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Functional Imaging Introduction: Gaining new insight from biophotonic imaging

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Over the past 2 decades, there has been an explosive growth in the range of measurement modalities available to the physiologist. Today, a very large part of the electromagnetic spectrum is used to probe and record physiological function from the level of the whole body to tissue, cell and even organelle. This use of photonic methods includes radio waves in the form of nuclear magnetic resonance imaging, through light in, for example, microscopy and video imaging, and on to real time fluoroscopy with Xrays. Even gamma rays from radioisotopes find some imaging applications with gamma ray cameras. Perhaps the most common use of photonics in physiological research is in the area of microscopy and cell biology. Here we see methods that employ almost most of the fundamental features of light itself to generate physiological images; for example polarization and interference are routinely used to help generate contrast within unlabelled cells.

The effort to clarify cell function has been aided by the development of a wide variety of probes that reveal elements of cell function that cannot be directly observed through other contrast enhancement methods. With these probes we can now image intracellular ion levels, vesicle trafficking and even protein synthesis and degradation within living cells. Although today we talk about "cell biology" , "neuroscience" and "developmental biology" as cutting edge disciplines, in reality they are simply physiological research repackaged to detach the term "physiology". It is possible that a part of this has arisen from the view that the more classical forms of physiology involved rather more quantification, mathematics and physics than is palatable for many students. This is a pity, for careful measurement can often be more revealing than cursory examination. For example, digital imaging microscopy is routinely used in all of the above disciplines but detailed analysis of the already digitized data is rarely performed. Unfortunately, the massive amount of real data contained in microscopic images is frequently ignored and image processing applied to only "beautify" images rather than provide feature extraction and quantification. I cannot help but wonder what Bernstein and Starling would have made of this "science".

Measurement within new imaging methods has given powerful insight in to cell function. As an example, our discovery and quantification of microscopic calcium sparks[1-3] has shown that signal transduction occurs in microscopic domains where behaviour cannot be deduced from whole cell measurements. Such experiments have firmly established the need for confocal microscopy in live cell imaging. Today, I am still amazed that we have been now been able to directly measure microscopic gradients of calcium within sarcomeres with a fast confocal line scanning method[4]. Two decades ago, such gradients could only be deduced from computer models of my rather poor quality calcium signals obtained using aequorin[5,6]. That such exquisitely sensitive methods can now be routinely applied to follow cellular processes promises great insight into cell physiology. This has been aided by the co-development of better probes for cell function (as well as instrumentation).

In the area of calcium metabolism, calcium imaging inside cells is not new; for example in 1928 Pollack[7] injected an amoeba with alarazin which precipitated as a calcium salt at the site of pseudopod formation. With increasing awareness of the multiple roles of calcium new methods were developed to image Ca and obtain sub-cellular resolution. Autoradiographic imaging of ^{45}Ca with electron microscopy was applied to muscle in the 1960's[8,9]. The development of calcium measurement techniques occurred quite rapidly around 1980 (see 10 for review). To improve temporal response and the ability to directly measure function in living cells required the development and application of more sensitive compounds (e.g. aequorin and metallochromic indicators) and ultimately fluorescent probes such as fura-2[11,12]. The high signal strength of the fluorescent probes made real time video fluorescence imaging possible[13,14]. The spatial resolution of the fluorescent microscope was been improved by the development and application of laser scanning confocal microscopy[15-18]. Such laser scanning methods promise resolution to the level of single molecules within cells – although it is always questionable whether the study of the interactions of single molecules really falls under the umbrella of "physiology". More uncommon microscopic imaging modalities are still developed in areas such as non-linear excitation in multiphoton microscopy, fluorescence energy transfer, quantitative birefringence and quantitative phase microscopy. These methods are bringing a new generation of physicists and engineers into the life sciences and helping to further accelerate our development and application of new methods.

That visible light has proved so useful for studying cell function is easy to explain. In order to measure something it must be probed by energy in some form and to maximize the signal to noise ratio, you need to employ the highest possible energy levels. Since the energy of the visible wavelength photon (~2 eV) is somewhat lower than that of a typical chemical bond, molecules can be repeatedly probed by visible wavelength photons without destroying them. Of course, the energy difference is not so large that damage can be ignored, but with careful experimental design we can record cell function and cell

responses to many stimuli and bracket responses with controls. In live cell imaging, noisy signals are to be expected and not to be confused with poor experimentation. Therein lies the true artistry of vital imaging: to achieve the clearest possible imaging results while treading carefully along the boundary of cell damage. In connection with this point, we should also not forget that introducing any probe carries the risk of changing cell function and that molecular biological manipulation to make the cell report it's own functions is also not without the risk of changing cell function/behaviour.

In summary, I suggest that the greatest advances in understanding physiological function have come about from the development and application of new measurement methods and the physiologist has always been quick and creative in applying new methods to problems in his/her area of research. Application of these new methods is always harder than using well established methods but the rewards are also greater. Thus while imaging is not new in physiological research, new imaging modalities are giving fresh insight into cell behaviour. The application of new imaging technologies within the life sciences is likely to continue to reveal unexpected complexity in physiology and life. In the following four papers presented in this symposium, we can see examples of the latest quantitative imaging techniques. As we continue this work, we can also take a moment to enjoy the beauty of the images that can be made.

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