Liquid Scintillation Counting

6/21/2011

General

Introduction

A scintillating liquid, referred to as the “cocktail,” serves as the detector.

The cocktail (perhaps 10 ml) is inside a plastic or glass vial that is transparent to the light emitted by the cocktail.

Ideally, the sample (e.g., 1 ml) is dissolved in the cocktail. Failing that, the sample might be suspended as an emulsion or suspended in a gel. In some cases, a large solid sample (e.g., a smear) is simply placed into the cocktail with no attempt at dissolving it.

Introduction

Liquid scintillation counting is primarily used to quantify pure beta emitters, e.g., H-3
Ni-63
C-14
S-35
P-32

It is also used to quantify alpha emitters and nuclides that decay by electron capture (e.g., Fe-55, Cr-51, I-125).

LSC incorporates elements of spectroscopy, but it is rarely used to identify radioactive material. As a rule, LSC is used to quantify the activity of a known nuclide.

Important Characteristics of LSC

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Three Important Characteristics of LSC:

1. High counting efficiency: 100% for many nuclides. Efficiencies as high as 70% for H-3

2. No backscatter

3. Low background

Important Characteristics of LSC

1. High Counting Efficiency

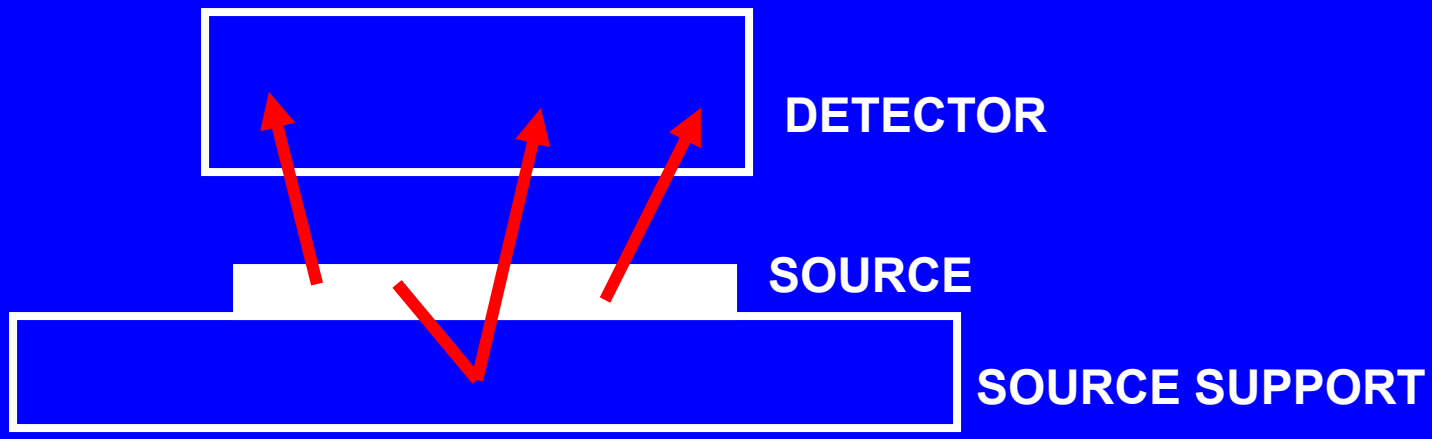
There are two reasons for the high counting efficiency:

- There is no window through which the radiation must pass in order to reach the sensitive part of the detector
- The system employs a “four pi” geometry. No matter which direction the radiation is emitted in, it will interact with the cocktail (assuming we are talking about charged particle radiation).

Important Characteristics of LSC

2. No Backscatter

When proportional counters or GM detectors count beta particles, backscatter directs some betas towards the detector that otherwise would have gone in a different direction.



While this increases the detector counting efficiency, it makes our estimate of the counting efficiency less certain!

LSC involves no source support in which backscatter can occur.

Important Characteristics of LSC

3. Low Background

The LSC vials (glass or plastic) are made of materials with very low levels of naturally occurring radionuclides.

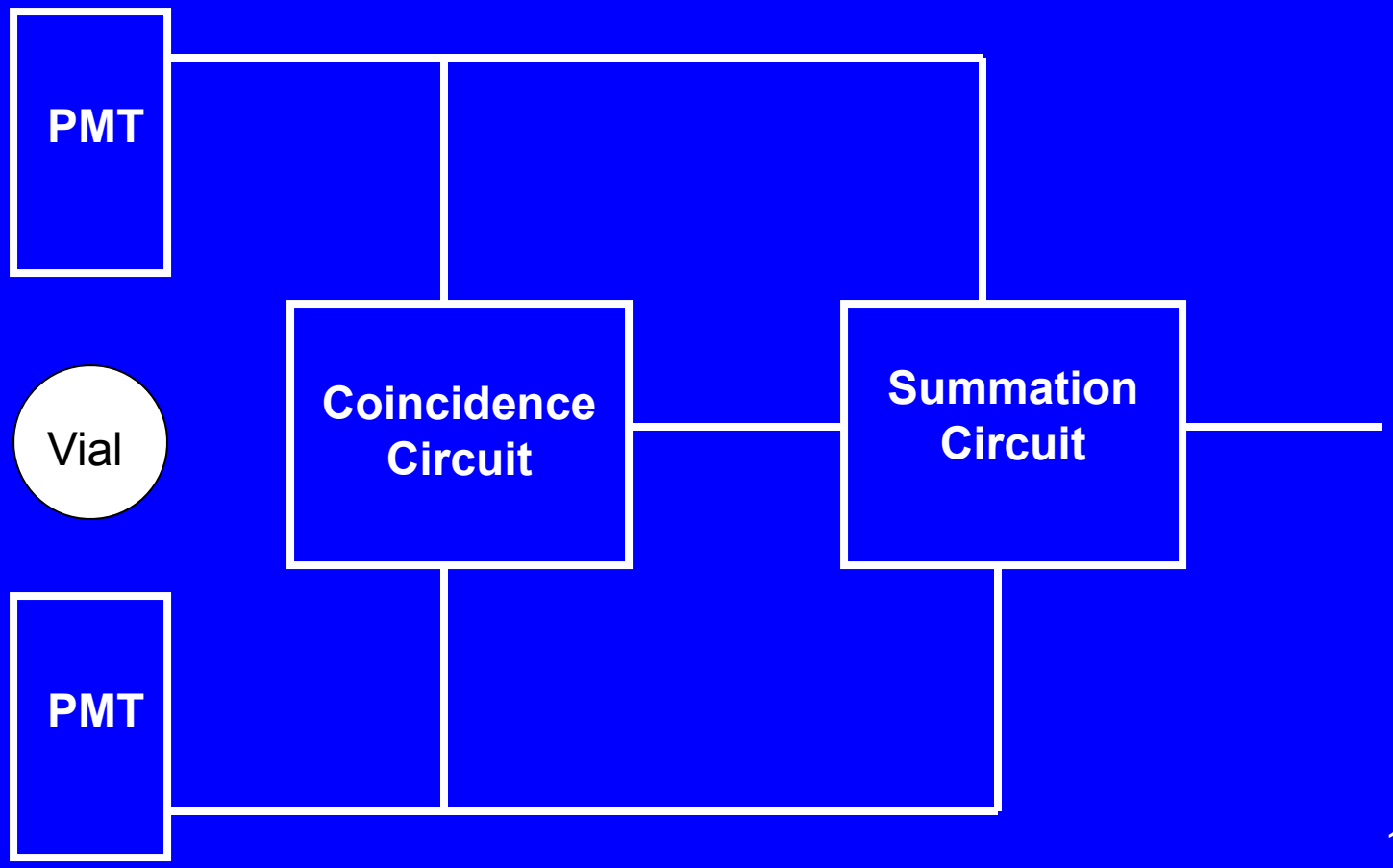
When positioned inside the counting chamber, the LSC vial is shielded from background gamma rays. Otherwise, these gamma rays could interact with the vial wall or the cocktail (primarily via Compton scattering) and transfer their energy to electrons which could produce a signal indistinguishable from that produced by the betas being counted.

The shield might be passive (e.g., lead) or active (e.g., an anticoincident BGO guard detector)

Important Characteristics of LSC

3. Low Background

LSC systems use coincidence counting to reduce the number of spurious counts produced by electronic noise.



Important Characteristics of LSC

3. Low Background

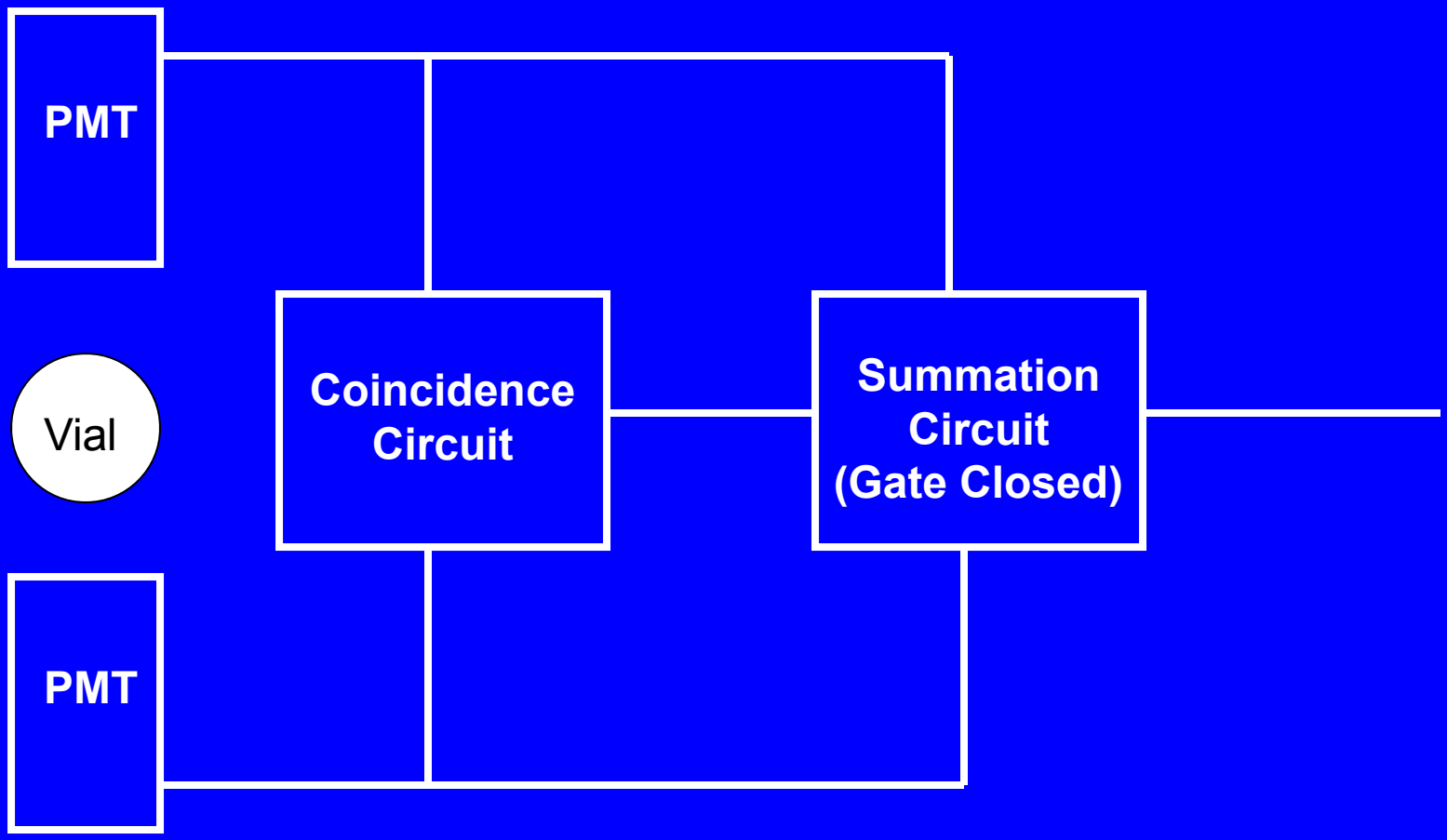
When a beta particle is emitted in the cocktail, a single scintillation event (flash) occurs in which light photons are emitted in all directions.

The two photomultiplier tubes react to the scintillation at the same moment in time (in coincidence) and each PMT generates a pulse that travels to the coincidence and summation circuits.

The coincidence circuit “recognizes” that this represents a legitimate decay event and it sends a logic pulse to the summation circuit telling it to sum the two pulses and open its electronic gate to allow the resulting pulse through.

Important Characteristics of LSC

3. Low Background



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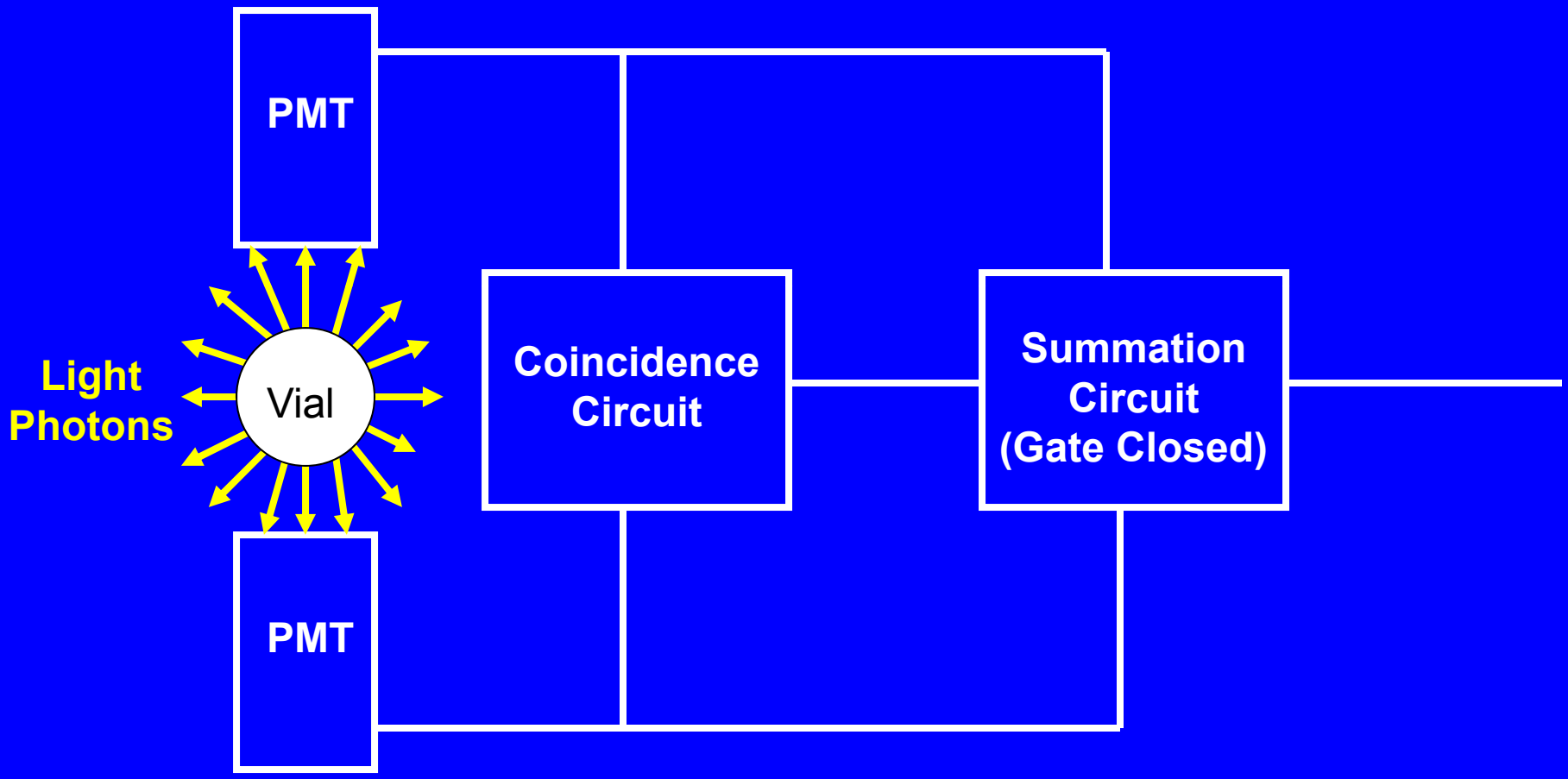

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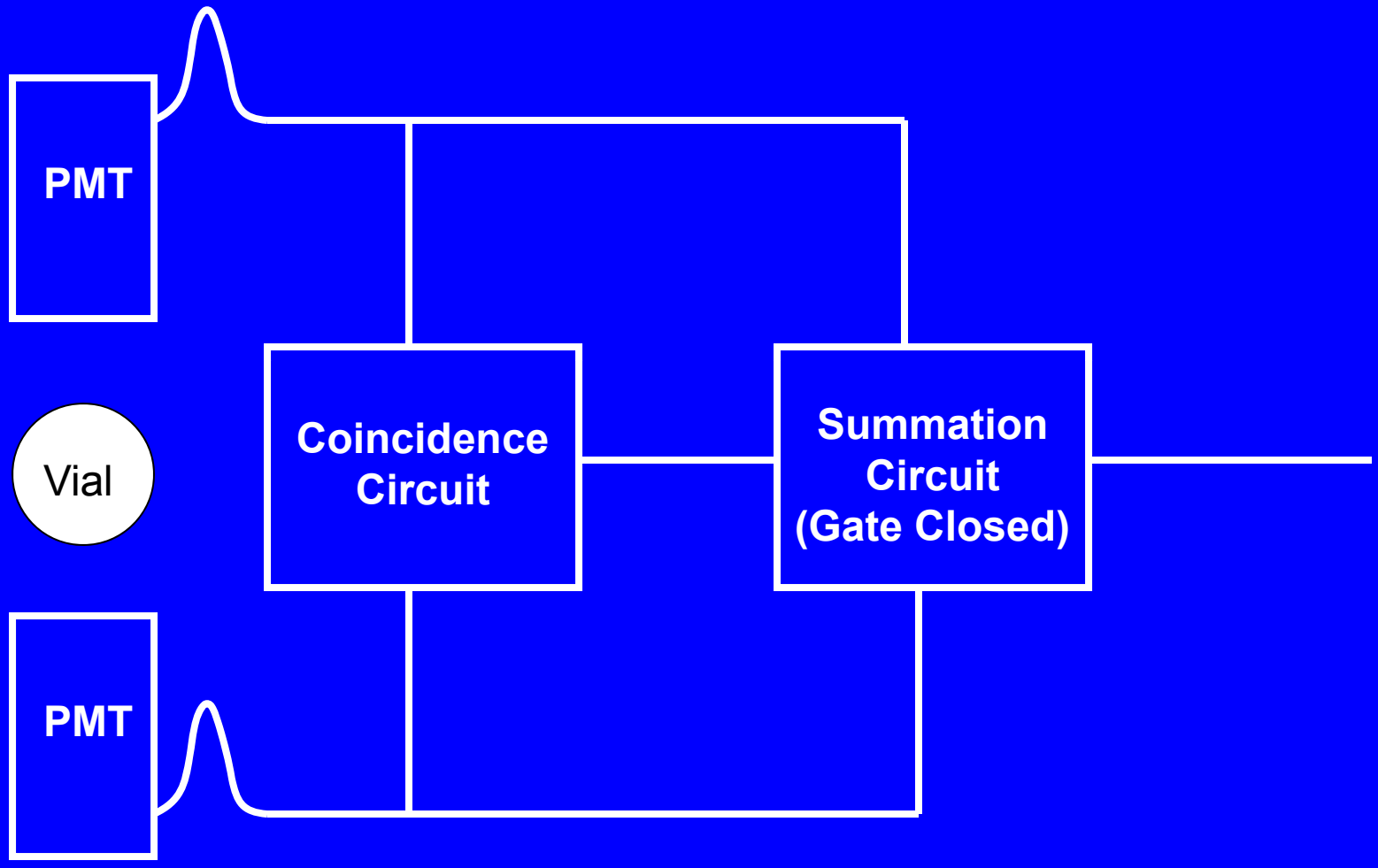

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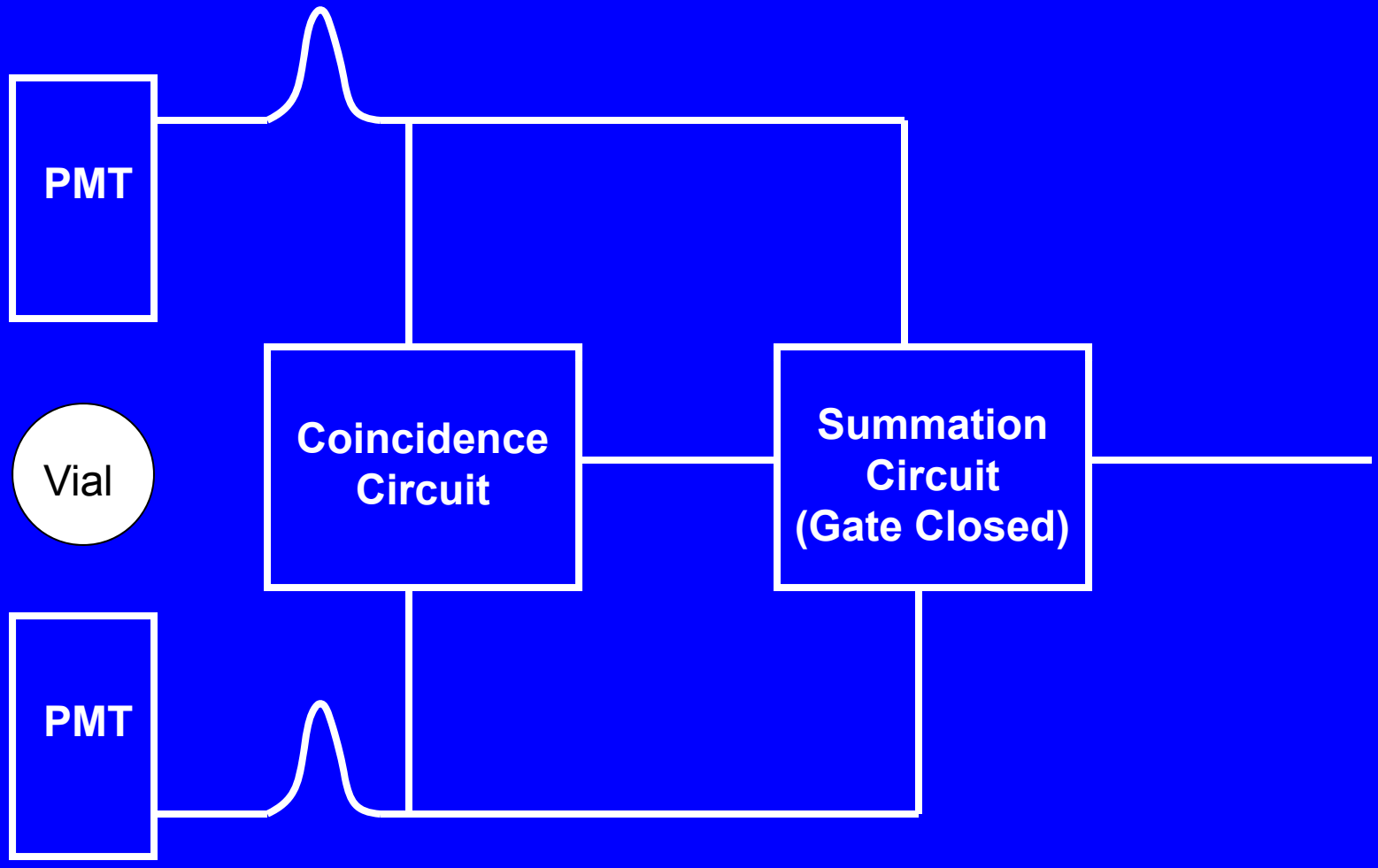

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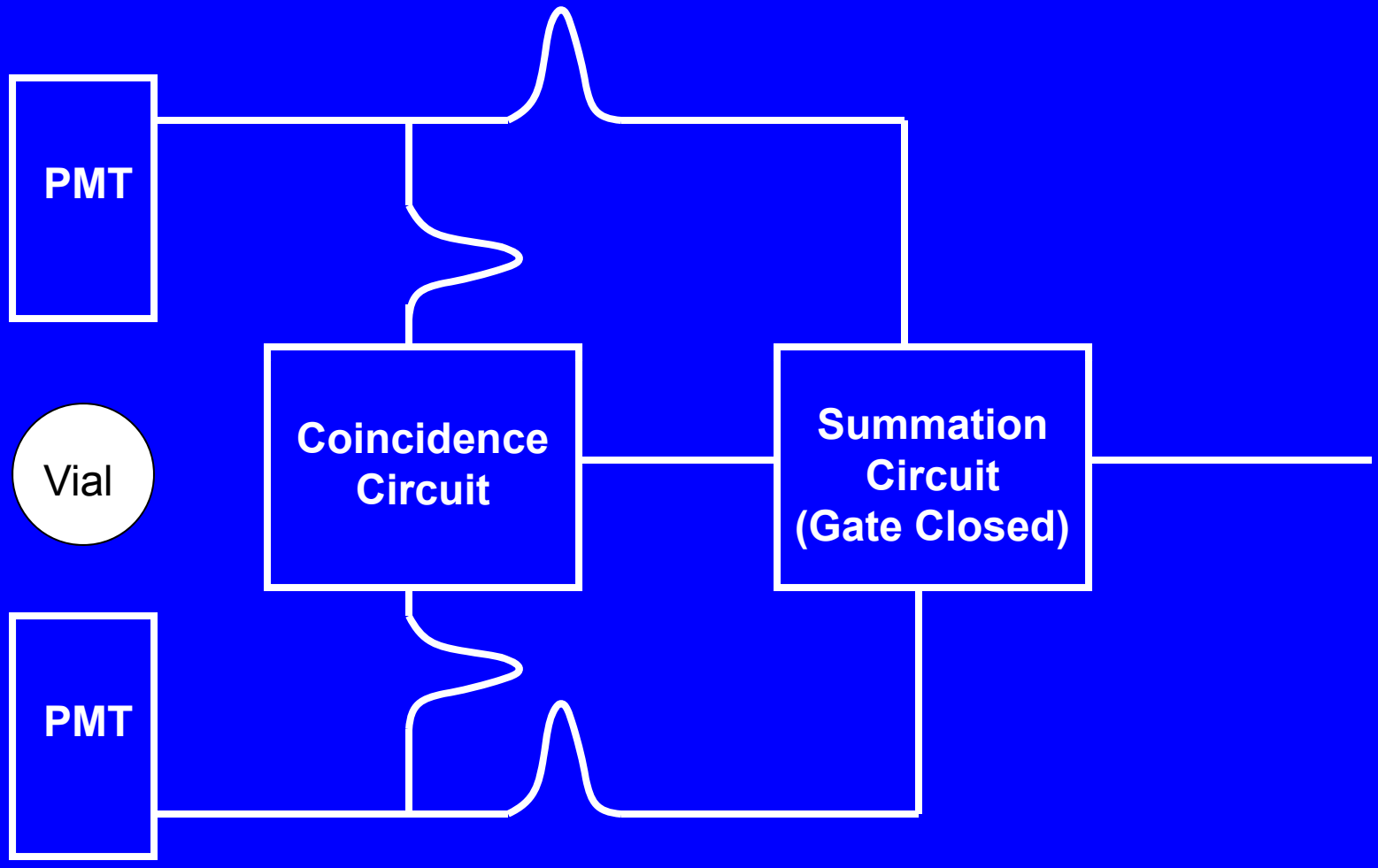

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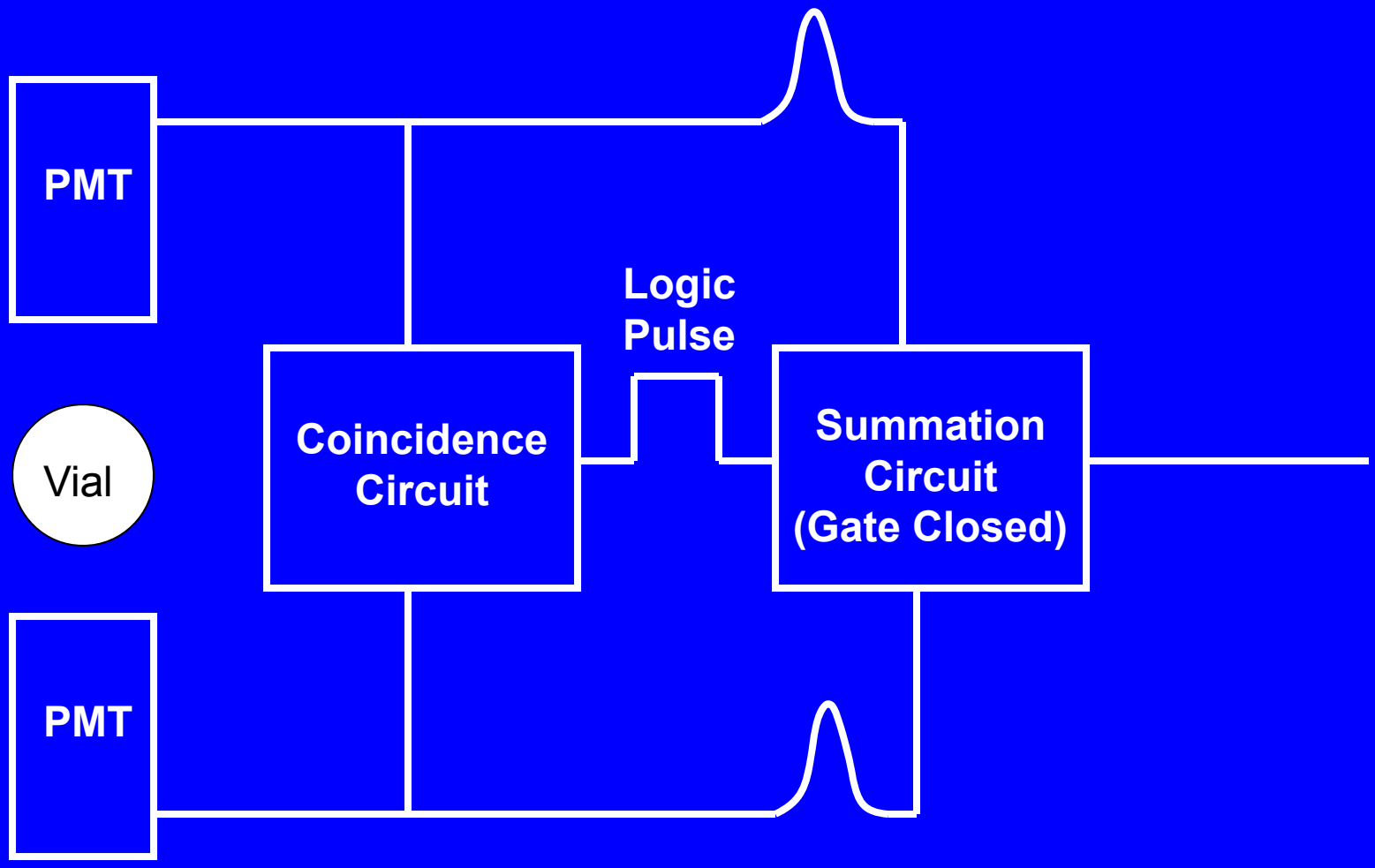

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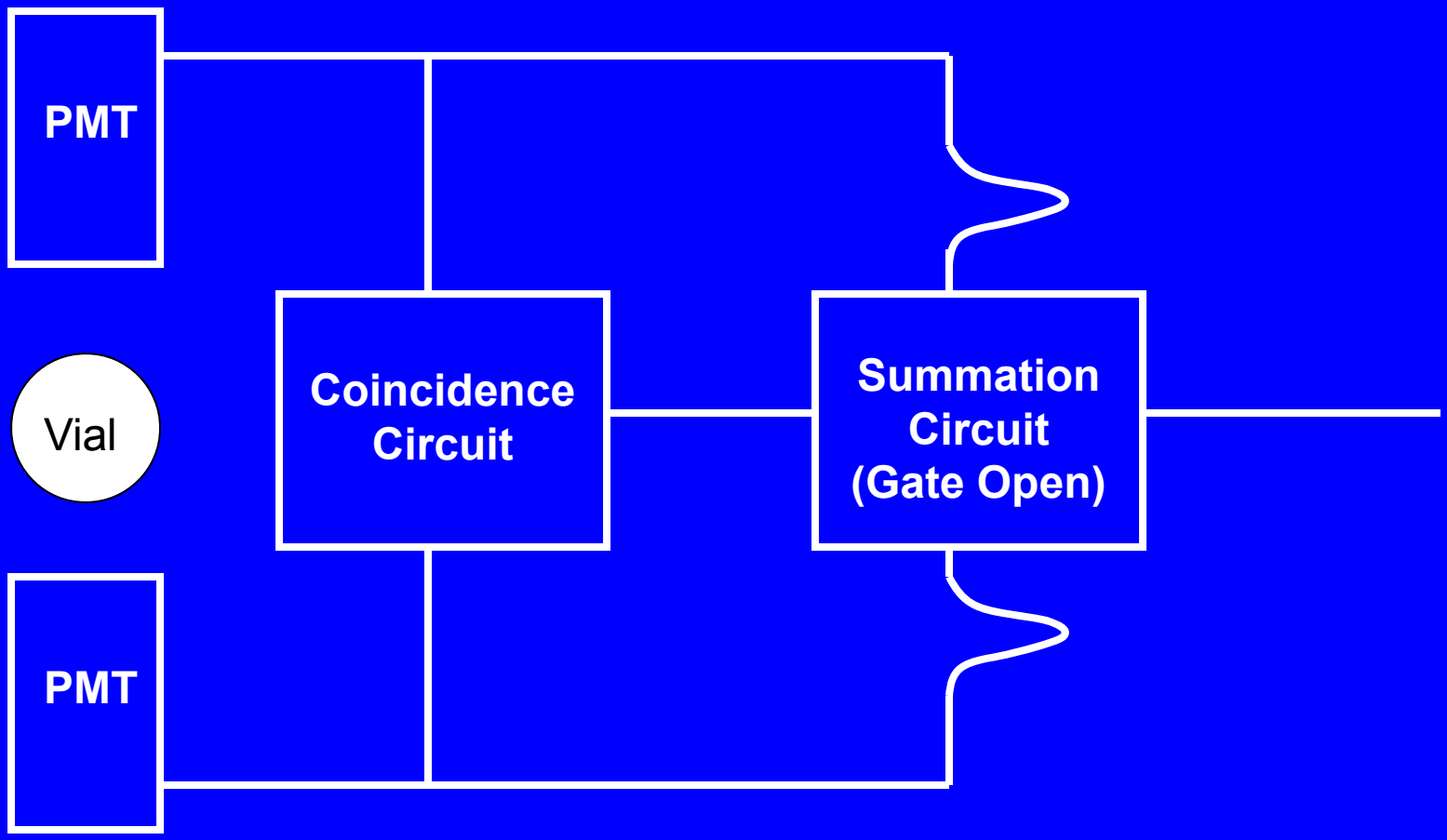

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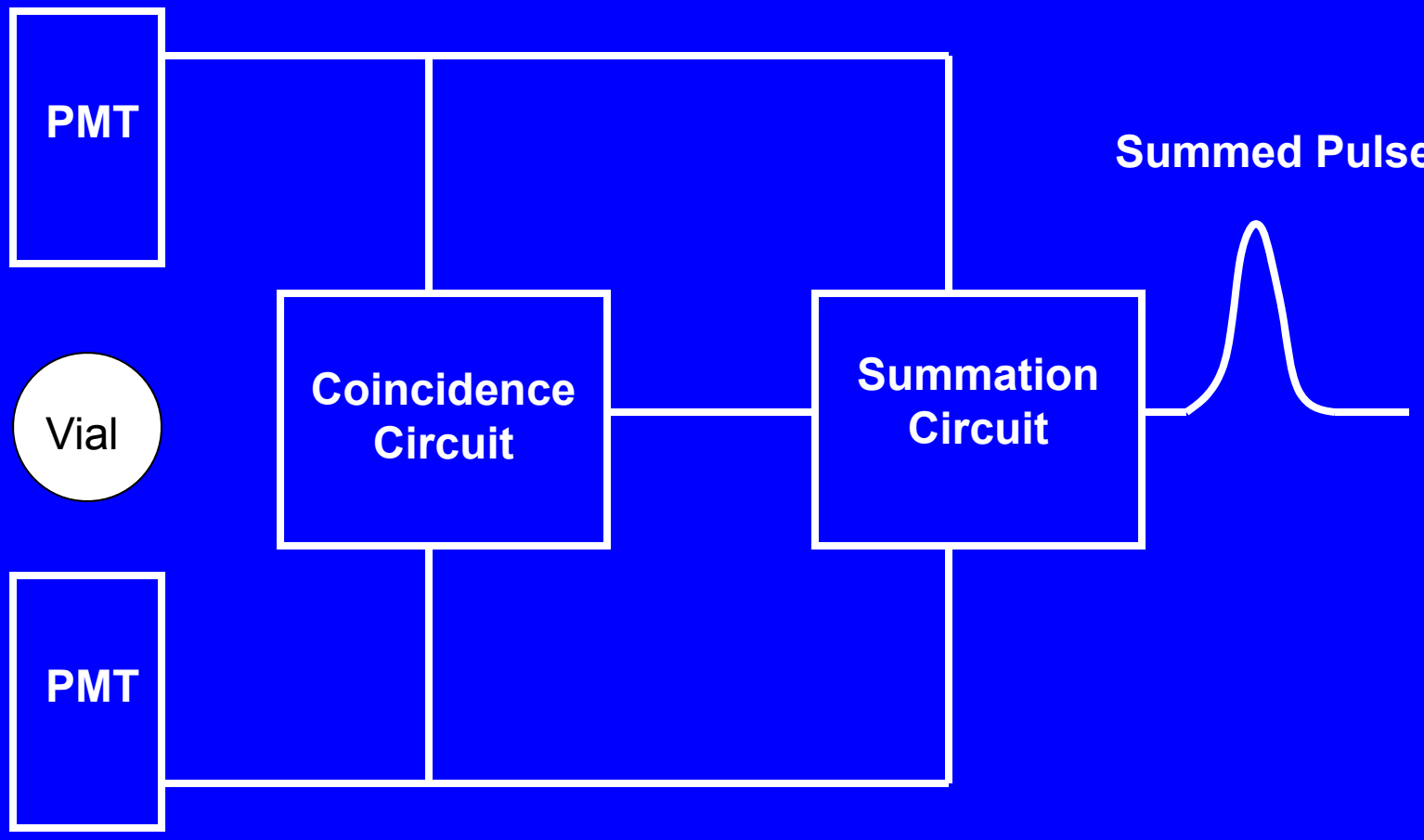

Important Characteristics of LSC

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Electronic noise from the PMTs (random fluctuations in the voltage) is a potentially significant source of background counts. Although such noise “pulses” consists of small voltage fluctuations, they still might be mistaken for small legitimate pulses of the sort produced by tritium.

Only infrequently are “noise pulses” produced by the two PMTs in coincidence. As such, in the absence of legitimate pulses, the coincidence circuit rarely produces the logic pulses required to open the summation circuit’s gate. If the latter is kept closed, spurious noise pulses will not get past the summation circuit for analysis.

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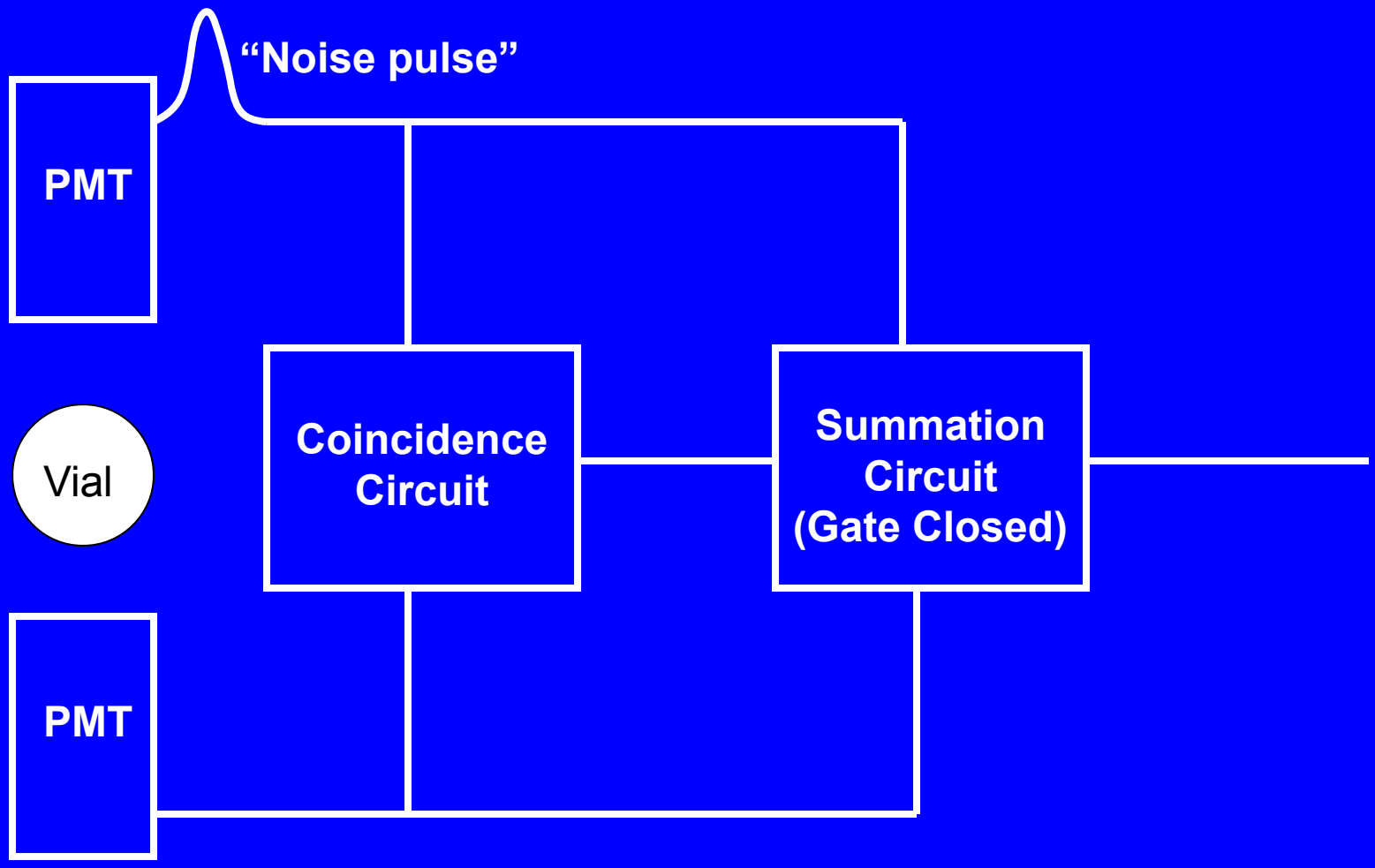

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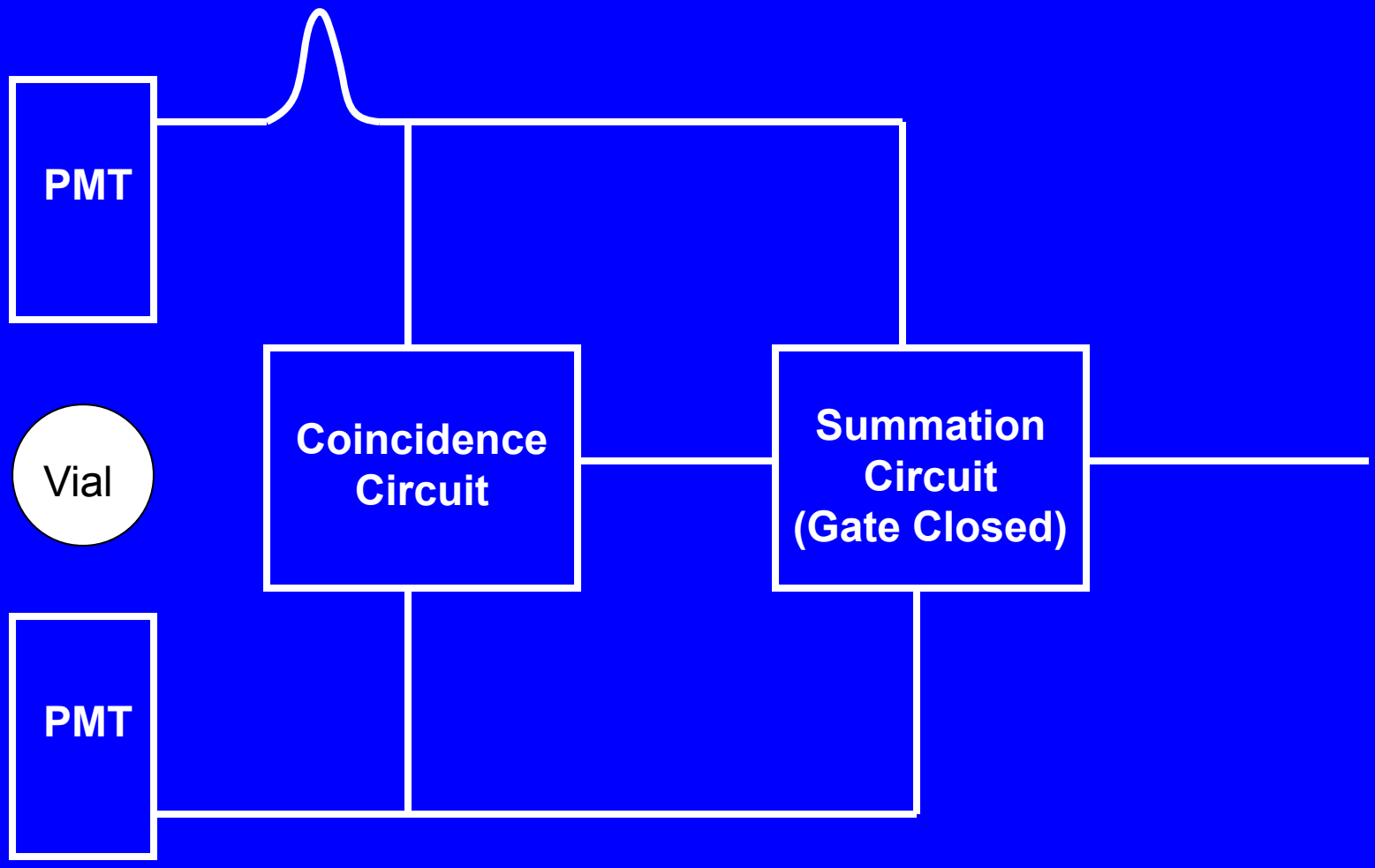

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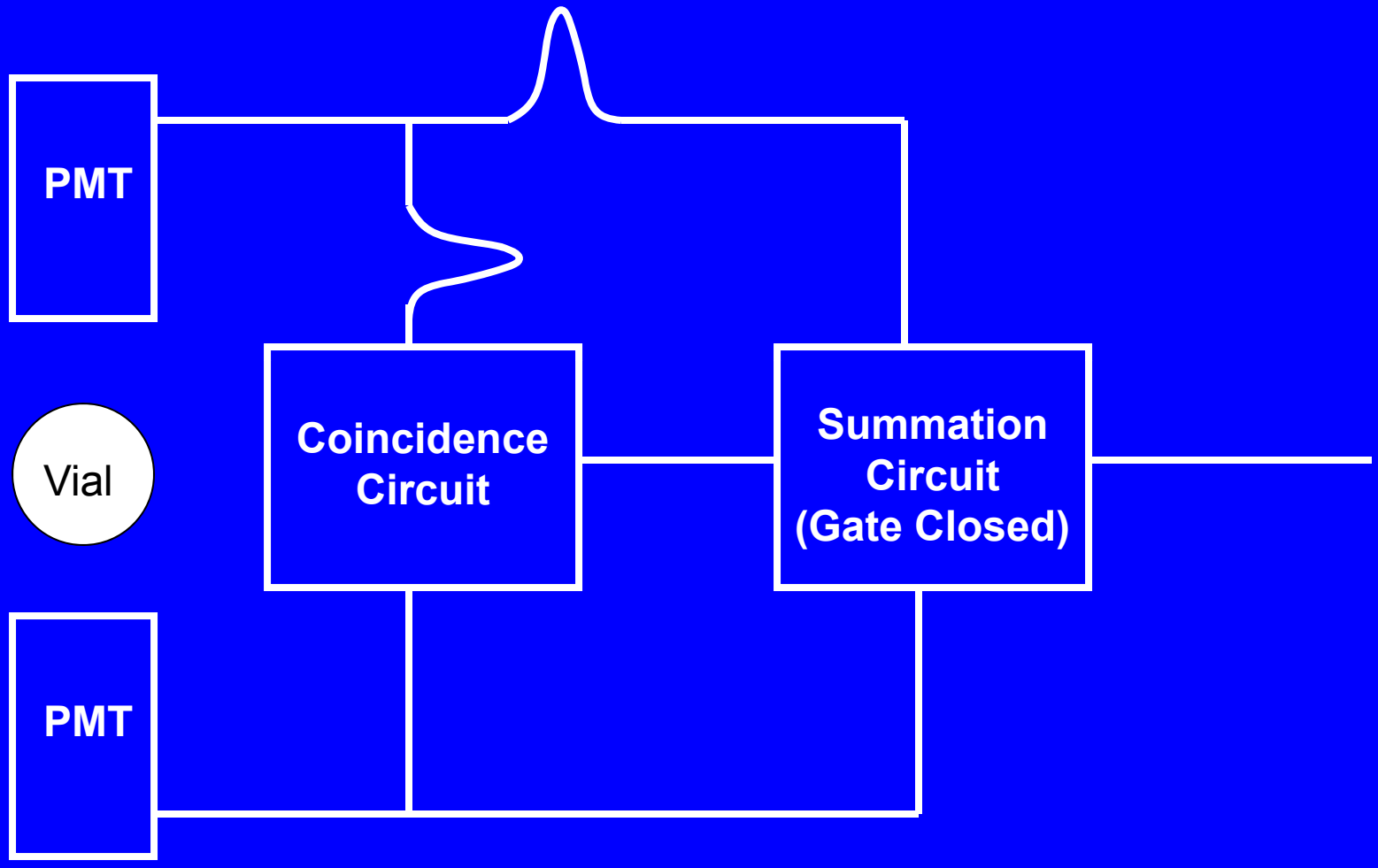

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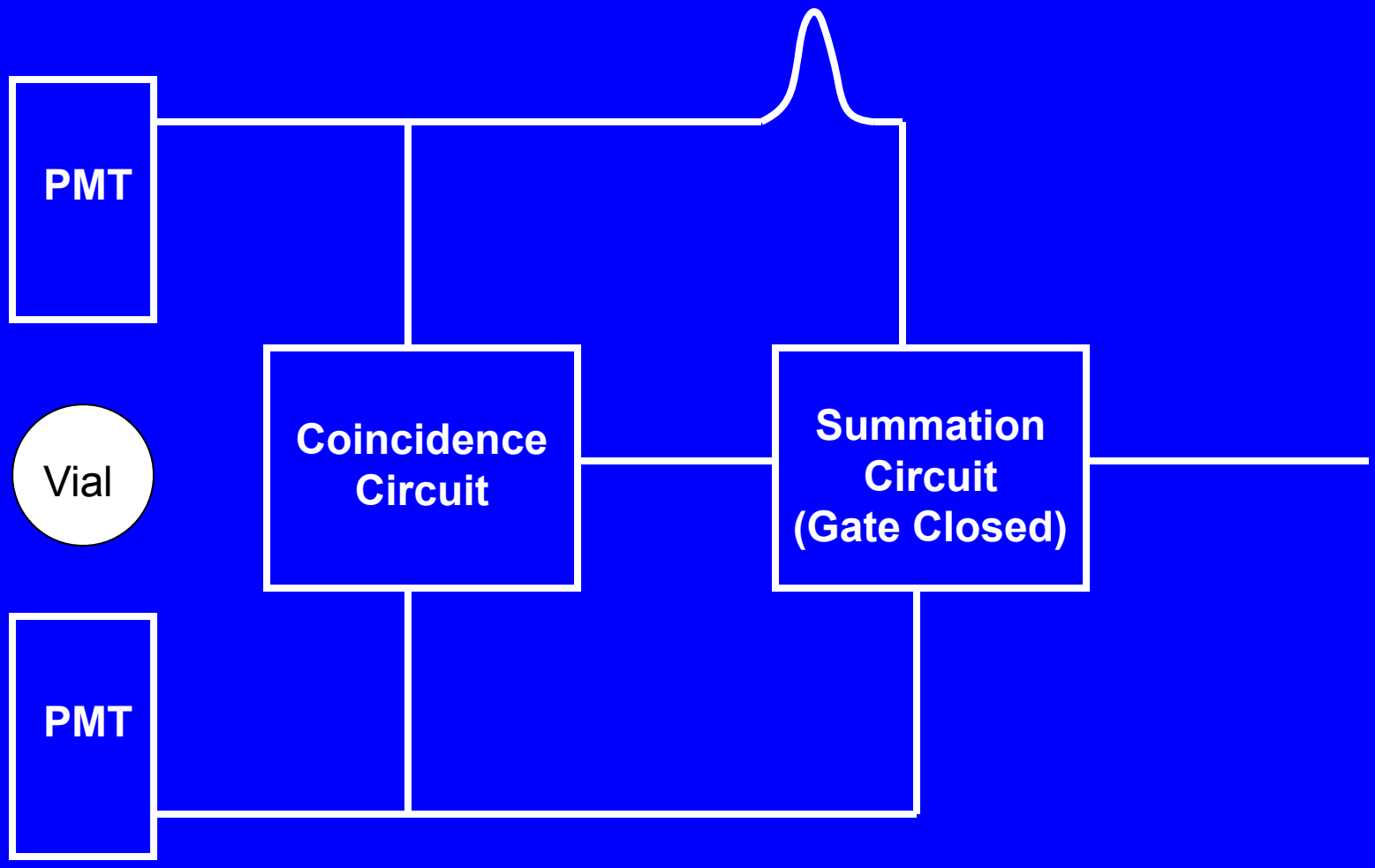

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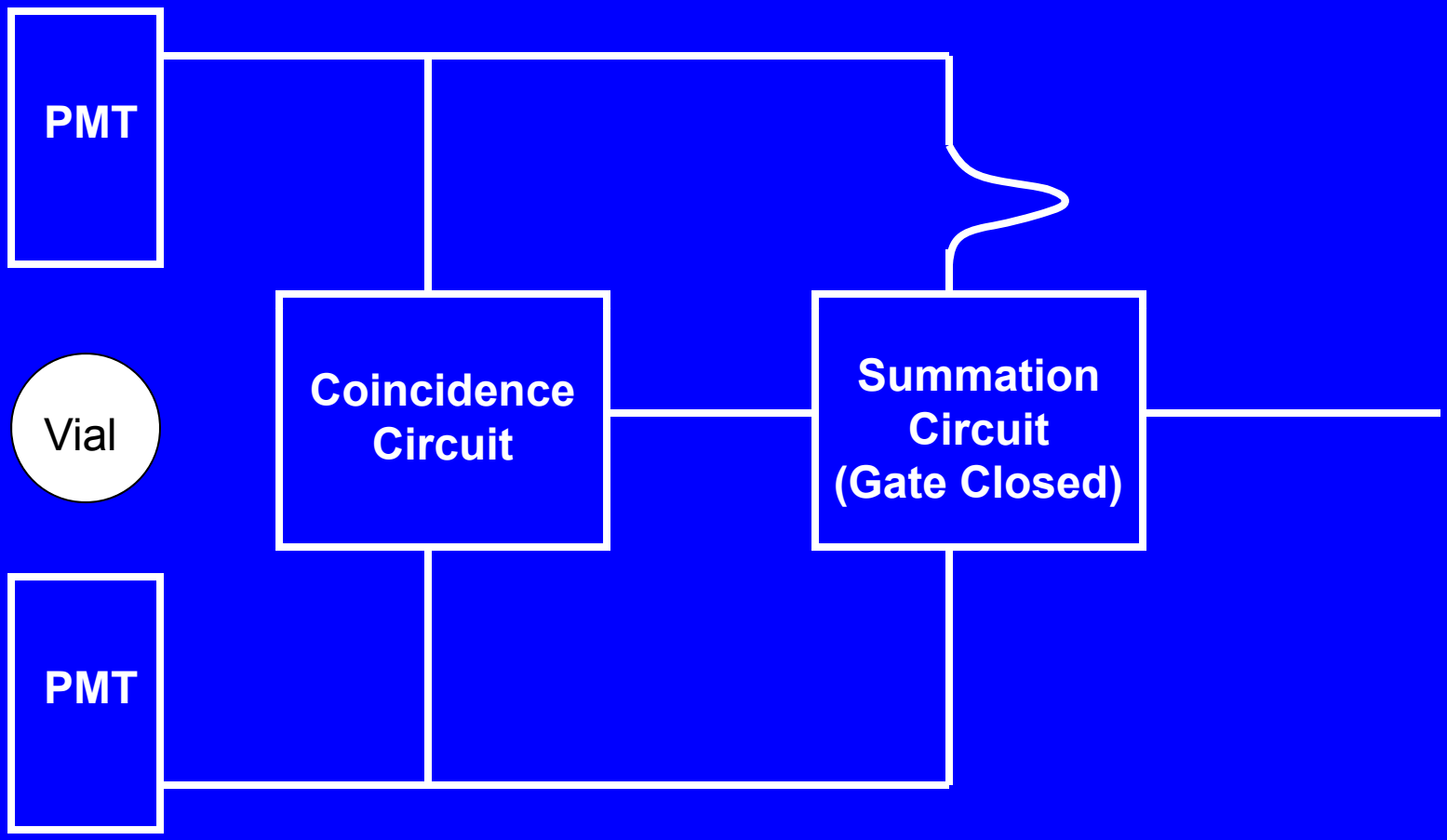

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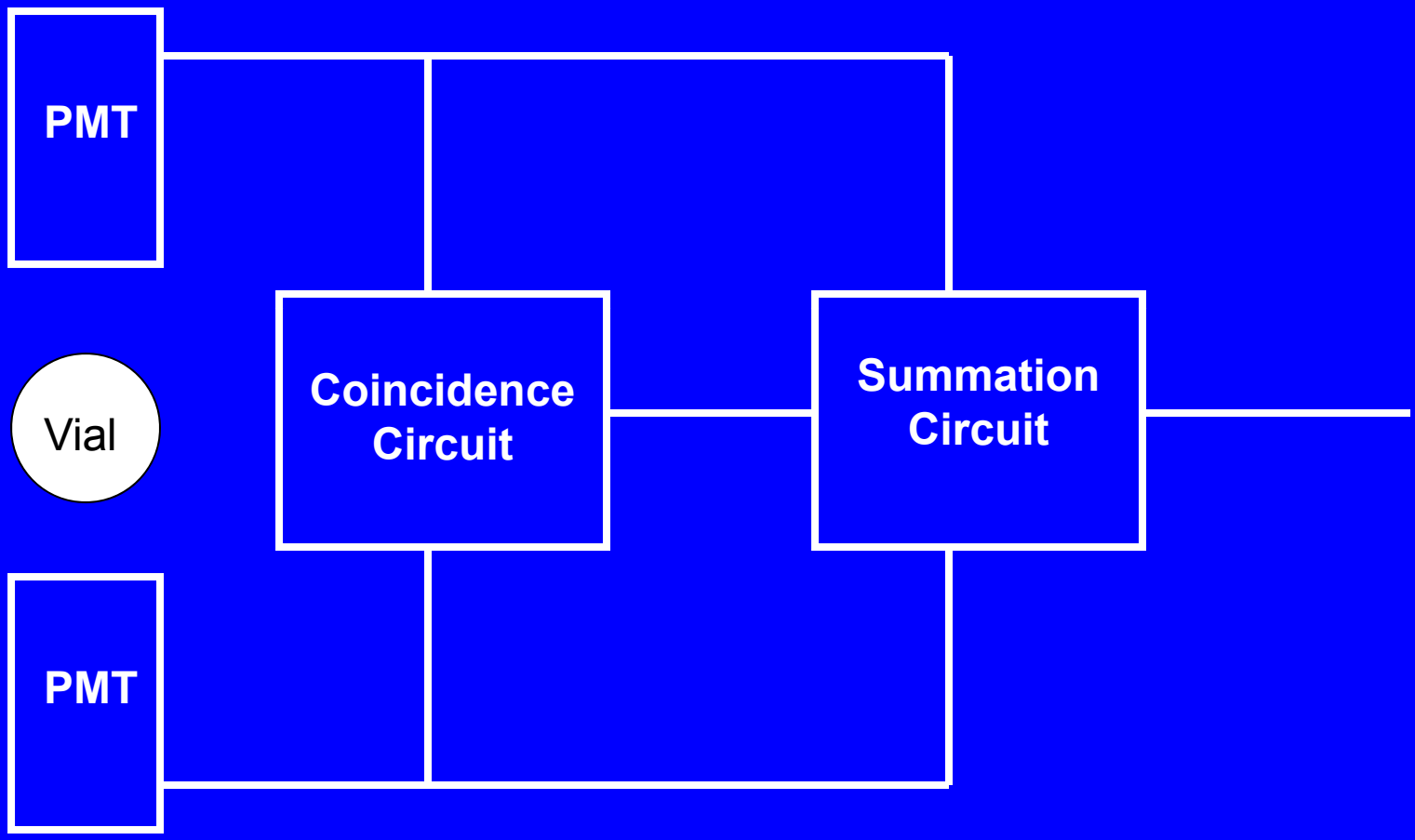


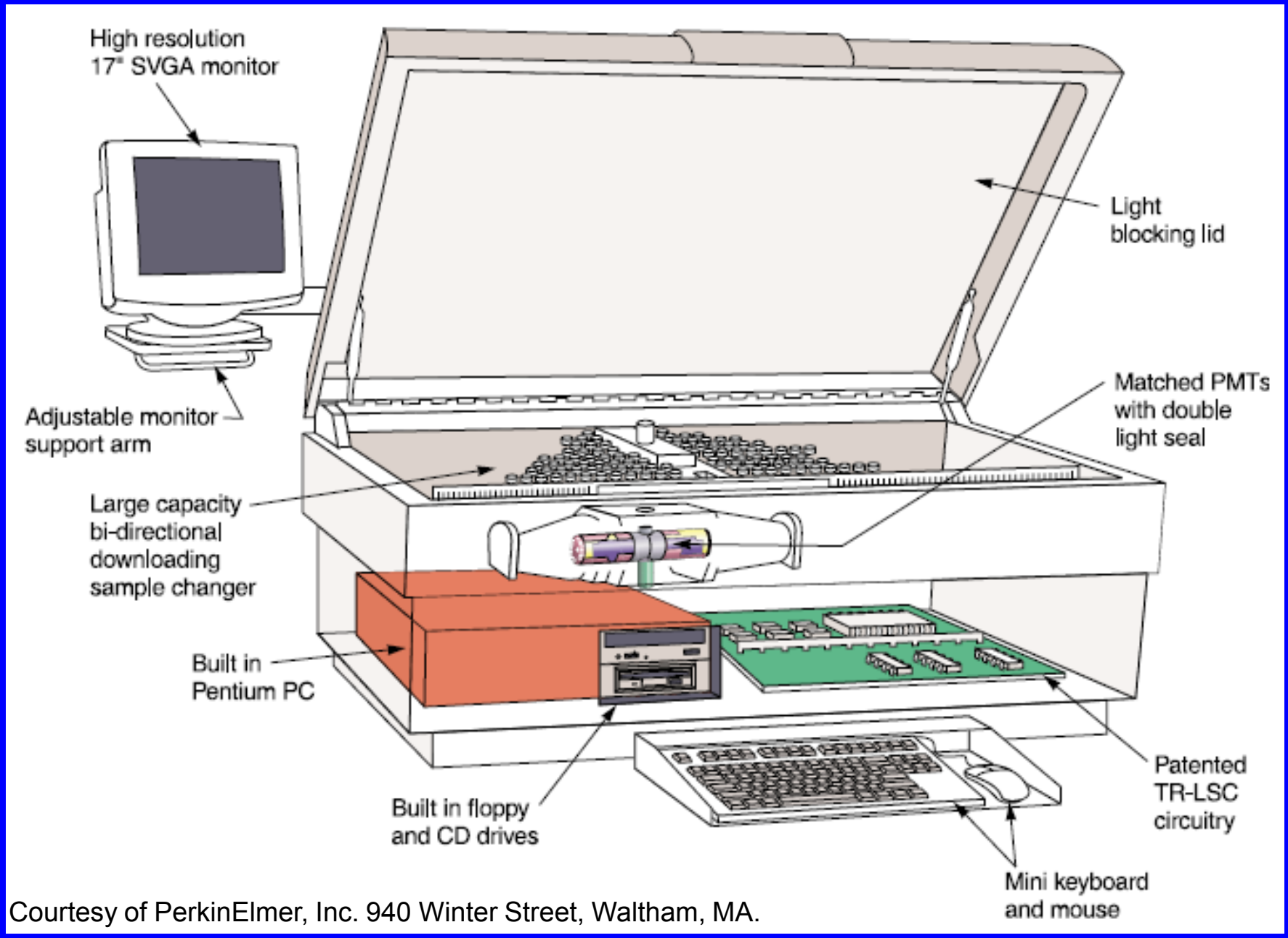
Maximizing Light Collection

A given scintillation event (flash) consists of many individual light photons. But even a high energy beta emitter such as P-32 emits some low energy betas that produce weak scintillations.

With the low energy beta emitter tritium (H-3), the brightest scintillations produced by its 18.6 keV betas consist of 200 – 250 photons. The average energy betas of H-3 only produce scintillations involving 70 – 100 photons.

Because these scintillations can be so weak, the LSC system is designed to maximize the transmission of light from the sample vial to the PMTs. It does so via reflectors positioned around the vial and PMTs. There might even be a reflective foil inside the cap of the sample vial itself!

Typical LSC System



Courtesy of PerkinElmer, Inc. 940 Winter Street, Waltham, MA.

The Liquid Scintillation Vial

The most common size of LSC vial is 20 ml (intended to hold 10 mls of cocktail). Smaller sizes include 6, 7, 8 ml.

- Glass (borosilicate). Low potassium versions are available. Transparency allows visual inspection of cocktail for color, inhomogeneity, etc.
- Plastic(polyethylene). Less expensive, lower background, but permeable to toluene, xylene and benzene

The LSC Cocktail and the Energy Transfer Process

The Liquid Scintillation Cocktail

The two major components of the LSC cocktail are the:

- Solvent - dissolves sample and fluor
- Fluor - emits light

Solvent

The solvent makes up 60-99% of the cocktail by volume. Solvents are almost always aromatic organics.

Properties of an ideal solvent:

- good solubility for sample
- high flash point
- low vapor pressure
- low toxicity
- biodegradable
- good solubility for fluor
- low photo and/or chemoluminescence
- high counting efficiency (tritium)
- chemical and color quench resistant

Solvent

Solvents can be characterized as:

- Aqueous solvent – for samples soluble in water
- Non-aqueous solvent – for samples not soluble in water
- Multipurpose solvent – for samples that are soluble or insoluble in water

Secondary Solvents - these might be added to the primary solvent to improve the solubility of the sample (sometimes referred to as a solubilizer).

Older Solvents

- Toluene
- Benzene
- Dioxane
- Xylene
- Pseudodocumene

Modern Biodegradable/Disposable Solvents

- Linear alkyl benzene (LAB), e.g., Packard's Opti-Fluor, RPI's Bio-Safe
- Di-isopropylnaphthalene (DIN), e.g., Packard's Ultima Gold, Wallac's Optiphase Hi-Safe
- Phenylxylylethane (PXE), e.g., Beckman's ReadySafe

Fluor

The fluor (aka, scintillator) might make up 0.3 to 1.0% of the cocktail by volume.

Its purpose is to absorb excitation energy from the solvent and emit some fraction of this energy as light.

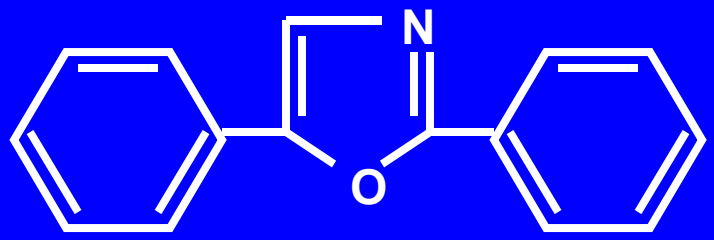
There are two categories of fluors:

- Primary Fluor – most primary fluors emit light in the UV range with wavelengths shorter than 400 nm

- Secondary Fluor (wave shifter) – the presence of the secondary fluor increases the wavelength of the emitted light to one more efficiently absorbed by the photomultiplier tubes.

Primary Fluor

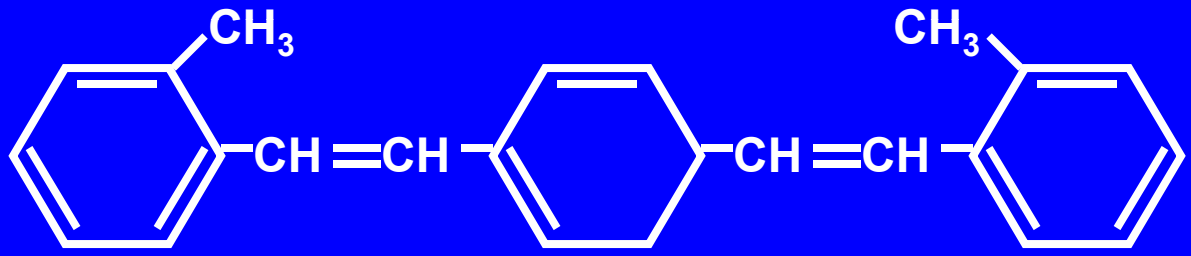
The most common primary fluor is 2,5 diphenyloxazole (PPO) whose peak emission wavelength is at 357 nm.



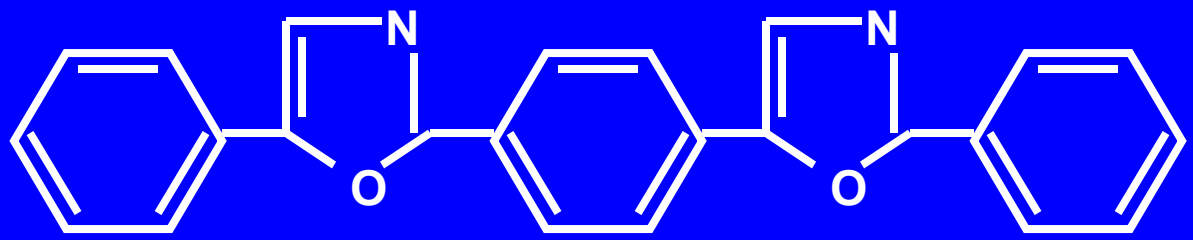
PPO

Secondary Fluor

The most common secondary fluors:



1,4 bis[2-methylstyryl] benzene (Bis-MSB)



1,4-bis[5-phenyloxazol-2yl] benzene (POPOP)

Energy Transfer Process in the Cocktail

Radioactive decay in the cocktail results in the emission of a charged particle (e.g., beta particle) that travels (a few mm at most) through the cocktail.

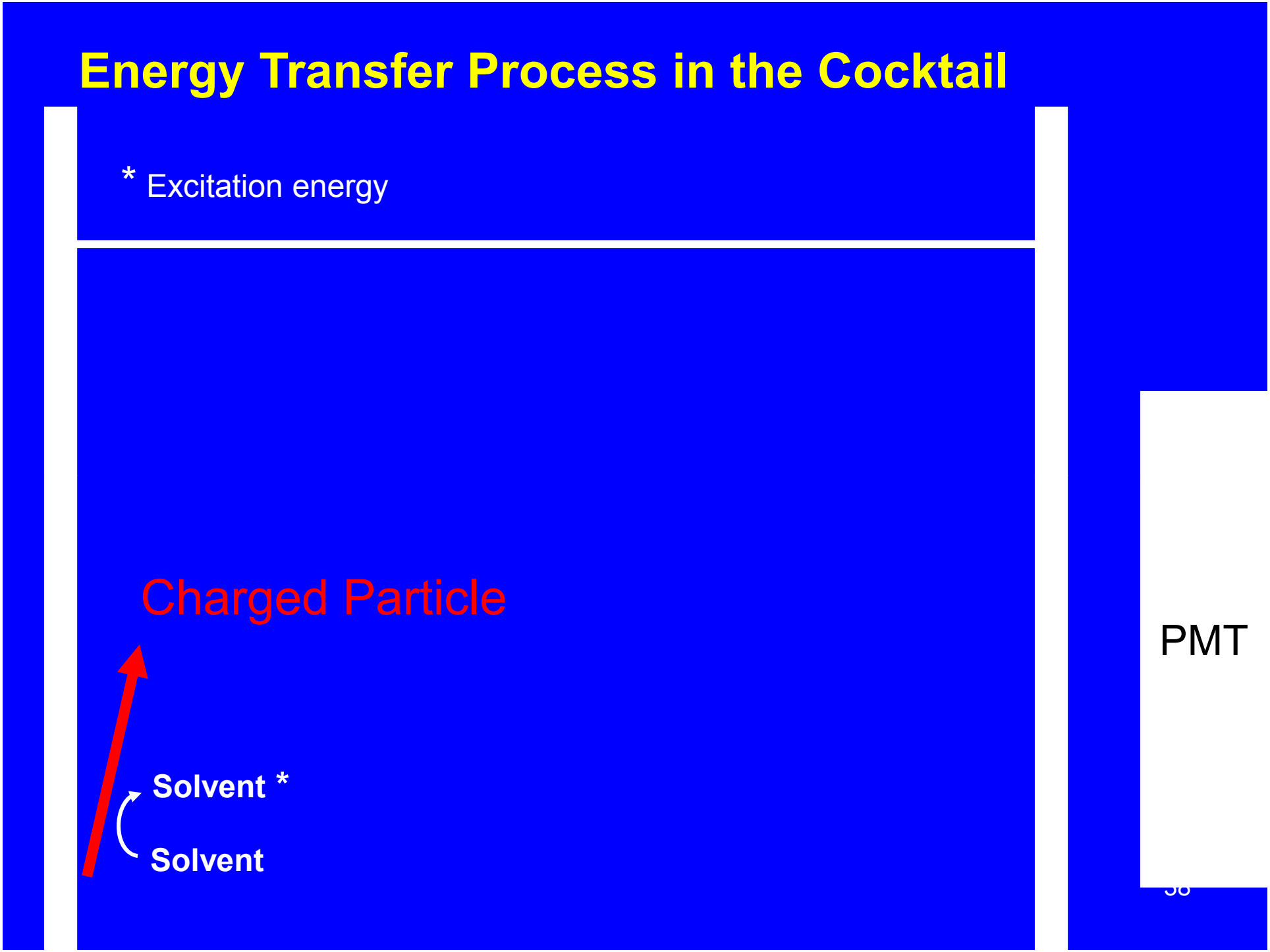
Pi electrons in the aromatic rings of the solvent molecules close to the charged particle tract are excited.

These excited solvent molecules collide with other solvent molecules and transfer the excitation energy to them.

Eventually a primary fluor molecule picks up the excitation energy from an excited solvent molecule – this does not require a physical collision but can occur at some distance.

By some mechanism, not completely understood, this energy can be transferred to a secondary fluor molecule which de-excites and emits a light photon.

Energy Transfer Process in the Cocktail



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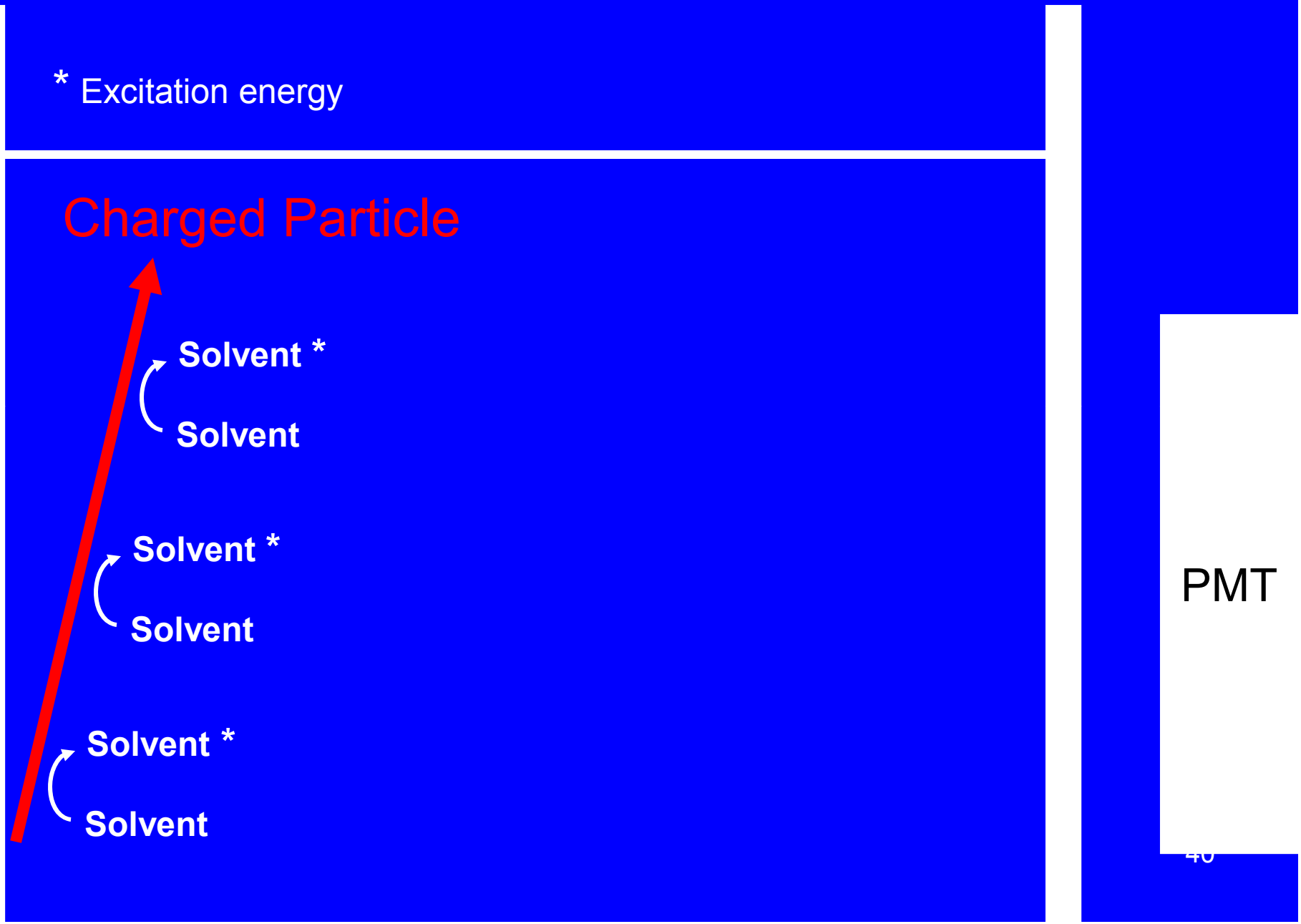
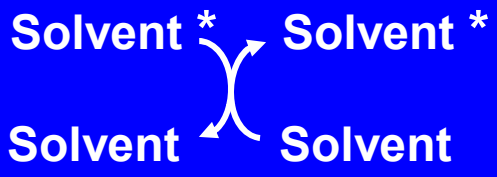
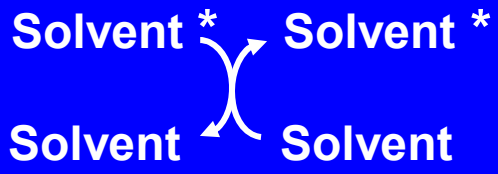
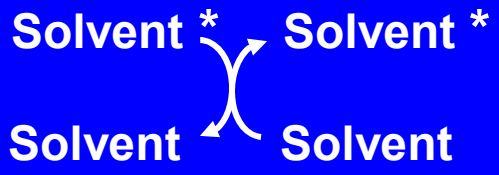


Energy Transfer Process in the Cocktail

* Excitation energy



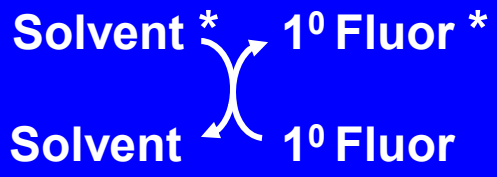


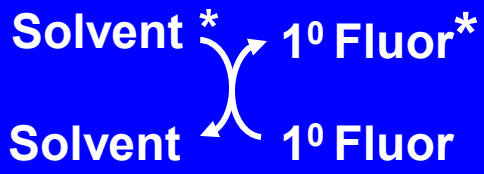


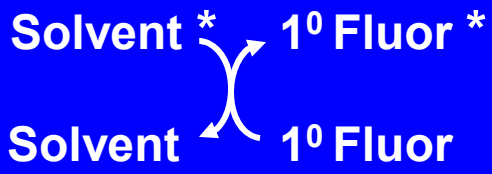
PMT

Energy Transfer Process in the Cocktail

* Excitation energy





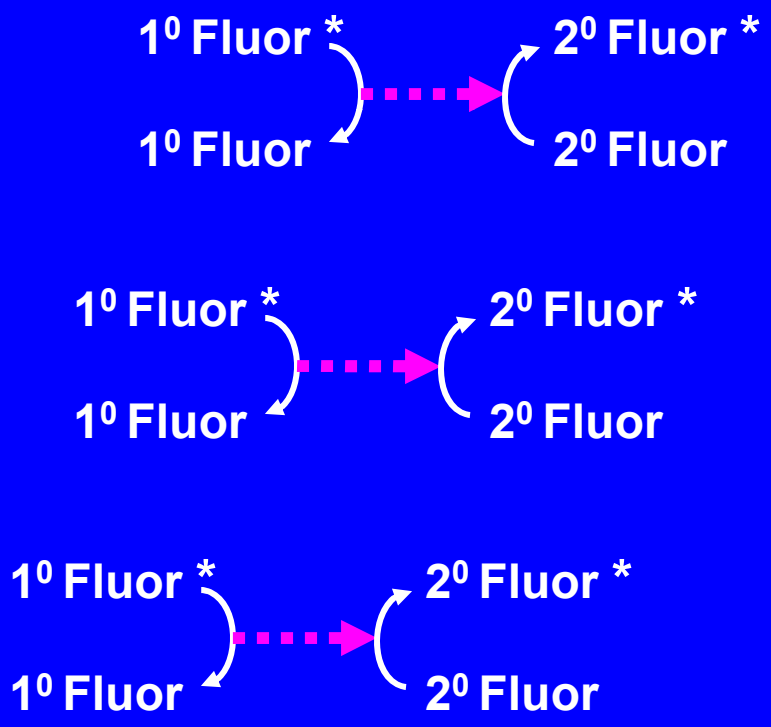


PMT

Energy Transfer Process in the Cocktail

* Excitation energy

Short wavelength light photon



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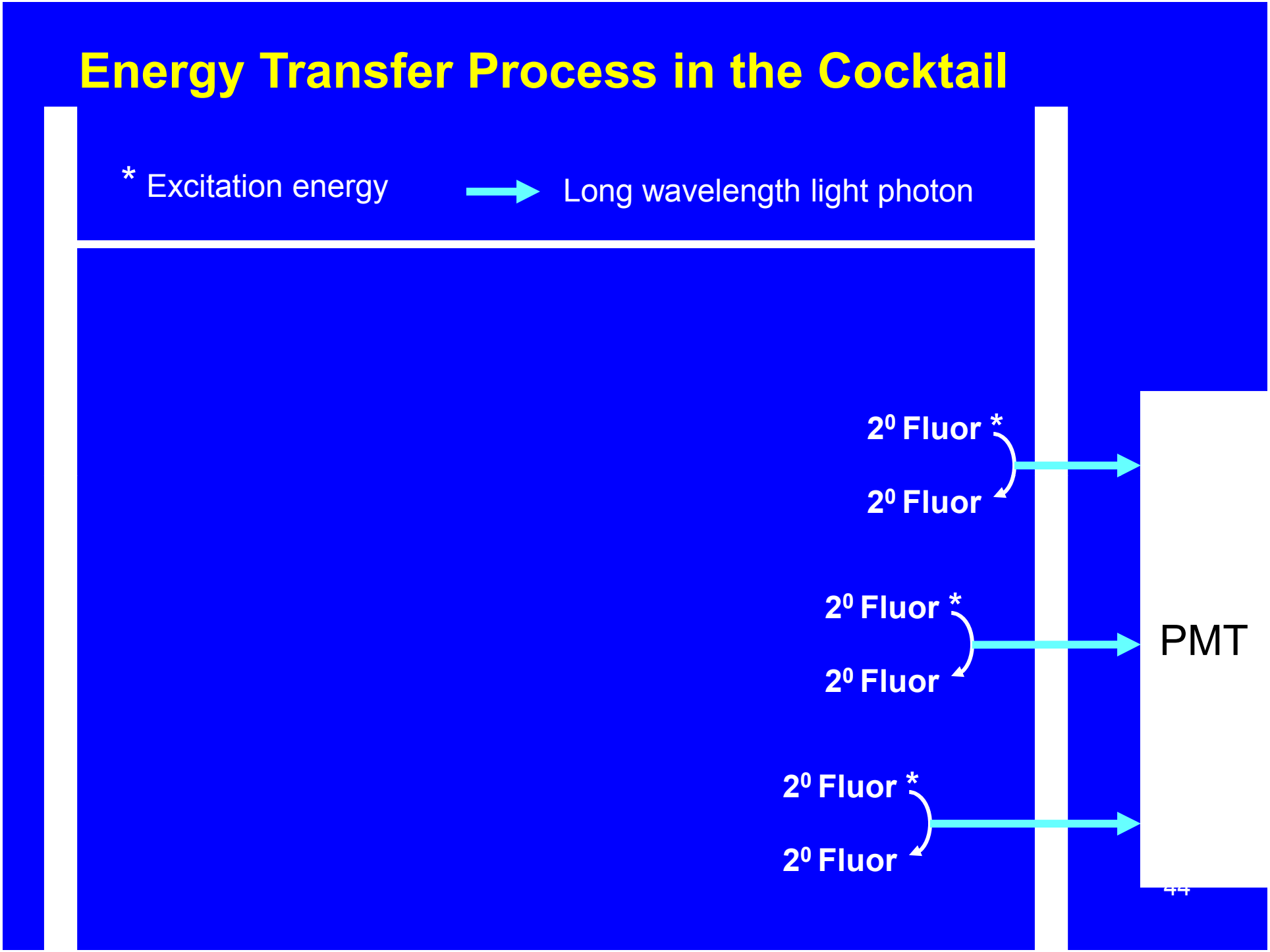

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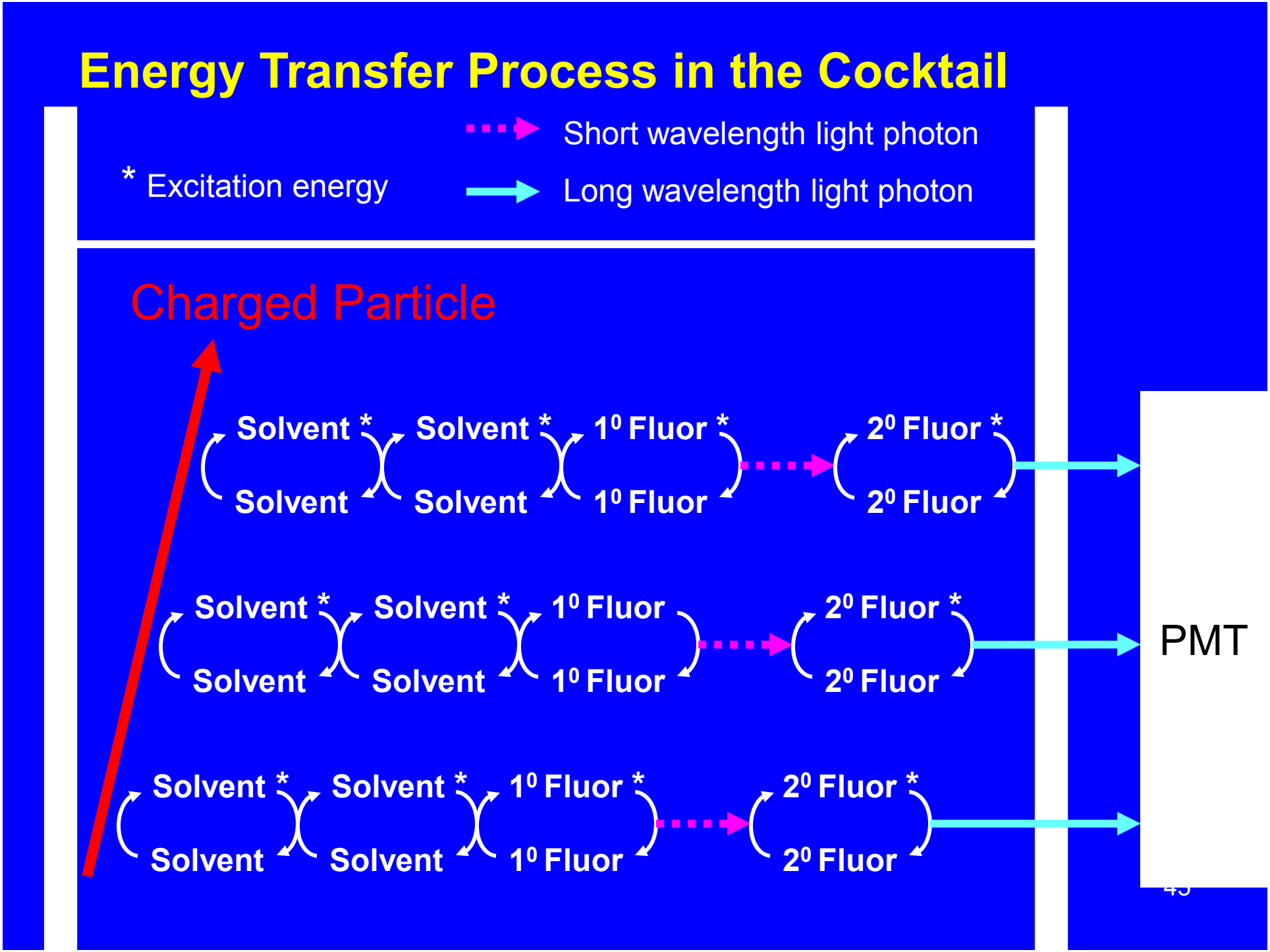


Energy Transfer Process in the Cocktail

The greater the energy of the charged particle, the greater the number of excited solvent molecules

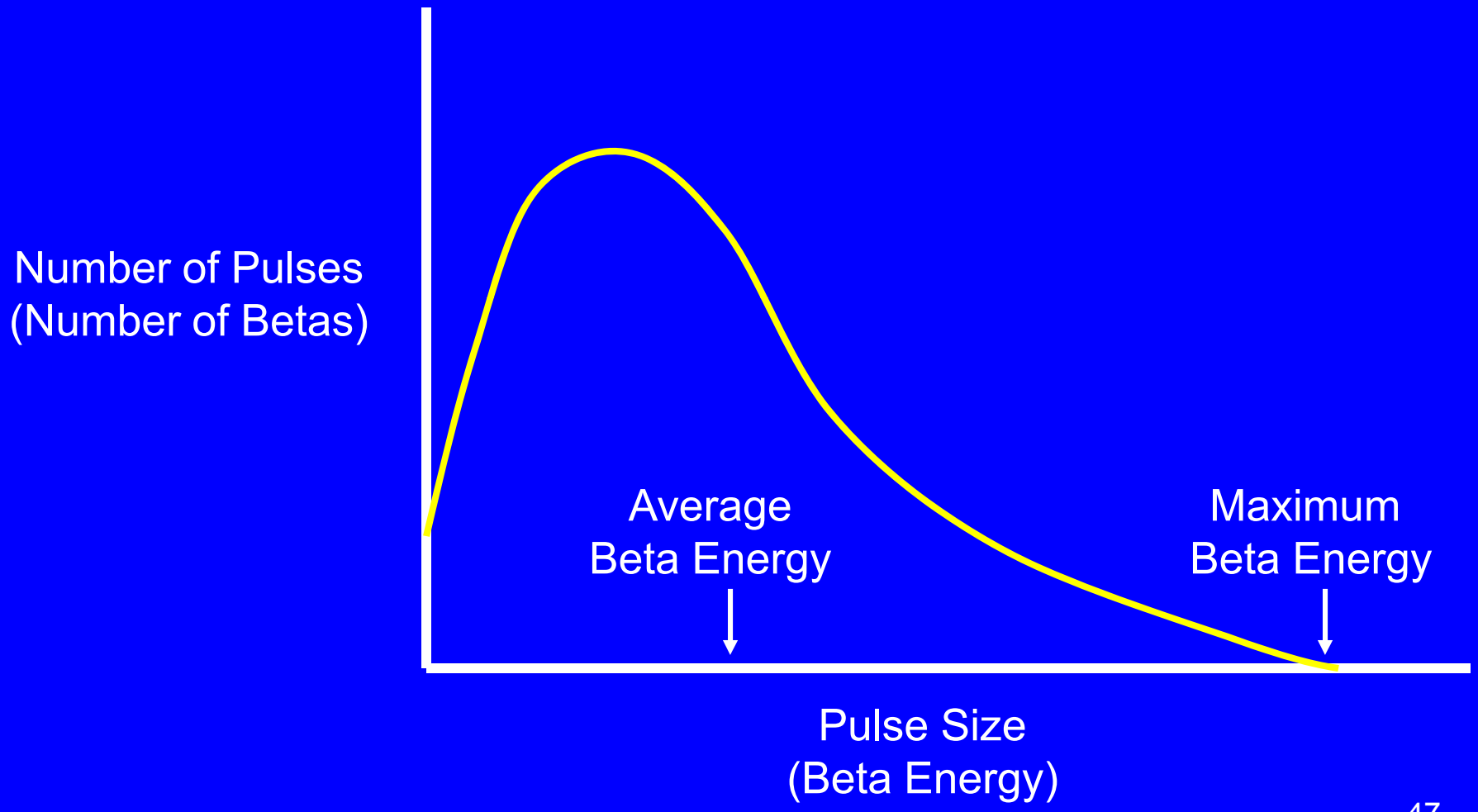
The greater the number of excited solvent molecules, the greater the number of light photons emitted by the cocktail.

The more light photons emitted by the cocktail, the greater the size of the pulse produced by the PMT.

A given beta emitting radionuclide emits beta particles with a range of energies up to some maximum value.

Beta Spectrum

The beta spectrum looks something like this:



Problems to be Addressed

Problems to be Addressed

There are several potential problems that must be addressed in LSC:

- Getting the sample into solution
- Static Electricity
- Photoluminescence
- Chemiluminescence
- Quenching
  - Optical
  - Color
  - Chemical

Getting the Sample in Solution

The goal is to get the sample completely dissolved in the cocktail or uniformly dispersed throughout the cocktail as an emulsion or gel.

In addition, the solution should be clear, colorless and of a neutral pH.

If the radioactive component of the sample is not in intimate contact with the cocktail, the emitted betas may lose some or all of their energy before the energy can be transferred to the solvent molecules. As such, phase separation of the cocktail components would be a major problem.

Getting the Sample in Solution

If the sample is not completely dissolved in the cocktail, the counts may go up over time as the radioactive material is gradually “taken up” by the cocktail.

If this might be a problem, a small amount (e.g., 500 microliters) of a solution that the sample is known to be soluble in can be added to the sample before the latter goes into the cocktail. Ideally this solution should produce minimal quenching. Water, ethanol, acetonitrile, ethyl acetate, hexane are relatively good in this regard.

A more thorough mixing of the sample in the cocktail (e.g., via ultrasonication) might help in some cases.

Getting the Sample in Solution

Organic samples are fairly easy to get into solution because the cocktail solvents are organic molecules. With such samples, the best efficiencies are obtained with cocktails that are only intended for organics.

Aqueous samples require cocktails employing emulsifiers. These cocktails can also be used for organic samples, and for convenience they might be used for all types of samples.

With aqueous samples, the trade off is between counting efficiency and the sample capacity, i.e., the amount of water the cocktail can hold.

Getting the Sample in Solution

Prior to counting, biological material might be homogenized, macerated or combusted and then treated with a solubilizer.

Solubilizers might be:

- alkaline (e.g., Soluene-350). These solubilizers might be used with blood, urine, muscle. Solubilization is accomplished by hydrolysis of the sample.
- acidic (e.g., perchloric acid, nitric acid). These might be used with bone, cartilage. Solubilization is achieved via oxidation of the sample.
- some other type (e.g., sodium hypochlorite). Plant material is often treated with sodium hypochlorite. Sodium hypochlorite works by oxidative bleaching.

Static Electricity

Static charges on the surface of the scintillation vial can result in the emission of random single photons. The maximum pulse size due to such static charge events would equate to 10 keV betas (i.e., H-3).

Plastic vials, especially the small vials in adapters, are more prone to developing static charges than glass vials.

Handling the vials in low humidity conditions with cloth gloves can exacerbate the problem.

Static eliminators are optional equipment for most LSC systems.

Photoluminescence

Excitation of the vial or cocktail by ultra-violet light results in spurious single photon emissions that can produce pulses similar to those of H-3.

The maximum height of photoluminescence induced pulses correspond to 6 keV betas. The vast majority of the pulses are in the 0 – 2 keV range.

Fortunately, photoluminescence decays away very quickly and can be completely gone within 5 minutes or so.

Photoluminescence is independent of quenching and can be reduced somewhat by cooling the sample.

Chemiluminescence

Chemiluminescence is the spurious production of light by chemical reactions that involve various components of the sample.

Unfortunately this light emission can last for up to a day or more!

Chemiluminescence is primarily a problem when:

- the sample is alkaline (high pH)
- the sample contains oxidizers
- the cocktail contains solubilizers

Chemiluminescence

To a large extend chemiluminescence, which consists of multiple single photon events, is minimized by the LSC counter's coincidence circuitry.

Chemiluminescence can also be reduced by ensuring that the cocktail has a neutral pH. This is often accomplished by adding acetic acid (alkaline cocktails are the most problematic). This can compromise the sample holding capacity however.

A simpler approach is to wait several hours to a day after the sample has been added to the cocktail to count.

Some folks heat the cocktail in order to drive the chemical reactions to completion, let it cool down, and then count.

Quenching

While quenching is a “good” thing in GM detectors, it is undesirable in liquid scintillation counting.

In essence, it is an interference in the energy transfer process that reduces the number of photons reaching the PMTs.

This handout will consider three types:

- optical quenching
- color quenching
- chemical quenching

Optical Quenching

This term is usually treated as a synonym for color quenching.

As used here, the term applies to the absorption of the emitted light by something other than the cocktail.

For example, putting a paper label on the wall of the vial or labeling the side of the vial (instead of the cap) with a marker.

Other examples of what we call optical quenching would be fingerprints on the vial wall or condensation.

Vials should be handled by the cap when possible. If gloves are worn or the vial is cleaned prior to counting, it should be done so as to minimize the generation of static.

Color Quenching

As used here, the term refers to the absorption of emitted light by color in the cocktail itself. This often a problem with biological samples, e.g., blood, urine.

A simple and effective approach is to decolor with ultraviolet radiation (UV). Exposing the samples to sunlight for a few hours might be all that is necessary.

Aqueous samples are often bleached by mixing 0.1- 0.3 ml of 30% hydrogen peroxide in 1 ml of sample. The O_2 that is produced is a strong quenching agent and must be driven off by heating to 50 ºC for and shaking occasionally.

Color Quenching

Non-aqueous samples and samples digested with organic solubilizers can be bleached with benzoyl peroxide.

Two mls of a benzoyl peroxide/toluene solution is used per ml of sample. The solution is prepared by dissolving one gram of benzoyl peroxide in five mls of toluene.

The sample is then incubated at for half an hour, allowed to cool to room temperature, and added to the cocktail.

Chemical Quenching

Chemical quenchers are components of the sample/cocktail that absorb excitation energy from solvent molecules (or directly from the charged particle) but fail to transfer this energy to the fluor molecules.

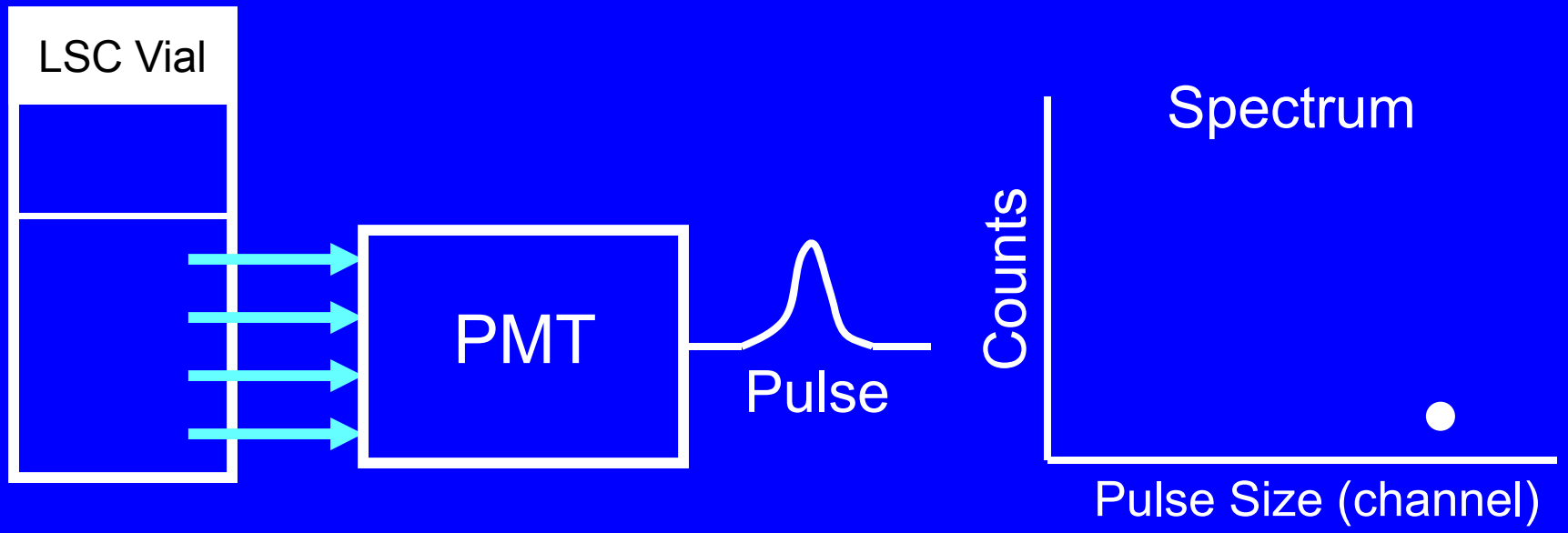
Common examples of chemical quenchers include oxygen and chloroform.

Consequences of Quenching

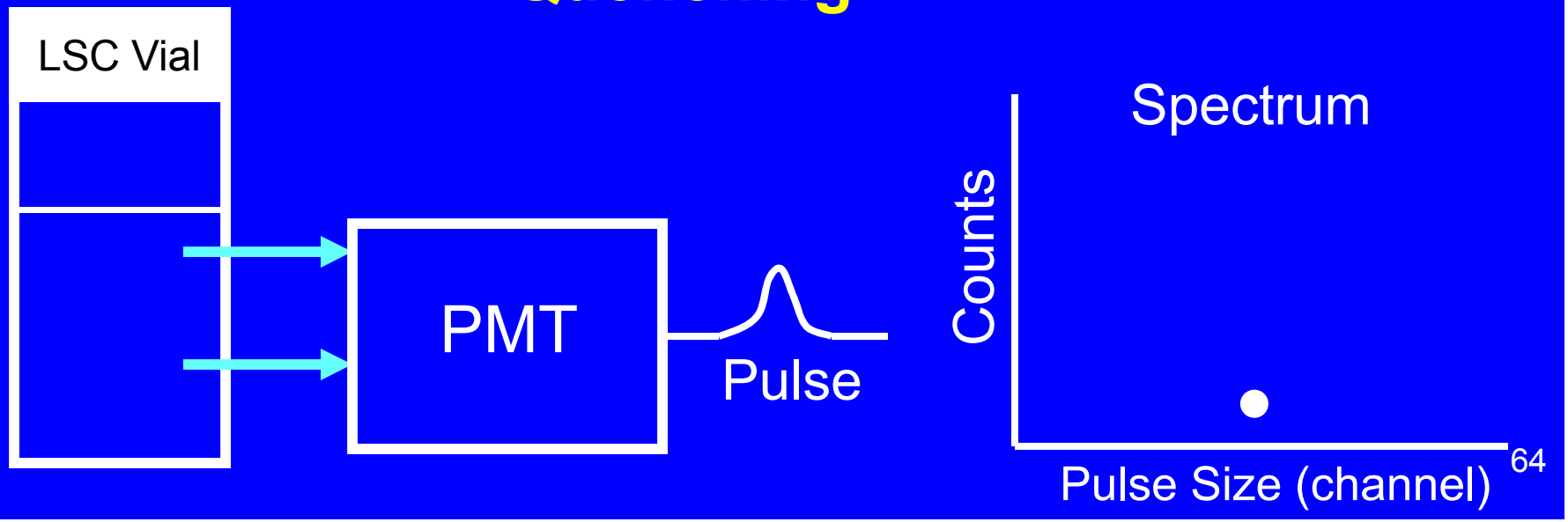
Quenching reduces the number of photons reaching the PMT in a given scintillation. This reduces the size of the pulse.

The result is that the spectrum shifts to the left.

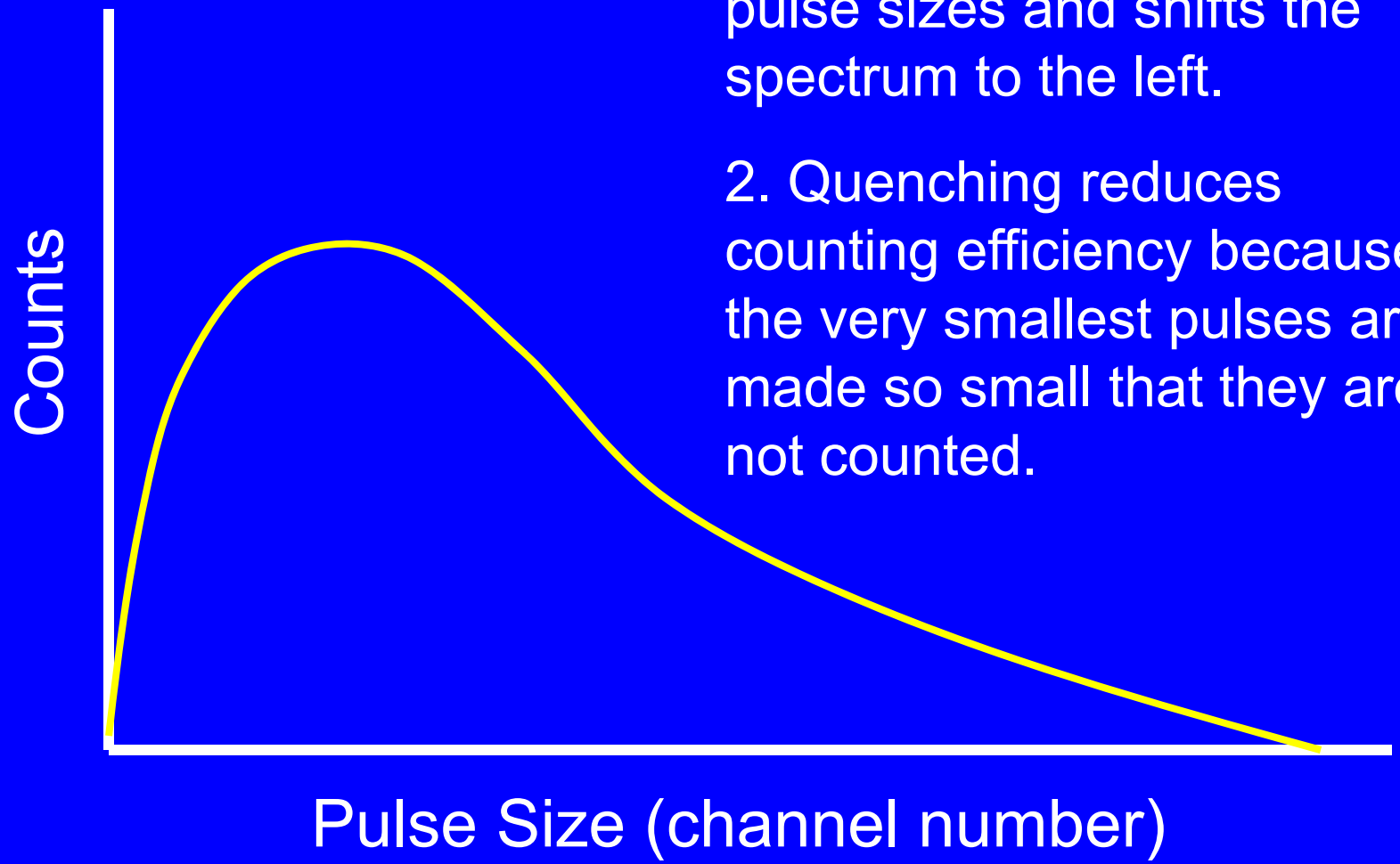
No Quenching



Quenching



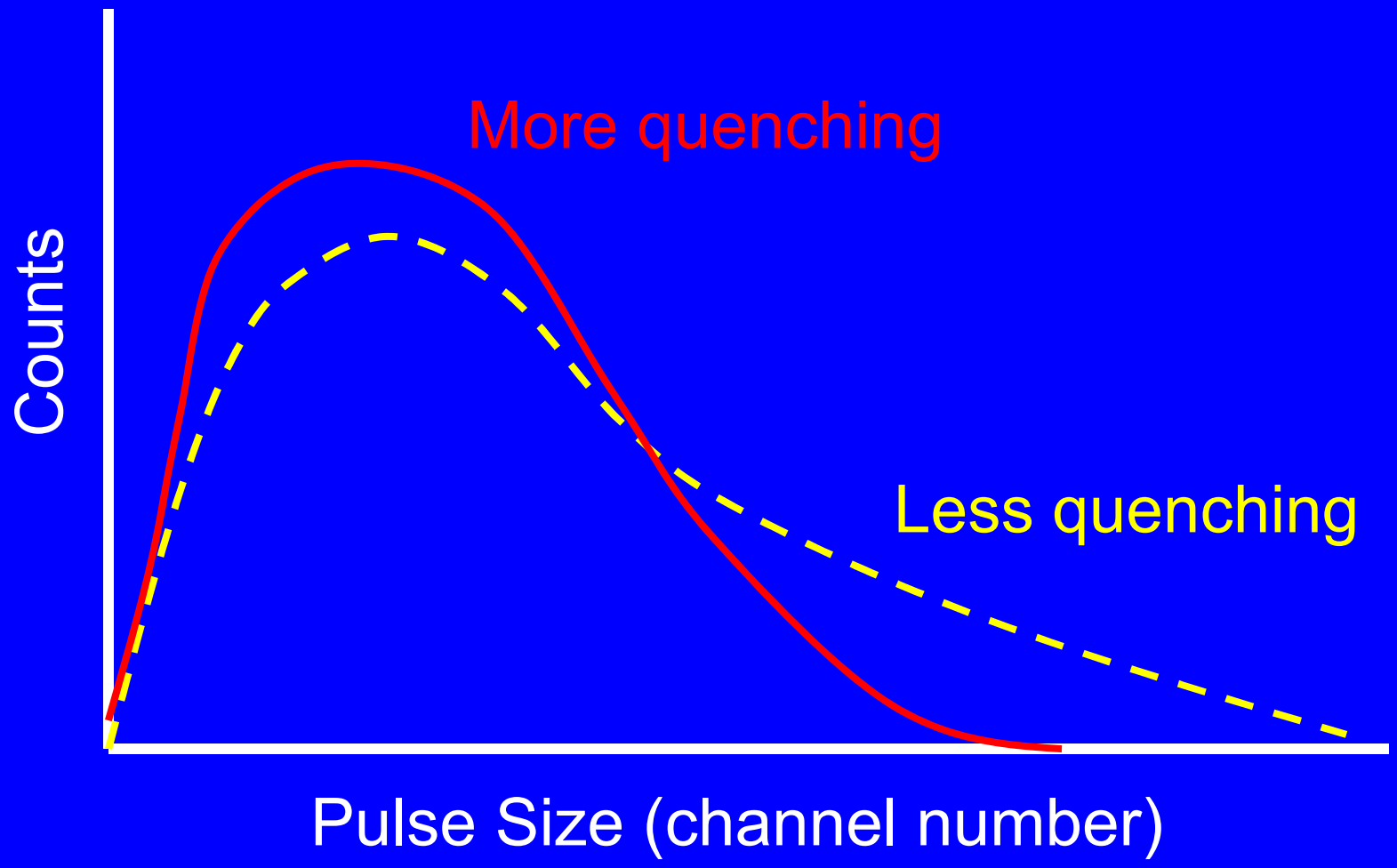
Consequences of Quenching



1. Quenching reduces the pulse sizes and shifts the spectrum to the left.

2. Quenching reduces counting efficiency because the very smallest pulses are made so small that they are not counted.

Consequences of Quenching



Quench Correction

Quench Correction

Since the amount of quenching affects the counting efficiency (counts per disintegration) and because the amount of quenching varies from sample to sample, the counting efficiency can vary from sample to sample.

Determining the counting efficiency for a sample is known as quench correction. There are several quench correction techniques that can be used, e.g.,

1. Internal Standard method
2. Channels Ratio method (or variant)
3. External Standard method (or variant)

Quench Correction

1. Internal Standard Method

This involves the use of a “spike.”

i. The sample is counted and the resulting count rate is R_1 (cpm or cps).

ii. A standard (the spike) containing a known activity (Q_k) of the radionuclide of interest is added to the cocktail. The fact that the volume of the spike must be small in order to minimize quench effects means that skilled personnel are required. This method is particularly inconvenient if many samples are being analyzed.

iii. The sample is counted again. The resulting count rate is R_2.

Quench Correction

1. Internal Standard Method

iv. The efficiency is calculated as follows:

$$E = \frac{R_2 - R_1}{Q_k}$$

v. The activity in the sample is then calculated as follows:

$$Q = \frac{R_1}{E}$$

Quench Correction

2. Channels Ratio Method

This technique employs a standard containing a known activity of the radionuclide of interest to determine the relationship between the counting efficiency and the shape of the beta spectrum.

Once we know this relationship, we then use the shape of a beta spectrum to determine a samples counting efficiency.

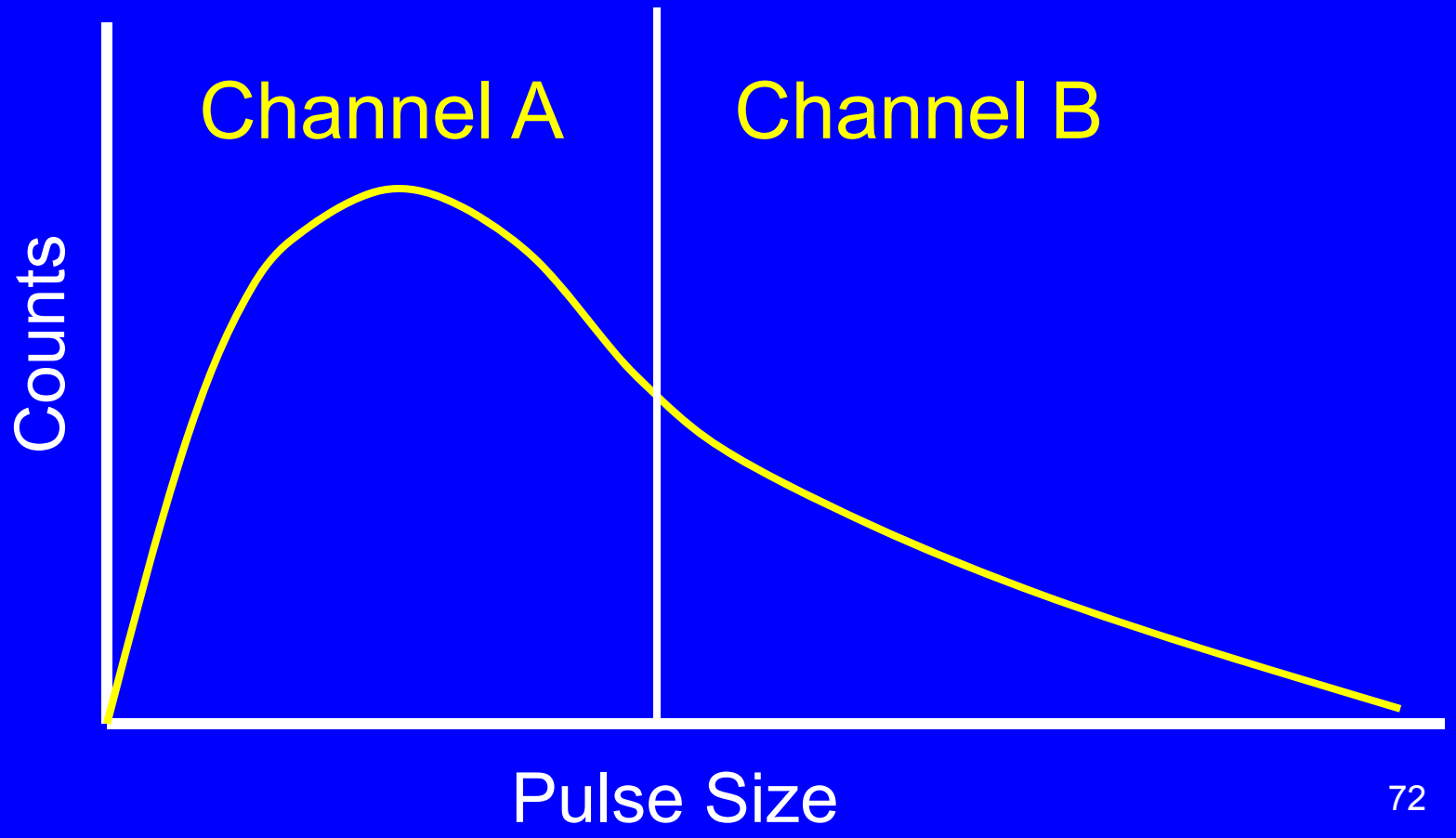
The shape of the spectrum is described via the “channels ratio” (CR).

Similar correction methods describe the spectrum shape via different parameters, e.g., the spectral index of the sample,

Quench Correction

2. Channels Ratio Method

In the Channels Ratio method we divide the spectrum into two “channels”: A and B.



Quench Correction

2. Channels Ratio Method

i. A set of standards is counted. Each of these contains the radionuclide of interest (e.g., H-3) at the same known activity (Q_k).

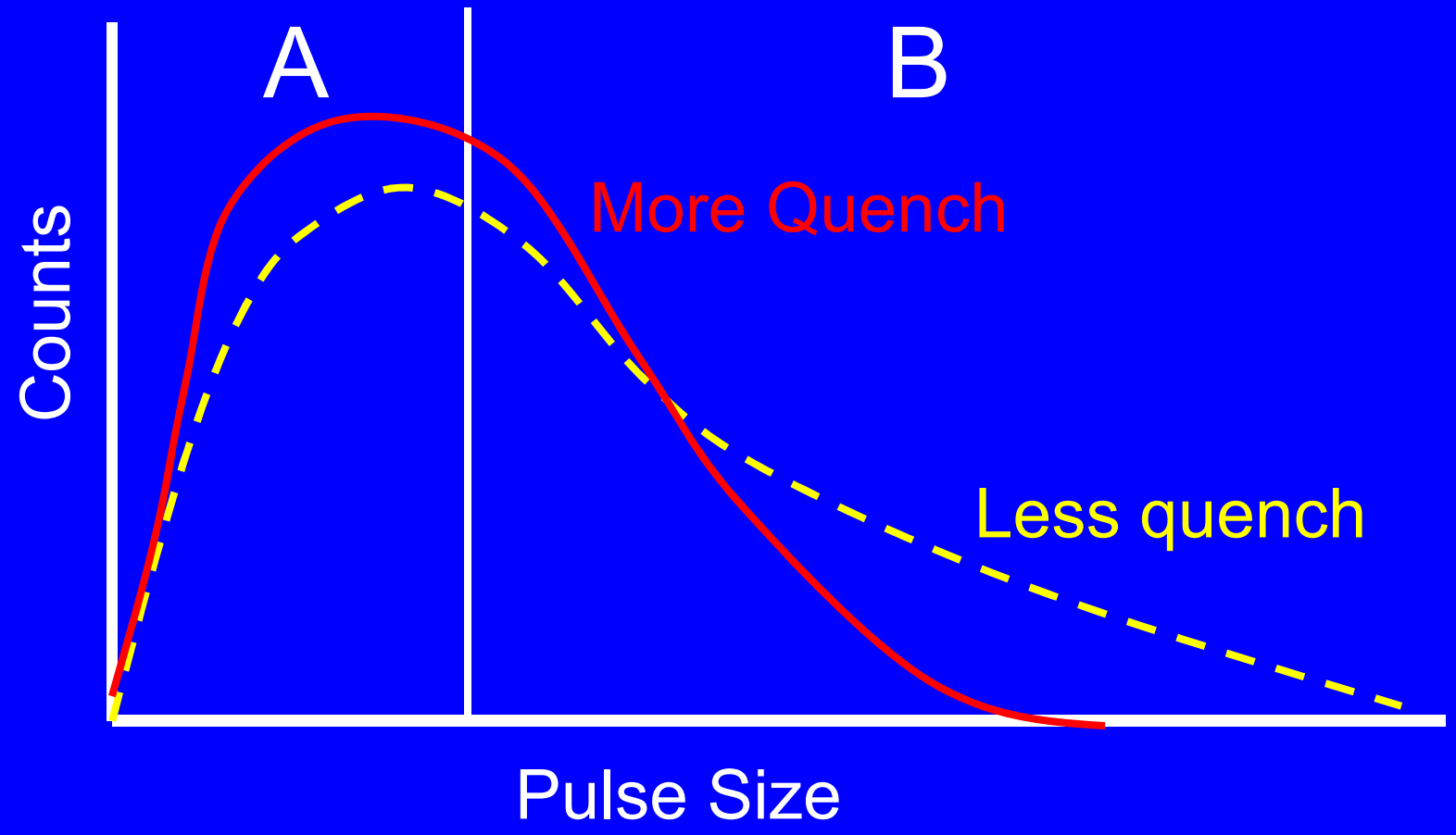
The standard vials and, if possible, the cocktail, should be identical to those that will be used to count the samples in.

Since each standard contains a different amount of a quenching agent, the spectra of these standards will have different shapes.

The greater the amount of quenching agent, the fewer the counts (i.e., the lower the counting efficiency) and the further to the left the spectrum is shifted.

Quench Correction

2. Channels Ratio Method



Quench Correction

2. Channels Ratio Method

ii. We integrate the counts in Channel A and Channel B. For each spectrum we calculate two values:

An efficiency (E)

$$E = \frac{Counts\ in\ B}{Known\ Activity} = \frac{B}{Known\ Activity}$$

A channels ratio (CR)

$$CR = \frac{Counts\ in\ B}{Total\ Counts} = \frac{B}{A + B}$$

The efficiency and channels ratios could be defined differently. The key is that we standardize the definitions.

Quench Correction

2. Channels Ratio Method

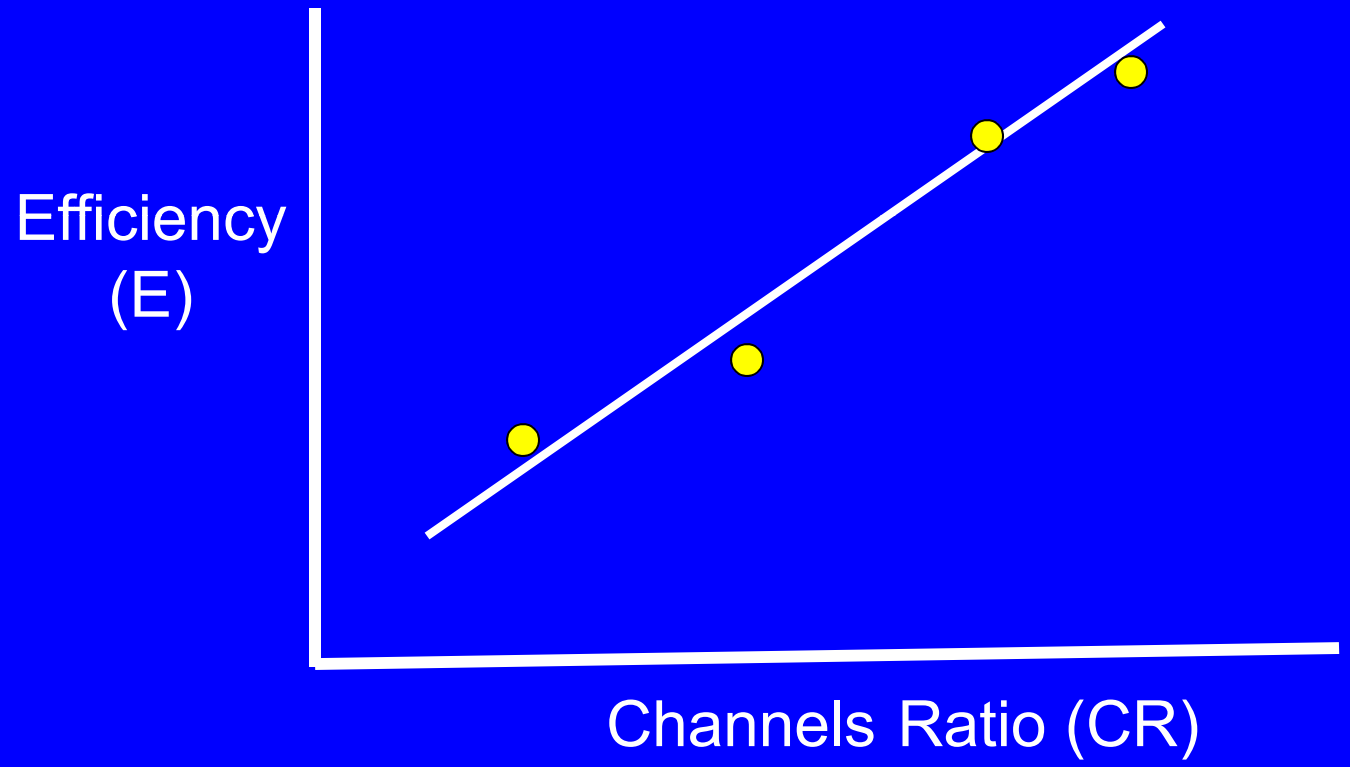
In general, as quench is added:

- the count in Channel B decreases
- the efficiency (E) decreases
- the channels ratio (CR) decreases

Quench Correction

2. Channels Ratio Method

iii. We then plot efficiency (E) as a function of the channels ratio (CR). This is our quench correction curve.

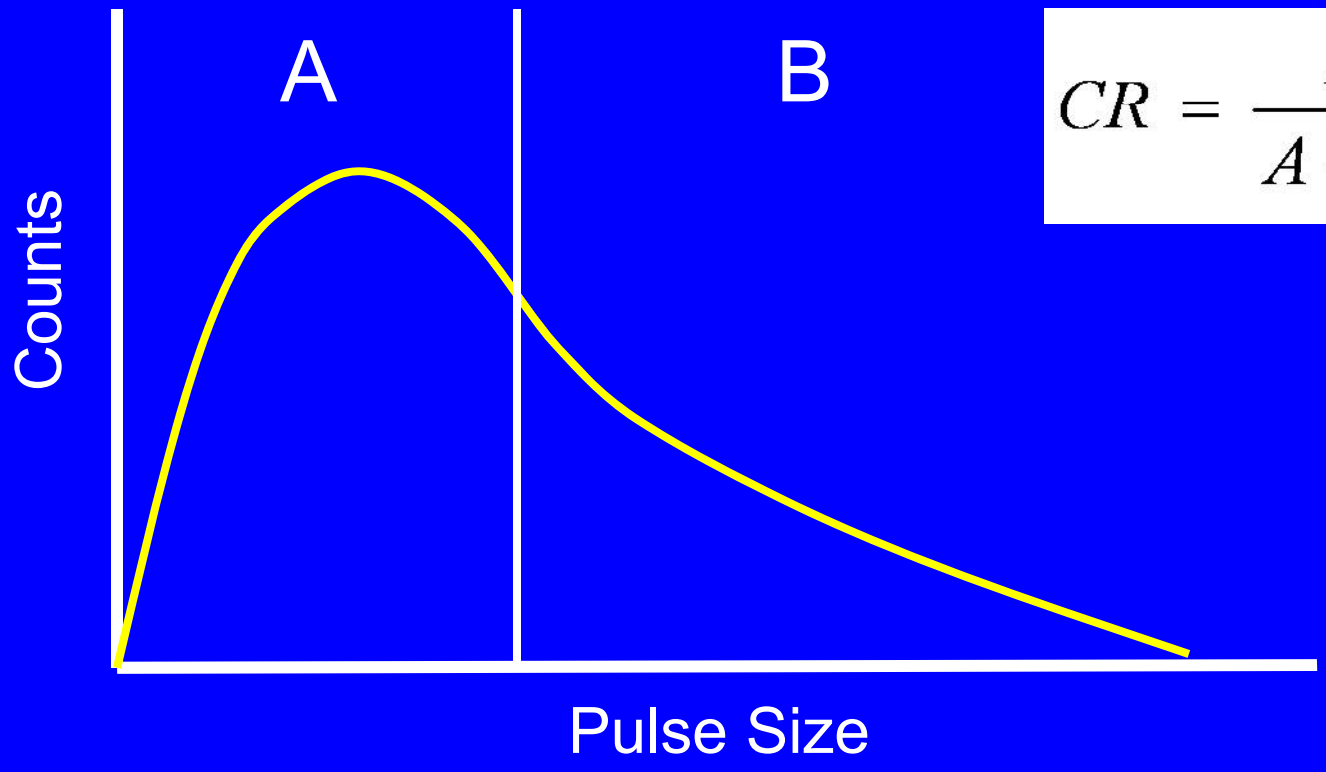


The channels ratio (CR) describes the spectrum shape.

Quench Correction

2. Channels Ratio Method

iv. We then acquire a spectrum for our sample, and calculate the channels ratio:

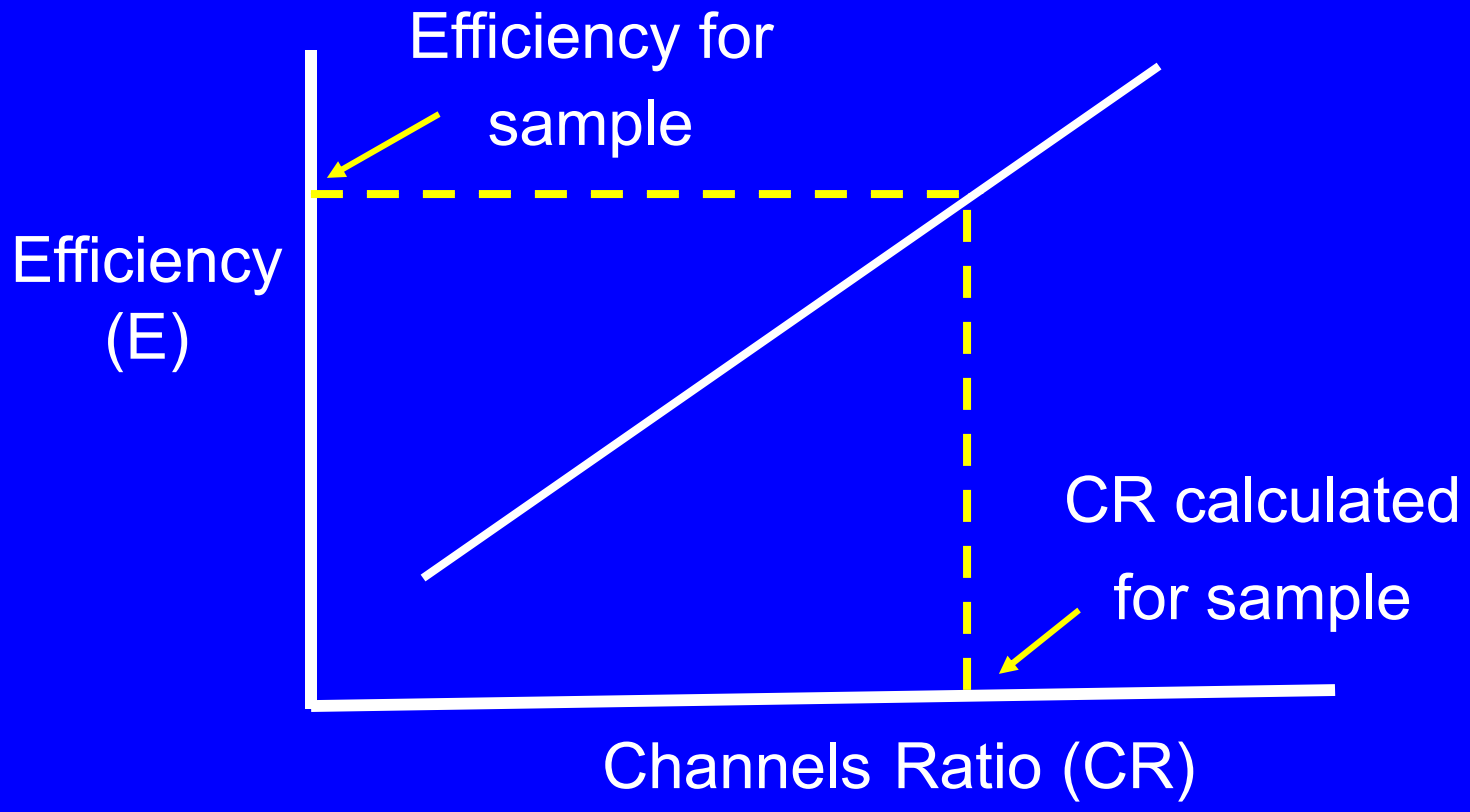


$$CR = \frac{B}{A + B}$$

Quench Correction

2. Channels Ratio Method

v. On the quench correction curve, we find the efficiency that corresponds to the channels ratio for the sample.



Quench Correction

2. Channels Ratio Method

vi. Using the efficiency (E) obtained from the quench correction curve, we determine the activity of the sample:

$$Activity\ of\ Sample = \frac{B}{E}$$

Quench Correction

2. Channels Ratio Method

The Spectral Index of the Sample (SIS) technique is very similar to the channels ratio method.

The difference is that the shape of the spectrum is described via the “spectral index of the sample” rather than the channels ratio. As such, the quench correction curve is a plot of the counting efficiency as a function of the SIS.

Mathematically, the calculation of the SIS is somewhat more complicated than the calculation of the CR since it involves the processing and weighting of each individual pulse.

An advantage is that the SIS method is more sensitive than the CR method. Nevertheless, neither method is suitable for low activity (e.g., < 500 dpm) samples.

Quench Correction

3. External Standard Method

Despite the name, this technique is unrelated to the internal standard method discussed earlier.

What it does is employ a clever “trick” to make accurate evaluations of the amount of quenching and the counting efficiency even when the sample activities are low and counting statistics are poor.

i. The sample is counted but no attempt is made to evaluate the spectral shape.

ii. A gamma emitting source (e.g., Ba-133 or Cs-137) is automatically brought into position underneath the sample vial and the sample is counted again.

Quench Correction

3. External Standard Method

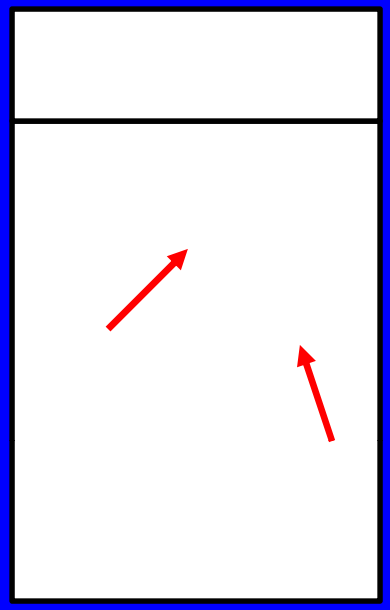
The low energy gamma rays/x-rays from the “external standard” interact with the vial and cocktail via Compton scattering. The resulting Compton scattered electrons travel through the cocktail mimicking beta particles.

iii. The counting efficiency is then determined from the shape of the spectrum generated by the Compton scattered electrons via a quench correction curve. This curve was produced using the same type of standards used in the CR and SIS methods to generate quench correction curves.

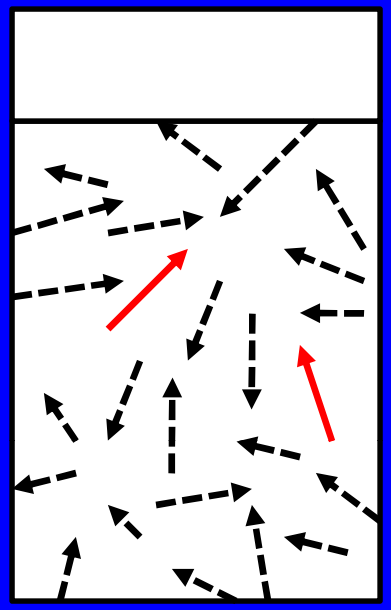
Quench Correction

3. External Standard Method

First Count of Sample



Second Count of Sample



Ba-133 Source

Low Counts (A_1 and B_1)

$CR = B_1 / (A_1 + B_1)$

High counts (A_2 and B_2)

$CR = B_2 / (A_2 + B_2)$

Large uncertainty in channels ratio means large uncertainty in efficiency (E_1).

Little uncertainty in channels ratio means little uncertainty in efficiency (E_2).

Sample Activity = B_1 / E_2

Quench Correction

3. External Standard Method

Because of the high count rates associated with the Compton scattered electrons, the spectral shape evaluation and efficiency determination can be highly accurate irrespective of the sample activity.

Some LSC systems use a “quench indicating parameter” known as the “transformed Spectral Index of the External Standard” (t-SIE) to describe the spectral shape. In these systems, the quench correction curve plots counting efficiency as a function of the t-SIE value which ranges from 0 – 1000. Like the CR and SIS values, the t-SIE values decrease with increasing quenching.

Cerenkov Counting

Cerenkov Counting

Cerenkov radiation, the blue light produced when electrons travel faster than light in a transparent medium, can be used to assay high energy (> ca. 500 keV) beta emitters.

The sample is simply dissolved in a transparent solution such as water or alcohol.

Although Cerenkov counting can be used to count any high energy beta emitter, its primary application has been to assay Sr-90/Y-90.

Advantages of Cerenkov counting:

- Low background
- No chemiluminescence
- No chemical quenching