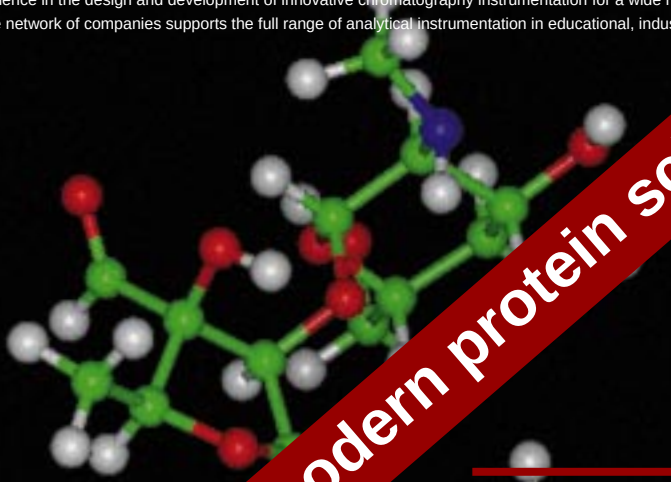
JASCO

As we enter the new millennium we can now look back at 42 years of innovation from JASCO.
In 1958, to meet the need for an Infrared Spectrophotometer at the Institute of Optics (now Tsukuba University), a group of researchers developed their own instrument. This was a great success with a highly reliable unit giving excellent optical performance. This led to other research groups requesting replicate instruments for their own laboratories and the foundation of the Jasco Corporation in 1958 to meet the growing demand for optical spectroscopy products.
Today, JASCO manufactures a wide range of UV/VIS/NIR, FT/IR, Fluorescence, Raman and related spectroscopic instrumentation. JASCO is the world leader in the field of Circular Dichroism Spectropolarimeters.
The experience gained by Jasco in both optical design and computer technology led to the production of spectrophotometric detectors for HPLC.
The move into the HPLC market continued with the production of solvent delivery systems, gradient elution devices and a complete range of detectors. Jasco now has 30 years experience in the design and development of innovative chromatography instrumentation for a wide range of applications.
A worldwide network of companies supports the full range of analytical instrumentation in educational, industrial, quality control and research laboratories.



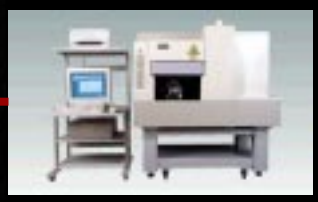
pioneering in modern protein science

UV/Vis

FTIR

Raman

CD



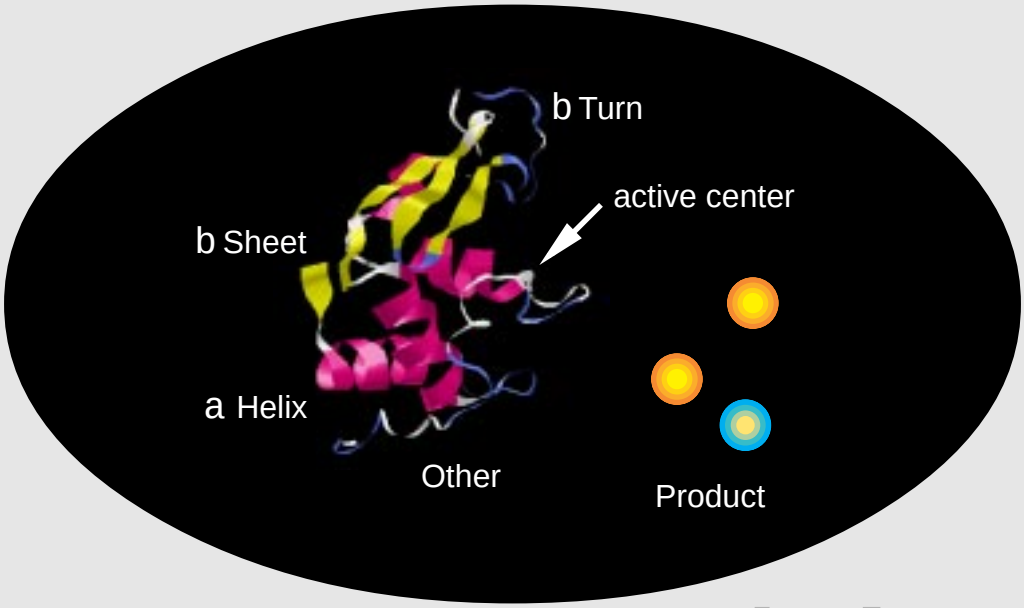
Equipments

CD

This device allows analysis of secondary protein structure using Circular Dichroism. CD uses mainly aqueous protein solutions diluted to less than 1% for the measurements. Combined with a thermo-regulation system, titrator/stopped flow, scanning fluorescence monochromator and other accessories, allow analysis of dynamic changes in secondary structure for each parameter.

FTIR

This device allows analysis of secondary protein structure using the amide absorption peaks of the infrared absorption spectrum. A characteristic feature of the FTIR is that it is not restricted to liquid samples but can also measure solid specimens (i.e. crystals, amorphous substances). Combined with a micro-sampling accessory the FTIR allows analysis of the secondary structure of specimens with an in-plane configuration. Examining peaks other than those for amides allows analysis of various mixtures such as substrates, products and inhibitors.



Raman

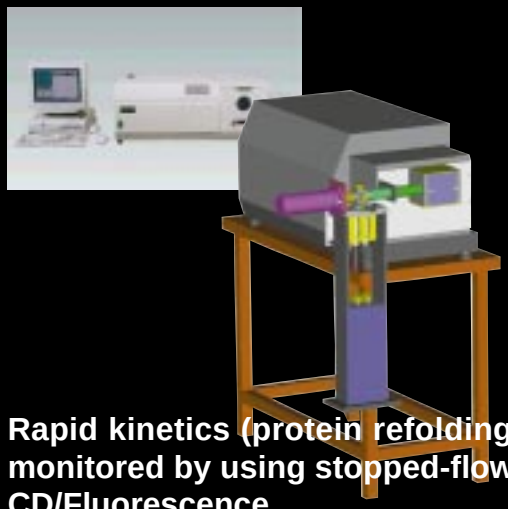
A Raman spectrum can be obtained when samples (proteins) are irradiated with a laser and the scattered light is detected using a Raman spectrometer. Moreover, utilizing the resonance Raman effect allows specific analysis of specific amino acids (Tyr, Trp, Phe) or active centers. This allows in vivo measurement of the scattered light emitted from the specimen of microorganisms in water, or measurements on cultivated cells without pretreatment.

UV/Vis

Using visible UV spectral absorption, this device allows the quantitative determination of proteins (280 nm absorption or Lowry method, etc.) or monitoring of substrates and products during enzyme reactions. Measuring the reaction rate for substrates and products allows reaction kinetic analysis of the enzymes. The UV spectrometer combined with a thermo-regulated cell further enables thermodynamic analysis or DNA melting measurements.

CD

Circular Dichroism Chiroptical Spectrometer



Rapid kinetics (protein refolding) monitored by using stopped-flow/ CD/Fluorescence

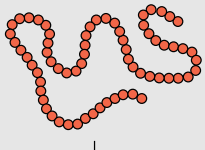
Refolding measurement of Cytochrome C
Cytochrome C in its unfolded state, denatured in the presence of guanidine hydrochloride, is refolded by dilution of the guanidine hydrochloride with a sodium phosphate buffer. This refolding process, which is completed in around 300msec, is monitored by simultaneous CD/Fluorescence measurement with stopped flow dilution.

Kinetic trace at 222nm (secondary structure region)

Figure 1 gives CD and Fluorescence spectra of Cytochrome c, showing the unfolded and refolded states, in the secondary structure wavelength region. A change in this region (225nm) is largely due to alpha-helical content. Figure 2 shows CD and Fluorescence kinetic traces at 220nm when Cytochrome C in guanidine hydrochloride (unfolded state) was mixed with sodium phosphate buffer using the Biologic uSFM-20 two syringe microvolume stopped-flow and JASCO CD/Fluorescence simultaneous measurement attachment.

Kinetic trace at 289nm (aromatic side chain region)

Figure 3 gives CD and Fluorescence spectra of Cytochrome c, showing the unfolded and refolded states, in the near UV (aromatic side chain) region. Changes in this region reflect changes in the local environment of aromatic side chains and tryptophan residues. Figure 4 shows CD and Fluorescence kinetic traces at 289nm. Cytochrome C is refolded in a mixture of guanidine hydrochloride and a sodium phosphate buffer.



Primary protein structure - Amino acid chain

Secondary protein structure - Sequence of Amino acids linked by hydrogen bonds

alpha-helix

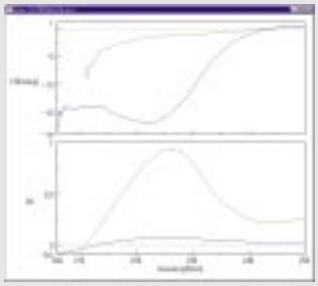


Figure 1

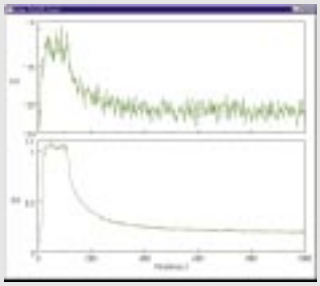
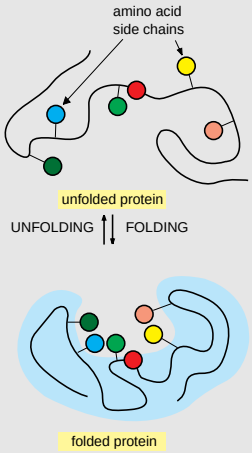


Figure 2

Unfolding state (random)
Refolding state (alpha-helix)



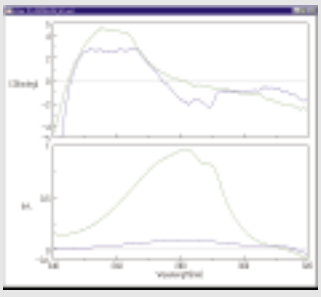


Figure 3

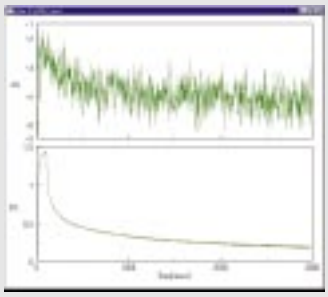


Figure 4

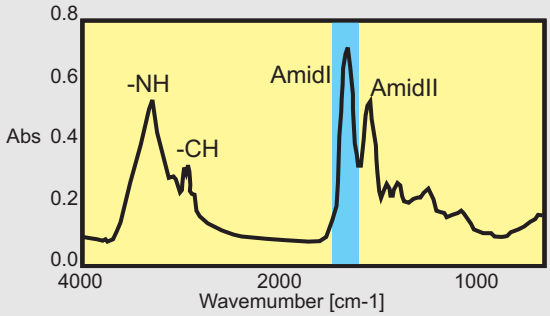
Unfolding state
Refolding state

FTIR

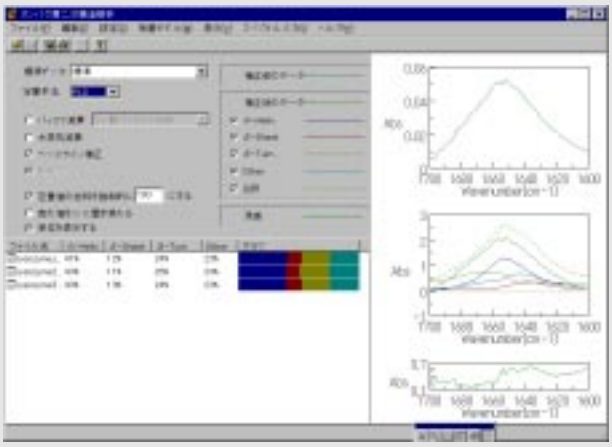
Fourier Transform Infrared Spectrometer

The amide I peaks of the IR spectrum (proteins) show minute variations associated with changes in secondary structure. To analyze these minute variations, the secondary protein structure analysis program for the FTIR utilizes the data from of x-ray structural analysis, regression analysis of the main components (PCR) and partial method of least squares (PLS).

IR, unlike CD or x-ray structural analysis allow measurement of both liquid and solid (i.e. crystals, amorphous substances samples) The FTIR and microscope allow mapping measurements for samples with specific localities thus making possible the analysis of in-plane distribution information of secondary structures.



IR spectrum of hair (proteins)

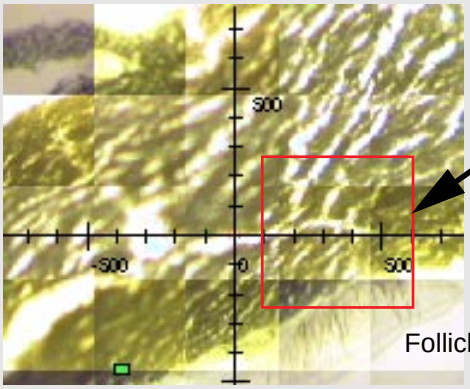


Secondary protein structure analysis program

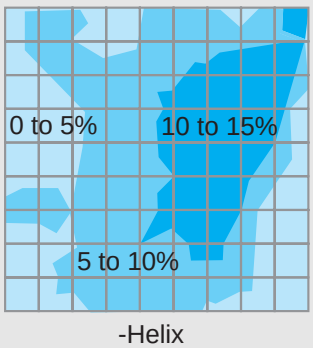
Application example: analysis of a hair follicle

Measuring conditions

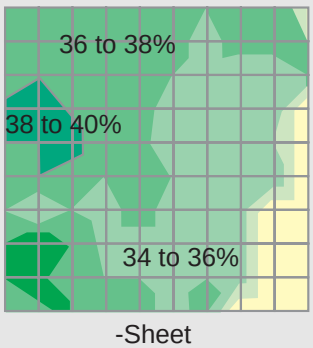
Equipment:
FT/IR-420+IRT-30+IR profile system
Measuring range: 500 x 500 mm
Number of measurement points:
100 points (10 x 10)
Cumulative repetitions: 16 times
Analysis algorithm: PCR



This figure shows an in-plane distribution of a secondary protein structure. Hair proteins are mainly rich in b-sheet keratin, however in the vicinity of hair follicles a slight increase in the ratio of a-helices can also been confirmed.



-Helix



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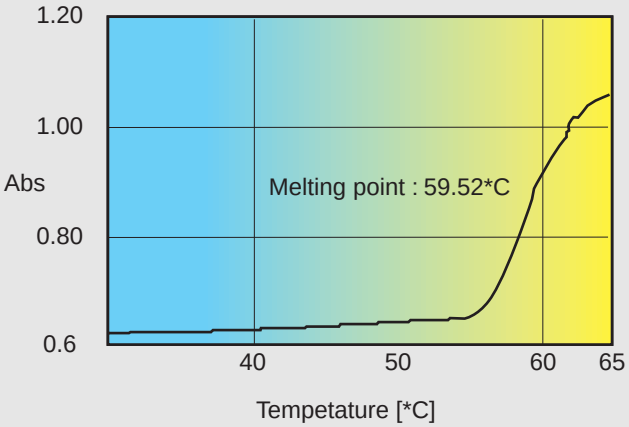
UV/Vis

UV/Vis Spectrophotometer

This UV/VIS spectrophotometer is used for quantitative determination of proteins, measurement of DNA melting, reaction analysis of enzyme reactions etc.

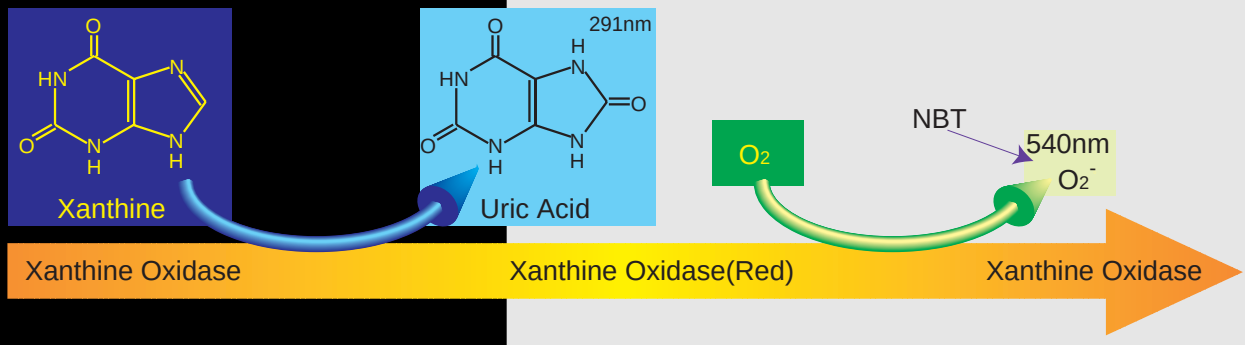
Equipped with a melting analysis program, a constant temperature cell holder and six types of cell changers, this device is simple and accurate allowing analysis of melting points or Km, Vmax and similar parameters.

Application example 1: DNA melting curve



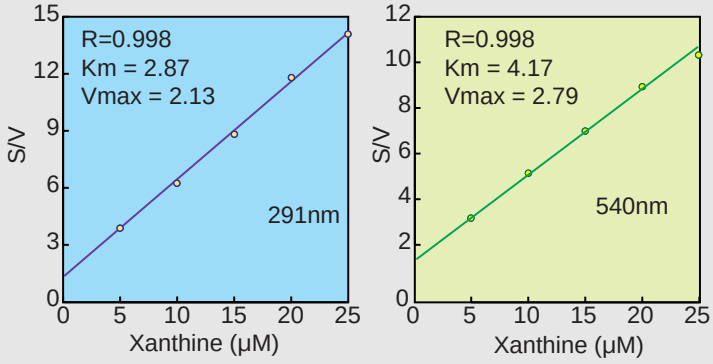
Increased light absorption at 260 nm is often used as a measure for the physical changes of DNA denaturation (increased light absorption effect). This is due to heat induced fusion of the double helix and thus can be used to calculate the base composition like the content of G-C base pairs from the melting temperature Tm.

Application example 2: Theoretic analysis of xanthine oxidase reaction rate



Xanthine oxidase is an enzyme forming uric acid and active oxygen (O2-) from xanthine and O2. (Two substrates, two products)

The rate of formation of uric acid can be measured at 291 nm and that of active oxygen, using NBT, at 540 nm. Analysis of these two different wavelengths allows an analysis of the Km and Vmax of two products.



Data was provided by professor Kohashi of the University of Shizuoka.

Raman

Laser Raman Spectrometer

UV/Vis Resonance Raman spectrophotometer

The Raman spectrum provides information about molecular vibration or rotation (similar to the IR spectrum) and is used for the identification of chemical compounds. A characteristic feature of the Raman spectrum is that it selectively provides information about specific chromophores because excitation with a laser in the vicinity of the absorption wave length of a chromophore - with an absorption at a given wave length - allows observation of the markedly pronounced Raman bands (resonance Raman effect).
For example, use of a UV laser allows specific measurement of only those amino acids within proteins that include the benzene ring (Tyr, Trp, Phe). Additionally, the use of a visible light laser allows specific measurement of the active centers of metal containing enzymes, etc. Because the Raman water band is relatively weak compared to the water band in the IR spectrum, this device also characterized by the ease of measurements of aqueous solutions.

Application example 1: Higher protein structural analysis using resonance Raman

The figure shows the differences in the spectra of ribunuclease A obtained by excitation with both visible laser and UV lasers. Using the visible laser the amide peaks related to the secondary protein structure and buffer peaks can be confirmed.

Conversely using the UV laser, the peaks of Tyr or Phe appear as strong Raman resonance effects and are thus markedly amplified. This enables higher structural analysis of the changes associated with protein tailoring.

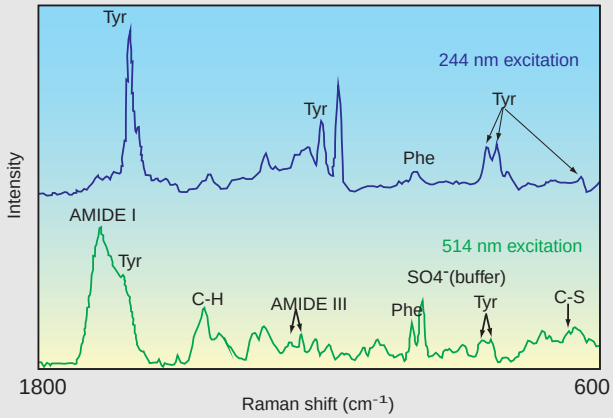
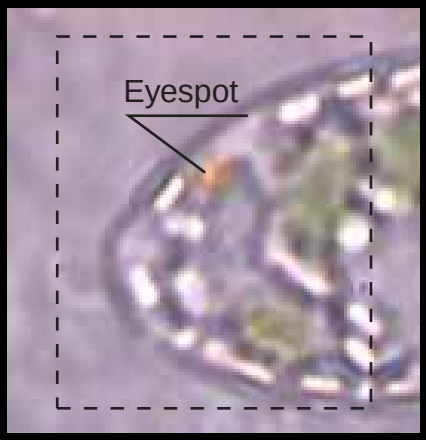


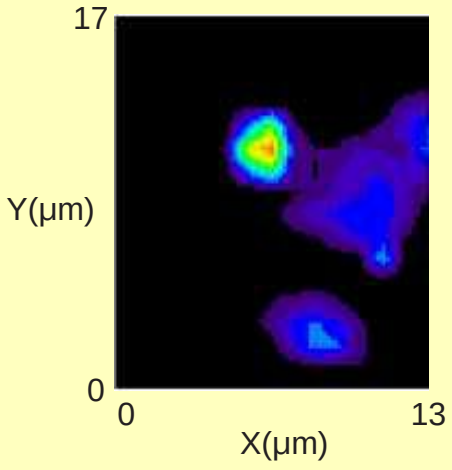
Fig. UV Resonance / Visible Raman spectra of ribunuclease A

Application example 2: Mapping measurement of single cells using Raman microscopy

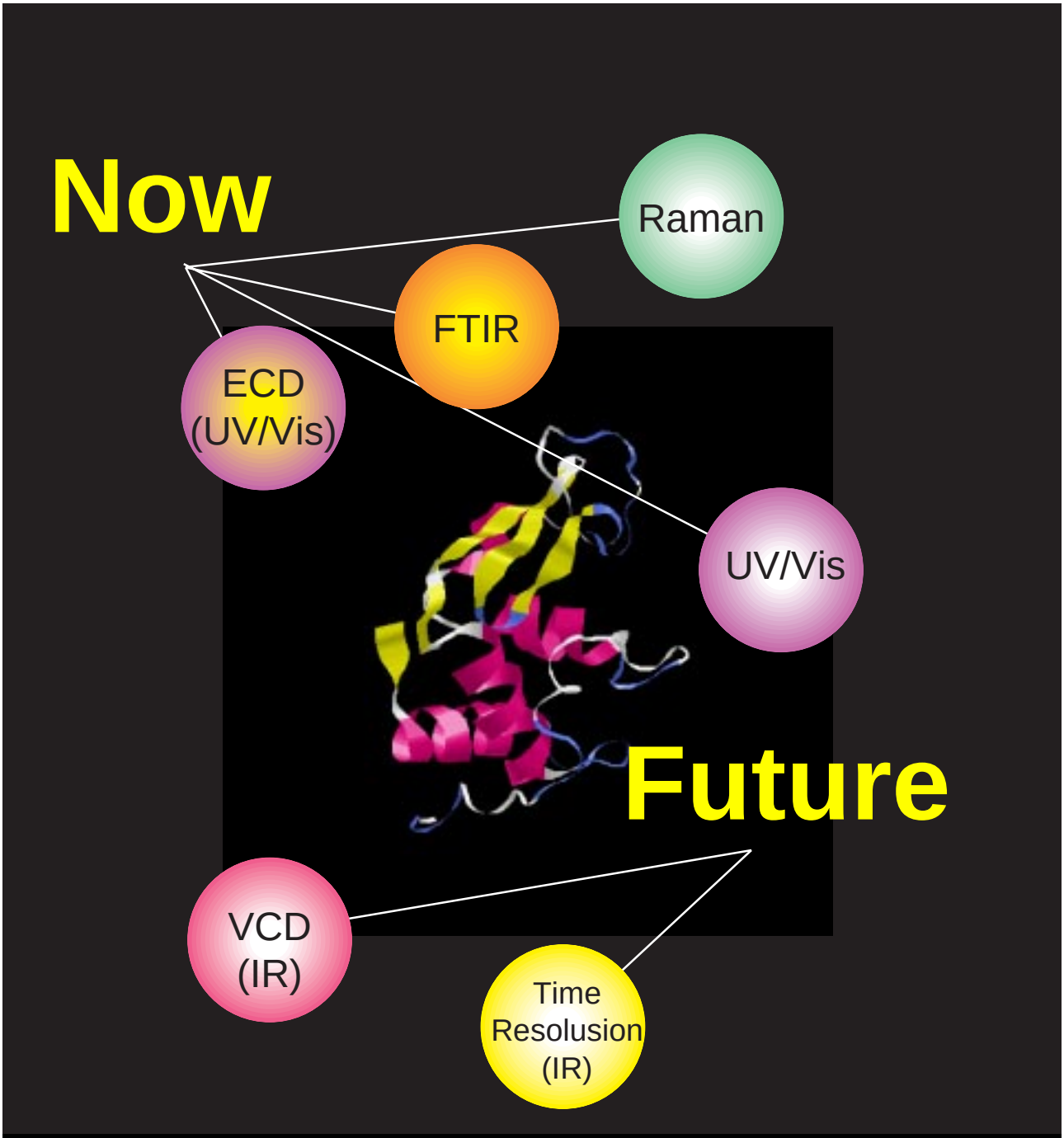


Microphotograph of Euglena

Raman micro-spectrometry allows the beam of the probe light to be focused to a diameter of less than 1 mm. Moreover, because it easily allows in vivo measurements it is effective for investigations of chemical distribution within single cells. This illustration shows an example of the carotene distribution within a single cell Euglena. The result shows an accumulation of carotene in the eyespot.



Carotene distribution within the Euglena
(the broken line in the left figure shows the mapping measurements)



Protein structure and functions are still being studied using spectral analysis. From now on, with VCD (IR-CD), it may be possible not only to study the secondary structure, but also the tertiary structure of proteins. Moreover, with the TRIR-1000 dispersive fast time-resolved infrared spectrophotometer, it may be possible to analyze the medium in enzyme reaction.
JASCO also pledges to provide tools from now on for the comprehensive study of proteins using light.