

AN049: PIPETTING – DISC PUMP APPLICATION NOTE

1. INTRODU	ICTION
	out this Application Note
3. THE VOL	UME CONTROLE MODULE
	Toduction to the VCM 3 chitecture 4 The Feedback Orifice 4
3.4. Pre	lculating the Volume of Fluid Aspirated
	M Pipetting process steps
3.6.2.	Orifice Selection
3.6.3.	Pump Selection9
3.7. Exa 3.7.1.	ample systems
3.7.2.	Design B11
3.7.3.	Design C12
3.8. Pitf 3.8.1.	falls to avoid



	3.8.2.	Leaks	14
	3.8.3.	Compliance	15
4.	DATA		16
4	.1. Dat	a with Pipette tips	17
4	.2. Dat	a with a Liquid Flow Sensor	18
4	.3. The	ermal Stability	20
	4.3.1.	Thermal Expansion	21
5.	USEFUL D	DESIGN ADDITIONS	21
5	5.1. Val	ving	21
	5.1.1.	Side Vent Valve	21
	5.1.2.	Feedback Valve	22
	5.1.3.	Multi-Volume Valve	23
5	5.2. Ter	nperature measurement	23
6.	USEFUL E	QUATIONS	24
6	5.1. Asp	pirating Against Gravity	24
6	6.2. Dis	pense / Deflation through an Orifice	25
6	3. Ori	fice Pressure/Flowrate vs. Pump Power	26
6	6.4. The	ermal Effects	27
6	6.5. Acc	counting for Compliance	28
7.	APPENDI)	(28
7	'.1. PD	C VS SDC	28
7	.2. PE	RFORMANCE TABLES FOR ALL PDC PUMP	29
8.	SUPPORT		32
9.	REVISION	HISTORY	32



1. INTRODUCTION

The disc pumps are a silent, high-performance piezoelectric micropump.

Owing to its operating mechanism, the disc pumps can be controlled with unmatched precision, yet at the same time respond to full-scale set point changes in a matter of a few milliseconds. The compact form factor means it can be tightly integrated into products, increasing portability.



Figure 1: A piezoelectric disc pump

1.1.About this Application Note

This Application Note introduces a novel pipetting scheme—known as the Volume Control Module (VCM)—that takes advantage of the high-precision pressure control provided by disc pump. The note covers:

- The basic design and operation of a VCM.
- Guidance on optimising VCM performance, including key considerations and pitfalls to avoid.
- Data from The Lee Company's own prototype systems.
- Possible design inclusions for increased utility.
- Useful equations.

Our intention is that this note provides a helpful starting point for customers considering implementing this scheme in their own products.

Please note that The Lee Company does not design/manufacture pipetting modules.



2. DISCLAIMER

This resource is provided "as is" and without any warranty of any kind, and its use is at your own risk. The Lee Company does not warrant the performance or results that you may obtain by using this resource. The Lee Company makes no warranties regarding this resource, express or implied, including as to non-infringement, merchantability, or fitness for any particular purpose. To the maximum extent permitted by law The Lee Company disclaims liability for any loss or damage resulting from use of this resource, whether arising under contract, tort (including negligence), strict liability, or otherwise, and whether direct, consequential, indirect, or otherwise, even if The Lee Company has been advised of the possibility of such damages, or for any claim from any third party.

3. THE VOLUME CONTROL MODULE (VCM)

3.1.Introduction to the VCM

When dosing controlled liquid volumes, a syringe pump or air displacement pipette are often used. These technologies offer excellent precision but are often large and expensive. An alternative approach to this method is proposed.

Due to the high responsiveness and precise control of disc pump, a method based on pressure control can be used. This is opposed to the "volume control" approach used by syringe pumps (i.e. by plunger position).

Pressure control is already widely used in dosing: the predominant approach, "pressure-time", involves applying a known pressure for a fixed time to a body of fluid. This has the disadvantage that it is sensitive to timings, viscosity of the fluid, and the pressure difference across fluid.

The system proposed herein also uses pressure control; however, it works more similarly to an air displacement pipette, by displacing a controlled amount of gas from the pipette tip into a reservoir. The amount of gas is regulated by controlling the pressure increase in the reservoir. In turn, the amount of fluid aspirated/dispensed can be controlled. This system is called a Volume Control Module (VCM).

The Lee Company has built a prototype VCM system as a proof-of-concept, enabling us to explore the capability of this approach, key design considerations and pitfalls to avoid. Section 4 provides a detailed



results collected with a prototype VCM. In summary, the system delivered a CV of less than 1% over the dosing range explored, from approximately 14 μ L to 387 μ L.



Figure 2: Left - Prototype VCM system, using The Lee Company Smart Pump Module and a custom pipetting manifold block. Right -Using the same prototype to dispense aliquots of increasing volume into successive wells of a 96-well plate.

3.2.Architecture

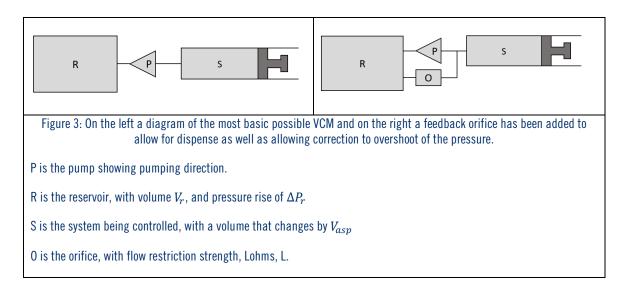
In its most basic form, the VCM comprises a pump driven into a reservoir (left-hand panel in Figure 3). As gas is pumped into the reservoir the pressure increases by an amount proportional to the amount of gas moved. Therefore, if the pump inlet is connected to a closed system, a known amount of gas can be removed from the system. This known amount of gas can be used to move a known amount of liquid, in the same manner as a conventional air displacement pipette. Unlike pressure-time dispensing, this setup does not require precise timing and is less dependent on gravity effects and fluid viscosity.

3.2.1. The Feedback Orifice

There is a problem with this simplified VCM system, however: disc pump is unidirectional, in that gas only flows freely through it in one direction; as a result, fluid can be aspirated but not dispensed. This can be solved by adding a feedback orifice between the reservoir and the system (as can be seen in the right-

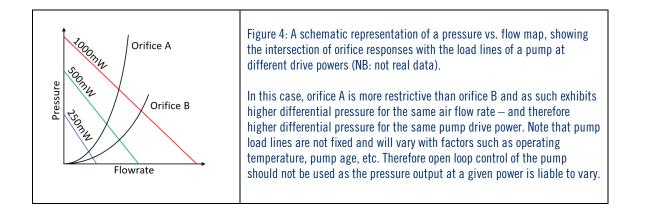


hand panel of Figure 3). This allows pressure in the reservoir to dissipate through the orifice, in turn enabling aspirated fluid to be dispensed.



Note that when using a feedback orifice, the pump must run continuously to maintain the desired pressure rise in the reservoir, as gas will escape through the orifice whilst the reservoir is pressurised. This in itself is not an issue, but does place importance on the correct selection of system components (e.g. pump, reservoir and orifice) for good operation. Selection of these components is considered in §3.6

There are additional benefits to having a feedback orifice. First, if the pressure setpoint is overshot, the orifice allows the pressure to dissipate so that the overshoot can be corrected. Second, orifices enable the full-scale pressure range of the system to be adjusted to suit the application requirements. This can effectively provide greater pressure control precision where the required range is smaller (see Figure 4).





3.3.Calculating the Volume of Fluid Aspirated

The volume aspirated by the system *S* can be calculated by using the Ideal Gas Law, PV = nRT, providing that the number of particles is kept constant (i.e. there are no leaks in the system). Therefore,

$$\Delta n_r = -\Delta n_s$$

Where *n* is the number of particles and *r* and *s* are reservoir and system, respectively. The result is that for a given aspiration pressure ΔP_r the volume aspirated is:

$$V_{asp} = -\Delta V_s = \frac{T_s}{T_r P_s} \cdot V_r \Delta P_r \left[= \frac{V_r \Delta P_r}{P_s} \text{ if } T_s = T_r \right]$$

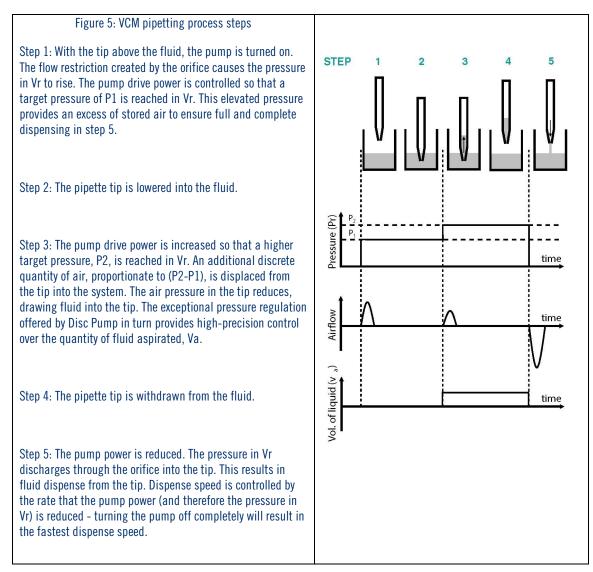
Therefore the volume aspirated is linear with the aspiration pressure. Note that where the temperature of the reservoir and the system are not the same, the volume aspirated will also depend on T_r/T_s . However, this factor has a modest effect: a 3°C temperature difference results in only 1% change in volume aspirated, and therefore may only be a concern for high-precision applications.

3.4. Pre-charging the VCM reservoir to enable complete dispense

To ensure complete dispense, and to enable the blow-off functionality often used in pipetting workflows, it is helpful to pre-charge the VCM reservoir. To achieve this, before lowering the pipette tip into the fluid, run the pump and control the reservoir to a set pressure. This provides an excess of stored air that enables complete dispense.



3.5.VCM Pipetting process steps





3.6.Component Selection

3.6.1. Reservoir and Pressure sensor selection

Using the equation from 3.3, Figure 6 shows how the liquid volume aspirated varies with reservoir volume and aspiration pressure.

Re	eservoir	L	iquid volur.	ne aspirated	for given as	piration pres	sure					
V	'olume	2 mBar	5 mBar	10 mBar	20 mBar	50 mBar	100 mBar					
	1 cc	2 µL	5 µL	10 µL	20 µL	50 µL	100 µL					
	2 cc	4 µL	10 µL	20 µL	40 µL	100 µL	200 µL					
	5 cc	10 µL	25 µL	50 µL	100 µL	250 µL	500 µL					
	10 cc 20 μL 50 μL 100 μL 200 μL 500 μL 1 mL											
	20 cc	40 µL	100 µL	200 µL	400 µL	1 mL	2 mL					
	50 cc	100 µL	250 µL	500 µL	1 mL	2.5 mL	5 mL					
	100 cc 200 μL 500 μL 1 mL 2 mL 5 mL 10 mL											
Fi	igure 6: A 1	table showir	ıg aspirated	volume for a	range of reserv	oir pressures	and volumes.					

As is evident, the larger the reservoir volume, the lower the aspiration pressure required to aspirate a given liquid volume. On one hand, selecting as large a reservoir as possible (given other constraints e.g. acceptable system size) is attractive, as it reduces the pump drive power required to maintain the aspiration pressure. On the other hand, dosing precision depends on how precisely the aspiration pressure is measured and controlled – clearly, a lower aspiration pressure will require a higher-resolution pressure sensor.

In addition, to ensure a good signal-to-noise ratio (SNR) on the pressure measurement, the maximum aspiration pressure should be a good fraction of the full-scale range of the pressure sensor.

3.6.2. Orifice Selection

When selecting an orifice, there is a trade-off to consider between the desired dispense speed, and the effect of self-heating due to the pump. On one hand, a larger orifice enables the pressure in the reservoir to dissipate more quickly, supporting faster dispense speeds. On the other hand, with a larger orifice, the pump will need to work harder to maintain pressure in the reservoir between the aspirate and dispense steps. As a result, the pump will generate more heat, which in turn will heat the gas flowing through the



pump, reservoir, and orifice. This will contribute to a temperature difference between the gas in the reservoir (T_r) and the gas in the pipette tip (T_s), which will impact pipetting precision (without further temperature compensation).

Dispense speed can be calculated as follows:

$$T_D = 15 L \sqrt{V_{asp} V_r}$$

Where T_D is the dispense time in milliseconds, L is the feedback orifice restriction in Lohms, V_r is the reservoir volume in litres, and V_{asp} is the fluid aspiration volume also in litres.

Using this equation, Figure 7 shows the maximum reservoir volume that should be used to achieve a desired dispense speed for a range of common orifice sizes, for an aspiration volume (V_{asp}) of 100µL.

Dispense	vispense Feedback Orifice Restriction - LOhms											
Time	4k	8k	12k	15k	20k	25k	30k	40k	45k			
20ms	1.1mL	277µL	123µL	79µL	44µL	28µL	20µL	11µL	8.8µ			
50ms	6.9mL	1.7mL	772µL	494µL	278µL	178µL	123µL	69µL	55µl			
100ms	28mL	6.9mL	3.1mL	2.0mL	1.1mL	711µL	494µL	278µL	219µ			
200ms 111mL 28mL 12mL 7.9mL 4.4mL 2.8mL 2.0mL 1.1mL 878												
500ms	694mL	174mL	77mL	49mL	28mL	18mL	12mL	6.9mL	5.5m			
1s	2.8L	694mL	308mL	198mL	111mL	71mL	49mL	28mL	22m			
2s	11L	2.8L	1.2L	790mL	444mL	284mL	198mL	111mL	88m			

Note that the orifice value itself does not directly affect V_{asp} , only the power needed to achieve the desired ΔP_r . As a result, that the exact value of the orifice is not critically important: this means that it is possible to use a lower-precision orifice if desired.

3.6.3. Pump Selection

The Lee Company recommends that VCM modules are made with parallel-configuration (PDC) pumps (this position is explained in the appendix). Pump models UBLB5400200A and UXPB5400200A are



therefore ideal. Figure 8 shows the performance supported by UBLB5400200A for a range of orifice sizes and drive powers. We recommend operating the pump at/below 250 mW drive power, to minimise the effect of pump self-heating (as described in 3.3.1).

flow) the pressures across different orifice sizes were calculated for a range of pump drive powers. The colour of the		Pre	Pressure (mBar) across orifice for UBLB5400200A										
20mW 4 6 7 8 8 9 9 9 9 50mW 8 13 15 16 16 17 17 18 18 100mW 15 22 25 26 27 28 29 30 30 250mW 32 44 49 51 53 55 56 57 57 500mW 57 75 82 85 88 90 91 93 94 1W 99 126 136 141 145 148 150 152 153		Drive Orifice - LOhms											
50mW 8 13 15 16 16 17 17 18 18 100mW 15 22 25 26 27 28 29 30 30 250mW 32 44 49 51 53 55 56 57 57 500mW 57 75 82 85 88 90 91 93 94 1W 99 126 136 141 145 148 150 152 153		Power	4k	8k	12k	15k	20k	25k	30k	40k	45k		
IOOmW 15 22 25 26 27 28 29 30 30 250mW 32 44 49 51 53 55 56 57 57 500mW 57 75 82 85 88 90 91 93 94 1W 99 126 136 141 145 148 150 152 153		20mW	4	6	7	8	8	9	9	9	9		
250mW324449515355565757500mW5775828588909193941W99126136141145148150152153Figure 8: Using the specification performance of a UBLB5400200A pump (160mBar stall pressure and 1.65slpm freeflow) the pressures across different orifice sizes were calculated for a range of pump drive powers. The colour of the rrepresents how likely the drive power is to cause problems due to self-heating of the pump (green equates to little se		50mW	8	13	15	16	16	17	17	18	18		
500mW 57 75 82 85 88 90 91 93 94 1W 99 126 136 141 145 148 150 152 153 Figure 8: Using the specification performance of a UBLB5400200A pump (160mBar stall pressure and 1.65slpm free flow) the pressures across different orifice sizes were calculated for a range of pump drive powers. The colour of the r represents how likely the drive power is to cause problems due to self-heating of the pump (green equates to little se		100mW	15	22	25	26	27	28	29	30	30		
1W99126136141145148150152153Figure 8: Using the specification performance of a UBLB5400200A pump (160mBar stall pressure and 1.65slpm free flow) the pressures across different orifice sizes were calculated for a range of pump drive powers. The colour of the r represents how likely the drive power is to cause problems due to self-heating of the pump (green equates to little se		250mW	32	44	49	51	53	55	56	57	57		
Figure 8: Using the specification performance of a UBLB5400200A pump (160mBar stall pressure and 1.65slpm free flow) the pressures across different orifice sizes were calculated for a range of pump drive powers. The colour of the r represents how likely the drive power is to cause problems due to self-heating of the pump (green equates to little se		500mW	57	75	82	85	88	90	91	93	94		
flow) the pressures across different orifice sizes were calculated for a range of pump drive powers. The colour of the r represents how likely the drive power is to cause problems due to self-heating of the pump (green equates to little se		1W	99	126	136	141	145	148	150	152	153		
	flow) the pressures	across differen ly the drive pow	t orific ver is t	ce sizes to cause	were ca e proble	alculate ms due	d for a to self-	range o heating	f pump ; of the	drive po pump (g	owers. T	he colour of the r	

3.7.Example systems

In this section, we work through the selection of components for three example systems with differing requirements and configurations.

	Design A	Design B	Design C				
Required	Aspirate 50-200µL	Aspirate 500µL	Aspirate 10µL with				
Performance	And dispense in 500ms	Dispense in 200ms	highest possible accuracy				
Resulting	UBLB5400200A	UBLB5400200A	UBLB5400200A				
Design	$V_r = 10 \text{mL}$	$V_r = 20 \text{mL}$	$V_r = 0.2 \text{mL}$				
	20kLohm Orifice	4kLohm Orifice	45kLohm Orifice				

Figure 9: Three example system requirements and configurations, considered in further detail below.



3.7.1. Design A

In our first example, the required performance is to aspirate fluid volumes of 50μ L to 200μ L, and to dispense the volume in half a second (500ms).

In this case, we start by considering the lower target aspiration volume of 50μ L. Using Figure 6, we select a reservoir volume of 10mL to give a low-but-measurable aspiration pressure of 5 mBar. Then, with the upper end of the target aspiration volume range of 200μ L, we use the equation in §3.6.2 to calculate the orifice size required to give the correct dispense speed:

$$T_D = 15 L \sqrt{V_{asp} V_r}$$

Therefore:
$$L = \frac{T_D}{15\sqrt{V_{asp}V_r}} = \frac{500}{15\sqrt{0.0002*0.01}} = 23570 \ Lohms$$

We choose the largest standard orifice Lohm rating below the calculated value. In this case, a 20kLohm orifice will be suitable. Consulting Figure 6 once again, we note that the pressure required to aspirate 200µL with a 10mL reservoir is 20mBar. Figure 8 shows that the pump drive power required to generate 20mBar across a 20kLohm orifice with UBLB5400200A is less than 100mW – this is lower than 250mW, which means that any effects due to self-heating should be small.

Finally, we should select a pressure sensor with an appropriate full-scale range: 30 to 50 mBar would be appropriate, given the maximum aspiration pressure of 20 mBar.

3.7.2. Design B

In the second example, the requirement is to aspirate 500µL and then to dispense this in 200ms.

In this case, we first identify the largest reservoir that will support the desired dispense speed. Using the equation in §3.6.2 and selecting a small orifice size of 4kLohm, we calculate that the reservoir size:

$$T_D = 15 L \sqrt{V_{asp}V_r}$$

Therefore:
$$V_r = \frac{T_D^2}{225 L^2 V_{asp}} = \frac{200^2}{225 \times 4000^2 \times 0.0005} = 0.022 L = 22 mL$$



Therefore, the reservoir should be ≤ 22 mL. We therefore choose a 20mL reservoir. Using the equation in 3.3, we can calculate the pressure required to aspirate 500µL:

$$V_{asp} = \frac{V_r \Delta P_r}{P_s}$$

Therefore:
$$\Delta P_r = \frac{V_{asp}P_s}{V_r} = \frac{0.005 \times 1}{0.02} = 0.025 Bar = 25 mBar$$

Finally, consulting Figure 8 we can see that the pump drive power required to generate 25 mbar across a 4kLohm orifice, using a UBLB5400200A pump, is less than 250mW; this means that any effects due to self-heating should be small.

This system uses a large reservoir to combat self-heating from the pump, however, note that large reservoirs will also be more susceptible to changes in ambient temperature.

3.7.3. Design C

In the third example, the requirement is to aspirate 10µL with the highest accuracy possible.

In this case, it is a good idea to operate with a high aspiration pressure so that the impact of pressure sensor imprecision is minimised. At the same time, we should be mindful of the drive power required to reach the aspiration pressure (given the orifice selected): we should still aim for this to be lower than 250mW to avoid significant self-heating.

Consulting Figure 8, we can see that an aspiration pressures of \leq 57mBar with a 45kLohm orifice will require drive power \leq 250mW for a UBLB5400200A, so this sets our upper pressure limit. We choose 50mBar to provide a little margin. Using the equation in 3.3, we can calculate the reservoir volume required to aspirate 10µLwith 50mBar:

$$V_{asp} = \frac{V_r \Delta P_r}{P_s}$$

Therefore:
$$V_r = \frac{V_{asp}P_s}{P_r} = \frac{0.00001 \times 1}{0.05} = 0.0002 L = 200 \,\mu L$$



Therefore the reservoir volume should be 200µL. We note that this is a small reservoir and will require manufacturing and system assembly precision; however, given pipette tips of this volume are available, we conclude that it should be possible to manufacture a system reservoir with similar (and therefore adequate) precision. We calculate that this setup will provide a dispense time of less than 50ms.

Note that while this configuration is suitable for aspirating 10μ L (and volumes lower than this), it is likely unable to aspirate volumes that are significantly larger than 10μ L without suffering from self-heating effects.

3.8.Pitfalls to avoid

There are a number of possible pitfalls to avoid when designing and operating the VCM as follows:

- 1. Self-heating that occurs when running the pump at higher drive levels, which can raise the temperature of the gas in the reservoir and cause drift in the volume of fluid aspirated.
- 2. Leaks from the reservoir to the outside world, which can result in a continuous increase in the aspirated volume in order to keep the reservoir pressure at the required setpoint.
- 3. Leaks from the system side (e.g. at the interface between the pipette tip and the module it connects to) causing the aspirated volume to drop whilst being held in the pipette tip.
- 4. Compliance of the reservoir and/or connecting pathways, resulting in expansion on pressurisation.
- 5. Environmental temperature changes

3.8.1. Self-heating

As the pump runs, a portion of the drive power goes directly to heat. In addition, as air returns through the feedback orifice more energy is lost to heat. As a result, the temperature of the air circulating through the reservoir, pump and orifice will increase.

As its temperature increases, the gas expands. Given that the reservoir volume is fixed, the expanding gas causes the pressure in the reservoir to rise. Given that pump is driven to control the reservoir to set pressure, the pump drive level is reduced, allowing gas to flow from reservoir side to the system side. This has the effect of gradually reducing the aspirated volume over time as the circulating gas heats up, as shown in Figure 10.

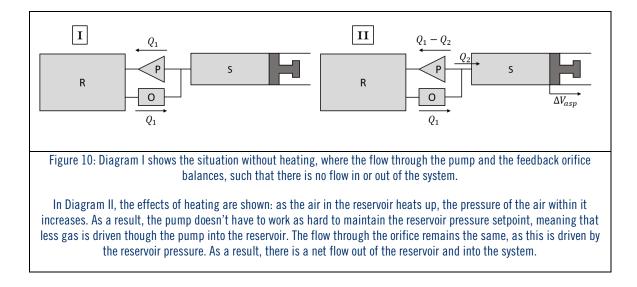
This can be countered by using a larger reservoir, as for a given aspiration volume, the aspiration pressure required will be lower; this means that the pump can be driven more gently, reducing the



amount of heat that the pump generates. In addition, the thermal mass of the circulating air will be larger, slowing the temperature rise.

Whilst a larger reservoir can counter self-heating, it does make the VCM more susceptible to ambient temperature swings (or heating from other active components that may be nearby) whilst pressure is being held in the reservoir. For the ambient thermal effects to be small it is needed that:

$$\frac{\Delta T_{therm}}{T_0} < \frac{\Delta P_{res}}{P_0}$$



3.8.2. Leaks

If the system is perfectly sealed, then when the reservoir pressure is held constant, the flowrate through the pump will be equal to the flow leaving the reservoir through the feedback orifice. This means that the amount of gas in the system will remain constant, as in diagram 1 in Figure 11.

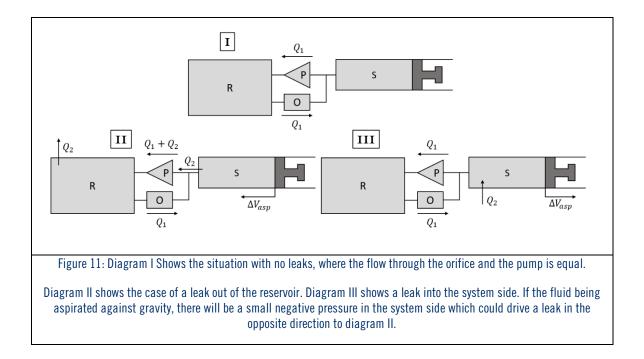
If there is a leak in the reservoir (or between the reservoir and pump, or reservoir and orifice, etc) then the flowrate of the pump will no longer be equal to the flowrate through the feedback orifice. If the reservoir pressure is kept constant, then the flow through the pump will increase to match the leak rate. This causes the fluid aspiration volume to increase gradually as in diagram 2 of Figure 11.

A leak on the system side would cause the volume aspirated to drop, due to extra gas entering the system, shown in diagram 3 of Figure 11. However, this kind of leak is likely to present less of an issue,



as the pressure in the system will (in most cases) be much closer to ambient pressure, causing the leak rate to be smaller.

Preventing leaks in the VCM critically important to ensure good, precise performance. This is especially true where the aspiration volume is small: for example, when aspirating 10μ L, it only takes 6 seconds for a 1μ L per minute leak to cause a 1% change to the volume aspirated. Leaks this small can be hard to measure—and indeed prevent.



3.8.3. Compliance

If the reservoir (or linking fluidic pathway) is made of a compliant material, then as the reservoir pressure (P_r) increases, the volume of the reservoir (and/or linking pathway) will also increase. As a result, the volume aspirated is no longer linearly proportional to the pressure in the system, given the volume of the system is now a function of the pressure.

This is easily prevented by using stiff materials and constructions that do not expand readily with pressure.



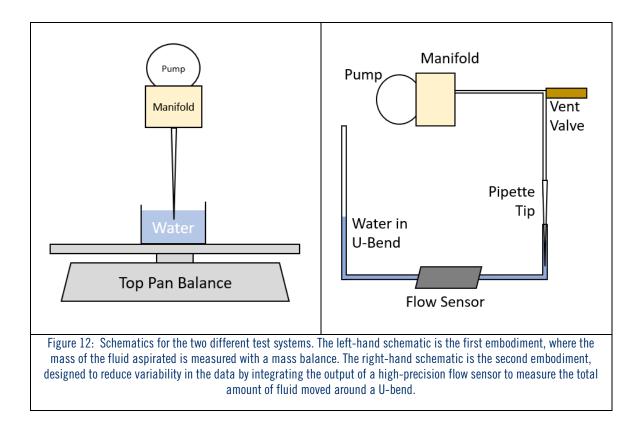
4. DATA

Two main embodiments of this system have been built and tested, as per the diagrams in Figure 12:

- In the first embodiment, the mass of water aspirated by a pipette tip was measured using a high precision mass balance. Two balances were used: one with 0.1 mg sensitivity, and one with 1 mg)
- 2. In the second embodiment, the total amount of fluid moved is measured with a high-precision liquid flow sensor.

The first embodiment uses a pipette tip, mimicking the real-world use case we anticipate for the VCM. However, with this approach we found inconsistencies in the interaction between the fluid and the pipette tip—such as the formation of droplets, liquid remaining on the interior sidewall of the tip, and other effects related to surface tension. We believe these inconsistences cause variation between pipetting cycles that appear to limit repeatability / precision.

Therefore, the second embodiment is designed to show the potential repeatability of the system by removing complications due to fluid-tip interaction.



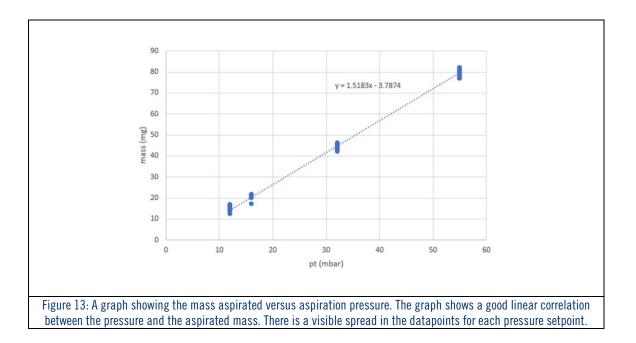


4.1.Data with Pipette tips

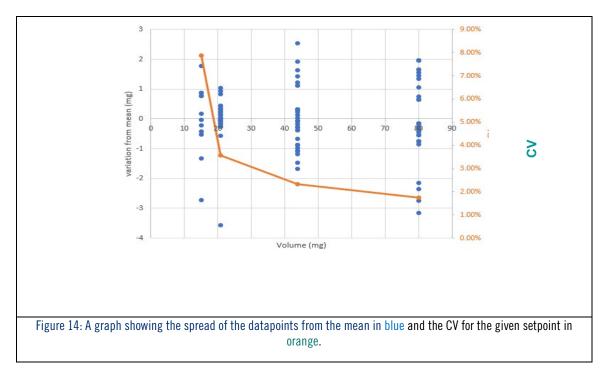
In this test a VCM with a reservoir volume of ~1.5mL and an *ART Molecular BioProducts 200L* tip was used. The amount of liquid aspirated was measured by lowering the pipette tip into a bath of water located on the mass balance. The water was then aspirated into the tip and the mass difference measured.

In the data presented in Figure 13, four different aspiration pressure setpoints were measured. There was a good linear correlation between ΔP_r and V_{asp} . Figure 14 shows the spread in the data more clearly, with the range being approximately ±2 to 3mg from the mean. This gives a lower limit on the aspirated volume that can be achieved with a Coefficient of Variation (CV) of less than 1%, of 100-200µL.

However, during this test we noticed inconsistencies in the interaction between the fluid and the pipette tip—such as fluid pinning in the tip, droplets remaining on the interior sidewall of the tip after dispensing, and other effects related to surface tension. We believe these inconsistences contributed to a significant fraction of the variation that limited repeatability between pipetting cycles.







4.2.Data with a Liquid Flow Sensor

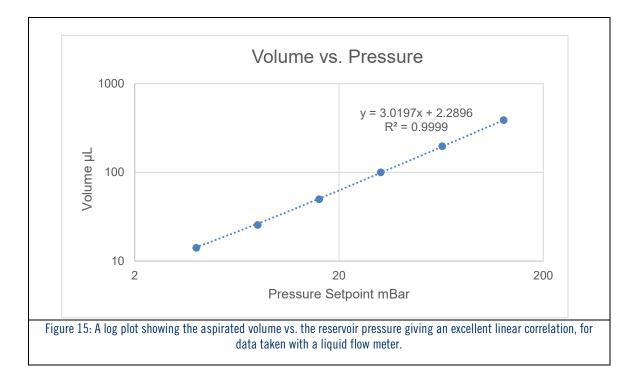
To determine the impact that inconsistent fluid-tip interaction has on repeatability, a second VCM embodiment was designed to remove fluid-tip interaction altogether. In this embodiment, the tip is not inserted into / removed from the fluid during each pipetting cycle. Instead, the tip is in permanent contact with a body of fluid in a 'U'-bend shaped tube, as shown in the right-hand schematic in Figure 12. The flow rate of fluid "aspirated/dispensed" was measured with a high-precision flow sensor, and then the flow sensor output was integrated to give the total volume moved through the U-bend.

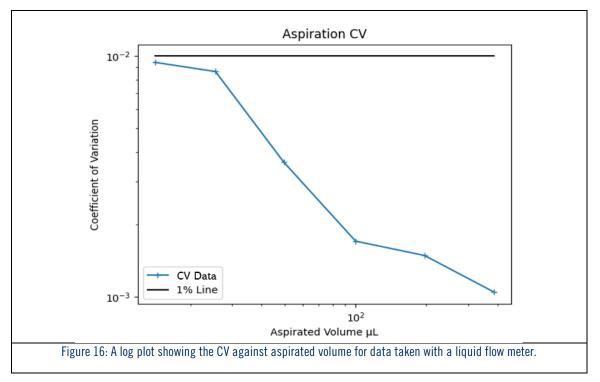
In this embodiment, there was an excellent linear correlation between the reservoir pressure and the volume "aspirated/dispensed" as can be seen in Figure 15. Note that the gradient of this curve is different to that in Figure 13, as the reservoir volume was different.

The standard deviation attained by this method were significantly improved, leading to better CV values, as can be seen in Figure 16. The measured CVs were lower than 1% across the full range of aspiration volumes, from \sim 15µL to \sim 400 µL.



The results for this measurement method were much more repeatable. This provides confidence that the VCM is capable of high-precision operation, and that real-world precision may be limited by fluid-tip interactions, and by the measurement precision of the mass balance used in the first embodiment.





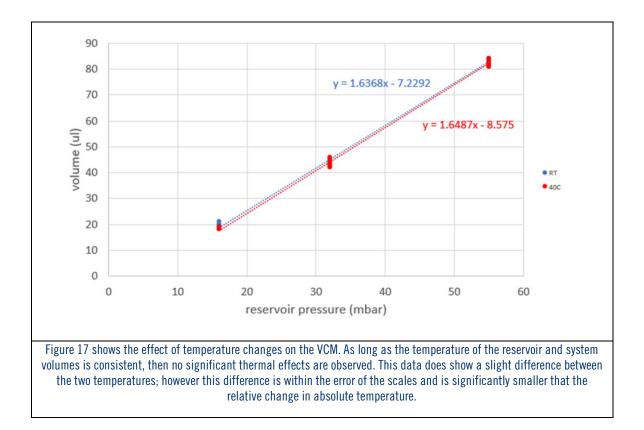
19



4.3.Thermal Stability

Using the first experimental setup, the effect of temperature on the VCM was tested. In theory, if the reservoir and system volumes are at the same temperature, there should not be any thermal effect on the pressure needed to aspirate a given volume.

The results of this test are presented in Figure 17. The test was conducted at two temperatures: room temperature, and 40°C. Despite the ~7% increase in absolute temperature, the gradient of the curve (for reservoir pressure versus aspirated volume) only increased by ~0.7%. This change is within the measurement noise of the scales and is therefore considered insignificant. This provides evidence that ambient temperature changes do not affect the VCM, providing that the reservoir and system volumes are at the same temperature.



The standard deviation of the mass aspirated was also unchanged by raising the temperature.



4.3.1. Thermal Expansion

Whilst the fundamental operation of the VCM has been shown to be independent of the ambient temperature, if the materials selected for the VCM exhibit significant thermal expansion, then changes in temperature may still have a significant effect on pipetting repeatability. Therefore, care should be taken when selecting materials, considering the range of temperatures that the system is designed to operate in.

In our thermal test the reservoir was a 3D printed in SLA part. The material used expands by approximately 0.5% when heated from room temperature to 50 C. If instead the reservoir was made from steel, the amount it would have expanded by would have roughly been 0.05%, i.e. 10x less, which would result in improved pipetting repeatability across temperature.

5. USEFUL DESIGN ADDITIONS

This document has covered a minimal viable VCM design. There are a several additions that can be made to the system to enable useful functionality.

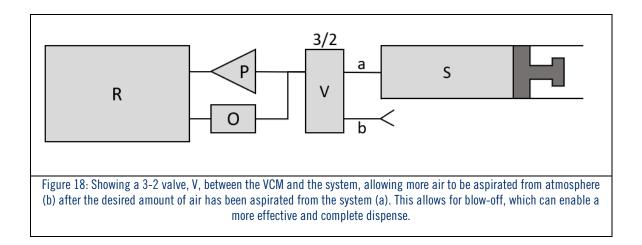
5.1.Valving

5.1.1. Side Vent Valve

Step 1 of the VCM process in §3.5 is a pre-charge step, intended to enable full and complete dispensing during Step 5. Whilst this technique works well, it does require the pump to run harder to maintain a higher aspiration pressure whilst holding fluid in the pipette tip. This will increase the amount of heat generated by the pump. For many applications, this temperature rise will be negligible. For more demanding applications that require higher pump drive levels, it may reduce pipetting accuracy and repeatability as discussed in §3.8.1.

To mitigate this issue, a side vent valve can be used as shown in Figure 18. This enables the VCM to draw air from the atmosphere rather than from the pipette tip, making it possible to increase the pressure in the reservoir without aspirating more air from the system (and therefore without aspirating more liquid). As a result, the additional 'charging' step required for full and complete dispensing can be carried out *after* aspirating the fluid, and immediately prior to dispensing. This minimises the length of time that the pump runs at higher power, reducing heating and maximising pipetting performance.





5.1.2. Feedback Valve

If the application requires the aspirated volume to be held for large periods of time, it may not be possible to limit the heating effects of the pump with the methods already described. This will lead instability in the aspirated volume.

One way to counter this is with a valve, either in parallel or series with the feedback orifice. If the valve is in series with the feedback orifice, then the valve may be closed and the pump turned off once the required pressure is reached in the reservoir. Then, when it comes to dispensing the volume, the valve can be opened and the gas allowed to escape the reservoir. This setup has the disadvantage that any backflow through the pump will need to be countered by turning the pump back on, so as to top up the reservoir pressure. Therefore the VCM should continuously monitor the reservoir pressure during the hold stage.

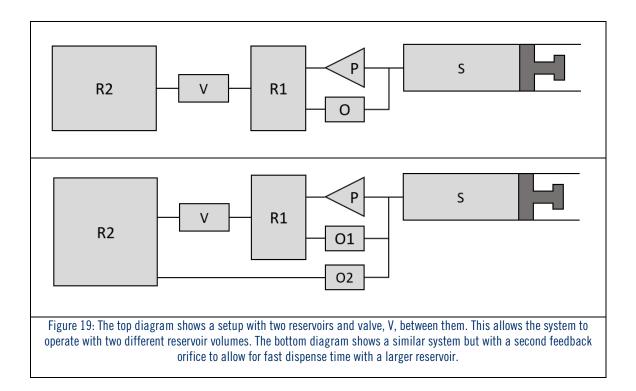
If instead the valve is placed in parallel with the feedback orifice, then a much more restrictive orifice can be selected than dispense speed would otherwise require (e.g. per Figure 7). This allows the pump to be operated at a much lower drive power than with a higher-flow orifice, thereby reducing heating. When dispensing, the valve is opened, allowing the reservoir pressure to discharge quickly, supporting rapid dispense speeds.



5.1.3. Multi-Volume Valve

A valve can be placed between the main reservoir and second reservoir (as shown in Figure 19), which allows for different fluid volumes to be aspirated while still running the pump at optimal pressure for self-heating and pressure sensor accuracy.

A second feedback orifice could also be fitted to the second reservoir to allow for a greater dispense speed if so required.



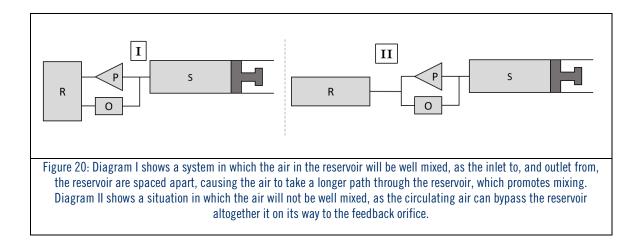
5.2.Temperature measurement

If it is expected that the ambient temperature of the system and the reservoir will be different (e.g. because the fluid is cooler or hotter than the ambient temperature, for example) it may be prudent to measure the actual gas temperatures of the VCM and system—for example with a pair of thermocouples. Any temperature difference between the VCM and system will have an impact on the volume aspirated per unit pressure. By measuring these temperatures, it is possible to calculate and apply appropriate temperature compensation.



It is also possible to use thermocouples to measure any self-heating of the module. Applying temperature compensation for this measurement may be more complicated, however, as it requires knowledge of the full internal volume of the system (e.g. including the internal volume of the pump and connecting fluidic pathway). In addition, the air in the pump chambers will be at a significantly higher temperature than the reservoir and so will account for a large portion of the thermal expansion for systems with a small reservoir volume. Nevertheless, in principle it is still possible to apply temperature compensation for self-heating where this is necessary.

Measuring the temperature of the air in the reservoir requires that it is well mixed. A good way to ensure this is to have the gas travel though most of the reservoir on its journey from the pump to the feedback orifice (Figure 20).



6. USEFUL EQUATIONS

6.1. Aspirating Against Gravity

If the liquid being aspirated is being lifted against gravity, then aspiration pressure will need to be higher to be able to hold the liquid against gravity. Unless the pipette tip is particularly long, the effects should be small. Nevertheless, it is easy to calculate and therefore compensate for:

$$V_{r}\Delta P_{r} = P_{0}V_{asp} + \frac{\rho g V_{asp}}{A} [V_{s} - V_{asp}]$$



Where *A* is the cross-sectional area of the pipette tip and ρ the density of the fluid. The terms in red are the addition terms relating to the interaction with gravity.

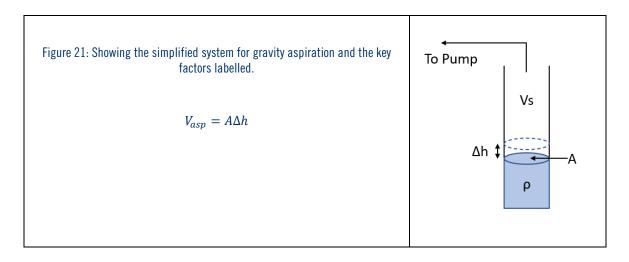
For the gravity effects to be large, these extra terms need to be significant when compared to the standard terms. Therefore, to be able to ignore gravity effects it is required that:

$$\frac{\rho g}{AP_0} \left[V_s - V_{asp} \right] \ll 1$$

An example system acting on water and consisting of a 10cm long uniform cross section pipette tip at atmospheric pressure would give:

$$\frac{\rho g}{AP_0} \left[V_s - V_{asp} \right] \approx \frac{\rho g V_s}{AP_0} = \frac{\rho g H}{P_0} \approx \frac{10^3 \cdot 10 \cdot 10^{-1}}{10^5} = 10^{-2} = 1\%$$

Where H is the height of the tube, and it is assumed that $V_s \gg V_{asp}$. In this system the effect is small, yet not insignificant for a high precision pipetting system.



6.2. Dispense / Deflation through an Orifice

For air at room temperature, if the units are:

- slpm for flowrate.
 - o Litres for volume
 - Minutes for time
- Bar for pressure.



Then the flowrate through an orifice can be calculated¹ as:

$$Q = \frac{8000}{L} \sqrt{P_{atm} \Delta P_r} = \frac{8000}{L} \sqrt{\Delta P_r} \text{ (if } P_{atm} = 1Bar)$$

This then gives a deflation time, for a given reservoir of volume V_r and initial pressure of ΔP_r , of

$$T_D = \frac{LV_r \sqrt{\Delta P_r}}{4000}$$

This can then be rewritten in terms of V_{asp} rather than ΔP_r , and with time units in milliseconds rather than minutes:

$$T_D = 15 L \sqrt{V_{asp} V_r}$$

Leading to the equation given in a previous section.

Therefore for a 10cc reservoir, a 4K orifice and an aspirated volume of 100µL.

- *L* = 4000
- $V_r = 0.01$ Litre
- V_{asp} = 0.0001 Litre

gives a T_D of 60ms.

6.3. Orifice Pressure/Flowrate vs. Pump Power

The pressure that a pump will be able to generate against an orifice is calculated by intersection of the orifice flow curve (shown in Figure 4). The orifice line is given in the previous section as,

$$Q = \frac{8000}{L} \sqrt{\Delta P_r}$$

And the pump load line at a given power, which if assumed to be a straight line in pressure vs. flowrate, is given by,

¹ See <u>How to Calculate Flow Resistance for Gases | The Lee Co</u>, using the subsonic equation.



$$P = P_{stall} - \frac{P_{stall}}{Q_{free}}Q$$

Where *P* is set to = ΔP_r , P_{stall} is the stall (maximum) pressure of the pump and Q_{free} is the flowrate with no back pressure. This can be solved to give the pressure that would be in the reservoir if the pump was run against a given orifice.

$$\Delta P_r = 2 \left(\frac{8000P_s}{2Q_f L}\right)^2 - 2 \left(\frac{8000P_s}{2Q_f L}\right) \sqrt{\left(\frac{8000P_s}{2Q_f L}\right)^2 + P_s}$$

 ${\it P_{stall}}$ and ${\it Q_{free}}$ can be approximated at different powers (II) using,

$$P_{\Pi} = P_{stall} e^{0.7\Pi}$$
 and $Q_{\Pi} = Q_{free} e^{0.55\Pi}$

6.4.Thermal Effects

If it is assumed that the gas in the reservoir is the only gas being heated then the fraction of the initial aspirated gas that subsequently flows back to the pipette tip is:

$$\frac{n_{therm}}{n_{asp}} = \frac{P_0 + \Delta P_r}{\Delta P_r} \cdot \frac{\Delta T_r}{T_0 + \Delta T_r}$$

This can be approximated as:

$$\frac{n_{therm}}{n_{asp}} = \frac{P_0 + \Delta P_r}{P_0} \cdot \frac{\Delta T_r}{T_0 + \Delta T_r} = \frac{\Delta T_r}{T_0} \cdot \frac{1 + \frac{P_0}{\Delta P_r}}{1 + \frac{\Delta T_r}{T_0}} \approx \frac{\Delta T_r}{T_0} \cdot \left(1 + \frac{P_0}{\Delta P_r}\right) = \frac{\Delta T_r}{T_0} \cdot \left(1 + \frac{V_r}{V_{asp}}\right)$$

As the increase in temperature for a given amount of heat will be inversely proportional to the volume of gas ($\Delta T_r = k/V_r$) that is heated, this means that the fraction de-aspirated due to heating is given by:

$$\frac{n_{therm}}{n_{asp}} = \frac{k}{V_r T_0} \cdot \left(1 + \frac{V_r}{V_{asp}}\right) = \frac{k}{V_r T_0} + \frac{k}{V_{asp} T_0}$$



The amount of heating is also proportional to the power of the pump that is roughly proportional to the square of the pressure, meaning that $k \propto (\Delta P_r)^2 \propto V_r^{-2}$ giving:

$$\frac{n_{therm}}{n_{asp}} \propto \frac{1}{V_r^3} + \frac{1}{V_{asp}V_r^2} = \frac{1 + V_{asp}/V_r}{V_{asp}V_r^2} \approx \frac{1}{V_{asp}V_r^2} ~(if~V_r > V_{asp})$$

This means that heating effects will get smaller with a larger reservoir.

6.5. Accounting for Compliance

If the material used is stiff enough that the expansion can be approximated as linear, then the volume aspirated is given by:

$$V_{asp} = \frac{T_s}{T_r P_s} \cdot V_r (\Delta P_r + \boldsymbol{\alpha}_{\Delta P_r} \boldsymbol{P_r})$$

Where $\alpha_{\Delta P_r}$ is the fractional expansion of the reservoir when pressurised to the target pressure. In this case V_r is specifically the initial reservoir volume, and P_r is the absolute pressure in the reservoir once it is filled.

It may be possible to calibrate a system if very high precision is needed, however it would likely be easier to use a material stiff enough to not expand. If aspirating to 20mBar, then for over-aspiration to reach 1% due to expansion, the fractional expansion $\alpha_{\Delta P_r}$ would need to exceed 0.0196, which would be a large expansion for such a low pressure for any reasonable material.

7. APPENDIX

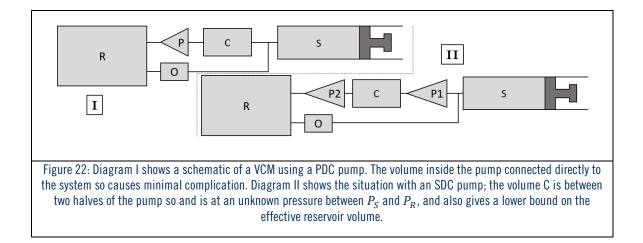
7.1.PDC VS SDC

As this is a pressure-limited rather than flowrate-limited system, it might appear tempting to try and use a higher pressure pump (e.g. an SDC or even a HP Series pump) instead of a PDC. However, this is not a good idea. This is because Disc Pumps have internal volume: in the case of a PDC pump this is a small volume before the valve, and adds to the system-side volume. In an SDC pump, this volume is between



the valves (as shown in Figure 22) and, during operation, is at a undefined and hard-to-measure pressure. This reduces control precision and places a minimum value on the effective reservoir volume.

This is also true for HP Series and UltraSlim Series pumps.



7.2.PERFORMANCE TABLES FOR ALL PDC PUMP

Values given are approximate values, calculated from pump minimum product specification and assuming a straight load line.

UBLB5400000	0000A: BL series, max power 1W												
		Stall Pressure (mBar) of an UBLB5400000A											
	Drive				Orif	ice - L(Dhms						
	Power	4k	8k	12k	15k	20k	25k	30k	40k	45k			
	20mW	2	4	6	7	8	8	9	9	9			
	50mW	5	10	12	14	15	16	17	18	18			
	100mW	9	17	22	24	26	27	28	30	30			
	250mW	22	37	44	48	51	54	55	57	58			
	500mW	40	64	75	80	86	89	92	95	96			
	1W	73	111	127	135	143	148	151	156	157			



UBLB5400200	BLB5400200A: BL series, max power 1W												
	Stall Pressure (mBar) of an UBLB5400200A												
	Drive		Orifice - LOhms										
	Power	4k	8k	12k	15k	20k	25k	30k	40k	45k			
	20mW	4	6	7	8	8	9	9	9	9			
	50mW	8	13	15	16	16	17	17	18	18			
	100mW	15	22	25	26	27	28	29	30	30			
	250mW	32	44	49	51	53	55	56	57	57			
	500mW	57	75	82	85	88	90	91	93	94			
	1W	99	126	136	141	145	148	150	152	153			

UBLX0500000A: XP series. Max power 1.4W intermittent, but not recommended for a VCM due to self-heating.

		Stall I	Pressu	re (mE	Bar) of	an UB	LX050	0000A			
		Orifice - LOhms									
	Power	4k	8k	12k	15k	20k	25k	30k	40k	45k	
	20mW	3	5	7	8	9	9	10	10	11	
	50mW	6	12	14	16	18	19	19	20	21	
	100mW	12	20	25	27	30	31	32	33	34	
	250mW	26	43	51	55	58	61	63	65	66	
	500mW	48	74	86	92	98	101	104	107	108	
	1W	88	128	146	154	162	167	171	176	177	
	1.4W	116	166	188	197	207	214	218	223	225	



UXPB5400200A: XP series. Max power 1.4W intermittent, but not recommended for a VCM due to self-heating.

-												
		Stall Pressure (mBar) of an UXPB5400200A										
	Drive		Orifice - LOhms									
	Power	4k	8k	12k	15k	20k	25k	30k	40k	45k		
	20mW	4	6	7	7	8	8	8	9	9		
	50mW	8	12	14	15	16	16	16	17	17		
	100mW	15	21	24	25	26	27	27	28	28		
	250mW	31	42	47	48	50	52	52	54	54		
	500mW	55	71	78	80	83	85	86	88	88		
	1W	95	119	129	133	137	139	141	143	144		
	1.4W	124	153	165	169	174	177	179	182	183		

The 1.4W intermittent powers for XP series could be used to generate large blowoff pressures in conjunction with a valve.



8. SUPPORT

The Lee Company website provides advice on:

- Getting Started
- Applications
- Development Process
- Downloads (including datasheets, application notes, case studies and 3D models)

The Lee Company is happy to discuss next steps beyond prototyping, including system design. If you would like to discuss this with us, or for any other additional support, please contact your Lee Sales Engineer.

9. REVISION HISTORY

Date	Version	Change
June 2023	06/23	Rebranded document
13 April 2022	220413	First release of detailed application note.