

# Precise low volume compound handling with the new Nanoliter Head of CyBi®-Well vario



Katrin Undisz and Peter Zimmermann,  
CyBio AG, Goeschwitzer Str. 40, D-07745 Jena, Germany, [www.cybio-ag.com](http://www.cybio-ag.com)

## Introduction

The possibility for low volume compound transfer with high reliability has become the major requirement for compound management and assay miniaturisation. It limits compound consumption, enables low solvent concentrations especially for cellular assays and avoids additional compound dilution steps. CyBio, the leading provider for highly precise simultaneous liquid handling devices, now introduces the new Nanoliter Head for the CyBi®-Well vario. This pipetting module is available with 96 or 384 ceramic tips that were especially designed for the Nanoliter transfer of test compounds dissolved in DMSO. It provides precise liquid transfer from 25 to 2500 Nanoliter. Mother plates with a residual volume as low as 2 µl can be used. This poster is demonstrating the specification and experimental data of the Nanoliter Heads 96 and 384 for the whole volume range and is discussing optimized procedures for a reliable pipetting and tips washing procedure.



Figure 1: CyBi®-Well vario workstation with 2 stackers

## Components of the CyBi®-Well vario Nanoliter Head

The Nanoliter pipetting tool consists of the CyBi®-Well vario Nanoliter Head 96 or 384 with tip magazine containing 96 or 384 specifically designed ceramic tips (maximum capacity 2.5µl, length 30mm). The aperture and front surface of the ceramic tips were designed to minimize the adhesion (pin tool effect) and to enable low volume liquid transfer with high precision. The exceptional performance of the Nanoliter Head is primarily designed for wet-to-wet transfer.



Figure 2: CyBi®-Well vario Nanoliter Head 96

## Materials and Methods to validate the CyBi®-Well vario Nanoliter Head 96 and 384

P-Nitrophenol (p-NP, Sigma, 104-8) dissolved in DMSO (Merck Darmstadt) was used as model compound solution in a concentration between 60mM and 240mM. The different volumes were transferred in one step into 96-well or into 384-well transparent flat bottom plates (Greiner bio-one, 655 101 or 781 101) prefilled with 150 µl or 50 µl 0.1 N NaOH solution (Sigma, S 8045), respectively.

After shaking (10 min at 1000 rpm) and centrifugation (2 min at 700 x 9 G) absorbance at 405 nm was measured earliest one hour after the liquid handling with a POLARstar (BMG Labtechnologies). The selected p-NP concentration should result in optical densities between 0.5 and 1.5 (linear range of the reader).

The tips were cleaned with an active CyBio Tip Wash Station using water with 10% DMSO as cleaning reagent (a higher DMSO content is also possible). The cleaning effect was measured after pipetting 1000 nl highly concentrated p-NP solution (240 mM) into waste and immediate pipetting of 1000 nl water into a 96-well test plate. The plate was measured and the remaining p-NP solution was determined via a calibration curve. This procedure was repeated after pipetting and washing with different numbers of washing cycles.

## Optimized procedures for liquid handling with the CyBi®-Well vario Nanoliter Head

- The immersion depth during aspiration as well as during dispensing was set at 1 mm to minimize the wetting of the tips outer surface (pin tool effect).
- Pre-wetting of the ceramic tips is recommended.
- The most precise results were achieved using incremental pipetting with reduced plate processing speeds and a pause of 1 s after every aspiration and dispensing step.
- The overstroke volume at the end of a liquid transfer step should be as high as possible.

## Results

### Precision of the Nanoliter Head 96 and 384 for different volumes

| Volume [nl] | Specification Precision (cv per plate in %) | Experimental data Precision (CV per plate in %) |          |
|-------------|---|---|----------|
|             |   | 96 Head   | 384 Head |
| 2000        | 3.00  | 0.83  | 1.47     |
| 1500        | 3.00  | 1.14  | 1.20     |
| 1000        | 3.00  | 1.44  | 2.29     |
| 500         | 5.00  | 1.86  | 2.89     |
| 200         | 7.00  | 1.88  | 2.63     |
| 100         | 7.00  | 2.36  | 2.60     |
| 50          |   | 4.10  | 3.92     |
| 25          |   | 9.82  | 4.51     |

Table 1: Nanoliter Head 96 and 384, specified precision values and experimental data in dependence on the handled volume. Values show means of 3 plates

Up to 200 nl precision values less than 2 % were measured, for the handling of 100 nl precision values less than 5 % are possible.

### Cleaning of the Nanoliter Head 96 ceramic tips

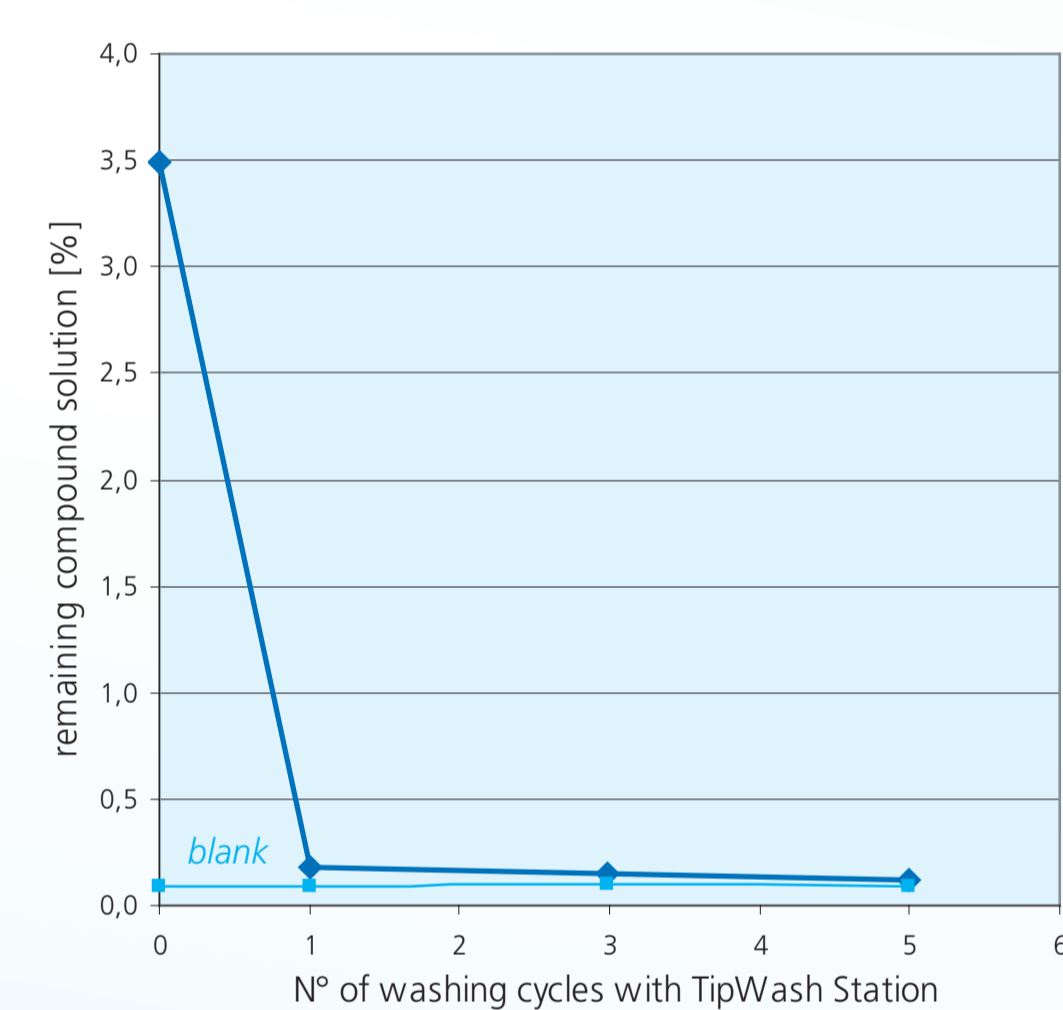


Figure 3: Remaining compound solution without washing and after different N° of washing cycles following the pipetting of 1000 nl 240mM p-NP solution with the Nanoliter Head 96 into a 96-well plate

The ceramic tips can be cleaned easily with the active CyBio Tip Wash Station that allows rinsing the tips also from outside.

After one washing cycle the remaining compound solution was less than 0.2 %. A tips cleaning procedure with 5 washing cycles ensures reliable decontamination with values in the range of the blank.

### Selected application examples

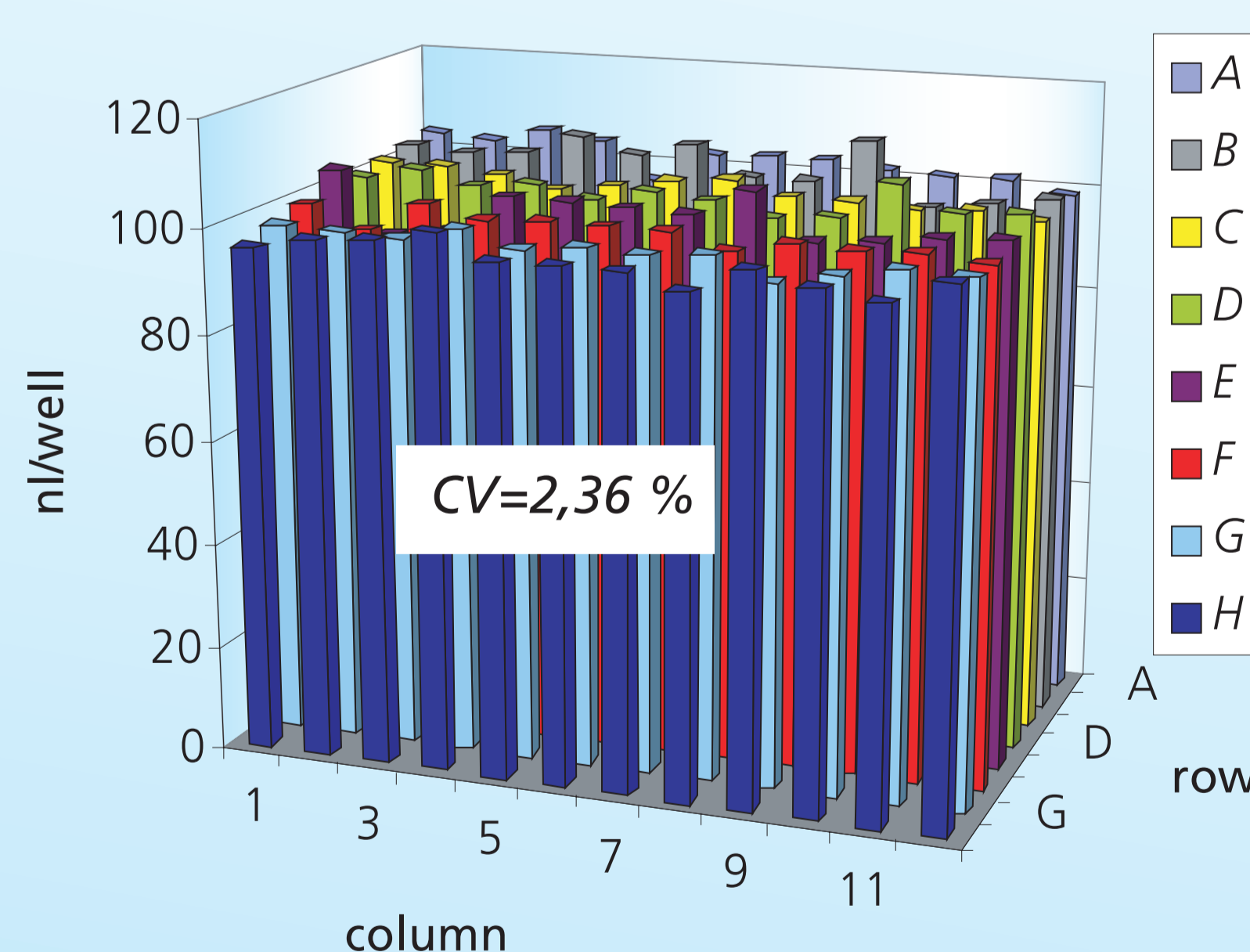


Figure 4: Precision of a 1 step transfer of 100 nl 240 mM p-Nitrophenol solution into a 96-well plate with the Nanoliter Head 96 (for details see materials and methods)

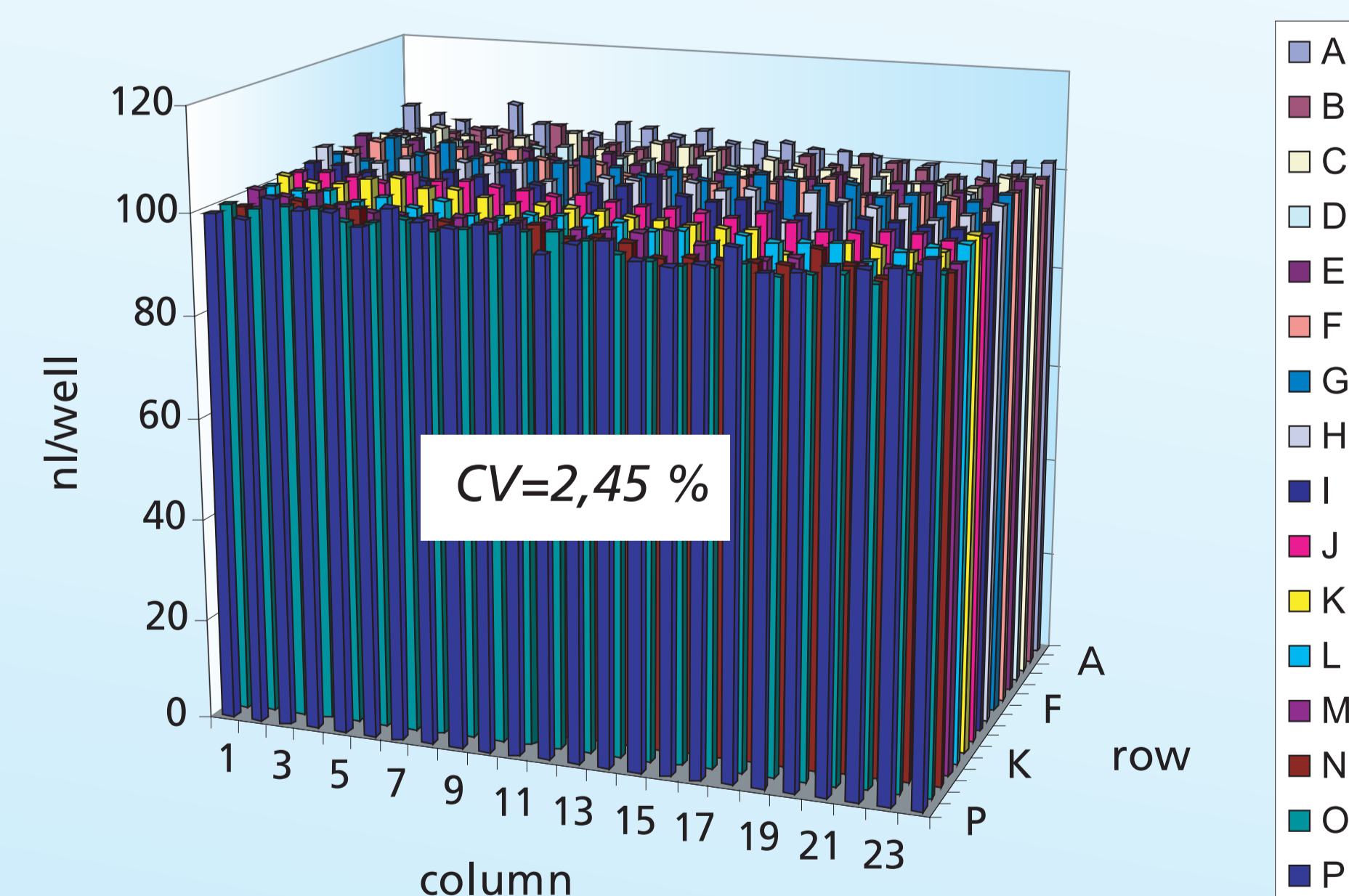


Figure 5: Precision of a 1 step transfer of 100 nl 60 mM p-Nitrophenol solution into a 384-well plate with the Nanoliter Head 384

Figure 4 shows graphically the result of transferring 100 nl 240mM p-Nitrophenol solution in DMSO in one step into a 96-well plate. Figure 5 shows the equivalent transfer but with a 384 channel head to fill a 384-well plate in one go. Both transfers were performed at high precision (CV = 2,36% and 2,45%, respectively).

## Conclusion

The new Nanoliter Head from CyBio completes the flexible pipetting platform of the CyBi®-Well vario and provides your solution for precise low volume compound handling.