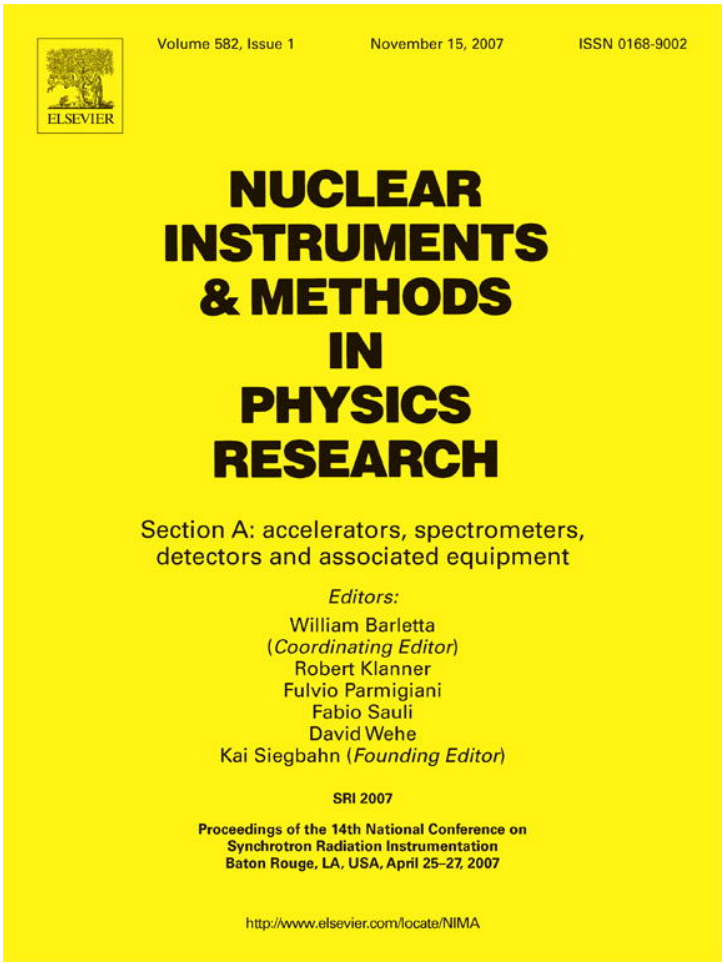

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The infrared microspectroscopy beamline at CAMD and its application in plant–pathogen interactions

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Abstract

At the beginning of 2006, the first infrared microspectroscopy beamline at the Louisiana State University, Center for Advanced Microstructures and Devices (CAMD) storage ring came into operation. The infrared microscope has recently been upgraded with a new liquid nitrogen-cooled mercury–cadmium–telluride detector, MCT-A, and a new dipole chamber to improve the signal-to-noise ratio and extend the beamline capability to far-IR region.

In this contribution, we report first results, by using the microspectroscopy beamline, in the investigation of plant–pathogen interactions: apple–*Venturia inaequalis* causing scab. The infrared spectra of the healthy plant leaves were compared to those obtained from the infected ones. These spatially resolved data are used to understand the dynamics of physiological modifications, which occur during pathogenesis.

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Keyword: Infrared microspectroscopy; Brightness; Signal-to-noise ratio; Pathogen; Plant

1. Introduction

Synchrotron radiation-based Fourier infrared microscopy has significantly improved the lateral resolution of infrared imaging with a superior signal-to-noise ratio compared to conventional globar sources [1,2]. This advantage has widened the applications of IR microscopy and makes it a unique analytical tool to probe the chemical composition of small samples and also small heterogeneous parts of large samples. The CAMD IR beamline has been used to investigate a broad range of samples from various research areas: from geological rocks and samples from rusty pipelines to polymer blends where the chemical compositions and interactions between macromolecular constituents have been elucidated.

2. Beamline performance

The original dipole chamber for the IR beamline at CAMD was designed to extract radiation from 70 mrad horizontal and 15 mrad vertical directions. These openings allow the measurements in the mid-IR region; however, it limits the performance in the far-IR region. To enhance the capability of the IR beamline, specifically, in the low frequencies (far-IR and THz regions), a new IR dipole chamber with $50 \times 50\,\text{mrad}^2$ opening angles, funded by Board of Regents of Louisiana State, was recently installed. In addition, we installed a MCT-A detector which possesses a higher sensitivity compared to the original MCT-B detector that leads to better signal-to-noise (S/N) ratio.

Fig. 1 shows the infrared spectra of a wheat leaf taken with synchrotron and globar sources at $15 \times 15\,\mu\text{m}^2$ aperture size. It illustrates how synchrotron radiation

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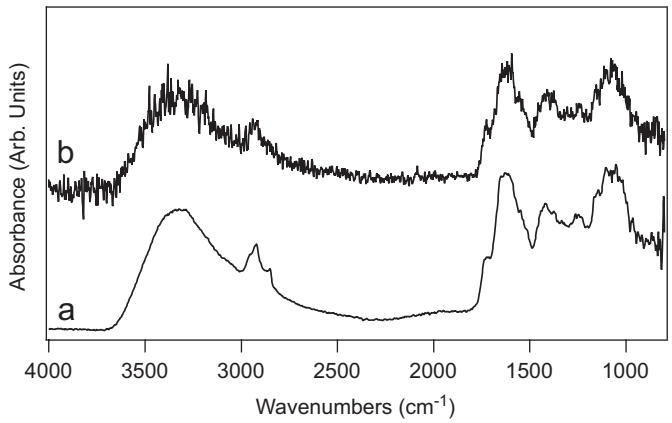

Fig. 1. Infrared spectrum of a wheat leaf measured with synchrotron radiation at 202 mA beam current (a) shows improved signal-to-noise ratio compared to one collected with a globar source (b).

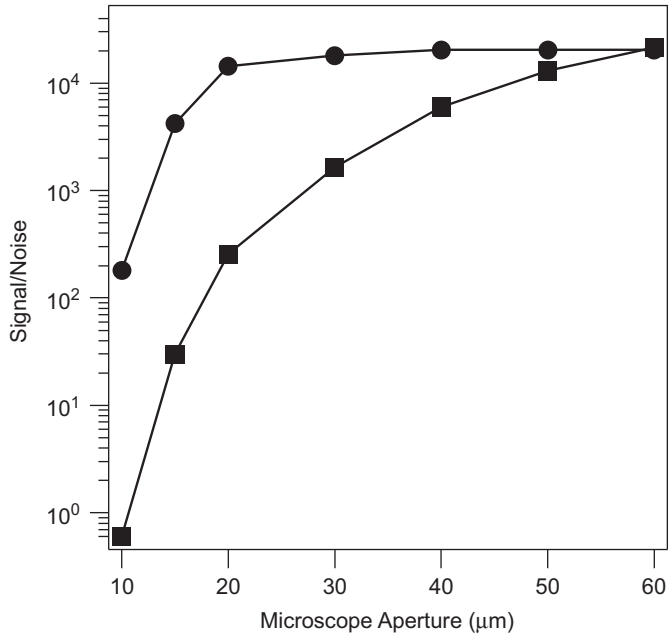

Fig. 2. Signal-to-noise ratio as a function of the microscope aperture size for synchrotron radiation after the installation of the new dipole chamber and the globar source.

improves the S/N ratio due to its superior brightness. As opposed to one measured with synchrotron radiation, not all the peaks can be discerned in the spectrum taken with a globar source.

The S/N ratio is calculated by obtaining the interferogram maximum value and root-mean-square noise value from the region of 2450–2550 cm^{-1} of the corresponding 100% transmission spectrum. An order to two orders of magnitude better S/N ratio is obtained for synchrotron radiation-based measurement (in the range of 100–200 mA beam current of storage ring) than the one obtained by the conventional thermal source. In Fig. 2, the measured S/N ratio for synchrotron and globar sources is plotted as a function of the microscope's aperture size. S/N measurements for synchrotron source were taken with 140 mA beam current. The brightness advantages of the synchrotron radiation greatly enhance the S/N ratio. At $15 \times 15\,\mu m^2$, synchrotron radiation provides 300 better S/N ratio than the one provided by the thermal source.

3. Application: plant–fungi interaction

Besides Asian soybean rust, that was discovered for the first time in the United States on November 9, 2004 in an experimental field in Louisiana, there are numerous other plant diseases caused by fungi that threatens agriculture in the US, e.g. head blight and the "take-all" disease that leads to major wheat crop losses, and powdery mildew and apple scab that damage fruits. In spite of all the damage caused by fungi, there is hardly anything known in detail about the mechanisms by which the plant metabolism is modified by infections in order to supply nutrients for pathogen development. There are hypotheses that the fungus accumulates trace elements that are important for a healthy growth and/or organic compounds that are crucial for the development of the pathogen. The second hypothesis was tested by spatially resolved Fourier IR spectroscopy at the CAMD IR beamline. For these experiments, apple leaves infected by *Venturia inaequalis* were investigated and IR spectra were measured in transmission mode

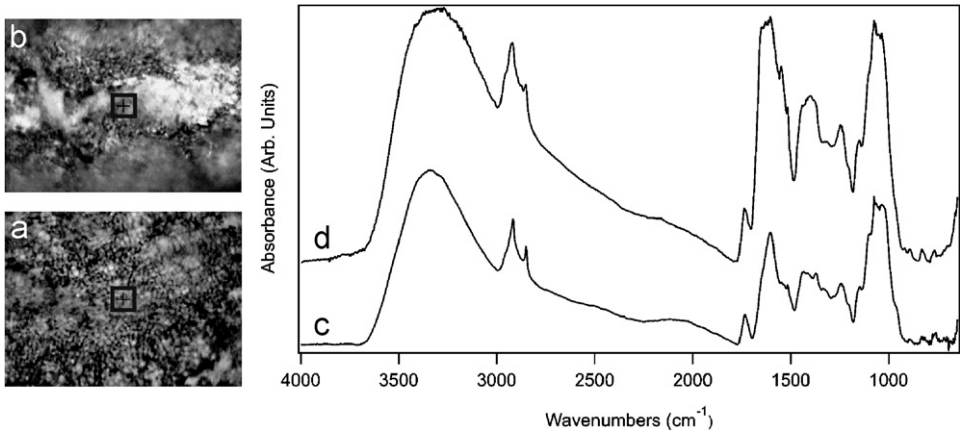

Fig. 3. The optical image of the healthy (a) and the infected apple leaves (b) and infrared spectra of healthy and infected samples are shown in (c) and (d), respectively.

for the healthy and infected leaves. Fig. 3 shows optical image and typical spectra from these samples. Both spectra are rather complex but typical for biological samples that are mixtures of proteins, nucleic acids, lipids and carbohydrates [3]. There are no distinguishable changes noticed in the lipid's features of both samples (2900–3000 cm^{-1}). The lipid ester peak, a sharp peak in the infrared spectrum of healthy leaf (C═O, 1730 cm^{-1}), becomes a shoulder in the spectrum of the infected sample. As noticed in other plant leaves, the most drastic changes are obtained in the features of proteins (1500–1700 cm^{-1}). This band is broadened and absorbance intensity is increased in the infrared spectrum of the infected leaves. This absorbance increase is also seen in the band of carbohydrates (1000–1200 cm^{-1}). Though not as obvious, there are also changes in the range between 1000 and 1250 cm^{-1}, indicating changes in the structure/concentration of nucleic acids and/or phospholipids. Although these studies need additional detailed analysis, it is already obvious that the fungus influences strongly the organic structure of the leaves.

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