

Multimedia Tools for Learning Fluorescence: A Spectrofluorometer Simulator

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A new teaching method, using multimedia software, is in progress at our university. Running on a PC computer, it allows self-formation in molecular spectroscopy. A new software package devoted to photoluminescence (ISPL) is currently in progress. The concepts are introduced by using numerous graphs, animations, and simulations. The software allows electronic book facilities (search function, glossary, and bookmarks, for instance) and self-assessment. Tedious and time-consuming training is always necessary to acquire experimental skill. This is particularly true in the case of luminescence because numerous artifacts are possible and the number of parameters to be optimized is very large. In this view, a spectrofluorometer simulator appears to be a valuable tool that makes this training easy and fast: after choosing a set of experimental parameters, the computer displays immediately the corresponding spectrum. The influence of every experimental parameter is simulated: monochromator slit width, excitation or emission wavelengths, xenon source type, high-voltage photomultiplier tube, etc. The nature of solvents, with first- and second-order Rayleigh and Raman scattering, is managed. Sample geometry and concentration, with a corresponding inner filter, are also simulated. Obviously, there is no risk of damaging the virtual photomultiplier tube.

KEY WORDS: Corrected spectra; simulation; polyaromatics; dyes.

INTRODUCTION

Simulation appears to be an attractive tool when the real experimentation is tedious, dangerous, expensive, or time-consuming. In spectroscopy, tedious and time-consuming training is always necessary to acquire experimental skill. In this way, the spectrometer simulator appears to be a valuable tool that makes this training easy and fast. After choosing a set of experimental parameters, the computer immediately displays the corresponding spectrum. The influence of every experimental parameter can be simulated: monochromator slit width, lamp emissivity, detector spectral response, etc. The first step in this

process is an extensive knowledge of the spectrometer, to model all physical parameters.

A simulator may be particularly useful in fluorescence because the number of parameters to be optimized is very large: excitation or emission wavelengths, xenon source type, single-beam or double-beam spectra, high-voltage photomultiplier tube, nature of solvents, sample geometry and concentration, scanning mode, etc. Besides, numerous artifacts are possible in luminescence: scattering, inner filter and autoabsorption, intensity deviation from uncorrected spectrometers, etc.

Thus, the modeling must reproduce inner filter and autoabsorption due to solutions that are too concentrated. Rayleigh scattering and Raman scattering must also be simulated to reproduce spectra without solvent subtraction. Obviously, uncorrected simulated spectra must be distorted by lamp emissivity and detector spectral response.

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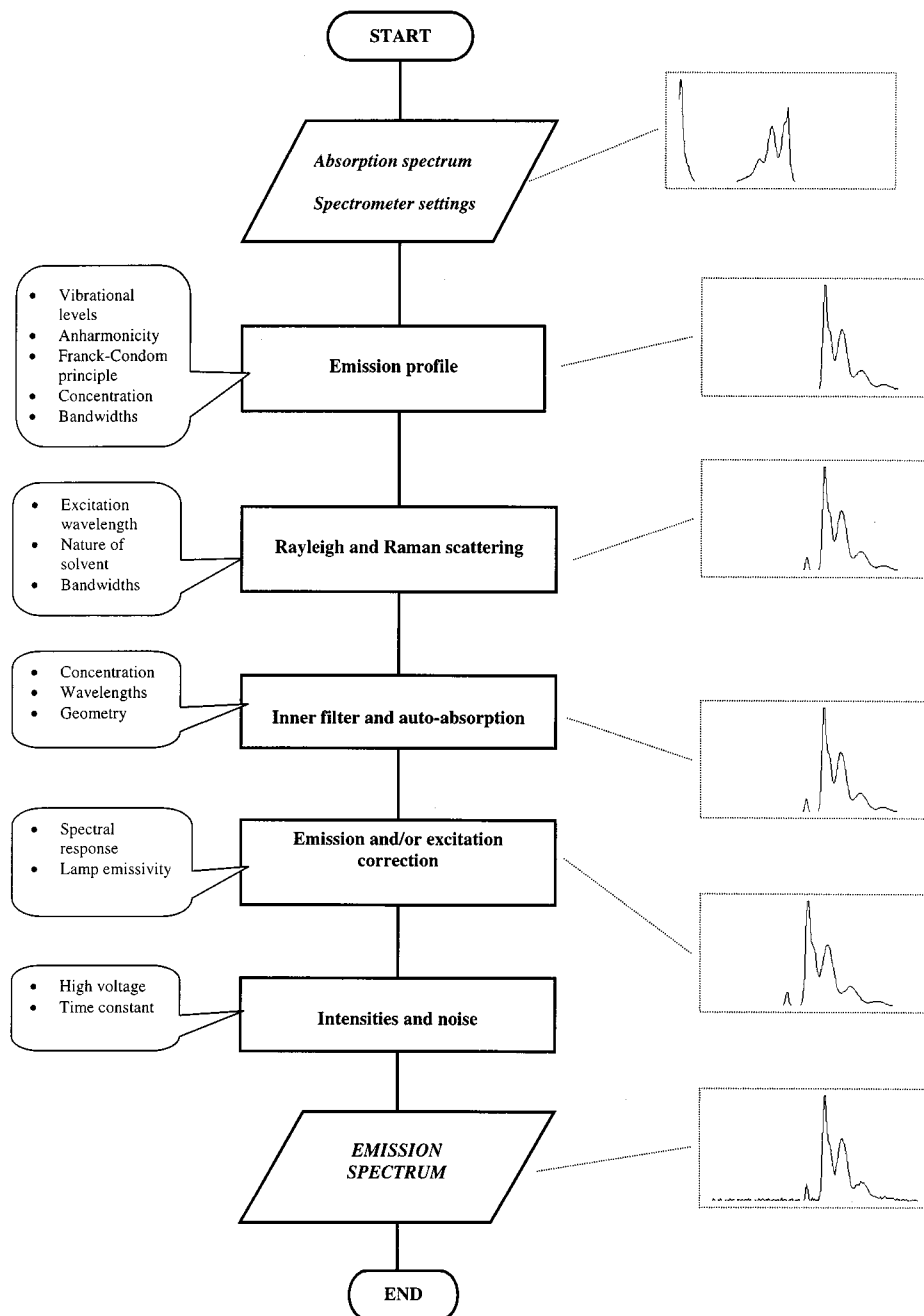


Fig. 1. Flowchart for simulation of an emission spectrum.

EXPERIMENTAL

The absorption spectra of significant model molecules (perylene, anthracene, and fluorescein) were recorded previously on an HP8452SA spectrometer.

The fluorescence spectra were recorded on a twin double-grating monochromator SPEX Fluorolog 212

spectrometer. The wavelengths were calibrated with a low-pressure mercury lamp. The uncertainty is ± 0.5 nm. The spectral bandwidths were calibrated to ± 0.1 nm with the low-pressure mercury lamp. The excitation correction data were recorded with a rhodamine B quantum counter reference assembly [1]. Perylene absorption spectra validated the excitation correction curve. The emission cor-

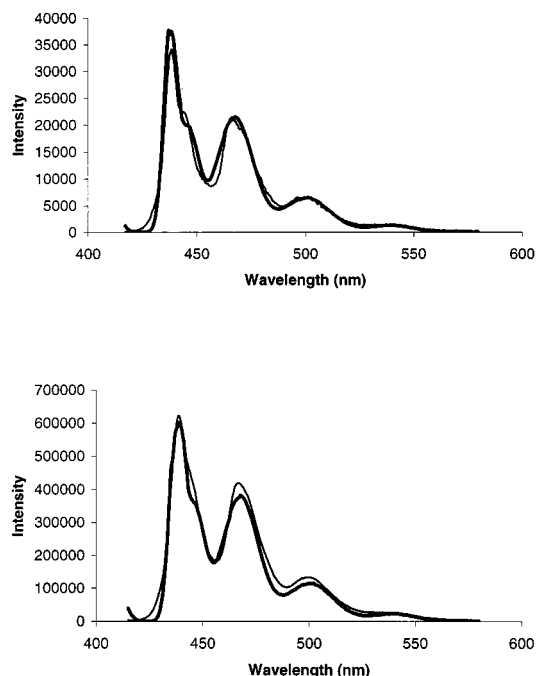


Fig. 2. Emission bandwidth effects on emission spectra of perylene. Top: SBS = 1 nm. Bottom: SBS = 4 nm. Thin line, experimental; thick line, simulated.

rection data were obtained using a calibrated tungsten lamp. Quinine sulfate emission spectra validated the emission correction curve [2].

Rayleigh scattering and Raman scattering of some typical solvents (water, cyclohexane, and ethanol) were recorded on the spectrofluorometer in the emission, excitation, and synchronous modes.

DISCUSSION

In spectroscopy, it is well known that the observed bandwidth spectrum (OBS) is the convolution product of the natural bandwidth spectrum (NBS) by the spectral bandwidth of the instrument (SBS) [3]:

$$\text{OBS} = \text{NBS} \otimes \text{SBS}$$

In absorption spectroscopy, it is generally acknowledged that a NBS/SBS ratio of 0.1 or less yields a correct absorbance measurement [4,5].

If the slit diffraction and the aberrations are not too large, this instrumental function can be approximated by a triangle. Computing the convolution product remains a problem, but this difficulty may be overcome by using the Fourier transform theorem [6]. This procedure implies two Fourier transforms: an inverse and a direct Fourier

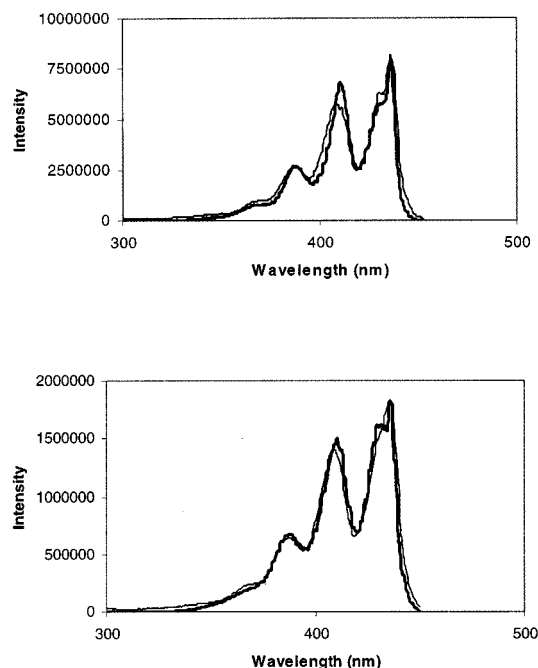


Fig. 3. Excitation bandwidth effects on excitation spectra of perylene. Top: SBS = 1 nm. Bottom: SBS = 4 nm. Thin line, experimental; thick line, simulated.

transform. It will, still today, be time-consuming, and some problems such as aliasing, apodization, and phase error remain. Also we have chosen to use simply a mathematical representation of spectrum by gaussian fitting [7]. Each band is characterized by its position λ_0 , its full width at half-height (FWHH), and its maximum height A_{\max} :

$$A = A_{\max} \cdot \exp[-\text{FWHH}(\lambda - \lambda_0)^2] \quad (1)$$

The fluorescence spectra will also be simulated using gaussian bands with similar parameters. Assuming that asymmetric bands are due mainly to unresolved vibronic structure, we decided to reproduce asymmetric bands by some gaussian lines rather than adding a cubic term to Eq. (1). A good fit was also obtained for scattering bands with a gaussian profile.

Simulation of an Emission Spectrum

The basis of the simulation is the well-known fluorescence intensity law [8,9]:

$$F = I_0 \Phi (1 - \exp[-2.3\epsilon cd]) \quad (2)$$

Step 1

First, some parameters are computed from the absorption spectrum: the vibrational energy level of the

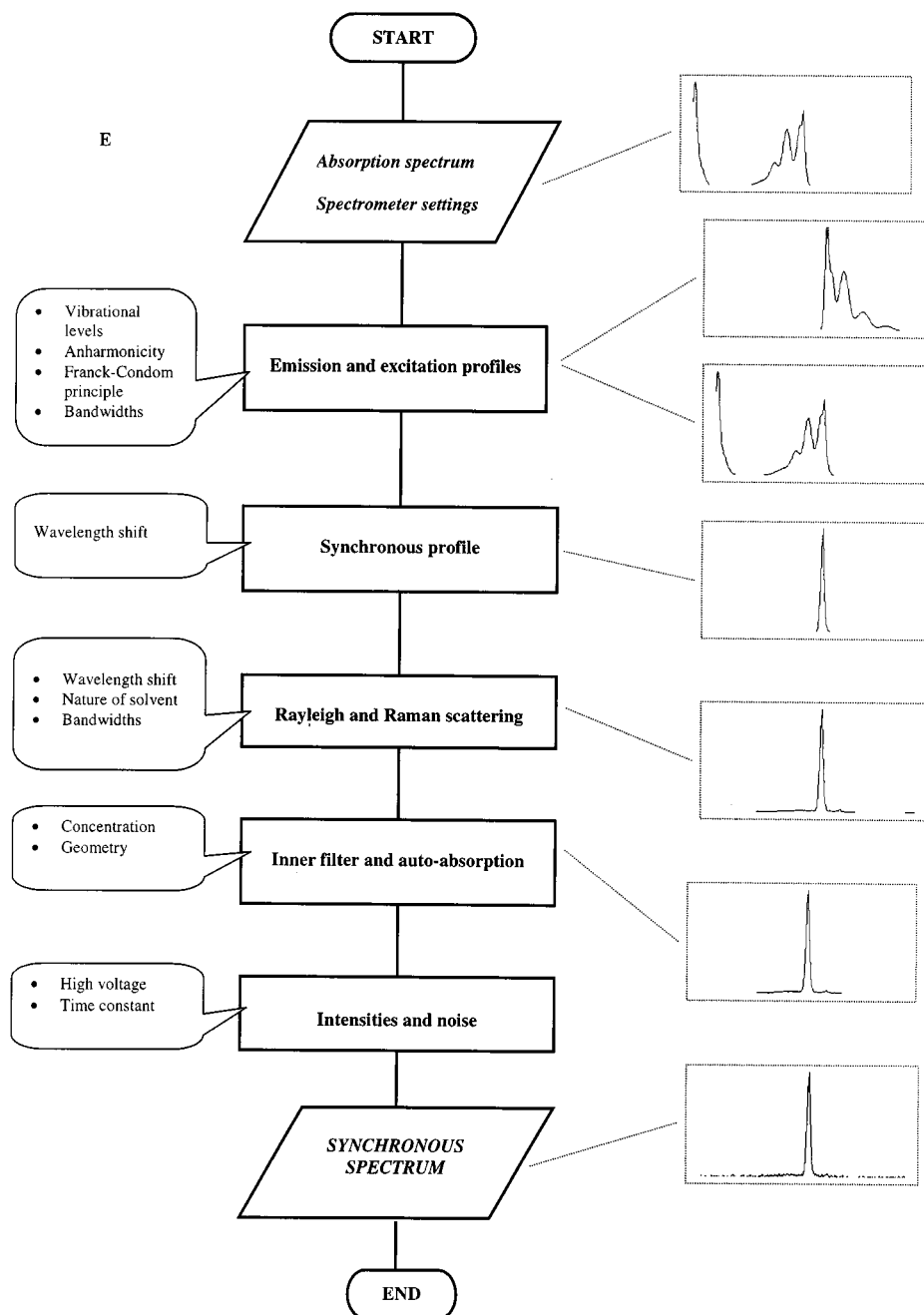


Fig. 4. Flowchart for simulation of a synchronous spectrum.

electronic excited state and the anharmonicity are estimated. Obviously, during this step wavenumbers are used, instead of wavelengths. The emission transition probabilities are then estimated using the Franck–Condon principle. Assuming a mirror between the first absorption band and the emission spectrum [10] and some solvent Stokes’s shift [11], the vibrational energy levels of the electronic fundamental state are predicted.

In this step, first-approximation fluorescence spectrum with some gaussian functions is computed. For each function, three parameters are required.

- The position: The peak position, computed previously, is used. In “analogical mode,” the peak may be shifted if the product “time constant·scanning speed” is too large.

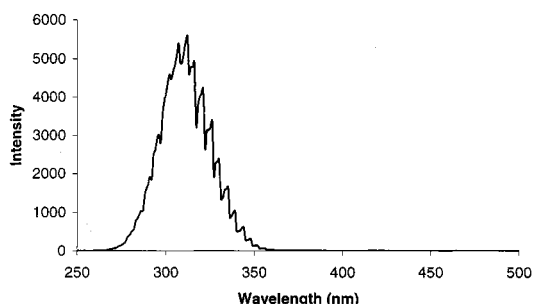
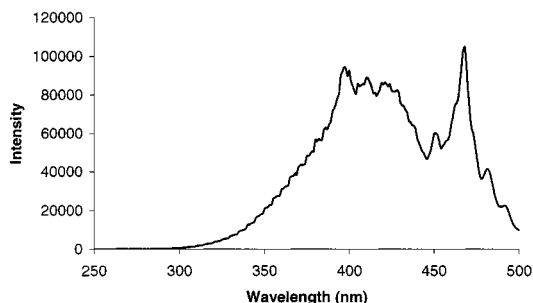


Fig. 5. Scattering patterns in synchronous spectra of cyclohexane. Top: $\Delta\lambda = 3$ nm, SBS = 4 nm. Bottom: $\Delta\lambda = 25$ nm, SBS = 4 nm. Thin line, experimental; thick line, simulated.

- The width: The OBS is predicted using a hyperbolic function of the NBS/SBS ratio.
- The intensity: The intensity is adjusted, in agreement with the absorption spectrum and the Franck–Condon principle.

The concentration c and the molar absorptivity ϵ at excitation wavelength are then introduced in Eq. (2) to compute F .

The excitation and emission bandwidths are used to predict intensities. For a continuous spectral source the throughput of a monochromator varies as the square of the slit width, but for a discrete source, the throughput varies as the slit width [12]. Experimentally, the fluorescence intensity was found to be

$$F \propto SBS^{1.8}$$

Step 2

Rayleigh scattering is then simulated with a gaussian band. Possibly, second-order Rayleigh scattering is introduced. According to the wavelength and the solvent, the Raman line λ_R is then added:

$$\lambda_R = 10^7 / [(10^7 / \lambda_e) + \nu_R] \quad (3)$$

where ν_R is the Raman shift (in wavenumbers).

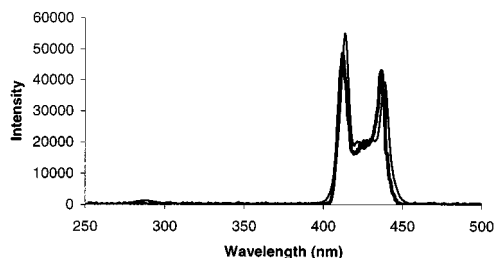
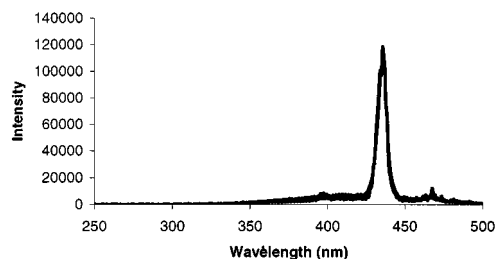


Fig. 6. Scattering patterns in synchronous spectra of perylene in cyclohexane. Top: $\Delta\lambda = 3$ nm, SBS = 4 nm. Bottom: $\Delta\lambda = 25$ nm, SBS = 4 nm. Thin line, experimental; thick line, simulated.

The excitation and emission bandwidths are used to predict scattering intensities. Experimentally, the scattering intensity was found to be

$$S \propto SBS^{1.4}$$

that is, a value between a discrete and a continuum source.

It was not possible to correlate the scattering intensity with wavelengths; also, we tabulate some typical values, and finally, the intensity is interpolated.

Step 3

According to the selected geometry, the inner filter and autoabsorption are then applied to the intensity [13]:

$$I'_0 = I_0 \cdot 10^{-\epsilon(\lambda_e)cd} \quad (4)$$

Equation (4) represents absorption of the excitation beam in the cell.

$$F' = F \cdot 10^{-\epsilon(\lambda_m)cd} \quad (5)$$

Equation (5) gives us the absorption of the luminescence spectrum.

Step 4

The spectral response of the detector and the spectral distribution of the source distort the observed spectra [1,2,14]. If the spectrum is not corrected, the correspond-

ing correction curve is then applied to distort the simulated spectrum. If the corrections options are selected, the previously computed spectrum is obviously used without distortions.

Step 5

Finally, the high-voltage value is used to set up the gain and the noise. The noise is then smooth according to the "time constant" or "measurement time" in accordance with the scanning mode selected.

Figure 1 summarizes these steps and Figure 2 presents a comparison between experimental and simulated spectra for two characteristic SBS values.

Simulation of an Excitation Spectrum

The flowchart used for the simulation of an excitation spectrum is quite similar to Fig. 1, with some minor differences.

In particular, the Raman line is observed according to Eq. (6) instead of Eq. (3):

$$\lambda_R = 10^7 / [(10^7 / \lambda_x) - \nu_R] \quad (6)$$

The second marked difference is that the order of correction, that is, steps 3 and 4, is reversed.

With these assumptions, good simulations were obtained for excitation spectra, as shown in Fig. 3.

Simulation of a Synchronous Spectrum

Figure 4 displays the flowchart used for synchronous spectra. In step 1, using previously described procedures, an emission profile and an excitation profile are computed. Using the wavelength shift $\Delta\lambda$, their product is then calculated in step 2:

$$I_s(\lambda) = I_m(\lambda) \cdot I_x(\lambda - \Delta\lambda)$$

Step 3 deals with scattering. Introducing scattering is more tedious than in previous modes. Two cases must be considered.

- The wavelength shift and the SBS are similar: a coincidence between the two Rayleigh profiles occurs and a characteristic pattern can be observed (see Fig. 5). The lines standing between 450 and 500 nm are coming from the xenon lamp.
- The wavelength shift is greater than the SBS: a coincidence between the Rayleigh profile and the Raman may occur and a rather broad band can

be observed in Fig. 5. This band being very weak, the signal-to-noise ratio is not very high.

Figure 6 displays some experimental and simulated synchronous spectra of perylene in cyclohexane.

Generalization

Using perylene results, the rules of the knowledge base were deduced and implemented in the software. These rules were then extended to fluorescein and anthracene. The comparison between their simulated and their experimental spectra was quite fulfilling. Thus, the user may now estimate the fluorescence spectra of any of these compounds under any set of experimental conditions. For pedagogic purposes, the method was extended: a series of fictitious absorption spectra of polyaromatic hydrocarbons or dyes is generated and the user may simulate their fluorescence spectra.

CONCLUSION

During the development of a software package for self-formation in luminescence, a spectrometer simulator was written to acquire the experimental skill necessary for fluorescence. The comparison between simulated and experimental spectra was quite fulfilling. Thus, using this software, the user may now estimate the fluorescence spectra of any similar compounds under any set of experimental conditions.

REFERENCES

1. W. Karcher, J. Devillers, Ph. Garrigues, and J. Jacob (1991) in *Spectral Atlas of polycyclic aromatic compounds Vol. 3*, Kluwer Academic Publishers, Dordrecht, pp. 10–11; R. M. Williams (1993) in *EPA Newsletter*, **49**, 3–8.
2. National Bureau of Standards (1979) Certificate Standard Reference Material No. 936.
3. J. M. Lerner and A. Thevenon (1988) in *The Optics of Spectroscopy*, Instruments SA, Inc., pp. 21, 25.
4. T. Owen (1996) in *Fundamentals of Modern UV-Visible Spectroscopy: A Primer*, Hewlett-Packard Company, pp. 52–55.
5. F. C. Strong (1976) *Anal. Chem.* **48**, 2155–2161.
6. A. G. Marshall and F. R. Verdun (1990) in *Fourier Transforms in NMR, Optical, and Mass Spectroscopy: A User's Handbook*, Elsevier, Amsterdam, pp. 38–56.
7. H-H. Perkanpus (1992) in *UV-VIS Spectroscopy and Its Applications*, Springer-Verlag, Berlin, pp. 220–223.
8. C. A. Parker (1968) in *Photoluminescence of Solutions*, Elsevier, Amsterdam, pp. 15–22.
9. D. Rendell (1987) in *Fluorescence and Phosphorescence Spectroscopy*, ACOL, John Wiley, London, pp. 88–99.
10. Ref. 8, p. 26.
11. Ref. 8, pp. 13, 14.
12. Ref. 3, pp. 34, 36.
13. Ref. 8, pp. 220–234.
14. Ref. 8, pp. 246–261.