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# Highly IR-transparent microfluidic chip with surface-modified $BaF_2$ optical windows for Infrared Microspectroscopy of living cells

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ABSTRACT

In this contribution we present the first example of a microfluidic chip based on $BaF_2$ for Infrared Microspectroscopy (IRMS) of living cells. The advantage in using barium fluoride as platform relies on its high IR transparency, especially in the spectral region below 1300 $cm^{-1}$, where the absorption bands of nucleic acids and carbohydrates are located.

Barium fluoride is slightly soluble in water (0.12 g/100 g water) and it is potentially harmful for living cells. To overcome these problems, here we exploit an approach whose feasibility has been demonstrated previously on $CaF_2$: the surface modification obtained by sputtering a thin Si layer on the surface. The Surface Modified Microfluidic Devices (SM-MD) hence obtained not only solve the $BaF_2$ drawbacks, but also provide a silicon-like substrate fully compatible with standard micro-fabrication processes. These potentialities are here further explored in the direction of chemical or topographical nano-patterning of the silicon-like surface. The silicon thin layer was structured in the shape of 300 nm wide grooves (500 nm pitch) with a thickness of 35 nm by using standard NIL and etching processes; chemical patterning was achieved by exploiting silane chemistry.

Finally, we tested the performances of these devices at SISSI beamline@Elettra, collecting IR spectra of single MDA-MB-231 living cells maintained either in physiological solution or complete medium. A comparison of the spectra of a single cell obtained in $BaF_2$ and $CaF_2$ MDs is reported.



## 1. Introduction

Understanding the sequence of biochemical events in a living cell when exposed to external stimulation is one of the major scientific challenges in biology, with a great impact on fundamental knowledge and relevant biomedical applications.

Coupling Infrared Microspectroscopy (IRMS) and Microfluidic Technologies represents a new frontier for this kind of study. IR spectroscopy, in fact, is a non-destructive and minimally invasive technique that allows a label free detection of biochemical information [1]. Using the microfluidic approach, fluidic chambers with path length less than 10 μm (in order to avoid the strong IR absorption of water) has been fabricated and physiological conditions for cell viability reproduced [2–5].

The major advantage in working with living systems is the possibility to follow their real-time evolution without any artifact induced by fixatives that alter the biological information, especially on the content and conformation of nucleic acids [6]. We have already proposed a microfabrication approach for the realization of fully sealed microfluidic devices (MD) employing $CaF_2$ as substrate [7,8] and recently we have reported the optimization of the fabrication protocol leading to an IR–Vis transparent microfluidic chip, suitable for exploring biochemical changes occurring in living-cells under different stimulation made on surface modified (SM) $CaF_2$ optical windows [9]. There we described the deposition of a thin silicon film on the optical window as a simple method to modify the surface properties of $CaF_2$, so as to increase its surface energy and wettability, to make all processes compatible with established nanofabrication processes and to separate cells from water soluble, potentially poisoning substrates.

Here we discuss further developments in the direction of: (a) the use of more IR-transparent materials (i.e. $BaF_2$); (b) silicon layer nanopatterning and/or chemical functionalization.

For the surface modification of $BaF_2$ optical windows a thin (10–20 nm) silicon layer has been deposited by sputtering. This layer does not affect appreciably the window transparency in the range of interest. The main advantage in using $BaF_2$ is its excellent IR-transparency in a wider spectral window compared to $CaF_2$ (see

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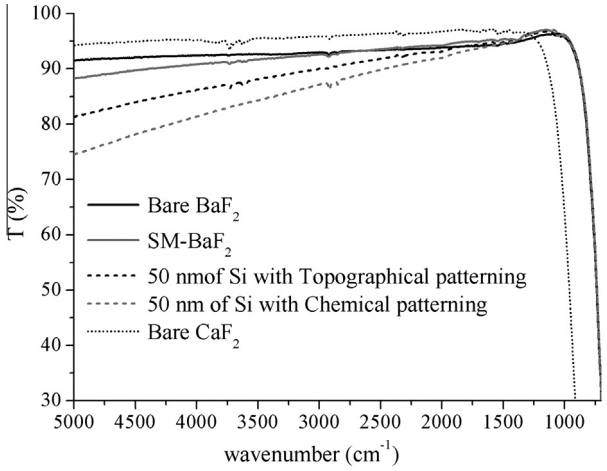


**Fig. 1.** Transmittance curves of $CaF_2$ (black dotted line) and $BaF_2$ (continuous black line) 2 mm thick windows in the 3500–800 mid-IR regime are plotted. SM-$BaF_2$ with 15 nm of silicon transmittance curve (dark gray continuous line) as well as topographical and chemical patterned SM-$BaF_2$ transmittance curves (black and gray dashed lines respectively) are reported. The lower transmittance of topographical and chemical patterned SM-$BaF_2$ is due to the higher thickness of silicon not to the patterning.

Fig. 1) [10]; however, $BaF_2$ is more soluble in water than $CaF_2$, strengthening the demand for a thin layer acting as a barrier.

We tested the new devices based on $BaF_2$ substrates by collecting IR spectra from MDA-MB 231 cells in different environments and compared the results with data obtained under analogous conditions but using devices made on $CaF_2$ substrates.

## 2. Experimental methods

IR-grade $BaF_2$ optical windows 25 × 13 mm (Crystran Ltd., UK), were purchased in two different thicknesses, 2 and 1 mm and used as microfluidic chip substrates and leads, respectively. Both 2 and 1 mm windows were sputter-coated with a thin (~15 nm) silicon film (Ar sputtered at $1.2 \times 10^{-3}$ mbar, 600 W DC for 15 s). UV photolithography was used to fabricate the fluidic circuitry on X-ARP 3100/10 positive tone resist (All Resist GmbH, DE), spin-coated to a thickness of 7.5 μm. The sealing of the device was achieved by clamping the two windows together in a homemade two-shell metal holder [8]. A heating strip with a closed-loop control system with a PT-100 thermocouple served to stabilize the device temperature at 37 °C, in order to preserve physiological conditions for living cells.

Nanopatterning of the silicon layer was obtained by NanoImprint Lithography (NIL) as follows: silicon was deposited with a thickness of 50 nm; the NIL resist MRI 7020 (MRT GmbH, DE) was spin-coated to a thickness of 145 nm on a SM-$BaF_2$ optical window and nanoimprinted at 90 °C and 5 MPa. After removal of the residual layer by oxygen plasma, the silicon was etched by dry etching in a fluorine-based ICP reactor (SPTS Technology). Surface topography was inspected by SEM (see Fig. 2).

For the patterning of chemical functionalities, silicon was deposited with a thickness of 50 nm; SM-$BaF_2$ windows were patterned by UV-photolithography using Shipley S1828 resist, spin-coated to a thickness of 3 μm. After development, the surfaces were exposed to vapor of octo-decyl-trichloro-silane creating a hydrophobic functionalization on the opened areas, the patterned resist preventing the contact between underneath silicon and silane moieties. After resist stripping using hot acetone, exposure to water aerosol revealed the pattern created. Optical image of the surface was collected using an optical microscope equipped with a CCD camera at a magnification of 10× (see Fig. 3).

MDA-MB 231 cells (mammary gland adenocarcinoma cell line) were chosen as model for device testing. Cells were maintained in the culture medium (Dulbecco's Modified Eagle's Medium, Sigma Aldrich, 0.01 mg/ml bovine insulin, fetal bovine serum 10%) at 37 °C in an incubator ($CO_2$ 5%). Cells were then harvested from the flask using trypsin and, after being washed twice, re-suspended in culture medium or physiological solution. The cell suspension was then dropped inside the device for measurements. IR spectra were acquired at SISSI beam line (Elettra Synchrotron, Trieste, Italy) [11] using a Bruker Hyperion 3000 IR–VIS microscope in transmission mode, setting the sampling area at 30 × 30 μ. The background was collected within the device in a region free of water and the water spectral contribution was subtracted as detailed in [6].

## 3. Results and discussion

### 3.1. Surface modification of $BaF_2$ windows

Barium fluoride is of widespread use in the IR-spectroscopy field due to the high transparency in the Mid-IR region, especially below 1300 $cm^{-1}$, where the IR features of nucleic acids and carbohydrates are detected [12]. However, its use as a substrate for IRMS of living cells is hindered by its water solubility (0.12 g/100 g water) and by the toxicity of its ions [13]. Surface chemistry of the culturing substrate is a key parameter that influences the fate of cells [14,15]. Therefore the possible interaction between adherent cells and surfaces (e.g. tuning of adhesion processes, chemical signaling, poisoning) has to be controlled.

We have recently demonstrated [9] a simple and effective method for the tuning of the surface–cell interface, consisting in the deposition of a thin layer of silicon on IR-transparent $CaF_2$. This technique produces silicon films strongly adherent onto the $CaF_2$

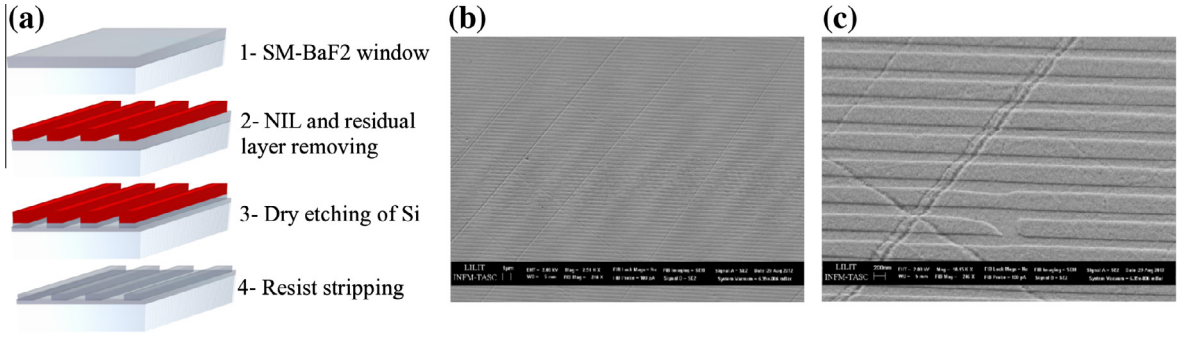


**Fig. 2.** (a) Scheme of the fabrication process based on NIL to obtain topographical patterning of the silicon layer; (b) SEM pictures of the resulting patterning: 300/500 nm width/period lines etched at a depth of 35 nm on 50 nm initial silicon layer; (c) SEM image at higher magnification showing details of the resulting grating.