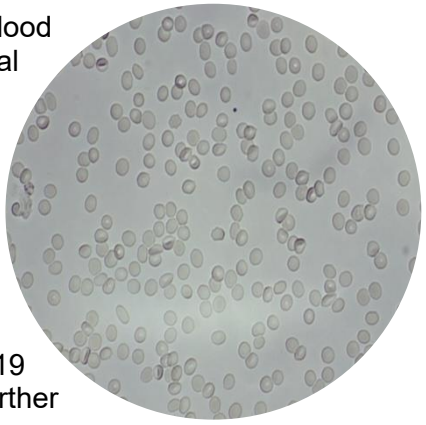
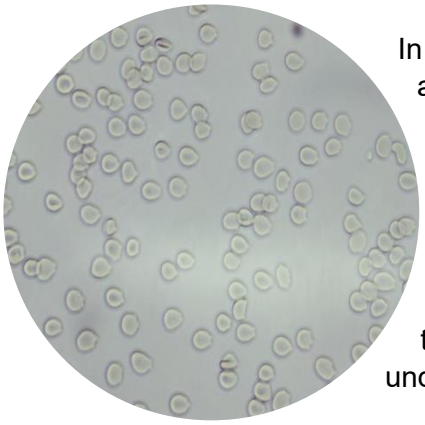
ASSESSING ERYTHROCYTE ELONGATION UNDER FLOW USING THE “FLOW & PATCH” CHAMBER AND AI-BASED IMAGE ANALYSIS



Flow induced erythrocyte deformation and stiffness is a key determinant of blood rheology and plays a crucial role in numerous physiological and pathological processes. Although simply described as a cellular resistance to shape change (stiffness), deformability is inherently complex and challenging to define with precision. As a biomechanical cellular property, it is most commonly characterized using physical methods that examine the red blood cell’s (RBC) response to controlled deforming shear forces. Impaired RBC deformability is often associated with a wide range of disorders, including sickle cell anemia, diabetes mellitus, malaria, sepsis, and ischemia-reperfusion injury, possibly even serving as an early indicator or contributing factor to organ dysfunction. Recent studies have also shown that COVID-19 infection may have an adverse affect on the mechanical properties of RBCs, further contributing to microvascular complications in COVID patients.



In this application note, we introduce a novel measurement system that assesses RBC deformability by analysing their elongation under controlled shear stress. The system captures high-resolution video of RBCs once at steady state (low-flow or no-flow) and once during exposure to elevated flow. These recordings are then processed and evaluated by an artificial intelligence (AI) platform to extract shape parameters and determine erythrocyte elongation, leading to a robust and reproducible determination of RBC mechanical properties. The “Flow and Patch” chamber provides the user with a biomimetic approach to studying red blood cell mechanics under physiologically relevant shear stress.

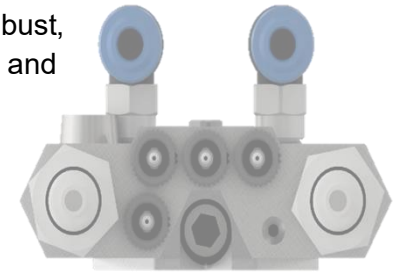
Keywords: Erythrocyte deformation, RBC elongation, RBC stiffness, elongation-index, artificial intelligence, flow chamber, shear stress

Advantages of the “Flow & Patch” chamber

- Fast analysis with a low-volume blood sample (finger prick)
- One platform for all: Experiment and evaluation under one software
- Non-invasive, label-free, time and cost effective
- Direct chemical addition via flow system without pre-incubation (physiologically relevant)
- Real-time AI evaluation with user feedback, interactive image review and correction
- Continuous AI learning and improvement
- Reversibility test with a simple wash-out step
- RBC stiffness testing in patient´s autologous blood plasma

How to use the “Flow & Patch” system?

The **“Flow & Patch”** system offers a user-friendly platform for quantifying red blood cell (RBC) deformability (stiffness) through precise measurement of the elongation index under physiologically relevant, and also customizable flow conditions. By simulating microvascular shear stress within a controlled chamber, the system enables real-time imaging of RBCs as they respond to dynamic flow. High-resolution images captured during both low-flow and elevated-flow states are analysed using AI-based image processing, which uses key morphological parameters such as cell length and width. This approach eliminates the need for pre-incubation or labelling, and allows for real-time substance testing, closely mimicking in vivo conditions. With built-in user feedback and correction capabilities, the AI continually improves in accuracy, offering a robust, adaptive solution for assessing RBC mechanical properties in both research and clinical settings.



The graphic (right) provides a visual representation of the four supply inlets to the flow chamber (middle). The administration of the control and substance solutions is facilitated by the utilisation of these discrete channels, which enable the movement of fluids to the flow chamber. This configuration allows for the examination of up to three distinct substances in parallel on a single red blood cell monolayer, with a dedicated channel allocated for the cell buffer. The buffer solution that generates the shear flow in the flow chamber is supplied and removed, repectively via the two large upper connections. This setup supports both comparative testing and reversibility studies by using the buffer inlet for washing steps. Test solutions may include chemical agents or modified buffer solutions with altered pH, osmotic pressure, or O_2 partial pressure, offering experimental flexibility. Measurements can also be performed using autologous patient plasma instead of buffer, for patient-specific conditions. The system also features a precision thermostat, allowing measurements under physiological conditions (37 °C) with a temperature stability of ±0.2 °C. Should the experiment demand it, a range of temperatures can be set continuously in order to simulate pathological environments and investigate their effects on the mechanics of erythrocytes.

1. Required Materials

- **Flow & Patch Chamber**
- **Microscope Camera**
- **Microscope Slides**
- **Blood Sample***
- **Buffer/Test substance****

*** Haematocrit <5%, recommended 1%**

**** May vary depending on the specific experimental conditions**

2. Blood Sample Preparation

- **Blood Collection and Haematocrit Check**

  Blood is drawn according to standard laboratory procedures (capillary, venous, or arterial). The haematocrit value is determined (i.e. using a hematocrit centrifuge) to ensure precise dilution.

- **Plasma Removal and Resuspension**

  Blood is washed at least twice to remove plasma. After centrifugation at 1000g for 5 minutes, the plasma is carefully removed. The process is then repeated. Washed cells are gently resuspended in PBS (without human albumin) at room temperature. The PBS volume is adjusted based on the desired haematocrit.
  Recommended: 1% HCT for optimal results

3. Flow & Patch setup

- Install the software for **"RBC mechanics – Erythrocyte Elongation"** and ensure all devices are properly connected.
- Place the flow chamber base (without the top cover) onto the microscope stage.
- Launch the RBC mechanics software to begin the setup process.
- Position the microscope slide onto the base of the flow chamber.
- Apply a drop of the blood sample (~20 µL) to the left side of the slide. Then carefully cover the slide with the top part of the flow chamber and lock the top and bottom parts together to ensure leak-proof sealing together with a guaranteed reproducible flow channel hight.

4. Step-by-Step measurement

i. Heating

The software will run a temperature check. If the internal system temperature is at **37±0.2°C**, next step can be started. User is free to skip the heating step if they are using temperature as a trial parameter.

ii. Position Camera

Ensure that the appropriate connection for docking the camera to the microscope is available (BRESSER MikroCam SP 5.0 microscope camera has a C-mount). Attach the camera to the microscope. If necessary, adjust the camera slightly (adjust the screws forwards or backwards) to ensure horizontal alignment with the flow chamber (tested with a short PBS flushing flow).

iii. Place slide inside the chamber

Open the flow chamber and insert the slides in the desired orientation. Before closing the flow chamber, ensure that the seal is positioned correctly. Place the flow chamber back in the holder.

iv. Fill Channels

User can utilize this step to ensure the contents of all four medium reservoirs, and will then use the pumps manually to fill the active channels and displace any air bubbles.

v. RBC Monolayer

This step will let the user turn on the flow from their desired reservoir, and use the fluid in this reservoir to wash the blood drop placed onto the slide to create an RBC monolayer. The user is free choosing the active channel and the duration of flow before starting the experiment.

vi. **RBC Sedimentation**

The software will start a 20-min timer to let the RBCs to sediment and form a monolayer. If desired to wait shorter, they can end before the timer goes off and skip to the next step.

vii. **Detach Non-adherent Cells**

In this step, the software will start a 0,25 Pa flow to wash any unadhered erythrocytes from the objective slide, and keep a clean monolayer.

viii. **Flow 1**

The first measurement will be taken in this step **(low-flow conditions).** A flow of 0,25 Pa will be started for 6 seconds, and an image will be taken at second 5 which will be saved into the software.

ix. **Flow 2**

The second measurement will be taken in this step **(increased-flow conditions, customizable)**. A flow of 3 Pa will be started for 6 seconds, and an image will be taken at second 5. This image will also be saved into the software.

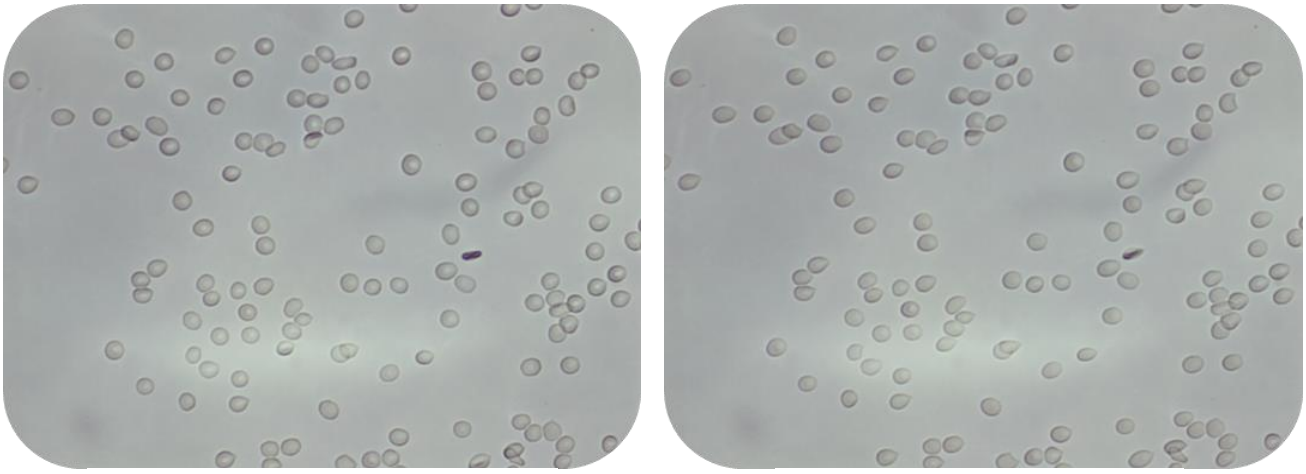
x. **Cleaning**

In the final step, the system will once again active the flow to wash and clean the slide and the chamber. For this step, preferably a reservoir with 70% ethanol is chosen.

Manual pump adjustment is available during the entirety of the measurement time. All steps are recorded onto the measurement log with a time stamp for easier control of the procedure.

5. Measurement evaluation

- Images that were taken at both flow steps are used for measurement evaluation (Figure 1).
- The results obtained from these images are documented in the following forms after the AI software has analyzed and evaluated each of it:



Flow 1 (no flow) **Flow 2 (low flow)**

Figure 1: Images of untreated RBC Monolayer at zero shear stress (left) and low shear stress of 0.25 Pa (right).

- **Graph 1: Average Elongation Index per image**
- **Graph 2: Distribution of the Elongation Index per image**
- **A log of recorded images with time stamps, average elongation index and standard deviation**

Recorded Images

1	**11:15:08 Uhr**	Elongation: **1.08**	Std. Deviation: **0.19**
2	**11:14:47 Uhr**	Elongation: **1.03**	Std. Deviation: **0.18**

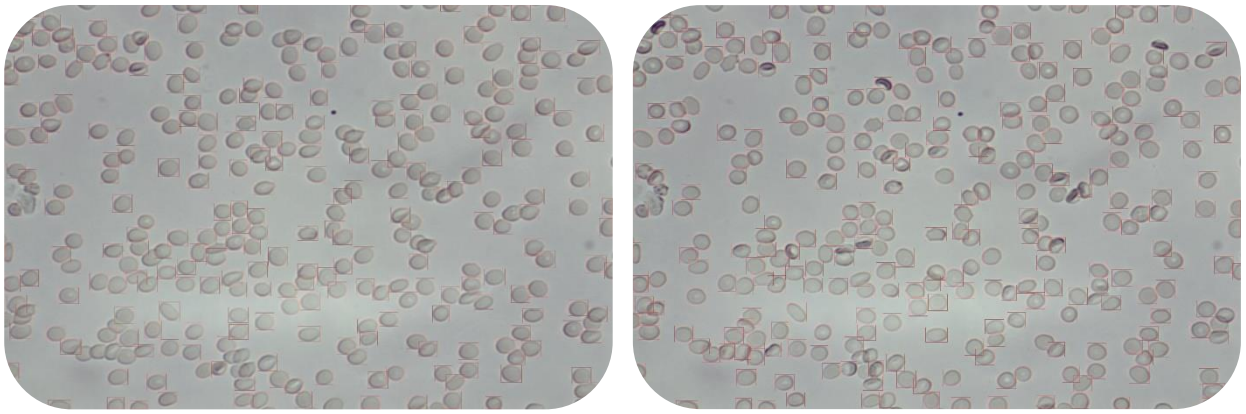


Figure 2: Images of untreated RBC Monolayer under increased 1.1 Pa shear stress (left) and low 0.25 Pa shear stress (right). As demonstrated in the above table, the elongation index values were calculated using artificial intelligence (AI) software. It is evident that higher elongation values are observed at higher shear stresses.

- Both images can be seen on the evaluation page using a toggle button.
- The results can be exported in the file formats **.pdf, .csv**

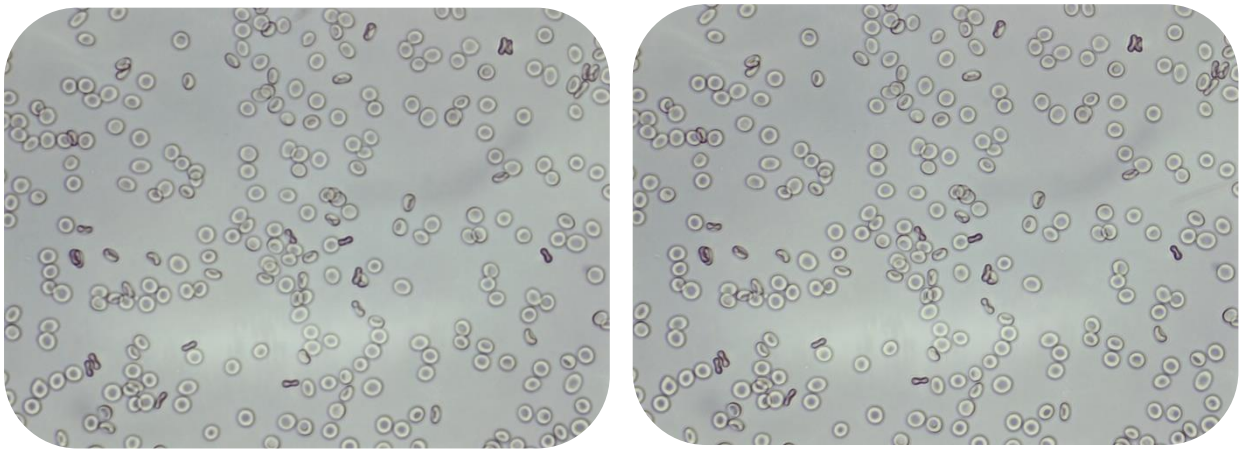
Ensure confidence from the start

Before putting your "Flow & Patch" system to work, a performance validation offers peace of mind by confirming the system is functioning as it should. This simple initial check helps verify proper operation, highlights any setup issues, and lets users get comfortable with the equipment. A quick trial run can uncover potential problems early, ensuring accurate results and smooth performance in every experiment that follows. During the trial run, users must perform two comparative measurements to verify device performance:

1. The baseline elongation index is established through measurement of a untreated blood sample.
2. A 2nd measurement is taken using RBCs treated with Glutaraldehyde (0.01–0.1%) incubated for 10–30 minutes. In the experiments described below, erythrocytes were incubated at 2.5% Hct with 0.1% glutaraldehyde for 15 minutes.
3. At increased flow no deformation of RBCs was detected.

Recorded Images

1	13:32:19 Uhr	Elongation:	1.00	Std. Deviation:	0.12
2	13:31:08 Uhr	Elongation:	0.98	Std. Deviation:	0.10
3	13:30:58 Uhr	Elongation:	0.99	Std. Deviation:	0.10



Flow 1 (low flow) **Flow 2 (high flow)**

Figure 3: Images of a RBC Monolayer treated with Glutaraldehyde (0.1% for 15 min at 2.5% Hct) under low shear stress of 0.25 (left) Pa and increased shear stress 1.1 Pa (right). No RBC deformation and/or significant change was detected in the RBC elongation index (see table above images). Image no. 3 was not included here showing a "no-flow image".

Interpreting Your Trial Run Results

As Glutaraldehyde exposure stiffens red blood cells, a noticeable reduction in deformability, and thus a lower elongation index is expected Figure 3 and table above). While exact elongation index values may vary depending on factors such as the blood source, Glutaraldehyde concentration, and incubation time, the key expectation in this trial run is a **relative decrease** in elongation index following Glutaraldehyde treatment as compared to non-treated RBCs. This quantitative shift confirms that the device is sensitive to mechanical changes in cells, even if specific numerical outcomes may differ

between samples. Comparing the elongation index values of Figure 1 and 2, a significant decrease in RBC elongation index can be seen in the Glutaraldehyde treated group ($E.I._{Glutaraldehyde}$=1.0) compared to the untreated group ($E.I_{Untreated}$ = 1.08).

Unlock More with Flow & Patch

Beyond its standard deformability assessment capabilities, the “Flow & Patch” system offers additional functionalities that can be tailored to specific research needs. The AI-based evaluation platform is continuously evolving and can be further customized or enhanced upon customer request. The same experimental setup can also be extended to analyse RBC relaxation times with minimal adaptation, simply by activating dedicated AI features*.

Furthermore, specialized test protocols such as those for investigating sickle cell disease, diabetes and haemolytic anaemia including the identification of RBC subpopulations are available upon inquiry. If you're interested in expanding the scope of your research, feel free to contact us to explore the full potential of the system.

***Requires higher hardware specifications than the classic Flow & Patch kit. Available upon inquiry.**

Ready to Get Started?

Your device is designed for precision, reliability, and ease of use. With a successful trial run, you're ready to explore its full potential in your research or clinical workflow. For further assistance, troubleshooting, or advanced applications, our support team is here to help.

Contact us:

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Customer Hotline: +49 171 414 7156

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