
Density Gradient Column Filler

Operating Manual



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Firmware version 5.03

H & D Fitzgerald Ltd.
www.density.co.uk

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1 Introduction

H&D Fitzgerald Ltd's microprocessor-controlled Density Gradient Column Filler allows you to build consistent and accurate density gradient columns repeatedly and quickly. Once the column has been filled, and a set of calibrated floats allowed to equilibrate in it, the instrument can calculate the density of any other object in the column from its position.

Features of the column filler include:

- more accurate, and more consistent than other filling methods, resulting in significantly smoother gradients than with conventionally filled columns;
- easy and automated filling;
- can use any two miscible liquids to give the lighter and heavier densities you need;
- can be set to work in either g/ml or kg/m³;
- column volume, and upper and lower densities, are fully programmable;
- can be set up to add variable buffer volumes at the top and bottom of the column;
- details of the previous filling routine are automatically stored for re-use in the next fill;
- can drive an optional column sweeping mechanism at variable speed.

1.1 Equipment also available

The following items are also available from the manufacturer:

- reference density gradient column floats with traceable calibration, produced in our ISO17025 accredited laboratory;

2 Warnings and safety precautions

This instrument has been made in accordance with European and International Regulations for the operation, electromagnetic compatibility, and safety requirements of electrical equipment. Improper use or handling could result in damage or injury.

Read these operating instructions thoroughly before you use your filler, to get the best performance out of your filler and to prevent damage to it.

To ensure safe and trouble-free operation:

- do not use this filler in a hazardous area;
- use a suitable mains lead;
- connect to a power supply of between 110 V (60 Hz) and 240 V (50 Hz) which has an earth. This appliance must be earthed;
- disconnect the mains lead from the power supply to switch the power off completely;
- The environmental conditions for temperature should be between 0-50°C, relative humidity should be between 0-95% and there should be standard atmospheric conditions.
- take care when using liquids close to the filler. The housing is designed to protect against liquid splashes, but it is not liquid tight.
- use a slightly moistened cloth to clean the outside of the filler. Make sure no liquid enters the filler housing while you are cleaning it.
- disconnect the instrument from the mains supply before connecting or disconnecting any tubes which could contain liquid.
- connect accessories only from H&D Fitzgerald Ltd that are designed to be used with the filler;
- contact H&D Fitzgerald Ltd if you have any problems with the filler—the address is given on the inside of the cover of this manual.
- if this equipment is used in a manner not specified by the manufacturer, protection provided by the equipment may be impaired.

3 Density gradient column techniques

To get the best performance from a density gradient column attached to H&D Fitzgerald's Density Gradient Column Filler, you must follow best practice in terms of the liquids used, preparation of the samples, temperature control, as so on. Even if you are experienced at using density gradient columns, we advise you read this chapter.

These tips apply when using any gradient column, and are in no way specific to this device.

3.1 Choice of liquids

There are a number of important factors to consider when choosing pairs of liquids, including: density; miscibility; shrinkage; effect on the sample; and safety.

The liquids chosen should be colourless, if possible, so that samples can be seen more easily. In addition, they should have as low a viscosity as possible, since settling time for the samples is a function of viscosity.

Density

The liquids you use must have densities that cover the range your measurement requires.

The following table gives some examples of useful liquids and their density ranges.

mixture	density range (kg/m ³)	density range (g/ml)
2-propanol & water	785 — 997	0.785 — 0.997
2-propanol & di(ethylene glycol)	785 — 1130	0.785 — 1.130
ethanol & water & zinc chloride	790 — 1700	0.790 — 1.700
water & sodium bromide	997 — 1400	0.997 — 1.400
water & calcium nitrate	997 — 1600	0.997 — 1.600
water & caesium formate	997 — 2350	0.997 — 2.350
ethanol & tetrachloroethylene	790 — 1620	0.790 — 1.620
toluene & tetrachloroethylene	870 — 1620	0.870 — 1.620
tetrachloroethylene & 1, 3-dibromopropane	1620 — 1985	1.620 — 1.985
1,3-dibromopropane & 1,2-dibromoethane	1985 — 2180	1.985 — 2.180
tetrachloroethylene & bromoform	1620 — 2890	1.620 — 2.890
1,2-dibromoethane & bromoform	2180 — 2890	2.180 — 2.890

In some circumstances, it will be necessary to use a three component mixture, normally because the sample is hydrophobic, and cannot be wetted by water. An example of such a system is the use of 8% of 1-methyl-2-pyrrolidinone in water/sodium bromide columns when testing certain plastics.

Miscibility

For the density gradient column to operate successfully, the two liquids must be completely miscible in the proportions in which they are going to be present in the column.

To test the miscibility of your liquids, try mixing small amounts of the two liquids in a beaker and check that they still form an homogeneous mixture after standing for an hour.

Shrinkage

In an ideal world, if we added a litre of water to a litre of 2-propanol (isopropyl alcohol) we would have a total volume of two litres, and the density of the mixture would be the average of the densities of water and 2-propanol. Unfortunately when we mix most pairs of liquids, the resulting volume is somewhat less than we might have predicted—shrinkage has occurred and the density of the mixture is higher than the average density (Some pairs of liquid expand when mixed, but they are unlikely to be used in density gradient columns).

When we mix two aqueous salt solutions, or water and an aqueous salt solution, such as sodium bromide or calcium nitrate, the amount of shrinkage is usually so low that it can be ignored for all but the very highest precision work (at a level unlikely to be needed in an industrial plastics laboratory).

On the other hand, when we mix water and 2-propanol, or two different strength solutions of 2-propanol in water, then considerable shrinkage will occur, and the density of the mixture will be higher than we might have predicted. This must be accounted for when programming the column filler.

The effect of shrinkage in water and 2-propanol mixtures can be mitigated in two ways: either by applying a mathematical correction to the parameters as entered into the Density Gradient Column Filler (see [Appendix A Shrinkage in 2-propanol / water mixtures](#)); or by pre-mixing solutions before using them with the Density Gradient Column Filler (see [Appendix B Preparing a 2-propanol / water column - worked example using pre-mixed solutions](#)).

Effect of the liquids on test specimens

The two liquids or solution in the gradient column must not alter the test specimens by chemical reaction or by absorption, otherwise there will be errors in determining the density of the sample being tested. Many plastics are affected by the liquids used in gradient columns, and—especially when testing thin films which have a high surface area to volume ratio—even slight water absorption can be sufficient to give slightly erroneous results.

To test if the sample is stable in the chosen liquid mixture, take a sample the same size as would be tested in the column; weigh it; soak it for 2 hours in a beaker containing some of the mixture; wipe dry; and then reweigh. If there is any change in weight after soaking, it is reasonable to assume that the sample is not stable in this mixture, and other liquids should be used in the column.

Safety

Many of the liquids suitable for use in density gradient columns are flammable, toxic, or both, so safety is a major concern when selecting a suitable combination.

Reduce the risk by choosing the least hazardous options, for example, methanol is more toxic than 2-propanol.

Carry out a risk assessment of the liquids you plan to use and implement the appropriate precautions.

3.2 Hints for filling the column

Removing dissolved air

One of the main sources of error when using density columns is dissolved air in the liquids. This will tend to come out of solution as micro bubbles which adhere to the test specimens and to the density floats. This increases their buoyancy, and therefore produces inaccurate calibrations and results.

The first step is therefore to de-air the starting solutions using degassers, boiling, or any other suitable method. Once you have de-aired the liquids you must minimize their exposure to the atmosphere to avoid allowing air to be taken back into solution. It is important to store de-aired liquids in gas tight containers, and to use them as soon as practicable after production to reduce the risk of reabsorption of air.

It is often easiest to de-air an aqueous solution by bringing it to a rolling boil under total reflux, and then immediately transferring it to a bottle chosen to minimise the air space above the liquid, securing the cap, and allowing it to cool. Suitable care must be taken to ensure safety when boiling liquids, especially flammable ones such as 2-propanol.

Experience with HPLC degassers suggests that they normally work well, but are rather slow and are relatively expensive. They work by passing the liquid through a tube made of gas-permeable membrane which is surrounded by a vacuum.

Trials using ultrasonic agitation to remove air have been relatively unsuccessful, with a large amount of air remaining dissolved in the solution.

Once the liquids have been de-aired, do not agitate them or stir them vigorously, as this will only encourage air to re-enter solution.

Before you start to fill the column, bring the liquids to within a couple of degrees of the column temperature, and check their densities with a density meter, hydrometer or pycnometer. Having the liquid temperature close to the column temperature minimizes mixing caused by convection currents during filling.

3.3 Sample preparation

Preparing samples carefully is a crucial part of density measurement. If your sample preparation is poor you will produce unreliable results—no matter how good your density column is. For process control it is important that there is a consistent approach to sample preparation.

Key points to pay attention to include:

- Samples must not contain voids. Trapped air in the sample increases buoyancy, which in turn will result in a lower density determination.
- Any conditioning procedures must be adhered to.
- Samples should not be handled, as grease from the skin might affect the density and will encourage bubbles to adhere to them. Use gloves or forceps.
- Samples must be thoroughly wetted with the lighter liquid before being placed into the column. It can be helpful to add a few drops of surfactant to the wetting liquid in order to discourage the adherence of bubbles.
- Samples should be as small as reasonably possible, since the smaller they are, the easier it is to estimate their centre when measuring their height in the column. The optimum size is typically between 2 and 6 mm long. Using small samples also minimizes the gradient deterioration which can occur when large samples fall quickly through the column, causing mixing.
- Outer surface needs to be smooth, so that bubbles of air are not trapped on the surface.

3.4 Introducing samples to the column

The sample must be carefully immersed in the top of the gradient column; a check made to ensure that there are no bubbles clinging to it; and then gently released.

Do not drop samples into the column: this is a sure way of causing mixing and destroying the gradient.

3.5 Determining when samples have come to rest

Samples can take quite a few hours to reach their final level in the column, especially if they have pores on the surface which initially contain light liquid, since this will slowly equilibrate with the liquid in the column by diffusion. On the other hand, if the samples are in the column for too long before their densities are read, they may absorb some of the liquid, and so change density. Only experience will allow you to decide at what stage a sample should be regarded as having reached its final position.

If the sample touches another sample or a calibration float as it descends through the column, it is very unlikely to reach its correct height. Samples can sometimes be separated by very slowly lowering a fine straight wire into the column to dislodge them.

3.6 Extending the life of your gradient column

The lifetime of a density column is partly dependent on the number and size of test pieces in the column when the column is swept. To allow a density column to be reused a number of times, it can be swept by very slowly raising a wire mesh basket up through the gradient, and capturing all the density floats and test specimens. The test specimens are discarded, and then the basket and density floats are lowered slowly down the column again. Although this procedure will always result in some deterioration of the gradient column, mixing can be minimized by having the basket travel as slowly as possible.

Even though sweeping causes some damage to the gradient, it is important not to wait until too many samples have gathered before you sweep, otherwise the open area of basket mesh fills with samples on its travel and acts more like a piston, and so destroys the gradient completely.

3.7 Temperature control

Like a Galileo thermometer, samples and density floats will shift in the column with temperature.

When the temperature rises, the solution in the column expands slightly and becomes less dense: the samples and density floats, on the other hand, have a minimal change in density with temperature, and so will settle further down the column.

If the temperature drops the opposite will occur, with the samples and floats rising up the column. For this reason, a column is normally held at a constant temperature in a water bath—typically 23°C for the plastics industry. Convection currents can quickly form in a column not held at a constant temperature, or which is subjected to heat from other equipment. These mix the column, and destroy the gradient. For the same reason, it is important that the liquids added to the column are both at the same temperature, and preferably at the column's operating temperature.

If the temperature of a density gradient column is only controlled to within $\pm 0.5^\circ\text{C}$, then there is the possibility that two consecutive calibrations are carried out at temperatures 1°C apart. This would result in a shift for all the density floats and samples, and would produce incorrect results. The extent of the error depends on the height and range of the column. To avoid this, you must either recalibrate the column before determining any density measurements, or gain tighter temperature control on the system. This is often achieved by surrounding the column with a constant temperature water jacket. Research columns are typically held within $\pm 0.02^\circ\text{C}$.

The temperature for polymer density measurements is usually 23°C, so the floats used for density measurement at 23°C must themselves be calibrated at 23°C.

If a float is to be used for density measurement at a different temperature ($t^\circ\text{C}$) than it has been calibrated for, a correction must be made to the float's density, as follows (where 0.028 is the expansion coefficient of glass):

$$\text{float density}_{t^\circ\text{C}} = \text{float density}_{23^\circ\text{C}} + ((23 - t) \times 0.000\,028) \text{ g/ml}$$

or

$$\text{float density}_{t^\circ\text{C}} = \text{float density}_{23^\circ\text{C}} + ((23 - t) \times 0.028) \text{ kg/m}^3$$

For example, if you need to operate your density gradient column at 6°C, but only have floats calibrated at 23°C, and one of these floats has a certified density of, say, 1.0199 g/ml at 23°C, we can use the formula above to calculate its density at 6°C, as follows:

$$\begin{aligned}\text{float density}_{t^\circ\text{C}} &= 1.0199 + ((23 - 6) \times 0.000\,028) \text{ g/ml} \\ &= 1.0204 \text{ g/ml}\end{aligned}$$

As a second example, if you need to operate your density gradient column at 33°C, but only have floats calibrated at 23°C, and one of these floats has a certified density of, say, 1562.5 kg/m³ at 23°C, we can use the formula above to calculate its density at 33°C, as follows:

$$\begin{aligned}\text{float density}_{t^\circ\text{C}} &= 1562.5 + ((23 - 33) \times 0.028) \text{ kg/m}^3 \\ &= 1562.2 \text{ kg/m}^3\end{aligned}$$

4 Preparing the column filler for use

Before you can use your column filler for the first time you must:

- ensure that you position the filler in a suitable place; see [4.2 Operating conditions](#)
- fit the tubing;
- switch the filler on;
- enter the volume of the tubing;
- enter your column parameters.

As the column filler is delivered, it will already have the pump calibration parameters stored in its memory. However, when you fit your tubing; you will need to recalibrate the internal pumps. Calibrate the pumps prior to use, see [5.2 Calibrating the pumps](#).

4.1 Description of the parts

The Density Gradient Column Filler has connectors on the back of the housing, and an operating panel on the front. You should familiarise yourself with what the various parts are called before attempting to use the column filler.

Note: the terms inlet and outlet are relative to the filler: inlet means from the stock bottles to the filler, and outlet means from the filler to your column.



View of Density Gradient Column Filler



View of Density Gradient Column Filler back panel

4.2 Operating conditions

The filler provides reliable column filling under normal ambient conditions. Bear the following points in mind when choosing where to place the filler:

- the filler must be operated on a solid even surface;
- ideally the stock bottles, column, and filler should be at the same height to prevent bubble formation, which would affect the measured volumes being pumped;
- the filler must not be sited near a heater or air conditioning unit;
- the filler should be situated within 1 metre of the column—if possible;
- there should be room for the bottles of light and heavy liquid, and the waste bottle, to be placed safely near the filler.

See [3.7 Temperature control](#) for more about the importance of controlling the temperature of density gradient columns.

4.3 Fitting the tubing

The filler is supplied with 2 x 400 mm peristaltic grade Viton tubing and an additional 5 m of standard quality Viton tubing. Use the peristaltic grade Viton tubing in each pump. See page 12-13 for tubing configuration.

Note: If the tubing is seated correctly the pump will run smoothly. If the tubing is seated incorrectly then stop the immediately and reset the tubing.



Lift the spring loaded pump lids.



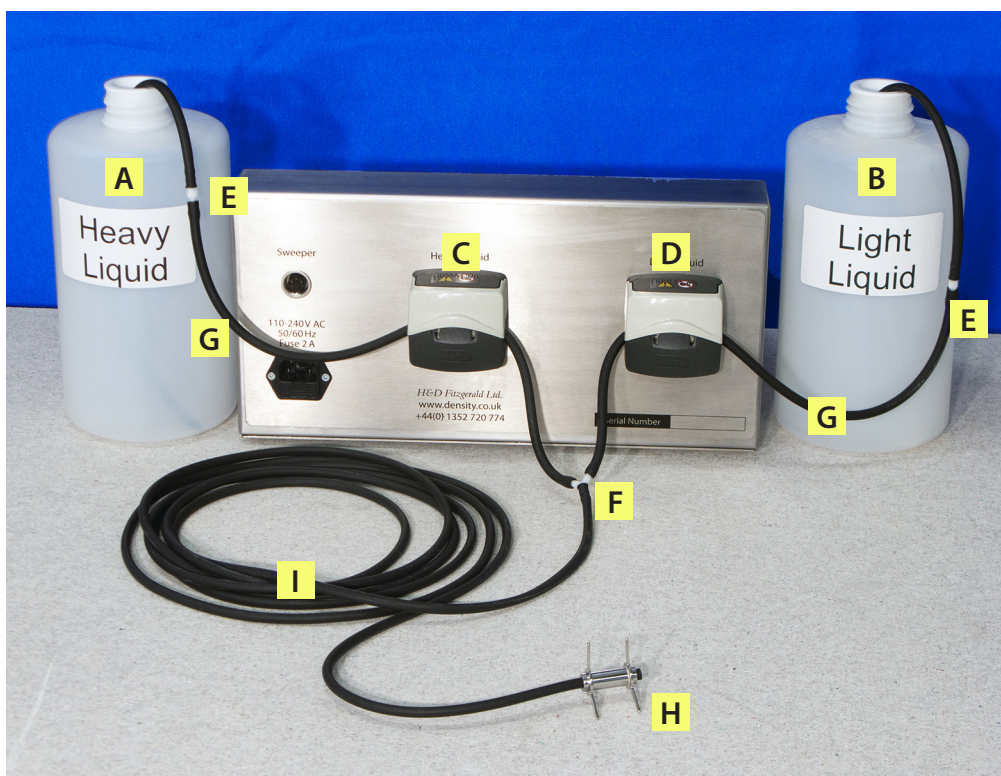
Lay the peristaltic tubing over the rollers and ensure it fits into the recesses on either side prior to securing. Ensure the tube length between the Y connector and the pumps is equal both side

Preparing the column filler for use



Press down the spring loaded pump lid to secure the tubing in place.

Column Filler tubing configuration



Column Filler tubing configuration.

- A** Heavy Liquid stock bottle (not supplied)
- B** Light Liquid stock bottle (not supplied)
- C** Heavy Liquid pump
- D** Light Liquid pump
- E** Tube barb straight connector
- F** Tube barb 'Y' connector
- G** Viton peristaltic grade pump tubing 2 x 400 mm
- H** Stainless steel outlet tube end
- I** Viton standard quality tubing 1 x 5 m

The filler is supplied with 2 x 400 mm lengths of peristaltic grade Viton tubing (**G**). The peristaltic grade Viton tubing for the heavy liquid pump (**C**) is labelled 'Heavy Liquid'. The peristaltic grade Viton tubing for the light liquid pump (**D**) is labelled 'Light Liquid'. The peristaltic tubing supplied has been calibrated to work with the column filler supplied. To fit the tubing into the pumps see 4.3 fitting the tubing.

The standard quality Viton tubing (**I**) comes fitted with a stainless steel outlet tube end (**H**). The standard quality Viton tubing (**I**) does not have the correct characteristics to work reliably in the peristaltic pumps.

Fit the two inlet ends of the peristaltic grade Viton tubing (**G**) into the tube barb 'Y' connector (**F**), shown in the picture opposite.

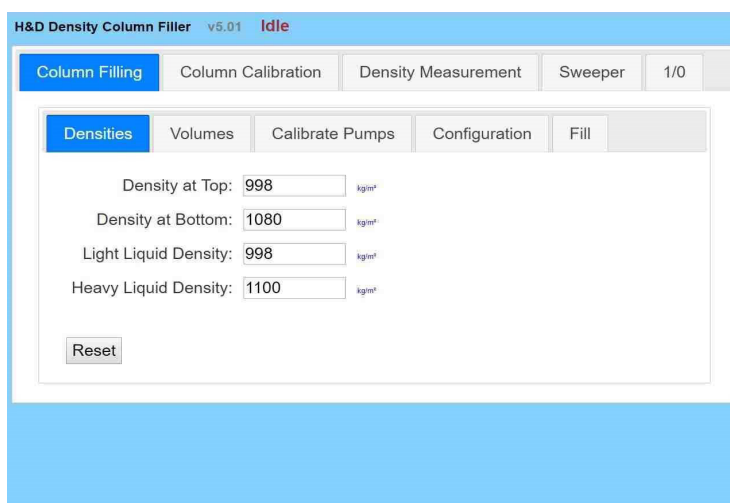
Cut the length of standard quality Viton tubing (**I**). This length needs to be long enough to reach from the bottom of the gradient column to the pumps. Connect the standard quality Viton tubing (**I**) to the bottom of the tube barb "Y" connector (**F**). Keep this length as short as reasonably possible.

Attach the tube barb straight connectors (**E**) to two cut lengths of standard quality Viton tubing (**I**) for delivering the light and heavy liquids (**A** and **B**) from the stock bottles to the peristaltic tubing (**G**).

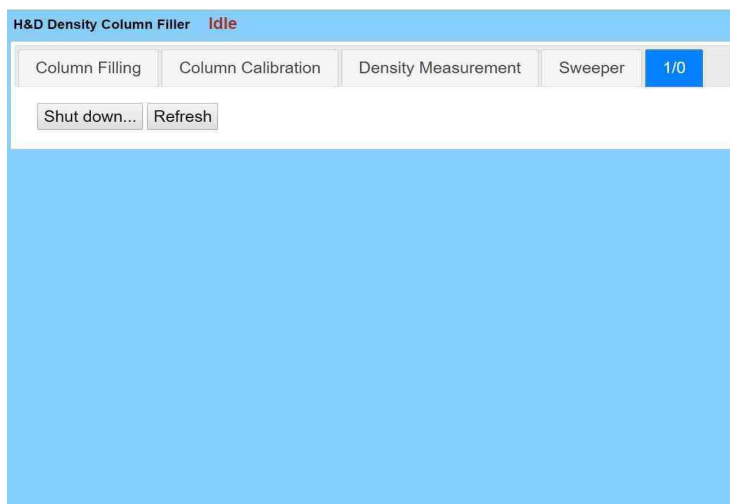
4.4 Switching the filler on and shutting the filler down

To switch on:

- push the IEC plug into the socket on the back of the instrument and insert the mains plug into the power supply;
- switch on the mains power—if applicable;
- press the on/off switch on the front of the instrument. After the start up screen has been shown, the main screen menu is displayed as below.
- to adjust the screen brightness tap the bottom middle of the display screen.



To shut down:



Select power tab (1/0) select Shut down, select OK to confirm shut down.

Note: The filler should be left for a minimum of one minute before switching on again, as the self-test screen will still be running.

4.5 Entering the volume of the tubing

This procedure should be followed before you first use your filler, and after changing the length of the inlet or outlet tubing.

For the filler to work correctly, the microprocessor must know what volume of tubing it has to flush before filling a column.

Note: if you notice air bubbles passing through the transparent connectors or appearing from the outlet tube towards the end of the flushing phase, you should increase the value entered as input tubing volume.

Using the Column Filler tubing configuration diagram on page 12 as a guide calculate the total tube volumes. The 4 mm diameter tubing has a volume of about 13 ml per metre of tube.

To ensure complete flushing and de-airing, use the following formulas:

$$\text{inlet tubing volume} = ((6 + ([A \text{ to } C + B \text{ to } D] \times 13)) \times 2$$

$$\text{outlet tubing volume} = ([C \text{ to } F + D \text{ to } F + F \text{ to } H] \times 13) \times 2$$

In both of the above calculations, the factor of 2 serves as a safety margin to ensure that both the inlet and outlet tubing receive a thorough flush. This helps to prevent cross contamination from previous fillings, and ensures that liquid of the correct density is dispensed as soon as column filling begins.

To enter the volume of the tubing:

H&D Density Column Filler v5.01 Idle

Column Filling | Column Calibration | Density Measurement | Sweeper | 1/0

Densities | Volumes | Calibrate Pumps | Configuration | Fill

Density at Top: 998 kg/m³

Density at Bottom: 1080 kg/m³

Light Liquid Density: 998 kg/m³

Heavy Liquid Density: 1100 kg/m³

Reset

Select Configuration.

Preparing the column filler for use

The screenshot shows the 'H&D Density Column Filler' software in 'Idle' mode. The 'Column Filling' tab is selected. The 'Configuration' sub-tab is active. The interface includes input fields for 'Light Pump Rate' (484.2 $\mu\text{L/hr}$), 'Heavy Pump Rate' (461.5 $\mu\text{L/hr}$), 'Inlet Tubing Volume' (empty), and 'Outlet Tubing Volume' (60 mL). The 'Density Units' are set to kg/m^3 , and the 'Filling Method' is set to 'From bottom'. A numeric keypad is visible on the right.

Enter the value you calculated into the Inlet Tubing Volume box

This screenshot is similar to the previous one, but the 'Inlet Tubing Volume' has been updated to 20 mL. The 'Outlet Tubing Volume' is now empty, and the cursor is flashing inside it, indicating it is ready for input.

Enter the value calculated into the Outlet Tubing Volume box.
Ensure the cursor is flashing in the value box to allow numbers to be entered.

The screenshot shows the 'H&D Density Column Filler' software interface. The top bar indicates the status is 'Idle'. Below the main menu, the 'Configuration' tab is selected. The interface includes several input fields for pump rates and tubing volumes, a dropdown for density units, and radio buttons for the filling method.

Parameter	Value	Unit
Light Pump Rate	484.2	$\mu\text{L/rev}$
Heavy Pump Rate	461.5	$\mu\text{L/rev}$
Inlet Tubing Volume	20	mL
Outlet Tubing Volume	60	mL
Density Units	kg/m ³	
Filling Method	<input type="radio"/> From top <input checked="" type="radio"/> From bottom	

If you want to switch between using kg/m³ and g/ml, select the Density Units drop down menu and select the desired units.

This is a duplicate of the screenshot above, showing the 'H&D Density Column Filler' software interface with the 'Configuration' tab selected. The parameters and their values are identical to the first screenshot.

Parameter	Value	Unit
Light Pump Rate	484.2	$\mu\text{L/rev}$
Heavy Pump Rate	461.5	$\mu\text{L/rev}$
Inlet Tubing Volume	20	mL
Outlet Tubing Volume	60	mL
Density Units	kg/m ³	
Filling Method	<input type="radio"/> From top <input checked="" type="radio"/> From bottom	

You can also select the filling method you desire here by selecting either From top or From bottom next to filling method at the bottom of the screen.

5 Filling the column

Use this procedure each time you need to create a new density gradient column.

If the column you are about to make is the same as the previous column, its parameters will be stored in the filler's memory.

Before you fill a column the following checks should be made:

- ensure the operating conditions are suitable, see [4.2 Operating conditions](#).
- ensure that the filler has been set-up in line with the instructions given in [4 Preparing the column filler for use](#).
- ensure that you have a set of calibrated density gradient column floats covering the density range you want to measure.
- ensure that the light and heavy liquids are at the same temperature, and preferably close to the operating temperature of the column.

5.1 Setting the column parameters

The column filler can be used with almost any liquids, but you will need to be able to measure their densities to ± 0.001 g/ml (1 kg/m^3). The two liquids must be completely miscible. See [3.1 Choice of liquids](#) for a discussion of some factors to consider when choosing liquids.

To program the filler you will need to know:

- the density that you want at the top of your column (allow for at least 2% of unused column above the point at which you expect to be making measurements);
- the density that you want at the bottom of your column (allow for at least 2% of unused column below the point at which you expect to be making measurements);
- the total volume of the column in millilitres;
- the volume of the layer (if any) of light liquid at the top of the column. This is known as the 'top buffer'. This is used in some situations to reduce column changes due to evaporation. However, tests have shown that the use of a top buffer can sometimes complicate the shape of the gradient: an alternative is to extend the gradient by 40 or 50 mm above the lightest density at which you expect to be taking measurements.
- the volume of the layer (if any) of heavy liquid at the bottom of the column. This is sometimes used where there is a particularly deep sweeping basket for collecting samples.
- the density of your heavy liquid. It must have a density which is the same as or greater than the density that you want at the bottom of your column.
- the density of your light liquid. It must have a density which is the same as or less than the density that you want at the top of your column.
- whether you intend to fill the column from the bottom or from the top. Unless there is good reason not to, it is recommended that filling from the bottom always be used as first preference.

Note: The volumes of the inlet and outlet tubing should not normally be altered, provided they have already been configured as per [4.5 Entering volume of tubing](#).

To set the appropriate values in the filler

Push the tube connected to light liquid pump to the bottom of the light liquid stock bottle.

Clip the tube to the bottle (clips are not provided).

Push the tube connected to heavy liquid pump to the bottom of the heavy liquid stock bottle.

Clip the tube to the bottle.

H&D Density Column Filler Idle

Column Filling | Column Calibration | Density Measurement | Sweeper | 1/0

Densities | Volumes | Calibrate Pumps | Configuration | Fill

Density at Top: kg/m³

Density at Bottom: kg/m³

Light Liquid Density: kg/m³

Heavy Liquid Density: kg/m³

[Help](#)

Select Densities tab. When you use the filler for the first time, the parameters will be those programmed in by the manufacturer. If you have previously used the instrument to fill a column, the parameters will be those for the last fill.

To change the column parameters:

The screenshot shows the 'H&D Density Column Filler' software in 'Idle' mode. The main menu at the top includes 'Column Filling' (selected), 'Column Calibration', 'Density Measurement', 'Sweeper', and '1/0'. Below this, a sub-menu has 'Densities' (selected), 'Volumes', 'Calibrate Pumps', 'Configuration', and 'Fill'. The 'Densities' sub-tab contains four input fields for density values, each with a unit of kg/m^3 : 'Density at Top' (empty), 'Density at Bottom' (1080.00), 'Light Liquid Density' (998.20), and 'Heavy Liquid Density' (1080.50). To the right of these fields is a numeric keypad with buttons for digits 1-9, 0, a decimal point, and a less-than sign. At the bottom left of the sub-tab are 'Reset' and 'Help' buttons.

If any of the densities need changing, select that item, and enter the new value.

The screenshot shows the same software interface, but with the 'Configuration' sub-tab selected. The main menu remains the same. The sub-menu now shows 'Configuration' as the active tab. The 'Configuration' sub-tab contains several settings: 'Light Pump Rate' (484.2 $\mu\text{L/hr}$), 'Heavy Pump Rate' (461.5 $\mu\text{L/hr}$), 'Inlet Tubing Volume' (20 mL), 'Outlet Tubing Volume' (60 mL), 'Density Units' (a dropdown menu currently showing kg/m^3), and 'Filling Method' (radio buttons for 'From top' and 'From bottom', with 'From bottom' selected).

If you want to switch between using kg/m^3 and g/mL , select the units menu item and choose the desired units. kg/m^3 is set as default on the system this can also be changed using the Configuration tab. The Configuration tab also shows the Light Pump Rate and the Heavy Pump Rate.

The screenshot shows the 'H&D Density Column Filler' software window. The 'Column Filling' tab is selected. Below it, the 'Volumes' sub-tab is active. The interface displays three input fields for volume in mL: 'Total Col. Liquid Vol:' with the value '1400', 'Top Buffer:' with '50', and 'Bottom Buffer:' with '50'. The status bar at the top right indicates 'Idle'.

Volume units may be changed, if so select Volumes tab.

This screenshot shows the same software window, but with a numeric keypad displayed on the right side of the input fields. The 'Volumes' sub-tab remains selected. The input fields for 'Total Col. Liquid Vol:', 'Top Buffer:', and 'Bottom Buffer:' are currently empty, and the status bar still shows 'Idle'.

To change the volume, select that item, and enter a new value.

5.2 Calibrating the pumps

The amount of liquid delivered by each pump during one complete revolution of the rotor is an important piece of information, without which the column filler cannot work properly. Although pumps of the same type deliver roughly the same volume per revolution, the precise volume will be unique to every individual pump, and will alter whenever the state of the pump is changed (e.g. by replacing the pump tubing).

The original pump rates are determined by the manufacturer as the column filler is undergoing its commissioning tests. These volumes are stored in the instrument's memory.

However, when fitting your tubing, you will need to recalibrate the pumps to determine the new pump rates and prior to each use.

Each pump is calibrated by measuring the amount of water actually discharged when the instrument is programmed to pump a nominal 90 ml. The pumping rate is defined as the volume delivered in one revolution of the pump.

To calibrate the pumps you need:

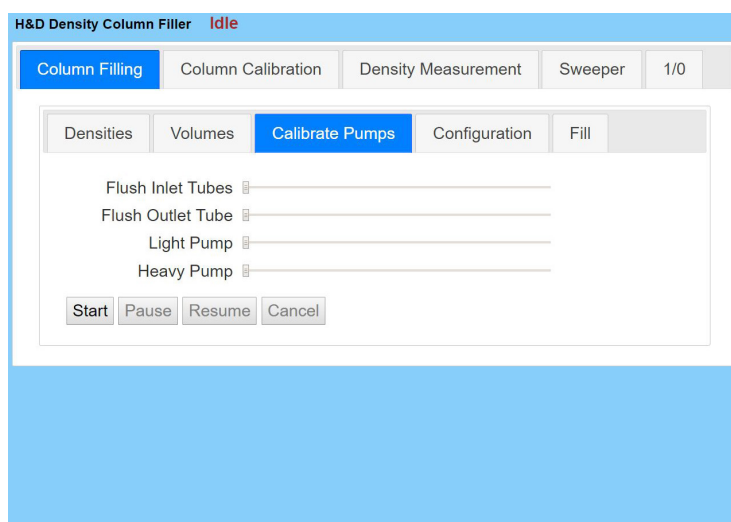
- either the 100 ml or 250 ml measuring cylinder provided;
- distilled water;
- a beaker with a minimum volume of 100 ml.

The first steps are to check that the filler is switched on, as described in [4.4 Switching the filler on](#); that the tubes are connected to the rear of the instrument, as described in [4.3 Fitting the tubing](#); and that you have entered the volume of the tubing, see [4.5 Entering the volume of the tubing](#).

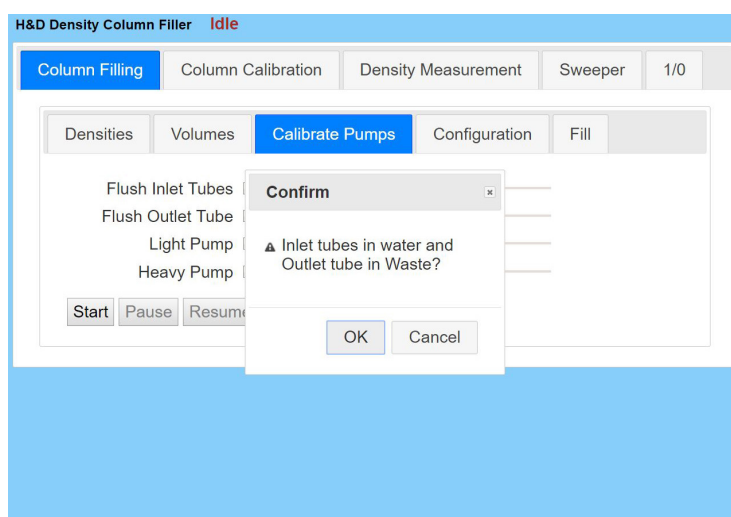
Note: It is recommended that the pumps are calibrated daily before use.

To calibrate:

Note: The filler will dispense a nominal 90 ml from the light pump and a nominal 90 ml from the heavy pump at room temperature. This volume is measured using the measuring cylinder and inputted into the program. The software will then calculate the volume delivered by the pump during one revolution.

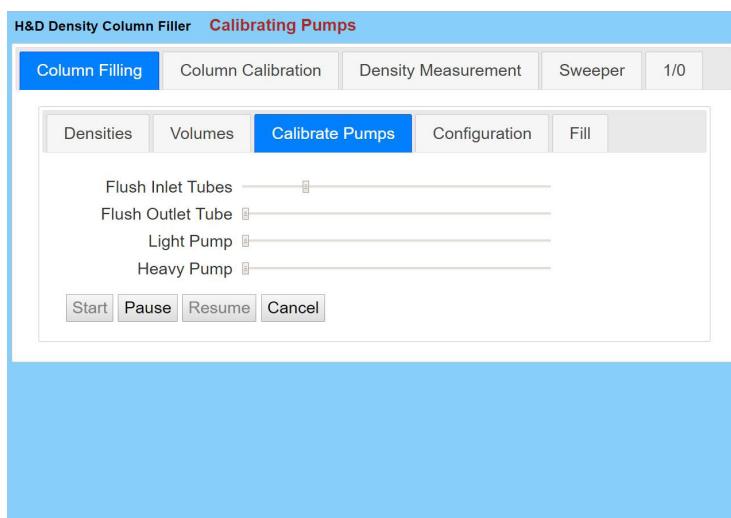


Select Calibrate Pumps tab, then select Start.



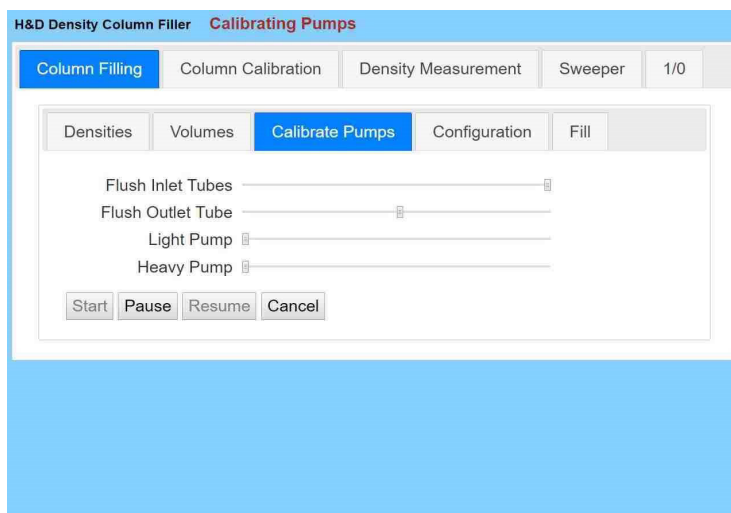
Select OK to confirm.

Note: The volume of the weighted end is 5 ml, if dispensing directly into the measuring cylinder this needs to be taken into account.

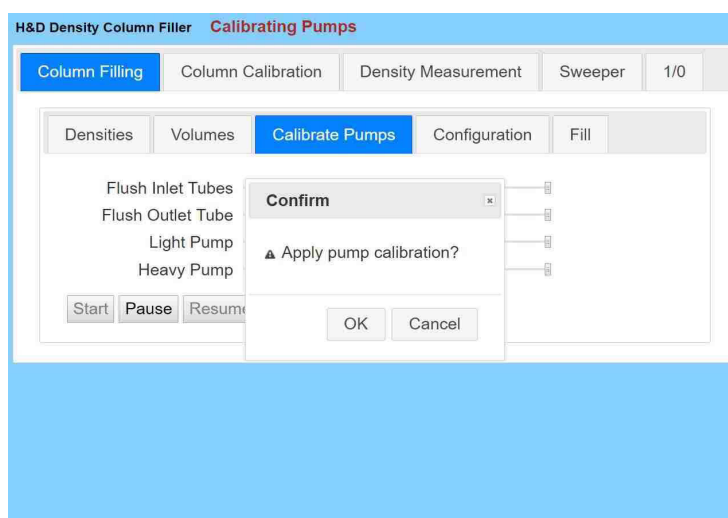


The inlet tubes will now flush as shown.

This process can be paused or resumed by selecting the relevant button.

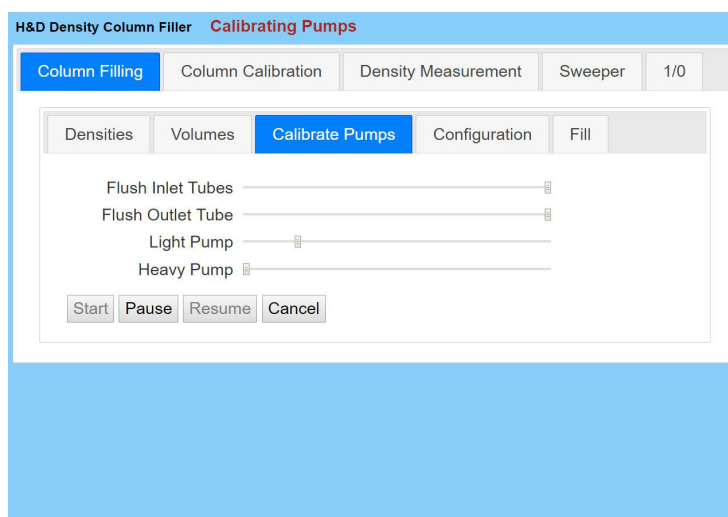


The outlet tubes will now flush as shown, again this process can be paused and resumed.

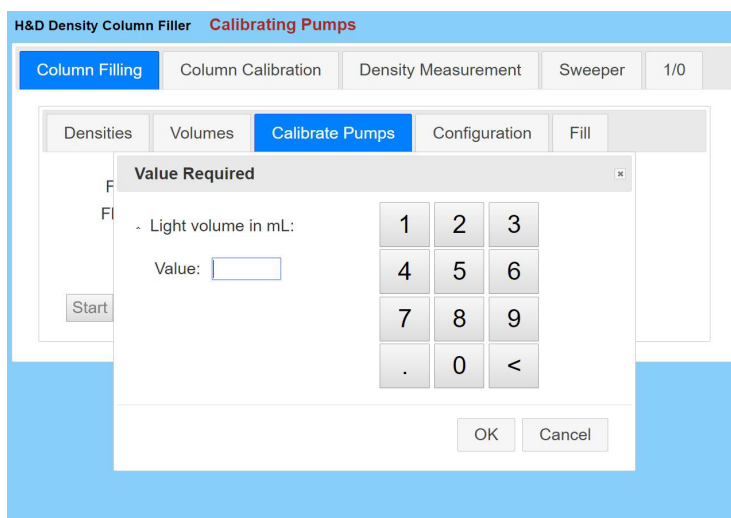


Follow the instructions in the pop up window and confirm by selecting OK.

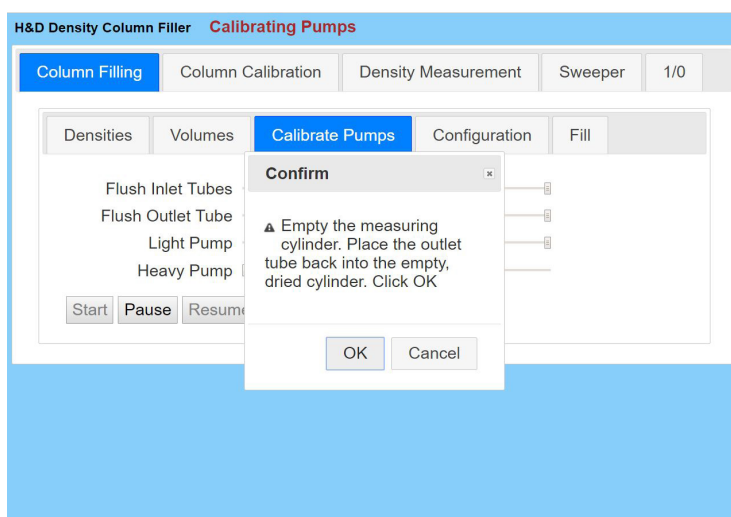
Note: If the weighted end piece is fitted, you may need to use a dry beaker. Once the liquid has been dispensed, pour the water from the beaker into a clean, dry measuring cylinder and note the volume.



The instrument will now dispense a nominal 90 ml through the light pump.

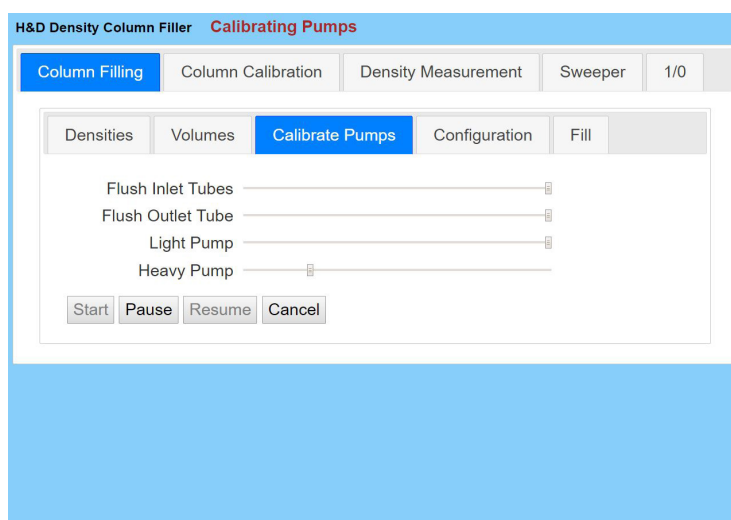


Enter the light volume dispensed in ml

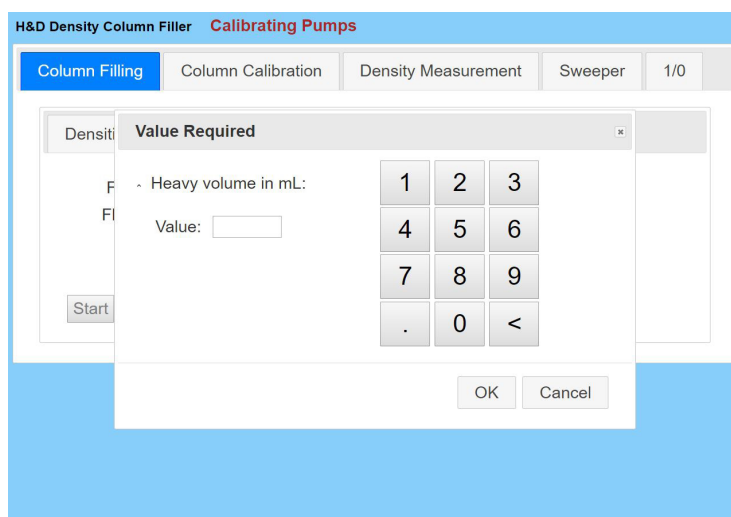


Follow the instructions in the pop up window and select OK to confirm.

Note: If the weighted end piece is fitted, you may need to use a dry beaker as before.

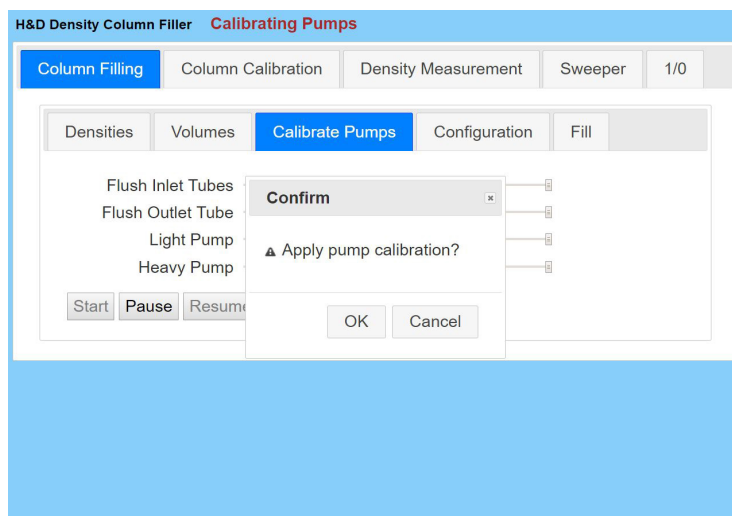


The instrument will now dispense a nominal 90 ml through the heavy pump.

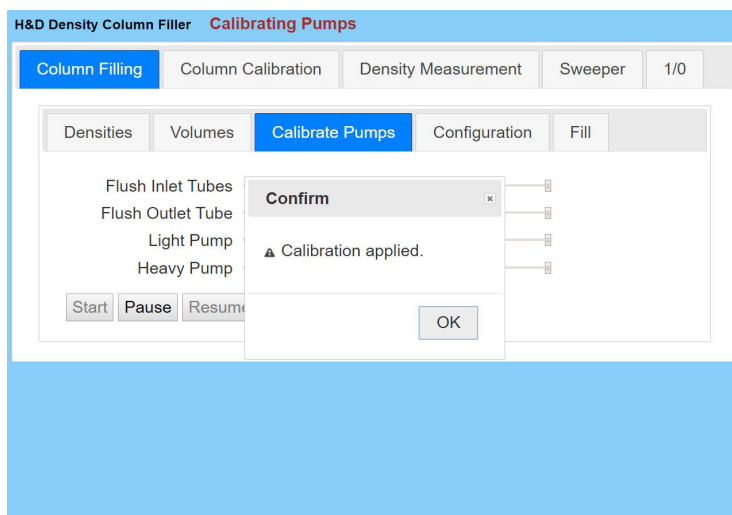


Enter the heavy volume dispensed in ml.

Filling the column

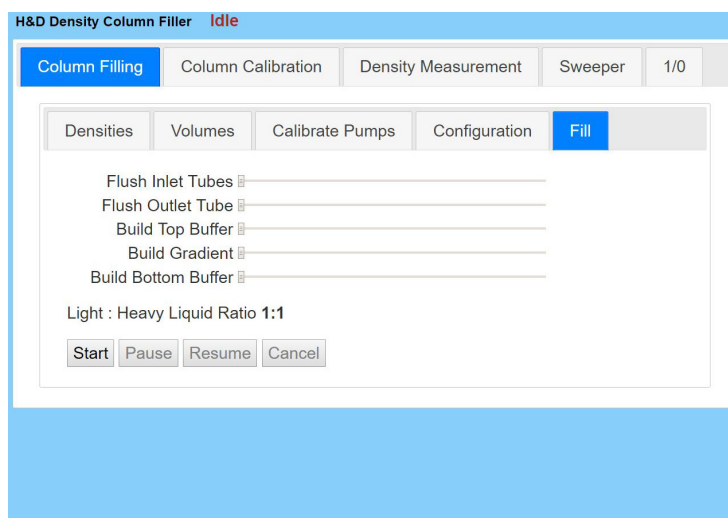


Select OK to confirm pump calibration



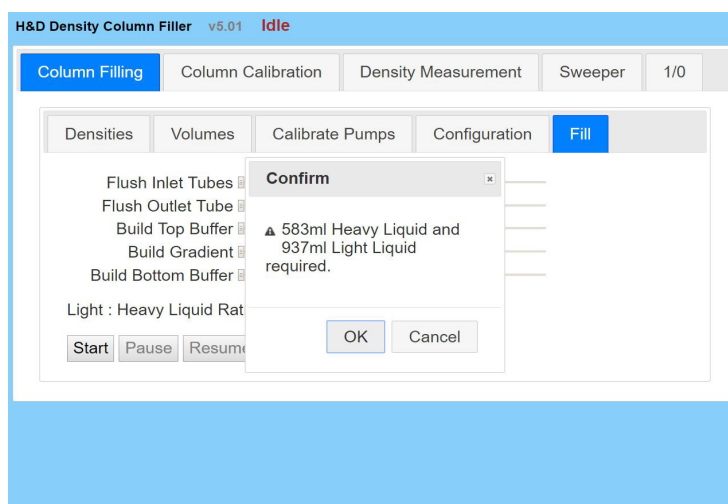
Select OK to confirm Calibration applied.

5.3 To fill the column



Select Fill.

The filler checks the stored data for consistency and reports any problems on screen. Press Start.

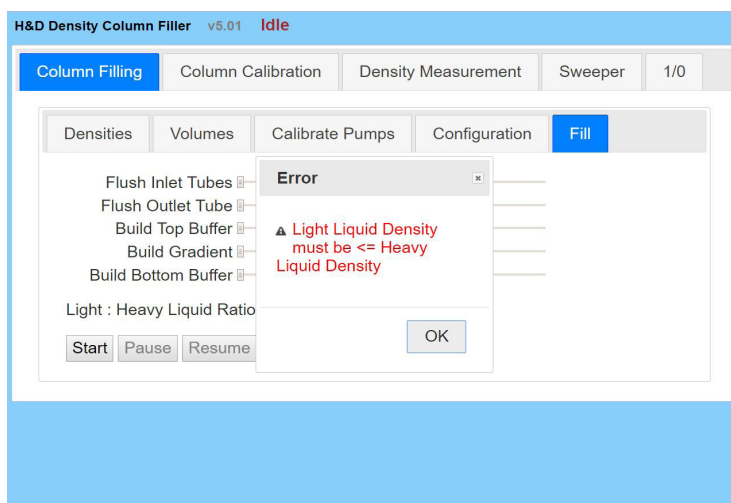


If you have the required volume of light liquid and heavy liquid available, then select OK.

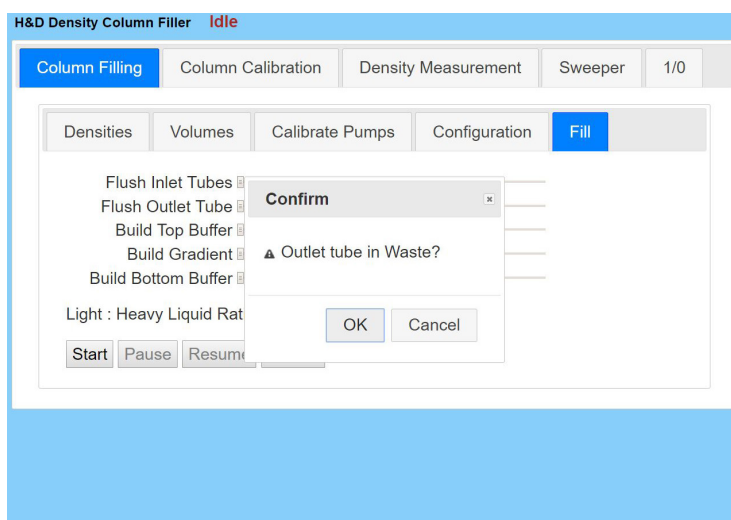
If you do not have the required volumes, either select **Cancel** to end column filling or provide more liquid.

Filling the column

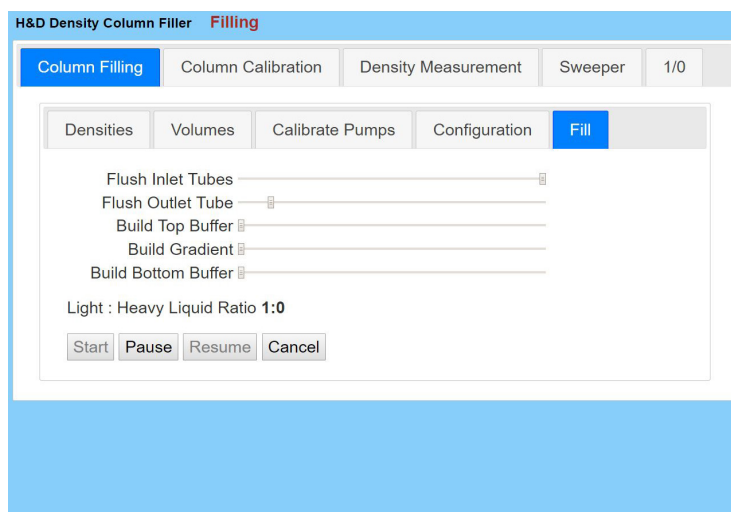
Note: If you set incompatible values, for example, if the density of the heavy liquid is less than the density required at the bottom of the column, the general purpose error screen is shown.



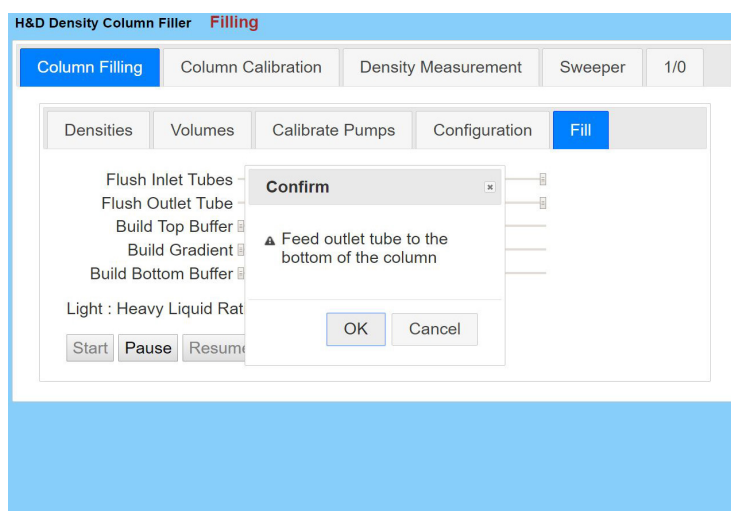
Example of general purpose error screen. Shown when incompatible values are entered.



Put the outlet tube in the waste bottle to collect the liquid used for flushing.
Select OK

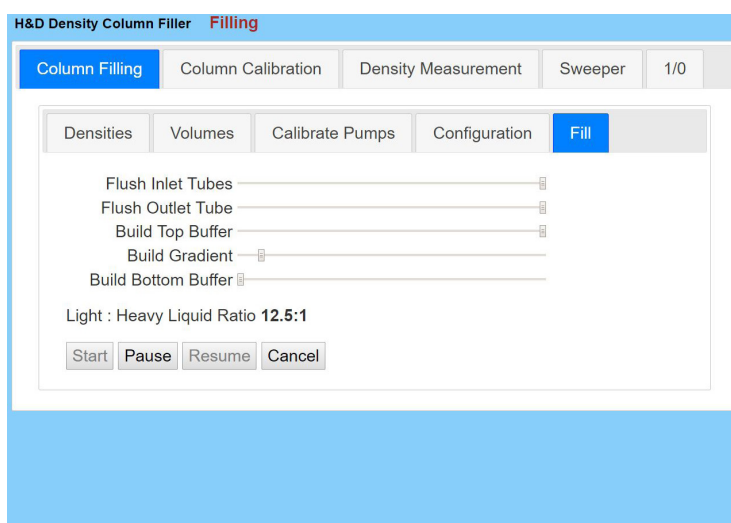


The flush cycle will commence. Wait for it to complete.
The progress bar labelled flushing indicates the progress.



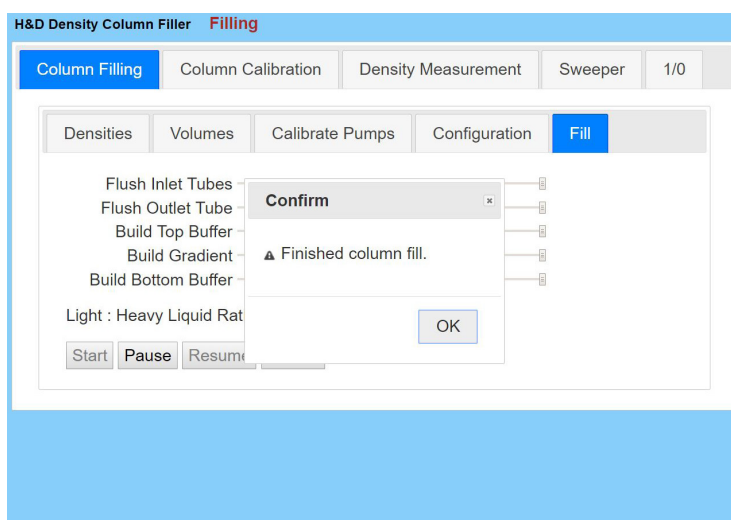
If you are filling the column from the bottom, you will now be prompted to feed the outlet tube (with the weighted end attached) to the bottom of the column.
If you are filling from the top, you will be prompted to attach the outlet tube (without the weighted end attached) to the top of the column.
Select OK

Filling the column



Filling will now commence. Wait for it to complete. The amount of time this takes depends upon the total volume of the column.

If you need to interrupt filling for any reason, select **Pause**, select **Resume** to continue.



When filling is complete this screen is displayed, the column is now ready for use.

Select OK to return to the main menu

Note: If you have been filling from the bottom, you will need to remove the outlet tube from the column before use.

This should be done using a slow, smooth and consistent motion so as to avoid damaging the gradient.

H&D Density Column Filler **Idle**

Column Filling | Column Calibration | Density Measurement | Sweeper | 1/0

Densities | Volumes | Calibrate Pumps | Configuration | **Fill**

Flush Inlet Tubes

Flush Outlet Tube

Build Top Buffer

Build Gradient

Build Bottom Buffer

Light : Heavy Liquid Ratio 1:1

Start | Pause | Resume | Cancel

It is possible to stop filling by selecting Cancel. Because this results in the loss of liquids already pumped into the column, you are asked to confirm that you really do want to cancel before the filler stops.

6 Calibrating the column

Before a completed column can be used to measure the density of solid samples, it must first be calibrated using reference floats.

The column filler stores a calibration table containing the heights in the column and densities of up to ten calibration floats. This table is retained when the filler is switched off. When the height of a sample is entered, the software uses linear interpolation to calculate its density from the values in the calibration table.

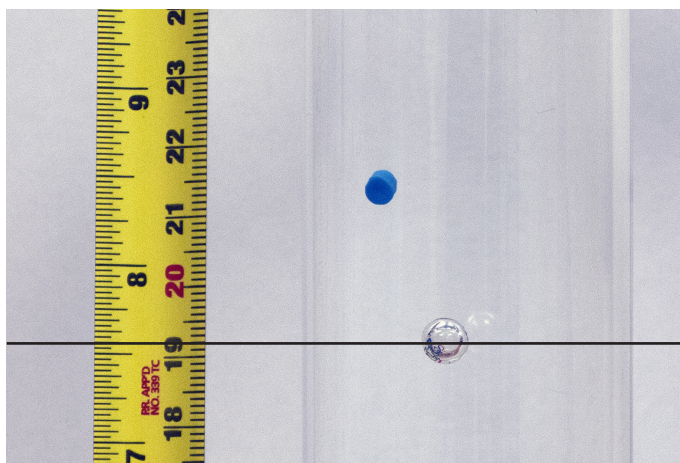
Keep the calibration floats in the column while it is in use, and check their heights whenever the heights of samples are measured.

Before you can successfully calibrate the column, the following checks should be made:

- ensure the operating conditions are suitable, see [4.2 Operating conditions](#).
- ensure that the filler has been set up in line with the instructions given in [4 Preparing the column filler for use](#).
- ensure that the column has been filled in line with the instructions given in [5 Filling the column](#).

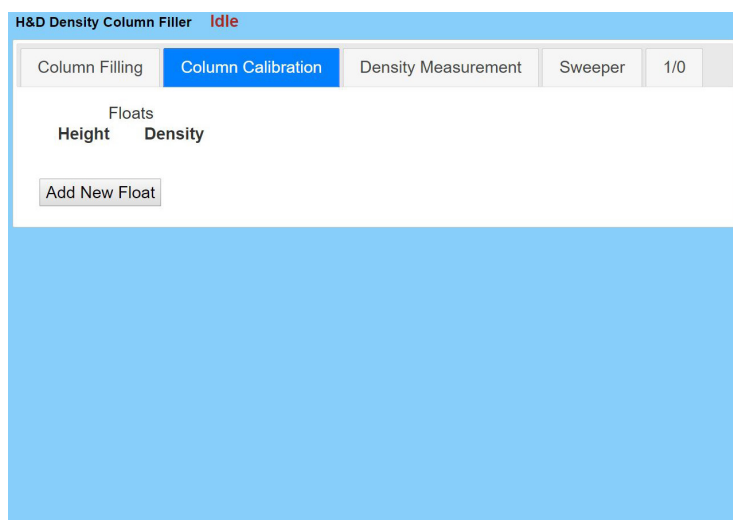
Measure the height of each float above your column datum in mm.

If any float is within 2% of the top or bottom of the column, then its reading should not be relied on. Consider increasing the buffer volumes to avoid this issue in subsequent columns.



Measuring the height of a float in the column.

Using the calibration table:



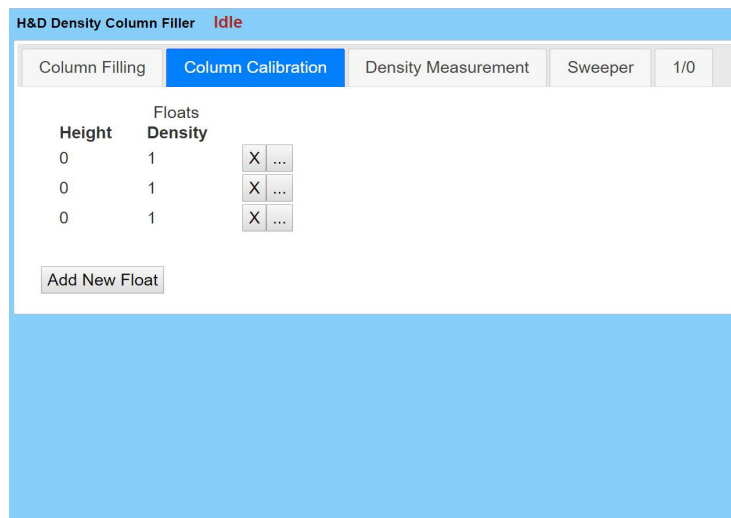
Select Column Calibration. The calibration table is shown.

Note: If the filler is being used with a new type of column, it may be necessary to delete an old calibration table in its entirety before entering a new one

H&D Fitzgerald Ltd. supply ISO 17025 calibrated density gradient column floats.
For more information, visit www.density.co.uk/products/gradient-column-floats/

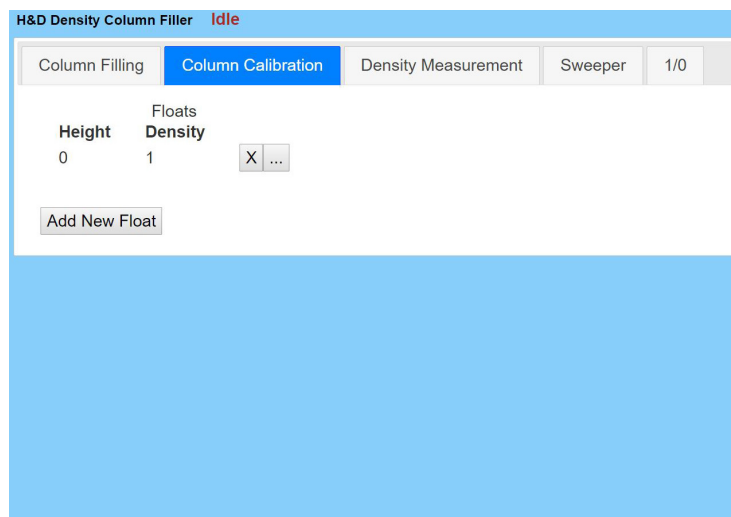
Calibrating the column

To delete an entry from the calibration table:



Select X alongside the required data point, repeat for each entry to be deleted.

To add an entry to the calibration table:



Select Add New Float, then select the ellipsis (set of dots).

H&D Density Column Filler **Idle**

Column Filling **Column Calibration** Density Measurement Sweeper 1/0

Height: 700

Floats Density:

Update Cancel

1	2	3
4	5	6
7	8	9
.	0	<

Enter height in box, enter density in box. Select Update.

H&D Density Column Filler **Idle**

Column Filling **Column Calibration** Density Measurement Sweeper 1/0

Height	Floats Density	
900	950	X ...
700	750	X ...
600	650	X ...
500	550	X ...

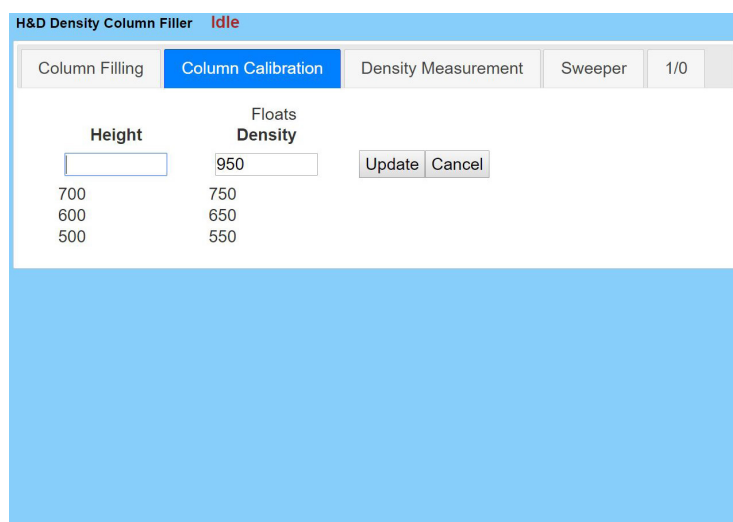
Add New Float

Repeat for each entry to be added.

To change an entry in the calibration table

If you have made a new column and are using the same floats whose values are stored in the filler, then this is the simplest way to update the calibration table, as the densities will be the same and only the height values will need changing.

You can also use this procedure to change the stored density of a float by entering its new density from its calibration certificate.



The screenshot shows the 'H&D Density Column Filler' software window. The 'Column Calibration' tab is selected. The window contains a table with two columns: 'Height' and 'Floats Density'. The 'Height' column has values 700, 600, and 500. The 'Floats Density' column has values 750, 650, and 550. There are input fields for 'Height' and 'Floats Density' at the top of the table. The 'Floats Density' input field contains the value '950'. To the right of the input fields are 'Update' and 'Cancel' buttons. The window also has a title bar with 'H&D Density Column Filler' and 'Idle', and a tab bar with 'Column Filling', 'Column Calibration', 'Density Measurement', 'Sweeper', and '1/0'.

Height	Floats Density
700	750
600	650
500	550

Select relevant line. Enter height value, enter density value. Select Update.

Repeat for each entry to be changed.

7 Measuring the density of a sample

Before you can successfully measure the density of a sample, the following checks should be made:

- ensure the operating conditions are suitable, see [4.2 Operating conditions](#).
- ensure that the filler has been set-up in-line with the instructions given in [4 Preparing the column filler for use](#).
- ensure that the column has been filled in-line with the instructions given in [5 Filling the column](#).
- ensure that column has been calibrated for use with the current floats, as described in [6 Calibrating the column](#).

Remember:

- The sample must not contain voids.
- Any conditioning procedures for the sample must be adhered to.
- Handle the sample with gloves or forceps.
- Thoroughly wet the sample with the light liquid before placing it in the column.
- Use a sample of between 2 to 6 mm long.
- See also the general points in [3 Density gradient column techniques](#).

To measure density:

Gently introduce the sample whose density is to be determined into the filled column, and allow it to reach equilibrium. This could take up to a few hours.

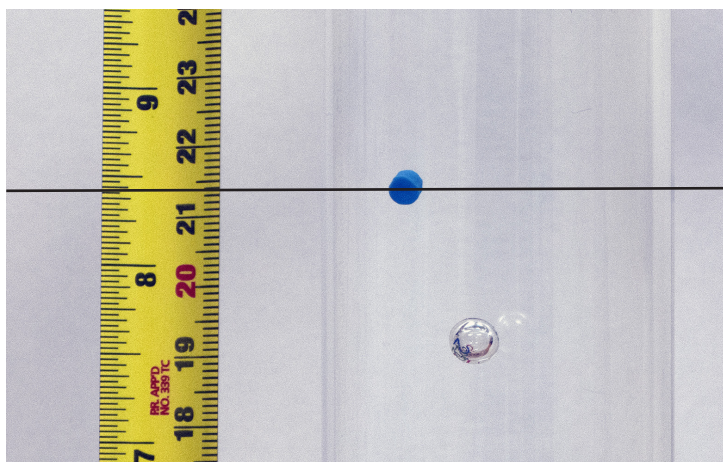


Introducing the sample into the column.

Measuring the density of the sample

Measure the height of the sample above your column datum in millimetres.

If the height is within 2% of the top or bottom of the column, then the sample's density cannot be reliably determined with this column.



Measuring the height of a sample in the column.

H&D Density Column Filler **Idle**

Column Filling	Column Calibration	Density Measurement	Sweeper	1/0												
Sample Height: <input type="text"/>																
Sample Density: <input type="text"/> <input type="button" value="Calculate"/>																
<table border="1"><tr><td>1</td><td>2</td><td>3</td></tr><tr><td>4</td><td>5</td><td>6</td></tr><tr><td>7</td><td>8</td><td>9</td></tr><tr><td>.</td><td>0</td><td><</td></tr></table>					1	2	3	4	5	6	7	8	9	.	0	<
1	2	3														
4	5	6														
7	8	9														
.	0	<														

Select Density Measurement.

H&D Density Column Filler **Idle**

Column Filling | Column Calibration | **Density Measurement** | Sweeper | 1/0

Sample Height:

Sample Density:

1	2	3
4	5	6
7	8	9
.	0	<

Enter the sample height just measured in millimetres, select Calculate.

H&D Density Column Filler **Idle**

Column Filling | Column Calibration | **Density Measurement** | Sweeper | 1/0

Sample Height:

Sample Density:

1	2	3
4	5	6
7	8	9
.	0	<

The filler displays the calculated density of the sample.

8 Using a sweeper

The column filler can drive a column sweeper.

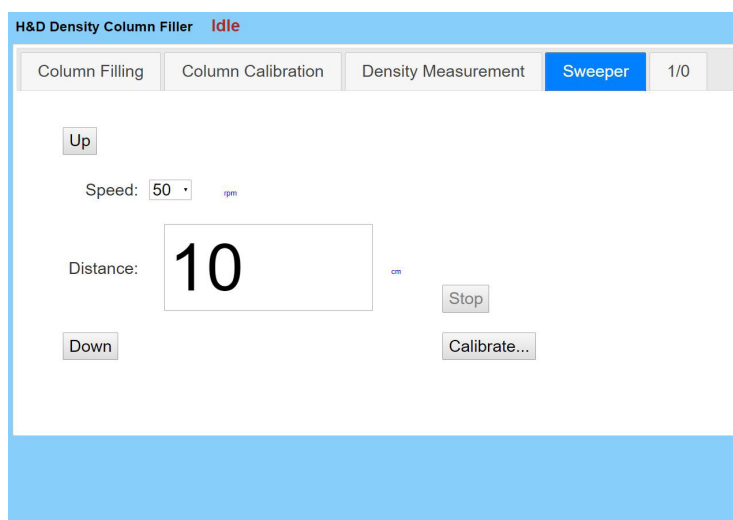
You can set the direction of travel; the distance to be travelled; and the sweeping speed (Remember: the slower the sweeping speed, the less you disturb the column gradient.)

To be able to accurately control the sweeping speed, you must first enter the sweep rate, that is, the distance the sweeper moves in one revolution of the sweeper's pulley (which is equivalent to the circumference of the sweeper's pulley).

You will need to calculate this value yourself. It cannot be preset by the manufacturer as it will depend upon the length of your column and the type of sweeper motor used.

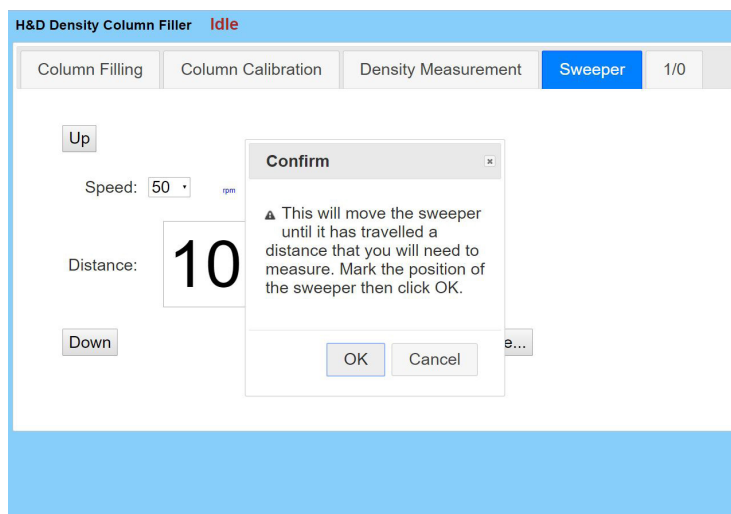
To set the sweep rate:

Select Sweeper tab

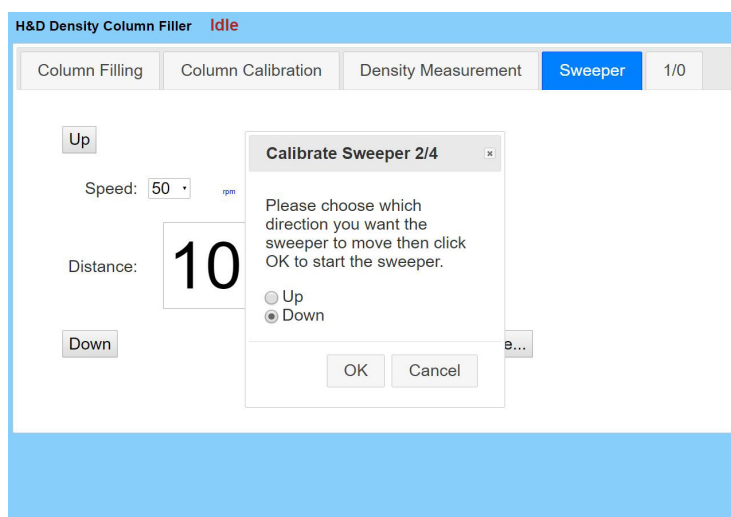


Select Calibrate.

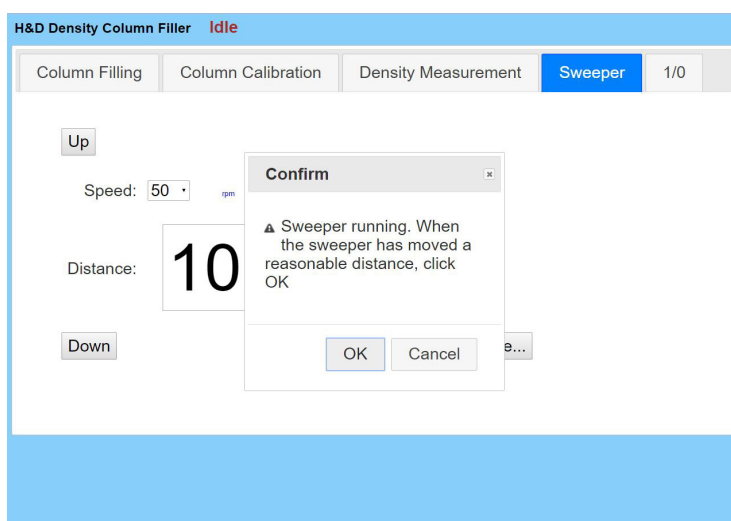
This will move the sweeper until it has travelled a distance that you will need to measure.



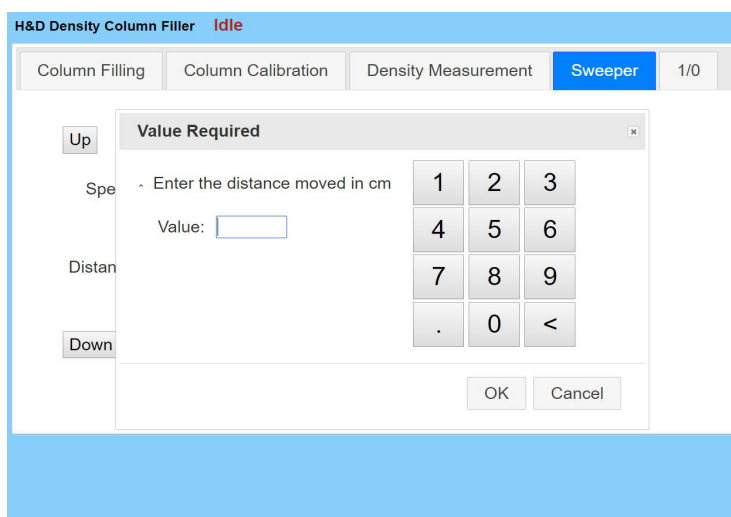
Mark on your column the start location of the sweeper, select OK.



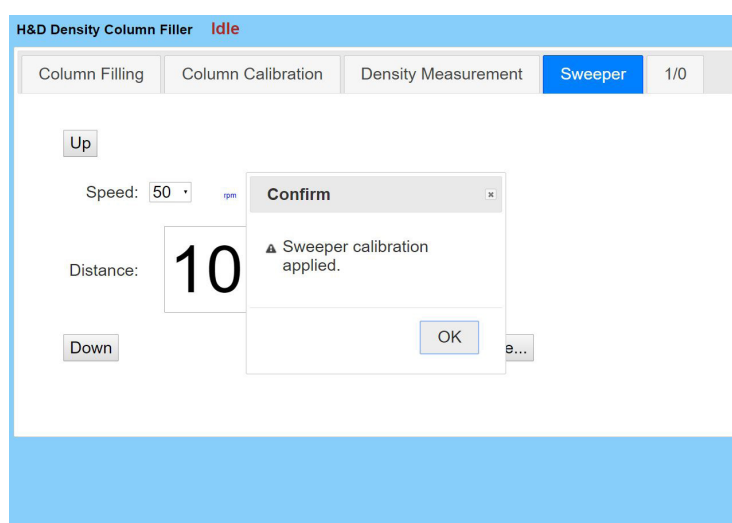
Select Up or Down, select OK.



When the sweeper has moved a reasonable distance select OK to stop the sweeper.

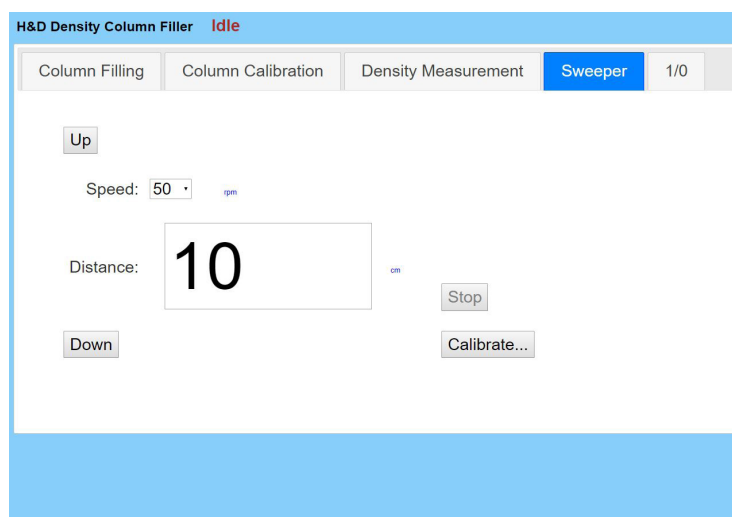


Measure the distance in cm and enter value, the filler will then calibrate the sweeper rate.



Sweeper calibration has now been applied. Select OK to continue.

To set the sweeper speed:



Select Sweeper.

Using a sweeper

H&D Density Column Filler **Idle**

Column Filling | Column Calibration | Density Measurement | **Sweeper** | 1/0

Up

Speed: 25 rpm

Distance: 10 cm

Down

Stop

Calibrate...

Select speed from the drop down menu

H&D Density Column Filler **Idle**

Column Filling | Column Calibration | Density Measurement | **Sweeper** | 1/0

Up

Speed: 100 rpm

Distance: 100 cm

Down

Stop

Calibrate...

Enter the distance that you wish the sweeper to move.

9 Care and maintenance

The filler should not be used if there is visible damage to the instrument; if the mains cable is damaged; or if it no longer functions properly in any way.

9.1 Cleaning

To clean the outside of the filler

1. switch off the filler and unplug the mains cable from the power supply;
2. carefully remove any liquid splashes with a cloth;
3. clean the filler using a piece of cloth which has been slightly moistened with either water, or water containing a small amount of detergent. Do not use any aggressive cleaning agents. Do not allow any liquid to come into contact with the touchscreen.
4. wipe down the filler and touchscreen with a soft dry cloth after cleaning.

To clean the pump tubing

1. set the column filler to pump a suitable solvent to flush the pump tubes, (remember that water is an excellent solvent for many liquids);
2. set the column filler to pump air.

9.2 Replacing the pump tubing

If needing to replace the tubing refer to section 4.3 Fitting the tubing.

Note: When tubing has been removed, moved or replaced and prior to use the pumps should be recalibrated, see section 5.2 [Calibrating the pumps](#).

9.3 Repairs

With the exception of fitting new lengths of tubing, repair work must be carried out by manufacturer authorised service technicians. Attempts by untrained persons to perform repairs may damage the instrument.

Any unauthorised removal or opening of the housing invalidates your warranty.

10 Filling example

In this section we present an example of the gradients specified and the gradient actually achieved. The column used in the example below had a volume of approximately 1250 ml.

10.1 Water and sodium bromide solution

In this example the light liquid was water and the heavy liquid was a sodium bromide solution. The operating temperature was 23°C.

The column parameters entered were:

Density at the Top 1248.5 kg/m³

Density at the Bottom 1282.5 kg/m³

Light Liquid Density 997.5 kg/m³

Heavy Liquid Density 1310.4 kg/m³

After filling the column, a set of 13 calibrated floats was dropped into the column. The uncertainty in the calibration of the floats was ± 0.10 kg/m³.

Using a quadratic equation to fit the densities to the heights of the floats resulted in a standard error of estimate of 0.13 kg/m³.

The actual density at the top of the column was 1248.2 kg/m³ (target was 1248.5).

The actual density at the bottom of the column was 1282.6 kg/m³ (target was 1282.5).

11 Specification

Filling speed	Approximately 65 ml per minute, depending on the selected density gradient and liquid used.
Density gradient	Any two miscible liquids covering the required density range can be used.
Selectable density units	kg/m ³ or g/ml
AC power supply	110 - 20V AC
Frequency	50 to 60 Hz
Power consumption	20 W when filling, 15 W when driving sweeper.
Operating temperature	Normal ambient conditions
Dimensions	180 mm high, 350 mm wide, 240 mm deep (excluding mains cable)
Weight	6.10 kg

A Shrinkage in 2-propanol / water mixtures

As mentioned in 3.1 [Choice of liquids](#), certain mixtures exhibit shrinkage and have a smaller volume when mixed than might have been expected.

The graph below shows the effect of shrinkage on 2-propanol / water mixtures at different densities.

Note: this graph should only be used for mixtures of water and pure 2-propanol: it does not apply to mixtures of water and 2-propanol solutions.

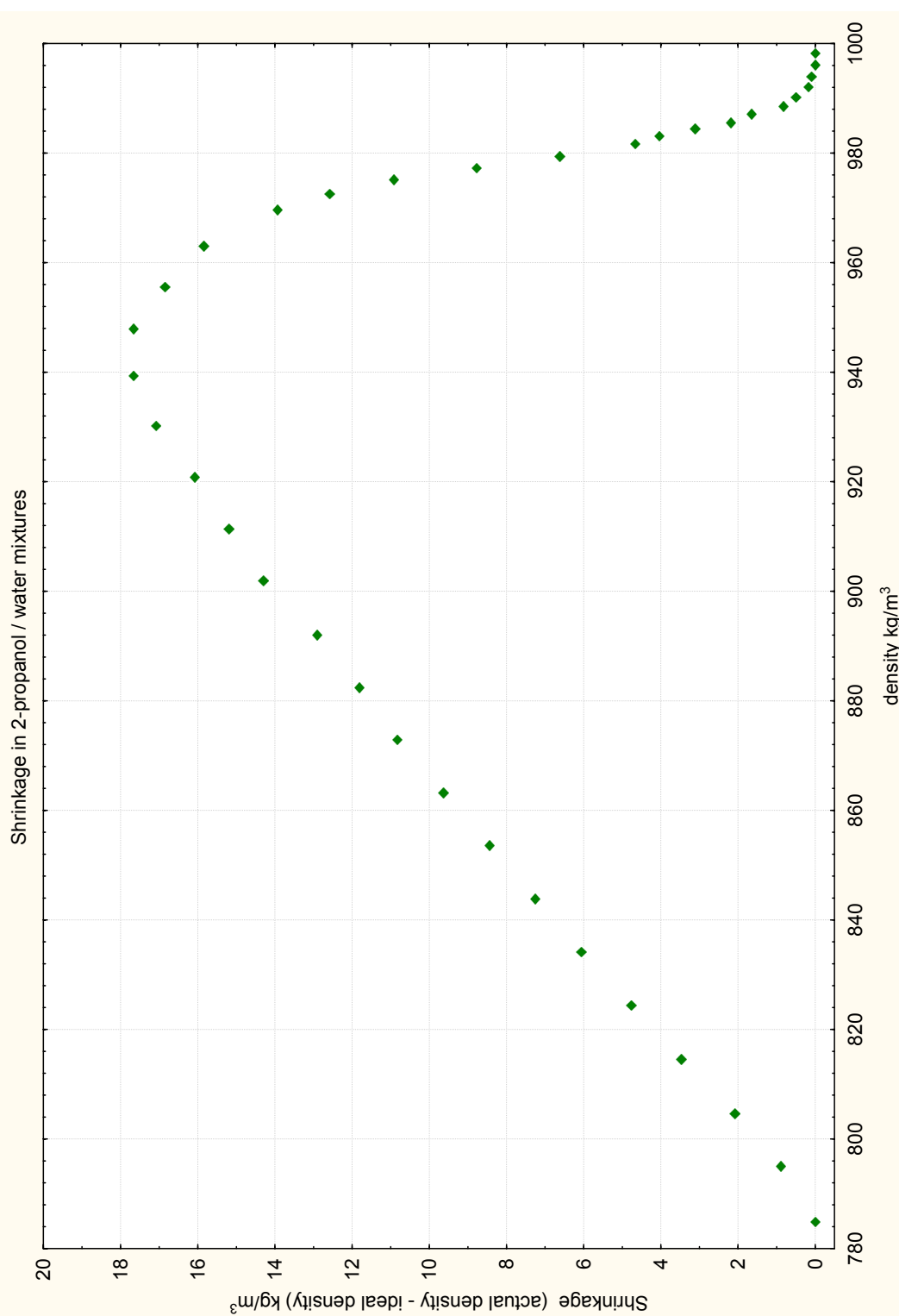
Example:

In this example we want to make a column with a bottom density of 940 kg/m^3 and a top density of 920 kg/m^3 using a mixture of water and pure 2-propanol.

From the graph we see that the shrinkage at 940 kg/m^3 is approximately 17.8 kg/m^3 ,

So we program 922.2 kg/m^3 ($940 \text{ kg/m}^3 - 17.8 \text{ kg/m}^3$) in the column filler as being the bottom density required.

Similarly, the shrinkage at 920 kg/m^3 is approximately 16 kg/m^3 , so we program 904 kg/m^3 ($920 \text{ kg/m}^3 - 16 \text{ kg/m}^3$) in the column filler as being the top density required.



B Preparing a 2-propanol / water column - worked example using pre-mixed solutions

It is also possible to mitigate the effect of shrinkage in 2-propanol* / water columns, by avoiding use of the pure liquids and instead starting with two different strength solutions of 2-propanol and water at the 'light' and 'heavy' components of the column.

The following method gives a worked example of how to prepare a density gradient column from two pre-mixed solutions of 2-propanol and water. It was developed through many years of experimental experience, and its authors consider it best practice.

Note: *2-propanol is also known by the synonyms Isopropyl alcohol (IPA), Isopropanol, and propan-2-ol.

Methodology for IPA / water column filling:

Propan-2-ol (isopropyl alcohol) naturally dissolves substantial quantities of air and when mixed with water the solubility falls so that air is released. There appears to be no sensible way in which the quantity of air can be measured – standard chemical methods are not possible because of the alcohol content. The amount of dissolved air can be reduced but not eliminated completely.

The following method allows a column to be prepared with a reduced amount of air, but even so care is needed when measuring the density of plastic samples (see later).

In general, when working with IPA / water columns, it is always best to prepare two pre-mixed IPA / water solutions where one has a density slightly lower than the lowest density required in the column, and the other has a density slightly higher than the highest density required in the column. This is preferable to working with pure IPA and pure distilled water as

- a) It is very difficult to de-air pure IPA safely.
- b) Working with premixed solutions will help to reduce the effects of shrinkage.

For the proportions of IPA to water to use to produce solutions of the desired density, consult a reputable reference guide such as the CRC Handbook of Chemistry & Physics for example.

If the bottom density of the column is reasonably close to that of pure distilled water, it is sufficient to prepare one pre-mixed IPA / water solution as the 'light' liquid and use pure distilled water as the 'heavy'. The use of pure IPA should be avoided wherever possible.

Remember to check the densities of both the 'heavy' and the 'light' liquid experimentally (ideally with a calibrated density meter, or otherwise with a calibrated hydrometer) before using them to build a column. It is important that an accurate density value is determined as the instrument needs to be programmed with accurate densities of both liquids in order to be able to fill the column properly.

Worked Example

This example assumes a desired column of 930-960 kg/m³ which is common in industry.

Selection of liquids:

The 'light' liquid is a pre-mixed solution of IPA and distilled water to give a density around 915 kg/m³.

In this column it is acceptable to use pure boiled distilled water as the 'heavy' liquid, as the target density is reasonable close to the density of pure water.

This choice of liquids keeps the quantity of IPA to a minimum. This is desirable both for ease of de-airing, and safety reasons.

The target density of the 'light' liquid is approximately 915 kg/m³. This can be obtained using IPA:water in the ratio 1:1 by volume.

Simple measuring cylinder technique is needed and is quite adequate because the density of the liquid will be measured prior to use, and the resultant the column will be accurately calibrated with density floats.

Assuming a column volume of 1600 ml (common in industry), around 1200 ml of 'light' liquid will be required. Therefore in order to allow for shrinkage. 750 ml distilled water with 750 ml pure IPA would be required to produce the mixture.

Equipment

- Measuring cylinder (ideally 1000 ml)
- Supplies of IPA and distilled water - approx 1500 ml water and 800 ml IPA (both in excess).
- **Air tight** containers for liquids – ideally 2 litre HDPE bottles with screw caps. A 'concertina' type bottle would be ideal.
- Some swarf or anti-bumping granules, porous pot, stainless steel, nuts or something similar.
- A simple electric kettle dedicated to the task of boiling pure distilled water.

Precautions

Pure IPA is very flammable and the vapour should be avoided.

Boiling the water allows heating of the mixture but avoids heating the IPA directly. However the 50% mix still has a flash point of ~180°C. Appropriate care is needed as the mix is readily flammable.

Method

'Light' liquid preparation

1. Measure 750 ml of distilled water into a kettle or suitable container to boil the water. It is useful to boil the water two or three times in quick succession to expel as much air as possible.
2. Put 750 ml of IPA into a suitable container for mixing (glass or polyethylene). Also add anti-bumping granules, stainless swarf / nuts or teflon swarf to provide nucleation sites encouraging bubble formation.
3. Boil the water, and immediately pour it into the cold IPA. The temperature of the mixture will be around 50°C which in itself reduces the solubility of air in the liquid. It is best if this mixture is held in a sealed glass or HDPE bottle which allows no air to enter as the mixture cools.
4. It is now essential that the mixture is stirred or in some way agitated. This seems to promote release of air by bubble formation. A magnetic stirrer works well.
5. Now seal the bottle and cool in cold water. The slight drop in pressure inside the bottle is beneficial.

On cooling to 23°C the density will be around 915 kg/m³.

6. Before using the liquid to build the column, measure its density using a calibrated density meter or hydrometer. This is the 'light' liquid density.

'Heavy' liquid preparation

7. Boil 750 ml of distilled water in the kettle as per step 1, and then pour into an airtight bottle, seal, and cool as per step 5.
8. Measure the density of the pure water as per step 6. This is the 'heavy' liquid density.

Column Filler Settings

Set up the column filler in the usual way (following the guidance in this manual), and use the following parameters.

Density at Top	927 kg/m ³ (allowing for some shrinkage)
Density at Bottom	960 kg/m ³
Light Liquid Density	Value as measured
Heavy Liquid Density	Value as measured
Total Col. Liquid Vol.	1600 ml
Buffers (Top & Bottom)	50 ml.

Follow the procedure for column filling as described in this manual.

Floats are added as normal – it is best to wash the floats with some of the 'light' IPA/water mixture to which a few drops of surfactant have been added.

Density Measurements

The column can be calibrated with suitable floats in the usual way. It is advisable to work quickly and measure the heights of both floats and the sample under test soon after they are introduced into the column and once they have stopped moving. If samples are left in the column for a long time before being measured there is always a risk that bubbles will start to form on them no matter how well the solutions have been de-aired. Immediate measurement is therefore sensible.