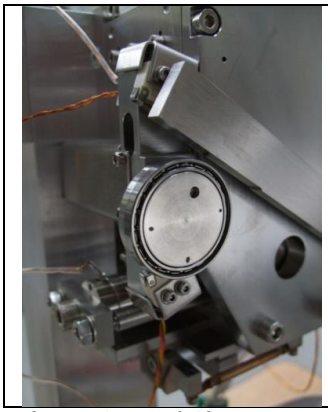
	Users should not be required to make any adjustment to this mechanism.

Figure 13. Near \ Clear motor and side arm.

2.5. Stroke Raising and Lowering of Specimen.

A second piezo actuator is used to automatically raise and lower the “stroke” position of the microtome’s Z position of the specimen. This is termed the stroke piezo and is used to automatically lower the specimen when the knife is reversing across the specimen. The 3View2XP software automatically ensures that the stroke is lowered during the following processes.

All Nearing and Clearing movements.
Diamond knife retraction.
Imaging as part of automated sequence.

The stroke piezo is raised only in certain conditions.

Cutting operation.
Manual raise request, outside of specimen Z advance requests.

As the stroke is only in the up position for cutting and not imaging, focusing of the image takes place with the stroke in the default lower position. The full stroke movement is set at a default value of 7microns. The Stroke LED on the front of the Microtome Stage controller is fully lit red when it is the UP position (and potentially dangerous) and fully extinguished when in the lower position.

Note the vibration of the piezo in the diamond knife holder can be configured in the software. When the oscillator box is ticked in the software, the vibration is automatically turned on when the stroke is high, i.e. when ready to cut.

Imaging is not meant to occur during the cutting or when the stroke is high. The vibrating piezo causes a high frequency electrical oscillation that will introduce artefacts in the image. This is standard behaviour.

IF THE STROKE IS UP AND THE PIEZO VIBRATION ENABLED, SEM IMAGING WILL NOT BE POSSIBLE. FOR IMAGING PURPOSES, UNTICK THE STROKE UP BOX IN THE APPROACH TAB OF THE 3VIEW CONTROL WINDOW. ALSO, DO NOT FOCUS THE SEM WITH THE STROKE UP.

2.6. Specimen Z advance motor.

The specimen Z advance motor controls the vertical height of the specimen with respect to the fixed plane of the diamond knife, and hence controls the cut thickness.

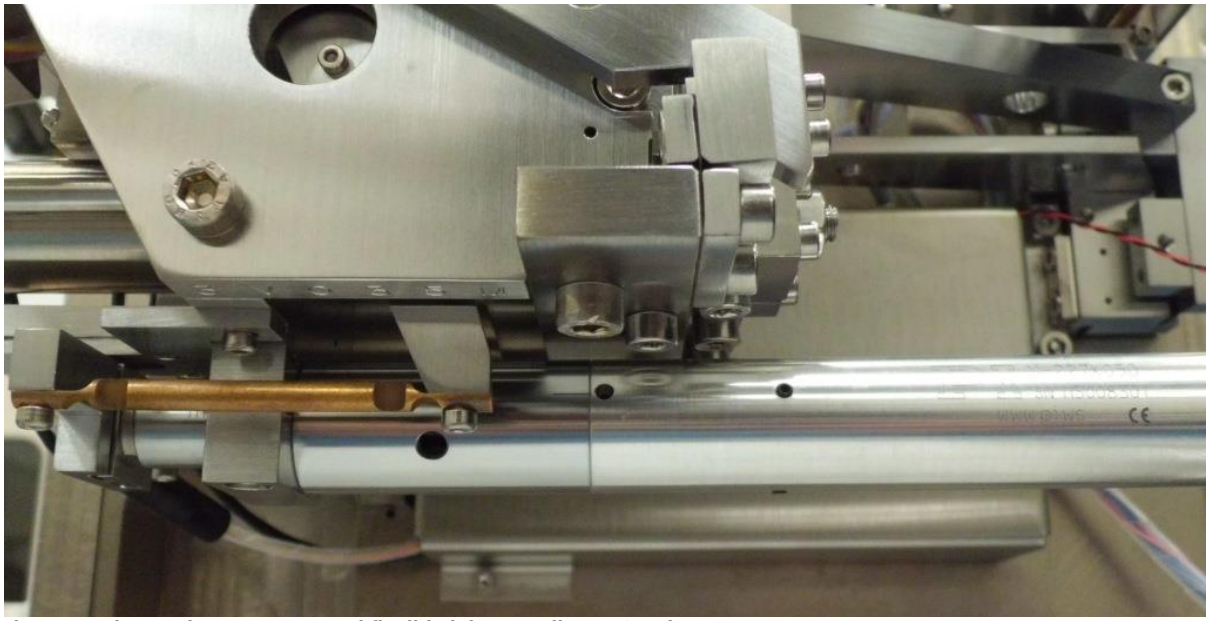


Figure 14 The Z-Advance motor and flexible joint coupling to 2 swing arms.

The advance and retract of the Z motor is controlled in the SPECIMEN STAGE section of the approach tab in the 3View control window. The travel limits are defined by the limit switches at the motor head. When DigitalMicrograph software is started, this motor does not move. The typical full range of the Z travel is approximately 700microns. When the Z motor moves, this is reported in the software. There is some visible / audible motion of the drive screw when the system is at air.

The specimen advance motor moves in the following instances.

- Manual specimen raise / lower requests. This is achieved using the Lower or Move to buttons.
- Automatic advance requests as part of a cut acquisition.

There are 2 types of automatic cut requests. With each the number of cuts, the cut thickness and cutting and retraction speed are configurable parameters.

(A) Use the Start button from the Approach panel. This cuts the specimen with no clearing of the knife and no imaging.
(B) Use the Start button from the Record panel. This cuts the specimen with automated clearing and imaging. Without this routine there are options for single ROI imaging, multi ROI imaging, and automated montaging.

2.6.1. **Software Procedure for Homing Z Advance Motor.**

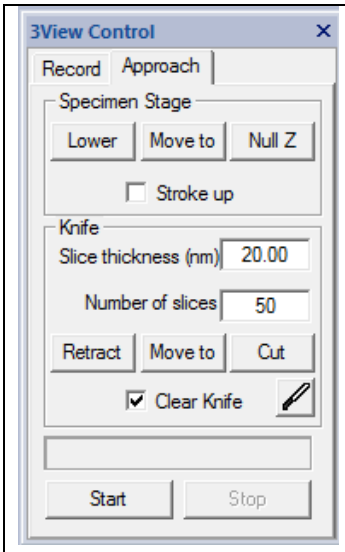
	Press Lower in the Approach Tab of the 3View Control panel. This drives the Z motor to its lowest position which increases the clearance between the top of the specimen and the knife cutting plane. At this position, press Null Z. This sets the lower position to Zero microns.

Figure 15 3View Control Approach GUI

2.7. The Diamond Knife.

Caution: ***There should be no reason to physically handle the sharp tip of the diamond knife.***

Caution: ***The diamond knife is easy to break if the correct procedure on cutting and the staged approach sequence is not followed. The diamond knives are not covered under warranty. The resharpening leadtime may be considerable.***

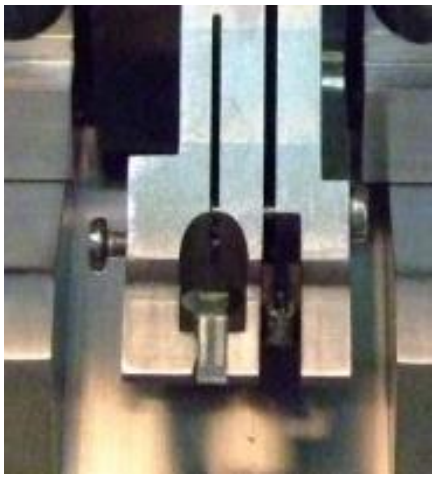
All mounted diamond knives are shipped from the factory under a strict documented quality control regime. Each mounted knife is observed and recorded as having cut a pristine surface using the 3View stereo zoom optical microscope. The 3View2XP certificate of quality which accompanies each 3View shipment testifies this.

Customers who purchase a resharpend knive will be provided with a swap out resharpened knive rather than their own unit unless specifically requested. However as the inventory of resharpened knives is not controlled, the lead time on this can vary considerably. If users are concerned about mitigating downtime, they should consider purchasing more than one spare mounted knive.

The diamond knife is a custom component designed specifically for the 3View knife holder and configuration in the SEM. The 3View diamond knife has a special coating to aid the process. For example it is not optimized for collecting the microtomed shavings from the block. The tip and presentation angle of the diamond knife is specially designed for cutting the block face, rather than collecting the cut sections. The diamond knife is held secure by clamping opposing symmetrical flat faces of the knife structure. 3View2XP is not designed to accept glass knives.

Users are not expected to mount, dismount or adjust the diamond knives in their holders. They are expected to clean them if required. Details of the mounting / unmounting process are given in the field service manual.

	The diamond knife holder is seated and clamped upside down in the secure mount. The clamping screw is shown and in this photograph is from right to left. The clamping screw should be tight to hold the

 **Figure 16. Diamond knife clamped in the holder.**	diamond secure. However, because the vice is a 3 point touch, and the screw exerting a closing force is closer to the flexure point, over tightening may result in weaker clamping.

Caution: ***The diamond knife is a fragile component which can be easily damaged. Only gas and a special polystyrene tool should be employed to remove debris.***

Caution: ***The clamping screw should be tight to hold the diamond secure. However, because the vice is a 3 point touch, and the screw exerting a closing force is closer to the flexure point, over tightening may result in weaker clamping.***

Caution: ***The diamond knife holder should only be removed from the microtome when the system is in the Clear position.***

Imaging of the knife tip and shavings are not recommended. In normal acquisitions, the beam is blanked when the knife passes over the specimen in order to avoid exposure of the knife tip and shavings to the electron beam. This is because the electron beam can burn very small volumes of the shavings onto the tip, which then serve to scratch the block.

Severe damage to the knife is normally clear if the tip is viewed in the optical microscope. Any damage is normally seen as a source of light from the tip, and will appear different to any residual sharp components.

Due to the fragility of the knives, they are consumables. However, with care they can last years. Users can purchase spare knives in their pre-mounted holders so that exchange is very simple. Gatan can re-sharpen knives in which case the knife needs to be returned in its holder.

2.8. Diamond Knife Holder.

In normal operation, the diamond knife holder sits securely on the diamond knife pivot arm and is held in place with 2 spring-loaded locking screws. Note, X,Y translation of the stage does not move the specimen independent of the knife and microtome.

Lateral translation of the knife holder to the support arms allows the user to choose which part of the knife to use, in case the specimen width is significantly smaller than the knife. This can extend the useful life of a damaged knife in case the damage is localized. The diamond knife holder should be flush with the defining ridge at the top of the knife arm when the spring-loaded screws are tightened. If this is not the case, then the knife can present an ill defined tilt angle to the specimen. The spring-loaded screws raise the diamond knife holder slightly when they are undone, and the holder can be simply lifted from the slotted holes.

The diamond knife holder contains grub screws on either side which adjust the tilt of the central section with regard to the main component. Users should not adjust these screws as they affect the off-axis tilt of the knife and this will affect whether the cutting plane is parallel to the focal plane of the microscope.

When attaching and detaching the diamond knife holder, e.g. for cleaning purposes, detachthe red / black cables at the connector. Ensure the flying lead does not get caught in an O ring when closing the door.

Figure 17**. The outer allen washer screws on the knife arm.**

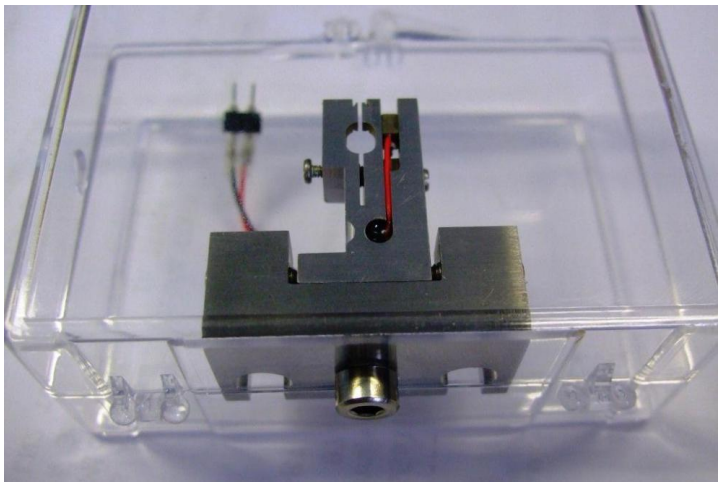


Figure 18. Diamond knife holder in plastic box

Note, the screw securing the unit to the plastic box is required as part of the assembly once removed from the plastic box.Please re-attach the central screw to use.

2.9. Secure Mounting Block.

A 3View2XP is supplied with two mounted diamond knife holders and a single secure block. The secure block allows the diamond knife and holder to be stored and shipped safely and fits snugly inside a plastic container with a screw top lid.

The diamond knife holder is mounted upside down and clamped in the secure block. This allows for safe access to the diamond knife for cleaning operations.

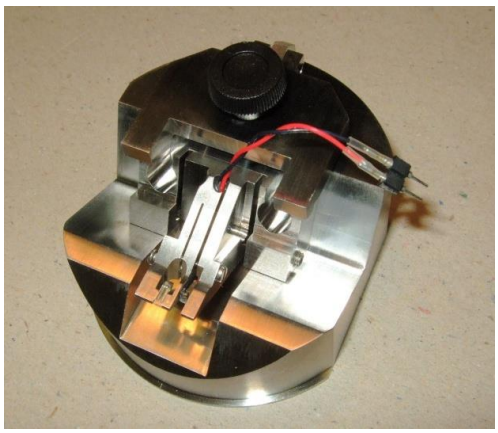


Figure 19. Diamond knife holder in the secure mount.

The diamond knife holder sits clamped upside

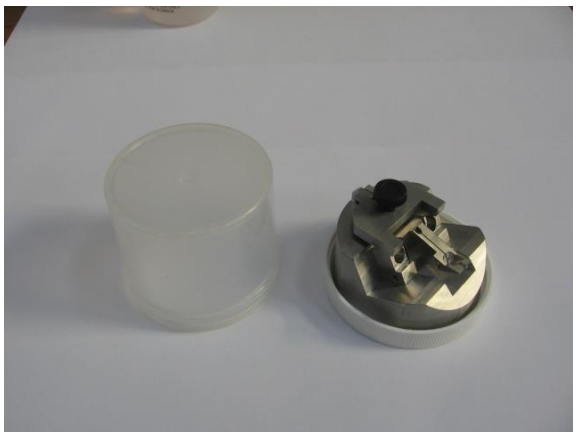


Figure 20. Shipping and storage container for the knife holder secure mount.

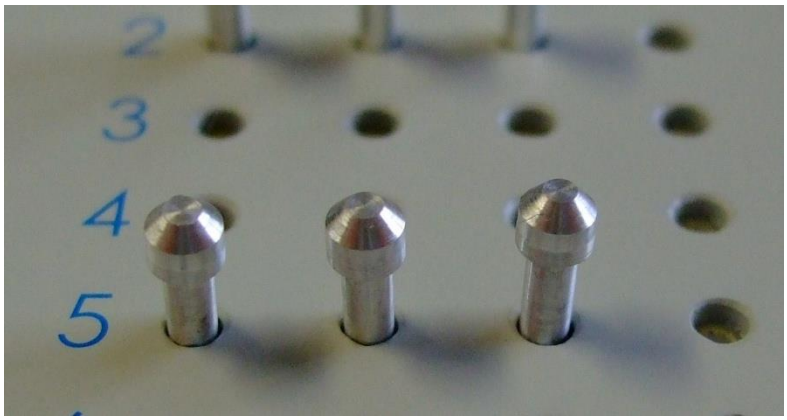
down in the secure mount. This provides access to the diamond knife and clamping screws.	The top opaque cover screws tight onto the white plastic lid base.

2.10. Specimen Holders

Gatan supplies two types of “rivet” style specimen holders to accommodate different height specimens. Both are flat headed. Plexi-glass rivets are an additional price list item from Gatan. These are transparent and suitable for illumination from below when performing the pre-preparation.

The user should choose rivets of type Gatan part number PEP6044 in order to work with relatively thin specimens, e.g. <700microns tall. The top face of this rivet sits higher in the holder appropriate to the Z movement of the holding carriage such that the specimen is at the correct cutting plane of the knife. The user should choose rivets of type Gatan part number PEP6590 in order to work with relatively thick specimens, e.g. >700microns tall. The top face of this rivet is a wider circular area. Such a wider base is appropriate for a taller specimen since an aspect ratio of a tall building would present excessive flexure during cutting.

The two types of rivet are distinguishable by their shape. Users should not confuse the two as this will lead to an inability to cut the specimen due to the limited Z movement of the holding carriage.

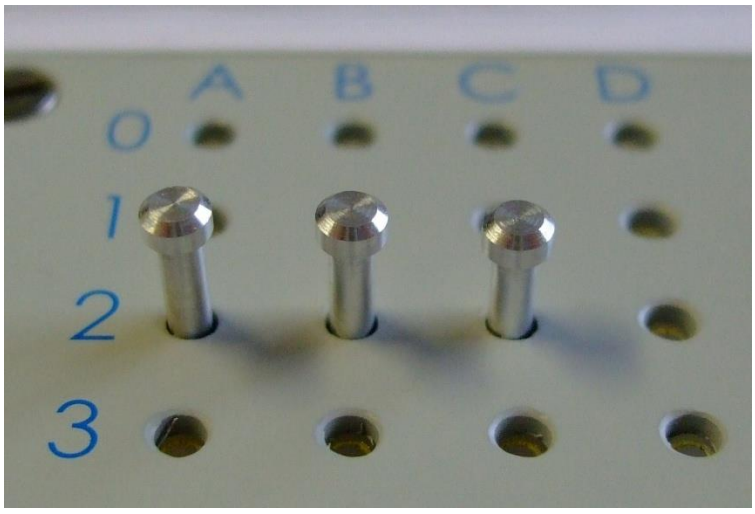


Gatan part number PEP6044

These rivets are designed for working with relatively thin specimens. They are more conical shaped and have a smaller area flat top.

Do no mount a tall thick specimen on these rivets.

Figure 21 Rivets for shorter, thinner specimens.



Gatan part number PEP6590

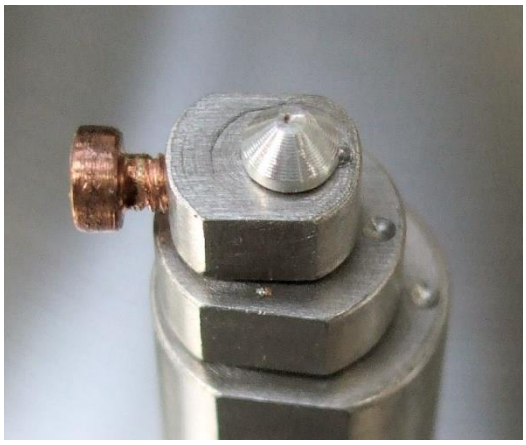
These rivets are designed for working with taller or thicker specimens. They are less conical shaped and have a larger area flat top.

Do not mount thin specimens on these rivets.

Figure 22 Rivets for taller thicker specimens.

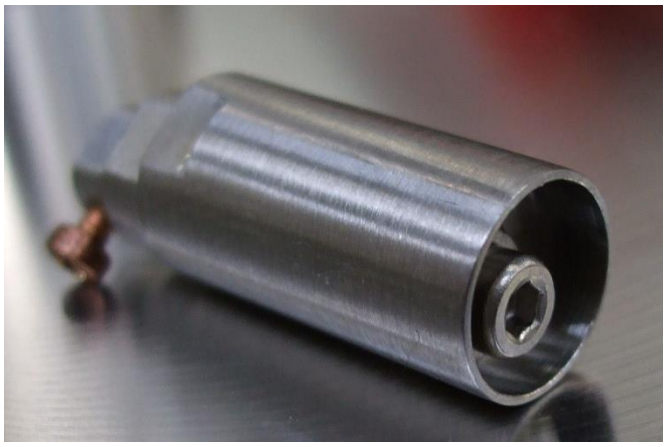
An Aluminium block for accepting rivets is an option in the price list. This is intended for baking operations.

A rivet is mounted in a rivet holder. This comprises 3 off-centric cylinders which allow lateral movement of the rivet with respect to the outer cylinder whilst keeping rotational symmetry of the specimen, and a fixed position of the rivet locking screw. The concentric cylinders are locked using a screw from the underside.



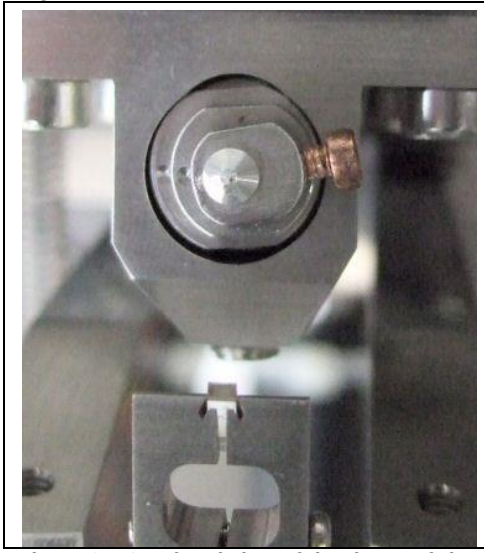
The 3 dots allow users to control the rotation of the off-set concentric rings.

The screw to the side clamps the rivet in place. This must be tight before cutting takes place. However, do not over tighten as the screw is relatively soft with limited thread.



The concentric rings of the holder are adjusted by slackening the locked screw on the underside. This must be tightened after adjustment.

Figure 23. The rivet holder.



This photograph is taken with the rivet holder secured in the microtome specimen mount. The blunt higher end of the diamond knife is visible in this photo as the knife is in the Clear position.

The specimen holder can be inserted with 180deg rotation symmetry depending on which configuration provides alignment with the zoom optic axis of the SEM as mapped by the bench top stereo zoom microscope.

Figure 24. Overhead view of the rivet and rivet specimen holder.

One additional conical shaped specimen holder is supplied with the system. This contains a grub screw to hold a rivet or other specimen in place.

The specimen holder is designed to grip a 3View2XP type rivet to allow trimming of the rivet on a bench top microtome. This specimen holder could be used in the 3View2XP system, but without concentric rings does not provide any horizontal translation adjustment.

A flat topped holder with a larger aperture and side grub screw for securing a standard Agar style specimen holder is included. This is typically used for gold on carbon resolution specimens.

Figure 25 Conical specimen holder.

⚠ **Caution:** ***The coarse adjustment and locking should not be used when the clearance between the knife and the specimen / specimen holder is not understood. Once it is understood, (i.e. the reflection of the knife can be seen in the specimen surface) then with practice it can be used for a controlled approach. However, it should not be employed for fine adjustments as this should only be performing using the computer controlled Z advance motor.***

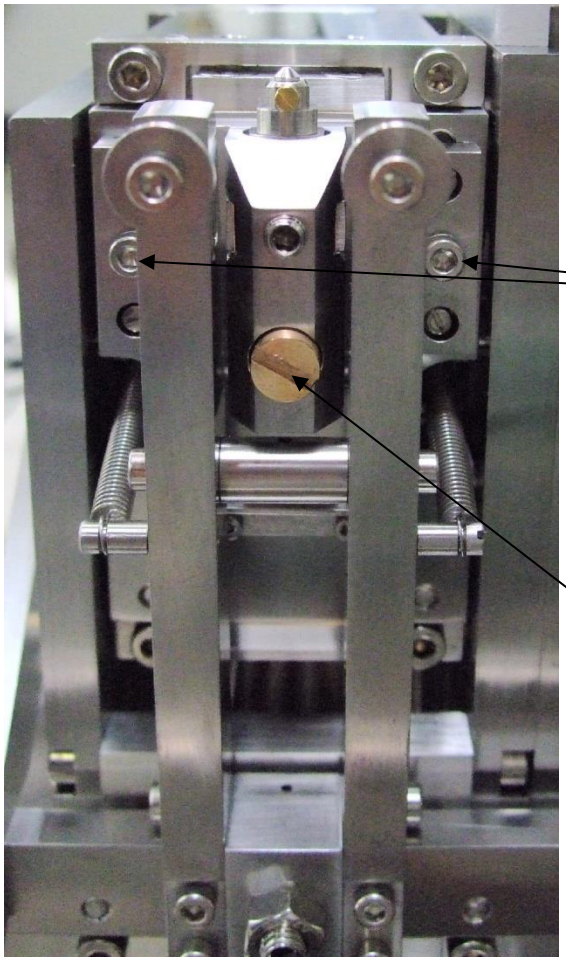


Figure 26. Adjustment and locking screws on the specimen holder mount.

Coarse Z adjustment and locking screws on the specimen holder mount. This mount is sprung loaded to allow precise movement without backlash.

⚠ ***Caution:*** **2 Allen screws MUST be slackened for adjusting the Z height of the mounting structure using the central flat bladed screw and tightened again once the correct coarse height is achieved. Failure to follow this protocol may damage the mechanism.**

Screw for adjusting Z height of specimen holder mount

Caution: ***When any cutting is performed it is very important that the specimen is secure on the rivet, the rivet clamped tight in the rivet holder, the rivet holder concentric rings are clamped tight using the underside clamping screw, and that the rivet holder is clamped securely in the specimen microtome mount. In case any of these are not secure then damage may occur to the knife in case the knife is presented with a specimen which can tilt with the force of the cutting blade. This scenario is dangerous for the knife and specimen. Cutting should only take place when specimen and knife are rigid and their respective positions finely controlled by the computer driven actuators.***

2.11. Stage door and X,Y translation motors.

The 3View2XP microtome is supplied on a custom made replacement SEM stage door which includes a special X,Y translation mechanism. The design and shape of this door depends on the SEM chamber in question. This replaces the SEM stage and door, but does not replicate the SEM's 5 axis X, Y,Z, tilt rotate specifications. Additionally, the 3View2XP door, X,Y stage and microtome are supplied in a custom made vacuum shipping vessel.

When closing the door for pump down, care should be taken to ensure that the O-ring and mounting surface is clean, and that no other impediments restrict the door from closing completely. Light pressure closing the door may be required in order to initiate successful pumping. As with all vacuum equipment, a shorter pump down time will be possible if the system has only been vented to dry nitrogen for a short period, and a longer pump down time is expected if the system has been vented for a longer period.

Caution: Caution may be necessary to ensure that the BSED is not damaged when closing the door. Ensure that the detector position has not altered from a safe clearance.

In some instances the 3View2XP may have been purchased with only the 3View2XP replacement door. In others the SEM manufacturer's standard door and stage may have been supplied.

The replacement door containing the microtome is shipped and stored in a dedicated vacuum vessel. This can also be used to store the SEM stage being replaced. Pump out fittings are supplied but not a pump. Customers are encouraged to keep the spare 3View2XP door, or SEM stage door in this vessel and evacuated when not being used. The absolute base vacuum level is not critical.

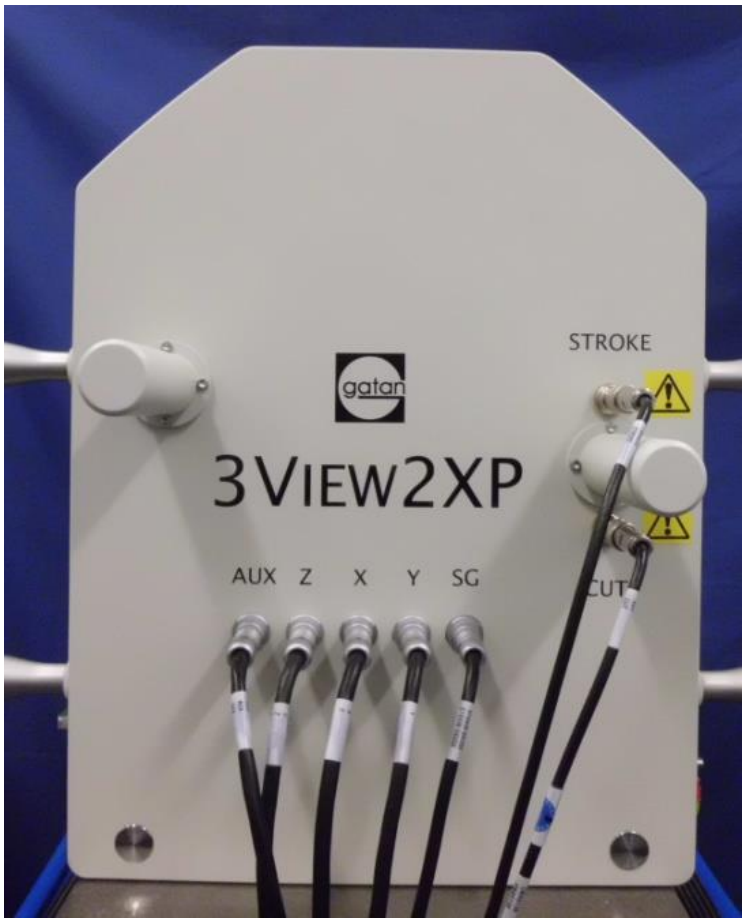


Figure 27. 3View2XP Door on bench top.

For some SEMs, the 3View2XP door will have rollers attached to the side. The heights of these rollers are adjustable and are intended to ensure that some weight is taken by these rollers when the stage is fully open. This provides more stability and protects the bearings. These rollers should not contact the SEM plinth or table top when the door is fully closed as this could affect the vibration isolation of the stage.

For some SEM manufacturers, the software on the SEM computer which controls the SEM stage (which is redundant for 3View2XP purposes) will not be aware that the SEM stage is not present inside the microscope unless the software is configured as such. Such configuration is often restricted to field service engineers. There is no option for the SEM manufacturer's software to control the X,Y position of the 3View2XP stage. Do not attempt to remove cabling to an SEM stage door unless advised by the SEM manufacture service engineer.

If a 3View2XP system is installed on an SEM where the SEM manufacturers own stage is present, the configuration can be swapped back to the default SEM configuration. To do this a certain protocol should be followed as dictated by the SEM service engineer.

⚠ **Caution:** ***For a 3View2XP replacement door which is engineered on rails, the extent of travel to allow the door to open is limited by a washer mechanism at the end of the rail. This is important to stop the unit from falling on the floor. In some microscopes, this washer may be at the rear of the unit. Only remove this washer when required in order to remove the door.***

⚠ **Caution:** ***The 3View2XP and SEM stage doors are heavy and should not be tempted by an individual. Seek help before attempting to move them.***

The stage translation has been optimised to provide the maximum amount of positional stability and location reproducibility, as required by performing multi ROI, or montaging acquisitions. The maximum translation is limited to approximately 1.2mm. This distance matches the width and translation distance of the diamond knife and so covers the maximum required stage movement in an automated experiment.

The X,Y stage translation is not strictly orthogonal and is not matched to the Cartesian space defined by the SEM door or knife. This is due to the lever design which allows large movements of the X,Y motors to map to smaller movements of the structure. The X and Y motors therefore cause the microtome, knife and specimen to describe arcs which are diagonal to the SEM door. When the X and Y motors increase according to the software, then the microtome stage is moved further away from the door of the SEM.

Although the software and this manual refer to the translation as X, and Y, this is not the same X,Y translation as defined by the SEM stage which has been replaced, or by the Cartesian coordinates of the scanned image. In most installations the X,Y translation of the motors is orthogonal to the Z translation (as required), but is rotated by approximately 45degrees. The scanned image shown on the DigiScan should be the same orientation as that shown on the SEM, whilst the X,Y movement is rotated ~45degrees to this geometry.

Backlash is automatically removed during automated montage and multi ROI settings. This ensures that the stage has extremely high reproducibility. Backlash is not automatically removed when the user manually moves the stage forwards and backwards.

2.11.1. Procedure for Homing the X,Y stage.

Like the Specimen Z-Advance Motor, the software remembers the X and Y motor positions when it is exited and restarted. The software gains knowledge of the stage positions when limit switches are referenced. Pressing the Home button performs an auto initialization routine which finds the central position between the limit switches for both motors. If the software is exited and restarted then the last known position is recalled. However, if the software malfunctions and does not exit correctly, then the home function may need to be repeated.

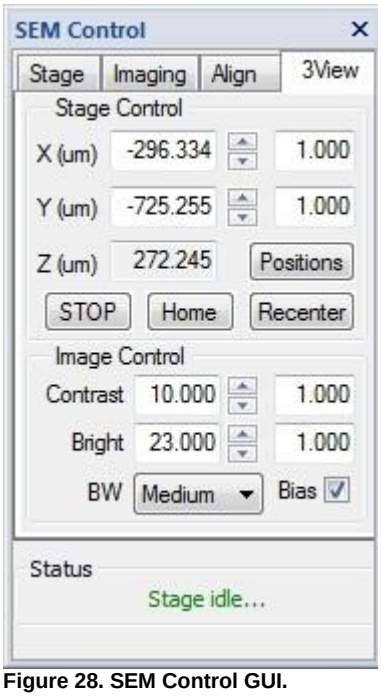


Figure 28. SEM Control GUI.

The 3View2XP system is designed and assembled such that the specimen rivet should be visible at low magnification in the SEM when the stage motors are approximately central. The concentric cylinders of the rivet holder are intended to allow the desired central working position to be set. This working position should not be close to the limit switches of the motors.

2.11.2. Vibration Isolation.

At installation the vibration isolation of the 3View2XP stage will have been checked. In many FESEMs this will simply involve checking that the gas pressure is suitable in the chamber vibration isolation mounts, and that the cable routing doesn't unduly impact the vibration isolation. When the 3View2XP door is closed, the chamber should float freely on the anti-vibration mount. The system is not necessarily expected to be vibration isolated when the door is open.

2.12. Stereo Zoom Optical Microscope.

The stereo zoom optical microscope is chosen to give the most appropriate depth of field as required by viewing the specimen and diamond knife with parallax. 2 sets of eyepieces are provided with the microscope, namely x10 and x15. With the x10 eyepieces the magnification ranges from x 10 to x70, and with x15 from x15 to x135.

Note, only the x10 eyepiece is fitted with a cross hair graticule which is used to map the specimen position on the holder when on the bench top stand. Users may wish to swap to using the x15 eyepieces when performing the approach sequence and viewing the initial cuts.

Figure 29. Optical Zoom Stereo Microscope mounted on SEM door.

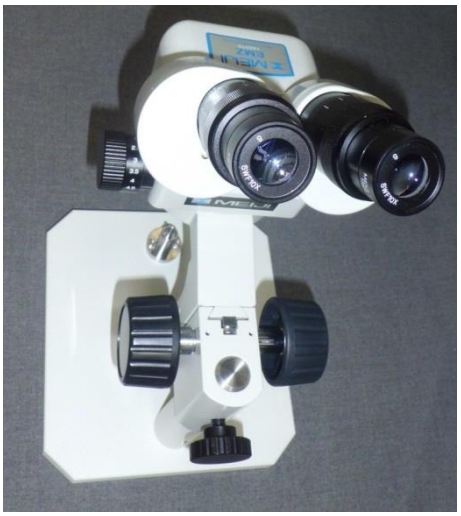


Figure 30. Optical Zoom Stereo Microscope attached to bench top mount.

The optical stereo zoom microscope is provided to be mounted in 2 set locations, attached to the fully open SEM door, and attached to the bench top stand. A fixed location on the bench top stand acts as a mapping tool so that the specimen can be confirmed to sit close to the zoom axis of the SEM once the stage is homed.

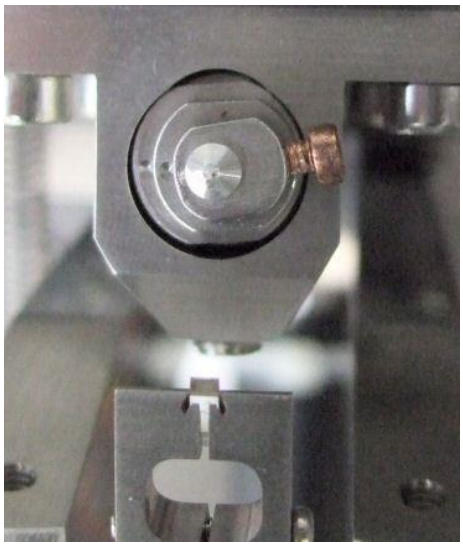
Note, the cross hair position in the x10 eyepiece does not represent the SEM zoom axis when the microscope is mounted on the SEM door. It only references the zoom axis when mounted on the bench top stand when this has been properly mapped. The mounting configuration on the SEM door is only to aid the specimen knife alignment protocol and to view the cutting process.

This mapping from the bench top configuration to the SEM zoom axis can only work if the rotation freedom of the specimen holder, which is inherent in the design, is controlled by the user. The user must ensure that the specimen holder is inserted into the microtome and into the bench top stand in the identical fashion each time. This is easiest to achieve if the rivet clamping screw is always pointing in a defined direction in the two locations. It is clear that if the specimen is not concentric to the specimen holder, then uncontrolled rotation of the specimen in the microtome or bench top will make the Cartesian mapping impossible.

If mapping position is lost, then an alignment procedure, normally initially carried out by field service at the installation may need to be repeated. This mapping routine should be required infrequently.

In case, the mapping from installation is lost, then the suggested method for mapping the bench top stand location correlation to the 3View zoom axis is as follows.

- Use a specimen of unusual shape with some distinguishing features that can be recognized at low magnification between the electron and optical images.
- Home the 3View stage and note where on the rivet (in 2D space) is the zoom axis of the SEM. This is your **Zoom Locator**.
- Move the specimen from the SEM to the bench top stand.
- Adjust the X,Y location of the specimen holder until the cross hairs map to the zoom locator position on the specimen. This may require a larger scale adjustment of the rotation of the post structure using the locking screw underneath.
- Now loosen the screw slightly on the base of the concentric ring holder.
- Rotate the concentric rings until the specimen is centered at the zoom locator. Ensure that the locking screw remains in the same place.
- Make a note of the position of the dimples as a reminder for the next specimen.
- Lock the base of the concentric ring specimen holder.
- Place the specimen in the microscope and confirm you have now mapped the specimen to your zoom axis. The idea is that the specimen is accessible within the stage travel limit.



Choose and record a preferred orientation otherwise the mapping will be lost because the specimen is not concentric to the holder.

Figure 31. The specimen holder in the bench top stand and microtome.

In the simplest manner of operation the microscope mount for the SEM door is designed to sit flush against the corner of the door with only the height of the mount as an adjustable parameter. The mount is locked in place with a screw handle. Do not use excessive force on this handle.

A bright white LED, is turned on automatically when angled forwards, and turned off by a microswitch when folded back. The LED should be off when closing the SEM door. On some microscopes, the LED will hit the pole piece of other detectors when not folded away. The software is not aware of the position of the LED arm.

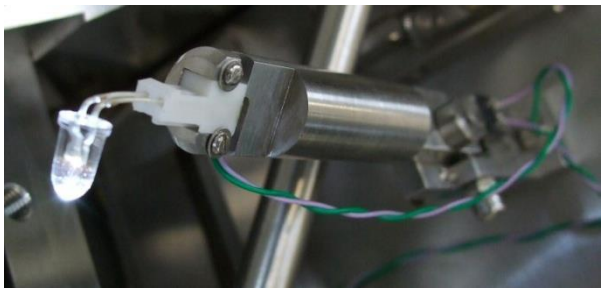


Figure 32 White LED

The bright white LED is on when unfolded to point at the specimen and knife.

This must be closed and therefore off, when closing the SEM door.

Please adjust the exact direction of illumination as required by bending the legs of the LED.

The bright white LED structure is designed to shine light at the rear of the diamond knife. This is in order for the reflection of the tip of the knife to be visible on the specimen surface when viewed with the stereo zoom optical microscope. The position of the LED can be adjusted by adjusting the wire legs of the LED. The voltage driving the LED illumination is not dangerous and connecting the device to the wrong polarity does not harm the light. However, the LED must be inserted in the correct polarity to work.

The stereo zoom microscope position on the SEM door is designed to provide an ideal view of the reflection of the diamond knife tip in a freshly cut specimen surface. (This fresh cut can be from a bench top cutting instrument). Imaging the reflection of the tip can depend on the angle of the illumination from the drop down white LED. The distance between the reflection of the knife tip and the knife tip is an accurate judge of the clearance of the knife from the specimen. However, if the clearance is large, the surface not reflective, or the knife too far forwards or backwards, the degree of parallax offered by this angle makes the clearance difficult to judge. For safe operation, users must be trained to recognize when the specimen is at a potentially dangerous height with respect to the knife, and to interpret the approximate clearance between the two from the reflection of the tip in the specimen's surface. See figure 60 for some example images.

No illumination is provided for the bench top stand. Any simple laboratory illumination will suffice for this microscope.

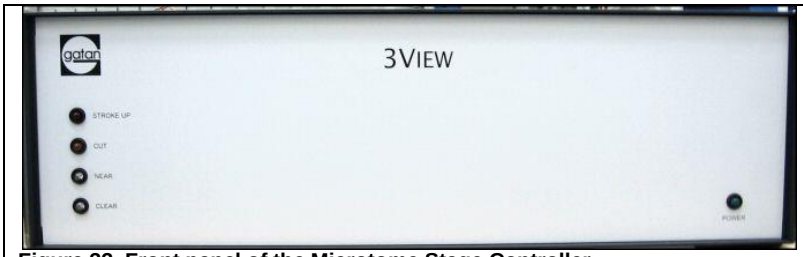
2.13. 3View Microtome Stage Controller.

Please be aware of safety advice regarding the 3View Microtome Stage Controller as detailed in Chapter 1.

Caution. ***Never connect or disconnect cabling from the controller to the door when the Microtome Stage Controller is powered on. This may damage electrical components and such damage will not be covered under warranty.***

The Microtome Stage Controller contains electronics to control the motors in the SEM. The unit is turned on using a switch on the rear of the unit and the power status is indicated by a green light on the front of the unit. It should be left powered on unless it is not to be used for extended periods. When the unit is powered, some of the other coloured LEDs on the left hand side may be lit depending on the status of the motor and actuator positions. Do not block the fan to the rear of the controller as this provides ventilation to the unit.

There are no user adjustable components internal to this unit. Some of the components are field service replaceable. These tasks should only be attempted by a trained service engineer.



A green LED indicates power on the right hand side.

4 coloured LEDs indicate the Stroke, Cut, Near and Clear status on the left hand side.

Figure 33. Front panel of the Microtome Stage Controller.

The figure below shows a basic schematic of the architecture of the 3View2XP system installed on an SEM. The whole 3View2XP cutting and acquisition system is powered by two main controller units, the DigiScan II and the Microtome Stage Controller, whilst a third modular 3VBSED controller controls the BSED pre-amp.

Figure 34. Schematic of the wiring configuration of the 3View2XP system .

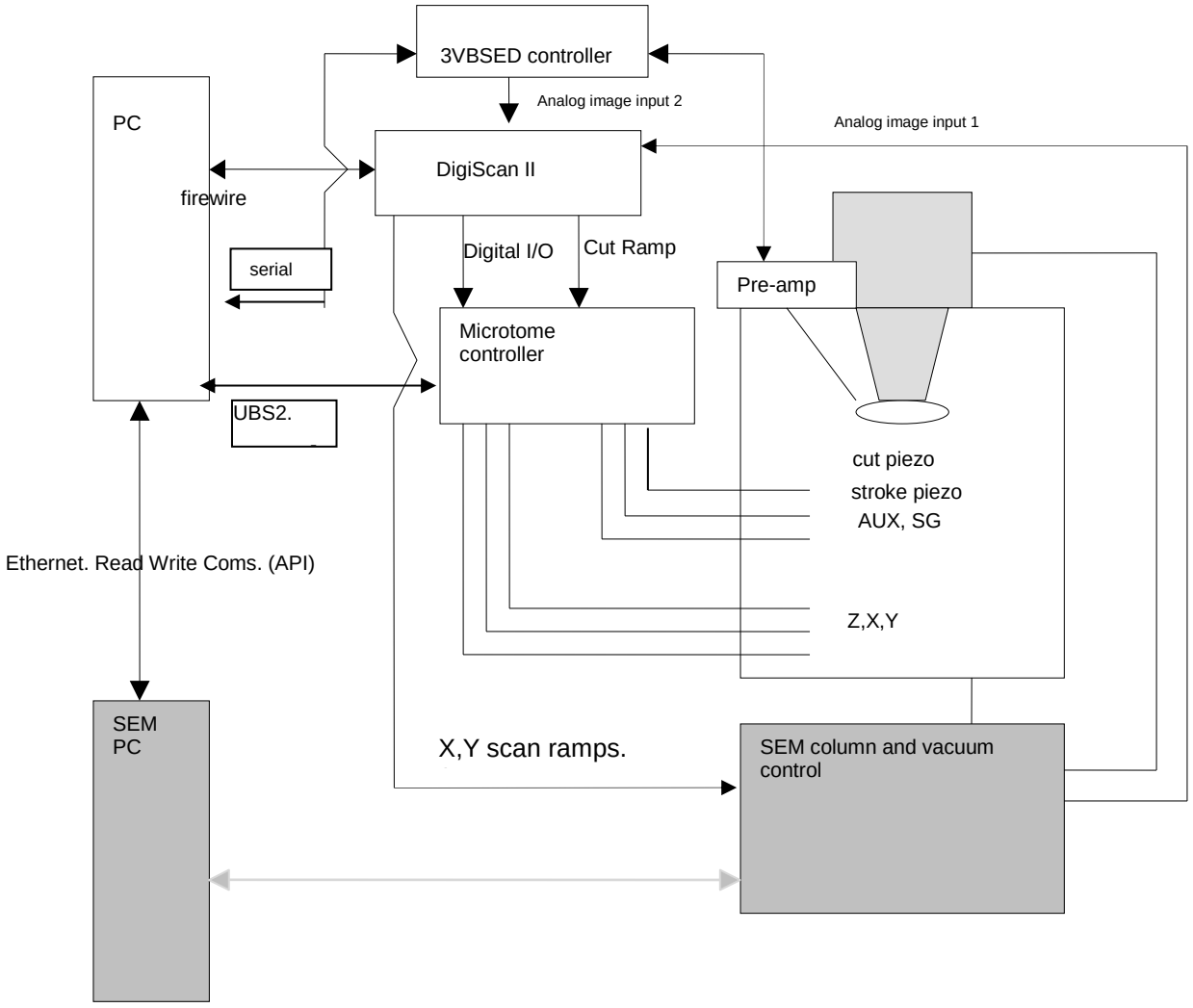


Table 1 Explanation of LED Lit Status on front panel of Microtome Stage Controller.

RED LED	**STROKE UP**	**STROKE Up when Lit. STROKE Down when not Lit.** Stroke Up only occurs when cutting, or by forced action in software.
ORANGE LED	**CUT**	**Knife in CUT position when Lit. Knife in RETRACT when not Lit.** LED brightness increases linearly as knife piezo actuator moves forwards. Only determines knife position in NEAR Status. CLEAR Status over-rides CUT / RETRACT Position.
BLUE LED	**NEAR**	**NEAR Status when Lit.**
BLUE LED	**CLEAR**	**CLEAR Status when Lit.**

The Microtome controller contains an internal USB expansion hub and this allows USB communication to the X,Y and Z motor controllers with a single USB lead from the PC. These serial ports are not visible in “windows device manager” unless the Microtome stage controller is turned on and the USB cable is connected.

2.14. DigiScan II.

DigiScan II is a common component in many scanning products from Gatan and forms the backbone of many analytical tools for STEM and SEM applications. The on status is indicated by a green light at right of the front panel near the on switch, and additionally by 4 green LEDs on the DDC. The DDC is a small box which interfaces between the black loom cable of the DigiScan and the connector for the SEM and Microtome Stage Controller.

Figure 35. Photo of the front of the DigiScan II unit.

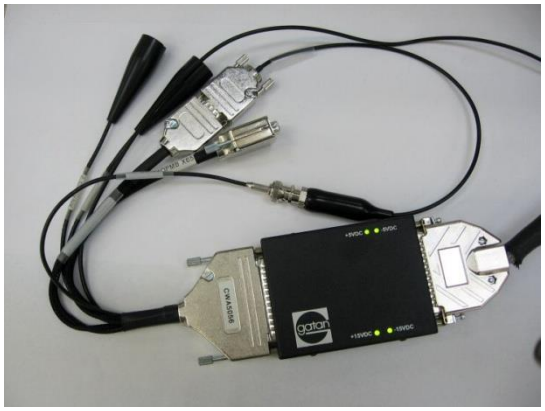


Figure 36. The DigiScan DDC and SEM specific Cabling.

The PC communicates with the DigiScan II via a Firewire connection. The presence of the Gatan Firewire Adapter (GFA) should be indicated in the Hardware section of the System dialogue in Windows Control panel. In case the PC is started and the Firewire cable is removed, and re-inserted Windows may request for the location of the Device Drivers for this Firewire adapter.

The functionality of the DigiScan can be divided into separate tasks as follows.

Signals to SEM External Scan interface.
Scanning Beam by providing 16bit analog voltage X and Y (Line / Fram) scan ramps to position beam.
Logic Signal, Switching SEM to External Scan mode. (SEM dependent).

Signals to Microtome Stage Controller.
16bit analog scan ramp volt to drive knife piezo controller.
Digital Output signals to control the stroke position.

Input Signals.
Image signal from SEM, e.g. SE detector.
Image signal from BSED.

The cable which connects the DigiScan unit to the SEM and Microtome Stage Controller is specific to the SEM model type and is manufactured appropriately. Drawings of the cable connections are supplied with this manual.

The DigiScan II does not control the beam blanking, which is required to minimize unwanted exposure of the focused beam onto the specimen or knife blade. Rather for most microscopes the Gatan PC, sends a blanking command to the SEM PC. The nature of this command depends on the microscope in question. There are no user adjustable components inside the DigiScan or the DDC box.

2.15. The 3View2XP PC.

The Gatan PC has been chosen as a high specification platform for the 3View2XP acquisition system. The PC contains a single hard-disk with no partition. No additional off-line PC is supplied for 3D data processing unless specifically ordered. The PC is shipped with software for 3View2XP pre-loaded. This includes 3D visualization routines. Hard copies of the software and licences are provided as back-ups. The PC does not have software associated with SEM PC communication pre-configured. This is accomplished at the installation. The 3View2XP PC is tested and shipped with a single administrator user account and no password. A different user account and domain may be required depending the protocol necessary to establish communication with the SEM PC. The PC is also set not to enter hibernation and has no additional firewalls configured. This is the recommended operational mode.

2.16. SEM PC Communication.

3View2XP software requires communications between the Gatan PC running DigitalMicrograph and the SEM PC running the column. This communication is set-up at installation, and typically is an Ethernet connection. To set this up may require administrator privileges on the SEM PC. In case of older generation microscopes, an alternative communication format may be required. The main communication parameters are as follows.

Read the following information from the SEM PC.
Gun HT
Spot size
Chamber vacuum / pressure.

Magnification / Field of View

Perform the following functions and control.
Set external scan mode to allow DigiScan to control beam
Blank or unfreeze the beam
Alter focus settings
Alter astigmatism settings

For ease of use, the Gatan – SEM PC communication may also involve the ability to share a mouse and keyboards between the 2 computers, whilst keeping the 2 monitors. This facility often allows limited share of data on a clip board between the 2 PCs. The possibilities for this option and exact configuration will depend on the SEM PC and monitor system in question.

2.16.1. FileServer, Internet and External Connections.

The customer is responsible for communication between the Gatan PC and the outside world or for connections to fileservers for storage. The presence of firewalls and anti-virus software etc, may impact the functionality of the 3View2XP computer to control the system, as well as record and save large amounts of data. During normal operation a large amount of memory may be allocated to a stack acquisition. In addition, all the USB, Firewire, and dedicated SEM PC communication are expected to take place without undue interruption. The PC supplied by Gatan includes 2 Network cards. This is intended to aid separation of communication between the Gatan PC and the SEM PC, and other connections to Fileservers or the outside world.

2.17. 3VBSED

The 3VBSED is developed specially for demanding 3View applications. Traditional back scattered detectors work at a higher kV, e.g. >5kV. The 3VBSED needs to work well at low kV which is important for limiting dose into resin specimens, and to achieve high resolution in 3D. This means that the 3VBSED diode is considerably more fragile than other solid stage BSED detectors, and operates at higher gain. Furthermore the working environment is harsh in that the cutting creates small shavings that can contaminate the diode surface and introduce noise.

3View applications also require extended stability. Contamination of the diode can manifest itself as providing higher noise or drift in the offset of the signal. For details on trouble shooting and maintenance please see Chapter 6.

The BSED consists of a silicon diode optimized for the highest quantum efficiency for low energy electrons and with a central circular aperture. The contrast polarity is inverted by default. This means that a higher signal associated with higher atomic weight or density areas provides a darker signal. This can be altered in the DigiScan configuration settings which is field service password protected, or in the DigiScan look up table which is not.

The BSED is ultra thin to allow the minimum working distance. The diode is connected to a chamber mounted pre-amplifier via an hermetic feedthrough. The mounted diode is detachable from the pre-amp electronics internally by means of a Fischer type connector. Low noise and high gain pre-amplification electronics are housed in a unit which is attached to the SEM flange. A separate 3VBSED controller provides additional signal processing and power and this is controlled from the 3View2XP PC.

There are two main types of diode design and these only differ in their mounting configuration. For some microscopes, a diode holder is attached to the pole piece. There is no adjustment to be performed and the central aperture should sit central to the zoom axis of the microscope. For some electron microscopes, optimum performance at very low kV requires the aperture to be closely concentric to the electron beam zoom axis. For these microscopes, the diode is attached to a side arm from a side chamber port. This provides fine ex-situ adjustment of the diode position in X, Y and Z directions.

The silicon diode is mounted on a thin fragile ceramic substrate. Fine gold wire bonds extend from the anode and cathode areas on the front surface to bond pads on the ceramic. These gold wires are visible with any eye piece. It is important that these bond pads do not get knocked. This is attached to an insulating plastic structure. This whole structure has a thin copper shroud for protection. There is an internal connection to an electrical feed through where the pre-amplifier is mounted on a chamber port.

For a 3View2XP system, a spare mounted diode is supplied which the user should be able to swap this out to ensure downtime is limited whilst diodes are replaced. Both diodes are shipped in dust proof protective shipping boxes and this should be used to return the units to the factory.

The user is expected to be able to swap out the mounted diodes on either the pole piece or on the side arm. The user should be trained to perform this action by a field service engineer at the time of the installation.

Please see chapter 6 for detailed advice.

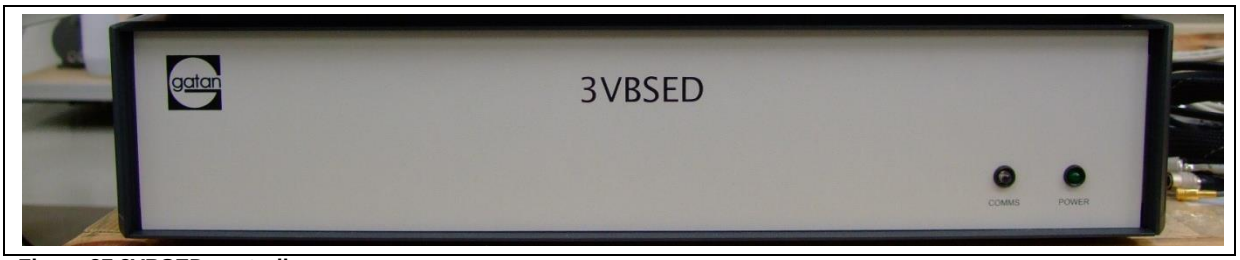
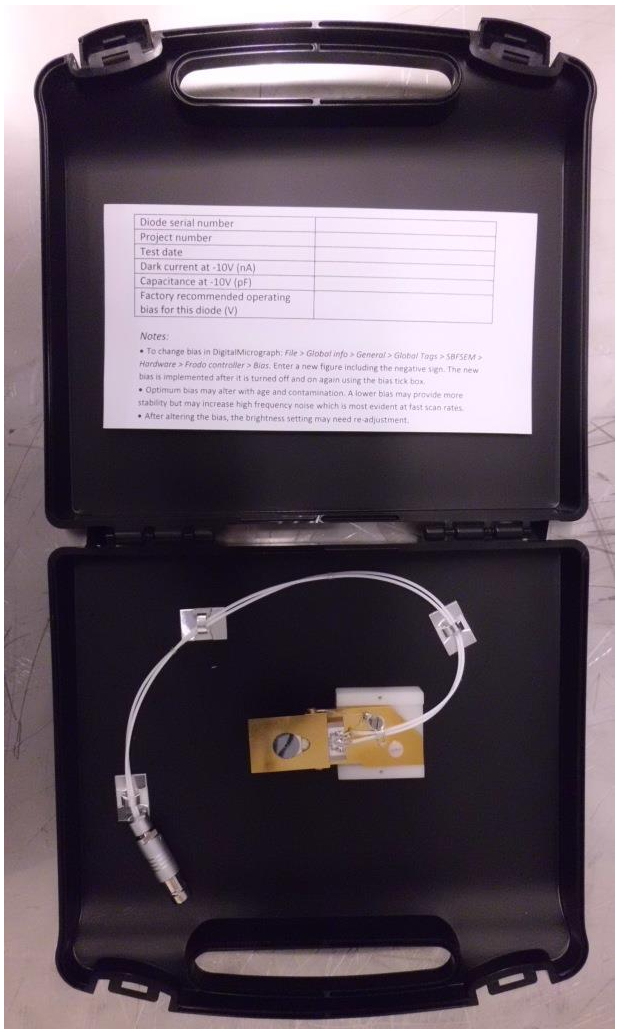


Figure 37 3VBSED controller.

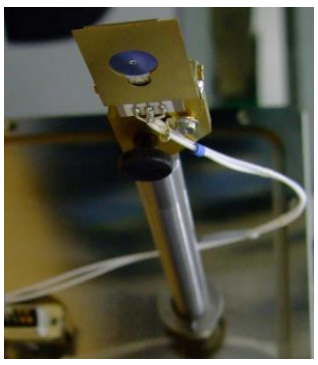


The diodes are shipped and stored in secure plastic boxes attached to a mount with a nylon screw. Please take case when removing the diode from the shipping box. This is achieved by unscrewing the single nylon central screw from the mount, and unclipping the cable / and connector.

The mounted diode structures should be returned to this box when being returned to the factory for replacement of the silicon / ceramic component.

The box records the factory test details of each diode.

Figure 38 Diode structures side arm mounting.



Slackening the thumb screw slightly allows the whole diode structure to be swung horizontally away from the pole piece when not being used. This allows the in-lens SE or BS detector to be employed without detaching the whole unit. Please ensure the thumbscrew is retightened to hold the diode in the correct “under the pole piece” or “swung out position”.

Fine adjustments to the X, Y and Z position can be made ex-situ using an Allen key into the orthogonally position grub screws. The back of the diode structure should be positioned very close to the bottom of the pole piece.

Figure 39 BSED diode which attaches to an ex-situ adjustable arm.

In all designs the diode is mounted with a small gap to a thin gold coated copper cover. Also, the microtome is positioned so that the diamond knife and diamond knife holder passes very close to the BSED structure. This allows the working distance during 3View2XP operation to be minimized and this helps the spatial resolution that can be achieved when working at low accelerating voltages.

There are no user adjustable components inside the pre-amplifier unit on the SEM flange. There are also no user adjustable components in the 3VSED controller unit. The following section gives details of the software controls of the 3VBSED system. These are all accessed from the 3View tab of the SEM Control software window.

2.18. Amplifier Gain and Offset.

Amplifier Gain and Offset is controlled via the Contrast and Brightness settings in the software. The maximum contrast value is 10. The Brightness value should be set by the user so that the waveform (red DigiScan line) is approximately central in the lower half of an image when the specimen is being imaged. It is important that the signal level does not saturate either the lower or upper values for particularly bright and dark parts of a specimen. If saturation is reached, then then like many high gain circuits, the amplifier can behave in an unstable manner and give unusual contrast features. If the signal level is set up with the beam blanked, then it is possible that the real signal will be outside the dynamic range of the amplifier.

It is common for the system to show a shift in brightness when the Bias tick box is on. This alteration in the brightness level is the “dark” offset of the diode, and the shift required will scale with the value of Contrast employed. The bias on / off status may also affect the focus of the image slightly, but this effect is column dependent.

Note, it is common for DigiScan imaging to be used with Autosurvey ticked on. Autosurvey automatically scales the constrast and brightness of the dynamic range in the signal to be shown. This is a useful imaging tool as the user doesn’t need to adjust settings each time the contrast or brightness of an image alters. However, it can be confusing especially when low contrast noise is amplified, or when signal ranges which are saturated at either a high or low value are shown as mid grey. Indeed if the brightness value is incorrect, this autoscaling can mean that a noisy grey signal is shown instead of the expected image. The key here is to utilize the red line waveform monitor to understand that the signal is indeed in the correct range.

Although the DigiScan autoscales the image when Autosurvey is ticked on, there is no option for the Contrast and Brightness of the amplifier to be automatically configured. It is the user’s responsibility to do this. Although the Contrast setting in the drop down menu scales from 0 to 10, this does not map to a gain range of zero to maxiumum. It represents a gain range of minimum to maximum. This means that gain 0 does not turn the detector off.

Use of maximum gain normally benefits the signal to noise in the image. Therefore users should consider reducing the Contrast only when the size of the injection conditions leads to saturation once the brightness has been correctly adjusted for the imaging conditions employed.

For details on care and swap out of the diode, please see Chapter 6.

3. Acquisition Software.

3View2XP software package runs on DigitalMicrograph GMS2.X. The protocol for installing the software is not covered in this User's Manual.

3View2XP software is designed to work on the Gatan supplied 64bit PC installed with Microsoft Windows 7. Software installed on one PC cannot be copied over to another PC. Software installation must follow the protocol of licence installation followed by the software "wizard" installation of the Gatan Microscopy Suite system.

The software installation will be configured specific to a particular SEM type, in which case this controls the communication protocol to the SEM PC. This is normally setup at installation. However, if the communication settings on the SEM PC are altered in any way, this may affect the communication protocol to DigitalMicrograph on the Gatan PC.

3.1. Off-Line Software.

An off-line licence involves a different version of 3View software for a PC not connected to any 3View hardware. Off-line licences are sold as options and this software does not search for hardware when started. The off-line software is used for opening, post processing, saving and exporting images and 3D data series. 3D visualization software is an option for off-line software packages.

3.2. On-Line Software.

The 3View2XP system is shipped with the software pre-installed on the Gatan 64bit PC. This is the PC that will have been used for testing the system in the factory. The software installation is controlled by a single licence which is specific to the PC used for running the hardware. This is termed the "on-line" licence. This term does not indicate connection to the internet.

The default software package for 3View2XP includes 3D visualization and a high specification graphics card to support this functionality.

DigitalMicrograph is started by clicking the icon on the desk top. For "on-line" installations, only do this once the relevant hardware has been turned on.

When DigitalMicrograph software is running 3View on-line and connected to powered hardware, it is important that the PC does not enter hibernation mode. This is normally configured from the power options within windows.

Certain configuration settings of the 3View2XP installation are detailed and controlled in a Tag setting menu. The majority of these settings should only be accessed at the time of installation and the values in this menu are saved as part of the preference files. Other Tags may be useful for the user to access. The Tag menu is accessed from the File menu under Global Tags. This contains sub menus pertinent to the microtome and controller, the SEM, and the DigiScan. Users unfamiliar with the tag menu are discouraged from altering settings without advice from Gatan service.

Once an installation is complete and all calibration values are correctly defined, it is good practice to make a back-up copy of the preference files in the "prefs" folder. This provides a useful back up of the setting.

3.2.1. GMS Windows Drop Down Menu.

Many of the 3View tools and windows are found in the windows drop down menu within DigitalMicrograph. Many of these windows has a specific name and can be opened, minimized, closed and moved around to suit the user's needs. Some windows have multiple tabs or panels, and these are brought to the front by clicking on the named tab. Some of these different tabs are explained in more detail below.

3.3. SEM Control and 3VBSED Control.

The SEM control window provides multiple functions associated with the SEM column, the X, Y translation of the 3View2XP stage, and the Gatan BSED. It does not provide control of a 3rd Party BSED. Note, the common layout whereby specific valves can be entered by typing the left hand boxes, or the values can be raised or lowered by the step size which is configurable in the right hand boxes.

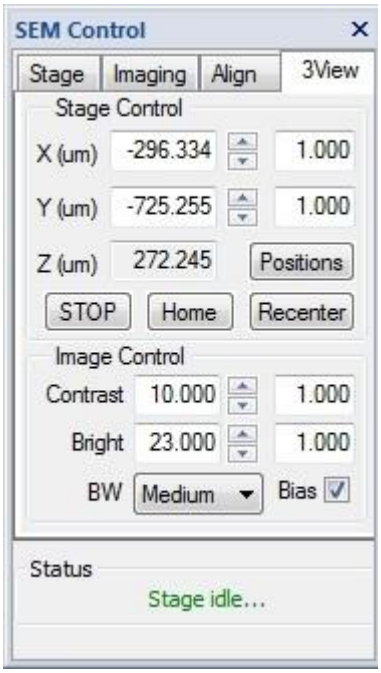
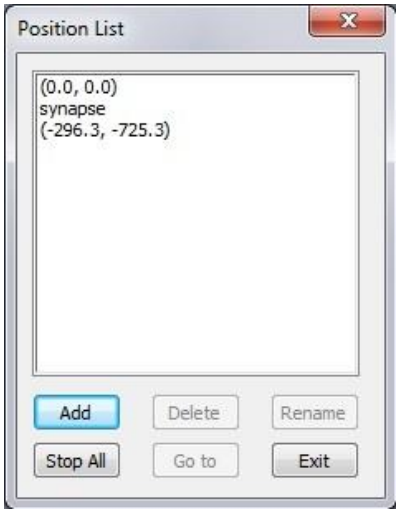


Figure 40 SEM Control and Positions List



3View tab.

X (um) Either type in a new value in microns, or else use the increment buttons which can be configured on the right.

Y (um) As above.

The X,Y stage movement is bipolar about the home position of 0.0, 0.0 microns. The absolution travel distance depends on the coarse positioning of the limit switches, but it is typically +-700microns. When movements are requested from this dialogue, no backlash is removed.

Z (um) The specimen height (Z stage) is only reported and not controlled from this palette. Use the 3View approach tab instead.

Positions: This allows users to define and optionally name stage positions. The default name for a position is the x,y coordinates.

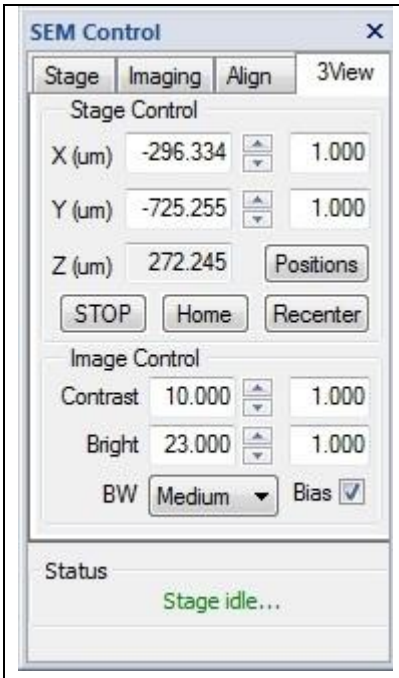
STOP: This aborts stage movement.

Home: This performs an auto-initialization routine in X and Y whereby the stage is driven first to the limits and then rests at the central point.

Recenter: Once this button is clicked the very next mouse click anywhere on the computer screen will denote the requested central location of the specimen with respect to the DigiScan image window which is front most. As this is anywhere on the screen, the point being denoted can be outside the field of view of the front most DigiScan image.

Note, for versions earlier than GMS2.3 the user must ensure that the magnification has not been altered. For GMS2.3 the magnification is continuously polled so this uncertainty is removed.

Status: When any of the motors are moving this is indicated in red font, otherwise the message Stage idle is shown in green font. This message is replicated on the other tabs from the SEM control window.



BSED Image Control

These controls affect the 3VBSED signal only. The boxes on the right hand side dictate the step size of the left hand up / down scroll functions.

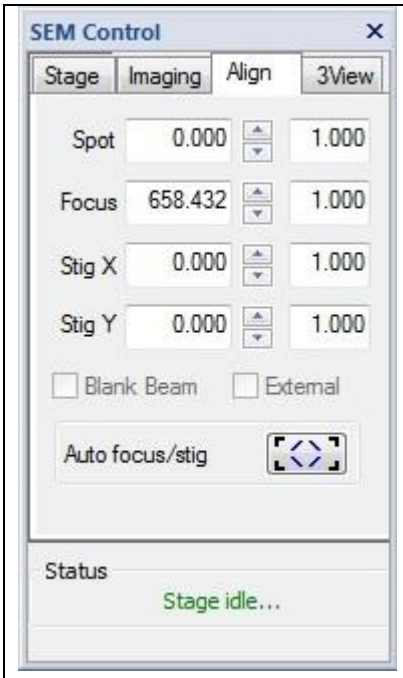
Contrast: Adjusts the gain of the Gatan BSED. The default condition is to use the maximum contrast setting of 10. However, if this leads to the signal saturating then it should be reduced. Note the brightness should be adjusted by the user to ensure that the signal is not saturating. Sometimes it is difficult to judge saturation conditions from an image, so please use the waveform monitor button ( ) in the DigiScan window.

Bright: Controls an offset (brightness) value of the Gatan BSED. This control is prior to the majority of the gain. Hence correct setting of the brightness will be required once a signal level has been established in order to set the correct gain and dynamic range in the image. The brightness range extends from -16 to +200 units. Once again use the waveform monitor on the DigiScan window to judge the correct settings and saturation conditions.

BW: This is a drop down menu of 3 different bandwidths (filtering speeds of low, medium, high) of the Gatan 3VBSED. A low bandwidth filters the signal and is not appropriate for shorter pixel dwell times. A high bandwidth setting should be chosen for the shortest pixel dwell times, e.g. <5us/pixel.

Bias: Tick box which turns on the bias to the Gatan BSED. The default mode of operation is with the bias on. The value of the bias cannot be altered from this interface. It can only be altered through the tags system. Note if the value is altered by running a script which alters the value in the tags. The new value is only applied once the bias is turned off and on again.

If the Image Control section is shown as grey, then communication with the 3VBSED controller has not been established.



Align Tab:

Spot: This is common terminology on some microscopes for the objective lens setting which controls the beam current. On some microscopes this is not a configurable value.

Focus: Users can choose whether to use this function. Any changes in the SEM focus using the SEM GUI, or focus knobs should update the value shown.

Stig X, Y: As above for focus.

Beam Blank: This function is only support if the beam blanking is supported as a software command. (SEM dependent). If it is not, then it will be shown as grey. If this command is active, then if the SEM is beam blanked from the SEM GUI, the tick box should update.

External: External refers to DigiScan being able to take control of the scan. If DigiScan has control but is not imaging, then the beam, if unblanked may be located in the center of the image and may damage the specimen. This button is only active if the external scan control is a software and not a hardware command. (SEM dependent).

For a correctly configured installation, the external scan control and beam blanking should take place automatically.

Auto focus / Stig: This button manually actives a refinement to the focus and then the stigmatism settings. This tool cannot be used as an automated protocol for large scale adjustments, it is only intended for minor adjustments. The settings used for the routine are taken from the DigiScan Preview settings, and so the length of time spent performing this depends on these settings. The area imaged for this operation is the existing field of view. Automated routines outside the field of view are only applied during automated cutting operations.

If the user presses the Auto focus / stig button a second time this cancels the operation.

Figure 41 SEM Control Align GUI

3.4. Diode Bias.

The diode is biased to reduce the capacitance of the device. This doesn't affect the speed, only the amount of high frequency noise in the signal. This is most apparent at the fastest scanning speeds. However, the bias can introduce leakage artefacts depend on the age and contamination of the diode. The optimum bias value is suggested in the test certificate inside the shipping box.This is duplicated below.

Note, although the dark current and capacitance of the device are listed at -10V, this is just a standard metric and doesn't suggest the recommended conditions. For new shipments, the software is preconfigured with the bias value for the diode included in the shipment.

Diode serial number	
Project number	
Test date	
Dark current at -10V (nA)	
Capacitance at -10V (pF)	
Factory recommended operating bias for this diode (V)	

Notes:

- Optimum bias may alter with age and contamination. A lower bias may provide more stability but may increase high frequency noise which is most evident at fast scan rates.
- After altering the bias, the brightness setting may need re-adjustment.

Figure 42. Diode shipping container label

The bias value should be set to that which is listed on the original diode shipping container unless it has been noted that a reduced bias is required, for example because of the age or contamination of the device.

The bias value can be altered by running the Change Bias script from the menu, and is applied when the tick box is turned on. DigitalMicrograph allows any script to be installed under custom menus. In the example shown below the Change Bias script is chosen from a Bias drop down menu.

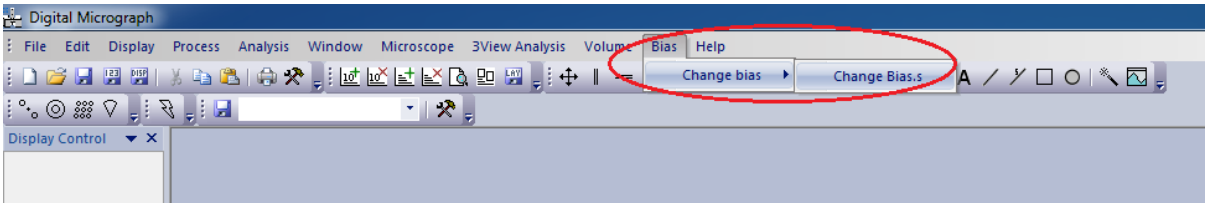


Figure 43 Change Bias Script

The bias value is recorded in a tag setting, and the value isn't shown to the user, and isn't configurable from the simple On / Off tick box.

If you have the change bias script, but it isn't installed and you wish to install it then;

Go to File > Install script, and create a new menu called 'Bias', and a sub menu called 'Change bias'.

If you do not have the script then an alternative method is manually altering the value in the tags;

To change bias in DigitalMicrograph: File > Global info > General > Global Tags > SBFSEM > Hardware > Frodo controller > Bias. Enter a new figure including the negative sign. The new bias is implemented after it is turned off and on again using the bias tick box.

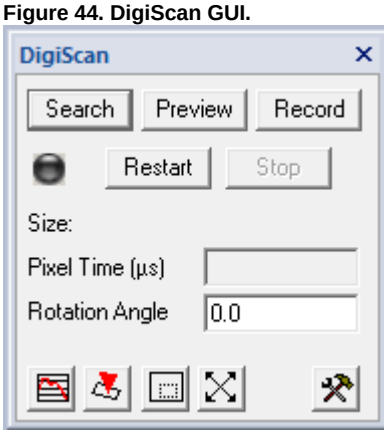
3.5. DigiScan Imaging Controls

The DigiScan window controls the functionality of the DigiScan for image acquisition. The DigiScan window presents the user with a choice of 3 configurable image acquisitions, namely Search, Preview and Record. Search and Preview are continuous scanning with constant refreshing, whilst Record is a single result acquisition. Search and Preview continue until a Stop request is made.

The Preview parameters determine pixel density and scanning speed used for 3View autofocus / autostigmation routine.

The 3View automated Record function for acquiring datasets takes its parameters from the Record settings in the DigiScan dialogue. These parameters are the choice of imaging signals, pixel values, dwell time and line-sync option. Multi pass acquisition can also be chosen.

Figure 44. DigiScan GUI.



Search, **Preview**, **Record, Restart** and **Stop** request buttons.

When the DigiScan is active, the button on the left is Green, and when not active it is Red.

If the **Restart** button is pressed, this performs the last Search, Preview or Record, but according to the last used pixel dwell time / rotate setting, not that defined in the DigiScan Dialogue Window.

The **Rotation Angle** is a DigiScan Rotation and not the SEM Scan coil rotation, or a physical rotation of the specimen.

The tools button opens the DigiScan Setup Dialog shown below for configuring the Search, Preview and Record Settings.

This button activates a Red line profile of the signal intensity across the bottom half of the DigiScan image window. This is an essential feature to monitor the BSED signal level without misinterpretation caused by the Autosurvey feature.

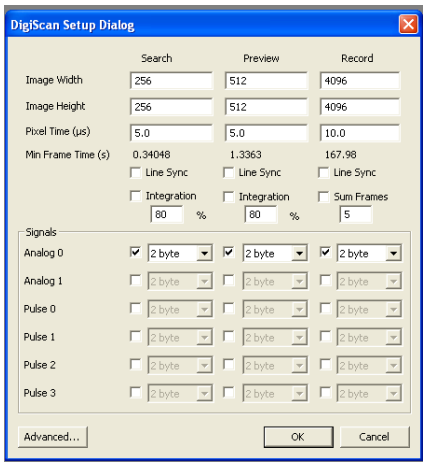
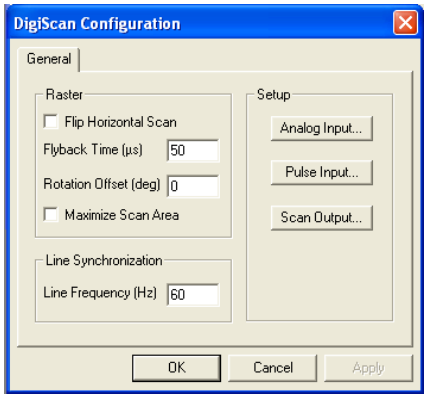
This button places the beam in a location defined by cross hairs on the DigiScan image. This function is not recommended for 3View2XP applications, because of the danger of burning resin.

This button acquires an image of a sub area defined as an ROI. This function uses the DigiScan zoom functionality. This functionality should be avoided for 3D data sets. If this button is used when an ROI has been drawn on a DigiScan image, then only that ROI is imaged. This is achieved without moving the stage and without altering the SEM magnification. Rather it is achieved by altering the DigiScan magnification. A DigiScan zoom factor is shown below the stop button. To cancel this zoom feature, double click on the zoom number shown.

This button increases the pixel density in a given acquisition. This functionality is not recommended as a 3View2XP tool.

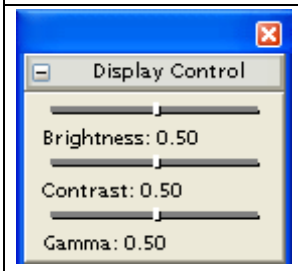
DigiScan Stop Requests.

Stop Button: Applies to Search, Preview, Record. Instantaneous. Image can be saved incomplete. Stop Button is inactive unless an acquisition is active. (Green button lit).
Esc Key: Same effect as Stop button, but DigiScan image must be front-most.
Space Bar: Image acquisition continues till the end of the present frame. DigiScan image must be front-most.
Close Window. Terminates the acquisition of that window.
Search, Preview, Record buttons. When these are pressed during an active scan, the scan is terminated.
Abort. This function call is only from the 3View2XP control window, not the DigiScan Dialog. When pressed the stack acquisition is aborted in a controlled manner and this involves

	terminating a DigiScan image at the end of a scan.
 **Figure 45 DigiScan setup dialogue**	**DigiScan Setup Dialog** allows users to choose the pixel density and pixel dwell times for the Search, Preview and Record Settings. The Frame Time is automatically calculated and reported. **Line Sync** will limit the shortest pixel dwell time and frame time. Line Sync will cause the beam to pause at the left hand side of the image and this can cause charging effects in 3View2XP mode. It should only be turned on if required to cancel stray fields at TV frequency which affect the image resolution. A **%** button allows the user to configure what percentage of an image is made up of the present image compared to previous scans. In the Record mode, this box is replaced by a Sum Frames number. Note, any drift in the image, e.g. associated with charging which extends by more than half a pixel wide will cause degradation in the spatial resolution. Sum Frames should only be employed when the user is confident it is the best method. The **Advanced** button is locked for general purpose. It can only be opened when the system is set to Service Mode. When pressed it opens the DigiScan Configuration Window shown below.
 **Figure 46. DigiScan Configuration Dialog.**	Clicking the advanced button opens this panel if it has been unlocked. This manual does not cover this configuration. Please refer to DigiScan installation manual for more details.

DigiScan Image Display.

 **Figure 47. Example Image**	The **Histogram** and **Display Control** windows are 2 useful standard tools within DigitalMicrograph. The Histogram window shows a histogram of the grey levels present in the front-most image window. The line (which in the box is a straight diagonal) represents the look-up table used for displaying the data. A useful feature is the ability to reset the dynamic range of the grey levels by dragging a region of interest across the histogram. A narrower region of interest increases the contrast. Double clicking the bottom panel of the histogram will apply the

Histogram Window.	AutoSurvey settings for the image display which can be configured as shown below.
 **Figure 48. Display Control Window.**	The **Display Control Window** can be used to alter the brightness, contrast, and gamma value of an image, or 3D data tower. When the slider bars are moved in the Display Control window, the brightness alters the height; the contrast the slope; and the gamma value, the curvature of the look-up table.

Any DigiScan image or 3D data series is accompanied by additional information. This information together with configurable display information can be accessed by right mouse clicking on any image.

Note, if the Line Profile tool feature is on, then the user must Right Click in the top half of the image. If the bottom half of the image is clicked, then the user can only configure the Line Profile Tool rather than the image properties.

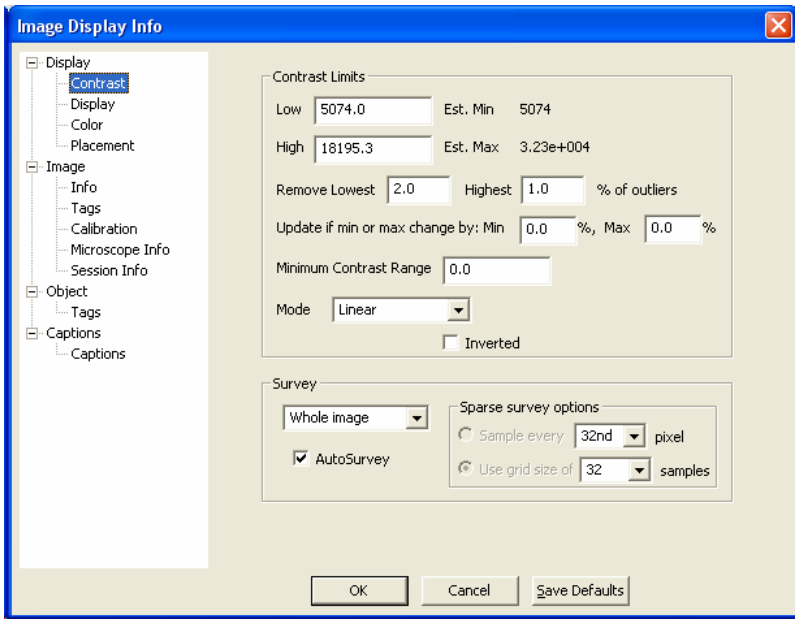


Figure 49. Example Image Display Info / Contrast GUI.

The Image Display Info panel (right mouse click on DigiScan image) contains a menu of display, image, object, and caption features.

The default contrast panel shows the contrast values and look-up table applied to the image.

The most important feature is the **AutoSurvey** Tick box. When AutoSurvey is on, the contrast values in the image are surveyed in a defined manner and the histogram treated for display purposes as shown. When not ticked, the display is controlled from the histogram / display control windows only.

Users should be aware that when the AutoSurvey feature is on, changes in the level or dynamic range of the signal will not always be apparent from the display because of the normalization taking place. In this case the Line Profile tool is recommended.

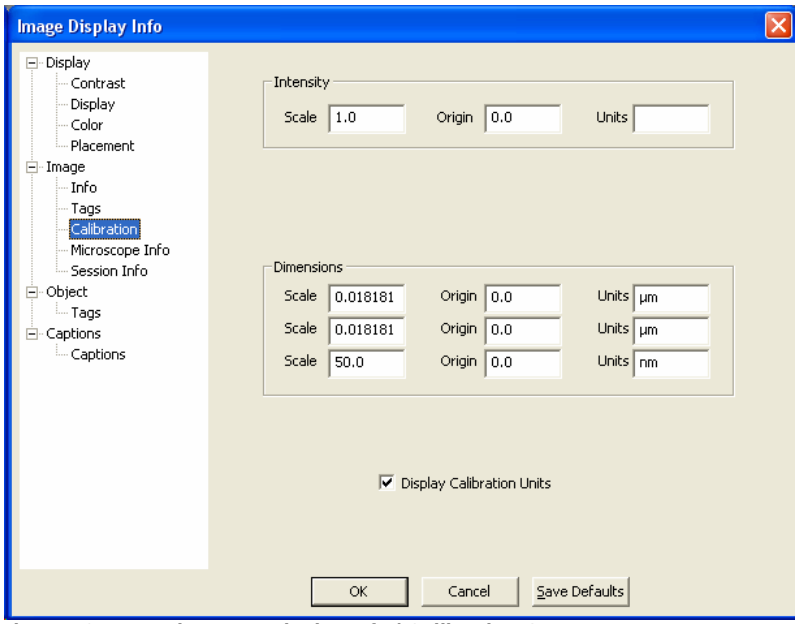


Figure 50. Example Image Display Info / Calibration GUI.

The calibration window in the Image Display Info shows the dimensions the pixels.

In case a 3D data stack is queried a 3rd entry details the Z dimensions of the pixels, which is the cut thickness of the experiment.

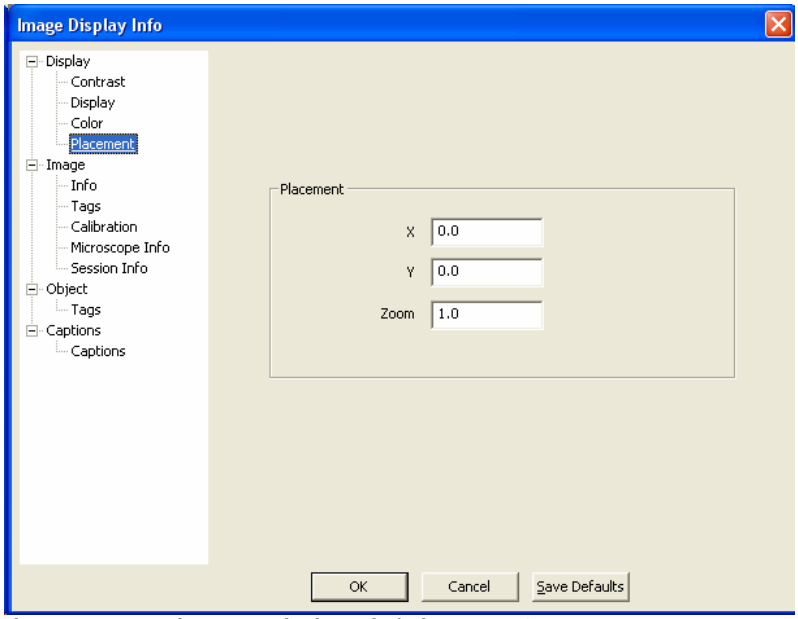


Figure 51. Example Image Display Info / Placement GUI.

The Placement window helps the user understand the zoom settings for the display. A zoom of 1.0 means that there is a 1 to 1 relationship between image and monitor display pixels. A value other than 1 means that interpolation is present.

Values of Zoom close to 1 or a simple fraction may result in a moiré pattern on the screen.

The display resolution does not affect the image quality when the data is exported. It only reflects the image display settings.

3.5.1. DigiScan image magnification.

Oversampling is common in many imaging fields, not least digital cameras where the pixel density can far exceed the information limit. In some cases this doesn't matter because memory is plentiful and the pixel size is irrelevant. For 3View experiments oversampling is more important because the density of dose injection into a resin specimen is important to control, and because memory usage is important to control when working with 3D datasets. The injection density is basically the size of each pixel and this is determined by both the SEM magnification (i.e. field of view), and the DigiScan pixel density. It is the combination of these settings which determine the real magnification and therefore the injection density on the specimen and whether oversampling is occurring.

The magnification in a displayed image depends on the pixel density on the specimen, and how these pixel dimensions are displayed in the viewing window. 3View software continuously polls the field of view from the SEM, and display calibrated images, for example with a calibrated scale marker bar.

On installation, DigiScan can be set to have a defined calibration which can be specific to the SEM kV. If an uncalibrated kV is chosen, the user is warned and an interpolation is made to the nearest appropriate kV setting.

DigiScan software provides a digital zoom feature. This is not a post-processing step and it does not alter the SEM reported magnification settings. Rather the DigiScan keeps the same number of pixels in an image but enlarges or reduces the pixel size. The lowest zoom ratio is typically limited to about 0.7, whilst the highest zoom depends on the pixel density currently being employed. If a low pixel density image is initially set, then the digital zoom in can provide a considerable zoom, e.g. of more than 100. However, the resolution in this image may not match what is achieved by increasing the SEM magnification by the same factor for a variety of reasons. Some SEMs alter the column conditions between lower and higher magnification, so the microscope may require refocusing when using the highest DigiScan zoom settings.

When DigiScan zoom is applied, the zoom factor is shown beneath the stop button on the DigiScan GUI. The zoom level is altered using the up and down arrows on the Gatan keyboard. To revert to zero DigiScan zoom, press the Home key, or double click on the DigiScan zoom setting text. When DigiScan zoom is applied, the scale marker bar and pixel dimensions are updated.

3.6. 3View Status Window.

This window reports the knife, stroke, and oscillator status. It is a reporting interface only, not an input panel. If any change is made to these parameters using other panels, they will be updated immediately in this window.

Figure 52. 3View Status Window.

Knife: The retracted status is shown as 0 and the cut status as 1200um. The knife is also reported as either clearing, or cleared.

Stroke: Reported as either up or down. When up, the Red LED on the controller is lit.

Oscillator: Reported as On or Off.

3.7. 3View Control: Approach.

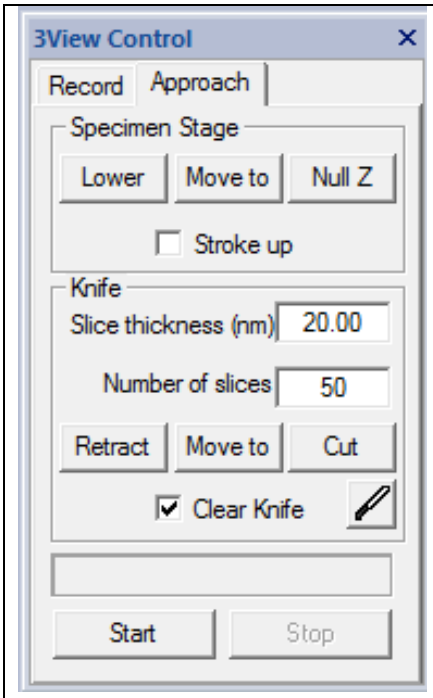
The 3View2XP control Approach tab is the most important user interface when performing the approach sequence and in setting up a 3D acquisition. Items configurable in the Approach panel should not be adjusted when a 3D stack is being recorded.

Slice Thickness: This number in nm defines the cut thickness during the Approach sequence. Numbers greater than 250nm should not be employed as this can damage the diamond knife. If larger movements are required, the Move to function should be used.

Lower: Sends the specimen to the lowest position by moving the Z-advance motor down until the limit switch is reached. The position of the motor with reference to its home position is reported in the status window, not the approach window.

Move to: Opens a dialogue window to enter a specimen height to move to. This should be performed with reference to an existing height as reported in the status window. The user is asked to confirm whether they really want to move this requested distance, or cancel the operation. This is intended to provide a level of safety as moving the specimen too high in an uncontrolled manner could damage the knife.

Null Z: When this button is ticked, the present specimen height



3View Control / Approach GUI.

Cutting during the Approach sequence is used to automatically and safely raise the specimen when the system is viewed at air under the optical stereo zoom microscope, and when at vacuum to prepare the specimen surface for a 3D acquisition.

The Manual Cut, Move to, Retract, and Start Stop software actions are potentially damaging as they initiate knife movement. The user must take responsibility of the relative position of a specimen when performing any of these actions.

and position of the Z advance motor is referenced to zero. Users should be aware that the reported software height is only referenced to the last position that was referenced as the zero origin.

Stroke Up: When ticked the stroke piezo actuator raises the specimen “stroke” up. This is the default height for cutting only, not imaging or other actions. The stroke up condition is also indicated by a red light on the front of the Microtome Stage Controller. The stroke distance is 5 microns.

Knife

Number of slices: This only defines the number of slices to perform from the Approach sequence, i.e. without imaging or the clearing of the knife. This does not control the number of slices during the Record process.

Retract: Sends the knife to retract position.

Move to: Opens dialogue requesting new knife position between 0 and 1200um where these extremities define the default cut and retract positions.

Cut: Sends the knife to the cut position. This does not function when the knife is in the Clear position.

Start / Stop: Buttons to start and stop the sequence of automated number of cuts as defined above. If Stop is pressed, the remaining cuts are cancelled and a further Start action will initiate again the specified number of cuts. There is no pause / resume function.

The bar above the Start / Stop buttons reports the progress of the sequence once initiated.

Retract Speed: As above.

Oscillator. This provides a software control for the option of high frequency piezo oscillation of the diamond knife. The oscillator is turned on during cutting only and turned off during imaging.

Cut Speed: This defines the cut speed in mm/s for the approach cutting only. The maximum value is 2.4mm/s. The cut speed influences the quality of the cut. The retraction speed has little influence.

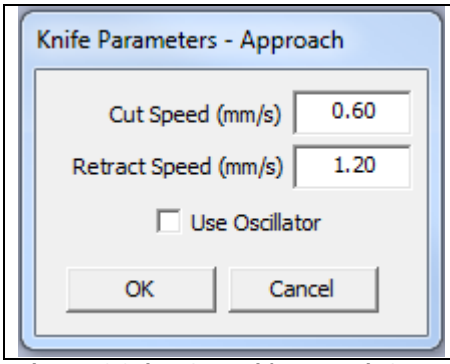


Figure 53 3View Control / Approach GUI.

3.8. 3View Control: Record.

The Record Panel in the 3View Control interface is normally the final panel to configure in setting up an experiment. Note, when a Record sequence is initiated, items in the Approach panel should not be adjusted.

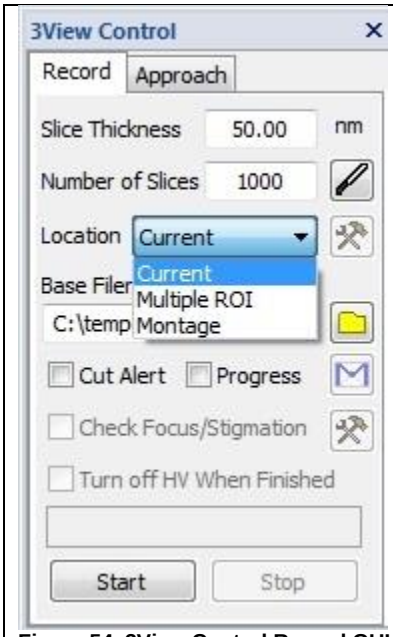


Figure 54. 3View Control Record GUI.

When the start button is pressed, the next step depends on the Location settings and the chosen settings in the configurable tools windows.

Slice Thickness: This value is configurable by the user in microns between 15nm and 200nm. Users should be careful not to oversample in Z, so the thinnest slices should only be attempted with the lowest of kV settings.

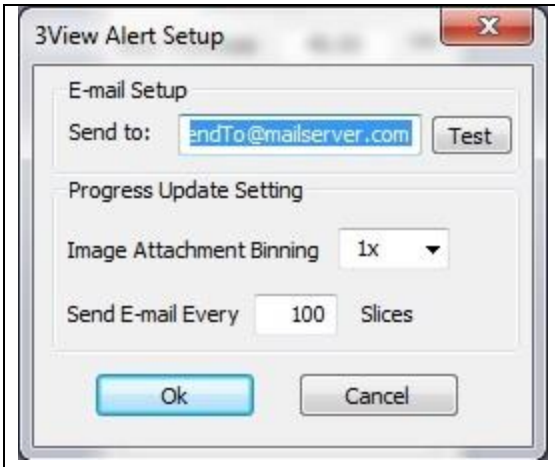
Number of Slices: This determines the length of the automated experiment. Users should be aware of the thickness of their specimen and the cut thickness when choosing this value.


This knife button configures the cutting and retraction speed and oscillator settings for the Recording sequence. Although the button and window appears similar, these are different values from the Approach sequence.

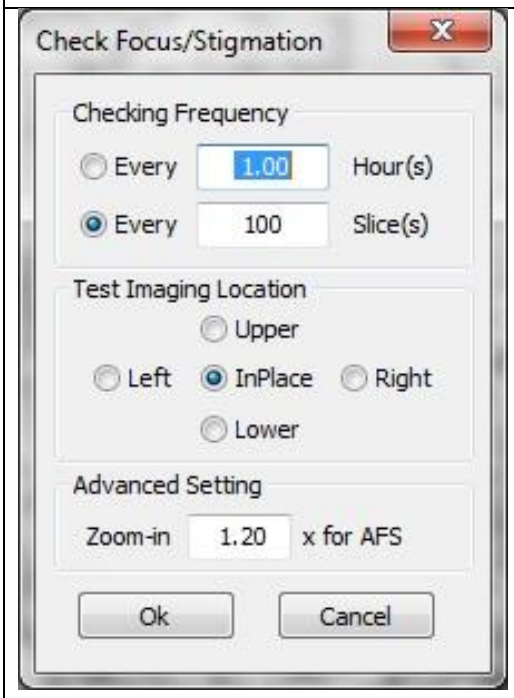
Location: Choose between current location, Multi ROI and Montage acqusition modes.


Tools button. This allows the user to configure the settings for the Multi ROI, and Montaged Image Location choices.

Base filename: Allows the user to name the files and location to save them.
Click on the folder icon to enter a new folder.

<table>
<tr>
<td> 
</td>
<td>Cut Alert and Progress Tick boxes.
These actions are individually configurable.

  : Click to configure the Cut Alert and Progress settings as well as to enter a valid email address.

The progress update settings allow the user to attach an image to the email being sent. The user can configured the binning ratio of the image in order to keep the images being sent to a manageable size.</td>
</tr>
<tr>
<td> 
</td>
<td>Check Focus/Stigmation tick box.
This routine is identical to the manual Autofocus / Stigmate request from the SEM control / align panel in that the imaging settings are taken from the DigiScan Preview settings. Furthermore the routine performs small iterative adjustments to the focus then the stigmation.
Click on the tools button to configure these settings as shown to the left.

If the user chooses Test Imaging Location as InPlace then the focusing takes place on the region of interest being imaged. If another location is chosen then the stage is moved automatically to this location. It is good practice to focus away from the central InPlace, but not to choose an area which the knife would cut prior to the imaged location. In addition the user may know that the region to one particular side may have a better area to use in case the specimen is not uniform.

Advanced Setting.
This increases the SEM magnification by the chosen factor as is common in fine focus / astigmatism adjustments.</td>
</tr>
<tr>
<td></td>
<td>Turn off HV when finished: Click to turn off HV after the experiment is complete. (This is only valid on certain SEM types)

Start: Button starts the automated experiment, imaging areas between each cut as defined by the Location settings.

Stop: Stops the acquisition once an image sequence is completed. If the acquisition is stopped then all data recorded so far is saved.

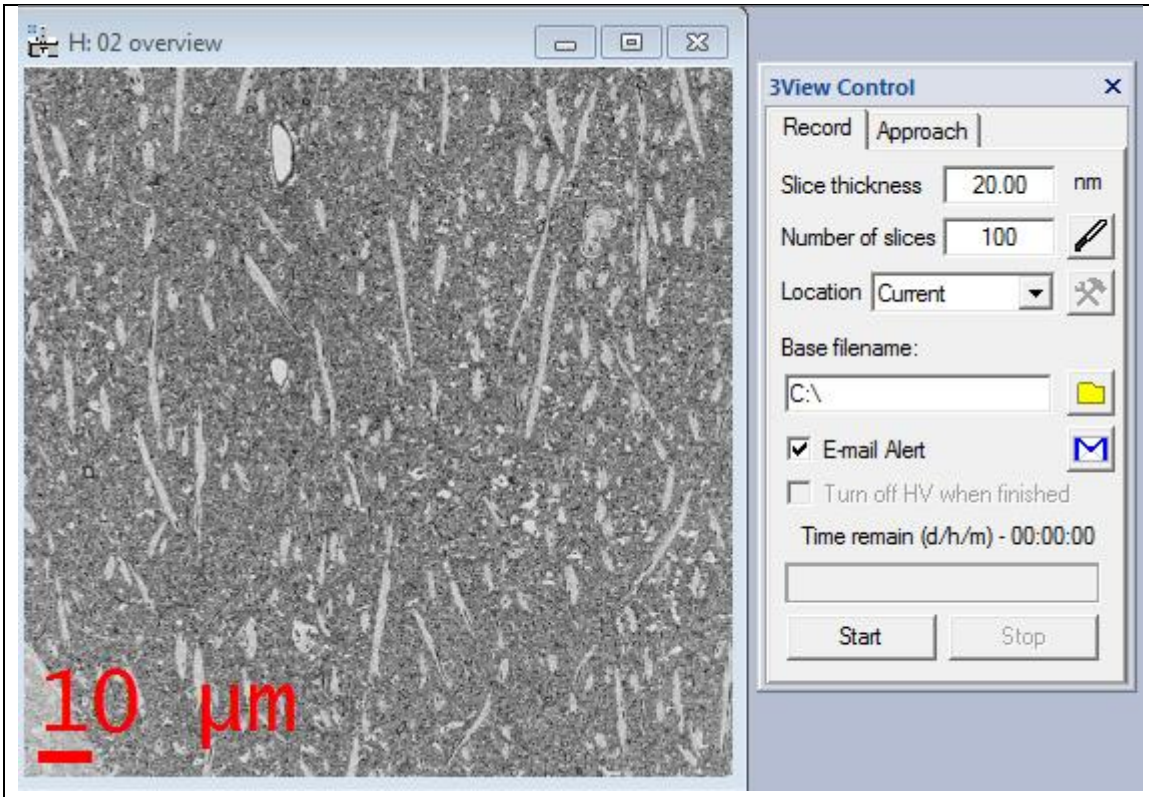
Users can also pause and resume the acquisition as required.

A progress bar and estimated time for completion is shown to the user when the experiment is live.</td>
</tr>
</table>

As each image in the stack is completed, the data is saved automatically with user defined prefix and an automatically generated sequential ending. Users should remember to close unnecessary 3D datasets which may be open in the software so as to reduce the memory load on the computer during acquisition.

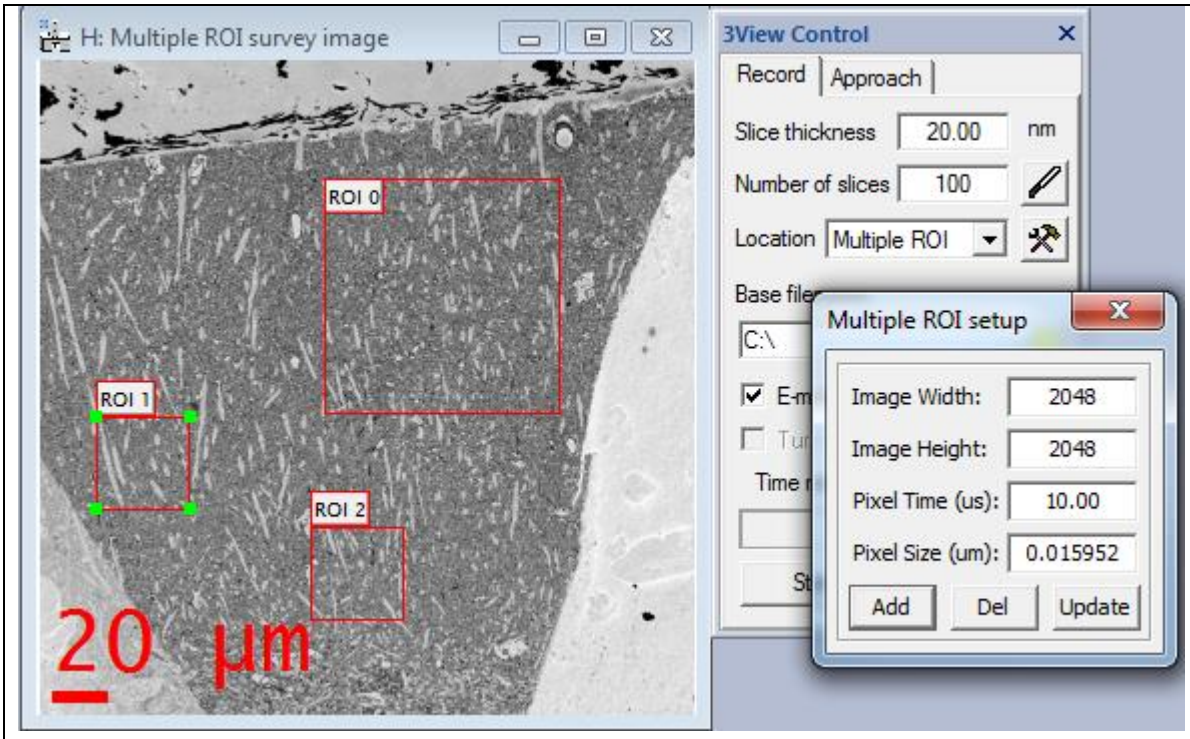
3.8.1. **Location Settings.**

If a simple “Current” location is chosen, then only the single field of view is recorded in the experiment. When the experiment starts, two DigiScan windows are shown per signal. One is constantly refreshed as a live 2D window. The other is a 3D dataset which comprises the stack of images which are gradually being built up. Each stack of images in the 3D dataset is limited by default to 1GB and once this limit is reached, the stack just shows a rolling buffer of the last 100 images. This means that for extended experiments, users cannot see data from an earlier period by default without specifically opening them from the saved file.



Current:

In the current location mode, the entire front image will be used to define the area of interest.

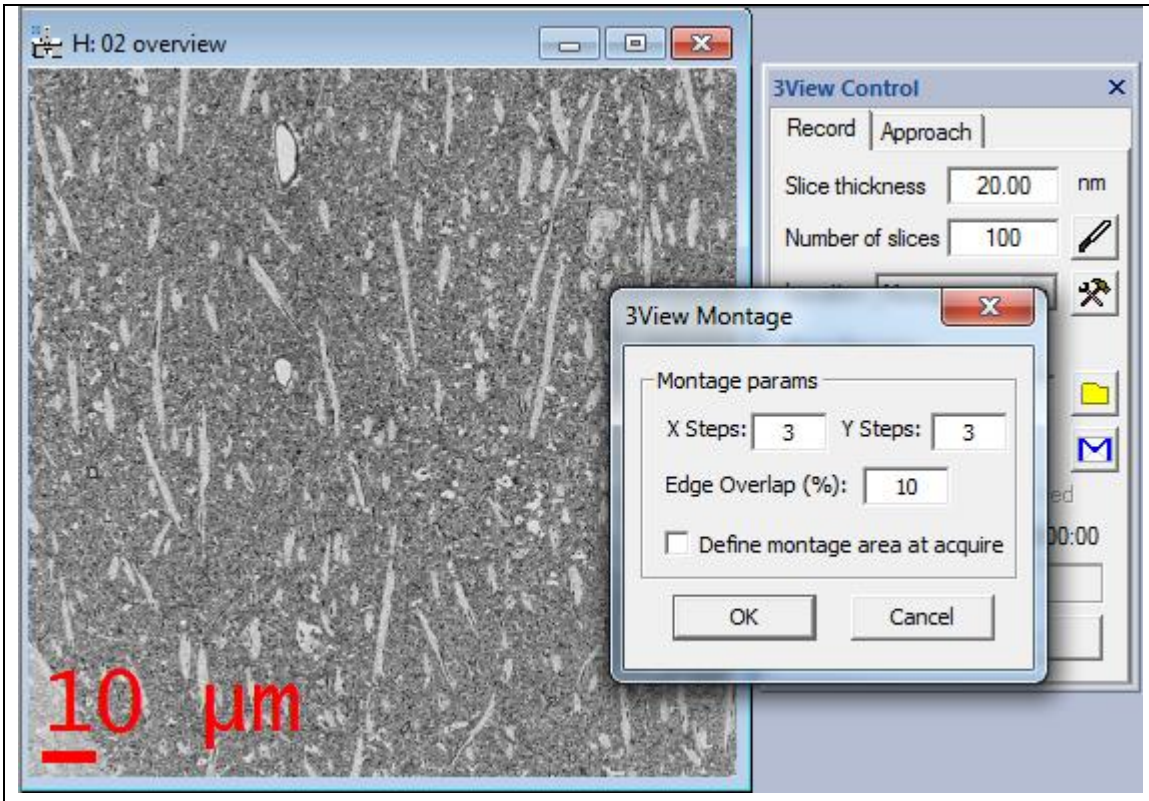


Multiple ROI:

 **:** Opens the Multiple ROI set up

The Multiple ROI set-up allows the user to add an ROI to an image. The survey image should be a lower magnification image to take full advantage of this function. Each ROI can have its own size and pixel dwell time. The set will calculate the pixel size for the ROI image. The user can modify the pixel size or image size for any of the added ROI, by altering the fields and selecting the update button. In addition the user can drag the ROI to a new size of aspect ratio, or delete it and selected ROI.

The Multi ROI function both moves the stage and adjusts the SEM magnification to provide the maximum flexibility in the experiment. The software allows the user to check the region of interest is correct for each ROI before accepting all conditions. In this process the user can fine tune the stage position for example by manually using the stage, or using the Recenter function.



3View Montage:

 **:** Opens the 3View Montage setup

The 3View Montage allows a user to acquire an array of adjacent images. The user can define the number of steps, and the image size is determined by the current DigiScan settings.

Edge Overlap (%): Determines the percentage overlap between each adjacent image.

Define montage area at acquire: There are two options to define the area of acquisition:

1- If this box is unchecked, the position of the current field of view (front image) will be used as the center of the montage area and the tiles with user specified number of X and Y steps will be built around it. Additionally, the magnification and field of view of each one of these tiles will be identical to the current settings of the SEM as seen in the front image.

2- If the box is checked, the user will be prompted to draw a region of interest to be montaged. For acquisition, this ROI will be divided by the number of X and Y steps defined by the user. Thus the field of view and Magnification in each tile is calculated by DM.

As stated above multiple windows are opened automatically when a 3D acquisition is requested. The display zoom factor for these displays is chosen so that the windows fit in the available space. Note that at zoom factors of less than 1, the software automatically bins neighbouring pixels such that the signal to noise in an image may appear better than is achieved when viewing at 1:1 pixel ratio.

3.8.2. **The Cut Alert Function.**

The cut alert function is triggered (if turned on) in case the system determines a poor cross correlation between successive images. These settings can only be configured from their default settings from the Global Tags menu. Select General/Global Tags/ SBFSEM/Record to display the appropriate tags. Cut alert max retry sets the number of times 3View will attempt to collects images if the alert trigger has been set. Cut Alert Threshold is the value of the two cross correlated images. With a Max retry value of 3, the system will take three more images and if the cross correlation value is still triggered the system will pause the experiment and send the user an email. This system is intended to detect problems with a 3D acquisition that are not self-corrected after a few more cuts, e.g. when debris is persistent on the specimen in the ROI. It is also helpful to spot problems associated with macroscopic drift, or spurious contrast conditions for example associated with charging.

If large cut thicknesses are being requested, and this sets off the cut alert function, then increase the cross correlation figure.

4. Post-processing Software.

Post-processing can be performed on “on-line” PCs and “off -line” PCs. The larger the datasets being processed the more beneficial it is to have larger RAM in the operating PC. Some data manipulation and rendering tools may be best performed by 3rd party software, and for this reason DigitalMicrograph allows the user to export 3D data in a flexible manner.

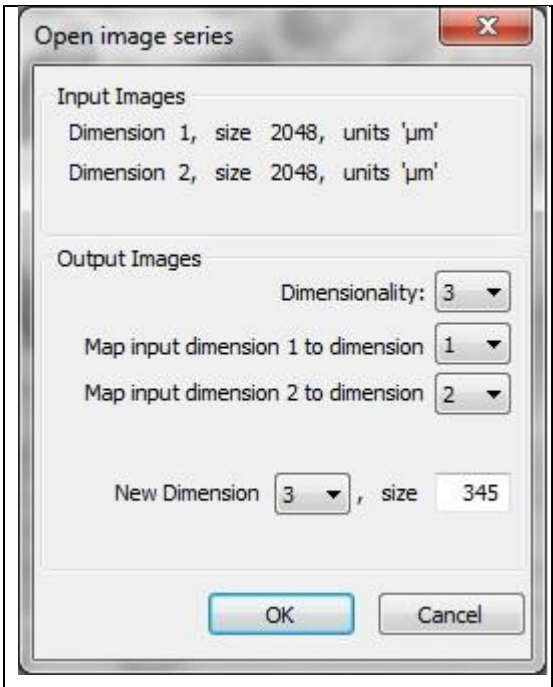
Note when opening files, then you need to specify the file type from the drop down menu (e.g. *.dm4) in order to see the data.

4.1. Open Series.

Extended 3View experiments collect sizeable data in an automated fashion whereby the file name ends in the format NNNN.dm4, starting at 0000,dm4 and increasing sequentially. The open series function only works when this format is utilized.

When the experiment is live then the live data tower presented to the user is restricted to 1Gb. The Open Series tool is found next to the Open function in the File drop down menu and allows the user to open larger datasets than this limit for post-processing. The Open file function is used to open just a single file.

If a dataset consists of a series of DigitalMicrograph, (or TIFF) images ranging from 1 to 4000, then Open series allows the user to specify opening a selected range, for example from 2000 to 3000. This may be necessary in case the dataset is larger than the memory capabilities of the PC being employed. To perform this function, it is necessary to select the image named prefix_2000.dm4 (where prefix is the name given to the file in the acquisition), and then select 1000 as the file size in the Open image series configuration tool shown below.



In this case, the user is requesting to open a 3D dataset consisting of images of dimension 2048 x 2048 in X and Y, and 345 in Z.

The dialogue does not allow you to specify the start or end image, only the number to start opening at. Therefore to open a subset, you must locate the first image in the data tower, and only specify the number to open.

Once OK is pressed, then a progress dialogue is shown where the image number out of the total is displayed.

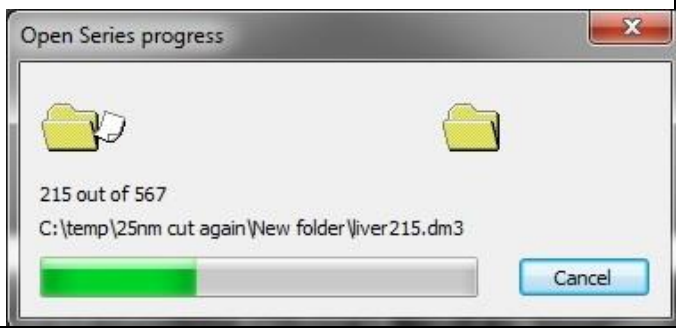


Figure 55 Open image series tool.

4.2. Slice Tool.

The Slice tool and Slice player are not restricted to post-processing functions and can be used to interrogate the live 3D dataset during acquisition. However, for simplicity the function is described in this chapter.

Figure 56. The Slice Tool.

Note, the Arrow to Slice tool from the 3View Tools drop down menu allows the user to move forwards or backwards by one slice using the forwards and backwards arrow keys on the keyboard.

The slice tool becomes active when a 3D dataset is the front-most image. If the front-most image is 2D, then it becomes inactive.

The top slider can be moved to select a particular slice (cut depth) from the 3D stack. Two numbers are provided because the slice tool provides the ability to bin data in the Z direction using the bottom slider. The movement of the slider from left to right increases the Z depth through the stack.

The bottom slider is the width slice which is shown in the image. With a 3View dataset, the minimum width is the cut thickness. If a larger width is chosen then two or more images are binned together. This can often lead to an improved signal to noise in the image, but a loss in resolution in case cutting or image movement occurred.

The slice tool is not suitable for automatically opening and examining data automatically saved in one experiment as multiple stacks. This would be too large a dataset to open. Instead the Stack Browser tool is used for this purpose as described below. A 3D dataset should be opened from the standard File / Open request menu.

4.3. Slice Player.

Figure 57. The Slice Player Tool.

If a 3D dataset is front-most, then the Slice Player can be used to automatically scan through the dataset in the Z direction.

The Slice Player is equivalent to manually moving the slice tools with the minimum width setting.

The small floppy disk button allows the user to create an AVI file from the Slice Player movie. Bear in mind that using any of the compression options will lose pixel detail.

4.4. Volume Tools Menu.

A range of Volume Tools for processing 3D datasets are found under the Volume drop down menu. These Volume Tools only apply to 3D datasets.

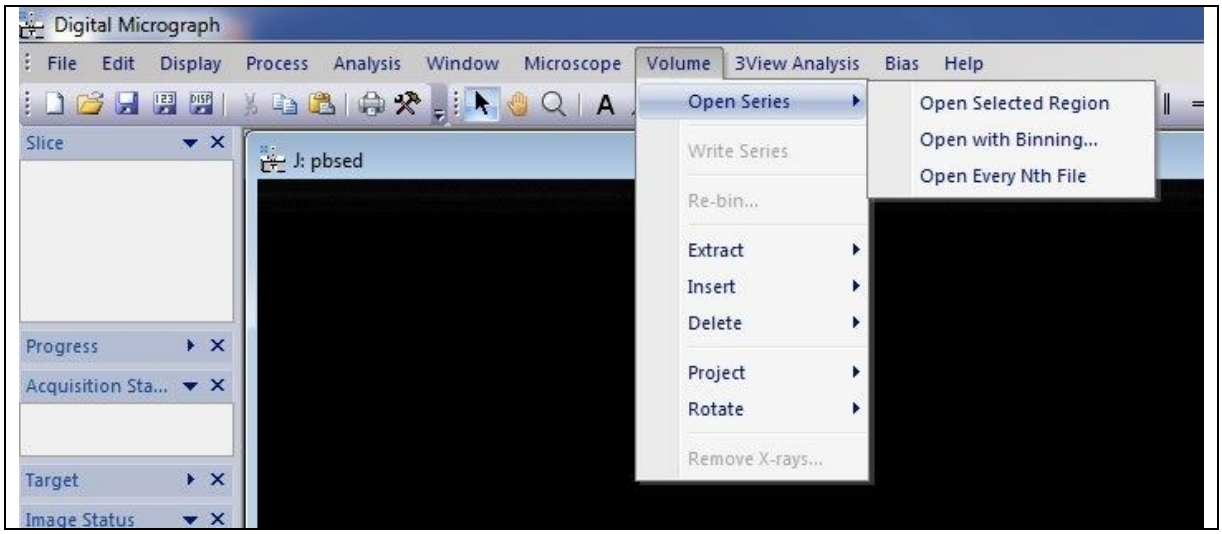


Figure 58. Volume Tools.

The Volume Tools provides a considerable functionality for handling and post-processing 3D datasets. The Open Series set of choices can be used in place of the normal File “Open series” command in case the user wishes to do open a 3D dataset under the following conditions.

- **Open only from a selected region in X,Y**
- **Open using binning to specified amount to average neighbouring pixels in any dimension.**
- **Open only every nth file in Z.**

As with all 3D data processing functions, the memory and CPU requirements can be significant, so some patience is required. The progress of the operation is reported in the Progress window which is found under the Windows drop down menu. Please do not attempt to use the slice tool or other operations until the progress is reported as complete.

The user can choose the rebinning function in the X, Y and Z dimensions. If a factor of 2 is chosen on all dimensions then the voxel volume increases by a factor of 8, and the memory requirements therefore drop. The rebinning action works by averaging neighbouring pixels, which increases the signal to noise ratio but decreases the spatial resolution. In the case of oversampled data (where the pixel size is smaller than the actual resolution), rebinning can be performed to a certain degree without losing spatial resolution, while retaining the benefits of increased signal to noise ratio and reduced memory requirements.

The Volume Tools also contains Volume Manipulation options. These include the ability to extract, insert, or delete planes within a 3D dataset.

Note that the Project Along Functions, (X,Y,Z) do not function on 3View datasets. This function is designed for spectroscopic 3D datasets and should be ignored.

4.5. 3View Analysis drop down menu.

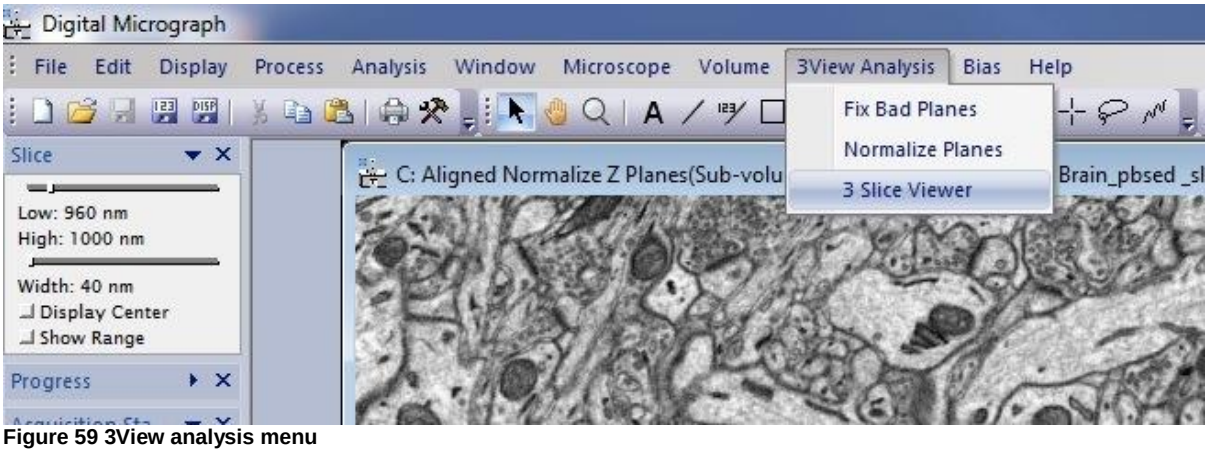
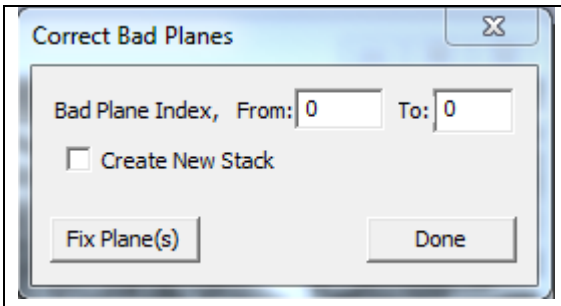


Figure 59 3View analysis menu

The 3View Analysis drop down menu contains three post-processing, or visualization items. Note, the drift correction function (which is commonly performed on 3View datasets prior to using the 3 Slice Viewer) is a menu function itself found under the Windows drop down menu.

4.5.1. **Correct Bad Planes.**



Correct Bad Planes: This is a tool for removing a bad image (slice) from a 3D dataset whilst removing the appearance of any discontinuity. When a slice is identified in the dialogue, the software replaces this slice with a new image whereby each pixel is the interpolation of the neighbouring information in the Z dimension. For example if slice 12 is identified as poor, then Correct Plane will delete the existing slice 12, and in its place insert a replacement which is an even mix of the information in slices 11 and 13.

The benefit of this approach is although some information is lost, the replication of a bad image with an interpolated image does not destroy the integrity of the data in Z. This tool is less useful if there are multiple bad images in a row.

Select the Create New Stack check box to generate an additional stack with the fixed planes.

Figure 60 Correct Bad Planes

4.5.2. **Normalize the grey scale intensity.**

The raw data may show unwanted variation in the average grey scale level between images in a stack - for example caused by small changes in the back scattered yield between slices. This may be due to charge build up on the specimen, rather than instability in the grey level from the detector. 3View software provides the ability to analyse the grey level and then provides options on the corrective methods that can be applied.

To perform this routine, select “normalize z planes”, as a menu from the windows drop down menu. If Use Calibration Region is selected a green ROI will be available to adjust and move anywhere in the image (the arrow tool must be selected in order to manipulate the calibration region box). After pressing OK, a blue line plot is created representing the average grey level inside the calibration region for each frame in the stack.

The calibration region should be chosen such that the contents will be representative of the sample you are interested in throughout the stack, and as large as possible to limit the effect of small objects with high contrast. If uncertain, expand the calibration region to fill the entire image.

If Average Adjacent Planes is selected, DM will perform a running average through the stack and will prompt the user for a value. Select the Create New Stack check box to generate an additional stack with the fixed planes. This action will consume additional RAM, but will leave the original stack unmodified.

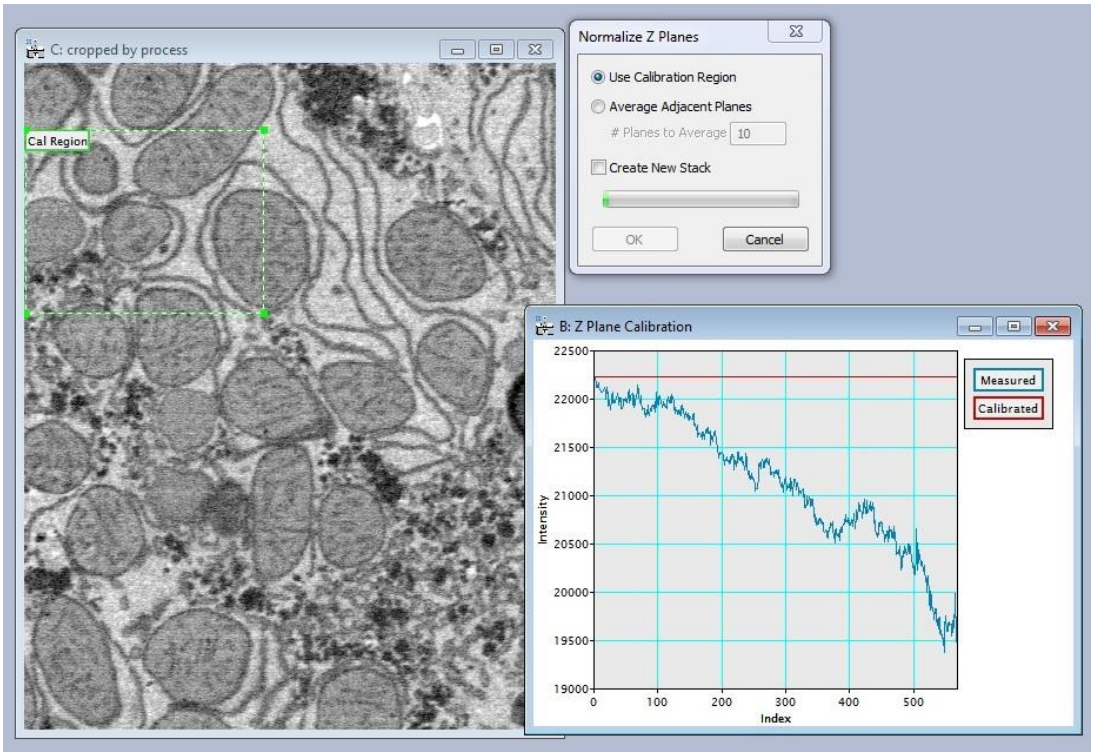


Figure 61 Normalize grey scale intensity

4.5.3. **3 Slice Viewer**

The 3 Slice Viewer is a very powerful tool for looking at 3 slices in XY, XZ and YZ simultaneously. As such it can be used to understand the integrity of the raw data, or can be used as a powerful visualization tool on data which has been post-processed to remove drift (jitter), or variations in grey scale. The slice position in each of the dimensions can be adjusted by using the slider tools at the base of the image, or by dragging the dotted line cursor on each of the images. The dotted line cursors therefore show the 3 slice positions through the 3D volume.

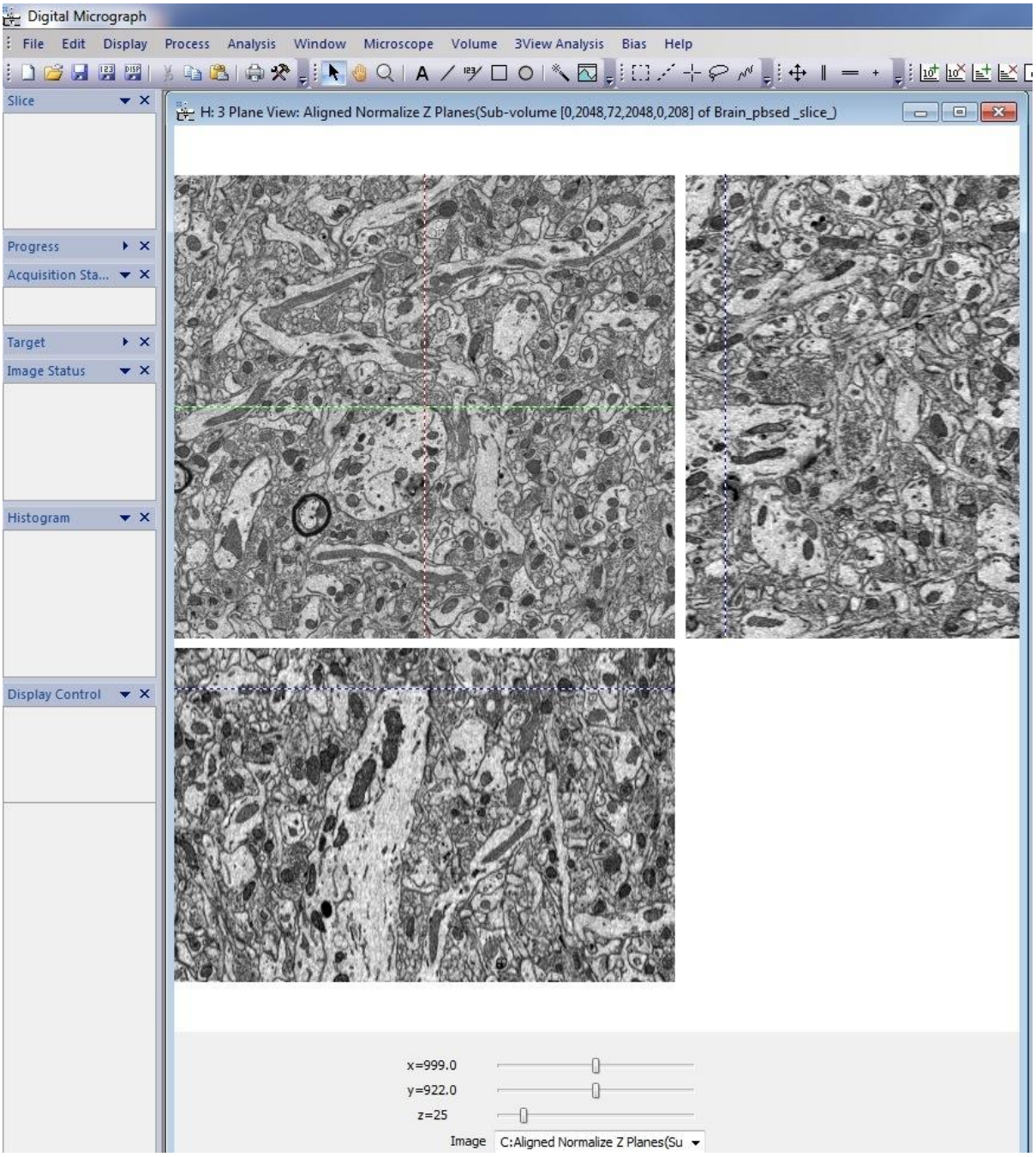


Figure 62 3 Slice Viewer.

4.6. Image Alignment Tool

Drift or jitter between sequential images in a 3D dataset can have multiple causes and sometimes these are difficult to diagnose. A gradual movement may be due to slow thermalization of the stage after it has pumped down, or if the room temperature is not stable over long periods of time.

Random movement can be caused by mechanical instability of the specimen as it is being cut, or by variations in the local charge environment. For example charged up debris can cause both defocusing and images shifts especially at high magnification.

In order to minimize any mechanical movement in the image it is essential that the specimen is very secure on the specimen holder, and that the specimen holder is locked secure. Squat flat topped pyramid specimens are optimum for specimen stability.

Drift correction on an open 3D dataset is performed by selecting the Image Alignment Tool from the Windows drop down menu. The tools button configures this process. The image filter algorithms are not recommended for 3View datasets. Once the Measure Spatial drift button is pressed, then two line graphs are plotted sequentially as the data from the whole field of view is analysed. The blue line is the shift in X and the red line the shift in Y. Note these coordinates are with respect to the image, not to the X,Y motors which are rotated to this axis. Also note that this routine can be performed on an open dataset, or as read from file as defined in the Source Images selection box.

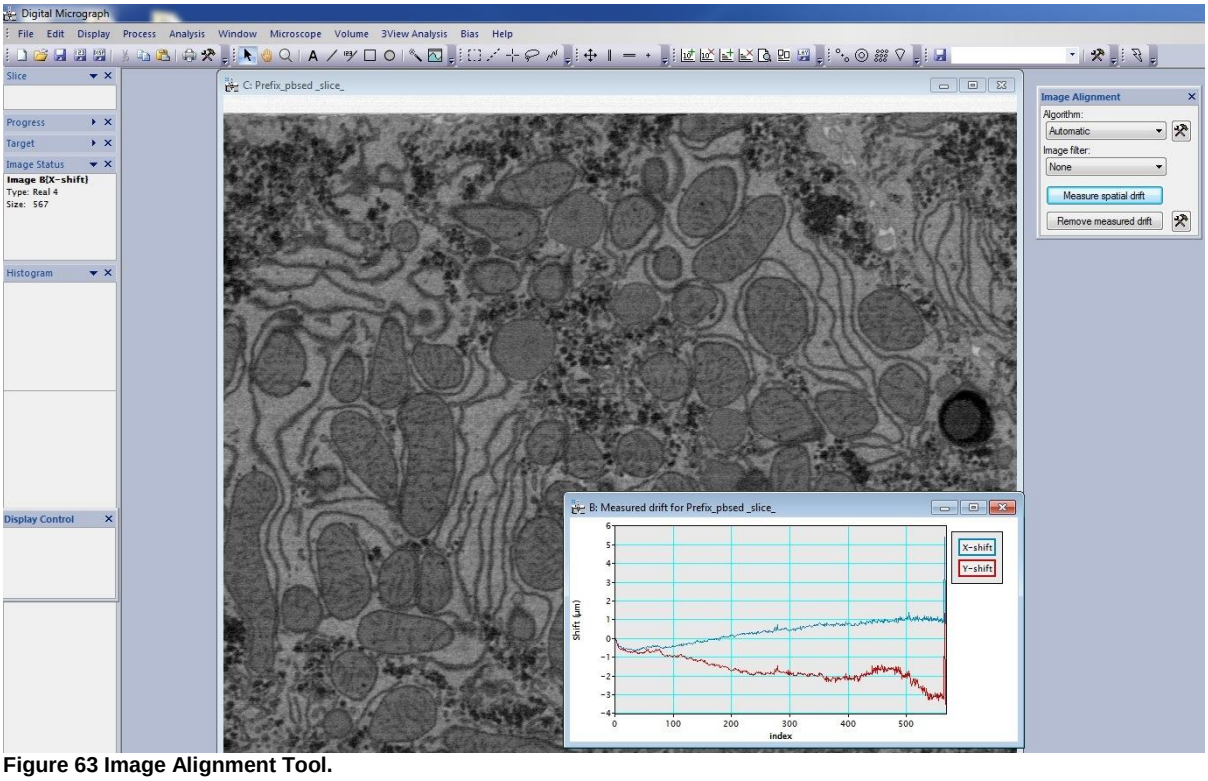


Figure 63 Image Alignment Tool.

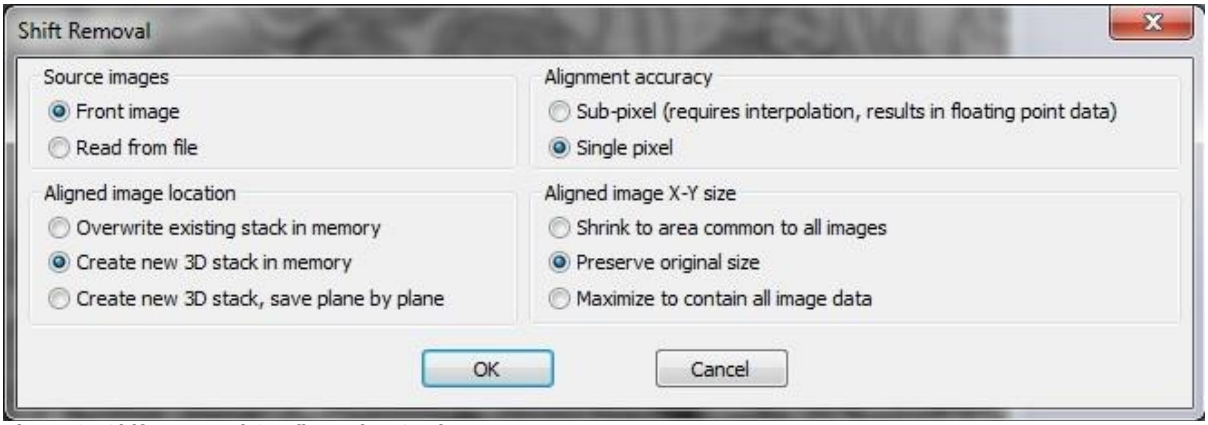


Figure 64 Shift Removal Configuration Settings.

It is recommended to analyse and understand the drift results in X and Y prior to performing an alignment routine. For example if just one or two images in a dataset show a considerable jump, then it may be worth sacrificing these images in order to avoid cropping issues. To do this it would be necessary to either manipulate the data using Volume tools, or if the misaligned images are at the beginning or end of a dataset, then to reopen the data without these images.

An alignment operation can only be performed on a dataset which has just been analysed. The single pixel accuracy is recommended for 3View images.

The aligned image X-Y size can be configured by the user. There is a choice to crop the data so there is no spare perimeter using the shrink to area common to all images. The alternatives are to preserve the original size, or else increase the size of the dataset to allow all data to be shown. Using the latter option creates a mid-grey level for all “spare” areas of the perimeter as the aligned image moves inside the larger sized area. Users can choose whether to overwrite the existing data in the memory, create a new dataset in the memory, or write this data one plane at a time to the hard memory. These different options take different periods of time and have different memory overheads.

Note that if the specimen contains texture which has a preferred orientation in Z, then this can be misinterpreted as drift when it is actually the true nature of the ultrastructure.

4.7. 3D Visualization

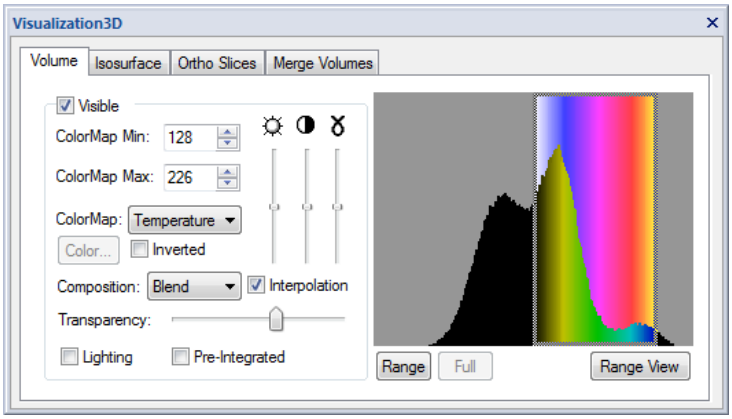
Digital Micrograph incorporates a raytracing engine capable of volume rendering large datasets on the high-spec GPU. This is an interactive display that allows the user to navigate around the volume, mapping colour and opacity to the raw data by value or local gradient. For additional detail, the user can also apply volumetric illumination or generate isosurfaces from the dataset.

By default, an image stack opens with the topmost slice visible like a standard image raster. In order to visualize the volume, right click on the image and select *Display type* > *Volume visualization*. A window should appear with a plan view of the dataset. Depending on the size of the dataset, this operation can take some time, so please be patient. Once the model appears, it then takes additional time to show the rendering option before the model can be manipulated.

The model can be rotated in 3D by clicking and dragging the mouse. The scroll button on the mouse is used for zooming in and out of the image.

Enable the Visualization 3D window to adjust the raycasting parameters: Select *Window* > *Floating Windows* > *Visualization 3D*.

The histogram shows the distribution of grayscale values in the raw data. The overlaid band is interactive and represents the mapping of the active colourmap to the raw data. The user should experiment to get the best rendering for their dataset.



The visualization can accommodate both Volume, Isosurfaces and Othoslices and the user is required to turn each of these options On and Off if required when switching between different tabs.

Figure 65 Visualization3D Software

4.8. Saving Tools

Saving tools are found under the File Menu. When a 3D series is being saved, the user is allowed to choose whether to save the data as a series of images (as are recorded automatically) with sequential file names. Alternatively the user can choose to save the 3D dataset as one file as long as this is a manageable size for the RAM to handle.

4.8.1. Saving as a TIFF

To save the original DM4 format series as a series of tiff images, click the folder button on the 3View control / record tab to bring up the file saving menu. Expanding the Save Image As menu will bring up several different file formats; select TIFF Format to save the entire series as tiffs. Note that none of the SEM or DigiScan information is saved in the tiff images.

4.8.2. Convert to TIFF

The Batch Convert Files option under the file menu can convert dm3 or dm4 file types to tiffs. The batch convert will convert all of the files in a folder as well as all of the sub-folders if the check box is selected. Select the files types to be converted, in the above image, dm4 files will be converted. Then using the Save Image As, select the file desired file format, in this example the file type is tiff. This is useful for exporting a dataset that is too large to hold in RAM, for use with 3rd party software.

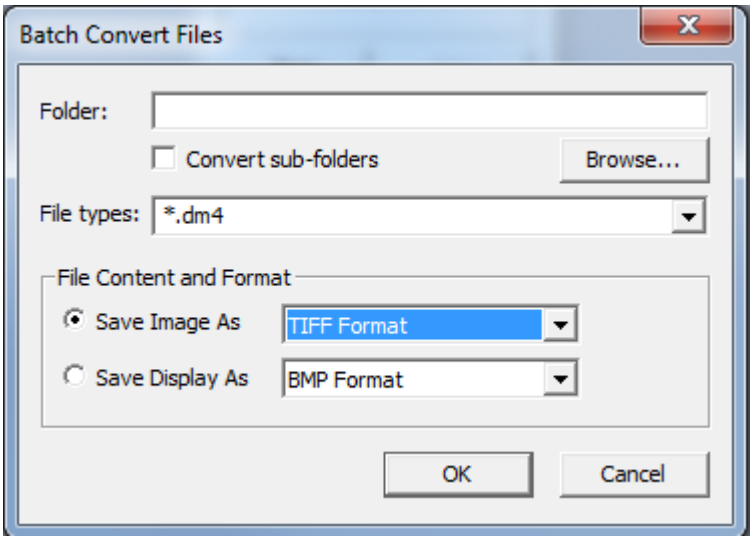


Figure 66 Batch Convert Files

5. Setting up protocol.

This section assumes that the system is installed, and the user is familiar with the operating software. This section provides a reminder of a typical working protocol.

5.1. Prerequisites

Before attempting 3View2XP experiments, the following pre-conditions form a useful check list.

5.1.1. **SEM.**

The SEM is on.
The Microscope can provide a high resolution image in high vacuum mode on a test specimen, e.g. gold on carbon, or the Gatan supplied test 3View specimen. This can be on the SEM stage, or the 3View2XP stage.
Any environmental effects (vibration and fields) are understood.
Any field cancelling system is on and functioning correctly.
If working in VP mode, then SEM can keep a stable low vacuum pressure.
The typical resolution of the microscope in low vacuum or high vacuum at a kV spot size suitable for 3View2XP applications is known. Gold on carbon results are important to understand.

5.1.2. **Specimen.**

The contrast features and region of interest within the specimen are known. For a biological resin embedded specimen, this includes location and depth from the surface and depth extent of the specimen showing heavy metal contrast (stain).

The specimen is securely mounted on a 3View rivet and has been trimmed to a suitable size with a microtomed flat top. An appropriate rivet has been chosen which can accommodate the specimen height and Z travel. The majority of specimens take the taller rivets. If working in high vacuum mode, then the edges of the specimen should be coated in conductive paint (which should be dry).

The specimen is known to provide contrast appropriate to the detection method. Under certain conditions secondary electrons can provide contrast but the contrast mechanism here can be a strong function of the exact dose, specimen and detection conditions. See Chapter on Specimen Preparation for more details.

5.1.3. **The 3VBSED.**

This assumes that the Gatan 3VBSED is installed, and that any other similar detector, if sometimes used on the microscope is withdrawn or detached.

The diode of the Gatan 3VBSED detector should be attached to the pole piece or, attached to an arm beneath the pole piece and the aperture. For diodes attached to arms, then the aperture should be adjusted to be central to the zoom axis. When in place, the low magnification field of view is restricted.

The diode wiring should be connected to the socket for the electrical feed through to the pre-amplifier which should be connected to the controller. The 3VBSED controller should be powered on and communicating with the software. The 3VBSED controller is connected to the PC using port number 1 on the PC. The bias should be configured to the correct level, as suggested in the storage box for the diode, and should be ticked on.

5.1.4. **3View2XP system.**

- The Firewire and USB connections between the PC and the controllers are secure.
- The PC has been on for a period to allow the Firewire device drivers to load.
- The Gatan PC can communicate with SEM PC.

- The DigiScan and Microtome Stage Controller are on and all electrical connections are secure.
- DigitalMicrograph Software is on there are no error messages on start up.
- DigiScan acquires images automatically taking control of the scan.
- The knife is clean of debris and securely fixed in the knife holder.

5.1.5. Optical Alignment.

Please read chapter 2.12 about the optical alignment. The purpose of the optical alignment is to cope with the limited X,Y movement of the stage. The bench top microscope acts as a positional mapping jig once it is correctly aligned to the SEM zoom axis on the microtome stage.

If alignment isn't performed then it is quite possible that once the beam is turned on, it won't be possible to drive the stage to the desired region of interest. It is the job of field service to aid with initial setting up the alignment at installation. However users are also required to engage and own this process for their own benefit.

There are 3 concentric rings on the specimen holder. This is to allow the user to fix the rotation of the specimen such that the correct facet faces the diamond knife, whilst keeping the rivet locking screw in a defined position away from the diamond knife holder and ensuring the specimen is approximately central to the zoom axis of the SEM. The screw should not point towards the diamond knife holder.

When the optical stereo zoom microscope is fixed in its defined, locked resting position on the bench top mount, the centre of the field of view as defined by the graticule cross hairs should map reasonably well to the zoom axis of the SEM when the specimen is inserted in its default manner in both the SEM microtome and the bench top stand. This zoom axis should be when the stage motors are homed. As the specimen to be cut does not have rotational symmetry in the holder, it is important to remove the rotational freedom by always using the same orientation of the holder in its two respective locations.

5.2. Experimental Protocol to Work on a New Specimen.

5.2.1. Initial Tasks.

- Click on Lower in the Approach tab to reach the lowest Z position. Press Null Z.
- Press Clear.
- Manually turn off the bias to the BSED.
- Turn off the Gun HT, Vent the SEM chamber and withdraw the stage fully.
- Lower the mount structure by slackening the 2 side locking screw clamps, and turning the large flat blade screw head anti-clockwise. This is to ensure that when a new specimen is inserted, it will be a safe distance from the knife cutting plane.
- Unclamp the rivet specimen holder if it is installed in the microtome specimen mount.
- Remove the specimen

5.2.2. Bench Top work.

- Place the rivet specimen holder in the bench top stereo zoom microscope mount.
- Insert a rivet containing the new specimen in the holder such that the desired face approximately faces the direction of the diamond knife if it were moved to the microtome.
- Unscrew the clamp at the bottom of the rivet holder slightly, and adjust the concentric rings if necessary to translate the specimen position such that it keeps its defined rotation, but is central to the cross hairs. Tighten the lower clamp, and the grub screw to ensure that the specimen and holder structure is completely secure and recheck the position.

5.2.3. Attach new specimen in Microtome.

- Place the specimen holder in the microtome facing the correct way. When ok, clamp it with the central Allen screw.

Caution: ***Be careful at this stage. The user is entirely responsible for ensuring that the specimen is not at a height which could hit the diamond, and that the diamond knife holder is attached carefully. Never attach a knife without checking the clearance status. Remove again if unsure, and if the work on the instrument is interrupted at this stage.***

5.2.4. The Approach Protocol.

The approach protocol takes place with the specimen holder locked in place and the diamond knife attached. It forms 3 distinct stages as explained below and is normally viewed with the optical microscope attached to the SEM door.

During the 1st two stages, it is important that the STROKE is UP. The software does not provide reminders and it is the users responsibility.

The illumination of the knife and specimen is important at this stage and the procedure is easier to perform if the specimen already has a reflective microtomed top. Gatan recommends using the Move to command from the knife section of the Approach panel (e.g. move to 600 places the knife mid-way) to place the knife tip directly above the specimen in order to be able to see the reflection of the knife tip on the specimen.

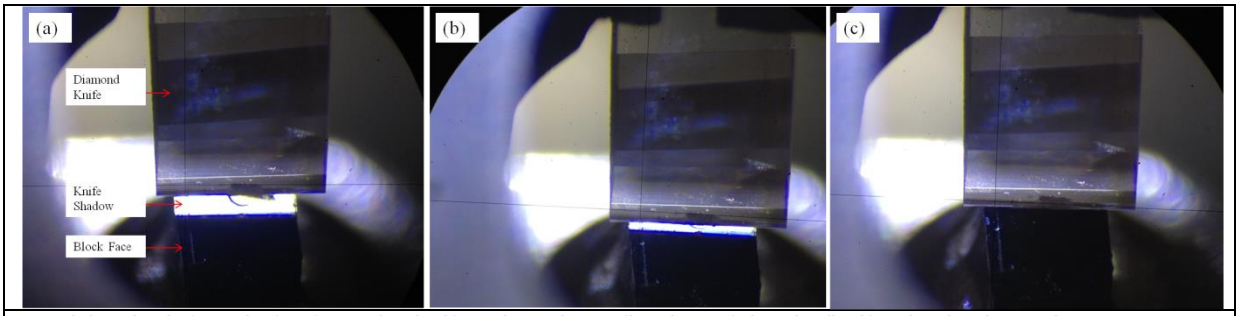
		STROKE MUST BE UP!
A	Coarse manual approach	Clockwise turning the flat bladed screw driver with the side screw clamps slacked provides accurate manual raising of the specimen to a safe height below the cutting plane.
B	Finer motorized manual approach	The sides screws are clamped tight. The user can request safe increments of the Z height so that the specimen is closer to the cutting plane. This stage is not essential but is safer than being over ambitious with the coarse approach, or spending too long waiting for the automated sequence C.
C	Automated Cutting the Approach software	Define the speed, thickness and number of cuts and observe the knife start to cut the specimen.

The user must be convinced that the clearance achieved with the manual approach allows the knife to pass over the top of the whole of the specimen without performing any cut. Manually moving the knife arm using finger pressure against the retraction springs is safer than using the piezo as then there is more control of potentially unsafe cutting forces.

When performing the Automated Approach, it is possible to pause the sequence and check on residual clearance optically. If many cuts have been performed, ensure that debris is blown away prior to pumping down the microscope.

One common mistake is to see the cutting take place on the specimen, but then to choose an ROI on the specimen which is away from the area which has been cut because of a tilt of the cutting plane with regard to the initial plane of the specimen. It is easier to judge whether the whole specimen is being cut through visual observation than using SEM imaging.

Do not remove the knife for cleaning and re-insert without re-performing the Z-advance and Cut Sequence protocol again as the exact height of the knife is not sufficiently accurate to within safe cutting heights of the diamond knife.



(a) Block face is far from the knife edge, the reflection of the tip (knife shadow) can be seen on the block face.
(b) Block face was raised using the flat bladed screw driver on the central brass screw; the width of the knife shadow (knife edge reflection) is reduced.
(c) Block face was raised further using the Manual Z-Advance Motor Approach, knife edge reflection can barely be seen in this position. The block face is now very close to the cutting plane and the user should be careful that there is a small clearance over the whole of the knife travel.

Figure 67 Images of the specimen being raised to the cutting height.

5.3. SEM Tasks.

- Set the Microtome into the Clear position with the Stroke down.
- Remove the stereo zoom microscope and mount structure from the SEM door.
- Fold away the LED light.
- Check the O ring is clean and not damaged and close the door.
- Ensure the BSED is correctly positioned, clean and that it is connected internally.
- Close the door and pump down the SEM to the chosen vacuum condition.
- Turn on the bias to the BSED using the tick box.
- Ensure that the gun HT is low and the beam current set for typical 3View2XP applications. High kV can burn resin such that out-gassing can damage the FESEM column.
- Turn on the gun HT when ready.
- Set the SEM to low magnification.
- Acquire BSED image. In this image the user should be able to judge which parts of the specimen have just been cut in the approach stage, and which parts of the specimen are of interest to acquire 2D images or 3D datasets from.

Note, although a cutting height has been achieved at air, several cuts need to be performed once at equilibrium under vacuum until resin is once more cut. If the correct depth of interest has not been achieved, then perform cutting using the Approach sequence for extra speed. It is better to be confident that cutting is taking place by taking relatively thick cuts, e.g. 200nm prior to attempting to reduce the cut thickness.

If the region of interest has been cut recently then it is at the 3View focal plane. If it is not being cut then the focal plane will keep rising. Hence it is important focus on regions of interest only once cutting is occurring in the region of interest.

5.3.1. Adjust and Optimize Imaging Variables.

The SEM Variables are:

- **KV**
- **Spot size, probe current.**
- **Chamber gas and pressure or high vacuum conditions.**
- **Focusing.**

- **Astigmatism.**
- **SEM Magnification.**

If the SEM column benefits from a degaussing procedure remember to do this once the kV and spot size has been fixed.

The BSED variables are

- **Contrast (gain)**
- **Brightness (offset)**
- **Bandwidth.**
-

The common configuration is to have the Contrast set to a maximum AND the brightness adjusted to give the correct dynamic range. Only scale back the contrast if the contrast in the signal is too large.

The DigiScan variables are

- **X,Y Pixel dimensions. (For a given magnification this affects the pixel density and hence dose per unit area on the specimen).**
- **Pixel dwell time.**

The options of Line sync and integrate multiple frames are rarely employed in 3View applications. All these variables will affect the quality of the image.

All focusing should take place with the knife cleared, and with the BSED bias on at the agreed level. On some columns the bias value affects the focusing slightly.

The action of focusing at high magnification often burns the resin. Users can decide whether to finely adjust the focusing and astigmatism setting of the SEM at a region of interest and cut away the damaged material, or else moving to a neighbouring less interesting area.

GMS2.3 provides an automatic focus / astigmatism function which can be configured to operate at different magnifications and stage locations if performed during the cut sequence. If not performed during a cut sequence then the routine performs the adjustment on the current field of view at the same magnification using the DigiScan preview settings.

Users may find focusing and astigmatism adjustment help using the Live FFT option within DigitalMicrograph. To use this, a user normally chooses to image at a pixel density of 256 x 256 or 512 x 512, and then the Live FFT option is requested. The field of view needs to be appropriate to use this facility. Full astigmatism correction needs to be confirmed as the focus is varied. When there is no astigmatism, going through focus should result in a fully symmetrical circular feature growing in size and reducing again as the focused is passed without any change to the symmetry. Astigmatism correction can be used to reduce the occurrence of elliptical diagonals on the FFT.

5.3.2. Adjust and Optimize 3View2XP Variables.

Users will typically experiment with manually recording 2D DigiScan images and cutting away exposed parts of the resin during the initial setting up of an automated sequence. It is important that the user is happy with the spatial resolution, field of view, depth of cutting and signal to noise in an image prior to starting an automated 3D stack acquisition.

However, users are reminded that injection conditions which provide the best possible 2D resolution in a single image on a freshly exposed block surface will not necessarily be compatible with an automated stack acquisition where resolution in 3D is important. This is because injection conditions for optimum signal to noise can burn the resin to the extent that cutting at the desired thickness is not possible after a few cuts.

The Main 3View variables for Automated Sequences are:

- *Pixel Dwell Time*
- *Pixel Dimensions in an image, which combined with the SEM field of view determine the pixel density on the specimen and hence injection dose per unit area.*
- *Cutting Thickness.*
- *Number of Cuts.*
- *Location choices of Current, multi ROI and montage.*
- *Choice of autofocus / autostigmate.*
- *Choice of Cut Alert and threshold values as well as progress reporting.*

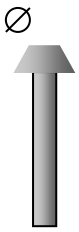
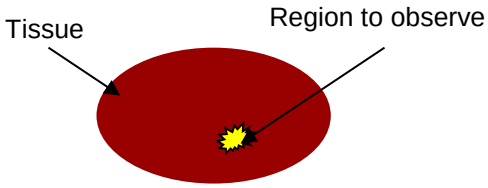
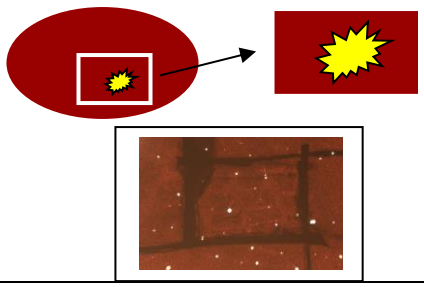
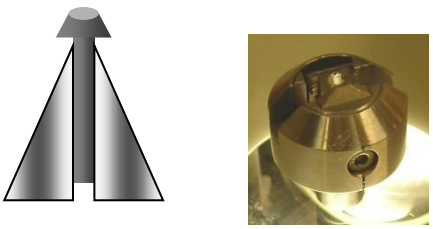
6. Advice on Specimen Mounting.

Caution: ***This protocol involves sharp objects and chemicals. Please consult your laboratory manager or safety officer for safety instructions***

This protocol concerns samples that have been processed for electron microscopy and are already embedded in resin. For material science specimens or those which are not resin embedded please address questions to Gatan.

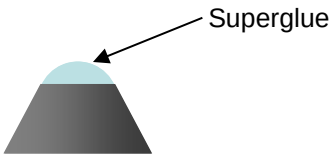
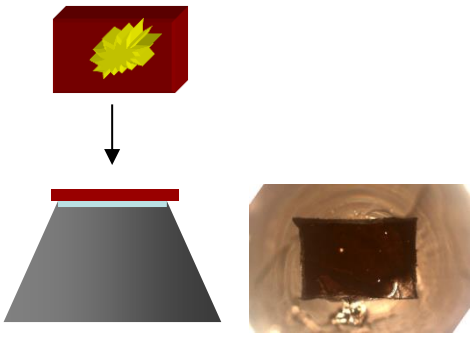
This protocol uses two different types of rivet shaped stubs which are the only specimen holders fitting inside the 3View2XP system. The choice of rivet type depends on the height / thickness of the specimen to be mounted. In addition to Al rivets, special versions can be ordered manufactured from Plexiglas. These are unique in allowing transmissive illumination from below during the preparation process.

<table>
<tr><td>Rivets as shown are in Al or in Plexiglas and are either a short version (PEP6590) for taller specimens, or a taller version (PEP6044) for shorter specimens.

Plexiglas allows observation in transmission with light microscope using bottom illumination.</td><td>  </td></tr>
<tr><td colspan="2">Preparation of the embedded sample</td></tr>
<tr><td colspan="2">On slides</td></tr>
<tr><td>• Determination of the piece of material that will be observed. It must not exceed a diameter of 1.2 mm.</td><td>  </td></tr>
<tr><td>• Cut off the piece of material you want to observe with a razor blade roughly.</td><td>  </td></tr>
<tr><td>• Place the stub in a specimen holder (the chuck of an Ultracut is well adapted)</td><td>  </td></tr>
</table>

<table>
<tr><td colspan="2">

- Rinse the surface of the stub and the resin with ethanol

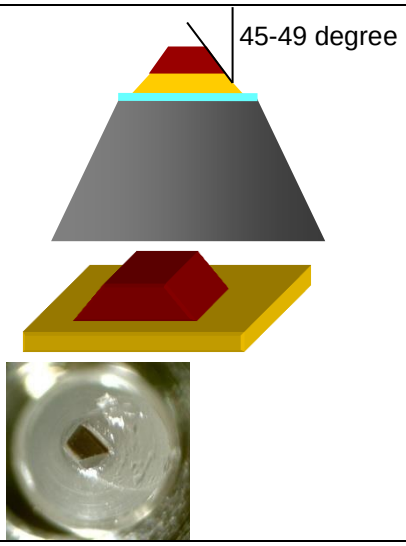
</td></tr>
<tr><td>Place a drop of super glue on the tip of the stub.
If necessary, remove the excess with a tissue</td><td> 
</td></tr>
<tr><td>Place the piece of material on the tip with a tweezer and let it dry</td><td>  </td></tr>
<tr><td>

- Trim the material in a trapezoid shape on the tip of the stub:

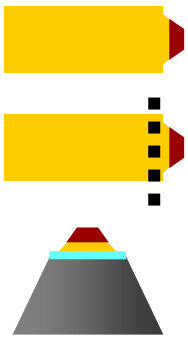
The large sides of the trapezoid must be parallel and at an angle of approx 45 degree

The small sides are cut at 90 degree

The trimming is better if performed on an ultracut because clean sides are required

The surface should stay smooth. This is clear in an optical microscope.</td><td> 
</td></tr>
<tr><td colspan="2">In capsules, 2 cases:</td></tr>
<tr><td>Case 1.
The sample is trimmed in the trapezoid shape on the classical block

a) The tip of the block must be cut off and glued as shown above on the tip of the stub. It can be cut roughly with a saw / blade and then trimmed thinner with a glass knife.

b) The trapezoid should be re-trimmed and adjusted on the stub.</td><td>  </td></tr>
</table>

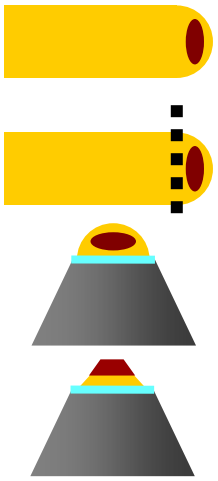
Case 2. **The capsule is intact** a) Cut off the tip of the capsule with the material using a saw / blade / glass knife. b) Glue the tip of the capsule on the tip of the stub with the material centered. c)Trim the sample in trapezoid as described above.	

Figure 68 Advice on Specimen Mounting

7. 3VBSED Diode Maintenance.

7.1. Background.

The diode is very fragile component and live the diamond knife can't be treated with the same warranty conditions as the rest of the equipment. The diode is exempt from extended warranties.

Gatan offers a service whereby the diode mount can be returned to the factory inside the shipping box, and the "silicon on ceramic" component can be swapped out.

As the signal to noise is difficult to quantify because of unknown signal sizes, a useful metric on the status of the detection system is the dark noise, e.g. images recorded but with the beam blanked. The dark noise is then the noise in the DigiScan BSED image for specified pre-amp Contrast / Brightness settings with the electron beam off, and the chamber in darkness under vacuum.

As different types of diode degradation occur, and different types of electronics problems can occur, these can manifest themselves in different dark images. Another key identifier is how the brightness alters as a function of bias on / off or magnitude. This is seen as a shift in the DigiScan red line profile over time or as a function of the bias.

At the maximum contrast setting of 10 it is normal to have to adjust the brightness when the bias is turned off. However, if the brightness value can't be adjusted sufficient to bring the signal into range at maximum contrast, then the diode has catastrophic leakage and normally can't be repaired.

It is recommended to qualify the diode performance before attempting to swap out or repair the unit. A first suggestion is to record dark images with the bias on and off and at different magnitudes. Remember that with autosurvey turned on, then DigitalMicrograph will always expand the available contrast (even if very small) to fill the dynamic range.

When contacting the factory or Gatan field service it is useful to be able to provide dark images under set conditions.

If a DigiScan image shows low frequency noise, or jitter in the brightness, then to diagnose the problem further, please record images with the beam blanked with the bias off and bias on.

Low frequency noise can occur if there is cutting debris on the surface of the diode. This can be difficult or even impossible to remove because of the welding action of the electron beam. It may be possible to blow some debris off the diode surface in situ. However, do not use dust off or similar compressed gases as these contain propellants containing hydrocarbonds. Also the device contains fine bond wires which can get damaged if using too strong a blast of air. Consider using the orange puffer supplied in the spare kits, or a jet of clean dry nitrogen gas. If it is not possible to do this in-situ consider removing the diode for closer inspection.

The silicon component mounted on the ceramic component can be swapped out on the diode mount if it is returned to the factory.

7.2. Swap out service.

Diodes which have become noisier normally cannot be repaired by following any cleaning process at Gatan. The normal repair procedure is to swap out the silicon on ceramic component once the customer returned the mounted diode in the shipping box to the factory. Please contact Gatan for a quote and the returns procedure.

7.3. Removing the diode from the SEM.

Always wear good fitting gloves when handling the mounted diodes. The user should be able to swap out the mounted diode with the supplied spare if there are question marks over the performance. For example if some cutting debris is on the surface of the diode, then this can cause noise in the image and it is often necessary to remove the diode in order to inspect and clean the device.

The protocol for swapping out the diode differs between pole piece mounted designs and side arm mounted designs. If diode is damaged then a repair often involves replacing the diode component in the mount. The diodes are very fragile and are used at extremely high gain for typical 3View operating conditions and so are more sensitive to damage than traditional higher kV back scattered detectors. For this reason the diodes should be considered as consumables with a limited lifespan.

For pole piece mounted designs, the structure should be a simple push fit onto the bottom of the pole piece. Please ensure that no pressure is ever exerted on the front face of the structure. It is important that the metal cover does not touch the silicon structure or the fine gold bond wires.

7.3.1. Side arm mounted diodes.

3View relies on short working distance to optimize the resolution performance of the SEM. This means there is only a small clearance between the backside of the diode's ceramic support and the bottom of the SEM pole piece. There is a smaller clearance between the front side of the diode and metal cover plate. There should be a safe clearance distance between the metal cover plate and the top of the 3View knife structure. This clearance distance usually can't be seen using a chamber scope and it may be necessary to remove a spare side port on the SEM to validate this clearance if the height of the Z position of the diode has been altered.

Caution:

- ***The back of the 3View diode should be parallel to the bottom of the pole piece but must not touch it. The suggested clearance is ~100um.***
- ***The metal cover of the diode must not touch the diode structure.***
- ***If any adjustment is made to the height of the diode on the arm, then you must check that there is still clearance to the 3View when inserted.***

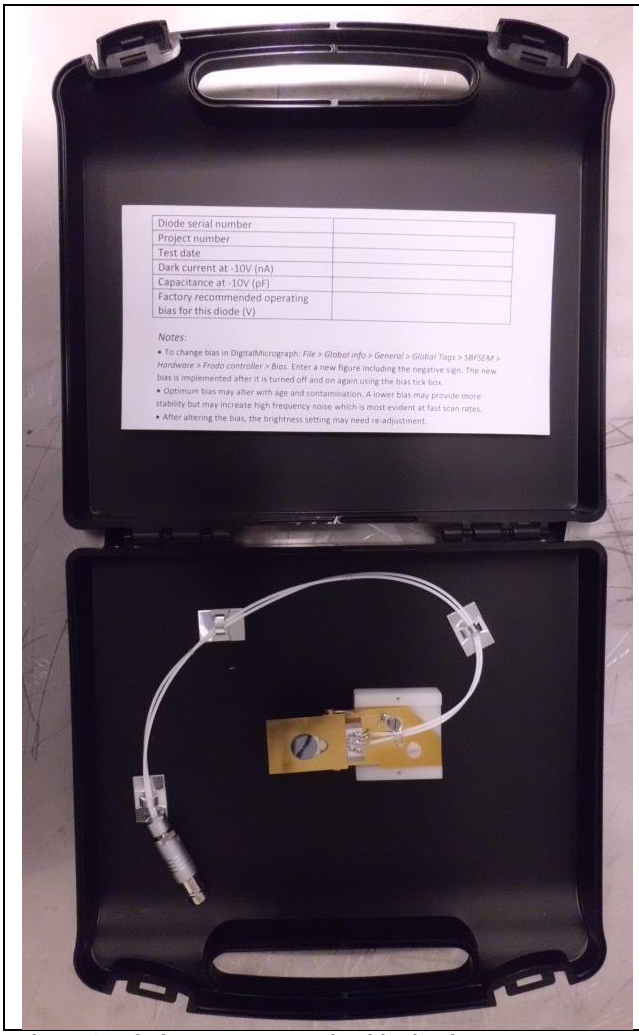
	Always secure the spare diode in the plastic box for shipping and storage. The connector should be held away with the clips. Ensure that the clips don't allow the connector free movement.

Figure 69 Diode secure mount in shipping box.

7.3.2. **Adjustment of the diode position.**

Mounted diodes are attached to a support arm which has adjustment on a fine pivot mechanism in Z and Y. There is additional adjustment in X (insertion) which is sprung loaded. This external adjustment can be performed with the microscope vented or at vacuum with the SEM operational. The adjustment is necessary to ensure that the diode aperture is central to the zoom axis of the microscope. When it is central, then the aperture will be central at low magnification and the microscope will have better performance at low kV with respect to astigmatism corrections.

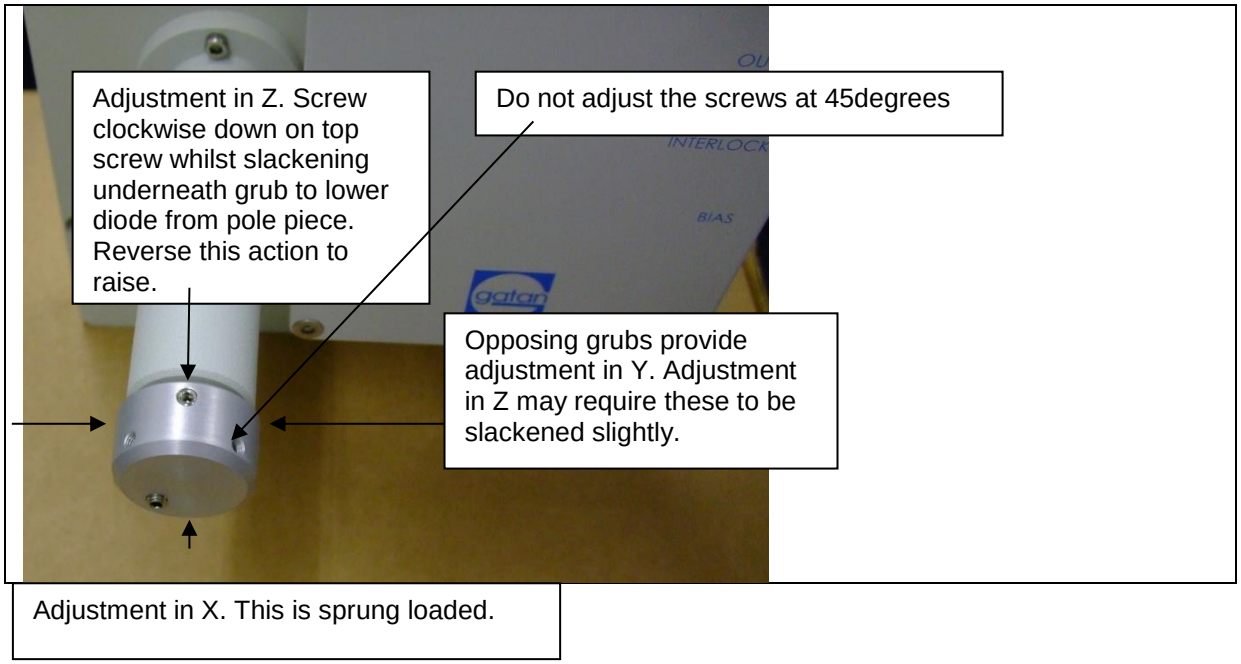


Figure 70 Adjustment of diode position on side arm.

This adjustment is best performed with the aid of a colleague to provide feedback on the clearance to the pole piece. Once the system is at vacuum and the microscope imaging at low magnification, iterative adjustment is required to ensure the diode is completely central. Ensure the screws are locked tight once you are happy with the position. Do not make any significant adjustments in Z when the microscope is at vacuum in case this makes the diode hit the pole piece or the 3View.

7.3.3. **Suggested Protocol for Swap Out.**

The 3View diode can be swapped out with the 3View door fully open but remaining attached to the SEM. Access can be awkward so it is necessary to be careful. Other options are to remove the 3View door from the microscope completely, but this should only be performed if you are familiar and confident with the protocol. Also it is possible to remove the whole pre-amp and support arm assembly from the SEM port. If this approach is followed then the user should take care when re-attaching the unit to avoid the diode clashing the pole piece. This should be performed by 2 people with the diode swung to one side and with a safe clearance distance from any other structure.

If the choice is to detach the mounted diode from the arm then the following is suggested.

- Slacken the Y grub screws on the side slightly.
- Unscrew the lower grub screw and then tighten the top grub screw to lower the diode from the pole piece to provide safety.
- Remove the cable connector from the flange. The Fischer barrel needs retracting to unlock the connector. Do not pull on the co-axial cable.
- Unscrew the thumbscrew which attaches the diode mount to the arm.
- Keep the thumbscrew and washers safe for re-use.

This photo shows the thumbscrew slightly undone, as the diode is not parallel to the bottom of the pole piece.

Hold the diode at the sides between finger and thumb where the side screws hold the cover plate. Do not grab hold of the fragile cove plate. Use the other hand to remove the thumbscrew.

A single thumbscrew is used to clamp the diode mount to the arm.

When this screw is slackened the diode can be swung away from the pole piece. A hard rotation stop allows the diode to be swung back into the same position.

The springiness of the co-axial cable may result the diode swinging back into position.

When attaching a new diode, ensure the thumbscrew is tight such that the diode mount is held very secure on the arm.

This photo shows that the diode cover is parallel to, but is not touching the diode.

There are two washers between the thumbscrew and the diode mount. This photo shows the ceramic mount of the diode is parallel to the bottom of the pole piece.

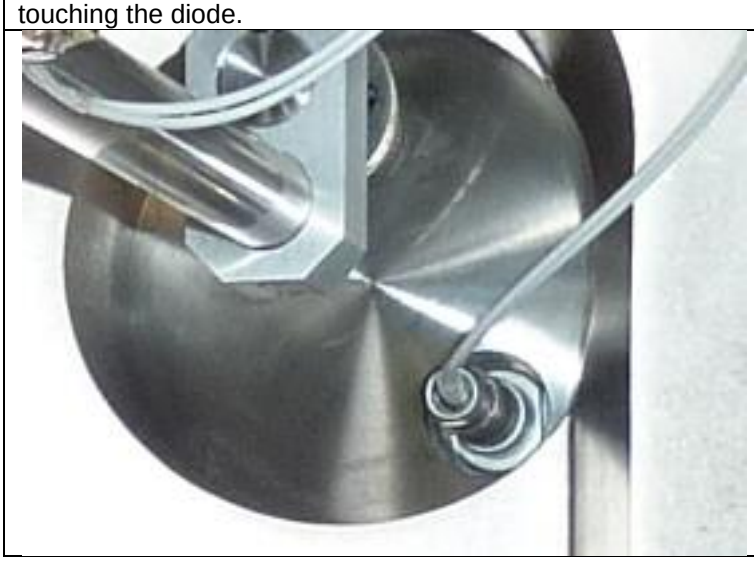


Figure 71 Photos to aid diode swap out procedure.

The diode is connected to the flange using a D type or a Fischer connector.

It is best to remove this connector prior to removing the diode from the arm. The cabling should be positioned such that the 3View doesn't hit the cable when inserted.

When attaching a new diode perform the reverse operation.
However ensure a colleauge can help with providing feedback when raising the diode close to the pole piece. If the diode has been secured in position and rotated to the end stop, then the aperture should be approximately central.

Once the microscope is at vacuum, then the diode aperture will need to be centralized with the microscope at the minimum magnification. This is an iterative process and it is normal that there is some cross talk in the finescale movement.

If sprung loaded grubs screw are employed, then it is normal for one side to be sprung loaded (requires flat bladed screw driver) and for the other side to be an allen key hard stop. The sprung loaded design is designed to make adjustment easier without recourse to slackening the other side. The sprung loaded grubs have limited travel range, and so some adjustment is necessary for any macroscopic movement.

Do not close the 3View door after any adjustment of the diode unless you are sure there is no chance of a clash. If in doubt, then remove a port from a side chamber and use a torch to confirm the clearance.

7.4. Visual diagnosis.

Use the optical zoom binoculars to examine the specimen surface and the bond wires.

When the diode is exposed to light, you should see a few tenths of a volt between the two outer contacts on the ceramic board. The central contact is the earth plane. If there are no volts present at all, there is a continuity break or a short. Check the fine gold bond wires under an optical microscope as these are the most fragile component. As multiple bond wires are used, the diode can still be functional as long as there is continuity to the anode and cathode.

7.5. Cleaning procedure

Users should attempt to clean the diode using the orange puffer supplied in the spares kit, or else a jet of clean nitrogen gas. Disassembly and cleaning using solvents has unknown benefits. If sonic baths are employed, then they should not be vigorous as they can shake off the fine gold bond wires. If solvent cleaning is attempted, then only the cleanest solvents should be used, and the surface should be blown dry using clean dry gas and not left to form a residue.

Do not touch the front surface with any cloth, cotton bud or implement.

- gold wires should only be making contact with the device at the contact pads.

8. Trouble-Shooting and Advice.

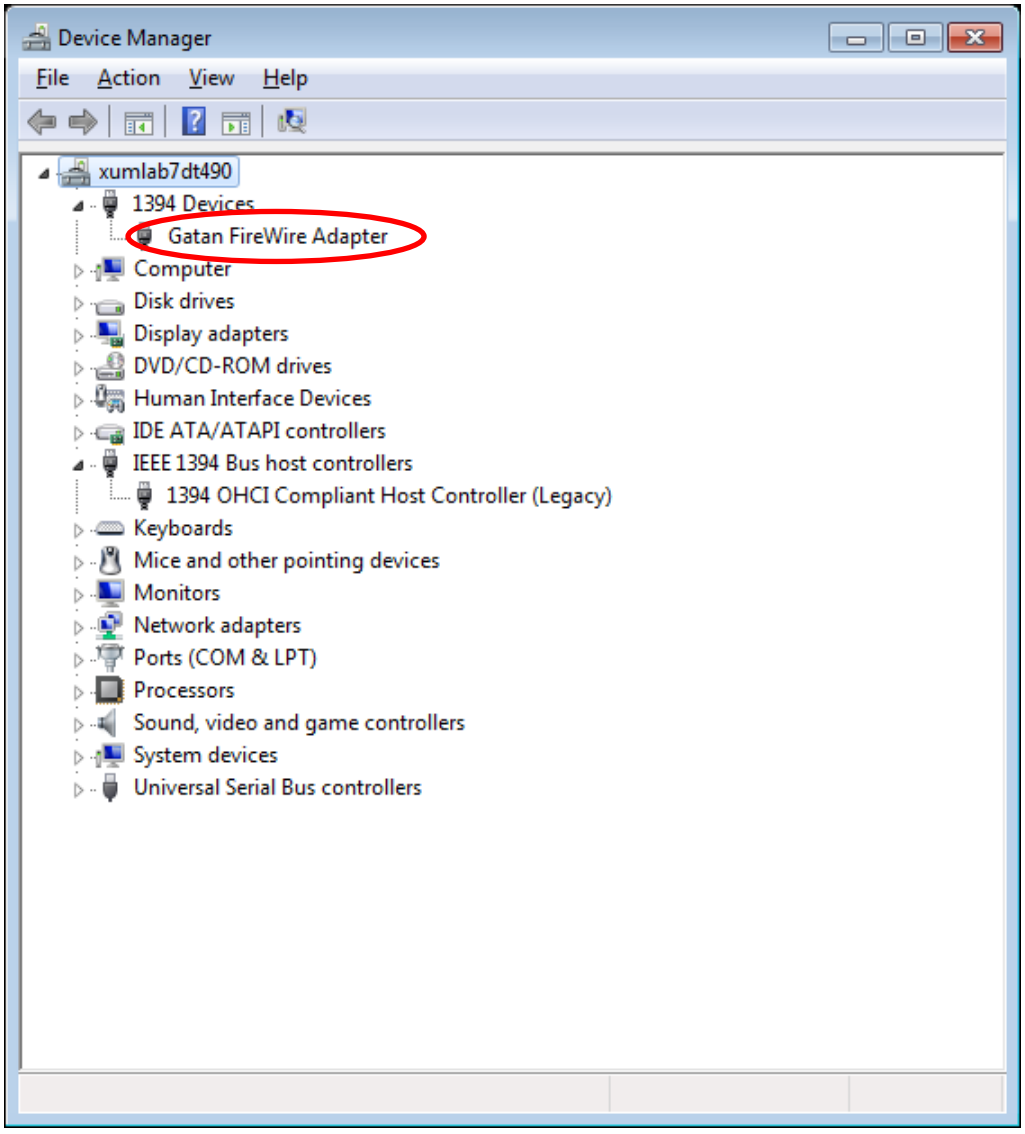
8.1. Procedure for Reloading the DigiScan Drivers

When the PC boots, Windows can take some time to discover the Firewire device driver, especially if the PC is configured in the BIOS to provide fast booting. Fast booting can be disabled by altering the BIOS settings. If not then Gatan recommends that users are patient after the PC is started prior to starting DigitalMicrograph.

If DigitalMicrograph does not detect the DigiScan, but Windows 7 does (i.e. the GFA evident as a 1394 device in device manager) try disabling and then re-enabling the device.

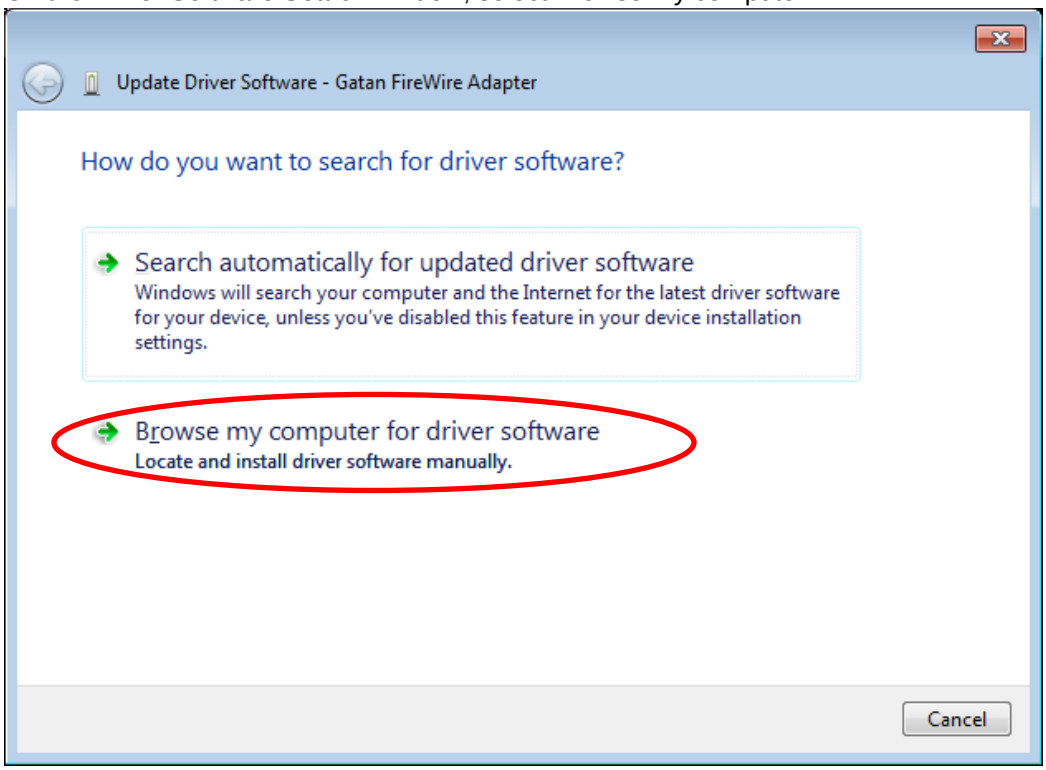
If this fails, try reloading the drivers as described below.

1. Open the Device Manager: Simultaneously press the Windows and Pause key. (The Windows key has the Microsoft flag logo on it.) This brings up the Control Panel/System window. Click Device Manager on the left side of this window. Verify that “Gatan Firewire Adapter” is seen under 1394 Devices.

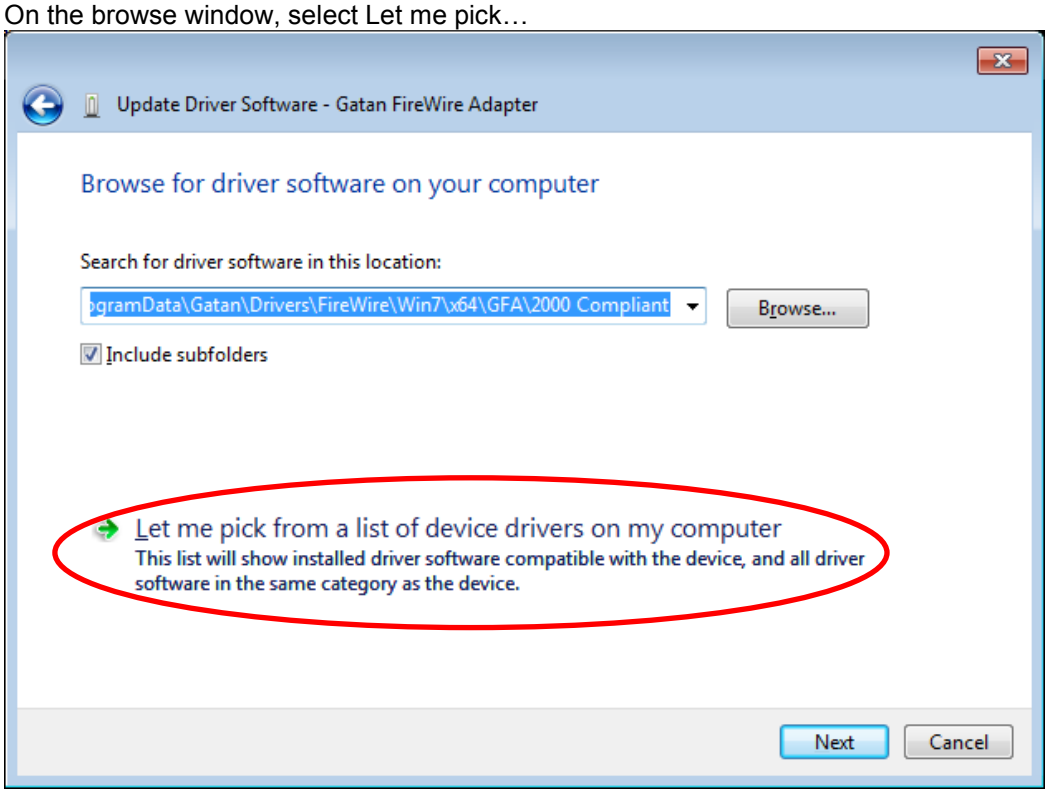


2. Right-click on the DigiScan and select Update Driver Software.

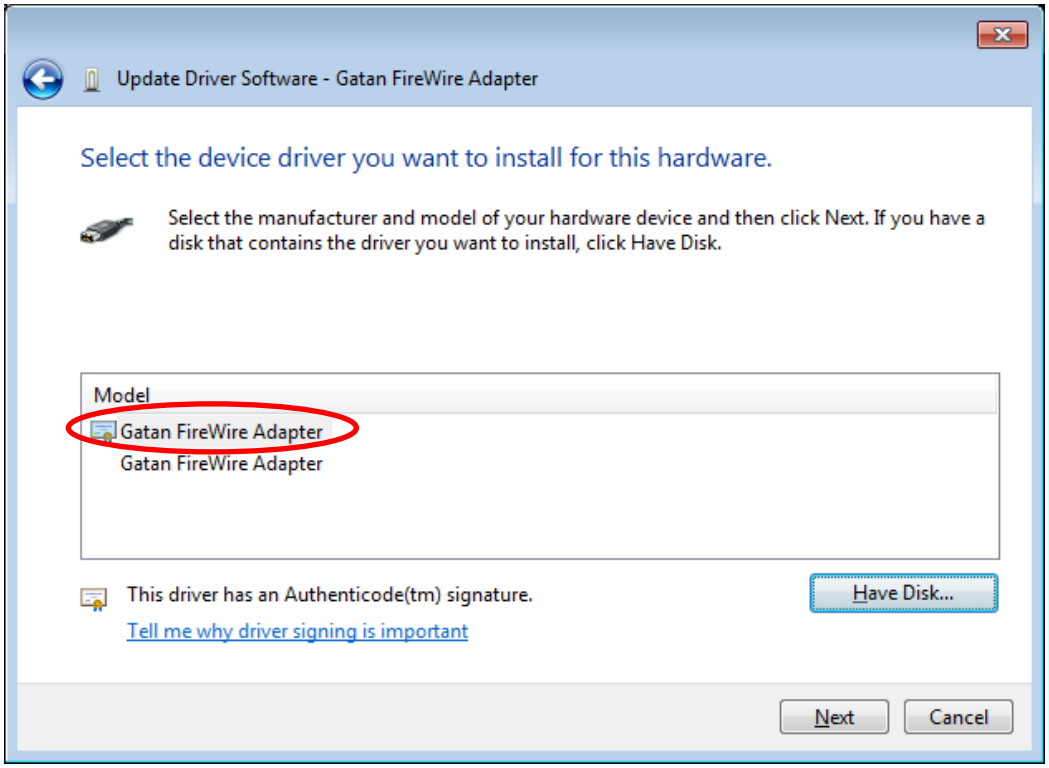
3. On the Driver Software Search window, select Browse my computer…



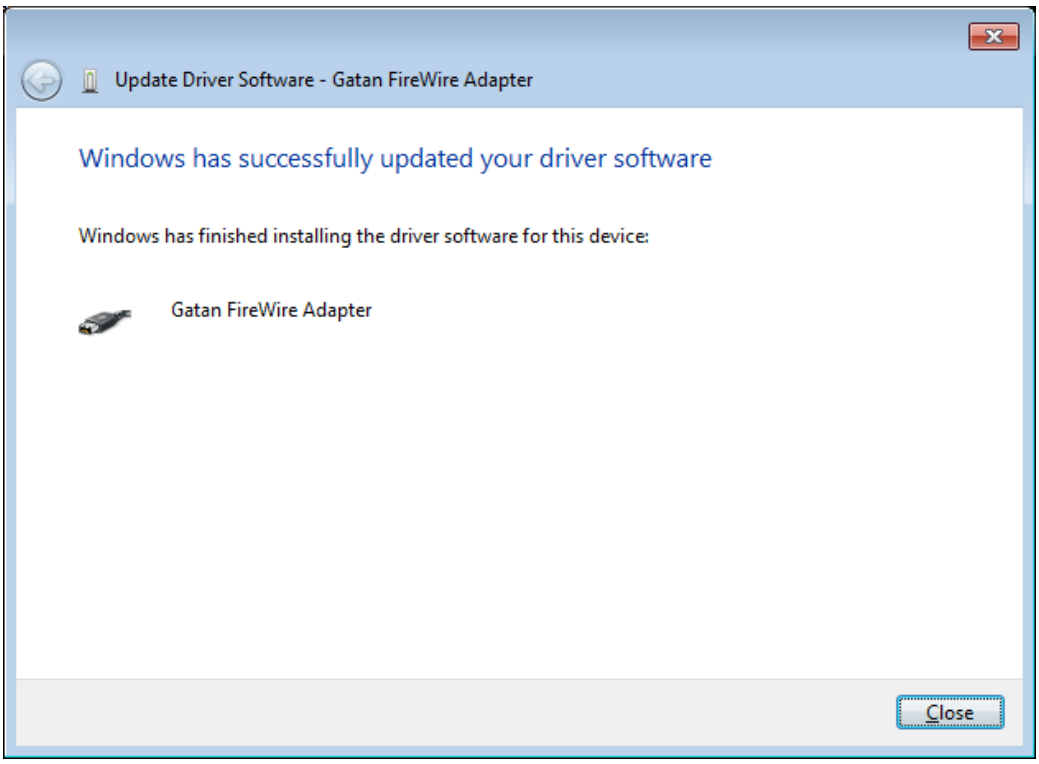
4. On the browse window, select Let me pick…



5. Select the Gatan Firewire Adapter Driver. If you have 2 options, select the one with the Authenticode signature symbol next to it.

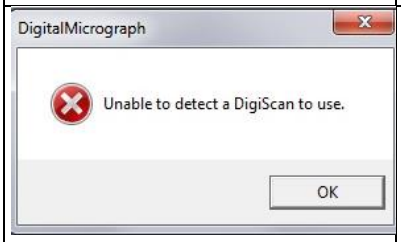


6. You're done. You should see a window confirming that the driver was loaded successfully. Restart DM and confirm that the DigiScan is found.



8.2.

Problem	Possible causes	Remedy.

When Software is started.		
X,Y or Z motor are not found.	dislodged USB cable	Close DM. With the USB connected to the powered 3View controller, check that there are ports allocated to the X,Y,Z (mercury) controllers as serial ports in windows device manager. Unlike earlier versions of software, (pre GMS2.1) DM does not require the COM ports to be specific fixed values (e.g.3,4,5).
	The software is not detecting the DigiScan. When Windows 7 is booted, it can take a few minutes for the device drivers to be correctly identified.	Wait for 1 minute after Windows starts before starting DM. Check Firewire cable is connected and GFA is present as a 1394 device in windows device manager. Try disabling GFA and then re-enabling in device manager. See section below. Check DigiScan is powered on and 4 LEDS are powered on the DigiScan DDC. This may not be visible to users but interfaces between the black loom cable and the SEM.

X,Y and Specimen Advance Information.		
The sample is not in the field of view as expected.	Diamond knife is in Cut position, but not Cleared. The specimen is not inserted.	Diamond knife must be retracted or cleared to view the specimen. The default position for focusing should be the cleared position.
The specimen can't be brought into the field of view using the X,Y translation as the limit of travel is reached.	Either the specimen hasn't been positioned correctly in the holder to the mapped position on the bench top stand, or else the mapped position is wrong.	Check the specimen located in the bench top stand is central to the cross hairs. This is achieved by adjusting the off-centric rings of the specimen holder, and re-clamping this tight. You may need to double check the reference alignment of the bench top stand and defined centrality of the graticule with respect to the X,Y stage. Ensure that the specimen holder in the bench top stand and the microtome is always inserted with the screw pointing the same way, and this is the configuration that the mapping was performed.

DIAMOND KNIFE		
The knife blade is visibly damaged.	The knife has been damaged by inappropriate usageusage or handling. The most common method is by the knife hitting the specimen with the specimen at the wrong height. All mounted knives are shipped with zero flaws as verified in a certificate of quality with photographic evidence from the factory.	The correct procedure for specimen height alignment must be followed to protect the fragile knives. There is no shortcut to this procedure. Some knives can be resharpened as a price list option by returning the mounted knife to the factory. A resharpening procedure will normally supply another swap out resharpened knife rather than the resharpened original. This is because the resharpening process has a considerable lead time. Users can purchase additional mounted knives as price list options. Knives are not covered under warranty.
The knife causes a visible scratch on the specimen.	All knives shipped from the factory are tested to cut without creating scratches visible in the optical microscope. Knives have a limited lifespan and this will depend on their usage.	The user can laterally alter the position of the knife on the arm so that scratches are away from the region of interest. 2 mounted knives are provided in each shipment to alleviate downtime.
Different knives cut on different planes.	The knives are aligned in the factory. As the cutting is thin, then a very small angular difference may have a noticeable effect on which side of the specimen starts cutting first. The cutting plane is normally within the depth of field of the electron microscope optics.	
The knife does not go to the clear position	There is either a communication problem with the Z motor controller, or else the near/ clear motor / and microswitches are at fault.	Check the status of the blue motor LEDs on the front panel of the controller. If none/both are ON, the motor is in an intermediate state and the software considers this to be a dangerous situation. Contact help. Check to see whether the Z motor moves as this controller also controls the near / clear logic status. Check whether the USB cable is dislodged and whether all 3 PI motors are evident in device

		manager as a serials coms device.
The knife isn't positioned correctly before the specimen in the retract, or after the specimen in the cut position.	The specimen dimensions must be less than 1.2mm max travel cutting distance of the knife. A knife arm position may need adjustment.	The user may need to manually adjust the coarse knife adjust position using the allen key at the bottom of the knife pivot arm. The user needs to ensure the knife travel will cover the whole specimen even as the specimen gets larger as it is sliced away. You must lock the nut which stops this position from moving over time.
The piezo vibration isn't audible.	The piezo vibration should be on when several conditions are met. The stroke is up. The oscillation tick box is on. The black and red wires are connected. The user can hear 12KHz The door is open.	Check these conditions. Try the spare knife as a diagnostic.
IMAGING		
A DigiScan image shows no contrast.	Beam may be off. Go to low magnification mode.	Check the HT is on and the column valve open. This may be controlled from the SEM software.
The imaging on the SEM is not blanked when DigiScan is in control.	On some microscopes this is normal.	
The 3VBSED image is not present on the SEM monitor.	The default condition is for the 3VBSED image to be on Channel 2 of the DigiScan only. Some users may have this installed to be shared with a spare imaging input on the SEM.	
The 3VBSED image can't be adjusted from the SEM controls.	This is true. The 3VBSED is only controlled by the user adjusting the contrast, brightness, bias and bandwidth boxes in DigitalMicrograph.	Use DigitalMicrograph to control the 3VBSED amplifier.
The image shows oscillation.	If the piezo vibration is on, and the stroke is up, then the image will wobble. The normal imaging condition is with the stroke down.	Only image with the stroke down.
There is some residual vibration in the image at very high magnification.	This is normal. The 3View microtome does not guarantee the same imaging resolution	It is important not to confuse residual vibration of the stage with stray fields. The imaging performance of the SEM is

	specifications as the standard SEM stage. However, the residual vibration measured at ~1-2nm.	normally compared to the imaging performance of the 3View stage at installation using a gold / carbon specimen. If high amplitude vibrations are seen, then examine the acoustics and vibration isolation of cabling in the room.
DigiScan image shows unusual contrast.	The signal may be overloaded because the brightness has not been correctly adjusted. If autosurvey is on, then it is possible to have the signal out of range, but this is still shown as a mid-grey image. Chamberscope may be on.	Go to lowest magnification. Check that “Autosurvey” and line trace profile in DM image acquisition is on. Alter gain and brightness settings to prove that signal level is not saturated. Ensure the chamberscope is turned off. A chamber scope may only introduce a small ripple and offset in the image.
The image has contrast but is difficult to understand.	A thin layer of debris may cover the region of interest. The BSED signal may be out of range.	Go to low magnification. Check that the diamond knife is Cleared. Examine the SE image. Check there are no communication problems to the SEM PC. (e.g. can you alter the SEM PC magnification from the Gatan mag: control window). Go to lower magnification to try to understand the image and check for debris on the image. Turn on the red line, lower the contrast, and adjust the brightness so that contrast is sensible.
More signal produces darker images.	This is correct. The default 3VBSED contrast is for opposite polarity contrast to a standard BSED image.	There is nothing to rectify. The polarity can be reversed in the display look up table, or can be reversed by default in the DigiScan acquisition but only when the software is in field service mode.
The image field of view is restricted by a white round shadow	You are imaging the hole in the middle of the BSED diode. This hole should be approximately central to the zoom axis of the microscope.	For diodes on retractable arms, alter the insertion position to make central.
The specimen is not visible in the field of view	The specimen is masked or out of the field of view.	Clear the knife. Move the stage to localize the sample. If this out of the travel range, then

		the specimen position will need to be adjusted by venting the chamber and removing the specimen. Remember to lower the specimen a safe distance.
The signal seems weak or poor signal to noise.	Contrast setting is too low. The BSED Bias is turned off. Specimen has weak contrast, or a depth in the specimen with suitable heavy metal contrast has not been reached. A low beam current (smaller spot size is being used). Too high a gas pressure is being used for a given kV so excessive scattering is reducing the signal size.	Use contrast value 10 and adjust the brightness so that the red DigiScan line is approximately central for a typical image. Bias is turned on using a tick box in the Microtome Stage Controller window. Check SEM conditions. Check specimen, perhaps swap out with known standard. It is normal to employ low kV and high vacuum conditions if the specimen is suitably stained. If low vacuum conditions are employed, then a higher kV and beam current will be required to have a similar signal to noise.
There are streaking effects on the image.	This is a charging effect and means that the gas pressure used is not fully able to compensate for the injected charge at the chosen operating conditions, (HT, spot size, scanning speed, cut thickness).	Increased gas pressure reduces charging but also reduces image contrast, such that a similar signal to noise in an image requires more beam current or slower scanning speed. The most important issue may be to alter the gas pressure or injection conditions such that image features of interest start to show no charging. If features in the image are not of interest and show some charging this is a worthy compromise.
The image is not symmetrical from left to right in terms of contrast.	This is a charging effect. The extremities of an image are subject to edge effects which are more pronounced when gas cancellation of the charging is borderline for the chosen injection conditions. The electron beam pauses for a different period at the right hand side of an image to the left hand side.	Edge effects are reduced on the left hand side by increasing the flyback variable in the DigiScan tools dialogue. The right hand edge effect has no similar control, other than the balance of injection conditions (including magnification) with gas pressure for charge cancellation. Consider whether this effect is detrimental to the image information. Some edge effects can be treated by post-

		processing algorithms as long as the features of interest are within the dynamic range of the input.
The image shows some streaking on the left hand side.	The linesync option in the DigiScan dialogue is on. This alters the period that the beam pauses at the left hand side of the image.	The linesync option removes environmental interference in an image at the mains frequency. Ideally this should not be required as there should be minimal field at the installation.
I cannot request a faster DigiScan image.	The line-sync option is ticked in the DigiScan image info window.	Untick this option to allow a wider choice of pixel dwell times.
The image resolution is poorer in a 3D acquisition than during initial setup.	Check whether multiple frame acquisitions are being requested. Sometimes frame integration can lead to some loss in resolution due to image shift between frames.	DigiScan Record has a tick box for Frame Integration. 3View Control Setup / Signal window is used to configure the Frame Integration in a 3D stack acquisition.
After a few cuts the surface and image quality degrades.	The specimen surface is being damaged by the beam to a greater depth and severity than is recovered by cutting. The beam dose into the resin causes shrinkage such that the actual cut thickness is less than that requested. In an automated acquisition there needs to be an equilibrium whereby the cut exposed resin which is suitably (if not completely fresh). The conditions required for optimising the spatial resolution in a 2D image will not necessarily map to those conditions required to optimise resolution and signal definition in a 3D series.	Beam dose is increased by the following factors. KV Pixel Size (therefore mag and density) Scanning speed. Focus and astigmatism Beam Current. (spot size) Gas pressure (influences probe current on specimen). The only microtome variables are cut thickness and cutting speed. A compromise in voxel size may be required with respect to maintaining required signal to noise in order to achieve stable cut condition throughout a 3D experiment. There is no single magic recipe as optimum conditions will depend on the specimen (resin characteristic) and contrast, as well as which parameters are being optimised.
There is debris on the image.	Debris on the image is more likely if the system is imaged in the retracted position than the clear position.	Does the debris clear by performing Cut / Retract without raising the specimen by a cut thickness? Does the debris clear by performing a short automated cut sequence, e.g. 3 cuts of 100nm?

		If debris cannot be removed, then vent SEM and observe specimen using stereo zoom microscope. Clean away debris from specimen and knife using the orange puffer.
There is a scratch on the specimen in the same location on all images.	The diamond knife is dirty or damaged at this point.	Try cleaning with the diamond knife cleaning solution on a sharpened polystyrene stick. Move knife laterally to use fresh part on region of interest. Repeat Approach protocol. Use the spare knife instead. Purchase a replacement or resharpened knife.
The knife cuts only part of the specimen.	The distance between the start and stop position controlled by the software and the knife arm central locking screw do not allow full coverage of the specimen when only Cut / Retract is employed. The specimen must not be wider than the knife or extend beyond the knife edge. Otherwise this performs uncontrolled tearing at the edge.	This is a dangerous scenario as a step has been created on the image, and if a Clear is then requested, this presents too much specimen thickness to the knife. Lower specimen. Consider retrimming top face flat in bench top microtome. Reset knife span coverage. Restart approach sequence. Re-trim specimen to be correct size.
There is some drift in the image between sections.	Some drift will be caused by temperature changes. These are more severe when the SEM is first pumped down. The rivet, specimen holder or Z stage is not properly clamped. The resin is soft, tall, or presents a poor shape to the front surface to the knife.	For maximum stability the SEM should be in a temperature controlled room, not exposed to direct sun light, and the system should stabilize to equilibrium internal temperature. Ideal image stability is achieved with the specimen size set to the best known shape and aligned with respect to the diamond knife. The Analyze drift software tool Shows a line plot of drift in X and Y between successive images.
The BSED image intensity changes.	The signal from the specimen is changing with the cut depth. The pressure conditions in the microscope are not stable. The BSED diode is	Understand your specimen. Understand the stability of the pressure in the microscope. Higher pressure will reduce the signal. Examine the diode for contamination, Follow cleaning

	contaminated.	protocol. Consider replacing diode as consumable.
There is instability in my BSED image.	Confirm the instability by imaging with the bias on and off with no beam on.	Use the Change Bias script to reduce the bias value. For example try halving the bias applied to see what impact this has.

9. List of Consumables and Options.

Gatan provides a comprehensive list of consumables with the standard system. Additional and extra consumables are available at a discount rate to users who have purchased a product maintenance plan. Please contact Gatan or your distributor for more information.

9.1. Consumables.

Metal rivets PEP6590 or PEP6044 Pack of 12 / 24 or 48.
Plexiglas rivets, pack of 6.
Diode repair service. Please send back mounted diode in plastic box for repair swap out of silicon / ceramic component.
Replacement diamond knife. (Mounted / aligned in factory on swap out knife holder).
Resharpening diamond knife. (Mounted / aligned in factory on swap out knife holder).
Trimmed and rivet mounted test specimen of stained tissue.
Microcentrifuge tubes 0.5mL
Razor blades
Tweezers
Polystyrene diamond knife cleaner sticks
Diamond knife cleaning solution

9.2. Options.

Rivet specimen holder (adjustable using concentric rings).
Rivet holder for bench top microtoming.
Additional spare mounted diode
Al block with 24 holes for supporting rivets. Suitable for oven baking.
Additional diamond knife holder with spare diamond mounted in factory.
Spare diamond knife holder secure mount.
Gold on Carbon Resolution standard mounted on holder
Off-Line PC suitable for processing 3View2XP data and 3D rendering. Includes monitor.
Desk for PC and electronics.
Off-line licences for 32bit or 64bit Windows 7 PCs.

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