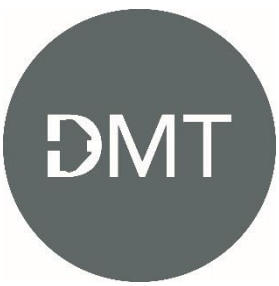
MYONORM MANUAL

USER GUIDE VOL. 1.0



MyoNORM Manual

DMT MyoNORM is a simple cross-platform (Win/Mac) application that simplifies the normalization procedure.

The Normalization procedure aims to determine for an artery mounted in a DMT myograph the internal circumference the artery would have when relaxed and under a given transmural pressure in-vivo. Normalization is the act of pre-stretching an intact vessel segment to an Internal circumference that produces a suitable physiologic resting transmural pressure for the experimental conditions being tested (i.e., it mimics the natural in vivo state of the vessel in terms of pressure and circumference). In short, this is a means of standardizing the vessel circumference in order to yield maximal responses to vasoactive agents and provide reproducible results.

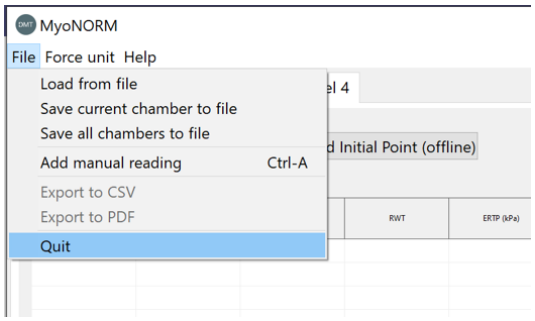
MyoNORM can be used in

a) Automated mode for the 620M, 720MO, 320A, 360CW, and 420A systems. In the automated mode, the force reading will automatically be transferred to MyoNORM, and only the micrometer position needs to be entered manually.
b) Manual mode for the 610M, 700MO, 310A, 410A systems or systems of other brands: The user must enter the micrometer reading and force manually. MyoNORM will calculate the optimal Internal circumference for a given mounted artery.

1. Menus

- File
- Force Unit
- Help

File menu



Load from file: Load a Normalization file save from an earlier experiment

Save current chamber to file: Save the normalization of the active channel

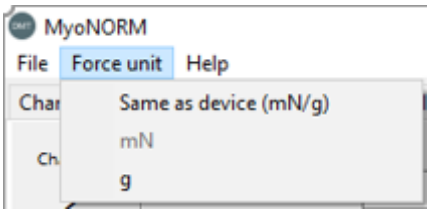
Save all chambers to file: Save all normalization data from all chambers to a file

Add manual reading: Gives the possibility to add a point.

Export to CSV: Export all data to a comma-separated file.

Export PDF: Export data and curves to a PDF file (see appendix A)

Force unit menu.



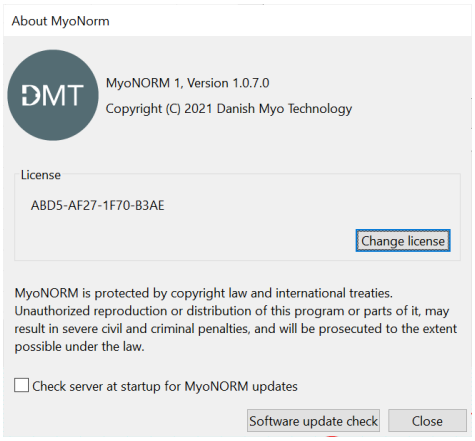
Same as device (mN/g): When the computer is connected to a myograph, it will automatically send the force to MyoNORM and use the force unit selected. Always select **Same As Device** if a myograph is connected to the computer with a USB cable.

> ***NOTE: The 'Same as Device' feature is ONLY possible for the following DMT myograph systems: 620M, 720MO, 320A, 360CW, 420A.***

mN: ONLY selected in the fully Manually mode of MyoNORM for older DMT systems, e.g., 610M, 700MO, 310A, 410A, 120CW, and non-DMT systems.

g: ONLY selected in the fully Manually mode of MyoNORM for older DMT systems, e.g., 610M, 700MO, 310A, 410A, 120CW, and non-DMT systems.

About menu



Here the License can be set, and the user can check for new MyoNORM updates.

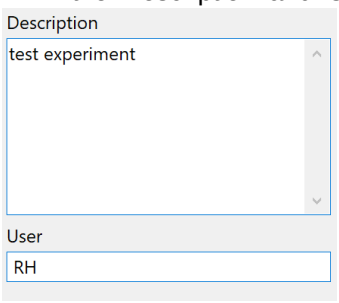
If the user wants to check for updates automatically tick mark the box 'Check server at startup for MyoNORM updates' mark the tick mark box

2. Setup and use of MyoNORM

1) Ensure the USB cable is connected to the Myograph interface for the DMT 620M, 720MO, 320A, 420A, and 360CW system.

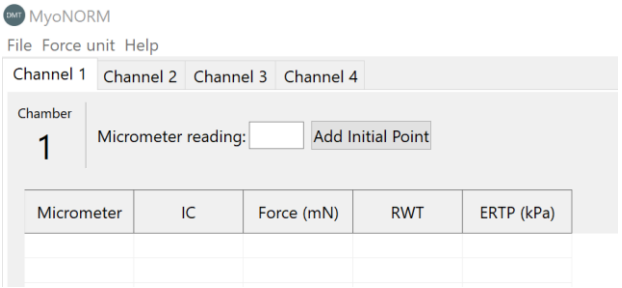
   NOTE: For Labchart Pro and DMT Device Enabler and Powerlab users, it is possible to run LabChart and the MyoNORM simultaneously getting the force trace data into LabChart and the normalization data into MyoNORM

2) Fill in the 'Description' and 'User'

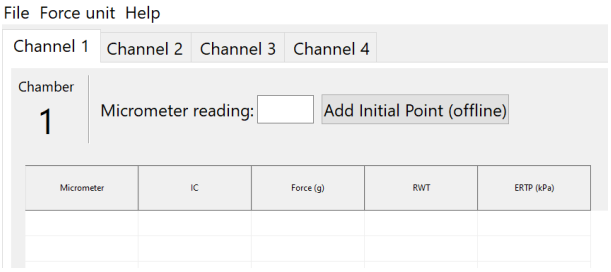


3) Select the Force Unit
4) MyoNORM will always show four Chambers, but depending on the DMT system connected, only the number of chambers of the system will be active on MyoNORM. Therefore, the normalization can be performed on up to four chambers simultaneously by clicking the different tabs. For example, if the one chamber 320A system is connected, only Chamber 1 will be active and the other three inactive. Select the chamber to normalize by clicking on the appropriate tab.

   Automated mode for DMT 620M, 720MO, 320A, 420A, and 360CW systems



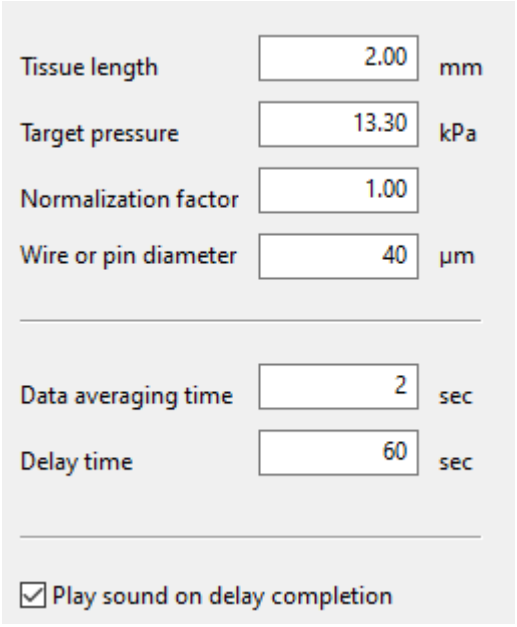
   Manual mode: Older DMT systems and non-DMT systems the 'Add Initial Point (offline)' will be shown



5) Enter the appropriate values for the selected chamber :
   a. **Tissue length** in mm
   b. **Target Pressure** in kPa. This is the normal mean blood pressure of the mounted artery. The default values of 13.3kPa correspond to 100mmHg. To convert mmHg to kPa, consult https://www.convertunits.com/from/mm%20Hg/to/kPa
   c. **Normalization factor**. The normalization factor has to be calculated using a combination of passive stretch and activation curve as described in the DMT Normalization Guide

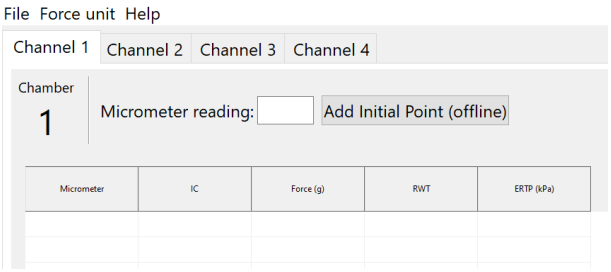
(https://www.dmt.dk/uploads/6/5/6/8/65689239/dmt_norm_guide_v2.1.41.pdf). The default value of 0.9 is for rat mesenteric arteries.

d. **Wire or pin diameter**. It is crucial to enter the correct dimension of the mounting wire or pin used to mount the tissue. DMT supply 40µm, 25µm, 15µm and 10µm wires and 200µm, 250µm, 300µm and 400µm mounting pins.
e. **Online averaging time**. The time in seconds MyoNORM will collect data from and calculate the average force values. For example, if the value is 2 seconds, MyoNORM will take all data 2 seconds before the **Delay Time** expire and calculate the mean force value.
f. **Delay Time**. The time it will take the force reading to stabilize after each stretch. It may vary a lot between different artery types. The default is 60 seconds and will be enough for most arteries.

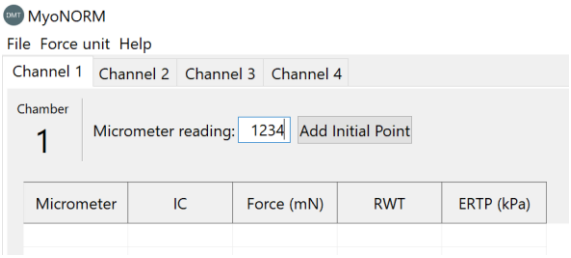


6) Make sure the mounting supports are as close together as possible without touching each other.
7) Zero the force of the appropriate chamber (Important the force is zero +/- 0.1mN or +/-0.01g).
8) Read the micrometer position, enter the number in the ‘Micrometer reading box, and press Add Initial Point. Consult **Appendix B** to learn how to read a micrometer positioner.

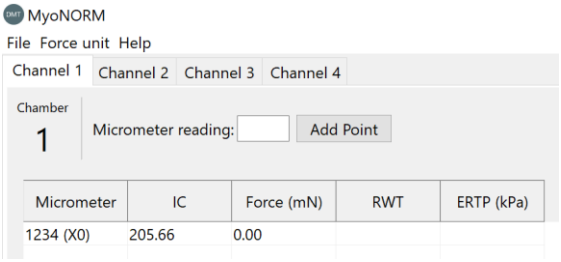
If MyoNORM does not recognize the system, it will show ‘Add initial Point (offline),’ and the user will have to enter all micrometer positions and force values manually



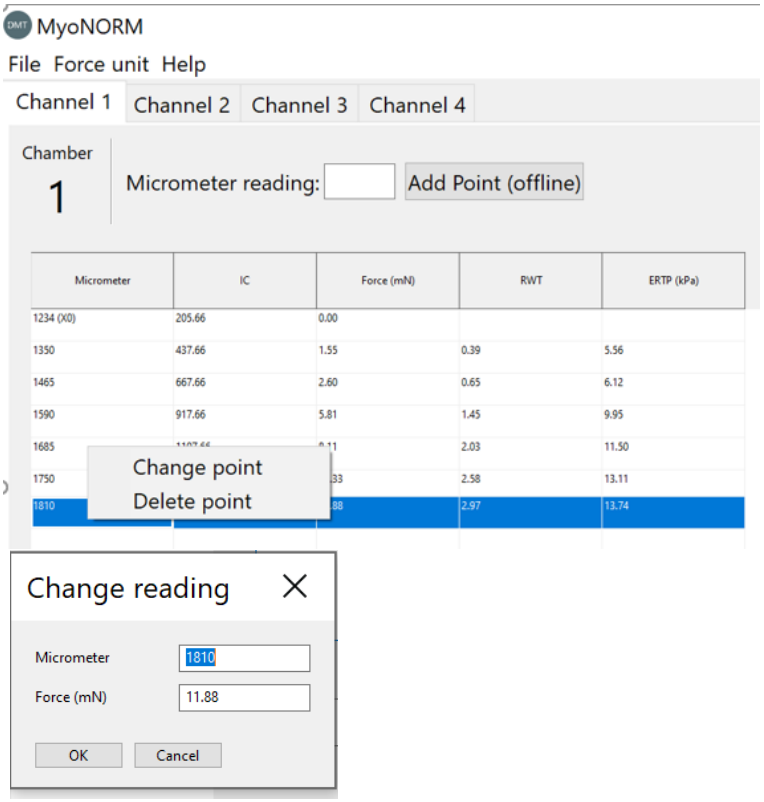
If MyoNORM has recognized the DMT system, the ‘offline’ will not be shown, and therefore MyoNORM will transfer the force automatically from the system.



9) After the Delay time, MyoNORM will enter the micrometer position, IC (Internal Circumference), Force, RWT, and ERTP (kPa) values as shown below.



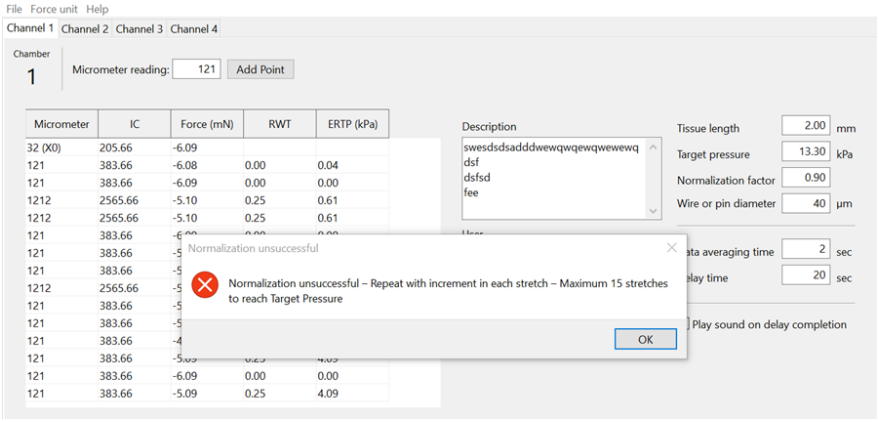
10) Make the first stretch by moving the micrometer positioner to a new position applying a small force to the mounted tissue. Next, enter the micrometer position in the Micrometer reading box and press ‘Add point.’
11) If a stretch/data point is entered wrong, it is possible to change the values or delete the point by marking the data point and right-click. A menu with ‘Change point’ and ‘Delete point’ will turn up. Clicking on ‘Change point’ will show the data for the given data point with the possibility to enter new values (see below)



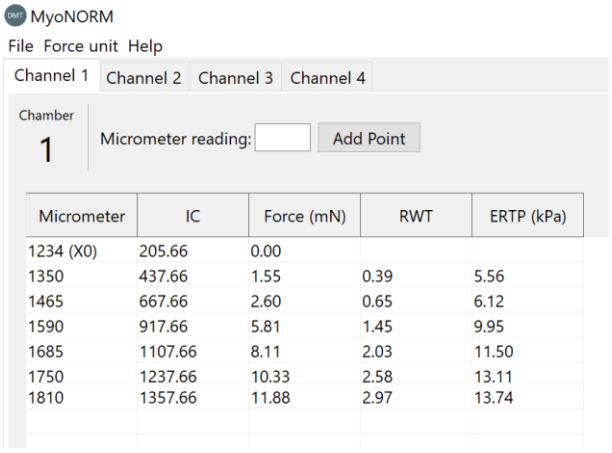
12) Repeat step 10 until the ERTP reaches the ‘Target Pressure.’

NOTE: It is important to have at least a minimum of four and a maximum of fifteen stretched before the target pressure is reached to obtain a good curve and good normalization calculation.

In cases where the target pressure is not reached after fifteen stretches, the information “**Normalization unsuccessful – Repeat with increment in each stretch – Maximum 15 stretches to reach Target Pressure”** will be shown below. The normalization has to be repeated with stronger stretches in each step.



When the ERTP (Effective Pressure) reaches the Target pressure (13.3kPa in this shown example), MyoNORM will return with text *Normalization successful. Go to micrometer position XXXX’



In the example below MyoNORM is successful and inform the user to set the micrometer positioner to position 1732 (red box)

13) In the example above, adjust the micrometer positioner to position 1732. The mounted tissue is now normalized and ready for your experiments.

⚠ ***WARNING: Do not overstretch the mounted artery during the Normalization. If the artery is over-stretched, the artery will be harmed and of no use for further investigations. It is important to stretch the artery until the ERTP value exceeds the Target pressure, BUT the ERTP should be very close but higher than the target pressure, as in Appendix A.***

Appendix A

MYONORM – NORMALIZATION REPORT EXAMPLE

DMT MyoNORM - Normalization report

Chamber: 1

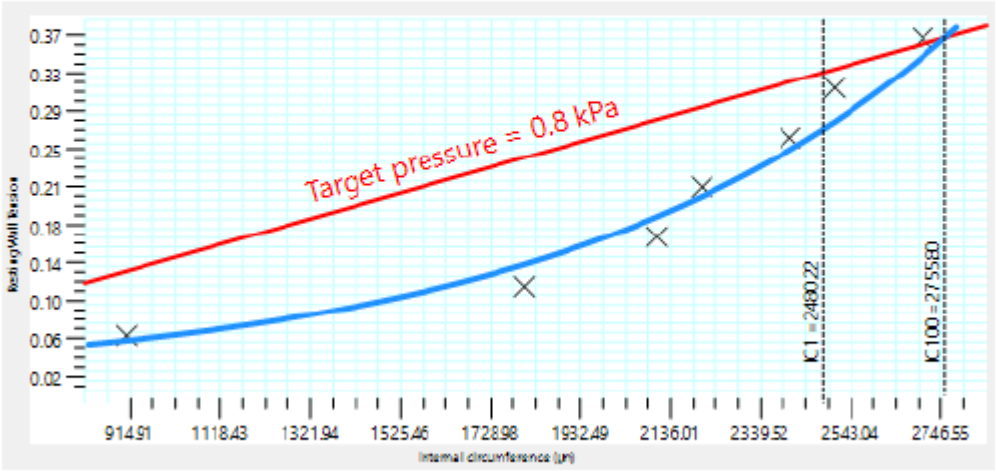
Normalization settings		Description	User
Tissue length (mm)	1.00		
Target pressure (kPa)	0.80		
Normalization factor	0.90		
Wire or pin diameter (µm)	40.00		

Micrometer readings

Micrometer	IC	Force (mN)	Resting Wall Tension	ERTP
8050 (X0)	205.66			
8400	905.66	0.10	0.05	0.35
8850	1805.66	0.20	0.10	0.35
9000	2105.66	0.30	0.15	0.45
9050	2205.66	0.40	0.20	0.57
9150	2405.66	0.50	0.25	0.65
9200	2505.66	0.60	0.30	0.75
9300	2705.66	0.70	0.35	0.81

Normalization preload. Set micrometer to 9187

Graph



Normalization of Mouse Portal vein. Work by Dr Naima Endesh in the Beech Group at Leeds, supported by Wellcome

APPENDIX B

READING A MICROMETER POSITIONER

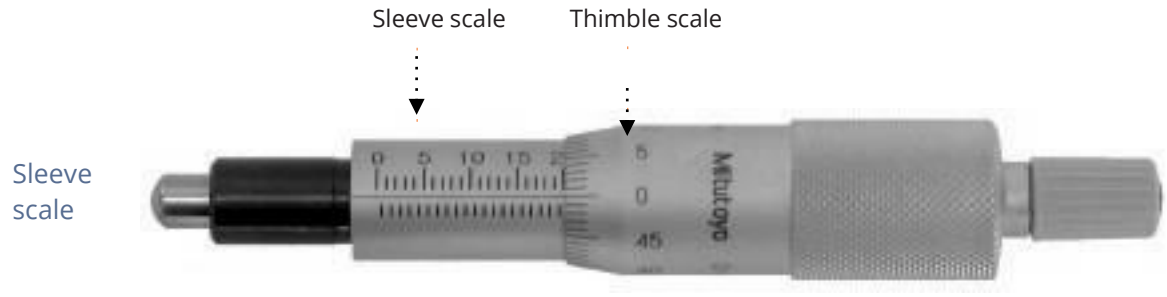


Figure A3.1 Overview of the micrometer parts (actual reading 20000 µm = 20 mm)

The micrometer sleeve scale has a total length of 25 mm divided into 50 equal parts. Each part of a division above the horizontal line represents 1 mm, where each 5th line is marked by a longer line and a number designating the length in mm. Each division below the horizontal line is placed between each 1 mm mark (scale above the horizontal line) and represents 0.5 mm.

Thimble scale

The thimble is divided into 50 equal parts, and one complete rotation of the thimble is indicated by the smallest division on the sleeve, which equals 0.5 mm. Each division on the thimble scale is 10 µm. If the thimble scale falls between two lines, then a number between 0 and 10 µm must be approximated.

Example 1

1. Note that the thimble has stopped at a point beyond “10” on the sleeve indicating 10000 µm (10 mm).
2. Note that there is no mark completely visible between the 10 mm mark and the thimble.
3. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

A.	Reading on sleeve:	10000µm
B.	No additional mark visible:	0µm
C.	Thimble reading:	380µm
	Total reading:	10380µm

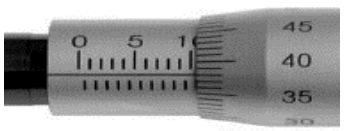


Figure A2.2 Example 1: reading = 10380um

Example 2

Note that the thimble has stopped at a point beyond “16” on the sleeve indicating 16000 µm (16 mm).

1. Note that this time a mark is visible between the 16 mm mark and the thimble indication 500 µm.
2. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve

A. Reading on sleeve: 16000μm

D. No additional mark visible: 500μm

E. Thimble reading: 280μm

Total reading: 16780μm

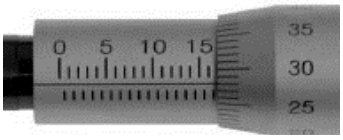


Figure A2.3 Example 2: reading = 16780um