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# Fringes in FTIR spectroscopy revisited: understanding and modelling fringes in infrared spectroscopy of thin films

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Abbreviations: Extended multiplicative signal correction (EMSC), Fourier transform (FT)

## Abstract

The appearance of fringes in the infrared spectroscopy of thin films seriously hinders interpretation of chemical bands because fringes change relative peak heights of chemical spectral bands. Thus, for the correct interpretation of chemical absorption bands, physical properties need to be separated from chemical characteristics. In the paper at hand we revisit the theory of the scattering of infrared radiation at thin absorbing films. While in general scattering and absorption are connected by a complex refractive index, we show that for the scattering of infrared radiation at thin biological films, fringes and chemical absorbance can in good approximation be treated as additive. We further introduce a model-based pre-processing technique for separating fringes from chemical absorbance by Extended Multiplicative Signal Correction (EMSC). The technique is validated by simulated and experimental FTIR spectra. It is further shown that EMSC, as opposed to other suggested filtering methods for removal of fringes, does not remove information related to chemical absorption.


Key Words: FTIR spectroscopy, fringes, thin film scattering, zero-filling, EMSC

## Introduction

Thin film transmission measurements are a frequently used sampling technique in infrared spectroscopy of biological materials. Thin film transmission measurements are for example applied in the infrared spectroscopy of microorganisms[1-5] and cells[6], where dried thin films of microorganisms and cells are prepared on infrared-transparent sample holders. Infrared spectra are obtained by transmitting infrared radiation through the thin films and the infrared-transparent sample holders. Thin films of the solid matter of liquids, such as blood or milk samples, can be obtained by drying the samples to form thin films on sample holders.[7, 8] Further, thin films are obtained when sections of tissues are placed on infrared transparent materials such as ZnSe or $CaF_2$[9].

The strongest non-chemical variations in the Fourier Transform Infrared (FTIR) spectroscopy of thin dried films are due to differences in the sample thicknesses of thin films or tissue sections. Since the penetration depth of infrared radiation into biological material is of the order of a few microns, thin films of typically 6-10 μm need to be prepared for infrared transmission spectroscopy. When samples with varying thicknesses are to be compared, the variation in sample thickness leads to variations in the effective optical path length, which can be effectively estimated and suppressed by EMSC[10-12]. Another non-chemical interference pattern that is frequently encountered in the FTIR spectroscopy of thin films are sinusoidal modulations in FTIR spectra called 'fringes'[13]. The fringes usually result from reflections inside the sample or sample holder or from reflections between the sample holder and the sample. These reflections lead to additional interferences between the two infrared beams inside an FTIR spectrometer. In an interferometer, the main interference appears at the so-called Zero Path Difference (ZPD) position of the interferometer and is called center burst. Center bursts occur when the two mirrors of the FTIR spectrometer are at the ZPD position. The fringes create additional spikes (side bursts) which are usually located close to the central spike, the center burst. These spikes appear in the interferogram space and are transformed to the sinusoidal waves in the spectrum domain by means of a Fourier Transform (FT)[13]. They can seriously hinder interpretation of the chemical bands in FTIR spectroscopy because they change relative peak heights of chemical spectral bands.

For the interpretation of chemical absorption bands, physical properties such as effective optical path length variations and fringes need to be separated from chemical characteristics. If the separation of physical and chemical information is omitted or done incompletely, physical characteristics might be misinterpreted

as biochemical information. The literature includes many different techniques for removing fringes. A commonly used approach is the so-called interferogram editing. Side bursts are removed from the interferogram via filling regions containing bursts with zeros or by applying straight line interpolation[14, 15]. However, interferogram editing has disadvantages. First, it is often difficult to locate the spikes in the interferogram without employing special procedures for identification of spikes and removing them. A more fundamental objection against zero-filling of regions containing spikes is a possible loss of spectral information. This is because each spike contains also relevant information, which is removed when the spike is zero-filled. Another approach for the removal of interference fringes was presented by Clark and Moffatt[16]. In their approach, fringes are fitted by the use of a sinusoidal function. A sinusoidal wave and a baseline are generated after manual determination of the required parameters and subsequently subtracted from the spectrum containing fringes. The disadvantages of this method are that parameters such as frequency and amplitude have to be estimated manually and that the algorithm is only applicable to regions of low absorptivity. The reason for the latter is that regions with strong absorptivity lead to a bias in the estimation of the fitting parameters. Further, it has been proposed to use a modified background interferogram to cancel fringes in the transmission spectrum[17]. According to this technique a synthetic background interferogram is created, which contains the same fringes as the recorded sample spectrum. It can be reached via editing the sample interferogram and replacing it with zeros except at side burst and center burst positions. This modified interferogram is then transformed into a background spectrum by FT. By subtraction of this background spectrum, a fringes-free transmittance spectrum is obtained. As for the zero-filling, the disadvantage of this approach is that relevant information is removed, since spectral information at the bottom of the side and center burst spikes is eliminated by subtracting the background spectrum based on the fringes. Faggin and Hines[18] suggested a method for handling interference fringes using a combination of digital filtering by the Savitzky-Golay algorithm[19] and a boxcar function in combination with Fourier analysis. After application of the Savitzky-Golay algorithm, spectral features are expected to show sharper features than the fringes and are separated by using a boxcar function. In a second step, further cleaning of the spectrum is performed by visually identifying regions of Fourier-transformed center and side bursts in the spectrum and by filling these regions with zeros. The drawback of this method is that, as for previously discussed methods, useful information is suppressed both by the filtering and by the zero filling.

Other techniques utilize the fact that fringes have much lower frequencies than the recorded spectra and use filtering techniques either in the spectral domain or in the Fourier domain. For example, series expansion in the spectral domain[20], or filtering in the Fourier (frequency) domain[21] has been employed to remove low frequency terms. While filtering in the Fourier domain has again the drawback that useful information is very likely to be removed, series expansion in the spectral domain suffers from the same insufficiency, namely that chemical information is removed together with the low frequency fringes.

Also instrumental techniques for removing fringes have been applied[22, 23]. In this approach, the sample is rotated to achieve maximum transmittance (minimum reflectance) for parallel polarization, which is achieved when the angle of incidence is the so-called Brewster angle[24]. The infrared beam is then polarized parallel to the plane of incidence. Under these conditions there is no reflection and, hence, no interference. However, the Brewster angle is absorptivity-dependent and will provide an accurate spectrum only in regions of low absorptivity[16].

While all above discussed approaches are based on filtering, Extended Multiplicative Signal Correction (EMSC) is a so-called model-based approach allowing the separation of different effects in infrared spectroscopy of biological materials[10]. EMSC allows quantifying different types of physical and chemical variations in spectra. It is thus enabling the scientists to study the different effects separately. It has been successfully used for removing variations due to the effective optical path length, scattering effects due to Mie scattering in single cell scattering[25] and variations due to water in thin films[26].

In the paper at hand, we introduce a technique for reducing fringes by EMSC. We will show how spectral information in spectra containing fringes is preserved by EMSC. In the Methods section, we introduce EMSC for fringe removal. To this purpose, we review exact electromagnetic calculations for the description of scattering of electromagnetic radiation at a thin film. In order to treat scattering and absorption at a thin film exactly, we will consider a non-constant and complex refractive index when establishing the electromagnetic theory. In the Results section, the method based on EMSC is tested with simulated and experimental FTIR spectra.

## Basic definitions

In Fig. 1 the transmission measurement of a thin film sample is illustrated. We denote the infrared radiation intensity that is incident on the film as $I_0$. When incident radiation at a given wavenumber hits the

film, it may be scattered as indicated by the red arrow to the left of the film, absorbed by an absorbing radiation sink as illustrated by the red area or transmitted as depicted by the red arrow to the right of the film. Therefore, the transmitted intensity is in general attenuated by both scattering and absorption. We denote the transmitted intensity by $I$ and the scattered intensity by $I_{sca}$. Experimentally the transmitted intensity $I$ is obtained by confining a measurement area by the aperture $G$ in front of the detector (see Fig. 1). The incident intensity or background intensity $I_0$ is obtained by removing the thin film out of the infrared radiation path. The transmittance $T$ is further defined as the ratio of the incident intensity $I_0$ and the transmitted intensity $I$

$$T = \frac{I}{I_0} \quad (1)$$

Finally the absorbance $A$ is calculated as

$$A = -\log_{10}(T). \quad (2)$$

When the incident radiation is attenuated by scattering and absorption, the transmittance $T$ is reduced at the respective wavenumbers. When analysing the obtained FTIR spectra, the scientist is at a loss to decide if the attenuation is due to scattering or absorption.

For the general case of scattering at a finite-size, arbitrarily shaped object, the quantity $I_{sca}$ is a vector, generally pointing in the radial direction and therefore not directly useful for studies concerned with the forward direction. In the case of the film however, $I_{sca}$ is naturally (anti-)parallel to the forward direction and therefore a useful quantity in the thin-film scattering case. The thicknesses of thin films, as denoted by $l$, are typically of the order of a few microns, since thicker films are opaque for infrared radiation . The absorbed intensity $I_{abs}$ has to be carefully distinguished from the scattered intensity $I_{sca}$. While the scattered radiation is emitted instantaneously, the absorbed radiation is converted into internal energy of the film. This internal energy might be conveyed by conduction to the environment, e.g. to the sample holder or to the air. It is true that the internal energy might also be re-emitted as thermal radiation with the same or a different frequency, described by Planck's radiation law [27]. Still, it cannot be counted as scattered radiation, because the re-emission happens with a time delay.

The scattering of infrared radiation at a thin film can be described exactly, even when the film is absorbing, i.e. not transparent in the infrared region of the radiation. Theoretical derivations can be found in appendix A. It can be shown that for non-magnetic materials the scattered intensity $I_{sca}$ can be written as

$$I_{sca} = I_0|r|^2, \quad (3)$$

where $r$ is the reflection amplitude given by

$$r = \frac{(1-\hat{n}^2)\sin(2\pi\hat{n}\tilde{\nu}l)}{(1+\hat{n}^2)\sin(2\pi\hat{n}\tilde{\nu}l)+2i\hat{n}\cos(2\pi\hat{n}\tilde{\nu}l)}. \quad (4)$$

For the transmitted intensity $I$ we obtain

$$I = I_0|t|^2, \quad (5)$$

where $t$ is the transmission amplitude given by

$$t = \frac{2i\hat{n}e^{-2\pi i\tilde{\nu}l}}{(1+\hat{n}^2)\sin(2\pi\hat{n}\tilde{\nu}l)+2i\hat{n}\cos(2\pi\hat{n}\tilde{\nu}l)}. \quad (6)$$

Here $\hat{n}$ is the refractive index, $l$ is the thickness of the film and $\tilde{\nu}$ is the wavenumber of the incident radiation. The chemical absorption is taken into account by the imaginary part of $\hat{n}$. Via Eqs. 1, 2, 5 and 6 the transmittance $T$ and the absorbance $A$ can be calculated. Contrary to the ideal conditions in our model, the front and back surface of the tissue sections are usually rough and not ideally parallel. Still, fringes are often observed in FTIR spectroscopy of biological materials. Other parameters such as the beam convergence and polarisation of the radiation may also influence the fringes pattern[28].

When we consider biological thin films the refractive index is a complex function. The complex refractive index is defined in the following way: $\hat{n}(\tilde{\nu}) = n(\tilde{\nu}) + in'(\tilde{\nu})$, where $n(\tilde{\nu})$ is the real part of the refractive index depicting the refractive properties of the material. The $n'(\tilde{\nu})$ function is the imaginary part of the refractive index and describes the absorptive properties of the material. The real and the imaginary parts of the complex refractive index $\hat{n}$ automatically fulfil the Kramers-Kronig relation

$$n(\tilde{\nu}) = n_0 + \frac{2}{\pi}\mathbf{P}\int_0^\infty \frac{s\cdot n'(s)}{s^2-\tilde{\nu}^2}\,ds, \quad (7)$$

where the symbol **P** denotes the Cauchy principal value of the integral. In general we cannot assume a constant and real refractive index. When chemical absorption bands are present, as in the infrared spectroscopy of biological materials, the refractive index $\hat{n}$, which enters into Eqs. 4 and 6, has a non-zero imaginary part and both, the real and imaginary parts, depend on the wavenumber. The imaginary part of the refractive index has the shape of an absorption band. In the case of a thin film, and neglecting the scattering, the absorbance $A$ is related to the imaginary part of the refractive index (see Eq.A21). The $n'(\tilde{\nu})$ can be calculated in the following way

$$n' = \frac{A \cdot \ln(10)}{4\pi l \tilde{\nu}}. \quad (8)$$

# Method

*Extended Multiplicative Signal Correction (EMSC)*

Extended multiplicative signal correction is a model-based pre-processing technique providing the possibility to separate physical effects and chemical information in infrared spectra and to investigate these separately. The main idea of EMSC is that in the first pre-processing step, every spectrum is represented with respect to a reference spectrum $m(\tilde{\nu})$ [10]. For the correction of fringes we suggest to extend the EMSC model by sinusoidal terms according to

$$A(\tilde{\nu}) = a + b \cdot m(\tilde{\nu}) + d_1 \cdot \cos(x\tilde{\nu}) + d_2 \cdot \sin(x\tilde{\nu}) + e \cdot \tilde{\nu} + \varepsilon(\tilde{\nu}) \quad (9)$$

where $b$ is the fitting parameter corresponding to $m(\tilde{\nu})$, describing the multiplicative variation of the sample spectrum with respect to the reference spectrum $m(\tilde{\nu})$. The parameter $a$ in Eq. 9 describes constant baseline variations. The term $\varepsilon(\tilde{\nu})$ accounts for random measured noise and unmodeled spectral structures. The terms $d_1 \cdot \cos(x\tilde{\nu})$ and $d_2 \cdot \sin(x\tilde{\nu})$ describe periodic baseline effects due to fringes, where $2\pi/x$ corresponds to one period in the wavenumber domain. The term $e \cdot \tilde{\nu}$ describes linear effects. In order to account for the unknown phase of the fringes, it is essential to keep both terms $d_1 \cdot \cos(x\tilde{\nu})$ and $d_2 \cdot \sin(x\tilde{\nu})$. The EMSC model in Eq. 9, may be further extended by a quadratic term when quadratic effects are present in the spectra[10]. We will in the course of the paper show that the extension by the sinusoidal terms is justified and in good agreement with the rigorous theory provided in the previous section.

An important comment concerns the reference spectrum $m(\tilde{\nu})$ as part of the EMSC model. Infrared spectra of biological materials have a very typical shape with visually not too strong shape variations when comparing samples of similar origin. For example, when comparing spectra of different microbial thin films[5], only tiny, but very reproducible differences are due to chemical differences. In fact, the major visual differences are due to variations in the effective optical path lengths and other physical differences. This justifies the model shown in Eq. 9. In this respect, the term related to the reference spectrum in Eq. 9, is contributing as a stabilizing part in the estimation of the fitting parameters. Chemical variations correspond to slight variations around the reference spectrum and are contained in the un-modelled residual $\varepsilon(\tilde{\nu})$. The reference spectrum assures that the parameters in the other terms in Eq. 9 are not, or only to a very small degree, biased by chemical absorption bands.

While the absorbance $A$ in Eq. 9 is written as a function of the wavenumber $\tilde{\nu}$, a measured spectrum is digitalized and available for discrete values of wavenumbers $\tilde{\nu}_j$. Denoting the vector related to the measured and digitalized absorbance spectrum by

$$\boldsymbol{a} = \begin{pmatrix} A(\tilde{\nu}_1) \\ A(\tilde{\nu}_2) \\ . \\ . \\ A(\tilde{\nu}_k) \end{pmatrix}, \quad (10)$$

defining the matrix of model spectra by

$$\boldsymbol{M} = \begin{pmatrix} 1 & m(\tilde{\nu}_1) & \cos(x\tilde{\nu}_1) & \sin(x\tilde{\nu}_1) & \tilde{\nu}_1 \\ 1 & m(\tilde{\nu}_2) & \cos(x\tilde{\nu}_2) & \sin(x\tilde{\nu}_2) & \tilde{\nu}_2 \\ . & . & . & . & . \\ . & . & . & . & . \\ . & . & . & . & . \\ 1 & m(\tilde{\nu}_K) & \cos(x\tilde{\nu}_K) & \sin(x\tilde{\nu}_K) & \tilde{\nu}_K \end{pmatrix}, \quad (11)$$

and collecting the fitting parameters in Eq. 9 into one vector

$$\boldsymbol{p} = \begin{pmatrix} a \\ b \\ d_1 \\ d_2 \\ e \end{pmatrix}, \quad (12)$$

we can write Eq. 9 as a matrix equation $\boldsymbol{a} = \boldsymbol{M}\boldsymbol{p} + \boldsymbol{E}$. Here we have used the convention commonly used in chemometrics, that vectors are denoted by bold, lower case letters and matrices by bold, upper case letters. The frequency $\tilde{\nu}_0 = 2\pi/x$ and consequently $x$ are determined by Fourier transform. When the period in the Fourier domain $\tilde{\nu}_0 = 2\pi/x$ is known, the parameter vector $\boldsymbol{p}$ can be found by least squares regression of each the spectrum vector $\boldsymbol{a}$ onto $\boldsymbol{M}$. After the vector of unknown parameters $\boldsymbol{p}$ has been estimated, the corrected spectra can be calculated according to

$$A_{corr}(\tilde{\nu}) = \frac{A(\tilde{\nu}) - a - d_1 \cdot \cos(x \cdot \tilde{\nu}) - d_2 \cdot \sin(x \cdot \tilde{\nu}) - e \cdot \tilde{\nu}}{b}. \quad (13)$$

In order to determine the period in the Fourier domain $\tilde{\nu}_0 = 2\pi/x$ we compute the Discrete Fourier Transform (DFT) $\tilde{A}(k)$ of the sampled signal according to

$$\tilde{A}(k\Delta x) = \sum_{n=0}^{N-1} A(\tilde{\nu}_n) \cdot \exp\left(-i\frac{2\pi nk}{N}\right), \quad (14)$$

where $N$ is the number of sampling points, $k = 0 \ldots N-1$.

Each $\tilde{A}(k\Delta x)$ is a complex number that encodes the amplitude of a sinusoidal component of the function $A(\tilde{\nu}_n)$. Its amplitude is calculated according to

$$\left|\tilde{A}(k\Delta x)\right| = \sqrt{\left[Re(\tilde{A}(k\Delta x))\right]^2 + \left[Im(\tilde{A}(k\Delta x))\right]^2}. \quad (15)$$

The frequencies in the Fourier domain are separated by a step

$$\Delta x = \frac{2\pi}{N \cdot \Delta\tilde{\nu}}, \quad (16)$$

where $N$ is the number of sampling points and $\Delta\tilde{\nu}$ is the wavenumber step.

For calculating the Inverse Discrete Fourier Transform (IDFT) we used

$$A(\tilde{\nu}_n) = \frac{1}{N}\sum_{k=0}^{N-1} \tilde{A}(k\Delta x) \cdot \exp\left(i\frac{2\pi nk}{N}\right). \quad (17)$$

## Materials and FTIR spectroscopy

For FTIR spectroscopy samples from beef muscle (*longissimus dorsi*) were embedded in an optical cutting temperature (OCT) compound (Tissue-Trek, Electron Microscopy Sciences, Hatfield, PA), and snap-frozen in liquid $N_2$. Samples were cryo-sectioned with 10 µm thickness, and subsequently thaw-mounted on infrared transparent ZnSe slides. Spectra were acquired with IR Scope II connected to an Equinox 55 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany), equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. IR spectra were acquired from single myofibers in transmission mode in the range from 6000 $cm^{-1}$ to 800 $cm^{-1}$. The details of the sample preparation and the spectral acquisition are described elsewhere[29].

## Results and discussion

In Fig. 2a an absorbance spectrum of a beef muscle tissue section is shown. The spectrum contains mainly pure chemical absorbance bands and is nearly scatter free. Only slight baseline variations are visible. In order to test the EMSC model in Eq. 9 for estimating and removing fringes, we simulated spectra containing fringes. For the case that no absorption bands are present, fringes can be easily simulated with the help of Eqs. 1, 2, 5 and 6. The result is shown in Fig. 2b, where for the calculation of the transmittance $T$ a real refractive index of $\hat{n} = n_0 = 1.33$ and a film thickness of $l = 4.3\ \mu m$ were used. Since according to Eq. A13 only $|t|^2$ enters into the computation of the absorptivity $A$, the periodicity of $A$ is immediately obvious from Eq. 6. Accordingly, the fringes in Fig. 2b are visibly periodic. However, what is not obvious from Eq. 6 is that, as stated in Eq. 9, the periodicity may be represented by simple sine and cosine functions.

This is motivated in Appendix B, where we show that replacing the exact electromagnetic result for the absorbance $A$ (see Eqs. 1, 2, 5 and 6) by simple sinusoidal functions in the EMSC model (see Eq. 9) is justified and results in only a very small error.

Another approximation, which is frequently employed, concerns the treatment of scattering and absorption as additive in the absorbance spectrum. By simply adding the simulated fringes (see Fig. 2b) to the measured absorbance spectrum (see Fig. 2a), an approximated absorbance spectrum with fringes can be obtained. The result is shown in Fig. 2c. We refer to this spectrum as the *absorbance spectrum with additive fringes.* It is important to note that the fringes contained in the spectrum in Fig. 2c are obtained according to Eqs. 1, 2, 5 and 6 employing a constant refractive index. This means that the periodicity of the fringes is exact and not an approximation. In the absorbance spectrum with additive fringes in Fig. 2c, fringes are clearly visible in regions without chemical absorption. Their effect on other regions is also obvious, such as the region between 1500 $cm^{-1}$ and 1000 $cm^{-1}$, where a maximum in the fringes spectrum causes a rise of the baseline.

As biological materials absorb infrared radiation, in general, the imaginary part of the refractive index is different from zero and has the shape of an absorption band. Starting from a practically scatter-free thin-film absorbance spectrum (see Fig. 2a), the imaginary part $n'$ of the complex refractive index was calculated from the absorbance spectrum $A$ according to Eq. 8. After $n'$ was determined, the Kramers-Kronig relation (Eq. 7) was used to calculate the real part $n$ of the refractive index. With $n'$ and $n$ at hand, the complex refractive index was determined for the whole wavenumber region and Eqs. 1, 2, 5 and 6 were used for the calculation of an absorbance spectrum simulating the scattering and absorption of infrared radiation at a perfect thin film (with equidistant surfaces). We refer to this spectrum as the *absorbance spectrum with dispersive fringes*, since a complex refractive index accounting for scattering and absorption was used as input for the exact electromagnetic theory according to Eqs. 1, 2, 5 and 6. As film thickness and constant refractive index, $l = 4.3\ \mu m$ and $n_0 = 1.33$ were used, respectively. The exact absorbance spectrum with fringes is shown in Fig. 3 (in red) together with the absorbance spectrum with additive fringes shown in Fig. 2c, which was obtained by treating scattering and absorption as additive. In addition, the difference spectrum between the exact spectrum and the approximated spectrum is shown in black. It can be seen that the agreement between the exact and the approximated spectrum is good. Still there are differences visible, especially in regions with high absorbance. These differences are due to dispersion generated by absorption.

Each absorption band results in a non-zero imaginary part of the refractive index and generates additional fluctuations in the real part of the refractive index according to the Kramers-Kronig relation in Eq. 7. These refractive index fluctuations generate the variations shown in the black curve in Fig. 3. It is obvious that the dispersive effect of the absorption bands is much smaller for fringes compared with what is observed in Mie scattering[30]. Since the dispersive effect due to absorption is small for the scattering of infrared radiation at thin biological films, the signatures of multibeam interference, scattering and absorption in fringes spectra are commonly considered as additive contributions. The EMSC model, which treats fringes and absorption as additive effects in Eq. 9, and all correction models discussed in the introduction of this paper, are justified within this approximation.

In order to separate fringes from pure absorbance, the parameters of Eq. 9 need to be estimated. This is accomplished in three steps. First, the frequency in the wavenumber domain $x$ is estimated in the Fourier domain in order to establish the EMSC model of Eq. 9. Then, the linear parameters in Eq. 9 are estimated by regressing the EMSC model spectra onto the spectrum with fringes. Finally, fringes are separated from the pure absorbance spectrum according to Eq. 13. All three steps will be illustrated in the following sections by simulated and actual experimental data.

*EMSC correction of a simulated fringes spectrum*

In order to estimate the period of the fringes in the wavenumber domain, we considered the Fourier transform (FT) (Eqs. 14-17) of the spectra shown in Fig. 2. The Fourier transforms of the spectra of Figs. 2a, b and c are shown in Figs. 4a, b and c, respectively. While the Fourier transforms of the spectra in Figs. 2a and 2c show non-zero amplitudes for a large range of frequencies (see Figs. 4a and 4c), the Fourier transform of the pure fringes spectrum of Fig. 2b, which is shown in Fig. 4b, shows only one distinct amplitude surrounded by a low magnitudes ringing effect at frequency $x \approx 0.007$. The low amplitudes are due to a finite signal. This frequency obviously corresponds to the frequency of the fringes shown in Fig. 2b. Since fringes shown in Fig. 2b are present in the approximate absorbance spectrum of Fig. 2c, we expect that the amplitude of the frequency $x \approx 0.007$ in Fig. 4c is elevated compared to Fig. 4a. Although a slight elevation of the amplitude of the frequency $x \approx 0.007$ can be seen in Fig. 4c, there are several other frequencies in Fig. 4c with higher amplitudes. Thus, the Fourier transform in Fig. 4c cannot be used for the identification of the frequency of the fringes. It stands therefore to reason that the Fourier transform of the

region from 6000 $cm^{-1}$ to 3800 $cm^{-1}$ of the approximate absorbance spectrum of Fig. 2c (red region) could reveal the frequency of the fringes. The Fourier transform of the region from 6000 $cm^{-1}$ to 3800 $cm^{-1}$ of the approximate absorbance spectrum of Fig. 2c (red region) is shown Fig. 4d. Since the region from 6000 $cm^{-1}$ to 3800 $cm^{-1}$ of the approximate absorbance spectrum of Fig. 2c does not show any absorption bands we may have expected that we obtain one single frequency which is related to the frequency of the fringes as in Fig. 4b. Yet, this is not the case. While most of the frequencies now show zero amplitudes in Fig. 4d, there are still more than one frequency with high amplitudes. The cause for this discrepancy is the so-called leakage effect[31-33]. The leakage effect appears, when the signal frequency coincides with the sample frequency. It results in a more complex Fourier spectrum with several non-zero frequencies, instead of a spectrum showing only one distinct peak. The FT algorithm assumes a periodic extension of the analyzed fragment of the signal. If the recording segment has an integer number of cycles, as in Fig. 2b, then a periodically extended signal is a continuous sinusoidal function and its amplitude spectrum contains only one non-zero frequency. If the number of cycles in the recorded segment is not integer as in Fig. 2c (in red), then the periodically extended signal is a discontinuous sinusoidal function and the spectral leakage effect occurs. Therefore, we obtain a range of frequencies when applying the Fourier transform to the truncated region (red) of Fig. 2c.

In addition, in Fig. 4d, a picket-fence effect[31-33] can be observed. In Fig. 4d we note that the expected frequency for the fringes ($x = 0.007$) is located between two neighboring peaks at $x = 0.0057$ and $x = 0.0086$. In order to overcome the picket-fence effect we change the number of points *N* in the recorded signal segment by adding zeros (zero-filling process) at the end of the signal before the FT is applied. This changes the locations of the FT spectral lines and reduces the interval between them. Following this approach, we increase the resolution in the frequency domain and can thus determine the frequency of the fringes with high accuracy. As a rule of thumb, the number of zeros should always be at least double the original number of signal samplings. Therefore, one should at least choose a zero-filling factor (ZFF) of two. Zero-filling increases the number of spectral lines simply by interpolation and increases the resolution in reciprocal space. After zero-filling we were able to estimate the period of the fringes in the simulated fringes spectrum. We observed new spectral lines (see Fig. 4e), which were hidden in Fig. 4d. The frequency $x = 0.0071578$ has the largest amplitude and is close to the exact value $x = 0.00725$ (see Fig. 4b) for the pure fringes spectrum. We can therefore consider the frequency at $x = 0.00716$ as the fringes amplitude. In

a pre-processing algorithm the fringes frequency can thus be identified as the maximum frequency in the FT (with zero-filling) of the region 6000 $cm^{-1}$ to 3800 $cm^{-1}$.

With the fringes frequency at hand we can now set up the EMSC model according to Eq. 9, estimate EMSC parameters and correct the absorbance spectrum with additive fringes (blue spectrum in Fig. 3) according to Eq. 13. As a reference spectrum, the spectrum shown in Fig. 2a was used. The result of the EMSC correction is shown as the orange spectrum in Fig. 5, together with the absorbance spectrum with additive fringes (green) and the pure absorbance spectrum (in blue). The black spectrum in Fig. 5 is obtained by filtering. Filtering is done by setting the amplitude at the frequency $x = 0.007245$ in the Fourier transform of Fig. 4c to zero (zero-filling) and by calculating the filtered absorbance spectrum by inverse Fourier transform according to Eq. 17. The aliasing effect due to zero-filling is clearly visible. Although filtering reduces fringes slightly in the region that is commonly considered, i.e. 4000 $cm^{-1}$ to 800 $cm^{-1}$, large aliasing effects are introduced in regions with absorption. This shows that filtering changes the chemical absorption bands and has to be applied with care. Surprisingly, the EMSC model, treating fringes as sinusoidal and non-dispersive, works very well for correcting fringes, which were simulated according to rigorous theory, i.e. taking into account the dispersive effect of absorption bands and employing the exact formula for fringes in Eq. 6.

As an algorithm for EMSC correction of fringes we suggest therefore to determine the fringes frequency in the region from 6000 $cm^{-1}$ to 3800 $cm^{-1}$, which is free from chemical absorption. For an accurate identification of the fringes frequency, zero-filling is applied as described above. The fact that for the determination of the frequency the region 6000 $cm^{-1}$ to 3800 $cm^{-1}$ is required, is not a limitation, since spectra can be obtained in this region by most commercially available FTIR spectrometers. When the frequency is determined, the EMSC model can be established according to Eq. 9 and the spectrum can be subsequently corrected according to Eq. 13.

Finally we consider an experimentally obtained FTIR spectrum with fringes. The spectrum is, as the pure absorbance spectrum shown in Fig. 2a, obtained from meat tissue sections and shown in Fig. 6a (green spectrum). The Fourier transform of this green spectrum is shown in Fig. 6b. In order to remove fringes we calculated the Fourier transform of the region from 6000 $cm^{-1}$ to 3800 $cm^{-1}$ of the green spectrum in Fig. 6a. To reduce the leakage effect (Fig.6c) we employed zero-filling before the Fourier transform. The obtained Fourier transform is shown in Fig. 6d. As fringes frequency, we obtained $x = 0.00636$. It was not possible

to identify this frequency component in Fig. 6b. This value was used in the EMSC model (Eq. 9) and fringes were corrected by Eq. 13 resulting in the blue spectrum in Fig. 6a. As reference spectrum we used the pure absorbance spectrum shown in Fig. 2a. A perfect agreement between the corrected spectrum (blue) and the reference spectrum (red) is not expected, since both spectra originate from different samples and thus chemical differences are expected to be present. However, we see that the measured spectrum with fringes was successfully corrected, since the sinusoidal oscillations are completely removed. The slight remaining oscillations in the region 6000cm$^{-1}$ to 3800 cm$^{-1}$ in the corrected spectrum may be due to non-parallel interfaces and beam convergence.

## Conclusions

In this paper we have presented an EMSC-based approach for correction of fringes in FTIR spectroscopy. We further showed that fringes exhibit dispersive effects as observed in Mie scattering, but within a good approximation they can be considered as non-dispersive and sinusoidal. We further compared our EMSC correction results after removal of fringes with filtering. The comparison shows that filtering introduces aliasing and leads to a loss of chemical information. We showed that it is not possible to remove one frequency amplitude completely in the Fourier transform without any loss of information, since each frequency component in the Fourier domain contains relevant chemical information. We have further demonstrated that frequencies can only approximately be identified in the Fourier domain. This is due to the leakage and picket-fence effects. In order to reduce these two effects, we used zero-filling. For the experimental spectra considered in this paper the identification of one frequency was sufficient for the correction of the fringes. In cases where more than one frequency is needed for the correction of fringes using an EMSC model, this can easily be achieved by following the same approach outlined in this paper.

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*Figures*

**Figure 1**

The evolution of a plane wave with intensity $I_0$ at a thin film. Part of the plane wave is reflected backwards with intensity $I_{sca}$, part of it is transmitted with intensity $I$. The absorption of infrared radiation by the thin film is indicated by the red area denoting a radiation sink.

**Figure 2**

a) Infrared absorbance spectrum of a meat tissue section.
b) Periodic fringes obtained by the exact model for fringes with a constant real refractive index $\hat{n}$=1.33. The fringes are considered for the spectral range from 6000 $cm^{-1}$ to 800 $cm^{-1}$. As thin film thickness we used $l$=4.3µm.
c) Approximated absorbance spectrum with fringes constructed as the sum of the pure chemical absorbance spectrum of a meat tissue section (Fig. 2a) and periodic fringes (Fig. 2b).

**Figure 3**

Absorbance spectrum with additive fringes in blue. The absorbance spectrum with dispersive fringes obtained by using a complex index of refraction and exact model for fringes (Eqs. 1, 2, 5 and 6) in red. The imaginary part of the refractive index was obtained by using Eq. 8. The real part of the refractive index was calculated via the Kramers-Kronig transform Eq. 7. Their deviation is shown in black.

**Figure 4**

Representation of spectra in the Fourier domain.
a) FT of the pure absorbance spectrum of Fig. 2a;
b) FT of the exact model for fringes spectrum of Fig. 2b;
c) FT of absorbance spectrum with additive fringes of Fig. 2c;
d) FT of absorbance spectrum with additive fringes, using only the region between 6000 $cm^{-1}$ to 3800 $cm^{-1}$ of Fig. 2c in red;
e) FT of the absorbance spectrum with additive fringes, using only the region between 6000 $cm^{-1}$ and 3800 $cm^{-1}$ of Fig. 2c in red after zero-filling;
f) Filtering in the Fourier domain of the spectrum of Fig. 2c.

**Figure 5**

The simulated absorbance spectrum with additive fringes (in green, constant real refractive index $\hat{n}$=1.33, thin film thickness $l$=4.3µm) was corrected by EMSC resulting in the orange spectrum. The result is shown together with the pure absorbance spectrum of Fig. 2a in blue. We used it as a reference spectrum. For comparison we also show a correction obtained by filtering the estimated frequency in the Fourier domain (in black).

**Figure 6**

a) The experimental spectrum with fringes (in red) was corrected by means of the EMSC model, resulting in the blue spectrum. The result is shown together with the pure absorbance spectrum (see Fig. 2a) in green used as a reference spectrum;
b) FT of the experimental spectrum with fringes of Fig. 6a in red;
c) FT of the experimental spectrum with fringes of Fig.6a in red using only the region between 6000 $cm^{-1}$ and 3700 $cm^{-1}$;

d) Zero-filling for FT spectrum of Fig. 6c.

## Appendix A

Consider a thin film with a complex dielectric constant[34] $\tilde{\varepsilon}_r$ as shown in Fig. 1. The left (front) and right (back) edges of the film are planes orthogonal to the $x$-axis, located at $x$=0 and $x$=$l$, respectively. The film naturally divides space into three regions, i.e. region I for $x<0$, region II for $0<x<l$ and region III for $x>l$ (see Fig. 1). We assume that the film is embedded in air or vacuum so that we have $\varepsilon_r \approx 1$ in regions I and III. Rigorous treatments for transmission measurements through thin cells with liquids can be found in the references[28, 35-37]. Suppose a plane wave of frequency $\omega$, propagating in the $x$-direction, is incident orthogonally on the front surface of the film at $x$=0. Our task, then, is to compute the infrared radiation intensity scattered back into region I and the infrared radiation intensity transmitted into region III. We accomplish this by solving for the electric field amplitude

$$\tilde{E}(x,t) = \tilde{E}(x)e^{-i\omega t} \quad \text{(A1)}$$

of the infrared radiation wave in regions I, II and III. From classical electrodynamics[34] we know that $\tilde{E}(x)$ satisfies the Helmholtz equation

$$\left[\frac{d^2}{dx^2} + \varepsilon_r k_0^2\right]\tilde{E}(x) = 0 \quad \text{(A2)}$$

where $\varepsilon_r = 1$ in regions I and III and $\varepsilon_r = \tilde{\varepsilon}_r$ in region II,

$$k_0 = \frac{\omega}{c} = 2\pi\tilde{\nu} \quad \text{(A3)}$$

and $c$ is the speed of light in vacuum. The solutions of Eq. A2 in the three regions are

$$\tilde{E}_{\mathrm{I}}(x) = E_0\left[e^{ik_0x} + re^{-ik_0x}\right] \quad \text{(A4a)}$$

$$\tilde{E}_{\mathrm{II}}(x) = E_0\left[Ae^{i\tilde{k}x} + Be^{-i\tilde{k}x}\right] \quad \text{(A4b)}$$

$$\tilde{E}_{\mathrm{III}}(x) = E_0te^{ik_0x} \quad \text{(A4c)}$$

where $E_0$ is the field strength of the incident radiation, $A$ and $B$ are complex constants, $r$ is the reflection amplitude, $t$ is the transmission amplitude and for non-magnetic materials

$$\tilde{k} = k + i\kappa = k_0\sqrt{\tilde{\varepsilon}_r} = k_0\hat{n} \quad \text{(A5)}$$

where $k$ and $\kappa$ are the real and imaginary parts of $\tilde{k}$, respectively, and $\hat{n} = \sqrt{\tilde{\varepsilon}_r}$ is the complex refractive index.

In order to determine the unknowns in Eqs. A4, we require that at the boundary surfaces of the film at $x$=0 and $x$=$l$ and for orthogonal incidence the field strength $\tilde{E}(x)$ is continuous with a continuous first derivative. These boundary conditions are appropriate for non-magnetic (biological) materials. They would

also be the boundary conditions for a scalar-field approach to the thin-film scattering problem[38]. These two conditions then yield the following four equations

$$1 + r = A + B \quad \text{(A6a)}$$

$$1 - r = \left(\frac{\tilde{k}}{k_0}\right)(A - B) \quad \text{(A6b)}$$

$$Ae^{i\tilde{k}l} + Be^{-i\tilde{k}l} = te^{ik_0 l} \quad \text{(A6c)}$$

$$Ae^{i\tilde{k}l} - Be^{-i\tilde{k}l} = \left(\frac{k_0}{\tilde{k}}\right)te^{ik_0 l} \quad \text{(A6d)}$$

Solving Eqs. A6a and A6b for *A* and *B*, we obtain

$$A = \frac{1}{2}\left[\left(1 + \frac{k_0}{\tilde{k}}\right) + r\left(1 - \frac{k_0}{\tilde{k}}\right)\right] \quad \text{(A7a)}$$

$$B = \frac{1}{2}\left[\left(1 - \frac{k_0}{\tilde{k}}\right) + r\left(1 + \frac{k_0}{\tilde{k}}\right)\right] \quad \text{(A7b)}$$

We may also solve Eqs. A6c and A6d for *A* and *B*. We obtain

$$A = \frac{1}{2}e^{-i\tilde{k}l}t\left(1 + \frac{k_0}{\tilde{k}}\right)e^{ik_0 l} \quad \text{(A8a)}$$

$$B = \frac{1}{2}e^{i\tilde{k}l}t\left(1 - \frac{k_0}{\tilde{k}}\right)e^{ik_0 l} \quad \text{(A8b)}$$

Taking the ratio *A*/*B* of Eqs. A7a and A7b, then taking the ratio of Eqs. A8a and A8b and equating the two ratios yields a single equation for *r*, where *t* is eliminated. Solving this equation for *r*, we obtain

$$r = \frac{\left(1-\hat{n}^2\right)\sin(2\pi\hat{n}\tilde{\nu}l)}{\left(1+\hat{n}^2\right)\sin(2\pi\hat{n}\tilde{\nu}l)+2i\hat{n}\cos(2\pi\hat{n}\tilde{\nu}l)} \quad \text{(A9)}$$

Since *r* is now known, we may now compute *A* and *B*, using Eqs. A7a and A7b. With *A* and *B* known we may now compute the transmission amplitude *t* from either Eq. A6c or Eq. A6d. The result is

$$t = \frac{2i\hat{n}e^{-2\pi i\tilde{\nu}l}}{\left(1+\hat{n}^2\right)\sin(2\pi\hat{n}\tilde{\nu}l)+2i\hat{n}\cos(2\pi\hat{n}\tilde{\nu}l)} \quad \text{(A10)}$$

The infrared radiation intensity incident on the film is

$$I_0 = \frac{c}{2}\varepsilon_0|E_0|^2 \quad \text{(A11)}$$

Defining the reflectance

$$R = |r|^2 \quad \text{(A12)}$$

and the transmittance

$$T = |t|^2 \quad \text{(A13)}$$

the final expressions for reflectance and transmittance in a case of real refractive index $\mu$ are given by

$$R = \frac{\left(1-\hat{n}^2\right)^2 sin^2(2\pi\hat{n}\tilde{\nu}l)}{4\hat{n}^2+(1-\hat{n}^2)^2 sin^2(2\pi\hat{n}\tilde{\nu}l)} \quad \text{(A14)}$$

and

$$T = \frac{1}{1+\frac{1}{4}\left(\frac{1}{\hat{n}}-\hat{n}\right)^2 \sin^2(2\pi\hat{n}\tilde{\nu}l)}. \qquad \text{(A15)}$$

In the case with complex refractive index $\hat{n}(\tilde{\nu}) = n(\tilde{\nu}) + in'(\tilde{\nu})$ the expressions for reflectance and transmittance are given by

$$R = \frac{\left(1-(n+in')^2\right)^2\left(1-\cos(4\pi n\tilde{\nu}l)ch(4\pi n'\tilde{\nu}l)+isin(4\pi n\tilde{\nu}l)sh(4\pi n'\tilde{\nu}l)\right)}{8(n+in')^2+(1-(n+in')^2)^2(1-\cos(4\pi n\tilde{\nu}l)ch(4\pi n'\tilde{\nu}l)+isin(4\pi n\tilde{\nu}l)sh(4\pi n'\tilde{\nu}l))} \text{ (A14a)}$$

and

$$T = \frac{8(n+in')^2}{8(n+in')^2+(1-(n+in')^2)^2(1-\cos(4\pi n\tilde{\nu}l)ch(4\pi n'\tilde{\nu}l)+isin(4\pi n\tilde{\nu}l)sh(4\pi n'\tilde{\nu}l))}. \text{ A(15a)}$$

The intensity of the scattered radiation is

$$I_{sca} = I_0 R \qquad \text{(A16)}$$

and the intensity of the transmitted radiation is

$$I = I_0 T \text{ (A17)}$$

The absorbance can be calculated according to Eq. 2 and because of the presence of the sin and cos functions shows considerable deviations from Beer's law[39], i.e. the absorbance is not a simple exponential function in the thickness of the sample. Therefore, it is important to note that only when neglecting the scattering, is the absorbance in the film simply related to the imaginary part of $\hat{n}$, where $\hat{n} = \sqrt{\tilde{\varepsilon}_r}$. This can be seen in the following way. According to Eq. A4b, neglecting the presence of the back wall of the film and the scattering off of the front surface of the film, a wave moving through the film in forward direction has the form

$$\tilde{E}(x) = E_0 e^{i\tilde{k}x} \text{ (A18)}$$

where $E_0$ is the field strength of the radiation just outside of the front surface of the film and $\tilde{k}$ is the complex wavenumber defined in Eq. A5. In analogy to Eq. A11, the radiation intensity at the back surface of the film is

$$I = \frac{c}{2}\varepsilon_0\left|\tilde{E}\right|^2 = \frac{c}{2}\varepsilon_0|E_0|^2 e^{-2\kappa l} \quad \text{(A19)}$$

which now, but only as a consequence of the simplifications introduced, is indeed of the form of Beer's law. Then, with Eq. A11,A17 and Eq. A19, we have

$$T = e^{-2\kappa l} = e^{-4\pi n' l\tilde{\nu}} \quad \text{(A20)}$$

from which we obtain the absorbance

$$A = -\log_{10}(T) = 4\pi n' l\tilde{\nu}/\ln(10) \qquad \text{(A21)}$$

For a more detailed description we refer the reader to the text book of Griffiths[34].

## Appendix B

According to rigorous theory fringes were easily simulated with the help of Eqs. 1, 2, 5 and 6. The result is shown in Fig. 2b, where for the calculation of the transmission amplitude a real refractive index of $\hat{n} = n_0 = 1.33$ and a film thickness of $l = 4.3\ \mu m$ were used. Although periodicity was not obvious from the formula presented in Eq. 6, the fringes obtained in Fig. 2b are visually periodic. In this Appendix we will show that the assumption of periodicity, i.e. replacing the exact electromagnetic result for the absorbance given by Eqs. 1, 2, 5 and 6, by a sinusoidal function in the EMSC model in Eq. 9, is justified and results in a very small error.

Let us consider the formulas Eqs. 1, 2, 5 and 6 in the rigorous theory for fringes. Using Eqs. 1, 5 and 6, we calculate the transmittance $T$

$$T = |t|^2 = \frac{4{n_0}^2}{(1+{n_0}^2)^2 \sin^2(2\pi n_0\tilde{\nu}l)+4{n_0}^2\cos^2(2\pi n_0\tilde{\nu}l)} = \frac{1}{1+\frac{1}{4}\left(\frac{1}{n_0}-n_0\right)^2\sin^2(2\pi n_0\tilde{\nu}l)}. \qquad \text{(A22)}$$

Now we use Eq. 2 to obtain the absorbance $A$

$$A = -\log_{10}(T) = -\log_{10}\left(1+\frac{1}{4}\left(\frac{1}{n_0}-n_0\right)^2\sin^2(2\pi n_0\tilde{\nu}l)\right)^{-1} = \frac{ln\left(1+\frac{1}{4}\left(\frac{1}{n_0}-n_0\right)^2\sin^2(2\pi n_0\tilde{\nu}l)\right)}{ln10}. \qquad \text{(A23)}$$

By means of the Taylor series for the natural logarithm

$$ln(1+x) = x - \frac{x^2}{2} + \frac{x^3}{3} - \cdots = \sum_{n=0}^{\infty}\frac{(-1)^n x^{n+1}}{(n+1)} = \sum_{n=1}^{\infty}\frac{(-1)^{n-1}x^n}{n} \qquad \text{(A24)}$$

where $-1 < x \leq 1$, we find the Taylor series for the absorbance $A$ in the following way

$$A(\tilde{\nu}) = \frac{ln\left(1+\frac{1}{4}\left(\frac{1}{n_0}-n_0\right)^2\sin^2(2\pi n_0\tilde{\nu}l)\right)}{ln10} =$$

$$\frac{1}{ln10}\left(\frac{1}{4}\left(\frac{1}{n_0}-n_0\right)^2\sin^2(2\pi n_0\tilde{\nu}l)\right) - \frac{1}{ln10}\left(\frac{1}{32}\left(\frac{1}{n_0}-n_0\right)^4\sin^4(2\pi n_0\tilde{\nu}l)\right) + \cdots =$$

$$\frac{1}{4ln10}\left(\frac{1}{n_0}-n_0\right)^2\frac{1-\cos(4\pi n_0\tilde{\nu}l)}{2} - \frac{1}{32ln10}\left(\frac{1}{n_0}-n_0\right)^4\frac{(1-\cos(4\pi n_0\tilde{\nu}l))^2}{4} + \cdots \qquad \text{(A25)}$$

To calculate $\sin^2(2\pi n_0\tilde{\nu}l)$ we used the half-angle formulas.

By estimating the first and the second terms in the Taylor series for absorbance for $n_0 = 1.33$, we have:

$$x_1 = \left(\frac{1}{4}\left(\frac{1}{n_0}-n_0\right)^2\sin^2(2\pi n_0\tilde{\nu}l)\right) = \left(\frac{1}{4}\left(\frac{1}{1.33}-1.33\right)^2\sin^2(2\pi\cdot 1.33\cdot\tilde{\nu}l)\right) \leq 0.083556$$

$$\frac{1}{ln10}x_1 \leq 0.0362878$$

$$x_2 = \frac{1}{ln10}\frac{x_1^2}{2} \leq 0.001516 \quad \text{(A26)}$$

We conclude that the second term $x_2$ of the series is about 4% of the first term and therefore the impact of all terms of the series from the second term on is not significant. This means that the replacing of exact electromagnetic calculations for the description of scattering of electromagnetic radiation at a thin film given by Eqs. 1, 2, 5 and 6 via sinusoidal functions is justified.

It is also important to note, that the series expansion is valid not for any refractive index. By estimating $x_1$ we conclude that the refractive index $n_0$ should satisfy the condition $n_0 \leq 2.4$. Calculations were done for a plane wave with phase zero. Thus, in general, both sine and cosine terms have to be taken into account in the EMSC model for the description of scattering effects of electromagnetic radiation at a thin film. It can also be shown that for refractive indices above 1.9 and below 2.4, additional terms have to be taken into account. This is beyond the scope of this paper and will be discussed in a subsequent paper.

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