



TIRF Labs

Total Internal Reflection Fluorescence

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TIRF Analytix
i-Diagnostics
u-TIRF stations

Super-resolution Microscopy

Single Molecule Detection

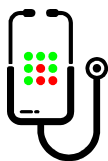
Single Ion Channel

**Single Molecule
Biology**

TIRF Slides
TIRF Lightguides
Fluorescence Illuminators

www.TIRF-Labs.com

*Detect and manipulate
single molecules*



Total Internal Reflection Fluorescence

**Single Molecule Biology, Single Molecule Detection,
Single-molecule-FRET, Super Resolution Microscopies,
TIRF Analytix, i-Diagnostics, Turnkey TIRF Microscopy Stations - uTIRF,
Real-time TIRF Microarrays for Molecular Diagnostics and Drug Screening,
Fluorescence Illuminators, Digital Fluidics**

TIRF Principles. TIRF has become a method of choice for single molecule detection, single molecule biology, super-resolution microscopies, plasma membrane studies, and numerous other applications, due to its exceptional spacial selectivity. The phenomenon of total internal reflection, which is part of TIRF, generates the evanescent wave with maximal intensity at the interface and an exponential decay of intensity with distance. The depth of penetration of the excitation light is ~20-100 nm -less than the wavelength, which allows for circumventing the diffraction limit of resolution. TIRF minimizes the background of irrelevant fluorescence and permits the detection of single molecules as well as performing of many other useful functions.

TIRF Geometries. Three geometries can be used to generate the TIRF effect: through-objective-, prism-, and lightguide-based optical schemes, or o-TIRF, p-TIRF, and Ig-TIRF, respectively. Because Nikon, Olympus, Zeiss, and Leica have strong expertise in developing microscope objectives, they have only marketed the o-TIRF geometry, which depends on specialized high NA lenses and other sophisticated optics. Typical oTIRF microscopy systems cost ~\$80,000 or more. Ironically, in the world of TIRF, expensive does not mean better quality. pTIRF and IgTIRF cost only \$15k-\$25k and demonstrate superior signal-to-background ratios - less than 0.001% and 0.1%, respectively - as opposed to the 10-15% signal-to-background ratio in the case of oTIRF [Axelrod D. *et al.*, *J Biomed Opt*, 2006, 11:014006A; Oheim M. *et al.*, *Biophys J*. 2014;106:1044]. pTIRF and IgTIRF geometries were not offered commercially until 2010, when TIRF Labs started to market them. Since 2010, our customers have generated unique TIRF data and have demonstrated the superior advantages of prism and lightguide geometries in a number of applications, including single molecule biology, the dynamics of lipid rafts, and real-time microarrays [1-4].

Selecting TIRF products. If you perform *in vitro* studies, prism-TIRF is the first geometry to consider, as it provides the cleanest TIRF effect. For studies with live cells that require open perfusion chambers, lightguide-TIRF will be the best geometry from the standpoint of signal-to-background ratio. If you intend to detect single molecules, you will need ~1kiloWatt/cm² excitation power, objectives with NA 1.0 or more to collect enough fluorescence, and a low light camera. Both pTIRF and IgTIRF are well-suited for single molecule detection, including sm-FRET and other multicolor TIRF experiments. Prism- and Ig-TIRF can be used for studies that require UV excitation. IgTIRF is compatible with AFM, electrophysiology, and electric field control. TIRF Labs supplies pTIRF and IgTIRF systems as factory-aligned add-on accessories. It takes no time to install/uninstall p-TIRF and Ig-TIRF, and they fit onto virtually all microscopes. See White Paper "Selecting the Optimal TIRF geometry...", application notes and references to articles of our customers at www.tirf-labs.com.

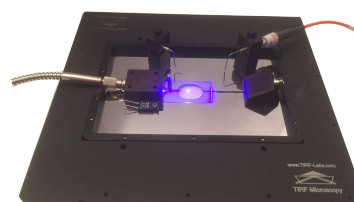
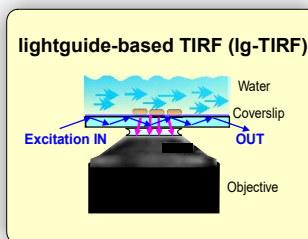
Compatibility with objectives, coverslips, illuminators, and XYZ microscope stages. pTIRF and IgTIRF systems are compatible with dry, water-, and oil-immersion objectives. They can be used with silica or glass coverslips or 1-mm slides. For SMD experiments, TIRF Labs offers low autofluorescence coverslips and slides. pTIRF and IgTIRF systems are mounted on 110 x 160 mm plates that are standard sizes for motorized XY stages. 110x160 mm plates can be nested into a 200 x 220 mm platform, well-suited for manual translation stages. >>> more: www.tirf-labs.com

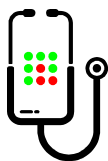
Literature cited:

[1. Lamichhane R, et al. SMD of G protein-coupled receptor β 2AR. PNAS. 2015;112(46):14254-9] [2. Lamichhane R, et al. Dynamics of DNA polymerase. J Am Chem Soc. 2013; 135(12):4735-42.] [3. Asanov A, et al. Ig-TIRF for lipid rafts dynamics. Biochim Biophys Acta. 2010;1801(2):147-55.] [4. Asanov A, et al. Ig-TIRF for combined DNA and protein microarrays. Sensors (Basel). 2012;12(2):1800-15.]

Product description

1. IgTIRF - lightguide-based Total Internal Reflection Fluorescence microscopy is a geometry, which provides a superior signal-to-background ratio and exceptional flexibility. In IgTIRF, the excitation lightpath and emission channels are independent. Excitation light enters one of the ends of the lightguide, as shown at the diagram, and escapes from the opposite end. IgTIRF is a factory-aligned system with fixed angles, which provides a reproducible intensity of the evanescent wave and is well-suited for multicolor TIRF; the depth of penetration can be changed by optical traps. IgTIRF is compatible with dry, water-, and oil-immersion objectives. It uses 0.13-0.17mm glass or silica coverslips as a TIRF lightguide, 1-mm microscopy slides, or Petri dishes with optical bottoms. IgTIRF system is available with open perfusion chambers and closed flow cells. It takes no time to install IgTIRF at XY-translation stage of an inverted microscope. The core unit is mounted at a K-frame (110 mm x 160 mm - standard for motorized XY translation stages). A 96-well SBS plate footprint 85x127 mm is available as special order. The K-frame can be nested into a 200 mm x 220 mm platform, which is well-suited for manual XY translation stages. The base model is supplied with one of the single-color illuminators: 405, 465, 520, 532, or 637 nm coupled into a fiber optics cable. Multicolor illuminators are available as options. IgTIRF also implements Shallow Angle Fluorescence Microscopy (SAFM) - a novel powerful method for cell biology studies. See Application Note "Multicolor Ig-TIRFM and SAFM for Cell Biology Studies" for more information.

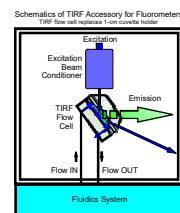




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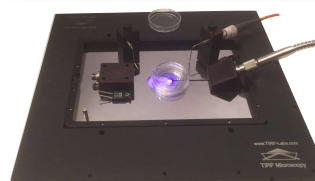
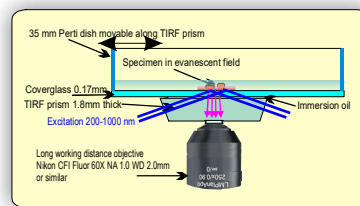
2. TIRF Flow System Accessory for Fluorometers TA1004

TIRF Flow System accessory TA-1004 is designed to replace the standard 1-cm cuvette holders that are common to fluorometers. The TA1004 accessory transforms a fluorometer into a super sensitive TIRF biosensor instrument. Supported fluorometers include Horiba-JY-SPEX Fluorolog and Fluoromax, PTI, ISS, Varian Eclipse, SLM, Shimadzu, and Hitachi fluorescence spectrophotometers. Please inquire about custom-designed TIRF if your instrument is not listed. The TIRF system TA1004 is supplied as a factory-aligned accessory. It takes no time to install/uninstall the TA1004. In a TIRF mode, the bulk of the solution is not excited, which reduces the background and allows for super-sensitive, real-time detection, down to single molecules. No other technique exists that can monitor fluorescence lifetime, polarization, anisotropy decay, quenching, resonance energy transfer (FRET), recovery after photobleaching (FRAP), and correlation spectroscopy (FCS) in real-time and with limit of detection at the level of single molecules. Base model of TA1004 is supplied with gravity-driven fluidics. TA1004 also can be used with external pumps. TIRF Labs' digital fluidics SmartFlow transforms a fluorometer into a computer-controlled TIRF biosensor instrument capable of performing unattended TIRF sensogram experiments. Electrochemical, dielectrophoresis and temperature control are available as options. Chemically modified TIRF slides and reagent kits for surface immobilization of biomolecules are available as consumables. See "TIRF Flow System Accessory" brochure at www.tirf-labs.com for additional information.



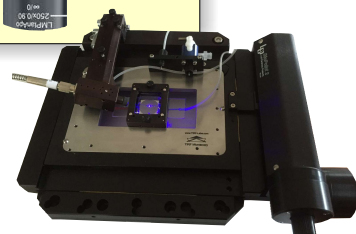
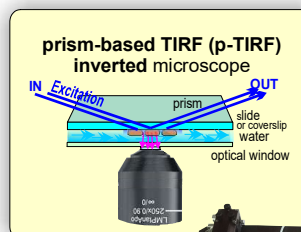
3. pdTIRF - prism-down Total Internal Reflection Fluorescence

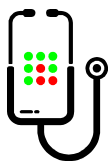
Fluorescence microscopy with open perfusion chamber and imaging through thin 2-mm prism. Prism-based TIRF is a geometry, which provides the best signal-to-background ratio. TIRF Labs offers imaging-through-the-prism - pdTIRF system, which uses 1.8 mm prism and 0.17mm thick coverslips, including Petri dishes with 0.2mm or thinner optical windows at the bottom. pdTIRF system is well-suited for live cell studies. It can be used with 36-mm Petri dishes. The dish can be XY translated over the prism. pdTIRF is compatible with microscope objectives with working distance 2 mm and larger. Such objectives with NA 1.0 and greater are available from Nikon, Olympus, Zeiss, and Leica. Visit webpage: tirf-labs.com/prismtirf.html and email at info@tirf-labs.com for more information.



4. puTIRF - prism-up Total Internal Reflection Fluorescence

microscopy with closed flow cell and TIRF imaging through the optical window. puTIRF system is well-suited for biomolecular studies that require closed flow cell for arranging flow of solutions along the TIRF surface. In puTIRF system a 6-mm prism and 1-mm slide are brought in contact by optically matching solution and are incorporated into a scheme with excitation in total internal reflection mode, as shown in the diagram. Imaging is performed through thin layer of solution and optical window, as shown. puTIRF system is supplied with embedded microfluidic system and fluidic cartridges that are well-suited for handling small liquid aliquots, down to sub-microliter volumes. puTIRF is compatible with dry, water-, and oil-immersion objectives; it uses silica prism and silica or glass slides, can use UV light for excitation. It is factory aligned arrangement that can be used as an add-on accessories for rapid installation on XY translation stages of different microscopes. Visit webpage: tirf-labs.com/prismtirf.html and email at info@tirf-labs.com for more information.

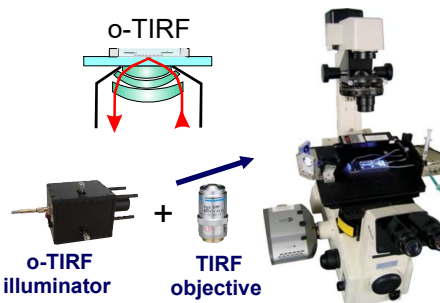




Product description

5. oTIRF - objective-based Total Internal Reflection Fluorescence

microscopy flow system. TIRF Labs offers o-TIRF flow systems with open perfusion and closed flow cell chambers. Closed flow cells are equipped with advanced fluidics, which is well-suited for handling sub-microliter aliquots of solutions. Base model is supplied with one of the single-color illuminators: 405, 465, 532, or 637 nm and 60X 1.49 NA TIRF objective. Multi-color illuminators and 100X 1.49 NA TIRF objective are available as options. TIRF Labs offers o-TIRF systems for Nikon Eclipse TE and Ti2 series, Olympus IX Series, and Zeiss Axiovert microscopes. Visit web site: turf-labs.com and email at info@turf-labs.com for more information.



6. Turnkey TIRF Microscopy Station

Turnkey TIRF Microscopy station is created on Nikon, Olympus, Zeiss, or Leica inverted microscope platforms. The station includes:

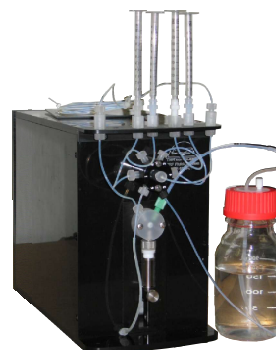
- Prism-based pTIRF microscopy flow system, and/or
- Lightguide-based IgTIRF microscopy flow system, and/or
- Objective-based oTIRF microscopy flow system
- Multi-color computer-controlled illuminator
- Low light CMOS or EMCCD camera
- Optional digital fluidics SmartFlow
- Optional temperature control system TC-40 25-40°C
- Optional filter-wheel EW-6 at emission channel
- Optional electrochemical control unit EC-1070
- Optional dielectrophoretic control unit EDC-407

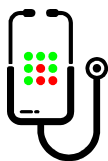
TIRF Labs supplies turnkey TIRF microscopy station with TIRF Studio software package, which controls MCI-7000 illuminator, Andor EMCCD cameras, digital fluidics SmartFlow, temperature control TC-40, filter-wheel EW-6, and electrochemical unit EC-1070. Microscope is not included. TIRF Labs will collaborate with microscope supplier of your choice to provide seamless integration of TIRF microscopy complex with the microscope of your choice.



7. Digital Fluidics SmartFlow

Computer-controlled TIRF Fluidics System SmartFlow® is equipped with precision digital syringe pump, computer-controlled valves, manifolds, containers for buffer solutions, and disposable plastic containers for bioanalyte samples. SmartFlow® fluidics is designed for delivery of bioanalyte and buffer solutions into TIRF flow cell or open perfusion chambers with profiled flow rates to minimize the effect of slow mass transfer and facilitate the measurement of true kinetics of association and dissociation and determination of rate constants k_{on} and k_{off} with minimum amount of bioanalyte solution. SmartFlow® fluidics is supplied with versatile software, which allows for configuring TIRF experiments for standard biosensor applications, as well as providing sophisticated flow profiles for injecting bioanalyte, buffer, and regeneration solutions for custom-defined experiments. The software contains flow profile wizard and set of fluidic programs for standard TIRF experiments.





Product description

8. TIRF BIOSENSOR INSTRUMENT FLUOROGAZER

TIRF Biosensor FluoroGazer® is a TIRF flow system equipped with integrated digital fluidics, multicolor illuminator, filter wheels, photon-counting PMT, or low light CMOS or EMCCD camera. The detection limit of FluoroGazer is at the level of single molecules. FluoroGazer is a cost-effective, upgradable system, with manual docking of TIRF sensor chips and automated delivery of up to six bioanalyte solutions. FluoroGazer is interfaceable with auto samplers for unattended analyses. Base model of FluoroGazer includes dual LED 480 and 530-nm illuminator and single-channel photon-counting PMT. High-end configurations includes 10-color illuminators covering the range from near UV to near IR - 280-1064 nm. The software package TIRF Studio controls multicolor illuminator, fluidics, filterwheel, and CMOS or EMCCD cameras. The software acquires the kinetics of TIRF response and derives k-on and k-off rate constants. Optional electrochemical polarization system, electric field control, dielectrophoresis, and temperature control can be used in FluoroGazer flow cell for manipulating with biomolecules and live cells, stimulating association and dissociation, or regenerating the TIRF sensor surface for next cycle of analysis.



9. PORTABLE TIRF BIOSENSOR

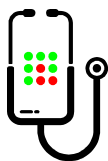
Portable Biosensor TIRF Sense® is totally autonomous (small-shoe-box-size) device for point-of-care molecular diagnostics. TIRF Sense® is capable of performing highly multiplexed analysis - simultaneously detect from a few to several hundred DNA, RNA, protein, and metabolite markers in a small 100-microliter sample. Rate of response is in the range from several seconds to a few minutes. TIRF Sense® biosensor requires no or minimal sample preparation. Whole blood, lysate of fine needle aspiration biopsy, or homogenated specimen of a tissue can be analyzed after minimal preparation procedures. TIRF Sense® measures real-time kinetics of microarray response, builds sensograms and derives k-on and k-off rate constants. The sensor is equipped with internal standards, positive and negative controls that are printed at the surface of TIRF sensor chips. TIRF Sense® uses silica, glass, and plastic microscopy slides (1 inch x 3 inches x 1 mm) as TIRF lightguides. TIRF Sense® also supports Electro-Chemi-Luminescence (ECL), bioluminescence, bead-based, and solution-phase fluorescence detection methods. The sensor is well-suited for real-time kinetic analysis of surface-immobilized gel-encapsulated bioassays. Custom-defined configurations of TIRF Sense® biosensor include multicolor illuminator, temperature, and electrochemical control.



10. i-Diagnostics - Cell Phone Based Molecular Diagnostics (i-TIRF arrays)

Molecular diagnostic device i-Diagnostics employs CCD cameras of smartphones to acquire the dynamic response of real-time TIRF microarrays that simultaneously detect protein, nucleic acid, and metabolite biomarkers. i-Diagnostics requires no or minimal sample preparation and is capable of detecting from a single to several thousands of molecular markers in a 50-microliter sample of biological fluids, including whole blood. Limit of Detection (LOD) for micro-RNA is 10^{-18} M. For proteins and metabolites LOD depends on the assay; for certain antibody-based assays LOD is at the level of $\sim 10^{-15}$ M. The broad dynamic range of i-TIRF sensor covers the entire spectrum of clinically significant concentrations. Classical TIRF microarrays operate with small, sub-monolayer amounts of antibodies and DNA probes immobilized on the surface; classical TIRF signal is small; a low light EMCCD camera is necessary. i-TIRF arrays are enhanced with silk fibroin that allows for immobilizing larger amounts of antibodies per unit area of bioassay spots. The i-TIRF signal is a thousand-fold greater than that in classical TIRF. CCD cameras of cell phones are sensitive enough to detect the signal from i-TIRF arrays. Because of several advantages, i-TIRF biosensors will become popular devices for hundreds of applications. If you are developing a diagnostic system based on luminescent assays, we invite you to collaborate to make your assays available to future users of i-Diagnostics sensors.



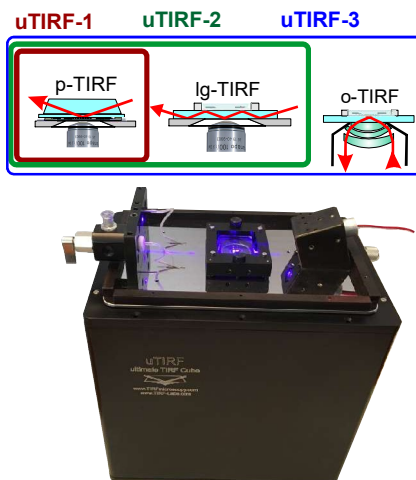


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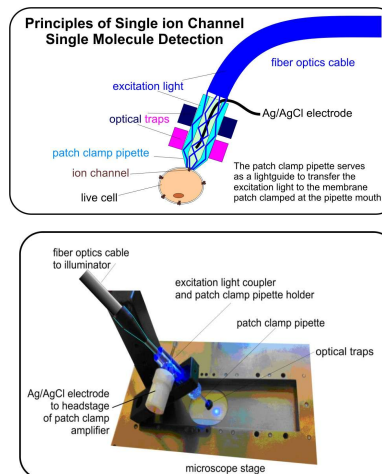
11. uTIRF - Ultimate TIRF Microscopy Station

Base model of the uTIRF station (uTIRF-1) consists of prism-based TIRF system, and includes three infinity objectives 4X, 40X, and 60X, three sets of optical filters for GFP, Cy3 and Cy5, a low light USB CMOS camera, and a three-color 465/532/638 nm illuminator. The model uTIRF-2 consists of prism- and lightguide-TIRF. The model uTIRF-3 - comprises all three geometries: prism-, lightguide- and objective-TIRF. Optional equipment includes additional infinity objectives, multicolor illuminators with UV, visible and near-IR excitation wavelengths, sCMOS, CCD, and EMCCD cameras, open perfusion and closed flow chambers, temperature control, electrochemical control, electric field and dielectrophoretic control.

Prism- and lightguide-TIRF feature optical schemes where the excitation lightpath is independent from the emission channel, which ensures small intensity of stray light, <0.01% and <0.1%, respectively. In the case of objective-TIRF geometry, stray light, at the level 10-15%, has been reported in the literature. One can use the uTIRF as a standalone turnkey station, or take prism-, lightguide-, and objective-TIRF as add-on accessories for inverted microscopes.



12. Single ion Channel Single Molecule Detection [Cell Calcium. 2015;57(1):1-13.] SC-SMD system combines the patch clamp method with fluorescence imaging of single molecules. It allows for parallel electrophysiological study of a single ion channel and simultaneous fluorescence imaging of single molecules that comprise the same ion channel. In SC-SMD the tip of the patch clamp pipette serves as a probe, which delivers spatially confined excitation of fluorescence to the micron-size area at the mouth of the pipette. The patch clamp pipette functions for electrophysiology (as usual) and also serves as a lightguide, which transmits the excitation light to the tip. This area is imaged by fluorescence microscopy and simultaneously probed by the patch clamp technique. Thus, the system allows simultaneously acquiring single channel electrophysiological recordings and detecting single molecule fluorescence within the same membrane patch. The SC-SMD system can be seamlessly integrated into existing patch clamp systems. Because the optical power is concentrated in a very small (1-micron diameter) area, the intensity of the excitation light is sufficient for single molecule detection experiments. Base model of the SC-SMD system consists of a modified patch clamp pipette holder, a fiber optics cable, and a fiber-coupled blue (465 nm), green (532 nm), or red (637 nm) fluorescence illuminator.



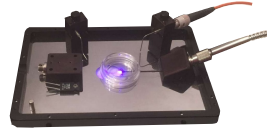
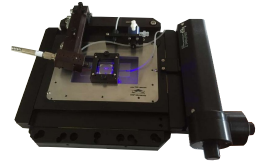
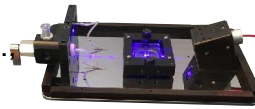
13. Single Color and Multi-color Fluorescence Illuminators

TIRF Labs offers broad range of multicolor and single color fluorescence illuminators to meet the requirements for sophisticated as well as simple fluorescence experiments. The price of an illuminator depends on your choice of colors and optical power. Contact us to request a price quotation for your set of colors and optical power. Tell us about your applications - we will help you to determine the best configuration of the illuminator. We offer fiber-coupled and free beam illuminators. Our base models feature SMA 905 or FC-PC output for coupling with single 100-micron fiber or with fiber bundle ~1.5 mm diameter. Optical adaptors for microscopes enable use of TIRF Labs' illuminators with virtually all fluorescence methods. Our base models are equipped with ports for TTL/analog modulation and 0-100% manual adjustment of power. USB, Ethernet, Wi-Fi, and Bluetooth com ports and embedded processors are available as options. LED and laser light sources and optical power: 100 mW - 5,000 mW; LEDs: 350, 405, 455, 465, 470, 505, 520, 530, 627, 800, 1270 nm; lasers: 405, 445, 450, 465, 470, 473, 520, 532, 637, 671, 1064 nm;



Prism- and Lightguide-based TIRF Microscopy Accessories

- Single molecule detection, single molecule biology
- Super-resolution microscopy: STED, PALM, STORM, ...
- Minimal stray light, crisp, high-contrast TIRF images
- Work with dry, water-, and oil-immersion objectives
- Use UV or visible excitation light 190-900 nm
- Use Petri-dish, open perfusion, or closed flow chamber
- Nested design - fits inside 96-well plate, K-frame, 4-inch round, or manual XY stages
- Optional temperature, dielectrophoresis, electric field control



Turnkey Single Molecule Detection TIRF Microscopy Station

Modular TIRFM stations include:

- Fluorescence microscope
- lightguide-, prism-, or/and objective-TIRFM
- Low light EM CCD or sCMOS camera
- Multi-color computer-controlled illuminator
- Digital fluidics SmartFlow
- Optional temperature and electric field control
- Software for instrument control and data analysis



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