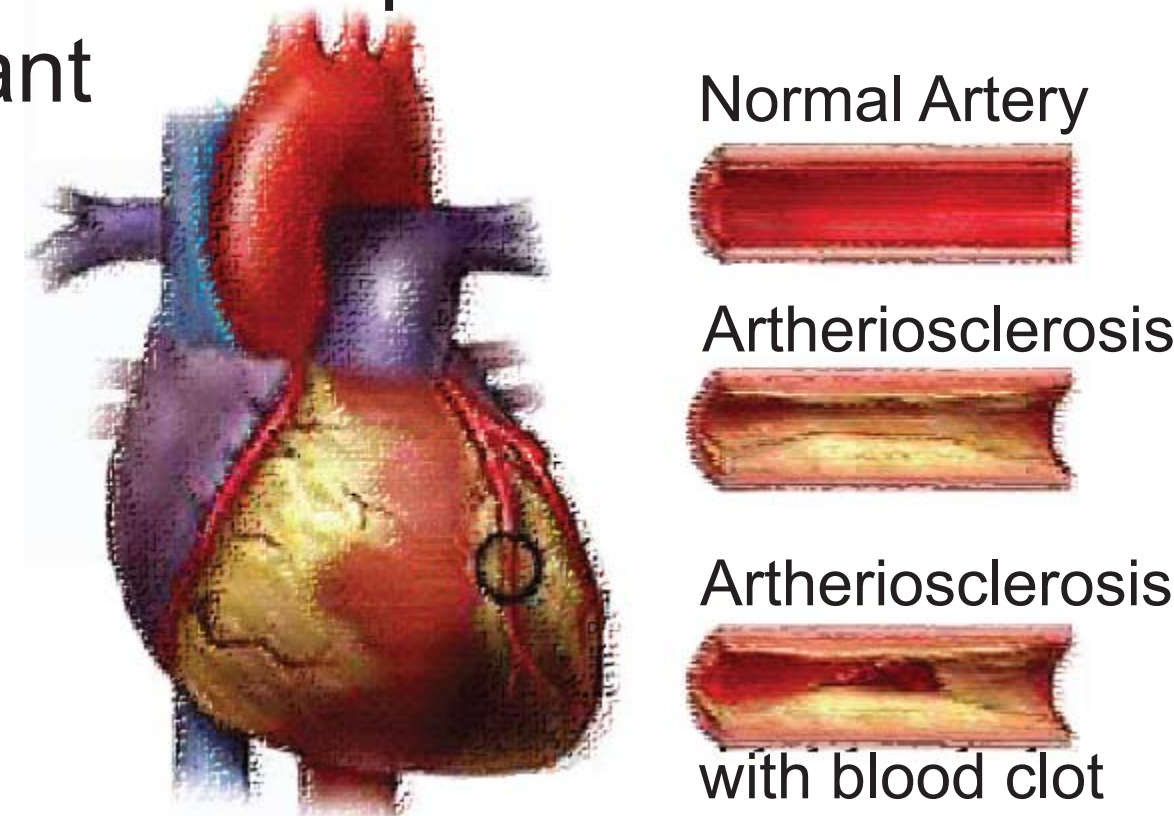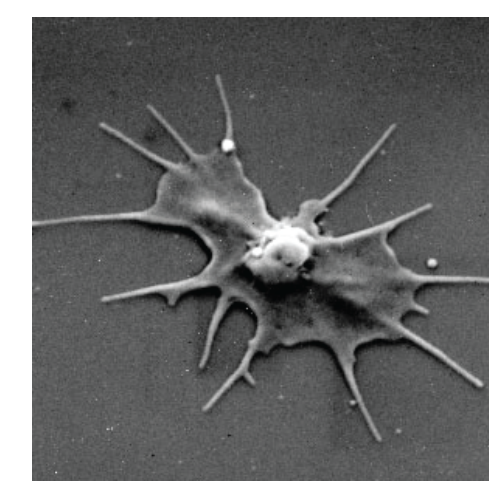# A PLATFORM TO STUDY PLATELET AGGREGATION AND THROMBUS GROWTH BASED ON STRAIN RATE CHANGES

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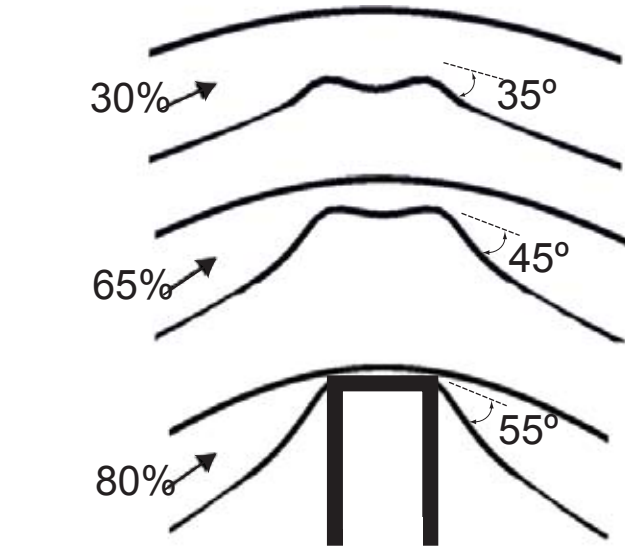
#These authors contributed equally to this work.

## INTRODUCTION

• Platelets are blood cells that are critical for the maintenance of normal blood flow.
• However abnormal platelet responses can lead to heart attack & stroke.
• Thrombi are formed by the aggregation of platelets.
• Current antithombotic drugs target chemical activators of platelets without considering mechanical blood flow effects.
• Microfluidics enable to test constant and dynamic stress with low blood sample.
• Recent studies have shown that dynamic stress generates important transient mechanical forces that promote platelet aggregation[1].



Normal Artery
Artheriosclerosis
Artheriosclerosis with blood clot

## RATIONALE

• Under constant shear stress, thrombi can be controlled using pharmacological inhibitors ( blocking chemical activators such as ADP, Thombin and Thomboxan (soluble agonists), see figure 0s.
• Under dynamic shear stress,  in vivo evidence[1] suggests the lack of effectiveness of the current pharmacological inhibitors, see figure 11.87s.
• A platform to test blood under controlled conditions of dynamic shear stress needs to be designed.



Mouse mesenteric artery

An induced stenosis generates dynamic stress and the effectiveness of the aggregation inhibitors is diminished.

## PRINCIPLE OF OPERATION
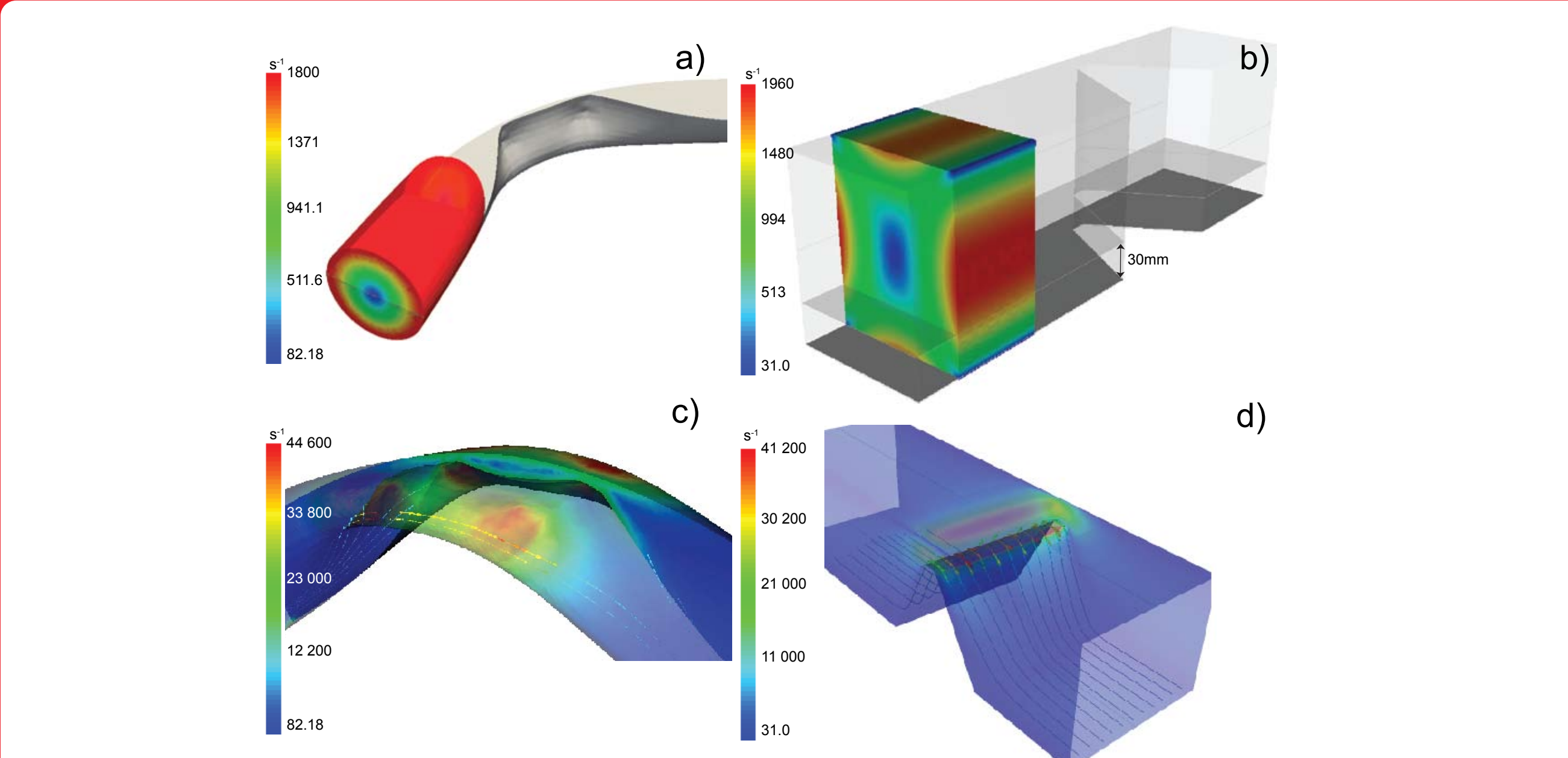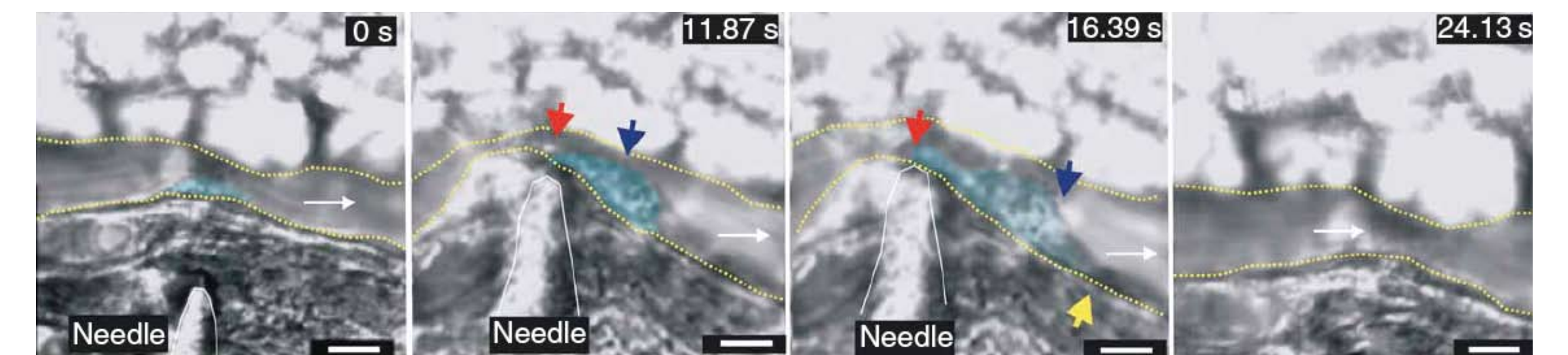


a) Nominal shear rate at a mouse mesenteric artery. b) Nominal shear at the microchannel.
c) Shear rates at the vessel contraction. d) Shear rates at the microcontraction.

Hydrodynamic analyses of the induced stenosis assist the mimicking process for a dynamic shear.
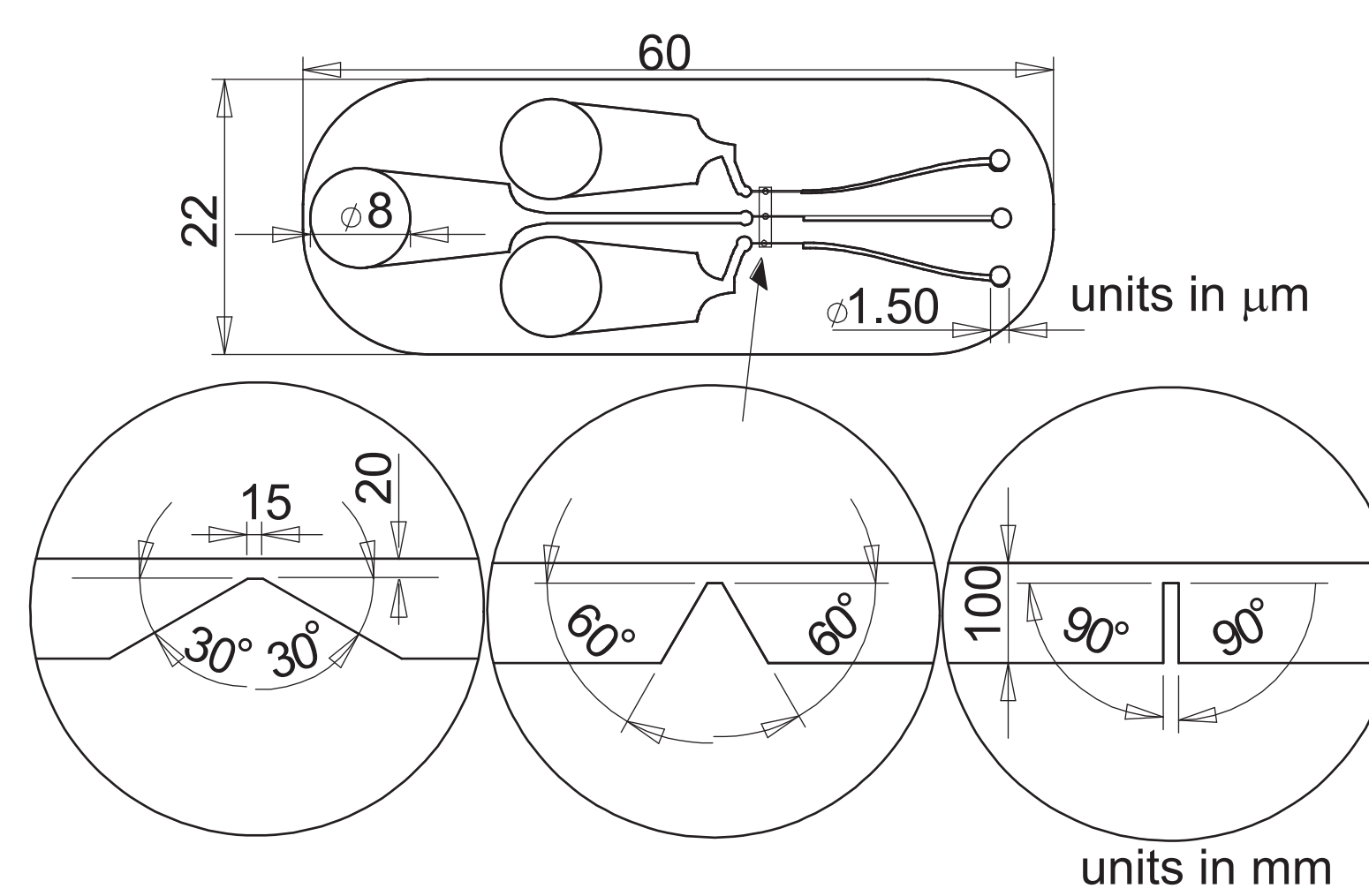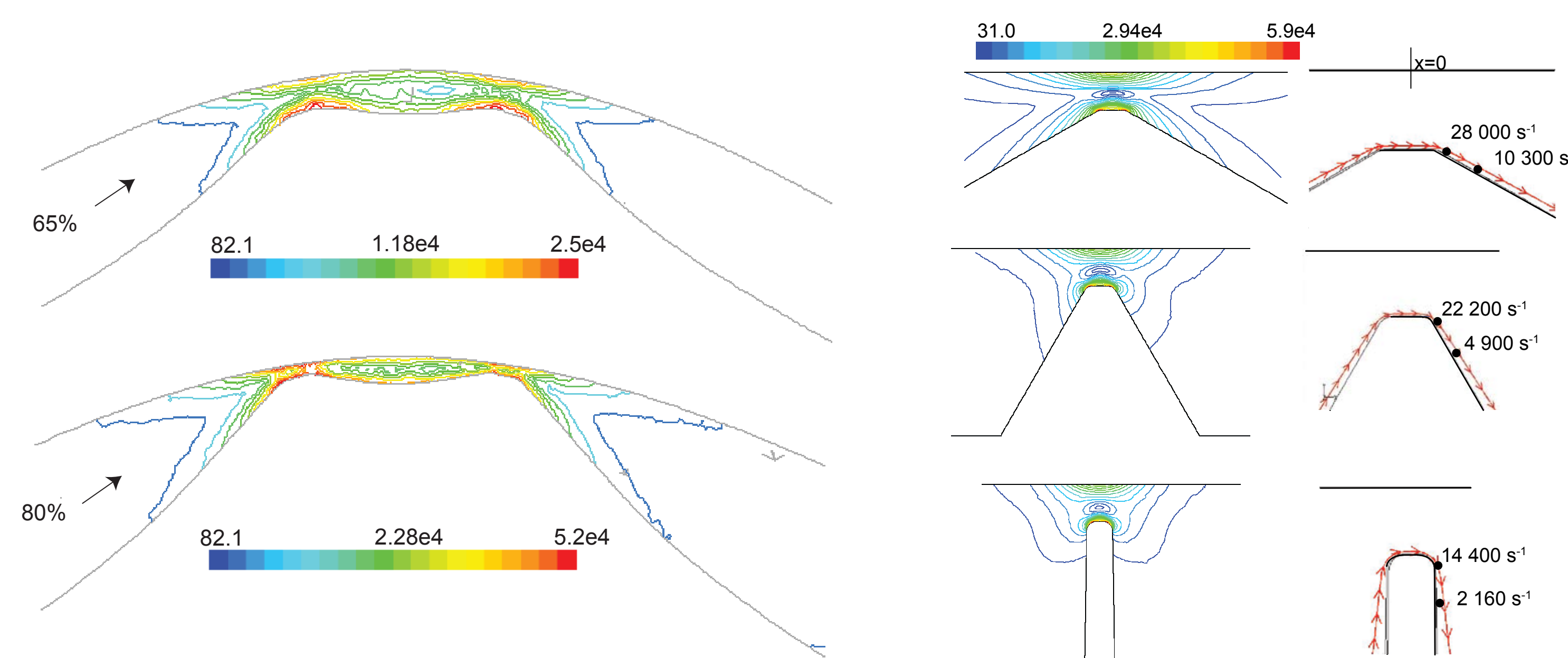


A microfluidic chip with idealized mimetic microcontractions was developed.
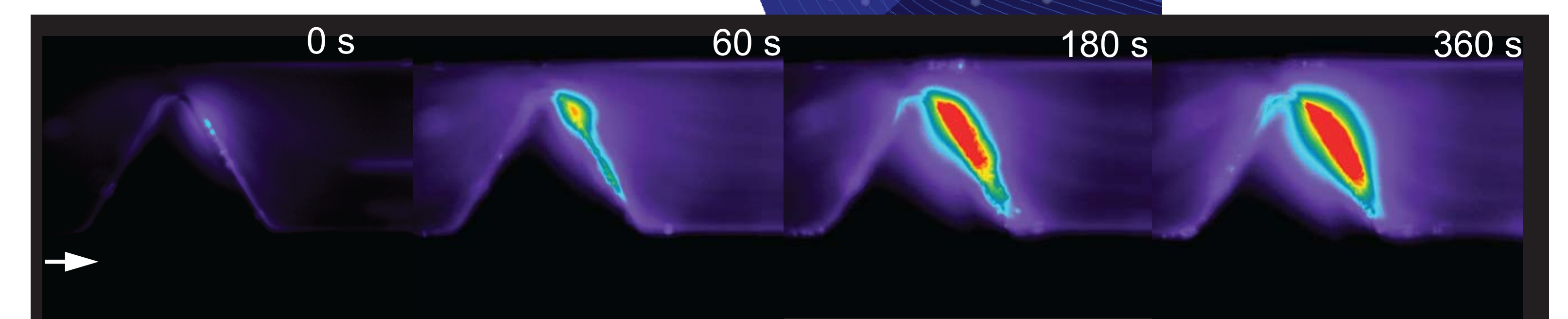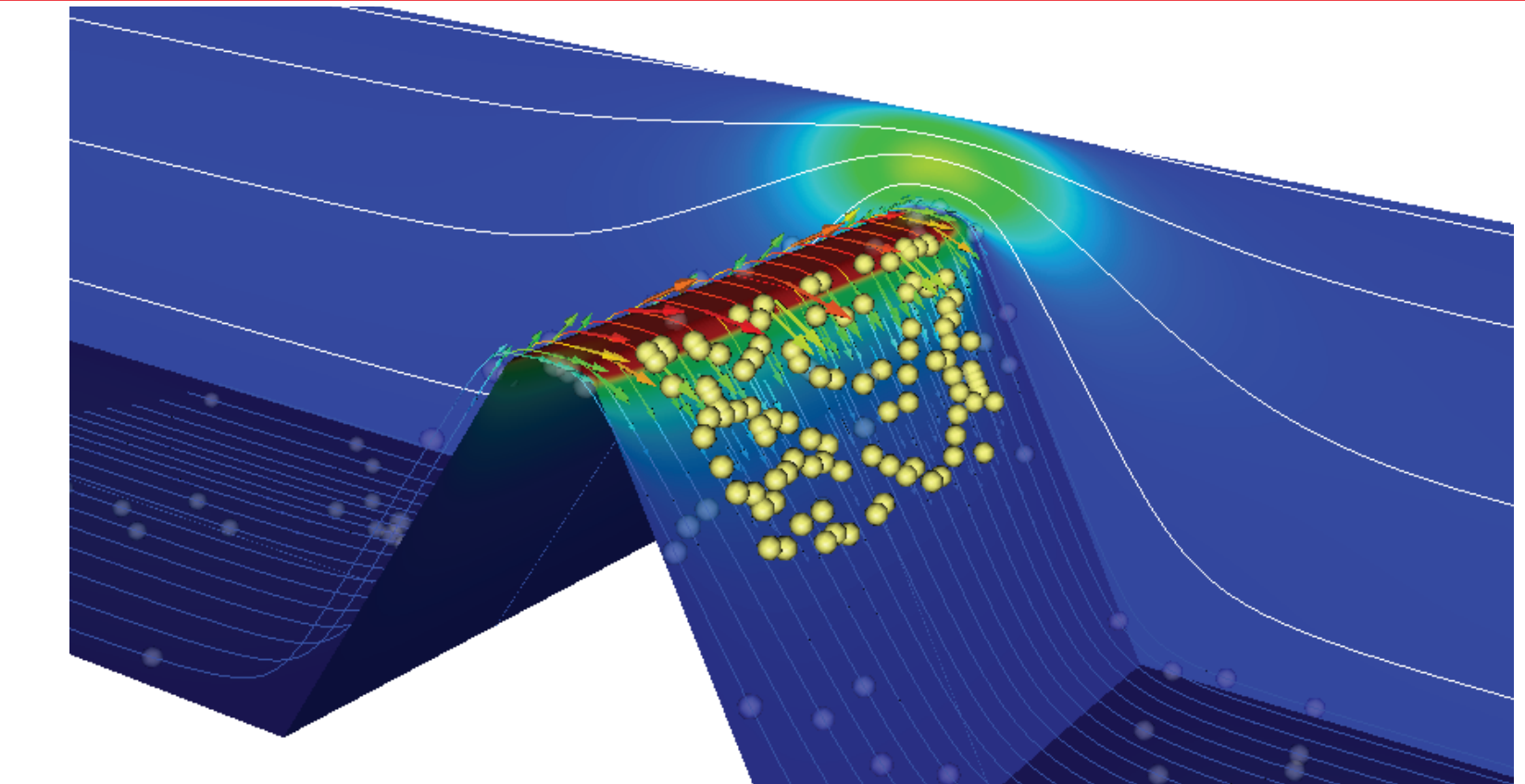


Platelet aggregation under dynamic shear is monitored and quantified using fluorescence.

## CONCEPTUAL DESIGN



65%
80%

*In vivo* stenosis

## METHODS



Microchannels

• CFD is used to study the shear rate relative to a platelet size.
• An estimation of the dynamic shear is determined.
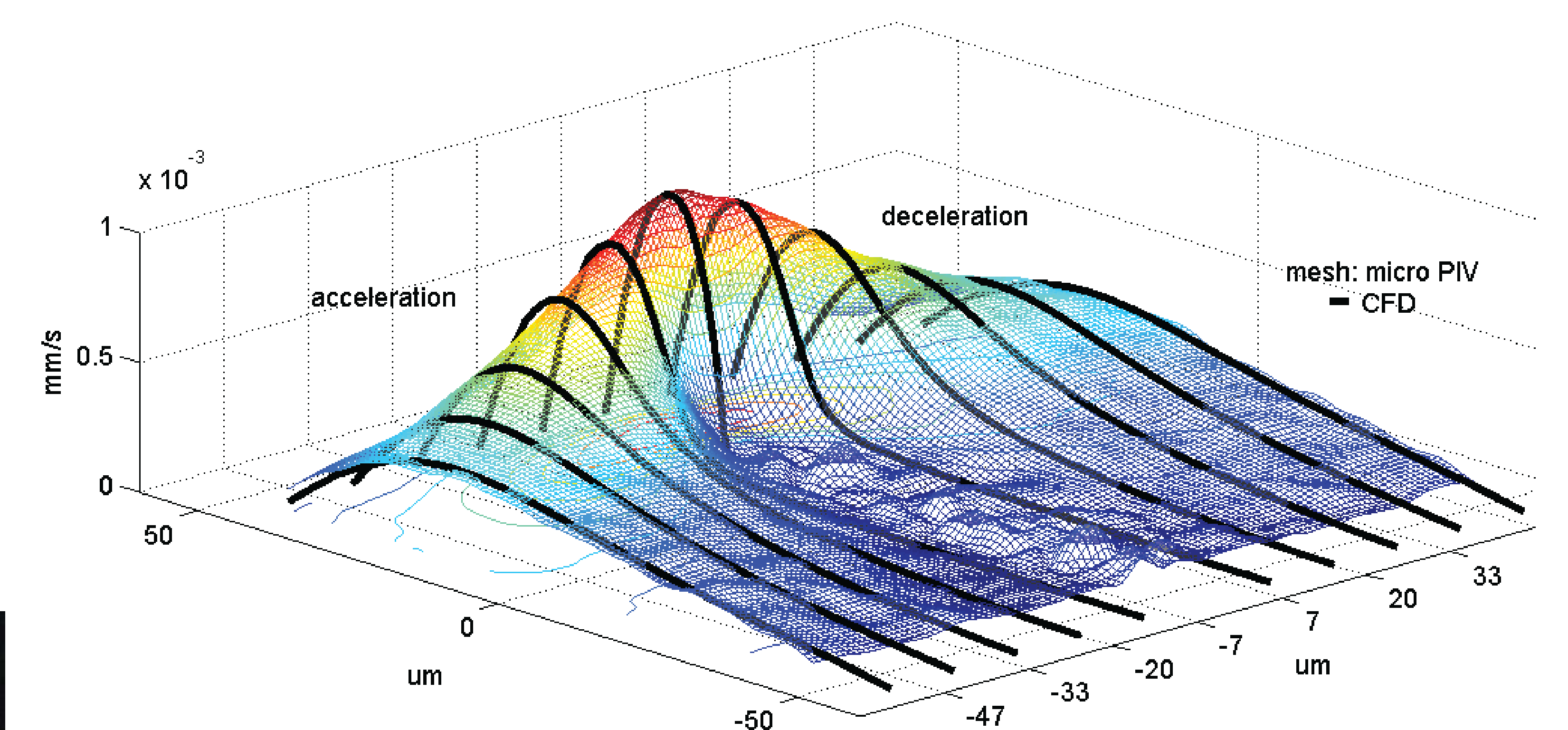
## MICROFABRICATION

• Standard photolithography to produce moulds.
• Channels of Polydimethyl-siloxane (PDMS).
• High resolution mimetic microcontractions relative to a platelet size can be fabricated.



## HYDRODYNAMICS



acceleration
deceleration
mesh: micro PIV
CFD
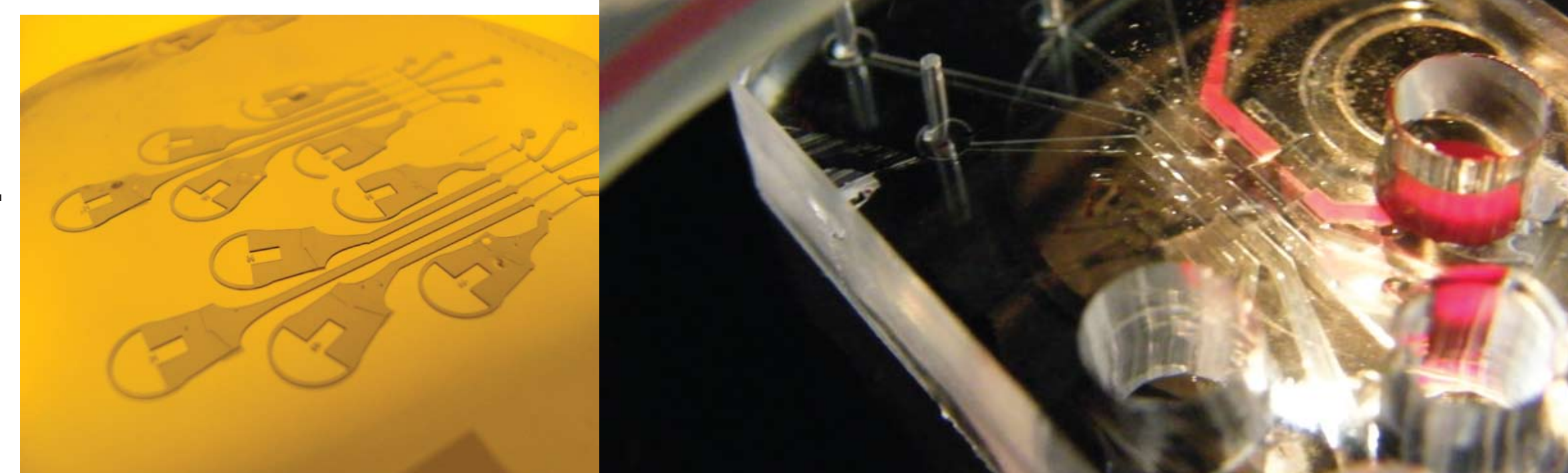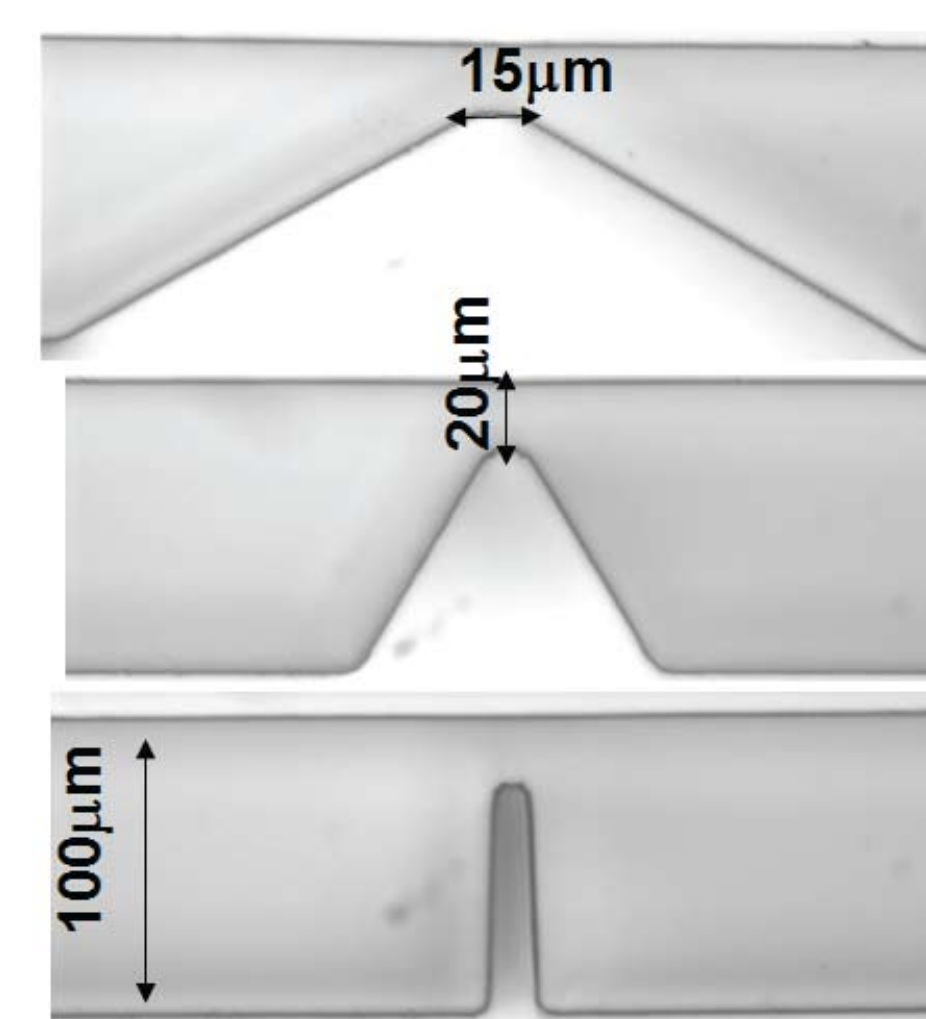
• Analytical solution of velocity is used to characterize the flow in sections of constant area.
• CFD is used to characterize the flow in the microcontraction, (mass continuity and momentum conservation equations).
• Micro Particle Image Velocimetry (µPIV) to validate the computations (1 µm particle size, LED backlight illumination)

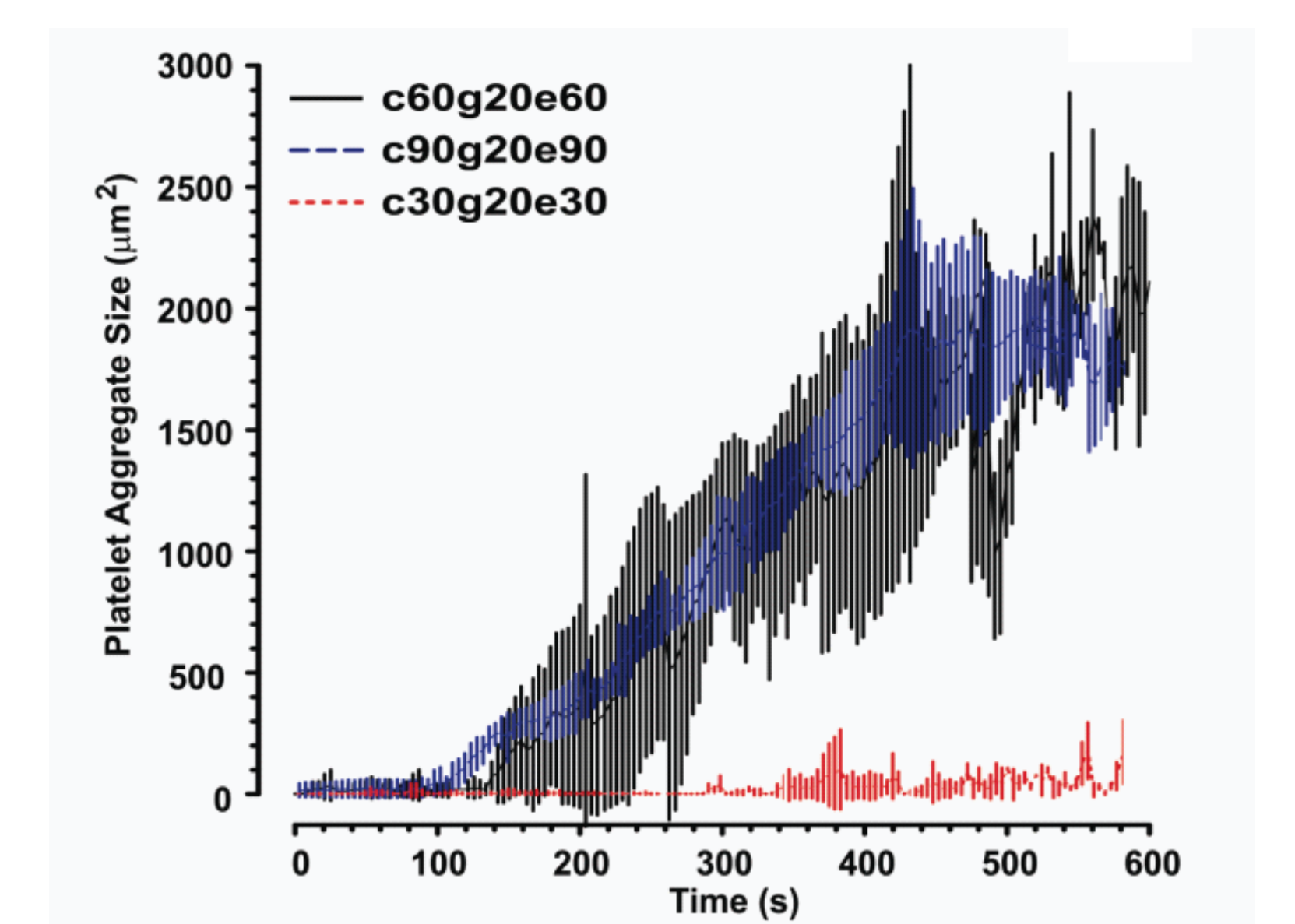## BIOLOGICAL EXPERIMENTS



c30g20e30
c60g20e60
c90g20e90

• Anticoagulated blood samples were obtained from healthy human donors.
• Platelets were stained with the fluorescent lipid dye DiCO6.
• Blood samples were treated with pharmacological inhibitors: Apyrase, 2Me, MRS Indomethacine to block the biochemical triggers of platelet aggregation, and study the effects of dynamic stress.
• Blood samples were perfused into the channel at controlled flow rate.
• Platelet aggregation dynamics was observed during 10 minutes.

### RESULTS

• Different aggregation response in function of the shear gradient was found.
• Platelet aggregation dynamics was monitored real-time and accurately quantified.



### CONCLUSIONS

• This platform technology may be useful as a clinical diagnostic tool and as a screen for new anti-platelet drugs.



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1. W. S. Nesbitt, et. al. "A shear gradient–dependent platelet aggregation mechanism drives thrombus formation" Nature Medicine, 15, pp. 665-73 (2009).

2. Tovar-Lopez FJ. et. al. Microfluidic platform to monitor platelet aggregation dynamics under blood flow. Lab Chip, 2010, 10, 291-302

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