Finnigan™ SpectraSYSTEM™

Fluorescence Detector
Reference Manual

Revision E
A0099-564

Thermo
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This manual and the instruments to which it applies have satisfied the requirements for CSA, FCC, the EMC, and Low Voltage Directives. Use of non-approved components and repair methods may reduce or invalidate the built-in protection that is required to meet the above certifications.

Technical information contained in this publication is for reference purposes only and is subject to change without notice. Every effort has been made to supply complete and accurate information; however, Thermo Electron Corporation assumes no responsibility and will not be liable for any errors, omissions, damage, or loss that might result from any use of this manual or the information contained therein (even if this information is properly followed and problems still arise).

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System Configurations and Specifications supersede all previous information and are subject to change without notice.

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Finnigan SpectraSYSTEM Fluorescence Detector Reference Manual — *Revision E A0099-564*

	Strongly Agree	Agree	Disagree	Strongly Disagree
The manual is well organized.	1	2	3	4
The manual is clearly written.	1	2	3	4
The manual contains all of the information I need.	1	2	3	4
The instructions are easy to follow.	1	2	3	4
The instructions are complete.	1	2	3	4
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The figures are helpful.	1	2	3	4
I was able to operate the system by using this manual. (If not, please comment below.)	1	2	3	4

Additional Comments: (Attach additional sheets if necessary.)

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Tear this sheet from the manual, fold it closed, stamp it, and drop it in the mail.

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Technical and Customer Support

This manual contains procedures for installing your equipment and verifying that it is operating within specifications. It will also help you understand how to use and care for your equipment. For additional support, contact one of the customer service offices listed below.

In North America

In North America, Thermo Electron San Jose Customer Service Engineers are available from the following offices:

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REPLACEABLE PARTS

Contact Customer Service Operations to order replaceable parts. The location and telephone and fax numbers for North America are as follows:

North America Customer Service Operations
1400 Northpoint Parkway, Suite 10
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Phone: [1] (800) 532-4752
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TECHNICAL SUPPORT

You can contact Technical Support at the following location, telephone and fax numbers, and e-mail address:

North America Technical Support Operations
1400 Northpoint Parkway, Suite 10
West Palm Beach, FL 33407

Phone: [1] (800) 685-9535
Fax: [1] (561) 688-8736

E-mail: techsupport.finnigan@thermo.com

In Europe

In Europe, customer support, replaceable parts, and technical support are available from each of the following offices.

Technical support is also available from North America Technical Support Operations at the following phone number and e-mail address:

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E-mail techsupport.finnigan@thermo.com

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Phone [61] (02) 9898-9000
Fax [61] (02) 9898-9800

Yokohama, Japan
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Fax [81] (45) 453-9110

Osaka, Japan
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Fax [81] (06) 6387-6641

Beijing, P.R. China
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Fax [86] (010) 6621 0851

For all other countries, contact your local Thermo Electron San Jose products dealer.

Safety and EMC Information

In accordance with our commitment to customer service and safety, these instruments have satisfied the requirements for the FCC and the European CE Mark including the Low Voltage Directive.

Designed, manufactured and tested in an ISO9001 Registered facility, this instrument has been shipped to you from our manufacturing facility in a safe condition.

CAUTION! This instrument must be used as described in this manual. Any use of this instrument in a manner other than described here may result in instrument damage and/or operator injury.

IDENTIFYING SAFETY INFORMATION

This reference manual contains precautionary statements that can prevent personal injury, instrument damage, and loss of data if properly followed. All statements of this nature are called to your attention through the use of bold type and the following icons:

CAUTION!

HOT SURFACE!

HIGH VOLTAGE!

Every instrument has specific hazards, so be sure to read and comply with the following precautions. They will help ensure the safe, long-term use of your system.

1. Before plugging in any of the instrument modules or turning on the power, always make sure that the voltage and fuses are set appropriately for your local line voltage.

2. Only use fuses of the type and current rating specified. Do not use repaired fuses and do not short-circuit the fuse holder.

3. The supplied power cord must be inserted into a power outlet with a protective earth contact (ground). When using an extension cord, make sure that the cord also has an earth contact.

4. Do not change the external or internal grounding connections. Tampering with or disconnecting these connections could endanger you and/or damage the system.

CAUTION! The instrument is properly grounded in accordance with regulations when shipped. You do not need to make any changes to the electrical connections or to the instrument's chassis to ensure safe operation.

5. Never run the system without the housing on. Permanent damage can occur.

6. Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage. Instead, disconnect the power cord and contact a Service Representative for a product evaluation. Do not attempt to use the instrument until it has been evaluated. (Electrical damage may have occurred if the system shows visible signs of damage, or has been transported under severe stress.)

7. Damage can also result if the instrument is stored for prolonged periods under unfavorable conditions (*e.g.*, subjected to heat, water, etc.).

8. Always disconnect the power cord before attempting any type of maintenance.

9. Capacitors inside the instrument may still be charged even if the instrument is turned off.

10. Never try to repair or replace any component of the system that is not described in this manual without the assistance of your service representative.

GOOD LABORATORY PRACTICES

Keep Good Records

To help identify and isolate problems with either your equipment or your methodology, we recommend that you keep good records of all system conditions (*e.g.*, % RSDs on retention times and peak areas, peak shape and resolution). At a minimum, keep a chromatogram of a typical sample and standard mixture, well-documented with system conditions, for future reference. Careful comparison of retention times, peak shapes, peak sensitivity, and baseline noise can provide valuable clues to identifying and solving future problems.

Chemical Toxicity

Although the large volume of toxic and flammable solvents used and stored in laboratories can be quite dangerous, don't ignore the potential hazards posed by your samples. Take special care to read and follow all precautions that ensure proper ventilation, storage, handling, and disposal of both solvents and samples. Become familiar with the toxicity data and potential hazards associated with all chemicals by referring to the manufacturers' Material Safety Data Sheets (MSDS).

Sample Preparation

Always consider the solubility of your sample in the solvent/mobile phase. Sample precipitation can plug the column, tubing and/or flow cell causing flow restriction. This obstruction may result in irreparable damage to the system. Particulate matter can be avoided by filtering the samples through 0.45 or 0.2 micron (or less) filters.

Solvent Requirements

Many chemical manufacturers provide a line of high-purity or spectro-quality reagents that are free of chemical impurities. Routine filtration of all solvents or eluents through a 0.45 or 0.2 micron (or less) fluorocarbon filter before placing them in the solvent reservoir will significantly prolong the life and effectiveness of the inlet filters, check valves and seals, injector, and column. Typically, HPLC-grade solvents do not require filtration.

Choose a mobile phase that is compatible with the sample and column you've selected for your separation. Remember that some solvents are corrosive to stainless steel. Inert, biocompatible versions of instruments are also available from Thermo Electron.

Degas the Eluents

Degas your eluent solvents using either the vacuum degassing or the helium sparging technique. A complete description of these techniques is found in separate documentation provided with degassing accessories.

Solvent Disposal

Make sure you have a solvent waste container or other kind of drain system available at or below the benchtop level. Most solvents have special disposal requirements and should not be disposed of directly down a drain. Follow all governmental regulations when disposing of any chemical.

High-pressure Systems and Leaks

LC systems operate at high pressures. Because liquids aren't highly compressible they do not store much energy. Accordingly, there is

little immediate danger from the high pressures in an LC system. However, if a leak occurs, it should be corrected as soon as possible. Finally, we recommend that you always wear eye and skin protection when working on an LC system and that you always shut down the system and return it to atmospheric pressure before attempting any maintenance.

Information sur la sécurité et la compatibilité électromagnétique (CEM)

Selon notre engagement à assurer à nos clients service et sécurité, ces instruments sont déclarés conformes aux normes de la FCC et à la réglementation européenne (CE), y compris à la directive sur les basses tensions.

Conçu, fabriqué et testé dans une installation homologuée ISO9001, cet instrument a été livré à partir de notre usine de fabrication dans le respect des règles de sécurité.

MISE EN GARDE ! Cet instrument doit être utilisé selon les instructions figurant dans ce manuel. Le non respect des consignes d'utilisation de cet instrument décrites dans le présent manuel risque d'endommager l'instrument et/ou d'infliger des blessures à l'opérateur.

IDENTIFICATION DES INFORMATIONS SUR LA SÉCURITÉ

Ce manuel de référence contient des précautions d'usage afin de prévenir tout dommage corporel ou matériel ainsi que toute perte de données lorsque l'opérateur se conforme aux instructions indiquées. Ces instructions sont accompagnées des icônes suivantes et sont affichées en caractères gras pour attirer l'attention de l'opérateur :

MISE EN GARDE !

SURFACE BRÛLANTE !

HAUTE TENSION !

Chaque instrument présentant des dangers spécifiques, il incombe à l'opérateur de lire les précautions suivantes et de s'y conformer, afin de maintenir la durée de vie et la sécurité du système.

1. Avant de brancher un module d'instruments ou de le mettre sous tension, toujours s'assurer que la tension et les fusibles sont réglés de façon à correspondre à la tension locale du secteur.
2. N'utiliser que des fusibles du type et du courant nominal spécifiés. Ne pas utiliser de fusibles réparés et ne pas court-circuiter le porte-fusible.

3. Le cordon d'alimentation accompagnant l'instrument doit être branché à une prise de courant avec mise à la terre. En cas d'utilisation d'une rallonge électrique, s'assurer que celle-ci comporte également une mise à la terre.
4. Ne pas modifier les connexions de mise à la terre internes ou externes. La modification ou le débranchement de ces connexions représente un danger pour l'opérateur et/ou risque d'endommager le système.

MISE EN GARDE ! Cet instrument est mis à la terre conformément aux règlements applicables lors de son expédition. Ne pas modifier les branchements électriques ou le châssis de l'instrument afin d'assurer un fonctionnement en toute sécurité.

5. Ne jamais faire fonctionner le système sans son boîtier. Des dommages permanents pourraient en résulter.
6. Ne pas mettre l'instrument sous tension si celui-ci a subi des dommages électriques. Débrancher le cordon d'alimentation de l'appareil et consulter un représentant du service technique pour procéder à un examen du produit. Ne pas essayer d'utiliser l'instrument avant qu'il n'ait été examiné. (Des dommages électriques peuvent s'être produits si le système montre des signes visibles d'endommagement ou si les conditions de transport ont été extrêmement difficiles.)
7. L'instrument peut également être endommagé s'il est entreposé pendant une période de temps prolongée, dans de mauvaises conditions (par exemple, s'il est exposé à la chaleur, à l'humidité, etc.).
8. Toujours débrancher le cordon d'alimentation avant d'effectuer n'importe quel type d'entretien.
9. Les condensateurs présents à l'intérieur de l'instrument peuvent toujours être chargés, même si l'instrument est hors tension.
10. Ne jamais tenter de réparer ou de remplacer un composant du système non décrit dans ce manuel sans obtenir de l'aide auprès d'un représentant du service technique.

BONNES PRATIQUES DE LABORATOIRE

Bonne tenue des dossiers

Pour permettre d'identifier et d'isoler les problèmes pouvant survenir avec l'équipement ou la méthodologie utilisés, il est recommandé de tenir correctement des dossiers de toutes les conditions du système (*p. ex.*, % CV sur les temps de rétention et les zones de pics, la forme et la résolution des pics). Il est recommandé tout au moins de conserver pour référence future un chromatogramme d'un échantillon type et d'un mélange standard, bien documenté et accompagné des conditions du système. Une comparaison précise des temps de rétention, des formes et de la sensibilité des pics ainsi que des bruits

de référence peuvent fournir des indices précieux pour l'identification et la résolution de problèmes futurs.

Toxicité chimique

Bien que l'utilisation et l'entreposage dans les laboratoires de grandes quantités de solvants inflammables et toxiques puissent représenter un danger, ne pas négliger les dangers potentiels posés par les échantillons. Veiller particulièrement à lire et à suivre toutes les précautions indiquées pour assurer la ventilation, le stockage, la manutention et l'élimination des solvants et des échantillons. Se familiariser avec les données sur la toxicité et les dangers potentiels associés à tous les produits chimiques en consultant les fiches techniques sur la sécurité des substances (FTSS) du fabricant.

Préparation des échantillons

Toujours considérer la solubilité de l'échantillon dans la phase mobile. La précipitation des échantillons peut boucher la colonne, les tubes et/ou la cellule de dilution, et en limiter le débit. Cette obstruction peut endommager le système de façon irréparable. L'accumulation de particules peut être évitée par la filtration des échantillons à travers des filtres de 0,45 ou 0,2 µm (ou moins).

Caractéristiques des solvants

Un grand nombre de fabricants de produits chimiques fournissent des réactifs de pureté élevée ou de qualité spectrographique dépourvue de toute impureté chimique. La filtration systématique de tous les solvants ou éluants à travers un filtre fluorocarboné de 0,45 ou 0,2 µm (ou moins) avant de les placer dans le réservoir de solvants prolonge de façon significative la durée de vie et l'efficacité des filtres d'entrée, des clapets et des joints d'étanchéité, de l'injecteur et de la colonne. De façon générale, les solvants pour chromatographie liquide sous haute pression ne nécessitent pas de filtration.

Choisir une phase mobile qui est compatible avec l'échantillon et la colonne sélectionnés pour la séparation. Noter que certains solvants sont corrosifs pour l'acier inoxydable. Des versions inertes et biocompatibles des instruments sont disponibles auprès de Thermo Electron.

Dégazage des éluants

Effectuer le dégazage des éluants selon la méthode de dégazage par le vide ou à l'hélium. Une description complète de ces méthodes est disponible dans la documentation fournie séparément avec les accessoires de dégazage.

Élimination des solvants

S'assurer qu'il existe un conteneur pour solvants à éliminer ou tout autre système de vidange au niveau de la table de travail ou au-dessous de celle-ci. La plupart des solvants doivent être éliminés dans des conditions particulières et ne doivent pas être évacués directement

par les canalisations. Respecter la réglementation en vigueur concernant l'évacuation des produits chimiques.

Systèmes à haute pression et fuites

Les systèmes de chromatographie liquide (CL) fonctionnent à des pressions élevées. Les liquides n'accumulent pas de grandes quantités d'énergie car ils ne sont pas hautement compressibles. Par conséquent, le risque d'un danger immédiat causé par les pressions élevées dans un système CL est faible. En revanche, si une fuite survient, il est nécessaire de la réparer le plus rapidement possible. Enfin, il est recommandé à l'opérateur de se protéger en permanence les yeux et la peau lorsqu'il travaille sur un système CL. De plus, il doit toujours mettre le système hors tension et le ramener à la pression atmosphérique avant de procéder à tout entretien.

Informationen zu Sicherheit und Funkentstörung

Wir sind dem Dienst am Kunden und der Sicherheit des Kunden verpflichtet. Diese Geräte entsprechen den Anforderungen für die FCC-Zulassung und für das CE-Zeichen sowie den Bestimmungen der Richtlinie für Niederspannungsgeräte.

Dieses Gerät wurde in einer nach ISO 9001 zertifizierten Fertigungsstätte entwickelt, hergestellt und getestet und hat unser Werk in sicherem Zustand verlassen.

VORSICHT! Dieses Gerät darf nur nach den Vorschriften dieser Bedienungsanleitung benutzt werden. Wenn dieses Gerät auf andere Weise als hier beschrieben benutzt wird, kann dies zu Schäden am Gerät oder zur Verletzung des Bedieners führen.

ERKENNEN VON SICHERHEITS-INFORMATIONEN

Dieses Handbuch enthält Warnhinweise, deren genaue Befolgung Personenschäden, Schäden am Gerät oder Datenverluste verhindern kann. Auf alle derartigen Warnhinweise wird durch Fettschrift und durch
Verwendung der nachfolgenden Symbole gesondert aufmerksam gemacht:

VORSICHT!

OBERFLÄCHE HEISS!

HOCHSPAN-NUNG

Jedes Gerät kann unter bestimmten Umständen gefährlich sein. Lesen Sie daher in jedem Fall die nachstehenden Sicherheitshinweise, und ergreifen Sie die entsprechenden Maßnahmen. Auf dieses Weise sorgen Sie für einen sicheren Betrieb und eine lange Lebensdauer des Geräts.

1. Bevor Sie eines der Gerätemodule einstecken oder das Gerät einschalten, überprüfen Sie in jedem Fall, ob die Nennspannung und die Sicherungen der Netzspannung der örtlichen Stromversorgung entsprechen.
2. Verwenden Sie nur Sicherungen des angegebenen Typs und der angegebenen Amperezahl. Verwenden Sie keine reparierten Sicherungen, und überbrücken Sie die Sicherung nicht.

3. Das mitgelieferte Netzkabel muß in eine Steckdose mit Schutzleiter eingesteckt werden. Wird ein Verlängerungskabel verwendet, muß auch hier der Schutzleiter durchgeführt sein.
4. Verändern Sie nichts an den externen oder internen Schutz- bzw. Erdungskontakten. Wenn Sie sich an diesen zu schaffen machen oder sie unterbrechen, können Sie sich selbst und andere gefährden, oder das Gerät könnte beschädigt werden.

VORSICHT! Das Gerät ist bei der Auslieferung vorschriftsmäßig geerdet. Es brauchen keine Veränderungen an der elektrischen Verkabelung oder am Gerätechassis vorgenommen werden, um einen sicheren Betrieb zu gewährleisten.

5. Nehmen Sie das Gerät nie mit geöffnetem Gehäuse in Betrieb, da dies zu irreparablen Schäden führen kann.
6. Schalten Sie das Gerät nicht ein, wenn Sie den Verdacht haben, daß an der Elektrik möglicherweise Schäden eingetreten sind. Ziehen Sie in diesem Fall den Netzstecker heraus, und lassen Sie das Gerät von einem Kundendiensttechniker untersuchen. Versuchen Sie bis zu dieser Untersuchung keinesfalls, das Gerät in Betrieb zu nehmen. (Eine Beschädigung der Elektrik kann z.B. eingetreten sein, wenn das Gerät äußere Schäden aufweist oder unter problematischen Umständen transportiert wurde.)
7. Schäden können auch eintreten, wenn das Gerät längere Zeit unter ungünstigen Umständen gelagert wurde (*z.B.* unter der Einwirkung von Hitze oder Wasser).
8. Ziehen Sie vor allen Wartungsmaßnahmen immer zuerst den Netzstecker aus der Steckdose.
9. Auch wenn das Gerät abgeschaltet ist, können die im Inneren befindlichen Kondensatoren nach wie vor unter Spannung stehen.
10. Versuchen Sie niemals, Gerätekomponenten zu reparieren oder auszutauschen, die nicht in diesem Handbuch beschrieben sind, ohne einen Kundendiensttechniker zu Rate zu ziehen.

GLP-VORSCHRIFTEN (GOOD LABORATORY PRACTICES)

Ordnungsgemäße Aufzeichnungen

Damit Probleme mit Geräten oder Methoden erkannt und eingegrenzt werden können, empfehlen wir Ihnen, ordnungsgemäße Aufzeichnungen sämtlicher Gerätezustände (*z.B.* % RSDs zu Retentionszeiten, Kurvenflächen, Kurvenformen und Auflösung). Archivieren Sie als Minimum ein Chromatogramm einer typischen Probe und

einer Standardmixtur mit umfassender Dokumentation der Systembedingungen zum späteren Vergleich. Ein sorgfältiger Vergleich von Retentionszeiten, Kurvenformen, Empfindlichkeitswerten und Hintergrundrauschen liefert wertvolle Hinweise für den Fall, daß zu einem späteren Zeitpunkt Probleme auftreten und eingegrenzt und behoben werden müssen.

Chemische Toxizität

Die großen Mengen an toxischen oder brennbaren Lösungsmitteln, die im Labor verwendet und aufbewahrt werden, können ein erhebliches Gefahrenpotential darstellen, doch darf man hierüber nicht die mögliche Gefährdung durch die Proben selbst vergessen. Achten Sie insbesondere darauf, sämtliche Warnhinweise hinsichtlich ausreichender Belüftung, Lagerung, Handhabung und Entsorgung von Lösungsmitteln ebenso wie von Proben sorgfältig zu lesen und zu befolgen. Machen Sie sich mit den Toxizitätsdaten und den möglichen Gefahren sämtlicher verwendeter Chemikalien anhand der betreffenden Sicherheitsdatenblätter vertraut, die von den Produktherstellern zur Verfügung gestellt werden.

Probenvorbereitung

Überprüfen Sie stets die Löslichkeit der Probe in der mobilen Phase. Durch das Ausfällen von Feststoffen können die Säule, die Leitungen oder die Durchflußzelle verstopfen und damit den Durchfluß hemmen. Durch eine solche Verstopfung können irreparable Schäden am System entstehen. Die Ablagerung von Partikeln läßt sich durch Filtrieren der Proben durch ein Filter mit einer Porengröße von 0,45 oder 0,2 µm (oder weniger) vermeiden.

Anforderungen an das Lösungsmittel

Viele chemische Hersteller bieten eine Produktserie hochreiner Reagenzien in spektroskopisch reiner Qualität an, die frei von chemischen Unreinheiten sind. Die routinemäßige Filtrierung aller Lösungs- und Extraktionsmittel durch ein Fluorkohlenwasserstoff-Filter mit einer Porengröße von 0,45 oder 0,2 µm (oder weniger) vor dem Einfüllen in den Lösungsmittelbehälter verlängert die Lebensdauer der Einlaßfilter, der Ventile und Dichtungen, des Injektors und der Säule beträchtlich. Spezielle HPLC-Lösungsmittel brauchen normalerweise nicht filtriert zu werden.

Wählen Sie eine mobile Phase, die zur Probe und zur für die Separation verwendete Säule kompatibel ist. Dabei ist darauf zu achten, daß Edelstahl durch bestimmte Lösungsmittel korrodiert wird. Reaktionsträge, biokompatible Geräteausführungen werden ebenfalls von Thermo Separation Instruments angeboten.

Entgasen des Lösungsmittels

Lösungs- und Extraktionsmittel sollten entgast werden, und zwar entweder durch Vakuum oder Heliumdurchperlung. Eine umfassende

Beschreibung dieser Techniken finden Sie in dem separaten Handbuch, das dem Entgasungszubehör beiliegt.

Entsorgung von Lösungsmitteln

Sorgen Sie dafür, daß ein Auffangbehälter für Lösungsmittel oder eine andere Auffangvorrichtung in Höhe des Arbeitstisches oder darunter zur Verfügung steht. Für die meisten Lösungsmittel gelten besondere Entsorgungsvorschriften; eine Entsorgung über die Abwasserleitung ist hier nicht zulässig.
Bei der Entsorgung von Chemikalien gleich welcher Art sind die einschlägigen Vorschriften streng zu beachten.

Hochdrucksysteme und Undichtigkeiten

Flüssigchromatographen arbeiten unter hohem Druck. Da Flüssigkeiten kaum komprimierbar sind, können sie nicht viel Energie speichern. Dementsprechend stellt der hohe Druck in einem Flüssigchromatographen auch kaum eine unmittelbare Gefahr dar. Jedoch sollten auftretende Undichtigkeiten umgehend beseitigt werden. Schließlich ist noch zu empfehlen, bei der Arbeit mit einem Flüssigchromatographen stets Augen und Haut zu schützen und vor allen Wartungsarbeiten darauf zu achten, daß das Gerät abgeschaltet und druckfrei gemacht wurde.

Startup Checklist

This is a brief summary of the steps that must be completed for the proper installation of your SpectraSYSTEM™ FL3000 detector. Complete installation information can be found in Appendix A.

- ❒ Unpack and inspect your instrument
- ❒ Read the Safety Information Card
- ❒ Position the detector appropriately
- ❒ Select voltage and check fuses
- ❒ Connect the power cord
- ❒ Make rear panel connections
- ❒ Connect the flowcell
- ❒ Turn on the instrument
- ❒ Check initial response to power-on
- ❒ Check operation with a test sample

This detector was installed by:

_____ _____

(Name) *(Date)*

List of Spare Parts and Consumables

Shown below is a list of spare parts and consumables available from Thermo Electron for use with your SpectraSYSTEM™ fluorescence detector. Contact your Thermo Electron representative for current local prices.

Options and Accessories

4802-0031	Extended Range PMT, 200 - 800 nm
9051-0143	Regulated Backpressure Accessory
3750-0075	340 Cutoff Filter
A3469-030	FL Test Mix

Manuals

A0099-564	Manual, Reference (English)
A0099-565	Manual, Field Repair

Maintenance/Repair Parts

5708-0091	PMT, Standard, 200 - 650 nm
4802-0031	PMT, Extended Range
5110-0051	Power Switch
5110-0058	Magnetic Proximity Switch
6040-0156	Cable, Display/Keypad
9051-0395	Slitwheel, Cell/PMT/Lens
A5256-010S	Flowcell, Square
9551-0144	Xenon Lamp
A5246-010	Xenon Lamp Socket
9551-0164S	PMT-Assembly Socket
9551-0165S	Power Input Module
9551-0198	Sensor, Magnetic Proximity Switch

Spare Parts and Consumables, continued

9551-0207-02	Transformer
B9010-010S	PCB, Digital
9851-0030	PCB, Analog
A9980-010	PCB, Mother
A5027-010	Lens, with Holder (ea) EX
A5028-010	Lens, with Holder (ea) EM

Contents

1

Getting Started

Introduction

This chapter provides you with the three basic rules you'll need for using your FL3000 fluorescence detector. It also introduces you to the instrument's command center and describes the conventions we'll use in this manual.

Before you start this chapter, be sure to read the Safety Information Card located at the beginning of this manual and to install your detector as described in Appendix A.

Throughout our explanations, we encourage you to explore the general architecture of the instrument's menus and screens. Use the Menu Tree in Appendix B as your guide if you wish.

Learning Your Way Around

AS EASY AS 1-2-3!

It's easy to learn your way around a SpectraSYSTEM™ detector. Just remember these three rules:

1. The arrow keys ([∧], [∨], [<], [>]) move the cursor in the direction printed on the key.

   HINT: Press [MENU] to jump quickly to the top of the menu structure.

2. The shape of the cursor determines how you make a selection:
   - If a triangular cursor appears, press [ENTER].
   - If a blinking square cursor (▮) appears, press the [+] or [-] keys to change values. Depending on the field, you will scroll up or down through preset choices, or change alphanumeric entries one letter or digit at a time.
3. There are four ways to accept (and automatically save) an entry. Just move the cursor out of the field by any of the following methods:
   - Pressing [ENTER]
   - Using the arrow keys
   - Pressing [MENU]
   - Pressing [STATUS]

   NOTE: You won't be able to leave a menu if errors are present or if you haven't filled in all the necessary entries.

VISUAL CLUES

The following conventions are used on the detector's display:

1. Top-level menu choices are displayed in all-capital letters.
2. A field's square cursor changes to an underscore cursor when you're scrolling through preset choices or entering numerical values and characters.
3. A solid down-arrow (♦) on the right side of some displays indicates that the current menu continues on additional screens. To access additional menu lines, press the down-arrow key, [∨].
4. The last line of a longer menu is frequently a blank display line (without a solid down-arrow).

Instrument Control

Take a look at the keypad and two-line display located on the front panel (Figure 1.1). This is the command center from which you'll access menus and control the instrument's operations. A brief explanation of the keys and the main menus and screens follows.

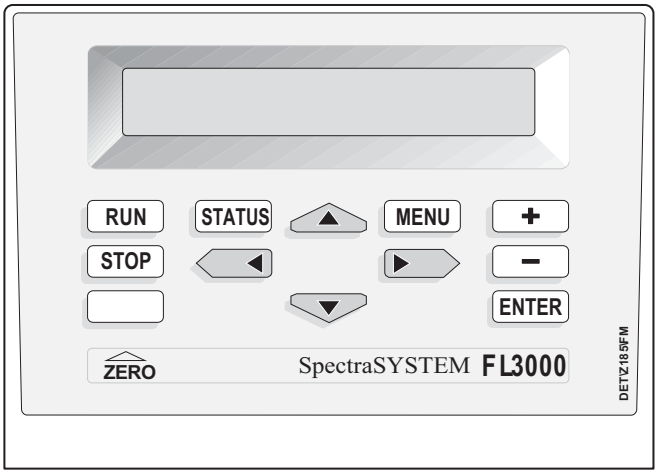


Figure 1.1 The Detector's Command Center

THE KEYPAD

The keypad of each SpectraSYSTEM instrument consists of twelve keys. Four keys directly control the instrument's operation: [RUN], [STOP], [STATUS], and, on the detector, a blank key called [ZERO]. The remaining keys either access commands ([MENU] and [ENTER]), or are used to set parameters and move around the display ([∧], [∨], [<], [>], [+], [-]). The function of each is explained below.

[RUN]

Pressing [RUN] starts a run. The detector must be in the READY state (or QREADY if a queue is loaded), indicating that the detector is stabilized and waiting to begin a run.

[STOP]

Pressing [STOP] halts a run, stops the internal clock, and returns the detector to a READY state. If a wavelength program is operating, pressing [STOP] halts the program and returns the detector to its initial conditions.

[STATUS]

Pressing [STATUS] displays the Status Screen (Figure 1.3). From the Status Screen you can monitor the run in progress. You can also access the Status Menu. See page 5 for more information.

[ZERO]

The unlabeled key is the only variable key in the whole SpectraSYSTEM family. On the detector, the blank key is the [ZERO] key. The key's name appears on the nameplate below the key.

Pressing [ZERO] resets the detector output to zero volts, plus or minus any offset.

[MENU]

Pressing [MENU] displays the Main Menu (Figure 1.2). See page 4 for more information.

[ENTER]

Pressing [ENTER] accepts a selected choice or menu entry. The [ENTER] key also advances the cursor to a new field, either on the same line of the display or in the line below.

[∧], [∨], [<], and [>]

Pressing any arrow key (up, down, left, or right) moves the cursor in the direction indicated on the key. The up- and down-arrow keys also move the cursor between menus and displays.

[+] and [-]

Pressing the [+] and [-] keys scrolls you through a field's available choices or changes the value of alphanumeric entries. Holding down either key will continuously scroll the list of choices forward or backward until you release the key.

In fields that require numerical entries, the value of each digit is increased or decreased by one unit each time you press the [+] or [-] key. In fields that accept *either* numeric or character entries, such as the File Name field, the [+] and [-] keys scroll through the alphabet from A to Z, then through the numbers 0 to 9, and finally to a slash, hyphen, and blank space.

In other fields, the [+] key advances you through a preset list of choices while the [-] key takes you back through the list.

MENUS, SCREENS, AND MESSAGES

Your detector's display can show you three kinds of information: menus, screens, and messages. Menus require you to make selections or enter specific values. Screens display information that cannot be edited. Messages confirm actions and point out errors. The Menu Tree in Appendix B outlines the structure and content of the detector's menus and screens, three of which are discussed here, three of which are discussed here.

Main Menu

The (Figure 1.2) is the top level of the menu structure. It gives you access to five menus: FILES, QUEUE, TESTS, COMMANDS, and OPTIONS. To see the Main Menu, press the [MENU] key at any time.

Figure 1.2 The FL3000's Main Menu

From the Files Menu you can edit, load, copy, or delete files. The Commands Menu lets you initiate spectral scanning, replay spectra, insert an event mark onto your chromatogram, short outputs, or shut down the detector. The Tests Menu lets you run built-in instrument tests and diagnostics. In the Options Menu, you can set up or change your instrument's configuration. From the Queue Menu you can edit or change the order of files in the sample queue. Refer to Chapters 3, 4, 5, and Appendix B for more information on any of the instrument's menus.

Status Screen

The Status Screen (Figure 1.3) displays the detector status, excitation and emission wavelength settings, and the fluorescence intensity reading. It automatically appears whenever the instrument is powered on or the [STATUS] key is pressed. No entries are made on the Status Screen.

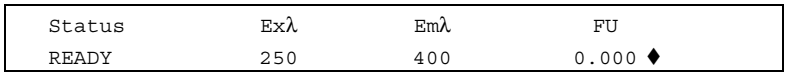


Figure 1.3 The Status Screen

Status Menu

Just below the Status Screen is the Status Menu. To access the Status Menu, press the down-arrow key from the Status Screen. The Status Menu lets you review and edit run parameters during a run. Chapter 3 discusses the Status Menu in more detail.

Messages

There are three kinds of messages that can appear on your detector's display: user messages, confirmation messages, and error messages.

User Messages

User messages, indicated on the display by double asterisks, tell you about an existing instrument condition or ask for further actions. Some of these will only appear on the display for three seconds. An example of a message requiring further action is shown in Figure 1.4.

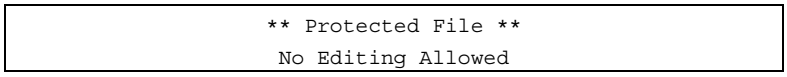


Figure 1.4 An Example of a User Message

Confirmation Messages

Confirmation messages (Figure 1.5), also indicated on the display by double asterisks, appear for one second after an operation has been carried out successfully.

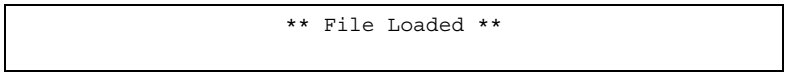


Figure 1.5 An Example of a Confirmation Message

Error Messages

Error messages (Figure 1.6), indicated on the display with capital letters and , are shown whenever an undesirable condition exists that prevents the instrument from carrying out an operation. Error remain on the display until you press a key.

```
!! RAM ERROR !!
```

Figure 1.6 An Example of an Error Message

Manual Conventions

This manual uses several conventions. Among them are menu displays, text conventions (brackets, slashes, etc.), standard words, and several different icons.

DISPLAYS

Figure 1.7 shows how we depict the two-line display. Note that, in menu illustrations, the triangular cursor location is indicated by a caret (>).

```
>  FILES                ❑ QUEUE                ❑ TESTS
              ❑ COMMANDS              ❑ OPTIONS
```

Figure 1.7 A Two-line Menu Display

Frequently the two lines shown on the display are only part of a longer menu. In this manual, menus having more than two lines are represented as in Figure 1.8.

```
   Zero on λ Change                  Yes
   Detection Type                  Fluor
------------------------------------------------------------
   Cursor Speed                   Medium
   Status Lock                       Off
```

Figure 1.8 A Menu Longer Than Two Lines

TEXT

Three typographic conventions are used to differentiate between keys, menus, and fields.

Brackets

Brackets, [ ], indicate instrument keys. For example: Press [MENU].

Slashes

Slashes, / /, are used around menu choices. For example: From the Main Menu, select /FILES/.

Capitalization

Capitalization is used to make field and menu names appear just as they do on the display. Generally the first letters of field names are capitalized. For example: Select /FILES/, /Copy/, Copy File #.

STANDARD WORDS

We have also standardized the meanings of two words: "select" and "enter."

select

The word "select" is used when you need to choose from among available options. For example, to "select" a particular menu choice, you would move the cursor to the appropriate choice and press [ENTER]. To "select" a field entry, move the cursor to the appropriate field and use the [+] and [-] keys to scroll to the desired preset value.

enter

The word "enter" is used when you need to specify individual alphanumeric digits. To "enter" a particular value, move the cursor to the desired field and use the [+] and [-] keys to increment or decrement each digit in the field until the desired value or letter appears.

ICONS

This manual uses the following icons to alert you to various situations. Each is called out by an icon in the left margin.

Caution!
A caution alerts you to situations that could result in personal injury. It also tells you how to avoid them.

High Voltage!
This icon alerts you to the presence of high voltage and to the potential injury that could occur from electrical shock were you to come in contact with a specific instrument area or component. It also tells you how to avoid contact with the high-voltage areas in your instrument.

Hot Surface!
This icon alerts you to potential injury that could occur from coming in contact with a heated surface or area on or in an instrument. It also tells you how to avoid contact with the heated surfaces in your instrument.

Note
Notes alert you to the correct operating or maintenance procedures needed to prevent equipment or data damage. They also alert you to important exceptions, side effects, or unexpected occurrences that may result from certain action(s).

Hint
Hints call out general rules or shortcuts. They specify ways to obtain the best performance and results from your instrument.

What's Next?

Now you're ready to try the practice example in Chapter 2, *A Quick Example*.

2

A Quick Example

Introduction

In Chapter 1, you read about the three easy rules for using your detector's command center and some of its menus and screens. In this chapter, you will find an example procedure that shows you how the rules and keys actually work as you move through the various menus.

This quick example uses only a fraction of the features available on your detector and is included only as a first step in becoming familiar with your new instrument.

After experimenting with this example, you'll want to turn to Chapters 3 and 4, which cover the detector's basic and more advanced operations. It is in those chapters that you'll learn about the full capabilities of your detector. First though, to give you a general understanding of the detector's capabilities and design, we'll briefly describe the features and benefits of the FL3000 here.

The FL3000 is a full-featured, time-programmable, fluorescence detector. The instrument offers an optical system design that provides high sensitivity and low baseline noise. With the advanced features of spectral scanning, multiple file storage, and file linking (with the Queue feature), the FL3000 provides increased versatility for your chromatography laboratory.

BEFORE YOU BEGIN

Before you begin this quick example, the detector should be fully installed in your chromatographic system according to the procedures described in Appendix A. You also should have completed the Start-up Checklist.

An Example

In this example, we will show you how to prepare a file and how to load the file into the detector's operating parameters. After a practice run, we will add a stop time.

HINT: You may wish to keep the Menu Tree in Appendix B on hand as you work through this example. If you lose your place at any time, you can:

1. *Press the [↖] key to move back to a previous screen.*
2. *Or, press [STATUS] to return to the Status Screen and retrace your steps.*

STARTUP

Set the power switch located on the detector's rear panel to **On**. After a series of power-up tests, the Status Screen (Figure 2.1) appears on the display. We will discuss the Status Screen after you have set up your operating parameters.

Status	Exλ	Emλ	FU
READY	250	400	0.000 ♦

Figure 2.1 The FL3000's Status Screen

SETTING PARAMETERS

To set your parameters, you need to prepare an edit file. The following steps will show you how to access the Edit Menu and prepare the file:

1. Press the [MENU] key. The detector's Main Menu appears on the screen (Figure 2.2).

> FILES	❑ QUEUE	❑ TESTS	
❑ COMMANDS	❑ OPTIONS		

Figure 2.2 The FL3000's Main Menu

2. Now select /FILES/ to display the Files Menu (Figure 2.3).

> Edit	❑ Load
❑ Copy	❑ Delete

Figure 2.3 The FL3000's Files Menu

3. Select /Edit/ to display the Edit Menu (Figure 2.4).

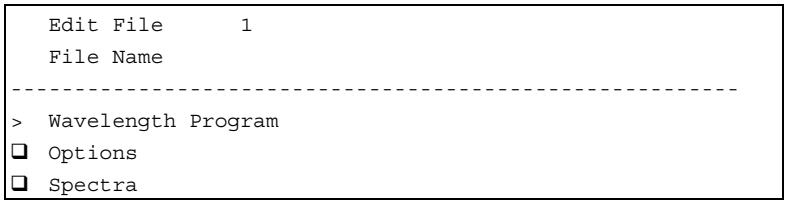


Figure 2.4 The FL3000's Edit Menu

For this example, we will use a file designation of 1 and leave the File Name field blank.

Wavelength

The excitation and emission wavelengths are examples of fields that require a numeric entry. To set each wavelength:

1. From the Edit Menu (Figure 2.4), select /Wavelength Program/ to display the Wavelength Program (Figure 2.5). The cursor will automatically be in the excitation wavelength field.

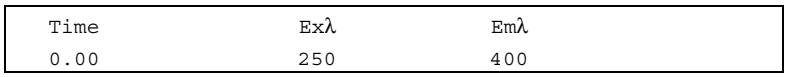


Figure 2.5 The FL3000's Wavelength Program

2. Using the [+] and [-] keys, set each wavelength field to the desired setting for your analysis. Remember that each digit must be edited individually.
3. Press [ENTER] to accept the new wavelength settings.

Lamp Status

Lamp Status is an example of a field that gives you a preset list of choices. To set the lamp status:

1. Select /Options/ from the Edit Menu (Figure 2.4) to display the Options Menu (Figure 2.6).

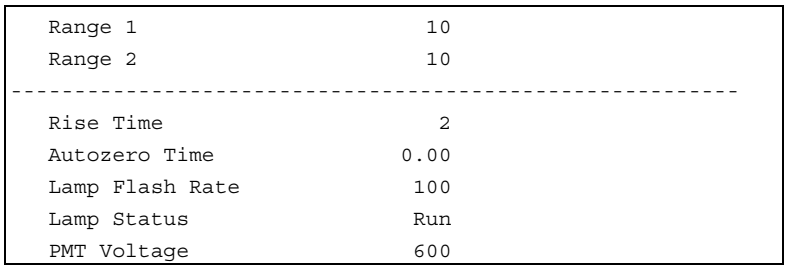


Figure 2.6 The FL3000's Options Menu

2. Using the [+] or [-] key, select **On** from the list of choices.
3. Press [ENTER] to turn the lamp on.

For this example, we will use the default settings for the remaining parameters. You will learn more about setting these parameters in Chapter 3.

Loading the File

You are now ready to load the settings from File 1 into the detector's operating parameters. To load the file:

1. Return to the Files Menu using the [∧] key.
2. Select /Load/. The screen in Figure 2.7 appears.

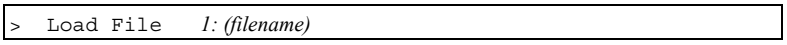


Figure 2.7 The Load File Command

3. Press [ENTER] to execute. The confirmation message shown in Figure 2.8 appears for one second.

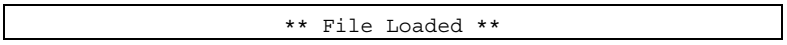


Figure 2.8 The File Loaded Message

You are automatically returned to the Status Screen and are ready to run your detector.

A PRACTICE RUN

Now you're ready for a practice run! Note that the Status Screen (Figure 2.1) now displays your excitation and emission wavelength settings, the detector's status, and the fluorescence intensity. If the Status reads READY, the program (file) is stabilized and ready to run.

When the detector is stabilized:

1. Press the [ZERO] key to zero the detector's analog output signal.
2. Inject your sample.

During setup, you may have noticed that there was no stop time entered in the detector's parameters. In this case, the detector stays in the READY state and continually monitors the column eluant. You do not need to manually start or stop a run with this set-up.

ADDING A STOP-TIME

To add a stop-time, you need to use the following steps to modify the detector's operating parameters. You will then start and stop a run, using the new setting.

1. From the Status Screen, press the [∨] key to move down to the Status Menu (Figure 2.9), which is the programming area below the Status Screen.

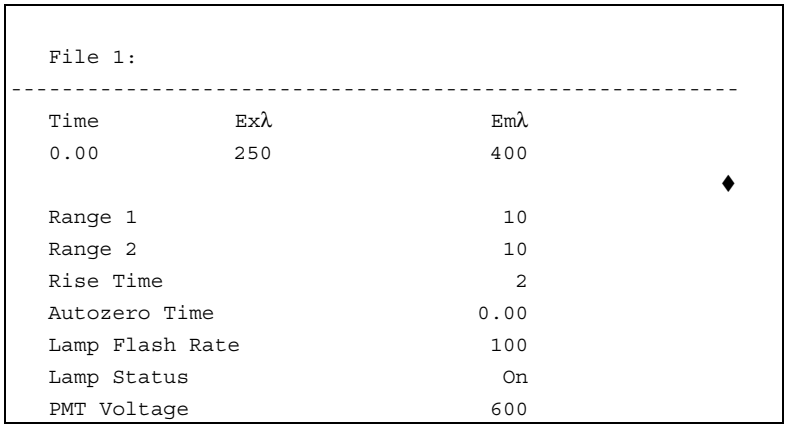


Figure 2.9 The FL3000's Status Menu

2. Using the [∨] key, move the cursor to the blank line below the 0.00 time line and press [+]. This adds a second line, with a time of 1.00 and the same wavelength settings as the first. Change 1.00 to the desired stop-time for the run, and leave the wavelengths unchanged.
3. To save your edits, scroll down to the words "Save File" (which now appear below PMT Voltage), and press [ENTER]. The message shown in Figure 2.10 appears and you are automatically returned to the Status Screen.

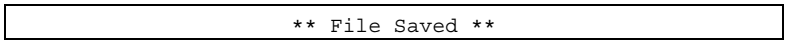


Figure 2.10 The File Saved Message

RUNNING WITH A STOP-TIME

Now that you have entered a stop-time, you will need to start the run with each injection. To do this:

1. Zero the detector's analog output signal by pressing the [ZERO] key.
2. When the detector is stabilized, inject your sample and press [RUN].

Notice that Status now shows the run time. If you wish to stop your run before the set stop-time, simply press [STOP].

What's Next?

Once you have completed this example and are comfortable with the keypad and display, proceed to Chapter 3, Basic Operations, to learn more about your detector.

3

Basic Operations

Introduction

This chapter provides you with step-by-step instructions for the most frequently used detector operations, including setup and run procedures for single (emission and excitation) wavelength mode, detector file management and protection, and analog output operations. You may wish to keep the Menu Tree and Field Reference Guide from Appendix B on hand as you work through this chapter.

NOTE: Your display's values may differ from those presented in this manual, especially if the detector has been previously programmed.

Before You Begin

Before you begin this chapter, your detector should be installed in a chromatographic system (see Appendix A), and you should have completed the Startup Checklist located at the front of this manual. We also recommend that you review Chapter 1, Getting Started, which gives general instructions for using the detector keypad and the conventions used throughout this manual.

Single-wavelength Operation

This section will show you how to perform single-wavelength operation. You'll learn how to identify and edit a file, how to load that file into the detector's current operating parameters, and how to start and stop a run. This section will also show you how to modify the detector's operating parameters.

SETTING PARAMETERS

Before you start setting any detector parameters, you need to identify the file you wish to edit. To do this, access the Files Menu.

To access the Files Menu, first press [MENU]. The Main Menu appears on the screen. From the Main Menu, select /FILES/. The menu shown in Figure 3.1 will appear.

Figure 3.1 The FL3000's Files Menu

Select /Edit/ from the Files Menu to display the Edit Menu (Figure 3.2).

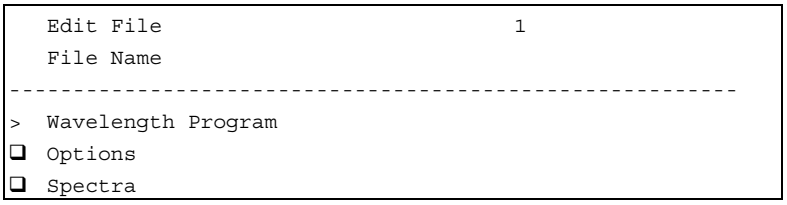


Figure 3.2 The FL3000's Edit Menu

File Identification

Enter the file number you wish to edit in the Edit File field. The FL3000 can store up to four files in memory, so file numbers from 1 to 4 are allowed. You may also enter a name of up to eight characters in the File Name field.

Wavelength Program

From the Edit Menu, select /Wavelength Program/. The Wavelength Program (Figure 3.3) consists of a table showing time and the excitation and emission wavelengths.

Time	Exλ	Emλ
0.00	250	400

Figure 3.3 The FL3000's Wavelength Program

You can operate with either a one-line or a two-line wavelength program. Using a one-line program, the detector is always in the READY state and you can continually monitor the chromatographic eluant. Using a two-line program, you can add a stop-line and you can start and stop the detector during a chromatographic run. (Stop-lines are useful, for example, in an automated series of runs where you want to autozero the detector's baseline after each injection.)

For a one-line program, enter the excitation and emission wavelengths for your analysis (in the Exλ and Emλ fields, respectively), that correspond to the start time of 0.00.

HINT: Always set the emission wavelength greater than the excitation wavelength by more than 1.5 to 2 times the monochromator's slit widths to minimize light-scattering effects. Also, avoid setting the excitation wavelength to exactly one-half the value of the emission wavelength. This condition creates second-order (Rayleigh) scattering that will require an optical filter in conjunction with the emission slit.

HINT: Switch to the optional Extended Range PMT if your emission wavelength setting is in the 600 to 800 nm range.

For a two-line program, add an additional line (the stop-line) by scrolling down to the blank line below and pressing [+]. The second line will automatically have a time setting of 1.00 and the same wavelength setting as the first. (You will learn more about time lines in Chapter 4.) Change 1.00 to the desired stop time for the run, and leave the wavelength values unchanged.

An example of a two-line wavelength program for a nine-minute run at an excitation of 250 nm and an emission of 400 nm is shown in Figure 3.4.

Time	Exλ	Emλ
0.00	250	400
9.00	250	400

Figure 3.4 An Example of a Wavelength Program with a Stop Time

Options

Select /Options/ from the Edit Menu to display the Options Menu (Figure 3.5). Use this menu to set the detector's ranges, rise time, autozero time, lamp flash rate, lamp status, and PMT voltage.

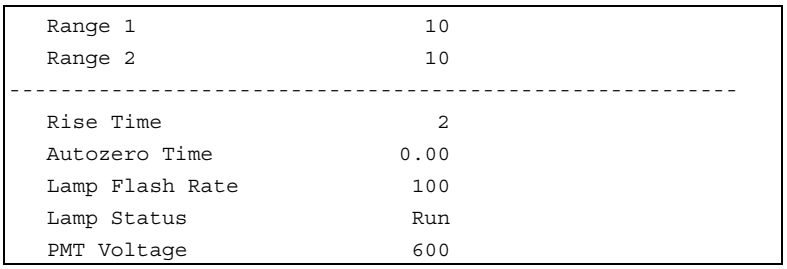


Figure 3.5 The FL3000's Options Menu (as selected from /Files/, /Edit/)

Range 1 and Range 2

Set each range value to an appropriate full-scale fluorescence intensity for your sample. For example, a peak of five fluorescence units will appear full-scale on a recorder at a range setting of 5 FUFS (fluorescence units full-scale). Note that Range 1 and 2 correspond to Analog Outputs 1 and 2 (labeled ANLG Outputs 1 and 2) on the rear panel of your detector. For more information on the use of ranges and analog outputs, see pages 24 and 68.

Rise Time

This field controls the detector's response time. Rise time is inversely proportional to the amount of baseline noise. For example, the longer the rise time, the less noise detected. The two-second default value is appropriate for most applications.

HINT: To minimize baseline noise while retaining maximum resolution, select a rise time that is at least one-tenth of the peak width at the base of the narrowest peak of interest.

Autozero Time

This parameter tells the detector when to perform an automatic zero of the baseline. If you don't want to set an automatic autozero and you are using a stop-line in your wavelength program, simply set the autozero time to a value greater than your stop-time.

HINT: It is good practice to zero the detector automatically at the start of each run. This will keep the detector output in range throughout an automated series of runs.

Lamp Flash Rate

Set the rate at which the xenon lamp should pulse on and off. The 20 Hz setting prolongs lamp life, while the 100 Hz setting results in greater sensitivity at the expense of the lamp life. Most likely you will use the 100 Hz setting for most applications.

Lamp Status

Set the lamp mode you wish to use. You can choose from the following selections:

- *On and Off*. These selections turn the lamp on or off, as soon as you accept the setting.
- *Run*. Choosing "run" automatically turns the lamp on at the beginning of each run and off at the end of each run.
- *Off@End*. This selection turns the lamp off at the end of a queue. (You will learn about the queue feature in Chapter 4.)

HINT: Lamp life is increased significantly by turning the lamp off when the detector is not in use.

PMT Voltage

Set the voltage to be applied to the photomultiplier tube (PMT). The PMT's sensitivity is proportional to the applied voltage, but higher voltages also cause a shortening of the PMT's service life.

HINT: The default value of 600 satisfies most applications' sensitivity needs, while providing an acceptable service life.

Loading a File

When you are ready to load a file, select /Load/ from the Files Menu. The screen will display the words "Load File 1:(filename)." Enter the desired file number and press [ENTER]. The message shown in Figure 3.6 will appear for one second. You are then returned to the Status Screen.

```
** File Loaded **
```

Figure 3.6 The File Loaded Message

RUNNING YOUR DETECTOR

Once you've set your detector parameters in the designated file and have loaded the file into the detector's operating parameters, you are ready to run your analysis. First check the detector's status by viewing the Status Screen. If you are using a stop-line in your wavelength program, you will start and stop the run with each injection.

Status Screen

You can check the detector's status, wavelength settings, and fluorescence reading from the Status Screen (Figure 3.7). To access the Status Screen, press [STATUS].

Status	Exλ	Emλ	FU
READY	250	400	0.000 ♦

Figure 3.7 The FL3000's Status Screen

If the Status Screen reads READY, the detector is stabilized and ready to run. The Exλ and Emλ fields display the current excitation and emission wavelength settings. The FU field is the current fluorescence intensity reading.

Inject Your Sample

When the detector is stabilized and you are ready to inject your sample, manually zero the detector by pressing the [ZERO] key.

If you are not using a stop-line in the wavelength program, the detector remains in the READY state throughout your chromatographic runs. If you are using a stop-line, you must start and stop the run with each injection, following the procedures below.

Starting a Run

If you're using a stop-line in your wavelength program, you need to start the run with each injection. There are two ways to start a run using the FL3000:

1. *Manually*, by pressing [RUN] each time you make an injection.
2. *Automatically*, by interfacing the detector with a remote run-signal from the injector (see Appendix A for details). In this scenario, a signal that is equivalent to pressing [RUN] is automatically sent from the injector to the detector with each injection.

During the run, you can monitor the run time from the Status Screen.

Stopping a Run

There are two ways to stop a run:

1. *Manually*, by pressing [STOP] before the programmed stop time.
2. *Automatically*, by allowing the run to finish at the preset time.

In either case, the detector returns to READY.

CHANGING RUN PARAMETERS

There are two ways to change the detector's run parameters:

1. You can use the Files Menu and follow the procedures outlined under "Setting Parameters" on page 16.
2. You can use the Status Menu, which is the programming area below the Status Screen.

Each has a distinct advantage. Programming in the Status Menu allows you to change the detector's current operating parameters, even while the detector is running. Programming in the Files Menu allows you to prepare a file containing the changes without altering the current detector settings. The file may then be loaded at a later time.

Status Menu

From the Status Screen, scroll down to the Status Menu (Figure 3.8),

which contains the loaded file identification (its number and name), Wavelength Program, Ranges, Rise Time, Autozero Time, Lamp Flash Rate, Lamp Status, and PMT Voltage.

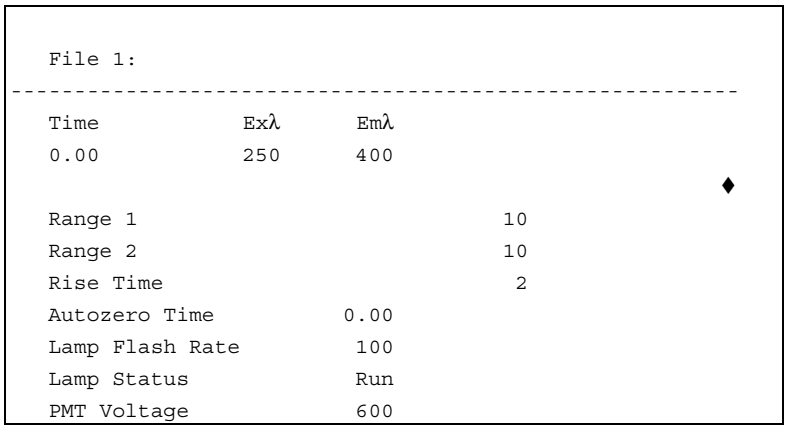


Figure 3.8 The FL3000's Status Menu

The detector's parameters are set following the same instructions previously given under "Wavelength Program" and "Options Menu," starting on page 17. However, you cannot modify the file identification while in the Status Menu.

NOTE: When you modify a file's parameters from the Status Menu, you do not change the contents of the same file number stored in the detector's memory. Only the copy of the active file is modified.

Saving the File

When you modify a loaded file from the Status Menu, each change is effective as soon as you leave the field. You are reminded of the file's changed status in two ways: the file name shown on the first line of the Status Menu (Figure 3.8) now reads "-changed" and a Save File command appears at the very end of the Status Menu (below PMT Voltage). Press [ENTER] at the Save File command to save the new values. The message shown in Figure 3.9 appears.

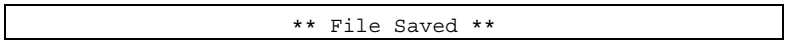


Figure 3.9 The Message That is Displayed When a File is Saved

If you wish to keep the original file without saving the changes, do not press [ENTER]. Instead, reload the unaltered file by using the Files Menu as follows:

1. Press [MENU].
2. Select /FILES/.
3. Select /Load/.
4. The words "Load File N: (filename)" will appear on the screen. Enter the desired file number and press [ENTER].

The confirmation message shown in Figure 3.6 will appear for one second. You are then returned to the Status Screen, where all settings will contain their original values.

More About Files

On page 16 you learned how to edit and load files from the Files Menu, but the Files Menu also allows you to copy and delete files in a few easy steps. This section will show you how. It will also show you how to protect files from being edited, copied to, or deleted.

COPYING FILES

To copy a file:

1. Press [MENU].
2. Select /FILES/ to display the Files Menu selections (Figure 3.10).

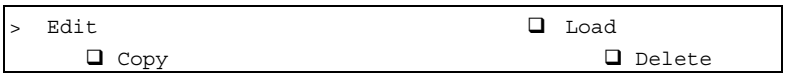


Figure 3.10 The FL3000's Files Menu

3. Select /Copy/. The Copy Menu will appear on the screen (Figure 3.11).

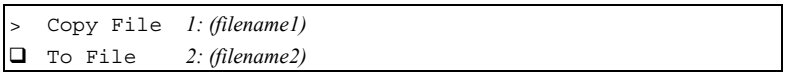


Figure 3.11 The FL3000's Copy Menu

4. Enter the identification number for the file you wish to *copy* in the Copy File field.
5. Enter the number of the file to which you wish to copy *to* in the To File field.

6. Press [ENTER]. The message shown in Figure 3.12 appears briefly, and you are returned to the Files Menu.

```
** File Copied **
```

Figure 3.12 The File Copied Message

If you attempt to copy to a protected file (see the section below, titled "Protecting Files"), you will get the message shown in Figure 3.13. If a file is not protected, make sure it's empty or unwanted before you copy to it, as it will be overwritten.

```
** Protected File **
Cannot Be Copied To
```

Figure 3.13 The Message That is Displayed When You Attempt to Copy to a Protected File

DELETING FILES

To delete a file:

1. Press [MENU].
2. Select /FILES/ to display the Files Menu (Figure 3.10).
3. Select /Delete/. The words "Delete File N:(filename)" will appear on the screen.
4. Enter the identification number of the file you wish to delete. When you press [ENTER], the message shown in Figure 3.14 appears briefly, and the display returns to the Files Menu. (The parameters in the file you have just deleted return to their default values.)

```
** File Deleted **
```

Figure 3.14 The File Deleted Message

If you attempt to delete a protected file (see the next section, "Protecting Files"), you will get the message shown in Figure 3.15.

```
** Protected File **
Cannot Be Deleted
```

Figure 3.15 The Message That is Displayed When You Try to Delete a Protected File

PROTECTING FILES

The FL3000 allows you to protect files from being edited, copied to, or deleted. To access the file protection operation, follow these steps:

1. Press [MENU].
2. Select /OPTIONS/. The Options Menu appears in Figure 3.16.

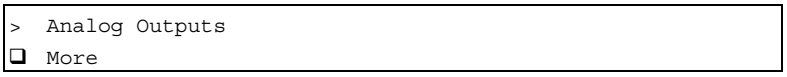


Figure 3.16 The FL3000's Options Menu

3. Select /More/ to display the More Menu (Figure 3.17).

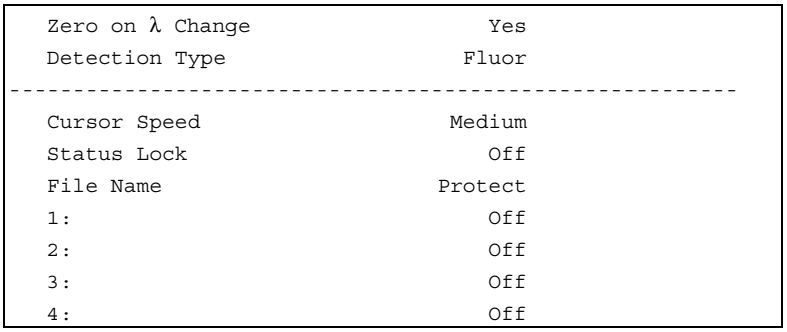


Figure 3.17 The FL3000's More Menu

4. Scroll down to the table containing the fields File Name and Protect. To protect a file from being edited, copied to, or deleted, select **On** in the Protect field that corresponds to the appropriate file number. To remove the file protection, select **Off**.

Analog Output Operations

The FL3000 has two outputs, Analog Output 1 and Analog Output 2. Labeled ANLG 1 Output and ANLG 2 Output on the detector's rear panel, these outputs are useful for monitoring analyses at two different sensitivity settings simultaneously. For example, analog outputs allow you to optimally detect very small peaks and very large peaks in the same sample run.

For information on how to make rear-panel connections for analog outputs, see page 68.

ANALOG OFFSETS

Analog offsets may be used when there is a high background fluorescence reading, or when there is considerable baseline drift from your chromatographic system and you are unable to keep your integrator's (recorder's) signal on-scale.

Because integrators have very limited capacity for handling negative signals, you may wish to set a small positive offset (1%) when using an integrator.

Use negative offsets with recorders, where you may wish to set the pen at either side of the strip chart.

```
The offset options are selectable from the Analog Outputs
Menu shown in Figure 3.0.Analog 1 Offset %              0
   Analog 2 Offset %                    0
-----------------------------------------------------------
   READY Output                 Active Hi
```

Figure 3.18 The FL3000's Analog Outputs Menu

To access the Analog Outputs Menu:

1. Press [MENU].
2. Select /OPTIONS/.
3. Select /Analog Outputs/.

HINT: We recommend a 1% offset setting for use with your data system or integrator.

4

Advanced Operations

Introduction

In this chapter, you will learn to use the FL3000's more advanced capabilities, such as wavelength programming, automatic autozeroing, scanning, and queues. You should be familiar with the instructions presented in Chapter 3, Basic Operations, before you begin.

Wavelength Programming

Your detector can change excitation and/or emission wavelength as a function of time, a feature we call Wavelength Programming. This feature gives you maximum detection sensitivity for each component in a mixture without making multiple injections of the sample.

BUILDING THE PROGRAM

In wavelength programming, you enter time lines into a "Wavelength Program." Each time line specifies the time at which you want a wavelength change to occur.

You can build a wavelength program in either the Status Menu or the Files Menu using the procedure outlined in this section.

Initial Conditions

Display the Wavelength Program (Figure 4.1) in either the Status Menu or the Files Menu.

Time	Exλ	Emλ
0.00	250	400

Figure 4.1 The FL3000's Wavelength Program

The initial time entry is 0.00. Move the cursor to the Exl and Eml fields, and enter the initial excitation and emission wavelengths for your analysis.

Entering a Second Time Line

To add a second time line, scroll down to the first blank line and press [+]. The second line will automatically have a time setting of 1.00 and the same wavelength settings as the first. Change the Time and corresponding Exλ and Emλ fields to the desired values.

Adding Subsequent Lines

A wavelength program may contain as many as ten lines for a single run. If you enter time lines out of sequence, the detector will automatically sort the lines and place them in chronological order.

The Stop-line

The last line of the program (the stop-line) lists the time at which the detector will automatically end the run and return to initial conditions. Since wavelengths are not important in the stop-line, they can be set to any value(s).

NOTE: Remember, the last line of the program is always the detector's signal to end a run; it is not a programmed wavelength change!

Deleting a Line

To delete an entire time line, place the cursor in the Time field and press [-] repeatedly until the value goes blank. When you leave the line, it will be deleted.

An Example

Figure 4.2 shows a completed wavelength program.

Time	Exλ	Emλ
0.00	250	400
2.50	280	375
6.00	280	375

Figure 4.2 An Example of a Completed Wavelength Program

In our example, the initial excitation and emission wavelengths are 250 and 400 nm, respectively. At 2.50 minutes into the run, the wavelengths change to 280 and 375 nm. The run ends at 6.00 minutes, and the detector returns to its initial wavelengths of 250 and 400 nm and to its READY state.

RUNNING THE PROGRAM

After you set the rest of your parameters, the detector is ready to run. It is good practice to zero the detector at the beginning of every run and at each wavelength change. See the next section, titled "Programmed Autozero," for details.

Once you start the run, you may edit any timed event (wavelength change, autozero, or stop time) that has not yet taken place. These changes can only be made from the Status Menu however! Each edit is entered immediately into the detector's operating wavelength program.

For example, for the program displayed in Figure 4.2, the stop time is 6.0 minutes. If, at 5.00 minutes into the run, you determine that the run should be 9.00 minutes long, you can edit the last line of the program and the current run will stop at 9.00 minutes.

Programmed Autozero

The FL3000 can be programmed to perform an automatic zero with each wavelength change during a run using the Zero on λ Change field. To access this feature:

1. Press [MENU] and select /OPTIONS/ to access the Options Menu (Figure 4.3).

Figure 4.3 The FL3000's Options Menu

2. Select /More/ to display the More Menu.
3. Place the cursor on the Zero on λ Change field. This field appears on the first line of the More Menu.
4. Select **Yes**, to automatically zero the detector response with each wavelength change during a run, or **No**, to turn this feature off.

You can also use this automatic zero feature to add autozeros into your wavelength program *without* changing the detector's wavelength settings. To do this, simply add additional time lines. Adding autozeros in this way is convenient in cases such as solvent programming, where the detector's baseline may drift due to changes in solvent background.

An example program is shown in Figure 4.4.

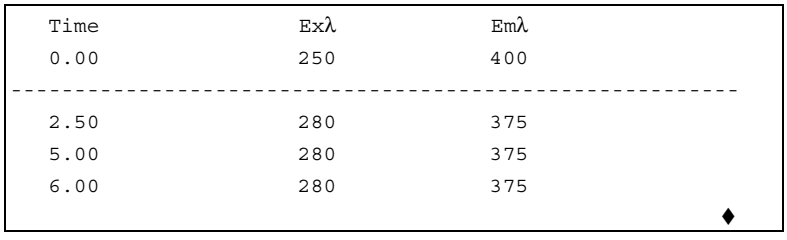


Figure 4.4 Example of Wavelength Program with Automatic Autozeros

With the Zero on l Change field set to Yes, the detector will autozero at 2.50 and 5.00 minutes into the run, even though the wavelength will only change once (at 2.50 minutes into the run).

Scanning

The FL3000 is uniquely capable of performing an excitation, emission, or synchronous (delta) spectral scan on eluting peaks without stopping the eluant flow. This ability greatly simplifies the determination of wavelength maxima for individual compounds in your sample during method development work.

HOW IT WORKS

When a scan is initiated, either manually or automatically, the monochromator moves from the run-wavelength to the scan's start-wavelength. The detector scans by stepping through the defined spectral range at specified wavelength increments and at a rate of 100 Hz. Individual fluorescence intensities are read at each increment until the monochromator reaches the last wavelength. The scan is then repeated in reverse, from the last wavelength to the first. Up to 32 scans can be averaged to minimize any effects from changing peak concentrations.

The number of data points in each scan determines the number of spectra that the FL3000 can collect and store for a single chromatographic run. Use the following equations to calculate the number of data points and the number of spectra you will be able to collect.

Equation 1. To calculate the number of data points for any scan between λ1 (the lower wavelength), and λ2 (the higher wavelength):

$$\#\ of\ data\ points < \frac{\lambda 2 - \lambda 1}{step\ size} + 1$$

Equation 2. To calculate the number of spectra you can collect:

$$\#\ of\ spectra < \frac{12{,}800}{(\#\ of\ data\ points * 4) + 14}$$

For example, if you want to scan from 200 to 400 nm in 2-nm steps, there would be 101 data points and the FL3000 would be able to store up to 30 spectra:

$$\#\ of\ spectra < \frac{12{,}800}{(101 * 4) + 14} = \frac{12{,}800}{418} = 30.62$$

Each spectrum is corrected for baseline fluorescence before being played back as individual data points or as a continuous curve.

PREPARING A FILE

To prepare a scan file, you need to set up both the detector's run parameters and its scan parameters.

Setting the Run Parameters

Use the following steps to set the detector's run parameters:

1. Press [MENU]. Select /FILES/.
2. Select /Edit/ to display the Edit Menu (Figure 4.5).

```
   Edit File
   File Name
------------------------------------------------------------
>  Wavelength Program
❑  Options
❑  Spectra
```

Figure 4.5 The FL3000's Edit Menu

3. Enter whatever file identification you wish to use in the Edit File and File Name fields.
4. Set the detector's run parameters in the Wavelength Program and Options Menus.

NOTE: To perform an excitation scan, the FL3000 uses the emission wavelength programmed in the file through the Wavelength Program (not the Spectra Menu). To perform an emission scan, the detector uses the excitation wavelength programmed in the file. For a delta scan, the monochromators move to the start wavelengths programmed in the Spectra Menu.

5. Select /Spectra/. The Spectra Menu, described in the next section, is where you'll set up your spectral scanning parameters.

Setting the Scan Parameters

Use the Spectra Menu as follows to set the detector's scan parameters:

1. From the Edit Menu, select /Spectra/. The Spectra Menu shown in Figure 4.6 appears.

```
   Scan Type                    Emission
   Start Excitation λ                250
------------------------------------------------------------
   Start Emission λ                  400
   Step Size                           8
   Scan Length                       100
   Number of Scans                     2
   Auto Spectra                      Off
   Auto Threshold                  0.010
   Scan Zero Time                   0.00
```

Figure 4.6 The FL3000's Spectra Menu

2. In the Scan Type field, select the scan mode you wish to run. For an excitation scan, the emission wavelength is held constant while the excitation monochromator performs the scan. For an emission scan, it is the opposite. In a delta scan, both monochromators move simultaneously, keeping the same wavelength span (delta) between them.
3. In the Start Excitation λ field, enter the excitation wavelength at which each scan should begin. When you are performing emission scans, this parameter is ignored.
4. In the Start Emission λ field, enter the emission wavelength at which each scan should begin. When you are performing excitation scans, this parameter is ignored.
5. In Step Size, enter the wavelength increment at which the detector will scan.

NOTE: If you chose starting and ending wavelengths that were not an exact multiple of your step size, the ending spike (event mark) on your chromatogram would be placed at the last multiple of the step size that falls within the scanning range. For example, with a starting wavelength of 200 nm, an ending wavelength of 364 nm, and a step size of ten, the end spike on your chromatogram would be at 360 nm, the last full step size within the range.

6. In Scan Length, enter the spectral range for each scan.
7. In Number of Scans, enter the number of times the monochromator should perform each scan (for averaging).
8. In Auto Spectra, select **On**, if you want the detector to scan automatically, or **Off**, if you choose to scan manually.
9. In Auto Threshold, specify the minimum peak fluorescence intensity that will signal the detector to perform an automatic scan. If you are scanning manually, disregard this parameter.
10. In Scan Zero Time, enter the run time at which the detector should perform an automatic baseline scan.

When you are finished with setup, return to the Files Menu. Load your scan file and you are ready to run.

WHILE THE SCAN FILE IS RUNNING

As was noted in the introduction, you can perform scans manually or automatically during a run. In either case, you will see a "wiggle" in the chromatographic trace each time a scan is taken. For this reason, quantitative analysis should never be performed when scanning.

Manual Scanning

To perform manual scanning, access Scan and Scan Zero in the Commands Menu as follows:

1. Press [MENU].
2. Select /COMMANDS/. The menu shown in Figure 4.7 appears.

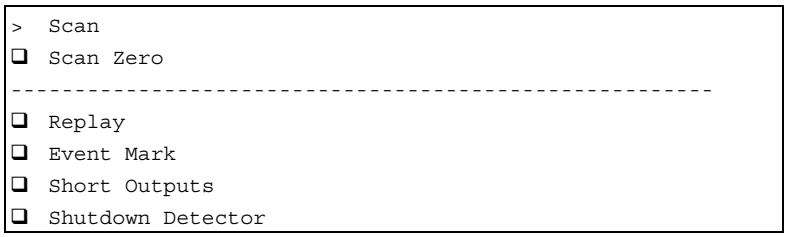


Figure 4.7 The FL3000's Commands Menu

3. Select /Scan/ or /Scan Zero/.

Scan

Select Scan whenever you want to perform sample scans of the chromatographic peaks. Press [ENTER] each time you wish to perform a sample scan.

Scan Zero

Use Scan Zero to perform baseline scans of the solvent background fluorescence. With the detector baseline stabilized and the cursor on the Scan Zero command, press [ENTER]. The FL3000 will perform and store a baseline scan using the parameters in your scan file. Each subsequent sample scan is corrected automatically for any background absorbance.

Baseline scans may be taken at any time during the run, as long as no peak is eluting at that time. Subsequent sample scans are corrected using the last baseline scan taken.

For example, let's say you perform a baseline scan before you initiate a run, and then again at 5.00 minutes into the run. You also perform sample scans of your eluting peaks at 2.4 and 5.6 minutes into the run. The sample scan taken at 2.4 minutes will be corrected using the baseline scan taken before the run began. The sample scan taken at 5.6 minutes will be corrected using the baseline scan taken at 5.0 minutes.

Automatic Scanning

If you have set the Auto Spectra field in the Spectra Menu to On (see the section, "Setting the Scan Parameters" on page 31), your detector will perform an automatic scan at 150 msec after a peak apex whenever the fluorescence intensity exceeds the value set in the Auto Threshold field. In our example chromatogram (Figure 4.8), a scan would occur automatically for Peak A, since it exceeds the value set in Auto Threshold.

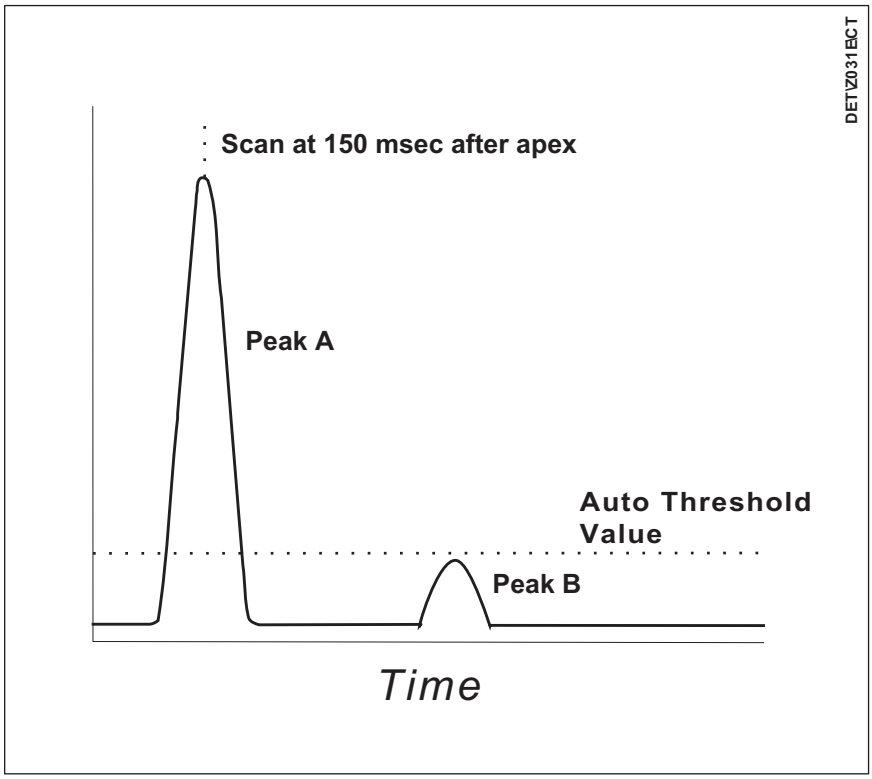


Figure 4.8 An Example of How Automatic Scanning Works

An automatic baseline scan will occur at the time specified in the Spectra Menu's Scan Zero Time field. Make sure that no peaks are eluting at the specified time.

REPLAYING YOUR SPECTRA

Once you've completed your run (either by pressing [STOP] or by the file having completed each time line), you can retrieve your stored sample spectra by selecting /Replay/ in the Commands Menu (Figure 4.7). If no spectra are stored in memory when you select Replay, the message shown in Figure 4.9 will appear on the display.

```
** No Spectra Available **
```

Figure 4.9 The message that is displayed when you try to replay spectra and none are in memory

The Replay Menu appears in Figure 4.10.

```
   Range 1                          10.0
   Range 2                          10.0
-------------------------------------------------------
   Replay Rate (nm/sec)               20
   Spectra Time                     0.10
❑  Replay Spectra
❑  Display FU, λ
```

Figure 4.10 The FL3000's Replay Menu

Setting Replay Parameters

From the Replay Menu (Figure 4.10), set the parameters for replaying your spectra as follows:

1. Set Range 1 and Range 2 for Analog Output 1 and Analog Output 2, respectively. If you are using only one output, disregard the appropriate range.
2. Enter the Replay Rate (nm/sec). This is the rate at which the detector will read out the spectral data to your integrator chart. You will use this value and an appropriate chart speed to calculate wavelength increments on your printed sample spectrum.

   For example, if your sample scan was taken from 250 to 350 nm (a span of 100 nm), a replay rate of 5 nm/sec would print the spectrum in 20 seconds. A chart speed of 30 cm/min would give you a scan of 10 cm in increments of 10 nm/cm.
3. Select the spectrum you want to replay by displaying its start time in the Spectra Time field. Each spectrum taken during the run is individually identified by the run time at which it was initiated.

When you have finished setting your replay parameters, you are ready to send the spectral data to your chart by selecting the Replay Spectra command in the Replay Menu (Figure 4.10).

Running Replay

To replay your stored spectrum, initiate the Replay Spectra command in the Replay Menu (Figure 4.10) by moving the cursor to /Replay Spectra/ and pressing [ENTER]. While the replay is occurring, the screen in Figure 4.11 appears on the display.

Replay	Exλ	Emλ	FU
0.50	250	400	999.999

Figure 4.11 The Display as it Appears While Running Replay

The screen's Replay field displays the start time of the spectra being played. The Exλ, Emλ, and FU fields display the individual data points being plotted. When the replay is finished, the display returns to the Replay Menu. You may stop a replay at any time by pressing [STOP].

Display FU, λ

You can display the individual data points of a stored spectrum by selecting /Display FU, λ/ in the Replay Menu (Figure 4.10). The screen shown in Figure 4.12 appears on the display.

Display	Exλ	Emλ	FU
0.50	250	400	999.999

Figure 4.12 The Display FU, λ Screen

The screen's Display field shows the time the spectrum was taken, the excitation and emission wavelengths (Exλ and Emλ), and the corresponding fluorescence intensity (FU). To scroll through the data, use the [+] and [-] keys. To return to the Replay Menu, press [∧].

SPECTRAL DATA STORAGE

Spectral data are stored in the FL3000's memory until the detector is turned off, or until you press [RUN] (after loading a new file or queue).

Sample Queue

Sometimes it's convenient to run groups of samples under different detector conditions in an automated run. For these occasions, the FL3000 offers a queuing feature. Using a queue, you can program the detector to load and run one file for your first group of samples, then automatically load a second file to run your next group of samples. The queue feature allows you to run as many as ten files in a single queue.

QUEUE MENU

To access the Queue Menu, follow these steps:

1. Press [MENU].
2. Select /QUEUE/.

When no queue is loaded, the Queue Menu appears as shown in Figure 4.13. On page 39, we'll see how the menu appears when a queue is loaded.

```
> Edit                    ❑ Load
                ❑ Delete
```

Figure 4.13 Queue Menu With No Queue Loaded

SETTING UP A QUEUE

To set up a queue, select /Edit/ from the Queue Menu. If the queue is empty, the display will look like Figure 4.14.

```
Order          File:Name            #Runs
  1
```

Figure 4.14 An Empty Queue

Entering a Line

A "1" is automatically placed in the Order field of the first file to be run. You can't change that, so the cursor appears under the first editable field, "File:Name." Scroll through the available files and press [ENTER] when your choice appears.

Enter the number of injections to be made in the #Runs field and press [ENTER]. You can have as many as 999 injections per file.

Adding Subsequent Lines

After completing the first line, a second line appears automatically. The Order field reads 2, and the rest of the line is blank. Select the proper file and the number of injections to be made for that file. You can have as many as ten groups for a single queue.

Deleting a Line

To delete a line, use the [-] key in the File:Name field until it goes blank. When you leave the line, it is deleted and the queue is resorted automatically.

An Example

An example of a queue appears in Figure 4.15.

Order	File:Name	#Runs
1	2:THEOPHYL	5
2	3:ABCD	25
3	1:BARBITUR	10

Figure 4.15 An Example of a Queue

In our example, we have programmed the detector to run File 2 for the first five injections, File 3 for the next 25 injections, and File 1 for the last ten injections.

LOADING A QUEUE

To load a queue, select /Load/ in the Queue Menu (Figure 4.13). When the words "Load Queue" appear, press [ENTER]. The confirmation message shown in Figure 4.16 appears for one second.

```
** Queue Loaded **
```

Figure 4.16 The Confirmation Message When a Queue is Loaded

When a queue is loaded, the letter "Q" appears at the extreme left of the Status Screen (Figure 4.17).

Status	Exλ	Emλ	FU	
Q READY	250	400	0.000	♦

Figure 4.17 The Status Screen When a Queue is Loaded

If you attempt to load a queue when no queue exists, the message shown in Figure 4.18 will appear on the display.

```
** No Queue Available **
```

Figure 4.18 The Message That is Displayed When No Queue is Available

RUNNING A QUEUE

When the detector receives its first start signal, it loads and runs the file designated in Order 1. It will continue to run this file each time it receives a start signal until the file has run the number of times specified in the #Runs field. The detector will then load and run the file designated in Order 2 and run it the number of times specified in that line, and so on, until the entire queue has run.

Viewing its Progress

To view the progress of a queue that is running:

1. Press [MENU].
2. Select /QUEUE/. Note that when a queue is loaded, the Queue Menu (Figure 4.19) looks different. The Load field has been replaced by "Pause," which we will discuss on page 40.

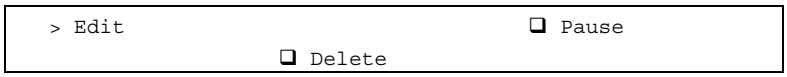


Figure 4.19 The Queue Menu With a Queue Loaded

3. Select /Edit/ to display the queue. (Refer to Figure 4.15 for an example queue.)

While the queue is running, the #Runs field automatically decreases by one with each injection. When a particular file's last injection is made, the queue is automatically resorted. In other words, the information for Order 2 is now moved up to Order 1, the information for Order 3 is moved up to Order 2, and so forth. This process continues until the queue becomes empty, is paused, or is deleted.

Loading other Files

When a queue is loaded or running, you may not load any other file from the Files Menu without first pausing or deleting the queue. If you try to load a different file without pausing or deleting the queue, you will get the message shown in Figure 4.20. You are then returned to the Files Menu.

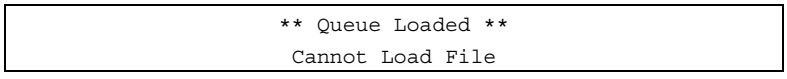


Figure 4.20 The Message That Appears When You Attempt to Load a File While a Queue is Loaded or Running

EDITING A QUEUE

To edit an existing queue, follow the procedures outlined in "Setting Up a Queue" on page 37. You are allowed to edit the queue while it is running, but if you want to edit anything in Order 1, you'll have to pause the queue first.

PAUSING A QUEUE

The following steps tell you how to pause a queue:

1. Select /Pause/ from the Queue Menu (Figure 4.19).
2. When the words "Pause Queue" appear, press [ENTER]. If a file is running, the run continues until it is completed, at which point the detector returns to its READY state. The letter Q no longer appears in the Status Menu.

To continue, you must reload the queue. When the detector receives a start signal, the queue will resume operation at the point where it left off.

DELETING/ STOPPING A QUEUE

Use the following steps to delete an existing queue or to stop a running queue:

1. Display the Queue Menu (Figure 4.19).
2. Select /Delete/.
3. When the words "Delete Queue" appear, press [ENTER]. If a file is running, the run continues until it is completed. The message shown in Figure 4.21 appears for one second and you are returned to the Queue Menu.

Figure 4.21 The Queue Deleted Message

You may delete or stop a queue at any time, but remember that the queue will be subsequently erased from the detector's memory. It is good practice to delete an existing queue prior to designing a new one.

Phosphorescence

Throughout this manual we have been talking about fluorescence, the virtually instantaneous and temperature-independent emission of light by a sample that has been excited by incident light energy.

But the FL3000 can also detect phosphorescence. Phosphorescence is defined as the delayed and temperature-dependent emission of light by a sample when that sample is excited by incident light energy. In both fluorescence and phosphorescence, the wavelength of light that excites the sample is typically shorter than the wavelength of light that the sample emits (i.e., the sample emission is a Stokes emission).To select the type of detection:

1. Press [MENU].
2. Select /OPTIONS/.
3. Select /More/.
4. Scroll down to Detection Type. Select **Fluor** for fluorescence or **Phos** for phosphorescence. [Fluor sets the PMT's (photomultiplier tube) voltage integration for immediately after the lamp flash; Phos sets it for approximately 20 msec after each lamp flash.]

Zero Order

You can enter a value of zero for the excitation wavelength, the emission wavelength, or both. This sets the respective monochromator to what is known as the "zero order" position. In the zero-order mode, the monochromator doesn't diffract light into its spectral components. Rather, it functions like a mirror, simply reflecting all wavelengths of incident light.

The zero-order mode is useful when you don't know the proper wavelength to monitor for your sample. To find the excitation wavelength, set the emission wavelength to zero and scan the excitation wavelengths for activity.

When the excitation monochromator is set to zero order, all wavelengths of light emitted by the xenon lamp are reflected toward the flowcell. When the emission monochromator is set to zero order, you may get a "PMT Overloaded" message, since all wavelengths of light emitted by the fluorescing compound are reflected toward the PMT.

Setting either or both monochromators to zero order may increase sensitivity, but selectivity will decrease and baseline noise can increase significantly.

HINT: You can improve sensitivity by installing a standard 1/2-inch (12.5 mm) diameter optical filter on the PMT, excitation, and/or emission slit assemblies. (One is included in your Accessory Kit.) The filter fits into the ½-inch diameters recesses machined in the slit holders. Contact your Thermo Electron representative for more information on filters.

If you plan to use zero-order settings on your detector's monochromators, try experimenting with various excitation and emission slit-width settings (see page 57) to determine the best conditions for your application.

Other Features

Additional features offered by the FL3000 include the abilities to lock the Status Screen, to short the detector outputs, to place an event mark on the chromatogram, and to send a ready signal to external devices. You can also control the display's contrast and cursor speed, and do a quick shutdown of the detector's lamps and motors.

STATUS LOCK

You can lock the detector's display by using the Status Lock field. This feature lets you prevent accidental changes to a file that is currently being run. With the lock on, only the Status Screen appears. You will not be able to move the cursor below the Status Menu's File Name field. You will still be able to access the Main Menu and the rest of the menu structure using the [MENU] key however.

To access Status Lock:

1. Press [MENU].
2. Select /OPTIONS/.
3. Select /More/.
4. Scroll down in the More Menu to Status Lock. Select **On** or **Off** to turn the lock on or off, respectively.
5. Press [STATUS].

SHORT OUTPUTS

When zeroing a readout device such as an integrator or recorder, it's convenient to be able to short the detector outputs. You can do this by using the detector's Short Outputs feature.

To access Short Outputs:

1. Press [MENU].
2. Select /COMMANDS/. The Commands Menu (Figure 4.22) appears.

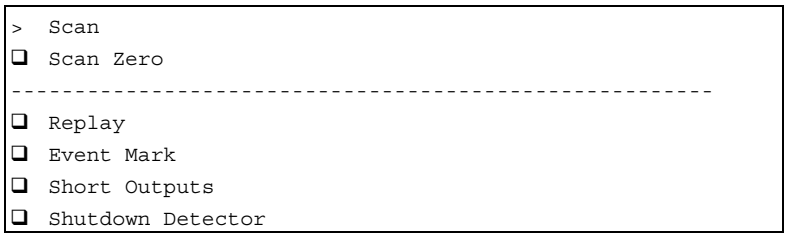


Figure 4.22 The FL3000's Commands Menu

When you select Short Outputs, the detector analog outputs are shorted together (zero volts) and the field changes to "Unshort Outputs." To remove the short and return the outputs to their normal operating state, select Unshort Outputs, and the field changes back to "Short Outputs." (When you leave this screen, the field returns automatically to Short Outputs.)

EVENT MARK

You can place an event mark on your chromatogram to note the occurrence of certain events, such as the turning of a sampling valve. The event mark is a spike (15% of full-scale for one second) on both of the detector's outputs.

To set an Event Mark:

1. Press [MENU].
2. Select /COMMANDS/.
3. Place the cursor on Event Mark (Figure 4.22) and press [ENTER] each time you wish to place an event mark on your chromatogram.

NOTE: You may not want to use event marks if your data will be analyzed by an integrator. Integrators can misinterpret event marks as peaks!

READY OUTPUT

Using the READY(Output) terminal on its back panel, the detector can send a signal to other devices each time it goes to its READY state. This feature is frequently used with autosamplers to signal that the detector is ready for the next injection.

To access the READY Output field:

1. Press [MENU].
2. Select /OPTIONS/.
3. Select /Analog Outputs/.

4. Scroll down to the READY Output field. Select **Active Hi** or **Active Lo** depending on which signal you wish to send.

*HINT: All SpectraSYSTEM instruments are set to receive high signals, so select **Active Hi** if you are hooking up to this type of chromatograph. For any other type of instrument, refer to the appropriate reference manual.*

For details on interfacing your detector with other devices, see page 71.

DISPLAY CONTRAST

You can vary the display's contrast to make it easier to read.

To change the display's contrast, first press [STATUS] to access the Status Screen. Then simultaneously press the [>] key and the [+] key to *increase* the contrast, or the [>] key and the [-] key to *reduce* the contrast.

CURSOR SPEED

The detector lets you control the cursor speed to make it easier to use.

To access Cursor Speed:

1. Press [MENU].
2. Select /OPTIONS/.
3. Select /More/.
4. Scroll down to the Cursor Speed field and select Fast, Medium, or Slow.

SHUTDOWN DETECTOR

This shutdown feature offers a quick shutdown and subsequent startup of the detector's lamp and motors. The electronics stay on to maintain the detector's memory.

To shut down the detector:

1. Press [MENU].
2. Select /COMMANDS/.
3. Scroll down to the Shutdown Detector field (Figure 4.22).
4. Press [ENTER]. The confirmation message shown in Figure 4.23 appears on the display.

```
** Detector Shutdown **
```

Figure 4.23 The Shutdown Confirmation Message

To start the detector up again, press any key on the keypad. The detector will come up under the same conditions present when the Shutdown field was activated.

5

Required Maintenance

Introduction

SpectraSYSTEM detectors are finely-tuned scientific instruments that we at Thermo Electron are proud to stand behind. Even so, routine maintenance is necessary to ensure peak performance, so we can only guarantee our detectors' performance if you follow proper care and maintenance procedures.

This chapter describes the routine maintenance procedures for your detector, including flowcell cleaning, and replacement of the xenon lamp and photomultiplier tube.

Remember that a fluorescence detector measures extremely small amounts of light. Therefore, any contamination on any parts of the optical system may affect system performance. While handling any of the optical components, such as the flowcell or lens assembly, never touch an optical surface because even clean hands have acids and oils that will affect system performance and may permanently damage the surface. Also, never breath on an optical surface. Lastly, never leave an optical surface exposed, such as to the lab environment, where dust or other contaminants may accumulate.

If you have any questions regarding proper maintenance or would like to arrange for a preventive maintenance program, please contact your local Thermo Electron representative.

The Flowcell

This section describes the changing and general cleaning of your detector's flowcell. For other flowcell problems, such as cracks or leaks that occur in locations other than at the inlet/outlet fittings, contact your Thermo Electron representative.

CHANGING THE FLOWCELL

You will need to remove the flowcell whenever you replace a broken cell or clean the cell's interior with nitric acid.

To access the flowcell, remove the front panel of the detector. The flowcell is premounted in a holder assembly that is easily identified by the inlet and outlet lines shown in Figure 5.1. The holder makes the cell easy to install and makes the assembly self-aligning.

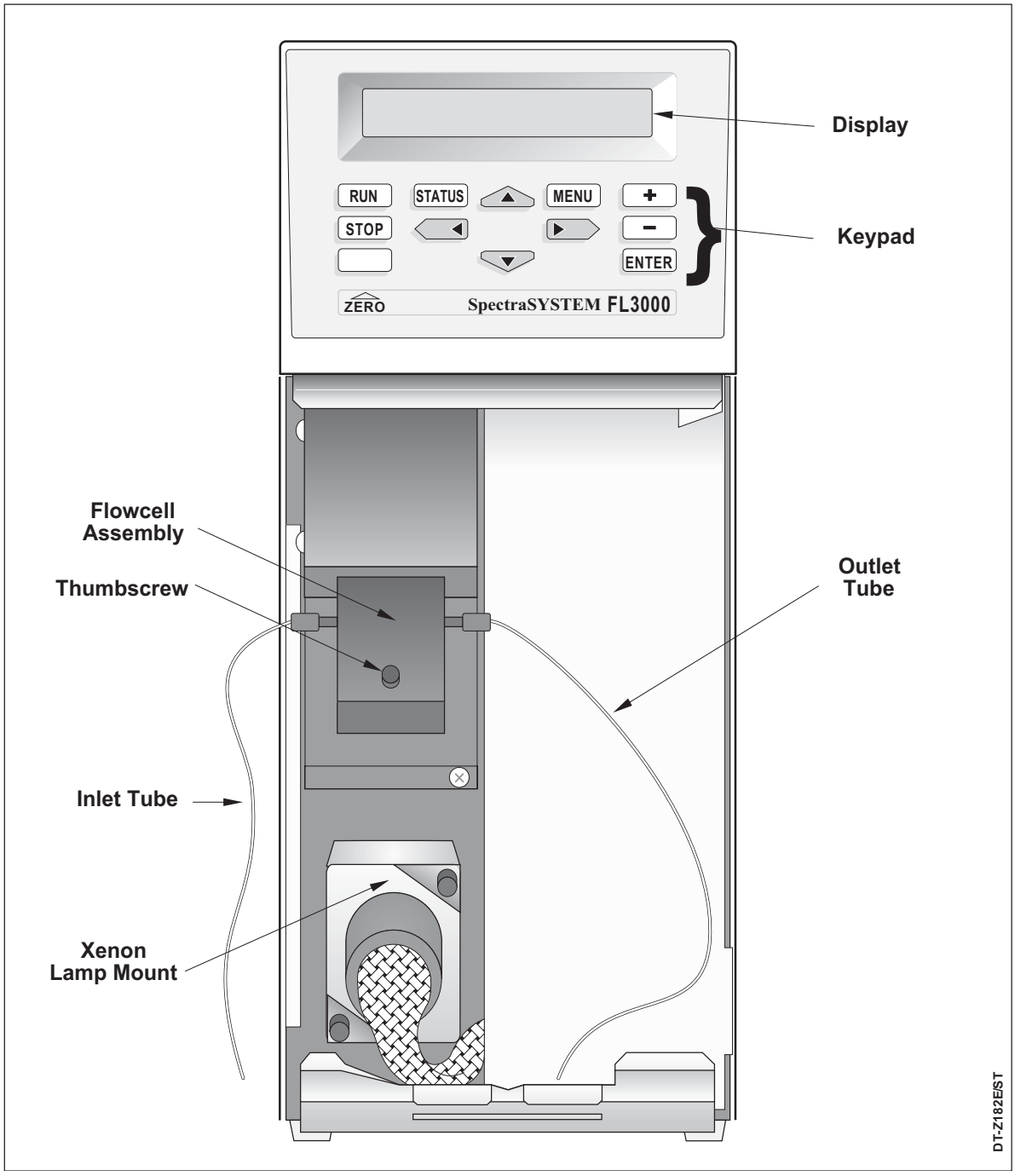


Figure 5.1 Flowcell Assembly and Xenon Lamp Locations

Removing the Flowcell Assembly

Use the following steps to remove the flowcell assembly.

NOTE: Avoid touching the flowcell's quartz tube, the lenses, the lamp's surface, or the photomultiplier tube, all of which are exposed during these procedures. The oils on your skin can be permanently etched into the quartz surface when exposed to UV light. The best policy is never to contaminate an optical surface because removing all of a contaminant may be difficult. If necessary, use an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to clean the contaminated surface, taking care not to drip methanol on the interior surfaces of the detector.

1. Disconnect the power cord from the detector's rear panel and turn the power switch off.
2. Unscrew and remove the small thumbscrew on the face of the flowcell assembly. Gently tilt the flowcell assembly downward and gently pull it away from the detector.
3. Carefully unscrew and remove the inlet and outlet fittings and their associated tubing from the flowcell assembly, taking care not to lose the ferrules.

NOTE: The inlet and outlet tubing may be removed as is convenient before or after flowcell removal.

Replacing the Flowcell Assembly

To replace the flowcell assembly, follow these steps:

1. With the flowcell assembly tilted back slightly (Figure 5.2), guide it so the alignment dowel pins at the base of the flowcell housing engage into the holes in the monochromator. Then tilt the assembly up and in until it is seated securely. Fasten the assembly in place with the small thumbscrew.
2. Replace the inlet and outlet tubing into their fittings, making sure that the tubing is fully inserted.
3. Connect the power cord to the detector panel.

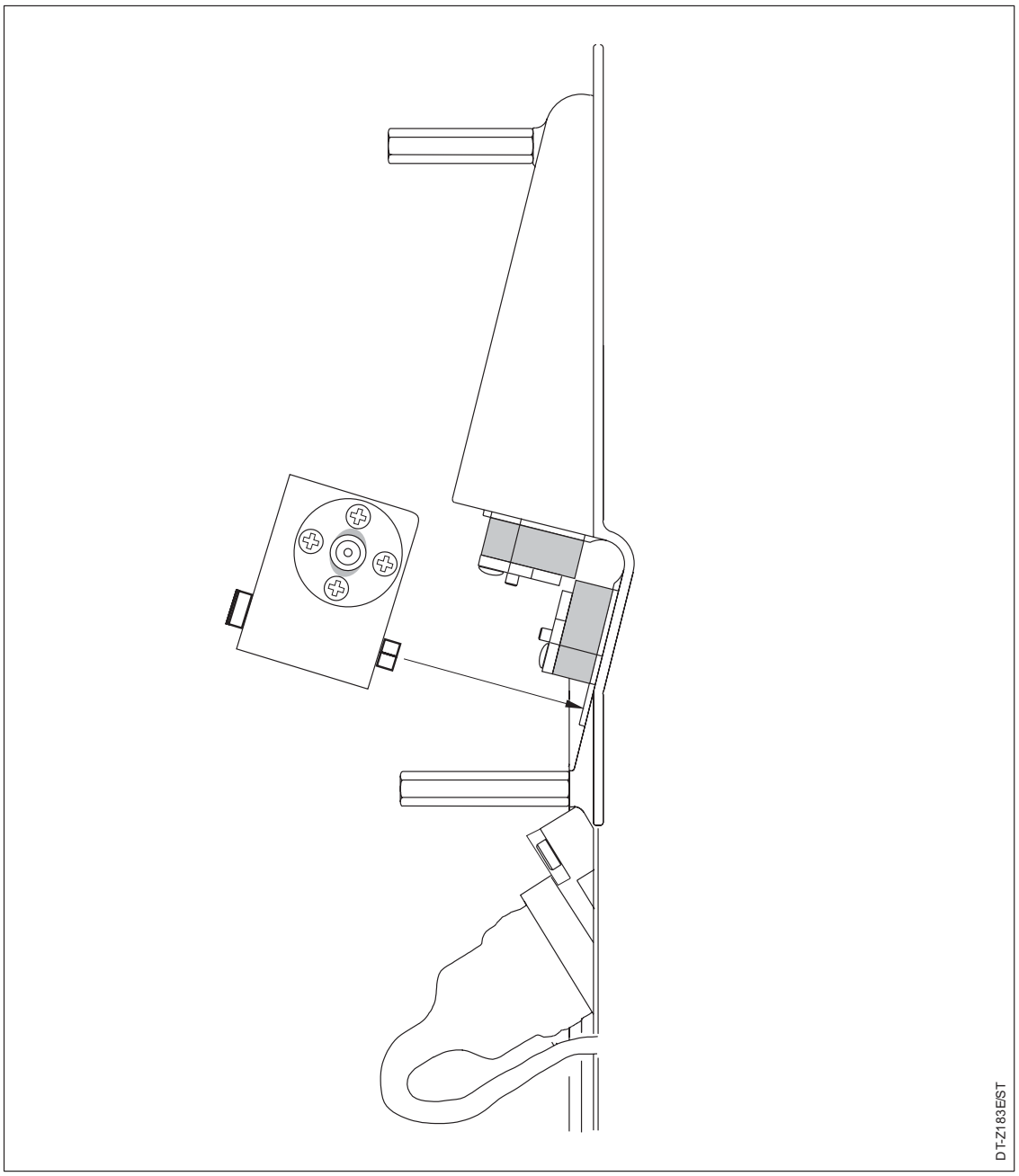


Figure 5.2 Side View of the FL3000 Showing the Location of the Flowcell Assembly and the Rocking Motion Recommended for Flowcell Removal

CLEANING THE FLOWCELL

The exterior and interior surfaces of the flowcell can become contaminated, depending on the quality of your mobile-phase solvents and the cleanliness of your samples. Signs of a contaminated flowcell include increased baseline noise, spikes in the

chromatogram, an erratic or drifting baseline, and increased back-pressure.As a first step toward resolving contamination problems, use the external cleaning procedure described below. If the problem persists, continue with the two internal cleaning procedures that begin below.

NOTE: Do not store or handle optics-cleaning solvents in plastic labware. Fluorescent compounds present in and/or on the plastic can contaminate the solvents, making them worthless for optical cleaning procedures. Store and handle cleaning solvents in glass containers only!

Cleaning the Exterior

Inspect the exterior surface of the quartz tube (Figure 5.3) carefully (with magnification if necessary) for evidence of fingerprints, dust, or other contaminants. If you see any contaminants, without taking the flowcell assembly apart, carefully rinse the outside surfaces of the quartz tube with a clean eye-dropper or Pasteur pipette filled with spectroscopic-grade isopropyl alcohol (IPA).

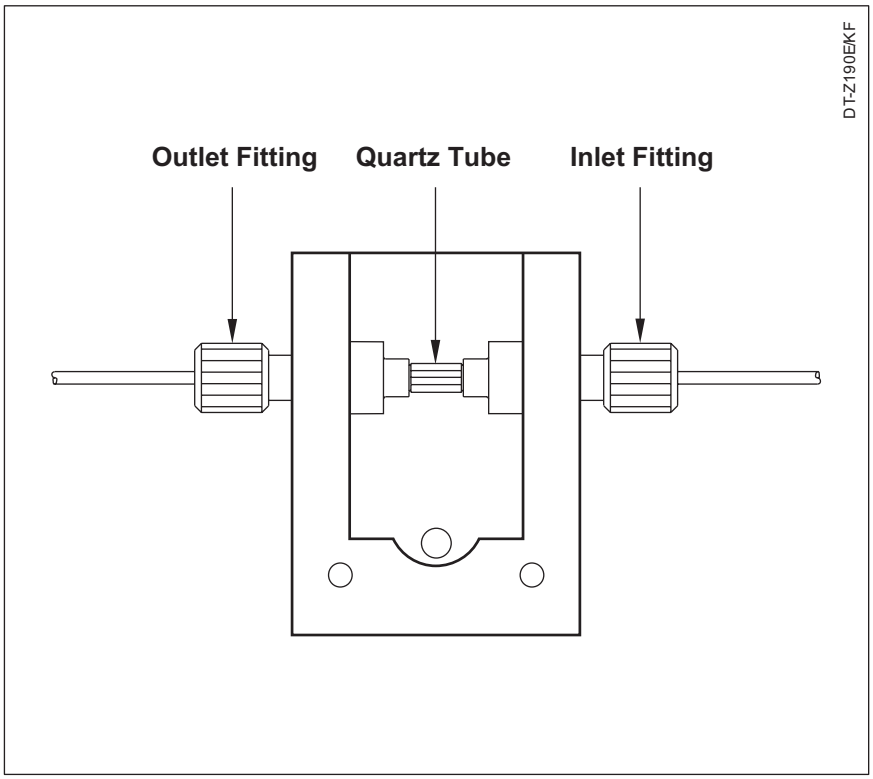


Figure 5.3 The Flowcell Assembly

NOTE: Don't clean the flowcell's quartz tube with methanol-dampened wipes or lens paper. Oils from your skin will be dissolved in the solvent and disbursed over the surfaces you're attempting to clean.

Cleaning the Interior

There are two ways to clean the interior of a flowcell. Try using organic solvents (see below) before resorting to the use of a nitric acid solution (see procedure on page 51).

Cleaning with Organic Solvents

If you suspect that your flowcell needs to be cleaned, start with the following procedure using organic solvents.

1. Make certain that the cleaning solvent(s) you plan to use is/are miscible with the solvent already present in the flowcell and pump. Isopropanol is a good choice for most applications.

HINT: If the last solvent in the pump was an aqueous buffer solution, pump 25 - 40 mL of HPLC-grade water (or equivalent) through the system to remove any salts before flushing with the cleaning solvent(s).

2. Flush the flowcell with 40 - 50 milliliters of solvent (HPLC-grade water, methanol, or isopropanol). You can either *pump* the solvent through the flowcell with the chromatographic pump, or you can *draw* the solvent through the flowcell using a large-volume syringe.

   If you use an LC pump to flush the flowcell, first remove the column from your chromatographic system to avoid column degradation. Replace the column with an appropriate length of tubing, ensuring that all connections are snug and leak-free. If you use a syringe, always draw the solution *through* the flowcell.

CAUTION! Never use a syringe to force solvent through a flowcell. Pressurizing the syringe could cause a leak or rupture that would result in an extremely dangerous, uncontrolled spraying of solvent.

Cleaning with Nitric Acid

Methanol or isopropanol is generally sufficient for cleaning a flowcell. However, if the flowcell is still contaminated after flushing with organic solvents, follow this nitric acid procedure.

CAUTION! Nitric acid is extremely corrosive and can react explosively with alcohols (especially methanol). Be sure to adhere to your company's safety procedures for handling and disposal of corrosive acids. Flush the flowcell with water to remove all traces of alcohol prior to flushing with nitric acid!

1. Remove the flowcell assembly from the detector housing (following the procedure on page 47) before cleaning with a nitric acid solution. This will prevent possible leaks from harming the mechanical or electronic components of the detector.
2. Flush the flowcell with water before proceeding. This step is very important!
3. Prepare a 20% (v/v) solution of nitric acid in HPLC-grade water.
4. Pump the nitric acid solution through the flowcell with the chromatographic pump or draw it through with a large-volume syringe.

   If you use an LC pump, replace your column with tubing and make sure water was the last solvent in the pump and solvent reservoir. If you use a syringe, always draw the solution *through* the flowcell.

CAUTION—Chemical Hazard! Take care when passivating with strong acids. Wear protective eye covering and protective clothing.

CAUTION! Never use a syringe to force nitric acid through a flowcell. Pressurizing the syringe could cause a leak or rupture that would result in an extremely dangerous, uncontrolled spraying of nitric acid.

5. After you have finished the cleaning procedure and before returning to the buffer solution, pump another 25 - 40 mL of water through the flowcell to remove all traces of nitric acid before returning to your chromatographic solvents. Reinstall the flowcell assembly.

NOTE: Flush the pump with water immediately after the nitric acid flush. Leaving nitric acid solution in the pump for prolonged periods can damage pump seals.

If you encounter contamination problems that are not remedied by either cleaning procedure, a flowcell maintenance kit is available. Contact your local Thermo Electron representative and ask for part number 9550-0231.

Changing the Xenon Lamp

The FL3000 has a pulsed xenon flashlamp that requires no warm-up time. This lamp provides exceptional performance and reliability across the entire UV/Visible spectrum. The lamp is aligned and permanently seated inside a slotted mount for easy installation and alignment.

As lamps age, there is a reduction in light output, resulting in decreased peak height. If you notice a reduction in peak height, to where sensitivity is no longer acceptable, you may need to change the lamp.

Removing the Lamp Assembly

To remove the lamp assembly from the detector:

1. Turn off the power switch and unplug the power cord from the detector's back panel.
2. Remove the detector's front panel. The lamp is located on the left side, at the bottom (Figure 5.1).
3. Loosen the two mounting screws that secure the lamp assembly and socket (Figure 5.4).

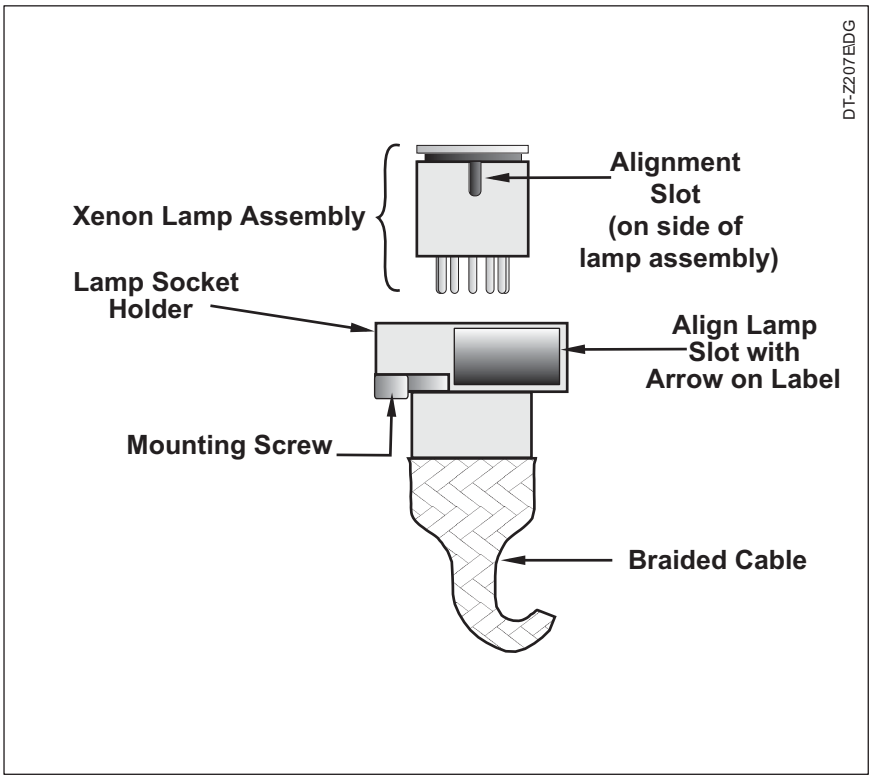


Figure 5.4 Side View of Xenon Lamp Assembly and Socket

4. Pull the socket (with the lamp assembly still attached) straight out of the detector.
5. Grasp the lamp assembly in one hand, and the braided cable (that attaches the socket to the detector) in the other. Gently pull the lamp assembly out of the socket.

Replacing the Lamp Assembly

Before replacing the lamp assembly, note that the pins on the base of the lamp are arranged in a C-shaped pattern that exactly match the holes in the lamp socket.

NOTE: The detector must be recalibrated any time the lamp is replaced. A recalibration procedure is provided on page 61.

1. Orient the pins and socket holes. Slide the pins into the socket holes until the lamp assembly is fully seated in its socket.

NOTE: The face of the lamp must be clean of all fingerprints and debris at the end of the installation procedure. Don't clean the optical surfaces with methanol-dampened wipes or lens paper. Oils from your skin will be dissolved in the solvent and disbursed over the surfaces you're attempting to clean. If necessary, use an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to flush fingerprints or debris from the newly mounted lamp.

2. Grasp the socket (with the lamp assembly seated firmly inside it) and insert it in the lamp recess. Make sure that the keyed slot in the lamp assembly aligns with the silver guide pin in the lamp recess.

HINT: Position the lamp assembly such that the slot in its side is towards the right-hand side of the detector as you're facing it. If the slot is perpendicular to the flat, right-hand side of the socket, it will slide easily into place in the lamp recess. To install easily, rotate the lamp in the socket so that the slot is aligned with the arrow on the lamp socket assembly label (Figure 5.4).

3. Align the mounting screws on the socket with the screw holes on the detector, and tighten the thumbscrews. (The part of the assembly that contains the mounting screws can be moved independently of the socket to make it easier to align the thumbscrews with the screw holes.)
4. Replace the external cover, tighten the mounting screws, and reconnect the power cord.

Tracking Lamp Life

The Lamp Count display found in the Tests Menu (see Appendix C, page 89) can be used to track the age of the xenon lamp. You should set the lamp count display value to zero when a new lamp is installed.

Changing the PMT

The photomultiplier tube (PMT) is a device that generates a current whenever photons of light strike it. The digital electronics of the FL3000 integrate and process this current to produce a voltage signal that drives your recorder/integrator.

If you still have baseline noise problems after cleaning the flowcell and replacing the lamp, you may need to change the PMT. However, bear in mind that under normal operating conditions the PMT is designed to perform to specifications for several years. If the instrument is less than three years old, it's more likely that the tube needs cleaning than replacement.

NOTE: Never touch the PMT's surface with your fingers. Contamination of the PMT's quartz bulb can cause increased baseline noise and diminished sensitivity. Use a lint-free tissue or clean cotton gloves to protect the PMT from contact with your skin during these procedures.

NOTE: A PMT is sensitized by exposure to room light or other bright light sources. When exposed to such light and installed in a detector, the PMT needs at least 30 minutes operating time to reach its optimum performance level. To minimize the effects of this sensitization, never leave the PMT exposed to room or other light. Keep the PMT stored in its box in the dark as much as possible.

Removing the PMT

The PMT is accessed from the left side of the detector. To remove it, use the following steps:

1. Turn off the power switch and disconnect the power cord from the detector's real panel.
2. Loosen the screw that holds the rectangular panel located on the detector's left side. Remove the panel.
3. Locate the 1/8-inch, Allen-head screw that secures the PMT slit-wheel holder in position. Remove the Allen-head screw and then pull the holder straight out toward you to expose the PMT.
4. Put on a pair of vinyl laboratory gloves to provide grip and to prevent your fingerprints from getting on the PMT in Step 5.
5. Grasp the PMT and gently pull it straight out from its socket with minimum force.

If the instrument is relatively new, you should first clean the PMT using an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to rinse the PMT's outer surfaces. Reinsert the PMT following the next procedure, and run an analysis. If problems still exist, install a new PMT.

NOTE: Don't clean the flowcell's optical surfaces with methanol-dampened wipes or lens paper. Oils from your skin will be dissolved in the solvent and redisbursed over the surfaces you're attempting to clean.

Replacing the PMT

To replace the PMT:

NOTE: The detector must be recalibrated any time the PMT is replaced. A recalibration procedure is provided beginning on page 61.

1. Grasp the glass portion of the PMT and insert the PMT fully into the socket.

   HINT: Note the guide-pins on the PMT before you install it. They make the PMT much easier to align in the socket.

2. Replace the slit-wheel holder and secure it in place with the 1/8-inch Allen-head screw.
3. Reconnect the power cord.

Changing Slit-wheels

The FL3000's signal-to-noise ratio is directly proportional to the square root of the available emitted light. Thus, if the emission light coming from the monochromators increases by a factor of four, the signal-to-noise ratio, and therefore the sensitivity, of the detector increases by a factor of two.

In some cases you can improve the detector's sensitivity by increasing the monochromators' bandwidths, thus allowing more light to pass through to the PMT.

Bandwidth is controlled by three slit-wheels: the excitation, emission, and PMT slit-wheels. Each slit-wheel has three manually-adjustable slit settings: 8, 20, and 30 nm. Your detector is shipped with each slit set to 20 nm. The excitation and emission slit-wheels are accessible when the flowcell is removed. The PMT slit-wheel is accessed from the left side of detector.

NOTE: Certain parts of the excitation and emission slit-wheel assemblies look almost identical, but are functionally different. These parts are not interchangeable; switching them will decrease detector performance. Specifically, the excitation and emission lens assemblies and excitation and emission filter holders are different. To differentiate between the excitation and emission parts, the emission parts have a notch. (Figure 5.5 shows how to remove the excitation and emission slit-and-lens assemblies; Figure 5.6 shows the emission assembly and Figure 5.7 shows the excitation assembly).

To change the emission monochromator's bandwidth, set both the emission slit and the PMT slit to the same setting. To set the

excitation monochromator's bandwidth, you need only change the excitation slit.

HINT: The FL3000 is designed to provide optimized performance for most applications when all three slit-wheels are set to 20 nm. Larger slit-widths increase sensitivity, but also decrease specificity, and you may start to see interfering compounds. Conversely, smaller slit sizes increase specificity but decrease sensitivity.

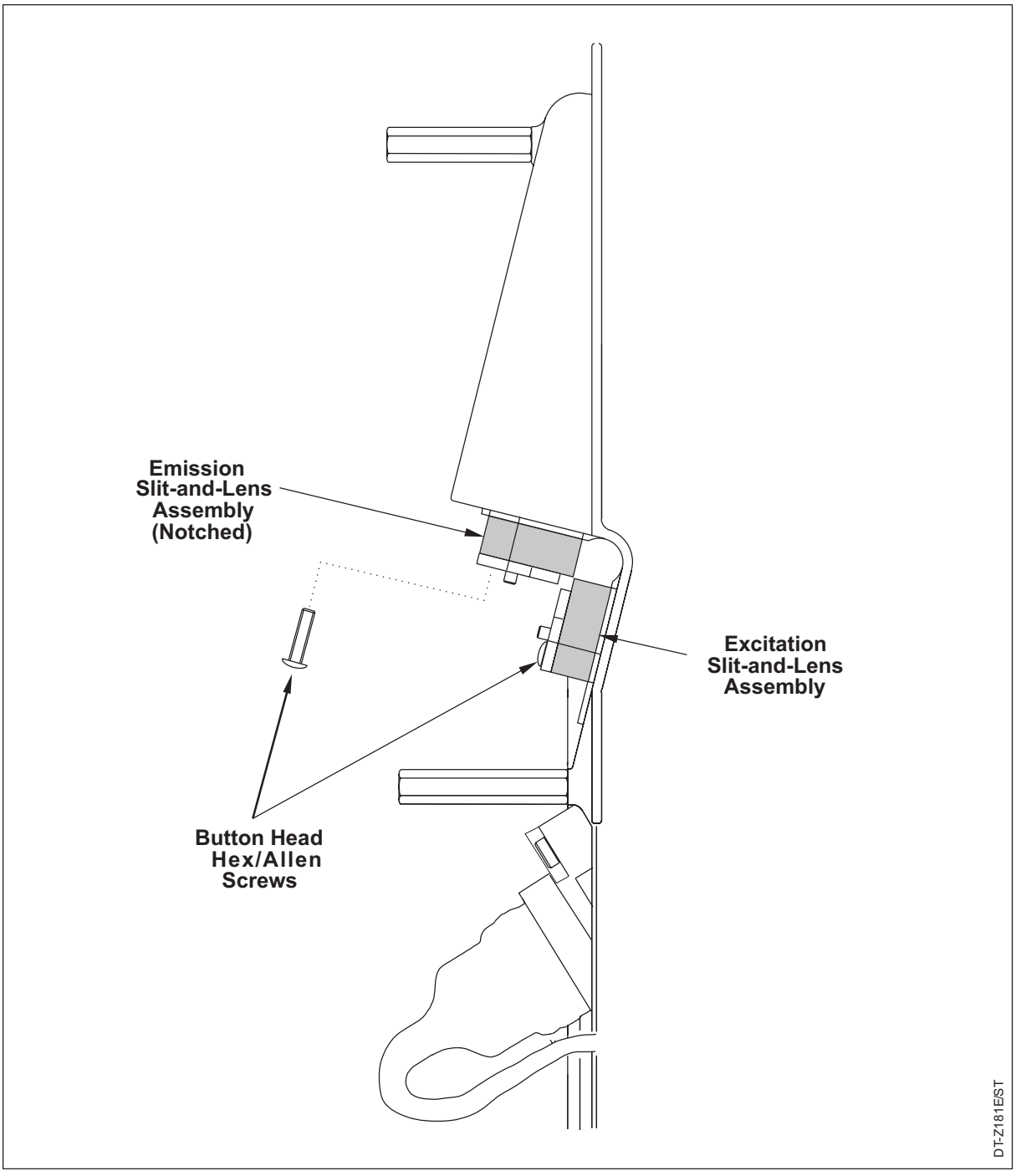


Figure 5.5 Removing the Excitation and Emission Slit-and-Lens Assemblies

Changing the Excitation and Emission Slit-Widths

Use the following steps to change either the excitation or emission slit-widths.

1. Disconnect the power cord from the detector's rear panel and turn the power switch off.
2. Unscrew and remove the small thumbscrew on the face of the flowcell assembly and pull the entire assembly away from the detector.
3. Carefully unscrew and remove the inlet and outlet fittings and their associated tubing from the flowcell assembly. Take care not to lose the ferrules.

NOTE: Never touch either lens surface with your fingers. Contamination of the lenses can cause increased baseline noise and diminished sensitivity. If necessary, use an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to rinse the lenses, taking care not to drip methanol on the detector's interior surfaces.

4. You can now see the assemblies that hold the slits and lenses. Remove the screw (Figure 5.5) that secures the slit-and-lens assembly to the monochromator.
5. Remove the lens-holder from the slit-wheel holder.
6. Lift the slit-wheel clear of the two guide pins.
7. Rotate the wheel (Figure 5.6 and Figure 5.7) until the desired slit is directly over the opening into the monochromator.
8. Replace the slit-wheel and lens-holder on the slit-wheel holder, and tighten the Allen-head screw.
9. Replace the flowcell following the procedure on page 47.

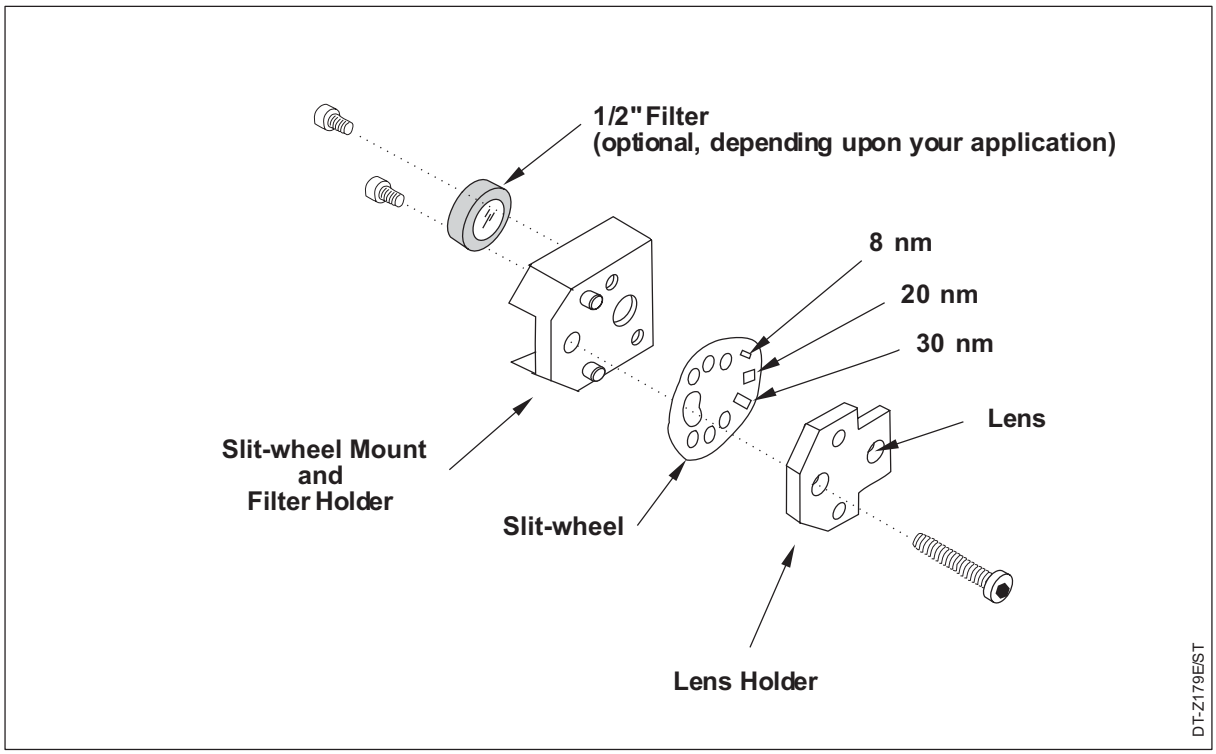


Figure 5.6 An exploded view of the emission slit-wheel assembly

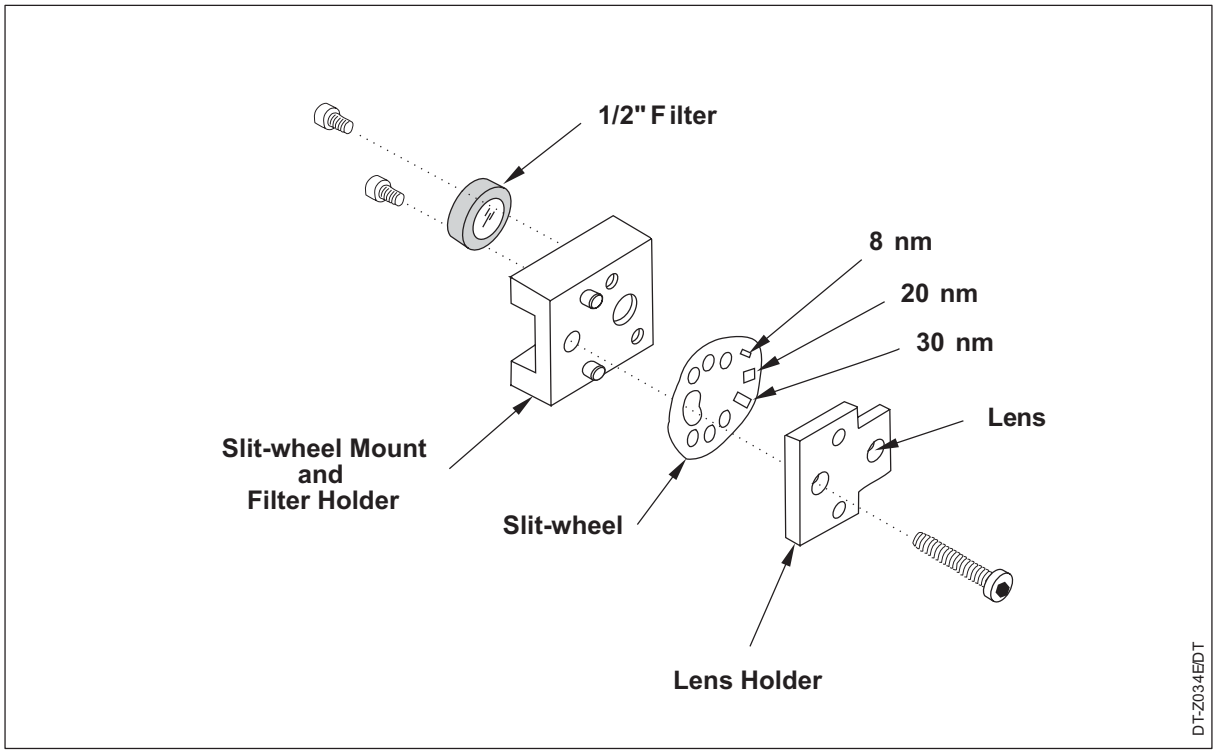


Figure 5.7 An exploded view of the excitation slit-wheel assembly

Changing the PMT Slit-width

Use the following procedure to change the PMT slit-width:

1. Turn off the power switch and disconnect the power cord from the detector's real panel.
2. Loosen the screw that holds the rectangular panel located on the detector's left side. Remove the panel.
3. Locate and remove the Allen-head screw that secures the PMT slit-wheel holder in position. Pull the holder straight out toward you to expose the PMT.
4. Next, remove the Allen-head screw that holds the slit-wheel to the holder and lift the slit-wheel from the two guide pins (Figure 5.8).

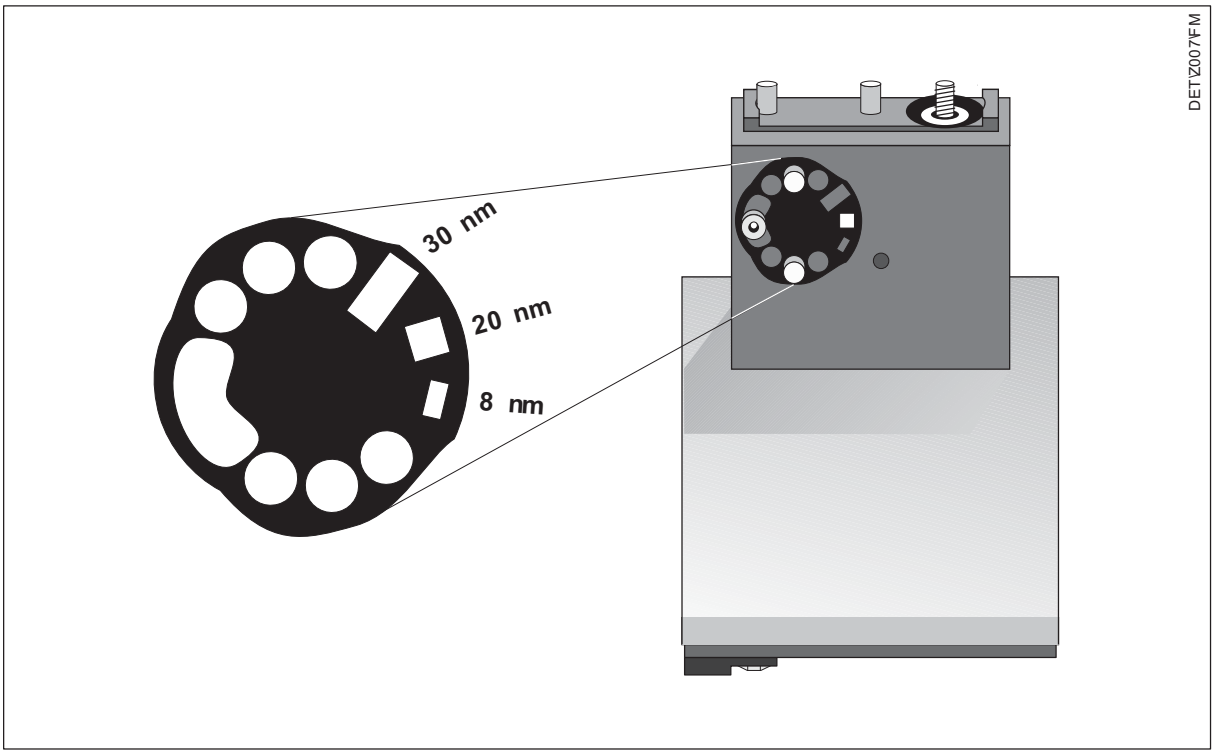


Figure 5.8 The PMT Slit-wheel and Slit-wheel Holder

5. Rotate the wheel until the desired slit setting is directly over the center opening on the slit-wheel holder.
6. Replace the slit-wheel and the Allen-head screw.
7. Replace the slit-wheel holder and secure it in place with the Allen-head screw.
8. Reattach the rectangular panel and secure it in place.
9. Reconnect the power cord.

Recalibrating the Detector

You should recalibrate the detector each time you replace the lamp and/or PMT. This process normalizes the difference in light output or PMT response that is certain to exist between the old component and its replacement. Although the procedure that follows uses anthracene, you can calibrate your detector with the standard of your choice.

NOTE: Recalibration also may be required if a user has inadvertently activated the fluorescence-response function (described on page 91).

NOTE: If you don't perform a calibration following a lamp or PMT change, the results of subsequent analyses—while comparable to each other—cannot be compared to data that you generated prior to the lamp/PMT replacement.

The standard anthracene calibration procedure requires the following equipment, tools, and materials:

- Precision micro-balance (accurate to ± 0.1 mg)
- LC gradient pump
- 1/8-inch Allen wrench
- HPLC-grade methanol and water
- Reagent-grade anthracene

Use the following procedures to prepare and use a standard anthracene solution to recalibrate the detector.

Preparing a Standard Anthracene Solution

This calibration procedure requires a 2.0 mg/L solution of anthracene in methanol. The solution should be created using scrupulously clean, particle-free laboratory glassware. Use these steps to prepare the standard anthracene solution:

1. Weigh out 0.002 gm of reagent-grade anthracene and transfer it quantitatively to a clean, one-liter, volumetric flask.
2. Fill the volumetric flask to the mark with HPLC-grade methanol.

NOTE: You must use methanol. If you use a solvent other than methanol, the emission and excitation maxima will shift.

3. Add a clean magnetic stir bar and place the flask on a stir plate. Allow the solution to mix at a moderate speed for 1 hour.
4. Pipette 1.0 mL of the resulting anthracene solution into a second, clean, one-liter, volumetric flask and dilute to the mark with HPLC-grade methanol to produce the working solution.
5. Transfer the solution just created to a clean, one-liter, screw-top bottle.

HINT: The anthracene/methanol calibration solution has a shelf-life of approximately one week under normal laboratory conditions. To extend the shelf-life, store the solution in the dark and refrigerate it.

Performing the Calibration

Once you've prepared the anthracene solution, use it and the following procedure to perform the calibration:

NOTE: If using a different calibration standard, make the appropriate changes to the items that are marked with asterisks(*) in the following procedure. Since these changes of solvent, standard, and wavelength settings are specific to your calibration standard, we can't document them here.

1. Fill a clean, one-liter, screw-top bottle with HPLC-grade methanol*. Place that bottle and the one containing the standard anthracene* solution near the gradient pump's inlet tubing.
2. Ensure that all three sets of optical slits (excitation, emission, and PMT) are set for 20 nm. Instructions for setting the slit-widths are provided beginning on page 58.
3. Verify that the excitation and emission wavelengths are set for 250nm* and 400nm,* respectively.
4. Place a flow restrictor on the outlet side of the detector to ensure adequate back-pressure.
5. Flush the detector's flowcell by running several milliliters of HPLC-grade water or methanol through it (your choice of solvent should be the same as the last fluid that was run through the flowcell). If your initial flushing solvent was water (due to analyzing salt samples), finish the flushing process by running several milliliters of HPLC-grade methanol* through the flowcell. Once flushing is completed, press [ZERO] and then end the run.
6. Begin a run using the standard solution. While the calibration standard is flowing, press [MENU] to select the Main Menu.
7. Press [>] until the cursor is in the /Test/ field, then press [ENTER]. Press [∨] to move the cursor to the /Fluorescence Response/ field, then press [ENTER].
8. Observe your display. It should look like Figure 5.9.

```
Fluor Response                          Active/Inactive
FU:   00.00                              Factor: 00.00
```

Figure 5.9 Fluorescence-response Display

9. Press [>] to move the blinking cursor to the /Active/Inactive/ field, then press [+] until the word "Active" appears on the display. Next, press [∨] to move the cursor to the /Factor/ field. You can press [<] or [>] to select the digit(s) you want to change and then press [+]/[-] to change the value of a selected digit. As you change the digit(s) you'll see the FU reading changing in real-time response to your modifications of the value displayed in the /Factor/ field. When the desired FU reading appears, press [ENTER] and go on to Step 10.

NOTE: Although the fluorescence-response factor set in Step 9 is stored in non-volatile RAM (NOVRAM) and is always available at power up, you must select "Active" on the Fluorescence Response screen for the factor to take effect.

10. Following completion of Step 9, dispose of all waste solvent in accordance with local regulations.

HINT: It's good practice to maintain a record of the response factor in the back of your reference manual.

A

Installation and Specifications

Introduction

This appendix covers the initial installation of your fluorescence detector, including hookup to other chromatographic instrumentation. As you go through unpacking and installation, you may want to use the Start-up Checklist located at the beginning of this manual. The checklist is an abbreviated version of this appendix and is supplied as a quick reference of how to conduct a successful installation. After installation, verify that the detector is working properly by running a standard FL test mix. (Refer to the Spare Parts List at the front of this manual.)

This chapter also includes a list of your detector's specifications.

Installation

UNPACKING

Carefully remove the detector from the shipping container and inspect both the detector and packing for any signs of damage. If you find any damage, immediately contact the shipping company.

The shipping container should contain the detector, an accessory kit, any options you ordered for your detector, and this manual. The accessory kit should contain the following items:

- 8-pin connector (p/n 2110-0354)
- 12-pin connector (p/n 2110-0353)
- 3-foot, 4-conductor cable (p/n A4095-010)
- Analog cable (p/n 6040-0103)
- External run cable (p/n 6040-0102)
- Finger-tight nut (2 each, p/n 3256-0024)
- Finger-tight ferrule (2 each, p/n 3256-0025)
- Union (p/n 3256-0026)
- Low-pressure nut and ferrule set (3256-0022)
- Teflon tubing (p/n 3256-0023)
- Cap screws (2 each, p/n 9550-0203)
- 340 nm emission filter (p/n 3750-0075)
- 5/64" Allen wrench (p/n 5401-0040)
- 7/64" Allen wrench (p/n 2556-0107)

Carefully check to make sure you received all the items listed on the packing list. If any items are missing, contact your Thermo Electron representative immediately.

You will need the following tools for installation:

- a narrow-tip screwdriver (2 mm wide)
- a #2 Phillips screwdriver

Place the detector on the benchtop as close as possible to the chromatographic column outlet, thus minimizing the length of tubing necessary for connection to the flowcell inlet. Allow at least five inches (13 cm) of clear space between the detector's rear panel and any wall or obstruction. This provides both access to the rear-panel connectors and a free flow of cooling air.

POWER CHECKOUT

The detector is shipped with the voltage and fuses preset for your location. Verify the setting by looking through the cut-out window on the voltage selector cover (Figure A.1). (The cover is located at the bottom left of the detector's rear panel but, if your instrument is new, it may be hidden behind a precautionary sticker.) If the voltage setting satisfies your local site requirements, skip to "Fuses" on page 66. If not, proceed to the next section, "Voltage Selection."

NOTE: Do not plug in the instrument without first verifying that the voltage is properly set for your location. And never *run the detector at more than 8% below the nominal line voltage!*

Voltage Selection

If the preset voltage does *not* satisfy your local site requirements, select the correct voltage by following these steps:

1. Insert a small flat-blade screwdriver into the slot at the top of the voltage selector cover (Figure A.1).

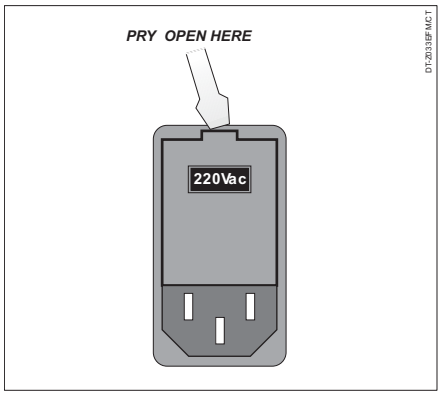


Figure A.1 Opening the Voltage Selector Cover (note the current voltage through the cut-out window)

2. Gently pry open the cover. Once unlatched, the cover will swing downward to reveal the voltage selector barrel and the fuses.

3. Remove the voltage selector barrel from the detector. The selector resembles a drum imprinted with four settings: 100, 120, 220, and 240 V (Figure A.2).
4. Rotate the barrel such that the desired voltage setting will be visible through the cut-out in the cover when it is replaced.
5. Replace the barrel in the detector. Before closing the cover, check the fuses according to the procedure below.

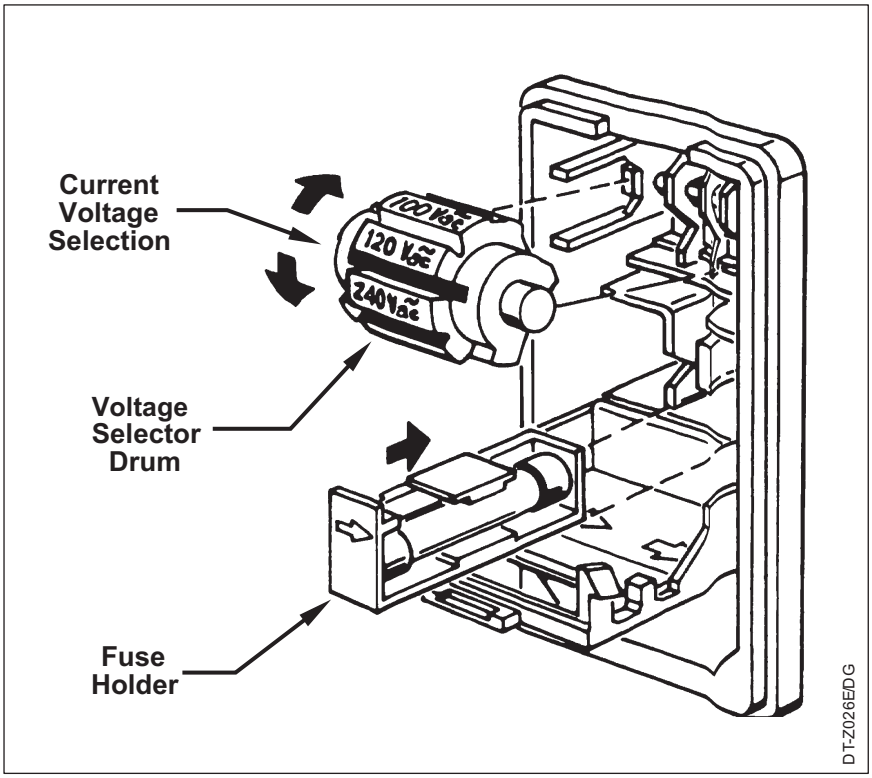


Figure A.2 Voltage Selector Barrel and Fuse Holders

Fuses

To verify that your detector is fitted with the correct fuses, follow these steps. (If you haven't already done so, first open the voltage selector cover according to step 1 in the "Voltage Selection" procedure listed above.)

1. Pull each fuse holder straight towards you. The fuse holders are the black squares with arrows located directly beneath the voltage selector (Figure A.2).
2. Remove each fuse from its holder. Check the fuse's amperage, voltage, and type according to the following description. You should have either:
    - two T2A/250V fuses, for 100 - 120 VAC operation, or
    - two T1A/250V fuses, for 220 - 240 VAC operation

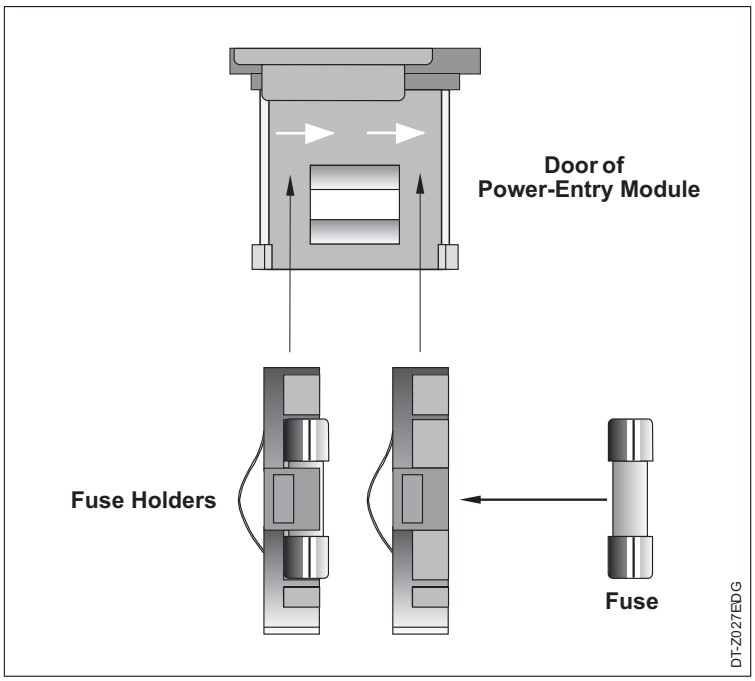


Figure A.3 Fuses

3. Assuming that you have the proper fuses on hand, reinsert the fuses and fuse holders, making sure that the arrows on the holders are oriented in the same direction as the arrow inside the cover panel (Figure A.3).
4. Close the cover panel by swinging it upward and pressing it in until it snaps closed. The correct voltage should appear in the cut-out panel.

NOTE: To avoid damaging the instrument, verify that the new voltage setting (displayed in the cut-out window) is correct before you turn it on!

Power Cord

Attach the power cord at the lower left of the detector's rear panel.

REAR PANEL CONNECTIONS

Locate the two in-line connectors (8-pin and 12-pin) in your accessory kit and insert them in the appropriate sockets on the detector's rear panel (Figure A.4). Note that the connectors are both labeled and keyed to the sockets, making it impossible to insert them incorrectly.

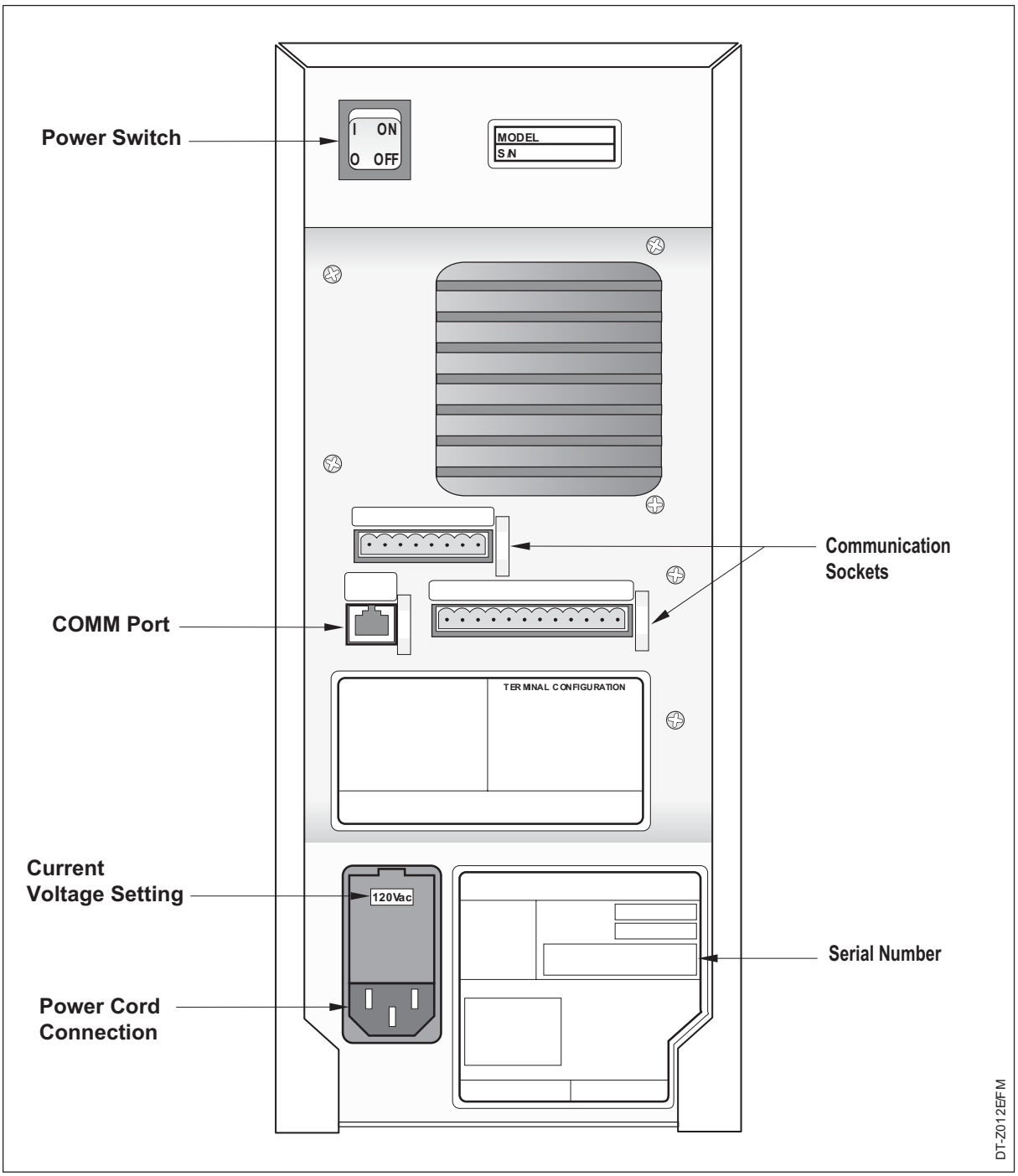


Figure A.4 The Rear Panel of the FL3000

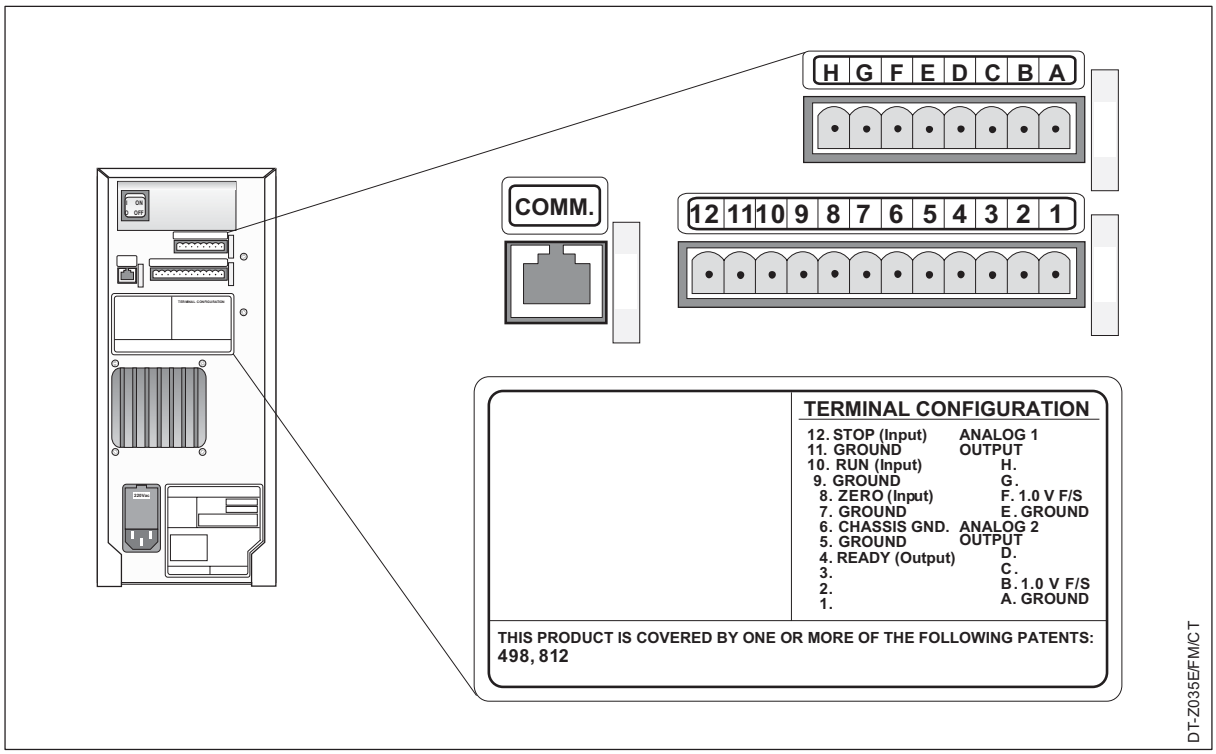


Figure A.5 Analog Output and Remote Communications Connectors

The upper connector (Figure A.5) holds the detector's two analog outputs (Output 1 and Output 2, labeled as terminals H through A). The lower connector allows the detector to communicate with other devices in your liquid chromatographic system (terminals 12 through 1). There is also a communications port, labeled COMM.

Use the cables supplied with your detector to complete the connections described in this section. For each connection, loosen the small setscrew located next to the appropriate terminal, insert the cable's bare wire, and hold it in place while you tighten the screw.

Analog Output Connections

The terminals on the FL3000's analog output connector are labeled ANALOG 1 Output and ANALOG 2 Output (Figure A.5). Each output has four terminals, labeled H through E for Output 1, and D through A for Output 2. These terminals correspond to:

- 0.01 V F/S, full-scale (H, D)
- 0.10 V F/S, full-scale (G, C)
- 1.0 V F/S, full-scale (F, B)
- Ground (E, A)

NOTE: Analog outputs are driven to twice their range. In other words, their maximum output is twice the selected range. To avoid clipping the voltage, be sure to connect integrators and data systems to the 1.0 V terminal and to use caution when connecting recorders to the 0.01 or 0.10 V terminal.

Connecting to an Integrator or Workstation

Connect your integrator/workstation to the 1.0 V F/S (F or B) and corresponding GROUND (E or A) terminals.connecting to analog outputs;

NOTE: The 0.01 and 0.10 V F/S terminals are provided for recorders and special applications. We recommend that you use only the 1.0 V F/S terminal for an integrator or workstation.

Connecting to a Recorder

Connect the positive input from your recorder to the full-scale voltage (0.01, 0.10, or 1.0 V) appropriate for your recorder. Connect the recorder's floating ground input to the corresponding GROUND terminal.

NOTE: Do not connect the detector's GROUND to any earth ground on your recorder. This would lead to an increased noise level and a subsequent decrease in sensitivity.

Remote Communications Connections

The FL3000 can accept inputs from, as well as send inputs to, remote devices through the remote communications connector (see terminals 1-12 in Figure A.5). For example, if your chromatographic system has programmable timed events (contact closures or TTL), you can use one to automatically zero the detector signal during a run.

The terminals available on the FL3000's remote communications connector are labeled STOP (Input), RUN (Input), ZERO (Input), and READY (Output), each with an associated ground terminal. The remote input terminals are labeled 12 through 1.

STOP(Input)

You can use a timed event from your chromatographic system to take the detector out of run by connecting the system's event to the detector's STOP(Input) and GROUND terminals (12 and 11).

RUN(Input)

You can use the remote-start event on your injector or autosampler to automatically put the detector into run whenever an injection occurs by connecting the event to the detector's RUN(Input) and GROUND terminals (10 and 9).

ZERO(Input)

You can zero the detector signal automatically by connecting a timed event on your chromatograph to the detector's ZERO(Input) and GROUND terminals (8 and 7).

READY(Output)

The detector is capable of driving one TTL load each time it goes to its READY state through the READY(Output) terminal. This ability to signal other instruments is particularly useful with autosamplers, where the detector can signal that it is ready for the next injection in an automated series of runs. To hook up the READY(Output) terminal, connect the input from the other instrument to the detector's READY(Output) and GROUND terminals (4 and 5). For information on accessing this feature through the detector's keypad, see page 43.

FLOWCELL CONNECTIONS

Use the following steps to connect the detector's flowcell to the rest of your LC system:

1. Remove the front panel of the detector.
2. Using the finger-tight nut and ferrule sets included with the installation kit, connect the column outlet directly to the detector's fluid inlet on the left side of the flowcell (Figure A.6). If additional tubing is required to reach the inlet, use a zero dead-volume union.
3. Connect the detector's fluid outlet to the supplied low-pressure union and waste tubing.

HINT: If you have several detectors hooked up in series, place the FL3000 last to avoid back-pressure problems that could damage the flowcell.

4. Replace the front panel of the detector, making sure that the tubing passes through the slots without being pinched.

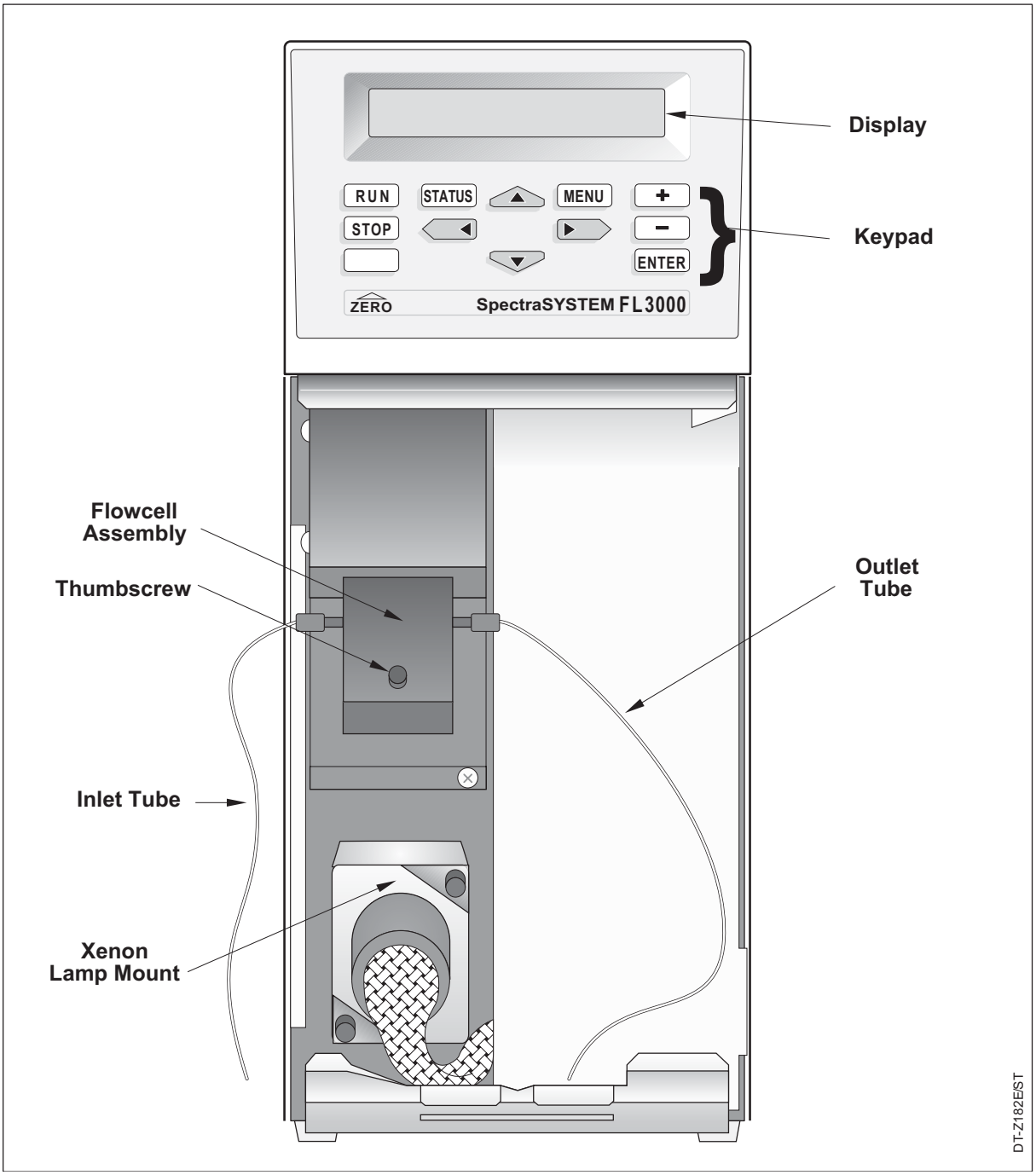


Figure A.6 The FL3000 with the front cover Removed

Specifications

Detector:	Photomultiplier tube, 200 - 650 nm
Wavelength Range:	200 - 650 nm excitation; 200 - 650 nm emission; 200 - 800 nm emission with optional extended range (red-sensitive) PMT
Wavelength Accuracy:	± 2 nm @ 248 EX/398 EM
Wavelength Precision:	< 0.5 nm
Spectral Bandwidth:	8, 20, or 30 nm, selectable
Sensitivity:	S/N >1500 for 2µg/L anthracene in MeOH 248 nm EX/398 nm EM
Lamp:	Pulsed xenon lamp
Lamp Frequency:	Selectable 20 or 100 Hz
Flowcell:	High-purity quartz, Teflon®, Kalrez®, and PEEK 8 microliter illuminated volume, maximum pressure 200 psi (14 bar)
Range Selections:	500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, 0.01 FUFS
Analog Outputs:	
Outputs 1 and 2:	Range-selectable over entire fluorescence range
Communications:	
Remote Inputs:	Run, Stop, and Zero
Remote Outputs:	Ready
Display:	2 x 24 character, high-contrast LCD
Dimensions:	14.5” (37.2 cm) x 6” (13.3 cm) x 18.5” (47 cm) (H x W x D)
Weight:	24 lb. (11 kg)
Power Requirements:	100/120/220/240 VAC; 50/60 Hz; 0.9/0.8/0.4/0.4A
Environmental	10-40°C 5-95%RH noncondensing

B

Menu Reference

Introduction

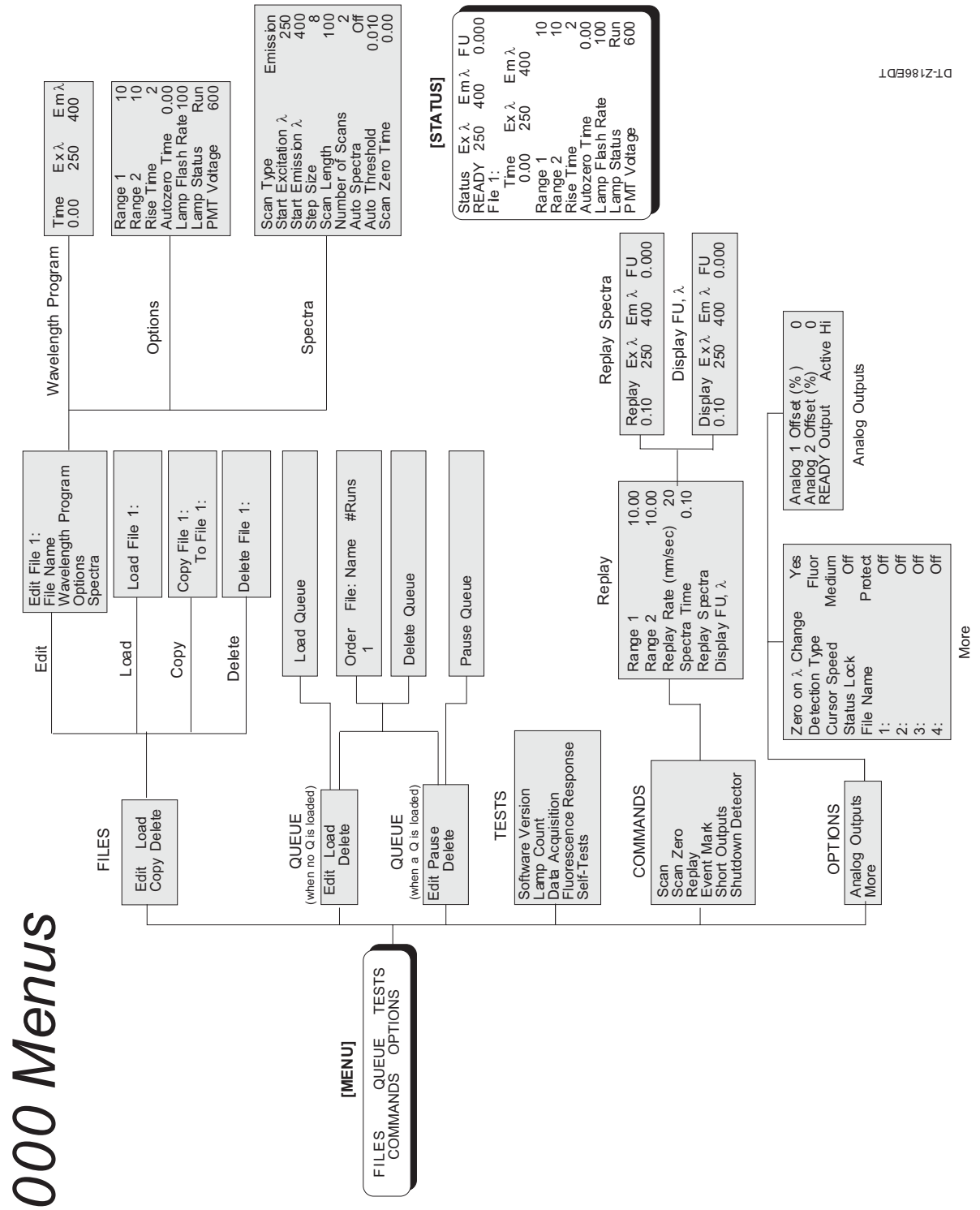
This appendix provides you with a Menu Tree and an alphabetical description of all the instrument's display fields. Fortunately, it is not necessary to read this appendix in order to learn how to use your detector. It is included in the manual simply as a quick reference and aid to using your instrument.

The Menu Tree is a representation of the detector's overall menu structure. It shows the location and interrelation of all the instrument's menus. This is a good reference to keep on hand while you work through the operating instructions in Chapters 3 and 4. It will also help if you become "lost" while moving through the detector's menus. A separate, removable copy can be found in the pocket at the front of this manual.

The Menu Reference is an alphabetical listing of each menu field and command. Included in each listing is the field's definition and, where appropriate, all allowable and default values for the field.

Menu Tree

The Menu Tree on page 74 is useful for learning your way around the detector. You may wish to keep it handy while you learn where each display is located in the overall menu structure.


FL3000 Menus
[MENU]
FILES QUEUE TESTS
COMMANDS OPTIONS
FILES
Edit Load
Copy Delete
Edit
Load
Copy
Delete
Edit File 1:
File Name
Wavelength Program
Options
Spectra
Load File 1:
Copy File 1:
To File 1:
Delete File 1:
Wavelength Program
Time Ex λ Em λ
0.00 250 400
Options
Range 1 10
Range 2 10
Rise Time 2
Autozero Time 0.00
Lamp Flash Rate 100
Lamp Status Run
PMT Voltage 600
Spectra
Scan Type Emission
Start Excitation λ 250
Start Emission λ 400
Step Size 8
Scan Length 100
Number of Scans 2
Auto Spectra Off
Auto Threshold 0.010
Scan Zero Time 0.00
[STATUS]
Status Ex λ Em λ FU
READY 250 400 0.000
File 1:
Time Ex λ Em λ
0.00 250 400
Range 1 10
Range 2 10
Rise Time 2
Autozero Time 0.00
Lamp Flash Rate 100
Lamp Status Run
PMT Voltage 600
QUEUE
(when no Q is loaded)
Edit Load
Delete
Load Queue
Order File: Name #Runs
1
Delete Queue
QUEUE
(when a Q is loaded)
Edit Pause
Delete
Pause Queue
TESTS
Software Version
Lamp Count
Data Acquisition
Fluorescence Response
Self-Tests
Replay
Range 1 10.00
Range 2 10.00
Replay Rate (nm/sec) 20
Spectra Time 0.10
Replay Spectra
Display FU, λ
Replay Spectra
Replay Ex λ Em λ FU
0.10 250 400 0.000
Display FU, λ
Display Ex λ Em λ FU
0.10 250 400 0.000
COMMANDS
Scan
Scan Zero
Replay
Event Mark
Short Outputs
Shutdown Detector
OPTIONS
Analog Outputs
More
Analog 1 Offset (%) 0
Analog 2 Offset (%) 0
READY Output Active Hi
Analog Outputs
Zero on λ Change Yes
Detection Type Fluor
Cursor Speed Medium
Status Lock Off
File Name Protect
1: Off
2: Off
3: Off
4: Off
More
DT-Z186EDT

Menu Reference

For quick reference, we have included this alphabetical list of each field, including a short definition, and allowable and default values. For a more detailed explanation of the functions of your detector, you should refer to Chapter 3, *Basic Operations*, and Chapter 4, *Advanced Operations*.

Analog 1 Offset % This field offsets the Analog 1 output signal by a positive or negative 50, 20, 10, 5, 2, 1, or 0 percent of the full-scale range. Default is 0%.

Analog 2 Offset % This field offsets the Analog 2 output signal by a positive or negative 50, 20, 10, 5, 2, 1, or 0 percent of the full-scale range. Default is 0%.

Analog Outputs This menu allows you to offset the analog output terminals located on the back panel of the instrument, and access the READY Output field.

Auto Spectra This field allows you to tell the detector to automatically scan a peak whenever the output signal exceeds the Auto Threshold value. Selections are Off and On. Default is Off.

Auto Threshold This field allows you to set the minimum signal at which the detector will automatically scan a peak. Allowable values range from 0 to 99.999 FUs. Default is 0.010.

Autozero Time This field tells the detector when to perform an automatic zero. Allowable values are 0.00 to 99.99 minutes. Default is 0.00 minutes.

COMMANDS The Commands Menu lets you perform a sample or baseline scan manually, replay spectra, put an event mark into your chromatogram, short detector outputs, and shut down the FL3000.

Copy This menu choice accesses the Copy File field.

Copy File This field, along with the To File field, allows you to copy *from* the specified file to another file designation.

Cursor Speed This field may be set to Slow, Medium, or Fast according to your need. Default is Medium.

Delete *Under the top-level menu FILES*, this field accesses the Delete File command.

Under the top-level menu QUEUE, this field accesses the Delete Queue command.

Delete File This field deletes the designated file, setting all fields to their default values. After pressing [ENTER], the message **File Deleted** appears for one second.

Delete Queue

This field deletes the designated queue. After pressing [ENTER], the message ** Queue Deleted ** appears for one second.

Detection Type

This field allows you to put the detector into the fluorescence (Fluor) or phosphorescence (Phos) mode. Default is Fluor.

Display

This field shows the time at which the displayed scan was taken.

Display FU, λ

This field displays a screen that shows the actual incremental wavelength versus fluorescence intensity data for the selected spectral scan.

Edit

Under the top-level FILES Menu, the Edit Menu allows you to set up or edit files. The edits do not change the current settings of the detector until the file is loaded.

Under the top-level QUEUE Menu, the Edit Menu allows you to set up or edit a queue. Edits may not be made to Order 1 while a queue is loaded or running unless you pause the queue first.

Edit File

This field allows you to identify the file you wish to edit. Allowable designations are 1 to 4. Default is 1.

This field shows the detector's emission wavelength.

Event Mark

The Event Mark field applies a 15% of full-scale spike on the detector's output signals.

Ex λ

This field shows the detector's excitation wavelength.

FILES

The Files Menu allows you to edit, load, copy, or delete files.

File Name

This field allows you to enter a file name for a designated file (numbered 1 to 4). The name can contain up to eight characters from the following list: A to Z, 0 to 9, /, -, and blank. Default is blank.

FU

This field gives the intensity of fluorescence in fluorescence units (FU). It is a six-digit number, ranging from 0.001 to 999.999 FUFS.

Lamp Count

This field allows you to track the total number of lamp flashes. The field must be reset when a new lamp is installed.

Lamp Flash Rate

This field allows you to set the pulse frequency applied to the xenon lamp. Allowable values are 100 and 20 Hz. Default is 100 Hz.

Lamp Status

This field allows you: to *manually* turn the lamp on (On) or off (Off) at any time; or to *automatically* turn the lamp on and off at the beginning and end of a *run* (Run); or to automatically turn the lamp off at the end of a *queue* (Off@End). Default is Run.

Load

Under the top-level menu FILES, the Load selection accesses the Load File command.

Under the top-level menu QUEUE, the Load selection accesses the Load

Queue command.

Load File The Load File command loads the designated file settings into the active run file. After pressing [ENTER], the message **File Loaded** appears for one second.

Load Queue The Load Queue command loads the designated queue. After pressing [ENTER], the message **Queue Loaded** appears for one second.

More The More Menu allows you to access the Zero on λ Change, Detection Type, Cursor Speed, and Status Lock fields, and the file protection feature.

Number of Scans This field designates the number of times the detector should scan for each spectrum. In scanning, the detector scans from the low to the high wavelength, and then from high to low. The total number of scans are subsequently averaged to produce the final spectrum. Allowable values are 2 to 32, in multiples of two. Default is 2.

OPTIONS Found in the Main Menu, the Options Menu allows you to access the Analog Outputs and More menus.

Options The Options selection in the Edit Menu of FILES allows you to edit Range, Rise Time, Autozero Time, Lamp Flash Rate, Lamp Status, and PMT Voltage.

Order This field designates the order in which the detector is to run the selected files in a queue.

Pause This field accesses the Pause Queue command.

Pause Queue This command pauses an active queue. If a file is running, the file continues until it is completed, and the detector returns to a READY state.

PMT Voltage This field designates the voltage to be applied to the photomultiplier. Allowable values are 0 (to turn it off), 500, 600, 700, 800, 900, and 1000 volts. Default is 600.

Protect This field, in conjunction with the File Name field, protects a specified file from being edited, copied to (overwritten), or deleted. The field toggles between On, allowing no changes to the file, and Off, where changes may be made. Default is Off.

QUEUE The Queue Menu allows you to edit, load, delete, or pause a queue. A queue is a series of files which are run in a specific order, and is typically used for automated runs.

Range 1 This field controls the full-scale output range for the Analog Output 1 terminal. Allowable full-scale ranges are 500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, and 0.01 FUFS. Default is 10 FUFS.

Range 2 This field controls the full-scale output range for the Analog Output 2 terminal. Allowable full-scale ranges are 500, 200, 100, 50, 20, 10, 5, 2, 1,

0.5, 0.2, 0.1, 0.05, 0.02, and 0.01 FUFS. Default is 10 FUFS.

READY Output This field is used to communicate with other devices through the READY(Output) terminal. This TTL terminal switches the transistor between high and low states whenever the detector starts a run. Select "Active Hi" or "Active Lo," for the high or low state, respectively. Default is Active Hi.

Replay *Under the top-level menu COMMANDS*, this field accesses the Replay Menu where you can set the parameters for replaying stored spectra.

During a replay, this field shows the time at which the scan was taken.

Replay Spectra This command initiates replay of the designated spectrum.

Replay Rate This field designates the rate at which you wish to replay a stored spectrum. Allowable values are 2, 5, 10, 20, and 40 nm/sec. Default is 20 nm/sec.

Rise Time This field controls the detector's response time. Rise time is inversely proportional to the amount of baseline noise. For example, the longer the rise time, the less noise detected. Allowable values are 0, 0.1, 0.2, 0.5, 1, 2, 5, and 10 seconds. The default value of 2 seconds is appropriate for most applications.

#Runs This field displays the number of runs to be made for each file in a queue.

Scan Length This field designates the wavelength span for each scan. Allowable values are 0 to 600 nm. Default is 50 nm.

Scan This command initiates a sample spectral scan.

Scan Type This field designates the type of scan to be performed. The choices are Emission, Excitation, and Delta. In a delta scan, the detector scans both the excitation and emission spectra, keeping a constant wavelength differential between the two monochromators. Default is Excitation.

Scan Zero This command initiates a background scan.

Scan Zero Time This field allows you to set the time at which the detector should perform a baseline scan automatically. Allowable values are 0.00 to 99.99 minutes. Default is 0.00 minutes.

Self-Tests This command tells the detector to run through its internal diagnostic tests.

Short Outputs This field is used to short the detector analog outputs together (zero volts). When the outputs are shorted, the field changes to Unshort Outputs. To remove the short and return the outputs to their normal operating state, select Unshort Outputs, and the field changes back to Short Outputs. When you leave this screen, the field returns automatically to Short Outputs.

Shutdown Detector This field shuts down the detector's lamp, photomultiplier, and motors, leaving the electronics on to preserve memory. Press any key to return the detector to the same settings as when this field was activated.

Software Version This field displays the E-PROM version of your detector's software.

Spectra The Spectra Menu allows you to set up the parameters for scanning.

Spectra Time This field contains a list of the scans that are currently stored in memory. Each scan is identified by the runtime at which it was initiated.

Start Emission λ This field defines the wavelength at which the detector should begin an emission scan. Allowable values are 0 and 200 to 800 nm. Default is 400 nm.

Start Excitation λ This field defines the wavelength at which the detector should begin an excitation scan. Allowable values are 0 and 200 to 650 nm. Default is 250 nm.

Status This field in the Status Screen gives the current condition of the detector. The possible conditions are: READY (the detector is stabilized and waiting for initiation of a run), and NRDY (Not Ready) the detector is not stabilized, is performing internal tests, or has a possible internal problem). The run time is displayed when the running file has a programmed stop-time. The letter Q appears at the beginning of this field when a queue is loaded.

Status Lock The Status Lock field limits accessibility to the Status Menu (the programming area below the Status Screen). When set to On, only the Status Screen is shown on the display and the down-arrow icon is not seen. Default is Off.

Step Size This field defines the wavelength interval at which the detector should perform scanning. Allowable values are 2, 4, 8, and 16 nm. Default is 8 nm.

TESTS The Tests Menu allows you to access the detector's software version, lamp-count, data acquisition, and fluorescence response screens, as well as its internal diagnostic tests.

Time, Emλ, Exλ The Wavelength Program is a table containing the Time, Emλ, and Exλ fields. It allows you to program changes in the detector's emission and excitation wavelengths as a function of time.

Time refers to the amount of time into the run, in minutes, when a specified wavelength change is to occur. Allowable values range from 0.00 to 999.999 minutes. Default is 0.00 minutes.

Emλ and Exλ show the emission and excitation wavelength, respectively, to be set at a specified time. You can program up to nine different wavelengths for a single run. Allowable values are 0 and 200 to 800 nm for emission wavelengths, and 0 and 200 to 650 nm for excitation wavelengths. Defaults are 400 and 250 nm, respectively.

To File This field, along with the Copy File field, allows you to copy a file *to* the specified file number.

Wavelength Program This command allows you to access the Wavelength Program. See the "Time, Emλ, Exλ" description above for details.

Zero on λ Change This field toggles between Yes, where the detector baseline automatically zeroes each time the wavelength changes during a programmed run, and No. Default is Yes.

C

Troubleshooting

Introduction

This appendix provides you with helpful information for troubleshooting possible detector and chromatographic system problems. We have divided it into four sections:

- a brief theory of operation
- a troubleshooting guide that lists symptoms, possible problems, remedies
- possible error messages
- a description of the detector's diagnostic tests

Theory of Operation

The FL3000 detector consists of a pulsed xenon lamp, an excitation monochromator, an emission monochromator, a flowcell, a photomultiplier tube (PMT), and an optical system, all coordinated by supporting software and electronics.

As shown in Figure C.1, a beam of light from the xenon lamp is directed through the excitation monochromator (diffraction grating). From there, a bandwidth of light passes through the excitation slit and lens into the quartz flowcell, illuminating the sample as it passes through. If the sample is fluorescent or phosphorescent, it absorbs energy from the excitation light beam and subsequently emits light of a different wavelength.

The emitted light passes from the flowcell, through the emission lens and slit, to the emission monochromator. User-selected wavelengths of the emitted light are reflected through the PMT slit to the PMT. The PMT and its supporting circuitry convert the transmitted light into a current and ultimately into a voltage signal that is proportional to the intensity of the light received. The voltage signal is read out to an integrator/recorder.

The emission side of the detector's optics is positioned at right-angles (90°) relative to the excitation side to minimize the amount of scattered excitation light reaching the PMT tube. As a result, the PMT receives virtually no incident light unless a fluorescent material is passing through the flowcell.

A PMT generates some current (called dark current), even when no light is present. Dark current can contribute to detector background noise, reducing the signal-to-noise ratio, and ultimately reducing sensitivity. The FL3000 only integrates the current from the PMT during a lamp flash, virtually eliminating the contribution of dark current to detector noise.

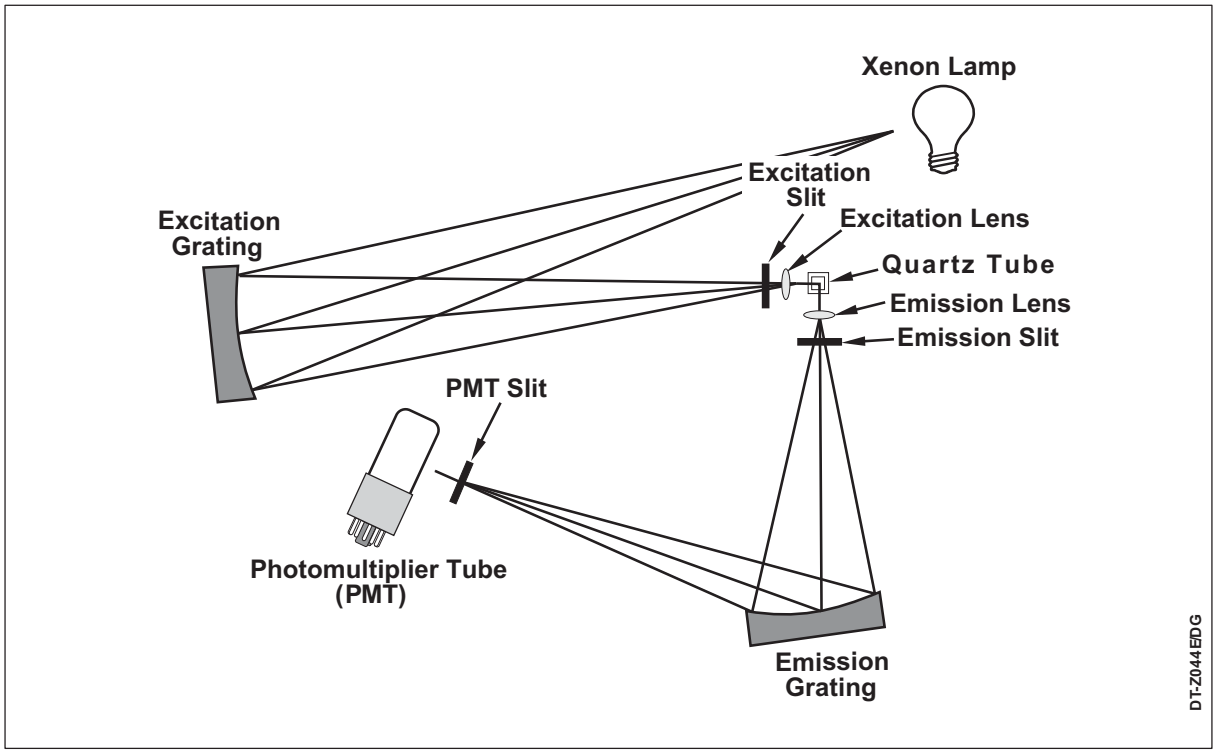


Figure C.1 FL3000's Optical System

Common Problems

This section contains a table of symptoms, possible causes, and remedies for some common problems you may observe in detector response. Many of the problems attributed to the detector may actually be due to other components in the chromatographic system, so we have included references to these types of problems and solutions as well.

Troubleshooting Table

Symptom	Cause	Remedy
1. No peaks, or peaks much smaller than expected.	a) Incorrect excitation and/or emission wavelength. Incorrect slit width(s).	a) Check excitation and emission wavelength settings. Ensure that the correct file is loaded. Check all slit widths (excitation, emission, and PMT).
	b) Lamp not lighted.	b) Check Status Menu to verify that the lamp is on. Run Self-Tests (Lamp) and replace lamp if necessary.
	c) Lamp power supply or connector defective.	c) Contact your Thermo Electron representative for assistance.
	d) Integrator/recorder input voltage mismatched with detector output voltage.	d) Reconnect positive lead of the integrator's or recorder's connecting-cable to correct terminal on detector. Verify correct integrator attenuation.
	e) Dissolved oxygen in mobile phase quenching fluorescence response.	e) Degas mobile phase.
	f) Defective PMT or PMT power supply.	f) Replace PMT and/or contact your Thermo Electron representative for assistance.
	g) Insufficient sample reaching the detector.	g) Check entire chromatographic system for leaks. Verify sample injection volume.

Troubleshooting Table (cont.)

Symptom	Cause	Remedy
2. Spikes on recorder baseline.	a) Bubbles in the flowcell.	a) Flush flowcell with solvent. Check fittings for leaks.
	b) Electrical interference.	b) Check electrical connections for good continuity. Check for RFI (Radio Frequency Interference) from nearby sources such as computers, monitors, printers, etc. Verify that detector's GND input isn't connected to the recorder's earth-ground terminal, creating a ground loop.
	c) Large AC-line voltage fluctuations.	c) Connect detector to a power outlet not shared with heavy-current-draw devices (refrigerators, large electric motors, etc.).
3. Random noise on integrator/recorder baseline.	a) Flowcell contamination.	a) Clean flowcell with solvents (see Chapter 5.)
	b) Leaking sample-inlet line.	b) Check all fittings from the column outlet and to the flowcell inlet for leaks. Tighten or replace fittings as necessary.
	c) Bubble trapped in flowcell.	c) Increase flowrate to dislodge bubble. Supply back-pressure device to flowcell (max. 500 psi).
	d) Flowcell leaking.	d) Replace flowcell.
	e) Ground-loop between detector and integrator/ recorder.	e) Verify that detector's GND input isn't connected to the recorder's earth-ground terminal, creating a ground loop. Ensure that both devices are connected to the same AC outlet.
	f) Dirty optics (flowcell, lamp, PMT, or lenses).	f) Clean appropriate system optics (see Chapter 5).

Troubleshooting Table (cont.)

Symptom	Cause	Remedy
	g) Integrator/recorder input voltage mismatched with detector output voltage.	g) Reconnect positive lead of integrator's or recorder's connecting-cable to correct terminal on detector. Verify correct integrator attenuation.
	h) Incorrect rise-time setting.	h) Determine and enter appropriate rise-time.
3. Random noise on integrator/recorder baseline, cont'd.	i) Mobile phase contaminated with fluorescent material.	i) Replace with fresh mobile phase made with high-purity solvents.
	j) Excitation wavelength too close to emission wavelength. Scattered light interferes with detection.	j) Adjust wavelengths and/or slit settings.
4. Excessive drift in recorder baseline.	a) Contaminated flowcell.	a) Clean flowcell with solvent (see Chapter 5). Check fittings for leaks.
	b) Contaminated mobile phase.	b) Replace with fresh mobile phase.
	c) Contaminated column.	c) Clean or replace column.
	d) Oxygen diffusing into degassed mobile phase.	d) Apply a continuous degassing technique (*e.g.*, helium sparging).
	e) Leaking flowcell.	e) Replace flowcell.
	f) Leaks in system.	f) Leak-test all fittings.
5. Detector won't power up.	a) Tripped circuit breaker at AC wall outlet.	a) Resolve problem, reset circuit breaker.
	b) Blown detector fuse.	b) Resolve problem, replace fuse.
	c) Incorrect voltage selected.	c) Reset detector for correct incoming line-voltage (see Appendix A).
	d) Power cord not connected.	d) Connect power cord.

Error Messages

Three types of errors may appear on your detector's display:

- System
- Real-time
- User-input

Each type is explained below in further detail.

SYSTEM ERRORS

System errors are indicated on the display by exclamation points (!! !!), and occur whenever an undesirable condition exists that prevents the detector from operating. If one of these messages appears, first try turning the detector's power switch off and on. If the message reoccurs, contact your Thermo Electron representative.

- SYSTEM RESET
- RAM ERROR
- ADDRESS ERROR
- BUS ERROR
- DIVIDE BY ZERO
- LOW L0 ERROR
- LOW L1 ERROR
- DISTANT QUEUE ERROR
- PARAM QUEUE ERROR

REAL-TIME ERRORS

The following real-time error messages may appear on the display of your detector.

PMT OVERLOADED

The PMT is saturated from too much incident light. This is usually caused by operating the instrument with a dry flowcell, leaving out the PMT slit-wheel, or operating in the zero-order mode with the wrong PMT slit-size selected and/or no optical filter installed.

CASE OPEN

The case of the detector is open, allowing stray light to enter. You must close either the PMT slit-mount access door or the front panel.

USER-INPUT ERRORS

The following error messages indicate improper use of the detector's menu system.

A File is Already Running
You cannot start a file while a different file is already running.

Invalid Parameters, Spectrum Not Allowed
Invalid scanning setup parameters have been entered, so the detector cannot perform a spectral scan.

No More Available Memory
All available system memory is full.

No Queue Available
You cannot load a queue if none has been set up first.

No Spectra Available
You cannot run the replay command when no spectra are available in memory.

Protected File, Cannot Be Copied To
You cannot copy to a protected file.

Protected File, Cannot Be Deleted
You cannot delete a protected file.

Protected File, No Editing Allowed
You cannot modify a protected file.

Queue Loaded, Cannot Load File
When a queue is loaded, you cannot load any other file.

Run In Progress, No Testing Allowed
You cannot run the detector's built-in diagnostics while a run is in progress.

Run Not In Progress, Spectrum Not Allowed
A spectral scan can only be performed when a run is in progress.

Diagnostic Tests

This section describes diagnostic tests you can use if you suspect that your detector is not working properly.

NOTE: Don't attempt to run any diagnostic test other than Software Version while you're collecting data (conducting a run). Conducting any of the other tests while a sample is running can cause your baseline to shift and thereby generate inaccurate data.

To access the detector's internal diagnostic tests, follow these steps:

1. Press [MENU].
2. Select /TESTS/.

The Tests Menu appears in Figure C.2.

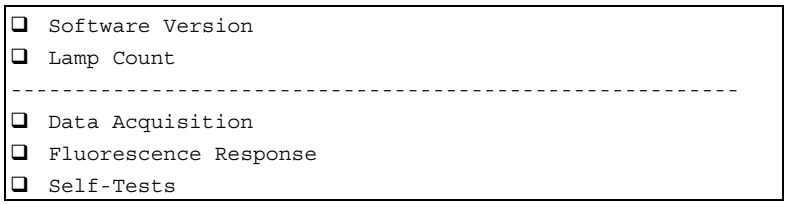


Figure C.2 The Tests Menu

SOFTWARE VERSION

Select this field to display the E-PROM version of your detector's operating software (Figure C.3).

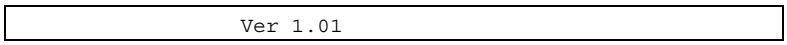


Figure C.3 The FL3000's Software Version

LAMP COUNT

The lamp count display (Figure C.4) shows the number of hours your detector's xenon lamp has operated. Access it by pressing [MENU], and selecting /Tests/, /Lamp Count/.

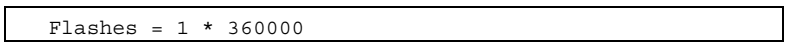


Figure C.4 Lamp Count Display

The detector's xenon lamp has an expected service life of approximately two billion flashes (about 28,000 hours at the 20 Hz strobe rate or 5,600 hours at the 100 Hz rate). The Lamp Count test shows the number of hours your detector's xenon lamp has operated (based on the 100 Hz strobe rate).

To calculate the number of times the lamp has flashed during its service life, multiply the number of hours of operation (the number to the immediate left of the asterisk in Figure C.4) by 360,000.

Be sure to reset the lamp count to zero each time a new lamp is installed. To zero the lamp-hour count, move the cursor to the leftmost digit and hold down the [-] key.

HINT: Before changing the lamp-hour count, write down the current value.

For example, if the display reads 2800, you'd place the underline beneath the 2, press the [-] key, and hold it down. The underlined digit would rapidly and automatically decrease until it reached zero. The cursor would then automatically move one digit to the right and decrement it to zero. When you've reached the last digit to the right, decrement it manually by repeatedly pressing the [-] key until only an underlined zero (0) is displayed.

DATA ACQUISITION

The FL3000's Data Acquisition test is the third test in the Tests Menu. Access it by pressing [MENU], and selecting /Tests/, /Data Acquisition/. The display shown in Figure C.5 appears.

NOTE: The words "(signal)" and "(offset)," shown in the center of Figure C.5, do not appear on the detector's display. We show them here only to help you differentiate the values.

PMT:	nnnnnnn	nnnnn	Hz
	(signal)	*(offset)*	
PHO:	nnnnnnn	nnnnn	Hz

Figure C.5 Data Acquisition Display

The values shown in the data acquisition screen represent the analog-to-digital (A/D) conversion frequencies of the photomultiplier tube (PMT) and the photodiode (PHO). These frequencies can vary between instruments and with different applications.

HINT: Be sure to run the data acquisition test at least once a month and keep a written record of the values!

FLUORESCENCE RESPONSE

The fourth test in the Tests Menu is Fluorescence Response. You can use this selection to activate/inactivate the response factor and to recalibrate the detector using the standard solution of your choice. To access this function, press [MENU], and then use the arrow and [ENTER] keys to select /Tests/ and /Fluorescence Response/.

The Fluorescence Response display that is shown in Figure C.6. If you move the blinking cursor to the /Active-Inactive/ field and press [+], you can enable or disable the response factor. If you move the cursor to the /Factor/ field while analyzing your standard calibration fluid, you can recalibrate your detector.

Using this display, the calibrant peak is set for the FU value of your choice. You do this by changing the value of the /Factor/ field, one digit at a time, beginning with the "tens" digit and moving toward the "hundredths" digit. You select the digit by pressing [<] or [>] and then you change its value by pressing [+] or [-]. As you change the value of the digit(s) you can watch the real-time effect that your changes have by viewing the FU value that's displayed to the right side of the /FU:/ field.

Complete details of the recommended calibration process are provided on page 61.

Figure C.6 Fluorescence Response Display

SELF-TESTS

The detector automatically runs five internal diagnostic tests every time the power is turned on. To initiate the tests at any other time, simply select /Self-Tests/.

If any test (other than the lamp test) fails, you'll see a message to that effect on the display. Clear the message and run the remainder of the self-tests by pressing [ENTER]. Repeat this process as many times as necessary until all self-tests are completed and the Status Screen appears. If any test has failed, the Status Screen will read "NRDY" (Not Ready).

Although you can frequently get back to the ready state on your own (*e.g.*, you can load a new file or queue after the failure of particular self-tests), the detector may not function properly and your results may be affected.

For troubleshooting purposes, the most likely solution to any failed self-test other than the lamp test, is to replace the appropriate motor or PCB. Motor replacements must be done at a Thermo Electron Service Center due to special calibration equipment needs. PCBs, however, may be replaced in the field.

The five self-tests are:

1. **RAM.** This test checks both non-volatile and volatile RAM with a read/write test. The "RAM" message only appears during self-initiated testing. On power-up, the test occurs without any special message. Instead, you'll see words like "Version No." on the screen.
2. **Excitation Motor.** This test verifies proper operation of the excitation-monochromator's motor and of the motor's optical encoders.
3. **Emission Motor.** This test verifies proper operation of the emission-grating's monochromator motor and of the motor's optical encoder.

NOTE: You may be able to hear the motors operating during both motor tests.

4. **Internal Voltages.** The internal-voltages test checks the circuitry-supply voltages that supply the detector's electronics. Voltages tested include:

   - Lamp off
   - Lamp on
   - PMT
   - ± 12 Vdc
   - Analog-output
   - Motor voltage

NOTE: You may hear "clicking" during the Internal Voltages test.

5. **Lamp.** The lamp test verifies that the xenon lamp is operating (flashing) properly. The lamp test does *not* check lamp intensity. If the lamp test doesn't pass, you'll get a "Fail" message. If so, replace the lamp and re-test.

   If the lamp test passes during power-up, a new screen (Figure C.7) appears.

```
** Calculating **
** Reference Table **
```

Figure C.7 The Calculating Reference Table Message

Although you will never see the reference table that's being calculated, the message lets you know that a table is being generated that will correct (offset) any changes in excitation energy across the operating spectrum.

TEST MIX RUN

You may wish to run a standard FL test mix when you first set up your system. We suggest that you keep the initial run results on file for later comparison and troubleshooting, if necessary.

D

Glossary

Introduction

We have included a glossary to define certain technical terms used throughout the manual's text. These terms should be consistent with standard definitions used throughout the analytical industry, and are added here as a quick reference only.

A - C

A/D Analog-to-digital. Converts a detector's analog signal to a digital signal.

analog offset A voltage applied to the output signal in order to keep the signal "on-scale" throughout a run.

background scan The reference spectrum of the mobile phase. It is subtracted from the sample spectral scans to correct for baseline absorbances.

bandwidth The width of a band, measured at its base. Also called peak width.

baseline The reference line at the bottom of a chromatogram from which measurements are made. A baseline represents the chromatogram that would be drawn if only the mobile phase (with no sample) were run through the column.

D

defaults The values or choices built into a system. If no specific choice is made, the detector will run using the default settings.

degassing The practice of removing air from the mobile phase, usually by helium sparging or applying a vacuum.

delta scan A synchronous scan of both the excitation and emission spectra that keeps a constant wavelength differential between the two monochromators.

diagnostics Ways of detecting and isolating instrument of software problems.

display The two-line screen on all SpectraSYSTEM instruments.

E

emission wavelength The wavelength of the light emitted from a fluorescing/phosphorescing compound.

error message A displayed message that notifies you of a problem.

excitation wavelength The wavelength of the light used to excite a fluorescing/phosphorescing compound.

F - L

FUFS Fluorescence units; range of analog output signal.

field The area in a display, screen, or menu where an entry is required or a choice must be made.

file A list of detector parameters that contains the desired settings for an analysis.

fluorescence The instantaneous and temperature-independent emission of light by a sample that has been excited by incident light energy; the excitation wavelength is always shorter than the emission wavelength.

gradient elution A liquid chromatographic technique where the mobile phase composition changes over time; changes may be continuous or in steps. Also called solvent programming.

ground terminal A terminal used to connect the ground or earth lead of a signal or contact closure cable.

keypad All of the keys which you use to communicate with your instrument or computer.

M - Q

menu A list of choices.

NOVRAM This abbreviation stands for Non-volatile RAM. Non-volatile RAM consists of Random-Access Memory (RAM) chips that retain data even if the instrument's power source fails. NOVRAM is used to store critical setup data in the FL3000 detector.

parameter A value or set of values used to define the characteristics of behavior of an instrument or system.

peak broadening The dilution of a peak as it moves through the chromatographic system.

phosphorescence The delayed and temperature-dependent emission of light by a sample that has been excited by incident light energy; the excitation wavelength is always shorter than the emission wavelength.

queue A set of items (*i.e.*, samples, files) in a prearranged order.

R - S

RAM Random Access Memory.

range A detector parameter that controls the full-scale range for the output signal.

replay Retrieving a stored spectrum, which can be played back as either individual data points or a smoothed spectrum.

rise time A detector parameter that controls the detector's response time. Rise time is inversely proportional to the amount of baseline noise.

run time The duration of a sample run from injection to detection.

signal-to-noise A measurement of the sensitivity of a detector, the ability to measure a very small sample response over the baseline noise.

spectral scan A sample spectrum.

status The current condition.

T - Z

timed event An instrument action triggered to occur at a specific, preset time during a run (*e.g.*, autozero, wavelength change, stop-time).

troubleshooting Refers to locating the cause of problems with equipment or procedures, and solving these problems.

wavelength programming Programming the detector to change the monitoring wavelength as a function of time during a run.

zero order In fluorescence, the monochromator is set to act as a mirror, reflecting all wavelengths of incident light.

INDEX

KEYS

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z