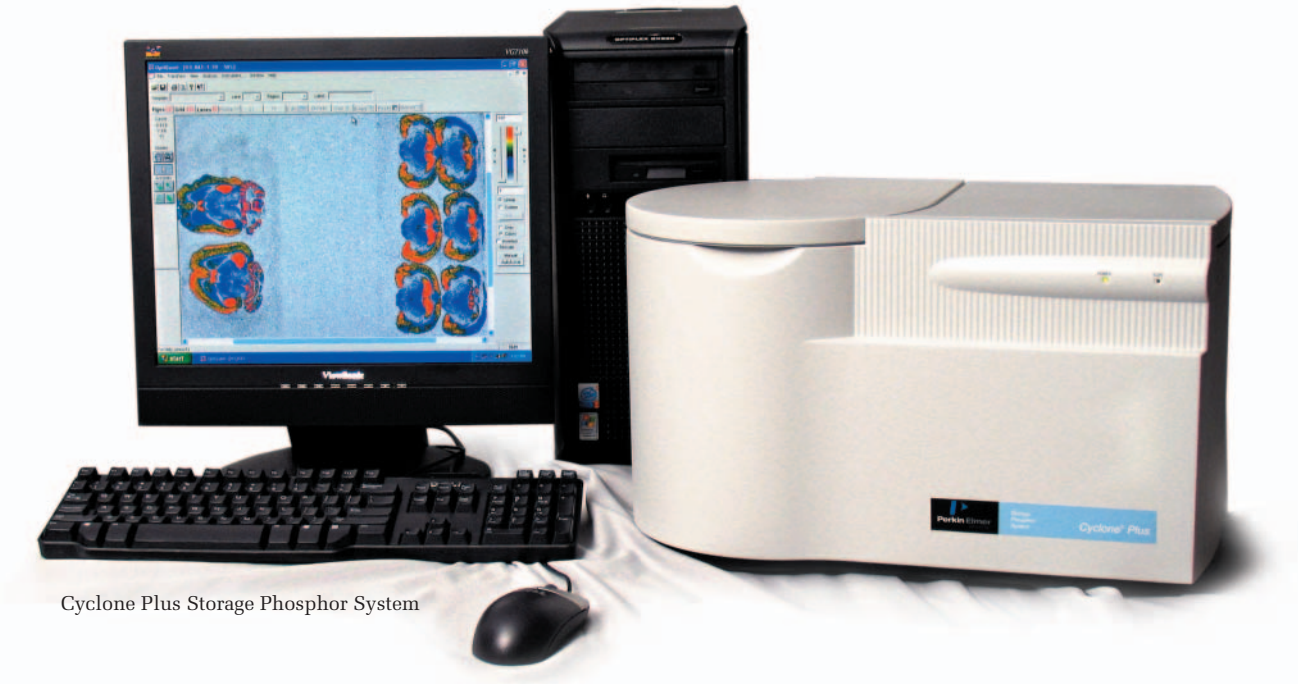
Cyclone Plus Storage Phosphor Screen Performance and Application Guide



Cyclone Plus Storage Phosphor System

Introduction

The Cyclone® Plus Storage Phosphor System is an affordable personal imaging system for quantitative image analysis, designed to replace the more qualitative methods in film autoradiography. The storage phosphor screens used to capture the activity of the sample have a number of advantages over film. Phosphor screens have a much longer linear dynamic range, improved response to isotopes for much shorter exposure times, the ability to be erased and used 1,000 times, and the convenience of scanning and obtaining data without developing chemicals.[1] Cyclone Plus screens are available in a variety of sizes designed to suit application and budget requirements. Smaller screens require a smaller investment and fit many smaller gels, blots, arrays and tissue sections. Longer screens cover the length of a sequencing gel, or fit multiple tissue sections or arrays.

Authors
Jeanne Raccio and Charles J. Passo, Jr.

PerkinElmer Life and Analytical Sciences

PerkinElmer® precisely.

Radiolabeled samples are exposed to the phosphor screens, which store energy in the photostimulable crystals (BaFBr:Eu2^{+}) by the mechanism shown in Figure 1. The energy of the radioisotope ionizes the Eu^{2+} to Eu^{3+} liberating electrons to the conduction band of the phosphor crystals. The electrons are trapped in the bromine vacancies, which are introduced during the manufacturing process to form temporary "F centers".[2] Exposure to stimulating laser light at 633 nm releases photons at about 390 nm, which are detected by a high quantum efficiency PMT, such as the one in the Cyclone Plus.[3]

Several types of storage phosphor screens are available for use with Cyclone Plus. The Super Resolution (SR) screen is formulated from a fine grain crystal, providing the best possible resolution. The Tritium Sensitive (TR) screen uses high grade crystals, but is also uncoated to allow the low energy emission of tritium to be detected. The MultiSensitive (MS) screen replaces the MultiPurpose (MP) screen, a durable all-purpose screen and the SuperSensitive (ST) screen. The MS screen has the sensitivity of the ST screen with better resolution and the durability of the MP screen.

Previous studies did not include the MS screen.[4] TR screens are used solely for the detection of tritium labeled samples, and were therefore not included in the comparative analysis of performance with high energy isotopes. The following describes the comparative performance of the SR and MS screens relative to the performance of the discontinued MP and ST screens and most appropriate applications for all screen types.

Methods

Sensitivity

To determine the comparative response to high-energy isotopes, 14 spots of relatively equal amounts of ^{32}P-labeled material were spotted onto vellum paper. Each screen type was exposed to the source for 10 minutes and then scanned in the Cyclone Plus at 300 dpi resolution. Quantitation was performed using OptiQuant software (group of regions templates). Fourteen identical elliptical regions were drawn around the spots of activity as well as 20 background regions of the same size. Signal, signal-to-noise ratios and comparative ratios were calculated.

To determine the comparative sensitivity with a lower energy isotope, each phosphor screen was exposed to ^{14}C microscales (Sigma Chemicals, St Louis, MO) for one hour and scanned in the Cyclone Plus at 300 dpi resolution. A template was created in OptiQuant to include rectangular boxes within each of the 16 microscale levels and 20 background regions. Net digital light units or DLU/mm^2 and signal-to-noise ratios for each screen were calculated.

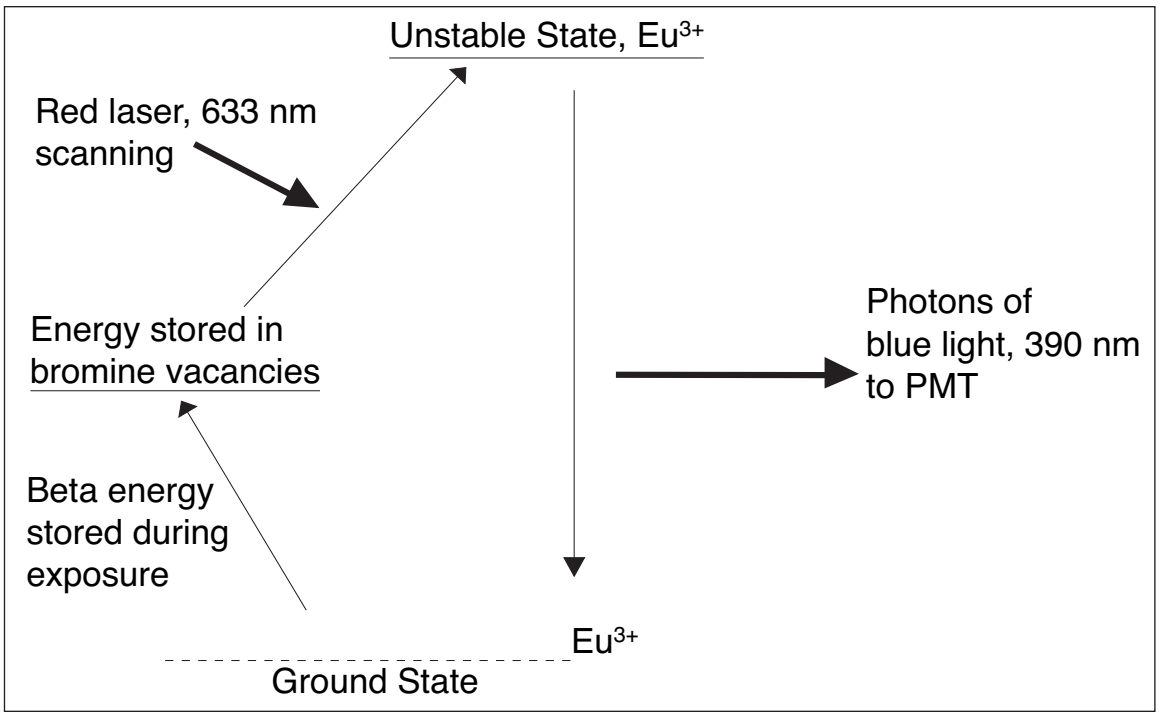


Figure 1. Schematic representation of the storage phosphor process.

Resolution

Each phosphor screen was exposed for one hour to a lined resolution test source containing a range of line pairs spaced at 1.2 line pairs/mm to 5.0 line pairs/mm, in both x and y directions. The test source was drawn with C^{14} ink on vellum paper. The images were analyzed by calculating the contrast transfer function, (CTF) in each direction using a one-pixel wide lane template and profile of activity. CTF defines the wave pattern that is generated by the parallel lines and spaces of equal width:

$$CTF = \frac{\text{ave. maximum - ave. minimum}}{\text{ave. maximum + ave. minimum}}$$

A CTF of 33% is generally considered "resolved" for quantification purposes.[1]

Results and Conclusions

MS, MultiSensitive Screens and ST, SuperSensitive Screens Provide Best Sensitivity

The MS screens accumulate about 12–15% more signal than the MP and SR screens, respectively and accumulate about 3% less signal than the ST screens for a ten minute exposure to ^{32}P. However, the signal-to-noise ratio provides a better measure of sensitivity and ability to detect low activity samples, because it takes into account the accumulation of background signal and the variation that exists in the background. The signal-to-noise ratio for the MS screen is about four times greater than the SR screen, about 1.5 times greater than the MP screen, and is about equal to the ST screen as shown in Figure 2. In addition the MS screen has the same durable coating available on the MP and SR screens.

For lower energy isotopes such as ^{14}C, the difference in sensitivity between the MS and the other screens is also significant The MS screen shows about a 50% higher accumulation of signal compared to the SR and MP screens, a signal-to-noise ratio of over 1.5 times that of the SR and ST screens, and over 1.8 times that of the MP screen as shown in Figure 3.

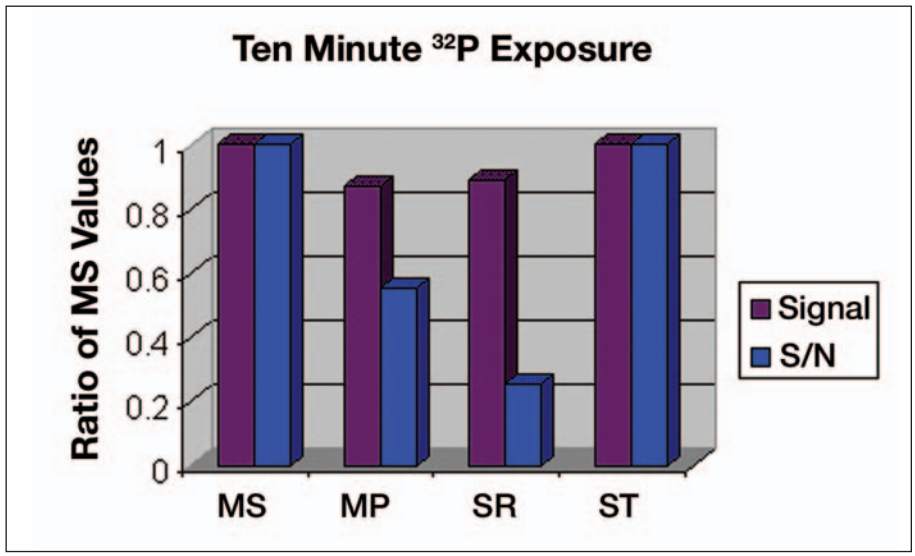


Figure 2. Graphical representation of signal accumulation and signal-to-noise ratios of MP, SR and ST screens as a ratio of MS screen values for high energy isotopes.

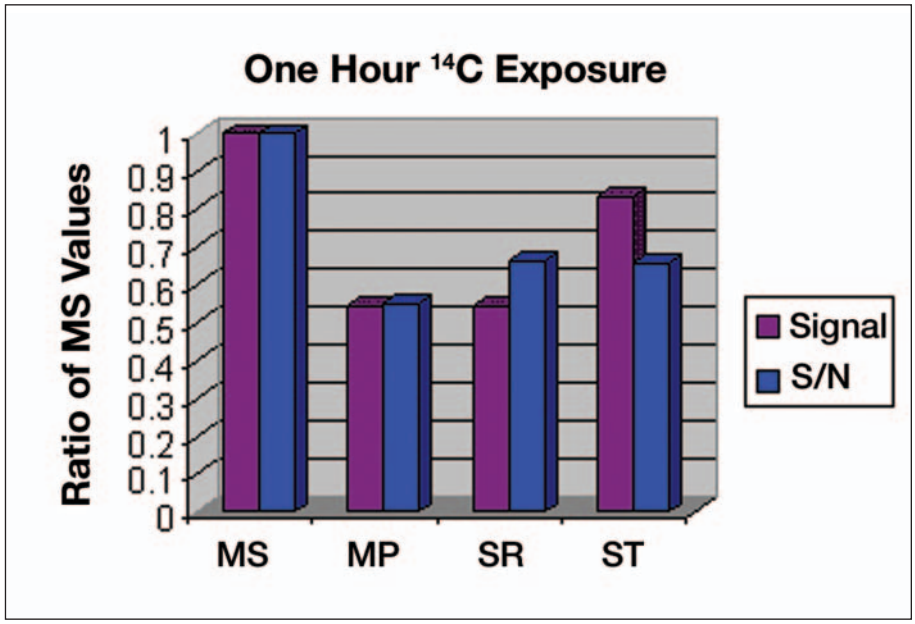


Figure 3. Graphical representation of signal accumulation and signal-to-noise ratios of MP, SR and ST screens as a ratio of MS screen values for lower energy isotopes.

SR, SuperResolution Screens Provide Best Resolution for Lower Energy isotopes

Qualitatively, the images produced by the MS and SR screens are very similar in terms of lines that can been seen (Figure 4). However, creating a lane and profile, and calculating CTF does show that the

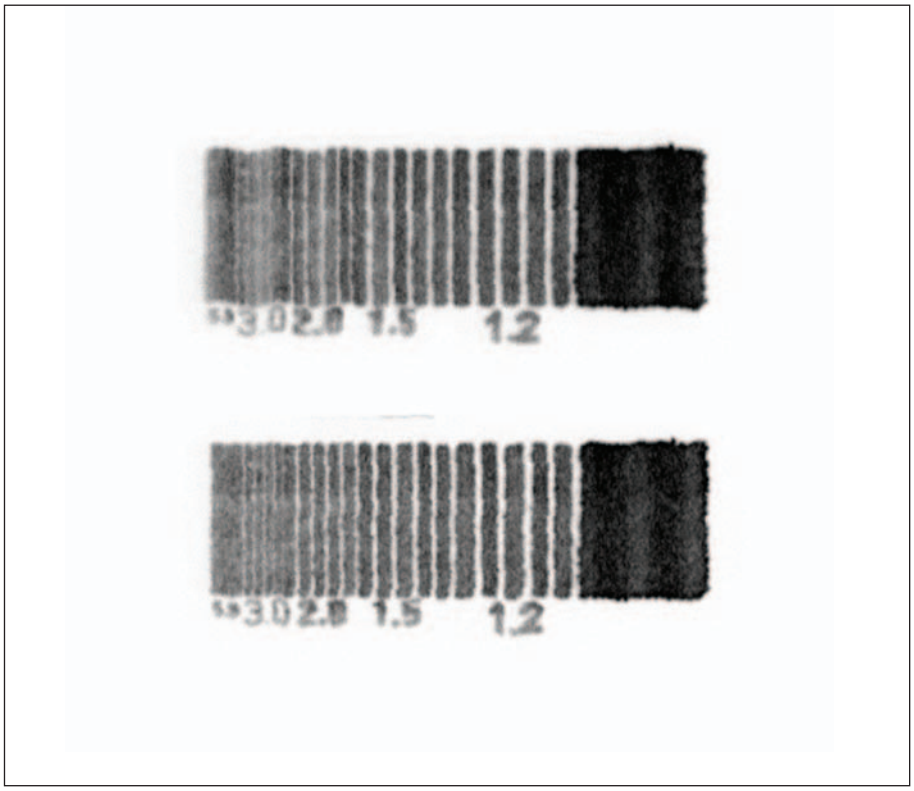


Figure 4. Qualitative images of line pair source as imaged with MS (top) and SR (bottom) screens show little difference in apparent resolution, although quantitatively the difference is significant as shown in Figure 5.

separation of lines is better for the same source using the SR screen (Figure 5). Contrast transfer functions were calculated as described above and graphed as shown in Figure 5. In general, more line pairs per mm are more difficult to resolve and have a lower CTF value. MS and MP screens exhibit very similar resolution, and the ST screen is less able to separate closely spaced line pairs. The SR, Super Resolution screen shows best separation of line pairs as calculated by using the ratios of peaks to valleys in the profile analysis of a single lane template using the CTF calculation.

Discussion

Based on these results, the MP and ST screens are discontinued and are only available as stock lasts. The data are preserved here for comparison to the MS Screen replacement.

The new MS screen is as durable as the MP, and exhibits the same sensitivity as the ST, and therefore is a good replacement for both MP and ST screens in terms of performance. The SR screen provides superior resolution with low energy isotopes and should be the choice for high-resolution samples. The TR, Tritium Sensitive screens are uncoated and are able to detect tritium-labeled samples. TR screens are not generally used for other applications because they are subject to potential contamination and cannot be cleaned as easily as the other screens. The following are applications specific recommendations for each screen type.

MS, MultiSensitive Screens

For applications with well-separated spots and bands using higher energy isotopes such as ^{32}P, ^{125}I and other gamma emitters, less resolution is typically required and more sensitivity would be useful. These isotopes have higher energy and spread out such that the resolution capability of the screen is not the limiting factor and does not actually provide better resolution of the sample. However, a higher sensitivity screen will detect the lower activity spots or bands more readily. MS screens provide the best sensitivity, especially for higher energy isotopes and exhibit resolution sufficient for applications with well-separated spots and bands. They accumulate more signal and exhibit lower backgrounds so that the signal-to-noise ratio is higher compared to other screens. Therefore, MS screens are recommended for ^{32}P-labeled macroarrays, Northern blots, Southern blots, TLC plates, preparative gels, ^{125}I-labeled Western blots, and other applications, such as RNAse protection assays.

SR, Super Resolution Screens

For applications with spots, tissues or bands that are very closely spaced and that use lower energy isotopes such as ^{14}C, ^{35}S and ^{33}P, higher resolution screens are useful. Lower energy isotopes have a shorter path length and are best used for imaging tissue sections, microarrays and whole body autoradiography applications. The sensitivity of an SR screen is not as great as the MS screen, so a longer exposure maybe required; however, closely spaced features will be easier to discriminate using an SR screen. SR screens are recommended for ^{33}P-labeled microarrays, ^{14}C-labeled tissue sections or whole body autoradiography, ^{35}S-labeled *in situ* hybridization or gel applications.

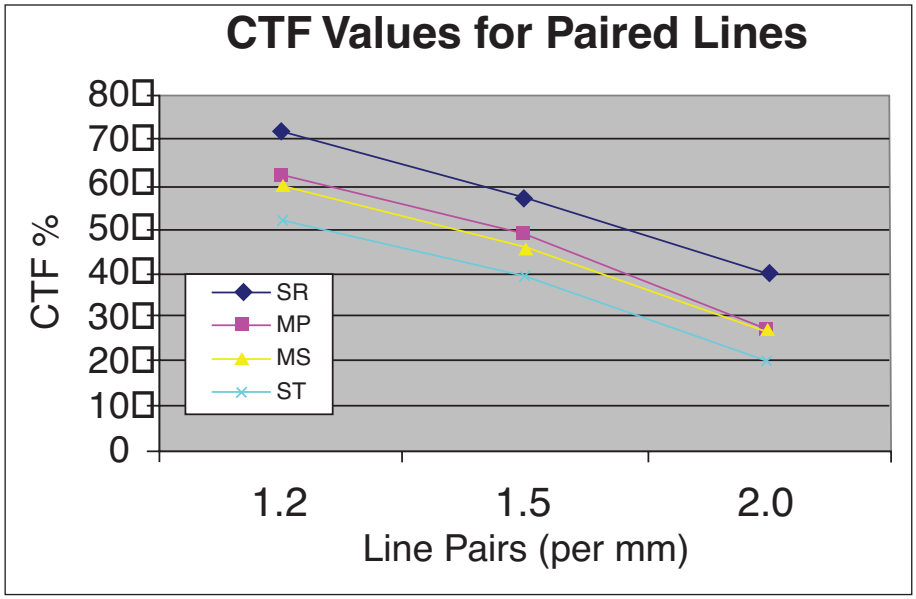


Figure 5. Each screen is able to resolve line pairs to different degrees as measured quantitatively by the CTF values. SR screens give the best resolution to the line pairs.

TR, Tritium Sensitive Screens

TR screens are used for applications that require the use of tritium for labeling, such as some whole body applications, receptor autoradiography, and some metabolism studies on TLC plates. These screens are created with the highest grade of $BaFBr:Eu^{2+}$ crystals and are uncoated so that the low energy beta particles of tritium can be detected. Because the TR screens are uncoated, they are subject to moisture accumulation and contamination with use. The following recommendations are useful for extending the life of a TR screen:

1. Baking in a 60 °C oven with a beaker of anhydrous calcium sulfate for three days removes moisture and improve background.[5]
2. Methanol can be used to wash silica particles from a screen that was exposed to a TLC plate. Hold screen vertically, pour methanol over the screen, and allow to air dry.[5]
3. Vapor fixation of samples prior to exposure in a sealed vessel containing paraformaldehyde powder eliminates the contamination resulting from the affinity of a ligand for the uncoated TR screen. This procedure does not reduce the signal relative to the samples prepared without fixation.[6]

General Guidelines for Phosphor Screen Use

1. Erase using white light box just prior to exposure, even if brand new. The screens are so sensitive in general that they accumulate cosmic radiation even in storage.
2. Keep stored in cardboard sleeve packaging, in a dry dark place. This is the best way to keep them from being misplaced or mishandled when not in use.
3. Do not use intensifying screens or freezer to enhance signal. Intensifying screens do not work with phosphor screens because they are not sensitive to light. Exposure in the freezer subjects the screen to moisture accumulation due to condensation. It can be done to improve signal marginally, but should be done in a container of desiccant.
4. Keep away from UV light, ethidium bromide or other fluorescent stains. UV light charges up the screen and makes it difficult to erase again fully. Ethidium bromide and other fluorescent stains appear like contamination on the screen. The laser scanning mechanism will detect these fluorescent stains as activity.
5. Wash with KODAK® intensifying screen cleaner (PN6011701). This can be used to remove oily fingerprints, dust and other material that accumulates with normal use.
6. Do not fold, scratch or otherwise abuse screens.
7. To check for contamination, erase and store for the amount of time typical exposures are performed. Scan directly after storing with no sample. Any markings will indicate the spot of contamination. Print out at 100% magnification and use the print out to mark areas of contamination on screen to avoid. This enables one to use uncontaminated areas of the screen for further imaging.

With typical use and minimal care, coated phosphor screens such as the MS and SR screens provide over 1,000 quantitative images. TR screens are the exception to this since they are uncoated. They may be contaminated on the first exposure, or might provide as many as ten quantitative images.

The following table illustrates the best features and applications of each type of screen:

Table 1. Summary of storage phosphor screen performance and some of the best applications.

	Relative Sensitivity	Relative Resolution	Moisture Resistance	^{3}H Sensitivity	Best Applications
MS	****	***	****	N/A	^{33}P macroarrays Northern and Southern blots TLC plates Preparative gels ^{125}I-labeled Western blots RNAse protection assays
SR	***	****	****	N/A	^{33}P-labeled microarrays ^{14}C-labeled tissue sections or WBA ^{35}S-labeled *in situ* hybridization
TR	****	****	N/A	****	Whole body applications Receptor autoradiography, Metabolism studies on TLC plates

**** = Highest *** = Excellent ** = Good * = Fair N/A = Not Applicable

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PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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