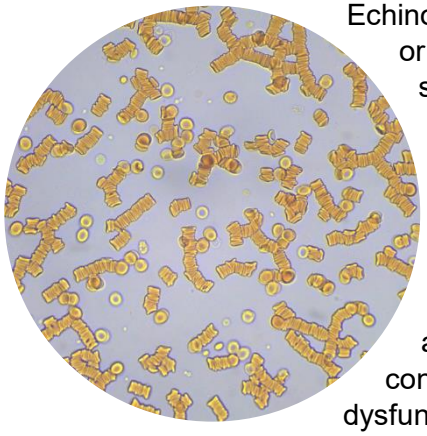
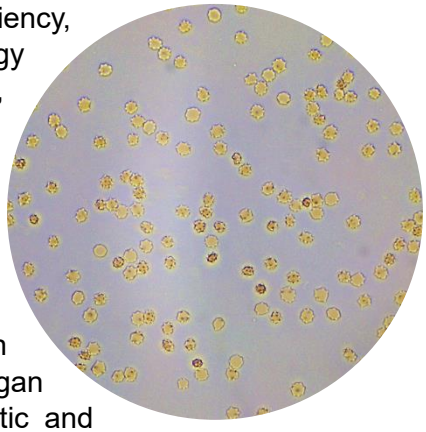
BIOEXP “FLOW & PATCH” - ASSESSING ERYTHROCYTE SHAPE AND AGGREGATION UNDER FLOW USING AI-IMAGE ANALYSIS

The application is intended for medical and biological research, in particular for hematologists, specialists in infectious diseases and hereditary blood disorders such as thalassemia and sickle cell anemia, as well as for transfusion medicine experts: Red blood cells (RBCs) typically appear as flexible, biconcave discs that ensure efficient oxygen delivery. However, distorted RBC morphologies, such as stomatocytes (cup-shaped cells with a slit-like central pallor), spherocytes (dense, round cells without a central pallor), and echinocytes (burr cells with uniform spicules), indicate pathology. Stomatocytes are seen in hereditary stomatocytosis or as artifacts. Spherocytes often indicate hereditary or autoimmune hemolytic anaemia.



Echinocytes arise in uremia, pyruvate kinase deficiency, or hypophosphatemia. Abnormal morphology signals disease and impairs RBC deformability, reducing microvascular perfusion [1,6,8-10]. Concurrently, RBC aggregation (Rouleaux), driven by plasma proteins, increases blood viscosity and disrupts flow. This is commonly observed in inflammation, diabetes, and multiple myeloma. Together, altered shape and heightened aggregation contribute to hemolysis, hyperviscosity, and organ dysfunction. These factors serve as vital diagnostic and prognostic markers in hematologic, renal, metabolic, and inflammatory disorders [2,8-10].



In this application note, we introduce a novel measurement system that assesses RBC morphology and aggregation. The system captures high-resolution images of RBCs either at steady state or during exposure to flow conditions. These recordings are then processed and evaluated by an artificial intelligence (AI) platform. It determines RBC morphology and morphological sup-populations, as well as rouleaux shape parameters, leading to a robust and reproducible determination of RBC responsiveness to specific drugs, conditions and more.

Keywords: Erythrocyte shape, Aggregation, Rouleaux formation, Echinocyte, Spherocyte, artificial intelligence, flow chamber, sickle cell disease, Thalassemia

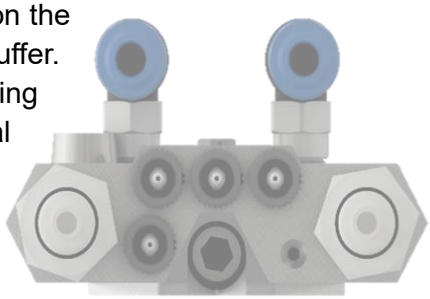
Advantages of the “Flow & Patch” chamber

- 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 step

How to use the “Flow & Patch”?

The **“Flow & Patch”** system offers a user-friendly platform for assessing RBC morphology and aggregation through precise measurement of shape parameters under physiologically relevant and customizable flow conditions, as well as during steady state. By simulating microvascular shear stress within a controlled chamber, the system enables real-time imaging of RBCs as they respond to dynamic flow. Additionally. the flow may be carried out utilizing RBC shape altering substances. High-resolution images captured during the experiment are analysed using AI-based image processing, which uses key morphological parameters such as cell shape parameters, colour and more. 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 erythrocyte morphology and aggregation in both research and clinical settings.

The chamber is equipped **with three separate inlets (red) and a singular outlet (yellow). as seen in the right figure**, enabling parallel testing of up to three different substances on the same red blood cell monolayer, while one channel is reserved for cell buffer. 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 O2 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 [8]. If desired, various temperatures can be tested to simulate pathological environments and study their effects on erythrocyte mechanics.



1. Required Materials

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

*** Haematocrit value <5%, recommended HCT = %1**

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

2. Blood Sample Preparation (Optional: Depends on experimental conditions)

- **Blood Collection and Haematocrit Check**
  Blood is drawn according to standard laboratory procedures (capillary (finger prick), venous, or arterial). The haematocrit value is determined (i.e. using a Hct 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.

3. Flow & Patch Setup

- In the BioExP application software, initiate SOP5 (RBC Form / Rouleaux Formation) and ensure all devices are properly connected. (see Original Operating Instructions)

- Place the flow chamber base (without the top cover) onto the microscope stage.
- 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.

4. Step-by-Step measurement

Disclaimer
This application note primarily serves as a tool for image capture and subsequent AI interpretation of the captured images. It does not adhere to specific protocols, as users are free to select individual experimental parameters, such as flow, temperature, substance usage, and more. The following steps are optional and may be ignored if not needed.

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 (can be tested with a short flushing process).

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.

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 has freedom to choose the active channel and the duration/intensity of flow before starting the experiment.

vi. **RBC Sedimentation**

The software starts a 20-min timer to let the erythrocytes to sediment and form a monolayer. If the user wishes to wait shorter, they can end the step 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. **Image capturing**

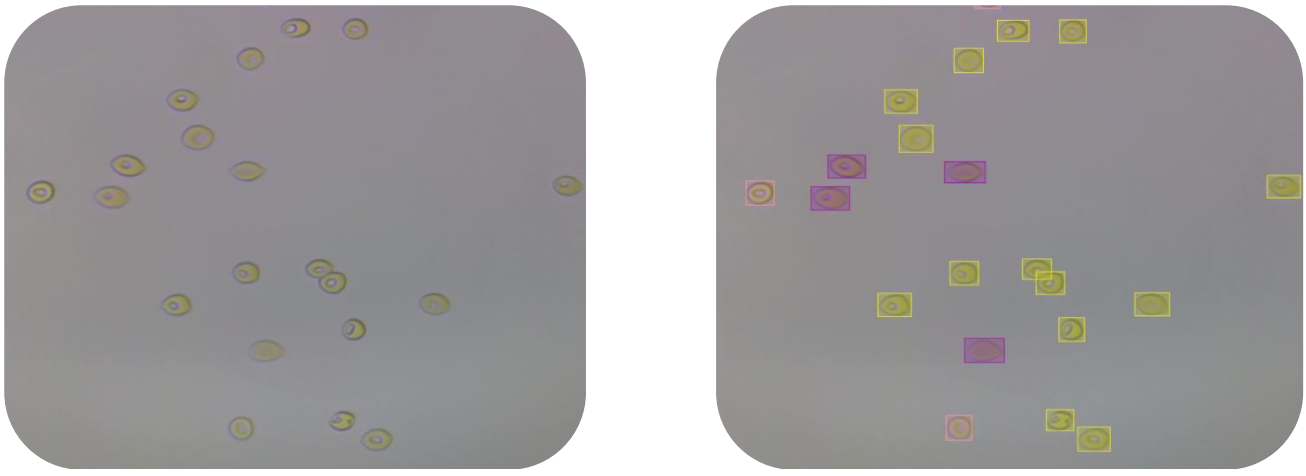
Images of the microscope slide can be taken manually throughout the entirety of the experiment. The images may be taken during steady state or during flow conduction.

ix. **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



Captured Image of RBC **AI detected RBC in Image**

Figure 1: Left side: Captured image of RBC in "Flow & Patch" Chamber, Right side: Same image after undergoing AI evaluation (red = RBC, yellow = spherocyte, purple = elongated RBC)

- Images that were taken are used for measurement evaluation.
- The results will be presented as indicated below after the AI software labels and evaluates each image:
  - **Bar chart with individual RBC morphologies listed on x-axis**
  - **The following RBC morphologies can be distinguished**
    - **Red Blood Cell (Discocyte) (Also in cross-sectional view!)**

- **Elongated RBC**
- **Echinocyte**
- **Red Blood Cell Ghost**
- **Red Blood Cell Rouleaux**
- **Red Blood Cell swollen (Spherocyte)**
- **Sickle and thalassemia cells**
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- All analysed images can be seen on the evaluation page using a toggle button.
- The results can be exported in the file formats **pdf and csv**

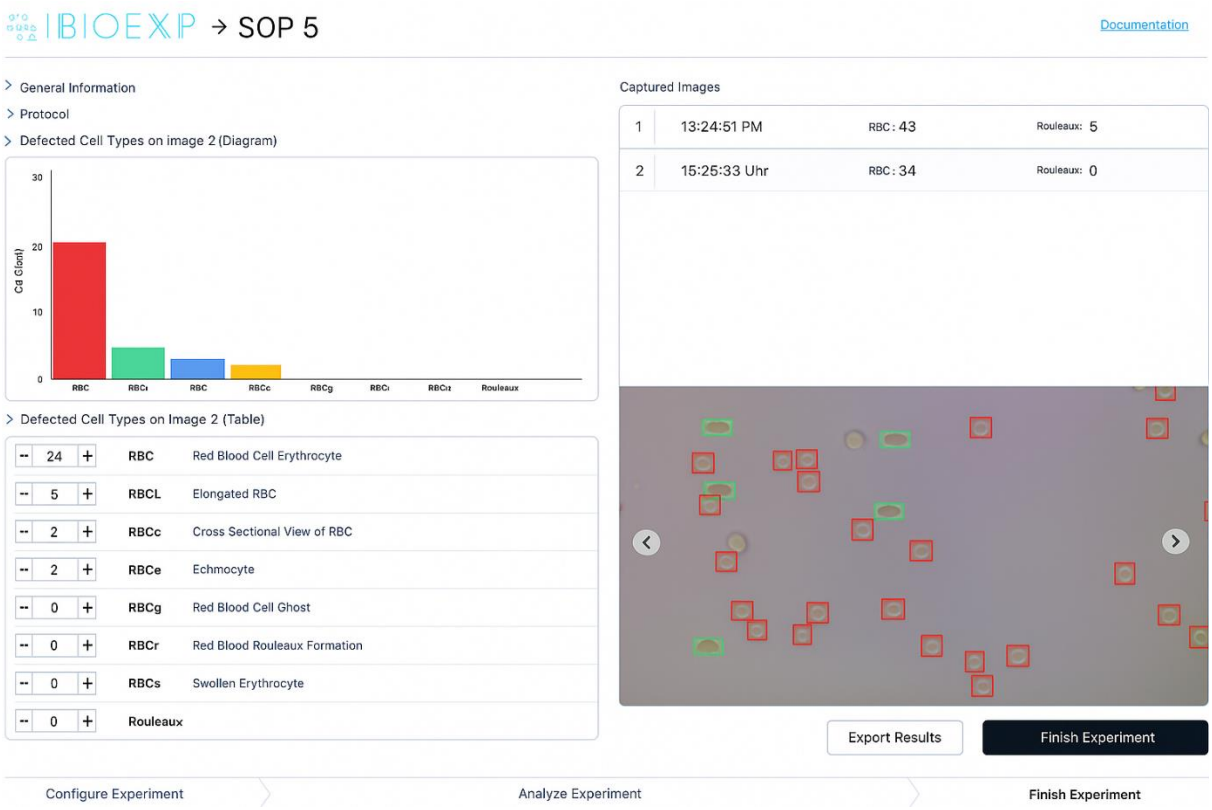

Figure 2: Image of AI evaluation interface in BioExP application software. Visible on the left side: Bar chart with evaluated RBC morphologies. Right side: Analysed image through AI with object detection boxes

The user is able to manually vary the number of cells in each RBC category after AI analysis is completed. This allows for manual correction of possible misinterpretation or for other uses.

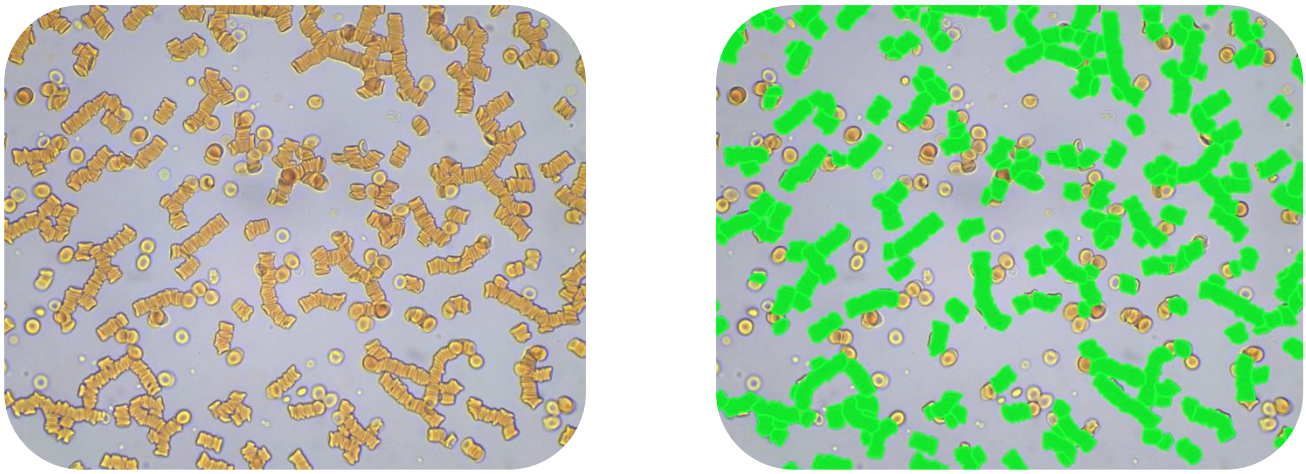

Figure 3: Left side: Captured image of aggregated RBC (Rouleaux) in "Flow & Patch" Chamber, Right side: Same image after undergoing AI evaluation, remaining non-aggregated cells not highlighted

Usage of the AI detected results

Analyzing RBC morphology and aggregation in a flow-chamber or microfluidic environment offers powerful tools for medical research. These systems are designed to replicate physiological shear stresses and microvessel geometry, enabling real-time imaging of how RBC deform, form rouleaux, or break apart under controlled flow conditions. Abnormal shapes, such as echinocytes and spherocytes can indicate various disorders, including hereditary spherocytosis, sickle cell disease, and malaria. The measurement of aggregation is essential for the quantitative analysis of rouleaux formation and disaggregation dynamics. This assessment is crucial for the evaluation of inflammation, fibrinogen levels, and storage lesions in the field of transfusion medicine as well as in hereditary genetical disorders [3, 8-10]

Potential further areas of research

- Studies of age-fractionation: Employ density separation to distinguish between young and old red blood cells. Then, assess their deformability and aggregation in microfluidic flow. This will allow to explore the links between RBC aging and vascular occlusion. [5]
- Flow-based platforms are an effective solution for testing anti-aggregation agents or membrane-stabilizing drugs. These platforms enable real-time observation of changes in rouleaux under shear, providing valuable insights into drug effectiveness and performance.
- Kinetics and reversibility of erythrocyte sickling al lowered partial oxygen pressure.
- Sickle cell/Thalassemia Erythrocyte sub-population analysis.

Unlock More with Flow & Patch

Beyond its standard 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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