Multi-beam Synchrotron FTIR Chemical Imaging: Impacts of Schwarzschild Objective and Spatial Oversampling on Spatial Resolution

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Abstract. IRENI (InfraRed ENvironmental Imaging) is a recently commissioned FTIR chemical imaging beamline at the Synchrotron Radiation Center in Madison, WI. This novel beamline extracts 320 mrad of radiation, horizontally, from one bending magnet. The optical transport separates and recombines the beam into 12 parallel, collimated beams to illuminate a commercial FTIR microspectrometer (Bruker Hyperion 3000) that is apertureless and equipped with a multiple element detector. The beams are partially overlapped and defocused, similar to widefield microscopy, homogeneously illuminating a relatively large sample area compared to single beam arrangements. The effective geometric size of a pixel at the sample plane is defined by the magnification of the optics, and as expected, objectives with varying magnification and numerical apertures (NAs) impact the diffraction-limited resolution that can be obtained in the resulting images. We demonstrate here that spatial oversampling for synchrotron-based infrared imaging is critical to obtain diffraction-limited images at all wavelengths simultaneously. In this article measured and simulated point spread functions (PSF), resolution criteria and results from raw and deconvoluted images for two common Schwarzschild objectives (36x, NA 0.5 and 74x, NA.65) are compared to each other and to prior reports for confocal microscopes. The resolution of the imaging data can be improved by deconvolving the instrumental broadening that is simulated with the PSF. The contrast resolution for IRENI without employing apertures is similar to dual aperture, confocal microscopes.

1. Introduction
Synchrotron-based FTIR Chemical Imaging has recently been developed at the Synchrotron Radiation Center in Madison, WI [1,2]. There are two main components of the beamline dedicated to this experimental technique that distinguish it from previous synchrotron based Infrared beamlines equipped with FTIR microspectrometers. The first unique component of the beamline is the large swath of radiation (320(h)×35(v) mrad²) that has been collected, divided and recombined to create a...
homogeneous wide-field illumination at the sample plane in the microscope. The extended area that is illuminated is approximately \(40 \times 40\) micrometers\(^2\) (64 \(\times\) 64 pixels with 74\(\times\) objective) for the central, homogeneously, illuminated section, but can also illuminate up to \(140 \times 140\) micrometers\(^2\) (128 \(\times\) 128 pixels with 36\(\times\) objective) with reduced signal levels in the outer most areas of the field of view. The second component of the beamline that is critical is a multi-element detector, which is employed to collect multiple spectra simultaneously, retaining spatial definition [3].

Diffraction-limited imaging is dependent on the wavelength of the probe light, and on the NA of the objectives. There is a common Rayleigh criterion to distinguish two objects that are close to one another (\(d=0.61\lambda/\text{NA}\) for full-field illumination) that is similar to the criterion for Schwarzschild objectives, which is not available in closed form. The midinfrared spectra are broadband; the wavelengths range from 2 to 10 \(\mu\text{m}\), and thus it is impossible to collect high definition data for all wavelengths simultaneously if one uses only apertures and a single point detector. One must compromise either signal or spatial definition. Spatial oversampling is one strategy to obtain diffraction-limited, spatially resolved images across this bandwidth. The contrast resolution for confocal, dual aperture systems is predicted to be superior to one aperture systems, since the point spread function (PSF) is a multiplicative effect of two Schwarzschild objective PSFs, and results in a narrower central peak and suppressed sidelobes at each wavelength [4]. Interestingly, reports of the contrast resolution for confocal microscope optics with no apertures illuminated with a synchrotron beam [5], in which the resolution is limited primarily by the electron beam source size, were similar to the predicted values with dual apertures [4]. For the widefield geometry employed in the present experiment, which is apertureless, the definition of the effective geometrical pixel size is based on the magnification of the objective.

In this paper we will compare PSFs and spectrochemical images from two Schwarzschild objectives with varying magnification and NA (36\(\times\), 0.5 NA and 74\(\times\), 0.65 NA) that can both be used in the Bruker Hyperion 3000 microscope. While the highest magnification is likely desirable in most cases, sometimes experimental constraints require the inherently larger working distance (i.e. environmental controlled stages) and or the lower numerical aperture (i.e. tomography) that are available with the lower magnification, lower NA objective. Thus a direct comparison of the characteristics of these two objectives is provided here. Moreover, we explore the feasibility of applying recently developed PSF deconvolution algorithms [6] to IR hyperspectral data sets acquired using the 36\(\times\), 0.5 NA objective. We show that the experimental contrast resolution is dependent on the NA of the objective and the spatial oversampling, can be improved with deconvolution, and is similar for both the widefield illumination, FPA and confocal, raster scanning beamlines.

2. Methods

We characterize the PSF of the imaging system using transmission measurements of a pinhole of 2 \(\mu\text{m}\) diameter. The performance of the 36\(\times\)-based imaging system and the corresponding PSF deconvolution are evaluated using test samples with strong mid-IR absorptions. The sample is a mixture of 1, 2.2 and 6 \(\mu\text{m}\) polystyrene (PS) beads embedded in a 10 \(\mu\text{m}\) polyurethane (PU) film. This film and independent PS beads are electrostatically bound to a polyimide (PI) sample holder.

The pinhole and sample are measured in transmission. They are illuminated with a defocused Schwarzschild condenser, with a matching NA to the focusing objective, thus defining the illuminated area by the defocused condenser, while the magnification of the geometric plane is defined by the parameters of the focusing objective. For example, when the 74\(\times\) Schwarzschild objective is used for focusing, a 20\(\times\) Schwarzschild condenser (.58 NA) is used to illuminate the sample. The objective effectively images 0.54 \(\times\) 0.54 \(\mu\text{m}\) \(^2\) onto each 40 \(\times\) 40 \(\mu\text{m}\) \(^2\) detector pixel, which is sufficiently spatially oversampled to obtain diffraction limited results for the entire mid-IR bandwidth [1,2]. Typically, lower magnification and smaller NA objectives provide larger working distances, which is also the case for Schwarzschild objectives. Thus, when experimental limitations require larger working distances than the limiting range of 1 mm for the 74\(\times\) objective, or require a smaller numerical aperture, these other options can be employed.
The data is collected as a hyperspectral cube (x,y, Abs(ν)). The deconvolution approach has been described in detail in a recent publication [6]. In brief, Fourier transform (FT) based image deconvolution methods have been developed, based on the measured and simulated point spread functions. Each image, as a function of wavelength, is deconvoluted independently and is subsequently rescaled by requiring equal transmitted light in the original and processed data. Finally, the images are reassembled in a hyperspectral cube.

3. Results and Discussion

3.1 Wavelength-dependent PSF of the 36× and 74× Objectives

To determine the instrument's PSF, a 2 μm diameter pinhole was placed on the image plane, and magnified onto the detector via the appropriate Schwarzchild objectives. The measured intensity distribution was fitted to an equation describing the diffraction pattern of an annulus, as described previously [6]. Figure 1 shows the measured PSFs, experimental and fitted cross sections at three different frequencies across the mid-IR range for both objectives. Several features are obvious: first, as the wavelength increases, the PSFs become broader and the side bands that represent the first “fringe” in each cross section due to diffraction are spread farther from the central maximum. It is also clear that the density of pixels for the 36× objective is not as high as the density of pixels for the 74× objective, and that the definition of both the central (zeroth) and first order maxima are more defined for the latter objective. Also, the measured PSFs for the 36× objective do not contain a uniform ring for the first order diffraction maxima as seen for the 74× PSFs. Rather, there is a nonuniform ring that appears superimposed with a fourfold-symmetric intensity distribution. The nonuniformity of the Airy ring is due to the assembly that holds the secondary mirror in place. The assembly consists of four tines that attach the secondary mirror to the housing of the primary mirror. Consequently, the 36× objective presents a significant deviation from the simplistic annulus model used previously [6] to describe the PSF of the 74× objective. Due to the asymmetry in the intensity distribution, the cross sections shown for the 36× in Fig. 1 are determined by taking the average of 4 profiles taken from the horizontal, vertical, and two diagonal directions through PSF. The observed four-fold intensity distribution is not expected to have any impact on the deconvoluted results because in the convolution operation, every point in the image is an multiplied with its corresponding value of the PSF from surrounding points in each direction. Thus, each pixel effectively sees the "average" of the PSF from surrounding points, which is represented by the averaged line profiles in Fig. 1.

3.2 Resolution of the 36× NA 0.4 and 74× NA 0.65 Objectives

In Figure 2 simulated resolutions based on the parameters determined from the experimental PSFs for both objectives are compared and overlaid with the experimentally-determined wavelength-dependent resolution of both objectives. We first consider the full-width at half-maximum (FWHM) of the PSF profiles of both objectives. As expected, the FWHMs trends display a monotonic increase with increasing wavelength. Furthermore, over the entire wavelength range observed, the FWHM of the 74× objective is significantly narrower than that of the 36×, ranging from a difference of ~0.75 to 1 μm, which is due to both the NA and effective geometric pixel sampling for the objectives. Next, the PSF contrast resolutions (PSFCR), similar to the Rayleigh criterion for the Airy function, for the objectives are compared. While this contrast resolution was initially derived from the Rayleigh criterion for unobstructed lenses [7], the objectives used here are obstructed, and there is no equivalent closed-form analog to the Rayleigh criterion. Nevertheless, the contrast criterion [7] is commonly used as a measure of the resolution for imaging systems with obstructed lenses despite the deviation of the PSF of such systems from the conventional Airy function [1,5,8]. Here, full width at 73.6 % maximum is calculated since two objects are considered resolved if the contrast difference between them exceeds 26.4 %. As for the case of the FWHM, the PSFCR is substantially smaller for the 74×
than for the 36× objective, particularly at the longest wavelengths. Finally, the experimental resolutions of the objectives were determined from transmission measurements of high-resolution United States air force (USAF) targets, as described elsewhere [1-2,6]. Results from the original experimental and deconvolved data were compared to the simulated PSFCRs. For the 74× objective, the experimentally resolved features (grey circles) from the original data agree well with the simulated PSFCR and therefore this effectively describes the resolution of the objective. Interestingly, the experimentally determined resolution for the original data for the 36× objective is noticeably poorer than that predicted by the PSFCR criterion. This is likely attributable to the coarser sampling used for this objective (1.1 μm effective pixel size), which precludes this setup from achieving its optimal resolution. Following deconvolution, however, both objectives yield resolutions exceeding the predicted contrast resolution criterion. This suggests that instrumental broadening is successfully removed, and a more faithful image is recovered.

Next, we compare these results to previously published works with analogous objectives. Carr [4] explored resolution limits using a confocal synchrotron-based IR microscope with NA=0.65, while Levenson et al. [5,8] used a similar approach, in which an apertureless confocal geometry with a focused synchrotron beam and spatial oversampling was used, to study spatial resolution limits with a 32× objective with NA=0.65. The PSF contrast resolution results presented here for the original data collected using the 74× (NA=0.65) objective show resolution limits ranging from comparable (3000-4000 cm⁻¹) to poorer (2000-2500 cm⁻¹) to those described in [4] for a dual aperture confocal setup. Upon deconvolution of IRENI’s PSF, the resolution exceeds the simulated PSFCR criterion and becomes comparable to the resolution reported for the confocal geometries [4,5]. However, as expected due to the smaller NA, the results for the 36× objective always demonstrate a poorer resolution. In sum, with adequate spatial oversampling, the resolution of raw FPA based
measurements approach the observed confocal mapping resolution, with the inherent speed advantage from parallel detection. Further, by applying deconvolution with accurate PSFs the resolution of all images across the mid-infrared demonstrate equivalent results as those recorded with confocal mapping.

![Image](image.png)

**Fig. 2: Extracted parameters from fitted PSFs.** FWHM resolution and contrast resolution of the PSF for the 36× and 74× objectives compared to the experimentally determined resolution limit for the 36× and 74× objectives before and after deconvolution. For comparison, we have added the experimental resolution from Ref. [5] and the Rayleigh criterion for each objective.

3.3 Hyperspectral Deconvolution of 36× Spectrochemical datasets

In Figure 3, visible and chemical images of the PS/PU and PI assembly obtained at IRENI using the 36× objective are shown. The visible images in Fig 3A-B show the front and back of the sample; images viewed from the back more effectively resolve PS beads that overlap with the PI sample holder. Fig. 3C-F includes chemical images generated from integrating over PU (C and D), PS (E and F) and PI (G and H) specific absorption bands from both raw (left, C,E and G) and deconvoluted (right, D,F and H) data. Also, spectra from points indicated in the images are shown (I,J) before and after deconvolution, demonstrating the quality of the spectra that are measured and retrieved from deconvolution. Comparing the PI images, before and after deconvolution, the edges of the structure are more clearly defined after deconvolution. For the PS images, a weak CH band is integrated, and the PS beads embedded in the PU are easily observed after the deconvolution. In contrast, PS beads that are co-located with the PI holder are more difficult to detect, which we attribute to the overlapping absorption bands for the PS and PI CH stretching vibrations. The PU images clearly show minima at all of the PS beads. The combination of all three images is useful to identify the positions of the weakly absorbing PS beads. We emphasize that there are no features in the image that present four-fold intensity artefact following deconvolution. These data confirm that the deviation from axial symmetry of the measured PSF is effectively removed in favour of the radially averaged behaviour of the PSF and that the fitted, axially symmetric PSF is appropriate to use for deconvolution. In Figs. 3K.
and L, stacks of spectra across an individual PS bead (along the line in Fig. 3C) are provided to compare detailed spectral results from the original and deconvoluted data. Moving from off to onto a PS bead, changes in the PS-specific band at 3026 cm\(^{-1}\) show a much more gradual onset and decay in the raw data, while in the deconvoluted data, a sharper more abrupt transition is observed. A quantitative analysis of this transition is provided in Fig. 3M, which shows the intensity of this band in both sets of data as a function of position along the line. Note that deconvolution causes a shift in spectral weight from the exterior of the bead to the center of the bead, resulting in increased absorption intensity in the center relative to the periphery. This is indicative of a reduction of the diffraction-induced blurring achieved by the PSF deconvolution. In Fig. 3N, we show an analogous analysis for data taken at IRENI from with the 74\( \times \) objective.

**Fig. 3:** Deconvolution of Chemical Images and Spectral Reconstructions of a PS/PU/PI sample. A,B) Visible images of the sample consisting of PS beads embedded in a PU film, supported on a PI holder as viewed from the front (A) and the back (B) the holder. (C) shows a chemical image generated integrating under a PU-specific band (1650-1710 cm\(^{-1}\)), with the same image generated from the deconvoluted data in (D) (scale bar=20 \( \mu m \), total area imaged is 70 \( \times \) 70 \( \mu m^2 \)). Chemical images generated by integrating the raw and deconvoluted data sets under a PS-specific band (3000-3050 cm\(^{-1}\)) are shown respectively in (E-F), and under a PI-specific band (1575-1610) in (G-H). Spectra from the original and deconvoluted data sets taken from a region containing only PU are shown in (I), and from a region on top of a PS bead in (J). In (K-L), we compare spectra taken from along a line spanning a PS bead from the original and deconvoluted data sets, respectively. Spectra from the hyperspectral cubes are extracted along the dashed black line indicated in (C). The top two spectra show the PS and PU standard spectra for comparison. We
compare the absorbance of the PS band at 3026 cm$^{-1}$ at the positions used for the spectral stacks of both the original and deconvoluted data in (M), and from an analogous region in a dataset from the 74× objective in (N).

**Conclusion**
The experimental contrast resolution for synchrotron based FTIR imaging is only slightly less than the contrast resolution for confocal, raster scanning microscopes and becomes equivalent when PSF deconvolution is applied, while benefiting from the rapid data acquisition with a multi-element detector. The experimental resolution is dependent on the characteristics of the focusing Schwarzschild objective, where different NA objectives show different results. Correct spatial oversampling is necessary to obtain diffraction-limited results for the entire mid-IR bandwidth. In this paper, we have compared measured and simulated PSFs for 74× (0.65 NA) and 36× (0.5 NA) objectives. We find that, based on the characteristics of the PSFs, the 74× objective intrinsically has a superior spatial resolution than the 36× objective. The as measured results with the former (latter) objective have a similar (slightly poorer) spatial resolution to reported values for confocal optics with similar NAs. Nevertheless, in both cases, PSF deconvolution can be applied to the data to further improve the spatial resolution, by removing instrumental broadening. Deconvolution techniques are successfully applied to a mixed polymer system measured with the 36× objective. A comparison of cross sections across polystyrene beads measured with the 36× and 74× objectives shows similar behavior with reduced intensity variation for the former objective.

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