790.1

Lab Techniques

for Analytical & Physical Chemistry

Jianbo Liu
Chem & Biochem
Queens College of CUNY

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Course Objectives

Discuss background and principles for s*elected* instrumental analysis

- Origin of chemical and physical properties need to be measured
- Instrument design and build
- Data acquisition and processing
- Relationship between instrument readout and property measurement

Required Textbook for Lecture

Principles of Instrumental Analysis, D. A. Skoog, F. J. Holler and S. R. Crouch, 7th ed., 2017.

Syllabus and lecture slides are available on line

http://chem.qc.cuny.edu/~jliu/Liu_page/teaching.htm

Topics to Be Covered

- Instrumentation, and Its Signal and Noise (2 Units)
- Optical Instruments and Methods (2 Units)
- Molecular Electronic Spectrometry (2 Units)
- Molecular Vibrational Spectrometry (2 Units)
- Mass Spectrometry (3 Units)
- Chromatographic Separations (3 Units)

1 Classification of Analytical Methods

1.1 Qualitative analysis (what?)

measured property indicates the presence of analyte in matrix

Classical	Instrumental
identification by colors,	chromatography, electrophoresis,
boiling points, odors	spectroscopy, electrode potential, etc.

1.2 Quantitative analysis (how much?)

the magnitude of measured property is proportional to the concentration of analyte in matrix

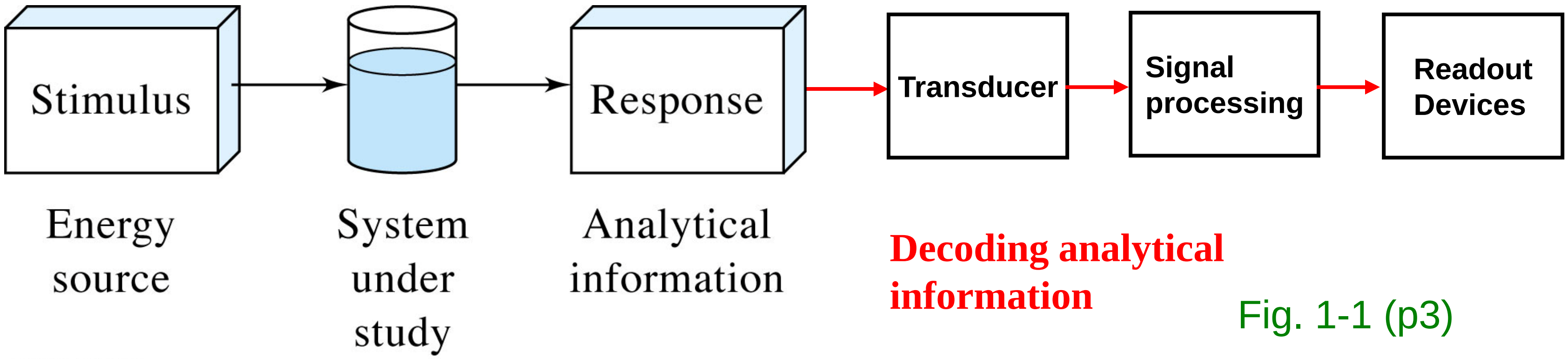
Classical	Instrumental
mass or volume	measuring property and
(e.g., gravimetric, volumetric)	determining its relationship to concentration

2 Types of Instrumental Methods

Table 1-1 (p2)

Properties	**Methods**
Radiation emission	Emission spectroscopy (X-ray, UV, Vis, electron, Auger, fluorescence, phosphorescence, luminescence)
Radiation absorption	Spectrophotometry and photometry (X-ray, UV-Vis, IR), NMR, ESR
Radiation scattering	Turbidity, Raman
Radiation refraction	Refractometry, interferometry
Radiation diffraction	X-ray and electron diffraction methods
Radiation rotation	Polarimetry, circular dichroism
Electrical potential	Potentiometry
Electrical charge	Coulometry
Electrical current	Voltammetry: Amperometry, polarography
Electrical resistance	Conductometry
Mass	Gravimetry
Mass-to-Charge ratio	Mass spectrometry
Rate of reaction	Kinetics, dynamics
Thermal	Thermal gravimetry, calorimetry
Radioactivity	Activation and isotope dilution methods

Block Diagram of Instruments and Signal



Decoding analytical information

Fig. 1-1 (p3)

1. Encoding in various Data Domains

	Spectrophotometer
Stimulus → elicit signal	monochromatic light source generated from a lamp
Response→ analytical information	light absorption

2. Decoding

Transducer→ convert analytical signal to an electrical signal	photomultiplier, produces voltage proportional to light intensity
Signal processing	amplification, discrimination to remove noise, AC-to-DC conversion, current-to-voltage conversion, Math, etc.
Readout devices	Transmittance (I/I_0%) or absorbance ($-\log(I/I_0)$) on meters and computer displays

3.1 Data domains

various modes of encoding analytical responses in electrical or non-electrical signals

Non-electrical Domains

physical (light intensity, pressure)
chemical (pH)
scale position (length)
number (objects)

Interdomain conversion

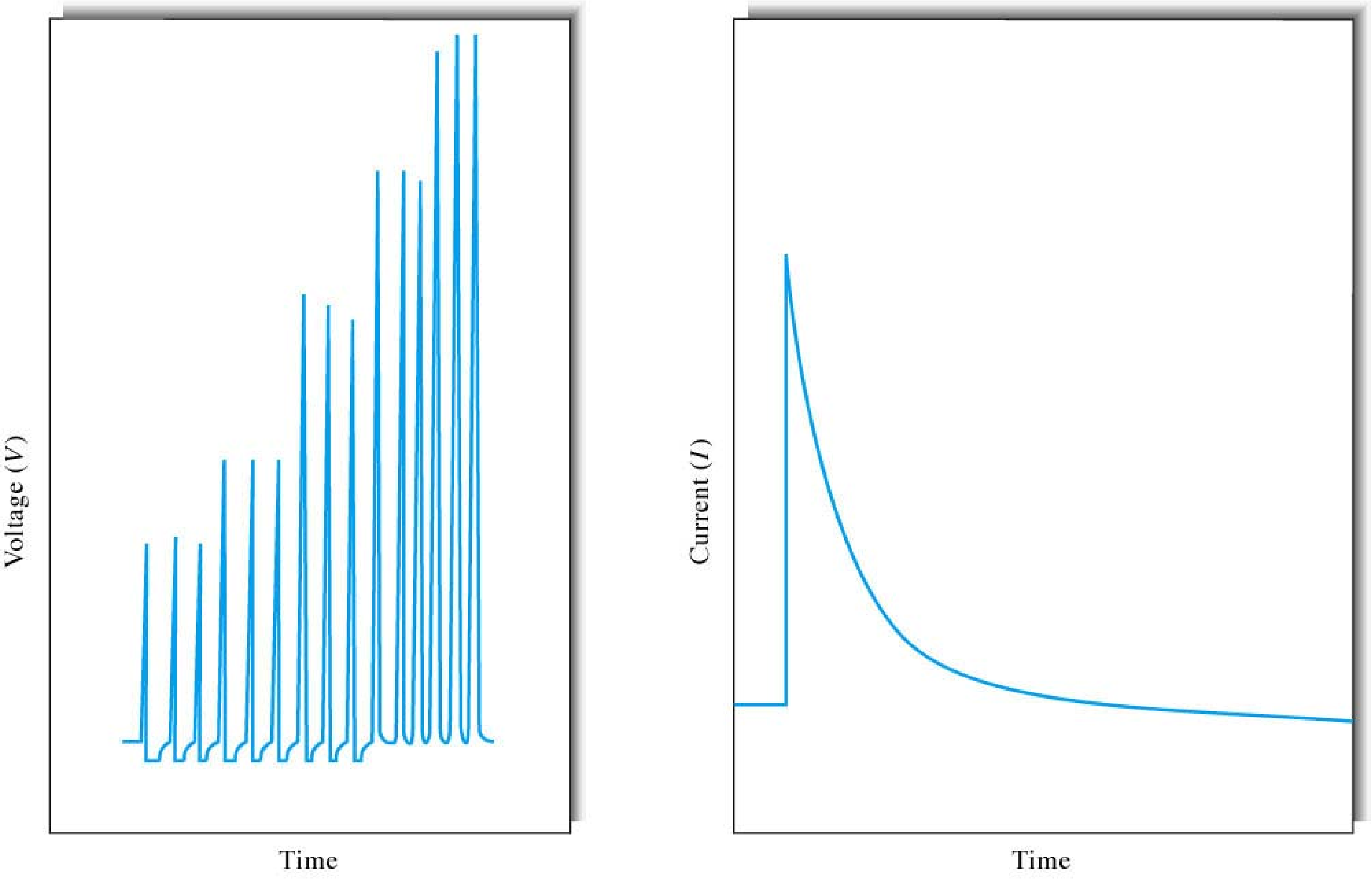
Electrical Domains

Analog domain: continuous in both magnitude and time (current, voltage, charge)
susceptible to electrical noise.

Time domain: frequency, period, pulse width
frequency: the number of signals per unit time
period: time required for one cycle
pulse width: the time between successive LO to HI transition.

Digital signal

Analog signals

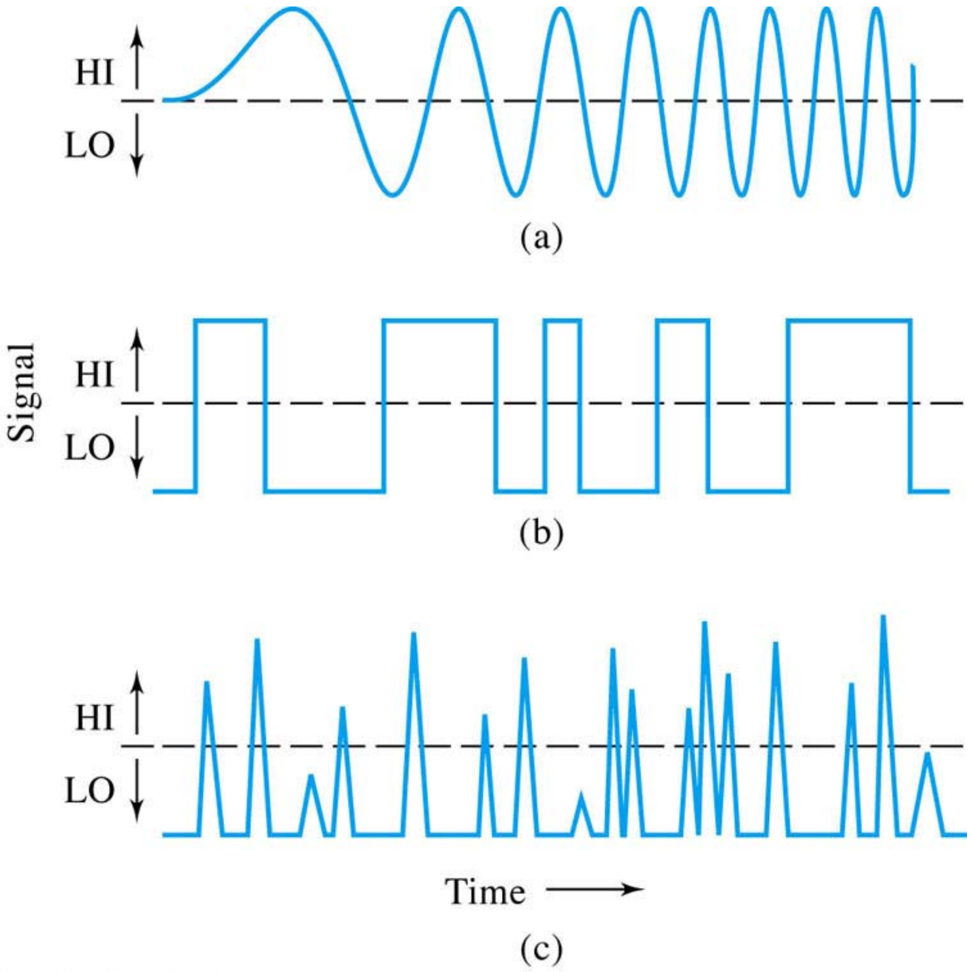


(a)

(b)

Fig. 1-4 (p6)

Time-domain signals



threshold

Fig. 1-5 (p7)

Example: Amplifier/Discriminator connected to an Electron Multiplier in Mass Spec

F-100TD Pulse Preamplifier
w/ TTL Output & Digital Threshold Monitor,

Advanced Research Instruments Corp
http://aricorp.com/f100td.htm

Spec's of F-100TD:

Input pulse rise time	2-3 ns
Minimum pulse width	10 ns
Maximum pulse width	1 µs
Pulse pair resolution	< 20 ns
Max. repetitive pulse rate	> 50 MHz
Output signal	+5 V (TTL)

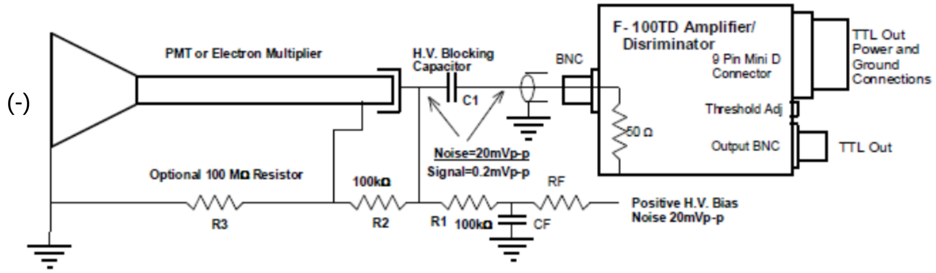


Figure 5a. Electron Multiplier Bias with Positive Supply

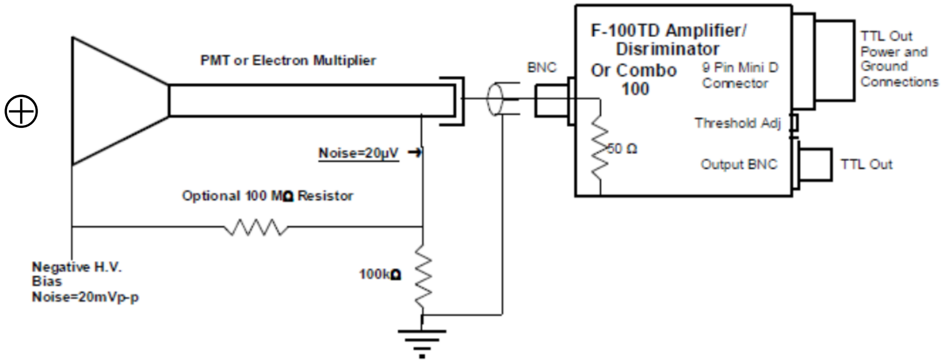


Figure 5b. Electron Multiplier Bias with Negative Supply

DeTech 411 Electron multiplier spec's :

Pulse rise time	> 3-5 ns
Pulse width	>10 – 20 ns
Gain at 2050 V	5×10^7
Dark noise	< 0.05 cps

Amplifier/Discriminator Set up

F-100TD Pulse Preamplifier w/
TTL Output & Digital Threshold Monitor

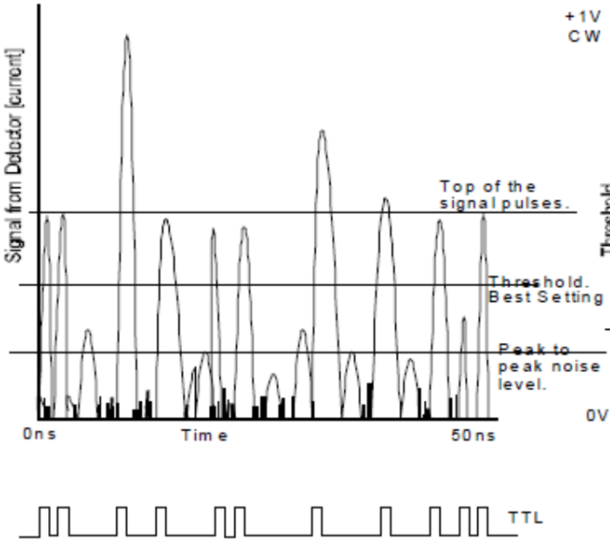


Fig. 1 (top) Typical signal from the multiplier and the threshold setting. (bottom) The TTL output from F-100TD.

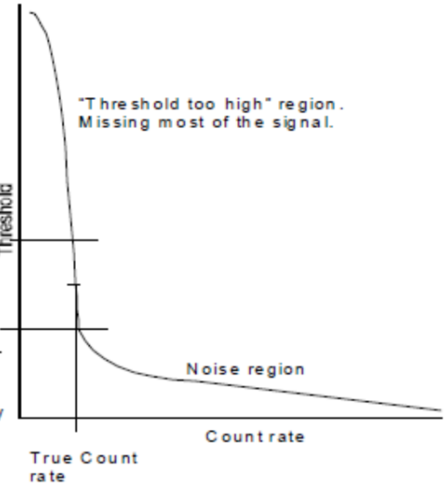


Fig. 2 Relationship btwn threshold and resulting count rate.

Fig 1 shows signal and noise coming from the amplifier just before it enters the discriminator for thresholding.
When the threshold dial is set to zero, the threshold is buried in noise (input noise plus preamplifier noise), and FT-100D **produces high count rate** pulses **even if no signal is present**.

Adjustment

1. With the electron multiplier turned on + ion source turned off, increase the threshold to reduce dark count rate to 1 cps (ideally 0.05 cps)
2. With the ion source on, increase the threshold and note the change in the count rate. There should be a minimal change within certain range, i.e. **the best setting** as shown in **Fig. 2**.
3. When reach a threshold setting that is too high, the count rate drastically drops off.

The best setting is approximately in the middle between the noise level and loss of the signal.
(An ideal electron multiplier produces signal pulses all of the same height; and in that case, there should be no change in the count rate when changing the threshold with the indicated range in **Fig. 1)**.

Digital signals

Digital: easy to store, not susceptible to noise

1. Serial data
2. Binary coding

to represent “5”

count serial data: 11111, 5 time intervals

binary: 101, 3 time intervals, $1x2^0 + 0x2^1+1x2^2 = 5$

With 10 time intervals:

In serial data count, we can only record numbers 0-10

In binary encoding, we can count up to $2^{10}-1 = 1023$ by different combinations of Hi or LO in each of 10 time interval.

1023/10 >100 times.

3. Serial vs. parallel signal

To use multiple transmission channels instead of a single transmission line to represent three binary digits.

Have all the information simultaneously.

Digital signals

serial data *vs*. binary

serial binary *vs*. parallel binary

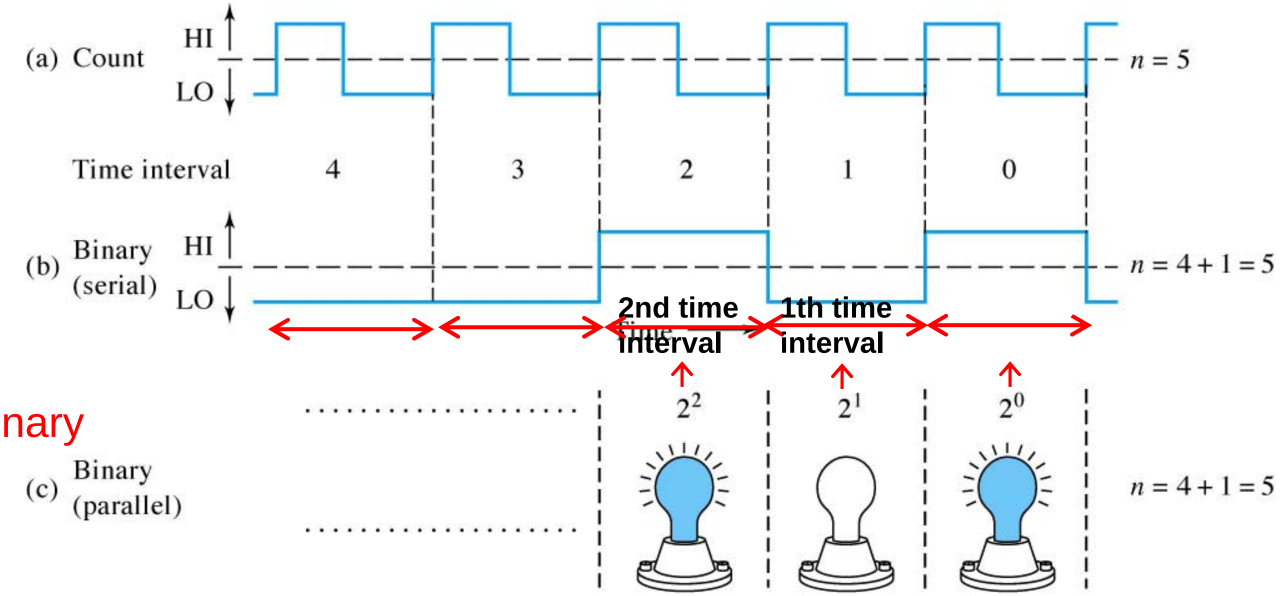


Fig. 1-6 (p8)

4 Instrumental Noise

What is noise?

any “unwanted” part of the analytical signal

noise always accompanies with signal

Signal-to-noise ratio (S/N)

for a set of data (replicate measurements)

$$\frac{S}{N} = \frac{\bar{x}}{s} = \frac{1}{RSD}$$

for a temporal-varying signal

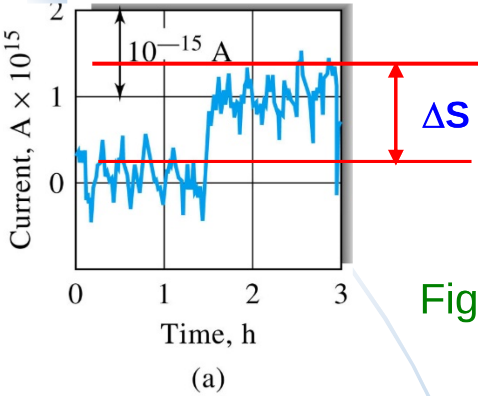


Fig. 5-2 (p111)

$$s = \frac{\Delta S}{5}$$

$$\frac{S}{N} = \frac{5\bar{x}}{\Delta S}$$

For meaningful measurements, S/N ≥ 3,

Sources of Noise (characterized by frequency)

4.1 White noise – amplitude invariant with respect to frequency

Thermal noise

-voltage fluctuation due to random electron motions in resistive elements

$$\bar{v}_{rms} = \sqrt{4kTR\Delta f}$$

k: Boltzmann’s constant

T: absolute temperature

R: resistance

Δ*f*: frequency bandwidth, $\Delta f = \dfrac{1}{3\tau_r}$

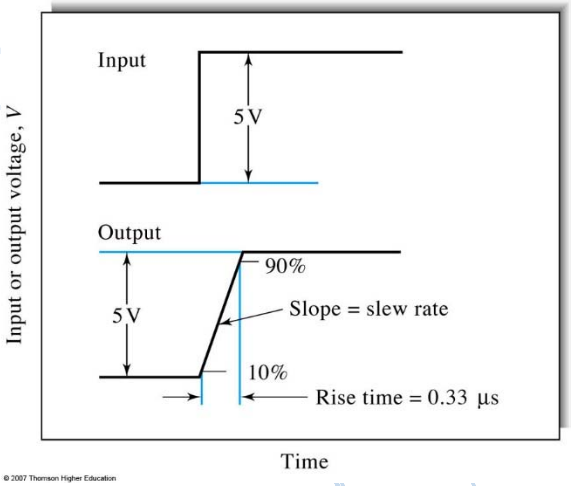


Fig. 3-9 (p65)

$$\Delta f = \frac{1}{3\tau_r}$$

$$\tau_r = 0.01\, s$$

$$\Delta f = 33\, Hz$$

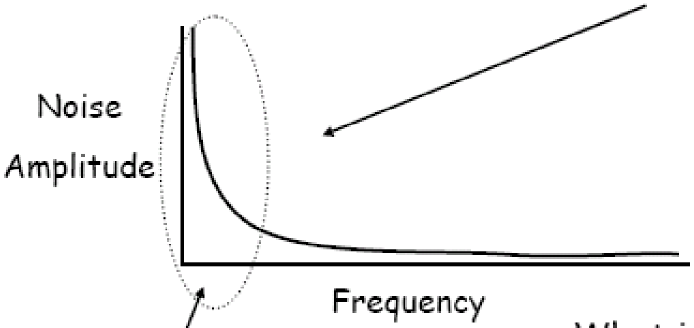
Shot noise

-current fluctuation due to random motions of electrons cross a junction (e.g., *PN* interface, space between anode/cathode)

$$i_{rms} = \sqrt{2Ie\Delta f}$$

I: average current
e: charge of electron

4.2 Flicker noise – amplitude varies with 1/f, appears as a drift in a measurement

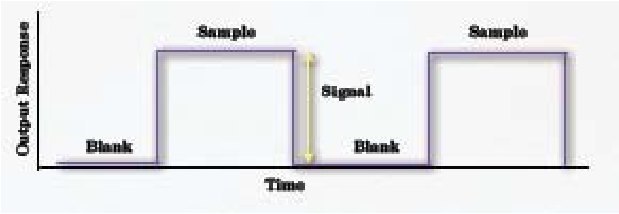


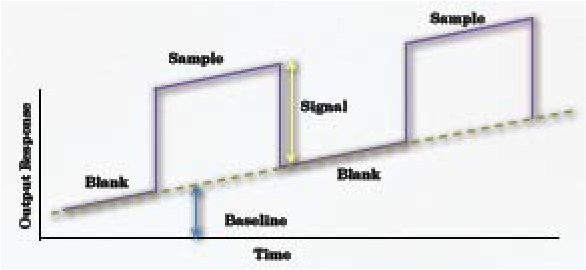
Most significant at low (<100 Hz) frequencies

What is it?

-drift

-*low freq.* signal fluctuations



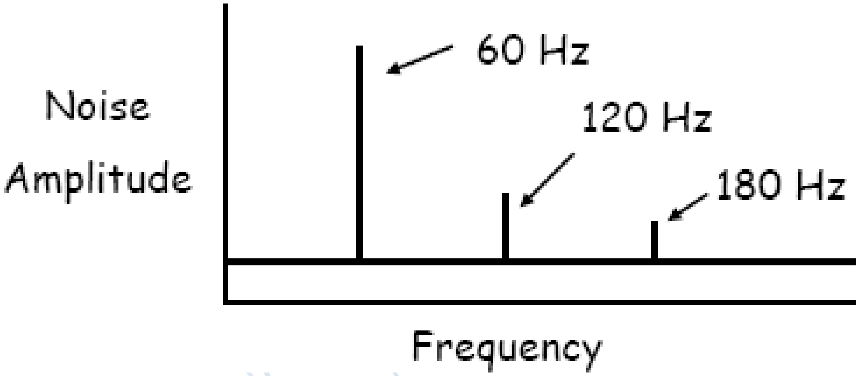


4.3 Environmental noise

- different forms of noise that arise from the surroundings

- some occur at known discrete frequencies

- some unpredictable, and difficult to correct
 (e.g., TV stations, computers, motors, etc.)



Example:

AC "hum"
from power-line

4.4 Composite noise spectrum

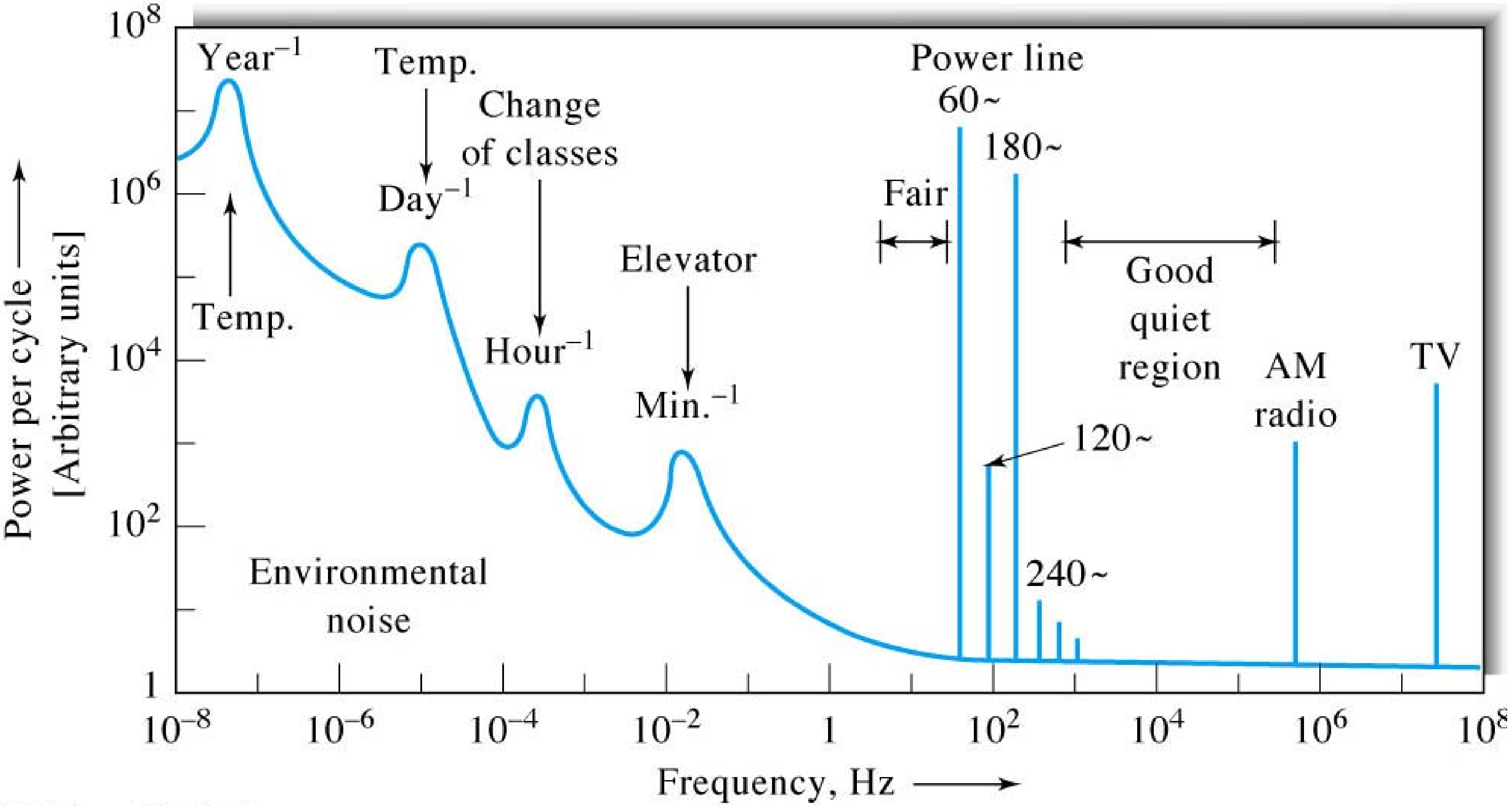


Fig. 5-3 (p113)

5 Strategies for S/N Enhancement

- **White noise → reduce Δf, temperature, resistance, and I**
- **Flicker noise → make measurements at frequencies >100kHz**
- **Shielding & grounding → absorbing electromagnetic noise**

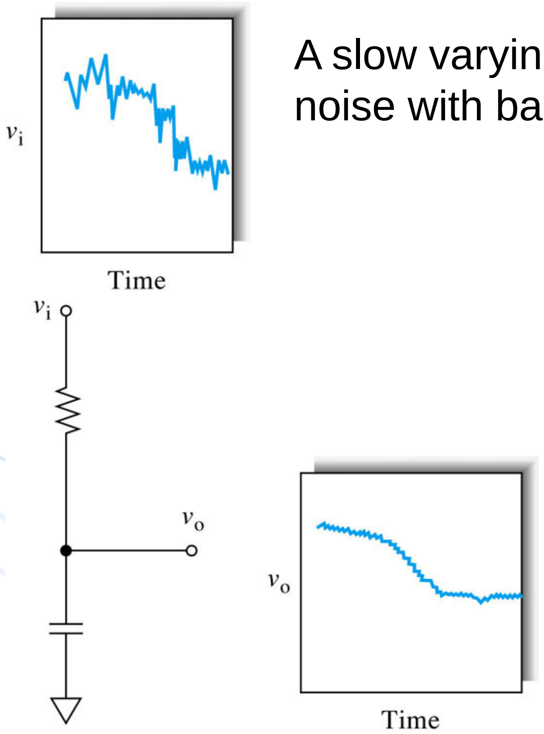
But signal

- often at or near dc (low frequency)
- often directly proportional to resistance
- often directly proportional to current
- often measured with transducers of high Δf (fast response, PMT Δf $>10^7$Hz)

5.1 Reducing Δf (white noise)

5.1.1 Analog filtering: low-pass RC circuit

A slow varying dc signal containing high-frequency noise with bandwidth extending over a wide range



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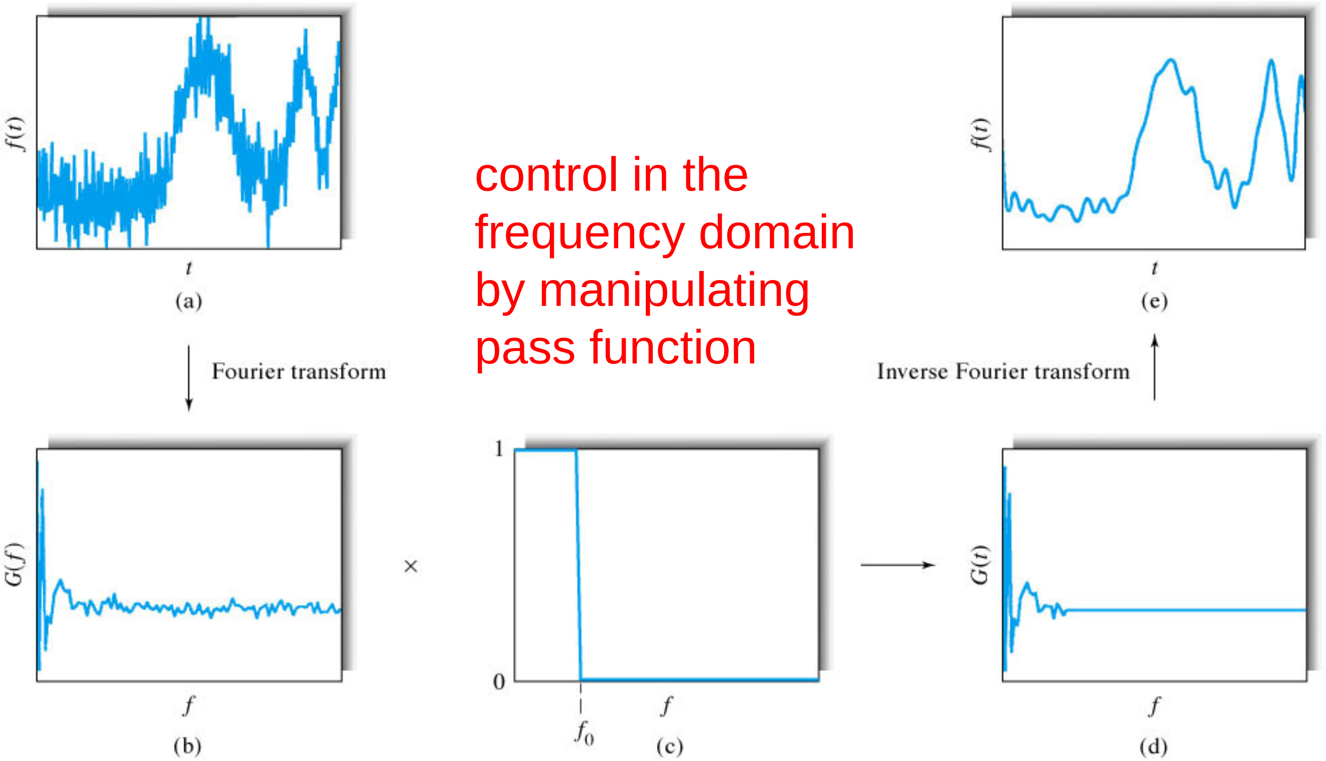
$$G_C = {}^{1}\!/_{R_C} = 2\pi f$$

Fig. 5-5 (p115)

High-frequency components are rejected, and Δf is reduced

5.1.2 Digital filtering: Fourier transform/smooth



control in the frequency domain by manipulating pass function

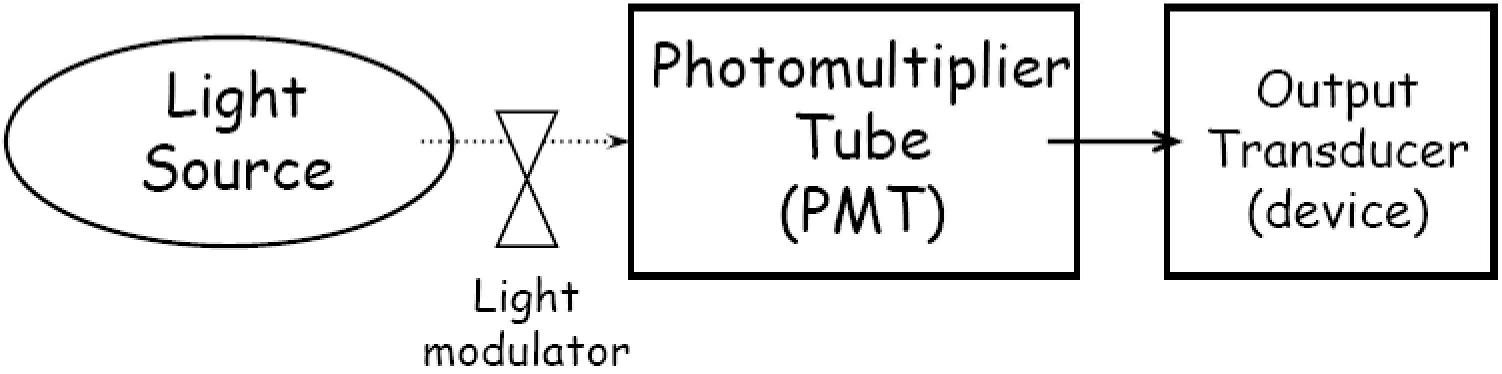
Fig. 5-12 (p121)

-It is easy to smooth/filter signal as well as noise. Make sure that result is not distorted

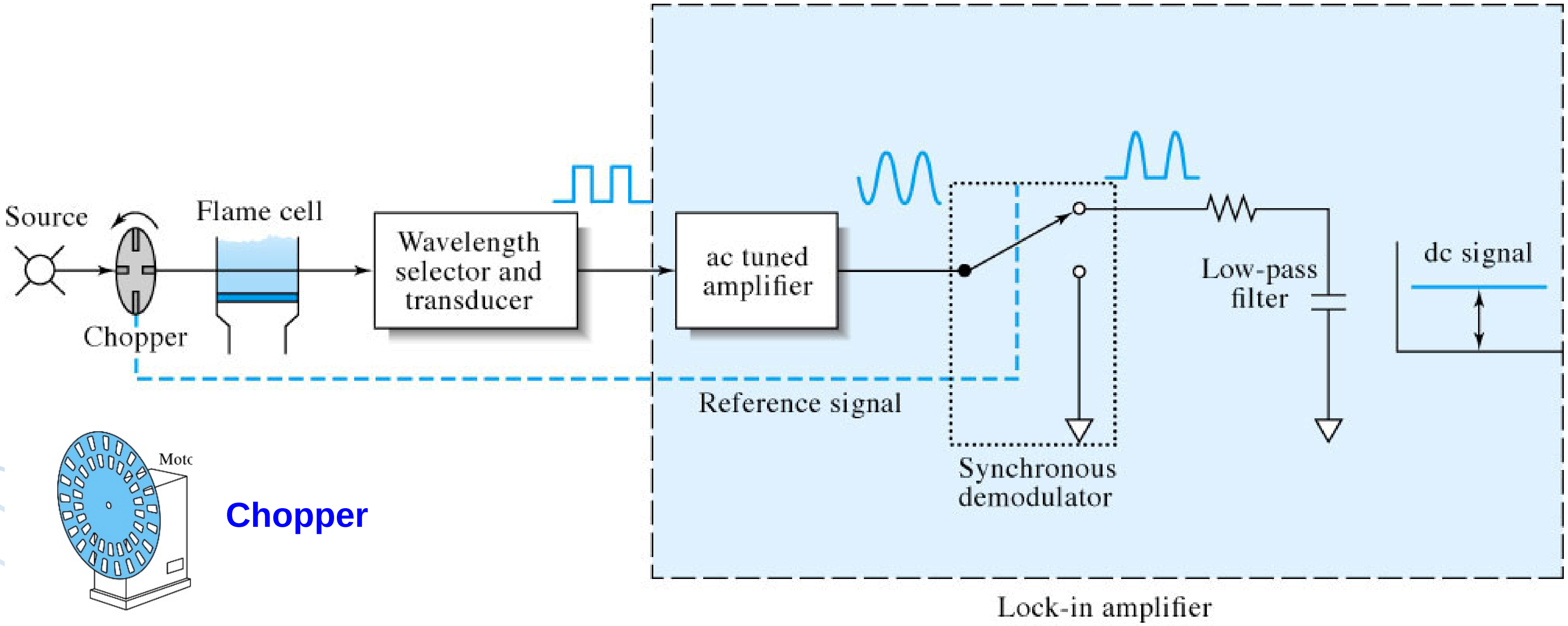
- trade-off between resolution and noise. Need high data density to prevent losing information.

5.2 Increasing *f* (flicker noise)

- **We need to move *f* to >100kHz…**
- **How?**
  - \- *Modulate*: encode analytical signal at a high frequency, where 1/f noise is negligible
  - \- *Amplify* the signal at the modulation frequency, while reduce the noise.
  - \- *Demodulate* the signal



Lock-in amplifier



Chopper

2. Amplify modulated signal

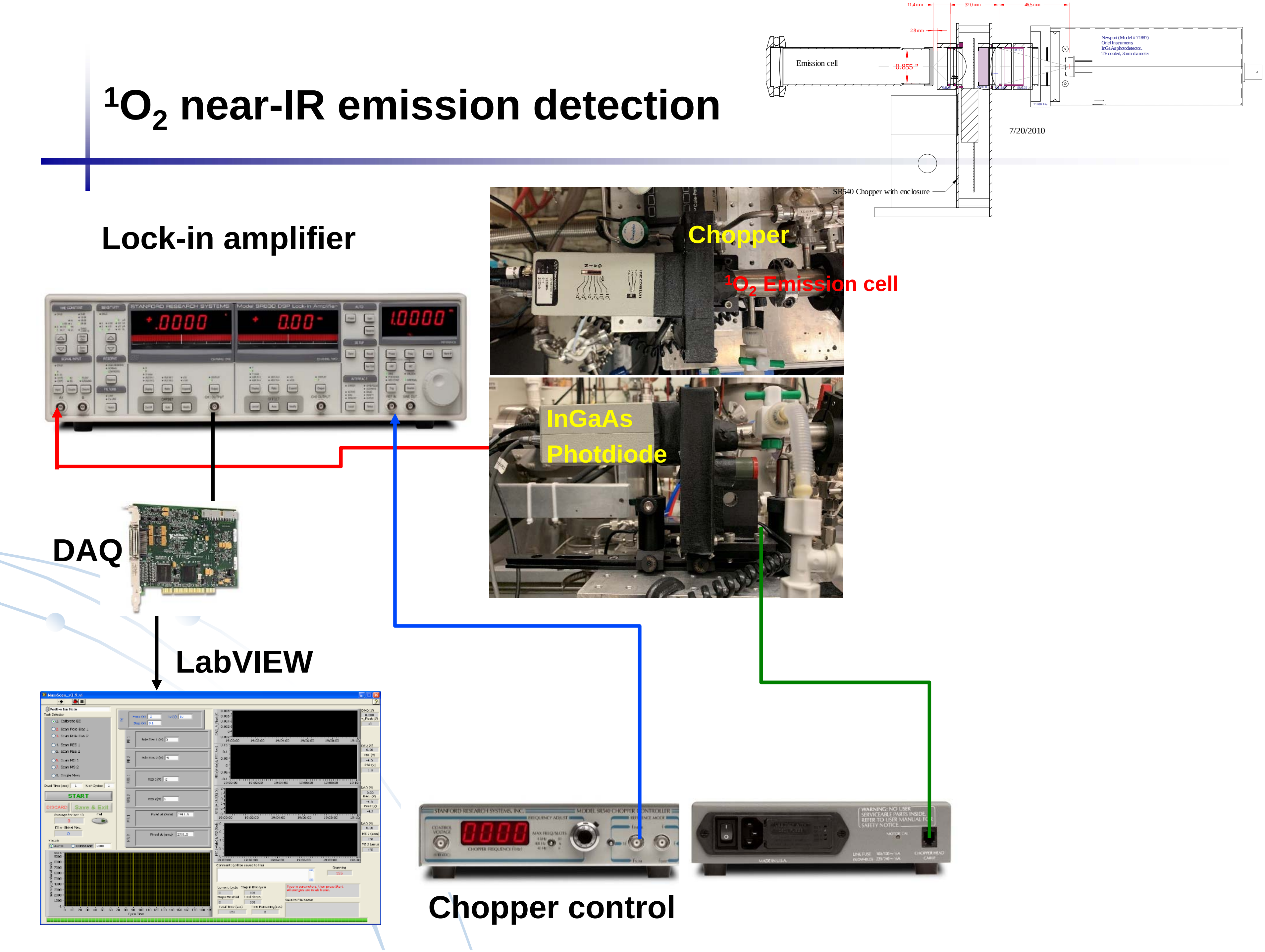
1. Modulate

3. Demodulate

Fig. 5-8 (p117)

1) Permits the recovery of signal even when the S/N is unity or less1.
2) Only those signals that are locked to the reference signal are amplified. All other frequencies are rejected by the system.

$^1O_2$ near-IR emission detection



5.3 Signal averaging

- **Total intensity of signal: increase linearly with the number (n) of replicate signals**

$$S_n = \sum_{i=1}^{n} S_i = nS_i$$

- **Noise: increase as $(n)^{1/2}$**

$$N_i = \sigma_i = \sqrt{\frac{\sum_{i=1}^{n}(S_i - S_x)^2}{n}} \qquad N_n = \sqrt{\sigma_n^2} = \sqrt{\sum_{i=1}^{n}\sigma_i^2} = \sqrt{n}\,\sigma_i = \sqrt{n}\,N_i$$

→ **S/N increase as $(n)^{1/2}$**

$$\left(\frac{S}{N}\right)_n = \frac{nS_i}{\sqrt{n}\,N_i} = \sqrt{n}\left(\frac{S}{N}\right)_i$$

5.3.1 An example for signal averaging

- Suppose we wish to *mass* a **10-mg** object on an analytical balance ($\sigma = 0.1\ mg$)

For a single (n =1) measurement:

$S = 10.\ \text{mg},\ N = 0.1\ \text{mg} \rightarrow \mathbf{S/N} = \underline{100}$

For n = 4:

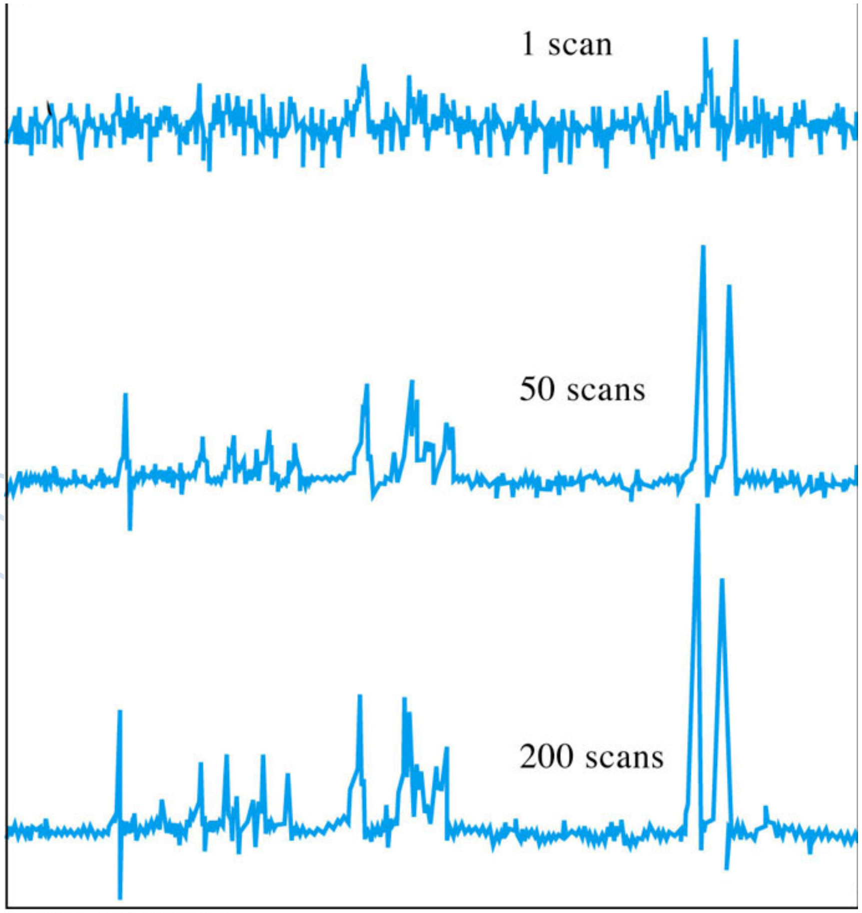
$\mathbf{S} = n \times 10.\ mg = 4 \times 10.\ \text{mg} = 40.\ mg$

$\mathbf{N} = \sigma_T = (n(\sigma)^2)^{1/2} = (4(0.1)^2)^{1/2} = 2(0.1) = 0.2\ mg$

$\mathbf{S/N} = 40./0.2 = \underline{200}$

For n = 16: $\mathbf{S/N} = \underline{400}$

5.3.2 Signal averaging for spectrum



Get S/N increased with $n^{1/2}$

Need good synchronization for replicate scans

Fig. 5-10 (p119)

5.3.3 Boxcar averaging

- A approach for smoothing irregularities

- A single –channel signal integrator
  select a single delay time

  integrated signal over
  selected gate time

  average signal for n-replicate

  repeat at new delay time

  S/N increases with (averaging time)$^{1/2}$

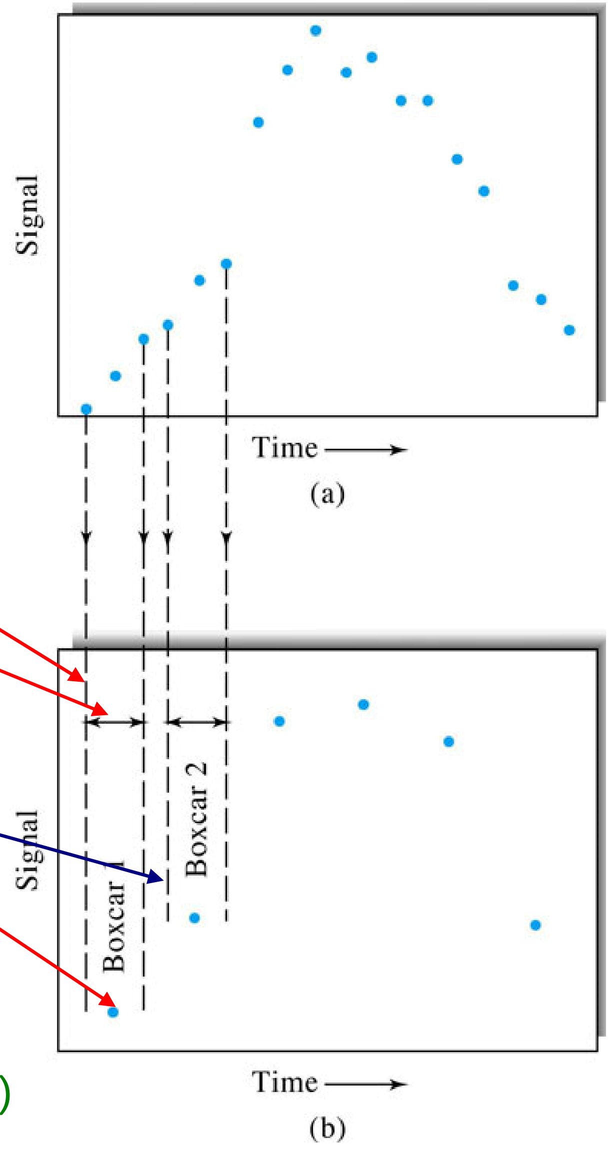


Fig. 5-11 (p119)

Performance Characteristics

- How reproducible? – Precision
- How close to true value? – Accuracy
- How small a difference can be detected? – Sensitivity
- What application range? – Dynamic Range
- How much interference? – Selectivity

6.1 Precision: Indeterminate or random error

absolute standard deviation: $$s = \sqrt{\frac{\sum_{i=0}^{i=N} (x_i - \bar{x})^2}{N-1}}$$

variance: s^2

relative standard deviation: $RSD = \frac{s}{\bar{x}}$

standard error of mean: $s_m = \frac{s}{\sqrt{N}}$

6.2 Accuracy: Determinate error, a measurement of systematic error

bias = $\bar{x} - x_{true}$

6.3 Sensitivity

calibration curves $S = kc + S_{bl}$

larger slope of calibration curve m means more sensitive measurement.

6.4 Detection limit

signal must be bigger than blank and random noise

commonly accepted for distinguished signal $S_m = ks_{bl} + S_{bl}$

ks_{bl}: *size of statistical fluctuation in the blank signal, k =3 at 95% confidence level*

$c_m = (S_m - S_{bl})/k$

6.5 Dynamic range

Limit of quantitation (LOQ): lowest concentration at which quantitative measurement can be made

Limit of linearity (LOL): the concentration at which the calibration curves departs from the linearity by a specified amount (5%).

Dynamic range: LOL/LOQ = 10^2 to 10^6

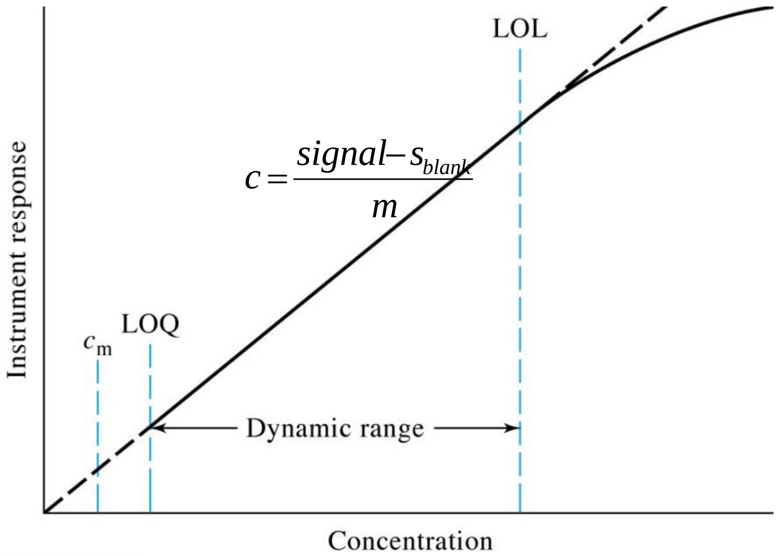


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Fig. 1-13 (p21)

6.6 Selectivity

Matrix with species A&B:

Signal = $k_A c_A + k_B c_B + S_{bl}$

selectivity coefficient : $K = k_B / k_A$

K = 0: no selectivity

K = larger number: very selective

Calibration curve (working or analytical curve): magnitude of measured property is proportional to concentration

$signal = mc + s_{bl}$