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Application of doubled-angle phase correction method to time-resolved step-scan FT-IR spectra

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Abstract

To increase the sensitivity with which time-resolved Fourier transform infrared (FT-IR) difference spectra are measured, the detector is often AC-coupled. Thus, the measured interferograms correspond to spectra with both positive and negative intensities. The presence of signed intensities presents problems for the standard Mertz and Forman phase correction methods. The Mertz Signed phase correction method was designed to handle signed intensities, but the smoothing inherent in calculating the phase angles at reduced resolution introduces other errors in AC-coupled spectra produced with this algorithm. These errors are evident as signal remaining along the imaginary axis after phase correction. A new approach to phase correction, the Doubled-Angle method, can directly correct the phases of transient AC-coupled spectra without the need for a DC interferogram [M.S. Hutson, M.S. Braiman, Appl. Spectrosc. 52 (1998) 974]. When this method was applied to the transient AC interferograms measured after photolysis of bacteriorhodopsin, the signal was fully rotated onto the real axis following phase correction. Here, we show that the Doubled-Angle method can be applied to time-resolved difference FT-IR spectra of halorhodopsin, a more demanding biological system due to its intrinsically small differential absorption signals.

Keywords: Double-Angle method; FT-IR spectra; Mertz Signed phase correction method

1. Introduction

Interferograms collected in FT-IR spectroscopy are never perfectly symmetric. Thus, upon Fourier transformation, a complex result is produced from the interferogram, i.e.,

$$F \cdot I(\delta) = b(\nu)e^{i\theta(\nu)}.$$

A phase correction method is then used to extract the intensity information, $b(\nu)$, from this complex representation of the spectrum. When $b(\nu)$ can be assumed to always be positive this is a straightforward task. However, if $b(\nu)$ is signed then the phase correction is ambiguous due to the equality:

$$(-A)e^{i\theta} = Ae^{i(\theta+\pi)}.$$

This is exactly the situation encountered when time-resolved FT-IR interferograms are collected in an AC-coupled configuration. AC coupling is typically employed to improve the sensitivity and accuracy with which small spectral changes can be measured and digitized.

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The Mertz phase correction method assumes that all of the signal intensity is positive [2]. Thus, for broad negative bands the calculated phase angle is π radians from the true phase angle. Applying the phase correction,

$$B(\nu) = \left[b(\nu) e^{i\theta(\nu)} \right] \cdot e^{-i[\theta(\nu)+\pi]}$$
$$= b(\nu) e^{-\pi} = -b(\nu),$$

produces a 'reflected peaks' pattern [3].

A modification of the Mertz method, the 'Mertz Signed', was proposed by McCoy and de Haseth for vibrational circular dichroism spectra with positive and negative bands [3]. Instead of assuming that $b(\nu) > 0$, the Mertz Signed method assumes that all true phase angles fall in the range $-\pi/2$ to $\pi/2$. Any frequency for which the calculated phase angle falls outside of this range is assigned to a negative real intensity. As McCoy and de Haseth showed with the phase correction of quarterwave plate/polarizer vibrational circular dichroism spectra, the Mertz Signed method correctly eliminates the reflection of negative peaks in spectra with broad bands [3].

While the Mertz Signed method can properly correct the phases of difference spectra with broad features, spectra containing sharp alternating bands of opposite sign pose a problem. This has been shown for a set of simulated difference spectra and the time-resolved step-scan difference spectra of the bacteriorhodopsin photocycle [1]. The problems encountered with the Mertz Signed method stem from using a reduced resolution for the phase calculation. For differential interferograms, this means that a discontinuous function is smoothed and intermediate phase angles are calculated. The restriction placed on the phase angle range by the Mertz Signed algorithm restores continuity, but in an incorrect manner, resulting in significant systematic errors.

To solve this problem, we devised the Doubled-Angle phase correction method [1]. In the first step of the phase calculation, the differential interferogram $\Delta I(\nu)$ is self-convolved, and the result is truncated. In spectral space, this is equivalent to squaring the complex spectrum, then reducing its resolution. Now, since $(b(\nu))^2 > 0$ and $e^{i2\theta} = e^{i2(\theta+\pi)}$, the phase angle discontinuities have been eliminated. An advantage of doing the angle doubling in interferogram space (rather than spectral space) is that it permits accurate determination of the point of Zero Path Difference (ZPD) of the interferogram. In differential interferograms, the ZPD is not necessarily at an extremum of $\Delta I(\delta)$. However, the ZPD is always at the maximum of the self-convolution, $\Delta I \otimes \Delta I$. The Fourier transform can be applied to $\Delta I \otimes \Delta I$ at reduced resolution without the introduction of intermediate phase angles. Once $\sin 2\theta'$ and $\cos 2\theta'$ have been calculated, the half-angle formulas can be used to find $\sin\theta'$ and $\cos\theta'$. The choice between the two possibilities for θ' at each frequency is made on the basis of continuity of the phase spectrum. This means that when the final phase corrected spectrum is produced, the bands will be oriented correctly relative to one another, but the global orientation is ambiguous. The choice of whether to 'flip' the spectrum must be made on the basis of some a priori knowledge of the difference spectrum being measured.

2. Materials and methods

Purified halorhodopsin (hR) samples were prepared as described previously from membranes of halobacteria [4]. The purified hR was washed with a 10 mM HEPES, 50 mM KBr, pH = 7 solution several times. After concentrating it by centrifugation, 25 μl (~ 250 μg of hR) were pipetted onto a BaF_2 window (25 mm diameter × 2 mm thick). The hR sample was then dried with a gentle stream of N_2. Once most of the water had been removed from the sample, a second BaF_2 window was coated around its edge with vacuum grease and pressed against the first to seal the sample. The hR sample is thus in a nearly saturating KBr solution.

Interferograms were collected on a Bruker IFS-66 FT-IR spectrometer in time-resolved step-scan mode with a photoconductive HgCdTe detector. The detector's preamplifier and the internal digitizer were used in an AC-coupled configuration throughout. The data were collected at 16 cm^{-1} resolution over a 0–3940 cm^{-1} bandwidth. A long-pass filter was placed in front of the detector to block the light from the laser flash and to prevent aliasing. A pulsed, frequency-doubled Nd^+:YAG laser (532 nm @ 3 Hz) was used to trigger the hR photocycle for the time-resolved FT-IR measurements. The signals from 20 flashes

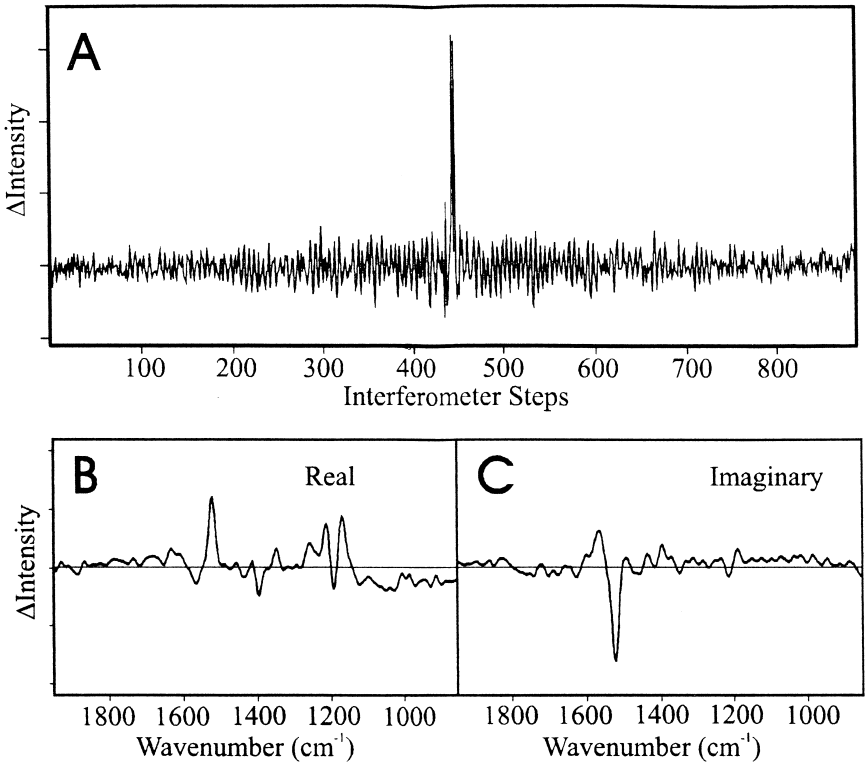

Fig. 1. Time-resolved step-scan FT-IR measurement of halorhodopsin photoreaction. (A) The differential interferogram shown was collected at 16 cm^{-1} resolution over the first 2 ms after a Nd^{+}:YAG laser flash (see Section 2 for more details). After identification of the ZPD, Fourier transformation of (A) yields the complex spectrum shown in (B) and (C).

were averaged at each mirror position to produce transient differential interferograms at intervals of 25 μs. The 80 interferograms corresponding to delay times of 25–2000 μs after the flash were averaged to produce the higher signal-to-noise-ratio (S/N) differential interferogram shown in Fig. 1.

3. Results and discussion

Fig. 1A shows the time-resolved differential interferogram obtained from transient signals collected during the first 2 ms after halorhodopsin photolysis. After Fourier transformation at 16 cm^{-1} resolution, but before phase correction, the spectral information is split almost evenly between the real (Fig. 1B) and imaginary (Fig. 1C) axes.

The Mertz, Mertz Signed and Doubled-Angle phase correction methods were then applied to this differential interferogram. For each method, we used phase spectra calculated at four different resolutions and then interpolated to the original 16 cm^{-1} resolution of the data. In the top 2 panels of Fig. 2, the phase correction at 16 cm^{-1} resolution rotates almost all of the spectral information onto the real axis regardless of which method is used, but the calculated spectra for the three methods are all different. The Mertz spectrum contains only positive bands due to the reflection of negative peaks discussed earlier. The Mertz Signed algorithm actually does a fine job of flipping the negative bands back to the correct orientation. The Doubled-Angle method produces a spectrum very similar to the Mertz Signed method, except for in the region above 1600 cm^{-1}. One can see in Fig. 3 that in this region at 16 cm^{-1} phase resolution, the poor S/N results in an error of π radian in the phase angle calculated with the Doubled-Angle method.

However, if the phase resolution is reduced to improve the S/N ratio, then the error in the Dou-

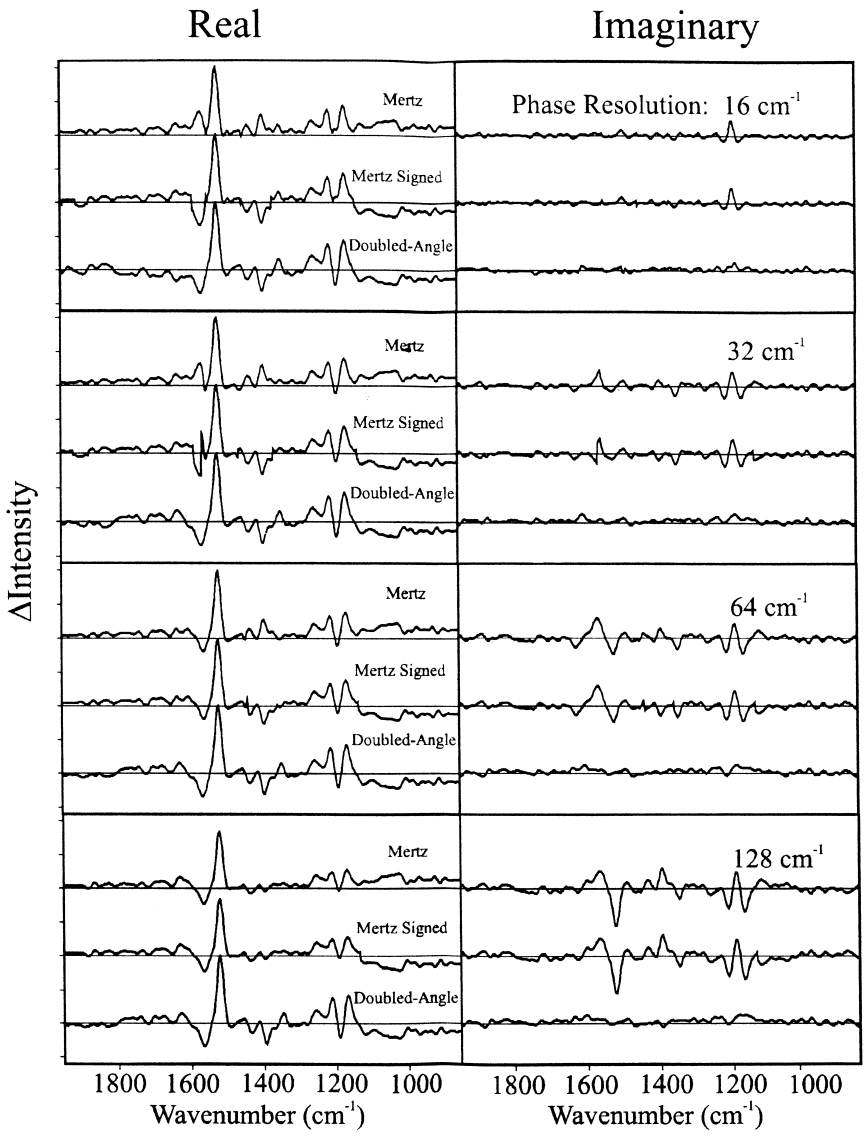

Fig. 2. Evaluation of three phase correction procedures applied to the time-resolved differential interferogram of the halorhodopsin photocycle shown in Fig. 1. The real and imaginary spectra after phase correction are shown for each method at four different phase resolutions.

bled-Angle phase calculation is eliminated (see e.g., Fig. 2, Doubled-Angle correction at 32 cm^{-1} resolution). At the same time, all of the spectral information continues to be aligned along the real axis. As the resolution is reduced even further, the real and imaginary spectra produced with the Doubled-Angle method change very little (See Fig. 2, 64 cm^{-1} and 128 cm^{-1} results).

On the other hand, the story is very different for the Mertz and Mertz Signed methods. As the resolution is progressively reduced, more and more spectral information remains imaginary. The Mertz algorithm properly reflects only a subset of the negative bands as the phase resolution drops towards 128 cm^{-1}, and the Mertz Signed algorithm produces odd-shaped bands of reduced magnitude along the

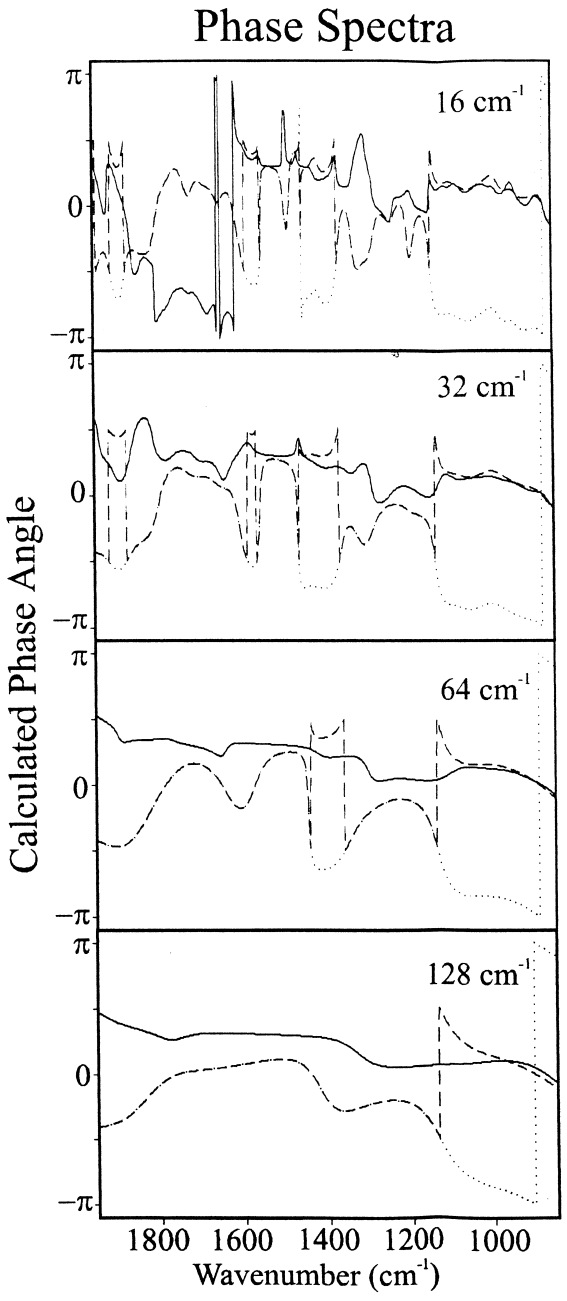

Fig. 3. Phase spectra calculated with the three different phase correction procedures applied to the time-resolved, differential interferogram of the halorhodopsin photocycle shown in Fig. 1. The phase spectra are shown for each method at four different phase resolutions: Mertz (dotted), Mertz Signed (dashed) and Doubled-Angle (solid). Between $-\pi/2$ and $\pi/2$, the Mertz and Mertz Signed algorithms produce the same spectrum. Thus, the Mertz curve is obscured by the Mertz Signed curve.

real axis. The negative band at ~ 1435 cm^{-1} is actually lost entirely. The phase spectra of Fig. 3 show that although the S/N increases, more intermediate phase angles are calculated with the Mertz and Mertz Signed methods as the resolution is reduced.

We first demonstrated the applicability of the Doubled-Angle phase correction method to time-resolved difference interferograms of bacteriorhodopsin (bR) photoproducts [1]. Halorhodopsin is a more demanding system for several reasons. First, it cannot be prepared in a concentration comparable to bR. Second, its photochemical quantum yield is reduced (2–5 ×) [4]. Third, it is more susceptible to irreversible photobleaching at high laser powers [4]. All of these characteristics play a role in reducing the absorption changes for hR by a factor of 5–10 × as opposed to bR. The S/N attainable in time-resolved difference spectra of halorhodopsin photoproducts is therefore considerably less than the S/N of similar measurements of bR. We demonstrate here that the Doubled-Angle phase correction method is able to properly phase correct even these lower S/N difference spectra. Only at a phase resolution equal to the full spectral resolution (16 cm^{-1}) is this method unable to produce the correct spectra. As the phase resolution is reduced towards 128 cm^{-1}, phase correction by the Doubled-Angle method improves, while phase correction by the Mertz and Mertz Signed methods does not. Thus, the Doubled-Angle method can be used on difference interferograms with moderate S/N, as long as the S/N of the phase angle calculation is improved by using a reduced phase resolution.

Acknowledgements

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