Cartridge Usage Recommendations for WOLF & WOLF G2 with WV3.3.4313

Updated Cartridge Set-up Recommendations – with WV3.3.4313

With respect to cartridge set-up, we NO longer recommend that customers "scan for bubbles" as a part of the normal workflow

- *Rationale for change:* Over the past few years, we have made a series of design improvements to both our cartridges and software priming algorithms. With these new improvements, we are much less likely to have an unwanted bubble within the chip. Furthermore, even if there is a bubble present, it may not negatively impact the ability to calibrate and sort.
- *Benefits of change:* streamlined customer experience, less time troubleshooting "bubbles" that may not actually cause a problem
- *New Recommended Workflow:* prime --> align --> analyze calibration beads --> calibrate.
    - If calibration passes, then you can confidently continue on to sample analysis and sorting.
    - If calibration fails and provides a prompt about possible incomplete priming or calibration error code 03, then you can scan for bubbles at that point.

If calibration fails and our software messages indicate that there may be a priming-related issue, please do NOT press the "re-prime" button or "flush" buttons, instead "prime" the cartridge a second time

- *Rationale for change:* The "prime" algorithm is different than the "re-prime" algorithm. In my recent experience, performing two "primes" is more effective than performing one "prime" and one "re-prime".
- *Benefits of change:* enhanced success rate
- *New Recommended Workflow:* If calibration fails and provides one of the following messages: error code 03 or possible incomplete priming, proceed to do the following: *open purge valve ---> new cartridge --> prime --> align --> analyze calibration beads --> calibrate*
    - Please perform this troubleshooting workflow even if you do not observe any bubbles, as the bubble could be out of the live camera field of view
    - Reminder - when performing the second "prime", please remember to open the purge valve prior to starting - otherwise it will not be as effective
    - Please remember to close the purge valve when prompted by the software at the end of the prime step.
    - Please proceed to calibrate the cartridge regardless of whether or not there is a bubble
- *Additional comments:*
    - Please do not attempt to re-prime or flush the cartridge to remove bubbles.
    - After performing the 2nd "prime" attempt, if calibration still fails with error code 03 or messages about possible incomplete priming, please switch to new cartridges and alert NanoCellect Technical Support.
    - If the failed cartridge is within warranty, NanoCellect will replace that cartridge.
    - Please do not attempt to "prime" the cartridge more than 2X. If the cartridge is not successful after two "primes", its unlikely that additional primes will improve the outcome.

Updated Cartridge Design Change

Improvements to our cartridge design

As part of our cartridge design optimization over the past few years, we have incorporated the use of a bubble within our cartridge to help effectively sort cells.

- *Rationale for use of dampening mechanism*: When the piezoelectric actuator of the cartridge pulses, it generates a pulse wave that will re-direct the fluid stream to either channel A or channel C. However, we also need a way to dampen the pulse wave, otherwise it'll accidentally sort additional, unwanted cells.
- *Disadvantage of previous design*: Our previous cartridge design utilized an elastic membrane layer to dampen the pulse wave. However, that membrane layer was a weak design point, and often a source of failure.
- *Design Improvements:* In order to make our cartridges more robust, we incorporated an air pocket to dampen the pulse wave. This air pocket is designed to be in a very precise location in the left chamber, please see image below. This bubble should be very far away from the main channel, and will not be shown in the normal "scan for bubbles" algorithm within our software. However, it can be visualized if the user manually moves the live chip image.
- *Additional Comments:*
    - If the sheath pump is turned off, this bubble will expand and may enter the field of view. However, this is normal behavior - after the sheath pump is turned back on the bubble will return to its normal position within a few minutes.
    - All aspects of our new cartridge design have undergone extensive validation and verification testing. The new cartridge designs performs as well if not better in all aspects.

Purpose: Echo Chamber Bubble serves to dampen the pulse wave generated by the PZT, to prevent off-target events from being sorted by secondary echo wave