

Feature Article

堀場雅夫賞 特別賞受賞者論文

Masao Horiba Award

Using Surface Plasmon Resonance Imaging to Address Cell-based Biological Issues

表面プラズモン共鳴イメージングを利用した細胞単位の生物反応分析

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Miniaturized systems are gaining increasing interest since the last two decades. In the specific case of food safety, environment issues and medical applications, Biosensors and Biochips are developed worldwide to propose new, fast, cheap, reliable and multiplexed tools for Physicians. Since 2005, I am leading several projects focused on the design and fabrication of Biochips yielding new kinds of data. To that end, we are proposing miniaturized systems suitable for real-time analysis - by Surface Plasmon Resonance (SPR) imaging- of living cellular sample. Direct capture, controlled release of specific cell type, and more recently secreting activities have been successfully carried out in our Laboratory on these miniaturized devices.

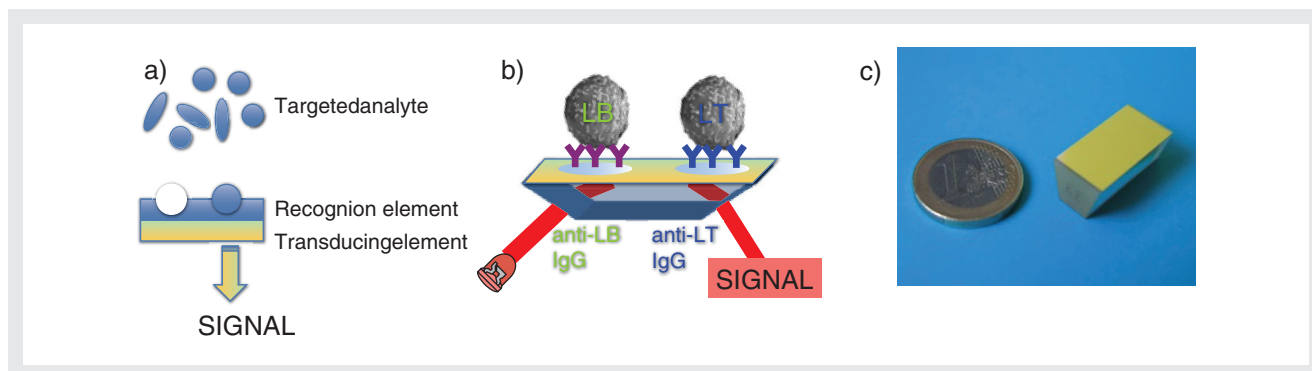
過去20年間、システムの微小化への関心はますます高まっている。特に食の安全、環境問題、医療機器の分野において、バイオセンサーやバイオチップが世界中で開発され、新規性に富み、高速、安価で、信頼性が高く多機能なツールが医療関係者に提供されている。2005年以来私は、リアルタイム測定データ等、従来法では得られなかった新たなデータがとれるバイオチップの設計及び製作を目的としたいくつものプロジェクトを率いてきた。その成果である、SPRイメージングを用いた、生きている細胞のリアルタイム分析に適した小型システムを提案する。我々の研究室では、その小型バイオチップ上で、細胞を直接捕捉する技術、また特定の細胞種をコントロールしながら放出させる技術に加え、細胞に分泌活動をさせることに成功した。

Introduction

Living organisms are highly complex machines. To be fully functional, they require the controlled operation of a tremendous number of chemical reactions. Any disorder or shift from this dynamic equilibrium may affect the whole organism and then trigger troubles or pathologies. For these reasons, the characterization of biological constants (linked to biomolecules or cellular analysis) along with the observation of other 'measurable' parameters (fever, local inflammation...) has been used as the main source of data by the physicians for centuries. Because the earlier a pathology is identified the more successful is the patient recovery, the search for fast, reliable, and easy-to-use devices may increase the success in many Health issues.

Biosensors are technological developments dedicated to the detection/characterization/monitoring of one biological target. They all rely on a common dogma putting all together a recognition element, highly specific to a biological analyte whose binding to the recognition element will fire a signal through a transducing element (Scheme 1a). Biochips are multiplexed Biosensors capable of simultaneous characterization of a large number of analytes. One of the main advantage of these analysis plate-forms is their reduced size requiring tiny amounts of biological sample but also enabling their easy integration in automated processes.

Since 2005, my research projects are focused on the development of Biochips dedicated to the detection of Blood cells. The basic architecture of our miniaturized system is composed of thin metallic gold film lying on a



Scheme 1 Biosensors and Biochips. a) General scheme of a biosensor. b) Scheme of a glass prism modified with antibodies specific to either B or T lymphocytes. The cell capture on the surface locally affects the optical index whose shift is monitored in real-time by SPR imaging. c) Microphotograph of a high-index glass prism recovered with a gold layer (50 nm) along with a One Euro coin.

high-index glass prism. The device surface is modified by one or several ‘molecular probes’ (DNA, proteins: antibodies, antigens, structural proteins...). One of the original aspects of our system is the optical detection carried out by monitoring Surface Plasmon Resonance (SPR) effects. Although less sensitive than fluorescence assays for molecular target analyte detection, the SPR imaging of the biochip allows real-time monitoring of the analyte capture by the surface-bound molecular probe (recognition element). An other significant interest of this technique is the lack of any labeling step, significantly affecting the cost and the operation of the analysis. In a very first attempt, antibodies specific to different Blood cells (i.e. B or T lymphocytes) have been used to functionalize Blood specific Biochips (Scheme 1).

Surface Chemistry: A bottleneck to get through

As shortly described in the introduction, the physical

coupling of the recognition element on the transducing element is a key step to produce fully operating Biosensors and Biochips. Our Laboratory proposed an original electrochemical process for biomolecule (mainly DNA) immobilization on electrodes.^[1] It is possible to remotely control the electropolymerization of pyrrole (a small water-soluble molecule) along with pyrrole-modified molecular probes. During the electrochemical process, linear insoluble polypyrrole molecules are synthesized and settled on the anode electrode (the gold layer in our case) (Figure 1).

Using this original grafting chemistry, we successfully immobilized antibodies specific to antigens surrounding cellular membranes.^[3] Our device was suitable for an real-time monitoring of the cell-capture on micro-arrayed features: SPR images allowed the easy detection of discrete micrometric objects (individual cells) bound on larger spots corresponding to features of electro-polymerized antibodies (500 to 800 μm in diameter) (Figure 2a). In collaboration with the CNRS-LAAS Laboratory (Toulouse, France), we managed to produce

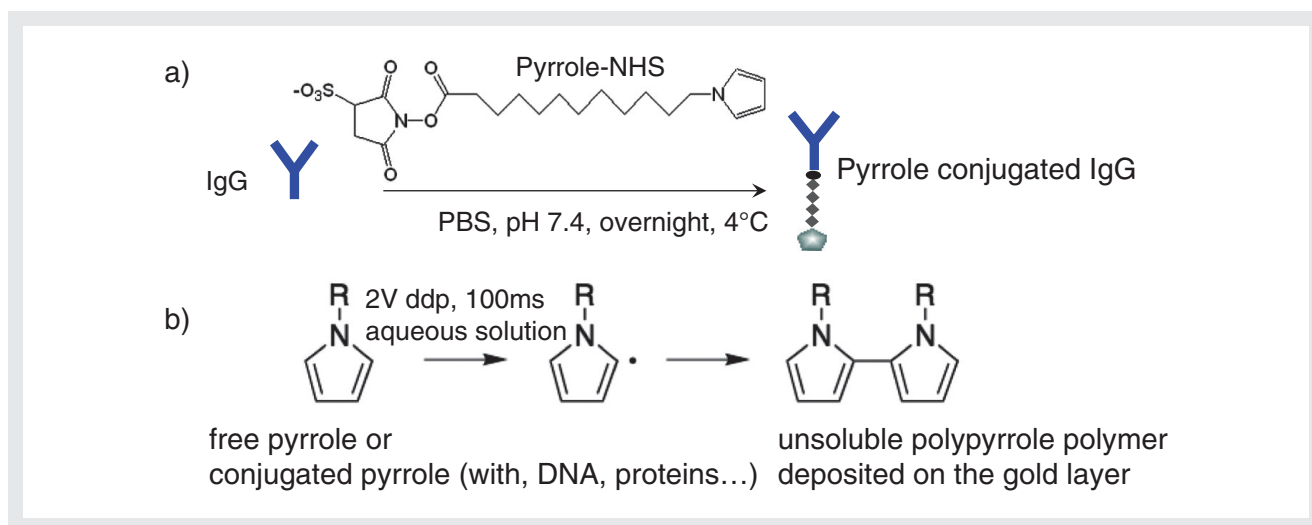


Figure 1 Electrochemical deposition of biomolecules on metallic electrodes. a) In the specific case of antibodies, an home-made pyrrole-modified molecule is used for covalent conjugation with Immunoglobulin G (IgG, i.e. antibody).^[2] b) Radical generation and polymerization of free pyrrole containing pyrrole-modified probes. After polymerization, the polypyrrole polymer remains tightly bound to the electrode surface.

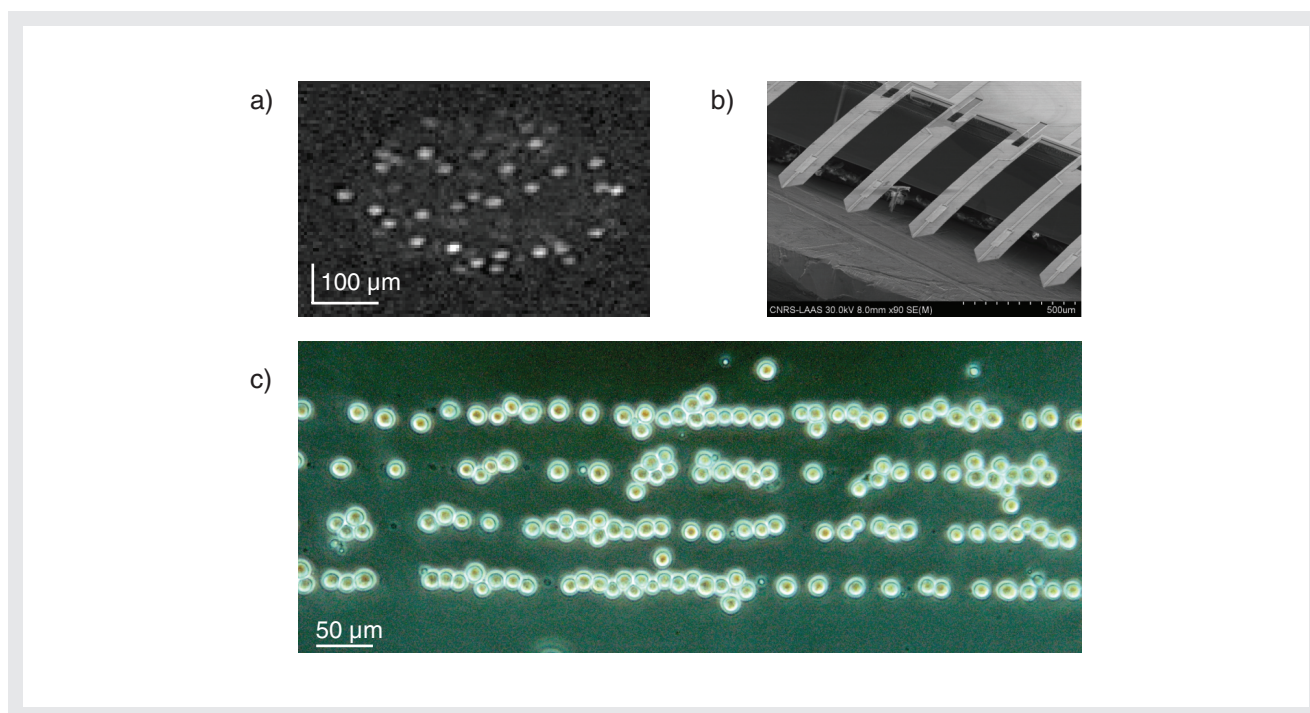


Figure 2 Trapping and seeing cells on a Biosensor. a) SPR image of an individual feature of immobilized antibody specific to B lymphocytes. Individual cells are easily seen as discrete objects captured on the spot. b) Scanning electron microscopy (SEM) image of cantilevers used for the protein electro-polymerization. c) Microscopic image of lymphocytes captured on micrometric features (20 μm pitch, 50 μm between each line).

micro-cantilever modified by a gold film lying in an open-channel (Figure 2b).

These silicon-based micro-tools were successfully used as ‘pencils’ to handle antibody aqueous solutions as ink to array micrometric spots on a gold layer (few microns in diameter). After incubation with lymphocytes solutions and gentle was off, lines of captured cells could easily be seen by standard transmission microscopy (Figure 2c).^[4]

DNA Grafting for Cell Capture and Release Monitored in Real-time

More recently, we proposed an alternative approach for antibody grafting on Biochips. Our method is based on the conjugation of antibodies with DNA fragments, hybridized on a DNA biochip (Scheme 2). We combined several Restriction Sites (i.e. specific DNA sequences that are cleaved by specific enzymes in mild conditions) with the different antibodies to allow the specific, sequential and controlled release of the cell by adding the proper Restriction Enzyme.

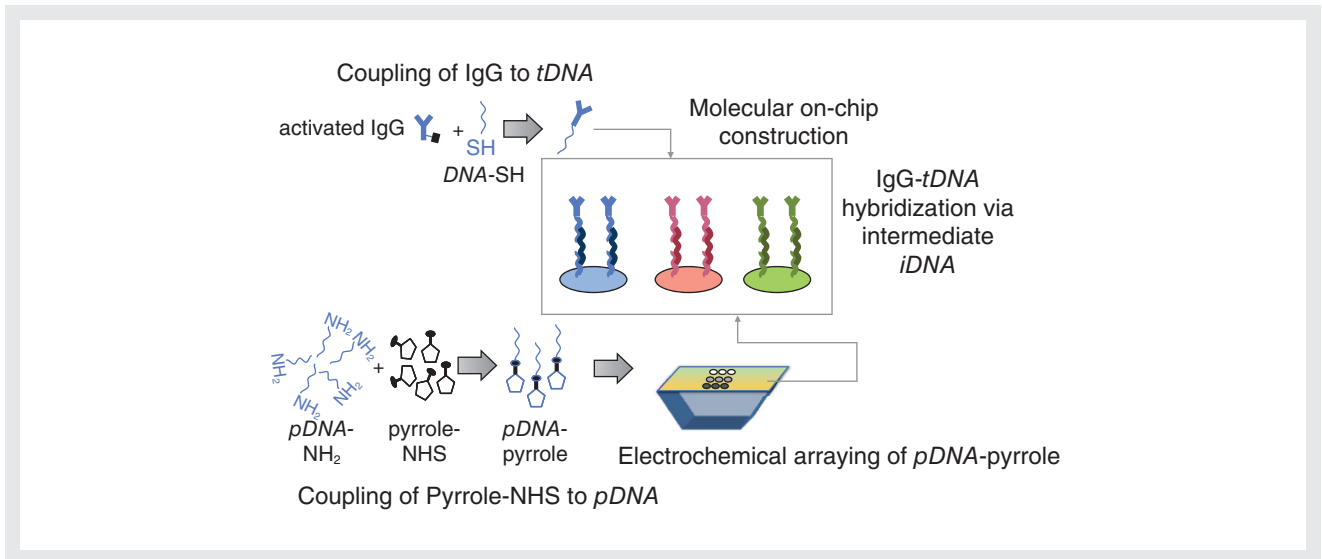
After completion of the molecular building up, biological samples containing B and T lymphocytes were incubated on the micro-array to assess the full operation of the Biochips. The whole process (molecular building up, cell

capture and controlled release upon action of Restriction Enzymes) was monitored in real-time, and in a label-free manner by SPR imaging. Because this optical technique is highly sensitive to any change of reflective index occurring in the vicinity of the gold layer (within few hundreds of nanometers), it has the unique property to be used to monitor molecular interactions (Biochip building up) along with cellular interactions (cell capture and controlled release) (Figure 3).

One More Step: Looking forward cellular activities

More recently, we focused our attention on the development of Biochips capable of better exploration of cellular insights.^[5] The underlying idea is to follow, in real-time, cellular events that are not –or poorly– accessible by usual techniques. As a model of choice, and because Blood cells are the most common cellular sample, we have decided to monitor cellular secretions from Lymphocytes.

Because Blood cells are non-adherent cells, the main communication mode is based on the production (and detection) of soluble molecules in the extra-cellular medium. These molecules (named Cytokines), are



Scheme 2 Turning a DNA Biochip into a Protein Biochip. This scheme describes the modification of the biochip surface with DNA to array DNA fragments at different locations on the biochip (lower part). The conjugation chemistry is based on the electropolymerization of pyrrole molecules. In the mean time, antibodies are modified with an other set of DNA sequences (upper part) to ensure proper hybridization of the conjugated products on the DNA microarray. After full completion, the biochip is made of double stranded DNA whose end is linked to a functional antibody (middle). Each color corresponds to a pair of antibody linked to a specific double stranded DNA containing a restriction site.

involved in a large variety of biological processes: inflammation, allergy, bacterial and viral responses... For these reasons, we chose as model secreting cells, hybridoma cells (i.e. engineered B lymphocytes) continuously secreting antibodies specific to a well-

defined and available antigen. For these specific assays, the antigens were chemically grafted on the surface, and secreted antibodies capture was monitored by SPR imaging.

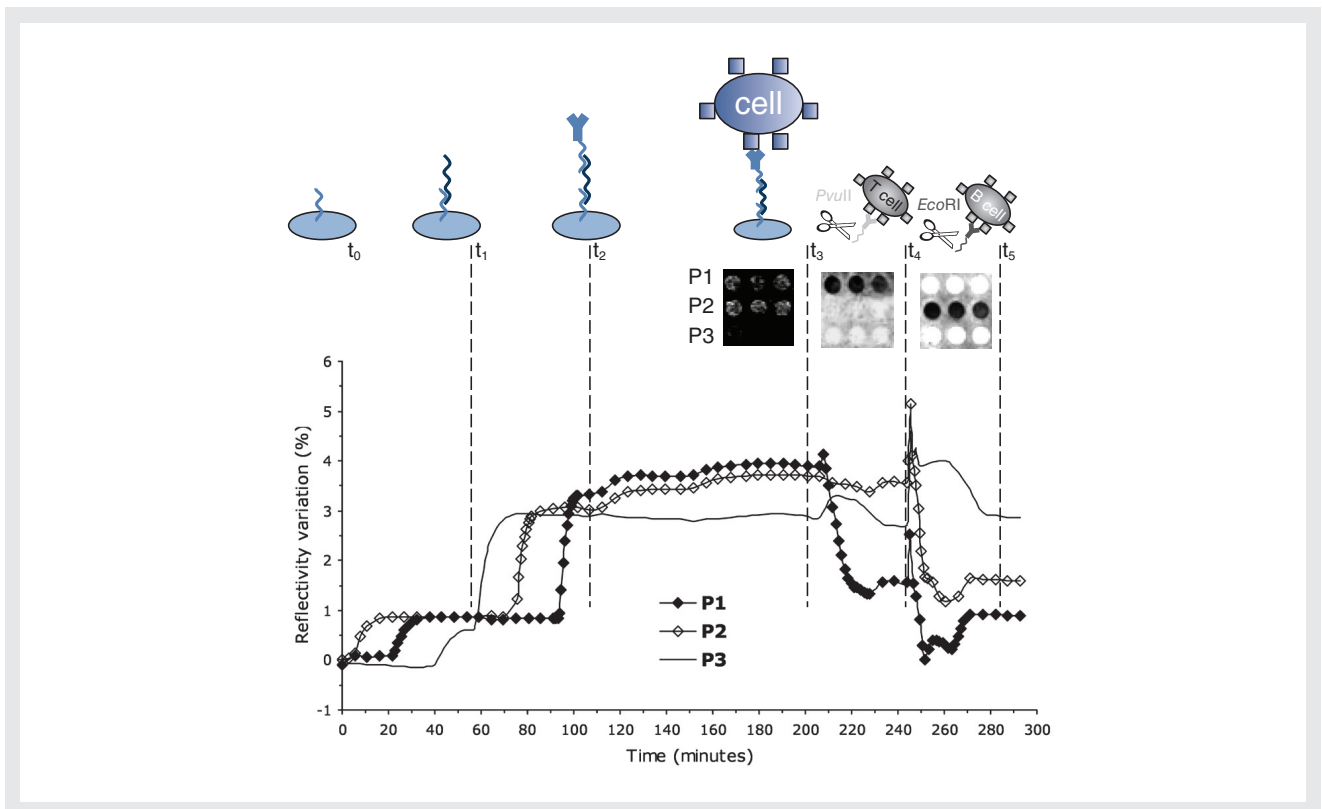


Figure 3 SPR traces corresponding to the real-time monitoring of Biochip building up, cell capture and release. t_0 : addition of long complementary strand; t_1 : addition of the DNA-antibody conjugated product; t_2 : addition the B and T cell sample, t_3 : addition the *PvuII* Restriction enzyme to specifically release T lymphocytes, t_4 : addition the *EcoRI* Restriction enzyme to specifically release B lymphocytes; t_5 : wash off the Biochip with buffer. P1, P2 and P3 traces respectively correspond to T cell specific, B cell specific and no-cell specific (negative control) spots. Images in inset are SPR images of spots arrayed in triplicate after cell capture, T cell release and B cell release.

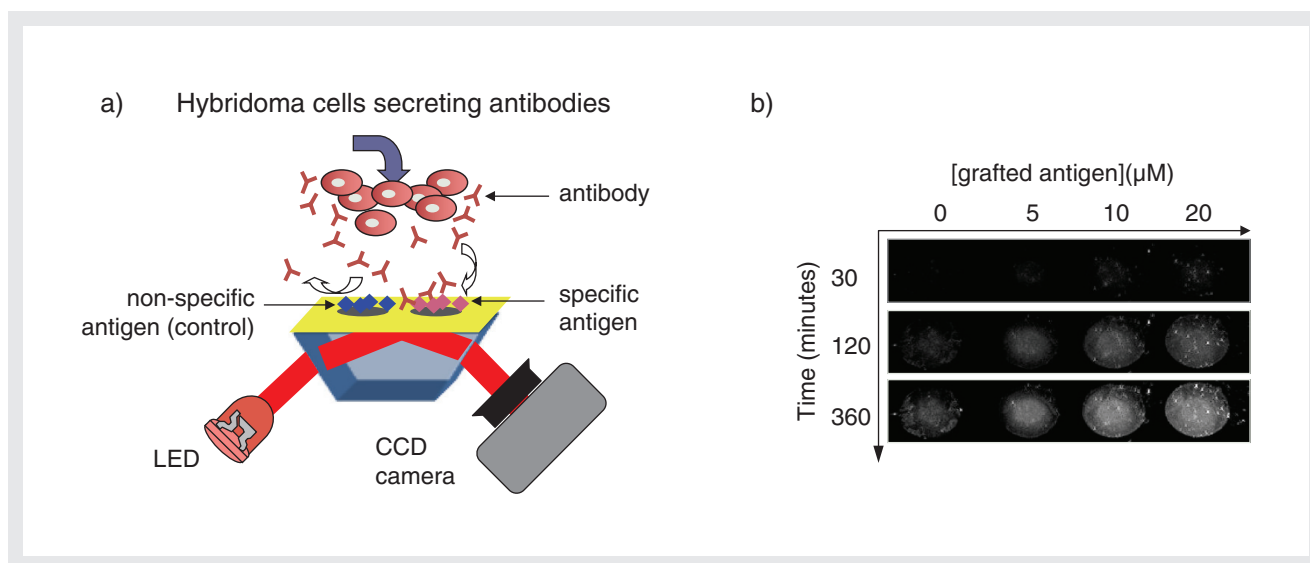


Figure 4 Real-time monitoring of antibody secretion using SPR imaging. a) In this specific set-up, antigens were grafted on the Biochip surface to ensure the capture of antibodies secreted by the hybridoma. b) SPR images monitored at different time laps, several concentration of antigens were used for the chemical grafting to assay the best concentration. The concentration effect was easily seen by SPR imaging.

For the very first time, we managed to show how cellular secretions could be monitored in real-time, on a multiplexed Biochip containing different probes and controls species (Figure 4).

Conclusion

These results paved the way to new generations of Biosensors and Biochips dedicated to cell analysis. The specific use of SPR imaging brings several significant advantages compared to alternative 'classic' techniques (such as the real-time detection and thus the access to kinetic data or the direct and label-free handling of the samples). Although encouraging, there is a long way to walk before proposing a packaged, easy-to-use, cheap device to the Society. To get one step closer, we are currently working on Biochips suitable for primary cell analysis (spleen and Blood cells from mice).

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