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Blood cell - vessel wall interactions probed by Reflection Interference Contrast Microscopy

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ABSTRACT

Numerous biophysical questions require the quantification of short-range interactions between (functionalized) surfaces and synthetic or biological objects such as cells. Here, we present an original, custom built setup for reflection interference contrast microscopy that can assess distances between a substrate and a flowing object at high speed with nanometric accuracy. We demonstrate its use to decipher the complex biochemical and mechanical interplay regulating blood cell homing at the vessel wall in the microcirculation using an *in vitro* approach. We show that in the absence of specific biochemical interactions, flowing cells are repelled from the soft layer lining the vessel wall, contributing to red blood cell repulsion *in vivo*. In contrast, this so-called glycocalyx stabilizes rolling of cells under flow in the presence of a specific receptor naturally present on activated leucocytes and a number of cancer cell lines.

Keywords: Reflection interference contrast microscopy; high speed tracking; cell-surface interactions.

1. REFLECTION INTERFERENCE CONTRAST MICROSCOPY

Monitoring interactions between objects and a nearby surface is a key aspect of soft matter studies ranging from wetting to complex fluid flow. In biophysics, these interactions govern cell adhesion or the flow of microswimmers or blood cells. The non invasive investigation of such short-range interactions is nevertheless challenging, in particular under flow where fast imaging is required: while TIRF has been used extensively for cell adhesion studies, it requires staining of the sample and the quantitative measurement of distances remains challenging. Here, we demonstrate the use of Reflection Interference Contrast Microscopy (RICM) to probe interactions between blood cells (or blood cell mimetics) and functionalized surfaces displaying mechanical and biochemical properties similar to the blood vessel walls.

RICM is an optical technique that can probe optical distances between two or more interfaces based on the interference pattern formed by the light reflected from these interfaces. In its simplest form, it consists in illuminating the sample with nearly parallel, partially coherent light that is reflected by the substrate (e.g. the upper face of a glass coverslip) and the object under investigation: a cell, bead or vesicle adhering or hovering over this substrate [1]. The intensity pattern is imaged on a camera and analyzed in terms of difference in optical path lengths between the two reflections that give rise to constructive or destructive interferences (Fig. 1). RICM imaging can provide information on the height profile of complex samples such as vesicles and cells. In this case, the shape of the object under investigation is unknown, and the intensity profile is used to determine the height of the object at different positions by use of *a priori* knowledge of the object optical properties or previous calibration (Fig. 5d). This approach has been successfully used for studying adhesion of unilamellar vesicles and cells, or protein or electrolyte layer deposition on surfaces [1,2]. Alternatively, RICM can be used to automatically track objects of well-defined shape and optical properties such as beads with an accuracy of ~5 nm in all directions.

Here, in order to investigate the mechanical and biochemical aspects of blood cell - vessel wall interactions, we have designed and built an original RIC microscope allowing multimodal imaging of our sample at high frame rate. Coupled

to a careful analysis of the data, this has allowed us to demonstrate experimentally the role of the softness of the vessel wall outer layer in the repulsion of non-interacting cells.

Our custom-built experimental setup is shown on Fig. 1. In contrast to previous implementations of RICM, it decouples polarisation optics from the objective, allowing complete freedom on the choice of magnification, immersion medium and chromatic aberration correction. Coupled to a broadband, continuous white light source this has allowed us to build a flexible setup allowing multicolour RICM images or its straightforward combination with other modalities such as brightfield, phase contrast or fluorescence imaging. We demonstrate its use on the precision tracking of functionalized microspheres at high speed that can be used as cell mimetics for *in vitro* studies.

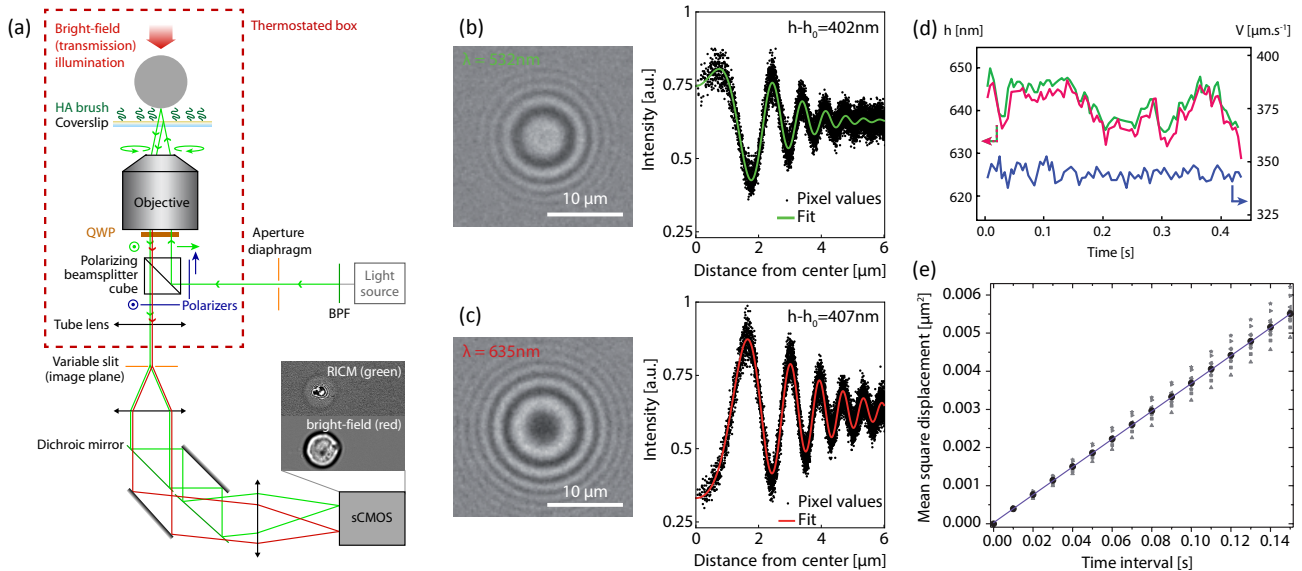


Figure 1. Multimodal RICM setup and bead tracking. (a), schematic view of the multimodal microscope. BPF, dichroic bandpass filters. QWP, imaging-flat quarter waveplate. For two- or three- color imaging at high speed, a home-built image-splitting setup is used to simultaneously acquire spectrally separated images on a sCMOS camera. Here, an example of combined RICM (green)/ brightfield (red) imaging is shown. (b-c), typical RICM images of 15- μm polystyrene beads at two different wavelengths. Azimuthal averages of the curves can be fitted to retrieve the bead-substrate distance with an accuracy of a few nm after localization of the pattern center that permits in-plane tracking of the bead. (d), example of the resulting height trajectories (green, 532 nm and red, 635 nm) and speed (blue) under flow. (e), analysing the mean square displacement of Brownian beads without flows permits quantifying the precision of in-plane localization as the offset of the curve (here ~ 5 nm rms). Adapted from [3].

2. IN VITRO PROBING OF CELL-VESSEL WALL INTERACTIONS UNDER FLOW

Next, we discuss the application of this method to the study of blood cell - vessel wall interactions under flow. This interaction is highly regulated as it is both critical in the flow of red blood cells and in the control of white blood cell adhesion to the walls e.g. at a site of inflammation. However, the biochemical and mechanical cues governing this regulation are still poorly understood. In particular, endothelial cells lining the blood vessels are covered with a thick and highly deformable polymer layer called glycocalyx that is first encountered by flowing cells when interacting with the vessel wall. To decipher the essential parameters controlling cell homing or repulsion, we have developed an *in vitro* platform allowing precise control of the surface functionalization (by a glycopolymer brush) mimicking the physiological glycocalyx and of the flow of cell mimetics that can display specific receptors of glycocalyx components, while permitting direct imaging of these mimetics at high speed [3].

Based on this platform, we have studied how the interplay between mechanical properties of the brush and specific biochemical interactions regulate cell-surface interactions under flow. First, we have shown that in the absence of specific biochemical interactions, the microspheres mimicking blood cells are repelled from the brush when submitted to a strong enough shear flow. The equilibrium distance can reach up to several hundreds of nanometers under physiological shear stresses, significantly contributing to limiting cell adhesion and to the margination of red blood cells

experimentally observed *in vivo* in microcirculation. These results are well explained by the theory of elastohydrodynamics that accounts for the effect of substrate deformation under hydrodynamic forces, providing the first experimental evidence of this "soft biolubrication" effect at play at small scale [4].

We then show how the introduction of specific binding to the hyaluronan brush serving as glycocalyx model through functionalization of the microspheres with CD44 receptors stabilizes bead rolling under physiologically-relevant flow fields. This rolling is strongly dependent on physical parameters of the brush (thickness and softness), providing insight into the mechanisms of control of cell homing by the glycocalyx in the initial stages of the process.

Finally, we show how these results can be extended to probing the interaction of real white blood cells with our model surface, confirming the mechanisms unveiled with our fully synthetic model.

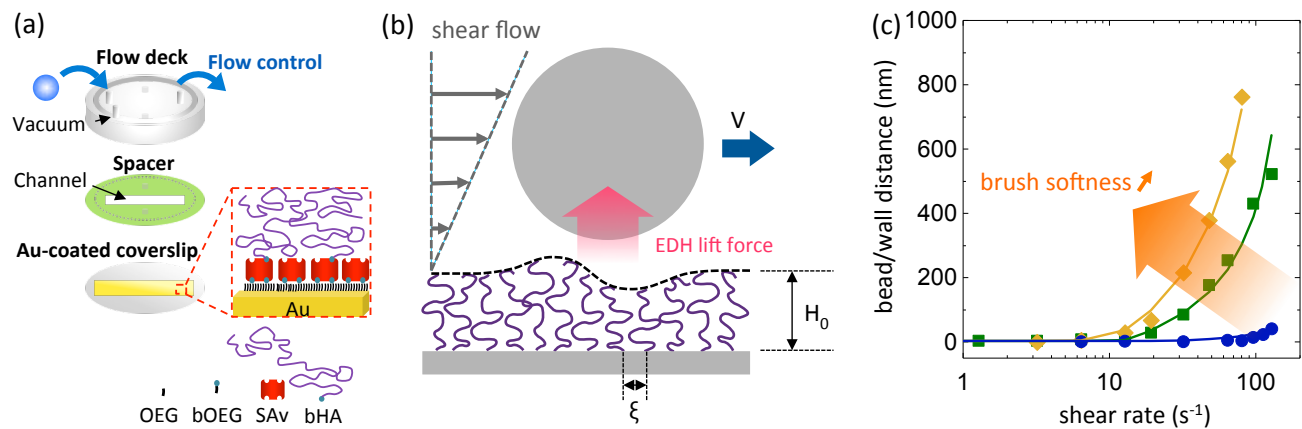


Figure 2. (a), *in vitro* platform for the study of glycocalyx model - cell mimetic interactions. A flow chamber coupled to a syringe pump is used for *in situ* surface functionalization and subsequent flow control. 15- μm microspheres are used as cell mimetics after functionalization with a well controlled surface density of CD44 receptors. (b) OEG, (biotinylated) oligo ethylene glycol; SAV, streptavidin; bHA, end-biotinylated hyaluronic acid of controlled molecular weight. Optical observation is possible through the bottom coverslip and the transparent flow deck. Adapted from [3]. (b), schematic representation of the bead-model layer interaction under a shear flow in the absence of CD44 functionalization. The magnitude of the elastohydrodynamic (EDH) lift depends on the shear stress but also on the brush parameters: thickness, grafting density. (c), experimental (symbols) and theoretical (plain line) EDH-induced equilibrium bead/wall distance vs. shear rate for different HA brushes of varying elastic properties (blue, 15 kPa; green, 40 Pa; Yellow, 8 Pa): softer brushes induce a lift of greater magnitude. Adapted from [4].

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