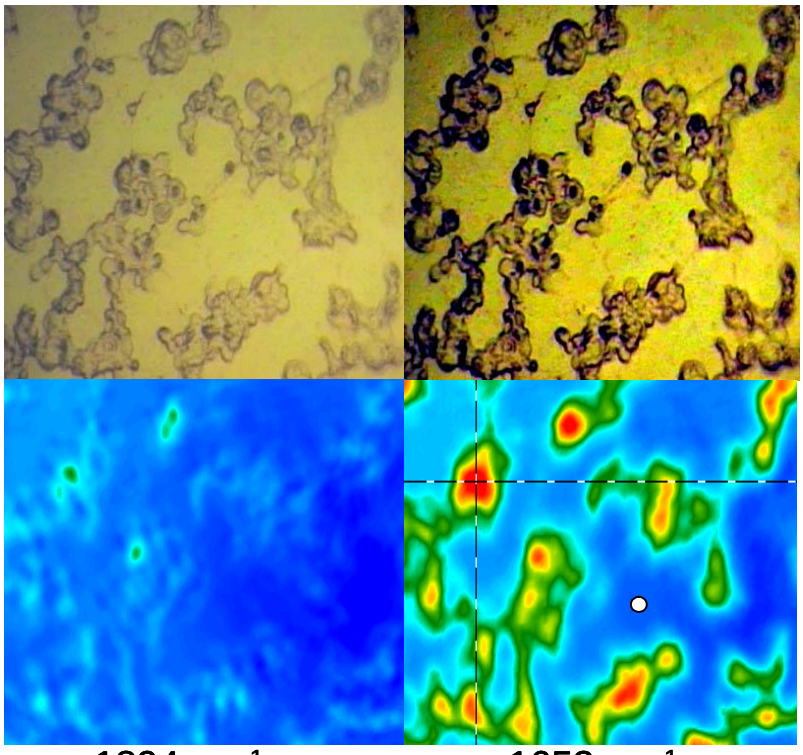
Hyperspectral Microscopic Infrared Imaging



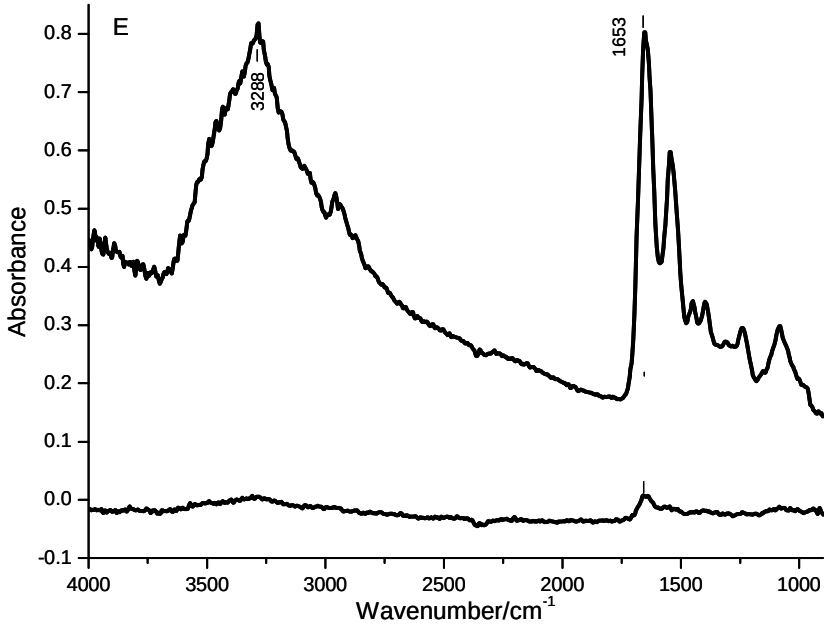


Figure 1: (A) Transmission visible image collected for vero cell monolayers that have been infected for 48 hours with HSV-1 at a MOI of 10. The cells were grown directly on ZnSe windows. After the specified time interval the cells were washed and fixed with acetone. (B) Same image as in (A) that has been modified using contrast enhancing filters in Adobe Photoshop 8.0. (C), (D) False color spectral images acquired at single frequencies of 1894 and 1652 cm^{-1}, respectively. Spectral resolution was set at 4 cm^{-1} and 64 spectral image blocks were coadded. A 128x128 MCT array was used. These spectral image cubes were collected using the step scan mode of the Varian 7000 FTIR spectrometer. The colors represent absorbance above a zero baseline at that pixel. (E) Spectra obtained at the single pixels marked with a cross (*top spectrum*) and white circle (*bottom spectrum*) in (D). The vertical lines in (E) show the frequencies at which the images in (C) and (D) were produced. So, at the cross/circle in (D) the absorbance is ~0.85/0.2, respectively. Spectral images and spectra generated using Resolutions pro software from Varian.

Figure 1A shows a visible image collected in transmission mode for vero cell monolayers that have been infected with HSV-1 at a MOI=10 for 48 hours. Figure 1B shows a contrast modified version of the image in (A). From the visible images one could conclude that the cell monolayers have been heavily decimated, with only patches of cellular material remaining. Figure 1D shows a spectral image at 1652 cm^{-1}. This frequency is in the range at which proteins absorb (the so-called amide I region), so this image should specify the protein distribution in the image. One only expects proteins in regions where cellular material is present, and clearly the infrared image is in good agreement with the visible image, with regions of high absorbance at 1652 cm^{-1} shown as red and low absorbance at 1652 shown as blue. However, the spectrum taken at the white circle indicates that cellular protein material is present at this location, albeit at a much reduced level compared to that at the position of the cross. In fact, the protein absorbance in the vicinity of the white circle in figure 1D is considerably below that expected for a monolayer of cells while the absorbance at the cross is considerably higher than that expected for a monolayer (~0.1-0.4 for a monolayer). Thus the IR images provides information that cannot be so directly gleaned from the visible images, that cellular protein material, probably debris, is present in regions that look like blank spaces in the visible images. In addition, the IR spectra indicate that cells appear to have piled up on top of each other, and do not maintain a monolayer type structure. This conclusion can also be drawn from the visible images.

A 128x128 focal plane array (FPA) was used to collect spectral image hypercubes. That is, we have collected 16388 spectra at 4 cm^{-1} resolution, covering the 4000-800 cm^{-1} region. Only two of these spectra are displayed in figure 1E. This rather large quantity of data overwhelms the digitizing electronics in our FTIR machine if one aims to collect data in rapid-scan mode. For this reason we collected all data in step-scan mode.

Given the large number of spectra the question arises as to methods for analysis. The simplest approach is to simply average spectra in various regions. Figure 2A shows the same infrared image as in figure 1D. Marked on the figure are 6 boxes. All of the spectra at each pixel within the boxed regions are averaged. For example, for box 1, each of the pixels is shown as a circle, of which there are 54 for box 1. In figure 2B the average of these 54 spectra is shown and labeled as spectrum 1. Thus the spectra labeled 1-6 in figure 2B are the average of all spectra within the boxed regions in figure 2A. Obviously, boxes 5 and 6 contain more spectra than boxes 3 or 4. Also shown in figure 2B is the average of all 16388 spectra. This averaged spectrum is labeled as A in figure 2B. The spectra in figure 2B HAVE NOT been baseline corrected, and the broad baseline undulations are due to scattering effects.

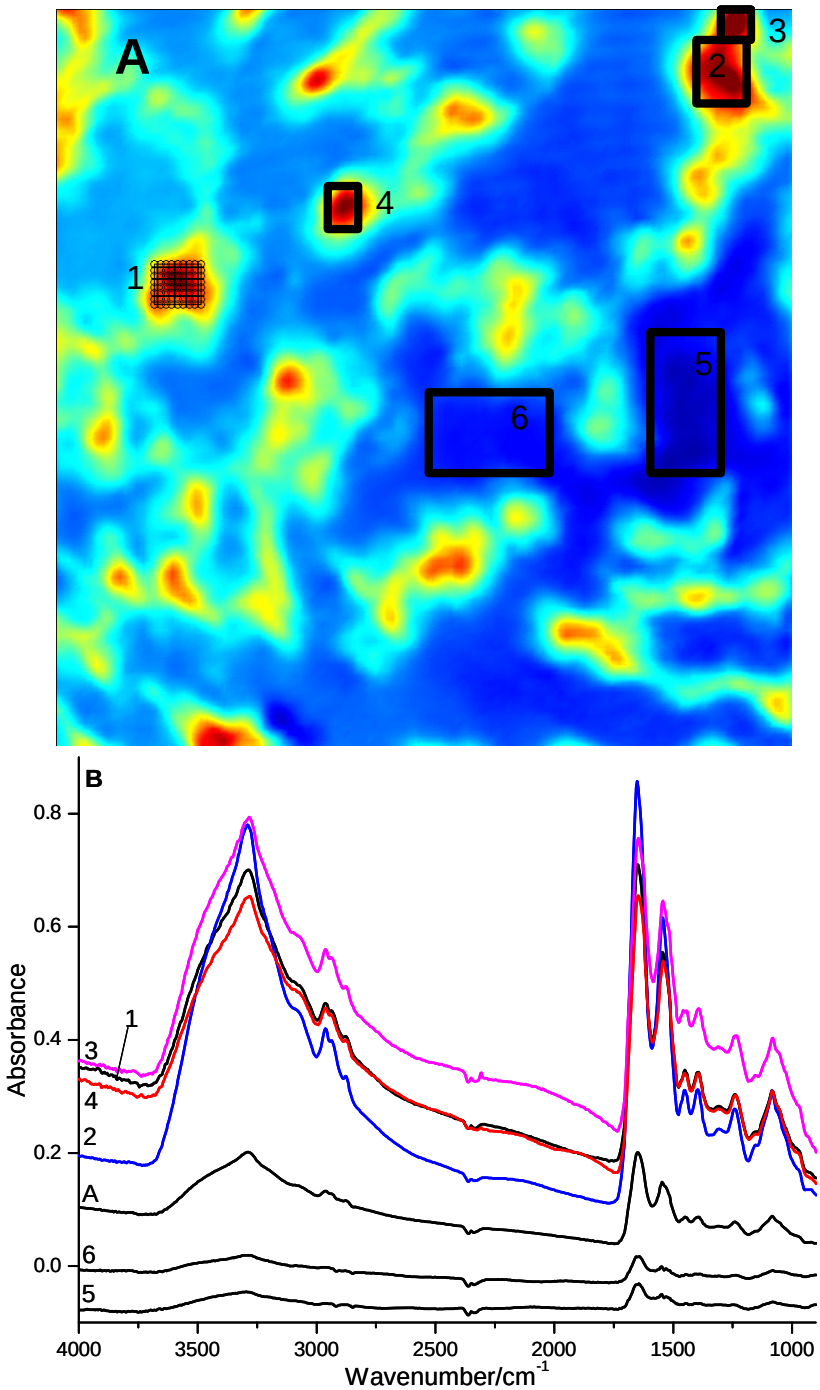


Figure 2: (A) 1652 cm^{-1} spectral image shown in figure 1. Each pixel in the image corresponds to a spectrum, and in the bow labeled 1 54 pixels are shown as circles. (B) Spectra obtained by averaging all of the spectra in the different boxed regions in (A). The number of spectra in each box varies. These averaged spectra are labeled 1-6, and correspond to the boxes labeled 1-6 in A. The spectrum labeled A is the spectrum obtained by averaging all 16388 spectra from all of the pixels (128x128). The spectra in (B) are the raw data, and have not been subjected to any processing. 3D image blocks and extraction of spectra from the blocks was undertaken using Cytospec software version 1.4.