Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Simple procedure to produce FDM-based 3D-printed microfluidic devices with integrated PMMA optical window

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Table SI1 – Optimized parameters settings to print 3D microfluidic devices

Parameter	Optimized setting
First layer height	0.20 mm
Layer height	0.1 mm
Outline printing speed	9.6 mm s^{-1}
Infill printing speed	9.6 mm s^{-1}
Overall printing speed	12 mm s^{-1}
Filament temperature	205 °C
Printer bed temperature	Off – Room temperature
Number of outlines	3
Infill	100%
First layer printing speed modifier	60%
Infill angle	-45° and 45°

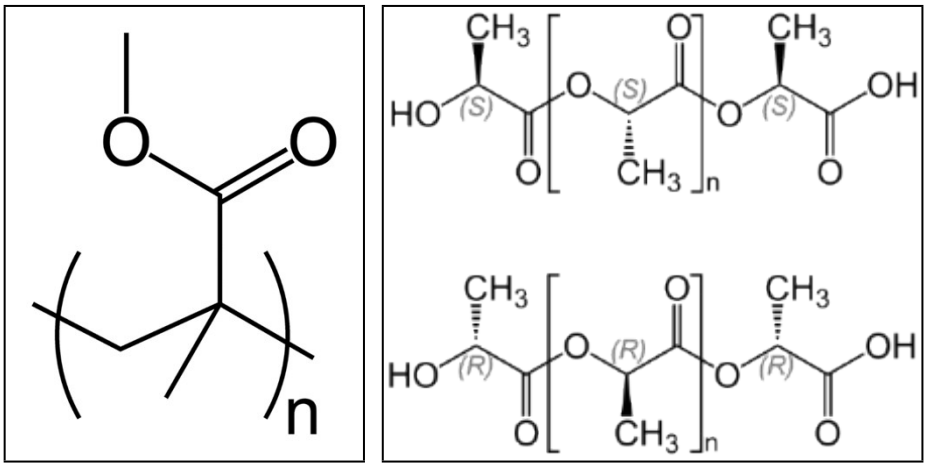


Figure SI1 – Chemical Structure of poly(methyl methacrylate) (PMMA) and poly(lactic acid) (PLA)

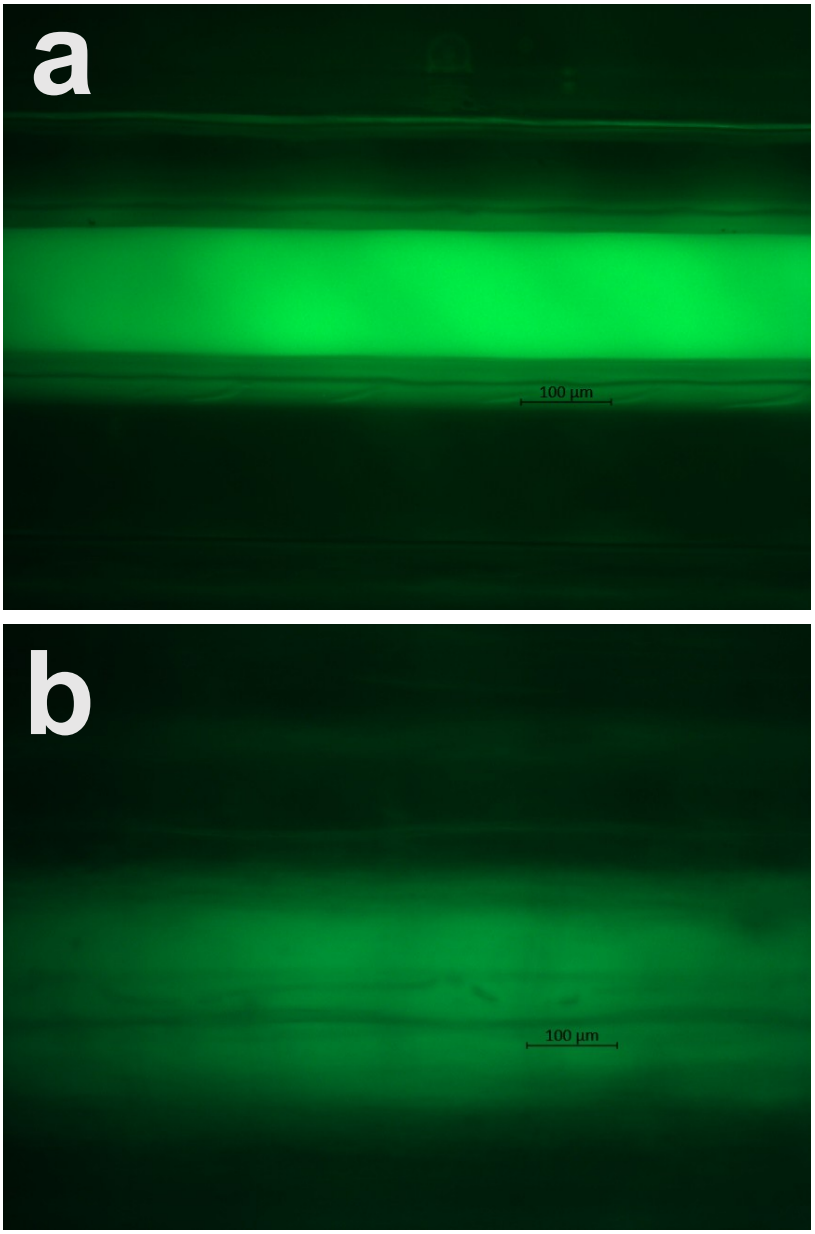


Figure SI2 – a) Fluorescence optical image obtained from a channel made of PLA and PMMA by the proposed method filled with 100 µmol L^{-1} fluorescein. b) Fluorescence optical image of a microchannel made completely from PLA filled with the same solution. Scale bars are 100 µm.

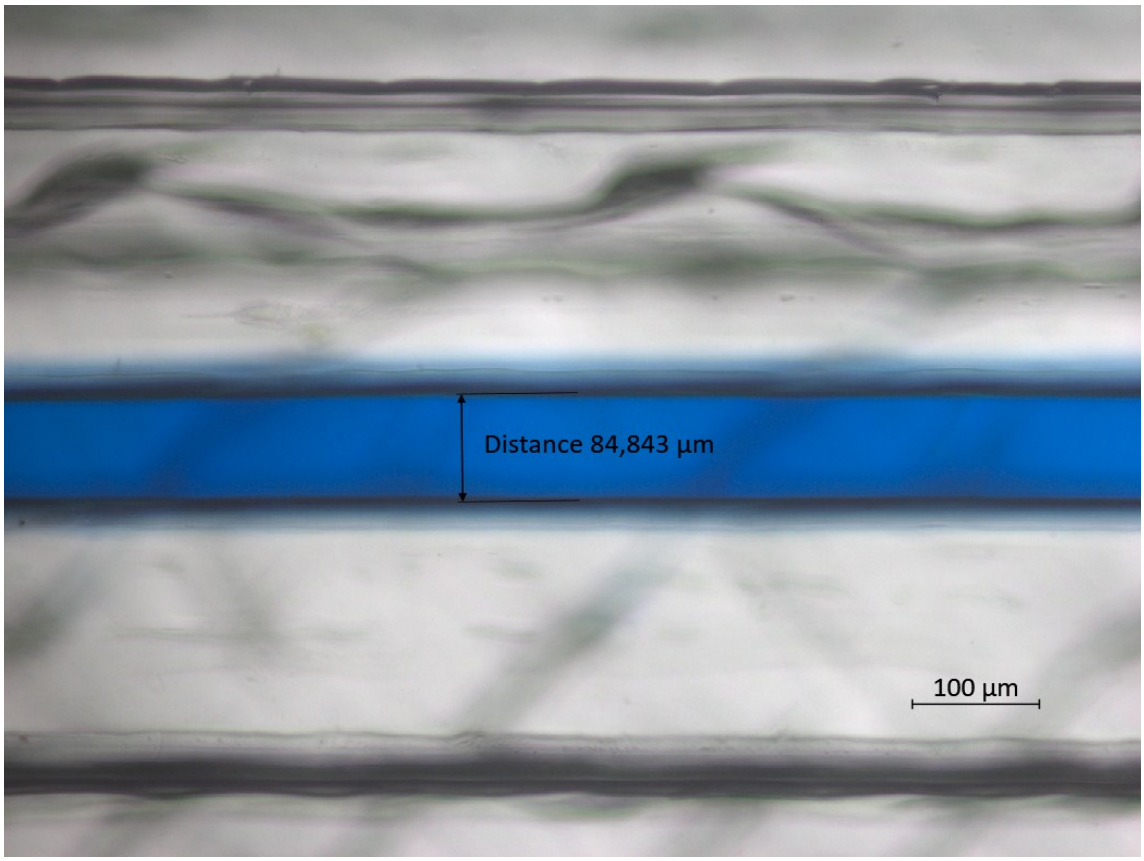


Figure SI3 – Optical image of a channel created by the proposed protocol with 85 µm width. The channel was filled with blue colorant.

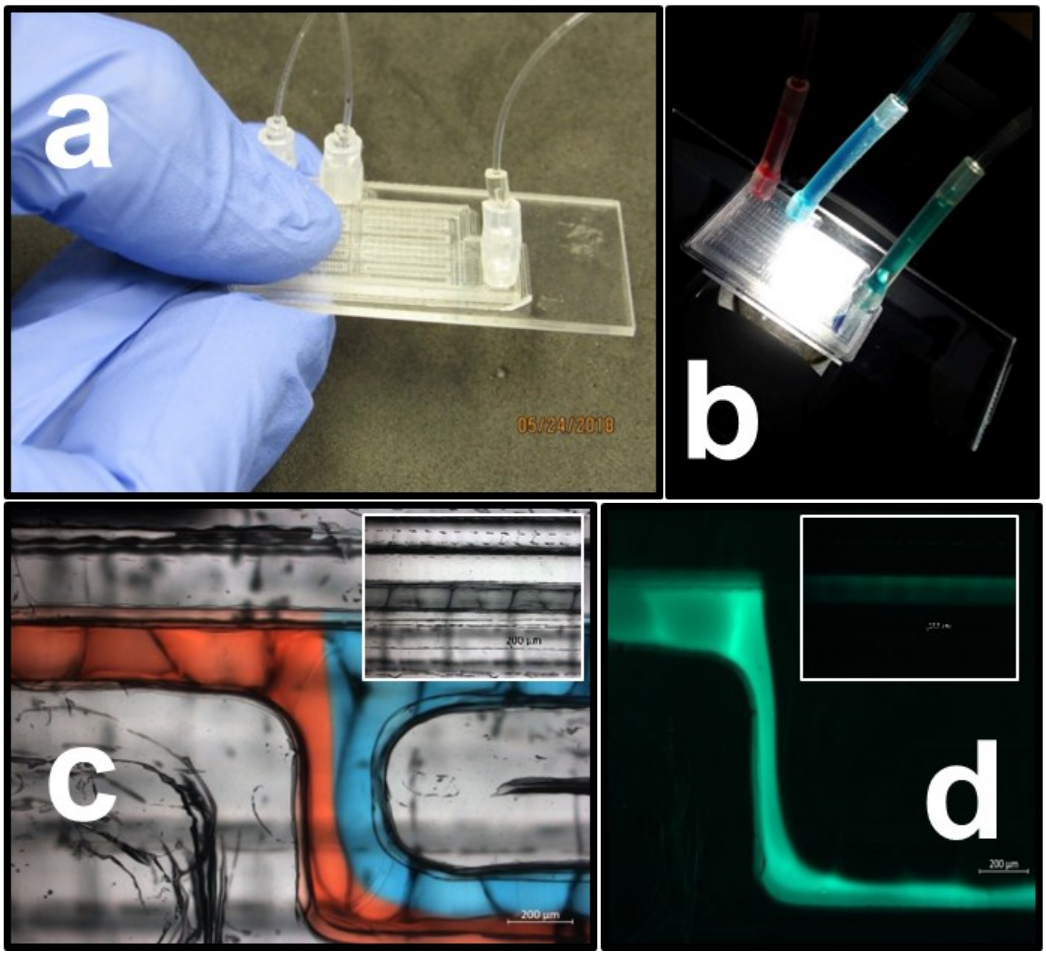
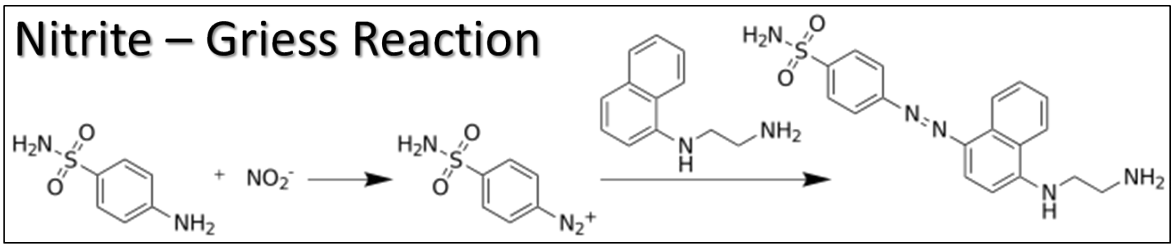
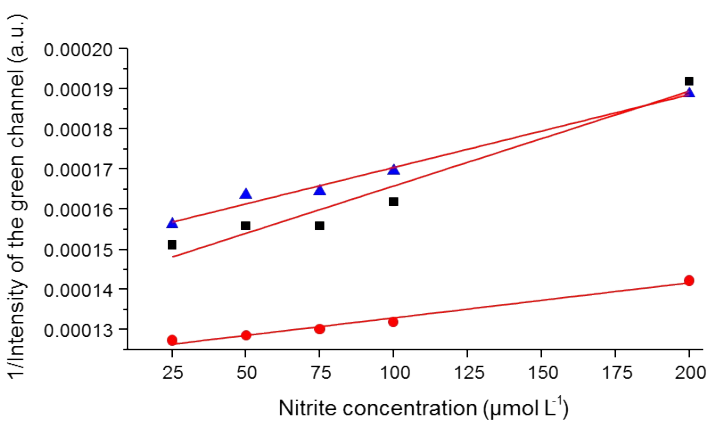


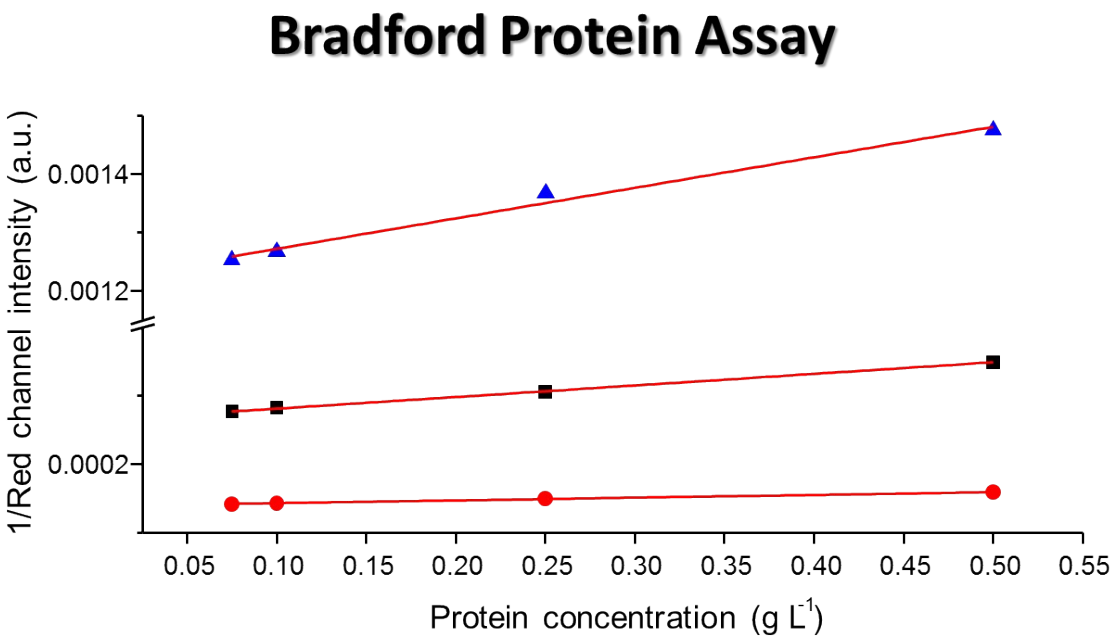
Figure SI4 – a) Photograph of the final device. b) Microfluidic mixer mounted over on the inverted microscope. c) Zoom of the confluence region (food dyes were used for contrast purposes) where the inset shows the two fluids fully mixed. d) Fluorescence photograph of the confluence region (a 100 µmol L^{-1} fluorescein solution was used) where the inset shows the two fluids fully mixed.





Equation	y = a + b*x		
Weight	No Weighting		
Residual Sum of Squares	$5.0 \cdot 10^{-11}$	$2.7 \cdot 10^{-12}$	$8.2 \cdot 10^{-12}$
Pearson's r	0.9764	0.9905	0.9932
Adj. R-Square	0.9379	0.9748	0.9821
		Value	Standard Error
Device 1	Intercept	$1.42 \cdot 10^{-4}$	$3.3 \cdot 10^{-6}$
Device 1	Slope	$2.37 \cdot 10^{-7}$	$3.0 \cdot 10^{-8}$
Device 2	Intercept	$1.241 \cdot 10^{-4}$	$7.6 \cdot 10^{-7}$
Device 2	Slope	$8.75 \cdot 10^{-8}$	$7.0 \cdot 10^{-9}$
Device 3	Intercept	$1.52 \cdot 10^{-4}$	$1.3 \cdot 10^{-6}$
Device 3	Slope	$1.81 \cdot 10^{-7}$	$1.2 \cdot 10^{-8}$

Figure SI5 – Colorimetric analysis (Griess Reaction) for five different concentrations of nitrite: 25, 50, 100, and 200 µmol L^{-1}. Calibration plots are presented for three different devices (microchip design as in Figure SI4).



Equation	y = a + b*x		
Weight	No Weighting		
Residual Sum of Squares	$2.4 \cdot 10^{-12}$	$3.7 \cdot 10^{-13}$	$3.9 \cdot 10^{-10}$
Pearson's r	0.9996	0.9990	0.9939
Adj. R-Square	0.9989	0.9971	0.9817
		Value	Standard Error
Device 1	Intercept	$2.645 \cdot 10^{-4}$	$9.3 \cdot 10^{-7}$
Device 1	Slope	$1.68 \cdot 10^{-4}$	$3.2 \cdot 10^{-6}$
Device 2	Intercept	$1.384 \cdot 10^{-4}$	$3.6 \cdot 10^{-7}$
Device 2	Slope	$4.1 \cdot 10^{-5}$	$1.2 \cdot 10^{-6}$
Device 3	Intercept	$1.22 \cdot 10^{-3}$	$1.2 \cdot 10^{-5}$
Device 3	Slope	$5.2 \cdot 10^{-4}$	$4.1 \cdot 10^{-5}$

Figure SI6 – Colorimetric analysis for four bovine serum albumin concentrations 0.075, 0.01, 0.25 and 0.05 g L^{-1} in three different devices. Calibration plots are presented for three different devices (microchip design as in Figure SI4).

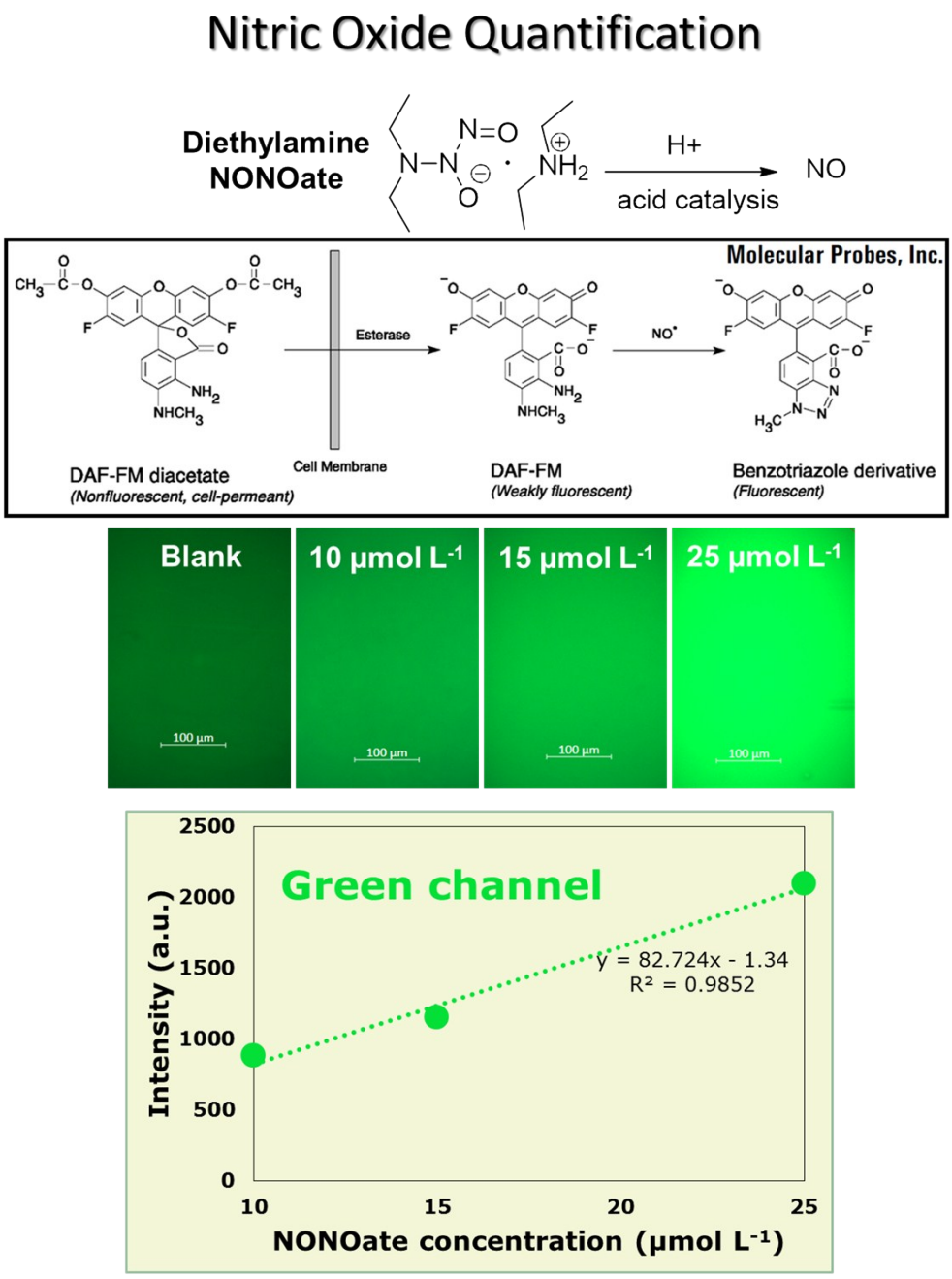


Figure SI7 – Nitric oxide measurement in the device from Figure SI4. Three different concentrations (10 to 25 µmol L^{-1}) of nitric oxide were measured from NONOate precursor. The scale bars are 100 µm.

Protocol of analysis for the applications

1 Nitrite analysis

We made nitrite solutions from 25 to 200 μmol L^{-1} through dilution with water of a 10 mmol L^{-1} stock solution. The Griess reactant was used as received. In the microdevice, we used a flow of 25 μL min^{-1} to fill the channels and to perform the analysis we used 3.2 and 0.8 μL min^{-1} flow to the reactant and standards, respectively. After five minutes of run, we acquired a snapshot to color analysis. We utilized the green and blue channel of the RGB space to perform the colorimetric analysis. The integration time of the image was set to 40 ms and the objective used was 10x. The blank values were subtracted from all images before performing the regression analysis. In this and all other cases, the images were captured in the last portion of the channel before the outlet.

2 Protein analysis

We made the solutions of BSA ranging from 0.075 and 0.5 g L^{-1} through the dilution of the stock solution in PBS. We used the Bradford reagent as received. Again, we filled the channels with a flow of 25 μL min^{-1} and used the reactant and standards flow of 20 and 5 μL min^{-1}, respectively. After five minutes of run, we acquired a snapshot and used the red channel of the RGB space to perform the colorimetric analysis. The integration time was set to 40 ms and the objective used was 40x. The blank values were subtracted from all images before performing the regression analysis.

3 Nitric oxide analysis

The procedure was based on that used by Mainz *et. al.*[1]. Briefly, a DAF-FM DA solution of 25 μmol L^{-1} was prepared in NaOH 10 mmol L^{-1} and kept away from light for 40 minutes to promote the hydrolysis. The solutions of Proli NONOate ranging from 20 to 100 μmol L^{-1} were prepared with equimolar concentrations of DAF-FM. A 10 mmol L^{-1} phosphate buffer with pH 2 was used to lower the reaction pH. Immediately, the system was filled with both solutions with a 25 μL min^{-1} flow. During the analysis, we reduced the flow of reactant and sample to 5 μL min^{-1} each. After five minutes, a snapshot was acquired to colorimetric analysis. We used the green channel to perform the colorimetric analysis. The integration time of the image was set to one second

and the objective used was 10x. The blank values were subtracted from all images to perform the calibration analysis.

4 Cell dyeing with fluorescein

We added the tip of a spatula of the Saf-Instant® yeast to 4.5 mL of a PBS solution and 500 μL of 100 μmol L^{-1} sodium fluorescein to a conical flask. This mixture was kept away from light for one hour. Following, we performed three washing cycles to obtain the dyed cells as follows: i) the tube was centrifuged at 4000 rpm for five minutes; ii) the supernatant was discarded and iii) the pellet was dissolved in 5 mL of PBS. The final solution was used to the analysis.

References

1 E. R. Mainz, D. B. Gunasekara, G. Caruso, D. T. Jensen, M. K. Hulvey, J. A. Fracassi da Silva, E. C. Metto, A. H. Culbertson, C. T. Culbertson and S. M. Lunte, *Anal. Methods*, 2012, **4**, 414.