Deconvolution Multi-Contrast Light Profile Microscopy (MC-LPM)

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Light profile microscopy (LPM), is a technique of optical inspection that returns direct cross-sectional images of layered and non-homogeneous thin films via illumination along an axis perpendicular to the optic axis of a microscope. LPM forms images via light emitted from a source beam intersecting a material, where illumination is delivered in a thin plane or a focused line. A cross-sectional edge prepared in the material, acts as a viewing surface for light emitted from the beam volume, and multiple modes of broadband and spectral contrast are used to form direct images of the interfacial structure from images of the intersecting beam. While the direct resolution of LPM images approaches the Rayleigh diffraction limit, the width of the illumination beam limits the image resolution through the contributions of out-of-focus light. However, with robust deconvolution schemes, it is possible to obtain resolution improvements of 2.5-4 times, and final images resolved just below the Rayleigh diffraction limit. This is now established in both broadband and spectral imaging with wide apertures (up to 0.7 NA in air). The optical EM-MLE (expectation maximization-maximum likelihood estimation) de-convolution algorithm is successfully applied in routine use, on a quasi-blind basis, using an initial estimate of the LPM point spread function (PSF) from diffraction theory. An evaluation of the image resolution under deconvolution is made through several performance indicators derived for imaging 1D layered systems. Conditions for divergence and aliasing limits of the algorithm are further identified. Deconvolution LPM demonstrates an unprecedented direct registration of the chemical and physical depth maps of fine interface details in thin films on the scale of 0.5-1.0 µm.

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Keywords: light profile microscopy, blind deconvolution, depth profiling, Raman imaging

Introduction

Light profile microscopy (LPM) [1-4] is an optical technique for the direct cross sectional inspection of layered and depth varying micro-structures. A thin film is inspected (Fig. 1(a)) by directing a source beam through the material along its depth ‘x’ axis, inside a cross-sectional viewing edge. This edge is used as a transmission window for image light emitted from the source beam volume, with edge defects held out of focus in the recorded image. A direct image of the beam emission is obtained by a microscope along the axis ‘z’, showing outstanding contrast for subtle features of interface morphology that are invisible to conventional microscopy [4]. Multiple contrast mechanisms [4] are available including elastic and inelastic (Raman) scatter, photo-, thermo- and chemi-luminescence. Imaging may be performed on a broadband (2D) basis and/or a spectral (1D) basis (in which a line depth (‘x’) profile through the test material is dispersed along a wavelength axis. The integration of multiple imaging modes in a multi-contrast (MC) light profile microscope [4] provides the analyst with a suite of maps of chemical composition registered to depth images of the sample morphology.

While LPM has the capability for inspecting 1-, 2-, and 3D objects, the present work is concerned with the inspection of thin layers. These structures are invisible to classical angle tomography, as a result of the ‘missing cone’ in the microscope’s optical transfer function (OTF) [5]. LPM overcomes the problems seen in Z-scan confocal microscopy where depth features of interest are translated axially through the object plane, but where refractive artifacts may severely limit axial resolution [6]. LPM also overcomes the stray light problems encountered in transversely scanned Raman micro-probes where internal reflection and wave-guiding effects have produced serious interpretation problems, even into images of micro-tomed thin sections [6].

In an LPM, the axial light intensity distribution in the neighborhood of the object plane determines the width of the system point spread function PSF [7]. From Fig. 1(a), we consider a microscope equipped with an objective lens operating at wavelength λ and numerical aperture NA0. The objective’s depth of field δ0, is given by δ0=λ/4NA02, and its diffraction limit by the Airy disk diameter δA=1.22λ/NA0. The LPM system PSF (in incoherent emission) is computed as the integral over the axis ‘z’ of the source point emission intensity through the object plane, at fixed field ‘x’ [7], in a layered material that is homogeneous along ‘z’. As the source beam diameter becomes axially small relative to the field depth, the PSF approaches the dependence of the Airy disk, as expected for a thin object. As the diameter increases relative to δ0, more out-of-focus light appears in the PSF. As the beam width becomes very wide relative to δ0, a condition termed ‘wide-beam’ or ‘infinity illumination’ applies, and the spread function approaches a limiting dependence denoted h0(x,y) [4,7]. At high aperture with standard objectives and broadband illumination this condition is usual. Notwithstanding, an experimental resolution of 2-3 times the Airy disk diameter, δ0 is typical. In spectral images, on the present platform, the ratio of the source beam width to the field depth varies depending on the power and aperture, and channel aberrations are more significant in setting the PSF dependence.
Figure 1. (a) Schematic of experimental MC-LPM geometry (b) Broadband (BB-LPM) image formed in planar illumination with white light, (c) Spectrograph LPM Image (d) Layered test material under study; (e) Raman spectra of individual layers against spectral references [4]. Reproduced with permission from The Society for Applied Spectroscopy.

Resolving below the limits of the experimental images requires de-convolution of the microscope PSF, but routine calibration of the LPM-PSF is not practical. The situation calls for a blind or quasi-blind de-convolution method that provides an adaptive estimate of the system PSF that is robust under experimental conditions, consistent with the optical system aberrations, and can routinely de-blur an LPM image to the Airy disk limit. Such an algorithm has been adapted in this work from the optical expectation maximization/maximum likelihood estimation (EM/MLE) deconvolution algorithms of T.J. Holmes [8-11]. The present adaptation operates on a quasi-blind basis in a regime limited by image aliasing. It is fully established here for the first time in LPM at wide apertures in air, and on the principal incoherent imaging modes used for materials analysis.

Theory: Algorithm and Performance Measures

The EM-MLE (blind) deconvolution algorithm [10] iterates object, $f_k(x,y)$ and PSF $h_k(x,y)$ estimates, from an input image $g(x,y)$. For the $k^{th}$ trial, a predicted image may be computed as $g_k(x,y) = h_k(x,y) \ast f_k(x,y)$, where $\ast$ represents a 2D convolution, from which a ratio image is defined as $r_k(x,y) = g(x,y)/g_k(x,y)$. The iterated object and PSF are then updated as:

$$f_{k+1}(x,y) = f_k(x,y) \{h_k(-x,-y) \ast r_k(x,y)\}$$

$$h_{k+1}(x,y) = \left( \frac{h_k(x,y)}{N_p} \right) \{f_k(-x,-y) \ast r_k(x,y)\}$$

where $N_p$ is the total photon count in the image. These equations are derived from a conditional probability framework in [10]. Iteration starts at $k=0$ with $f_0(x,y) = g(x,y)$ (the object is initially approximated by the image) and $h_0(x,y) = h_{LPM}(x,y)$ (the initial PSF is approximated by a wide beam diffraction model of the LPM PSF [4,7]). We note that as convergence of the iteration is approached, $r_k(x,y) \rightarrow 1$ (a unity valued field). The bracket enclosed convolutions in Eqs. (1a) and (1b) respectively approach constant values of unity and $N_p$, requiring $f_{k+1}(x,y) \rightarrow f_k(x,y)$ and $h_{k+1}(x,y) \rightarrow h_k(x,y)$. The error in the object, $\varepsilon_{conv}$, thus minimizes at nominal convergence:

$$\varepsilon_{conv} = \frac{\max|f_{k+1}(x,y) - f_k(x,y)|}{\max|f_k(x,y)|}$$
while the root-mean-square error of the object also minimizes, albeit slowly:

\[ \varepsilon_{m}\text{est} = \sqrt{\frac{1}{ \max(g(x,y)) } \int I_i(x,y) - g(x,y) \, dx \, dy} \]  

(3)

Image resolution may be tracked with iteration number to identify a best quality object. The following indicators are applied to images of vertical layers. The width of a reconstructed edge, \( \delta x_{0k} \), is measured as the ‘x’ interval bracketing 90% of the edge signal transition [4]. Resolution is also tracked through the high frequency content in the object Fourier transform \( F_k(o_x,o_y) \) [8] for the 1D case \( o_y=0 \). The indicator \( A_k \) measures the object amplitude at frequency \( o_{REF} \) relative to the aliasing level at \( o_{\text{alias}} \):

\[ A_k = \frac{|F_k(o_{REF},0)|}{|F_k(o_{\text{alias}},0)|} \]  

(4)

In images of vertical layers, resolution may be assessed by correlating edges to a set of model images ([9] and op cit). From a well resolved estimate of the object, \( f(x,y) \) (near nominal convergence, Eq. 2), a 1D depth profile of edge/interface contrast is sampled as \( f_k(x,0) \) (\( y=0 \) by default, at the image center). One or more image edges are selected for analysis. If an edge consists of a single rising step, it may be modeled ideally as the profile \( O(x) = I_x U(x-x_i) \) where \( U(x) \) is the unit step function, \( x_i \) the interface position, located at the inflection point of the edge profile and \( I_x \) the transition in contrast. A more complex interface may contain several step levels approximated by the staircase \( O(x) \) relative to the aliasing level at \( x \) from a set of PSFs having the form of the Airy disk, \( h(x) \). The \( O(x) \) profile may be modeled graphically or using a segmentation algorithm. The profile is augmented to a 2D image of the model layers as \( O(x,y) = O(x) \times U(y) + U(-y) \). A reference image set, \( g_{\text{ref}}(x,y) \), is formed from a set of PSFs that focused spectral line images through a spectrograph, and compensated astigmatism. Full details of the layout are provided in Reference [4]. All computations were conducted with Matlab source code written by the author (The Math Works, Natick MA). Computations directly implemented Eqs. 1(a)-1(b) without constraints.

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Deconvoluted frames were 256X256 elements, zero padded to 512x512. Iteration loop time was limited by the time needed to execute three FFT and inverse FFT calculations. Imaging was demonstrated on a 5-ply test material (Fig. 2) prepared by blow extrusion [12] and consisting of (Fig. 1(d)): outside ‘skin’ layers of low density polyethylene (LDPE); adhesive ‘tie’ layers of LDPE chemically modified at levels near 10% w/w (LDPE2); a core of poly(ethylene terephthalate) (PET). A 5-ply material consisting of 5 \( \mu m \) layers of LDPE and polypropylene (PP) [13] was also examined (Fig. 3).

**Implementation and Evaluation of the Deconvolution Algorithm**

Chemical and morphology mapping of extruded thin films

Figure 1 shows the principal LPM image modes [4] on which EM-MLE deconvolution is demonstrated on the test material (Fig. 1(d)). The broadband BB-LPM mode (Fig. 1(b)) is formed from scatter of white light with plane sheet illumination. It gives a primary map of the cross-section morphology through incoherent elastic scatter. A focused laser beam (4 \( \mu m \) diameter) excites a one dimensional depth profile of Raman/luminescent emission through the structure, resulting in the spectrograph image of Fig. 1(c). The spectral channel disperses the laser beam emission image along...
wave number (vertical) and depth (horizontal) axes, yielding a map of Raman transitions appearing as thin horizontal lines running across the depth (‘x’) field. These superimpose onto a background (baseline) image of anomalous scatter and luminescence. Raman spectra are recovered as plots of intensity versus wave number, by averaging spectral line emission over individual layers and correcting for an underlying baseline (Fig. 1(e)). The spectrograph image provides a direct visualization of composition with subsurface depth. While an experienced analyst may recognize common industrial polymers from a simple inspection of this image, a comparison of the layer spectra with references (Fig. 1(e)) provides definitive identification [4]. The minor chemical differences between the LDPE tie and skin polymers were not resolved at the spectral resolution of ca. 10 cm$^{-1}$.

A Raman LPM depth profile analysis is made by imaging individual spectral line groups through the structure at higher magnification and plotting the average Raman line intensities with depth, ‘x’. Since the intensity of normal Raman scatter is linearly proportional to the local polymer density, the core polymer density (concentration) is well represented by the PET group frequencies near 1615/1730 cm$^{-1}$ while LDPE CH stretching frequencies near 2850-70 cm$^{-1}$ track the LDPE tie/skin polymer density [4]. Figure 2 shows imaging of these Raman frequency ranges at M40X/0.65NA, registered to the broadband (BB-LPM) image (Figs. 2(b) and (e)). In each case a light profile of Raman emission was determined by averaging vertically over the spectral lines at 1615 cm$^{-1}$ and the doublet at 2850 cm$^{-1}$.

Local background emission was averaged just above each spectral line and subtracted to yield the baseline Raman emission depth profile. This is superimposed onto each cross section as a white trace.

The results of EM/MLE de-convolution on the experimental images appear in Fig. 2, with the original (raw) images presented at the left, and the converged object estimates (in the range of 40-75 trials), at the right. Image signal-to-noise ratio and dark current effects [8-9] were not found to be significant in the present examples. The central image channel that references morphology and sample dimensions is the BB-LPM image (middle: Figs. 2(b) and (e)), onto which a raw image of coherent elastic scatter from the source laser beam is superimposed (Fig. 2(e)). The laser beam image indexes the precise sampling point at which the spectral images (upper and lower) were acquired. The incoherent (BB-LPM) light profile of elastic scatter through this region (white trace) is shown offset along ‘y’, just below the beam center. Note that the BB-LPM image, which arises from right-angle elastic scatter inside the edge, differs markedly from conventional backscattering micrographs, recorded directly on the edge: the layer structures in conventional

![Figure 2: Original LPM spectral and broadband images (a),(b),(c) at M40X/0.65NA compared to respective EM-MLE de-convoluted forms in (d),(e),(f); Broadband images are (b) and (e); Raman spectral images are recorded in the CH stretching range near 2900 cm$^{-1}$ in (a),(d) and in the group frequency range, near 1600 cm$^{-1}$ (c),(f).](image-url)
imaging were completely invisible [4]. The spectral images recorded at mean wavelengths of 625 nm and 580 nm show details of the LDPE doublet transition near 2850 cm\(^{-1}\) and the PET core lines near 1615/1730 cm\(^{-1}\).

The effects of de-convolution in these images are obvious as a significant sharpening of the interface detail and spectral features. Resolution along the ‘x’ axis improves from about 2-3 \(\mu\)m (full width) in the original images, to near 1 \(\mu\)m, after deconvolution. The slight left to right (‘x’ variation in the spectral line width near at 1615 cm\(^{-1}\) arises from a slight variation in the source beam waist size across the field. Although the coherent elastic scatter was not deconvoluted, the raw image provided a well resolved reference profile. In this case the beam diameter was small or comparable to the microscope field depth so that beam width effects on the PSF were absent, although geometric aberration residuals remained.

The detailed interface structure that emerges under deconvolution is readily cross-validated over this image set. Laser scatter occurring in thin interface sheets, i and ii in Fig. 2(e), is consistent with the core/tie layer interfaces seen in the broadband (Fig.2(c)) and spectral images of the channel depth structure formed by the LDPE layers (Fig. 2(d)). These interface details clearly derive from local features at the edge. An elastic scattering defect present in the tie layer, iii, shows up as a phantom edge in both the LDPE (Fig. 2(d)) and PET spectral line images (Fig. 2(f)). A slight grazing of the core surface by the sampling laser beam, iv, produces a minor but visible perturbation in the PET spectral image and depth profile, while a trace of spectral bleed, v, at the edge of the PET core (Fig. 2(f)) can be assigned to a scattering defect in the LDPE phase that also appears broadband in Fig. 2(e), and spectrally in Fig. 2(d).

Deconvolution along the spectral dimension provided modest resolution improvements, as seen from the sharpening of vertical detail in Figs. 2(d) and (f). This aspect was of secondary interest because these spectral features are relatively slowly varying on the present wave-number scale. Compensation of the spectral blur in the images occurred approximately independently of the ‘x’ dimension, consistent with focusing of the spectral channel’s cylindrical optics. Resolution enhancements occurred without serious distortions of the line doublet near 2850 cm\(^{-1}\), although a residual baseline oscillation is just visible in the PET line image near 1615 cm\(^{-1}\). The latter is of computational origin, the result of a slight over-iteration in the reported image, as discussed below.

The detailed micro-structural cross-referencing of images made possible by deconvolution LPM allows morphology-related features to be directly registered to Raman scatter depth profiles on micron scales and below. In extruded thin films, elastic scatter with its sensitivity to polymer morphology, gives bright BB-LPM contrast for sample regions containing crystallites, (such as the LDPE layers of the test structure) while amorphous regions (such as the central PET core) appear dark [4]. While the spectral (Raman/ luminescence) LPM channels depth profile chemical composition through the layers, some of this information is morphology related through primary scattering of the source laser beam. Secondarily, Raman/luminescence emission may be elastically re-scattered, refracted or guided by local interfaces/defects/ morphologies. Both mechanisms may now be identified through the laser beam LPM image and the de-convoluted BB-LPM image, which correlate the defects between Raman and broadband LPM frames and also map the extended 2D structures responsible for the laser scatter. This allows an unprecedented direct differentiation of image contrast of true chemical origin from that produced by optical mechanisms.

Spectral imaging is restricted, on the present platform to apertures up to 0.65 because of the limited working distances of standard optics (the use of which significantly controls component costs). Broadband imaging on the main channel may be performed up M60X/0.85NA but with a wide illumination beam. Figures 3(a) and (b) illustrate the reconstruction of a 5 \(\mu\)m wide layer of LDPE in a reference material comprised of alternating layers of LDPE and poly(propylene) (PP). The latter polymer scatters weakly and thus appears dark in the image. An improved spatial resolution from ca. 2.5 \(\mu\)m (raw) to near 800 nm was obtained.

Figure 3: BB-LPM images at M60X/0.85NA: (a) original and (b) de-convoluted images of the core/tie structure of 5 \(\mu\)m single layer LDPE (middle) laminated with outside PP layers [13]; (c) converged spread function reconstruction on BB (main) channel at M40X/0.65NA, plotted as solid line: \(h_{\infty}(x,y=0)\) and dotted line: \(h_{\infty}(x=0,y)\).
The converged system PSF, $h_{90}(x,y)$ is shown in Fig. 3(c), at M40X/0.65NA on the main channel. Past work has shown that such PSF estimates are generally consistent with experimental optical aberrations. The iteration starts from an initial PSF obtained from wide beam diffraction theory, $h_{00}(x,y)$. The Airy disk limit is shown for reference. Under deconvolution, the full width at half maximum (FWHM) in $h_{90}(x,y)$ is nearly unchanged from the initial estimate while the glare tail [7] arising from defocused object energy is strongly modified. There is a slight asymmetry that suggests residual coma in the optical system, but computational effects cannot yet be ruled out. The narrowing of the experimental PSF relative to the $h_{00}(r)$ reference may be the result of increased off-focus aberrations of the objective lens, an effect that remains un-evaluated in theory. The width of $h_{90}(x,y)$ bracketing 90% of the energy with respect to the center was evaluated by numerical integration of the profile and interpolation. The average PSF width on the main channel was 2-2.5 times the width of the Airy disk, consistent with the resolution seen in the raw images. Similar trends were observed on the spectral channel, but with more directional variation in the PSF along $x,y$ and an effective width of 3-3.5 times the Airy disk width. This is attributed to the cylindrical optics used by the spectral channel, to somewhat increased aberration residuals, and to the greater complexity of focusing of the spectral optics [4].

Image (depth) resolution evaluation

Based on the equations present in the Theory section, a number of 1D image resolution measures was applied to experimental layer structures and used to track algorithm performance with iteration number.

The first of these, $\delta_{90}$, was sampled at multiple edges in the individual images, and mean values, with associated standard errors, are reported in Table I. Figures are expressed as multiples of the Airy disk diameter $\delta_{90}$ at each nominal aperture and magnification. The values reported at M40X/0.65NA were sampled directly from the image profiles in Fig. 2, At M20X/0.4NA, replicate image records were analyzed to obtain the reported values. From this, it is seen that the average $\delta_{90}$ resolution of the raw broadband channel and spectral (Raman) channel images, over all apertures, was 2.3$\delta_{90}$ and 2.6$\delta_{90}$, with a standard error of determination near ±20%. With deconvolution the respective figures improved to 0.7$\delta_{90}$ and 1.0$\delta_{90}$. At similar uncertainty levels, these figures are statistically consistent with resolution at the Airy disk diameter or slightly below. An average resolution improvement of 2.5-3 times was obtained. The directness of the $\delta_{90}$ measurement is an obvious advantage, but unbiased sampling requires images of clean, well resolved edges, free of oscillations. When such structure was present, $\delta_{90}$ was estimated as a total width of the peak to valley transition of the leading edge. When the blur in an edge is large, the floor and ceiling features may not settle to accurate stationary levels, whereas in the presence of complex interfaces, such as very thin unresolved layers running parallel to an edge, apparent blur may be increased. The identification of best resolved interfaces is a matter of careful application of this method, and interface non-ideality accounts for the observed standard error.

An alternative resolution measure that does not directly rely on the quality of a sampled interface is obtained from the frequency content of the object [8-9] as assessed through increases in the density of the real part of the 2D object transform magnitude. Figure 4(a) plots $|F_{x,y}(k,0)|$ for the spectral image at 625 nm (Fig. 2(a),(d)) at the indicated iteration numbers. At ca. 30-40 iterations, a high frequency shoulder appears in the transform that attenuates linearly with frequency ($k$=64 and 75). This feature is associated with the resolution of thin layers. While it remains to be analytically established, the triangular form is suggestive of the transform of the 1D line spread [14] of an ideal

<table>
<thead>
<tr>
<th>Channel</th>
<th>M</th>
<th>NA</th>
<th>λ (nm)</th>
<th>$\delta_{90}$ (μm)</th>
<th>$\delta_{90}$ (μm)</th>
<th>$\delta_{x,y}$ (μm)</th>
<th>$\delta_{x,y}$ (μm)</th>
<th>$\delta_{x} \pm \delta_{y}$ (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB 20x</td>
<td>0.4</td>
<td>550</td>
<td>2.1</td>
<td>1.9</td>
<td>0.7 (±0.1)</td>
<td>0.8 (±0.1)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>BB 40x</td>
<td>0.65</td>
<td>550</td>
<td>1.34</td>
<td>2.3</td>
<td>0.6(0)±0.2</td>
<td>0.8 (±0.1)</td>
<td>0.7(5)</td>
<td></td>
</tr>
<tr>
<td>BB 60x</td>
<td>0.85</td>
<td>550</td>
<td>0.96</td>
<td>2.8</td>
<td>0.8(3)(±0.2)</td>
<td>0.7 (±0.1)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Sp/R 20x</td>
<td>0.4</td>
<td>580</td>
<td>2.21</td>
<td>2.0</td>
<td>0.9(0)±0.1</td>
<td>1.0 (±0.3)</td>
<td>1.1</td>
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<tr>
<td>Sp/R 40x</td>
<td>0.65</td>
<td>580</td>
<td>1.34</td>
<td>3.1</td>
<td>0.8(5)(±0.2)</td>
<td>1.1 (±0.1)</td>
<td>0.9</td>
<td></td>
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<tr>
<td>Sp/R 60x</td>
<td>0.85</td>
<td>625</td>
<td>2.38</td>
<td>2.0</td>
<td>0.8(1)(±0.1)</td>
<td>1.2(6)±0.2</td>
<td>0.9</td>
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<tr>
<td>Sp/R 80x</td>
<td>1.52</td>
<td>625</td>
<td>2.8</td>
<td>0.7(6)(±0.2)</td>
<td>0.8 (±0.2)</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. BB (broadband) or Sp/R (spectral-Raman); 2. Magnification; 3. Working aperture is 80% of nominal; Standard errors directly determined from multiple image records appear without brackets; reference values are enclosed in brackets.

In the incoherent optical system, with pupil cutoff at frequency $\omega_{\text{max}}$, where the shoulder intersects the baseline. From this, an equivalent 1D edge width in object space may be interpreted as: $\omega_{\text{max}} = 2\pi / \omega_{\text{max}}$. The shoulder feature was prominent in broadband and long-wavelength spectral LPM images where the experimental resolution obtained by $\delta_{90}$ was in the range 0.7-0.75 $\delta_{90}$ (Table I), with a standard error of 0.1$\delta_{90}$. In some of the spectral image records, the shoulder in
[F(ω₀,0)] tended to show more curvature and sometimes to be modulated by an oscillatory factor, that was suggestive of the features seen in (incoherent) optical system modulation transfer functions (MTFs) in the presence of defocus and geometrical aberrations [14]. In such cases a tangent line was fitted to the roll-off curve and extrapolated to the baseline to establish ω₀, max. Curvature related ambiguities in the shoulder extrapolation resulted in a larger uncertainty in δR (0.2-0.3 δR) and a typical image resolution of 1-1.1 δR.

The third indicator used to evaluate image resolution was the best-correlated Airy-disk diameter, δx_{min}, representing the closest equivalent resolution of the object layers by an ideal microscope. The tabled figures were just below δR. At complex edges O(x) contained multiple steps, whose precise location and number were sometimes difficult to discern un-ambiguously. To compensate this, independent estimates of O(x) were made and used to evaluate an uncertainty in δx_{min}. This was determined to be about ±20%, with object correlation values ρ_{mk} in the range of 0.992-0.9999.

Convergence of the Iteration

The identification of an object estimate of best quality and of a stopping point for the iteration were addressed by tracking several derived indicators with iteration number. In Fig. 4(b) the convergence error ε_{conv} tracks the similarity of (k+1)^th to the k^th object iterate. The RMS error ε_{RMS} tracks similarity of the predicted to the original image. δx_{min} follows edge resolution through correlation to a model while A_k tracks growth in the object high frequency content. The evolution of these parameters over k clearly marks off three distinct regions, typical of all images studied in this series. At small iteration numbers (k=0 to 20) an adaptation region is defined where both f_{k}(x,y) and h_{k}(x,y) vary rapidly. δx_{min} linearly decreases as ln(k), consistent with prior work [9]. The PSF adapts to a form approximating experimental validity, while f_{k}(x,y) shows only modest resolution improvements. Next, (k= 40-80) a convergence region occurs where ε_{conv}, ε_{RMS} and δx_{min} broadly minimize. Because this problem is to some extent ill-conditioned, some of these indicators tend to be insensitive to high frequency object information. Significant increases in the object transform at high frequency are seen in the growth and peaking of A_k near 65-70 iterations. Past the convergence zone (k>80), de-blurring continues but spectral leakage (oscillations, starting at the frame edges) and aliasing, begin to corrupt f_{k}(x,y) and h_{k}(x,y). Attenuation of A_k as occurs as aliasing increases and the cutoff frequency extrapolated in |F(ω₀,0)| (Fig. 4(a)) starts to exceed the aliasing frequency. Full aliasing of the transform shoulder occurs above k=100 where ω₀, max→∞. Promptly past this point, f_{k}(x,y) becomes buried in leakage oscillations (Gibb’s phenomenon), there is a rapid loss of any object similarity to the original image, and a divergent increase in ε_{conv} suddenly appears.

This iteration behavior shows marked differences from the prior, fully blind EM/MLE de-convolution works in wide field and confocal microscopy [10-11]. These works showed that, with the imposition of constraints of circular symmetry and bandwidth on the PSF/OTF reconstructions could be made fully blind to the initial PSF estimate.

![Figure 4](image-url)  
**Figure 4.** Evolution of the object frequency content with iteration number k (as indicated); (b) Evolution of several error and resolution indicators as a function of iteration number.

Following an adaptation region of several hundred iterations, h_{k}(x,y) approximated a final, converged form that showed experimental validity. This was followed by a slow convergence of the object over 5,000-10,000 iterations. A super-resolution of f_{k}(x,y) in the range of 0.2-0.3 δR was attainable, limited by iteration numbers [11]. However, a key distinction of the prior from present work was that previously, the width of h(x,y) was a larger fraction of the total field size. The optical system sustained more empty (surplus) magnification, as appropriate when an isolated, highly blurred feature of interest is to be super-resolved. In an LPM, where the primary goal is wide field imaging, much less empty
magnification is used. The PSF is sparsely resolved (with 6 pixels spanning the Airy disk). This leads to a resolution limited by aliasing (cf Fig. 4(a)) before any band-limit or symmetry constraints on the model become operative.

The present results establish reliable EM/MLE deconvolution in a multi-contrast (spectral and broadband) LPM in a regime of high aperture. This is significant because of the numerous non-idealities that appear in this regime, including the breakdown of paraxial optical models, and possible field dependent variations in optical aberrations and edge topography. The present microscope operates at apertures near 75% of the physical maximum in air. This is equivalent to NA 0.75-1.1 for inspection in a high index immersion medium, with an equivalent (full width) limiting resolution of 500-650 nm. These limits are set by the low cost standard objective lenses used by the present platform. The presumption of an initial PSF model from LPM diffraction theory [7] is shown to be adequate to resolve features to just below the Airy disk limit reliably in less than 100 iterations, and to cross-validate this information directly in multiple, registered broadband and spectral images.

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References