Infrared Imaging of High Density Protein Arrays

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Question: High throughput studies of proteins by infrared spectroscopy are still difficult to perform because of the rather poor quality and slow reading of multi-well plates, handled either in transmission or reflection modes. We suggest here that, using a dedicated spotter and an IR imaging system, it would be possible to read efficiently large arrays of protein spots deposited on an infrared transparent or reflective surface.
Methods: Spots of 160 pl protein solutions (1 or 10 mg/ml) were deposited as regular arrays of protein solution drops. Spectra were recorded on an Agilent 128x128 FPA mid-IR imager. No binning was applied. Spectra were collected between 3903.8 and 898.7 cm^{-1} at a nominal resolution of 8 cm^{-1}. Each spectrum was the mean of 64 scans.
Results: One image covering about 1.5x1.5 mm^2 was obtained by assembling 4 unit images. Together it contained 65536 pixels, each being an infrared spectrum of the sample. Such an image contained about 110 protein spots, each covered by ca 384 pixels. The entire image could be acquired in less than 15 minutes. The mean protein amount present in one spot was 1.6 ng/spot or 160 pg/spot for 10 and 1 mg/ml protein solutions respectively. The mean amount of protein per pixel is therefore respectively 4.8 pg and 0.48 pg respectively. High quality spectra were obtained in these conditions as assessed by evaluation of the signal-to-noise ratio for each pixel. Information about protein secondary structure could be easily retrieved.
Conclusion: Reading protein arrays by infrared spectroscopy is now possible. It provides structural data on the proteins and does not require any labelling, a clear advantage over other techniques presently used in this field of research.