6-12-2017

Droplet-Microfluidic Device for the Characterization of Perfluorinated Emulsions

Daniel Horvath
Santa Clara University, dhorvath@scu.edu

Nam Ahn Tran
Santa Clara University, nvtran@scu.edu

SANTA CLARA UNIVERSITY

Department of BioEngineering

I HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER MY SUPERVISION BY

Daniel Horvath, Nam Anh Tran

ENTITLED

Droplet-Microfluidic Device for the Characterization of Perfluorinated Emulsions

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

BACHELOR OF SCIENCE

IN

BIOENGINEERING

Paul Abbyad — **June 12th, 2017**

Thesis Advisor (Paul Abbyad) — Date

Yuling Yan — 6/14/17

Department Chair (Yuling Yan) — Date

Droplet-Microfluidic Device for the Characterization of Perfluorinated Emulsions

By

Daniel Horvath, Nam Ahn Tran

SENIOR DESIGN PROJECT REPORT

Submitted to
the Department of BioEngineering

of

SANTA CLARA UNIVERSITY

in Partial Fulfillment of the Requirements
for the degree of
Bachelor of Science in BioEngineering

Santa Clara, California

Spring 2017

Abstract

Microfluidics is being used throughout academia and industry today to perform large numbers of experiments with extremely small volumes of fluids. By doing this, those that study microfluidics hope to raise through-put, lower cost and limit the environmental impact of scientific research.[2] Complementing the increased use of microfluidics, the use of perfluorinated emulsions in the field of droplet-based microfluidics is also experiencing large growth.[3] However, many of the products available today are either proprietary and/or poorly understood. While some chemical structures are known, some of the most scientifically intriguing perfluorinated oils and surfactants do not have their chemical structures or characteristics available to the public research community. During the course of our senior design project, we isolated one particular perfluorinated oil and surfactant mixture and performed a number of tests on the stability of the emulsions over a variety of different biologically relevant variables. To achieve this, we were required to design multiple iterations of droplet-microfluidic chips to create the simplest emulsions for study. The resulting designs gave us an excellent platform to study not only our emulsion system, but many future emulsions systems in a novel manner. We proved its efficacy by using the system to show great variance in the stability and surface energy of different pH droplets in QX100 perfluorinated oil with surfactant as well as a characterizable change in stability when changing the concentration of phosphate and media constituents in the droplets. Finally, we further analyzed the emulsion with the help of Dr. Gerald Fuller's coalescence lab in Stanford University's chemical engineering department and observed changes in the viscoelastic nature of aqueous-perfluorinated oil interfaces over a variety of biologically relevant conditions. Though much was accomplished, there is still a need to further characterize both more aqueous conditions and more perfluorinated systems to truly shed light on the field. Additionally, more detailed viscoelastic analysis needs to be performed on these new conditions and oil-surfactant mixtures as well as a thorough pendant-drop style surface tension experiment on all experiments performed.

Acknowledgements

We would like to thank our advisor, Dr. Paul Abbyad, for the countless times he provided us with much needed advice and assistance.

We would also like to thank Dr. Gerald Fuller of Stanford University's chemical engineering department for assisting our research and providing us with a Ph.D. student to help us run interfacial rheometry experiments. Also, a big thanks to Simone Bochner de Araujo, the Ph. D. student who helped us run all of the experiments at Stanford.

Finally, we would like to thank the school of engineering for the funding and opportunity to perform a project like this in our senior year of Bioengineering.

Table of Contents

List of Figures

List of Tables

Chapter 1 – Introduction and Background

Introduction

The modern-day advancement of life science research is largely limited to the amount of time and resources allocated.[1] Much of the research done around the world today has the potential to be both more time efficient and reduce reagents used via an alteration of how the research is done. By shrinking traditional biochemical procedures down to a micro-scale, researchers are able to have greater control of the environment in which reactions occur, produce less waste, and increase efficiency with implementation of large scale microfluidic experiments.[2] Within the study of microfluidics, there are several categories that are separated based on the mechanism that drives flow and the nature of the devices. One of these categories, droplet-based microfluidics, uses discrete aqueous-phase droplets suspended in an immiscible oil phase. Due to this, droplet-based microfluidics offers a variety of benefits as entire experiments can be compartmentalized into each droplet, and the droplets can be created and manipulated at an astounding rate.[3] However, the use of microfluidics is typically hindered by the devices' reliance on large, expensive and specialized laboratory equipment. One of the many cumbersome pieces of equipment required in droplet-based microfluidics is the use of a high-energy external stimuli to induce droplet coalescence. We aim to remove the need for some of this large equipment by the characterization of emulsion stability of relevant compounds used in droplet-microfluidics and on the lab bench. Not only will the characterization of these reagents' effects on emulsion stability assist in developing large-scale biological assays, but it will also greatly contribute to the understanding of emulsion science which has applications all over the modern world.[8]

We fabricated a droplet-microfluidic device capable of characterizing the stability of droplet emulsions to coalescence. In this device, we can vary many different important parameters that control emulsion stability including aqueous phase contents, aqueous phase pH and surfactant concentration in the flow phase. The device uses an array of wider channel sections that trap droplets via lowering of their surface energy such that tens of droplet pairs can be trapped and isolated in a roughly 1mm x 1mm region. Feeding this array region are two

functional regions of the chip, the first being the geometry that creates droplets and the second being the geometry which enables the mixing of two components in a laminar flow environment. By doing this we hope to observe how different aqueous phase components and parameters affect the stability of the emulsions. Once, many relevant chemicals to biological assays have been characterized, the data will allow it to be unburdened by the often costly inclusion of external, high-energy devices for inducing coalescence. Additionally, the further understanding of complex emulsions could assist a variety of industries including petroleum and cosmetics in performing expensive, and complex processes involving emulsions.[6]

Background and Significance

Microfluidics, itself, is the flow of liquids less than a milliliter in volume through micrometer sized channels and has applications in multiple fields. For the purposes of our project, we apply this system to chemical and biological studies where we control different parameters of the flowing system such as: channel geometry, flow rates, concentration, mixing, shear stress, viscosity, and surface properties. As a whole, microfluidics uses smaller volumes of reagents which promotes cheaper, greener, and safer chemical reactions.[4] Additionally, we confine all of our reagents into droplets, which are ideal vessels through which to manipulate reagents and have been shown to be a promising platform through which to perform experiments. Additionally, chemically induced coalescence has been shown to work selectively in multiple other microfluidic systems and perfluorinated oils' role in research and industrial application is projected to grow rapidly.[4,3]

Understanding emulsion science is critical in order to effectively design or evaluate new or existing processes in multiple industries. For example, the petroleum industry for decades has been seeking to improve various methods of demulsification with respect to varied influential parameters such as temperature, pH, salinity, droplet size, dispersed phase content, and emulsification concentration. Although research on effectiveness of various emulsification methods has been inconsistent, surfactants have been the primary contributor to emulsion stability.[5] The nature of certain surfactants (ionicity, hydrophobicity, and concentration) may prove beneficial to the industry by increasing efficiency in the demulsification process while imposing the least economic burden.

Emulsion science is also important in certain areas of the pharmaceutical industry. Nanoemulsions are being increasingly investigated as potential drug carriers.[6] While there is still research to be done on nanoemulsion formulation, method of preparation, and characterization, it is a very promising as a drug delivery vehicle for disease diagnostics and therapies such as cancer treatment. Further characterization of various biochemical reagents has the potential to help the pharmaceutical industry formulate processes to develop drugs primarily carried through nanoemulsion technology.

Another important feature of microfluidics is the numerous biological and chemical applications, microfluidics is used as a tool to speed up and reduce cost of performing hundreds

to thousands of individual experiments at once.[7] This has been implemented to provide accurate, and fast analysis for laboratory research such as live-dead cell assaying, 3D cell culture, digital PCR, and proteomics.[2] However, a sub-type of microfluidics, known as droplet-based microfluidics, is shown to be of more use in experimentation of single cell analytical assays than traditional, Microfluidic large-scale integration devices.[3] In a large part, this is due to the isolation of each from one another and the ability to treat each droplet as its own test-tube or well. This being said, droplet microfluidics is hindered by a few cumbersome necessities. First of all, it requires the use of complex and expensive flow inducing devices such as syringe pumps.[2] This is required due to the need for very precise and small volume flows through the chip. Secondly, it is often limited by the reagents that can be used in the chips as the most common material used to fabricate them is Polydimethylsiloxane (PDMS), which absorbs most organic solvents and swells when doing so.[3] Finally, the droplets require an energy source to combine them together in a phenomenon known as coalescence. This requires even more expensive and cumbersome laboratory equipment because the droplets are usually stabilized by surfactants to keep them from coalescing uncontrollably and high-energy external stimuli such as electro-coalescence or optical heating elements are necessary to induce it.[8] Because the droplets offer built-in compartmentalization to control which reagents are able to interact with others, coalescence is absolutely necessary in the field of droplet-microfluidics to allow researchers to combine droplets at will.

In the past, many different emulsions have been used to create immiscible phases to keep the droplets from interacting.[5] However, due to typical microfluidic device fabrication materials (namely PDMS), the use of most oils proves difficult due to their unwanted interaction with the PDMS.[6] A variety of methods are used to avoid this problem including coating the inside of the chip with chemicals such as parylene-c or making the device out of a different material, however these processes prove to be both timely and less suited for cellular applications in comparison to PDMS.[9] A recently implemented class of immiscible phase fluids have been shown to overcome the problematic interactions of flow-phase oils and provide several other benefits in microfluidic devices. Perfluorinated oils, not only avoid permeating PDMS, but are also completely bio-neutral and readily absorb oxygen making them the ideal flow-phase for biologically-focused, droplet-based microfluidic devices.[10]

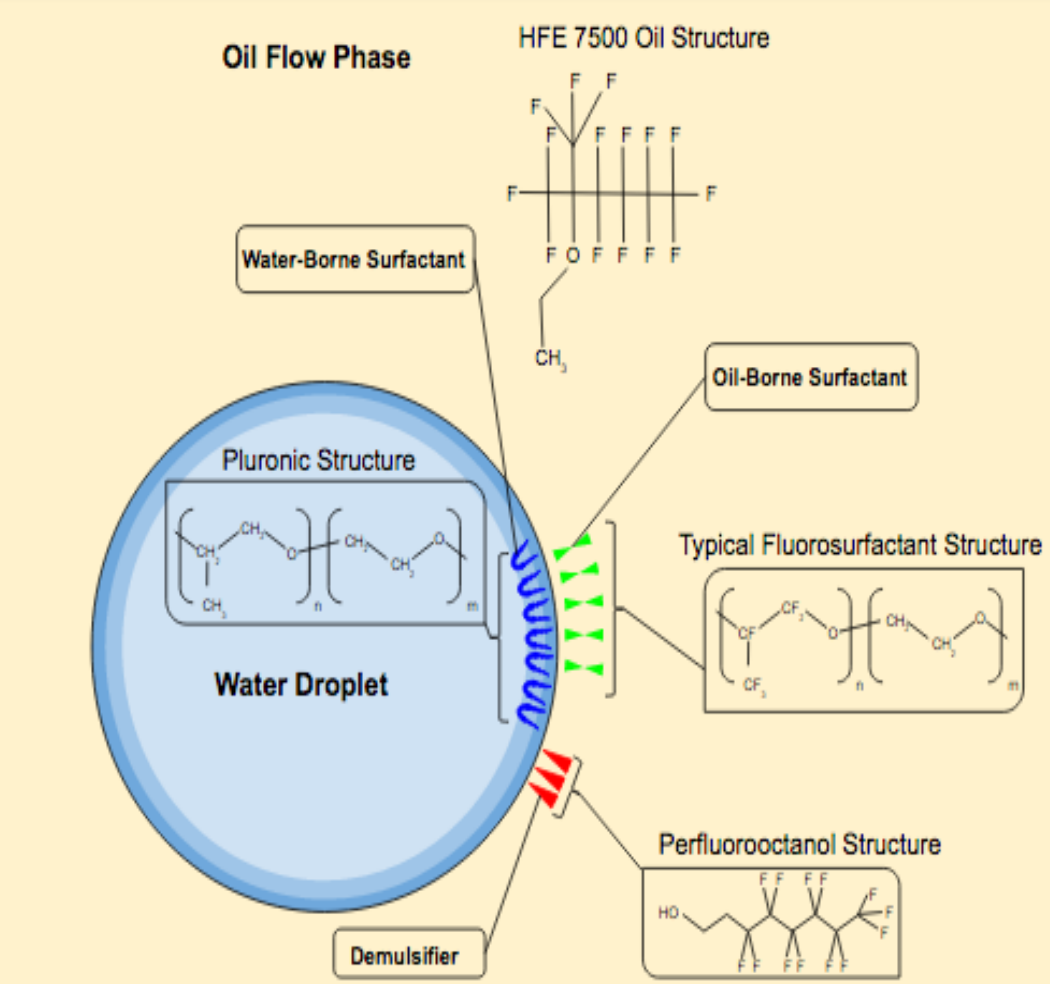


Figure 1. *Droplet in Oil with relevant chemical structures of the perfluorinated oils and surfactants our project attempts to characterize.*

In addition to this, very few compounds are able to dissolve in the perfluorinated oils, removing 'cross-talk' or transfer of reagents between droplets. This is important to ensure that each droplet remains in total isolation, acting as its own experiment. In our device, not only does the flow-phase fall under this class of perfluorinated oils but also, the surfactants within them as is shown in Figure 1. Most importantly, multiple scientific fields are moving towards a perfluorinated, droplet-microfluidic solution to replace traditional well-plate techniques. Some perfect examples of this are digital and quantitative PCR, scientific tools with an overall market value of 3.28 billion in 2016.[11] Within the last decade, Bio-Rad's Qx-series digital PCR machine has grown massively and represents a significant portion of that market.[12]

Emulsion science is traditionally a tedious study, due to the stochastic nature of coalescence and emulsion breakdown. Our chip has the potential to make a significant impact by providing a better understanding of emulsion stability as it is the ideal environment for precise control of the components of an emulsion. This is achieved via the use of many anchors to create many coalescent pairs in a single experiment, allowing up to 60 data points to be created in a single experiment. Finally, the device is unique in comparison to tradition emulsion study as it creates the simplest form an emulsion; two equal sized droplets, suspended in an immiscible phase. In this, we aim to achieve a much higher level understanding of perfluorinated emulsions and surfactants than has previously been available to the commercial or academic world.

Chapter 2 – Microfluidic System Components

Basic Microfluidic Geometries

The term "geometry" in reference to microfluidics refers to the two-dimensional top view of a channel or cluster of channels that serve a particular purpose in the manipulation of fluids. In our case, our chips rely on three basic geometries that allow for all the functions we desire. The first and arguably the most important geometry is known as a flow-focuser. It consists of a single channel of aqueous phase with two merging channels of continuous phase oil being forced through a constriction considerably smaller than the channels to create droplets.

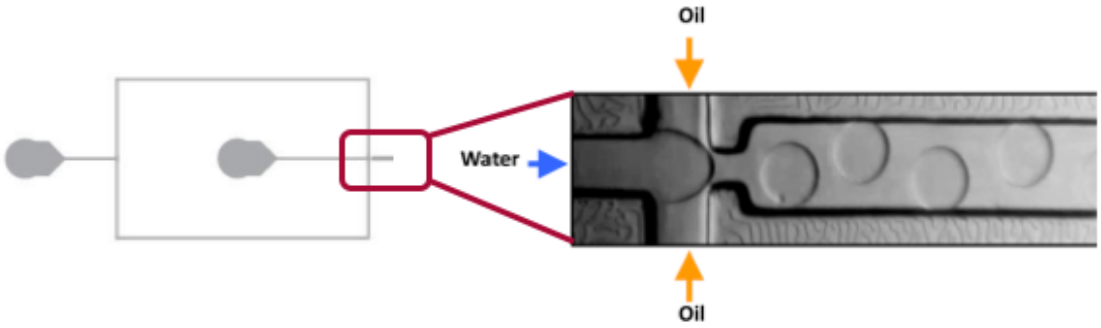


Figure 2. *A top view of a flow-focuser geometry expanded into a real-life image of a flow focuser producing droplets on one of the microfluidic chips used in Dr. Abbyad's lab.*

The second geometry one will see in our systems is an anchor array. This is the region of the chip mentioned in the background and significance section that is used to trap aqueous phase droplets by allowing them to become more spherical. They are effectively circular regions of the channel that have greater height than the main channel, thus allowing pancake-shaped droplets to become slightly more spherical in shape. Due to the small scale of the droplets, this slight difference in surface energy allows them to stay at a lower surface energy state which is strong enough to hold them in place even when continuous phase oil flow reaches high speeds. It also allows us to capture multiple droplets in one anchor, thus we can perform coalescence experiments as done in our senior design project.

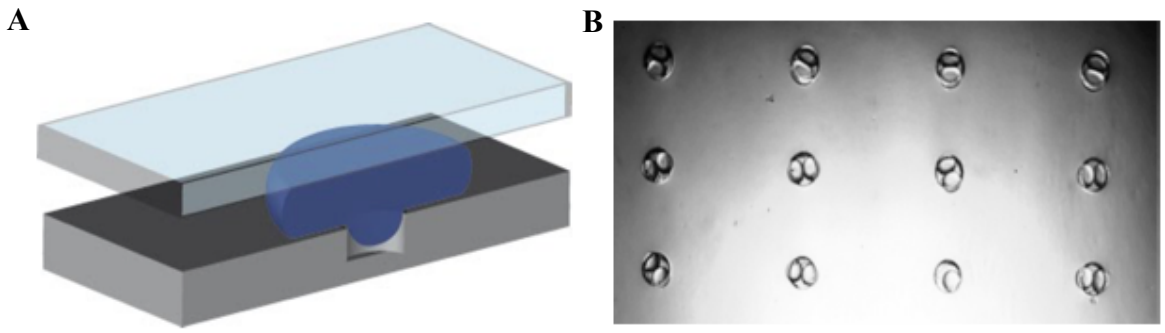


Figure 3. *A demonstration of how the anchor geometries work in our systems. (A) A computer-generated image of the cross-section of a single droplet stuck in an anchor. (B) A real image of the droplet pair ability utilized by our anchors.*

The final geometry that only our first experimental design utilizes is the laminating mixer. As was brought up in the background and significance section, it is difficult to mix things that are flowing laminar as they are in microfluidic systems. Thus, there is a need to either induce turbulent flow or create a scenario where diffusion can facilitate mixing. In our system, nine layers of oil with differing surfactant concentration are layered on top of each other and the large increase in surface area to volume ratio allows diffusion to make the two oils have the same homogenous surfactant concentration in a matter of seconds. To verify this worked, fluorescent surfactant was introduced to our system and images of the laminating mixer geometry are shown in the figure below.

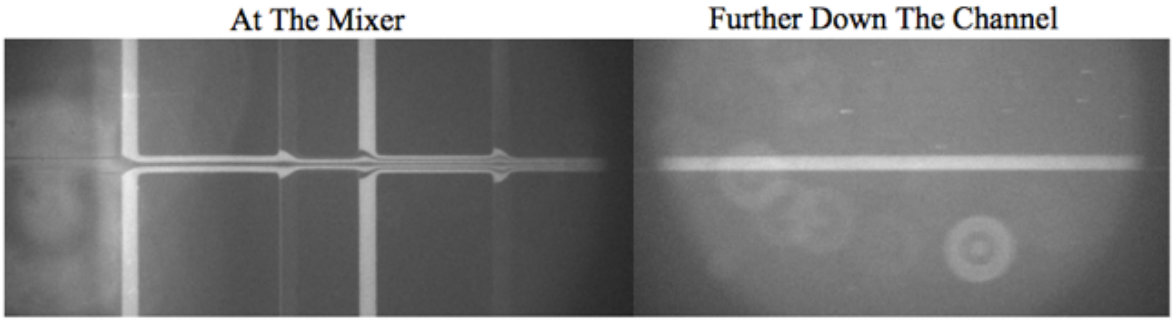


Figure 4. *Two fluorescent microscope images of an oil with no surfactant present and an oil with fluorescent surfactant in it mixing via diffusion in a laminating mixer.*

Design of First Experimental Chip

The first experimental chip we created utilized all three geometries described in the prior section. The idea behind the experimentation was to vary the concentration of surfactant in the continuous oil phase until droplets coalesced. By slowly lowing the concentration over a 10 or 20-minute ramp, and knowing the lag time from oil to get from their inlets to the anchor array where the droplets were stored, the exact concentration of surfactant in the oil could be calculated when each coalesce event occurred. Thus, we could draw conclusions on how stable an emulsion was, the less surfactant required to keep the droplet pairs from coalescing, the greater the stability of the emulsion.

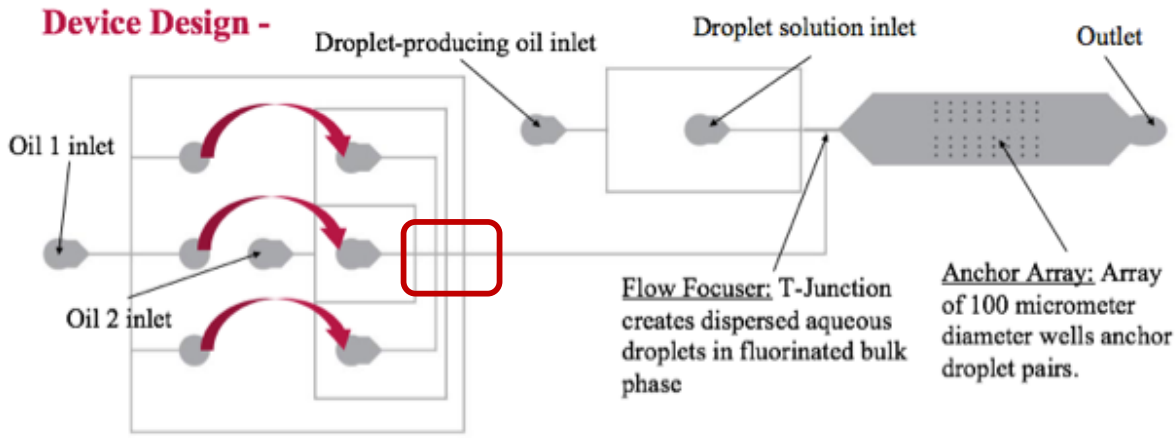


Figure 5. *Image of the 2-dimensional (top view) design of our first experimental chip designed to vary the concentration of surfactant in the oil over time. In general, fluid flows from left to right in the device. The red arrows denote where fluid would climb to a second layer of the device in order to bypass channels on the first layer. The oil 1 and oil 2 inlets can be interchangeably used to flow either the high surfactant or low surfactant oils through the laminating mixer. The red square shows the laminating mixer geometry from the previous figure.*

Design of Second Experimental Chip

The second chip we created due to various difficulties with the first design discussed in the discussion section. This chip is much simpler than the first and utilizes only two of the chip geometries form earlier in this section. Instead of flowing in oil with slowly declining surfactant concentration, this chip simply creates uniform droplet pairs and then flows in surfactant-free oil. For that reason, the chip only requires a flow focuser and anchor array portion without any sort of laminating mixer. These experiments were designed to measure time to coalescence in a surfactant free environment instead of concentration of surfactant at the time of coalescence. Though a much less sophisticated method and experiment, the correlation between emulsion stability and time should still exist, as the longer the droplets have existed in the surfactant free environment, the more stable they are because more surfactant has been pulled off of.

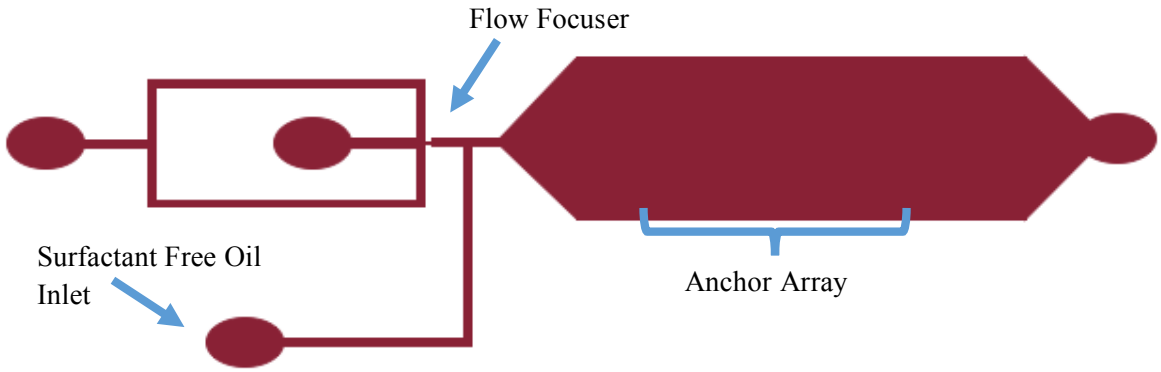


Figure 6. *Top view of the 2D mask design of the second generation device. All the dimensions are identical to the dimensions of the first device except for the removal of the laminating mixer which has simply been replaced with an inlet. Due to the lack of the laminating mixer, there is only one layer of the device.*

Chapter 3 - Experimental Protocols

Note: The control aqueous solution used in all of our experiments were 0.25 weight percent pluronic dissolved in pure ddi water.

Note: All microscopic work was completed using a Moticam 2.0 hooked up to an Olympus Inverted microscope using the latest versions of ImageJ Micro-Manager plugin. All Syringe pump work was done with a Nemesys syringe pump system and corresponding Nemesys software. Details for operating both of these pieces of equipment and their respective software can be found in Appendices D and E.

Experimental Protocol 1

Our first protocol was created in fall of 2016 and played an integral role in the development of our second system. However, only small portions of our data were obtained using this protocol.

1. Tape down the chip onto the inverted microscope, turn on the microscope using the green switch at its base. Also, turn on the syringe pumps using the large red switch.
2. Cut a hole in the lid of a microcentrifuge tube and a 5 inch piece of tubing. Insert one end of the tubing into the tube and the other into the waste outlet of the chip.
3. Fill and insert syringes into the chip in the following order:
    a. The first syringe will contain pure QX100 droplet generation oil and will be utilized in the flow focuser region of the chip.
    b. The second syringe contains your higher surfactant oil for the mixer section of the chip.
    c. The third syringe contains either pure Novec 7500 (surfactant free oil) or some extremely dilute surfactant-oil mixture.
    d. The final syringe will contain the aqueous mixture with whatever variable components mixed in.
4. Start a live frame of Micro-Manager to view the chip while setting up.

5. Run all oil syringes at a flow of 5 microliters per minute and then insert the aqueous phase syringe. Flow the all syringes at 5 microliters per minute until all the air at the ends of the tubing has been pushed out. (Oftentimes, light tapping on the chip will assist with particularly stubborn air bubbles in the chip).
6. Produce droplets by synchronously starting the aqueous phase syringe and the surfactant-rich syringe at flows of 0.3 microliters per minute and 7 microliters per minute respectively. (Surfactant-free oil syringe is run at 0.5 microliters per minute during this whole process.)
7. Once the anchor array is full, run the aqueous syringe at a negative flow of 1 microliter per minute until it recedes back up the inlet to ensure none will leak into the chip during the experiment.
8. Synchronously start the recording of video in Micromanager and a pumping scheme programmed into Nemesys shown in the figure below. For details on how the pumping scheme was created, consult Appendix D.

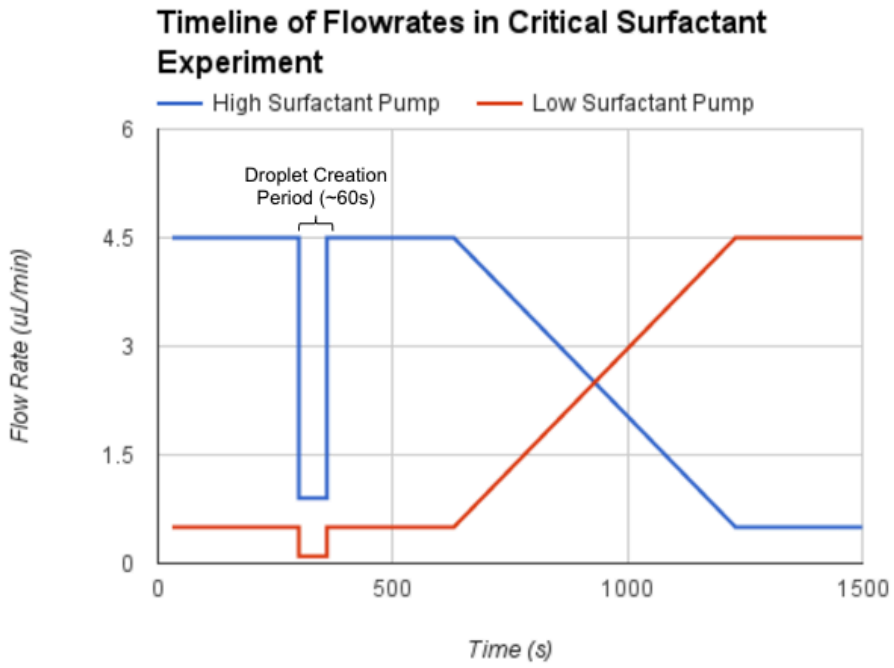


Figure 7. *Demonstration of Nemesys system flow profile for original experimental design. For more information regarding the low and high surfactant concentration pumps, consult section 2.2 on experimental chip 1.*

9. Stop video recording and pumps when all droplet pairs coalesce or when the experiment is deemed complete for any other reason. Open up a live window of Micro-Manager and run the droplet producing oil at 20 microliters per minute to wash out all the droplets and reset the chip for the next experiment.

Experimental Protocol 2

This protocol outline second experimentation method that was settled upon during winter quarter 2017. The vast majority of data was obtained using this protocol.

1. Tape down the chip onto the inverted microscope, turn on the microscope using the green switch at its base. Also, turn on the syringe pumps using the large red switch.
2. Cut a hole in the lid of a microcentrifuge tube and a 5 inch piece of tubing. Insert one end of the tubing into the tube and the other into the waste outlet of the chip.
3. Fill the syringes, one with surfactant rich oil, one with surfactant free HFE 7500, and one with the aqueous phase of interest. Attach about ten inches of new PTFE tubing to all three syringes. Open up the Nemesys Syringe-pump software and set all the syringe pumps to the volume in each of the recently filled syringes. Finally, clamp in the syringes.
4. Repeat for all three syringes. Run the syringe until the reagent begins dripping out, at that point, it is inserted into the chip in their respective inlets. Always insert oil syringes first to avoid wetting the surface treatment of the chip.
5. Run both oil syringes at 5 microliters per minute and the aqueous syringe a 1 microliter per minute until all the air has been pushed out of the chip. Oftentimes, light tapping on top of the chip assists in removing stubborn air bubbles.
6. Produce droplets by synchronously starting the aqueous phase syringe and the surfactant-rich syringe at flows of 0.3 microliters per minute and 7 microliters per minute respectively. (Surfactant-free oil syringe is run at 0.5 microliters per minute during this whole process.)
7. Once the anchor array is full, run the aqueous syringe at a negative flow of 1 microliter per minute until it recedes back up the inlet to ensure none will leak into the chip during the experiment.
8. Synchronously start the video recording via micromanger imageJ software as well as the surfactant-free oil syringe at a specified flow rate (noted in your lab notebook) and cease the surfactant -rich oil syringe. (Ensure that the microscope tray is positioned so that the anchor array is visible when starting the video.)
9. Stop the video once all droplet pairs coalesce.

Chapter 4 - Materials and Methods

Chip Fabrication Protocols

Photolithographic Mask Design -

Masks used in this project were created in Adobe Illustrator, as of now, all future masks are being created in autoCAD. For the purposes of this document, this will describe Adobe Illustrator mask making techniques.

Start: Open up an file created since 2015, as you will use the general formatting as the formatting for your masks. Ensure the document has a total of 12 masks on it (2 columns and 6 rows).

Note: When making masks, make sure to open the windows layers and transform. To do this go into the drop down menu labeled Windows and check next to layers and transform

Using the Transform Window -

- When you select an element in Adobe with the transform window open an X coordinate, Y coordinate, Height, and width will appear in the window.
- On the left of the window is an array of 9 squares, arranged in a square. These squares correspond to the different reference points of the selected element, top left, top center top right, left center etc. The displayed X,Y coordinate corresponds to whichever reference point is selected.
- Adjusting any of the values in the transform window changes the position or shape of the object accordingly.
- You can rotate objects by entering an angle in the rotate window, the window with the angle symbol next to it, or by selecting an angle from the drop down menu.

Mask Making Standards -

- All anchor arrays should be centered at the X center of the mask +9 and the Y center of the mask.

- Anchor channels should be 10mm wide 3 mm high .
- Masks should be labelled with your initials, the date, and your full name at the top of the sheet. Each individual mask should have a label at the bottom describing the mask.
    - example: Medium mixer on 10 mm channel or 100 um circular anchors
- For the purposes of emulsion characterization experiments, flow focuser constrictions must be 30 microns wide to maintain consistent droplet sizes across all experiments.

Labeling Mask Files -

- Dates should be in the same format as when saving files on the microscope, with the year first. (Example: 150702 is 2015, July second.)
- DO NOT label files with just dates, there is some incongruity with old mask files which may be confusing, files should be labeled with your initials as well in the top right corner of the document as well as the top right corner of all the individual masks.

Note: Once mask files are complete, email to Dr. Abbyad. All mask printing is performed by CAD/Art services of Bandon Oregon and were printed on photomask material (often referred to as “transparency”).

Mold Fabrication -

1) **Turn on** UV lamp and Laminator (100C, Speed 3).
2) Cut piece of blue film smaller than glass slide. Ensure that the film you are using is 50 microns this as that is both the channel and anchor depth in all emulsion stability experiments. Also, Cut piece of cardboard that is bigger than blue film.
3) **Take off protective film** on side that curls UP using scalpel. (see figure below).
4) Place glass slide on piece of cardboard. Then blue film (with unprotected side towards slide).**Note:** Sometimes cutting the film smaller than the glass slide, so it doesn't lay over the edge, is helpful to avoid crinkling in the laminator.

Figure 8. *Demonstration of dry-film photoresist natural curl. Due to the difficulty removing the thin plastic film off of the photoresist film without damaging it, there is a need to standardize the approach to removing it that results in the smallest amount of damage possible.*

5) Put through **laminator** and **Remove other protective film** once it comes out.
6) **Put mask** over film. Gentle pressure can be used to make it stick a bit.
7) **Irradiate** with UV lamp (100% power, 5 sec per layer)
8) If multiple layers need to be placed repeat steps **3-8** for each layer
9) Develop with solution of **1% Potassium Carbonate** in ddi water. Immerse entirely the slide. Squirt with disposable pasteur pipette the most critical areas (ie: flow focuser)
10) Check under microscope, develop more if necessary.
11) Briefly wash mold with acetone followed isopropanol. Dry with stream of air. Make sure no visible residue if left on slide. Dry and ready to pour PDMS on top.

PDMS Pour/Soft Lithography -

1. Find the tub labeled “Silicone Elastomer Base” and the smaller bottle labeled “Silicone Elastomer Curing Agent”. They are both located in a secondary container in between the oven and the hood.
2. Take one weight boat, place onto balance and tare.
3. If there is already gel on the chip molds, extract about 10 mL of “Silicone Elastomer Base” with the giant plastic syringe located in the same general area. Transfer onto the weight boat. If making new chips, extract about 40 mL of “Silicone Elastomer Base” and transfer onto weight boat.
4. Use a disposable pipette to extract a 10:1 (by weight) amount of “Silicone Elastomer Curing Agent” and transfer onto weight boat. For example, if the balance reads 40 g, tare whatever is on the balance and transfer 4 grams worth of the curing agent onto the weight boat.
5. Take the weight boat off the balance and find a glass stirring rod. With this, you will make sure that the curing agent is evenly dispersed in the silicone base. With nice and broad circular hand motions, this will hopefully do as such. Cedar employs a sort of “egg beating” method, which may also work. *Pro Tip: Tilt the weight boat on its side just enough so that the gel can slide over to one side, and this will make it easier for you to mix. Cup the weight boat in your hand so that it does not spill.*
6. Mix for about 5 minutes (just to be safe). Make sure you do not get any of the gel onto your clothes unless you like having permanent PDMS stains on your clothing.
7. After mixing, place the weight boat into the vacuum. Turn the vacuum on, make sure that the valve is set so that no air is escaping from the vacuum. Let it sit for about 2-3 minutes.
8. Come back and turn the valve so that air goes back into the vacuum container. If air bubbles are still present, repeat step above until all air bubbles are gone.
9. Transfer the gel onto the trays containing the chip molds.
10. There may still be some bubbles left on the freshly poured gel, so take the rubber hose that should be attached to the air valve, turn air on, and force air gently over the bubbles. They will pop.

11. Place the chips to their designated area in the oven. The oven should always be set around 70 °C.
12. Wipe glass stirring rod with a kim wipe and place back into beaker. Toss the disposable pipette and weigh boat into the waste bin.

Chip Cutting and Bonding -

1. Remove molds out of the oven and bring them to the cutting station to the left of the hood. **Let them cool** slightly before starting to cut them (PDMS will take about 1.5 hours to solidify in the oven)
2. In the drawer labeled OFFICE SUPPLIES, you will find scalpels and hole punchers.
3. Place the mold over a light and begin to cut the gels. Press firmly into the molding and circle your scalpel around once. Make sure that the scalpel does not come near any of the inlets or wells. *Pro Tip: Go around the cut again to make sure that it is detached from the rest of the mold.*
4. Slide the scalpel tip under the mold and place onto cutting board.
5. Take the hole-puncher and punch holes through each inlet.
6. Use tape to make sure that the side the gel is going to be bonded onto the microscope slide is debris-free and clean. Repeat as many times as you see fit.
7. **Clean** area up.

Start: Plasma Bonding

8. **Turn power switch to "ON"**, (bottom right red switch)
9. **Clean microscope slides** (as many as you have chips that need to be bonded) with kim wipes (with a bit of ethanol or isopropanol) and water if necessary. Dry under stream of air. It is important that the microscope slide is absolutely debris-free in order to promote the best bonding possible between the slide and the gel.
10. **Place both the microscope slide and the PDMS chip into the plasma cleaner.** Usually, you can fit about 2 or 3 of each in the space.
11. **Close the door.**
12. On the plasma door make sure the **vent is completely closed** (turn upright knob fully clockwise) and the **toggle should have the arrows pointing towards the right** (to the gas pressure gauge).
13. **Turn on the pump on.** You will start hearing a loud rumble.

14. On top of the plasma cleaner is a device that reads the pressure inside the plasma cleaner. **Wait till it reaches 300** (this may take a minute or two). Turn pump off once 300 is reached.
15. Turn the **RF level to HI**.
16. A**llow a small amount of air inside until the intensity of the purplish light reaches a maximum.**
17. There should now be a plasma (glowing) inside the chamber. **Wait with the plasma for 40 seconds** (the stopwatch should be next to the plasma cleaner.)
18. After 40 seconds, turn the **RF level to OFF**, turn the **toggle on the door so that the arrow points to the left**, and **vent the chamber slowly by turning the top nozzle counterclockwise.**
19. Afterwards, it reaches atmospheric pressure, **open the door**. Take out a microscope slide and the gel. Try to place the gel very gently onto the microscope slide. It should stick very well. MAKE SURE THAT YOU ARE BONDING THE CHIP ON THE CORRECT SIDE.
20. Check the bonding by holding up the slide to a light. If you see air bubble, the gel has not bonded properly to the microscope slide. If this is the case, try pressing down firmly on areas that have not been bonded but be careful not to push too hard, as this may force the channels to bond to the glass slide as well.
21. The bonding is permanent and should not come off. If you still have small bubbles remaining, either toss away your chip or place back into the oven (at your own judgment). Remember, air bubbles are a physical chemist's worst nightmare in microfluidics…
22. A better bond can be obtained by placing the chip in the oven for 10 minutes.
23. After bonding all devices make sure to **Turn power switch to "OFF"**, (bottom right red switch). The plasma will no longer make noise.

Surface Treatment -

1720 Treatment: A superhydrophobic surface treatment that is appropriate for use of aqueous droplet in fluorinated oils.

Note: Surface treatment may be better if performed on the same day as the bonding of the PDMS to the glass (that way the surface of the PDMS is still in the activated state).

1. Find the bottle located below the hood labeled "**1720 SURFACE TREATMENT**". It should be a clear bottle with electrical tape wrapped around the top in the cabinets under the hood.
2. Pour a small quantity of solution into a small plastic vial. **DO NOT PIPETTE DIRECTLY FROM THE CONTAINER.**
3. **Close bottle immediately** and **seal carefully with electrical tape.**
4. Use a small plastic syringe with tubing to extract of the solution out of the small plastic vial. Make sure the bottle is closed properly while transferring, as the liquid is volatile.
5. Insert the tubing into one of the exit of the chip and dispel out the 1720 into the entire chip (a bit of liquid should be seen at outlets). Make sure the important parts of the chip are treated (anchors).
6. Put the chips in the oven at **150C** for 15-20 **minutes DO NOT PUT PETRI DISHES IN OVEN, THEY WILL MELT!** (The 150C oven is in the general chemistry lab, DS124, in the back left of room).
7. Remove chips from oven with mitts. Let chip cool before use.

Materials and Pricing

Note: All materials on this list are consumables used for the project. All Instruments (including inverted microscope, UV-exposure lamp, laminator and ovens) were supplied to us via Dr. Paul Abbyad in the Department of Chemistry and Biochemistry.

Item	Scientific Name	Point of Purchase
Sylgard 184 Silicone Elastomer Kit	Polydimethylsiloxane	Dow Corning
Microscope Slides	Borosilicate Glass Slides	Thermo-Fisher
50 Micrometer Photoresist Film	Alpho NIT250 Photosensitive PCB Photoresist	International Electric Components
008 Fluorosurfactant	Proprietary	Ran Biotechnologies
HFE 7500	3-Ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-(trifluoromethyl)-hexane	3M
QX100 Droplet Producing Oil	Proprietary	Bio-Rad
1.0 mL Syringe	Gas-Tight Luer-Locking PTFE Syringe	Hamilton Company
Thin Wall Tubing	Polytetrafluoroethylene Tubing	Cole Parmer

Table 1. *List of Materials, their scientific names and their Sources.*

Item	Unit Price	Quantity	Subtotal
Sylgard Silicon Elastomer Kit 0.5 kg	$42.99	2	$85.98
Novec 7500 Engineered Fluid 0.5 kg	$60.00	1	$60.00
Bio-Rad Oil For Droplet Generation (QX100) 70mL	$270.00	1	$270.00
Ran Biotechnologies 008 Fluorosurfactant 50g 2% wt.	$500.00	1	$500.00
Hamilton Gas-Tight 1.0 mL Syringe	$42.00	5	$210.00
Cole Parmer PTFE Thin Wall Tubing (100ft) (4 pack)	$24.25	1	$24.25
1/32” PEEK Tubing (5 ft)	$49.01	3	$147.03
Bio-Assays Systems EnzyLight Cytotoxicity Assay Kit	$189.00	1	$189.00
Combined Shipping Cost	$100.00	1	$100.00
		Total	$1343.26

Table 2. *List of Materials and their pricing. A brief budget of the project’s consumables*

Chapter 5 – Results

First Device Results

These results reflect the data obtained using our first experimental device. To create them, the lag time of the chip as well as the start time was subtracted from the length of the videos. Videos were recorded at one frame per second thus, by subtracting the same number of frames as seconds, the frame at which the droplets coalesced accurately reflects the time during the decreasing surfactant concentration ramp that they coalesced. Then, using the MATLAB code provided in addendum A, the following figures were made.

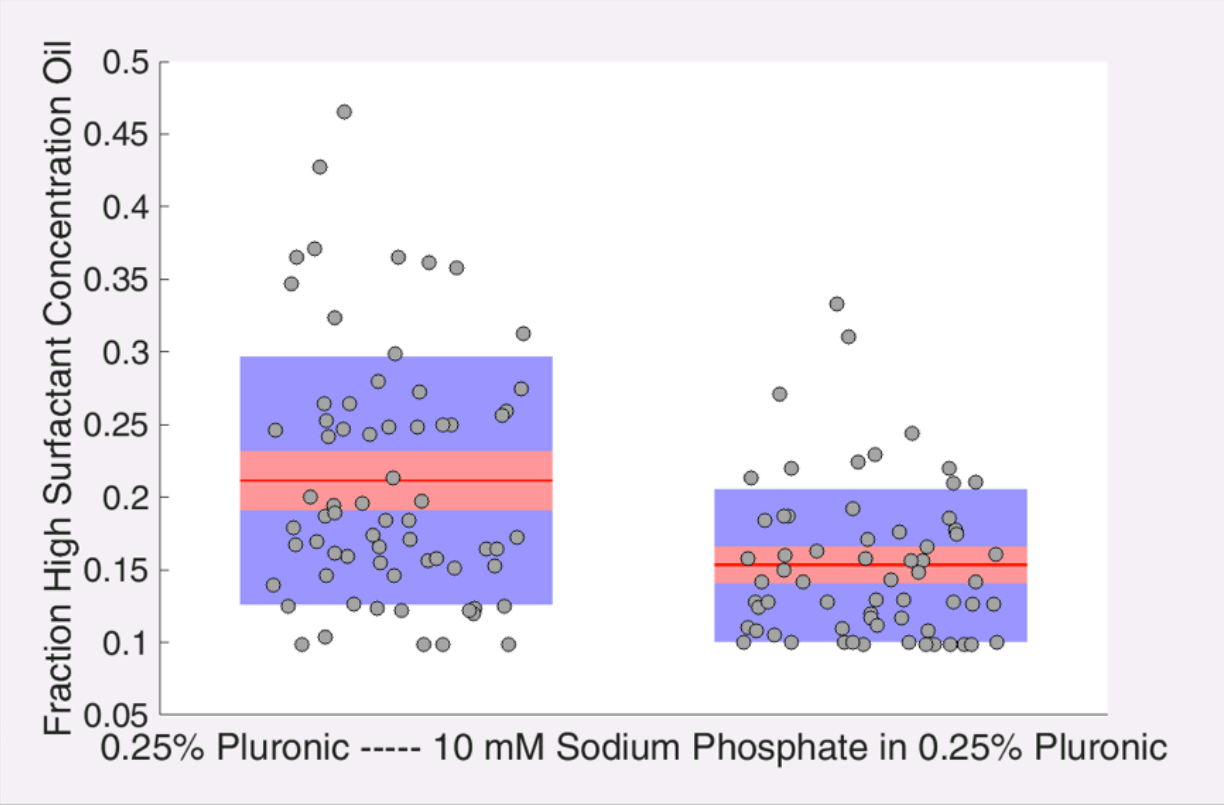


Figure 9. *First device surfactant concentration experiment with varied sodium phosphate concentration within the droplets. Each grey dot represents a coalescence event. The dark red line shows the mean, the light red region shows the standard error of the mean and the light blue region is a 90% confidence interval. The data in this graph represents experiments performed in triplicate and shows that the presence of sodium phosphate lowers the amount of surfactant required to keep droplets stable.*

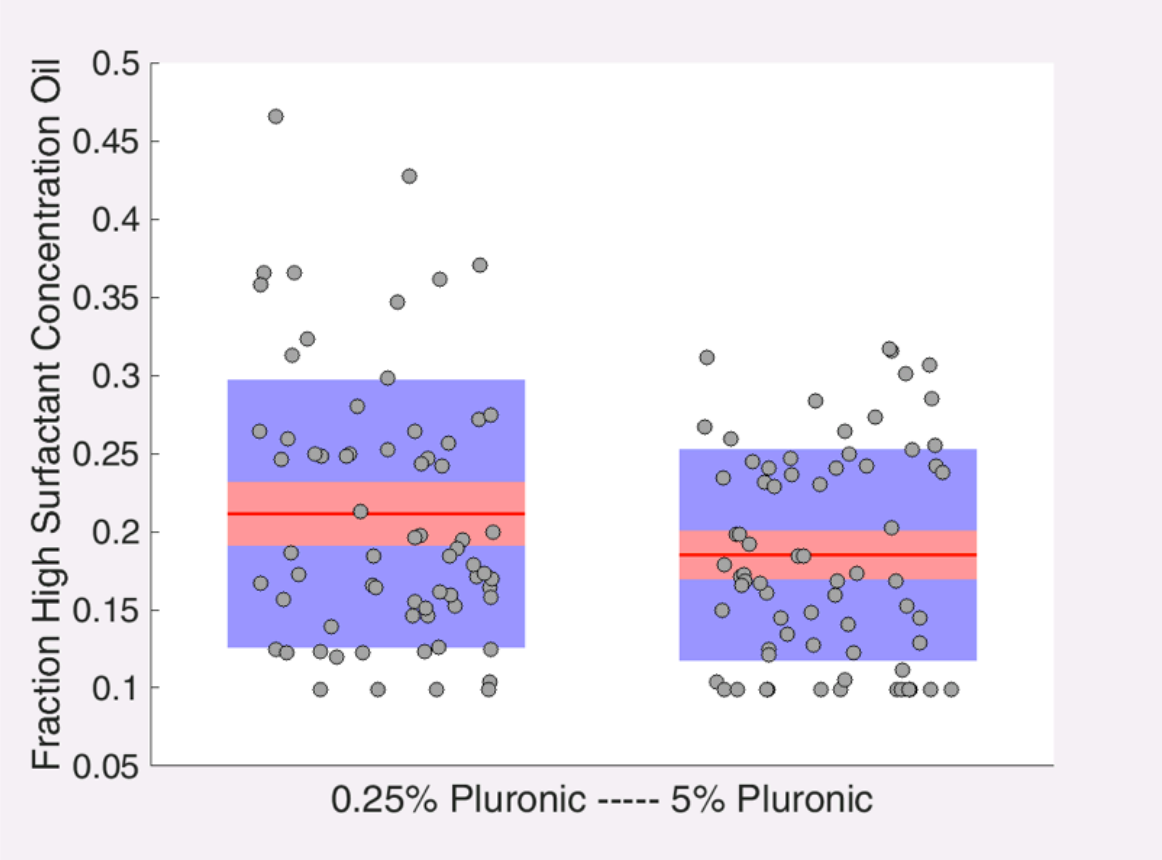


Figure 10. *First device surfactant concentration experiment with varied pluronic concentration. Each grey dot represents a coalescence event. The dark red line shows the mean, the light red region shows the standard error of the mean and the light blue region is a 90% confidence interval.*

Second Device Results

The results for this device were calculated by simply subtracting the start time and the chip lag time from the start of the video. The video was taken at one frame for second so by starting the video at the number of frames after frame zero that are the summation of the chip lag time and the start time after the video began filming this was achieved without data analysis. It is important to recognize the different scaling of the time on the x-axis for different experiments, some reagents stabilized the emulsion to such an extent that the scaling of the x-axis had to be changed. All graphs were made in excel. Unless otherwise noted, the flow rate of surfactant free oil surrounding these droplets was 2 microliters per minute.

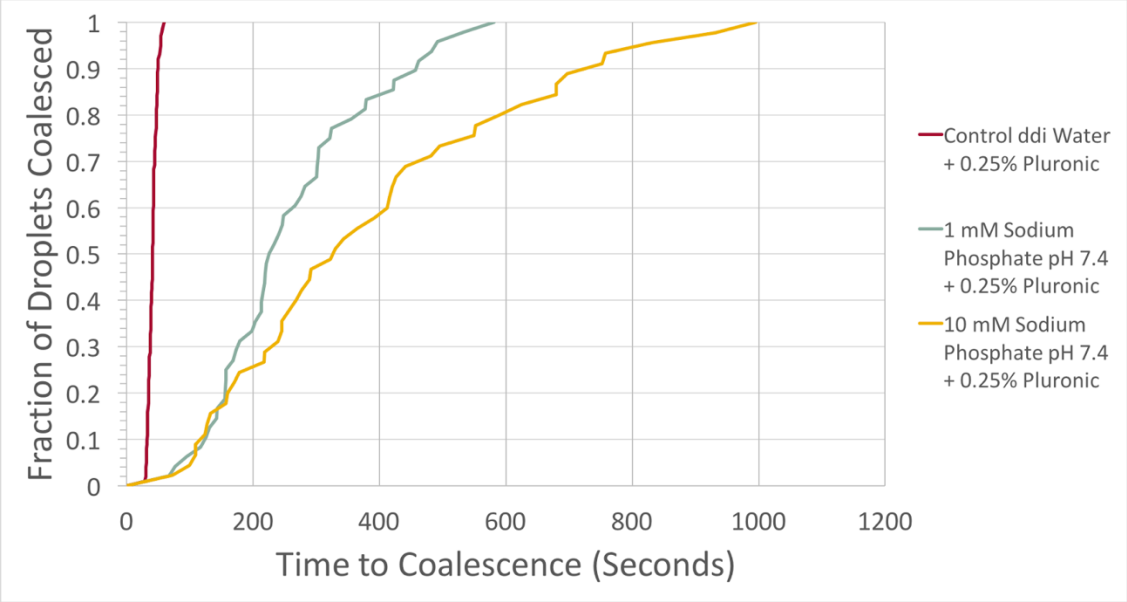


Figure 11. *Second device time to coalescence experiment with varied sodium phosphate concentration. All details of the figure can be found on the legend to the right of the figure.*

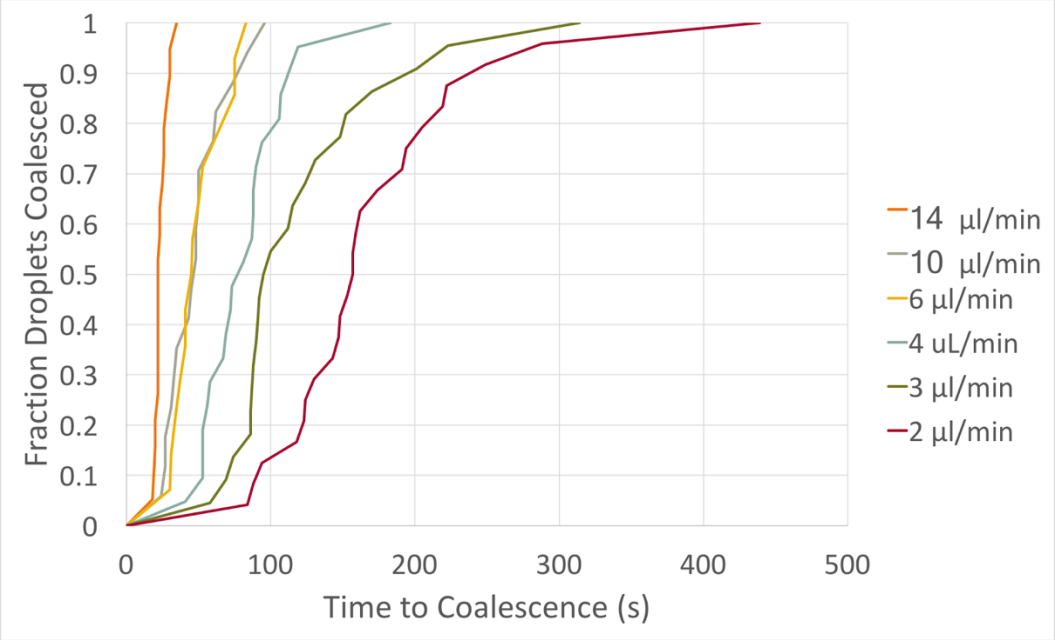


Figure 12. *Second device time to coalescence experiment with varied surfactant-free oil flow rate. It is important to note that this is the only experiment not performed at a continuous phase flow rate of 2 microliters per minute.*

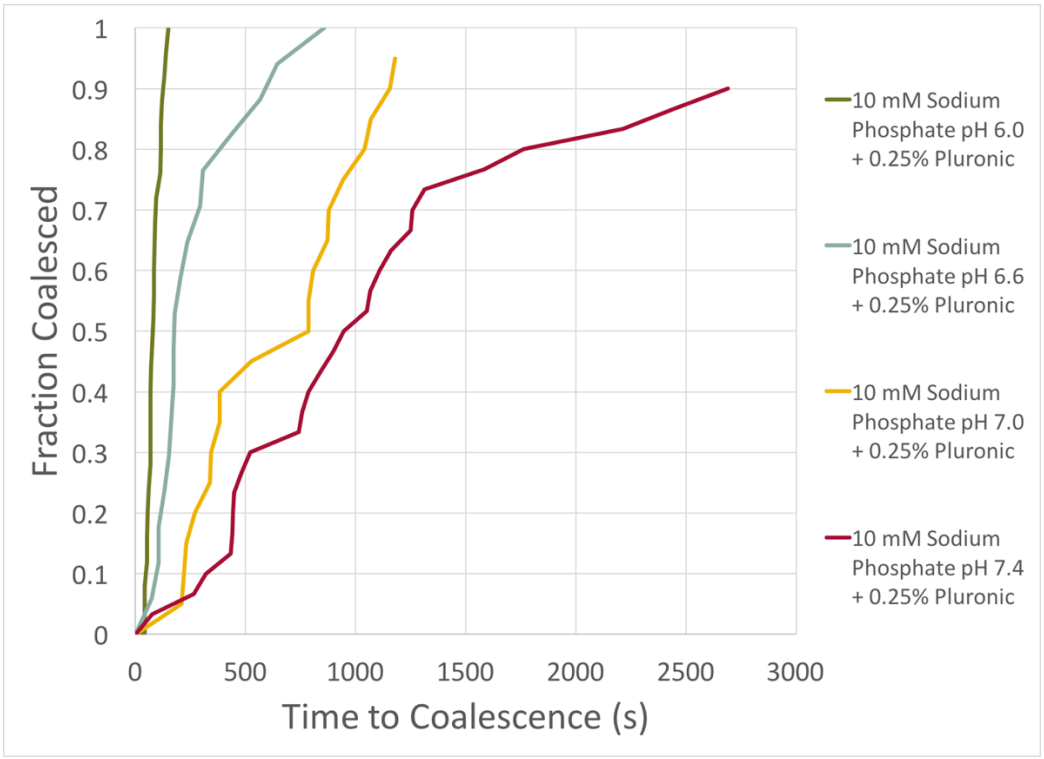


Figure 13. *Second device time to coalescence experiment with varied droplet pH at consistent Sodium Phosphate concentration.*

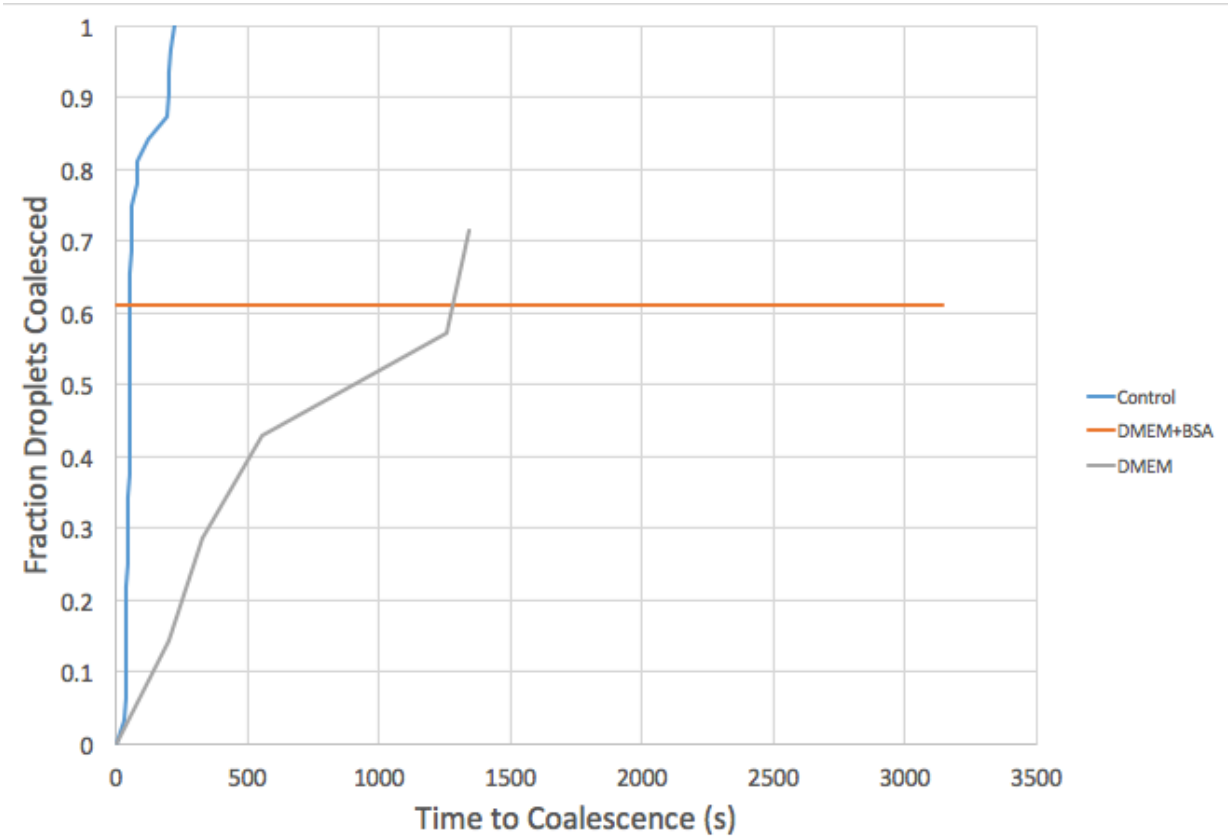


Figure 14. *Second device time to coalescence experiment with varied presence of discrete, minimal media and bovine serum albumin. It is important to note that lines which stop short of the top of the graph never saw complete coalescence and flat lines represent no coalescence. If a line starts above the 0,0 mark, that means coalescence occurred before the video and flow of surfactant free oil started.*

Stanford Experimental Results

These results reflect the experiments performed at Dr. Gerald Fuller's Lab in Stanford's Chemical Engineering department. The experiments were performed on a Double-Wall-Ring Interfacial Rheometer (Model: TA-AR2000) and were done with the assistance of a Ph.D. student by the name of Simone Bochner de Araujo. For information on how the experiments were performed, consult appendix B. Significance and interpretations of the data are present in the discussion section, chapter 6. All data analysis and figure design was completed by Simone Bochner de Araujo. The percent strain on the x-axis represents how far the ring is rotated at the interface.

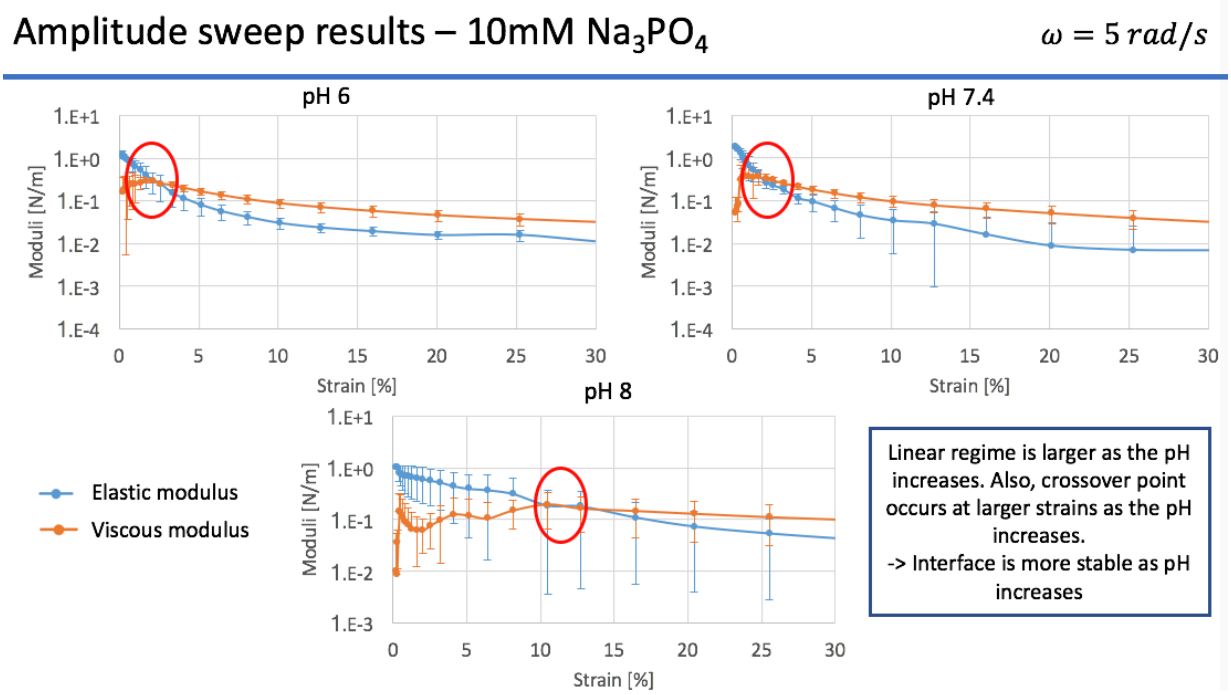


Figure 15. *Stanford interfacial rheometer experiment with varied pH at constant sodium phosphate concentration. It is important to note the point at which the viscous modulus overcomes the elastic modulus in each experiment (circled in red). Brief discussion can be seen in the bottom right corner of the figure.*

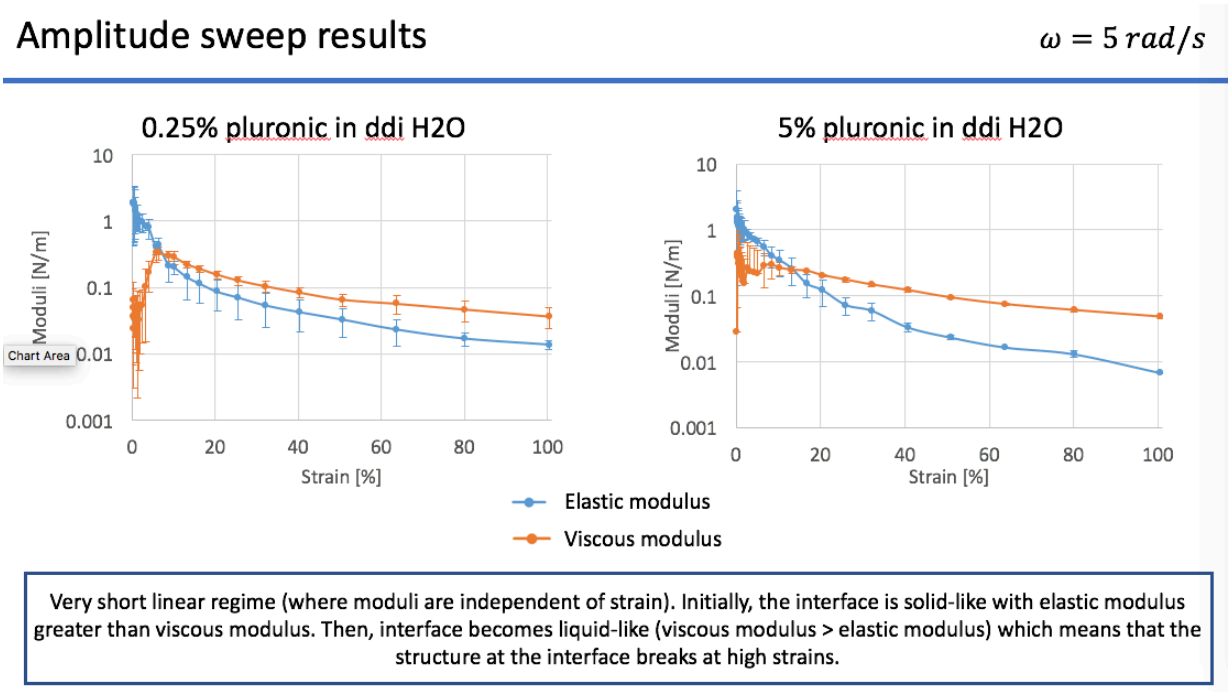


Figure 16. *Stanford interfacial rheometer experiments with varied concentration of pluronic. It is important to note the point at which the viscous modulus overtakes the elastic modulus. Additionally, brief discussion can be found at the bottom of the figure.*

Chapter 6 – Discussion

Before discussion specific experiments, it is important to understand why these experiments were performed and what each reagent represents. Especially in the case of Pluronic F-68, a reagent used in all our experiments, it is imperative to know why it is there and what it is doing. Pluronic serves two purposes in our experiments, one is biologically relevant, while the other is imperative to the functioning of our system. Due to what we speculate is the size of the perfluorinated surfactant molecules, there is a second-regime time period after a droplet is created where it is not stable and will coalesce with another droplet if it comes in contact. Pluronic serves as an aqueous phase surfactant which stabilizes the interface of droplets much faster than the perfluorinated surfactants that we use. In order for our chips to consistently produce droplets with no early coalescence issues, Pluronic is a necessity and is even included in our control experiments at 0.25 weight over weight percentage. The second reason pluronic is present has to do with the biological relevance of the system. Though most perfluorinated surfactant are poorly understood, and their toxicity is uncertain, it is generally agreed upon that the presence of Pluronic in cells that are encapsulated in droplets within a perfluorinated emulsion perform better than when Pluronic is not present.[13] Pluronic is therefore needed for any perfluorinated emulsion that intends to use cells, and is required if we wish to keep our system biologically relevant. All other reagents used in our experiments are part of common biological systems such as sodium phosphate in the pH ranges and concentrations we tested, discrete media and, bovine serum albumin.

Starting with the data from the first device, we managed to test both sodium phosphate and Pluronic concentration variance. In the case of Figure 9., we show that the presence of sodium phosphate does stabilize the interface and thus the emulsion as there are lower concentrations of surfactant required to keep droplet pairs from coalescing. Of all the experiments successfully performed on this device, this result shows by far the largest margin in emulsion stability. In Figure 10., we tested to determine if varied concentrations of Pluronic beyond our control concentration had any major effects on emulsion stability. To our surprise, even a twenty times increase in Pluronic concentration did not have that major of an effect meaning that the interface likely saturated with aqueous phase surfactant at relatively low concentrations. Both of these experiments were confirmed to have the same results both in our second device experiments as well as in the Stanford interfacial rheometer experiments.

In order to continue to discuss our second device experiments, it is important to understand why the switch to the second, simpler device design was made. Though we did manage to get a couple good experiments performed on our first device, we quickly ran into trouble when we started introducing more complex variables such as varied pH or salt concentration. It is fully understood that coalescence and emulsion stability in general follows a stochastic nature.[15] However, the extent to which is behaves stochastically exceed the dynamic range of our device. The laminating mixer could only function properly if the ratio of flow between either channel never exceeded 1:9. This created a situation where we were able to lower the surfactant concentration in the chip by less than an order of magnitude. When performing experiments with more complex reagents, we continually ran into issues involving large amounts of coalescence events either before or after the decreasing concentration of surfactant ramp. To obtain good data with reasonable confidence, the coalescence events largely have to occur during the ramp function so as not to weigh the average concentration of surfactant to either the maximum or minimum value of the experiment. After realizing this we quickly switched to the time-to-coalescence experiments after consulting with Dr. Gerald Fuller and seeing a similar approach being used at Stanford in a non-microfluidic application.

Looking at the data from the second device, we successfully managed to get several experimental conditions tested. In Figure 13. there is a varied pH trial showing that as pH rises, so does the emulsion's stability. This is incredibly important to anyone hoping to use a biological system in a perfluorinated emulsion as it can have serious consequence both in an out of microfluidic environments. In the Figure 12., as one would expect, increasing the flow rate of surfactant free oil around the droplets speeds up the rate at which droplets coalesce. Although this data is likely just measuring the time it takes the for the surfactant to be removed from the interface, it is still relevant to understanding the mechanism through which emulsions are stabilized by surfactant because it gives us a sense of how strongly bound the surfactant is to the interface and how mobile the surfactant is on the surface of a droplet. The fact that the curves begin to decrease in slope towards the lower flow-rate shows that the surfactant is either able to reposition itself to the interface (given time i.e. lower flow rate = surfactant removal at a lower rate) of the droplets or that it is able to adhere better in certain environments.

On Figure 11., though not labelled, the control run's pH is roughly 7.2. While that may seem like bad data as both the solutions containing different concentrations of sodium

phosphate have a pH of 7.4, the discrepancy in the time to coalesce far exceeds the difference seen between the pH 7.0 and pH 7.4 trials from Figure 13. This shows that increasing the concentration of sodium phosphate does indeed result in greater emulsion stability which confirms our findings from the first device.

Looking at Figure 14., it is clear that Bovine Serum Albumin (BSA) has an effect on the emulsion that our system struggles to reveal. The control group behaved as expected, coalescing relatively rapidly and the trials with just DMEM in them also behaved quite well. However, the DMEM group completely stopped coalescing about 20 minutes in and none of them coalesced in the following 20 minutes of the trial. We believe this is due to some reagent inside the DMEM a very slowly creating a very stable interfacial structure inside of the droplet. Even less expected is the BSA and DMEM trial. Almost all of the droplets coalesce in the first 10 seconds of the video (even while surfactant-rich oil is flowing) and then completely cease to coalesce after that. The only hypothesis we have for this is that the pluronic is interacting with something inside the BSA, keeping it from stabilizing the droplet off the bat. Then, some other reagent inside the BSA is acting as a super-stable, aqueous-phase surfactant that takes somewhere between 20-30 seconds to assemble on the surface of the droplet. However, it is clear more experimentation and potential different types of experiments are required to understand this phenomenon.

Finally, both Figures 15. and 16. show data from Gerald Fuller's Lab. This data was obtained from a double-wall-ring interfacial rheometer. To understand and interpret the data, it is first important to understand the device and viscoelastic materials. The rheometer operates by rotating a ring right at the interface of two fluids and measures the response of the fluids as the force that is exerted back on the ring.[14] When the interface is stable it behaves more like a solid than a fluid, because its elastic modulus dominates its viscous modulus and the fluid is able to quickly conform back to the previous shape it was in.[14] When the interface is less stable, the viscous modulus dominates and it behaves more like a fluid, not able to reform to its previous shape.[14] The important piece of information gathered by this device is the point at which the percent strain applied on the interface cause it to behave more like fluid. I.e. the viscous modulus overtakes the elastic modulus. The longer the elastic modulus dominates, the more stable the interface. This data shows that two of our results are very consistent with the previous results in both other devices. For the varied pH experiments in Figure 15., the general

trend of increasing pH causes increasing emulsion stability is certainly preserved as the percentage strain that the elastic modulus dominates the viscous modulus of the pH 8.0 solution is much greater than that of the pH 8.0 solution. However, the pH 7.4 solution does not show much of a difference, we believe this is either do the pH of the solution drifting from atmospheric carbon dioxide, or a viscoelastic phenomenon that is not closely linked to emulsion stability. In the case of Figure 16. It is clear that the trend of increasing pluronic concentration causing an increase in emulsion stability is preserved from both of our other devices. In this data, the elastic modulus of the 5% Pluronic solution continues to exceed the viscous modulus at greater percentage strain than is maintained in the 0.25% pluronic solution. Demonstrating that both of our previous conclusions from both prior devices is correct.

Chapter 7 – Core Requirements and Professional Concerns

Ethical Significance

Section I: Senior Design and the Ethical Justification of the Project

The fundamental moral reason for this project is to advance life science research and expedite discoveries in the field of surface chemistry. By studying the interactions, energetics, and properties of different compounds of interest submerged in perfluorinated oils, we are able to engineer a microfluidic system that serves the purpose of making experiments faster, cheaper, and more controlled while reducing amount of waste produced. Our model system serves as a skeletal platform ready to be adjusted for specific applications including, but not limited to, study of cell-cell interactions, enzyme-linked immunosorbent assaying (ELISA), and drug testing.

We embarked on this project with a vision of creating a platform as well as an approach to replacing traditional biochemical experimentation into a better, simpler model. Experimentation can typically involve manually and repeatedly pipetting substances between petri dishes or 96-well microtiter plates under a fume hood. Conducting equivalent experiments with a PDMS microfluidic chip, a computer, and a syringe system can allow greater efficiency, control, and programmability which would significantly conserve the principal resources of time, money, and labor.

Although these key benefits may be subjectively concealed to the everyday member of society, our system could be an instrument behind the curtain that allows a new breakthrough drug to reach market months faster, or discover and characterize novel chemical interactions between biologically relevant compounds that breeds a new generation of medical implants. By focusing on the potential to improve the quality of life for other human beings, we begin our project by knowing what we ought to do, thus owning ethical foundational groundwork upon which meaning and direction is given to our roles as scientists and engineers that interpret data and construct devices.

Section II: Senior Design Project and the Virtues of a Good Engineer

We can draw from a plethora of different engineering codes a reoccurring central idea: do no harm. With a utilitarian approach of ethics towards the development of our system, we retain a source of ethical standards in which we must consider future development of compounds with mal-intent be constructed with our system. However, the potential consequences are quickly diluted when considering the potential beneficial results: development of new drugs, new diagnostic methods, and new biochemical processes.

Although we lack sight into the future to see what our device will be used for and therefore are unable to reflect on the outcomes and explore alternative systems, we are still guided by virtue ethics outlined in Charles Harris' paper "The Good Engineer: Giving Virtue its Due in Engineering Ethics"[16]. He states on the first page of paper, "…virtue ethics are the greater place it gives [rather than preventative ethics] for discretion and judgment and also for inner motivation and commitment".[16] Drawing upon the liberal arts education we have received at Santa Clara University, we have the skills as intelligent communicators to take a step back from our narrow research and view our project as discerning citizens.

Our ethical approach was also sculpted as we gained techno-social sensitivity to the fields of surface chemistry, microfluidics, and surfactant emulsions. Under the guidance of Dr. Paul Abbyad we were able to gain critical awareness into the impact our system could have on these fields.

Lastly, our ethical significance is in part derived from a communal respect for nature. In a vision that is consistent with the virtues outlined in the SCU Strategic Vision and the School of Engineering Mission Statement, we have engineered a system that can vastly reduce the amount of chemical waste generated by traditional bench top biochemical procedures. Ending a day of experimentation with several milliliters, rather than liters, of chemical waste will undoubtedly have economic benefits that also drive sustainable laboratory practices.

The combination of these influences provides our team with an excellent source of ethical legitimacy for our project as we strive to push the evolution of technology and research forward.

Health and Safety

Safety is always a priority in the lab. Although there ought to be the same laboratory attire standards when fabricating the microfluidic chip or conducting chemical experiments, there are less safety risks associated with experimentation using our system due to the vast reduction of volume in reagents. A stable emulsion of an aqueous solution of interest or biological components within a fluorinated oil environment upon a hydrophobic surface treatment of substrate (Novec 1720) permits experimentation with only microliters of reagent.

Our device in particular is made of polydimethylsiloxane (PDMS), which is a widely used organic polymer that is inert and non-toxic[17]. Although the final device (unused) is safe, fabrication of the chip can be hazardous and requires technical skill. Basic training is required to be able to safely operate the UV lamp, laminator, vacuum container, and plasma cleaner as well as handling of silicone elastomer base, silicone elastomer curing agent (cross-linker). Although the silicone elastomer base is not a hazardous material as defined in the OSHA Hazard Communication Standard[18], the curing agent needs to be stored properly, as contact with water, moisture, and acid/bases can generate hydrogen gas[19]. These risks are usually recognized in even the most rudimentary lab settings and are mitigated by standard Good Laboratory Practice (GLP).

Used chips and materials should not be made available to the public. Model (unused) microfluidic chips may be developed for demonstrations, but risk is completely mitigated by ensuring only trained personnel come in contact with used chips, chip material, or experimental fluids. The principle of informed consent applies both to lab colleagues and untrained spectators. Proper labeling (date, concentration, status) of used chips, reagents, and syringes is strongly recommended to avoid contamination. Disposal of aqueous waste, solid waste, and sharp objects should be separate and in containers that are compliant with state regulations and standard chemical laboratory protocols.

Science, Technology, and Society

The effect of implementation of our system has the potential to expedite a wide variety of chemical and biochemical experimentation once the model system and procedures are specifically catered to a certain application. This first requires characterization of the physical properties and behavior of reagents in the chip environment. However, this initial investment in resources will also in and of itself serve as research as characterization often reveals trends as associations that are worthy of publication. The community of microfluidic systems engineers and surface chemists are able to benefit from such characterization and associations even if they do not implement our system.

Our system and any work done on it can provide substantial benefits to society. Drug discoveries can be expedited and time to reach market can be significantly reduced, potentially saving companies millions and allowing a breakthrough drug to see the light of day. Fluorescent biological assaying can be implemented with real-time control of parameters such as pH, flow rate, and concentration of reagent. New surfactants can be made and tested for slow-release of an angiogenesis-inhibiting drug on the surface of a medical implant at a local tumor site.

Although our device may be significantly altered for each application, the immense flexibility of our basic model system serves as a starting point for a variety of experiments in numerous fields. The science and technology behind our system is meant to further advance science and technology by making experiments more controlled and programmable. Society will indirectly reap the benefits of our system as it can be implemented by researchers and developers to make and test new drugs, devices, or diagnostic capabilities.

Civic Engagement

Our microfluidic chip and experimental protocol is not intended for use by the public, but rather in-laboratory researchers, developers, and engineers. To succeed in the market, results from several common biochemical procedures such as ELISA assaying and live/dead cell viability assaying need to be accurately replicated and published in professional journals. Professional societies such as the Biochemical Society or Royal Society of Chemistry may gain exposure to our system in peer-reviewed journals such as the Biomicrofluidics Journal or Lab on a Chip Journal.

Chemists and pharmaceutical engineers that plan on developing a drug or a medical device may use our device for initial testing, but our device is not intended for FDA-compliant clinical testing. However, characterization of biomaterials or various reagents may assist in qualitative assessments and comparative associations.

Manufacturability

The microfluidic chip system can be manufactured by hand with minimal training, although materials and instruments are required investments. Currently, manual chip fabrication takes about 5 hours to fabricate and dry for use. This requires instruments such as a UV lamp, laminator, and plasma cleaner, consumables listed in Table 1. and Table 2., and existing photoresist masks and chip molds.

Successful design and marketing of a completed specialized chip that has near-perfect replicability of a certain popular chemical or biochemical procedure may warrant investment in automation for chip fabrication.

Chapter 8 – Conclusion

Starting with our desire to further characterize the fascinating word of perfluorinated emulsions, and continuing with the introduction of these emulsions into droplet-microfluidic devices, we effectively designed a way to characterize them in droplet-microfluidic devices. Starting with a device to measure the concentration so surfactant at the moment of coalescence for 36 droplet pairs, we managed to successfully gather data on two biologically relevant conditions that will likely be used in these devices in the future. Though the first device struggled to complete the tasks we demanded of it, we quickly formulated a back-up plan and executed the design and fabrication of a second device which proved to be just as useful if not more so than the first device. With this second device we managed to measure the effect of many more biologically relevant conditions than was initially possible with the first device. In addition, we continued the investigation into the stability of perfluorinated emulsions beyond the means of this institution by collaborating with a Chemical Engineering lab at Stanford University. With the assistance of a Ph.D. student, we measured viscoelastic properties of the interface between aqueous phases and perfluorinated oil-surfactant mixtures. Despite the correlation between viscoelasticity and emulsion stability not being fully understood, we found trends to support the data we had gathered on a previous two devices. Overall, our ability to use our system to further our understanding of these emulsions was largely a success. However, due to our time constraints there is still a large amount of work to be done to fully characterize and understand these emulsions. Also, as a greater number biological reagents are introduced to the scientific community, even more characterization in perfluorinated emulsions will need to be performed.

In terms of our contribution to the scientific community, we achieved our goal in that we managed to perform scientific procedure which had never be performed before and managed to get valuable knowledge from it. Due to the major attributes of perfluorinated oils (optically clear, high oxygen diffusion and absorption, biocompatibility and immiscibility), their role in the study of droplet-microfluidics should only increase. However, in order to use them in a manner that actually provides use, the community will require an understanding of their characteristics. We've shown that both phosphate concentrations and pH play a vital role in the stability of these emulsions right in biologically relevant ranges. If the community ever

hopes to use these oils to their full capacity in biology, they will have to understand the behavior we documented in our senior design project.

Bibliography

1. Owens, Brian. "Slow science." *Nature* 495.7441 (2013): 300.
2. Sackmann, Eric K., Anna L. Fulton, and David J. Beebe. "The present and future role of microfluidics in biomedical research." *Nature* 507.7491 (2014): 181-189.
3. Teh, Shia-Yen, et al. "Droplet microfluidics." *Lab on a Chip* 8.2 (2008): 198-220.
4. Zhu, Ying, and Qun Fang. "Analytical detection techniques for droplet microfluidics—A review." *Analytica chimica acta* 787 (2013): 24-35.
5. Zolfaghari, Reza, et al. "Demulsification techniques of water-in-oil and oil-in-water emulsions in petroleum industry." *Separation and Purification Technology* 170 (2016): 377-407.
6. Shah, P., D. Bhalodia, and P. Shelat. "Nanoemulsion: a pharmaceutical review." *Systematic Reviews in Pharmacy* 1.1 (2010): 24.
7. "Microfluidics." *Wikipedia*. Wikimedia Foundation, n.d. Web. 12 Feb. 2017. <https://en.wikipedia.org/wiki/Microfluidics>
8. Akartuna, Ilke, et al. "Chemically induced coalescence in droplet-based microfluidics." *Lab on a Chip* 15.4 (2015): 1140-1144.
9. Toepke, Michael W., and David J. Beebe. "PDMS absorption of small molecules and consequences in microfluidic applications." *Lab on a Chip* 6.12 (2006): 1484-1486.
10. Lowe, Kenneth C., Michael R. Davey, and J. Brian Power. "Perfluorochemicals: their applications and benefits to cell culture." *Trends in biotechnology* 16.6 (1998): 272-277.
11. Rohan. "HOME › Press Releases › Digital PCR (dPCR) and QPCR Market worth 4.94 Billion USD by 2021." *Market Research Firm*. Marketsandmarkets, 2017. Web. 28 Feb. 2017. <http://www.marketsandmarkets.com/PressReleases/digital-pcr.asp>.
12. "Global Digital PCR Market By Components And Services (DPCR Instruments, Reagents And Consumables, DPCR Software And Services), By Applications (Clinical, Research, Forensics) - Growth, Future Prospects & Competitive Analysis, 2016 - 2023." *Market Research Reports*. Credence Research, Dec. 2016. Web. 28 Feb. 2017. <http://www.credenceresearch.com/report/digital-pcr-market>.

13. Brouzes, Eric, et al. "Droplet microfluidic technology for single-cell high-throughput screening." *Proceedings of the National Academy of Sciences* 106.34 (2009): 14195-14200.
14. Franck, A. J. "Understanding Instrument Inertia Corrections in Oscillation." *TA Instrument* (2005).
15. Yeo, Leslie Y., et al. "Film drainage between two surfactant-coated drops colliding at constant approach velocity." *Journal of colloid and interface science* 257.1 (2003): 93-107.
16. Harris, C.E. Sci Eng Ethics (2008) 14: 153. doi:10.1007/s11948-008-9068-3
17. "Polydimethyl Siloxane." Dimethicone. N.p., n.d. Web. 10 June 2017
18. "Material Safety Data Sheet." Sylgard® 184 Silicone Elastomer Base Http://www.cam.ac.uk/. Dow Corning Corporation, 13 Nov. 2001. Web. 10 June 2017.
19. "Material Safety Data Sheet." Sylgard® 184 Silicone Elastomer Curing Agent Http://www.cam.ac.uk/. Dow Corning Corporation, 15 Mar. 2011. Web. 10 June 2017

Appendix A: MATLAB Examples:

The function used to graph plots in MATLAB, notBoxPlo,t was made for open source use by Rob Campell

notBoxPlot Function

```
function varargout=notBoxPlot(y,x,jitter,style)
% notBoxPlot - Doesn't plot box plots!
%
% function notBoxPlot(y,x,jitter,style)
%
%
% Purpose
% An alternative to a box plot, where the focus is on showing raw
% data. Plots columns of y as different groups located at points
% along the x axis defined by the optional vector x. Points are
% layed over a 1.96 SEM (95% confidence interval) in red and a 1 SD
% in blue. The user has the option of plotting the SEM and SD as a
% line rather than area. Raw data are jittered along x for clarity. This
% function is suited to displaying data which are normally distributed.
% Since, for instance, the SEM is meaningless if the data are bimodally
% distributed.
%
%
% Inputs
% y - each column of y is one variable/group. If x is missing or empty
%     then each column is plotted in a different x position.
%
% x - optional, x axis points at which y columns should be
%     plotted. This allows more than one set of y values to appear
%     at one x location. Such instances are coloured differently.
% Note that if x and y are both vectors of the same length this function
% behaves like boxplot (see Example 5).
%
% jitter - how much to jitter the data for visualization
%          (optional). The width of the boxes are automatically
%          scaled to the jitter magnitude.
%
% style - a string defining plot style of the data.
%       'patch' [default] - plots SEM and SD as a box using patch
%             objects.
%       'line' - create a plot where the SD and SEM are
%             constructed from lines.
%       'sdline' - a hybrid of the above, in which only the SD is
%             replaced with a line.
%
%
% Outputs
% H - structure of handles for plot objects.
%
```

```
%
% Example 1 - simple example
% clf
% subplot(2,1,1)
% notBoxPlot(randn(20,5));
% subplot(2,1,2)
% h=notBoxPlot(randn(10,40));
% d=[h.data];
% set(d(1:4:end),'markerfacecolor',[0.4,1,0.4],'color',[0,0.4,0])
%
% Example 2 - overlaying with areas
% clf
% x=[1,2,3,4,5,5];
% y=randn(20,length(x));
% y(:,end)=y(:,end)+3;
% y(:,end-1)=y(:,end-1)-1;
% notBoxPlot(y,x);
%
% Example 3 - lines
% clf
% H=notBoxPlot(randn(20,5),[],[],'line');
% set([H.data],'markersize',10)
%
% Example 4 - mix lines and areas [note that the way this function
% sets the x axis limits can cause problems when combining plots
% this way]
%
% clf
% h=notBoxPlot(randn(10,1)+4,5,[],'line');
% set(h.data,'color','m')
% h=notBoxPlot(randn(50,10));
% set(h(5).data,'color','m')
%
% Example 5 - x and y are vectors
% clf
% x=[1,1,1,3,2,1,3,3,3,2,2,3,3];
% y=[7,8,6,1,5,7,2,1,3,4,5,2,4];
% notBoxPlot(y,x);
%
% Note: an alternative to the style used in Example 5 is to call
% notBoxPlot from a loop in an external function. In this case, the
% user will have to take care of the x-ticks and axis limits.
%
% Example 6 - replacing the SD with bars
% clf
% y=randn(50,1);
```

```
% clf
% notBoxPlot(y,1,[],'sdline')
% notBoxPlot(y,2)
% xlim([0,3])
%
%
% Rob Campbell - January 2010
%
% also see: boxplot




% Check input arguments
error(nargchk(0,4,nargin))
if nargin==0
   help(mfilename)
   return
end


if isvector(y), y=y(:); end

if nargin<2 || isempty(x)
   x=1:size(y,2);
end

if nargin<3 || isempty(jitter)
   jitter=0.3; %larger value means greater amplitude jitter
end

if nargin<4
  style='patch'; %Can also be 'line' or 'sdline'
end
style=lower(style);

if jitter==0 && strcmp(style,'patch')
   warning('A zero value for jitter means no patch object visible')
end


if isvector(y) & isvector(x) & length(x)>1
   x=x(:);

   if length(x)~=length(y)
      error('length(x) should equal length(y)')
```

```
    end

    u=unique(x);
    for ii=1:length(u)
        f=find(x==u(ii));
        h(ii)=notBoxPlot(y(f),u(ii),jitter,style);
    end


    %Make plot look pretty
    if length(u)>1
        xlim([min(u)-1,max(u)+1])
        set(gca,'XTick',u)
    end

    if nargout==1
        varargout{1}=h;
    end

    return

end




if length(x) ~= size(y,2)
    error('length of x doesn''t match the number of columns in y')
end







%We're going to render points with the same x value in different
%colors so we loop through all unique x values and do the plotting
%with nested functions. No clf in order to give the user more
%flexibility in combining plot elements.
hold on
[uX,a,b]=unique(x);

h=[];
for ii=1:length(uX)
    f=find(b==ii);
```

```
    h=[h,myPlotter(x(f),y(:,f))];
end

hold off

%Tidy up plot: make it look pretty
if length(x)>1
    set(gca,'XTick',unique(x))
    xlim([min(x)-1,max(x)+1])
end


if nargout==1
    varargout{1}=h;
end



%Nested functions follow


%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%
function h=myPlotter(X,Y)

SEM=SEM_calc(Y); %Supplied external function
SD=nanstd(Y);  %Requires the stats toolbox
mu=nanmean(Y); %Requires the stats toolbox

%The plot colors to use for multiple sets of points on the same x
%location
cols=hsv(length(X)+1)*0.5;
cols(1,:)=0;
jitScale=jitter*0.6; %To scale the patch by the width of the jitter

for k=1:length(X)
    thisY=Y(:,k);
    thisY=thisY(~isnan(thisY));
    thisX=repmat(X(k),1,length(thisY));

    if strcmp(style,'patch')
      h(k).sdPtch=patchMaker(SD(k),[0.6,0.6,1]);
    end

    if strcmp(style,'patch') || strcmp(style,'sdline')
      h(k).semPtch=patchMaker(SEM(k),[1,0.6,0.6]);
```

```
      h(k).mu=plot([X(k)-jitScale,X(k)+jitScale],[mu(k),mu(k)],'-r',...
          'linewidth',2);
    end

    %Plot jittered raw data
    C=cols(k,:);
    J=(rand(size(thisX))-0.5)*jitter;


    h(k).data=plot(thisX+J, thisY, 'o', 'Markersize', 10, 'color', C,...
                'markerfacecolor', C+(1-C)*0.65);
end

if strcmp(style,'line') | strcmp(style,'sdline')
  for k=1:length(X)
    %Plot SD
    h(k).sd=plot([X(k),X(k)],[mu(k)-SD(k),mu(k)+SD(k)],...
              '-','color',[0.2,0.2,1],'linewidth',2);
    set(h(k).sd,'ZData',[1,1]*-1)
  end
end

if strcmp(style,'line')
    for k=1:length(X)
        %Plot mean and SEM
        h(k).mu=plot(X(k),mu(k),'o','color','r',...
            'markerfacecolor','r',...
            'markersize',10);

        h(k).sem=plot([X(k),X(k)],[mu(k)-SEM(k),mu(k)+SEM(k)],'-r',...
            'linewidth',2);
        h(k).xAxisLocation=x(k);
    end
end

function ptch=patchMaker(thisInterval,color)
    l=mu(k)-thisInterval;
    u=mu(k)+thisInterval;
    ptch=patch([X(k)-jitScale, X(k)+jitScale, X(k)+jitScale, X(k)-jitScale],...
          [l,l,u,u], 0);
    set(ptch,'edgecolor','none','facecolor',color)
end %function patchMaker
end %function myPlotter
end %function notBoxPlot
```

T-interval Calc Function

```
function tint=tInterval_Calc(vect, CI)
% tInterval_Calc - confidence interval based on the t-distribution
%
% function tint=tInterval_Calc(vect, CI)
%
% Purpose
% Calculate the t-interval about the mean to a given confidence
% level (CI). Note that nans do not contribute to the calculation
% of the sample size and are ignored for the SD calculation. Output
% of this function has been checked against  known working code
% written in R.
% Inputs
% - vect: Calculates the two-tailed 95% t confidence limits for the mean.
% - CI [optional]: a p value for a different 2-tailed interval. e.g. 0.01
%
% Example - plot a 1% interval [rather than the default %5]
% r=randn(1,30);
% T=tInterval_calc(r,0.01);
% hist(r)
% hold on
% plot(mean(r), mean(ylim),'r*')
% plot([mean(r)-T,mean(r)+T], [mean(ylim),mean(ylim)],'r-')
% hold off
% Rob Campbell - 12/03/08
% Also see - SEM_calc, tinv
error(nargchk(1,2,nargin))
if isvector(vect)
  vect=vect(:);
end
if nargin==1
  CI = 0.025; %If no second argument, work out a 2-tailed 5% t-interval
  stdCI=tinv(1-CI, length(vect)-1);
elseif nargin==2
  CI = CI/2 ; %Convert to 2-tail
  stdCI=tinv(1-CI, length(vect)-1); %Based on the t distribution
end
if stdCI==0
  error('Can''t find confidence iterval for 0 standard deviations!')
end


tint =  ( (nanstd(vect)) ./ sqrt(sum(~isnan(vect))) ) * stdCI ;
```

Sample Data Analysis Code

```
highConcPluronic = [0.170940171 0.098619329 0.252465483 0.160420776 0.301117686
        0.231426693 0.255095332 0.247205786 0.244575937 0.098619329 0.17357002
        0.098619329 0.236686391 0.098619329 0.249835634 0.098619329 0.285338593
        0.228796844 0.315581854 0.202498356 0.184089415 0.105193951 0.149901381
        0.103879027 0.240631164 0.098619329 0.241946088 0.198553583 0.264299803
        0.230111769 0.311637081 0.134122288 0.172255095 0.098619329 0.144641683
        0.159105851 0.168310322 0.122287968 0.240631164 0.241946088 0.273504274
        0.316896778 0.284023669 0.098619329 0.166995398 0.098619329 0.165680473
        0.098619329 0.168310322 0.098619329 0.234056542 0.127547666 0.259040105
        0.238001315 0.266929652 0.168310322 0.152531229 0.12886259 0.178829717
        0.184089415 0.124917817 0.111768573 0.148586456 0.306377383 0.191978961
        0.120973044 0.198553583 0.098619329 0.144641683 0.14069691]
lowConcPluronic = [0.152531229 0.170940171 0.156476003 0.272189349 0.427350427
        0.465483235 0.24852071 0.252465483 0.164365549 0.169625247 0.186719264
        0.199868508 0.103879027 0.165680473 0.159105851 0.259040105 0.280078895
        0.370808679 0.256410256 0.274819198 0.155161078 0.166995398 0.19460881
        0.17357002 0.197238659 0.1617357 0.098619329 0.249835634 0.264299803
        0.365548981 0.247205786 0.264299803 0.123602893 0.365548981 0.124917817
        0.189349112 0.123602893 0.139381986 0.098619329 0.357659435 0.213017751
        0.24852071 0.241946088 0.184089415 0.172255095 0.184089415 0.122287968
        0.145956607 0.145956607 0.361604208 0.098619329 0.243261012 0.195923734
        0.312952005 0.245890861 0.164365549 0.124917817 0.11965812 0.347140039
        0.3234714 0.126232742 0.249835634 0.098619329 0.151216305 0.178829717
        0.157790927 0.298487837 0.122287968]
lowConcLine=zeros(size(lowConcPluronic));
highConcLine=ones(size(highConcPluronic));
figure(1);
xlabel('0.25% Pluronic ----- 5% Pluronic','fontsize',32);
ylabel('Fraction High Surfactant Concentration Oil','fontsize',32);
set(gca,'fontsize',24);
set(gca,'Xtick',[]);
notBoxPlot(lowConcPluronic,lowConcLine,[0.55]);
notBoxPlot(highConcPluronic,highConcLine,[0.55]);

lowConc1=[0.152531229
0.156476003
0.427350427
0.24852071
0.164365549
0.186719264
0.103879027
0.159105851
0.280078895
0.256410256
```

0.155161078
0.19460881
0.197238659
0.098619329
0.264299803
0.247205786
0.123602893
0.124917817
0.123602893
0.098619329
0.213017751
0.241946088
0.172255095
0.122287968
0.145956607
0.098619329
0.195923734
0.245890861
0.124917817
0.347140039
0.126232742
0.098619329
0.178829717
0.298487837
0.122287968]

lowConc2 = [0.170940171
0.272189349
0.465483235
0.252465483
0.169625247
0.199868508
0.165680473
0.259040105
0.370808679
0.274819198
0.166995398
0.17357002
0.1617357
0.249835634
0.365548981
0.264299803
0.365548981
0.189349112
0.139381986

0.357659435
0.24852071
0.184089415
0.184089415
0.145956607
0.361604208
0.243261012
0.312952005
0.164365549
0.11965812
0.3234714
0.249835634
0.151216305
0.157790927]

lowconc1line=zeros(size(lowConc1))
lowconc2line=ones(size(lowConc2))

figure(3);
xlabel('1st 0.25% Pluronic ----- 2nd 0.25% Pluronic');
ylabel('Volume Percentage High Surfactant Concentration Oil');
notBoxPlot(lowConc1, lowconc1line, [0.55]);
notBoxPlot(lowConc2, lowconc2line,[0.55]);

control = [
0.144
0.298666667
0.174666667
0.125333333
0.1
0.149333333
0.134666667
0.270666667
0.177333333
0.118666667
0.162666667
0.1
0.177333333
0.24
0.149333333
0.1
0.1
0.1
0.214666667
0.125333333
0.1

0.1
0.1
0.201333333
0.153333333
0.1
0.1
0.177333333
0.205333333
0.108
]

PBS = [0.165680473
0.142011834
0.126232742
0.127547666
0.156476003
0.219592373
0.157790927
0.098619329
0.11965812
0.157790927
0.210387903
0.176199869
0.117028271
0.098619329
0.098619329
0.143326759
0.186719264
0.142011834
0.098619329
0.110453649
0.170940171
0.149901381
0.160420776
0.098619329
0.117028271
0.098619329
0.127547666
0.148586456
0.213017751
0.124
0.12933333
0.184
0.20933333
0.128
0.108

```
0.27066667
0.244
0.22933333
0.12666667
0.1
0.22
0.224
0.192
0.17733333
0.14133333
0.10533333
0.16
0.10933333
0.17466667
0.18533333
0.108
0.1
0.112
0.31066667
0.156
0.12933333
0.16266667
0.1
0.1
0.1
0.18666667
0.128
0.33333333
0.1]

PBSline=ones(size(PBS));
controlLine=zeros(size(control));
figure(2);
xlabel('0.25% Pluronic ----- 10 mM Sodium Phosphate in 0.25% Pluronic','fontsize',32);
ylabel('Fraction High Surfactant Concentration Oil','fontsize',32);
set(gca,'fontsize',24);
set(gca,'Xtick',[]);
notBoxPlot(lowConcPluronic, lowConcLine, [0.55]);
notBoxPlot(PBS, PBSline,[0.55]);



PBS1=[0.124 0.12933333 0.184 0.20933333 0.128 0.108 0.27066667 0.244 0.22933333
0.12666667 0.1 0.22 0.224 0.192 0.17733333 0.14133333 0.10533333 0.16 0.10933333
0.17466667 0.18533333 0.108 0.1 0.112 0.31066667 0.156 0.12933333 0.16266667 0.1 0.1 0.1
0.18666667 0.128 0.33333333 0.1]
```

```
PBS2=[
0.165680473
0.142011834
0.126232742
0.127547666
0.156476003
0.219592373
0.157790927
0.098619329
0.11965812
0.157790927
0.210387903
0.176199869
0.117028271
0.098619329
0.098619329
0.143326759
0.186719264
0.142011834
0.098619329
0.110453649
0.170940171
0.149901381
0.160420776
0.098619329
0.117028271
0.098619329
0.127547666
0.148586456
0.213017751]
x=mean(PBS1);
y=mean(PBS2);
x1=sqrt(size(PBS1,2));
y1=sqrt(size(PBS2,1));
x2=std(PBS1);
y2=std(PBS2);
x3=(x2/x1);
y3=(y2/y1);
PBS1line=zeros(size(PBS1));
PBS2line=ones(size(PBS2));

figure(4);
ylabel('Volume Percent Pure QX100 at Coalescence');
xlabel('Different PBS runs');
notBoxPlot(PBS1, PBS1line, [0.55]);
notBoxPlot(PBS2, PBS2line, [0.55]);
```

```
a=mean(PBS);
a1=sqrt(size(PBS,1));
a2=std(PBS);
a3=(a2/a1);
b=mean(lowConcPluronic);
b1=sqrt(size(lowConcPluronic,2));
b2=std(lowConcPluronic);
b3=(b2/b1);
```

Appendix B: Stanford Rheometer Protocols

Obtained from TA Instruments:

Mazzeo, Fred. *Guide to Using an AR Series Rheometer*. New Castle, DE: TA Instruments, 2015. Print.

INTRODUCTORY GUIDE TO USING AN AR SERIES RHEOMETER
USING RHEOLOGY ADVANTAGE SOFTWARE* (VERSIONS ≤ 4.1)

Fred Mazzeo, Ph.D.
TA Instruments, 109 Lukens Drive, New Castle, DE 19720, USA

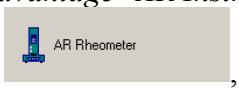
1. Turn on the computer controller.
2. Turn on the air supply to the rheometer. Pressure requirements are as follows: AR2000: 30 psi, AR1000: 25 psi, AR 550/500: 37.5 psi.
3. Remove the black bearing lock by holding it in place while turning the draw rod knob at the top in a counter-clockwise direction. Once the bearing lock is removed, make sure that the spindle rotates freely.

   NOTE: For an AR2000 system with a drive-shaft slide lock, please ensure that the drive-shaft slide lock is pulled-out.

   NOTE: If air supply is interrupted while bearing lock is off, **DO NOT TURN the DRIVE SHAFT**, this will cause damage to the bearing. Locate another gas source, attach it to the gas port on the rheometer, and then attach bearing lock while the air bearing is floating.
4. **Turn on the Power to Rheometer**

   Switch the power button, located on the rear of the electronics control box, to the *ON* position.

   For AR1000/550/500 systems equipped with an ETC, turn on the power to the ETC electronics box.

   NOTE: If step 4 is performed before step 3, an alarm will sound and the instrument controller display will read 'optical init. fail'. At this point, turn off the power and perform step 3.
5. When using a Peltier control device, please ensure that the water supply is turned on. If using a pump and tank system, ensure that the water is clean and the pump is fully submerged.
6. When the instrument has finished the system check, turn on the instrument control software: *Start>Programs>TA Rheology Advantage>AR Instrument Control*.
7. Choose the *Instrument Status Page* icon, 

, to make certain that communication has been established between the computer and the instrument. If communication is not established, the page will appear blank. The Instrument Status Page can also be used to manually control the instrument.
8. **Instrument Inertia**

   Determine the instrument inertia by selecting *Options>Instrument>Inertia* and press the '*Calibrate*' button.

   This value is unique for each air bearing assembly. An acceptable range for this value is ~14-16 μNms^2 for the AR1000 and AR2000, and ~26 μNms^2 for the AR500 and AR550. The value for the instrument should not change by more than

15% of the original Inertia value. _____ ± ___
If you notice a continual drift of this value, check the quality of the air used, as it could be indicative of a poor quality supply. If the problem persists, contact the instrument service department.

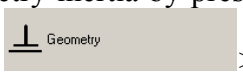
9. **Geometry**

Attach test geometry by sliding it up the drive shaft and hold it stationary while turning the draw rod knob at the top in a clockwise direction. If the geometry file was previously created, choose the appropriate geometry (*Geometry>Open…*), or create a new geometry by selecting *Geometry>New*, and follow the New Geometry dialogue window.

NOTE: If testing a solid sample, attach the appropriate spacers based on the sample's dimensions before calibrating the geometry. Refer to 18.c. for determining spacer size.

10. **Geometry Inertia**

The value of the inertia for each measuring system differs because they all have been uniquely engineered and have different masses. It is important to calibrate the inertia value for every geometry, particularly if high frequency oscillations are being used, or if low viscosity fluids are being measured.

Calibrate the geometry inertia by pressing the *'Calibrate'* button that is found in the *Geometry Page* 

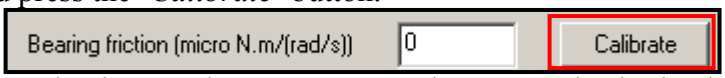
>Settings>Inertia: Calibrate.

11. **Bearing Friction Correction**

An air bearing is used to set the drive shaft afloat and provide virtually friction free application of torque to the sample. However, there will always be some residual friction. With most test materials, this is insignificant, but in about 1% of low viscosity samples, this inherent friction causes inaccuracies in the final rheological data. To overcome this, the software has an air bearing friction correction that should be activated.

NOTE: Please ensure that the Instrument (**Step 9**) and Geometry inertia (**Step 10**) has been calibrated before determining the 'bearing friction correction' value.

If using Rheology Advantage 3.0 or higher, go to *Options>Instrument>Miscellaneous*, check the '*bearing friction correction*' box and press the '*Calibrate*' button.



If using Rheology Advantage 2.0 or lower, uncheck the bearing friction correction box located within *Options>Instrument>Miscellaneous.* Go to *Help>Index,* type in the phrase '*friction: calibration>determining bearing friction correction*' and follow the steps.

This value is unique for each air bearing assembly and rheometer model. An acceptable range for this value is ~0.5-1.1 µNm/(rad/s). This value should be within ~20% of the original BFC value. _____ ± ___

12. **Temperature System Selection**

For an AR2000 system, attach the appropriate Smart Swap lower geometry temperature stage/system. (See Smart Swap operational instructions by going to *Start>Programs>TA Rheology Advantage>Rheology Advantage Manuals*: AR2000 pg 5-6)

For an AR1000/550/500 system, attach the appropriate lower geometry temperature stage, not necessary if using the Peltier plate. The temperature read and control must be set to the appropriate temperature system. This is accomplished by choosing *Options>Instrument>Temperature: Temperature Read* and *Temperature Control* setting both to 'Peltier', if using the Peltier Plate, or 'Temperature System' if any other system is being used, such as a water circulator, ETC or ETM.

NOTE: If the rheometer is equipped with an ETC and it is being used for testing, set the ETC flow meter to 10 lpm.

NOTE: If using Rheology Advantage 4.0 or greater and using an ETC equipped with low temperature fittings, checking 'purge gas only' (located in *Options>Instrument>Temperature*) will convert a low temperature flow meter to use only the ETC purge gas instead of Liquid Nitrogen to control temperature. Recommended lower temperature range when using this setting is 45°C. For tests that require lower temperatures using ETC, attach LN_2 feed and disable this setting.

13. **Mapping**

a. **Rotational Mapping**: Due to the micron-level tolerances needed to make an air bearing work, any bearing will have small variations in torque behavior around one complete revolution of the shaft. They are consistent over time unless changes occur in the air bearing.

By combining the absolute angular position data from the optical encoder with microprocessor control of the motor, these small variations can be mapped automatically and stored in memory for subsequent real-time corrections in the test. To create a map, the software rotates the drive shaft at a fixed speed, monitoring the torque required to maintain this speed through a full 360° of rotation. This results in a very wide operating range of the bearing without operator intervention - a confidence check in bearing performance.

Perform a rotational mapping on the geometry when the test procedure will be applying either a flow or transient (Creep or Stress Relaxation) mode of deformation. Begin the rotational mapping by pressing the icon  or go to *Instrument>Rotational Mapping*.

If using Rheology Advantage 3.0 or higher, select either the number of iterations or mapping type within the icon dialog window.

If using Rheology Advantage 2.0 or lower, go to *Options>Settings>Mapping* and *Options>Instrument>Miscellaneous: Mapping Type.*

The number of points in the map and the speed of rotation used are dependent upon the mapping type used. When mapping the geometry, the recommended settings are **'one'** iteration and '**standard**' type.

i. The mapping type can be set to fast, standard, precision (AR500/550/1000/2000) or extended (AR2000 only). For critical low-torque measurements, using <10 µNm, 'precision' mapping is more suited, unless 'extended' mapping is available.

ii. If 'extended' mapping is available (AR2000 only) and chosen, the number of iterations is not an option. When using fast, standard or precision mapping the number of iterations should be set greater than one. If performing a Creep procedure and using fast, standard or precision mapping, the number of iterations should be set to **four**, if the Recovery step is set to **zero**. Otherwise, setting the

number of iterations greater than three has diminishing returns in the mapping performance.

b. **Oscillation Mapping** [AR2000 only, using 4.1]: This mapping will perform a baseline subtraction only when using the continuous controlled strain mode and will improve the performance for low torque, low displacement data. To access in the software, go to *Instrument>Oscillatory mapping*. Refer to *TA Rheology Manuals* found on the computer controller's hard drive for more information.

14. **Zero the Geometry Gap**

Choose the zero gap icon  , or select *Instrument>Gap>Zero Gap* and follow the directions on screen.

NOTE: The upper geometry should be at the testing temperature before zeroing the gap. This will account for the change in dimensions due to the coefficient of thermal expansion of the testing geometry/system.

If equipped with normal force, there are two options can be used to zero the gap, deceleration or normal force. These options are located in *Options>Instrument>Gap>Gap Zero Mode: Deceleration or Normal Force.* If using *Normal Force*, set the value equal to 1 N.

NOTE: If using Rheology Advantage 4.0 or greater and using the torsion rectangular system, move the clamps within 5 mm. Manually, align the fixed raised surface of each clamp and go to *Instrument>Bearing Lock* or depress the Bearing lock icon  . This will electronically inhibit any rotation of the geometry and allow one to zero the gap without the geometry rotating.

15. **Gap Compensation**

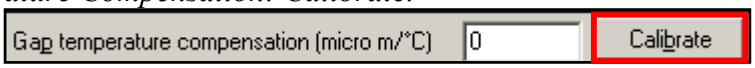
NOTE: Predetermined values can be entered by going to *Help>Index: 'compensation'*.

NOTE: The gap compensation check box must be activated, which is located within the *Options>Instrument>Temperature* dialog window, to account for the correction.

NOTE: Gap Compensation needs only to be used when testing over a temperature range.

NOTE: If controlling normal force throughout an experiment, the gap compensation value should be activated.

If using Rheology Advantage 3.0 and have the normal force transducer or 4.0 or greater with or without the normal force transducer, use the gap compensation button located in the *Geometry Page>Settings>Gap Temperature Compensation: Calibrate.*

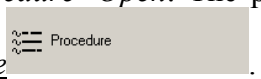


If using Rheology Advantage 2.0 or lower and have the normal force transducer, perform an oscillatory temperature ramp procedure (Step 16). Using a torque of 0.1 μNm, a frequency of 1 Hz conducted at a ramp rate of 2°C/min under global normal force control (1N +/- 0.1, 1000 μm up/down, compression, set initial value) with a conditioning step temperature equal to the starting value of the temperature range of the experiment with an equilibration time equal to 5 minutes. Plot the data and then fit a straight line to the graph of Gap vs. Temperature. The slope must be then entered in the *Options>Instrument>Temperature: Temperature Calibration* region within

the cell located to the right of the appropriate temperature system.

NOTE (version 2.0 or lower): The gap compensation value must be set to zero before this step is performed. Also, zero the normal force before running this gap compensation test.

16. **Procedure**

Create a new procedure by selecting *Procedure>New* or open a previously created procedure by selecting the appropriate file, by choosing *Procedure>Open*. The procedure can be viewed and adapted in the *Procedure Page* 

.

17. **Experimental Notes**

Enter sample information within the *Notes Page* 

or by selecting *Notes>New.*

18. **Sample Loading**

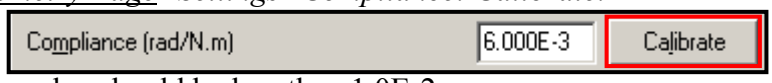
The amount of sample volume that is required, based on the dimensions entered in the *Geometry page>Dimensions* tab for cone, parallel and concentric cylinder systems, can be found in *Geometry page>Settings: Approximate sample volume.*

a. When testing a dispersion or polymer melt when using the Peltier or Parallel Plate ETC, go to *Help>Video Clips: Loading and Trimming a Sample* or review **Steps 19 & 20**.

b. When testing a polymer in pellet form, use the 25mm plates in conjunction with a melt ring, supplied in the Reusable Plate ETC kit, to contain the pellets. Place the melt ring around the lower 25mm stepped plate to form a well into which the pellets are placed, close the ETC, set a melt temperature  , allow sufficient time for the pellets to melt, and then lower the gap appropriately, then follow **Step 19 & 20**.

c. When testing a solid torsion sample using the ETC Torsion Kit: choose *Help>Content and Index>Index>Torsion>Guide to Sample Preparation.*

NOTE: Make sure that you run the compliance sample to determine the instrument compliance. This is determined by pressing the compliance calibrate button, located within the *Geometry Page>Settings>Compliance: Calibrate.*



This value should be less than 1.0E-2.

NOTE: Close the left ETC door and bend the upper moveable thermocouple (not the braided thermocouple attached to the heating elements) approximately 1.5 – 2 mm away from sample. Ensure that the thermocouple does not contact the geometry or the sample when the doors are fully closed.

d. Concentric cylinder: Use the volume of material specified in the *Geometry page>Settings: Approximate Sample Volume.*

e. Disposable ETC: Attach the plates loosely to the upper shaft and lower stage, in order for the plates to slide without slipping out of the holder, and then lower the plates to within 5mm. Activate normal force control  when viewing the *Instrument Status Page*, with settings: Normal force: 5N, Tolerance: 1N, Limit Up/Down: 100µm, Set initial value: On, Return to Window: On, Compression mode. Once the normal force has

reached 5N, then tighten screws, this will to ensure that the plates are parallel. Then raise the upper geometry and load the sample following 18.a.

f. Solid Sample Submersion System: Follow the Submersion Clamp Loading Wizard found in *Geometry page>Dimensions: Load sample*

19. **Gap Closure**

After loading a sample, the gap is closed by three different methods.

NOTE: When using the cone geometry, the gap set must be equal to the truncation gap value that is scribed on the geometry shaft.

NOTE: When using the parallel plate geometry, the gap is variable, and should be between 750 microns and Geometry Diameter (microns)/10.

a. Manually enter the desired gap by selecting the enter gap icon  or by selecting *Instrument>Gap>Enter Gap…*

b. Automatically have the instrument go to the gap value entered in the *Geometry Page>Dimensions* by using the go to gap icon  or select *Instrument>Gap>Go To Geometry Gap.*

NOTE: When using 19.a or 19.b, the compression settings (type, speed and distance above the geometry gap) can be adjusted by going to *Options>Instrument>Gap: Sample Compression.*

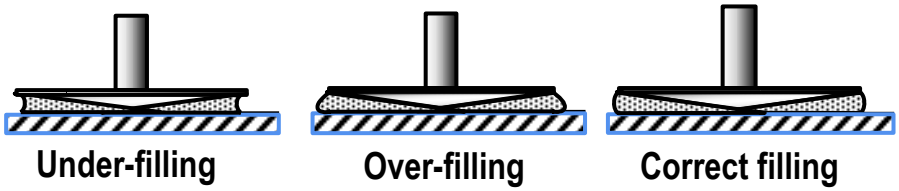
c. Manually raise or lower the gap by using the  icons.

NOTE: These icons are only available when viewing the *Instrument Status Page*.

20. **Trimming the Gap** (cone or plate geometry systems)

Load extra material and close the gap to a value of 5% larger than the required gap, so that excess material is expelled from between the upper geometry, and lower the plate, i.e. overfilled state. Then hold the spindle knob with one hand, in order to keep the geometry from rotating, and trim the excess material using a right edged tool with the other hand. Then lower the gap to the final test gap, i.e. by 5% to allow the correct filled state to be achieved.

NOTE: If using Rheology Advantage 4.0 or greater, go to *Instrument>Bearing Lock* or depress the Bearing lock icon  . This will electronically inhibit any rotation of the geometry and allow one to trim the sample without holding the spindle knob.



Proper loading of sample after closing the gap for cone and parallel plate geometry systems

21. **Run Test**

Run test by selecting the run experiment icon  or choose *Experiment>Run*.

* To upgrade to the latest version of Rheology Advantage, please request the upgrade form from TA Instruments

Table 1. Calibration Guide

Instrument Inertia (Step **8**)	Monthly Recommendation: Keep a log of this value
Geometry Inertia (Step **10**)	Once during the geometry setup Verification of value is recommended daily, but not required
Bearing Friction Correction (Step **11**)	Monthly Recommendation: Keep a log of this value
Rotational Mapping (Steps **13.a.i & 13.a.ii**)	Once daily (if same geometry) Every time the geometry is changed
Oscillation Mapping [**AR2000**] (Step **13.b**)	Only when using continuous strain control requiring low torque and displacement data
Zero Gap (Step **14**)	Every time geometry is removed or replaced
Gap Compensation (Step **15**)	Once if using same geometry/heating system

Support and Service

Website: www.tainst.com
TA Instruments Applications Support: 302-427-4070 (M-F 8:00-4:30pm EST)
TA Instruments Instrument Service Support: 302-427-4050 (M-F 8:00-4:30pm EST)

Appendix C: Excerpts from the Nemesys Software User Manual

Obtained from Nemesys Systems:

"Nemesys Original Instructions – Manual: Software and Hardware", *Model: Base 120*. Korbussen, Germany. **Cetoni Systems**. (January, 2014) Web.

NEMESYS®

Original Instructions – January 2014

Manual

Software

cetoni®

NEMESYS

The information and data contained in this documentation can be amended without notice. No part of this documentation can be duplicated or transferred for any purpose without the express written consent of cetoni GmbH, irrespective of the nature or medium of distribution - electronic or mechanical.
The general terms and conditions of cetoni GmbH apply. Alternative agreements must be in written form.

Software License

The software and the documents provided are protected by copyright. Installation declares your acceptance of the conditions of the licensing contract.

License Agreement

cetoni GmbH grants the purchaser the non-exclusive and non-transferable license right to use the software on a standalone computer or a networked computer system (LAN). It is expressly forbidden to copy or duplicate the software in part or in whole in any way. The same applies to mixing and combining the software with other software. The purchaser may create one back-up copy of the software for archival purposes. cetoni GmbH reserves the right to alter, develop and improve the software or develop new software to replace it. cetoni is not obliged to inform the purchaser of any alterations, new developments or improvements to the software or make these available to him. No legally binding assurance of certain features is given. cetoni is not liable for damages unless the damages are as a result of wilful intent or gross negligence on the part of cetoni GmbH or its employees. Any liability for indirect, collateral, or consequential damages is excluded.

cetoni GmbH
Am Wiesenring 6
D- 07554 Korbußen, Germany
Phone: +49 (0) 36602 338-0
Fax: +49 (0) 36602 338-11

e-mail: info@cetoni.de
Internet: www.cetoni.de

1 Summaries and directories

1.1 Table of contents

1.2 Change history

Ver	Date	Change
2.0.2	26.09.2008	• First version of neMESYS 2.0.2 software manual
2.0.3	07.11.2008	• Configuration of pressure sensor and over-pressure action inserted
2.2.0	11.03.2009	• Description of continuous flow generation added
2.4.0	25.01.2010	• Description of continuous flow generation updated (“cross flow”)
2.4.0	25.03.2010	• Description of shortcut menu added
2.5.0	01.06.2010	• Description for new features of continuous flow updated • Description of inverted syringe orientation added
2.5.1	15.09.2010	• Description of sensor configuration • Adaption of some formulations
20140131	14.01.2014	• Updated manual revision number to software version number

2 Introduction

2.1 Foreword

Thank you for deciding to purchase a cetoni product. We would like to support you as much as possible in your interaction with the neMESYS UserInterface software with this manual. We are directly available for any questions or suggestions that you may have.

You should not use the neMESYS dosing system before you have carefully read and understood this manual. We wish you much success in your work with the highly precise neMESYS dosing system and the neMESYS UserInterface software.

2.2 Symbols and Key Words Used

The following symbols are used in this manual and are designed to aid your navigation through this document:

IMPORTANT

Indicates tips for users and other especially useful information on how to act in dangerous or harmful situations.

NOTICE

Indicates a potentially harmful situation. Failure to avert this situation may result in damage to the product or anything nearby.

CAUTION

Indicates a potentially dangerous situation. Failure to avert this situation may result in light or minor injuries and property damage.

3 Installation

3.1 Installing the software

IMPORTANT

In order to ensure that the time-critical control processes of the neMESYS software are not affected, no applications needing high processing power should be executed on the control PC.

IMPORTANT

*Install the neMESYS software and device drivers **before** using the USB port to connect the unit to the PC.*

To install the software, insert the neMESYS CD-ROM into the CD drive. After you have done this, run the *"neMESYS UserInterface Setup.exe"* file from the CD. The installation wizard will then guide you through the process of installing the neMESYS software and the hardware drivers.

IMPORTANT

The user must be logged into Windows as the administrator in order to carry out the installation of the hardware driver.

During the installation, the hardware device drivers will be installed. This step is only necessary if the drivers have not already been installed on the computer. If the hardware drivers are already installed, please deactivate the *Hardware Device Drivers* (Figure 1) *components .*

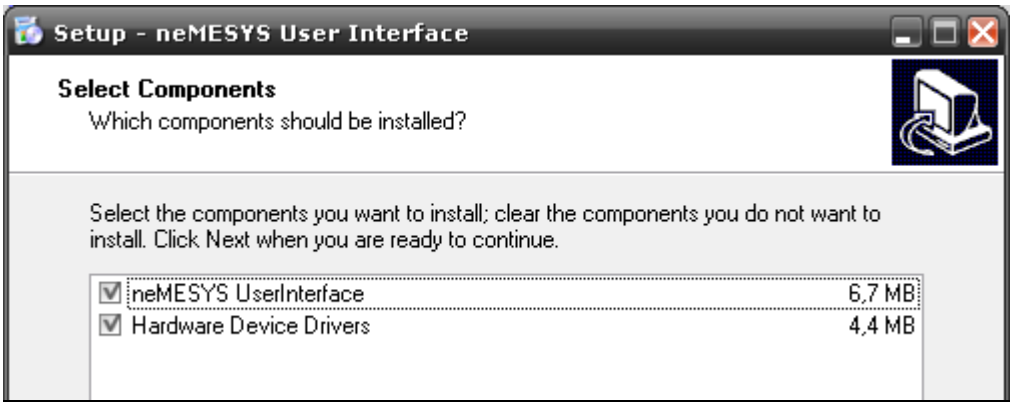


Figure 1 - Hardware Device Driver Installation

Your computer must fulfil the following system requirements in order to use the *neMESYS UserInterface*:

- PC with Pentium processor (or better) - min. 1.2 GHz
- at least 512 MB RAM (recommended 2 GByte)
- free hard disk space of approx. 20 MByte
- at least 2 free USB (1.1 or 2.0) interfaces
- Operating system Windows XP or Windows Vista
- Scroll wheel mouse

3.2 Installing the USB device drivers

When you connect the neMESYS dosing platform to the PC via a USB port for the first time, or if a different USB port is used at a later date, the Windows hardware wizard will recognise a new USB device and then automatically install the necessary drivers.

(1) The hardware wizard recognises a new unit on the USB port

Figure 2 - Windows Hardware Wizard

(2) The following dialog appears; this should be configured as shown on the image and confirmed with "Next".

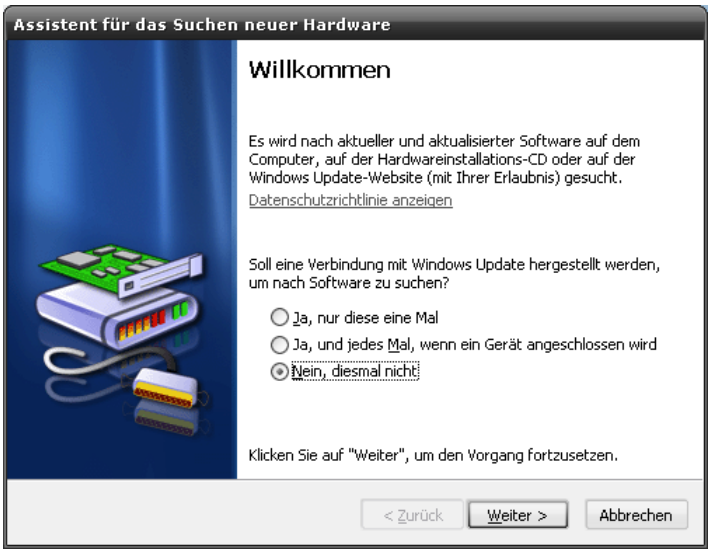


Figure 3 - Hardware Wizard - Welcome

(3) In the dialog that follows, please select automatic installation and again confirm this dialog with "Next”.

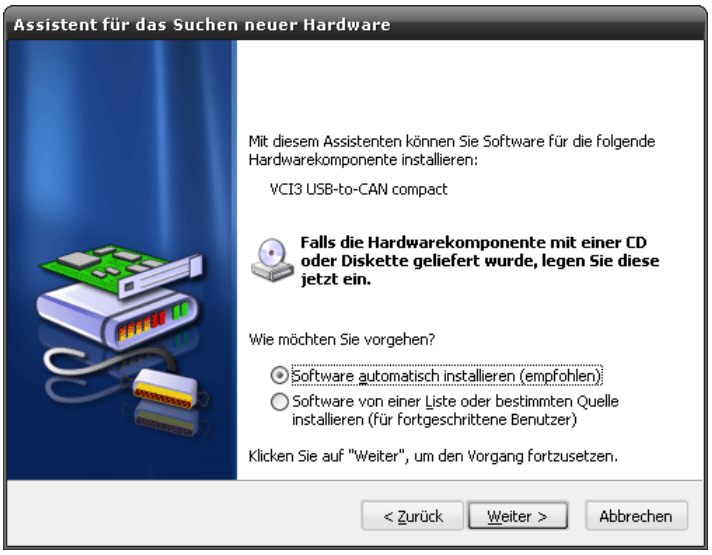


Figure 4 - New USB-to-CAN Compact Detected

(4) Windows detects a driver for the new device and the following dialog appears

Figure 5 - Driver Found

Complete the installation by clicking "Finish”.

IMPORTANT

The user must be logged into Windows as the administrator in order to carry out the installation of new hardware.

You can start the neMESYS UserInterface after the driver has been successfully installed. Please observe the points in section 4 - Setup and configuration in order to correctly configure the dosing unit.

NOTICE

Risk of data loss due to switching off in an uncontrolled manner!
First *exit the neMESYS UserInterface software* ***before*** *switching off the unit!*
This is the only way that all settings will be correctly saved and that configuration data will not be lost.

3.3 Software update

The neMESYS user interface is subject to continuous development. Threrefore new features are added and known bugs are fixed. For this reason it is always recommended to use the latest software version which can be downloaded from our website. Please visit our homepage from time to time to check if there is a new software release available.

To install the latest version just launch the setup program which is located on the attached software CD or which can be downloaded from our homepage. It is not necessary to remove an installed version because it will be replaced automatically with the one. After a successful installation the program can be started as usual.

4 Setup and configuration

4.1 Overview

The neMESYS UserInterface software enables you to conveniently control all unit parameters, the uncomplicated programming of differing flow profiles for each individual syringe pump, and the graphic visualisation of the unit condition for each individual axis.

Sleep mode must be deactivated on the laptop when operating the software, as the system entering sleep mode can cause the hardware device driver to malfunction.

NOTICE

Operation of pumps:

Risk of malfunctions / data loss caused by standby / sleep mode.

Deactivate standby / sleep mode on your PC or laptop to prevent the hardware driver from malfunctioning.

4.2 Initial Commissioning (Quick-start)

This chapter contains the quick-start instructions for commissioning of your new neMESYS modules. Further information are given in chapter 4.3 and 4.4.

NOTICE

Initial commissioning:

*Only **one** non-configured dosing unit with factory default settings (not displayed when physically connected) can be connected to the Base module for initial adding process.*

(1) Connect the neMESYS Base module with **one** plugged dosing module with the PC

(2) Start neMESYS UserInterface software and follow the instructions if requested (Important: the plugged dosing module won't be displayed yet!)

(3) Select *„Setup → Add Dosing Unit"* to add the already plugged dosing module to the platform

NOTICE

Use of further pump modules:

*Only add **one** additional module to your system and configure this before you add more modules.*

(4) Adding of further dosing modules

- Select *„Scan Devices"*
  (already configured dosing modules will be displayed)
- Select *„Setup" → „Add Dosing Unit"*

(5) Removing of dosing module(s)

- Select the corresponding module from the device list or main window
- Afterwards select *„Setup" → „Remove Dosing Unit"* and follow the instructions

4.3 Adding Dosing Units

Certain steps must be taken if you would like to add additional dosing units to the dosing system. All dosing units are supplied to you with factory settings. This means that all dosing units have the same address. For this reason, it is not possible to immediately connect and use all dosing units. Please observe the following steps to add new dosing units to the dosing platform.

NOTICE

Risk of incorrect configuration and damage to the dosing units!

*Only add **one** additional module (single module or double module) to your system and configure this before you add more modules.*

(1) In the *"Setup"* menu, please select the *"Add Dosing Unit"* menu item in order to configure your connected dosing unit and for the software to recognise this. A dialog will appear, follow the instructions in the dialog step by step:

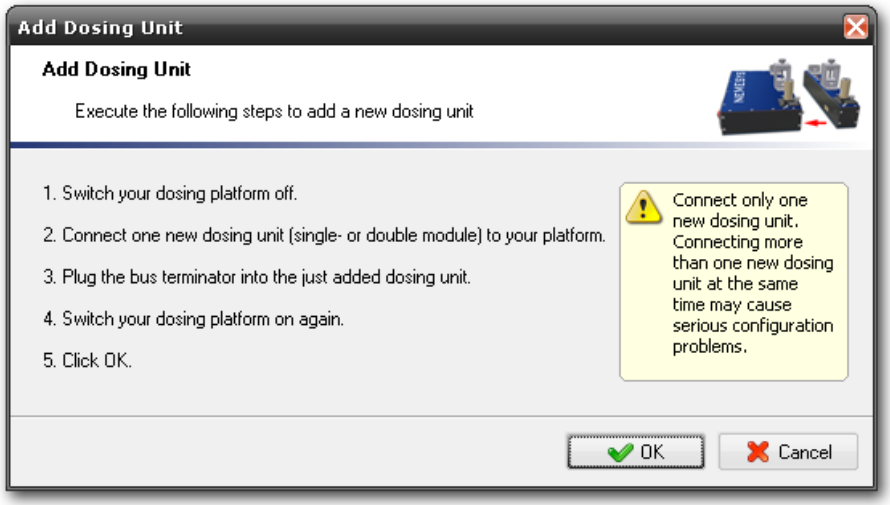


Figure 6 - Add Dosing Unit Dialog

(2) Switch your dosing platform off.

NOTICE

Risk of data loss due to switching off in an uncontrolled manner.

Do not switch off the dosing platform whilst the neMESYS UserInterface software is in use, except in an emergency or if the software clearly stipulates that this should happen. This is the only way that all settings will be

correctly saved and that configuration data will not be lost.

(3) Now connect the next module to your dosing platform as described in the hardware manual and don't forget to attach the terminating plug to the last dosing unit.

(4) Switch the dosing platform on again and click *OK*.

NOTICE

*Only **one** non-configured dosing unit with factory default settings (not displayed when physically connected) can be connected to the Base module for initial adding process.*

The software will now configure and add the units independently. To begin with a search is made to detect dosing units that have already been configured and connected. When the search is carried out for the first time and several Starter or Base Modules are connected with the PC, a hardware selection dialog will appear in order for you to select the unit with which the PC should connect. Select an entry from the list and click *OK*: If the list is empty, then no Starter or Base Module is connected to your PC. No hardware selection dialog will appear if only one Starter or Base module is connected to the PC. If this is the case, the software will connect with the module automatically.

The search will now start.

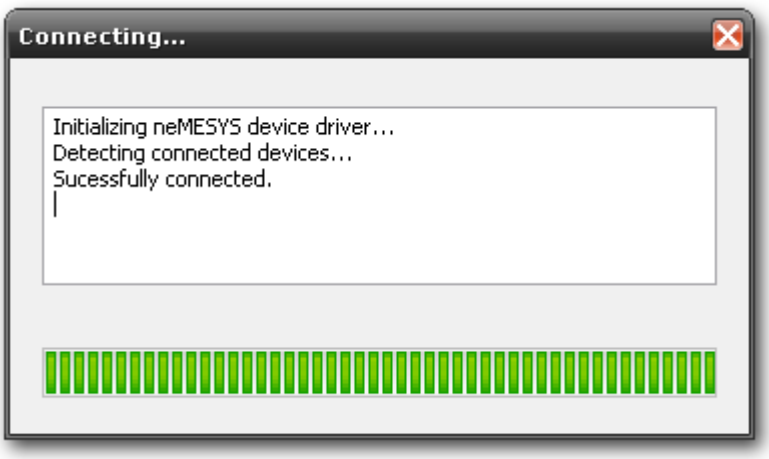


Figure 7 - Search Dosing Units

When the search is completed, user panels will be superimposed for all detected dosing units. The software will then search for newly connected dosing units with factory settings:

Figure 8 - Search for Dosing Unit Using Factory Settings

When the software has recognised the connected dosing unit, this will be automatically configured and will receive a unique unit address in the dosing platform.

On completion of the configuration, the software will conduct a further search in order to identify the properly configured dosing units. The newly configured dosing unit should now appear with the previously configured units.

When the newly installed dosing unit has been identified, the software will inform you that a calibration of the dosing unit is necessary.

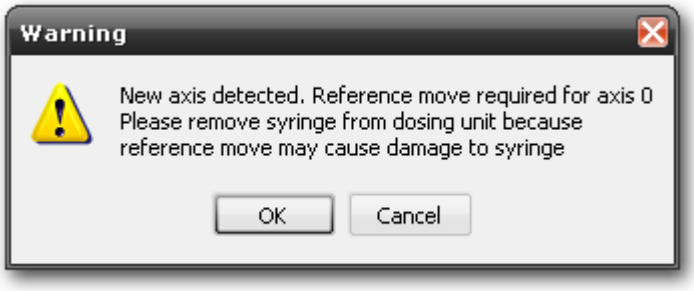


Figure 9 - Reference Move For New Dosing Units

Please carry out this calibration by clicking *OK* to confirm the dialog. During calibration, all drives are moved to their lower end position and zero position.

NOTICE

Risk of damaging syringes!

The system may only be calibrated if there is no syringe installed on the dosing unit.

On completion of the calibration, the connected dosing unit is operational and can be used by you. If you have purchased further dosing units, please connect the next dosing unit with the dosing platform (previously configured units must not be removed); to do so and for each further unit repeat steps 1 - 4. Repeat this process until all dosing units are connected to the dosing platform and have been configured by the software.

IMPORTANT

The order in which the dosing units are connected and configured is also the order that they will be displayed in the software; for this reason do not change the order of the dosing units after configuration.

4.4 Detecting Dosing Units Automatically

A search must be carried out each time the neMESYS UserInterface software is started. This is so that all the dosing units that make up your dosing platform are detected. Click on the *Scan* button in the toolbar, or click the *"Search Devices" menu item in the Setup menu.* At the same time, connection to the unit will be initialised.

When the search is carried out for the first time a hardware selection dialog will appear so that you can select the unit with which the PC should connect. (e.g. in case you use multiple starter modules or base modules at the same time on one PC). Select an entry from the list and click *OK*: If this list is empty, then no Starter or Base Module is connected to your PC.

The software will now run a search for any attached dosing units and identify them (Figure 10).

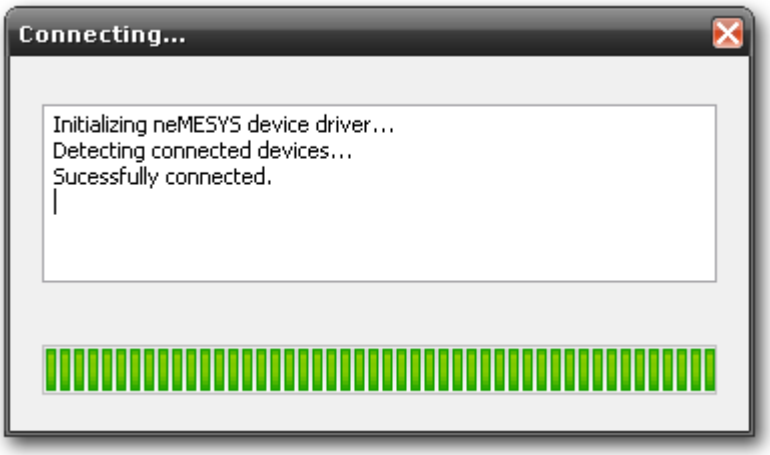


Figure 10 - Status Window Initialisation and Search for Dosing Units

A user panel for all correctly configured dosing units will be superimposed and the dosing unit appears in the list of dosing units (section 6.3).

4.5 Removing Dosing Units

NOTICE

Risk of damage when removing the plugged-together dosing units through tilting!

When you remove the dosing unit, ensure that the units are separated from each other in as parallel a manner as possible.

Please follow the following steps in order to remove dosing units from your dosing platform:

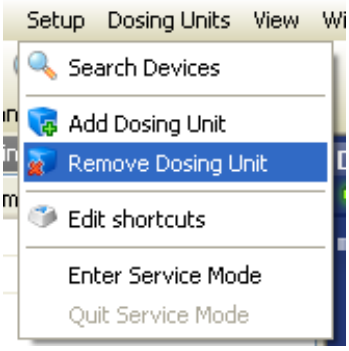


Figure 11 - Remove Dosing Unit Menu Item

(1) In the *Setup* menu, select the *Remove Dosing Units* (Figure 11) menu item, to remove a dosing unit from the dosing platform. A dialog will appear - follow the instructions step by step:

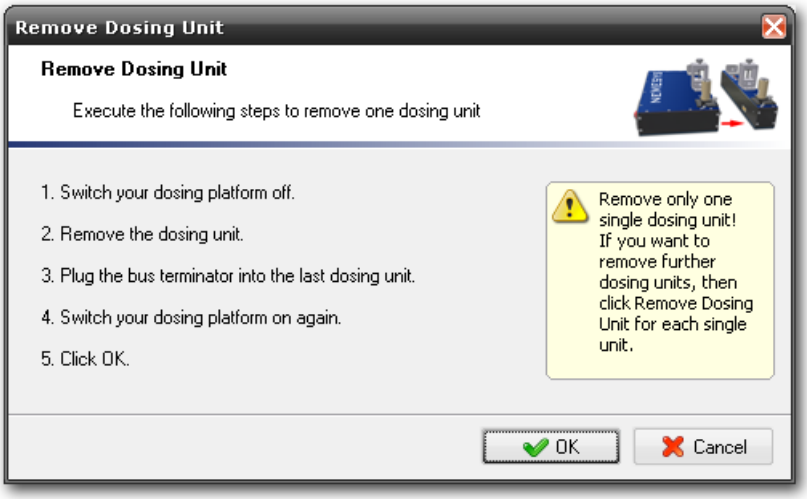


Figure 12 - Remove Dosing Unit Dialog

(2) Switch your dosing platform off

(3) Remove all dosing units that are no longer required or those you do not wish to use at present.

(4) Connect the bus terminating plug into the socket of the last dosing unit of your dosing platform.

(5) Switch the dosing platform on again.

(6) Close the Remove dosing units dialog by clicking *"OK"*. A new search will be run in order to detect the new configuration and number of dosing units in the dosing platform.

NOTICE

Risk of damage with an incorrect configuration!

*Follow the steps above for **each** dosing unit which you remove. Never remove a dosing unit from your dosing platform without following this procedure.*

NOTICE

Risk of damage with an incorrect configuration!

*Only remove **one** dosing unit at a time with the above procedure, repeating the entire procedure for each further module that you would like to remove.*

IMPORTANT

After removing a dosing unit, place the bus terminating plug into the last connected dosing unit again.

5 Operating the software

5.1 Entering numeric values

5.1.1 Structure of the entry fields

The software's "Value entry fields" are available to input values e.g. volumes and flow rates; these are controlled by the simple input and amendment of numeric values. These fields have the following structure:

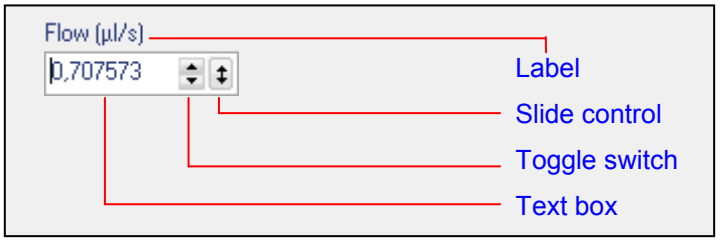
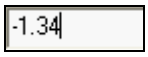


Figure 13 - Value Entry Field Structure

The following 4 possibilities are available for the input and amendment of values:

5.1.2 Entry of values



Direct input in the text box

To begin the input, click the left mouse key in the entry field and enter the value. Valid characters are all numbers from 0 – 9, the decimal separator (dot) and the minus character. The input is completed by pressing the return key or by clicking outside the entry field. In many cases values are entered (e.g. flow rates) that are dependant on the technical conditions (actuators, syringes). Upon completion of the input, the program calculates the values that are technically possible. Instead of the value you have entered, the display then contains the value calculated by the program. This value will often be slightly different to the value entered by you (e.g. input of a flow rate of 10 µl/s will be amended by the application to 10.0014 µl/s).

Using the "toggle switch" to make an adjustment

Two buttons can be found directly to the right of the text box; these enable an extremely precise and fine adjustment of the values. By clicking a button, values can be increased or decreased by the smallest technically possible increment. If the button is clicked and the mouse key held; the application will begin to run through the values after a short pause. This enables an increase of several steps without having to repeatedly press the buttons.

Using the "slide control" to make an adjustment

A button is located next to the "toggle switch" that enables the adjustment of values in a similar manner to that of a slide control. To change the values, use the left mouse key to click on the button, hold the mouse key down and move the mouse upwards or downwards.

Copy and paste

You can also transfer some of the software values by copying and pasting between the individual value entry fields. Using the right mouse key, click in a text box; a menu will appear that will enable you to select the desired action (copy, paste…).

5.1.3 Multiplication keys

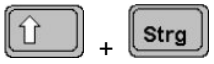
When using the "toggle switch" or slide control to adjust values, adjusted values will be increased by pressing certain keys. The following multiplication keys are available to you:

Control key

If this button is pressed when the slide control / toggle switch is used, the value amendment is larger by a factor of 10. This means that a click on the "toggle switch" will result in a value amendment 10 x larger than normal. This enables a slightly quicker value amendment.

Shift key

If this button is pressed, the value amendment is larger by a factor of 100. This enables a quicker value amendment over a larger range.



Shift key + control key

If both these buttons are pressed together, the value amendment is larger by a factor of 1,000. This enables a fast run-through of the value range.

5.1.4 Value Range

Values that are entered by you, e.g. flow rates, already have a certain value range that is limited by the technical conditions e.g. maximum flow speed, syringe volume. Even when inputting values, the entry fields monitor the values to ensure that you do not erroneously leave this value range. When the toggle switch or slide control is used to enter values, the value adjustment ceases when a value range limit has been reached. If you enter values using the keyboard, your input values will be checked upon completion. If the input value falls outside the value range, the program will automatically correct the value to the maximum or minimum possible value. You will be informed of this correction by a warning message (Figure 14).

Figure 14 - Example of a Warning

5.1.5 Critical Values

When data entered into the input fields lies within a range that could be classified as critical, e.g. very low flow rate, the user is alerted to this by a red colouring of the text (Figure 15). Red coloured data in the input fields are valid values, but also fall within a range where the fulfilment of certain requirements, parameters or limit values, e.g. a continuous flow, cannot be guaranteed.

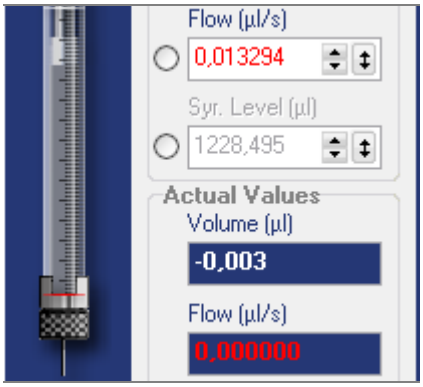


Figure 15 - Indication of Critical Values (red)

6 UserInterface Reference

6.1 Overview

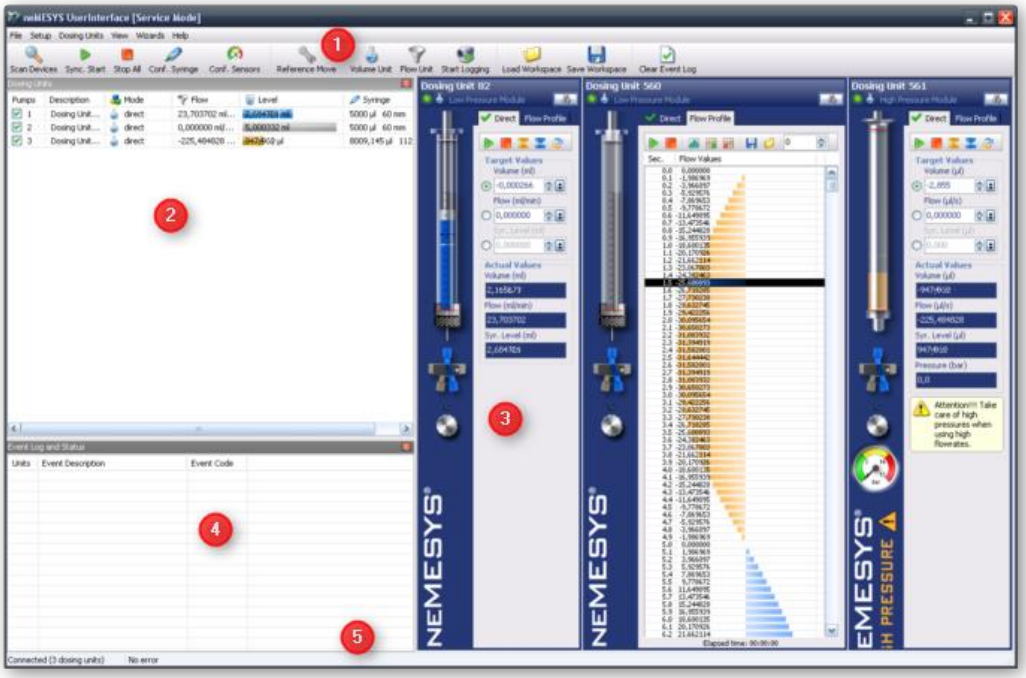


Figure 16 - Operating Software Overview

The application for controlling the dosing units gives you a clearly arranged interface for selecting, configuring and controlling the dosing units. The user interface consists of the following components:

- (1) Menus and toolbar
- (2) List of dosing units
- (3) Control panel for each dosing unit
- (4) List of device errors which have occurred (error history).
- (5) Status line

6.2 Menus and toolbar

6.2.1 Overview

The menu bar and toolbar with the main menu of the application are located at the top of the application window. Here you will find services and actions that you can use in various parts of the application.

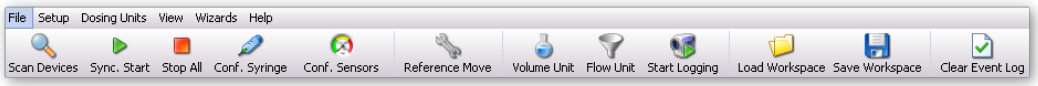


Figure 17 - Toolbar

6.2.2 Menus

The following menu items are available in the main menu of the application:

6.2.2.1 Setup

This menu contains services for the configuration and setup of the dosing platform and individual dosing units.

Search Devices	All the dosing units that are currently connected and active are detected.
Add Dosing Unit	Adds a dosing unit to the dosing platform and configures it.
Remove Dosing Units	Click this menu item to delete dosing units from your dosing platform
Restore Factory Settings	Resets the configuration settings of a dosing unit back to the default values.
Edit shortcuts	Set keyboard shortcuts for activating individual software functions.

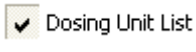
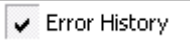
6.2.2.2 Dosing Units

In this menu, you can change various configuration settings for the selected dosing unit:

Configure Syringe	Configuration of syringe parameters
Reference Move	Calibration of the drive of the dosing unit
Select Volume Unit (nl, µl, ml…)	Configuration of the unit for volume values
Select Flow Unit (nl/s, µl/s…)	Configuration of the unit for flow rates
✔ Inverted orientation	Enable/disable inverted GUI orientation
Set Operational	Switch on the drive unit's power supply
Disable Drive Voltage	Switch off the drive unit's power supply

6.2.2.3 View

You can use this menu to configure the graphical interface, e.g. to display or to hide windows.

	Shows and hides the list of active dosing units
	Shows and hides the error list

6.2.2.4 Help

You can access the software's online help from this menu.

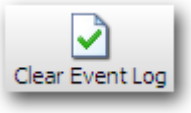
6.2.3 Toolbar

The toolbar contains the following buttons:

	Initialize the connection to the dosing platform and search for dosing units
	Start several dosing units synchronously
	Stop all dosing units
	Configure syringe parameters for the selected dosing unit
	Configure sensors for the selected dosing unit
	Calibrate the drive of the selected dosing unit
	Configure SI unit for displaying volume values
	Configure the SI unit for displaying flow rates
	Start logging the flow rates

Load the workspace

Save the workspace



Clear event log

6.3 List of dosing units

The list of dosing units displays all the connected dosing units in a clear list format. You can place the list freely within the application, i.e. by clicking the mouse in the title bar and then dragging the window with the mouse button held down, you can "dock" the list to the left, right or lower side of the application or release it as a separate window.

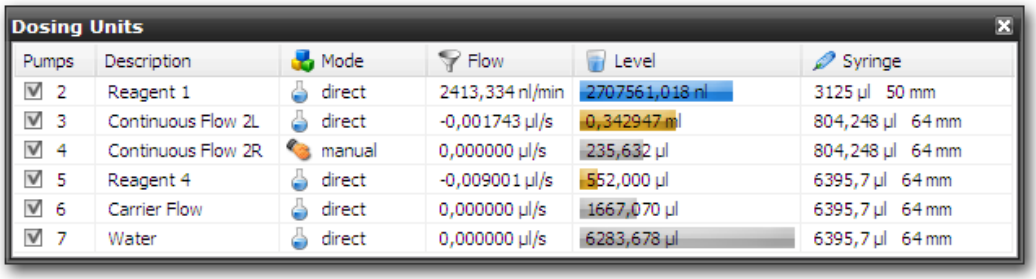


Figure 18 - List of Dosing Units

By selecting the "*Dosing Unit List*" menu item in the "*View*" menu, you can also completely hide the list of dosing units. This would be meaningful, for example, if you were working with only a few dosing units (1 - 4).

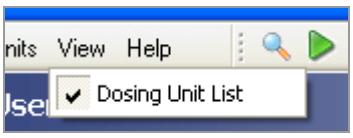


Figure 19 - Show and Hide the List of Dosing Units

You can select a dosing unit by clicking the list with the mouse. The name of the selected dosing unit will be highlighted in green in the operating panel.

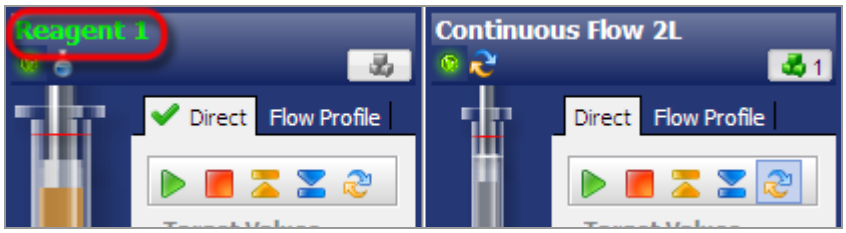


Figure 20 - Dosing Unit Selected

If you double-click a dosing unit in the list of dosing units, the operating panel of the dosing unit is scrolled into the visible area. This is very useful when you are working with many dosing units and often have to switch back and forth between the individual dosing units.

The list of dosing units displays the various important parameters of each dosing unit. The following parameters are displayed:

Units

Displays the device ID (1- 32) of the dosing unit in your dosing platform. By clicking on the checkbox before the device-ID, you can show or hide the operating panel of that dosing unit. If, for example, you have several dosing units, but at the moment are only working with 2 dosing units, you can hide all the dosing units not being used from the graphical interface.

Description

Displays the name of the dosing unit that you have assigned to it in the software.

Mode

The current operation mode of the dosing unit is displayed here. A distinction is made between the following operation modes:

- *direct* – Direct control, dosing at a constant flow rate
- *flow profile* – flow profile, dosing at a flow rate that varies over time
- *manual* – Manual control of dosing with the mouse wheel
- *cont.* flow – Generation of a continuous flow with two coupled dosing units

Flow

Displays the current flow rate of the dosing unit.

Level

The current fill level of the syringe is displayed here. By colouring the bar, an indication is provided whether a reagent is currently

- being taken up (orange)
- delivered (blue)
- or whether the dosing unit is currently inactive (grey).

Syringe

The configuration parameters, length and volume, of the syringe used are displayed here.

6.4 Event Log

Operation errors of a dosing unit will be stored in the event log and can be read there by you. An error in the dosing unit is indicated by red flashing of the status LED and one or more entries in the error list. You can also freely place this list in the list of dosing units (see section 6.3) and show or hide in the *View* menu.

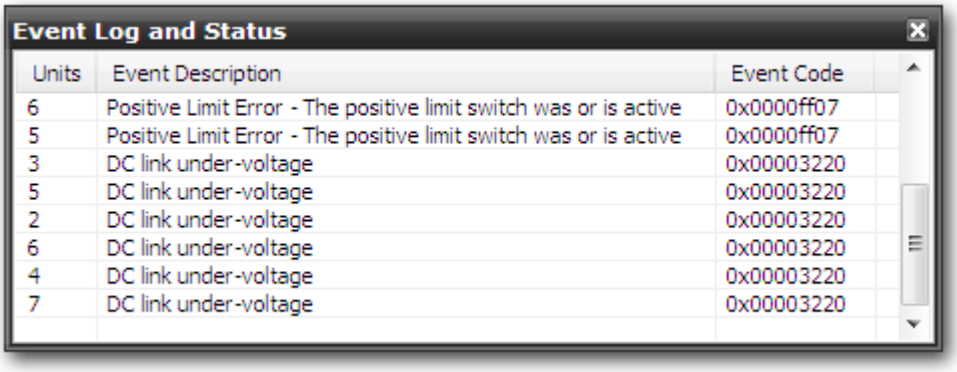


Figure 21 - Window Error History

The errors are chronologically sorted in the list. This means that the most recent error is at the top of the list. There are three columns in the event log:

Units

Indicates the number of the dosing unit that caused the event / error and/or which is in an incorrect condition. If you double click the row in the list the operating panel of the relevant display will be scrolled into the visible screen section. In the case of errors that cannot be attributed to a particular dosing unit or for software faults, this field is not filled out.

Event Description

In this column you will find a short description of the event or error that occurred.

Event Code

This column contains an event / error code which you can use for consultation with the technical hotline if necessary.

You can delete the entire error list by pressing the *Clear Event Log* icon in the toolbar (Figure 22). By deleting the error list the latest error message in the status line of the application is also deleted:

Figure 22 – Deleting the Error List

6.5 Status line

The status line contains information about the connection status of the software to the dosing platform, the number of connected metering units and the latest error. The error shown in the status line is the top entry of the error history (see section 29).

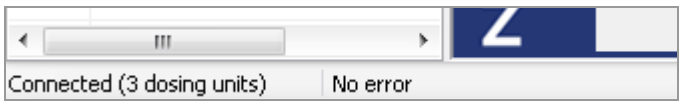


Figure 23 – Status Line

Deleting the error list also clears the last error in the status line.

6.6 Shortcuts

Using shortcut keys on the keyboard, it is possible to run several commands of neMESYS user interfaces. All keyboard shortcuts are available in the menu *"Edit shortcuts"* within the menu *"setup"* and if necessary they can be customized by the user.

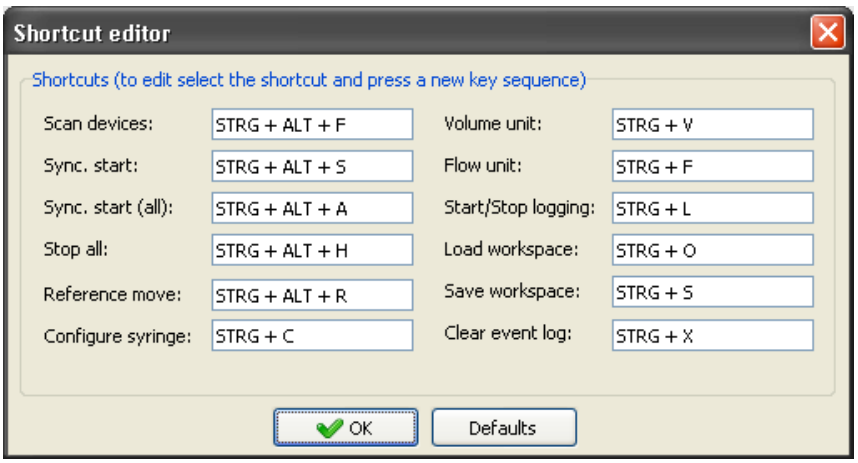


Figure 24 – Edit shortcuts menu

At the first start of the software, all available keyboard shortcuts are set to a default value. To customize a keyboard shortcut, click in the box next to the desired command and

press a new key sequence. For example, for re-assigning the command *"Scan devices"* to the shortcut CTRL-F, click in the box labeled "Scan devices", then hold down the button Strg and press the button F. The command was now assigned to the new key combination and can always be used in neMESYS user interface.

If at least two shortcuts are identical, then the following warning message will appear:

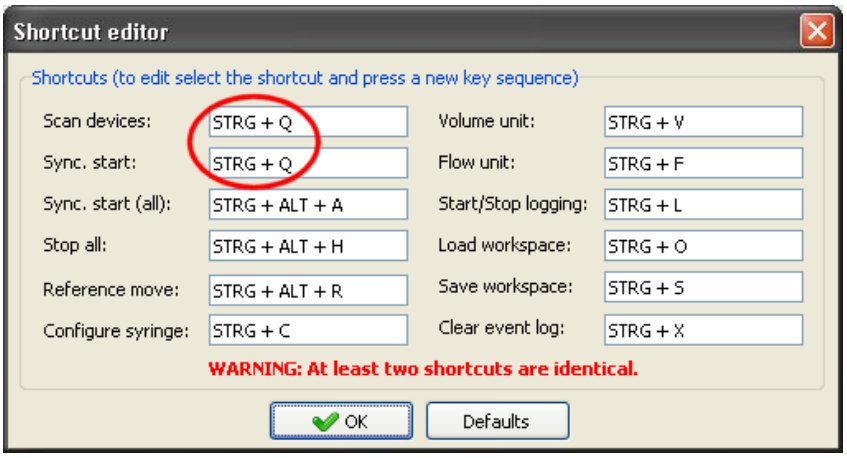
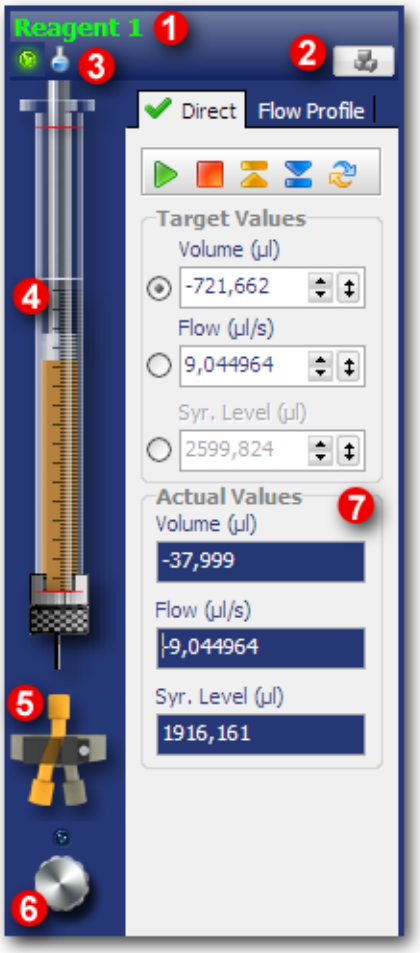


Figure 25 – Warning message

Any changes of the shortcut keys are stored in the Windows user profile. To restore the default hotkeys, click on the button "Default".

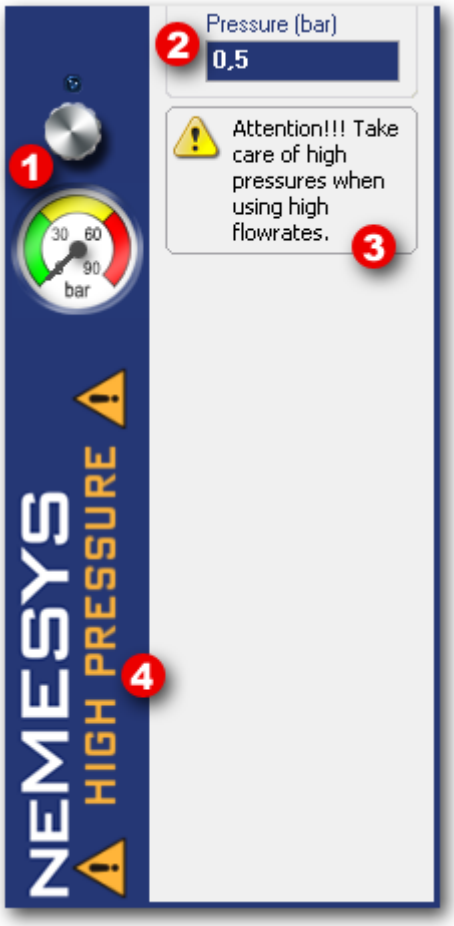
6.7 Dosing Unit Control Panel

6.7.1 Overview



- (1) Name of the dosing unit
- (2) Button for group selection
- (3) Status display (LED) of the drive and display icon for actual operating mode
- (4) Level indicator
- (5) Valve switching and status
- (6) Regulator for changing the manual flow rate with the mouse wheel
- (7) Controls (depending on operation mode)

6.7.2 Operating panel for high pressure dosing unit



If you have connected a high pressure dosing unit, the syringe in the level indicator will look like a stainless steel syringe.

Additional display elements will be displayed in the lower area of the operating panel for this pump.

- (1) Pressure gauge
- (2) Numeric pressure display
- (3) High pressure warning message
- (4) High pressure warning sign

6.7.3 Name of the dosing unit

You can assign an individual name to each dosing unit. You can thus identify, for example, which fluid is dosed with it or for what purpose the dosing unit is used. To assign a new name, please double-click the left mouse button on the name of the dosing unit.

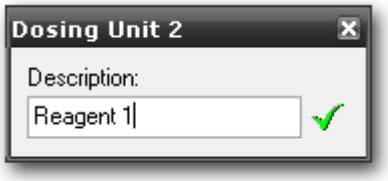


Figure 26 - Change the Dosing Unit Name

You can assign the new name in the input window that opens (Figure 26). You can end the input by pressing the *Enter* key or clicking the green check mark.

IMPORTANT

The name of the dosing unit is not stored in the device, but on the PC. If you use the dosing unit to another PC, you must name it there again.

6.7.4 Status indication of the drive

The status indication provides information about the current status of the drive of a dosing unit. The following operating states are displayed:

- **continuously green** – the drive unit is ready for operation
- **flashing red** – the drive unit is in a fault state

A fault state can occur owing to different events, such as reaching the limit switch, positioning error, hardware defect or faulty communication. As soon as an error occurs, you will be informed about it in a message and the status indicator flashes red. If a drive is in a fault state, no more dosing can be carried out with that drive.

At certain error events, such as reaching the limit switch, it is possible to set the drive back to an operational state. For this purpose, click the right mouse button on the Status LED. In the menu that opens, select the *"Set Operational"* menu item (Figure 27).

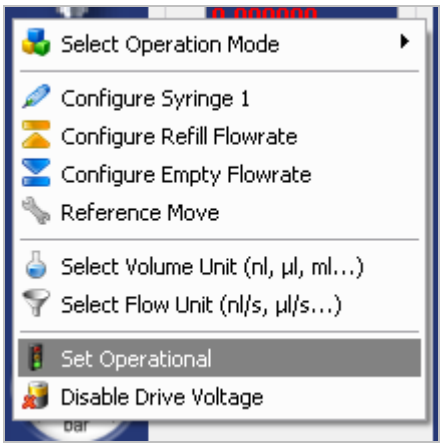


Figure 27 - Resetting the Fault State

If this action is successful, the status indication of the drive unit glows green continuously. If it is not successful, please terminate the application and switch off your dosing platform; then switch it on again after about 10 seconds. Then start your application again and initiate a search for the connected dosing units. Should the drive unit continue to indicate a fault state, please get in touch with cetoni technical support.

IMPORTANT

If a limit switch is reached, you should remove the syringe from the dosing unit and carry out a reference move.

6.7.5 Display of operating mode

This indicator displays the icon corrsponding to the operating mode. The following icons indicate the following operating modes:

	**Direct control** – Dosing of specific volumes, creation of continuous flows.
	**Flow profiles** – Change of flow rate over time using pre-configured value tables (flow profiles).
	**Manual control** – Interactive, manual control of flow rate using mouse wheel and rotary dial in control panel.
	**Continuous flow** – Creation of continuous flows over very long time periods by coupling two dosing units.

6.7.6 Syringe level indicator

6.7.6.1 Overview

The level indicator of the syringe always shows the current position of the drive unit and hence the current filled level of the syringe used. In addition, the current movement of the dosing unit is indicated by colouring the syringe. The following colours are possible:

Grey

The drive unit is currently inactive.

Blue

The piston of the syringe is being moved towards the syringe clamping (reagent delivery).

Orange

The piston of the syringe is being moved away from the syringe clamping (reagent take-up).

This coloured indication of the direction of movement is also used in other parts of the software (e.g. in the list of dosing units – section 6.3).

IMPORTANT

The colouring of the syringes is especially helpful for recognising the movement direction when flow rates are very low, as it is very difficult to recognise a change in the position of the syringe piston.

The thickness of the syringe indicates the approximate size of the syringe that is currently clamped. You should use large flow rates with caution for large syringes, as they can quickly give rise to high pressure which can damage the device (valve) or your application.

NOTICE

Risk of the fluid connection technology being damaged by high pressure. Never perform a reference move with a clamped high pressure syringe or damage may occur due to high pressure.

If you move the cursor above the fill level indicator, a notification window opens to show the parameters of the current syringe (Figure 28). The maximum pressure is displayed only for high pressure syringes.

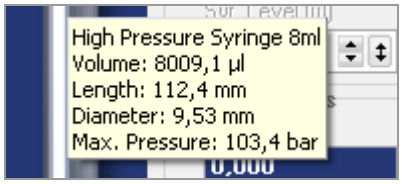


Figure 28 - Display Syringe Parameters

6.7.6.2 Setting the dosed volume

The fill level indication of the syringe enables a rough setting of the volume that is to be delivered. For this purpose, click with the left mouse button in the fill level indication and move the mouse up or down with the button held down to select the desired delivery quantity.

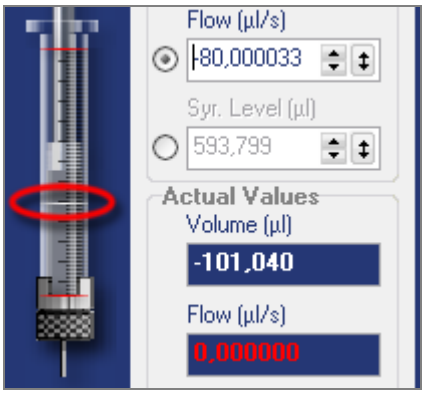


Figure 29 - Setting the Dosed Volume

The volume is then calculated relative to the current position of the syringe piston. If the target marking is below the piston, this means a reagent delivery (positive volume value). If the target marking is above the piston (reagent take-up), a negative volume value is displayed. The target marking can only be set between the two limit values marked in red.

6.7.6.3 Software limits

The software limits the path of a syringe with minimum and maximum values in order to avoid the syringe being destroyed or the piston being pulled out of the syringe body. These values are displayed in the form of two red markings on the fill level indicator (Figure 30), and must be defined in the syringe configuration (section 6.8) for each syringe.

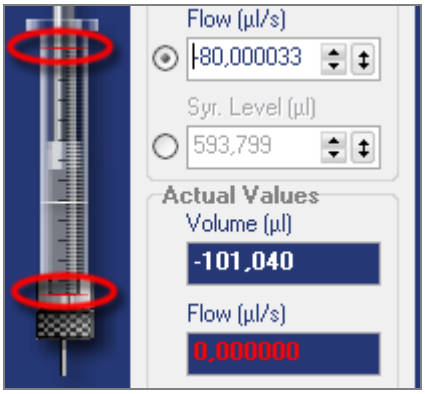
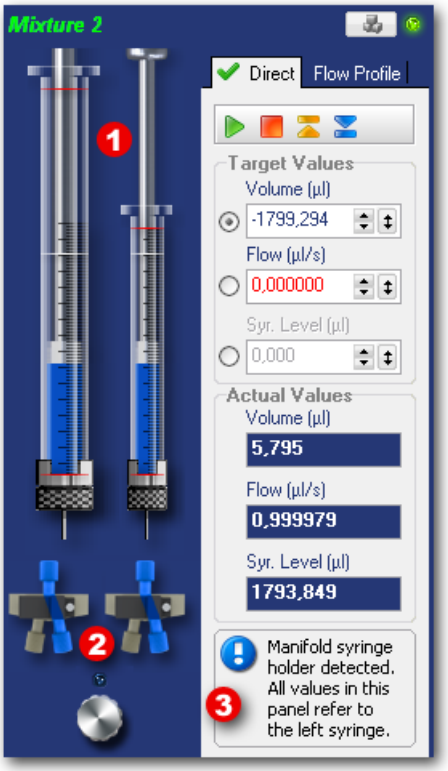


Figure 30 - Software Limits

6.7.6.4 Dual syringe adapter



If you use a dual syringe adapter, the fill level of the dosing unit will display two freely configurable syringes (1). If the module has two valves, the software will display this as well (2).

All values such as flow rates, volumes and fill levels always refer to the left syringe. An additional notification box (3) will inform you of this fact.

The movement range of the dosing unit is limited by the shortest syringe in the syringe adapter (see Figure 31). The software limits (red markings) can be used to limit the movement range further.

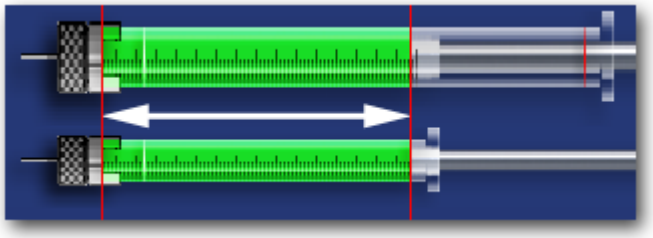


Figure 31 - Movement Range with Dual Syringe Adapter

IMPORTANT

When using a dual syringe adapter, all target values and actual values always refer to the left syringe.

6.7.7 Valve switching

6.7.7.1 Overview

If your dosing unit has a 3-way valve, you can switch the valve in this part of the operating panel or configure automatic valve switching. This section also displays the valve's switch state.

To toggle the valve, simply click with the left mouse button on the valve icon. You should hear a soft click and the representation of the valve should change to the other switch state. The current switch state of the valve is indicated by the following two graphics:

Blue - Valve is switched to reagent delivery

Orange - Valve is switched to reagent take-up

The coloured marking (orange and blue) corresponds to the colour of the syringe fill level indicator. Thus, by means of different colouring (for example, valve orange, syringe blue), it can be easily recognized whether the direction of movement of the syringe piston corresponds to the switching state of the valve and whether it is necessary to toggle the valve.

6.7.7.2 Valve automation

Valve automation makes it possible to automatically switch the valve. In order to configure valve automation, right click on the representation of the valve.

Figure 32 - Configuring Valve Automation

You can activate or deactivate individual points by clicking on them in the menu (Figure 32).

The following configurations are available:

- **Automatic Valve Switching** – activates automatic valve switching. The valve will be switched as soon as the dosing unit changes direction. If the dosing unit begins to dispense, the valve will be switched to the outlet and if the dosing unit begins to suck, the valve will be switched automatically to the inlet.

- **Inverted Valve Automatic** - this setting causes the valve to automatically switch in the opposite direction to the dosing unit. This means that when fluid is discharged, the valve switches to the inlet. When fluid is absorbed, the valve will switch to the outlet. This can be useful if you are taking up fluid very slowly and then dispensing it very quickly. The valve can be switched inversely to avoid the valve heating up when fluid is being absorbed. This setting has no effect on the manual switching of the valve.

- **Automatic Valve Turn Off** - this setting causes the valve to power off automatically after fluid is absorbed. This prevents the valve from heating up.

6.7.8 Manual flow rate control

The operating panel of every dosing unit has a regulator for manual regulation of the flow rate. To regulate the flow rate manually, click the left mouse button on the regulator. The green LED starts to glow, showing that manual regulation is active:

Manual control active

Manual control inactive

You can now control the flow rate of the corresponding dosing unit by turning the mouse wheel. By pressing the mouse wheel or the left mouse button (which deactivates manual control, if it is active), the flow rate is immediately set to 0 and the dosing unit drive is stopped.

The change in the flow rate that is produced by one click of the mouse wheel can be configured in the application. You can open the configuration window by placing the cursor over the regulator, right-clicking and selecting the *Configure slider increments* menu item (Figure 33).

Figure 33 - Calling up Configuration of Manual Flow Control

Move the mouse wheel to put the change to the flow rate in the entry field (Figure 34). One click of the mouse wheel corresponds to the value in the *flow rate* field.

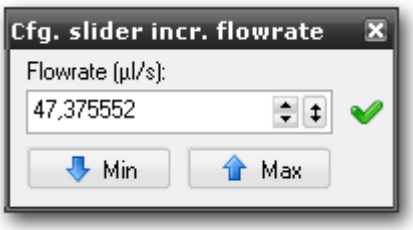


Figure 34 - Configuration of Manual Regulator

6.7.9 Pressure indicator (high pressure pump)

6.7.9.1 Overview

If the software detects a high pressure injection pump, the operating panel of this pump also displays a pressure indicator in the form of a manometer.

Figure 35 - Pressure Indicator (Manometer) For High Pressure Pump

The value range of this pressure indicator depends on the maximum permitted pressure of the high pressure pump. The maximum permitted pressure is limited by the maximum power of the drive unit, maximum permitted pressure of the syringe used and the pressure limit configured by the user. Limited by these three values, the lowest value is the maximum permitted pressure.

The limit value for the maximum pressure is the limit between the yellow and red range on the scale in the graphical depiction (Figure 35). If you move the cursor above the pressure indicator, the limit value is displayed in a small notification window (Figure 36).

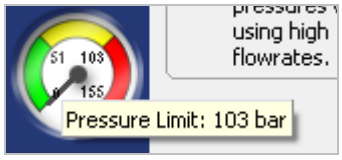


Figure 36 - Display Pressure Limit Value

6.7.9.2 Configure pressure limit and over-pressure action

If you right click the pressure indicator, you can configure the pressure limit value and over-pressure action in the menu displayed.

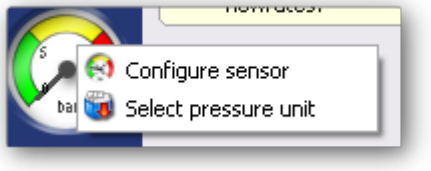


Figure 37 - Configure Pressure Limit

If you select the *Configure sensor* menu option, a configuration dialogue is displayed.

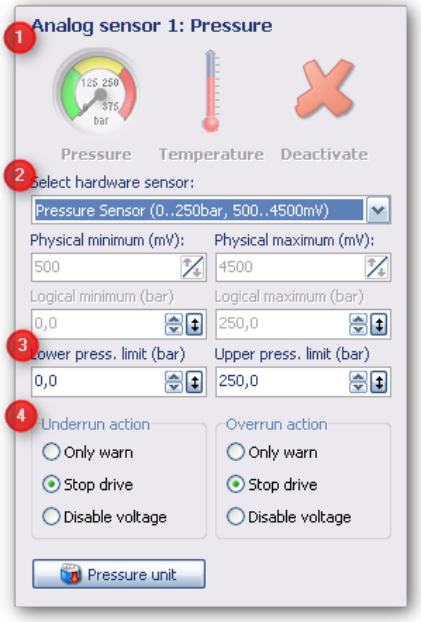


Figure 38 - Configuration Dialogue for Pressure Limit / Over-pressure Action

In this configuration dialogue, you can configure the under- and over-pressure action (*Underrun Action, Overrun action*) (4) and the pressure limit (*Pressure Limit*) (3). Additionally you can also configure the connected pressure sensor with the combo box (2). The selection of the sensor type is disabled if a high pressure module is used.

The limit values for the pressure set the minimum and maximum permitted pressure for the selected high pressure pump. All pressure values smaller or larger than these limit values are considered as under- and over-pressure. If the limit value is exceeded, the action configured as the under- or over-pressure action is executed.

The following actions can be configured to achieve the pressure limit value:

- **Only warn** – Only a warning message is displayed by the software on the status window. This does not affect the dosing process,

- **Stop high pressure drive** – The drive unit is stopped but retains its current position, i.e. the pressure is not released when a syringe is relieved. As long as there is over-pressure in the system, the software can only move the injection piston out of the syringe. In this status, it is no longer possible to dose towards the syringe any more, as it would lead to higher pressure.

- **Disable voltage** – The drive's power supply is disconnected. This means that the drive does not exercise any more power on the syringe, the system can relax and the pressure is removed.

Since up to max. 200 ms can elapse between the time an over-pressure is detected and over-pressure action is released, it is advisable to plan an appropriate reserve while configuring the pressure limit. This delay may result in pressures that are higher than the limit value set; especially for dosing processes with quick movements by the drive unit.

IMPORTANT

Configure the pressure limit with an appropriate reserve to avoid pressures that are too high even for quick dosing processes.

6.7.10 Control elements (depending on operation mode)

In this area, depending on the operation mode or according to the parameters that are to be configured, the corresponding control elements are displayed. You can switch between the individual operating panels by selecting the corresponding tab (Figure 39):

Figure 39 - Selection of the operation mode-dependent Control Elements

At the moment, there are operating panels available for direct control *(Direct)* and carrying out flow profiles *(Flow Profile)*. Switching between the individual operating elements does not toggle the operation mode of the dosing unit. I.e. while, for example, you directly dose a constant flow rate with one dosing unit, you can simultaneously generate a flow profile in the operating panel of this dosing unit and edit it, without the dosing process being influenced thereby. Only when you start the flow profile or switch the operation mode to *Flow Profile* will the current dosing process end.

The current operation mode is displayed at all times in the list of dosing units (section 6.3).

6.7.11 Inverted orientation

By selecting the entry *inverted orientation* in the menu *Dosing Units*, any dosing units are displayed in reverted order with inverted syringes and valves. This option can be useful for a better optical relation between the dosing panels and the devices. By selecting this option again, all dosing modules will be displayed in the default view.

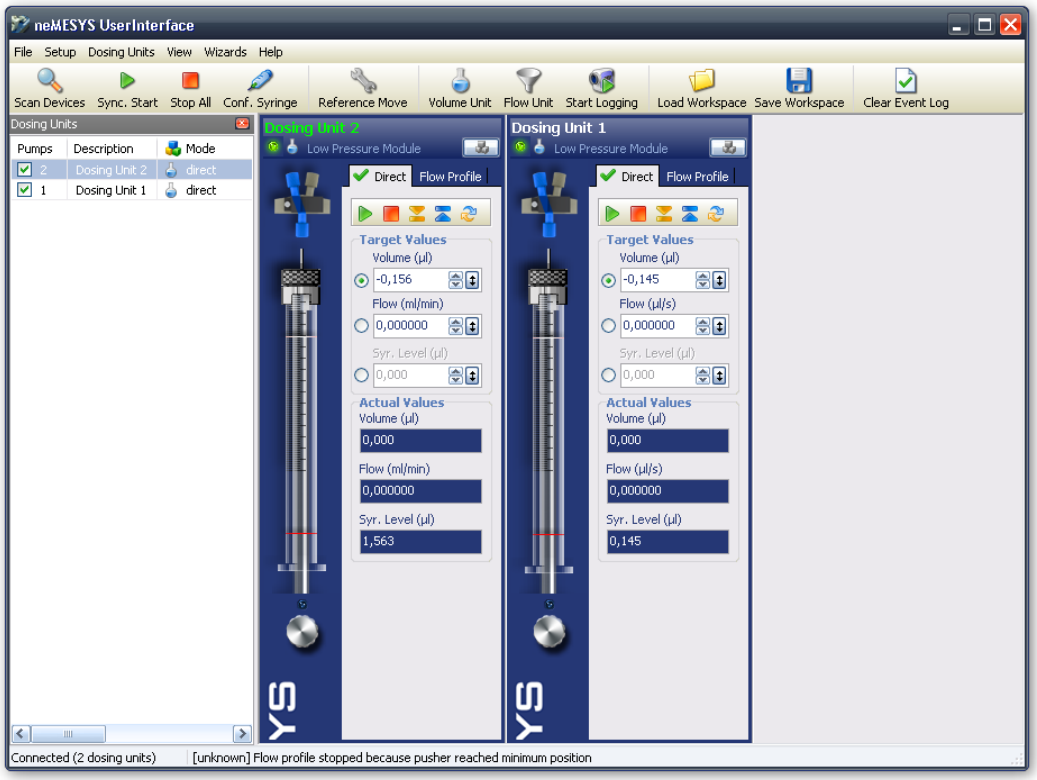


Figure 40 - Inverted orientation activated

6.8 Configuring Syringes

6.8.1 Introduction

The software allows you to configure and manage your own syringes quickly and easily. Using the software to configure and use specific syringes fulfils two tasks:

1. All flow rates and volumes in the software are calculated on the basis of the configured syringes.

2. The configured syringe lengths and the set limits restrict the piston's movement.

6.8.2 Calling up configuration

The operating software always shows the current flow rate for each of up to 32 dosing units. To be able to calculate the flow rate, the program requires the characteristics of the syringe used in each case. You must properly configure these values whenever you change the syringe.

To do so, right click in the depiction of the syringe on the axis that you wish to configure. In the menu that is displayed, select the *Configure Syringe* menu item. Then choose which syringe you would like to configure, as shown in Figure 41.

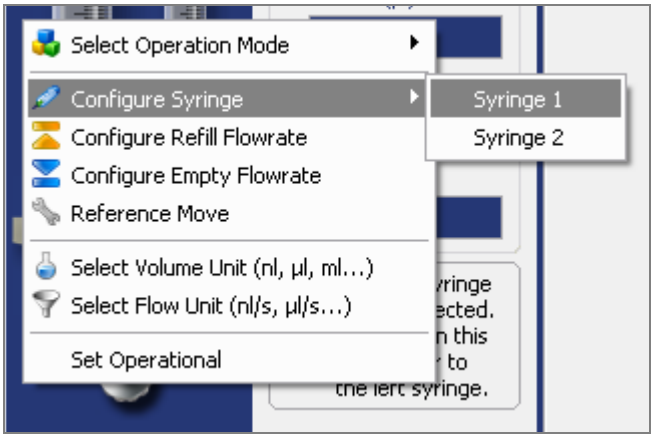


Figure 41 - Start Syringe Configuration

Alternatively, you can select a dosing unit in the list of dosing units and click on the *Conf. Syringe* button in the menu bar of the application. Only the first syringe in a multiple syringe adapter can be configured in this way.

IMPORTANT

When changing the syringe ensure that any existing flow profile is deleted and that the pull-out speed and emptying speed of this dosing unit are reset to very low, safe values.

6.8.3 List of available syringes

When you choose the syringe, a dialog box is opened (Figure 42), in which you can carry out the following steps to select the syringe:

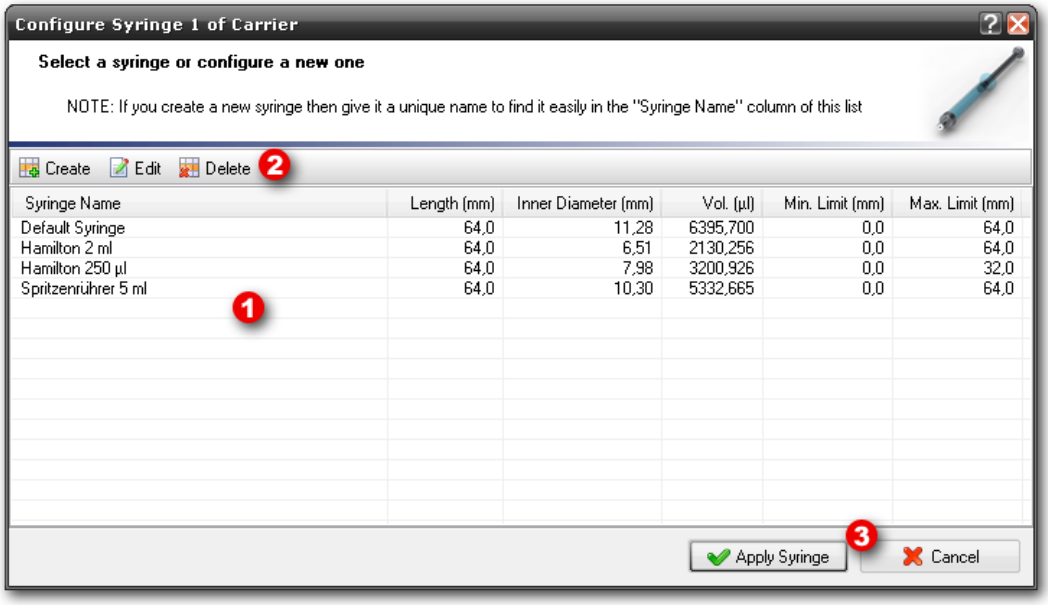


Figure 42 - Dialog for Syringe Configuration

(1) The list view displays all the syringes that you have created in the past. If the list contains a suitable syringe, it can be selected and the dialog box closed by clicking "*Apply Syringe* ". Alternatively, you can select a syringe by double clicking it.

(2) If there is no suitable syringe in the list, a new syringe can be created by clicking the *Create* button. Clicking the *Edit* button opens a window where you can edit the syringe parameters for the marked syringes. By clicking the *Delete* button, you can delete the selected syringe from the list. Deleting is also easily possible by pressing the *Del* button.

(3) The selection can be ended at any time by clicking *Apply Syringe*, or cancelled by clicking *Cancel*.

IMPORTANT

The list of available syringes contains pre-configured high pressure syringes. You can not delete these syringes.

6.8.4 Configure syringe parameters

If you click on the *Create* or *Edit* buttons, the wizard will open to allow you to configure the syringe parameters (*Syringe Configuration Wizard*). Follow the following steps to configure the syringes:

IMPORTANT

You cannot configure your own high pressure syringes but rather only use the existing high pressure syringes.

(1) First, enter a unique name for the syringe in the *Name* (Figure 43) field. When you have selected a syringe for editing and only change its name you can create a copy of the selected syringe. Click *Next* to move to the next step.

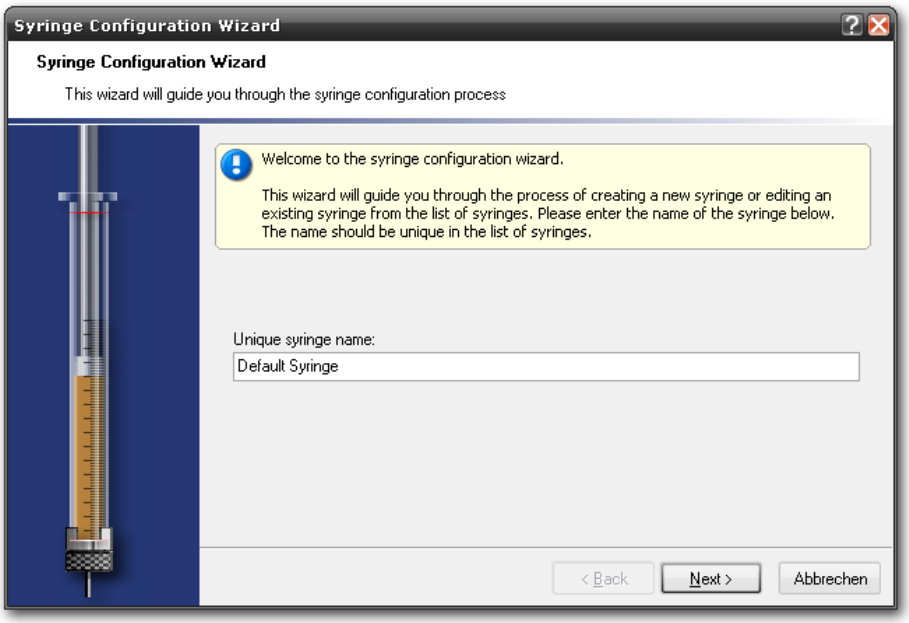


Figure 43 – Syringe Configuration Step 1 – Enter Name

(2) You should now configure the internal diameter of the syringe. The internal diameter of the syringe is required to calculate flow rates and dosage amounts. If you do not have the internal diameter you can define it as an alternative via the scale length in mm (*Scale Length*) and the volume it contains in µl (*Scale Volume*). The software calculates the internal syringe diameter from this. Select this step if you want to configure the syringe internal diameter (Figure 44). Depending on the selection continue the configuration at Point 3a or 3b. Click *Next* to move to the next step.

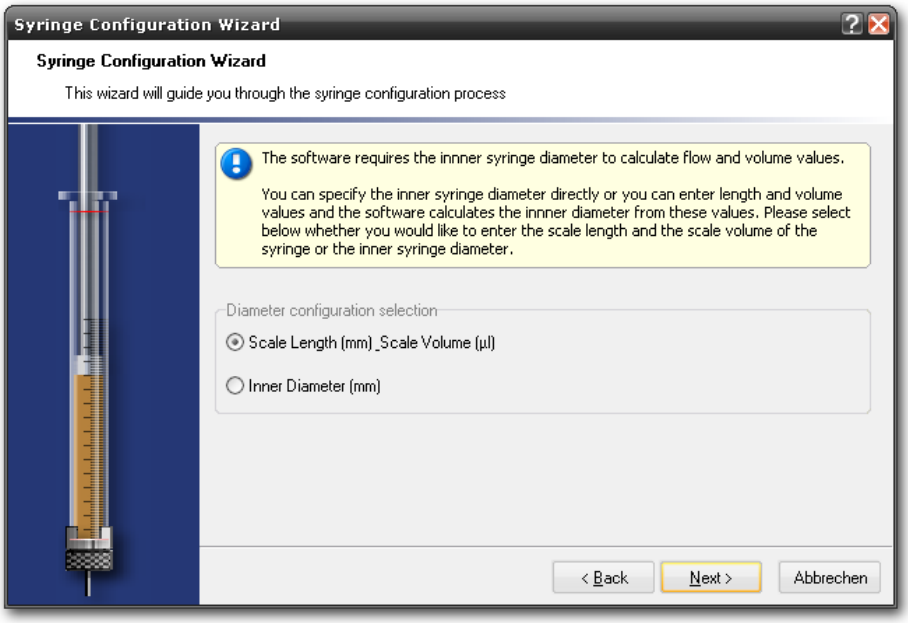


Figure 44 – Syringe Configuration Step 2 – Select Method

(3a) Configure the internal diameter of the syringe by stating the scale length and volume within this scale length (Figure 45). To do so measure the scale with an appropriate measuring tool (slide rule) and take the volume from the scale label. Then click *Next* to move to the next step and continue reading at Point 4.

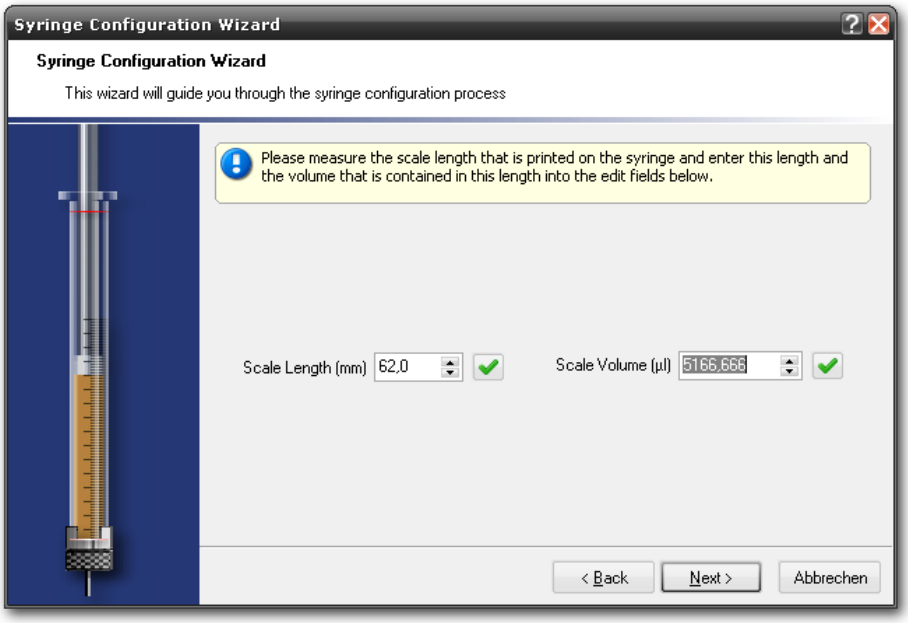


Figure 45 - Syringe configuration Step 3a - Enter Volume and Length

(3b) Configure the internal diameter of the syringe by calculating it with a slide rule. Enter the value in the *Inner Syringe Diameter* field and then click *Next* to move to the next step.

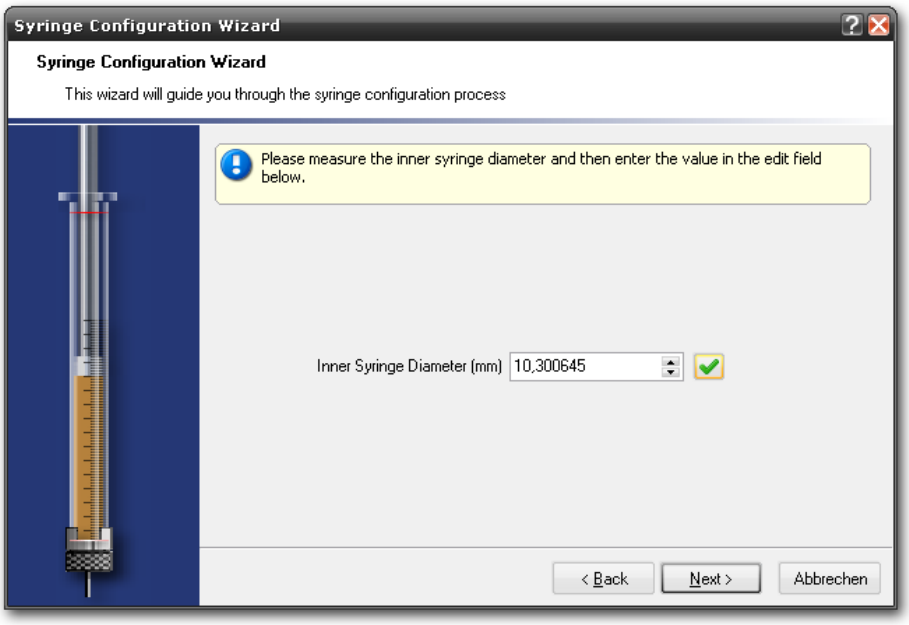


Figure 46 - Syringe Configuration Step 3b - Internal Diameter of the Syringe

(4) Now configure the syringe's piston stroke (*Piston Stroke*) (Figure 47).. The piston stroke is the maximum length which the piston can be moved into the syringe without it leaking and fluid coming out. We recommend that you use the length of the scale on the syringe as the maximum piston stroke.

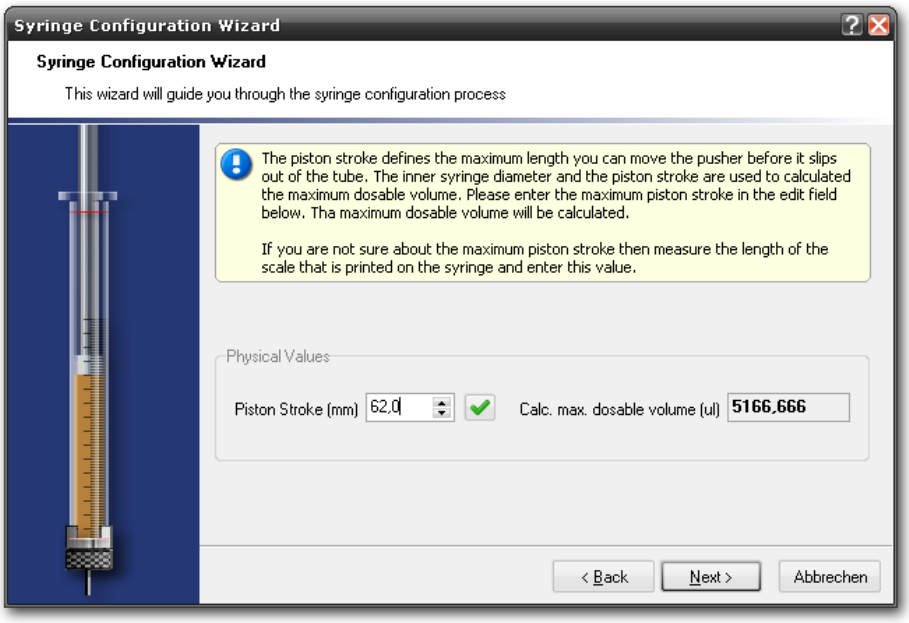


Figure 47 – Syringe Configuration Step 4 – Piston Stroke

If you are using multiple syringes of different lengths in a multiple syringe holder, the syringe with the smallest piston stroke will limit the drive's movement range. When you have stated the piston stroke, the software uses it and the internal diameter to calculate the maximum dose volume (*Volume µl – calculated*).

(5) Finally, set the limits or the maximum and minimum syringe fill level (Figure 48). This allows you to use two additional software limits to set the limits for the piston's movement range. You can enter these parameters in either microlitres or millimetres. If you are using a syringe stirrer, you should use these values to limit the movement range. If you enter the value in a unit (e.g. millimetres) the software calculates the value of the other unit (e.g. microlitres).

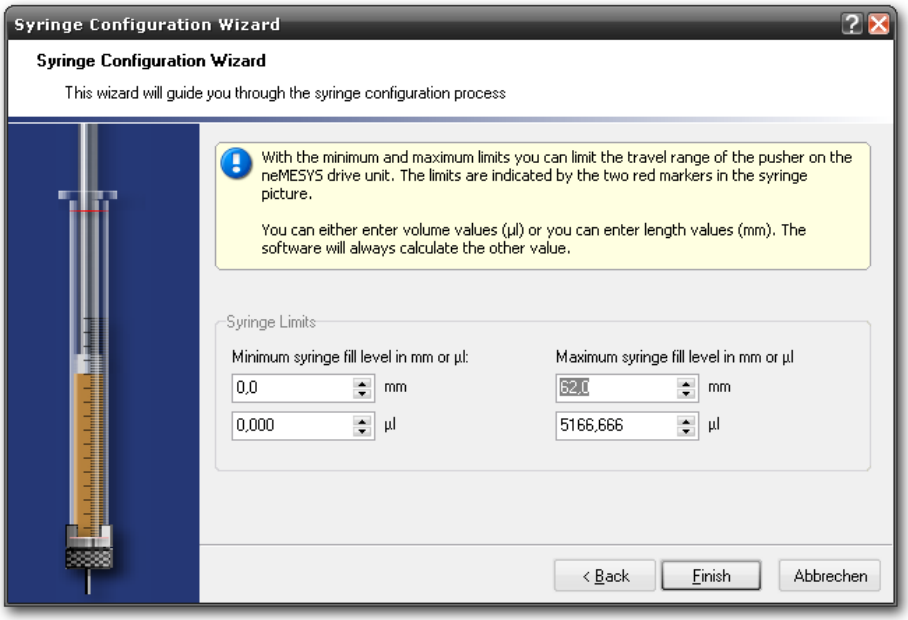


Figure 48 – Syringe Configuration Step 5 – Limits

(5) The syringe preview on the left shows how the syringe is currently configured. The width of the syringe changes in line with the internal diameter. The length of the syringe changes in line with the maximum piston stroke and the two red markings on the syringe indicate the two additional limits. The software's representation of the syringe will be based on the preview.

(6) Close the syringe parameter configuration by clicking the *Finish* button. You can cancel the configuration at any time by pressing the *Cancel* button. You can use the *Back* button at any time to return to the previous configuration step.

6.9 Configure Sensors

For each dosing unit you can configure up to two independent sensors, excepted the high pressure module with only one configurable sensor because one sensor is always used as a pressure sensor. There are two different types of sensors: pressure- and temperature sensors.

IMPORTANT

Make sure to use dosing modules with sensor hardware and to have the sensors configured correctly to ensure that the software operates with the correct values.

To configure a sensor just click on the icon for sensor configuration in the toolbar. Afterwards a dialog with all sensor settings will appear.

Figure 49 – Sensor configuration icon

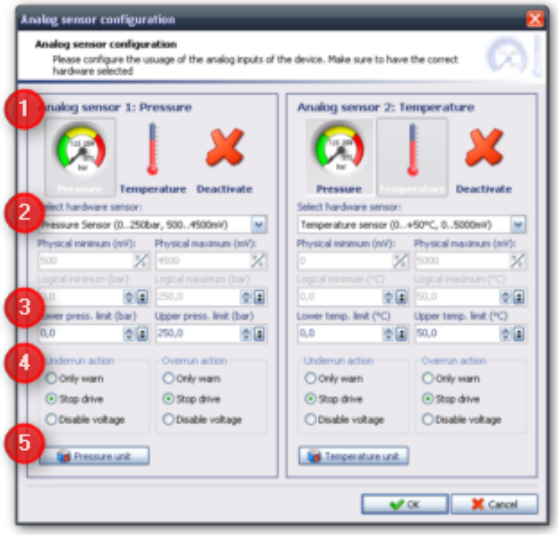


Figure 50 – Sensor settings dialog

To configure a sensor correctly please select a sensor type first. You can coose between pressure and temperature sensors (1). To delete a sensor via the software please click on the red cross icon. In the next step the sensor parameters have to be set. Please select the correct sensor from the list (2). If it is not available then select the entry *custom* and enter the correct sensor parameters (3). To limit the value range of the sensor you can enter an upper and a lower limit (4). If these values are below or above the limit

values then the selected action will be executed. For each sensor one of multiple units can be selected (5). To confirm the sensor settings please click on OK.

Figure 51 – graphical controls for displaying pressure and temperature

6.10 Configuring SI Units

In the case of dosing fluid flows, the flow rates used and the fluid quantities to be dosed or already dosed are the important parameters. For optimum customization to the respective application, the user can individually configure the SI units to display flow rates and volumes for every individual dosing unit. To configure the units, please click the right mouse button anywhere in the free area of the operating panel of the corresponding dosing unit to open the following menu (Figure 52):

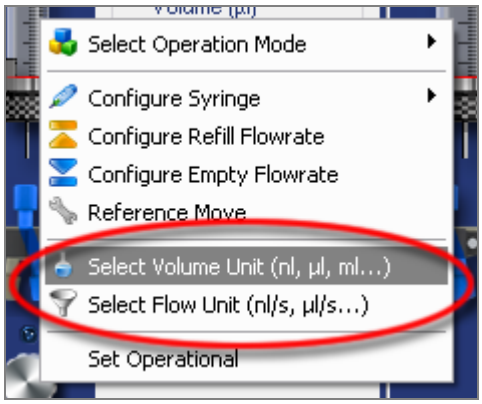
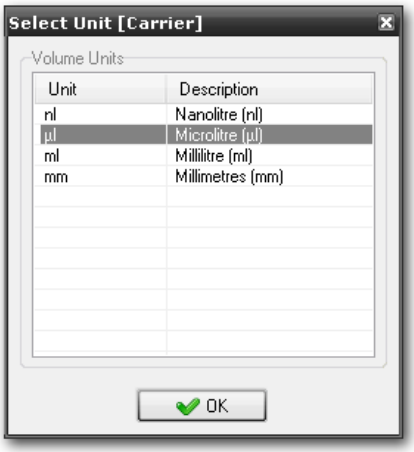


Figure 52 - Selection of SI Unit Configuration

In the menu, select whether you wish to configure the unit for the volume values (*"Select Volume Unit"*) or for flow rates (*"Select Flow Unit"*) of this dosing unit.

6.10.1.1 Configuration of the volume unit



You can select whether volumes for the corresponding dosing unit should be displayed in

- nanolitres (nl),
- microlitres (µl),
- millilitres (ml)
- or millimetres (mm)

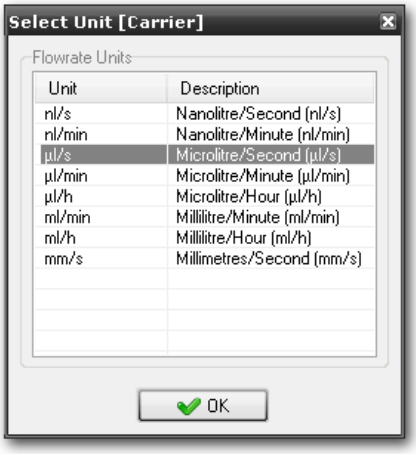
.

6.10.1.2 Configuration of the unit for flow rates

Flow rates can be displayed in

- nanolitres per second (nl/s),
- microlitres per second (µl/s),
- microlitres per minute (µl/min),
- microlitres per hour (µl/h),
- millilitres per minute (ml/min),
- millilitres per hour (ml/h),
- or millimetres per second (mm/s)

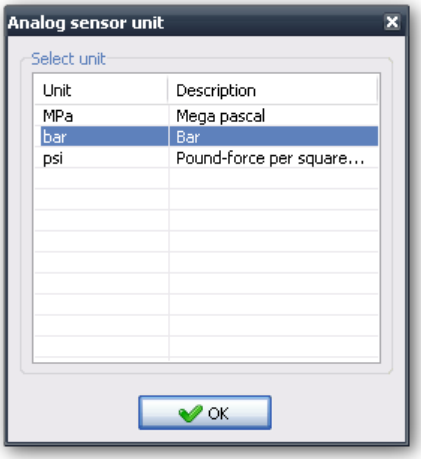
.



6.10.1.3 Configuration of the unit for pressures

Pressures can be displayed in

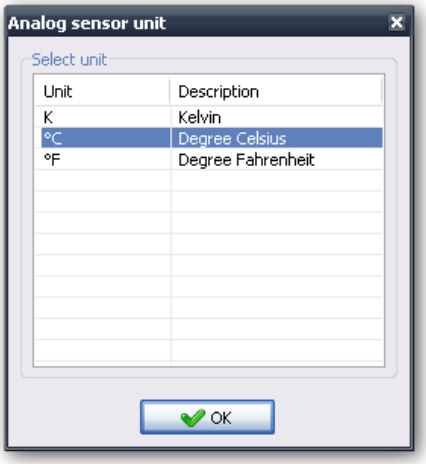
- Mega pascal (MPa),
- Bar (bar),
- PSI (psi),



6.10.1.4 Configuration of the unit for temperatures

Temperatures can be displayed in

- Kelvin (K)
- Degree Celsius (°C)
- Degree Fahrenheit (°F)



6.11 Carrying out a Reference Move

The drives of the dosing unit are monitored and controlled by a digital positioning unit. To calibrate this positioning unit, you can carry out a reference move of the dosing unit. A suitable time for this is, for example, when the syringe is being changed, since the syringe has to be taken out of the dosing unit for the reference move. During the reference move, the dosing unit moves to its lower end position and upon reaching the end position, calibrates its zero position.

IMPORTANT

You should always carry out a reference move if you operate the pumps on a different PC, as the calibration data is saved on the PC, not the dosing units.

To start a reference move, choose the unit which you would like to calibrate from the list of dosing units. Next, right click on the list. This will bring up a pop-up menu. Now choose the *Reference Move* item.

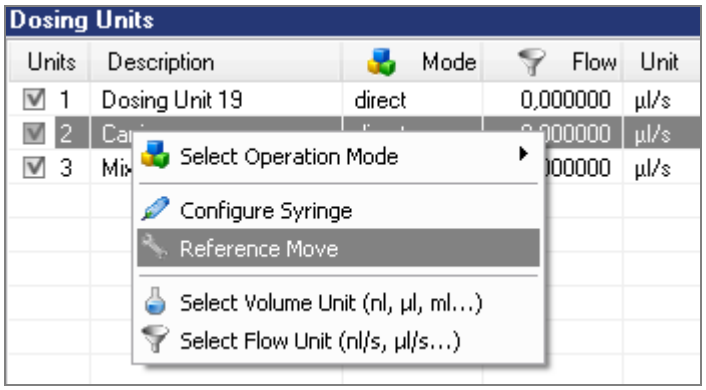


Figure 53 - Start Reference Move

Alternatively, you can also click the right mouse button in the operating panel of the corresponding dosing unit and select the *Reference Move* menu item.

NOTICE

Risk of damaging syringes!

The system may only be calibrated if there is no syringe installed on the dosing unit.

NOTICE

Risk of the fluid connection technology being damaged by high pressure.

Never perform a reference move with a clamped high pressure syringe or damage may occur due to high pressure.

6.12 Selecting operation mode

You can run the dosing units in different operation modes. One operation mode is *direct control*, in which constant flow rates or specific volumes are delivered. In the *Flow Profile* operation mode, the flow rate is altered in a fixed time pattern of 100 ms or a multiple of 100 ms. The basis for alterations to the flow rate is a previously defined flow profile which the software cycles through. The operation mode is activated when you click the button for the relevant operation mode on the dosing unit's operating panel (Figure 54).

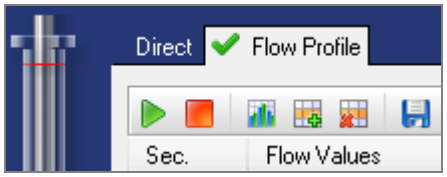


Figure 54 - Display operation mode in the Operating Panel

The operation mode of the dosing unit is displayed in the list of dosing units in the Mode column (Figure 55). You can also see which operation mode is active by looking for a green tick in the individual operating panel (Figure 54).

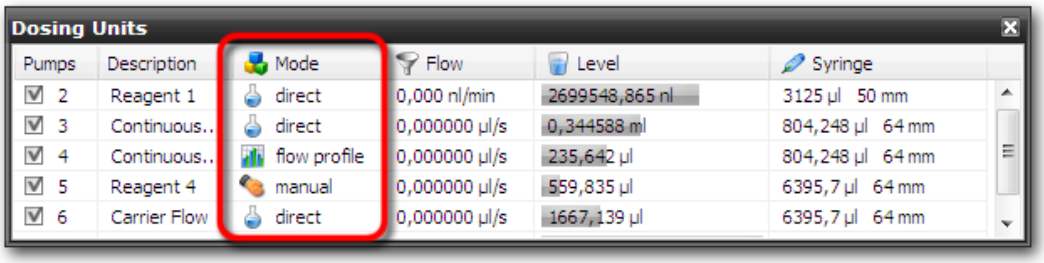


Figure 55 - Display operation mode

However, if you wish to start several dosing units synchronously, even before pressing the Start button, you must set the correct operation mode for all dosing units. For this purpose, right click in the list of dosing units on the unit whose operation mode you wish to set and in the menu, choose the *Select operation mode* item to set the operation mode.

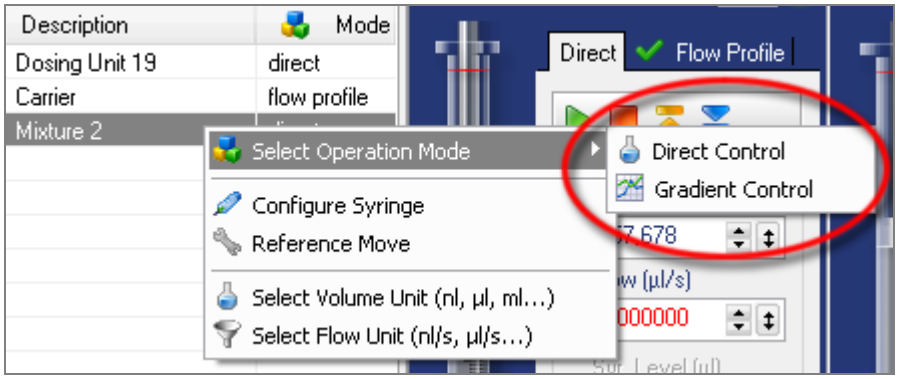


Figure 56 - Select operation mode

6.13 Grouping dosing units

6.13.1 Overview

Dosing units can be arranged in groups. Each dosing unit can be included in one of 8 groups. Certain actions, which are released together, can be specified for each group of dosing units. This means, if a certain action is released for a dosing unit (e.g. *start dosage* or *take reagent up*), then this action will be performed at the same time for all dosing units in this group. That makes it possible to control axes synchronously. So you can control e.g. multiple dosing units manually by turning the regulator at the same time.

6.13.2 Specify groups

The button for group selection is located next to the status indication of the dosing unit:

Figure 57 – Button for Group Selection

The current grouping of the dosing unit is indicated by the status of the button:

Button not pushed and grey

The corresponding dosing unit is in no group at the moment

Button pushed and green

The dosing unit is part of a group. The number of the group is displayed in the button.

If you left click the button a menu will appear and you can decide on the group for the chosen dosing unit:

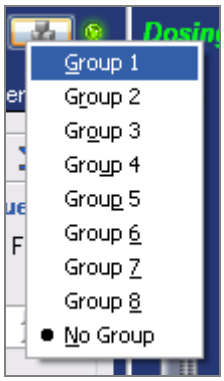


Figure 58 – Menu for Selecting the Group

When the dosing unit is part of a group, group actions (section 6.13.3) will be performed for all dosing units in one group at the same time.

6.13.3 Define group actions

For each of the eight groups individual group actions can be stipulated, which are performed for all dosing units at the same time. To open the configuration dialog for the group actions, click with the right mouse button on the icon and select *Configure Group Actions* in the menu (Figure 59).

Figure 59 – Menu for Opening the Group Actions

After selecting the item in the menu a dialog pops up where you can define group actions for the selected group.

Figure 60 – Configure Group Actions

In the configuration dialog you can select the actions, which are performed for all dosing units of the group at the same time. The following actions can be chosen:

Manual control	Allows the joint control of all dosing units of the group with the mouse wheel.
Start dosing	Starting a dosing process starts all dosing units in the group simultaneously. (This has no consequences for the gradient operations)
Stop dosing	When pressing the Stop button all dosing units will be stopped simultaneously.
Refill syringe	Select this mode to fill several dosing units simultaneously.
Empty syringe	This mode initiates the collective complete emptying of several dosing units.
Switch valve	This mode makes the common switching of the valves possible.

6.13.4 Example application

To control e.g. two dosing units manually with the mouse wheel and to move both dosing units in opposite directions, proceed as follows:

1. Please select the same group for both dosing units. There should be no other dosing units in this group (section 6.13.2).

2. Please configure the *Manual control* action for both dosing units.

3. Please define a positive value for the adjusting control of one dosing unit and a negative value for the other dosing unit.

4. Turning the adjusting control causes a manual change of the speed of the gear units in oppositional directions.

6.14 Logging the Flow Rates

6.14.1 Configuring Logging

If you press the *Start Logging* button in the toolbar, you can activate and configure the logging of flow rates and sensor values:

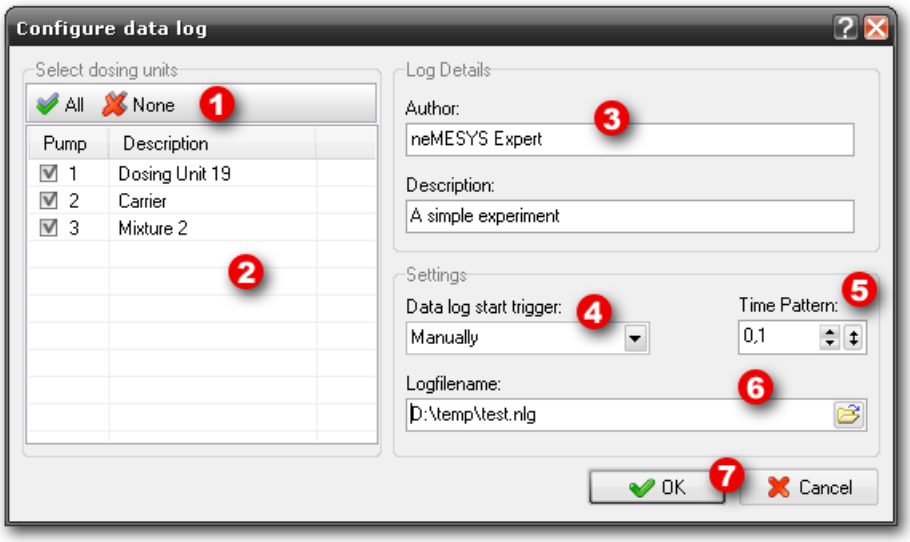


Figure 61 - Configuration Dialog for Flow Rate Logging

In this dialog box (Figure 61), you can configure for which dosing units the flow rate and the sensor values is to be logged, which event starts the logging and at which frequency values should be logged.

All the available dosing units are shown in the list (2). Here you can select the dosing unit from which flow rates and sensor values are to be logged. You can use the two buttons above the list to select either all or none of the dosing units in the list (1). You can enter additional information for this log into "Log Details" (3). With the checkbox (4) you can determine how logging the flow rates is started. The following options exist for starting the logging:

- **Manually** – The flow rates start to be logged immediately after pressing the *OK* button. This is meaningful when you have already started your dosing units and you subsequently wish to start logging the flow rates.

- **Single Start** – This is where logging the flow rates starts, as soon as the dosing process is started afresh in at least one dosing unit, i.e. when the start button in the flow profile operating panel, in the direct control operating panel or in the general toolbar is pressed.

- **Synchronous Start** – The logging is only started here if several dosing units are to be started synchronously, i.e. only upon pressing the start button in the general toolbar.

In the "*Time Pattern"* entry field (5) select the time pattern for the logging. The time pattern is a multiple of 100 ms. You can enter the complete filename of the log file into the *Logfilename* (6) entry field ". Alternatively you can click on the yellow folder icon, to choose a path and a file name for your log file. End the configuration process by pressing the *OK* button (7) or cancel the input by pressing *Cancel*.

IMPORTANT

Select as small a value for the time pattern as necessary and as large as possible, to keep the quantity of logged values and amount of data as small as possible.

IMPORTANT

For each of the selected dosing units the values of the configured sensors (i.e. pressure, temperature) are getting logged besides the flow rate. Note that the current unit of the sensors is being used. To change the unit please change the current unit of the sensor.

You can recognise that logging the flow rates is configured from the fact that the button in the toolbar is shown as if it were pressed. As soon as logging starts, the button is displayed with a red Stop symbol. The following states of the button are possible:

Logging of flow rates not active

Logging of flow rates configured – wait for trigger event

Logging of flow rates active

When the logging of the flow rates is active, you can stop the logging by pressing the button and save the logged values to a file. The logged values are then taken from the temporary log file stored in the final log file (Figure 62).

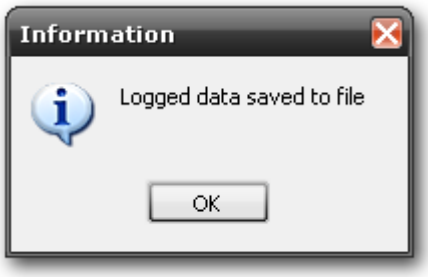


Figure 62 - Store Logged Flow Rates

6.14.2 Format of the log

The values of the individual dosing units are logged in a list in which the individual columns are delimited from one another with tabs (Figure 63).

Time(s)	Water µl/s	Reagent1 µl/s	Reagent2 µl/s
00000.0	00000.000000000	00000.000000000	00000.200054870
00000.1	00000.000000000	00000.000000000	00000.200054870
00000.2	00000.000000000	00000.000000000	00000.200054870
00000.3	00000.068696845	00000.007462277	00000.200054870
00000.4	00000.135637860	00000.014924554	00000.200054870

Figure 63 - Example: Logging Gradient Values

The log contains a column with the time in seconds and columns with the flow rates and sensor values for every dosing unit from which values were logged. The operation mode of the dosing unit has no role to play here - it is immaterial whether the dosing unit was active, manually controlled or running in gradient operation. The first line of the log contains the description of the respective dosing unit in the column headings and the unit in which the flow rate for that dosing unit was logged.

6.14.3 Recovering log data in case of an error

The values of the dosing units are constantly stored with active logging into a temporary file on your hard disk. If the software has been shut down incorrectly by an error, e.g. a software fault, a power breakdown or similar, then it is possible to produce a valid log file from the temporarily recorded data after restarting the software. Thus valuable results are not lost even in such an exceptional case.

To save the temporary data in a valid log file please select the *Save logged data* item from the *File* menu:

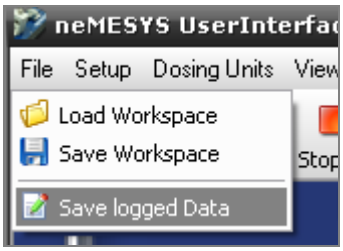
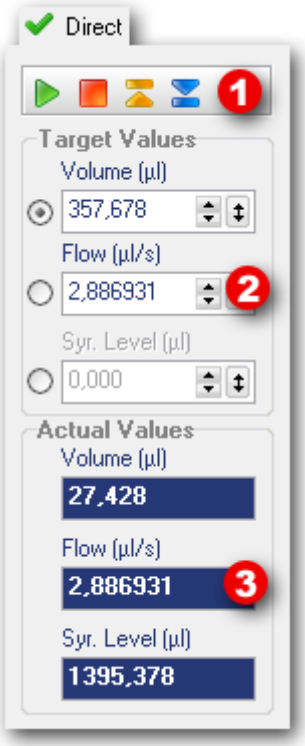


Figure 64 - Save Logged Data

Please select the directory and the file name from the directory overview that appears, and confirm the saving by clicking *OK*.

7 Direct Control

7.1 Overview



Use direct control to control the dosing units interactively. You can use it to dose precise amounts of liquids at defined flow rates or produce constant fluid flows.

Moreover, the current values for the flow rate, dosed volume and fill level of the syringe are displayed in the direct control. If you use a high pressure module, the pressure sensor will also display the pressure here.

The following operating and display elements are available:

- (1) Toolbar
- (2) Target values of the dosing unit
- (3) Actual values of the dosing unit

7.2 Toolbar

The toolbar contains buttons for starting the dosing procedures. The following buttons are available:


Start the dosing process with the target values that have been set


Stop the dosing process


Take up reagent – dosing unit is moved up to the maximum position


Empty syringe – dosing unit is moved up to the minimum position


Configure continuous flow

NOTICE

Danger of damage to the syringe while emptying!

When emptying the syringe, the dosing unit is moved at high speed towards the lower end position according to how it was configured.

7.3 Target values

7.3.1 Selecting the type of dosing

You can set the type of dosing with the selection boxes on the left side (Figure 65).

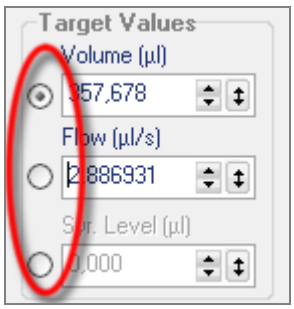


Figure 65 - Select the Type of Dosing

The following choices are available:

1. **Volume** - choose this mode to dose a defined volume at a defined flow rate.

2. **Flow** - this mode lets you create a constant fluid flow. Dosing will continue until an end position is reached or you stop the dosing process yourself.

3. **Syringe Level** - this mode allows you to define a syringe fill level to be reached at a defined flow rate.

7.3.2 Setting the flow rate and delivery volume

To input the flow rate, delivery volume or syringe fill level use the *Volume*,*Flow* and *Syr. Level* value entry fields. For inputting and modifying values, please follow the procedure described in section 5.1. Setting the target values does not immediately result in starting the drives or changing the current flow rate. Only when a dosing process starts are the new values transferred to the dosing unit by pressing the *Start* button in the toolbar.

If you wish to dose a certain volume, you must input the values for the volume to be dosed and the flow rate. The volume value is entered relative to the current position of the syringe piston. This means, for taking up reagent (aspirating), enter a negative volume and for delivering reagent (dispensing), a positive value. In this operation mode, the flow rate is always a positive value and marks at what flow rate the reagent take-up/ delivery should take place.

In order to produce a continuous fluid flow, only the flow rate is required, and the volume entry field is blocked for inputs. In this operation mode, the flow rate can be positive as well as negative. A negative value marks reagent take-up and a positive value reagent delivery.

If you wish to reach a precise syringe fill level, you can input the values for the syringe fill level and the flow rate. The syringe fill level must be a positive value between the minimum and maximum value of the syringe fill level (see section 6.8 - Configuring Syringes). In this operation mode, the flow rate is always a positive value and marks the flow rate used for reagent take-up/ delivery.

NOTICE

There is a risk of damaging the valve or fluid connections

High flow rates can give rise to very high pressure which can lead to the valve, fluid connections or your application being damaged.

If you use very small flow rates, you may be working in a critical range. In this critical range the generation of a pulsation free fluid flow can not be guaranteed. If you enter flow rates which lie in this range, the value in the input field turns red.

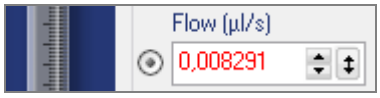


Figure 66 – Display of Critical Flow Rates (Target Values)

This case occurs frequently if you try to achieve very small flow rates by syringes with a large internal diameter. For the generation of these small flow rates the drive unit must work at a very low frequency range. In order to leave this range and to achieve the desired small flow rate, you should use a syringe with a smaller inner diameter.

IMPORTANT

Choose syringes with the smallest internal diameters when dosing small flow rates in order to guarantee pulsation free dosing.

7.4 Actual values

The current values reported by the device are displayed in this area. The *Flow* field indicates the flow rate that is realized by the dosing unit at the time. The *Volume* field displays the volume that has been delivered since the dosing unit started last time. If the drive is stopped and started afresh, the actual value for the delivered volume is reset to 0. The *Syr. Level* field displays the current level of the dosing unit in the configured SI volume unit.

Also for actual values critical flow rates will be displayed in red in the value field. In this case the use of smaller syringes for generating pulsation free fluid flows is recommended.

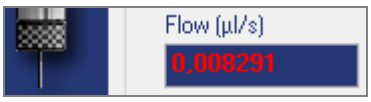
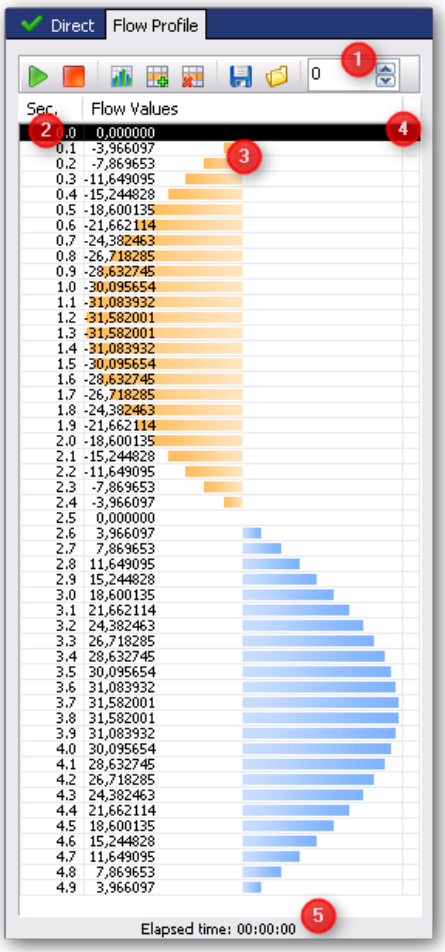


Figure 67 – Displaying Critical Flow Rates (Actual Values)

8 Flow profiles

8.1 Overview



- (1) Toolbar
- (2) Time scale
- (3) Fluid profile (list of fluid rates)
- (4) Valve switching list
- (5) Display of elapsed time

Flow profiles make it possible to run through a list of flow rates in a particular time pattern and thus implement certain changes in the flow rate over time (flow profiles). The time pattern can be freely selected as a multiple of 100 ms.

You can individually configure, for all dosing units, both the individual flow rates as well as the number of cycles that a flow profile should run through.

With the help of flow profiles you can create a continuous fluid flow with 2 dosing units mutually dispensing and taking up reagent.

8.2 Toolbar

Start a flow profile

By pressing this button, you can start the flow profile for this dosing unit. The profile is then run through in the set time pattern.

Stop the flow profile

Press this button to abort the processing of the flow profile and to stop the dosing unit.

Generate mathematical flow profile

Pressing this button opens a new window in which you can input all the parameters for generating a flow profile on the basis of a mathematical function.

Insert new value in the flow profile

This button inserts a new value in the list of gradient values. The value is inserted before the currently selected value in the list.

Delete value from flow profile

To delete an individual value from the fluid profile, please click this button.

Save the flow profile

You can use this button to save the flow profile of a dosing unit. On clicking the button, a *Save As* dialog box is opened. Select the location for saving the flow profile and assign a filename.

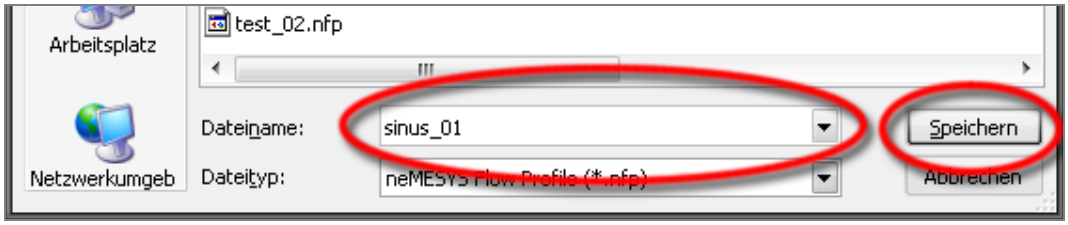


Figure 68 – Save Flow Profile Dialog

Then press *Save* to save the flow profile to the file.

Loading and saving flow rates is a simple method of copying flow rates from one dosing unit to another. To do so, save the profile of one dosing unit and then load them into another.

Load the flow profile

Press this button to load a flow profile. When you choose this action, a file selection dialog opens that supports you in selecting the file with the flow profile. You now have the option to load a flow profile that you have saved previously (file extension *.nfp*) for the current dosing unit. Here, you can also load a list of values that you have generated yourself (for example, with Microsoft Excel), if the list has the correct format.

When a flow profile is saved, the flow rates are saved. When you later try to load the flow profile again, the following warning message may be displayed:

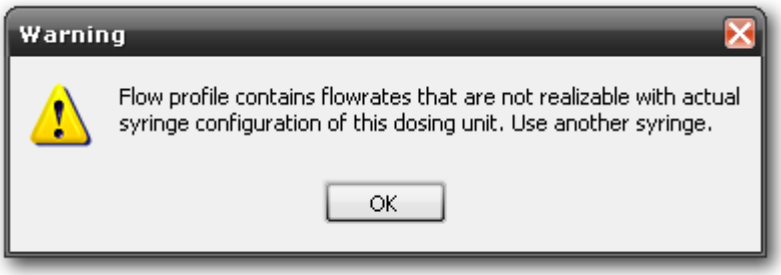
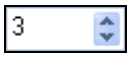


Figure 69 – Warning: Flow Rates When Loading Gradient

This is the case, for example, if, in order to generate a flow profile, you have used a syringe with a greater inner diameter than the syringe that is configured at the moment for this dosing unit. With this smaller syringe, it is then not possible to realize the greater flow rates of the other syringe. In such a case, you should use a larger syringe or generate a new gradient.



Set number of cycles

Here, you can set how often the flow profile should be run through. After the number of cycles is reached, the dosing unit is stopped. If you set the number of cycles to the value 0, the flow profile is run through an infinite number of times (till the flow profile is stopped manually or an end position of the dosing unit is reached).

8.3 Graphical representation of flow profile

8.3.1 Overview

The graphical representation of the flow profile provides a visualisation of the flow rates in the form of a bar chart. The flow rates are shown in the minimum and maximum flow rate area. This means that the offset of the function cannot be seen from the graphical depiction. The depiction of the values is mainly used to visualise the profile rather than to depict the specific individual values. The list consists of three columns. In the first column *Sec.*, you can see the time in seconds at which a certain flow rate becomes active. The time given is relative to the beginning of the flow profile.

The second column *Flow Values* displays the flow profile. Orange values indicate reagent take-up (negative flow rates), while blue values indicate reagent delivery (positive flow rates). The flow rate is also given as a numerical value on the left margin of this column. The value will be displayed in the SI unit for flow rates configured for this dosing unit.

Valve switching instructions are shown in the third and right column. If this column is blank for a particular entry, the valve setting is not changed. If this column contains a numerical position value, the valve will be switched into the set position at the start of this flow rate.

If the display of the gradients does not completely fit into the drawing area, a scrollbar is displayed on the right margin; this scrollbar can be used to show the hidden parts of the display.

8.3.2 Changing values

If you click the left mouse button in the gradient display, or drag the mouse with the left mouse button held down across the display, you can select individual values. If you would like to use the toolbar to delete or insert individual values, you can use it to choose the corresponding value.

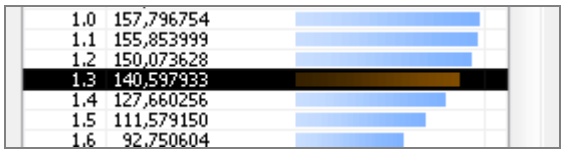


Figure 70 - Selected Gradient Value

When gradient operation is active, the marked line (Figure 70) always shows the current active gradient value. If values outside the visible area are active, the display automatically scrolls to those values.

Double-clicking a value in the gradient list selects that value for processing and a window is opened for editing the flow rate (Figure 71):

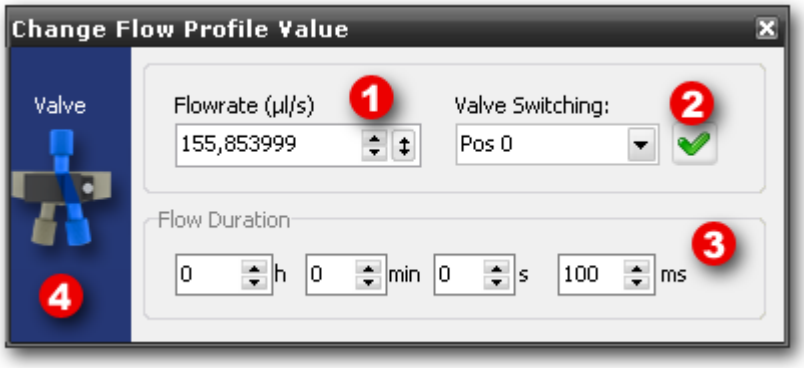


Figure 71 - Setting the Flow Rate for Gradient Values

In the window, you can set a new flow rate for the selected gradient value. The following control elements are available to you:

- (1) **Flow Rate** - you can set the flow rate you would like for the entry highlighted here.

- (2) **Valve Switching** - you can set the switching position of the valve in this selection list. If you select the *No switching* item, the valve position will remain unchanged.

- (3) **Flow Duration** - set the duration of the flow here. The minimum time period is 100 ms.

- (4) **Valve** - The valve preview indicates the switching position of the valve if you alter the value in the *Valve Switching* box.

You can end the input by clicking on the button with the green check mark.

8.4 Generating Mathematical Gradients

If you press the button for generating mathematical gradients on the toolbar, a window (Figure 72) opens, in which you can input all the parameters for generating a gradient on the basis of a mathematical function.

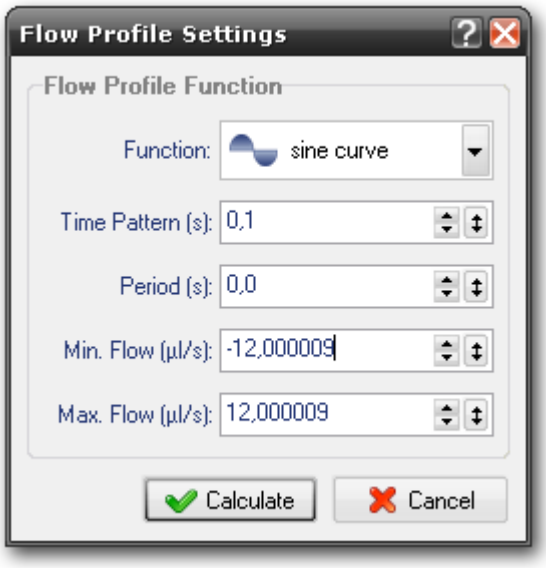


Figure 72 - Mathematical Gradient Values

Since flow profiles can be repeated cyclically, what you generate here is a period of a certain waveform, which is then repeated at a certain frequency. You can change the following parameters:

Selection of the mathematical function

Here, select the mathematical function that is used for calculating a period. At present, the waveforms *sinus*, *cosine*, *saw tooth*, *triangle* and *rectangle* are available. The *user-defined* option is also available as the last entry in the list. This is used to generate a flow profile with the corresponding period, in which all the values are given the flow rate 0. You can then manually edit and alter this profile.

Set time pattern

The time pattern defines the time interval for which the individual flow rates of the flow profile are calculated. The minimum value is 100 milliseconds.

Setting the period

The software calculates the number of values in the fluid profile from the time pattern that is entered and the period. Then, on the basis of the mathematical function, the corresponding flow rate is calculated for every individual flow profile value.

Minimum flow rate

Here, set the minimum flow rate that can be reached during the processing of a flow profile. You can also input negative values. Negative values result in a reagent take-up. The flow rate is displayed and entered in the unit that was configured for the selected dosing unit.

Maximum flow rate

Here, you can set the maximum flow rate that should be reached during the processing of the flow profile. Here, too, negative values result in reagent delivery. Here, too, the value is input with the configured unit for flow rates.

Your selection of the minimal and maximal flow rate determines the amplitude and offset of the mathematical function. The program allows you to set smaller values for the maximum flow rate than for the minimum flow rate. This gives results that reflect the function on the X-axis. Experiment with the values for minimum and maximum flow rate to get an idea of the effects and results.

Pressing the *"Calculate"* button starts the calculation of the flow profile with the settings that you have made for the selected dosing unit. You can recognize the success of the

calculation from the fact that the flow profile is displayed in the graphical display and the list of flow rates is filled with values.

8.5 File format of flow profiles

Flow profiles are not only generated in the application, but also loaded from external sources. You can, for example, generate your flow profile in spreadsheet programs, such as Microsoft Excel, and then import them into the software. The gradient files are simple text files with the **.nfp,* file extension that contain a list of values. These files can also be generated relatively easily with other applications.

Every gradient list contains the flow rate unit as the first value. The following units are allowed: *nl/s*, *µl/s*, *µl/min*, *µl/h*, *ml/min*, *ml/h and mm/s*. The number of cycles follows thereafter. All whole numbers equal to or greater than 0 are permitted, whereby 0 stands for an unlimited cyclical processing of the flow profile. There follows a list of data records that each represent a time period, a flow rate and a value for the valve setting.

The time value should be a multiple of 100 ms. The gradient value is a real value with a comma as a decimal point. The value setting is a whole number and is in the range of 0 to 255. The number 255 corresponds to the *No switching* command in the software.

An example of a flow profile saved from the program is displayed in Figure 73. The unit used is µl/s, the flow profile will be repeated ad infinitum and in the first database a flow rate of -19.9999 will be dosed for 500 ms, while the position of the valve will remain unchanged (value 255).

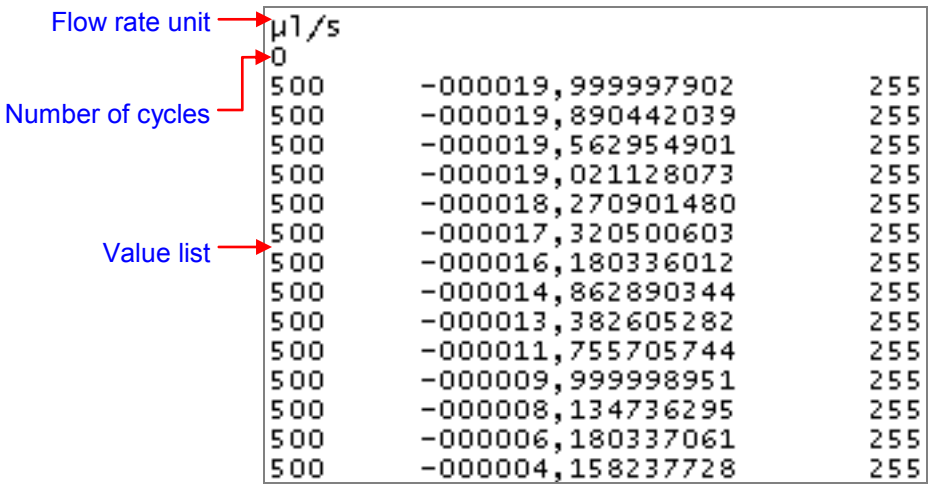


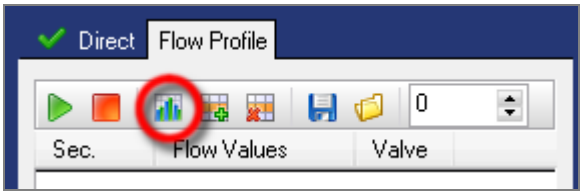
Figure 73 - Example of Flow Profile

To generate gradient lists with Excel, for example, save the table there as a simple text file and select the file extension *nfp*.

8.6 Continuous dosing

You can also use the flow profile in addition with two dosing units by mutual uptake and release of reagent and automatic valve reverse to produce a continuous fluid stream for a long period. To realize this you should use two dosing units with the same configuration (gear, screw thread) or a neMESYS double module. Insert identical syringes into both dosing units. One of the two syringes should be completely filled; the other syringe should be empty or only partly filled. Attach each syringe to the particular 3 way valve of the appropriate dosing unit. In order to realize a mutual uptake and release, proceed as follows:

1. Select the *Flow Profile* operating profile for the dosing unit with the filled syringe and click the button to mathematically generate a flow profile.



2. The configuration dialog will appear. Please select the *square wave* function (Figure 74). In the *Time Pattern* field, enter the number of seconds that every dosing unit should emit the reagent. Now enter a value into the *Period* field that is twice as big as the value in the *Time Pattern* field. The value in this field will define the time for a complete cycle of a syringe (uptake and release). The times you have to enter in *Time Pattern* can be calculated from the fill level in the syringe and the desired flow rate. Fill the *Max. Flow* field with the desired flow rate and the *Min. Flow* field with its negative value.

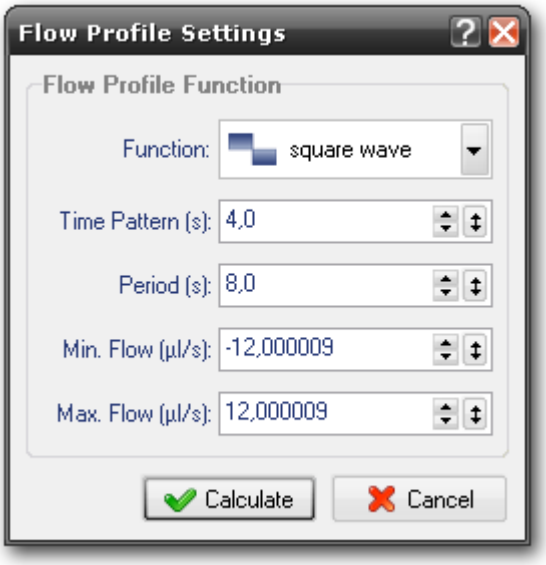


Figure 74 – Settings for Continuous Flow - Dosing Unit 1

3. Now repeat this entry for the second dosing unit. Make sure you now swap over the values for minimum and maximum flow, i.e. use the positive value for the minimum flow and the negative value for the maximum flow. This causes the second dosing unit to operate in exactly the inverse fashion to the first dosing unit. Both flow profiles of both dosing units should look similar to the lists in Figure 75:

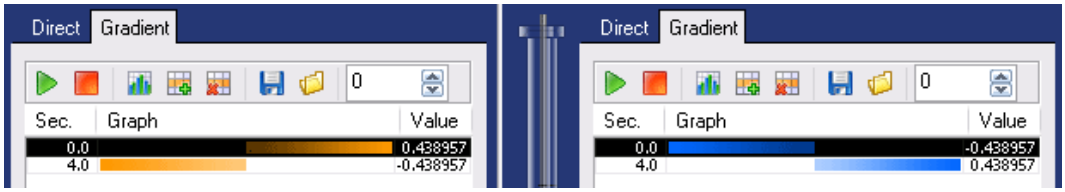


Figure 75 – Gradient Lists for Continuous Flow

4. Please set up the same cycle number for both flow profiles, or choose value 0 for both lists for a continual release until the user selects stop. Activate the automatic valve switching (section □), set the *operation mode* for both units to *Flow Profile* and click the start symbol in the main menu panel for a synchronized start of both dosing units. The dosing units will now generate a continuous flow by mutual uptake and release of reagent.

With this technology it is also possible to produce a continuous flow on the basis of a dynamic flow profile. In this case, use an inverted value list of the first dosing unit for the second of the two dosing units: in other words, positive values become negative and negative values become positive.

9 Continuous Flow

9.1 Introduction

In the *Continuous flow* operating mode, you can couple sets of two dosing units with each other in the software to create a continuous flow over a long period of time using reciprocal reagent uptake and delivery. For this, one of the two pumps doses the application with a particular flow rate while the other pump in the meantime takes up reagent from the reservoir .

When the dosing pump has emptied the syringe, the software automatically switches to the second dosing unit with the full syringe and continues the dosing process with this pump. This creates a continuous, uninterrupted flow that you can dose for a virtually unlimited period of time with a constant flow rate.

9.2 Control and Display Elements

9.2.1 Continuous flow operating mode

If continuous flow has been configured for a dosing unit, you can recognise this by the displayed icon for the *continuous flow* operating mode in the dosing unit's operating panel and the fact that the button with the icon for continuous flow is highlighted in the direct control area. (Figure 76).

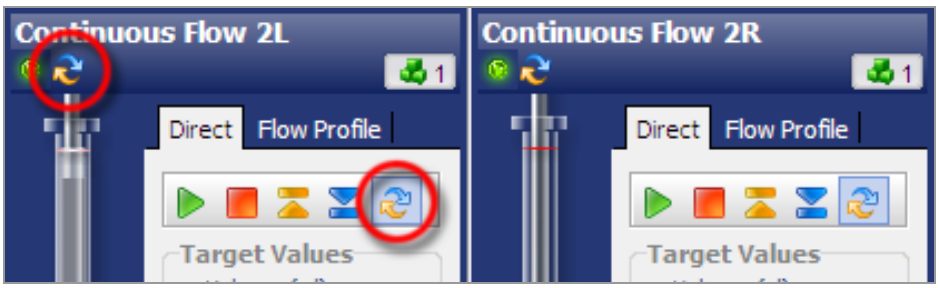


Figure 76 - Display Continuous Flow Operating Mode

To exit this operating mode, press the button with the continuous flow icon again. This places both dosing units in the *Direct control* operating mode.

As soon as you change the syringe configuration of one of the two connected dosing units or initiate a movement of the drive unit (reference move, fill syringes, empty

syringes etc.), the *continuous flow* operating mode is automatically terminated. In this case you have to re-configure all parameters.

9.2.2 Status indicators for continuous flow

If continuous flow is active for a dosing unit, the display elements (*Cont. Flow State*) are shown in the operating panel to indicate the status of the continuous flow (Figure 77).

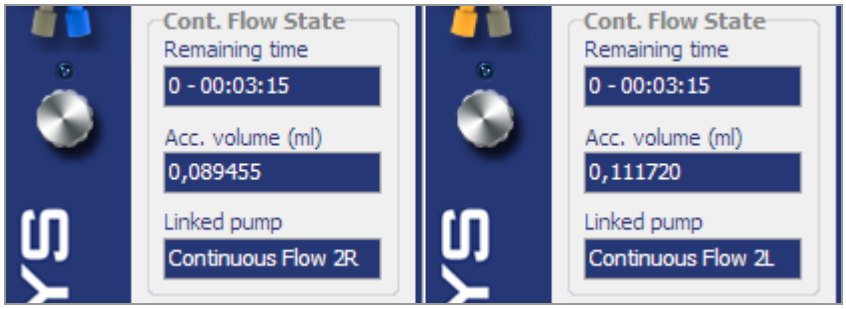


Figure 77 -Status Indicators for Continuous Flow

During this time, the following display elements are visible:

- **Remaining time** - Remaining time indicates how much time is left in the continuous dosing. When this indicator reaches zero, the continuous flow is stopped automatically.

- **Acc. volume (ml)** – The accumulated dispensed volume indicates the total volume in millilitres that was dosed by both pumps up to that time.

- **Linked pump** – The linked pump is the second pump that is linked with this pump to create the continuous flow. If you double click this field, the pump is highlighted in the list of dosing units.

9.3 Start configuration

To create a continuous flow, you require at least two neMESYS dosing units with the same configuration. Both units must have a valve and the same syringes must be installed in both dosing units.

To configure the continuous flow, click the right-hand icon with the blue and orange arrow in the toolbar of the direct control's operating panel. (Figure 78).

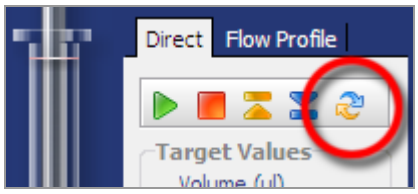


Figure 78 - Configure Continuous Flow

The dosing unit that you use to open the configuration of the continual flow is the first of the two dosing units that are connected with each other. As soon as you have pressed this button, a configuration dialog for the configuration of all the necessary parameters is displayed .

9.4 Select second dosing unit

9.4.1 Selection dialog

Once you have opened the configuration of the continuous flow, a dialog is displayed that guides you through the configuration of all the parameters. First select the second dosing unit for the continuous flow from the list of available dosing units (Figure 79).

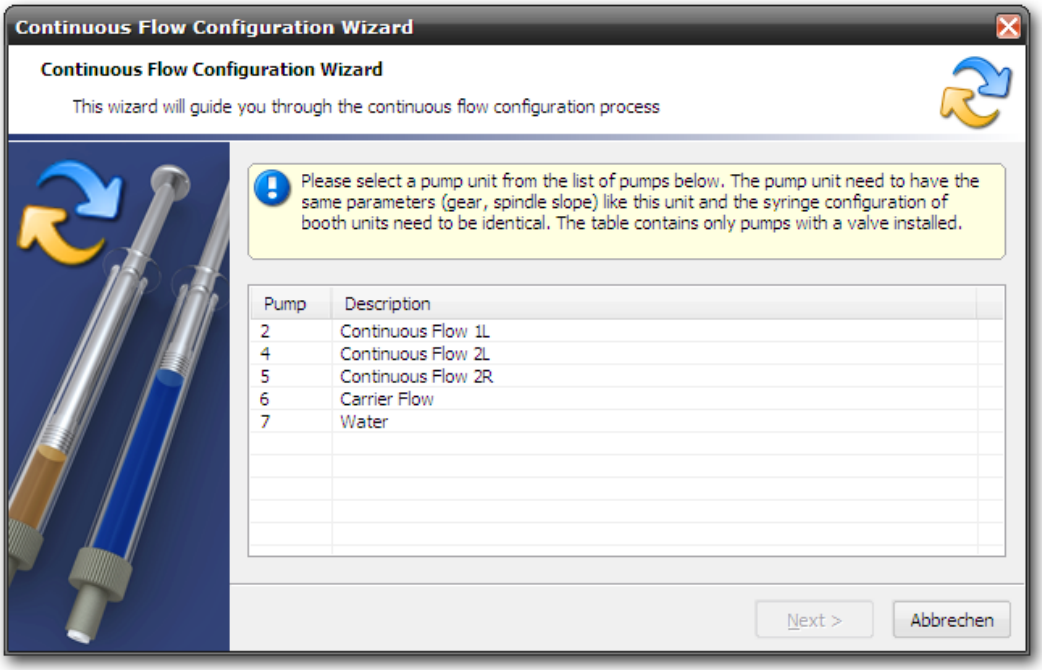


Figure 79 - Select Second Dosing Unit

The dosing units that are equipped with a valve are now displayed in the list of dosing units. Select the dosing unit by clicking the name in the list. Then click the *Next* button to continue the configuration.

IMPORTANT

For the continuous flow you should always select two dosing units that are directly connected with each other and shown next to each other in the software.

9.4.2 Possible error or warning notifications

9.4.2.1 Overview

To connect two dosing units to each other for the continuous flow, they must both meet certain requirements. If these requirements are not met, warning notifications indicate that this is the case and you will be unable to continue the configuration.

9.4.2.2 Different syringe configurations

The syringe configuration of the two dosing units must be identical, i.e. both syringes must have the same internal diameter, the same piston stroke, and the same limits must be configured for both syringes. You can ensure this by selecting the same syringe in the syringe configuration for both dosing units.

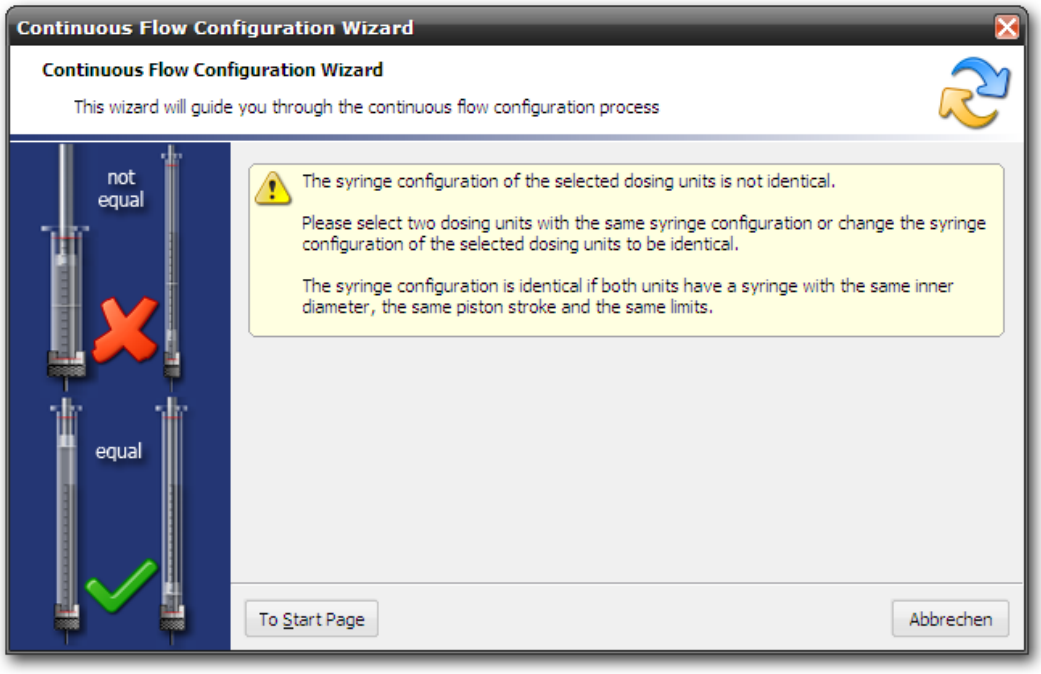


Figure 80 - Warning Notification for Different Syringe Configuration

If the syringe configurations are different, a warning notification is displayed (Figure 80).

If you see this notification, select a different dosing unit or end the dialog by clicking the *Cancel* button. Now change the syringe configuration and restart configuring the continuous flow.

9.4.2.3 Travel range too small

In order to obtain ideal results for the continuous flow and to reduce switching between reagent uptake and delivery to a minimum, you should use the complete travel range of the dosing units, i.e. when you start the configuration of the continuous flow, one syringe should be full and the other syringe completely empty.

However, this is not absolutely necessary, i.e. you can also generate a continuous flow that does not use the full travel range. But if the travel range is too small, a warning (Figure 81) will request that you increase the travel range.

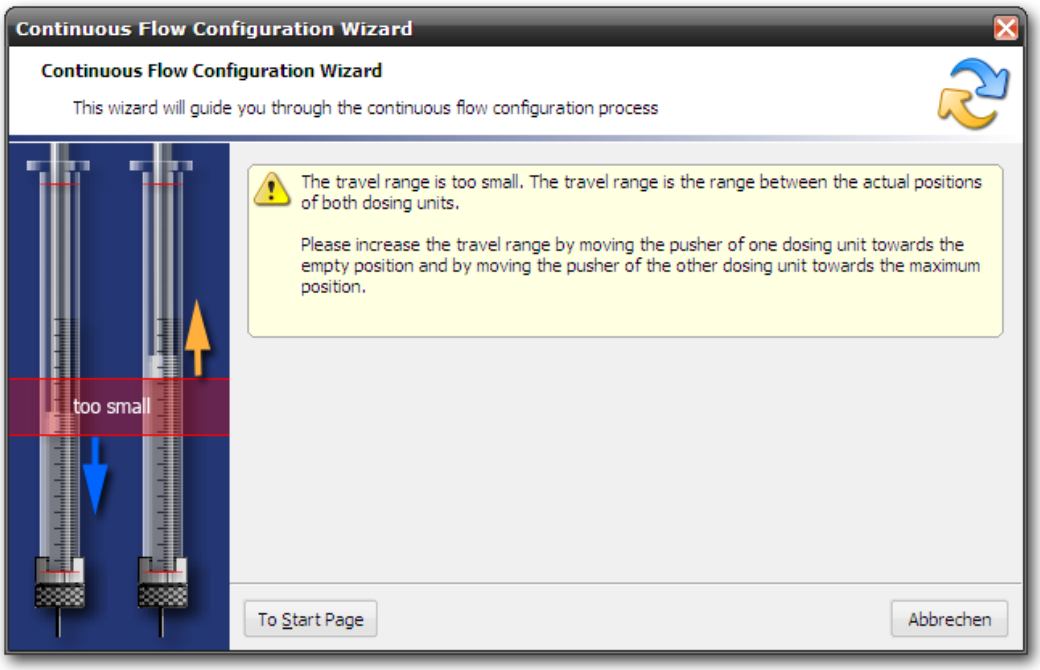


Figure 81 - Travel Range Is Too Small Warning

If you see this notification, select a different dosing unit or end this dialog by clicking the *Cancel* button. Then increase the travel range by pulling a syringe further up and/or emptying the other syringe more. Then restart the configuration.

9.4.2.4 Position not reachable

All dosing units have minimal differences to their maximum traversing ranges. When you have fully pulled up one of the two dosing units (maximum position) then this position may be further than the maximum position of the other axis, i.e. one axis can never reach the maximum position of the other axis.

If this the case, one of the following warning notifications is displayed (Figure 82).

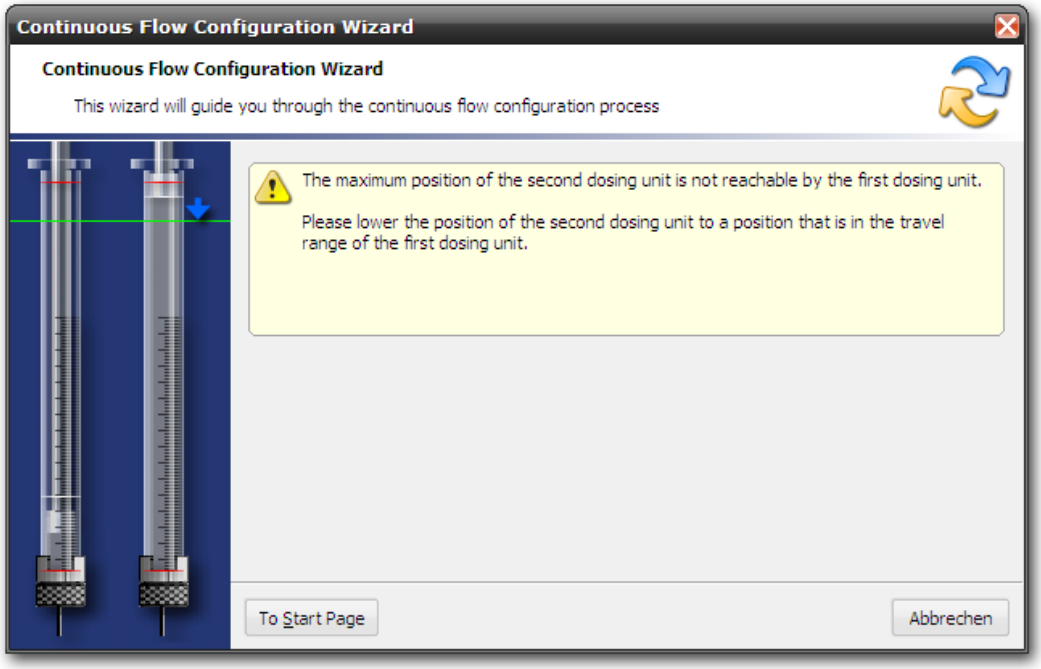


Figure 82 - Maximum Position Not Reachable Warning

In this case close the dialog by pressing the *Cancel* button. Now move the raised axis a few millimetres towards the zero position to reduce its maximum position. Then restart the configuration to enter the other parameters.

9.4.2.5 Syringe position not at upper limit

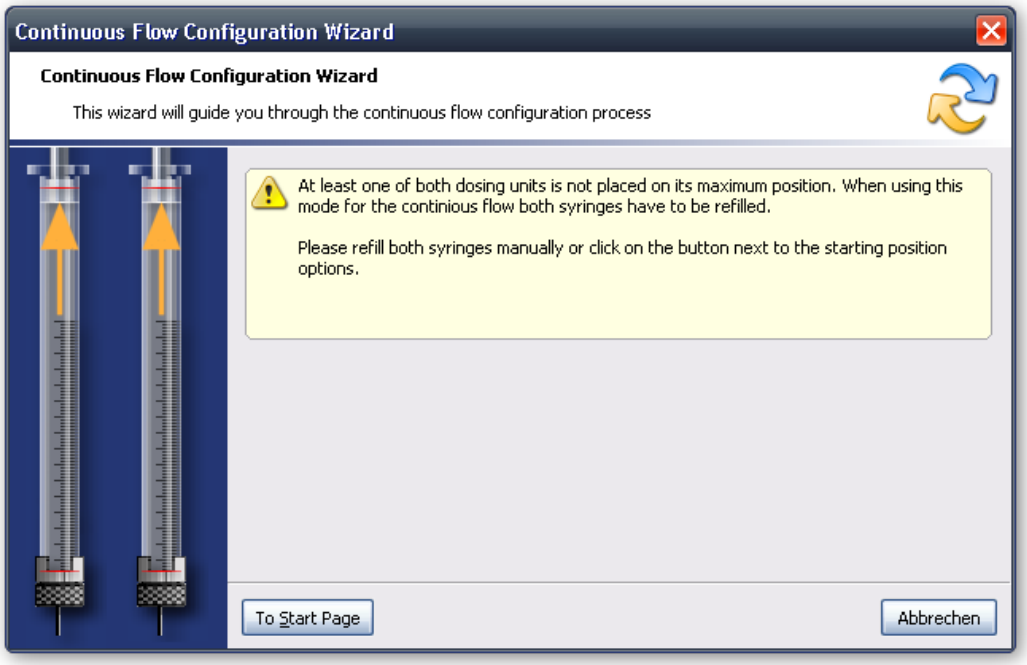


Figure 83 - Syringe position not at upper limit

This message can only appear with the starting mode *Both syringes on maximum position* selected. It indicates that at least one of both syringes is not at the upper limit

position. Please use the automatic positioning button or refill the syringe manually until it reaches its upper limit.

9.5 Select starting position

Before starting the continuous flow one of the following starting positions can be selected:
Start with different syringe positions: the syringe position of both dosing systems is different. The travel range of the continuous flow is defined by both current syringe positions. When starting, the syringe with the lower position is getting refilled while the other one is dispensing.
Start with both syringes on the maximum position: both syringes are refilled up to the maximum position. The travel rage is defined by the lower and the upper limit of the syringes. When starting, one syringe will dispense. When reaching the lower limit this syringe is getting refilled while the other one is dispensing.

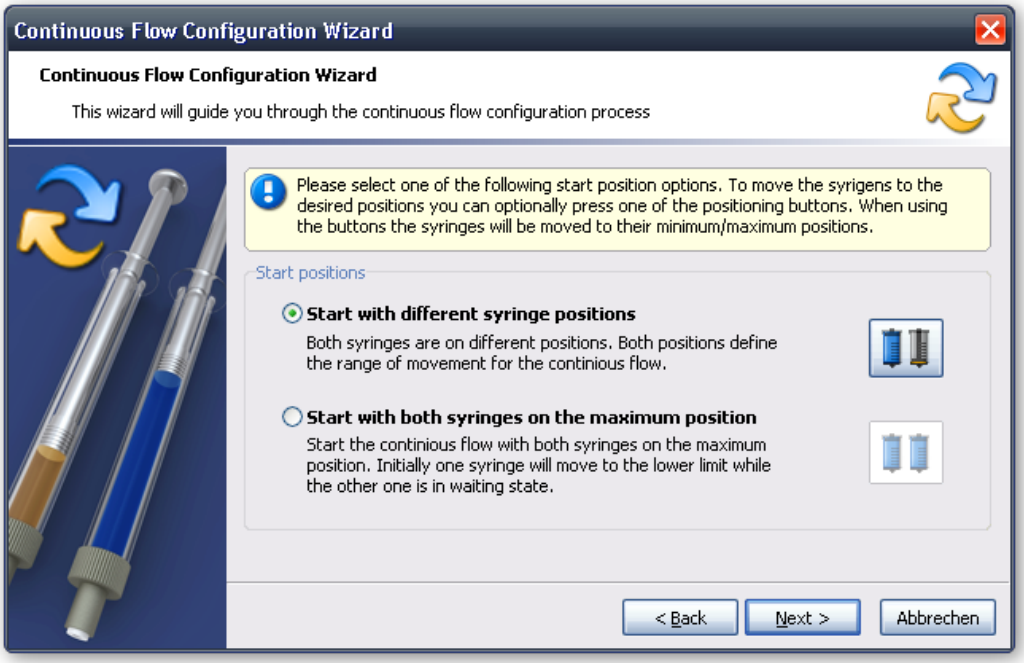


Figure 84 - Selection of the starting position

The starting position can be initialized by clicking on the corresponding positioning button. After applying the warning message both dosing modules will be moved to the desired limit positions.

9.6 Configure parameters

9.6.1 Configure continuous flow rate

When you have selected the dosing unit and the starting position, the next step is to configure the continuous flow rate. The SI unit of the flow rate is the unit that is configured for the dosing unit in whose operating panel you have started to configure the continuous flow.

Enter the value for the flow rate in the field *continuous flow rate* (Figure 85). You can set the flow rate to minimum or maximum values by pressing the *Min* and *Max* buttons. To minimize the switching impulse of the pumps there is the possibility to adjust the time for acceleration and deceleration during the switching. The value *crossover duration* defines this time. Larger values will cause smooth crossovers to reduce switching impulses but will also decrease the maximum possible flow rate. Please note that the native acceleration and deceleration values of the pumps are used for starting and stopping the continuous flow

Confirm each entry in the entry field by clicking the button with the green check tick.

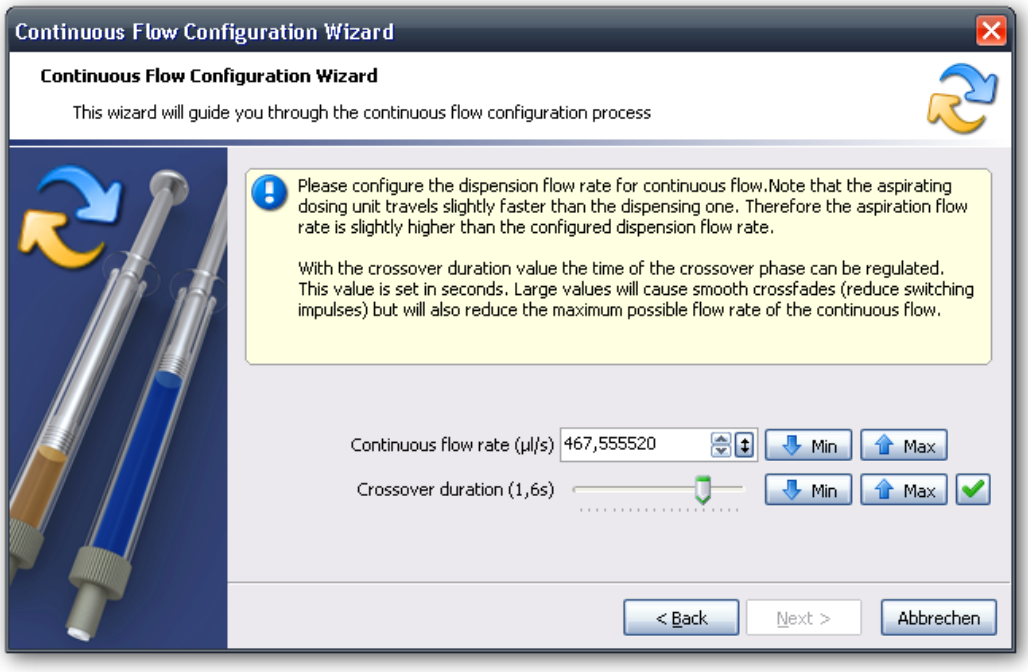


Figure 85 - Configure Continuous Flow Rate

When you have completed the entry, click the *Next* button to move to the next step.

9.6.2 Duration of the continuous flow

9.6.2.1 Overview

In this window you can limit the duration of the continuous flow. If you do not want to limit the duration, leave the *No limit* default value in place. With this setting the continuous flow continues until dosing is stopped manually.

To limit the duration of the continuous flow, you must configure a time after which the dosing is stopped automatically. To configure this time, this window offers two different options. You can either enter the time directly or alternatively enter a target value for the volume to be dosed and the software then automatically calculates the duration of the flow from this value and the flow rate.

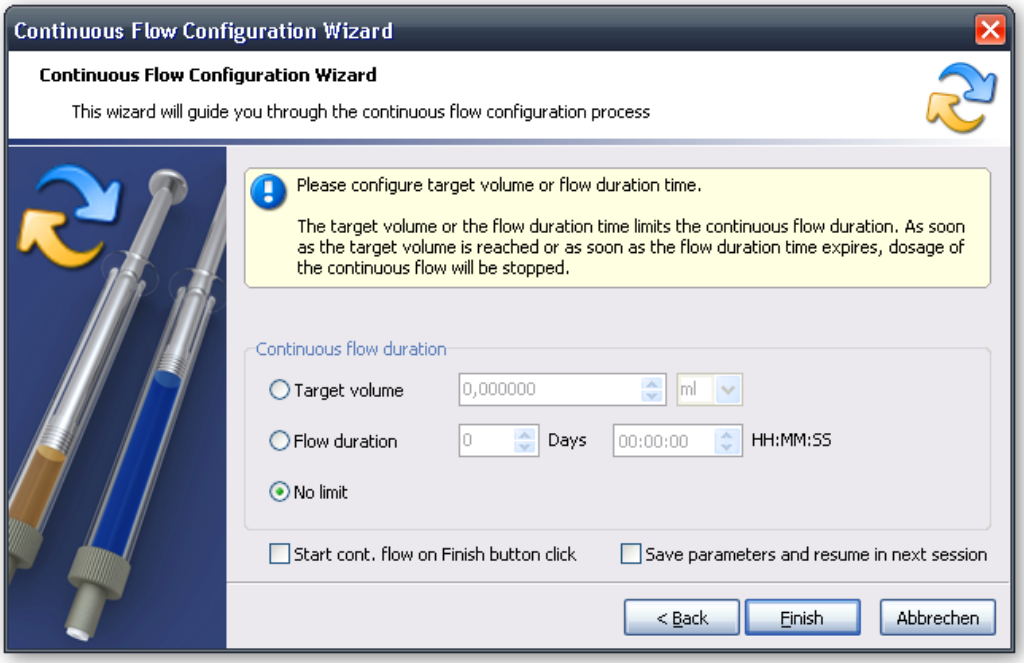


Figure 86 - Configure the Duration of the Continuous Flow

9.6.2.2 Enter duration directly

To enter the duration directly, activate the *Flow duration* selection field (Figure 86). As soon as the field is active, the relevant entry fields are released. Enter the number of days in the *Days* field and then configure the number of hours, minutes and seconds in the *HH:MM:SS* field. In the *Target volume* field, the software automatically calculates the expected target volume from the flow rate set and the the time configured. Close the entry by pressing the *Next* button.

9.6.2.3 Enter target volume

To set the duration using the target volume, activate the *Target volume* selection field. As soon as the field is active the relevant entry fields are released. Now select the SI unit for entering the volume and the value for the target volume. The software calculates the duration of the continuous flow from this value and the flow rate. Close the entry by pressing the *Next* button.

9.7 Start/stop continuous flow

9.7.1 Start individual flow

If you activate the *Start continuous flow on Finish button click* selection field in the window for dosing duration configuration (Figure 86), the continuous flow will start immediately after completing the configuration. If you only want to start one continuous flow, activate this selection field.

9.7.2 Synchronous start of several continuous flows

If you want to start several continuous flows synchronously, deactivate the *Start continuous flow on Finish button click* selection field in the continuous flow configuration dialog. You can now start several continuous flows synchronously by pressing the button to start several units synchronously in the application's main toolbar.

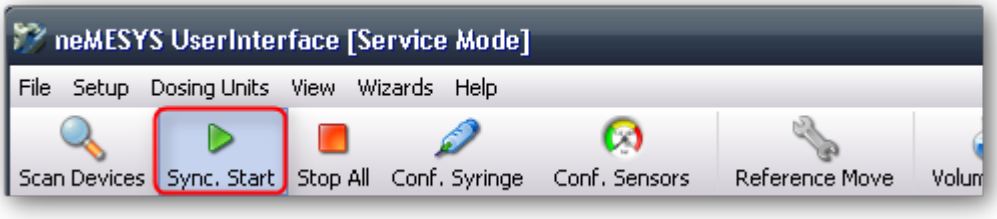


Figure 87 - Synchronous Start of Several Continuous Flows

Alternatively, as described in Chapter 6.13- Grouping dosing units, you can group several dosing units and then start and stop them synchronously once you have configured the relevant group actions.

9.7.3 Stop the continuous flow

If you press the stop button in the direct control area, you can stop an individual continuous flow. If you have grouped the dosing units, pressing the stop button stops all

of the dosing units in the same group. If you press the stop button in the application's toolbar, you can stop all of the dosing units.

If you stop a continuous flow dosing unit, the linked dosing unit is also always stopped. If you have configured the continuous flow for a particular duration, stopping the dosing unit pauses this duration. I.e. when you restart the dosing unit, the continuous flow continues with the duration that was current when it was paused. If the time which was set for the continuous flow has expired, both linked dosing units are stopped. When you start the continuous flow again, the dosing restarts with the same duration as the previous dosing process.

IMPORTANT

After pausing t a continuous flow, it continues when it is restarted. If you want effect a complete restart you have to reconfigure the continuous flow.

9.7.4 Resume the continuous flow

After the continuous flow was stopped with the *stop* button, it can be continued with the *start* button. If a time period or a target volume was specified for the continuous flow it will be resumed with the values at the time of the interruption. To resume the continuous flow in the next session please select the *Save parameters and resume in next session* checkbox during the last configuration step. If the software is then closed if one or more continuous flows are active then the current parameters will be saved and reloaded in the next session. So the continuous flow can be resumed directly with the same settings.

9.7.5 Change the flow rate

The flow rate of a continuous flow can easily be changed without repeating all steps from the configuration dialog. To change the flow rate please stop the current continuous flow. Next change the flow rate value of the field *flow* in the section *target values* of one of the linked dosing modules. It does not matter in which one because the new value will automatically be set for the second module. When clicking the start button the continuous flow will be resumed with the new flow rate. All other parameters keep unchanged.

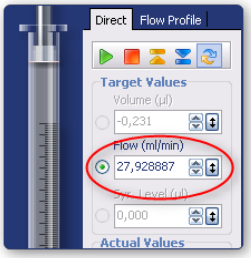


Figure 88 - Changing the flow rate of a continuous flow

10 Firmware Update

10.1 Introduction

The dosing is controlled with great precision by a microcontroller with a digital signal processor for each individual neMESYS dosing unit. Each of these controllers contains its own software, the firmware for the device and a set of configuration parameters. This firmware is stored on a rewritable hard disc on the controller and can be replaced with newer versions with expanded functionality and improved performance parameters.

From neMESYS UserInterface version 1.20 you can update the device firmware yourself. This allows you to bring the software of your dosing units up to the latest version and benefit from new features and functions in future firmware versions.

Important

All units are shipped with the latest stable firmware version. The latest firmware and compatible previous versions are included in the installation package of the neMESYS software. After setup they are located in the installation folder.

10.2 Preparation

Updating the device firmware is a delicate process, as updating the firmware on the controller can on occasion (e.g. if a power cut occurs during the update) lead to the device firmware being lost and the dosing unit not functioning any more. If you are not confident about carrying out the firmware update yourself, please get in touch with cetoni GmbH about the possibility of cetoni carrying out the update for you.

NOTICE

Updating the firmware can occasionally lead to the device firmware malfunctioning.

Before you start updating the firmware, you should take note of the following to minimise the risk of the firmware for the device malfunctioning:

1. Make sure that all of the dosing units which you want to update are connected correctly. An operating panel for each dosing unit should be visible in the application.

2. Make sure that none of the attached dosing units are set to their factory settings.

3. Close all other programs before starting the neMESYS UserInterface software to minimize the risk of your computer crashing or experiencing performance bottlenecks during the firmware update.

4. Make sure that your PC is unable to switch into standby or power down mode during the update.

5. Remove all syringes from the dosing units to avoid damage.

6. It takes around 1.5 minutes to update an individual unit - leave enough time to update all of your units.

NOTICE

Follow the firmware update instructions above. You may experience errors and device firmware malfunctions if you do not follow the instructions.

10.3 Execution

To begin the firmware update, select the *Firmware Update Wizard* menu item in the *Wizards* menu.

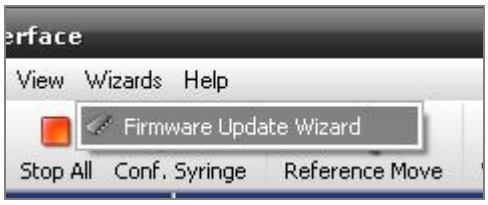


Figure 89 – Firmware Update Wizard Menu

The Firmware Update Wizard dialog (Figure 89) will appear to guide you through the firmware update process. Confirm the first step by pressing the *Please confirm that you've read and understood the warning* button. Then click on the *Next* button to proceed to the next step of the update.

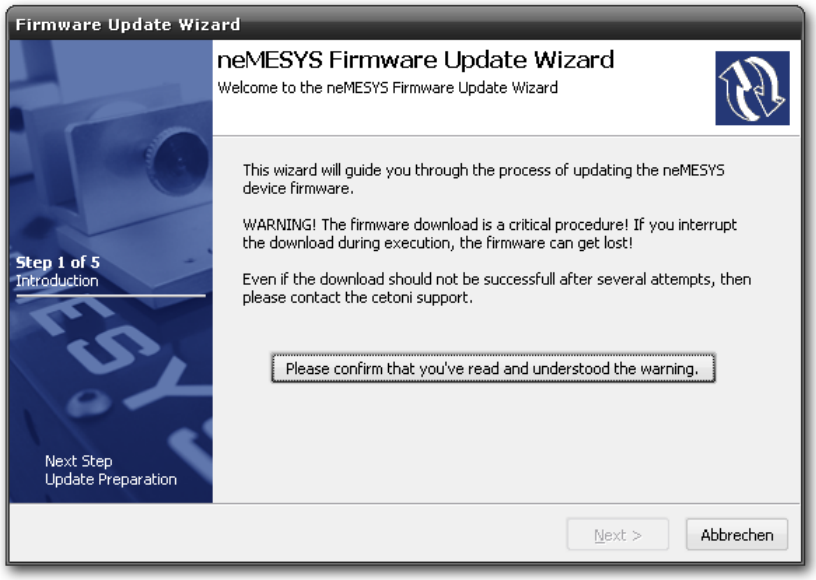


Figure 90 – Firmware Update Wizard - Step 1

Read through the instructions in the next dialog box carefully, and confirm that you done so by pressing the *Confirm that you've properly prepared your dosing platform and your PC* button. Press the *Next* button to go to the next step.

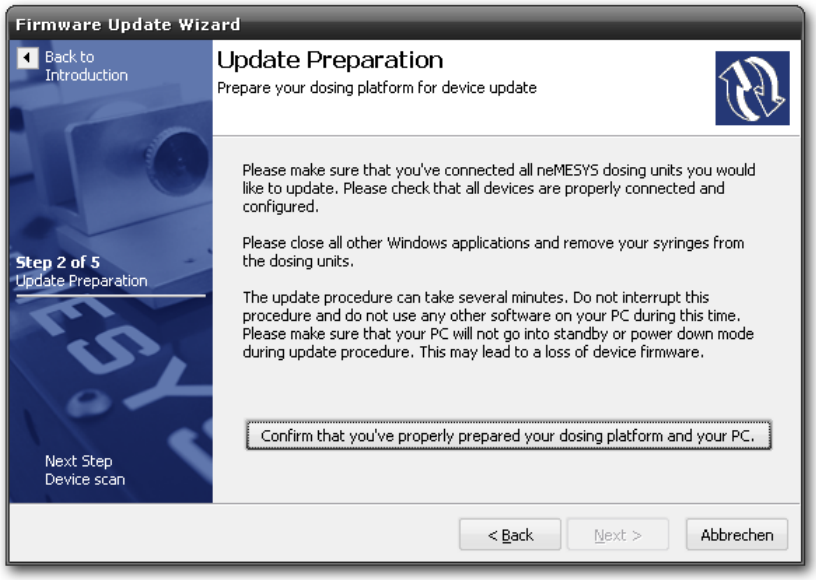


Figure 91 – Firmware Update Wizard - Step 2 - Prepare Update

This step will give you information about the firmware versions of the attached dosing units and which version the individual units will be updated to. If the *Update to version* (Figure 91) column contains the *Firmware is up to date* text, the dosing unit already has the latest firmware version and will not be updated. If the column contains the *unknown* text, it was not possible to find out the firmware version and no update will be carried out. If this is the case, get in touch with cetoni GmbH's technical support.

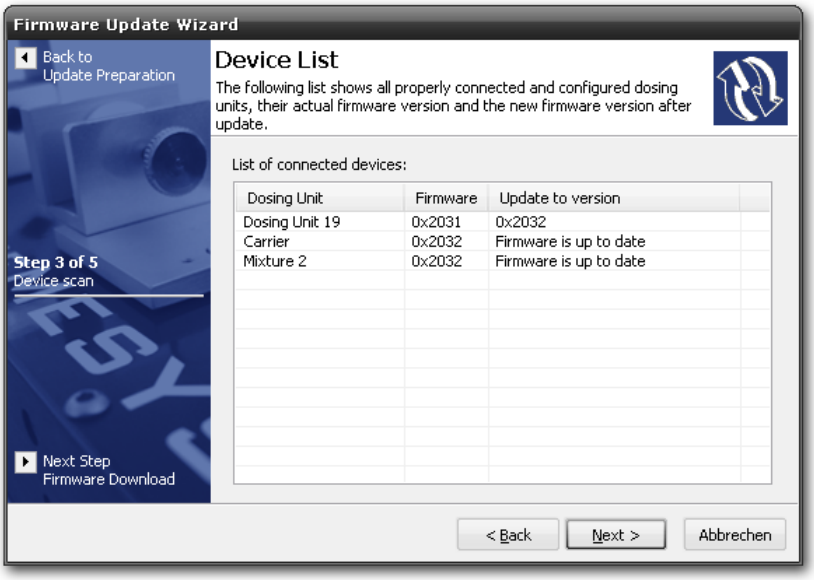


Figure 92 – Firmware Update Wizard - Step 3 - List of Dosing Units

Press the *Next* button to go to the next step.

In step 4, Firmware Download, you can start transferring the new firmware into the connected dosing units by pressing the *Start Firmware Update* (Figure 93) button. After starting the process you should not carry out any other operations on your PC until the transfer is complete. It can take approx. 1.5 minutes to update an axis. With up to 32 axes in play, make sure you leave enough time for the update to complete.

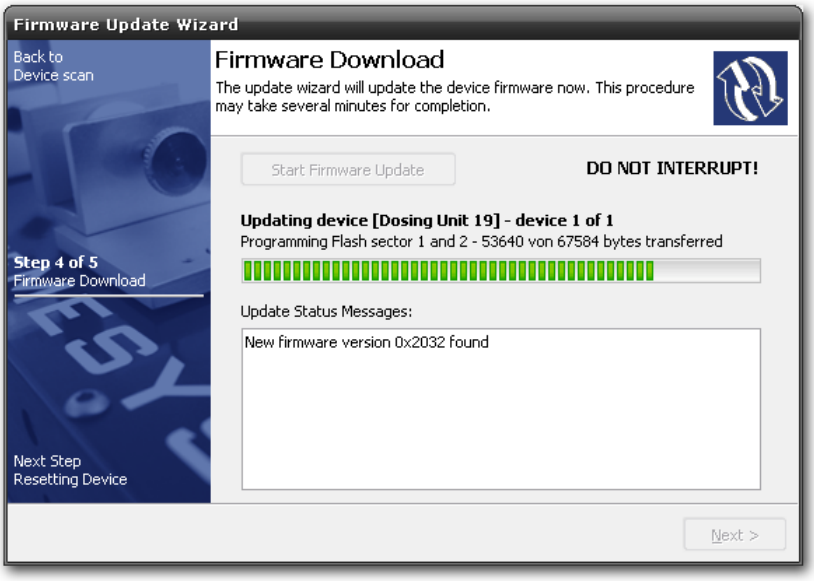


Figure 93 – Firmware Update Wizard - Step 4 - Firmware Download

The *Update Status Messages* status window gives you reports on the status of the firmware update. If the firmware update encounters problems, they will also be displayed in this window. If you do encounter an error during the update, you should select the entire content of the status window with the mouse, copy it onto the clipboard by pressing

Ctrl+C and then insert it into a text file. These status reports can help cetoni's technical support to find the cause of the error.

If the download ends successfully or with an error, the *Next* button will take you to the fifth and final step of the update (Figure 94). Follow the instructions in this window - turn your dosing platform off and then on again after a few seconds to reset the controllers. Click on the *Finish* button to end the firmware update.

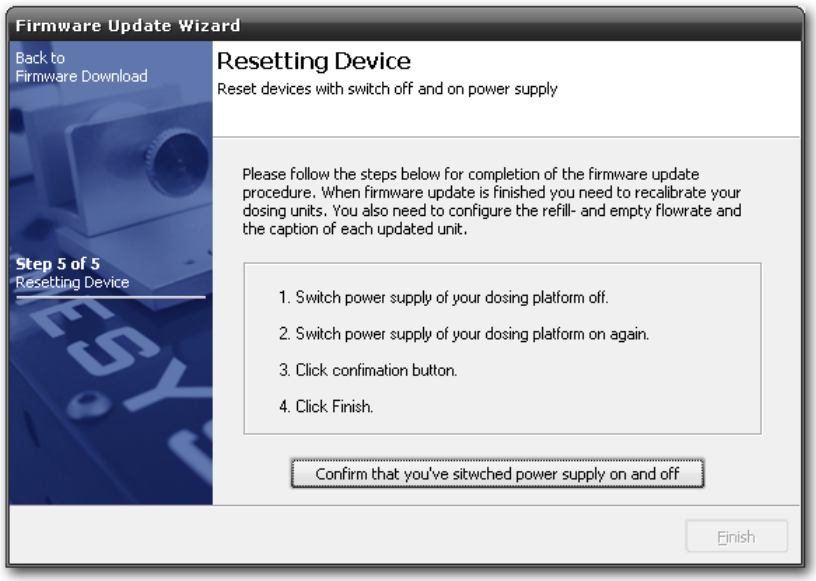


Figure 94 – Firmware Update Wizard - Step 5 - End Update

Please note that the update will overwrite different configuration parameters with default values, such as the flow rates for filling and emptying the syringe or the name of the dosing unit. You will have to reconfigure these values after the update. Carry out a reference move for each dosing unit after the update is completed to calibrate them.

IMPORTANT

The firmware update will overwrite configuration parameters. You will need to recalibrate the dosing units.

11 Troubleshooting

11.1 Introduction

In this section, we attempt to support you in solving problems that may occur when working with the neMESYS-dosing platform:

Hardware selection dialog empty

When I wish to carry out a search or add a new dosing unit, the hardware selection dialog is displayed. But it is empty and I cannot select any hardware.

Procedure

Please check whether you installed the hardware drivers for the device. These drivers are installed as the last step in the installation of the neMESYS UserInterface Software. If you look under *"Settings", "Control Panel"*, there should be an icon there for *IXXAT Interfaces* (Figure 95).

Figure 95 - Control Panel Icon for IXXAT Interfaces

If the drivers are properly installed, please check whether your computer is correctly connected to the dosing platform via the USB cable that is a part of the delivery kit. If necessary, try out another USB slot in your computer.

Error message "Error initializing hardware driver…"

I get an error message *"Error initializing hardware driver…"* (Figure 96) when the connection to the device is initialized.

Figure 96 - Hardware Driver Error Message

Procedure

Check whether you have opened another instance of the application that is already connected with the device. If that is not the case, restart your computer.

No dosing units detected

When the software searches for connected dosing units, no dosing units are detected.

Procedure

Please check whether you have correctly connected your dosing units to the dosing platform and whether the dosing platform is switched on. Before you can use the dosing units, you must first configure them, as described in section Installation.

Dosing units do not start when synchronously starting several units

When several units are started synchronously, some units do not carry out any dosing at all.

Procedure

Check whether you have correctly configured the values for the volume and flow rate for the corresponding units and that the units are not in their end positions.

For a synchronous start the gradients are not carried out

When several units are started synchronously, the programmed flow profiles are not executed by some units.

Procedure

Check whether, for the corresponding dosing unit, you switched the operating mode to *Flow Profile*. Only in this case are the flow profiles carried out. Also check whether the corresponding dosing units are not already in their end positions. If, for example, you take up reagent with your programmed flow profile in the first step, but the dosing unit is already in its maximum end position, the flow profile is stopped or not executed any further.

When inputting flow rates and volumes, the program changes the values

If values are input in the software for flow rates and volumes, the program independently changes these values.

Procedure

The position values of the drive units are recorded through a digital position evaluation. I.e. there is only a discrete number of steps available for every revolution of the drive. If you input a flow rate or a volume, the software automatically calculates the next value that the drive unit can realize.

Upon turning the manual regulator, the direction is not correct

If the speed of the drives is changed manually via the regulator, the direction change at some dosing units did not tally with the direction of rotation of the mouse wheel.

Procedure

In the operating panel of the direct control, you can determine the speed changes of the drives when rotating the mouse wheel. Here, you can also use negative values to reverse the direction of rotation and to match it to your requirements. In this case, check the prefix sign for the regulator.

Upon switching off the device, I get the error message "DC Link under voltage"

When I switch off the dosing platform, I get the error message "DC Link under voltage".

Procedure

Do not switch off the dosing platform while the neMESYS UserInterface software is running. You should switch off the dosing platform only in an emergency (e.g. emergency stop) or if the software expressly prompts you to do so.

Error message *"This program requires Windows NT Version 5.0.2195 or later"*

When installing the software, the error message *"This program requires Windows NT Version 5.0.2195 or later"* **appears.**

Procedure

Install the software on a computer with Windows 2000 or Windows XP. All other versions of Windows NT are not supported.

Not all connected dosing units are detected by neMESYS UserInterface

The software does not detect both dosing units of a certain Double Module nor all following dosing units which are connected to this Double Module.

Procedure

Check if the service switch on this particular Double Module is in its ON position. If the switch is not in its ON position then turn your dosing platform off, move the switch into its ON position, turn your dosing platform on again and then reconnect the software neMESYS UserInterface.

The syringe level is not displayed correctly

The syringe level which is displayed in the software is not matching the syringe level of the module.

Procedure

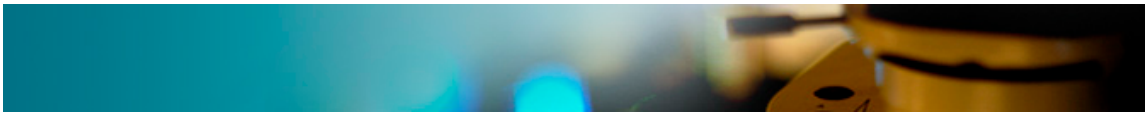
Select the module with the syringe level which is not displayed correctly. Then execute a reference move.

Appendix D: Micro-Manager User Guide

Micro-Manager is open-source software developed by scientists for scientists.

Credits, License and Copyright:

Micro-Manager is an Open Source software package for controlling automated microscopes on multiple platforms (Windows, Mac and Linux). The software is being developed in the **Vale Lab** at the Unversity of California San Francisco and funding was provided by the Sandler Foundation and a grant from the NIH. The original software design was by Nenad Amodaj, and the software is currently developed by Arthur Edelstein and Nico Stuurman. Many individuals contributed source code or other types of help (including this documentation). The Micro-Manager source code is distributed under the BSD license for the user interface and the LGPL license for the MMCore (control module). Most supplied device drivers ('adapters') for cameras and other devices are covered by the BSD license. Copyright for some of the adapters is owned by other parties.



documentation

User's Guide

Hardware Configuration Guide

Plugins

Tutorial (PDF) (http://valelab.ucsf.edu/publications/2010EdelsteinCurrProt.pdf)

Advanced Methods (http://www.jbmethods.org/jbm/article/view/36/29)

FAQ

More Help Topics

Video Screencasts

Micro-Manager User's Guide

Contents

Micro-Manager User's Guide

Introduction

Imaging cells has become an essential technology in many laboratories working in the life sciences. The components needed for such image acquisition consist of a microscope, cameras, light sources, mirrors, filters mounted in motorized filter wheels, x-y-z stages, and shutters. In practice, many complicated sequences of

motorized operations are needed to achieve the imaging strategy desired by the researcher (e.g. acquiring images from multiple wavelengths). Thus, computer control of image acquisition is an integral part of contemporary microscopy.

From the user's standpoint, the whole setup (a microscope equipped with camera, stage, filter wheels, etc.) ideally should appear as an integrated, coherent system -- an "appliance." This appliance should allow for effective and comfortable interactive work, as well as unattended, completely automated operation.

Available commercial packages more or less provide the needed functionality for controlling automated microscopes, but, because they are proprietary and closed, they are difficult to extend and customize. New devices cannot be added to the system unless the software vendor chooses to provide appropriate drivers. Sometimes the cost of configuring the system with all necessary software and drivers can be quite high.

In contrast to virtually all of the available commercial solutions, Micro-Manager supports multiple platforms (Windows, Mac and Linux) and provides an open plugin interface for adding new devices. In addition, Micro-Manager is compatible with the widely used image processing package **ImageJ (http://rsb.info.nih.gov/ij/)**, which is also available in the public domain. Combined with **ImageJ (http://rsb.info.nih.gov/ij/)**, Micro-Manager provides a full-featured microscope management and image processing package, comparable in capabilities to commercial solutions. It is distributed free of charge and under an Open Source license. Its code base and functionality can be customized, extended and re-used with practically no restrictions.

Installation

After you install Micro-Manager according to the instructions for your platform (below), you can test-drive the software in demo-mode. The application will start in demo-mode by default after initial installation. To use Micro-Manager with your microscope, you will need to create a configuration file for the hardware components that are part of your setup.

The configuration process is described in the **Configuration Guide**. You will need to create a configuration file specific to your system.

Installation on Windows

Run the installer (MM_Setup_1_x_yy.exe) and follow the prompts. The package includes a copy of ImageJ; it may be necessary to install or update **Java (http://java.com)** on your computer. Besides the manufacturer's low-level drivers for your camera and possibly other equipment, no other software is required to fully use Micro-Manager. Micro-Manager will be installed in C:\Program Files\Micro-Manager1.x.

There are no particular hardware or software requirements. However, for practical use we recommend Windows XP, 1.7 GHz or better processor and at least 512 MB of RAM. Micro-Manager works on Window 7 running as either 32-bit or 64-bit. However, some devices will only work using 32-bit drivers. Micro-Manager has also been reported to work with Windows Vista.

Installation on Mac OS X

Double click on the file Micro-Manager1.x.yy.dmg. This will open a drive called 'Micro-Manager'. The drive is on the desktop and in every Finder Window. Open the drive (by double-clicking). In the drive there is a folder called Micro-Manager1.x. You can install the application by dragging this folder to your Applications folder, or you can launch the application by opening the folder and double clicking the 'ImageJ' icon (to run a 32-bit version of Micro-Manager) or by clicking the ImageJ64 icon (to run the 64-bit version).

If you already use ImageJ

Micro-Manager is packaged as an **ImageJ (http://rsb.info.nih.gov/ij/)** plug-in and contains a copy of the entire ImageJ application, together with the Java run-time environment. Starting Micro-Manager will automatically start ImageJ as well. If you already use ImageJ and have it installed on your machine, the original installation will not be affected. Micro-Manager installs and uses its own copy of ImageJ. However, if you have any existing ImageJ macros or plugins that you want to use with Micro-Manager, you will have to copy them manually to the appropriate sub-folders within the Micro-Manager folder. Migrating your favorite ImageJ plugins to Micro-Manager installation should not cause any problems.

Memory settings

It is often necessary to adjust memory settings in order to optimize Micro-Manager performance and prevent errors. See the **Configuration Guide** for guidelines.

Getting started

On startup Micro-Manager displays a splash screen that allows you to choose a particular hardware configuration file. If you click OK without changing anything, the last successfully used configuration file will be loaded. The Demo configuration (software simulator) will appear as default the first time you start the application. This configuration can be used to explore Micro-Manager features, train novice users and as a reference point for troubleshooting.

Configuring Micro-Manager to work with your specific microscope setup is covered in detail in the **Configuration Guide**. Micro-Manager has made configuration easy with, the Configuration Wizard utility (**Tools | Hardware Configuration Wizard**), which provides a step-by-step workflow for creating and managing your hardware configuration. We recommend that you try out the Demo configuration to get familiarized with the program. All examples in this guide refer to the Demo configuration.

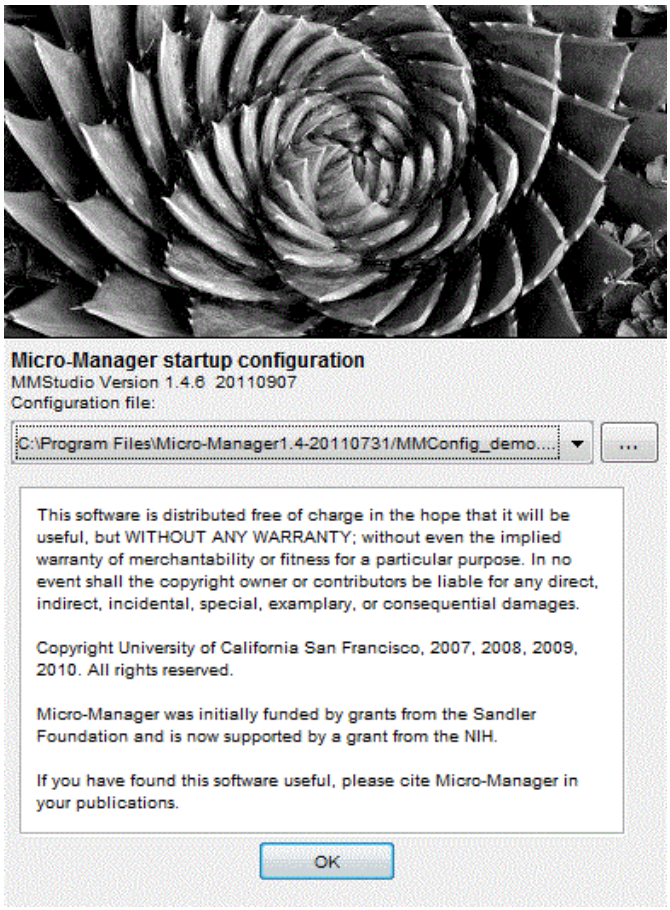


Figure 1. Startup screen: selection of the configuration file.

You can also choose to skip loading a configuration file (select "none" in the drop-down list) in which case the application starts without any devices attached. At anytime while using Micro-Manager under the **Tools** menu you have the options to Load, Reload, Switch and Save your **Hardware Configuration** files.

The Micro-Manager Main Window

Starting Micro-Manager will also start ImageJ (in fact, ImageJ starts up first and runs Micro-Manager as a plugin). Both ImageJ and Micro-Manager have their own menus (on the Mac you will need to click on either window to display the menu in the main menu bar). Unless specified differently, menu commands in this manual will refer to the Micro-Manager menu. The Micro-Manager and ImageJ windows appear differently on different computer systems; examples from multiple systems are used in this manual.

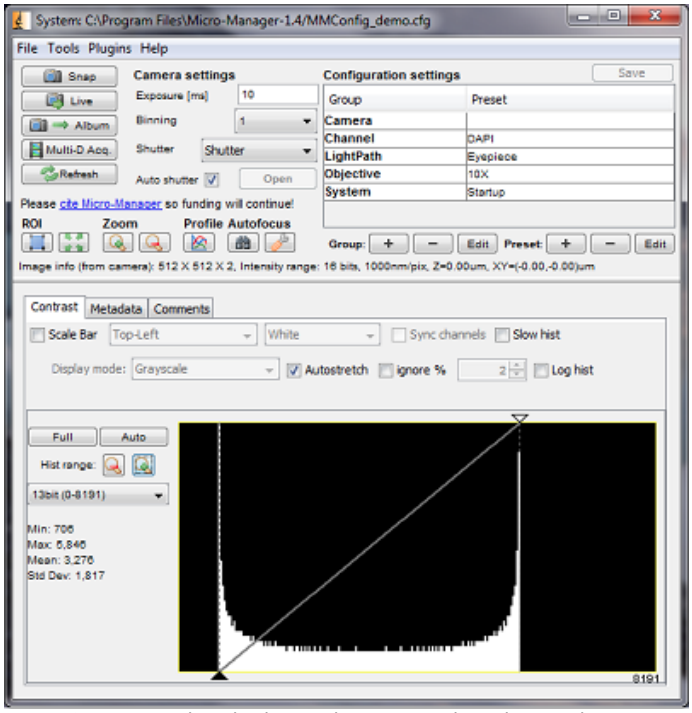


Figure 2. Main Window displaying device controls and image histogram

After the configuration file is loaded, the Main Window displays the current settings and shows the status of selected devices.

Snapping single images

To obtain a single image from the camera, press the "Snap" button. A display window will pop up with the acquired still image. You can use any of the available ImageJ tools to analyze, save or edit the image. In addition, at the bottom of the window there are shortcut buttons to save the image, enter live mode or send images to album.

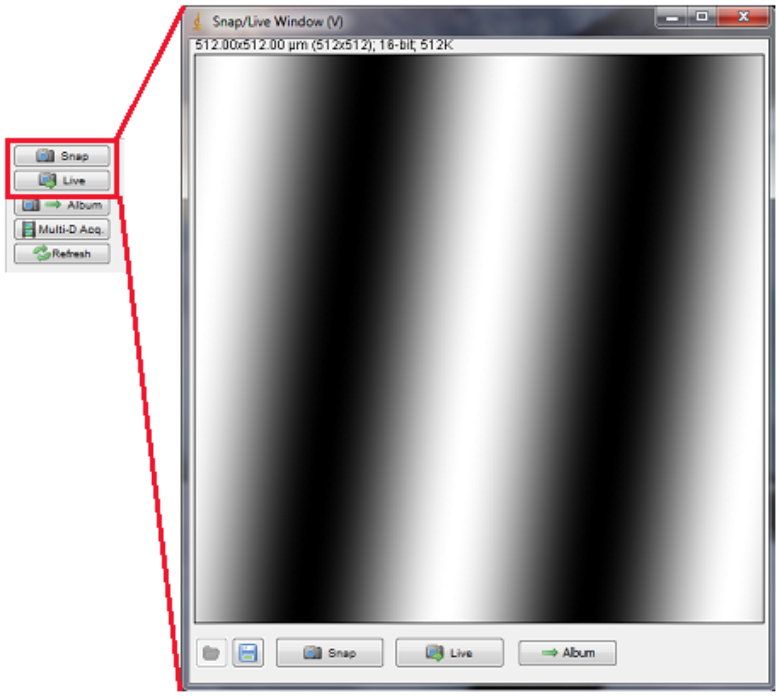


Figure 3. Display window for Snap and Live mode.

Each time you press the Snap button, the image in the display window will be updated. The Demo camera driver generates a spatial sine wave that shifts each time you snap a new image. The Main Window displays a histogram of the image; any changes to brightness and contrast will be immediate reflected on the display window.

Live image mode

To see a continuously updated, "live" view from the camera, press the "Live" button. The images will be displayed in the "Live" window. Pressing this button again, stops live mode. Settings in the Main Window can be changed during live mode and the effects on images in the "Live" window will be immediate.

Acquiring a series of images

With the "Album" button, you can collect a series of still images (snaps) in an image series window. The first time you click the "Album" button, a new series window will open, with a fresh image obtained from the camera. Every time you click the "Album" button thereafter, a new image will be added to the series. Click the "Save" button to write all images in the series window to disk.

Histogram, Brightness and Contrast

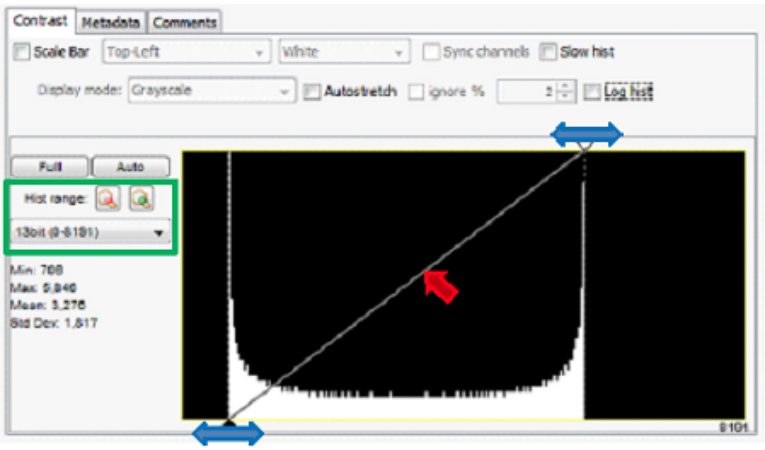


Figure 4. Histogram in Main Window. Brightness and contrast controls are shown in red and blue. Histogram range adjustment shown in green.

A histogram of the current (display window) image's pixel intensities is presented in the graph in the lower part of the Main Window. The histogram is automatically updated each time a new image is received. The histogram range can be adjusted, by clicking on the zoom icons or selecting a pixel range from the drop down menu shown in **green**. Selecting the "Camera Depth" from the drop-down menu will automatically adjust the histogram range to the hardware bit depth of the image. Some statistical information (min, max, mean, stdev) about the image is also provided.

The "Log hist" checkbox will change the y-axis of the histogram to a log scale.The histogram is overlaid with a graph showing the relation between pixel intensities in the image and display of the image on the screen. This graph allows manipulation of brightness, contrast and gamma of the displayed image.

Brightness and contrast

The end-points of this graph (black and white triangles, boxed in **blue** in Figure 4) set the lower and upper clipping levels (i.e. pixels values lower than the black triangle will appear as black, pixel values higher than the white triangle will appear as white). The steeper this line, the higher the contrast: moving the line to the right will darken the image, while moving it to the left will brighten the image. The "Full" button sets the lower and upper clipping levels to your camera's full intensity range. The "Auto" button will adjust the lower and upper clipping levels automatically, based on the extreme pixel values in the image. For "Live" imaging you can perform this adjustment continuously by checking the "Auto-stretch" box; once checked you can set a percentage of outliers to ignore.

Gamma Function

The gamma function allows you to change the relation between pixel value in the image and display from linear to hyperbolic. This gamma correction makes it possible to visualize both bright and dark objects in an image simultaneously, which can be beneficial, especially with cameras that have higher dynamic range than the display (for further explanation see the **wikipedia article on gamma corrections (http://en.wikipedia.org/wiki/Gamma_correction)**. The adjustments can be made by clicking and dragging the line just as is shown with the **red** arrow in figure 4.

Displayed vs. Original Image

Since the original image is saved separately from the displayed image, any adjustments made will apply only to the displayed image - the original image with the actual pixel values will not be affected. Also, because Micro-Manager displays images received from the camera in a standard image window, any ImageJ command can be used to adjust the appearance of the image, including the standard ImageJ Brightness and Contrast dialog.

Refresh

The Micro-Manager main panel will not necessarily be updated when a setting in your microscope changes. This behavior saves resources and avoids continuous polling of the hardware. To bring Micro-Manager in sync with the current state of the hardware, use the 'Refresh' button.

Region of Interest (ROI)

Most of the cameras used for microscopy can be configured to image only a Region of Interest (ROI) instead of the full frame. To select an ROI use the rectangle tool (from the ImageJ window) on the image window. By pressing the "ROI" button while the selection is active, you will apply the current rectangle to the camera. Some cameras will internally adjust the rectangle dimensions slightly to fit within specific hardware constraints. To return to the full frame imaging, press the "Full" button (right next to the ROI button).

Zoom

The "Zoom" buttons apply a software zoom to the topmost image window. Software zoom here implies zooming in on the captured image.

Line profile

The "Profile" button opens up a live line profile window. Unlike the regular ImageJ "profile" utility, this window will update each time a new image is displayed in the Live window. The profile is computed from the currently active line drawn over the image. To draw a line, use the standard line tool from the ImageJ toolbar.

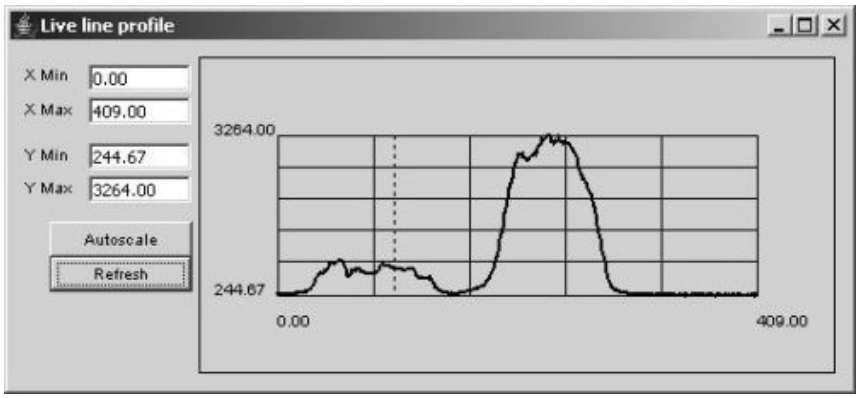


Figure 5. Line Profile window.

Camera Settings

A few camera settings found in all systems are directly accessible from the main window:

- Exposure Time: You can set the exposure time of your camera.
- Binning: Apply binning ('pooling' of pixels in both x and y direction).
- Active Shutter: You can change the 'active shutter': that is, the shutter that Micro-Manager will open before taking an image and close once the image is made.
- Deactivating Auto Shutter: This behavior of automatically opening and closing the shutter can be defeated by unchecking the 'Auto shutter' checkbox. Doing so will let you open and close the active shutter with the 'Open/Close' button in the Main Window.

Controlling devices

Exploring devices: Device/Property Browser

The Main Window provides control of only a small subset of the device properties loaded in the system. To view and control all devices and all settings available in the currently loaded hardware configuration, use the "Device/Property Browser" (available under the Tools menu). The Device/Property Browser displays a list of all devices and associated "properties" (settings available for each device). Since this list will often be overwhelmingly long, you can restrict the list of displayed devices using the checkboxes in the top left corner of the window. You can inspect and change any setting on any device. Read-only properties are shown as a line with a dark background. To change a setting, change it in the 'Value' column and then leave the field using the 'tab' key or by clicking the mouse elsewhere in the window.

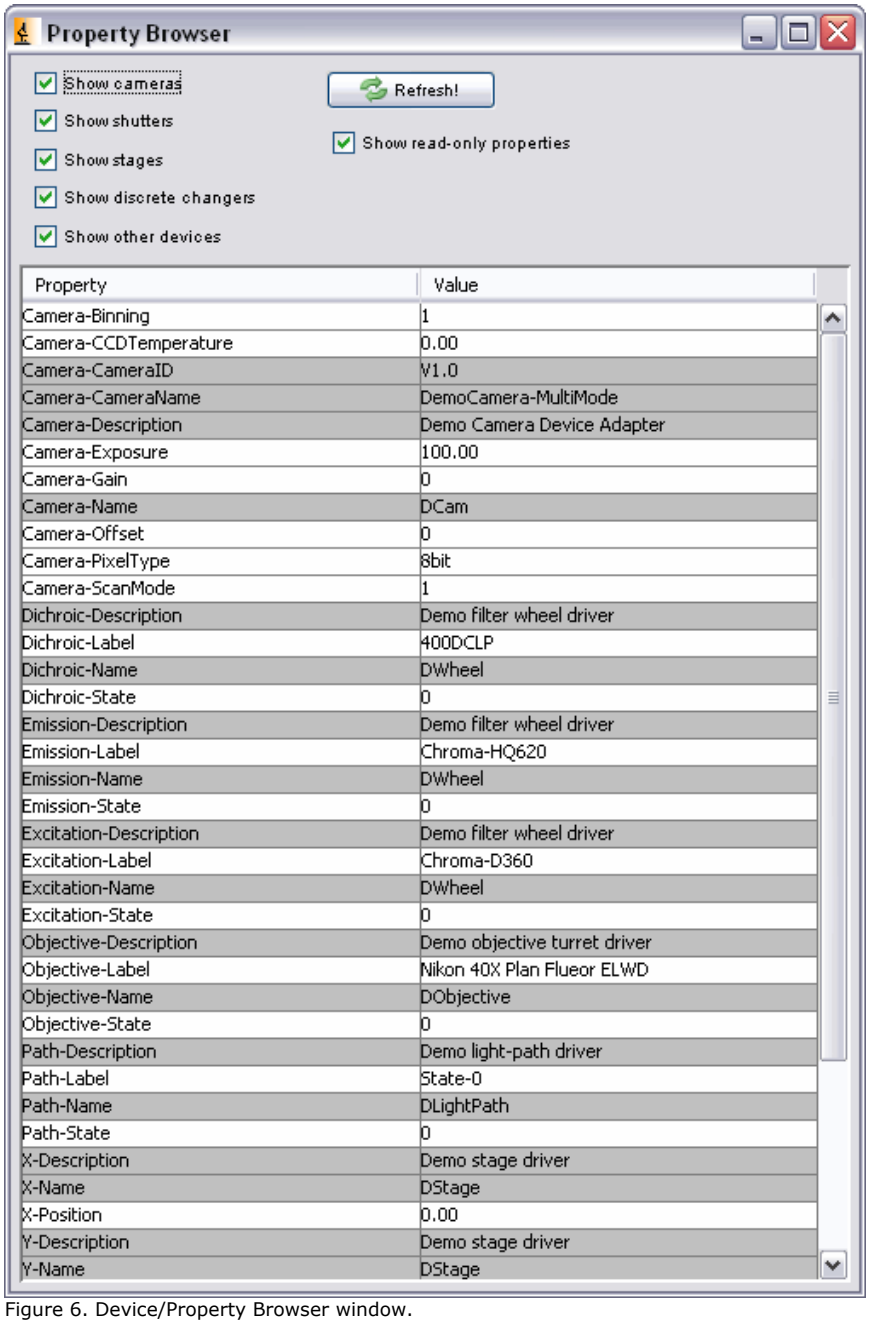


Figure 6. Device/Property Browser window.

Grouping device properties: Configuration Presets

Obviously, changing individual properties using the Device/Property Browser quickly becomes cumbersome. Micro-Manager therefore provides a way to generate 'shortcuts' that let you quickly set groups of device properties. Setting up these Configuration Presets is an important part of configuring Micro-Manager and is described in the **Configuration Guide**. Placing the cursor over the current preset will display a tool-tip window and show which devices will be affected by the command. In the example in the configuration guide, choosing the "DAPI" channel preset command will move three filter wheels to the appropriate positions.

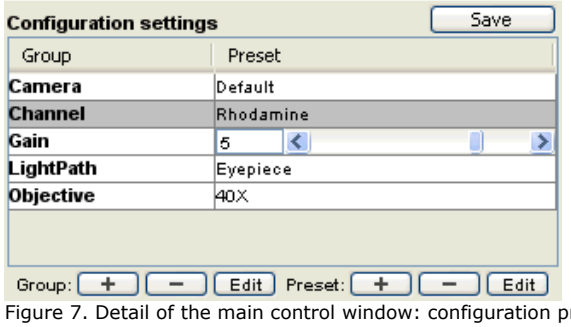


Figure 7. Detail of the main control window: configuration preset.

There is no limit to the number of devices or number of different properties you can include in one configuration group. You can set objectives, filters, stage positions, camera parameters in a single configuration command. The Configuration Presets shown here are just an example (used in the demo configuration). In some cases, device properties can show up as a slider, making it easy to set things like camera gain or temperature.

Recording Images

Multi-dimensional acquisition

To acquire multi-image stacks, press the "Multi-D Acq." button to open the Acquisition Control dialog (this dialog can also be reached through the **Tools | Acquisition** menu entry). Micro-Manager allows you to create a stack as multi-channel (wavelength coordinate), multi-frame (time coordinate), multi-slice (Z coordinate), multi-position (XY coordinate), or any combination of these.

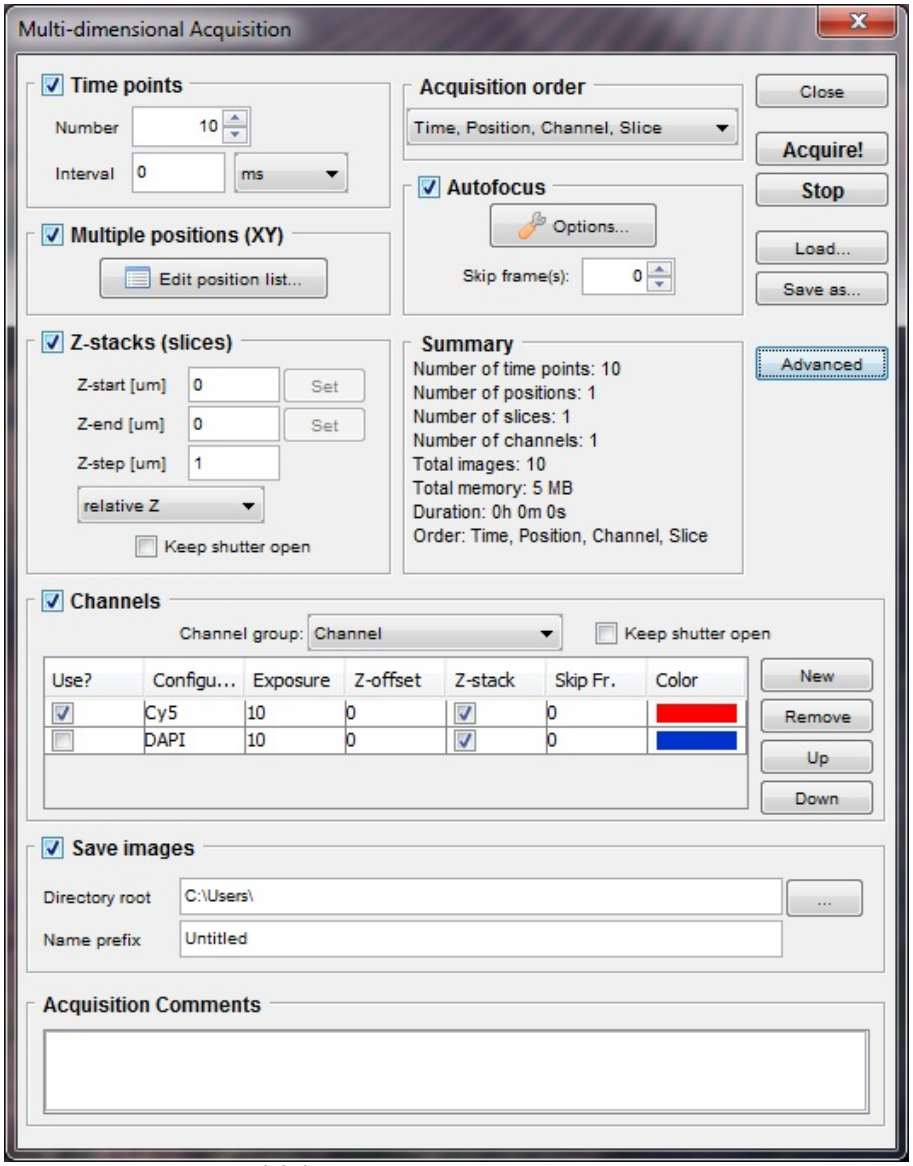


Figure 8. Acquisition control dialog.

After defining channels, slices and frames by using controls in the dialog, press the "Acquire" button. The acquisition starts immediately and an **Image Viewer** window will open displaying the progress. During and after acquisition you can use controls at the bottom of the 5D-Image window to play-back the sequence, browse channels, slices, frames, or positions, or save the entire image stack to disk.

Time points will allow you to define the number of frames you would like to acquire and the duration of the interval time between each frame. If you would like to acquire a continuous acquisition then simple specify the interval time as "0". The rate at which you acquire images will then be limited only by the speed at which your camera will acquire an image (hardware specific) and the exposure time you specify in the Main Window. For more complex time lapse setup click on the 'Advanced' button in the acquisition control dialogue.

Z Slices can be set either as relative to the current position (you will need to type in the start and end position) or as absolute positions. If absolute positions are selected, the 'Set' buttons will become active. Clicking these will set the current position to start or end.

Channels can be selected from the active 'Channel group'. Channel groups are the Configuration Preset groups that are defined in the configuration settings section in Micro-Manager's Main Window. You will need to set the desired exposure time for each channel. You can also set a z-offset for each channel, which can be useful when the

main object in one of the channels is in a different focal plane from the other channels. Setting 'Skip Frame' to a number other than 0 will cause the acquisition to 'skip' taking an image in that channel (after taking the first image) for the indicated number of frames. The 5D-Image Viewer will 'fill in' these skipped frames with the previous image. In some situations it may be desirable to acquire certain channels at lower sampling rates, to reduce photo-toxicity and to save disk space. Clicking inside the 'Color' column will open up a Color selector that lets you select the color to be used for that channel in the 5D-Image Viewer. (You can also change colors later on in the **Image Viewer**.)

Acquisition Order lets you choose between carrying out z-stacks with each channel (Slices first) or switching channels at each z-position (Channels first). 'Time first' mode will take a complete sequences of frames at a single position before moving on to the next, whereas 'Positions first' mode will cycle between all position at each time point, in effect acquiring time lapse sequences at all positions.

Checking the **Use XY list** option will cause the acquisition to be executed at each position defined in the **Position List**. **Autofocus** options are described below. If the **Save images** option is selected, images will be saved to disk continuously during the acquisition. If this option is not selected, images are accumulated only in the 5D-Image window, and once the acquisition is finished, image data can be saved to disk. However, saving files automatically during acquisition secures the acquired data against an unexpected computer failure or accidental closing of image window. Even when saving to disk, some of the acquired images are still kept in memory, facilitating fast playback. If such behavior is not desired, check the 'Conserve RAM' option (**Tools | Options**).

Fast Time Series Acquisition (Burst)

If you would like to acquire 'bursts' of images as fast as possible, Micro-Manager's Multi-dimensional Acquisition engine is designed to intelligently take care of this. You will need to:

- Set the time interval between frames to zero
- Deactivate Z Stacks
- Choose Time First
- Use no channels or a single chnanel

Thus you will have removed any delay that would get in the way of the fast acquisition. In previous Micro-Manager versions this was known as Burst Mode.

Split View

Split View functionality (compatible with such devices as the DualView or OptoSplit) is now available as a **plugin**.

Autofocus

Micro-Manager has a single, integrated user interface for hardware- and software-based autofocus. In the main window, press the **Set autofocus options** button (wrench icon) to open the Autofocus Properties dialog box. Here you can choose which autofocus mechanism to use among available hardware- and software-based options. You can then edit the settings for your chosen autofocus mechanism. The **Duo** autofocus option allows you to combine two autofocus mechanisms, so that they operate sequentially, one after the other.

The **Autofocus Now** button (icon with binoculars) on the Main Window will cause the autofocus mechanism you have chosen to execute its focusing protocol once.

Hardware Autofocus

Hardware-based autofocus support is provided for the Nikon Perfect Focus (PFS), ASI CRIFF and CRISP, Zeiss Definite Focus, and Olympus Z Drift Correction (ZDC) devices. These devices bounce an infrared reference beam off a surface of the coverslip, and use this reflection to adjust the focal position. To activate one of these devices in Micro-Manager, you need to include it in the Hardware Configuration and select it in the Autofocus Properties dialog.

Software Autofocus

Software-based autofocus plugins are also available in the Autofocus Properties dialog. They should work with any camera and Z-stage. These autofocus plugins iteratively adjust the focal position and acquire images to optimize a specific image feature. For example, Pakpoom Subsoontorn & Hernan Garcia (Caltech) contributed a Java plugin module ("JAF H&P") for auto-focusing based on the relative strength of gradients in the image (edge 'sharpness'). See **here** for further documentation of this autofocus plugin.

Autofocus in Multi-D Acquisition

The Multi-Dimensional Acquisition window offers integration with the autofocus system. Check the checkbox in the **Autofocus** section to activate autofocus during the multi-dimensional acquisition. The **Options** button (wrench icon) in the **Autofocus** section allows you to choose the autofocus mechanism and configure it. You can also specify how many frames (time points) will be skipped between autofocus events. If you have a hardware autofocus, it will be activated just before image acquisition and then be switched off again. If you rather have the hardware autofocus switched on continuously, do not check the autofocus checkbox, but be sure that the hardware autofocus is switched on before you start the acquisition.

Positioning

Position List Dialog

The Position List dialog can be reached from the menu (**Tools | XY List**), and from the **Multi-dimensional Acquisition** window (button next to 'Use XY list'). The Position List is used to record the positions of stages in your system. At the bottom of the dialog is a list of available stage axes, any of which you can select to use for recording positions.

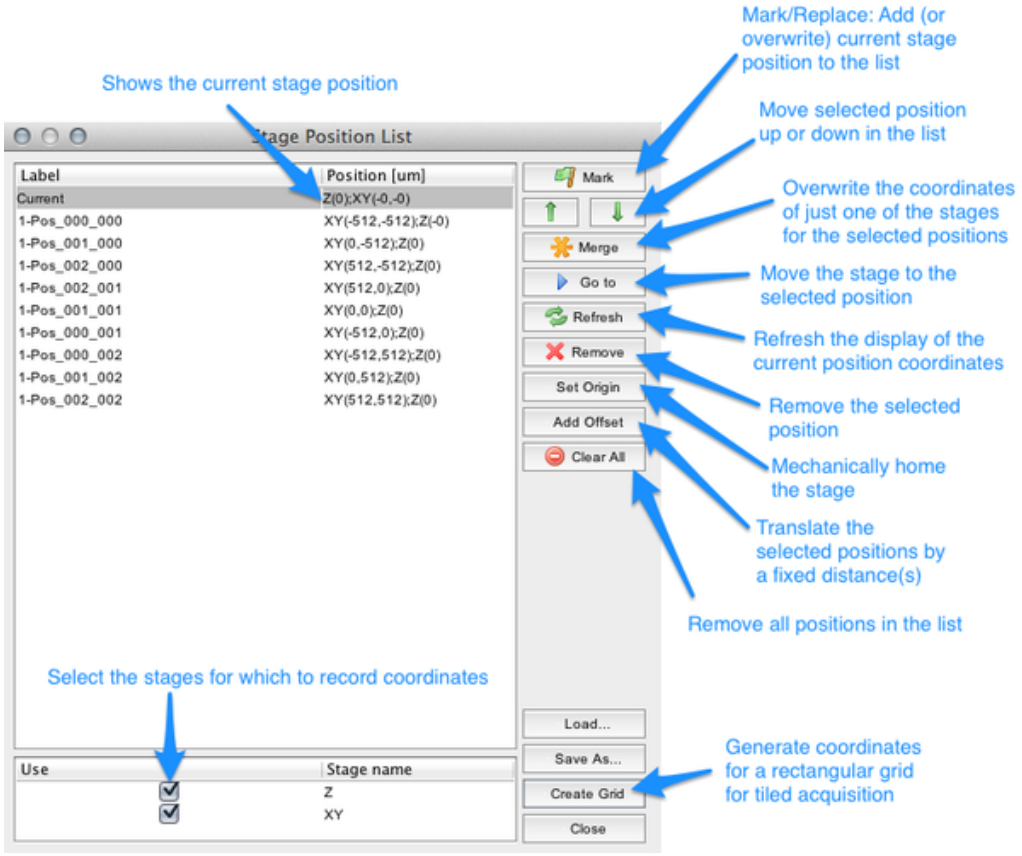


Figure 10. Position List dialog.

Use the **Mark** button to record the current stage position for all stages you have selected to use. When a position is selected the button will be named **Replace** and pressing the button will overwrite the selected position. Positions can be de-selected by clicking on them.

You can revisit a site by selecting the position and pressing the **Go to** button. The **Refresh** button will update the stage positions shown in the **Current position** pane. You can change the name of each position.

Position lists can be saved (**Save As** button) and re-used later (**Load**). When saving and re-using position lists, you should make sure that the coordinate systems of your stages are reproducible between invocations of the program, possibly using the **Set Origin** button. This will drive all stages to their origin and set this as the base of their coordinate system. Be extremely careful when doing so, since it is possible to run your XY stage into one of your expensive objective lenses!

Tile Creator

The **Create Grid** button in the Position List Dialog will open the Tile Creator Dialog. The purpose of this dialog is to quickly create a position list covering the area of an object (larger than a single image) in the microscope specimen.

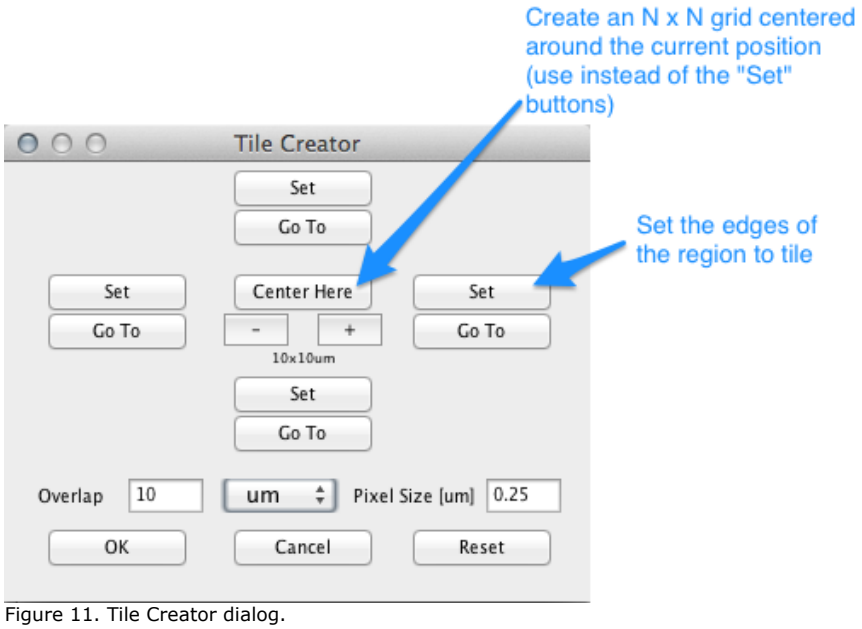


Figure 11. Tile Creator dialog.

Method 1: Use the **Set** buttons of the Tile Creator Dialog to mark at least two of the corners of the object that you are interested in. Pressing **OK** will generate a Position List that covers a bounding box around the corners that you set.

Method 2: Use the **+** and **-** buttons in the center to select the size of an N-by-N grid, then press **Center Here**. Then press **OK**.

For either method, the dialog needs to know the correct **Pixel Size**. If your system is **calibrated**, the correct pixel size will appear here automatically; otherwise you will have to enter the correct number. It is also possible to specify an **overlap** between the images generated from this position list (to specify a space between them, use a negative overlap).

Interactive Stage Movements

It can be cumbersome to exactly position a microscope stage. Micro-Manager has an option (**Tools | Mouse Moves Stage**) that might make this easier. To use this tools, your Micro-Manager configuration must be **calibrated**. When **Mouse Moves Stage** is enabled, double-clicking in the "Live" window (the window in which the "Snap" and "Live" buttons display images) will move the stage such that the place you double-clicked will move to the center. It is possible that the stage moves the wrong direction. If so, use the camera properties 'TransposeMirrorX', 'TransposeMirrorY', and 'TransposeXY' (accessible in the Device/Property browser) to achive the correct stage movement (you might want to save these settings in a 'System-Startup' configuration group). You can also move the stage by dragging the mouse through the image (a kind of 'Google Earth' effect).

When the "Live" window is open, you can also use the keyboard to move the stage (Mouse Moves Stage must be enabled):

Commands	**x,y modifiers**	**z modifiers**
+x: Right arrow	Normal: Move 10 pixels	Normal: Move 3 z-steps
-x: Left arrow	Control: Move 1 pixel	Control: Move 1 z-step
+y: Up arrow	Alt: Move half field of view	Shift: Move 10 z-steps
-y: Down arrow	Shift: Move whole field	
+z: 1, U, Page Up		
-z: 2, J, Page Down		

Multi-Dimensional Image Viewer

The Multi-Dimensional Image Viewer is an extension of the ImageJ HyperStack. Therefore, most ImageJ commands will work as expected on this window.

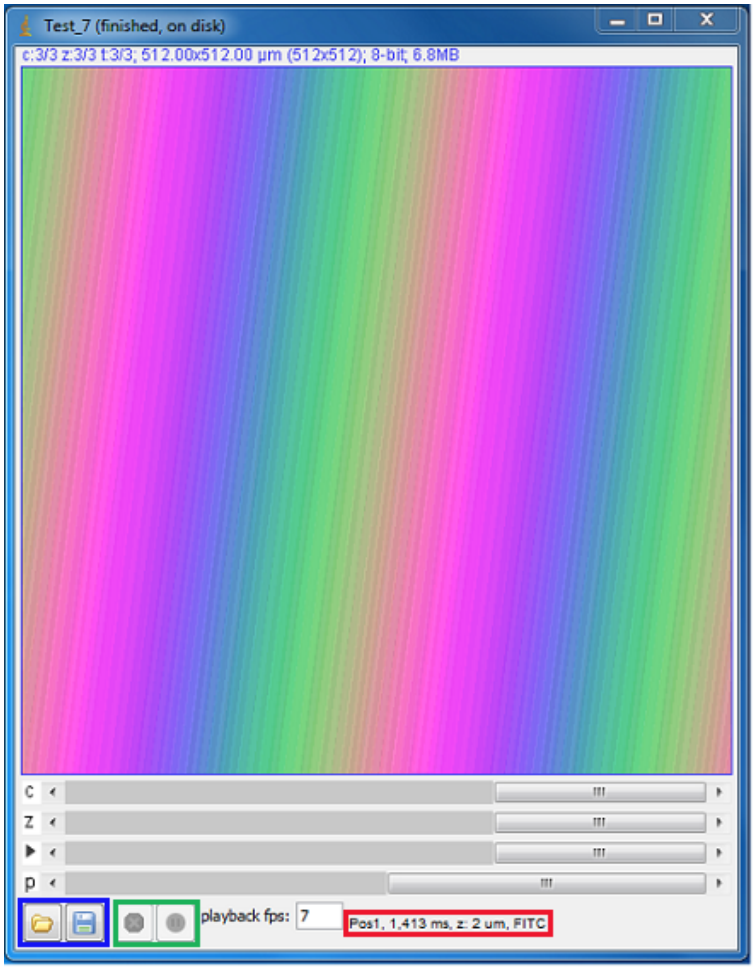


Figure 12. Acquisition data displayed in the 5D image window.

The viewer has sliders for all the dimensions you choose on the Multi-D Acq. which include: Channels, Z-slices, XY-Positions and Time points of the acquired images. You can playback Time Lapse sequences and adjust the speed using the text box (set in frames/second). The **red** box in Figure 12 shows where position, time, z-position, and channel for the current image are displayed. The buttons boxed in **green** allow you to Stop, Start and Pause during acquisition. Buttons boxed in **blue** allow you to open the folder in which the original files are located and also save the current image along with any adjustments to display brightness and contrast.

Channels, Metadata, and Comments

While using the 5D-Image Viewer the 'Histogram' section on the Main Window has three tabs for Channels, Metadata and Comments.

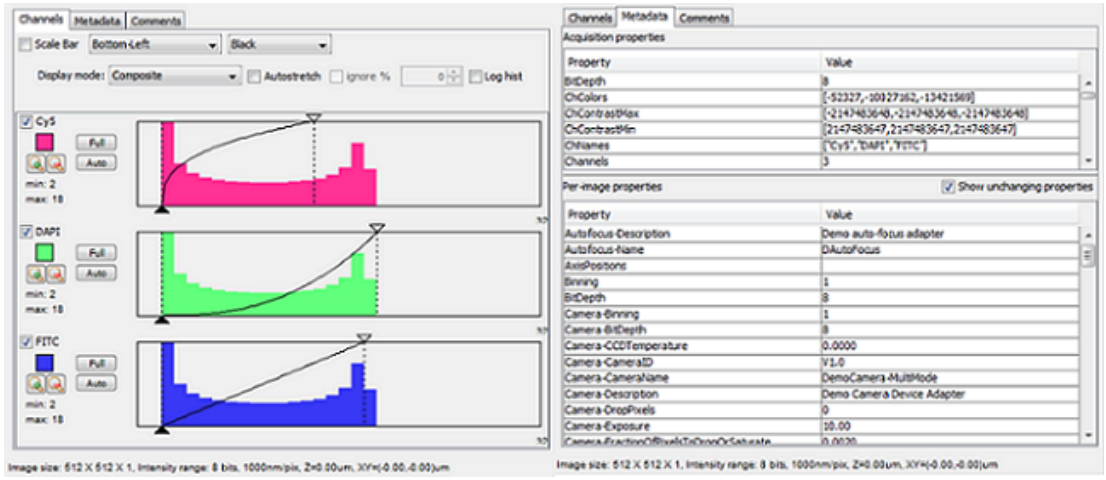


Figure 13. Channels and Metadata tabs for 5D-Image View in Main Window.

Channels

Under the **Channel** tab you are given various options which include:

- Scale Bar: You can choose to display a scale bar with a set position and color.

- Display Mode:
  - Composite: Displays image and histogram of all channels at the same time. You also have the option to turn off any channel by unchecking the box.
  - Color: Displays a single channel, depending on the position of the slider in the viewer.
  - Grayscale: Displays a grayscale image of a single channel, depending on the position of the slider in the viewer.
- Histogram of Each Channel:
  - You can choose to make adjustments a each channel as described in the **Histogram, Brightness and Contrast** section above.
  - The only difference is that "Autostretch" in this case makes adjustments to all channels while in composite mode.

Metadata

In the **Metadata** tab you will find a box of "Acquisition properties", which lists metadata common to the complete data set. Below are the "Per-image properties", which lists the complete state of the microscope system at the time the image was taken. Most of these will be the same for all images, you can hide these by un-checking the box that says "Show unchanging properties".

Comments

The **Comments** tab allows you to comment on the whole acquisition or an individual image. You can choose the image you would like to comment on by using the sliders in the 5D-Image Viewer. These comments will be automatically saved in the metadata and can be used to store your observations/annotations.

Files on Disk

Micro-Manager can save files in two formats, which referred to as "separate image files" and "Image file stack".

Separate image files

Acquired images are saved to disk as separate TIFF files, each containing a single grayscale image. The file naming convention is "img" prefix followed by frame number, channel name and slice number (img_00000000t_channel_00z.tif). In addition, the folder will contain a file named "metadata.txt" that contains the metadata in **JSON (http://www.json.org/)** format.

Image file stack

A TIFF file or group of TIFF files that contain multiple acquired images per a single file. These files conform to the **OME-TIFF (http://www.openmicroscopy.org/site/support/file-formats/ome-tiff)** specification, allowing them to be easily imported into a variety of analysis applications or anything that utilizes the **Bio-Formats importer (http://loci.wisc.edu/software/bio-formats)**.

Image file stacks are designed to be easily imported into ImageJ without the need for any special reader plugins. A stack file can be dragged onto the ImageJ toolbar and will automatically open as a hyperstack with the same contrast settings used in Micro-Manager. Any acquisition comments typed into the Multi-Dimensional Acquisition window or the comments tab of the main MM GUI can be viewed by pressing "i" with one of these files open in ImageJ.

By default, one file is created per an XY stage position (up to a maximum of 4 GB per file). In the tools-options menu, this can be changed to save all XY positions in a single file. This is especially useful for acquisitions using a large number XY positions. Since OME-TIFFs require that an identical String of XML metadata be embedded in each file in an acquisition, acquisition that have a large number of XY positions with a small amount of data at each waste space on disk by writing the same String of metadata in each file at the acquisition's conclusion.

Writing to these files results in faster performance than writing to **Seperate Image Files**, in part because it minizes the number of system calls to create new files. This can be advantageous in situations where disk write speed is a limiting factor (i.e. writing to a server or collecting data at a high rate).

More on File Formats...

Credits, License and Copyright

Micro-Manager is an Open Source software package for controlling automated microscopes on multiple platforms (Windows, Mac and Linux). The software is being developed in the **Vale Lab (http://valelab.ucsf.edu)** at the Unversity of California San Francisco and funding was provided by the Sandler Foundation and a grant from the NIH. The original software design was by Nenad Amodaj, and the software is currently developed by Arthur Edelstein and Nico Stuurman. Many individuals contributed source code or other types of help (including this documentation). The Micro-Manager source code is distributed under the BSD license for the user interface and the LGPL license for the MMCore (control module). Most supplied device drivers ('adapters') for cameras and other devices are covered by the BSD license. Copyright for some of the adapters is owned by other parties.

Updated for version 1.4.8, April 2012 (by Ziah Dean)
Updated for version 1.4.6, Sept. 2011 (by Ziah Dean)
Updated for version 1.3.40 on July 24, 2009.
Updated for version 1.3.22 (beta) on February 11, 2009
Updated for version 1.2.35 on August 23, 2008
Updated for version 1.0.61, on January 31
Updated for version 1.0.54 (beta) on December 4, 2006
Nenad Amodaj, April 2006