Feature Article

Introduction to Ultrafast Spectroscopy

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Abstract:
Two dimensional infrared spectroscopic technique is a novel method to study structure and dynamics of biological molecules. Understanding the complicated submicroscopic structure and their dynamics are beyond our real life imagination. It is obvious that the 2DIR technique is as complicated as the understanding of molecular structure and dynamics. But the basic ideas are hidden under the simple real life experiences. Here we make an analogy with those simple phenomena to explain the complicated 2DIR method.

1. Introduction

We can imagine a story, when young James Watt, about two and half century ago, sitting by the five place in his mother's cottage, intently watching the steam rising from the boiling tea kettle, such a small phenomena, but it is true that this was the beginning of the idea of a great discovery of mankind "The Steam Engine".

We are all familiar with the story behind the discovery of Penicillin. Alexander Fleming went on vacation halfway through an experiment with bacteria. He left a dirty petri dish in the lab sink. When he got back, he found bacteria had grown all over the plate, except in an area where mold had formed. This observation later lead to medicines producing anti biotics that could kill certain types of disease-causing bacteria inside the body.

These two great revolutionary discovery of mankind were possible only with great observation and deep understanding of these two phenomena.

In the twenty first century, we are getting information from Mars, we are watching live cricket match at Melbourne from Kolkata almost with out any time lag, but still people don't know the answer why people are dying from cancer or some other disease. Still people don't know the exact reason behind these disease and don't have exact explanation about these, though people have very good observation power to detect the disease and following up its stages in. But some dramatic changes occur in the molecular level, in human body , which may be the cause of these disease, but due to the lack of proper observation and understanding still people are not able to get rid of these. Now the question is how and what we need to observe in the molecular level, and why we can not observe them even in twenty first century when science and technology is advanced in such a high level.

2. Protein Dynamics

We will discuss the difficulties gradually, before that we try to draw a outline of some physical and biological relevant concept. Protein is one of the basic building blocks of human body, making up about 16 percent of our total body weight. Actually the word "protein" comes from the Greek word proteios, which means "first" or "foremost," reflecting the importance of this molecule in living body. The protein molecule is built mainly with carbon (C), oxygen (O), hydrogen (H) and nitrogen (N) atoms. These atoms are arranged in a susajita pattern and build a long chain of protein molecules, but the patterns are not fixed. It may undergo reversible structural changes when performing any biological

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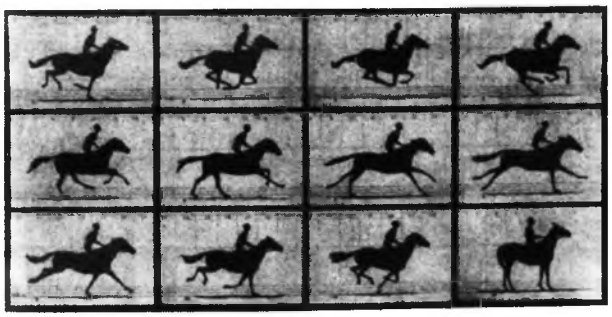
function. These alternative structures of the same protein are known as different conformations, and transitions between them are called conformational changes. Within a single protein structure the connecting bonds between two or more atoms are vibrating or rotating continuously. Although these vibrational and rotational motions are random, but normally they always maintain their structure. But accidentally sometime they misfold, just like if you move a long cotton thread randomly, normally there will be no knot, but some time a knot may occur. Accumulation of misfolded proteins can be the cause for abnormality in human body and that results in some disease. Unfortunately this kind of random molecular motions are very fast, much much faster than our normal sense of fast. A typical molecular bond vibration or rotation takes place in the range of couple of hundred femto second.

3. Observation of Motion

Before going to molecular motion let us first think about normal motion. How can we observe and understand the motion of an object? Imagine the motion of a swing in a park. When children swing in back and forth, we can follow their motion properly, because the motion of the swing is much slower than our eye resolution. But when the motion is faster than our eye resolution, we can not follow the intermediate states, only we can identify the first and last state of motion or position. Let us think about a falling coconut from a tree, when it falls from the tree, we only can identify how it was hanging and how it is lying on the ground, but it is hard for our eyes to understand the intermediate state when it is in air. If we have a camera with suciently high shutter speed and if we can take couple of snaps during the fall of the coconut, we will be able to understand the motion of the coconut during its falling time, whether it rotates with respect to its axis or it falls linearly. Just this idea lies behind the observation of molecular motion. But the total process took quite a long time to establish.

4. First Motion Picture

The idea of taking series of snaps to understand the fast motion started at the beginning of 1870's. At that time there was a controversy about the foot steps of a running horse, whether all four feet of a horse were of the ground at the same time while trotting. The same question had arisen during the gallop of a horse. Human eye could not break down this action of the trot and gallop because a horse's legs are moving so fast, it is impossible to tell just by looking. There was photographic technique to take a snap of a static object. The picture was taken when the photographer removed the lens cap for several seconds in order to expose the film and capture an image. During the open exposer, the target object need to be stand still, otherwise the resulting photograph would be blurred. In order to capture very fast action like a galloping, the exposure time would have to be very short and that time it was not available. In 1872, the former governor of California Leland Stanford, a businessman and race-horse owner, appointed a famous British photographer, Eadweard Muybridge, to solve this galloping arguments with photographic studies. Muybridge tried various methods of making the shutter movement faster and faster so as to shorten the exposure time, and as he did the quality of the image began to improve. Finally he set up 24 glass plate cameras in a line along the edge of a track parallel to the horse's path. The shutter of each camera was controlled by trip wires triggered by the horse's legs. The pictures were taken in succession at one thousandth of a second. After eight years of doing this experiment, he could able to manage a sequence of 12 images, and one of them clearly showed that all four of the horses hooves were o the ground at the same time. If one places those 12 photograph in a sequence and make a slide show in a speed that each photograph stay in screen 0.1 second, then it seems like a running horse, instead of individual photograph. It was the concept of movie, and the famous "Muybridge horse" is the first motion picture in human civilization.



The Horse in Motion

It is clear from the Muybridge experiment that to understand what happened during motion one

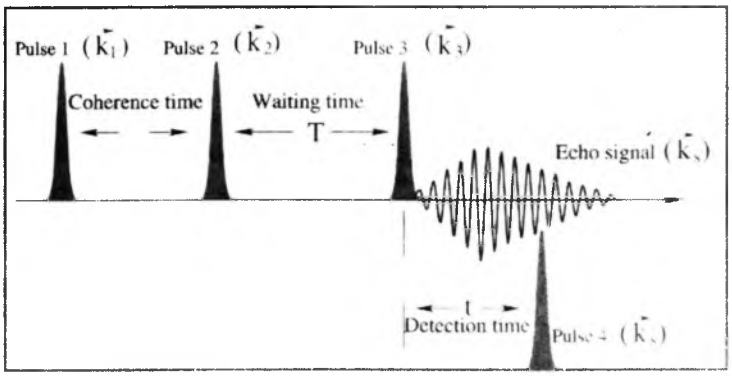
need to take a couple of snap during the motion. In the same manner we want to take the snapshot of molecular vibration and rotational motions, to understand their behavior. Before explaining the molecular movie let us have a little thought about Muybridge's experiment. The photographic experiments reveals that they depend on signals (typically light at same frequency), a device for recording them (a photographic film), and most important, a precisely sequenced timing of two or more events (the trip-wire, stroboscopic lighting). Muybridge's experimental setup would not have resolved Standford's bet unless the trip wires indeed tripped the shutters expeditiously, the shutter speed were short enough to define the horse and the light level were sufficient to record the event on the film used. The circumstances of the measurement, the distance between the trip wires, the shutter speed, the light - all had to be matched with the speed of the galloping horse.

5. Time Scale

Now come to the point to snapshot the molecular vibration. A typical vibrational life time of the vibration of a molecule is vary from couple of hundred "femto second" to few "pico second". What does it mean "femto second"? 1 fs is 10^{-15} sec on the other word, if we divide 1 sec by 10^{15}, we will get 1 fs. How long is this time 1 "fs"? Lets make it more sensible to our real life sense. If we divide 1 hour by 60, we get 1 minute. When we divide 1 minute by another 60, we get 1 second. How small is 1 second in compare to 1 hour? Just imagine. Now divide 1 second by 1 crore (1,00,00,000), you will get 100 nano second. Now if you divide further to 100 nano second by another 1 cror, you will get 1 femto second. Can you make a sense how small is 1 femto second? Take another example of motion picture. If we move more than 10 to 12 consecutive pictures in a series in 1 second, our eyes can not distinguish them as individual pictures, rather see them as a movie. Which means for a series of consecutive photos, if each picture stay about 0.1 sec in front of our eyes, we can not see them as individual picture. 0.1 sec is still 10^{14} (1 crore crore) times larger than a femto second, which is beyond our real life imagination. Then how we can make a snapshot of the molecular motion which takes place in femto second time scale?

6. Snap Shot of Molecular Motion

There is neither a mechanical shutter which can open and close such a high speed, nor a film which can capture angstrom (typical bond length is 1-2 A) level of resolution. Such kind of imagination is become possible after the development of femto second pulse laser. A well known femto second laser experiment is pump-probe experiment, which require two laser pulses, the first laser pulse pumps the molecule, means excite the molecule and starts the reaction going. After a time interval a second laser pulse is applied to the same molecule, to extract the information (what changes has been happened within the molecule due to the first laser pulse) from the molecule at that time.



Pump-probe experiment is a very successful method, but not sufficient for giging information about the conformational changes of protein (mentioned in introduction). It can only make a rough estimation about a particular vibrational band and its initial and final state. So we can judge the molecule roughly. A good example in real life might be the cricket end. It is very difficult to take the right decision of a critical run out for an umpire, who is standing behind the second stump. A second umpire must be needed whose position should be at the same line as three wickets. The combined judgement of two umpires is more efficient for this critical situation. A similar concept we apply to this newly developed technique which is called Two Dimensional InfraRed spectroscopy (2DIR) to observe the molecular motion or rather molecular vibration.

7.2 Dir Spectroscopy

In 2DIR spectroscopy four laser pulses are required. The first laser pulse excite the molecule and put it to the first excited state. The second pulse is applied on the sample, typically couple of fs later than first pulse, which bring back the molecule to the ground state or push up to the second excited state. Both the process is quite slow and take place in couple of hundred fs to couple of ps or even longer. After a sufficient waiting, a third pulse is placed on the sample, which stop both the processes. Interestingly, in a time interval a signal come out from the sample, neither the direction of the any of those three laser pulse, rather a different direction from them. Since this signal generates some time later than all those three laser pulse, it is called echo signal (just like a common sense of echo in sound). This echo signal carry all the informations of the molecule changes during those three pulses. Now changing the time between second and third pulses, we can take different snap shot of the molecule just like the Muybridge's snapshot of the gallop. All those snap shots give us the complete picture of molecule to understand its structure and structural changes.

Now if we move all those snapshot in a speed of 15 snap per second, we can see the motion of atoms in molecule. But unfortunately the realization is not as easy like the horse motion. For molecular motion, situation is more complicated. We will discuss that in the next issue.

Further Readings:

1. P. Hamm and M. Zanni. Concepts and Methods of 2D Infrared Spectroscopy. Cam-bridge University Press, 2011.
2. S. Borman. A new dimension in spectroscopy. Chem. Eng. News, 78(6):4150, 2000.
3. M. Robin Hochstrasser. Two-dimensional spectroscopy at infrared and optical fre- quencies. Proc. Nat. Acad. Sci., 104(36):1419014196, 2007.