Supplementary Materials

Supplementary Methods

Photometry platform assembly

The required materials for complete setup and use of the photometry platform are provided in Table S1. Figure S1 may be used as a visual guide for assembly and approximate size. Note that the system shown in Figure S1 utilizes Thorlabs' 30 mm cage system for additional alignment of optical components: this is not strictly required for assembly or operation and is entirely optional. The system is mounted to an aluminum optical breadboard (MB3030/M, Thorlabs) using optical posts (TR50/M, Thorlabs). Depending on application requirements, other optical mounting approaches may be preferred.

To assemble the platform we prefer to follow the direction of the beam path, inserting components as follows.

- 1. Connect one end of the fiber optic patch cable (M59L01, Thorlabs) to the 430 nm LED (M430F1, Thorlabs).
- 2. Attach the other end of the patch cable to the fiber-coupled collimator (F671SMA-405, Thorlabs).
- 3. Mount the collimator within the collimator adapter (AD15F, Thorlabs) using the provided set screws. The collimator should sit approximately in the middle of the adapter.
- 4. Connect the collimator adapter to one of the filter holders (SM1-QP, Thorlabs).
- 5. Place the excitation filter (65-682, Edmund) into the filter holder.
- 6. Separately, insert the dichroic mirror (86-332, Edmund) into the optical filter cube (CM1-DCH/M).

NOTE: The orientation of the dichroic mirror in the filter cube will dictate the position of the other optical components. The dichroic mirror will reflect wavelengths below 488 nm and transmit light above 515 nm. As a result, we assemble the platform such that light delivered by the LED (~430 nm) is reflected to the objective and light received from the objective (~520 nm) is transmitted to the CMOS camera.

- 7. Connect the collimator assembly to the assembled optical filter cube such that the delivered light is reflected in a preferred direction.
- 8. Attach the 20X objective (1-U2B225, Olympus) to the receiving side of the reflected light. A thread adapter may be required depending on the objective used, such as an RMS to SM1 thread adapter (SM1A3, Thorlabs).
- 9. Opposite to the objective, connect the other filter holder to the optical filter cube. Place the emission filter (FB520-10, Thorlabs) into the filter holder. Emitted light collected from the objective will be transmitted through the dichroic mirror to this filter.
- 10. Position the achromatic doublet (AC254-035-A-ML, Thorlabs) after the emission filter, at the tube length of the objective. We use Olympus objectives with a tube length of 180 mm; note that depending on the brand of objective used, the tube length will vary.
- 11. Place the CMOS camera (BFS-PGE-31S4M-C, FLIR) at the focal distance of the achromatic doublet. For our system, the CMOS sensor should be located approximately 35 mm from the doublet.

- 12. Separately, connect the 1-to-7 fiber cable bundle (F671SMA-405, Thorlabs) to the kinematic mount (KC1T, Thorlabs). Align the proximal end of the fiber bundle with the objective lens.
- 13. Adjust the position of the kinematic mount such that the proximal end of the fiber bundle is located at, or slightly further than, the objective working distance.

Optical alignment

- 1. Loosen all adjustment screws on the kinematic mount.
- 2. Turn on the 430 nm LED on low power. We use 10 mA power from the 4-channel LED driver (DC4104, Thorlabs).
- 3. Start a live recording with the CMOS camera. We use SpinView software (Spinnaker SDK, FLIR) for live recording and automated image capture.
- 4. Place the distal end(s) of the optical fibers facing a dim light white light source or an autofluorescent plastic slide.
- 5. Adjust the camera gain until an image appears. Decrease the gain if saturated pixels are observed. If no image appears, adjust the position of the kinematic mount and/or the CMOS camera. Starting at the objective working distance and at the objective tube length will typically yield an acceptable starting image.
- 6. Use the adjustment screws on the kinematic mount to sharpen the recorded image. The excitation light should be centered on the fiber cable(s) and fill all fibers once focused appropriately.
- 7. If the recorded image cannot be resolved to acceptable levels, carefully adjust the CMOS camera position until improved.
- 8. When properly adjusted, all fibers should be visualized as equivalently sized circles. If the fiber ends are ovular or misshapen, further adjustment with the kinematic mount is required.

Automated recordings

We use an Arduino microcontroller (Mega 2560 Rev3, Arduino) to automate light delivery and image capture. TTL triggers sent to the LED driver and CMOS camera are aligned to capture fluorescence emission at 10 minute intervals. Adjustments to the script will enable full customization of recording intervals, excitation exposure times, and excitation intensity (GitHub link: https://github.com/LNNR-and-ABNEL/In-situ-monitoring-of-barrier-inflammation-on-chip).

Table S1: Complete parts list required for assembly and use of the photometry platform. The reported number of parts needed is reported for independent, dual-channel sensing of apical and basal compartments described in the main manuscript. If only a single sensing channel is required, all parts quantities may be halved except for the LED driver, LED connector hub, and Arduino microcontroller. Between 2 and 14 parts may be used depending on the sensing requirements of the system. Each additional set of indicated components allows for one additional fiber end to be used for sensing, up to a maximum of 14.

Part	Part Number	Vendor	Number Needed*
430 nm LED	M430F1	Thorlabs	2
Fiber-coupled collimator	F671SMA-405	Thorlabs	2
Collimator adapter	AD15F	Thorlabs	2
Fiber optic patch cable	M59L01	Thorlabs	2
1-to-7 fiber optic bundle	BF74LS01	Thorlabs	2
Excitation filter	65-682	Edmund Optics	2
Emission filter	FB520-10	Thorlabs	2
Filter holder	SM1-QP	Thorlabs	4
Dichroic mirror	86-332	Edmund Optics	2
Optical filter cube	CM1-DCH/M	Thorlabs	2
Achromatic doublet	AC254-035-A-ML	Thorlabs	2
Kinematic mount	KC1T	Thorlabs	2
Low-autofluorescence patch cable	MAF3L1	Thorlabs	2-14
SMA to FC/PC adapter	ADAFCSMA1	Thorlabs	2-14
Quick-disconnect ferrule	ADAF2	Thorlabs	2-14
Fiber optic stub	CFMC54L10	Thorlabs	2-14
20X Objective	1-U2B225	Olympus	2
CMOS camera	BFS-PGE-31S4M-C	FLIR	2
4-channel LED driver	DC4104	Thorlabs	1
LED connector hub	DC4100-HUB	Thorlabs	1
Arduino microcontroller	Mega 2560 Rev3	Arduino	1



Fig. S1: Top-down (left) and side view (right) of the fiber photometry platform used for real-time, non-invasive barrier function sensing. Duplicate optical components are used to enable independent readout of apical and basal flow compartments. Base plate dimensions are 30 cm x 30 cm.

Optical readout analysis

For further analysis of Caco-2 and HUVEC optical sensing data, CSV files exported from our custom Python scripts (GitHub link: https://github.com/LNNR-and-ABNEL/In-situ-monitoring-of-barrier-inflammation-on-chip) were used. Data was collected from at least one control sample and at least one experimental sample per independent experiment.

To normalize the sensed data, control dF/F values were subtracted from trial-matched experimental data. To account for signal-to-noise mismatch and intrinsic chip-to-chip differences, the data was further normalized by shifting the baseline sensed dF/F to zero. For both Caco-2 and HUVEC experimental samples, all data points collected between 0 and 12 hours of recording were averaged. The average value was then subtracted from all control-normalized values to obtain experimental values as a normalized difference from control, adjusted to a baseline dF/F of zero.

To obtain time-specific sensed differences from control samples, as shown in Figure 4C, a 2-hour average window was applied to each experimental timepoint. For example, the average sensed dF/F difference from control between 23 and 25 hours was used as the value for the 24 hour timepoint. This approach was applied to all sensed data for all experimental samples.



Fig. S2: Fiber photometry system for dual-color sensing on-chip **A**) The optical setup consists of high-powered LEDs, optical filters, fiber optic cables, and CMOS cameras that simultaneously deliver excitation light and record emission from fluorophores on-chip. **B**) Top-down view of the fiber photometry platform used for real-time, dual color diffusion sensing. Emitted light from each fluorophore is directed to independent CMOS cameras to separate the emission signal. Base plate dimensions are 30 cm x 30 cm.

Individual Fiber Intensity Variation



Fig. S3: Distribution of 430 nm intensity values measured from each of the 6 sensing fibers used for non-invasive optical sensing on-chip. Individual data points are shown with mean +/- standard deviation.



Fig. S4: Relative fluorescence measured by our optical platform at known concentrations of lucifer yellow in blank organ-chips. Data shown as circles, with line of best fit from linear regression. n = 3, m = 5 replicates per concentration.



Fig. S5: Relative permeability of HUVEC monolayers exposed to the cytokine cocktail (TNF-a, IFN-g, IL-1b) on Day 0 measured by fluorescence plate reader. One-way ANOVA with Tukey's multiple comparisons. n = 1, m = 4 control, 8 experimental replicates.



Fig. S6: Representative immunocytochemistry results from control (left column), 24-hour cytokine exposure (center column), and 48-hour cytokine exposure (right column) groups. Comparison of tight-junction expression (ZO-1) shows more diffuse expression in inflamed monolayers compared to control. Images taken at 40X magnification, maximum intensity projection of 10-slice z-stack. Scale bar = 40 um.



Fig. S7: Representative immunocytochemistry results from on-chip control (left column) and 48-hour cytokine exposure (right column) groups. Comparison of tight-junction expression (ZO-1) shows more diffuse expression in inflamed monolayers compared to control. Images taken at 40X magnification, maximum intensity projection of 10-slice z-stack. Scale bar = 40 um.