Red-shifted Liquid Scintillation (RS-LSC).

An Opportunity to Progress The Technology?

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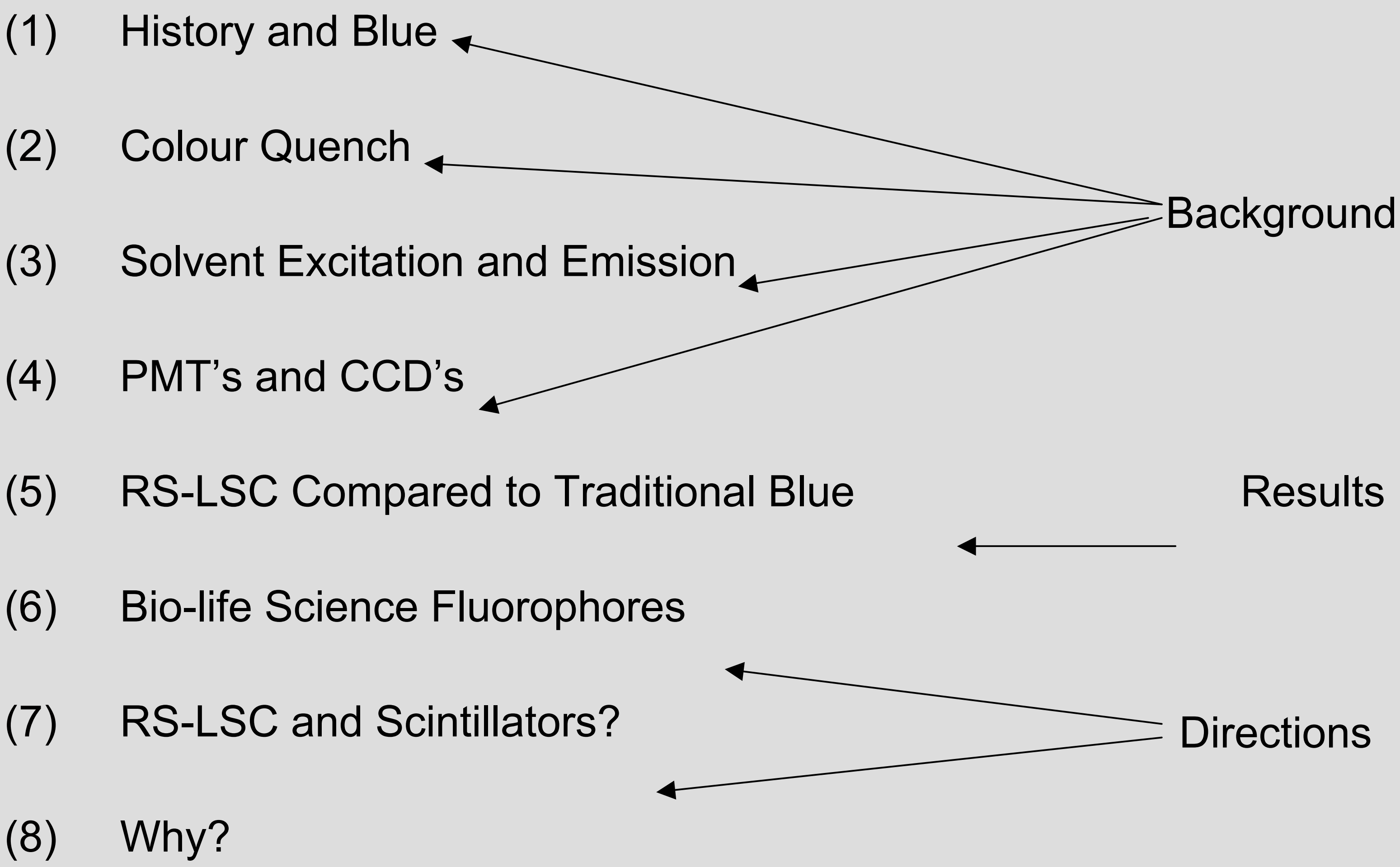
Using information from solid scintillators can we see an opportunity to progress LSC?



(1) History and Blue

(2) Colour Quench

(3) Solvent Excitation and Emission

(4) PMT's and CCD's

(5) RS-LSC Compared to Traditional Blue

(6) Bio-life Science Fluorophores

(7) RS-LSC and Scintillators?

(8) Why?

Background

Results

Directions

History and Blue Scintillators

6. Other primary solutes

Many other chemicals have been suggested as liquid scintillation solutes: several are substituted oxazoles (e.g. PBO, 2-phenyl-5-(4-biphenyl)oxazole), and others are *p*-oligophenylenes (e.g. BIBUQ—a butylated quaterphenyl). None of these alternatives has been adopted for general use.

F. The design of new solutes

The major aim in the synthesis of a new solute is to reduce its liability to quenching but retain good fluorescence properties. This can be approached in two ways, (i) by destroying the ability of the chromophore to retain a coplanar configuration relative to the rest of the solute molecule and (ii) by shielding the chromophore with a bulky substituent group. Both these approaches try to prevent close contact between the solute and quencher.

A passing reference can be made to attempts to produce a substance combining both solute and solvent properties. The material l-methylnaphthalene has these desirable properties but is not compatible with water and has not been available commercially in sufficient purity until comparatively recently.

G. Scintillator solution figure of merit

Birks[44] has presented a useful concept for the assessment of combined solvent–primary solute efficacy by the definition of the scintillator solution figure of merit *F* as

$$F = sfqm$$

where s is the solvent conversion energy, f is the solvent–solute energy transfer efficiency, q is the fluorescence quantum efficiency of the solute and m is the spectral matching factor. F is a function of the PM and for a bialkali photomultiplier instrument 3 g/l PPO in toluene has $F = 73$ and 10 g/l PBD in xylene has $F = 81$.

H. The secondary solute

Secondary solutes have been included in cocktails to obtain a better match between the scintillation emission and the response range of the PM tube. Small concentrations ($\sim$0.4 g/l) of other fluorescent solutes may be added to create a further, high efficiency, energy transfer stage between the primary solute and this additional secondary solute.

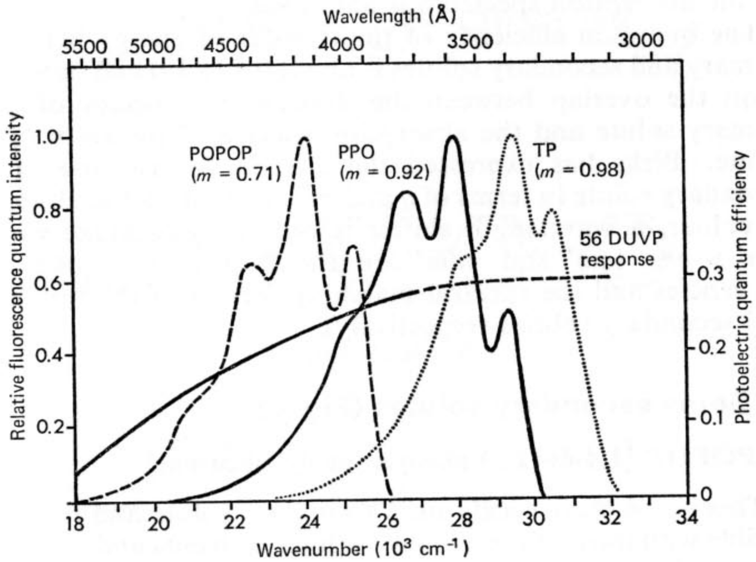


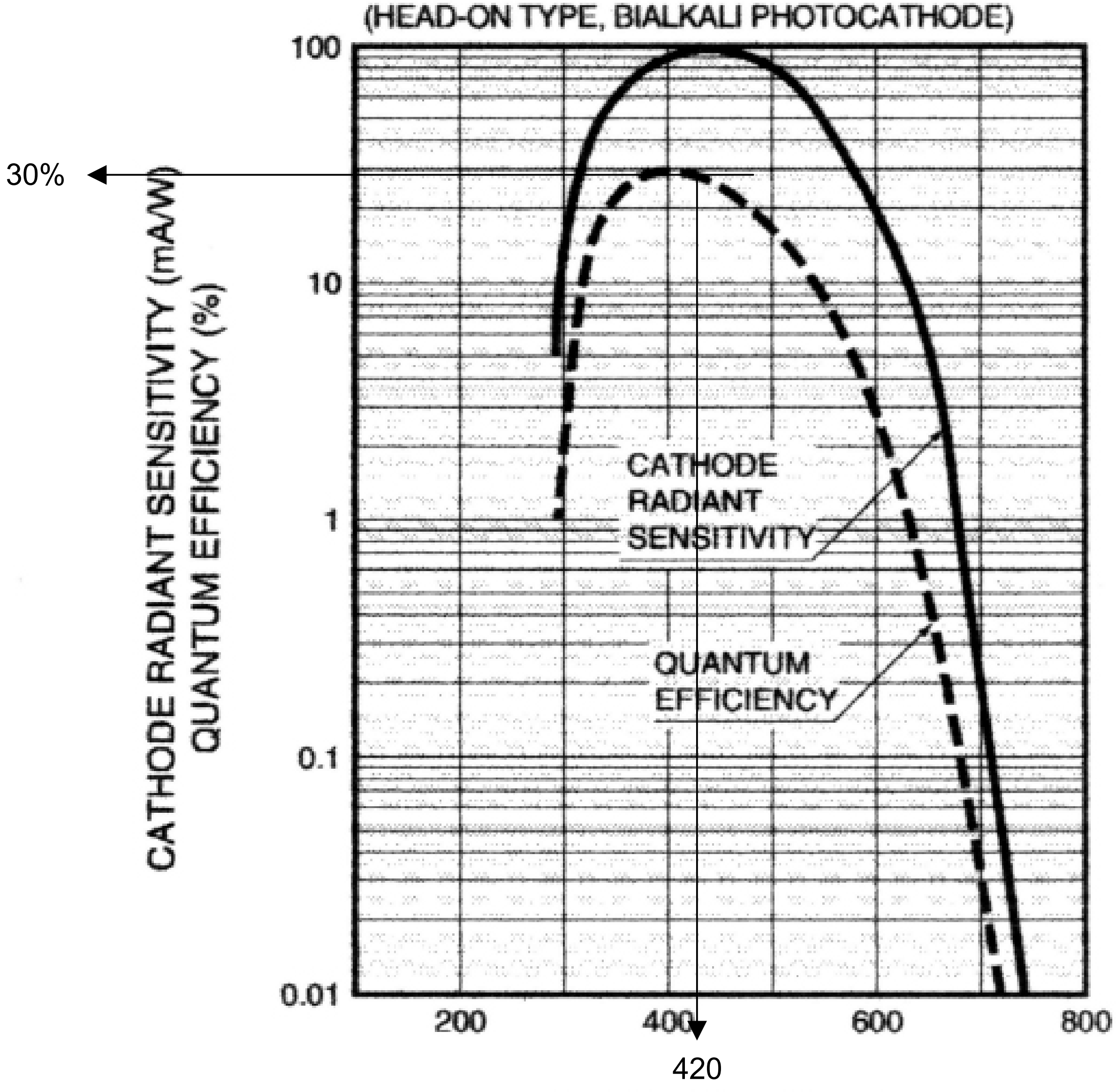
FIG. 5. Fluorescence spectra of TP, PPO and POPOP with spectra response of a bialkali PM. (m = spectral matching factor).[44]

The final scintillation principally is that from the return of excited secondary solute molecules to their ground state. These secondary solutes were chosen so that their mean fluorescence wavelength was more in accord with the caesium + antimony photocathodes of the PM tubes in the earlier instruments (hence their description as "wavelength–shifters" in earlier publications).

Modern instruments contain bialkali PM tubes which have a wider spectral response range which should eliminate the need for a secondary solute (Fig. 5). Despite this fact several

An Introduction to Liquid Scintillation Counting A. Dyer, Heyden & Son Ltd., 1974

Figure 4: Typical Spectral Response of Bialkali Photocathode



Hamamatsu

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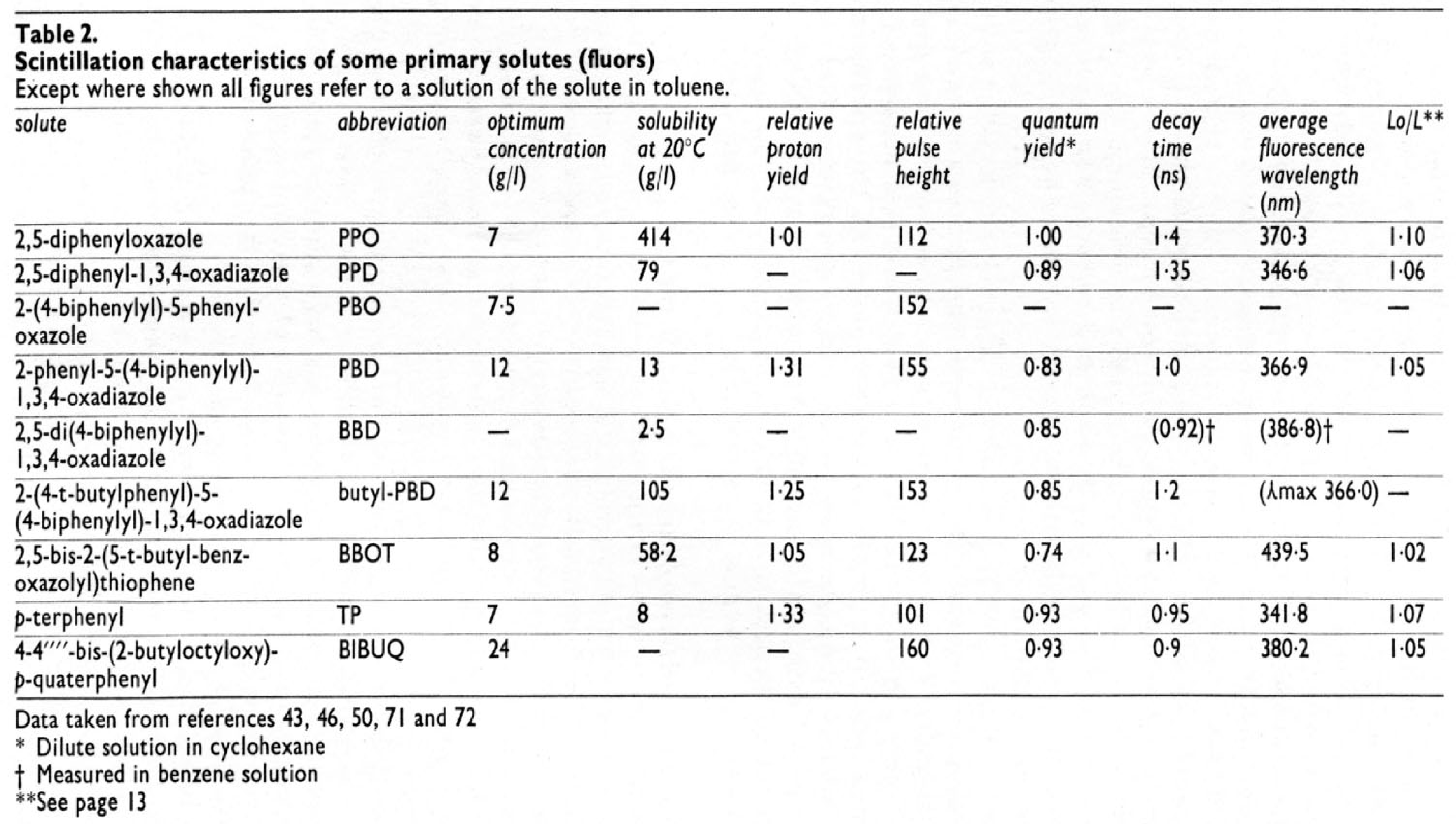


Table 2.
Scintillation characteristics of some primary solutes (fluors)
Except where shown all figures refer to a solution of the solute in toluene.

solute	*abbreviation*	*optimum concentration (g/l)*	*solubility at 20°C (g/l)*	*relative proton yield*	*relative pulse height*	*quantum yield**	*decay time (ns)*	*average fluorescence wavelength (nm)*	*Lo/L***
2,5-diphenyloxazole	PPO	7	414	1·01	112	1·00	1·4	370·3	1·10
2,5-diphenyl-1,3,4-oxadiazole	PPD		79	—	—	0·89	1·35	346·6	1·06
2-(4-biphenylyl)-5-phenyl-oxazole	PBO	7·5	—	—	152	—	—	—	—
2-phenyl-5-(4-biphenylyl)-1,3,4-oxadiazole	PBD	12	13	1·31	155	0·83	1·0	366·9	1·05
2,5-di(4-biphenylyl)-1,3,4-oxadiazole	BBD	—	2·5	—	—	0·85	(0·92)†	(386·8)†	—
2-(4-t-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole	butyl-PBD	12	105	1·25	153	0·85	1·2	(λmax 366·0)	—
2,5-bis-2-(5-t-butyl-benz-oxazolyl)thiophene	BBOT	8	58·2	1·05	123	0·74	1·1	439·5	1·02
p-terphenyl	TP	7	8	1·33	101	0·93	0·95	341·8	1·07
4-4′′′′-bis-(2-butyloctyloxy)-*p*-quaterphenyl	BIBUQ	24	—	—	160	0·93	0·9	380·2	1·05

Data taken from references 43, 46, 50, 71 and 72
* Dilute solution in cyclohexane
† Measured in benzene solution
**See page 13

Sample Preparation in Liquid Scintillation Counting, C.T. Peng workers have shown that, under certain conditions, it can still be an advantage to include a secondary solute in a liquid scintillation cocktail. These conditions are when either (i) the sample to be counted is coloured or opaque or (ii) the volume of the scintillator being used is large enough to cause the problem of self-absorption of the primary solute fluorescence. In regard to (i) it should be noted that a visual inspection for colour can be misleading and a check on the absorption spectrum is advisable.

The quantum efficiency of the transfer of energy between primary and secondary solutes is about 0.5 to 1.0 and depends upon the overlap between the fluorescence spectra of the primary solute and the absorption spectra of the secondary solute. Birks has expressed the criteria for the use of a secondary solute in terms of q and m such that, in the absence of colour, a wavelength shifter is only an advantage when $q'm' > qm$. (q,q' and m,m' are the fluorescence quantum efficiencies and the spectral matching factors of the primary and secondary solutes respectively).

J. Some secondary solutes (Fig. 6)

1. POPOP [1,4-di-(2-(5-phenyloxazolyl))benzene]

This is the most widely used secondary solute and is compatible with most of the primary solutes, solvents and samples commonly encountered.

2. DMPOPOP [1,4-di-(2-(4-methyl-5-phenyloxazolyl)) benzene]

The dimethyl derivative of POPOP suggested as an alternative to POPOP due to its greater solubility in toluene but it needs to be used in a higher concentration to achieve an equivalent efficiency.

3. bis-MSB [*p*-bis-(σ-methylstyryl)benzene][54]

This solute has a good solubility in toluene and is less liable to quench effects than both POPOP and DMPOP. A

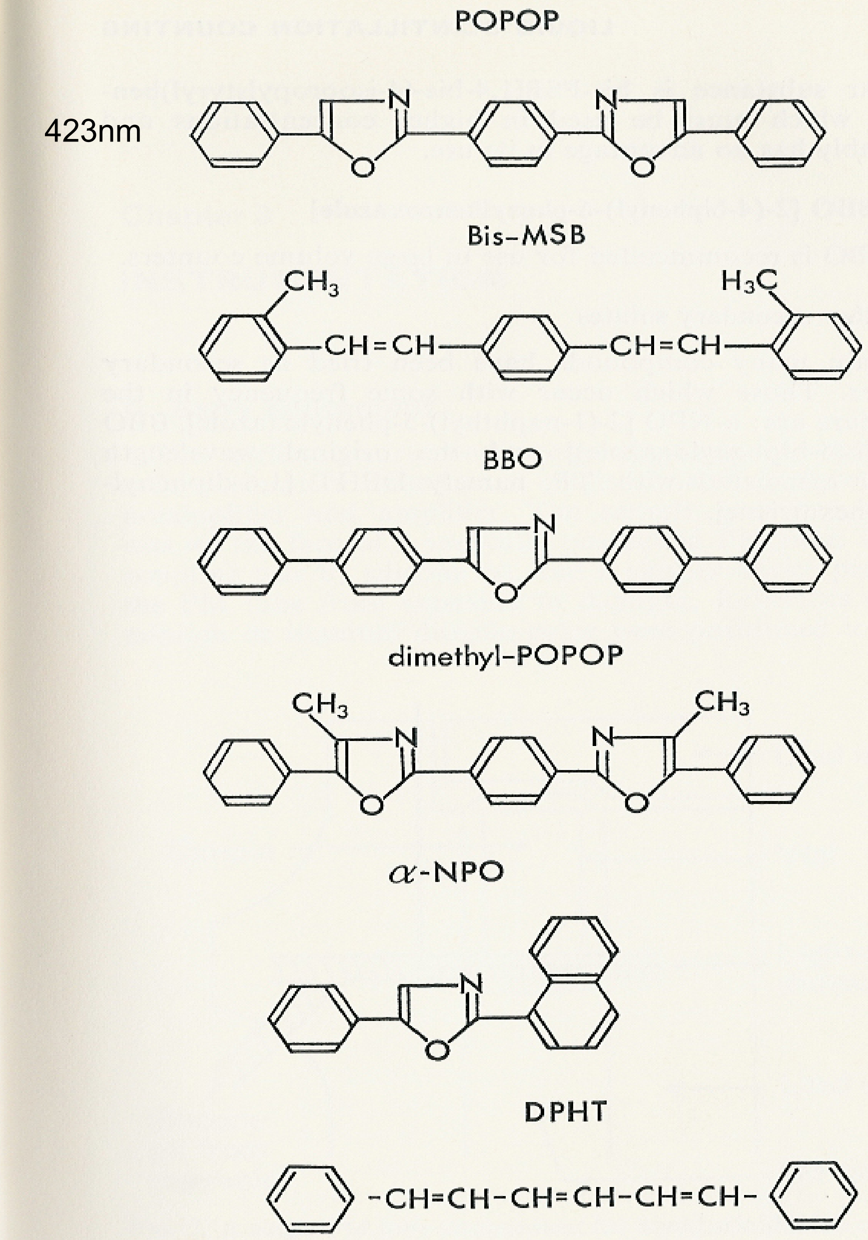


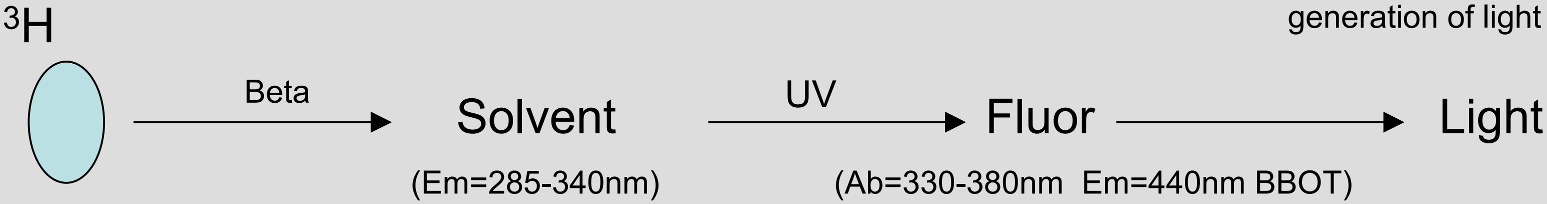
FIG. 6. Structural formulae of some secondary solutes.

An Introduction to Liquid Scintillation Counting A. Dyer, Heyden & Son Ltd., 1974

Energy of the excited solvent is emitted as UV light and the solvent molecule returns to ground state. The UV light is absorbed by fluor molecules which emit (blue) light flashes upon return to ground state. Nuclear decay events produce approximately 10 photons per KeV.

Packard Instrument Company

Colour quenching
occurs after the
generation of light



^{3}H

Beta

Solvent

UV

Fluor

Light

(Em=285-340nm)

(Ab=330-380nm Em=440nm BBOT)

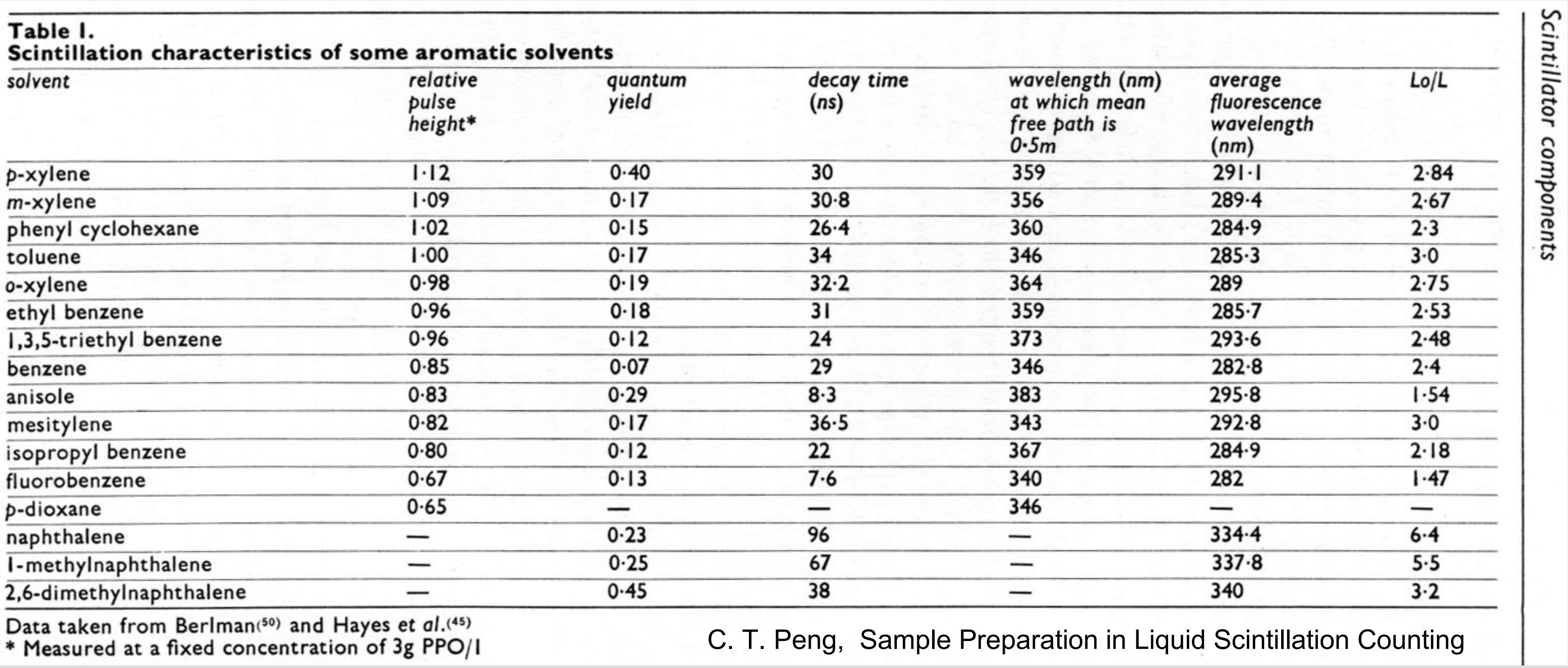


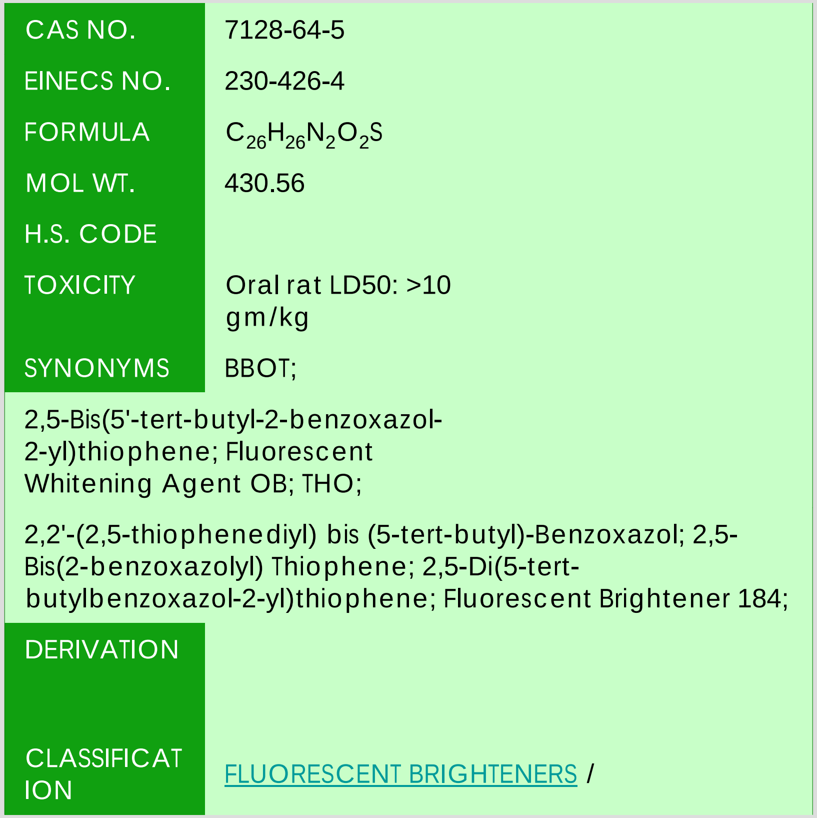
Table I.
Scintillation characteristics of some aromatic solvents

solvent	*relative pulse height**	*quantum yield*	*decay time (ns)*	*wavelength (nm) at which mean free path is 0·5m*	*average fluorescence wavelength (nm)*	*Lo/L*
p-xylene	1·12	0·40	30	359	291·1	2·84
m-xylene	1·09	0·17	30·8	356	289·4	2·67
phenyl cyclohexane	1·02	0·15	26·4	360	284·9	2·3
toluene	1·00	0·17	34	346	285·3	3·0
o-xylene	0·98	0·19	32·2	364	289	2·75
ethyl benzene	0·96	0·18	31	359	285·7	2·53
1,3,5-triethyl benzene	0·96	0·12	24	373	293·6	2·48
benzene	0·85	0·07	29	346	282·8	2·4
anisole	0·83	0·29	8·3	383	295·8	1·54
mesitylene	0·82	0·17	36·5	343	292·8	3·0
isopropyl benzene	0·80	0·12	22	367	284·9	2·18
fluorobenzene	0·67	0·13	7·6	340	282	1·47
p-dioxane	0·65	—	—	346	—	—
naphthalene	—	0·23	96	—	334·4	6·4
1-methylnaphthalene	—	0·25	67	—	337·8	5·5
2,6-dimethylnaphthalene	—	0·45	38	—	340	3·2

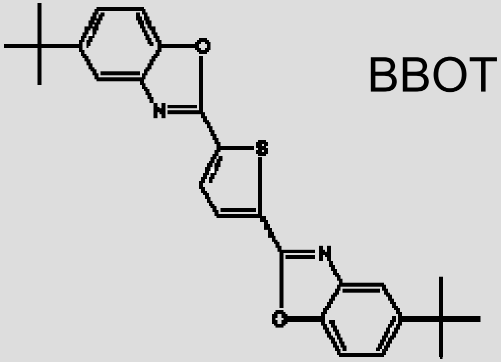
Data taken from Berlman(50) and Hayes *et al.*(45)
* Measured at a fixed concentration of 3g PPO/l

Scintillator components

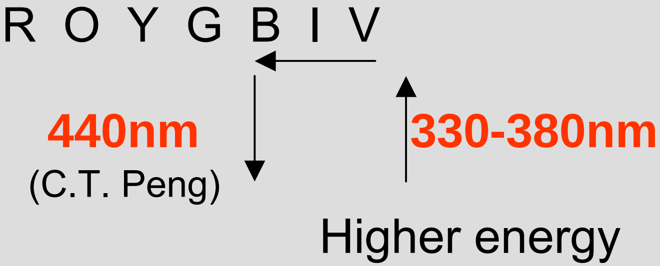
C. T. Peng, Sample Preparation in Liquid Scintillation Counting



CAS NO.	7128-64-5
EINECS NO.	230-426-4
FORMULA	$C_{26}H_{26}N_2O_2S$
MOL WT.	430.56
H.S. CODE	
TOXICITY	Oral rat LD50: >10 gm/kg
SYNONYMS	BBOT;
2,5-Bis(5'-tert-butyl-2-benzoxazol-2-yl)thiophene; Fluorescent Whitening Agent OB; THO; 2,2'-(2,5-thiophenediyl) bis (5-tert-butyl)-Benzoxazol; 2,5-Bis(2-benzoxazolyl) Thiophene; 2,5-Di(5-tert-butylbenzoxazol-2-yl)thiophene; Fluorescent Brightener 184;	
DERIVATION	
CLASSIFICATION	FLUORESCENT BRIGHTENERS /



BBOT



R O Y G B I V

440nm
(C.T. Peng)

330-380nm

Higher energy

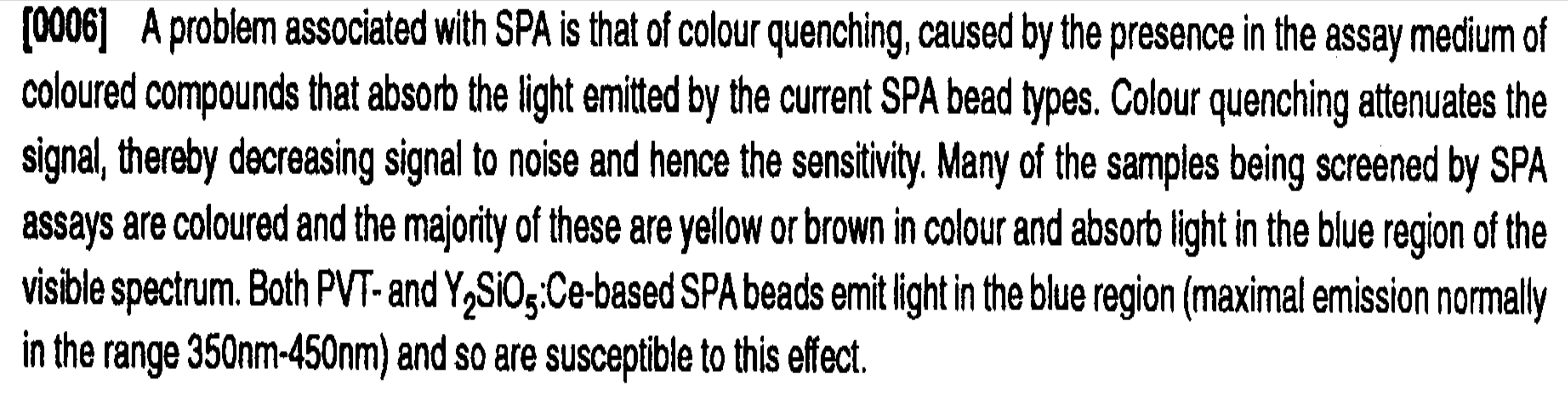
Fluorescent Whitening Agents, FWA's (also called optical brightener) absorb high energy radiation in the ultraviolet to violet region (330nm-380nm) on the part of characteristic molecules and emit lower energy radiation in blue region in visible spectrum (400nm-450nm), which yields the counteracting the yellowing appearance. 2,5-Bis(2-benzoxazolyl) thiophene is used as a fluorescent brightener for thermo plastic resins of PVC, PE, PP, PS, ABS, SAN SB, CA, PA, PMMA, acrylic resin, polyester fiber, paint, coating and printing ink.

Yttrium silicate

Yttrium silicate is a solid scintillator used for low energy beta. Chosen for the following reasons:

- Blue fluorescence that was complementary with the commonly used bi-alkali photomultiplier tubes (PMT's).
- Efficient.
- Price.
- Availability.
- Purity.
- Short afterglow after photoluminescence activation.

Colour Quench



[0006] A problem associated with SPA is that of colour quenching, caused by the presence in the assay medium of coloured compounds that absorb the light emitted by the current SPA bead types. Colour quenching attenuates the signal, thereby decreasing signal to noise and hence the sensitivity. Many of the samples being screened by SPA assays are coloured and the majority of these are yellow or brown in colour and absorb light in the blue region of the visible spectrum. Both PVT- and Y_2SiO_5:Ce-based SPA beads emit light in the blue region (maximal emission normally in the range 350nm-450nm) and so are susceptible to this effect.

Ref: EP 1 007 971 B1

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

Liquid Scintillation Counting Recent Applications and Development, Volume 2,
Edited by: Peng, Horrocks and Alpen. Academic Press 1980.

RECENT ADVANCES IN SAMPLE PREPARATION

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Paterson Laboratories
Christie Hospital and Holt Radium Institute
Manchester, U.K.

The art of good sample preparation is the ability to detect beta-particle emissions efficiently and reproducibly with the minimum of preprocessing.

Instrument design and development has advanced to a greater degree than sample preparation technology over the last decade, and any limitations of liquid scintillation counting appear to be associated more often with the preparation of the counting sample rather than a deficiency in instrumentation. There has however been little apparent progression in the development of new solvents or primary solutes in recent years. Toluene, p-xylene and dioxane-naphthalene are still the most population electron trapping solvents. A useful advance was made by Krumbiegel and Schmidt (1973) who claimed that 97% perdeuterated toluene allows tritium to be assayed almost 90% higher than with normal toluene. A further development in solvent modification was the introduction of organic lead and thallium compounds into scintillant mixtures, first suggested by Ashcroft (1969) as density-increasers to determine γ emitting isotopes in liquid scintillation spectrometers. Such solutions can be readily made by shaking saturated lead acetate with a mixture such as Ready Solv VI (Beckman Instruments Ltd) (Helman and Spiehler, 1974) and using the upper layer. Although these scintillants are used by immersing into it a small minivial containing the sample, a more recent development is the direct use of organic thallium or lead loaded scintillant solutions to improve the separation of ^{125}I and ^{3}H materials mixed directly with them (Lundqvist *et.al.*, 1976). This combination of isotopes is useful in nucleic acid biochemistry using ^{125}I-iododeoxyuri-

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TABLE I. Shows the recommended composition of Triton X-100: Toluene for optimal counting of different aqueous samples of biological interest

Sample	*Scint.comp. Triton X-100: Tol (v/v)*	*PPO g/L*[a]	*Counting Mixture: Scint (ml)*	*Sample (ml)*	*Merit Value*[b] *(MIV)*
Water	*1:1*	*8*	*6*	*4*	*1231*
8M urea	*1:1*	*8*	*6*	*4*	*1142*
5% sucrose	*2:3*	*6*	*5*	*5*	*989*
2M NaCl	*7:3*	*8*	*7*	*3*	*989*
Am.formate(.03M)	*2:3*	*5*	*5*	*5*	*778*
" " (1.0M)	*3:4*	*8*	*7*	*3*	*736*
TCA (5%)	*13:7*	*10*	*8.5*	*1.5*	*662*
PCA (5%)	*3:1*	*3*	*6*	*4*	*1148*
Formic Acid (0.1N)	*6:11*	*10*	*8.5*	*1.5*	*706*
HCl (1.0N)	*2:5*	*8*	*7*	*3*	*1030*
HCl (3.0N)	*5:11*	*5*	*8*	*2*	*748*
Fischer's Medium (+ 20% Horse serum)	*7:9*	*6*	*8*	*2*	*456*
Tryptone:yeast glucose (TYG)	*1:1*	*10*	*8*	*2*	*536*
Nutrient broth	*1:1*	*4.5*	*6*	*4*	*448*
Eagle's MEM	*1:1*	*3.3*	*8*	*2*	*313*
Cow's Milk	*3:5*	*3.3*	*8*	*2*	*664*
Human urine	*1:1*	*4.5*	*6*	*4*	*965*
Human plasma	*2:7*	*6*	*9*	*1*	*388*

[a] *The concentrations of PPO are not optimised for maximum efficiency in all cases.*

[b] *The Merit Value (MIV) is standardised for the instrument efficiency and is equivalent to:*

$$\frac{\%\ \text{counting efficiency} \times 100}{\%\ \text{sample value in mixture} \times \%\ \text{efficiency of machine references standard}}$$

The values of MIV are given for tritiated water only.

sensitivity could be affected by poor spectral matching and finally geometry changes within the vial could lead to light loss. It was shown by Takine and Ishikawa (1974) that the degree of colour quenching experienced corresponded to the degree of overlap of the quencher absorption spectrum and the scintillator emission spectrum. Impurity quenching on the other hand could not be related to any structural features of 30 or more quenchers examined, but some similarities between isomers were noted. Some interesting data was obtained by ten Haaf (1974) however, who showed that the 350-450nm maximal absorption by carotenoids did not correspond with the most powerful quenching effect and that other geometry parameters were also involved.

Liquid scintillation counting is clearly being employed in increasingly diverse fields associated with archaeology, climatology, hydrology, the movement of lubricating fluids in engineering, leaks in gas containing equipment, marine biology and in psycho-pharmacology. The study of bio- and chemiluminescence has been advanced by the development of the automated spectrometer, and I feel that it is in these latter fields we may see a future increase in the development of the spectrometer applications.

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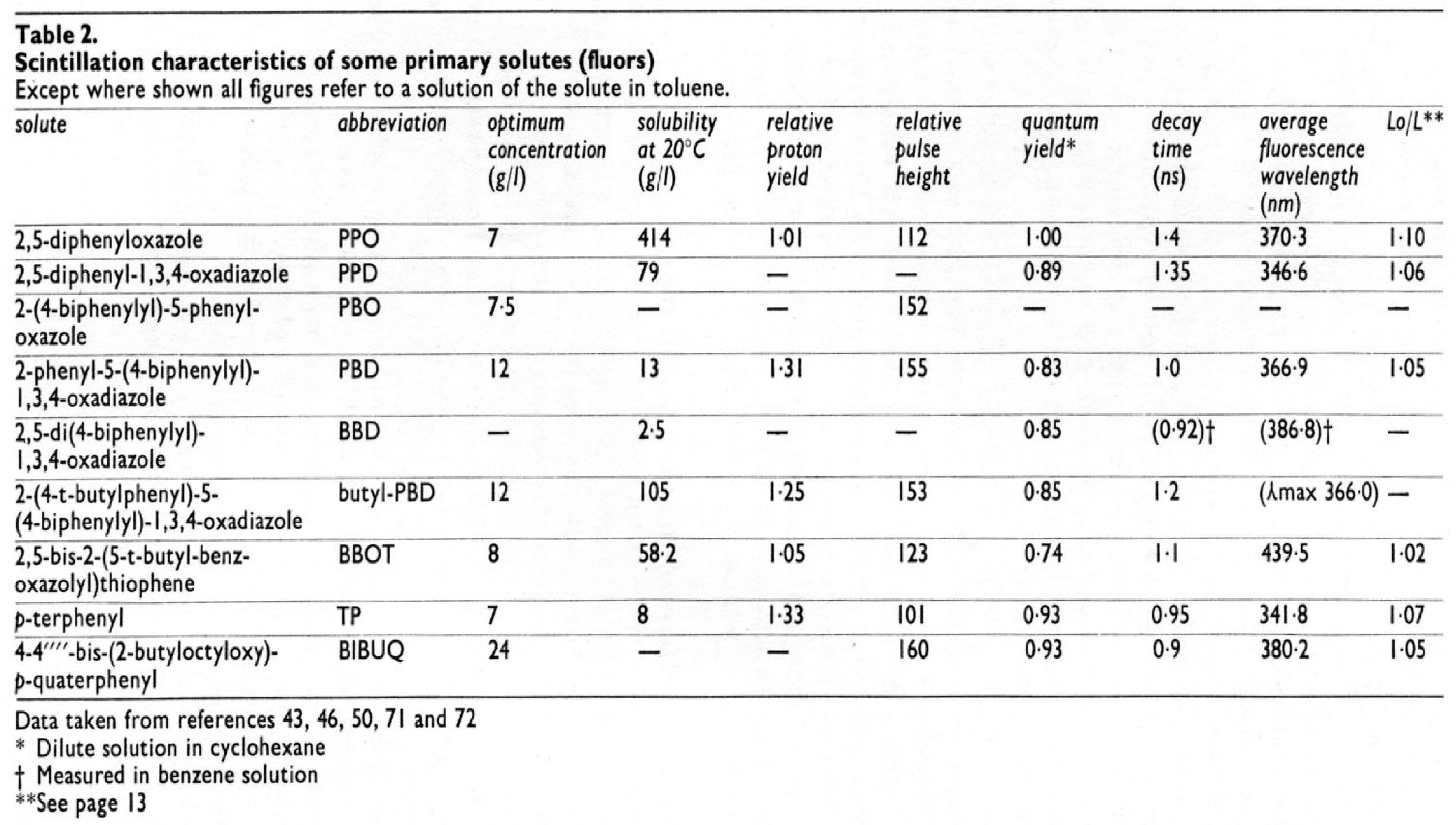

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p-terphenyl	TP	7	8	1·33	101	0·93	0·95	341·8	1·07
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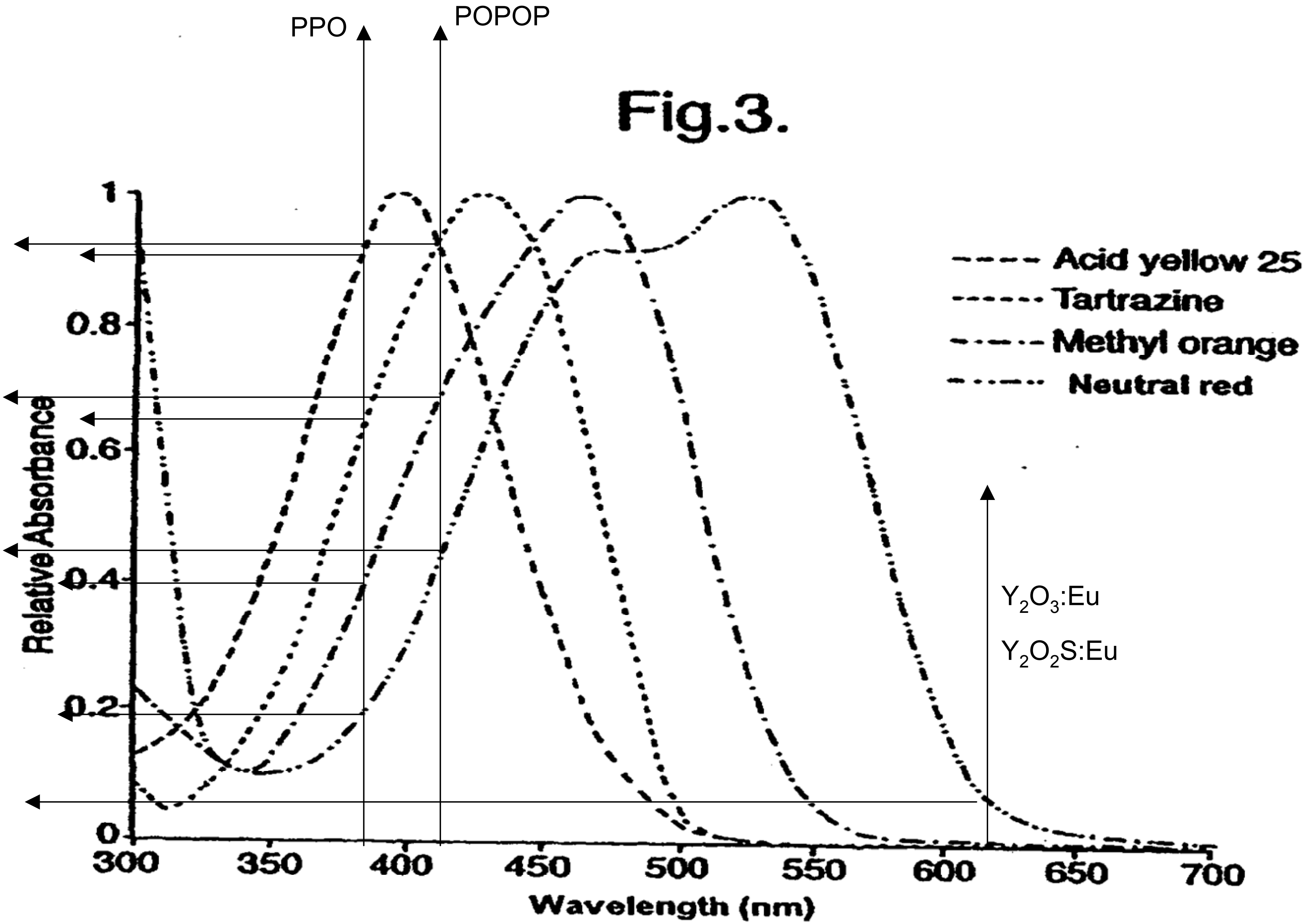
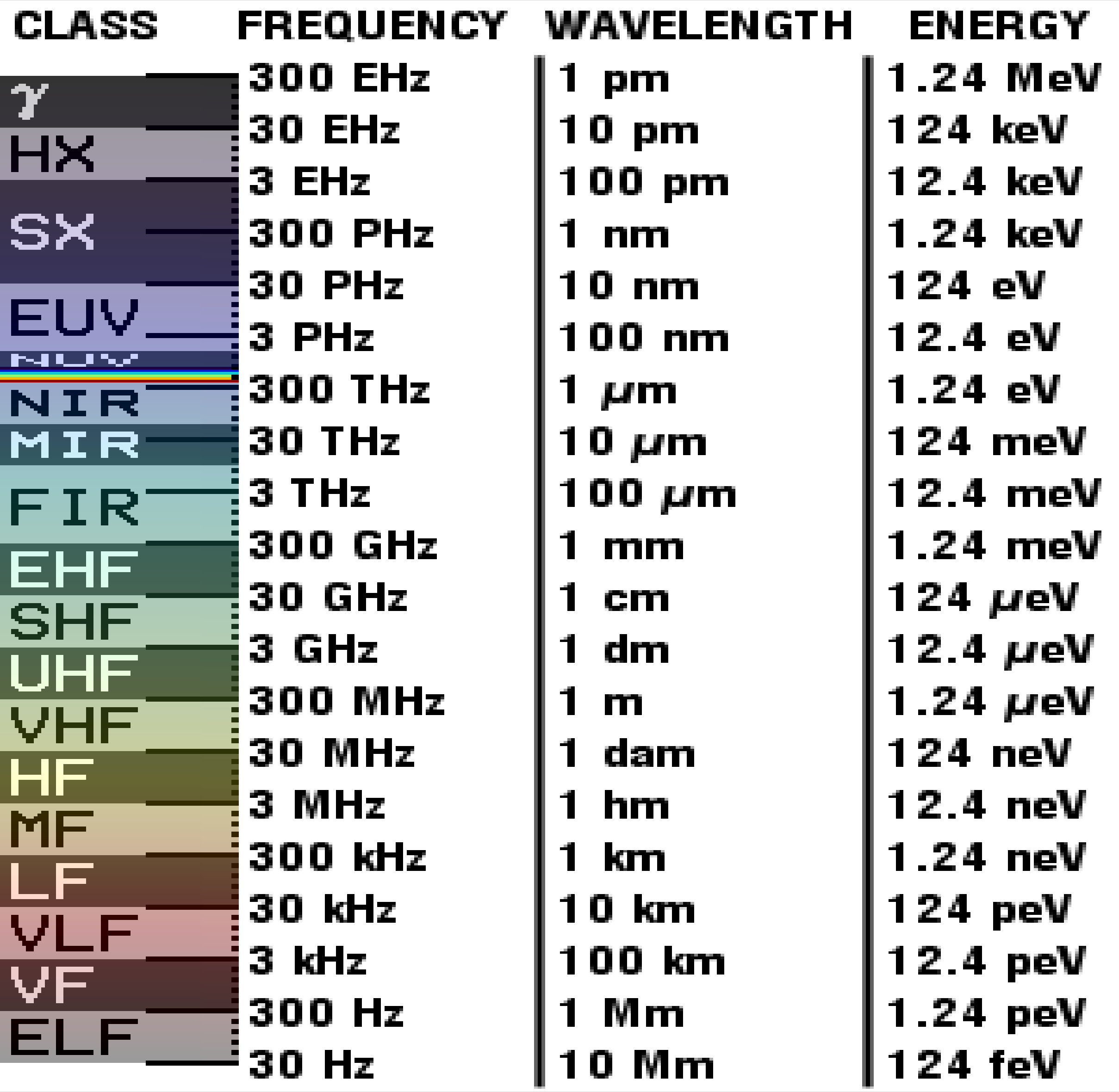
Data taken from references 43, 46, 50, 71 and 72
* Dilute solution in cyclohexane
† Measured in benzene solution
**See page 13

Sample Preparation in Liquid Scintillation Counting, C.T. Peng

Fig.3.


PPO
POPOP
Relative Absorbance
1
0.8
0.6
0.4
0.2
0
300
350
400
450
500
550
600
650
700
Wavelength (nm)
Acid yellow 25
Tartrazine
Methyl orange
Neutral red
Y_2O_3:Eu
Y_2O_2S:Eu



CLASS	FREQUENCY	WAVELENGTH	ENERGY
γ	300 EHz	1 pm	1.24 MeV
HX	30 EHz	10 pm	124 keV
SX	3 EHz	100 pm	12.4 keV
	300 PHz	1 nm	1.24 keV
EUV	30 PHz	10 nm	124 eV
NUV	3 PHz	100 nm	12.4 eV
NIR	300 THz	1 μm	1.24 eV
MIR	30 THz	10 μm	124 meV
FIR	3 THz	100 μm	12.4 meV
EHF	300 GHz	1 mm	1.24 meV
SHF	30 GHz	1 cm	124 μeV
UHF	3 GHz	1 dm	12.4 μeV
VHF	300 MHz	1 m	1.24 μeV
HF	30 MHz	1 dam	124 neV
MF	3 MHz	1 hm	12.4 neV
LF	300 kHz	1 km	1.24 neV
VLF	30 kHz	10 km	124 peV
VF	3 kHz	100 km	12.4 peV
ELF	300 Hz	1 Mm	1.24 peV
	30 Hz	10 Mm	124 feV

Legend:

γ = Gamma rays
HX = Hard X-rays

SX = Soft X-Rays

EUV = Extreme ultraviolet
NUV = Near ultraviolet
Visible light
NIR = Near infrared
MIR = Moderate infrared

FIR = Far infrared
Radio waves:
EHF = Extremely high frequency Microwaves
SHF = Super high frequency Microwaves

UHF = Ultrahigh frequency
VHF = Very high frequency
HF = High frequency

MF = Medium frequency
LF = Low frequency
VLF = Very low frequency

VF = Voice frequency

ELF = Extremely low frequency

From Wikipedia

Law of Conservation of Energy

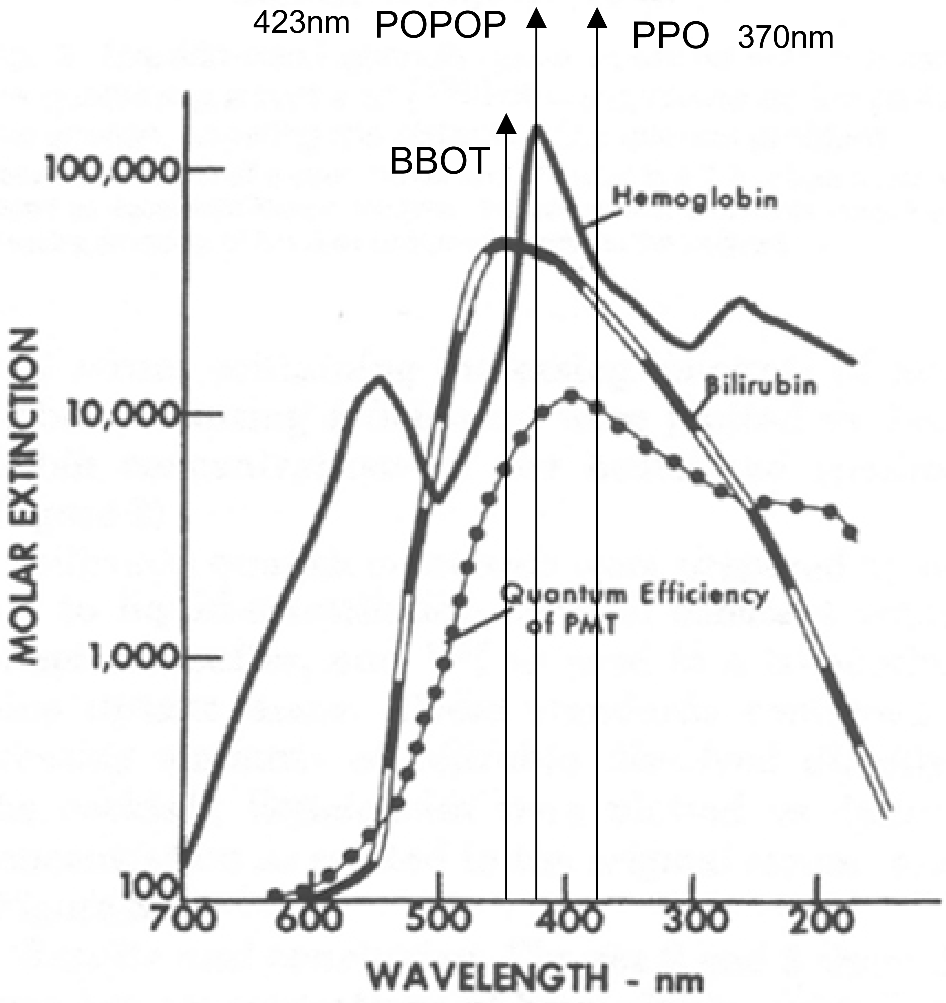
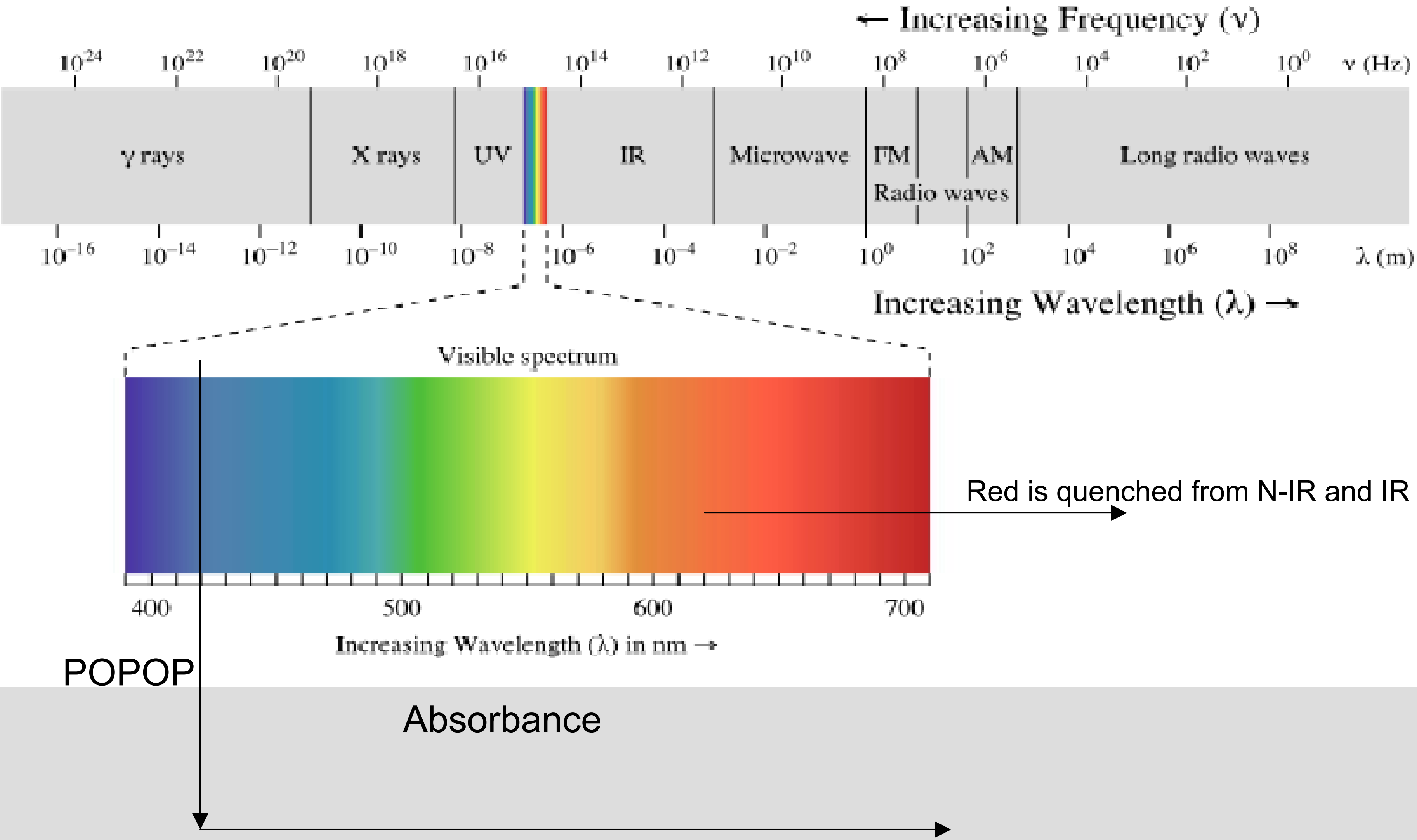


Fig. 1. Absorption spectra of hemoglobin and bilirubin compared to the wavelength dependence of the quantum efficiency of the liquid-scintillation counter photomultiplier tube (*PMT*)
Quantum efficiency is not drawn to scale. The absorption maxima of the colored compounds coincide with the sensitive region of the *PMT*, resulting in significant interference



Red is quenched from N-IR and IR

POPOP

Absorbance

Quenching is due to the overlap of scintillator emission spectrum with quencher absorption spectrum.

From Wikipedia

A. Dyer: An Introduction to Liquid Scintillation Counting.

Heyden & Sons Ltd., 1974, Page 15

- Modern Instruments contain bialkali PM tubes have a wider spectral response range which should eliminate the need for a secondary solute. Despite this fact several workers have shown that, under certain conditions, it can still be an advantage to include a secondary solute in a liquid scintillation cocktail. These conditions are when either, (i) the sample to be counted is coloured or opaque, or (ii) the volume of the scintillator being used is large enough to cause the problem of self-absorption of the primary solute fluorescence.

PMT’s and CCD’s

PMT’s for LSC

1) High quantum efficiency

2) Low thermionic noise

3) Fast response time

4) High pulse linearity

Hamamatsu models: R331, R331-05

Hamamatsu

Title:
Very high quantum efficiency PMT's with bi-alkali photo-cathode

Authors: Mirzoyan, R.; Laatiaoui, M.; Teshima, M.

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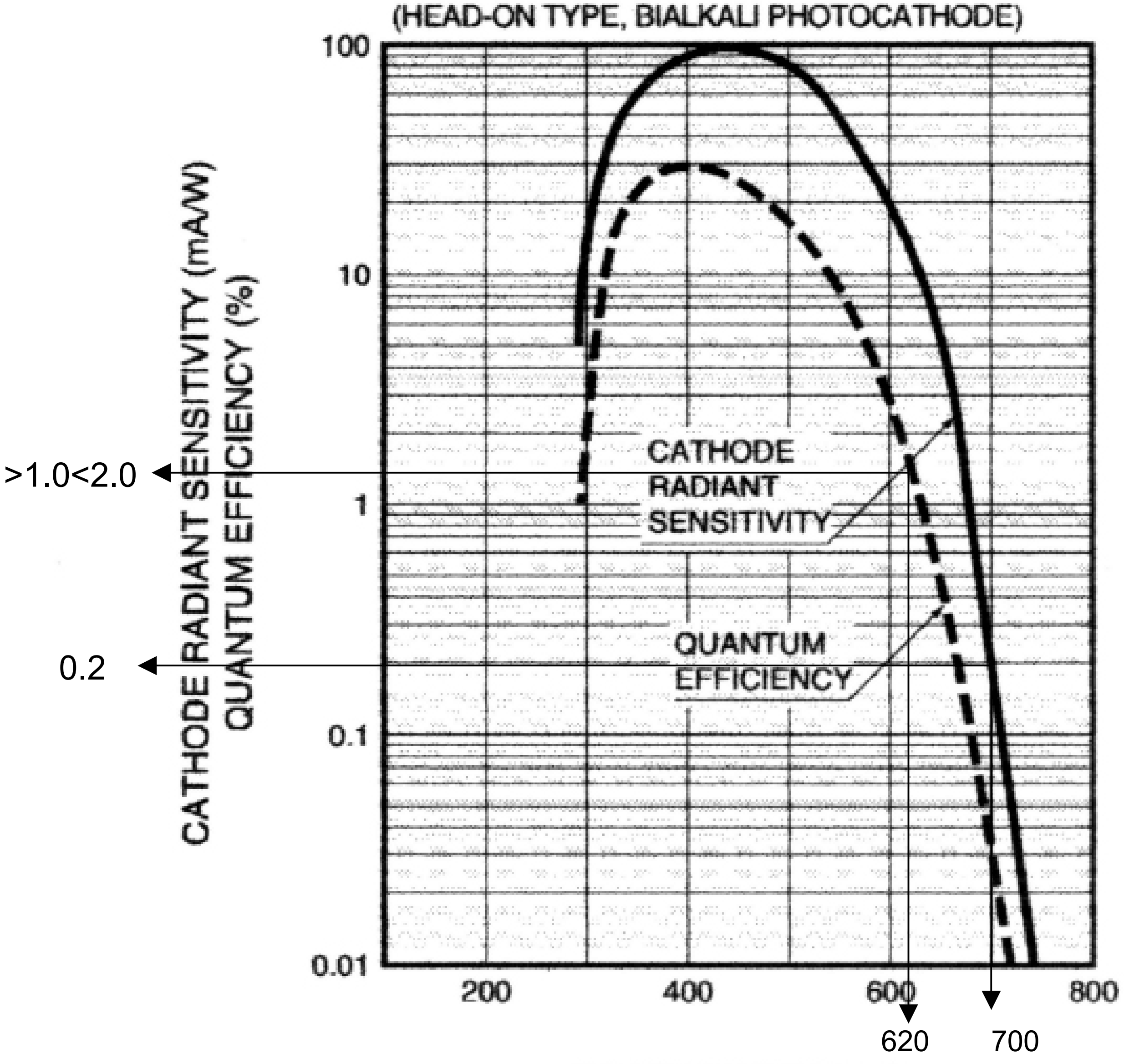
Nuclear Instruments and Methods in Physics Research Section A, Volume 567, Issue 1, p. 230-232.

Since the mid-1960s and until today the classical PMT's with semitransparent bialkali photo-cathode provide peak Quantum Efficiency (QE) of ca. 25 %.

About 2 years ago we started a program with the PMT manufacturers Hamamatsu, Photo and Electron Tubes for boosting up the QE of bi-alkali PMT's.
In the mean time we have obtained several batches of experimental PMT's from the above mentioned manufacturers and measured few samples with QE values as high as 32-36% in the peak. Also, we want to report on the modest (5-7) % increase of the QE of the PMT's with flat input window after sandblasting. Earlier we have reported that by coating the hemi-spherical input window of bialkali PMT's with a milky layer we could enhance their QE by ca. 10-20 % for wavelength ca. 320 mm. Assuming that the industry can reliably produce PMT's with 32-35% QE in the peak, by applying the milky layer coating technique to the PMT's with hemi-spherical input windows one shall be able to achieve peak QE values of 35-40 %. Being by an order of magnitude cheaper and providing a matching level of QE such PMT's will become strong competitors for hybrid photo-diodes (HPD) with GaAsP photo-cathode.

Figure 4: Typical Spectral Response of Bialkali Photocathode



Hamamatsu

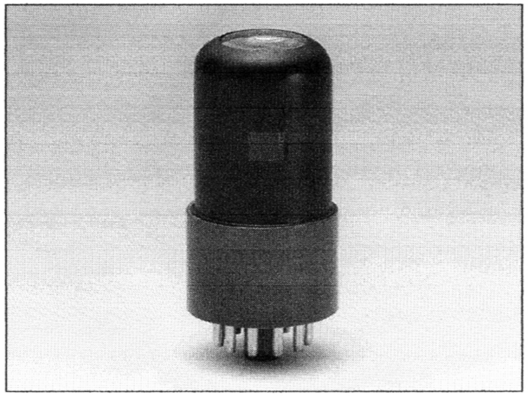
HAMAMATSU

PHOTOMULTIPLIER TUBE R9110

High Sensitivity and Lower Dark Current
Wide Spectral Range with Low ENI, 8 mm × 6 mm Photocathode Area Size

FEATURES

- Low Dark Current .... 5 nA (after 30 minutes)
- Wide Spectral Response .... 185 nm to 900 nm
- High Cathode Sensitivity
  - Luminous .... 525 µA/lm
  - Radiant at 450 nm .... 90 mA/W
  - QE at 450 nm .... 24.8 %
- High Anode Sensitivity (at 1000 V)
  - Luminous .... 10 000 A/lm
- High Signal to Noise Ratio

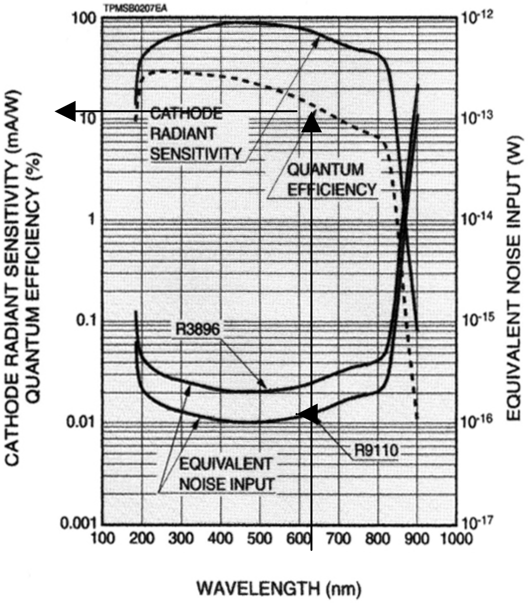


The R9110 is a 28 mm (1-1/8 inch) diameter, 9-stage, side-on type photomultiplier tube having an extended red multialkali photocathode same as the R3896. The R9110 features very low dark current, extremely high quantum efficiency, high gain, good S/N ratio and wide spectral response from UV to near infrared.
The R9110 is directly interchangeable with the R3896.

GENERAL

Parameter		Description/Value	Unit
Spectral Response		185 to 900	nm
Peak Wavelength		450	nm
Photocathode	Material	Multialkali	—
	Minimum Effective Area	8 × 6	mm
Window Material		UV glass	—
Dynode	Secondary Emitting Surface	Multialkali	—
	Structure	Circular-cage	—
	Number of Stages	9	—
Direct Interelectrode Capacitances	Anode to Last Dynode	Approx. 4	pF
	Anode to All Other Electrodes	Approx. 6	pF
Base		11-pin base JEDEC No. B11-88	—
Weight		Approx. 46	g
Suitable Socket (sold separately)		E678-11A	—
Suitable Socket Assembly (sold separately)		E717-63	—
Operating Ambient Temperature		-30 to +50	°C
Storage Temperature		-30 to +50	°C

Figure 1: Typical Spectral Response and Equivalent Noise Input



OPM™ Photomultipliers -- Model 77361

SPECIFICATIONS

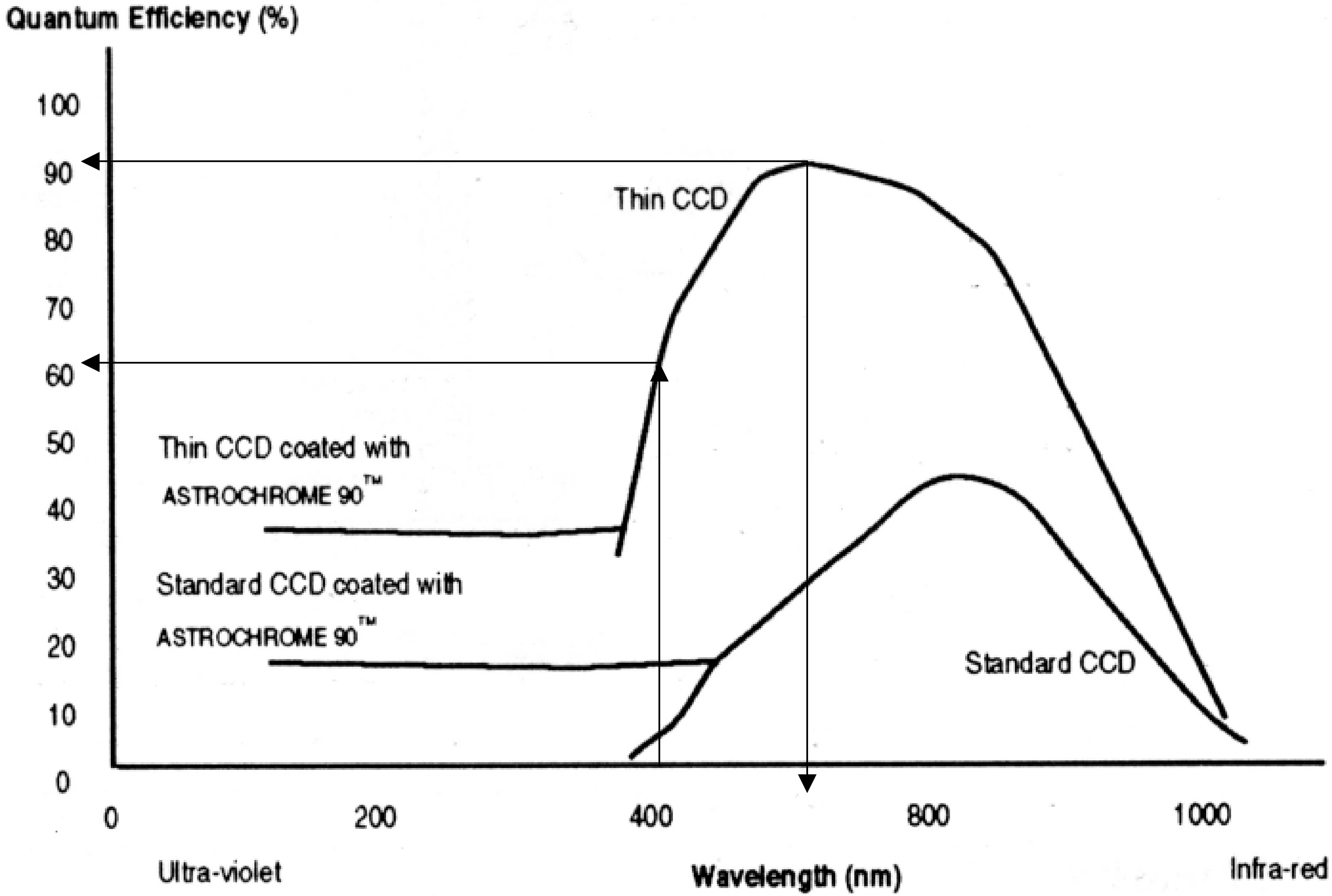
Photomultiplier Type	End-on
Photomultiplier Design	
Active Area Diameter or Length (mm)	25.00
Active Area Height (mm)	
Number of Dynodes	
Photomultiplier Performance	
Wavelength Range (nm)	400 to 1100
Peak Sensitivity Wavelength (nm)	800
Sensitivity (A/W)	950
Rise Time (ns)	10.00
Applied Voltage (volts)	1250
Gain	
Photomultiplier Noise	
Dark Current (nA)	1000
Noise Equivalent Power (W/Hz^{½})	4.20E-13
Photomultiplier Packaging	
Notes	High sensitivity UV detectors, easy to use, low light level measurement

Available from:

Newport Corporation

CCD’s

CCD QE at different wavelengths



The Role of Charge Coupled Devices in Low Light Level Imaging, A Technical Guide from Astromed, Nov 1991, Issue 1.

CCD’s are significantly more sensitive at the red end of the spectrum with a (max) QE>90%

Cooled CCD’s have the lowest noise.

Bi-alkali PMT’s have a wide spectral response but have a (max) QE of typically 25-30%.

Red shifted bi-alkali PMT’s are available, however they will exhibit thermal noise.

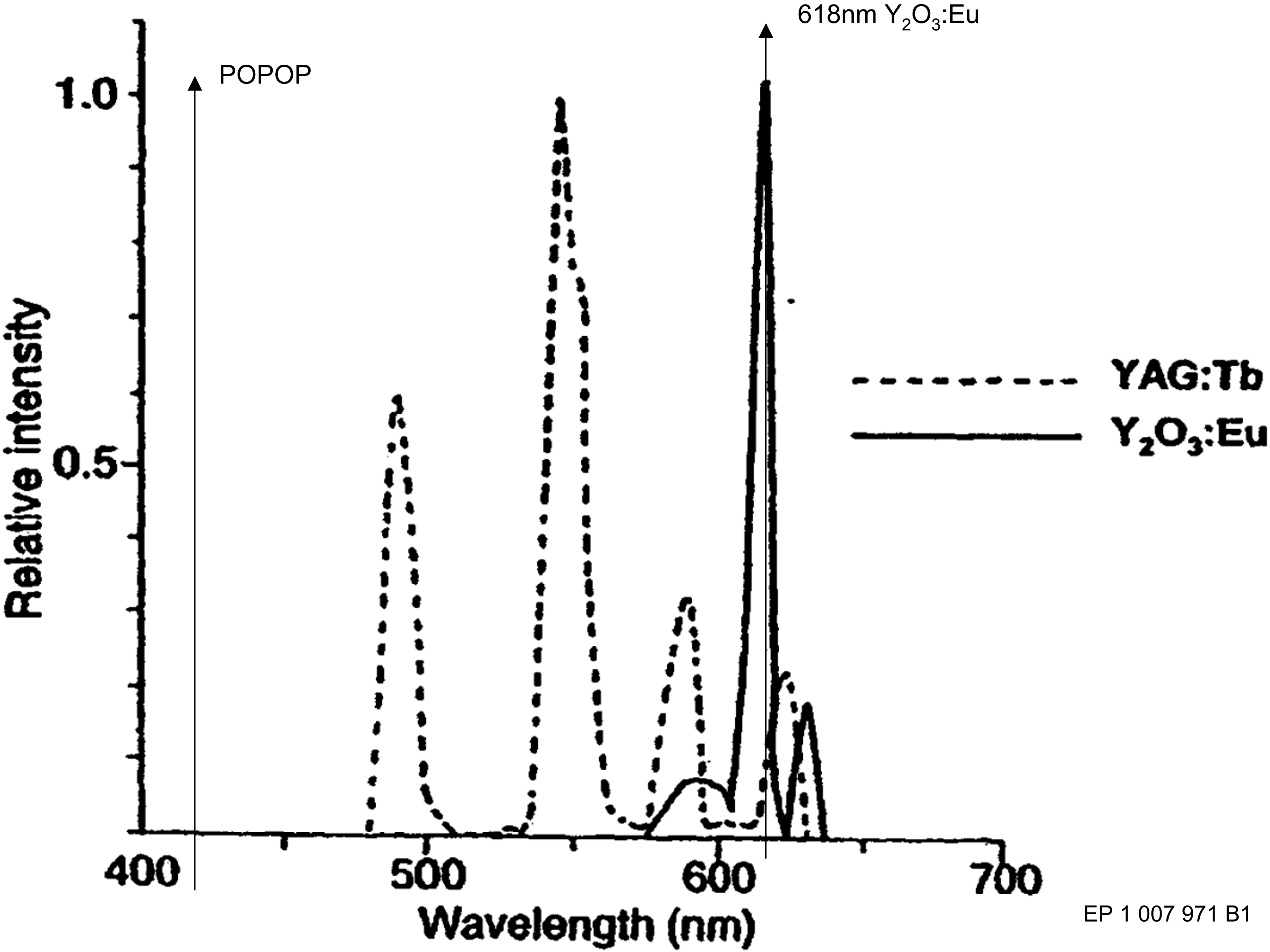
Problems of detector temperature and sample temperature.

RS-LSC in Comparison With Traditional Blue.

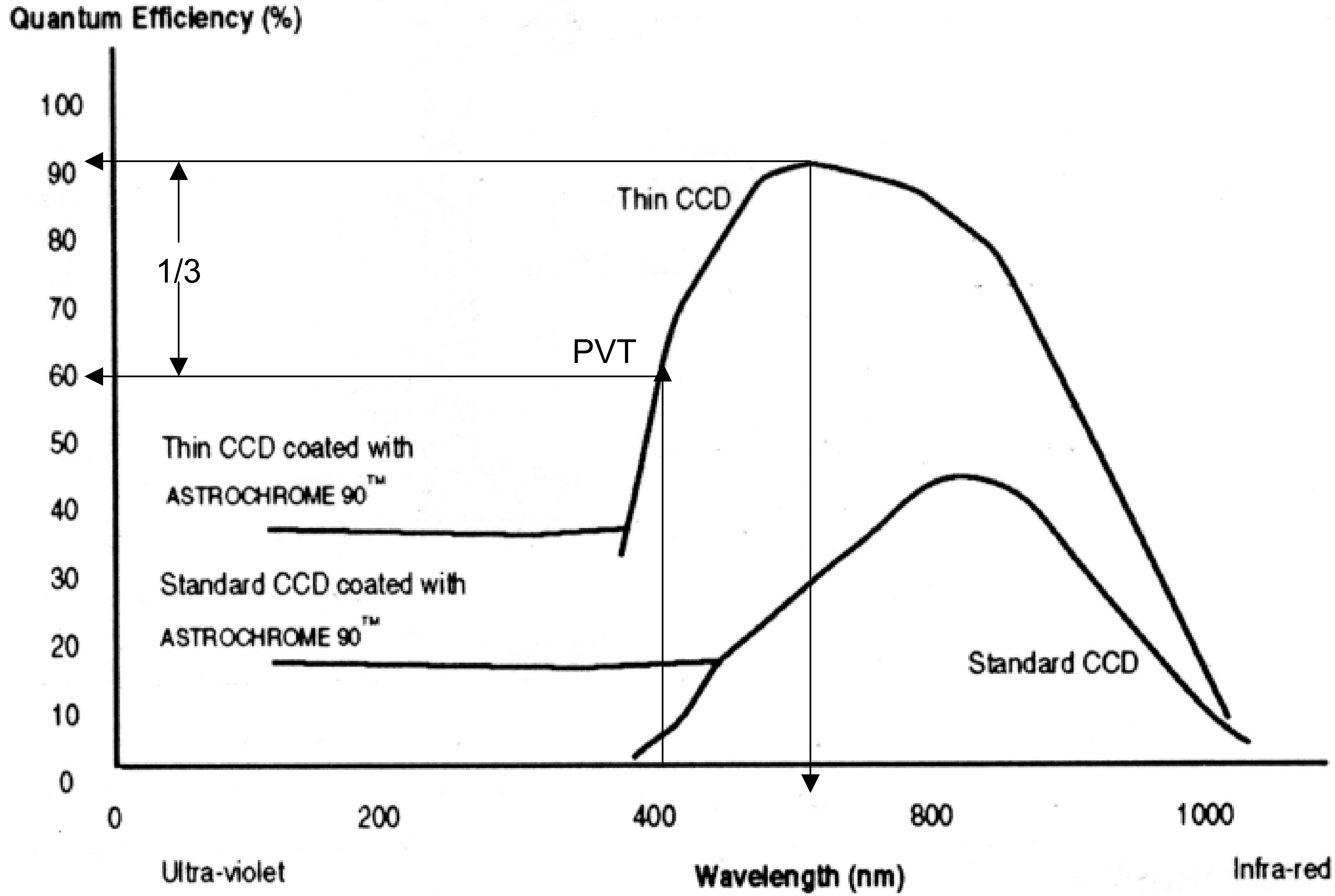
ALP-1 and ALP-7

Inorganic phosphors Y_2O_3:Eu, Y_2O_2S:Eu and YAG:Tb and organic chelate beads prepared from polystyrene containing tris(2,2,6,6-tetramethyl-3,5-heptanedionato)terbium III-diphenyl-phosphonimido-triphenylphosphorane (hereafter called ALP-1) or tris(napthoyltrifluoroacetonato)europium III-(diphenyl-phosphonimido-triphenyl phosphorane)1 or 2 (hereafter called ALP-7 or ALP-7-diphos respectively) were surface-coated with strptavidin and were compared with streptavidin coated polyvinyl toluene (PVT) SPA beads and streptavidin coated yttrium silicate particles in a [^{3}H]biotin binding assay. Coating of particles with proteins, such as streptavidin and other bioreactive species either covalently or by physical adsorption is accomplished by traditional methods known to those skilled in the art.

EP 1 007 971 B1


618nm Y_2O_3:Eu
POPOP
1.0
0.5
Relative intensity
400
500
600
700
Wavelength (nm)
YAG:Tb
Y_2O_3:Eu

CCD QE at different wavelengths



The Role of Charge Coupled Devices in Low Light Level Imaging, A Technical Guide from Astromed, Nov 1991, Issue 1.

In Fig.1, (solid scintillator), ^{3}H detected efficiently by Y_2O_2S:Eu (by CCD). Sensitivity approx.18 times higher than that achieved with Butyl-PBD in PVT.

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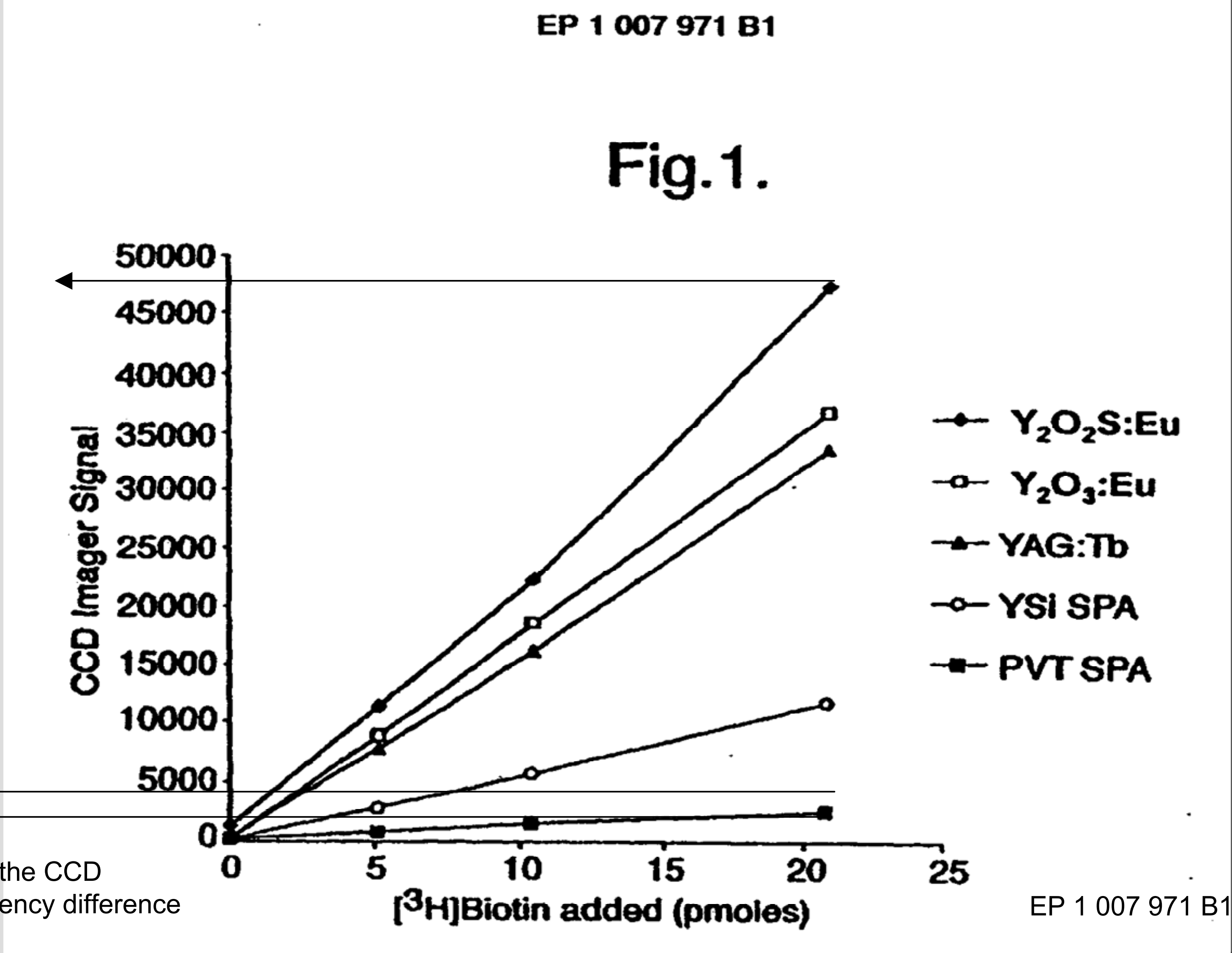
Fig.1.



2200x3/2

3300

2200

Compensate for the CCD wavelength efficiency difference

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EP 1 007 971-B1

Colour quenching occurs after the generation of light

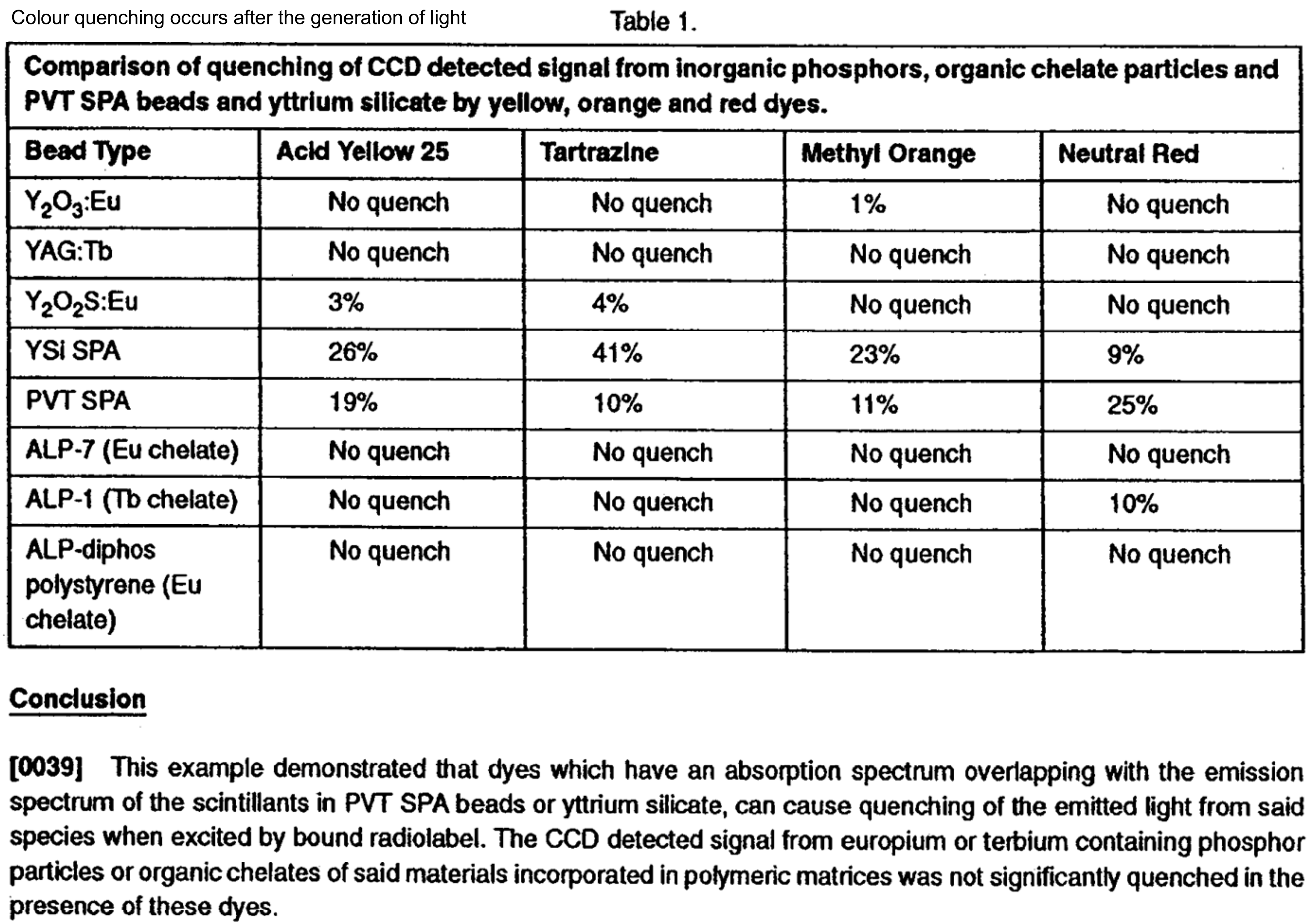


Table 1.

Comparison of quenching of CCD detected signal from inorganic phosphors, organic chelate particles and PVT SPA beads and yttrium silicate by yellow, orange and red dyes.				
Bead Type	**Acid Yellow 25**	**Tartrazine**	**Methyl Orange**	**Neutral Red**
Y_2O_3:Eu	No quench	No quench	1%	No quench
YAG:Tb	No quench	No quench	No quench	No quench
Y_2O_2S:Eu	3%	4%	No quench	No quench
YSi SPA	26%	41%	23%	9%
PVT SPA	19%	10%	11%	25%
ALP-7 (Eu chelate)	No quench	No quench	No quench	No quench
ALP-1 (Tb chelate)	No quench	No quench	No quench	10%
ALP-diphos polystyrene (Eu chelate)	No quench	No quench	No quench	No quench

Conclusion

[0039] This example demonstrated that dyes which have an absorption spectrum overlapping with the emission spectrum of the scintillants in PVT SPA beads or yttrium silicate, can cause quenching of the emitted light from said species when excited by bound radiolabel. The CCD detected signal from europium or terbium containing phosphor particles or organic chelates of said materials incorporated in polymeric matrices was not significantly quenched in the presence of these dyes.

Table 2.

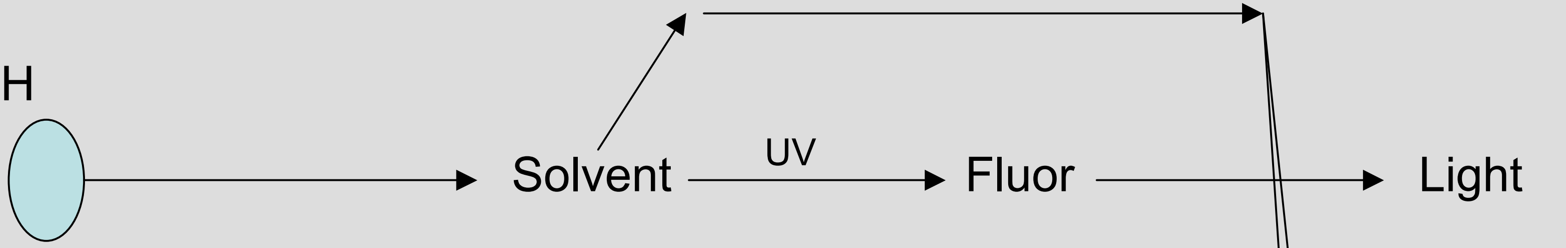
Quenching emission of SPA particles by tartrazine at 4 different levels of dye. Comparison of quenching of signal detected by CCD detection and by a scintillation counter.

	Tartrazine concentration, µg/ml			
CCD Detection	**5.1**	**10.2**	**20.4**	**40.8**
Y_2O_2S:Eu	No quench	No quench	No quench	No quench
Y_2O_3:Eu	No quench	No quench	No quench	No quench
YAG:Tb	No quench	No quench	No quench	3%
Ysi SPA	33%	44%	52%	58%
ALP-7-diphos polystyrene	No quench	1%	4%	No quench
PVT SPA	2%	4%	6%	15%
Microbeta, SPA mode	**5.1**	**10.2**	**20.4**	**40.8**
Ysi SPA	54%	61%	73%	82%
PVT SPA	37%	46%	69%	87%

Traditional Bio-life Science Fluorophores

Energy of the excited solvent is emitted as UV light and the solvent molecule returns to ground state. The UV light is absorbed by fluor molecules which emit (blue) light flashes upon return to ground state. Nuclear decay events produce approximately 10 photons per KeV.

Packard Instrument Company



^{3}H

Solvent

UV

Fluor

Light

Colour quenching occurs after the generation of light

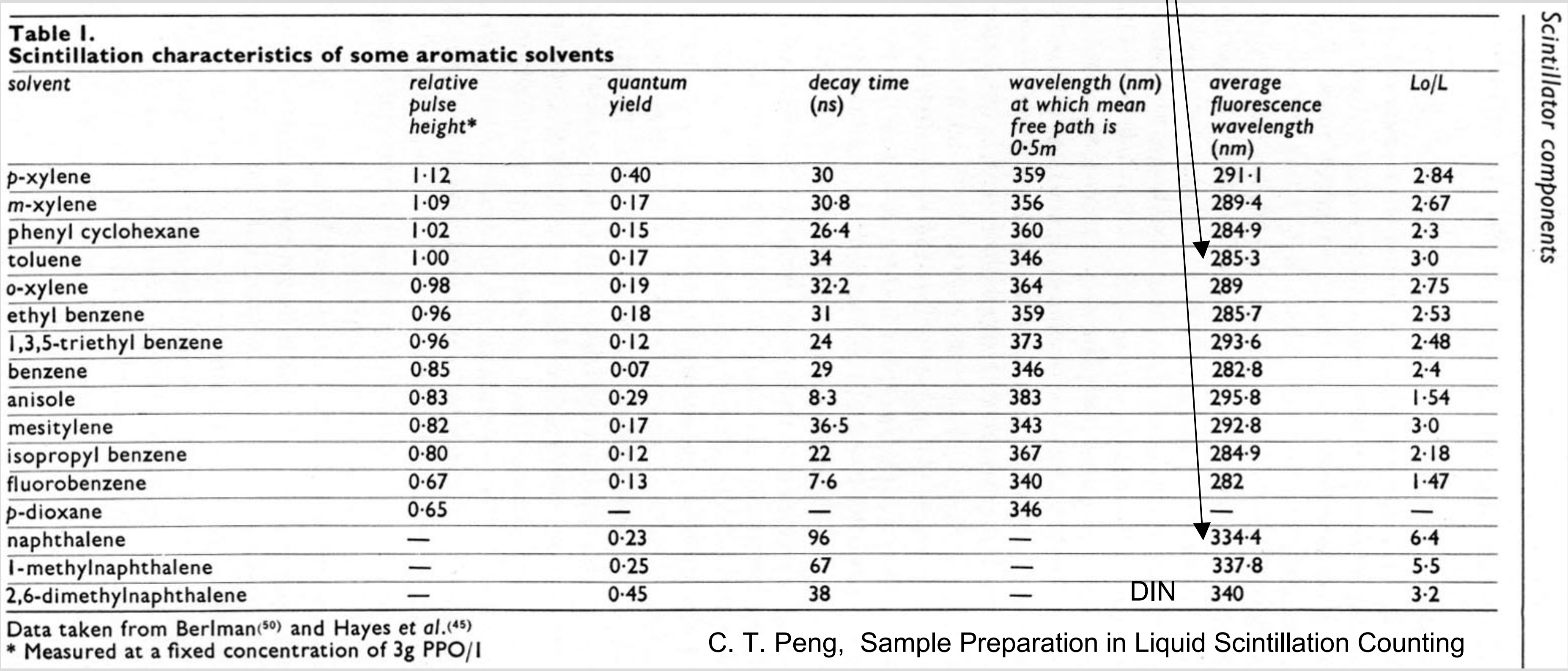


Table I.
Scintillation characteristics of some aromatic solvents

solvent	*relative pulse height**	*quantum yield*	*decay time (ns)*	*wavelength (nm) at which mean free path is 0·5m*	*average fluorescence wavelength (nm)*	*Lo/L*
p-xylene	1·12	0·40	30	359	291·1	2·84
m-xylene	1·09	0·17	30·8	356	289·4	2·67
phenyl cyclohexane	1·02	0·15	26·4	360	284·9	2·3
toluene	1·00	0·17	34	346	285·3	3·0
o-xylene	0·98	0·19	32·2	364	289	2·75
ethyl benzene	0·96	0·18	31	359	285·7	2·53
1,3,5-triethyl benzene	0·96	0·12	24	373	293·6	2·48
benzene	0·85	0·07	29	346	282·8	2·4
anisole	0·83	0·29	8·3	383	295·8	1·54
mesitylene	0·82	0·17	36·5	343	292·8	3·0
isopropyl benzene	0·80	0·12	22	367	284·9	2·18
fluorobenzene	0·67	0·13	7·6	340	282	1·47
p-dioxane	0·65	—	—	346	—	—
naphthalene	—	0·23	96	—	334·4	6·4
1-methylnaphthalene	—	0·25	67	—	337·8	5·5
2,6-dimethylnaphthalene	—	0·45	38	—	340	3·2

DIN

Data taken from Berlman[50] and Hayes *et al.*[45]
* Measured at a fixed concentration of 3g PPO/l

C. T. Peng, Sample Preparation in Liquid Scintillation Counting

Traditional Fluorescent Labels for Molecular Biology

Fluorophore	Excitation (nm)	Emission (nm)
Acridine Orange	502	526
BODIPY	503	512
CY 3	554	568
DiO-Cn-(3)	485	505
Fluorescein Isothiocyanate (FITC)	490	525
Propidium Iodide	536	617
Rhodamine 123	511	534
snarf 1	563	639
Tetra Rhodamine Isothiocyanate (TRITC)	557	576
TexasRed	596	615

Properties of the DyLight NHS-Ester Fluors.

DyLight Fluor Dyes	**Ex/Em***	ε†	**MW (g/mol)**	**Spectrally Similar**
488	493/518	70,000	1011	Alexa Fluor 488, Cy2
549	550/568	150,000	981	Alexa Fluor 555, Cy3
649	646/674	250,000	1,008	Alexa Fluor 647, Cy5
680	682/715	140,000	950	Alexa Fluor 680
800	770/794	270,000	1,050	IRDye 800

* Excitation and emission maxima in nanometers
†Molar extinction coefficient (M-1 cm-1)

Pierce Biotechnology

Absorbance max at approx 660nm
Emission max at approx 680nm

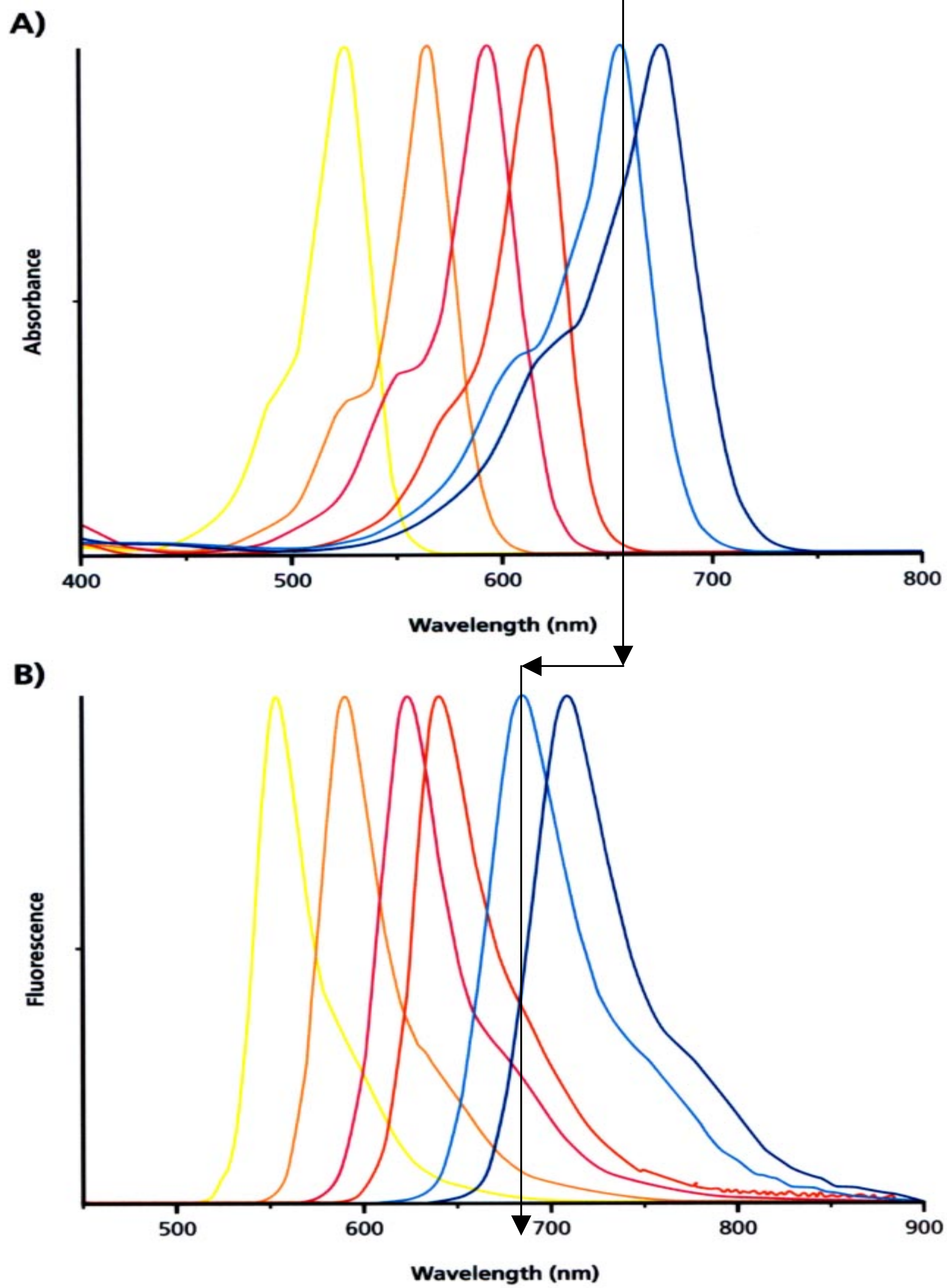


Figure 1. Normalized absorption (A) and fluorescence (B) spectra of Atto dyes measured in ethanol. *From left to right side: Atto 520 (yellow), Atto 565 (orange), Atto 590 (fuschia), Atto 610 (red), Atto 655 (aqua), Atto 680 (blue).*

Atto Dyes
Sigma-Aldrich Corporation,
Buchs,
Switzerland.

Absorbance (Ex) needs to be in the 340nm region

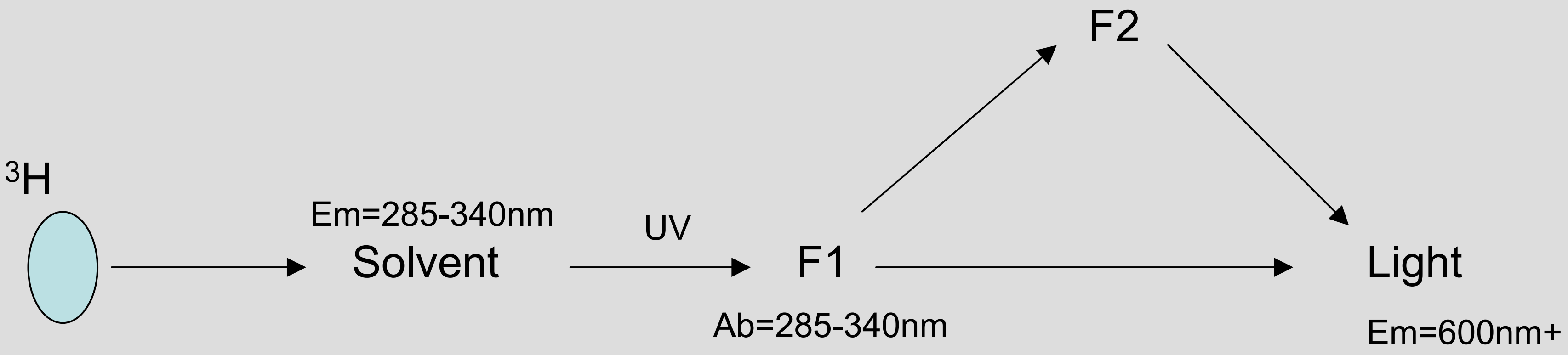
	Ex	Em		
Alexa Fluor 350	346	445	410	
Alexa Fluor 430	430	545	701	
Alexa Fluor 488	494	517	643	
Alexa Fluor 532	530	555	724	
Alexa Fluor 546	556	573	1079	
Alexa Fluor 555	556	573	1250	
Alexa Fluor 568	578	603	792	
Alexa Fluor 594	590	617	820	
Alexa Fluor 633	621	639	1200	
Alexa Fluor 647	650	668	1250	
Alexa Fluor 660	663	690	1100	
Alexa Fluor 680	679	702	1150	
Alexa Fluor 700	696	719		
Alexa Fluor 750	752	779		

Lucifer yellow	425	528		
NBD	466	539	294	NBD-X
R-Phycoerythrin (PE)	480;565	578	240 k	
PE-Cy5 conjugates	480;565;650	670		aka Cychrome, R670, Tri-Color, Quantum Red
PE-Cy7 conjugates	480;565;743	767		
APC-Cy7 conjugates	650;755	767		PharRed
Red 613	480;565	613		PE-Texas Red
Fluorescein	495	519	389	FITC; pH sensitive
FluorX	494	520	587	(AP Biotech)
BODIPY-FL	503	512		
TRITC	547	572	444	TRITC
X-Rhodamine	570	576	548	XRITC
Lissamine Rhodamine B	570	590		
PerCP	490	675		Peridinin chlorphyll protein
Texas Red	589	615	625	Sulfonyl chloride
Allophycocyanin (APC)	650	660	104 k	
TruRed	490,675	695		PerCP-Cy5.5 conjugate

Cy2	489	506	714
Cy3	(512);550	570;(615)	767
Cy3.5	581	596;(640)	1102
Cy5	(625);650	670	792
Cy5.5	675	694	1128
Cy7	743	767	818

DsRed, DsRed2 (RFP)	558	583	~110k	(*Clontech*) Quantum yield 0.79. Tetramer.
DsRed monomer	556	586	~28k	(*Clontech*)
AsRed2	576	592	~110k	(*Clontech*) Tetramer
mRFP1	584	607	~30k	(*Tsien lab*) Quantum yield 0.25
HcRed1	588	618	~52k	(*Clontech*) Dimer

Strategy for RS-LSC Cocktail



^{3}H

Em=285-340nm

Solvent

UV

F1

Ab=285-340nm

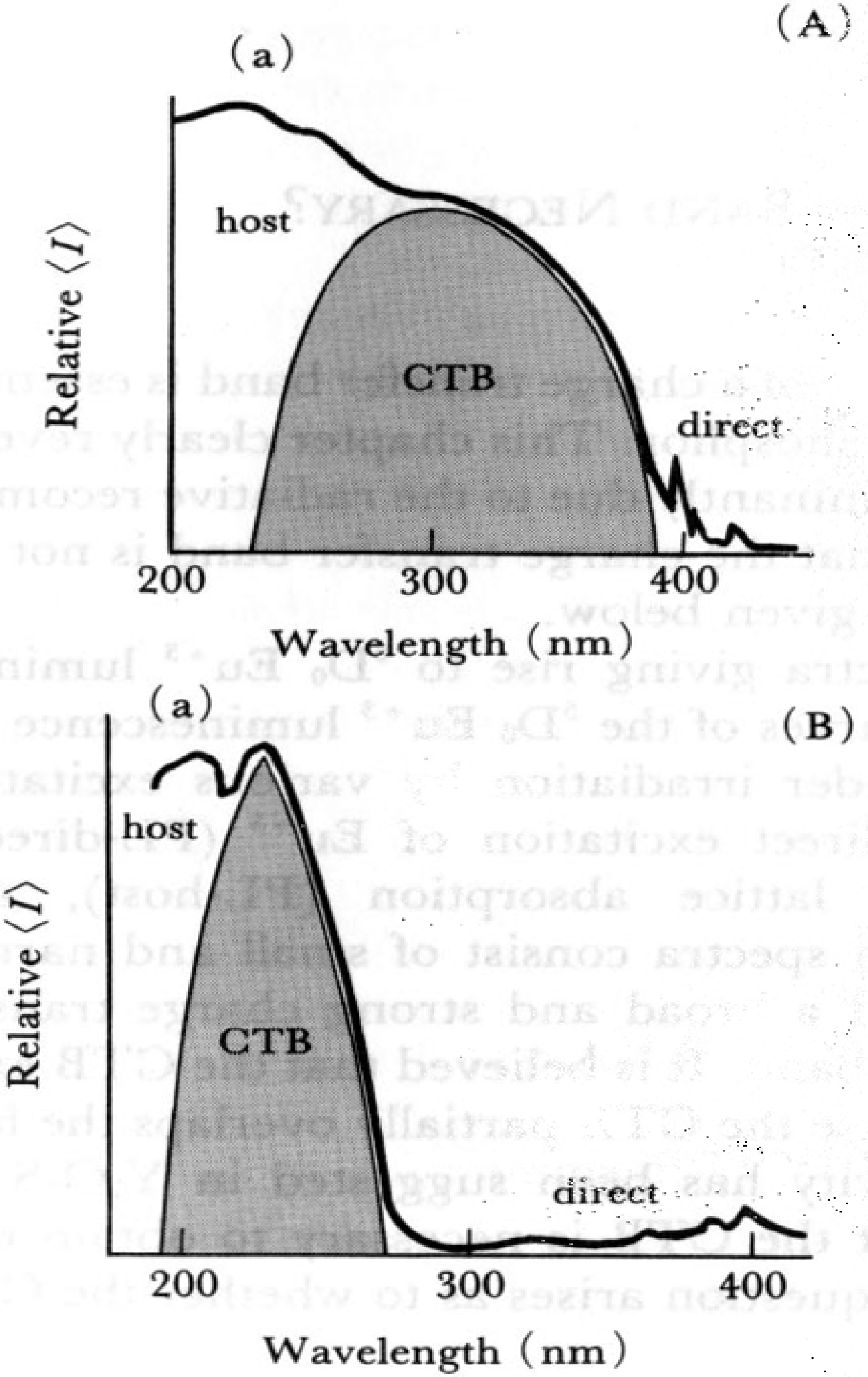
F2

Light

Em=600nm+

PXE (phenylxylylethane)

DIN



Excitation spectra of Eu^{3+} luminescence

(A) Y_2O_2S:Eu

(B) Y_2O_3:Eu

CTB (Charge Transfer Band)

Lyuji Ozawa, Cathodoluminescence

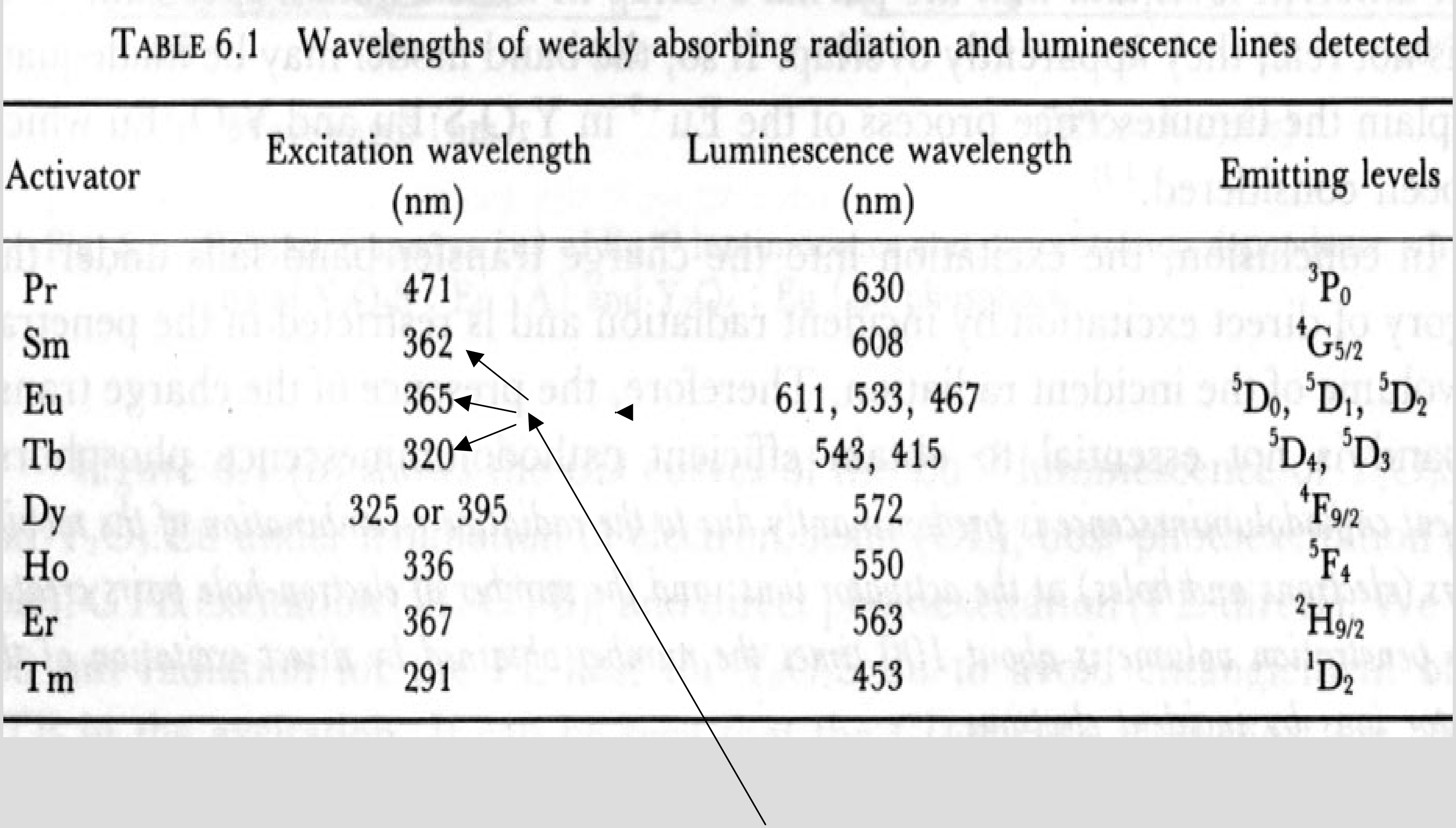


TABLE 6.1 Wavelengths of weakly absorbing radiation and luminescence lines detected

Activator	Excitation wavelength (nm)	Luminescence wavelength (nm)	Emitting levels
Pr	471	630	$^3P_0$
Sm	362	608	$^4G_{5/2}$
Eu	365	611, 533, 467	$^5D_0$, $^5D_1$, $^5D_2$
Tb	320	543, 415	$^5D_4$, $^5D_3$
Dy	325 or 395	572	$^4F_{9/2}$
Ho	336	550	$^5F_4$
Er	367	563	$^2H_{9/2}$
Tm	291	453	$^1D_2$

Solvent emission (2,6-dimethylnaphthalene) 340nm

Lyuji Ozawa, Cathodoluminescence

Why Red Shift?

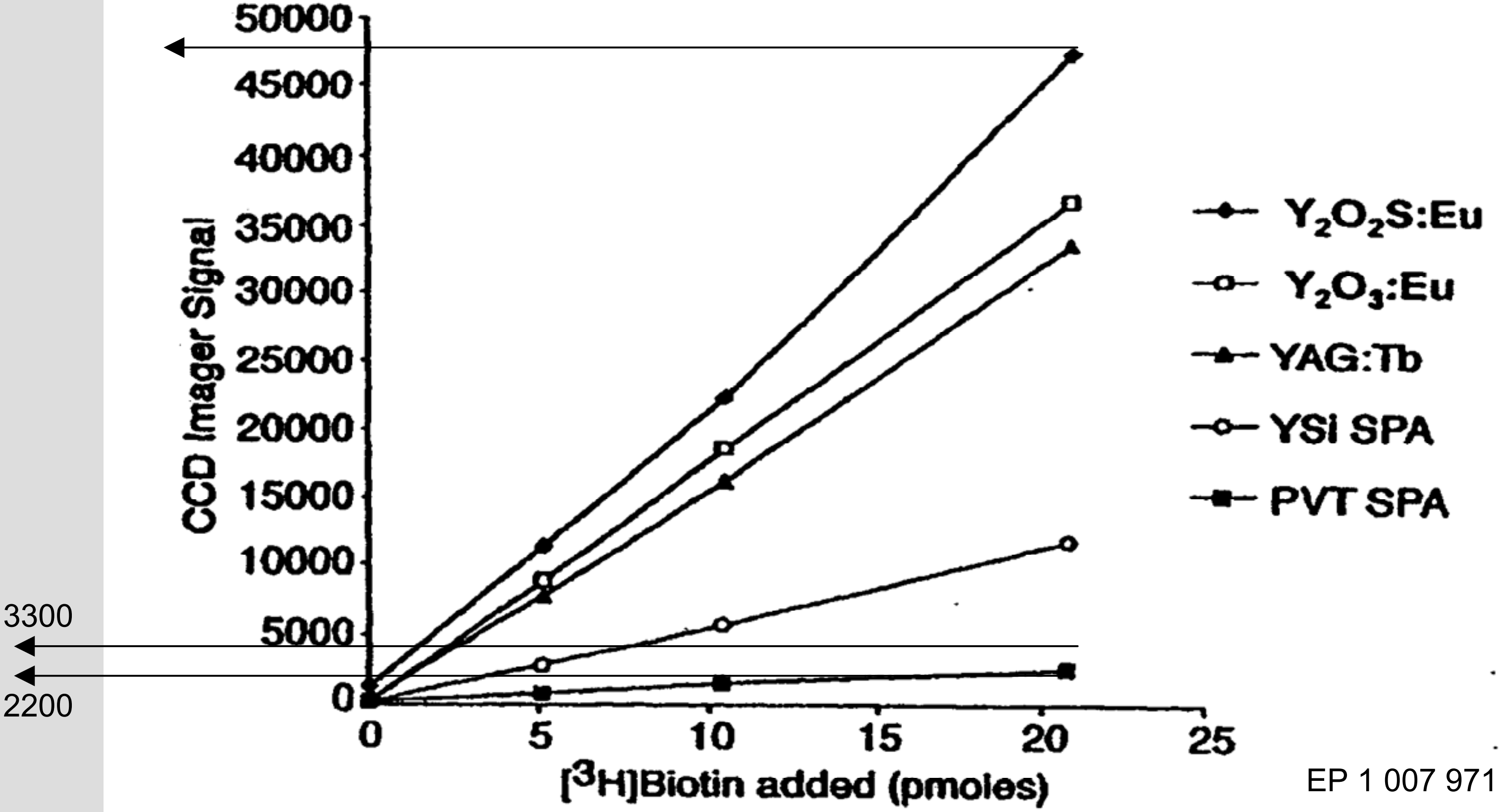
- Minimise, or eliminate the problem of colour quench.
- Higher detection sensitivity.

(solid scintillator Y_2O_2S:Eu), ^3H detected efficiency by CCD.

Signal 18 times higher than achieved with Butyl-PBD in PVT.

EP 1 007 971 B1

Fig.1.



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3300

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EP 1 007 971 B1

Thank you for your time

Phil Harrison