Supplemental information

Dynamic full-field infrared imaging with multiple synchrotron beams

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1. Spinal cord preparation

A non-symptomatic wild-type SOD-YFP mouse was sacrificed at 16 months of age. The mouse was anesthetized and perfused with PBS. To extract the spinal cord, the vertebral column was removed, and a syringe filled with PBS was used to push the spinal cord out of the column. The spinal cord was frozen immediately over dry ice, and stored in a freezer until sectioned. The frozen specimen was embedded in optimal cutting temperature (OCT) (Tissue-Tek, Sakura, Japan) and sectioned to 5 μm thick using a cryo-microtome (Leica, Germany) at -15 °C. Once the section was positioned on to a CaF_2 slide (Korth Kristalle, Germany), it was removed from the cryotome and quickly warmed to room temperature, drying-out in the process. Cross sections were rehydrated with water and stained with 200 μL of aqueous eosin Y solution (Sigma-Aldrich) that had been acidified with glacial acetic acid to a final concentration of 0.2% acetic acid. The acidified eosin was incubated on the sample for 30 seconds and then carefully rinsed with water to remove excess stain. IR images were acquired with 8 cm^{-1} resolution with 32 scans per frame (total time ca. 80 min).

2. Number of source segments calculation

The goal of this work was to configure the synchrotron source into an optimal format for FTIR imaging with a focal plane array detector. An additional constraint is delivering the light as a near-collimated beam through the interferometric spectrometer that analyzes the spectroscopic content at each detector pixel. The approach here was to optimize the collimation of individual source segments without sacrificing the intrinsic source brightness and then rely on diffraction-blurring at the sample plane to achieve reasonably homogeneous illumination.

We start by identifying the synchrotron source as a continuous sequence of nearly ideal diffraction-limited segments arranged along the arc of the electron beam as it traverses a dipole bending magnet. Each electron emits continuously in the forward direction as it traverses the orbit arc, forming a swath of light in the far-field. Thus, an optical extraction collecting an angle $\boldsymbol{\theta}$ of this swath views a source arc segment having length (or depth) $\boldsymbol{\rho\theta}$ where $\boldsymbol{\rho}$ is the arc trajectory's radius of curvature.[1] For the NSLS VUV-IR storage ring, $\boldsymbol{\rho}$=191 cm and the U10 beamline's collects 85 mrad horizontally, which corresponds to a source arc length of about 16 cm. In contrast, the source's lateral extent (ignoring diffraction) is only 0.17 cm. Thus the source has considerable depth that must be managed by an optical system intended for imaging planar samples. If ignored, only a segment of the source arc will be in focus. Due to the curvature of the source, the out-of-focus segments will extend off to one side, giving the appearance of coma aberration.

But this is not coma in the usual sense of an optical aberration but rather a projection of the out-of-focus beam. The degree to which this problem should be resolved is given by the diffraction of light.

Though the far-field intensity distribution of dipole synchrotron radiation differs from that of a Gaussian (especially in the horizontal direction), we can make useful approximations by assuming Gaussian beam optics where the Rayleigh range serves as a measure of the minimum discernible source depth. For a Gaussian beam passing through a focus:

$$W_0 = \lambda/\pi\theta_o$$

$$Z_0 = (W_0)^2\,\pi/\lambda$$

where θ_o is the RMS emission angle, W_0 is the RMS focus width, Z_0 is the Rayleigh range, and λ is the wavelength. Approximate expressions for dipole synchrotron radiation that obey these relationships are:

$$\theta_{sr} = (2/\pi)(\lambda/\rho)^{1/3}$$

$$W_{sr} = (1/2)(\lambda^2/\rho)^{1/3}$$

resulting in:

$$Z_{sr} = (\pi/4)(\rho^2\lambda)^{1/3}$$

Note that, although these are not true RMS values (since the beam is not an actual Gaussian), they are sufficiently accurate to serve our purpose. Also, the RMS value is smaller than the distribution's half-width at half-intensity (HWHM), therefore we use twice these quantities as a more practical value for the diffraction-limited source dimension.

We now consider the Rayleigh range for dipole radiation from the NSLS VUV-IR ring over the spectral range of interest. The variation with wavelength is fairly weak so we consider a wavelength of $\lambda = 6$ µm (frequency of about 1670 cm^{-1}) since it is intermediate to the overall spectral range of interest, lies in the fingerprint spectral region, and corresponds to some important vibrational spectra features, e.g. the Amide I vibration in proteins and various polymers. We find Z_{sr} = 2 cm and a minimum discernible source depth of 2 × 2 cm = 4 cm. This implies that dividing the 16 cm long source into four 4 cm segments, each segment will not have a geometric depth greater than this diffraction limited value. Indeed, we can define the number of source segments in terms of either emission angle or source depth:

$$\#_{segments} = (\theta_{coll}/2)\theta_{sr} = (\pi/4)(\rho/\lambda)^{1/3}$$

or, in terms of depth:

$$\#_{segments} = (r_{coll}/2)Z_{sr} = \rho(\theta_{coll}/2)Z_{sr} = (2/\pi)(\rho/\lambda)^{1/3}\theta_{coll}$$

which are approximately equal. Thus, the source depth issue is managed through our criteria for the number of source segments to divide the overall angular collection simultaneously. For the case of the IRENI beamline (collecting 320 mrad horizontally) the above equations yield approximately 14 individual segments.

3. Infrared extraction efficiency (figure-of-merit) calculation

For an application where an extended source is beneficial (such as illuminating a sample for infrared microscopic chemical imaging), the quality of the source is determined by both the number of available source segments and the brightness of each segment. The following section provides the reader with a straightforward approach to estimate the efficiency of the infrared light extraction for a given beamline.

As described above, the number of segments ($N_{segments}$) is the ratio of the actual horizontal extraction ($\theta_{collected}$) to the natural opening angle (θ_v). The intrinsic brightness of each segment is determined solely by the beam current (I). As an incoherent source, that dependence is linear, i.e.

$$\text{Brightness} \sim I$$

Thus, a useful figure of merit (FOM) for a given infrared beamline's optical extraction efficiency is the product of the number of segments and the current, i.e.

$$\text{FOM} = I \cdot \theta_{collected} \cdot \rho^{1/3}$$

where the wavelength dependence has been dropped since it does not depend on the particular synchrotron facility. **Table 1** summarizes FOM values for several synchrotron sources where infrared beamlines are operational.

References

1. Duncan, W. D., Williams, G. P. *Applied Optics* **1983**, *22*, 2914-2923

Table 1. Machine parameters and infrared extraction angles for a representative selection of synchrotron infrared beamlines.

Facility	Bend Radius [m]	Avg. Current [mA][a]	Hor. Angle [mrad]	FOM
NSLS VUV-IR	1.91	670	90	75
NSLS-II	25	500	50[b]	73[b]
SRC	2.083	200	320	85
ALS	4.9	500	69	59
CLS	7.14	500	50	48
BESSY	4.355	300	60	29
SOLEIL	5.39	400	78	55
ELETTRA	7.257	280	65	35
ANKA	5.559	100	45	8
DIAMOND	7.128	300	50	29
Australian Synchrotron	5	200	60	21
UVSOR	2.2	300	215	84
SPRing-8	39.3	100	36	12

[a] An average value is given for facilities that do not operate in top-up mode.

[b] The proposed InfraRed Imaging (IRI) beamline at NSLS-II will extract synchrotron radiation from two dipoles, resulting in a total horizontal acceptance of 100 mrad and a FOM=146.