Thermo Scientific™

Multidrop™ PicoIT Patterning Software

User Guide

Software version 1.3.0

Cat. no. 1509080 Rev. 1.0

thermo scientific

Copyright © 2020 Thermo Fisher Scientific Inc. All rights reserved.

Microsoft and Windows are registered trademarks of the Microsoft group of companies.

All other trademarks and registered trademarks are the property of their respective holders.

Thermo Fisher Scientific Inc. provides this document to its customers with a product purchase to use in product operation. This document is copyright protected and any reproduction of the whole or any part of this document is strictly prohibited, except with the written authorization of Thermo Fisher Scientific Inc.

The contents of this document are subject to change without notice. All technical information in this document is for reference purpose only. System configurations and specifications in this document supersede all previous information received by the purchaser.

Thermo Fisher Scientific Inc. makes no representations that this document is complete, accurate or error free and assumes no responsibility and will not be liable for any errors, omissions, damage or loss that might result from any use of this document, even if the information in the document is followed properly.

This document is not part of any sales contract between Thermo Fisher Scientific Inc. and a purchaser. This document shall in no way govern or modify any Terms and Conditions of Sale, which Terms and Conditions of Sale shall govern all conflicting information between the two documents.

About This User Guide

Warnings and other markings used in the documentation

The following symbols and markings appear in this user guide.

Warning / Caution Risk of injury to the user. Risk of damage to the instrument, other equipment or loss of performance or function in a specific application.

Note Marks a tip, important information that is useful in the optimum operation of the system, or an item of interest.

Tip Gives a helpful hint for getting the most out of the software functionality.

Contents

About This User Guide **3**

Chapter **Dispensing Software User Guide** **8**

Chapter **Safety** **9**
- General safety rules 9
- Personal protective equipment 9
- Possible inhalation hazard 9
- Ergonomic safety 10
- Additional assistance 10

Chapter **System specifications** **11**
- Intended Use 11
- Software 11
- Compatible fluids 11
- Additional specifications 11

Chapter **Software overview** **12**
- Layouts 12
- Targets 12
- Patterns 13
- Protocols 13
- Starting view 14
- Protocol view 15

Chapter **Building a simple protocol** **16**
- Creating a new protocol file 16
- Opening an existing protocol file 17
- Protocol fluids 17
- Applying fluid patterns 18
  - Importing a pattern 19
  - Completing the pattern apply 19
  - Pattern tooltip 20
- Changing pattern volume 20
- Removing fluid 21
- Adding a layer 21
- Delay after layer dispense 21
- Saving and Closing the protocol 21
- Running the protocol 21

Chapter **Additional protocol edit features** **22**
- Protocol patterns 22
- Dispense order 22
- Move layer 22

Protocol layout 23

Chapter **Advanced pattern volumes 24**
Volume gradient across targets 24
Volume gradient across spots within patterns 25
Calculate values 26
Specify values 26
Advanced tooltips 27

Chapter **Normalizing target volumes 29**

Chapter **Layout and pattern libraries 31**
Using a library layout in a protocol 31
Exporting a protocol layout to the library 31
Adding a pattern to a protocol 31
Exporting a protocol pattern to the library 32
Removing a layout or pattern from the library 32

Chapter **Editing a layout 33**
Saving a layout 33
Adding targets 34
Removing targets 35
Shifting targets 35
Target numbers 35

Chapter **Editing a pattern 37**
Saving a pattern 37
Pattern dimensions 38
Changing the spot pattern 38
Ellipse guide 38

Chapter **Examples 39**
Creating a circular gradient dispense pattern 39

Chapter **Best practices 40**
Dispensed spot alignment 40
Spot size and surface interaction 40
Multiple fluids onto same spot location 41

Chapter **Device utilities 42**
Cassette Check 42
Set Device ID 42
Alignment 42

Chapter **Running a protocol 43**
Dispense region limitation 43
Recommended dispense spot volume 43

Delay after layer dispense 44

Chapter **Reports 45**
Excel Compatible XML 45
Patterns 45
Layers 45
Tabular Detail 45
Target Volumes 46

Chapter **Settings 47**
Default values 47
Display 47
Reports 48
File locations 49
Run 50

Chapter **Appendix A – Dispense Specifications 51**

Chapter 1
Multidrop PicoIT Patterning Software User Guide

This Multidrop PicoIT Patterning Software User Guide is a companion guide to the **Multidrop PicoIT Software User Guide**. General information about the Multidrop Pico Dispenser, specifications, and best practices are found in Multidrop PicoIT Software User Guide.

Information in the Multidrop PicoIT Software **User Guide** is referenced in this user guide rather than duplicated.

Chapter 2
Safety

The Multidrop Pico dispenser should be used in accordance with all applicable safety rules that are in effect in the lab in which it is installed. Use of the Multidrop Pico dispenser should also follow common-sense safety guidelines for chemical, mechanical, and electrical safety.

Note If this unit requires servicing, or you are unsure about any aspect of operation or safety, contact the Service Help Desk. The contact information can be found in the Multidrop Pico dispenser **Service and Support** document.

Caution Do not remove any of the Multidrop Pico dispenser covers. Contact your local Thermo Fisher Scientific service representative if the Multidrop Pico dispenser is not working properly. Do not attempt to repair the Multidrop Pico dispenser yourself.

General safety rules

- Place the Multidrop Pico dispenser on a work surface that supports all four feet securely.
- Plug the Multidrop Pico dispenser into a standard, grounded, electrical outlet. Note the power requirement listed on the back label of the Multidrop Pico dispenser.
- Avoid the moving parts of the Multidrop Pico dispenser during operation.

Personal protective equipment

The same safety precautions and personal protective equipment (PPE) specified by your employer for fluid transfer operations, such as pipetting into bio-assay well plates, should be used when operating the Multidrop Pico dispenser and handling these materials.

Possible inhalation hazard

The Multidrop Pico dispenser was designed with chemical safety in mind; however, it does dispense picoliter droplets of fluid and generates a small amount, less than 1% by volume, of very small aerosol droplets that might not be trapped within the well plate that you are dispensing into. If the fluids are known or suspected to be an inhalation or contact hazard, use the Multidrop Pico dispenser in an enclosure, with an exhaust, such as a lab fume hood.

Ergonomic safety

The Multidrop Pico dispenser typically requires a single manual pipetting operation for each dispensed compound. In most situations, using the Multidrop Pico dispenser in the intended manner decreases the frequency of shoulder, arm, and wrist motions and so decreases the stress on these joints compared to traditional methods.

To minimize ergonomic risks associated with the use of the Multidrop Pico dispenser, consider the following work area design ideas:

- Place the Multidrop Pico dispenser on a work bench at a height that allows for proper visual and physical access for operators. Having the Multidrop Pico dispenser too high can lead to greater shoulder elevation when pipetting into the cassette and having the Multidrop Pico dispenser too low can lead to increased neck and back flexion.
- If an operator is too short to comfortably access the Multidrop Pico dispenser, consider moving the Multidrop Pico dispenser to a lower bench height. Alternatively, use a footstool or high lab stool that adjusts to the proper work height.
- Place the Multidrop Pico dispenser close to the front of the work surface to minimize the distance the operator must reach to pipette into the cassette.
- Place the pipette tip disposal receptacle close to the Multidrop Pico dispenser.
- Place the computer keyboard close to the Multidrop Pico dispenser to minimize reach distance.
- Adjust the computer monitor height and screen angle for good physical and visual access during Multidrop Pico dispenser operation.
- Perform data analysis at a separate ergonomically designed workstation whenever possible rather than at the Multidrop Pico dispenser computer.

Additional assistance

Contact the Service Help Desk for additional assistance. The contact information can be found in the **Service and Support** document.

Chapter 3
System specifications

Intended Use

The Multidrop Pico dispenser and cassettes are intended only for the following, wherein all three conditions must be met:

- Dispensing of DMSO solvent or water containing surfactant with or without small molecule compounds or biomolecule(s).
- For general laboratory use.
- Single-usage (i.e., non-reusable) operation of a cassette.

In addition to the requirement of Intended Use, the products should not be used for any of the following: (a) applications in Bio Safety Level 3 or 4 (BSL-3 or-4), food, animal or human testing or therapeutic delivery (except solely for therapeutic delivery for in vitro research uses) (b) dispensing cells, biofluids (unless otherwise specified on product packaging), bacteria, viruses, pathogens, contagions, infectious agents, potent toxins or oncogenes, radioactive substances; and (c) in uses that are unsafe, bypassing any product safety features, and/or disobeying any safety instruction.

Software

- Microsoft Windows 7 or greater.
- Microsoft .Net Framework 4.5.1 or greater. .Net Framework 4.5.1 is included on the install disk.
- Adobe Acrobat Reader – www.adobe.com/downloads

Compatible fluids

See *Appendix E – Dispense Specifications* in the **Multidrop PicoIT Software User Guide** for details of compatible fluids.

Additional specifications

See the Multidrop Pico Dispenser **Operating Manual** for more system specifications.

Chapter 4
Software overview

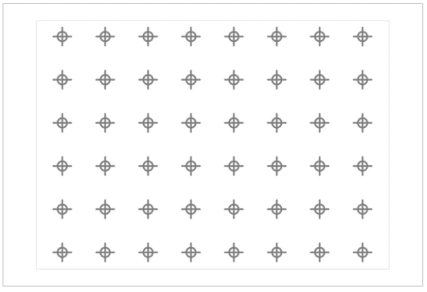
The Multidrop PicoIT Patterning software enables dispense of patterns at locations in the dispense region. Multiple fluid layers can be defined and will be dispensed in layer order.

Layouts

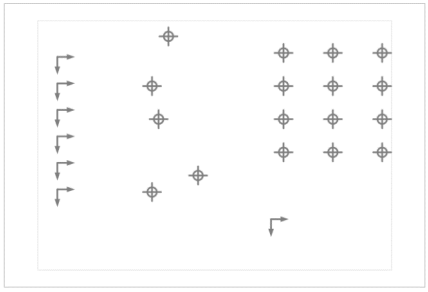
A layout specifies **target** locations that are available for dispensing **patterns**. The Multidrop Pico dispenser was designed to dispense to well plates and the layout area is based on the dimensions of a standard well plate. However, it is not possible to dispense to the entire well plate area, so the **dispense region** is smaller than a well plate. The dispense region is drawn on layout and protocol views as a pale rectangle inside the layout border.

Dispensing is not limited to well plates, and targets can be placed anywhere within standard well plate dimensions.

Note The **dispense region** is inset from well plate edges by 10 mm horizontally and 7 mm vertically. Any pattern spots outside the dispense region will not be dispensed.



Well plate layout

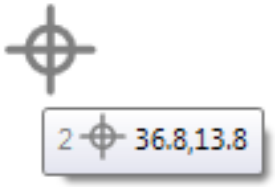


Freeform layout

See **Editing a layout** for more detail.

Targets

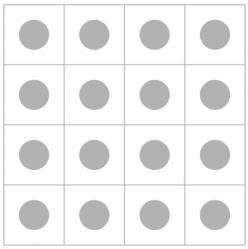
Layout targets define locations where **patterns** will be applied. Patterns will be centered at the target location or aligned to the upper-left corner. Hover the mouse over a target to display information about the target.



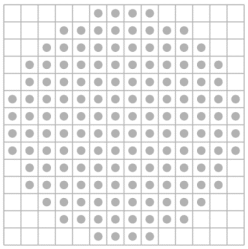
See **Adding targets** for more detail.

Patterns

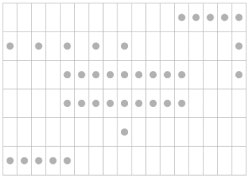
A pattern specifies how fluid will be used to create a **spot** pattern. Patterns can be created any size, but when they are applied at a **target** location, spots outside the dispense region will not be dispensed (see **Layouts**).



Rectangular pattern



Circular pattern

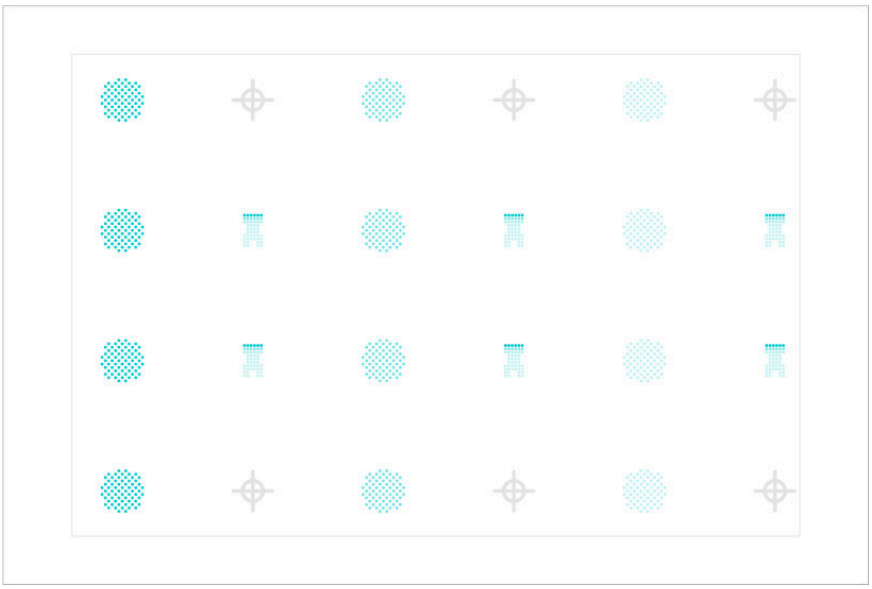


Irregular pattern

Protocols

A protocol specifies how **fluids** will be applied to the dispense region.

A single **layout** is used for all **layers** in the protocol. Fluids are dispensed in layers, each layer using a single fluid. On each layer, **patterns** are applied to **target** locations at specified volumes or volume gradients. The same pattern can be applied to different targets with different volumes.

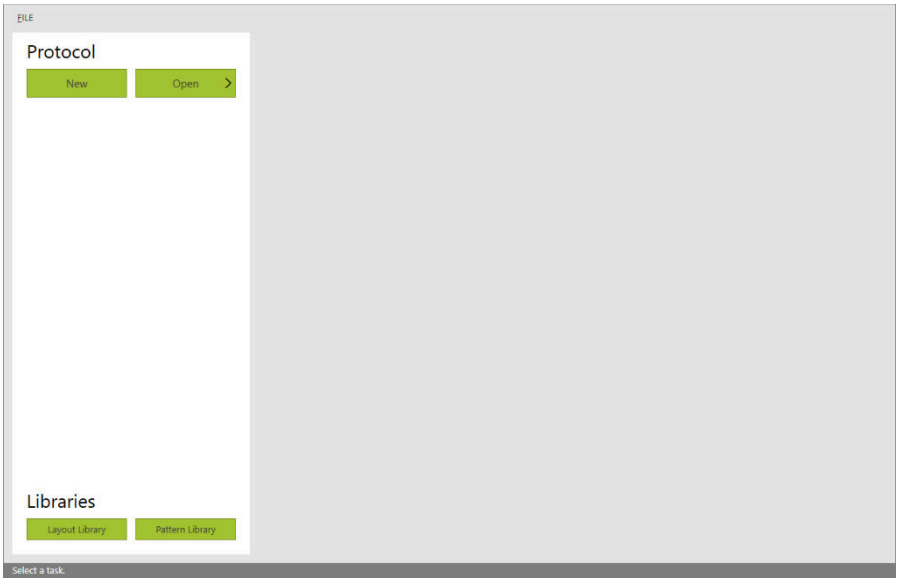


Protocol layer using 24-well layout

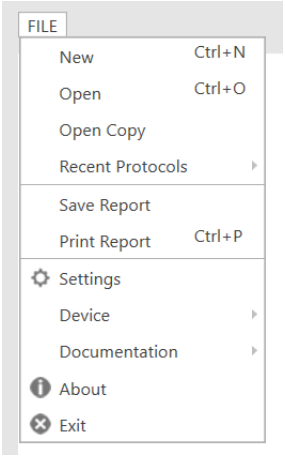
See **Editing a pattern** for more detail.

Starting view

When the Patterning software is started, the initial screen provides buttons to create a **New** protocol or **Open** an existing protocol. There are also buttons to access **Layout and pattern libraries**.



Additional options are provided in the **FILE** menu. The **Documentation** section provides access to Service and Support Contact Information.



Protocol view

When a protocol is open for editing, features of interest include:

- The main edit area, which includes buttons and controls on the left and right edges.
- A menu at the top.
- Buttons on the far left to Close, Save, or Run the protocol.
- A status bar at the bottom which includes zoom controls and information.

Chapter 5
Building a simple protocol

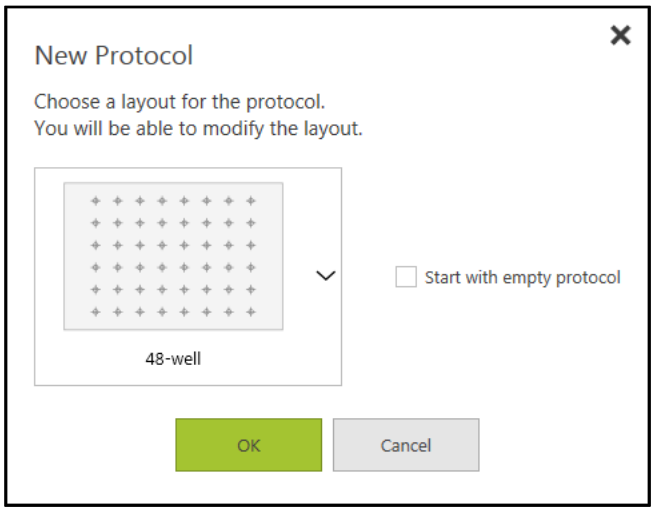
Creating a new protocol file

You can create a new protocol in two ways:

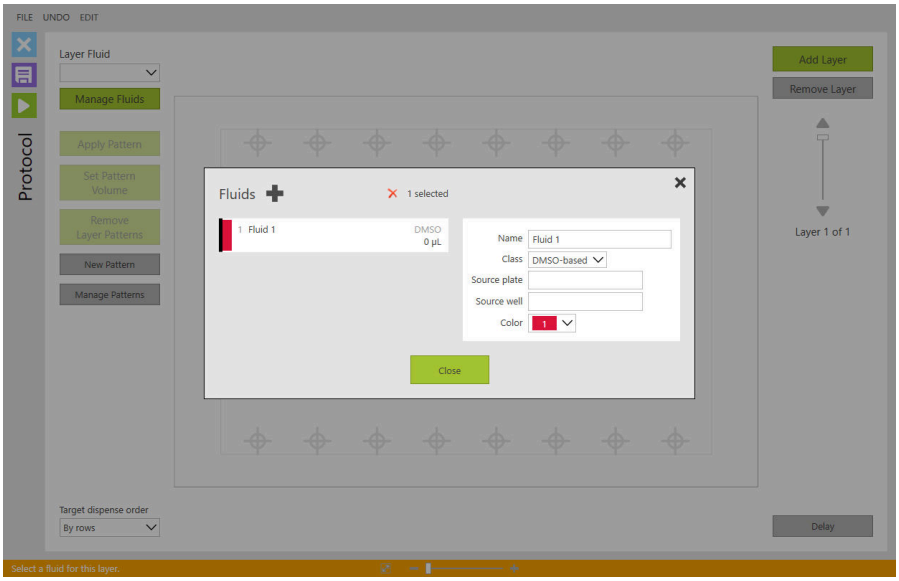
- Click the **New** button in the Protocol section of the Startup view.
- Select **New** in the FILE menu.

Each protocol uses a single layout for all layers. You can start the protocol with a copy of a layout from the Layout Library or with an empty protocol layout. If starting with an empty layout, targets will need to be added to the layout before applying patterns (see **Editing a layout**).

The layout is stored in the protocol file, so even when a protocol is created using a library layout, the protocol layout can be modified without affecting the library layout (see **Layout and pattern libraries** for information about libraries).



A new fluid is automatically created for the protocol.

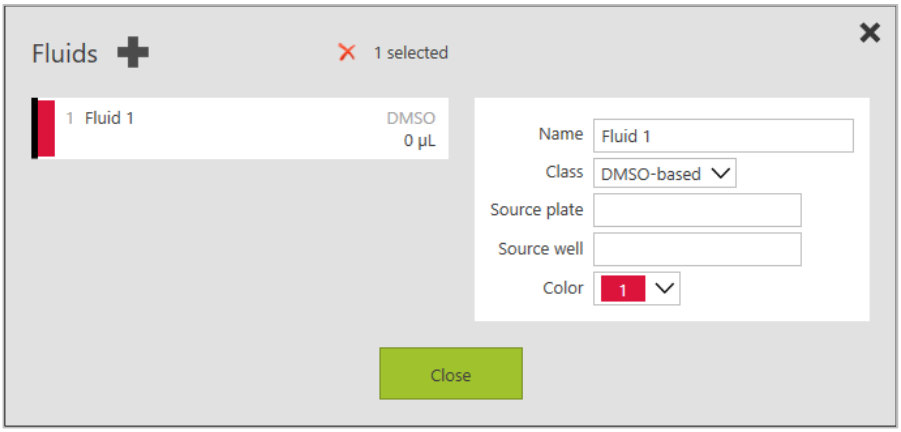


Opening an existing protocol file

To open an existing protocol:

- Click the **Open** button in the Protocol section of the Startup view. You can open recent protocols by clicking the arrow at the right edge of the Open button and selecting a file from the list.
- From the FILE menu, select **Open** or **Open Copy**. Open Copy will prompt for a file name to use for the protocol copy.

Protocol fluids



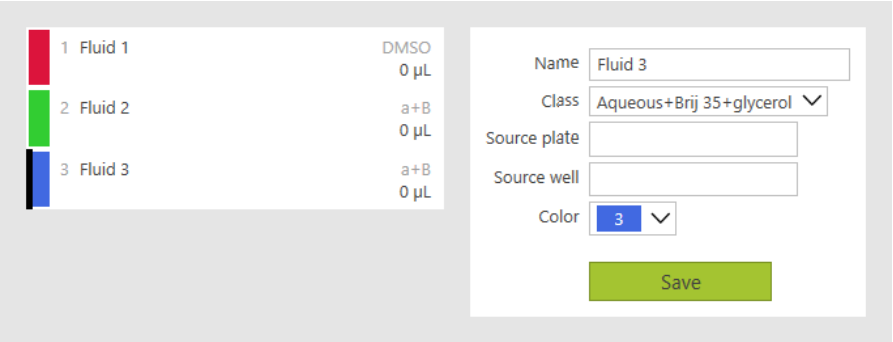
The fluid **Name** and optional **Source** plate and well can be specified. The **Color** can be changed. The fluid **Class** is used to adjust dispense characteristics for different types of fluids. In addition to DMSO fluids, there are additional classes based on aqueous fluids. Refer to *Appendix E – Dispense Specifications* in the Multidrop PicoIT **Software User Guide** for fluid class details.

Note Text Dispensing is always by volume, so the fluid stock concentration is not requested. This is different from the Multidrop

PicoIT Software. Because the stock concentration is not known, however, it is recommended that highly concentrated fluids not be dispensed with the Multidrop PicoIT Patterning software.

The *Dispensing Considerations* section of *Appendix E – Dispense Specifications* in the Multidrop PicoIT Software User Guide provides more detailed information.

Additional fluids can be created by clicking ✚ at the top of the fluid list. If changes are made to a fluid, the **Save** button will save the changes.

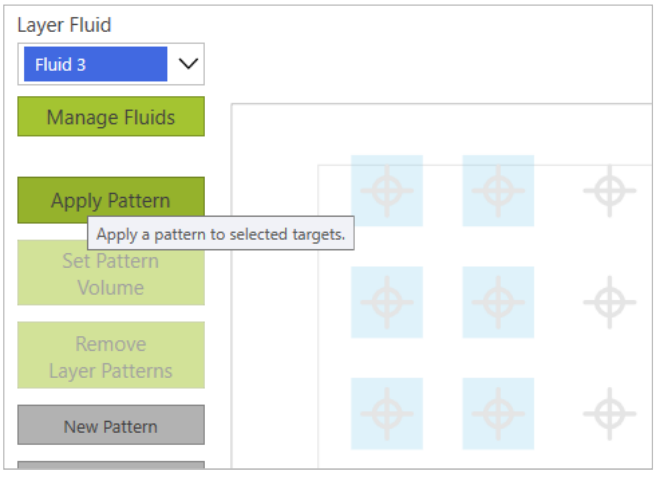


Click **Close** when done adding or editing the fluid list.

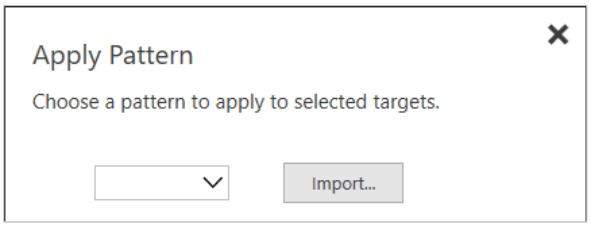
Applying fluid patterns

Select target locations using the mouse.

- Click and drag to select a group of targets.
- Ctrl+click to add or remove individual targets from the selection.
- Click on a selected target and drag to remove a group of targets.



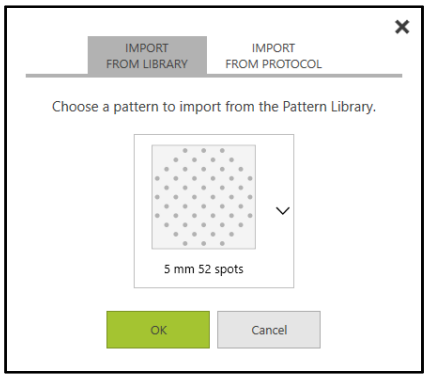
Click **Apply Pattern** (or select from the right-click context menu).

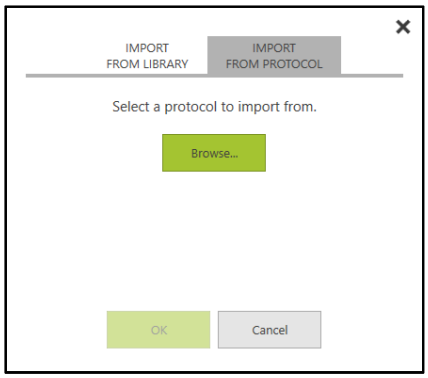


A new protocol does not contain any patterns, so the drop-down target list is empty. You can **Import** a pattern from the Pattern Library or another protocol. You can also create a **New Pattern** from the protocol edit view (see **Editing a pattern**).

Importing a pattern

Click **Import** to import a pattern from the Pattern Library or another protocol.



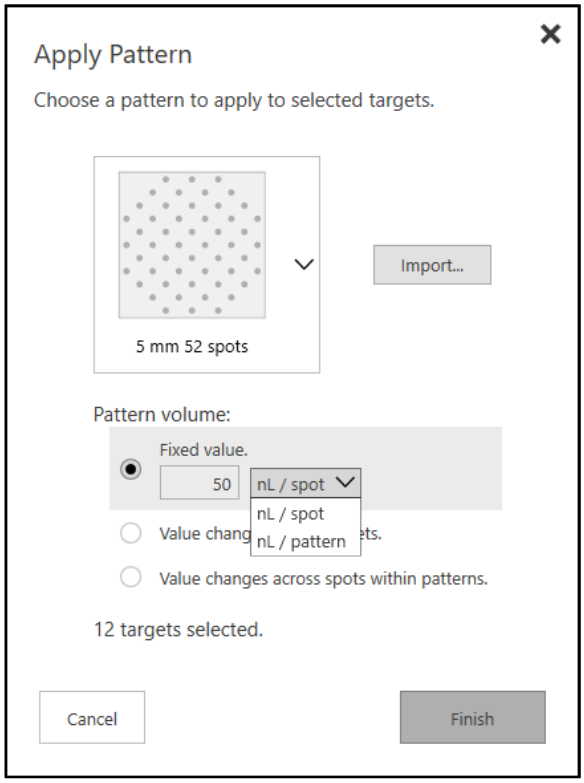


If importing from another protocol, you will **Browse** to select a protocol file and select a pattern from that file.

Completing the pattern apply

After a pattern has been selected or imported, the fluid volume must be specified. A fixed volume is the simplest way to specify pattern volume, and can be specified as:

- Per-spot volume. Total volume for the pattern will be the specified volume multiplied by the number of spots in the pattern.
- Per-pattern volume. Each spot volume will be the specified volume divided by the number of spots in the pattern.

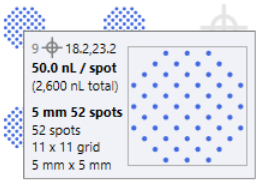


Note If a pattern is edited, and the number of spots changes, volumes change depending on how the pattern was applied. If per-spot volume was specified, the volume for each spot remains the same; if per-pattern volume was specified, the total pattern volume remains the same.

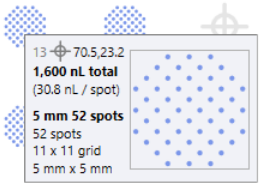
Advanced methods for specifying pattern volume are described in the **Advanced pattern volumes** section.

Pattern tooltip

Once the fluid pattern has been applied to targets, you can hover over a target to see details about the target, pattern, and fluid volume.



Volume per-spot



Volume total per-pattern

Changing pattern volume

To change the volume for existing patterns, select patterns to change and click **Set Pattern Volume**.

The same pattern volume options are available as with **Apply Pattern**, however, only existing patterns will be affected. Some pattern volume options may not be available depending on selected patterns.

Removing fluid

You can remove fluid patterns from a layer by selecting the patterns to remove and clicking **Remove Layer Patterns** (or select from the right-click context menu).

Adding a layer

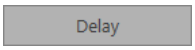
Click **Add Layer** to add a new layer to the protocol.

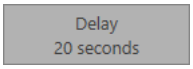
Use the control at the right edge of the edit region to move between layers. You can also use the Page Up and Page Down keys to change layers.

Note If adjacent layers use the same fluid, dispensing of those layers will be planned to minimize the number of cassette. Layers will be dispensed in layer order for all cases.

Delay after layer dispense

During the protocol run, a delay can be added after a layer is dispensed. Each layer has a separate delay setting (default is no delay). Click the Delay button to set a delay up to 5 minutes.





Note It is not recommended to delay between layers using the same aqueous fluid because of evaporation.

Note When adding a layer, the delay setting for the current layer is used for the new layer.

Saving and Closing the protocol

Press the button at the left edge to **Save** the protocol. You can also select **Save** or **Save As** from the FILE menu.

Press the button at the left edge to **Close** the protocol. You can also select **Close** from the FILE menu.

Running the protocol

Press the button at the left edge to **Run** the protocol. You can also press F5 to run the protocol.

See the **Running a protocol** section for more information about running a protocol.

Chapter 6
Additional protocol edit features

Protocol patterns

New Pattern is used to create a new pattern for the protocol. See **Editing a pattern** for more information about creating a new pattern.

Protocol patterns can be edited, removed, or added by clicking **Manage Patterns**. Remember that the protocol patterns are separate from the Pattern Library patterns (see **Layout and pattern libraries** for more information about libraries).

Dispense order

The order that patterns are dispensed can be modified using the **Target dispense order** selection. The selected dispense order is used for all layers in the protocol.

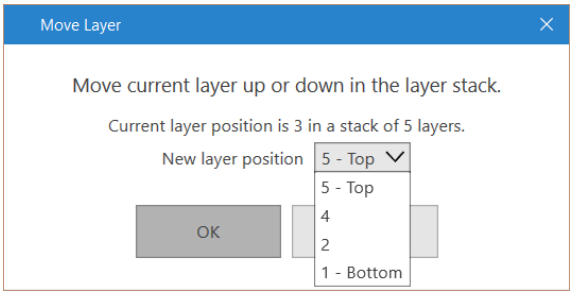
Available dispense order options are:

- **By rows**. Starting at the top of the dispense region, targets at the same vertical offset will be dispensed from left to right. Targets at the next vertical offset are then dispensed, continuing down the dispense region.
- **By target number**. Targets are dispensed in target number order; lower numbers are dispensed before higher numbers. Dispense order can be changed by editing target numbers in the layout (see **Target numbers**).

Note At protocol run, the actual dispense order may be adjusted if patterns with different volume/spot are applied on a layer. To ensure optimal dispensing, a pattern with the largest volume/spot will always be dispensed first.

Move layer

The current layer can be moved up or down in the layer stack by selecting **Move Layer** from the protocol EDIT menu or right-click context menu.



Protocol layout

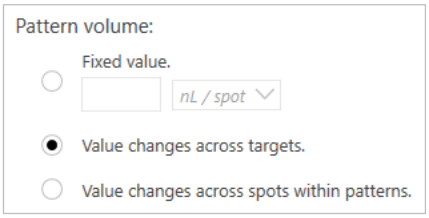
To edit the protocol layout, select **Edit Layout** from the protocol EDIT menu or right-click context menu. See **Editing a layout** for more information. Remember that the protocol layout is separate from Layout Library layouts.

Chapter 7
Advanced pattern volumes

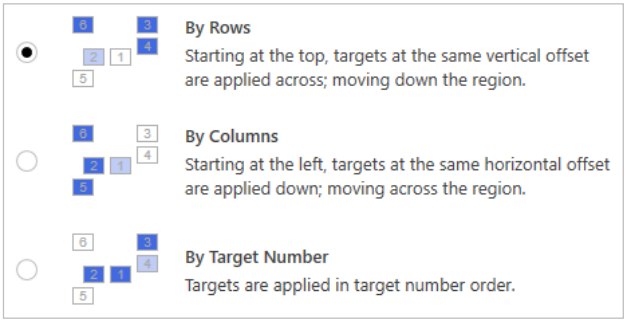
The method for specifying fixed per-spot and per-pattern volumes was described in the **Completing the pattern apply** section. There are also methods to create a gradient of volumes across a group of patterns or across each pattern.

Volume gradient across targets

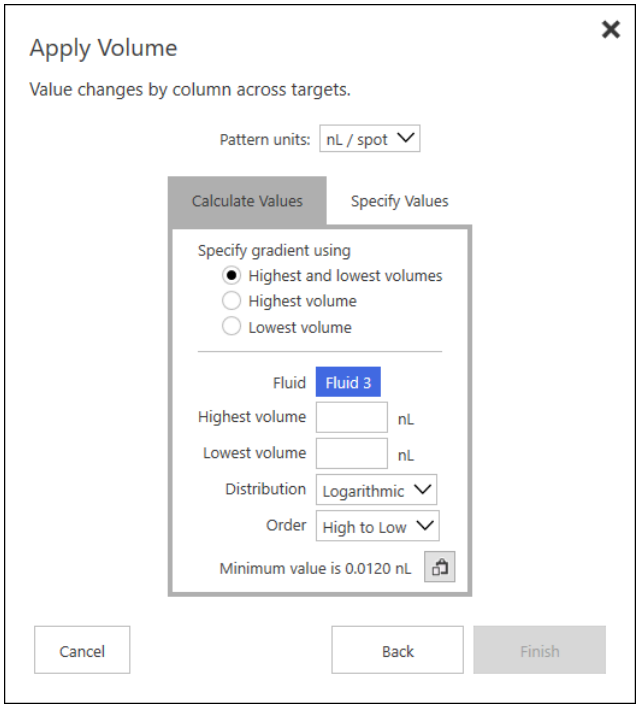
If multiple targets or patterns are selected, you can apply a gradient of values across the targets or patterns. Each individual target will have a single volume (per-spot or per-pattern).



After clicking **Next**, a selection is made for how to apply the volume gradient.



On the final screen, a per-spot or per-pattern selection is made and the volume gradient is defined.



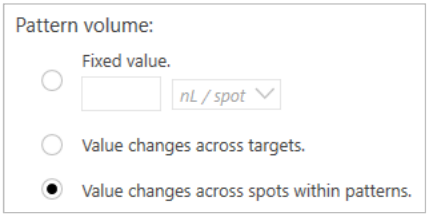
See the **Calculate values** and **Specify values** sections below for more information about defining the volume gradient.

Note Once the calculate or specified volumes have been applied to the patterns, the result is identical to defining a per-spot or per-pattern fixed volume to each pattern.

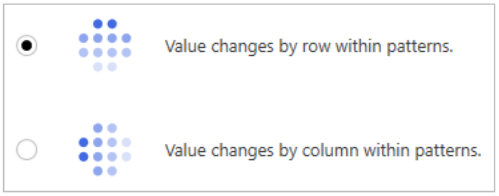
This features simply provides an alternate way to apply volumes to a group of patterns.

Volume gradient across spots within patterns

A gradient of values can be applied across the spots of each pattern. All patterns will use the same calculation or specified values.



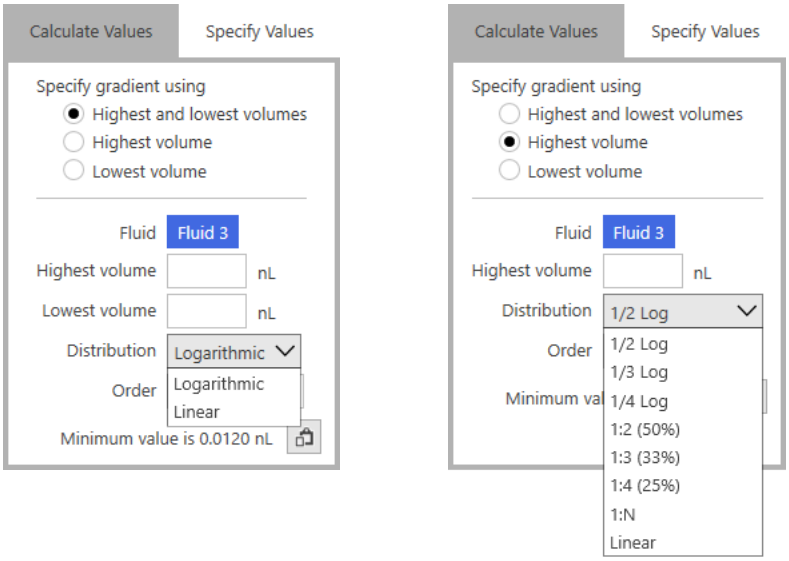
After clicking **Next**, a selection is made for how to apply the volume gradient, by rows or columns within the pattern.



Volumes are always specified as a per-spot volume and are calculated or specified per row or column.

Calculate values

The **Calculate values** tab allows the volume gradient to be specified similar to a **Titration** in the **Multidrop PicoIT Software User Guide**.



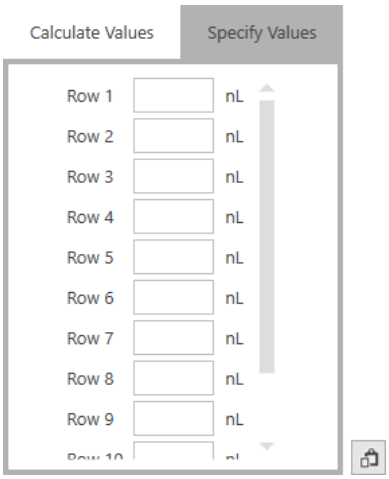
Volumes can be distributed logarithmically or linearly between a high and low volume. If volumes are calculated starting from a high or low value, there are many different **Distribution** options.

Note When **Calculate Values** is used to apply a gradient of volumes across pattern spots, the gradient is calculated separately for each pattern. If the patterns have different dimensions (different number of rows/columns), the spot values may differ when calculated.

Note In addition, if a pattern is modified later, gradient values will be re-calculated.

Specify values

The **Specify values** tab allows the user to specify a specific value for each row or column in the patterns. This option is not available if selected patterns do not have the same number of rows/columns during **Set Pattern Volume**.



Individual values can be set for each row, column, or target (when applying a gradient across targets).

The (Paste) button can be used to paste values from the clipboard.

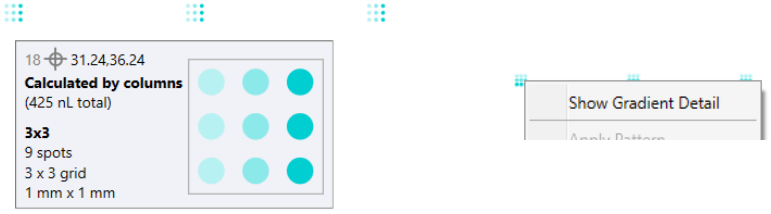
Note When **Specify Values** is used to apply a gradient of volumes across pattern spots, values are specified for a specify number or rows or columns.

Note If a pattern is modified later and the number of rows/columns reduced, some specified values will be removed. If the number of rows/columns is increased, the new rows/columns will have a 0 (zero) value.

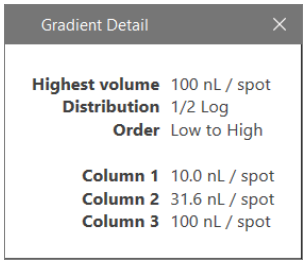
Advanced tooltips

For patterns with fixed volumes, the tooltip displays the per-spot or per-pattern volume, as indicated in the **Pattern tooltip** section. Advanced gradients cannot be shown in a simple tooltip, which indicated general information about the volume.

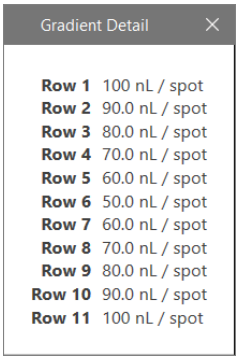
Detailed information about the volume gradient can be viewed by selecting **Show Gradient Detail** from the right-click context menu.



A popup message will show detail about the volume gradient.



Calculated values



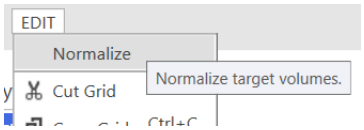
Specified values

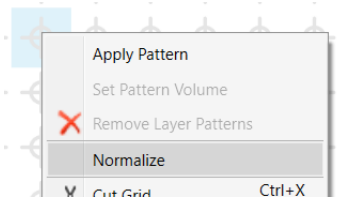
Chapter 8
Normalizing target volumes

When dispensing patterns to a well plate, it may be advantageous to normalize the volume dispensed to each target well. The **Normalize** feature will calculate the volume required to normalize total target volume for a group of targets.

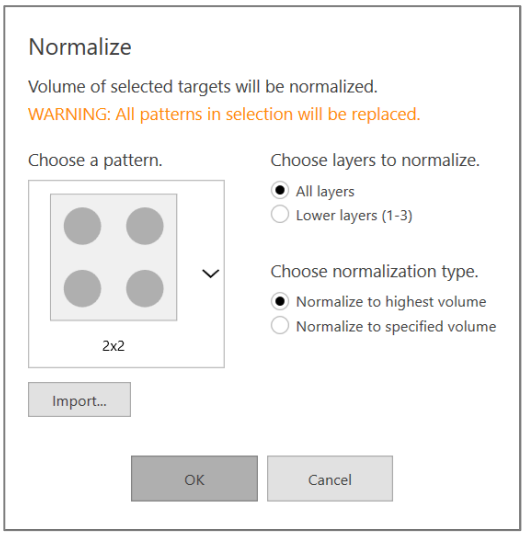
To normalize target volumes:

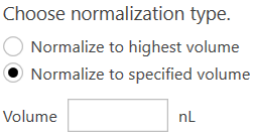
- Select the targets to be normalized. If there is no selection, all targets will be normalized.
- Choose Normalize from the protocol **EDIT** menu or the right-click context menu.





There are options in the Normalize dialog.





- The pattern to use for normalization fluid must be selected or Imported.
- Normalization volume can be calculated using all layers (above and below the current layer) or only using lower layers.
- Volume can be normalized to the highest total target volume or it can be normalized to a specified volume.

It is typical to add normalization on a new, empty layer. Any existing patterns in the selection will be replaced.

Note **Normalization** creates patterns with fixed pattern volume. If changes are made to other layers, the **Normalization** process may need to be repeated.

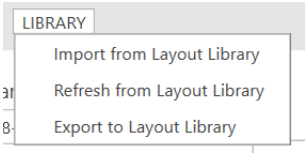
Chapter 9
Layout and pattern libraries

Libraries are groups of layouts or patterns that are stored on a specific computer and can be imported into protocols. When a layout or pattern is imported into a protocol, a separate copy is created in that protocol. Editing the protocol copy will not change the library copy and vice versa.

You can view or edit the libraries by clicking the **Layout Library** or **Pattern Library** buttons from the **Starting view**.

Using a library layout in a protocol

The most common way a library layout is imported into a protocol is by selecting the layout from the New Protocol procedure (see **Creating a new protocol file**). However, a library layout can be imported into a protocol at any time by clicking **Edit Layout** and then selecting **Import from Layout Library** from the LIBRARY menu.



If a layout in the Library has been modified, **Refresh from Layout Library** can be used to re-import the updated layout into the protocol.

Exporting a protocol layout to the library

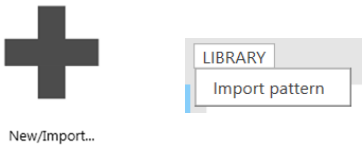
You may want to export a protocol layout to the library for use in other protocols, either because an existing library layout has been modified or a new layout has been created.

To export the protocol layout, click **Edit Layout** and then select **Export to Layout Library** from the LIBRARY menu. If a layout with the same name is already in the library, you will be prompted to confirm that you want to replace (update) the existing library layout.

Adding a pattern to a protocol

Library patterns can be imported while using the **Apply Pattern** feature (see **Applying fluid patterns**). However, patterns can also be imported into a protocol at any time by clicking **Manage Patterns** and then using the **New/Import** button or selecting **Import pattern** from the LIBRARY menu.

Patterns can also be imported from another protocol in the same manner.

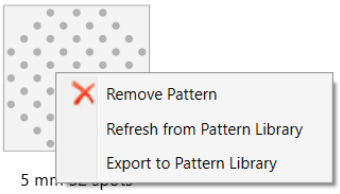


If a pattern in the Library has been modified, click **Manage Patterns** and right-click on a pattern. From the context menu, select **Refresh from Pattern Library** to re-import the updated pattern into the protocol.

Exporting a protocol pattern to the library

You may want to export a protocol pattern to the library for use in other protocols.

To export a protocol pattern, click **Manage Patterns** and right-click on a pattern. From the context menu, select **Export to Pattern Library** from the LIBRARY menu. If a pattern with the same name is already in the library, you will be prompted to confirm that you want to replace (update) the existing library pattern.



If a pattern in the Library has been modified, **Refresh from Pattern Library** can be used to re-import the updated pattern into the protocol.

You can also **Export** or **Refresh** a pattern from the LIBRARY menu while editing a protocol pattern.

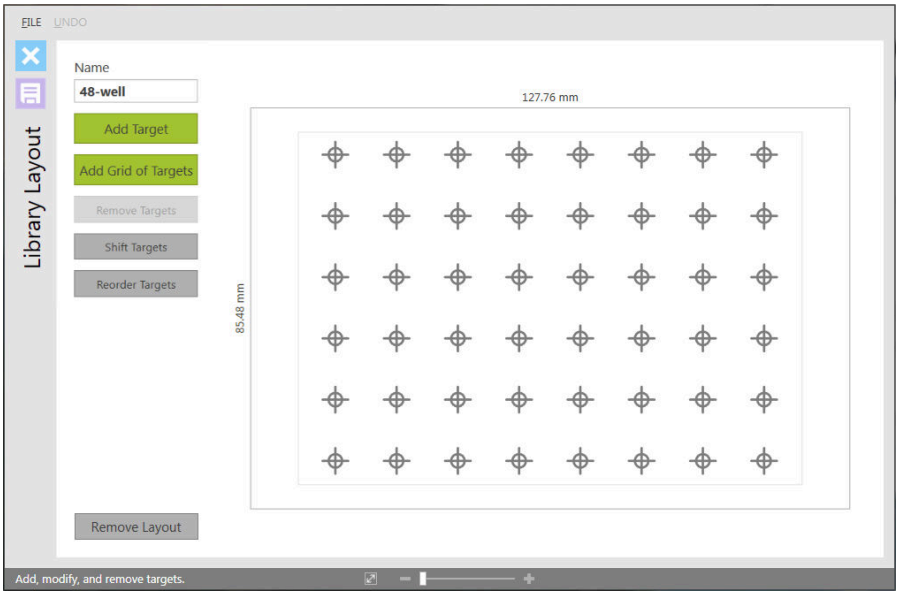
Removing a layout or pattern from the library

From the library view, right-click on a layout or pattern and choose **Remove Layout** or **Remove Pattern** from the popup menu. Alternately, you can click the **Remove** button at the lower-left while editing a layout or pattern.

Chapter 10
Editing a layout

View **library layouts** by clicking **Layout Library** from the **Starting view**. Click on a layout to edit the layout, or click the **New** item to create a new layout.

New...



Edit the **protocol layout** from the **Protocol view** by selecting **Edit Layout** from the protocol EDIT menu or right-click context menu. The same layout is used for all layers in the protocol.

Editing a protocol layout differs somewhat from editing a library layout:

- A layout **Name** is only required when exporting to the library.
- The menu includes a LIBRARY section for import or export (see **Layout and pattern libraries**).
- There is no **Remove Layout** button.
- The FILE menu does not include **Save As**.

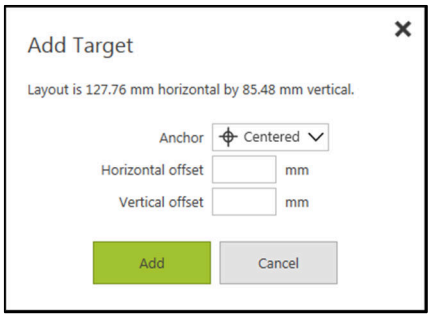
Saving a layout

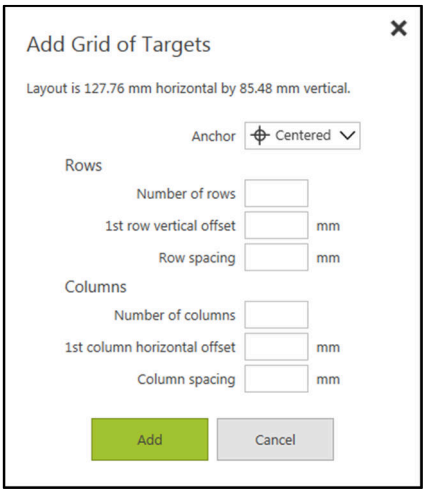
Click the button at the left edge to save a layout. You can also select **Save** or **Save As** from the FILE menu.

Note A protocol layout is not saved permanently until the protocol itself is saved.

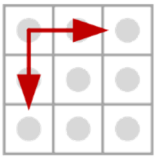
Adding targets

To add a single target, click **Add Target**. To add a grid of targets, click **Add Grid of Targets**.



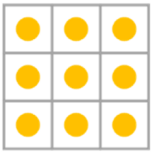


Anchor specifies how patterns will be aligned to the targets, and can be either **Centered** or **Upper-Left**.



Note When patterns are aligned Upper-Left, they align with the center of the upper-left spot.

The anchor type is significant when applying fluids to the same target location on different layers using different patterns. For example, if you have two simple patterns with different sizes, but the same pattern spot spacing:



When aligned on the same target, the resulting pattern alignment is quite different when the layers are viewed together. Interactions are even more complex when pattern spot spacing is different for the layered patterns.

Centered target anchor

Upper-Left target anchor

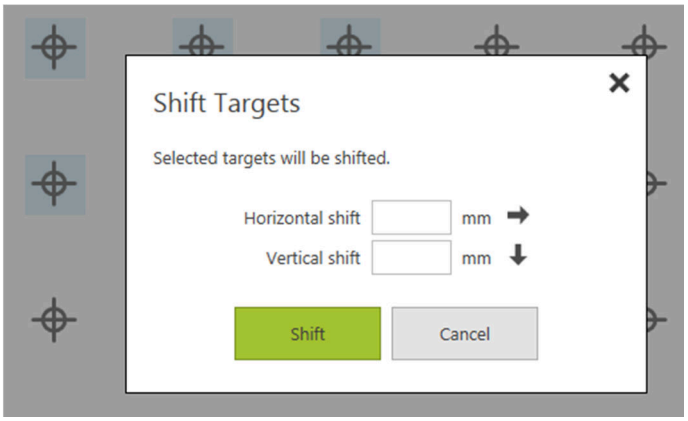
Horizontal and Vertical offsets are distances from the edge of the dispense region.

Removing targets

Select a group of targets, then click **Remove targets** (or press the Delete key).

Shifting targets

When working with a layout, it may be necessary to move a group of targets. Select a group of targets, then click **Shift Targets**.

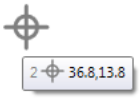


Selected targets can be moved in a positive or negative direction horizontally and vertically.

Do not use Shift Targets to correct for device alignment issues (see **Alignment**).

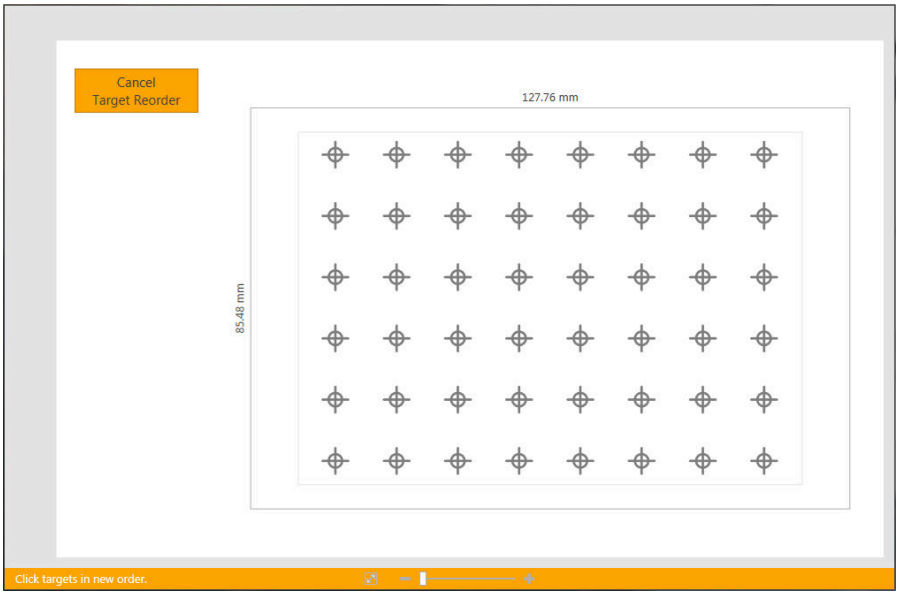
Target numbers

Each target is assigned a unique sequential target number when it is created. You can see the target number in the tooltip when hovering over a target.



Target number is 2

Target numbers are used in reports, and are also used to determine pattern dispense order if "By target number" is selected (see **Dispense order**). Target numbers can be changed by clicking **Reorder targets**. The target reorder view is displayed.



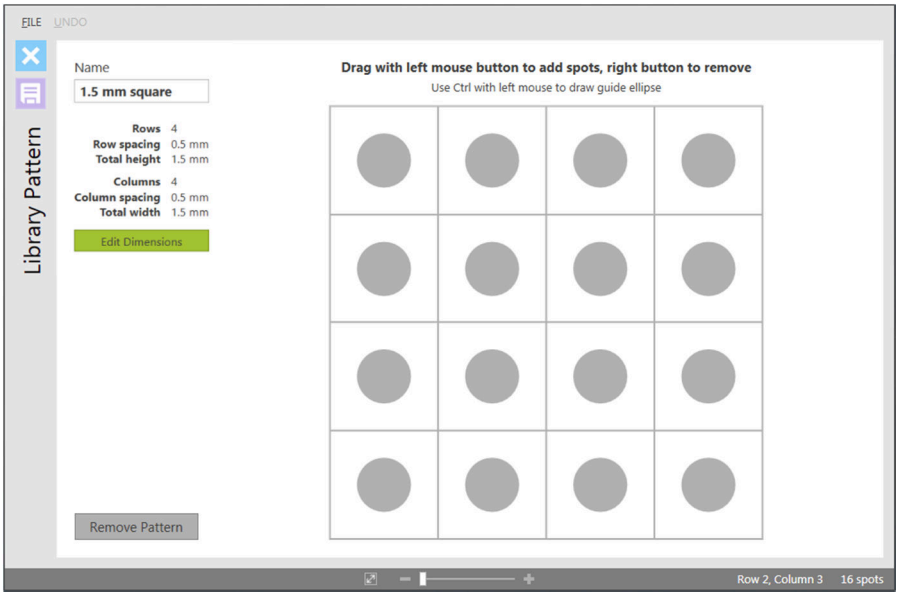
Each target in the layout must be clicked in the desired order. When all targets have been ordered, the target reorder view is automatically exited. You can cancel the target reorder by clicking the **Cancel Target Reorder** button.

Note Dispense order may be adjusted if patterns with different volume/spot are applied on a layer. To ensure optimal dispensing, a pattern with the largest volume/spot will always be dispensed first.

Chapter 11
Editing a pattern

View **library patterns** by clicking **Pattern Library** from the **Starting view**. Click on a pattern to edit the pattern, or click the **New** item to create a new pattern.

New...



View **protocol patterns** by clicking **Manage Patterns** from the **Protocol view**. You can also click **New Pattern** to create a new pattern.

Editing a protocol pattern differs somewhat from editing a library pattern:

- A new pattern will not require a **Name** until it is saved.
- The menu includes a LIBRARY section for import or export (see **Layout and pattern libraries**).

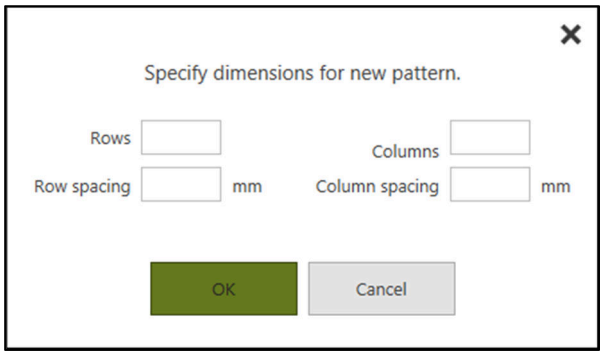
Saving a pattern

Click the button at the left edge to save a pattern. You can also select Save or Save As from the FILE menu.

Note A protocol pattern is not saved permanently until the protocol itself is saved.

Pattern dimensions

When creating a new pattern, the dimensions must be specified.



You can also edit the dimensions of an existing pattern by clicking the **Edit Dimensions** button.

When designing a pattern, **Best practices** for spot placement should be considered.

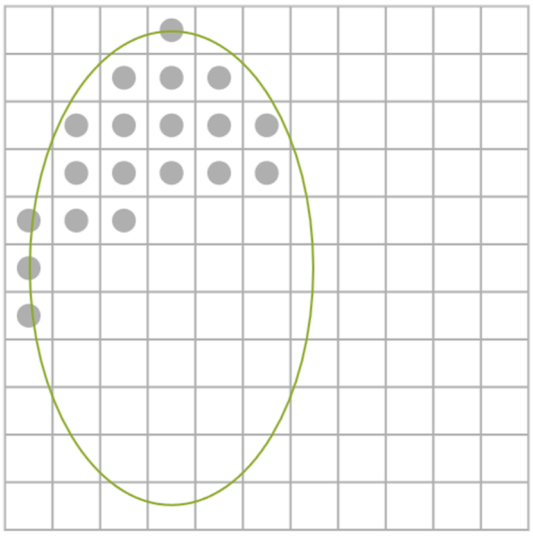
Changing the spot pattern

Use the left mouse button to click or drag and add spots.

Use the right mouse button to click or drag to remove spots.

Ellipse guide

Use Ctrl with the left mouse button and drag to draw an ellipse guide. This can be used as a visual reference when drawing patterns.

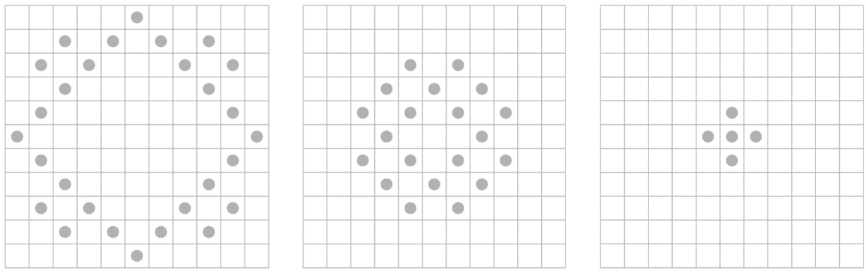


Chapter 12
Examples

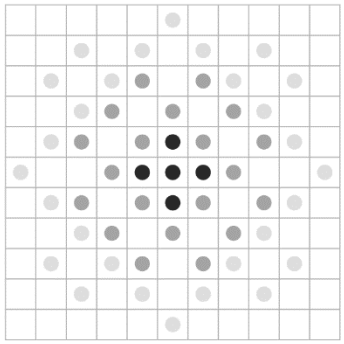
Creating a circular gradient dispense pattern

It is possible to create a vertical or horizontal volume gradient across pattern spots using Volume gradient across spots within patterns. However, if it is desired to create a gradient that varies from the center of the targets outward, the gradient must be created manually.

First, create a group of patterns with the same dimensions but different spots. An easy way to do this is to create the first pattern, then use Save As (see **Saving a pattern**) to create similar patterns.



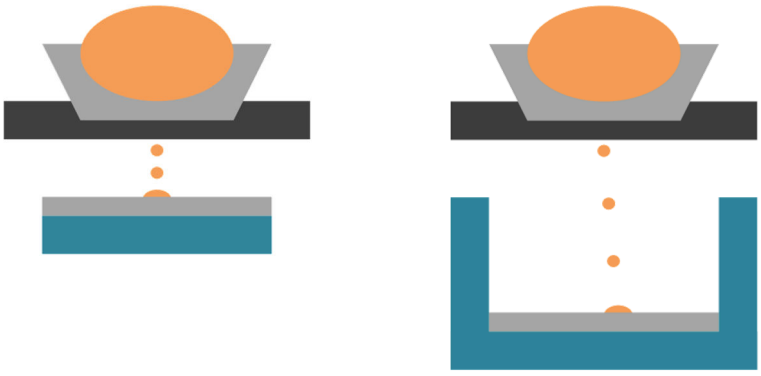
In this example, 3 layers are used. Each pattern in sequence would be applied to the same target location on sequential layers using a different volume/spot for each layer. The combined result is a pattern with high volumes in the center.



Chapter 13
Best practices

Dispensed spot alignment

The dispensing stage accuracy is within 125 µm, however, total dispense accuracy is affected by surface holder tolerance and cassette variation. The location of dispensed spots will typically be within 250 µm of the targeted location under best practice conditions, where the distance between the cassette and the dispense surface is minimized. Best practice examples are dispensing onto flat surfaces such as microscope slides, or onto the top of a well plate lid. When dispensing into recessed surfaces, such as into deep well plates, placement variation will be greater.

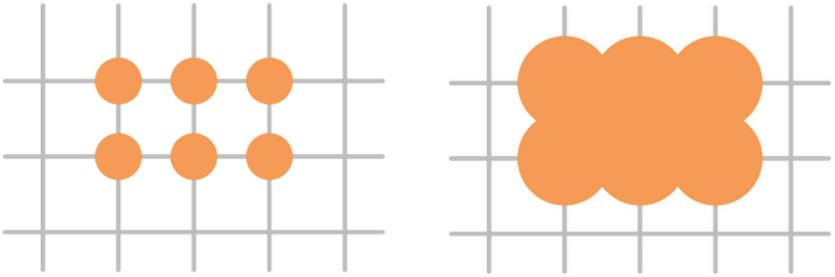


Best practice **Not optimal**

Note that the 250 µm guidance depends on good **Alignment** and accurate positioning of the targeted surface relative to the dispensing system.

Spot size and surface interaction

Spot size is a function of the interaction between dispensed fluid and target surface, as well as the volume of the fluid dispensed. Large volume spots dispensed with small pattern spacing will not necessarily remain discreet.

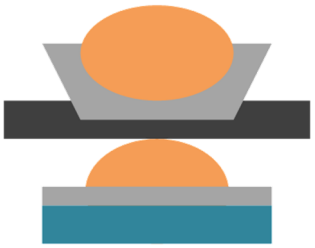


Discrete **Not discrete**

Note Spot size is dependent on the fluid dispensed and the interaction between the fluid and the dispense surface. While this interaction can be greatly influenced by the surface, some typical spot size numbers are:

- 30 pL (0.03 nL) aqueous spot on a clean glass surface is about 50 µm diameter.
- 1 nL aqueous spot on a clean glass surface is about 300 µm diameter.

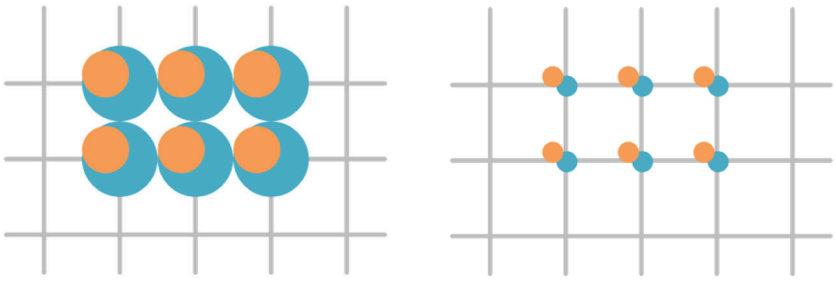
When dispensing larger volumes onto non-wetting surfaces, the spotted fluid may bead up, and in extreme situations (100s of nL or greater) the spot height may be large enough for the spot to touch the bottom of the cassette or the instrument deck. The amount of fluid needed for this to happen is dependent on fluid-surface interaction. If this occurs, one solution is to place the dispense surface into a slightly recessed fixture that will increase the spacing between the surface and the cassette.



Large volume spots

Multiple fluids onto same spot location

When dispensing multiple fluids to the same location, variation in spot placement may not ensure overlap between the fluids. In this case, increasing the volume per spot can help ensure overlap.

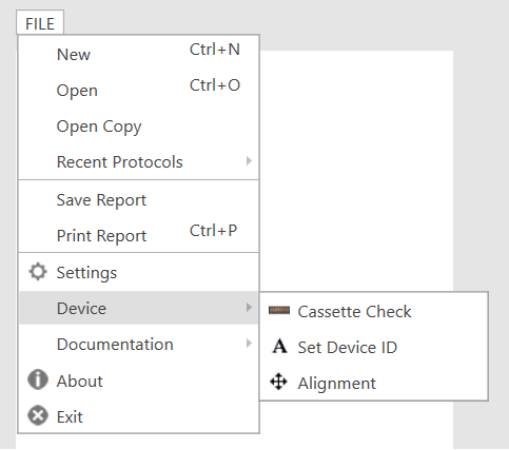


Good overlap

Less overlap

Chapter 14
Device utilities

The **FILE** menu in the Startup and Protocol views provides access to several Device utilities.



You must have a device connected to use these features.

Cassette Check

You can check to see which dispenseheads are available on a cassette by pressing the **Cassette Check** button. From the dialog box press Check to test a cassette and display results.

Set Device ID

Set Device ID allows you to give a unique name to the device which is included in reports run on that device.

Alignment

Alignment is used to adjust the precise position for a specific device. An example protocol is provided that will dispense a single spot in the center of corner wells on a 384 plate to assist with alignment verification. See the A1 alignment procedure in the **Multidrop PicoIT Software User Guide** for more information.

Chapter 15
Running a protocol

Refer to Running a protocol in the **Multidrop PicoIT Software User Guide** for detailed information about running a protocol.

Running a Multidrop PicoIT Patterning protocol is the same as running a standard Multidrop PicoIT Software protocol with a few exceptions.

Start a protocol run

Press the button at the left edge to run the protocol. You can also press F5 to run the protocol.

Saving a report

Select **Save Report** from the FILE menu to save a report from the most recent protocol run.

Printing a report

Select **Print Report** from the FILE menu to print a report from the most recent protocol run.

Plate load

Since Patterning protocols only support a single plate (or dispense surface), you will only be prompted once for plate load.

Layers

The software will automatically start with the lowest layer (Layer 1) and dispense that entire layer before dispensing the next layer. The visualization of the plate shows the current layer being dispensed.

Dispense region limitation

Pattern spots outside the dispense region (see **Layouts**) will not be dispensed. The initial run windows will display a warning if spots are outside of the dispense region.

Recommended dispense spot volume

When preparing for a protocol run, a Dispense Warning may be displayed indicating that at least one spot per layer is recommended at a minimum value for optimum performance.

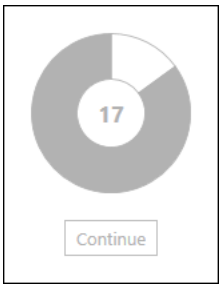
One way to avoid this warning is to add a single, higher-volume "junk" spot to a layer.

Select a target and use the **Applying fluid patterns** method, applying the One Spot pattern (or another pattern) with a volume/spot at or above the recommended value. If it is not appropriate to use an existing

target location, add a target to any location in the dispense region by editing the protocol layout (see **Editing a layout**).

Delay after layer dispense

If **Delay after layer dispense** has been set for a layer, the protocol run will pause and a countdown popup displayed. If **Continue** is pressed, protocol dispense will immediately move to the next layer.



Chapter 16
Reports

Report file formats, and much of the report itself is similar to the reports for Multidrop PicoIT Software protocols (see the Reports appendix in the **Multidrop PicoIT Software User Guide**). **Summary**, **Fluids**, and **Event** sections are almost identical to Dispensing software reports.

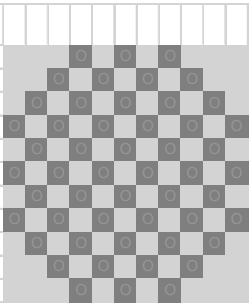
Excel Compatible XML

These sections are new or significantly different from Multidrop PicoIT Software reports.

Patterns

The **Patterns** tab shows information for all patterns in the protocol. A grid representation of the pattern is also included.

Pattern name	Rows	Columns	Row Spacing	Column Spacing	Spots
0.5D5.5-52	11	11	0.5	0.5	52



Layers

The **Layers** tab shows information for all layers in the protocol. The first section shows each layer, the fluid dispensed on that layer, and the dispense start and end times.

Layer	Fluid name	Start time	End time
2	Fluid 1	11:09:08.4	11:09:09.9
1	Test Fluid	11:09:07.9	11:09:08.2

The second section shows detail for each pattern applied to each layer; the target number and pattern name is included with alignment and location.

Layer	Fluid name	Target	Pattern name	Alignment	Horiz mm	Vert mm
1	Test Fluid	2	One Spot	Centered	12.13	8.99
1	Test Fluid	25	One Spot	Centered	115.63	8.99
1	Test Fluid	362	One Spot	Centered	12.13	76.49

The **Target** number can be used to correlate information on the **Layers** tab with detailed spot information on the **Tabular Detail** tab.

Tabular Detail

The **Tabular Detail** tab show dispense detail for all spots in all patterns on all layers.

For each spot, the target number, pattern name, and spot number are included with spot location. Fluid information for each spot is also

indicated with the specific cassette and dispense head used for the spot dispense. Dispense start time and dispensed volume are indicated for

Layer	Target	Pattern name	Spot	Horiz mm	Vert mm	Fluid	Fluid name	Cassette	Dispense head	Start time	Dispensed volume	Volume units
1	2	One Spot	1	12.13	8.99	1	Test Fluid	1	1	11:09:07.9	50	nL
1	25	One Spot	1	115.63	8.99	1	Test Fluid	1	1	11:09:07.9	50	nL
1	362	One Spot	1	12.13	76.49	1	Test Fluid	1	1	11:09:08.2	50	nL
1	385	One Spot	1	115.63	76.49	1	Test Fluid	1	1	11:09:08.2	50	nL
1	80	2mm square	1	38.38	21.74	1	Test Fluid	1	1	11:09:07.9	50	nL
1	80	2mm square	2	38.88	21.74	1	Test Fluid	1	1	11:09:07.9	50	nL
1	80	2mm square	3	39.38	21.74	1	Test Fluid	1	1	11:09:07.9	50	nL
1	80	2mm square	4	39.88	21.74	1	Test Fluid	1	1	11:09:08.0	50	nL
1	80	2mm square	5	38.38	22.24	1	Test Fluid	1	1	11:09:07.9	50	nL
1	80	2mm square	6	38.88	22.24	1	Test Fluid	1	1	11:09:07.9	50	nL
2	45	0.5D5.5-52	4	96.63	10.99	2	Fluid 1	1	2	11:09:08.6	2	nL
2	45	0.5D5.5-52	6	97.63	10.99	2	Fluid 1	1	2	11:09:08.6	2	nL
2	45	0.5D5.5-52	8	98.63	10.99	2	Fluid 1	1	2	11:09:08.7	2	nL
2	45	0.5D5.5-52	14	96.13	11.49	2	Fluid 1	1	2	11:09:08.6	2	nL
2	45	0.5D5.5-52	16	97.13	11.49	2	Fluid 1	1	2	11:09:08.6	2	nL
2	45	0.5D5.5-52	18	98.13	11.49	2	Fluid 1	1	2	11:09:08.7	2	nL
2	45	0.5D5.5-52	20	99.13	11.49	2	Fluid 1	1	2	11:09:08.7	2	nL

each spot.

If more than one dispensehead is used on a single spot, the **Dispense head** column will indicate "multiple."

Target Volumes

The **Target Volumes** tab shows the total volume dispensed at each target location on all layers as well as the total volume dispensed on all layers at each target location. This tab is most useful for verifying the result of **Normalizing target volumes**.

Dispensed volume (nL) Target	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5	Layer 6	Layer 7	All Layers
57				168				168
58			23.968	144.032				168
59				168				168
60				168				168
69				168				168
76	23.968			144.032				168
77				168				168
78	6.048			161.952				168
79				168				168
80				168				168
81				168				168
82			23.968	144.032				168
83				168				168
84				168				168
92				144.032			23.968	168
93				168				168
100	23.968			144.032				168

In this example, Layer 4 was used to normalize target volumes on all layers.

The **Layers** tab will indicate which pattern was dispensed at each layer target.

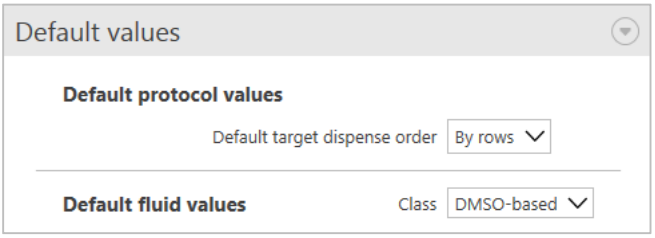
Chapter 17
Settings

In the Main Operating Window **File Menu**, click **Settings** to assign **application-wide** parameters and values.

- Each section is opened by clicking on the section title region. Some sections will scroll to access all settings.
- Edit the settings via specified drop-down menu choices, manual entry, check box/radio button, or browsing.
- Once you have updated the settings, click **OK** to save them or **Cancel** to ignore all changes made in the current Settings session.

Default values

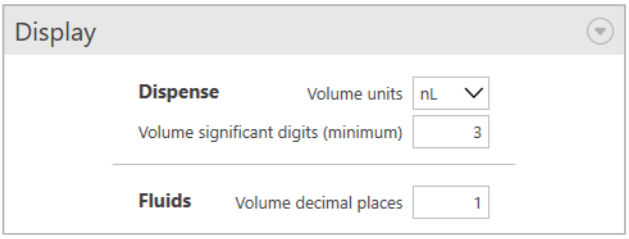
There are several values which are automatically filled in with defaults that may be changed. For instance, when you create a new protocol, the default settings are used. However, any of the values can be changed when you first set up a protocol or later on in the protocol preparation process.



Item	Measure	Choices
Default protocol values	Volume units for new protocols	Drop-down menu
	Target dispense order for new protocols	Drop-down menu
Default fluid values	Fluid class for new fluids	Drop-down menu

Display

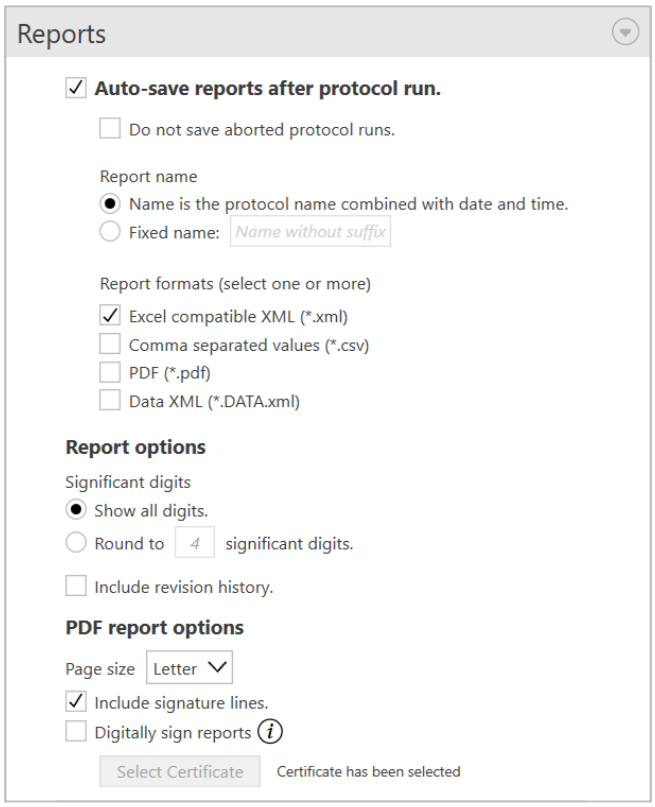
The display menu allows you to control how values are displayed

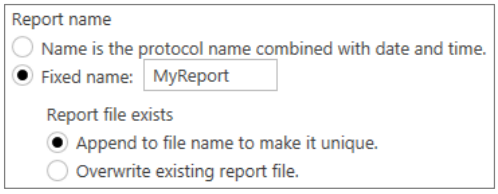


Item	Measure	Choices
Dispense	Volume units	Drop-down menu
	Volume significant digits	Manual entry – **2 to 8**
Fluids	Volume decimal places	Manual entry – **maximum of 4**

Reports

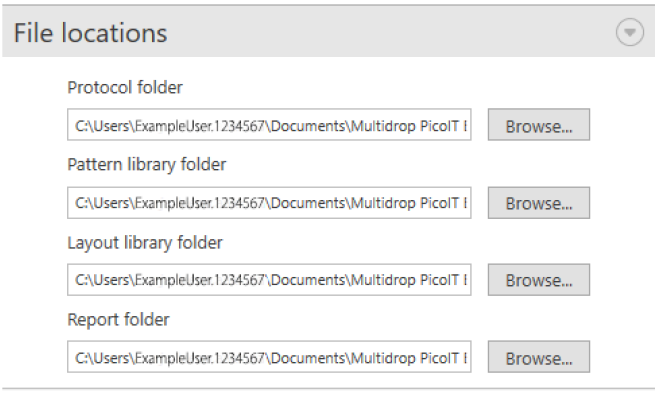
The reports menu allows you to set system defaults for report generation.



Item	Value
Auto-save reports after protocol run	If the box is ***un-checked***, reports must be manually saved after each protocol run.
	If the box is **checked**, you can set the auto-save report parameters and you will not be prompted for report save after each run. • **Do not save aborted protocol runs**: Check this box so that reports are not saved after an aborted run. • **Report name**: o **Name is the protocol name combined with date and time.** Click this to produce unique report names after each run. o **Fixed name.** Selecting this option will give the same name to each protocol report. Manually enter the desired report filename. Select whether a new file is saved with an appended name – e.g., ReportName(1) or if older files are overwritten.  • **Report formats:** Select one or more output formats.
Report options	• To round report data, click the **Round to** radio button and enter the number of significant digits. • Check to **include revision history** in reports.
PDF report options	• PDF reports can be written for Letter or A4 **page size**. • Check to **include signature lines** in PDF. • Check to **digitally sign reports** using a certificate. If checked, you can **select a certificate** to use for signing.

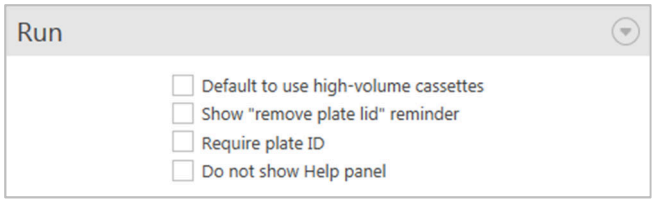
File locations

The file locations menu allows you to set system defaults for protocol and report folders.



Item	Value
Protocol folder **Pattern library folder** **Layout library folder** **Report folder**	Use the system-assigned defaults, or click the **Browse** button to search your computer hard drive and assign a different folder.

Run



Item	Choices
Default to use high-volume cassettes	The **Use high-volume cassettes** checkbox on the protocol Run screen will default to this setting.
Show “remove plate lid” reminder	If checked, a reminder will display each time a plate is loaded.
Require plate ID	If checked, a plate ID is required when the plate is loaded.
Do not show Help panel	If checked, the help panel will not be displayed.

Chapter 18
Appendix A – Dispense Specifications

Refer to *Appendix E – Dispense Specifications* in the **Multidrop PicoIT Software User Guide** for fluid class details. However, there are a few different specifications for the Patterning Software.

	DMSO dispensing	Aqueous dispensing
Minimum dispense volume	20 pL	28 pL