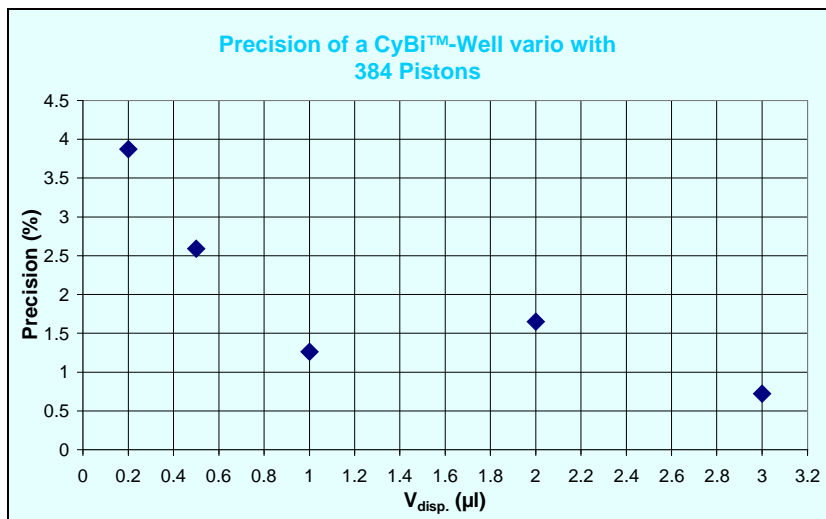# Application Note

Results of Pipetting of Small Volumes using a 384 CyBi™-Well

Handling pipetting volumes below 1 µl with a precision better than 10 % is currently one of the major needs in the present automated drug discovery. CyBio has complete an initial round of testing demonstrating that the CyBi™-Well vario and CyBi™-Well 2000 with 384 tips are capable of pipetting 0.2 µl into a wet plate with less than 5% CVs. The following data are results of precision and accuracy tests with a CyBi™-Well vario and a CyBi™-Well 2000 with 384 pistons according to the procedure described below.

Precision

Figure 1 and 2 show the photometric determined values of the precision and the deviation of the single wells.

Fig. 1: CV values of a 384 CyBi™-Well vario. Pipetted into a 384 Greiner clear bottom plate with 25 µl CyBi™-Tips in an aqueous solution as described below.

Volume (µl)	CV ** (%)
0.2	3.9
0.5	2.6
1.0	1.3
2.0	1.7
3.0	0.7

** These values were obtained by the below described procedure with a CyBi™-Well vario. For a reasonable check all parameters must be kept strictly. The values are not guaranteed.

Application Note

Fig. 2: The deviation of the extinction of the single wells from the mean extinction of a 384 Greiner microplate with a **CyBi™-Well 2000**.
Pipetted volume is 3 µl.
CV value is 0.75 %.
The **maximum deviation is 1.70 %,** the **minimum deviation is -1.80 %** measured according the below described procedure.
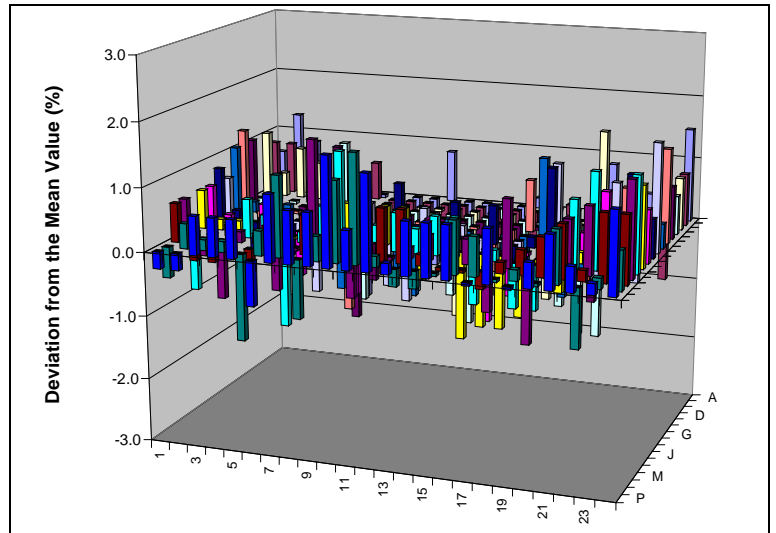
Accuracy

Figure 3 shows the accuracy values for the range of 0.2 µl to 3 µl.
The accuracy of the **CyBi™-Well 2000** with 384 channels was determined by weighing the microplates after pipetting.
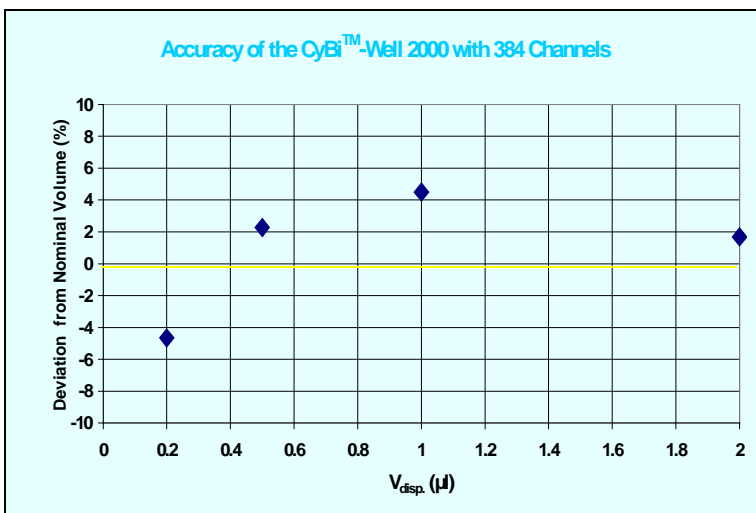
Volume [µl]	Accuracy *** [%]
0.2	- 4.7
0.5	2.3
1.0	4.5
2.0	1.7

*** The density of the solution was determined by a pycnometer. To evaluate losses by evaporation a sealed and a non sealed plate were compared.

Fig 3: Accuracy of a 384 CyBi™-Well 2000. Pipetted into a 384 Greiner microplate with 25 µl CyBi™-Tips into an aqueous solution.

Application Note

The results are affected by some methodological parameters as described in Table 1.

Table 1

Parameter	Influence
Type of microplate	Precision: - No difference between "normal" and "small volume" for 384 plates - CV better when dispensing into 384 plates than in 1536 plates (with the same volume)
Dispensing into dry plate or a liquid	Precision: - When dispensing into dry wells, the deviation from the mean value of the dispensed volume is higher than into a liquid due to the surface tension Accuracy: - When dispensing into a liquid, the deviation is positive due to the reduced outlet resistance
Flatness of the microplate	Important for dry dispensing (CyBi™-Well is able to touch very small volumes at a flat bottom)
Detection process*	Linear range must be ensured
Concentration of DMSO	Precision: - Decreases below 1 µl pipetting volume with increasing concentration Accuracy: - Increases slightly with increasing concentration
Volume of pipetting tips (10 µl/ 25 µl)	Precision: - Especially for dry pipetting of samples containing high amounts of water 10 µl CyBi™-Tips give better performance than 25 µl CyBi™-Tips Accuracy: - No significant difference
Evaporation*	Accuracy: - Mass reduction of less than 0.6 % per minute (25°C, 40 % humidity) - Evaporation is inhomogeneous
Pre-filled volume	Should be as low as possible because high volumes of pre-filled liquid enforce the diffusion of the indicator and the liquid in the tip into the pre-filled liquid. This effect is promoted by a lower density of the pre-filled liquid.
The depth of immersion	Should be as low as possible to avoid carry-over
Time of immersion	Should be as short as possible to avoid effects of diffusion (s. "pre-filled volume")
Concentration of p-Nitrophenol and NaOH*	A pH-value of ≥ 8 (color transition of p-Nitrophenol) and the linear range of the detector must be ensured.

* Highly importance on the significance of the measuring method, not on the precision or accuracy of the device

Application Note

The results shown in Fig. 1 and 2 prove that the CyBi™-Well 2000 and the CyBi™-Well vario in the 384 tip format are able to achieve CV values less than 5 % for 200 nl pipetting volume and less than 2 % for 1 µl pipetting volume when pipetting into a liquid in the 384 plate format.

The values shown in Figure 1 and 2 were obtained by the following procedure.

- The measurement was taken in Greiner 384-well clear bottom microplates with 25 µl CyBi™-Tips.
- The concentration of the tested solution were adjusted so that the resulting extinction at 405 nm was between 0.4 and 1.2. The concentration was 417 mg p-Nitrophenol per liter for 0.2 µl and 0.5 µl and 139 mg p-Nitrophenol per liter for 1.0 µl, 2.0 µl and 3.0 µl in 0.1 N NaOH.

- 50 µl 0.1 N NaOH per well were placed in the above mentioned microplate with a CyBi™-Well 2000.
- Only new tips were used for the precision measurements and they were rinsed with the p-Nitrophenol solution having the following settings:

Piston Speed:	100 rpm
No. of rinse cycles:	10
Rinse volume:	10µl

- Measurement took place by pipetting the test volume into the 384 microplate with the already placed NaOH-solution. During dispensing the test volume the pipetting tips were immersed about 1 mm into the NaOH-solution. The remaining volume was dispensed back into the reservoir, supply vessel, resp.
- Testing took place in the manual mode with an aspiration volume of 10 µl. A first pipetting cycle run back into the reservoir, supply vessel, resp.
- A single channel vertical photometer with the option for 384 microplates -Tecan Spectra Flour Plus - was used. Previous to the measurement, the geometric parameter of the well grid has been defined. Measurement of extinction in the vertical photometer.
- Due to the less favorable miscibility of the p-Nitrophenol-solution with the NaOH-solution in 384 microplates, the following process were executed before the measurement:
  The substances were mixed in an orbital shaker for 15 minutes.

  Mixing in the orbital shaker for 10 minutes at 1000 rpm

  Waiting 30 - 45 minutes

  Repeat mixing for 10 minutes at 1000 rpm
- The microplate were sealed after the addition of the NaOH-solution, after pipetting and during the mixing process.

The Precision is defined as follows:

$$CV = \frac{\sigma}{S_m} \, 100 \, (\%)$$

Where CV is the coefficient of variation,

σ the standard deviation $\quad \sigma = \sqrt{\dfrac{\sum\limits_{n=1}^{n=N} (S_n - S_m)^2}{N-1}} \quad$ where S_m is the mean extinction with $\quad S_m = \dfrac{\sum\limits_{n=1}^{n=N} S_n}{N}$, S_n the extinction of a single

well and N the number of wells.

The accuracy is defined as follows:

$$Accuracy = \frac{V_m - V_{adj.}}{V_{adj.}} \, 100 \, \%$$

Where V_m is the volume determined by weighing the microplate accordant $\quad V_m = \dfrac{m_{Plate}^{disp.} - m_{Plate}^{0}}{N\rho_{liquid}(T)} \quad$ and $V_{adj.}$ is the volume adjusted

by the CyBio Control software.