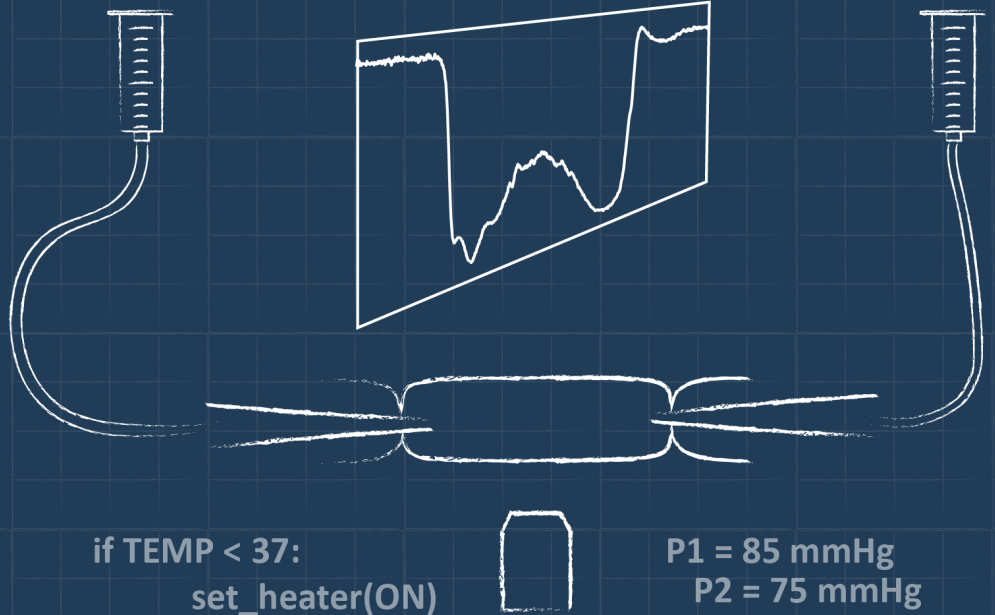


VASOTRACKER 2

BLOOD VESSEL DIAMETER
MEASUREMENT SOFTWARE



VasoTracker 2

Data Acquisition & Blood Vessel Diameter Analysis Software User Manual

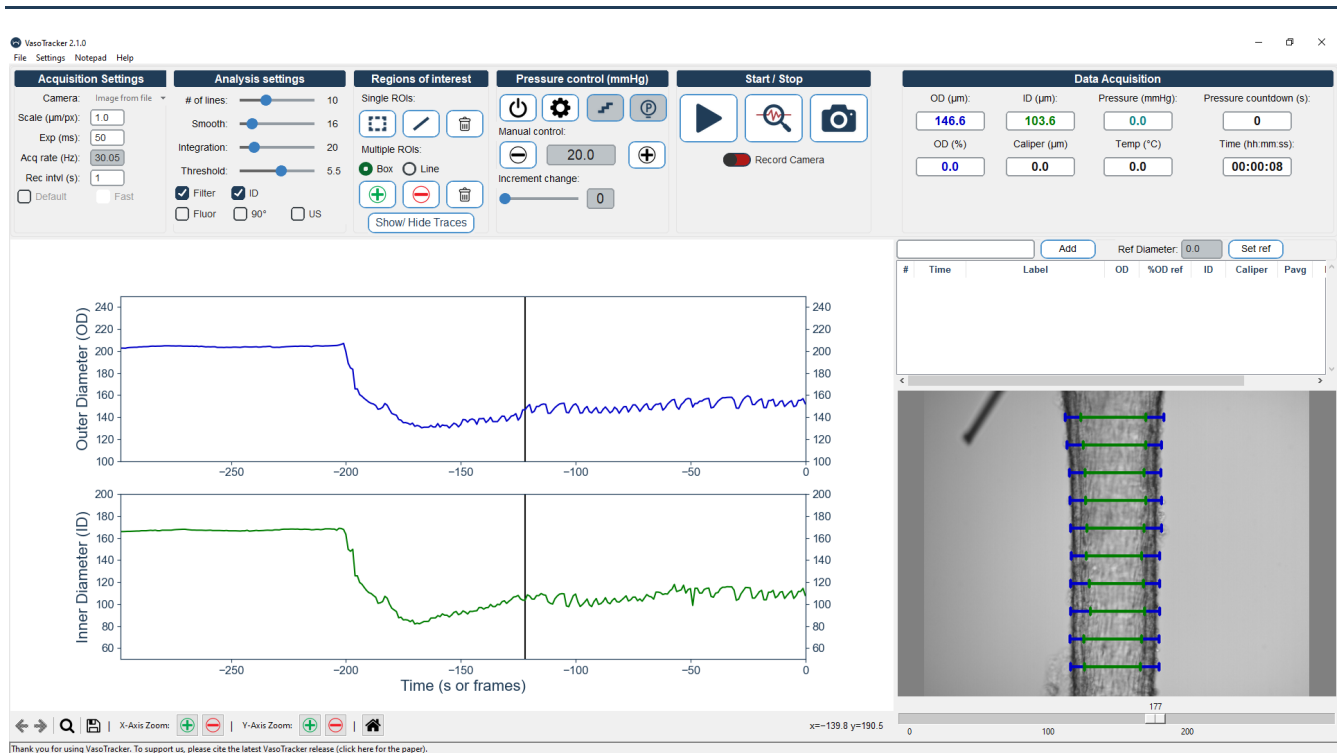
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Overview



The VasoTracker 2.0 Data Acquisition & Analysis Software

VasoTracker 2.0 is an open-source, Python-based software suite developed for tracking blood vessel diameter in pressure myography experiments. When paired with the VasoTracker Pressure Myograph Bath Chamber, VasoMoto Pressure Servo Controller, and Pressure & Temperature Monitor, it provides real-time vessel tracking, environmental monitoring, and robust analysis tools. VasoTracker was written by vascular physiologists for vascular physiologists. It does exactly what it needs to do—and does it well.

Key Features

- **Real-Time Diameter Tracking:** Track vessel diameter in real-time during live recordings or from pre-recorded images.
- **Offline analysis:** Track vessel diameter in pre-recorded images.
- **Multiple tracking algorithms:** Track outer and inner diameters in brightfield and fluorescence images.
- **Selective Tracking Options:**
 - Define multiple regions-of-interest (ROIs) for targeted edge detection.
 - Utilize user-defined scan lines for focused tracking.
- **Outlier Detection and Filtering:** Integrated filtering options to ensure data accuracy by detecting and excluding outliers.
- **Environmental Monitoring:**
 - Real-time pressure and temperature monitoring.
 - Automated pressure control using the open source VasoMoto Pressure Servo Controller (or commercial alternatives).
- **Event Marking and Tracking:** Record and track experimental events for precise data analysis.
- **Python 3-Based Software:** Developed in Python 3 for robust performance and extensibility.
- **µManager 2.0 Compatibility:** Fully compatible with µManager 2.0, ensuring seamless integration and control.

Citing VasoTracker

VasoTracker began life with the support of the Wellcome Trust and the British Heart Foundation to facilitate research on blood vessel function. Our ability to continue supporting, developing, and maintaining VasoTracker depends on further grant funding. If you use VasoTracker in any way, please cite VasoTracker in your scientific publications. For citation details, please visit:

<https://vasotracker.com/publications/>

Installation

Hardware Requirements



VasoTracker is only compatible with 64-bit Windows operating systems (well, we have only tested it on 64-bit operating systems).

- Microsoft Windows 10 64-bit
- 8GB RAM minimum (preferably 16GB)
- Compatible USB camera (see below)

Prerequisites

1. Install uManager:

- Visit the [uManager Downloads Page](#) and download the latest nightly build of uManager for your operating system.
- Follow the provided instructions to install uManager on your computer.

Steps

1. Download the latest VasoTracker release:

- Visit the [VasoTracker Releases Page](#) and download the latest zip file for your operating system.

2. Extract the Zip File:

- Locate the downloaded zip file on your computer.
- Right-click the file and select "Extract All..." or use your preferred extraction software.
- Choose a destination folder to extract the files and confirm the action.
- You should probably move the "vasotracker_2_0" folder to your C: drive.

3. Run VasoTracker 2.0

4. Navigate to the extracted folder.

5. Double-click the executable file to start the application.

Running from Source Code or Editing VasoTracker

VasoTracker is written entirely in the Python programming language. Anyone may edit it in any way they see fit.

[Full details for source code installation are on the VasoTracker 2.0 GitHub Repository](#)

Camera & System Setup

Basler Cameras

Basler Ace 2 (a2A1920-160umBAS)

- Download and Install Basler Pylon 7.1
- Configure uManager to use the camera and save the configuration file as “Basler.cfg”
- Replace the configuration file in the VasoTracker installation directory (this is mandatory, as VasoTracker must know the serial number of your camera).

Thorlabs Cameras

Zelux CMOS Camera (CS165MU/M)

1. Download and Install Thorcam 3.7.0
2. Follow the instructions on the µManager Thorlabs Scientific Imaging Cameras driver webpage (copying/pasting files is required here).
3. If installed correctly and working in µManager, VasoTracker will automatically connect when the camera is selected.

[Obsolete] CMOS Camera (DCC1545M)

1. Download and Install Thorcam 3.7.0
2. Follow the instructions on the µManager Thorlabs Scientific Imaging Cameras driver webpage (copying/pasting files is required here).

Any other µManager compatible camera

1. Download and install any required software/drivers.
2. Set up the camera in µManager
3. Create a µManager System Configuration with Startup and Shutdown Presets to set any specific settings required by your camera (VasoTracker will only control the exposure) - Follow the instructions here
4. Save your configuration file as “MMConfig.cfg”
5. Replace the configuration file in the VasoTracker Installation directory (VasoTracker will not be able to control your camera if you fail to do this).

Running VasoTracker

Setting up a VasoTracker shortcut

To set up a desktop shortcut:

- Navigate to the VasoTracker installation folder on your computer.
- Right click on the VasoTracker.exe
- Click “Send to”
- Click “Desktop”
- This action creates a desktop shortcut to the file or folder on your desktop.
- VasoTracker will run in administrator mode (this ensures VasoTracker can save data files wherever you specify).

Maximizing VasoTracker performance

We run VasoTracker on several different computer systems, on our less-able computers the following actions help performance:

- [Using the “High Performance” Windows 10 power plan](#)
- [Disabling Windows USB selective suspend](#)
- [Preventing Windows shutting off power to USB root hub](#)

Starting the VasoTracker Data Acquisition Software

VasoTracker can be launched by any of the following methods:

- Double-clicking the desktop shortcut (created above).
- Double-clicking VasoTracker.exe in the installation folder
- **From source code:** Running “vasotracker_2_0.py” from the python command line.

VasoTracker defaults to be run as administrator in order to save files. Upon launching the executable, Windows will ask the following:

Do you want to allow this app from an unknown publisher to make changes to your device?

Please click "Yes" to proceed and allow VasoTracker to launch.



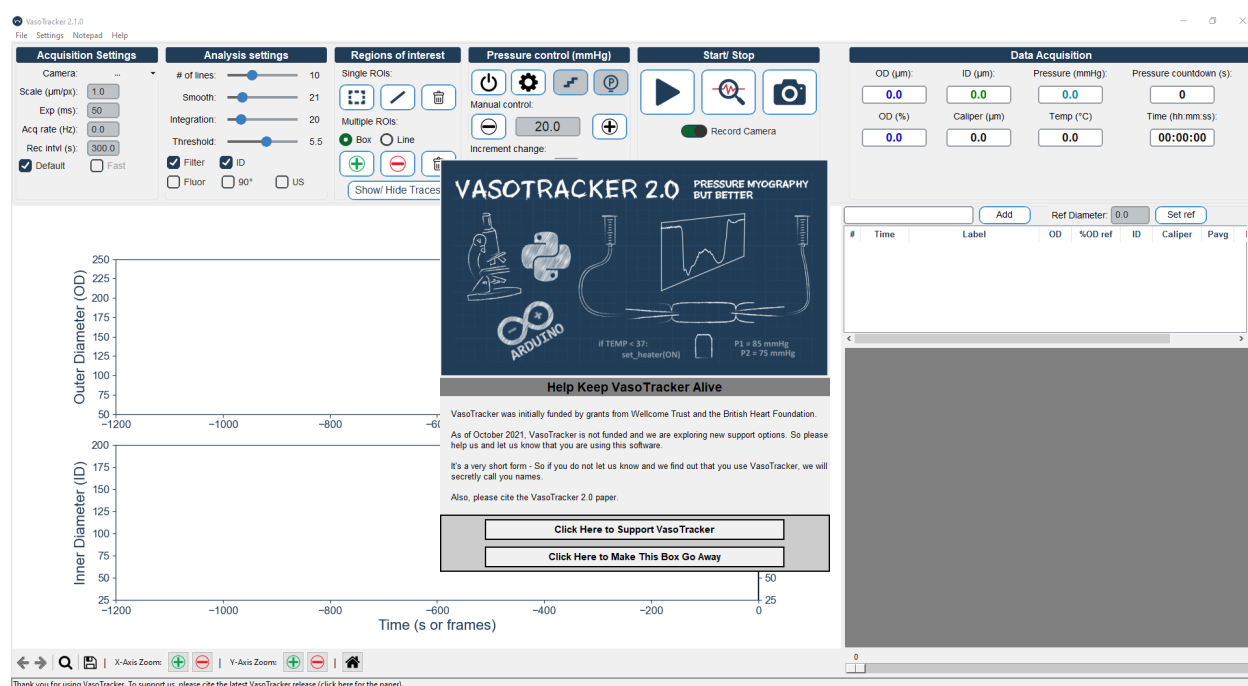
Note that signing applications can be quite expensive, which is why VasoTracker is currently not signed with a publisher certificate. We appreciate your understanding and assure you that the software is safe to use. But we also accept no liability.

Registering Your VasoTracker Installation

Upon launching, the VasoTracker software will prompt you to register your use by filling in a brief User Registration Form. Clicking the “Click Here to Support VasoTracker” button will open up the [VasoTracker User Registration Form](#) on Microsoft Forms in your web browser. Please fill this in.

This registration helps us track how many people use VasoTracker, and for what purposes, which is valuable for reporting to potential funders. If the computer on which you install VasoTracker does not have internet access, please register your use here:

VasoTracker User Registration Form



VasoTracker User Registration Screen

Analysing a Pre-recorded Dataset

VasoTracker 2.0 includes an analysis mode that lets users analyse pre-recorded datasets, including bundled example data (in the **SampleData** folder). To familiarize yourself with the software, you can analyse this sample data as follows:

1. Open Analysis Mode

- Go to the **File** menu and select **Analyse File**.

2. Confirm Analysis

- Click **Yes** in the confirmation box to proceed.

3. Select Sample Data

- Navigate to the **SampleData** folder within the VasoTracker installation directory, and open one of the available sample files.

4. Save Results

- Choose a location to save the analysed data. By default, VasoTracker will create a new folder within the **Results** folder located in the default Windows "Documents" directory. Click **OK** to confirm and begin processing the file with default settings.

5. Explore and Adjust Analysis Settings

- To explore the software's full functionality, try adjusting the analysis settings and reanalysing the dataset by clicking the **Track** button.

6. Manage Multiple Results

- Each new analysis overwrites previous results. To save different versions for comparison, create a new save file by selecting **File -> New File**.



In analysis mode, VasoTracker will run using default settings and will automatically adjust the graph axis limits to display the resulting trace. Default settings can be changed by editing the ".toml" file in the installation folder.

Live Mode

To use VasoTracker in live mode (acquiring images directly from a camera), follow these steps:

1. Load Camera Driver

- In **Acquisition Settings panel** select the appropriate camera driver for your setup.

2. Start the Camera

- Click the **Start Camera** button in the **Start/Stop panel** to begin acquiring images.

3. Begin Data Acquisition

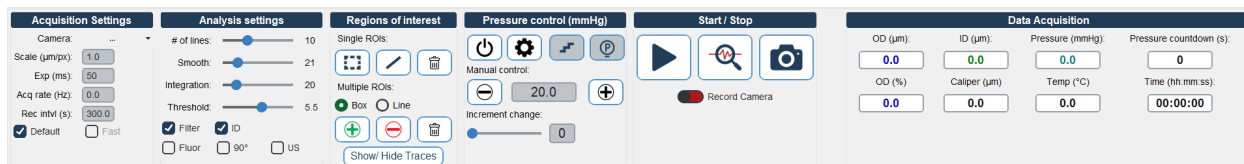
- Start a new experiment by selecting **File -> New File** and confirming.
- Click the **Track** button in the **Start/Stop panel** to begin tracking diameter.
- By default, outer and inner diameter will be plotted automatically in the **Graph Panel**.
- To ensure accurate tracking, adjust the **Analysis Settings** as needed.
- If required, limit tracking to specific regions (up to 5) by defining ROIs using the tools in the **ROI panel**.
- Add details to the **Data Entry Table** (e.g. drug additions) to record information and add cursors to the traces.

4. Ending an Experiment

- VasoTracker continuously saves data, so you can simply exit the programme when an experiment is finished.
- To start a new experiment, reset the data by selecting **File -> New File** and then confirming. Ensure you create a new file to avoid overwriting previous data (VasoTracker automatically generates filenames to help prevent this).

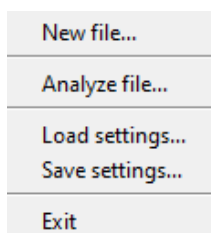
VasoTracker Settings

All VasoTracker acquisition and analysis settings can be altered in the **Menu Bar** and the **Settings Bar**. The settings bar also displays some handy information. Default settings can be changed by editing “default_settings.ini” in the root folder.



The Menu Bar

File Menu



New File: Creates a new experiment file to record data for a session.

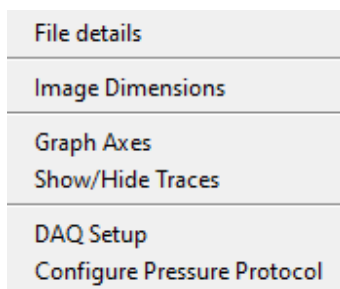
Analyse File: Opens a pre-recorded dataset for analysis, allowing you to test settings or analyse experimental data

Load Settings: Loads previously saved settings configurations to quickly set up common parameters for experiments.

Save Settings: Saves the current settings configurations, including camera, analysis parameters, and tracking options, to use for future experiments.

Exit: Closes the VasoTracker application.

Setting Menu



File Details: Displays information about the files in use..

Image Dimensions: Displays information about the current camera.

Graph Axes: Opens a new window to adjust the graph axes, allowing precise customization of axis scales, as an alternative to using the zoom buttons below the graph.

Show/Hide Traces: Provides options to show or hide traces for individual ROIs on the graph.

DAQ Setup: Opens a new window to configure Data Acquisition (DAQ) settings (e.g. National Instruments Device and Channel). Used when controlling a commercial Pressure Servo system.

Configure Pressure Protocol: Enables setup of specific pressure protocols for experiments utilising a Pressure Servo (VasoMoto or commercial alternative).

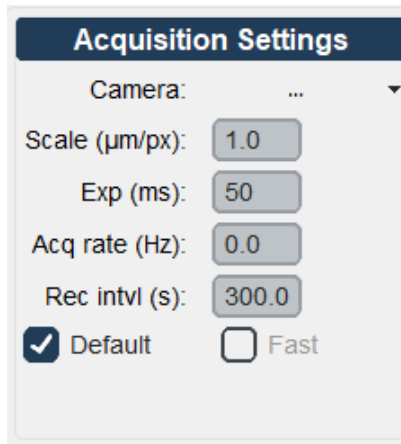
Notepad (available after starting an experiment)

Open Notepad: Opens a text file, allowing users to record additional details.

Help Menu: Contains link to various useful resources.

The Settings Bar

Acquisition Settings Control Panel



The Acquisition Settings Control Panel is a window with a dark blue header labeled 'Acquisition Settings'. It contains several settings: 'Camera:' with a dropdown menu showing '...'; 'Scale (µm/px):' with a text input field containing '1.0'; 'Exp (ms):' with a text input field containing '50'; 'Acq rate (Hz):' with a text input field containing '0.0'; 'Rec intvl (s):' with a text input field containing '300.0'; and two radio buttons at the bottom, 'Default' (which is selected with a checkmark) and 'Fast'.

Camera: Select your camera here. For non-standard cameras, select **µManagerCam** (requires a µManager config file in the VasoTracker installation folder).

Scale: Set the image calibration factor here; a default value of 1 outputs diameter values in pixel units.

Exp (ms): Adjust the camera exposure time here, in milliseconds.

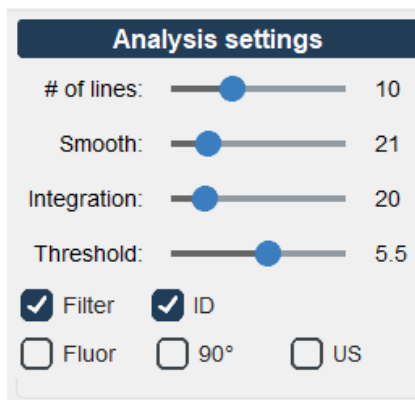
Acq rate (Hz): Displays the current acquisition rate.

Rec intvl (s): Set the interval (in seconds) for exporting images.

Default: Uncheck to enable custom editing of acquisition settings; displays the current acquisition rate.

Fast: Enable this option to set VasoTracker to run at maximum speed (experimental feature).

Analysis Settings Control Panel



The Analysis Settings Control Panel is a window with a dark blue header labeled 'Analysis settings'. It contains several settings: '# of lines:' with a slider ranging from 10 to 21; 'Smooth:' with a slider ranging from 21 to 20; 'Integration:' with a slider ranging from 20 to 5.5; and 'Threshold:' with a slider ranging from 5.5 to 10. At the bottom, there are four checkboxes: 'Filter' (checked), 'ID' (checked), 'Fluor' (unchecked), and '90°' (unchecked). There is also an unchecked checkbox labeled 'US'.

of lines: Set the number of scan lines used for tracking vessel diameter.

Smooth: Adjust the smoothing level for intensity profiles to enhance wall detection.

Integration: Specify the number of pixel lines over which diameter values are integrated.

Threshold: Set the threshold value to identify and exclude outliers in the data.

Filter: Toggle on/off to enable or disable outlier filtering.

ROI: This feature currently disabled.

ID: Toggle on/off to enable or disable detection of the inner diameter

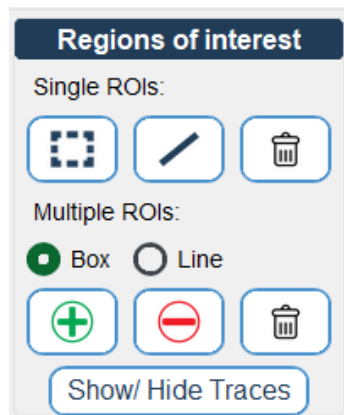
Fluor: Toggle on/off to activate a slight modification to the tracking algorithm for fluorescence imaging.

90°: Toggle to rotate the scan lines by 90 degrees.





After making adjustments to your acquisition and analysis settings, remember to save them to avoid losing your custom configurations. Use the **Save Settings** option in the File menu to store your preferences for future experiments. This allows for quick setup in subsequent sessions and ensures consistency in your measurements.

ROI Control Panel



ROI Section (for defining single ROIs)


 **ROI Button:** Click this button to pause image acquisition, enabling you to draw a Region of Interest (ROI) directly on the Camera Window for targeted analysis.

 **Caliper Button:** Click to pause the acquisition and manually draw a line for measuring distance

 **Delete Button:** Click to delete the last ROI.

Multi Section (for defining multiple ROIs)

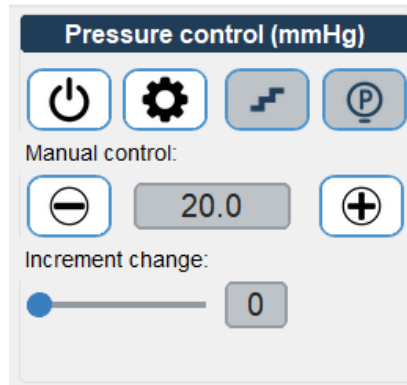
☒ Rect ☐ Line **Radio Selection:** Choose between drawing rectangular Regions of Interest (ROIs) or custom scan lines.


 **Add Button:** Click this button to pause image acquisition, enabling you to draw a Region of Interest (ROI).

 **Remove Button:** Click to delete the last drawn ROI.


 **Delete Button:** Click to delete all multi-ROIs.

Pressure Control Panel



 **Connect Button:** Click this button to open the DAQ Setup menu and configure the settings.

 **Start Protocol Button:** Click to begin an experimental pressure protocol (defined in the *Pressure Protocol Menu*)

 **Pressure Button:** Click to set the experimental pressure to that defined under *Manual Control*.

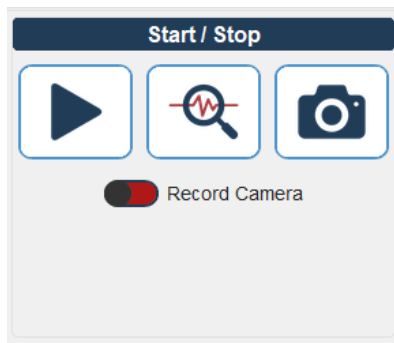
Settings Button: Click this button to open the *Pressure Protocol Menu* and configure the settings.

Manual Control: Edit using the buttons/slider to set the desired experimental pressure. Confirm using the *Apply Pressure Button*.



The Pressure Control Panel will be visible only if National Instruments software is installed on your computer.

Start/Stop Control Panel



► **Start Camera Button:** Click this button to initiate or pause image acquisition from the camera

🔍 **Track Button:** Click to begin or pause the tracking of vessel diameter in real time.

📷 **Snapshot Button:** Click to capture and save the current frame from the live image feed as an image file.

Record Camera: Enable to save images (with/without diameter tracking overlays) at intervals defined in *Acquisition Settings*.

Data Acquisition Panel

The image shows a software interface titled "Data Acquisition". It contains eight input fields arranged in two rows of four. The first row fields are labeled "OD (µm):", "ID (µm):", "Pressure (mmHg):", and "Pressure countdown (s):". The second row fields are labeled "OD (%)", "Caliper (µm)", "Temp (°C)", and "Time (hh:mm:ss):". The values displayed in the fields are 0.0, 0.0, 0.0, 0, 0.0, 0.0, 0.0, and 00:00:00 respectively.

OD (µm): Displays the current average outer diameter measurement in micrometres.

ID (µm): Displays the current average inner diameter measurement in micrometres.

Pressure (mmHg): Indicates the current experimental pressure value in millimetres of mercury, the average pressure value from the two VasoTracker Pressure Monitor sensors.

Pressure Countdown (s): Displays the countdown timer for the current pressure protocol.

OD (%): Shows the current outer diameter as a percentage of the reference, allowing for easy reference.

Calliper (µm): Displays the length of the manual calliper tool in micrometres.

Temp (°C): Indicates the current temperature measurement in micrometres, measured using the VasoTracker Pressure Monitor.



The Data Acquisition Panel will display zero by default if transducers or other measurement tools (e.g. manual callipers, inner diameter detection) are not connected or in use.

Data Management & Export Features

Data export

All experimental data is saved automatically to the output file defined at the beginning of each experiment. After selecting the output CSV file and starting an experiment, all relevant parameters - such as time, average diameter, pressure, temperature, and more - are continuously appended to the CSV file. This ensures that all data collected during the experiment is easily accessible for further analysis.

Individual scanline measurements:

The outer and inner diameter measurements for all scan lines are stored in a single column within the output CSV files. To access the data from individual scan lines, you can separate the measurements by using the "Text to Columns" function in Microsoft Excel.

Spurious measurements:

Whether or not the filtering algorithm is active, all scanline measurements are checked for spurious results. Indicators are also saved in the output csv file (1 indicates a good measurement, 0 indicates a spurious measurement). If the filtering algorithm is activated, spurious measurements are not included in the average calculations.

Data entry table

VasoTracker also includes a data entry table. The table allows the user to enter details of any experimental manipulations and provides a convenient measure of arterial tone at the time of each entry. Any data entered into the Data Entry Table (See below) is saved as an additional .csv file, "...table.csv".

PE			Add		Ref Diameter: 0.0		Set ref		
#	Time	Label	OD	%OD ref	ID	Caliper	Pavg	P1	
1	00:00:06	PE	193.6	-	141.7	0.0			

The Data Entry Table

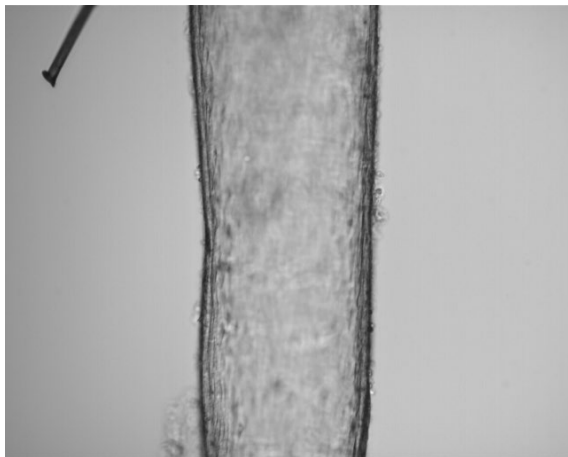
Image Export

Snapshots

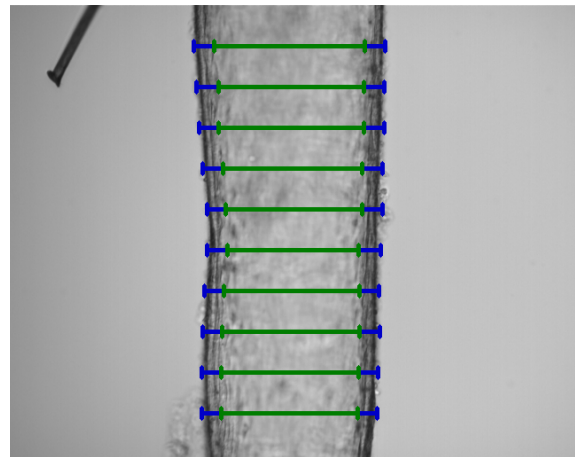
VasoTracker allows users to capture snapshots of the current camera feed. These images can be saved manually by clicking the Snapshot button, enabling users to document specific moments during experiments for further analysis or presentation. All snapshots are saved in a separate **Snapshot** folder within the output directory, and each snapshot includes diameter indicators for easy reference.

Experimental Recordings

When the "Record Camera" function is enabled, VasoTracker automatically saves multi-page TIFF images at intervals defined by the **Rec Intvl (s)** setting. This feature creates two files for each recording: one with diameter indicators overlaid and another without. This dual-file approach ensures that users have access to both raw images and annotated data.



Example Image



Example Image with Diameter Indicators



www.vasotracker.com