Noise of optical imaging of membrane potential with MiCAM01

The picture signal obtained from electronic cameras, such as CCD, has various noises. A shot noise influences in measurement most strongly in those noises. Since the cause of a shot noise is the quantum nature of light, it is impossible to remove shot noise. Next, what is influenced is a dark current noise and this noise occurs by heat fluctuation or electric instability. Since a dark current noise is smaller than a shot noise, it should be cautious only of a shot noise in the optical imaging of membrane voltage.

A shot noise is generated during the conversion from light photon to electron on the sensor, which is known as photoelectric conversion. For example, if the average of photon per pixel is 10,000, the number of photon in each pixel differs in the range of proportion to the square root of the number of total photon, since there is a probable distribution. Namely, the square root of 10,000 is 100, the number of photon in each pixel should be between about 9,950 and 10,050. Since this is a physical phenomenon, the shot noise appears in any sensors. Therefore, when there are few amounts of change like voltage-sensitive dye, very big influence appears in a result. In order to record 0.1% of change exactly, 1,000,000 photons per pixel are required at the lowest.

Then, in order to increase the number of the photons per pixel, the increase of the capacity to accumulate the electron, which appears after the photoelectric conversion, per pixel is effective. The noise of large pixel can be small because the accumulation capacity is related to pixel size, since the capacity of semiconductor is proportional to area size. That is, big sensor and bright lighting can reduce shot noise. In other words, unless the lighting is enough, it is impossible to catch up such as slight change in high spatial and time resolution. But there is limit to increase the lighting. Especially it is common in fluorescence imaging that the lighting is not enough to large sensor, because of the insufficient brightness even with the high wattage for light source. Usage of huge lamp such as more than 300W in order to increase brightness may break filters and lens in optics. It is very difficult to increase fluorescent intensity at 10 times in normal optics.

The mainstream view about the optical imaging was linear to minimize shot noise by increasing the lighting and making sensor large, so far. It was easy to achieve with absorb type of voltage-sensitive dye and the system with MOS sensor which developed in Electrotechnical Laboratory in Tsukuba Japan on 1991. It was not impossible but difficult to use this MOS sensor system efficiently with fluorescence type of voltage-sensitive dye because optic equipment and staining method should be concerned very well. And last problem is bleaching of dye. In the case of RH-795, recording chance was only once per sample because it was difficult to record in stable in the condition of quick bleaching even in 1 second recording. In short words, the illumination should be not so bright for stable optical recording. And it is hard to utilize the benefit of big sensor enough.

Is it possible to record fluorescence at both high spatial and time resolution? The methods to answer the question are averaging and signal processing. Even the feeble signal which is difficult to distinguish noise or signal in single recording can be distinguishable by 16 or 64 times averaging. Why the averaging method cannot be accepted is only that unrepeatable neuronal activities cannot be recorded.

Although averaging method cannot be adopted when single recording is needed, if it is possible to average “happening of neuronal activity” even it cannot be triggered, averaging method can work in many case.

For example, in the case of the recording of spontaneous signal, the aim is the imaging of peripheral neuronal activities related to single cell with spontaneous signal. It seems that the averaging method cannot work in this kind of just single action. But if the cellular activity can be monitored using electrode in unit, the averaging method can work in this case. Amplified record in unit can be input to imaging system as recording trigger. The images before and after the trigger can be averaged. And then asynchronous noise can be reduced and the signal synchronized to single cell can be emphasized. The important is timing before and after the trigger. The image before the trigger may capture the trigger for the single cell action, and the images after the trigger may capture the influence of the single cell action. It is a turning point how to use Pre-post trigger function, which mean recording before and after trigger.

Signal processing is also important. Noise reduction algorithm based on the character of shot noise can reduce shot noise a little.

Standard CCD camera of MiCAM01 can accumulate about 100,000 electrons per pixel. Then the shot noise level will be 0.3% in case of saturation is 100%. Optical recording with around 16 times averaging and signal processing has been check as it is possible to capture the voltage-sensitive fluorescent signal in many kinds of samples. Optical and illumination equipments should have very simple structure, and this is important matter. For the optical equipment, if more than 5 times magnification is needed, commercial available fluorescent microscope can be used for the recording. 150W halogen type light source is enough for illumination. Illumination can be long term because of the brightness is not so strong without a shutter system, although shutter system is still recommended. At low magnification recording, high performance fluorescent macroscope should be used, and such macroscope is able to made by setting of commercial lenses easily.

It is clear that MiCAM01 can work in most of optical imaging situation and has good balance as a recording system, although it may have too small CCD sensor in some case, and its electrical specification is not best of the world. MiCAM01 provide most of researcher with the solution near the target experiment because it has unified the function required for physiological experiment.

Please remember that if 10 times S/N ratio is needed for MiCAM01 recording, 100 times illumination is needed at the same time. And the illumination should be stable at 10 times, and breaching runs at the speed of 10 to 100 times. If NA value can be 3.2 times more, illumination become 10 times more and fluorescence became 1,000 times more!