

## Environmental and Earth Science

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### Probing Individual Living Cells with Synchrotron-Based FTIR Spectromicroscopy

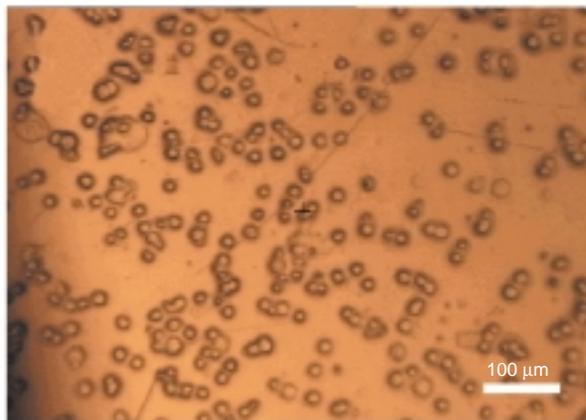
*Can infrared light from a synchrotron be used to study changes in single, living human cells? If so, it would open up possibilities for the eventual use of synchrotron infrared light in medical diagnostics or environmental health research. Some day, it may be routine to study a tiny sample of tissue from a patient and, by using synchrotron infrared spectroscopy, identify a single cell that has been subtly altered by exposure to a chemical. The two experiments reported here by a Berkeley Lab/ALS team, infrared microscopy studies of human cells throughout their life cycle and of cells exposed to the toxic chemical dioxin, bring this day closer. However, using a scientific technique in a way in which it hasn't been used before calls for verification by comparison with other methods. The strong correlation the researchers observed between infrared and known biochemical techniques shows that the group was indeed measuring real changes associated with the cell cycle and with dioxin.*

In this highlight, we first report the use of synchrotron-radiation-based Fourier-transform-infrared (SR-FTIR) spectromicroscopy to observe distinct spectral changes that occur in individual living human lung cells as they progress through the normal cell cycle and finally die. Second, we demonstrate that SR-FTIR spectromicroscopy can be useful for examining dioxin-induced changes in individual living human liver cells, thereby providing new clues about what happens to human cells exposed to dioxins (polychlorinated aromatic hydrocarbons that can cause cancer, birth defects, and altered

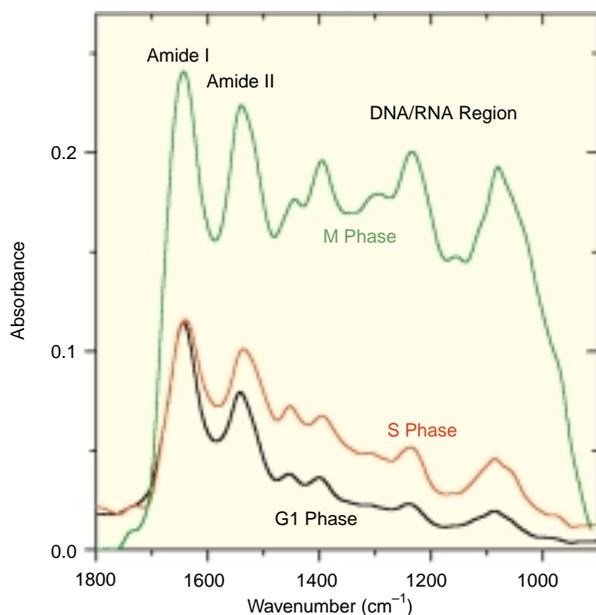
hormone levels, among other things). For all work, we used the FTIR microscope at ALS Beamline 1.4.3.

For the cell-cycle studies, we used living human lung fibroblast cells that had been placed onto gold-coated glass slides (Figure 1). We found that these cells showed clearly different infrared spectra throughout the cell cycle. Figure 2 shows spectra in the 1800 to 900  $\text{cm}^{-1}$  region for typical individual cells in three cell-cycle phases. During the S phase, the DNA is undergoing replication and we observe that the absorption in the DNA/RNA spectral region increases relative to the G1 phase spectra by approximately a factor of two. When a G2/M-phase cell is measured, we observe a large increase in the overall absorbance, as is expected since the cell has roughly doubled in material in preparation for mitosis. Absorption in the DNA/RNA region is significantly increased relative to the protein peaks.

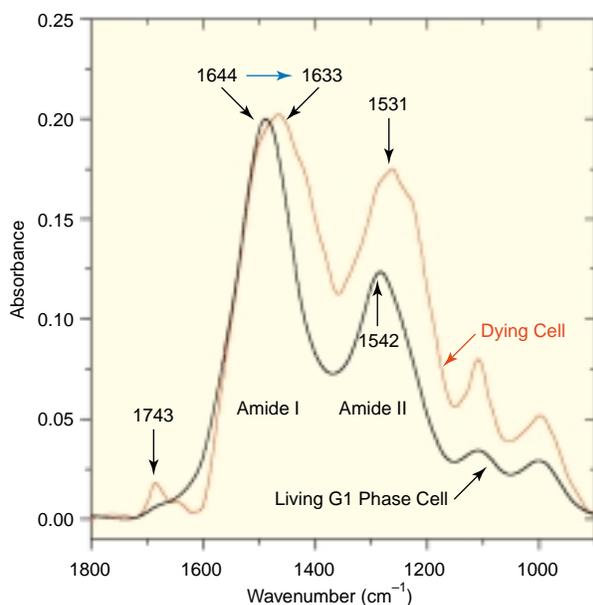
Occasionally some cells exhibited signs of dying or death even though visually the cells' morphology did not appear different. The spectrum of one such cell is shown in Figure 3 along with the spectrum of a normal living G1 phase cell. The "dying" cell shows two characteristic spectral signatures indicative of death. First, the centroids of the protein Amide I and II peaks shift from 1644 to 1633 and from 1542 to 1531  $\text{cm}^{-1}$ , respectively, indicating a change in the overall protein conformational states within the cell. Second, we observe the appearance of a peak around 1743  $\text{cm}^{-1}$ . These observations can now be used as signatures of cell death in future studies.



**Figure 1** Cells placed on gold-coated slides ready for SR-FTIR analysis. Spot sizes for spectromicroscopy were 10  $\mu\text{m}$  or less, allowing study of individual cells.



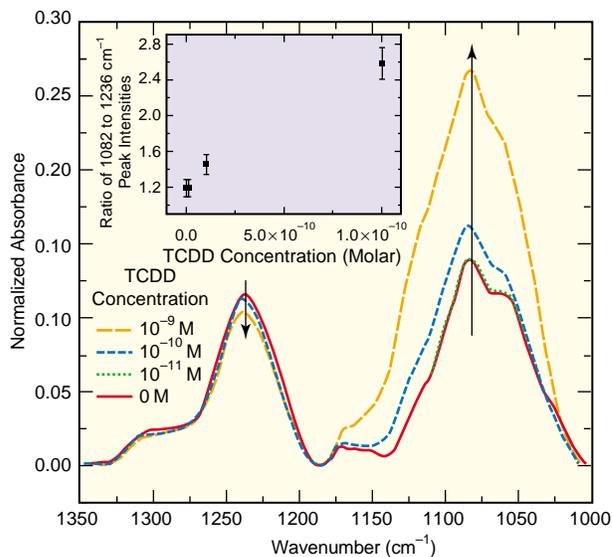
**Figure 2** IR spectra in the 1800 to 900  $\text{cm}^{-1}$  region for typical individual cells in the gap 1 (G1), synthesis (S), gap 2/Mitosis (G2/M) phases of the cell cycle showing clearly different infrared spectra. Spectra were not normalized, but a linear baseline was subtracted over the range of 2000 to 650  $\text{cm}^{-1}$ .



**Figure 3** IR spectra comparison of a cell exhibiting signs of dying along with the spectrum of a normal living G1-phase cell. Spectra were normalized to the Amide I peak, and a linear baseline was subtracted from 1800 to 1350  $\text{cm}^{-1}$ . Changes in the spectra can now be used as signatures of cell death in future studies.

For the dioxin studies, we examined the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on liver cells. In the body, particularly in liver cells, TCDD induces increased production of cytochrome P4501A1, which is involved in metabolizing foreign compounds, such as aromatic hydrocarbons. In the study, three experimental groups of human hepatocellular carcinoma (HepG2) cells (cells from a liver tumor) were exposed to TCDD at different concentrations for 20 hours, while cells in a control group were kept in an incubator for 20 hours with no TCDD exposure.

In the mid-IR spectra we recorded, significant absorption differences between the treated samples and the control samples were evident for wavelengths associated with stretching vibrations in two bond types, phosphate and C-H. For phosphate (Figure 4), the intensity for the symmetric stretch band increased relative to that for the asymmetric stretch band with increased TCDD concentration but did not shift in wavelength. The band representing methylene ( $\text{CH}_2$ ) stretch decreased and that



**Figure 4** Infrared spectra of HepG2 cells near phosphate bands at 1236  $\text{cm}^{-1}$  (asymmetric stretch) and 1082  $\text{cm}^{-1}$  (symmetric stretch) show intensity differences for cells treated with varying concentrations of TCDD. The unchanging wavelengths at which the phosphate vibrations were found show that phosphate has weak hydrogen bonding that does not change with TCDD exposure.

for methyl (CH<sub>3</sub>) stretch increased with increasing TCDD concentration. The increasing number of methyl groups relative to the number of methylene groups with TCDD exposure may indicate increased DNA methylation, which some researchers have suggested may cause gene inactivation—one possible mechanism by which TCDD could take its toxic toll.

Some cells from each group were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) to track the effect of TCDD on expression of the CYP1A1 gene. The RT-PCR results did in fact show the expected increase in CYP1A1 gene expression with increasing exposure to TCDD. This strong correlation between spectroscopic and RT-PCR data shows that the spectromicroscopy measurements succeeded in tracking real changes that are associated with induction of the CYP1A1 gene.

#### **INVESTIGATORS**

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#### **PUBLICATIONS**

1. H.-Y.N. Holman, M.C. Martin, E.A. Blakely, K. Bjornstad, and W.R. McKinney, "Infrared spectroscopic characteristics of cell cycle and cell death probed by synchrotron-based FTIR spectromicroscopy," *Biopolymers (Biospectroscopy)*, **57**(6), 329 (2000).
2. H.-Y.N. Holman, R. Goth-Goldstein, M.C. Martin, M.L. Russell, and W. R. McKinney, "Low-dose responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin in single living human cells measured by synchrotron infrared spectromicroscopy," *Env. Sci. & Technol.* **34**(12), 2513 (2000).