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Live imaging in *Drosophila*: The optical and genetic toolkits

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ABSTRACT

Biological imaging based on light microscopy comes at the core of the methods that let us understanding morphology and its dynamics in synergy to the spatiotemporal distribution of cellular and molecular activities as the organism develops and becomes functional. Non-linear optical tools and super resolution methodologies are under constant development and their applications to live imaging of whole organisms keep improving as we speak. Genetically coded biosensors, multicolor clonal methods and optogenetics in different organisms and, in particular, in *Drosophila* follow equivalent paths. We anticipate a brilliant future for live imaging providing the roots for the holistic understanding, rather than for individual parts, of development and function at the whole-organism level.

1. Introduction

Organisms are made of cells and cells undergo dramatic dynamic rearrangements and changes in shape and motility during our lifetimes. Development is the result of the action of functionally interlaced hierarchical levels, genes, proteins, cells, tissues and organs. Each of them are just elements of a program used to build organisms. Knowledge on genes' expression and function without understanding cellular behaviors development, morphogenesis or physiological functionality offers no convincing concepts or methods to grasp how system properties are built. Imaging thus arises as an essential tool to fully appreciate the cellular architecture and the dynamic functions and cooperative behavior of cells in this integrative context.

Approaching a developing organism by imaging constitutes a great challenge. During development, proteins become expressed, traffic, change their subcellular localization or disappear, cells divide, specialize, differentiate and move or die, tissues aggregate, expand or fold and organs grow, shape and become physiologically active. Further, developing organisms display unique inhomogeneous optic properties. They can be opaque, refringent or autofluorescent, with these properties changing with time or locally. Thus, for each specific application the imaging protocols must be appropriately tailored. In synthesis, the correct optical tools with precise spatial and temporal resolution capabilities and the suitable structural or functional probes to employ must be carefully chosen and tuned.

In this review, we will not comment on basic approaches or methodologies aimed to extract imaging information on fixed tissues or cultured cells, which are well covered throughout the literature. Our aim has been to present the latest methodological advances been employed in understanding *Drosophila* development.

Our organism of choice, *Drosophila*, has constituted one of the major objects for basic research over the last 100 years. Many, if not most, of the major qualitative breakthroughs in the advances of biological sciences have taken place by using *Drosophila* as a model system. Its genetics and accessibility for imaging and physiological analyses make it ideal for a systems integrative approach.

First, we present a thorough survey on the major optical techniques at place to undergo live imaging acquisition. Secondly, we review the major advances from the genetic point of view that have provided us with probes and methods to interrogate the organism by imaging means.

2. The optical toolkit

While bright field linear microscopy has brought a wealth of information for the understanding of morphology, cell and tissue behavior, it falls short accomplishing detailed information at the molecular level. In particular it is not suitable to detect those fast molecular events occurring in developmental or physiological

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processes. Fluorescence microscopy has become the method of choice to trace the molecular actions at the core of biological outcomes. Small intrinsically-fluorescent molecules, compounds derivatized with fluorescent reporters and modified genetically engineered protein chimeras all can be directly tracked in living cells and serve as sensitive sensors for expression, traffic or function of specific cellular components in a wide range of biological applications. A key advantage of using fluorescence in biological samples is that multiple protein species can be labeled and monitored concurrently in live specimens [1].

2.1. The classic approach

Fluorescence microscopy requires intense, near-monochromatic, illumination that in epifluorescence microscopes is focused through an objective lens that collects back the emission of the fluorophores present in the specimen. Although fluorescence microscopy can be extremely sensitive, the intrinsic design of classic fluorescence microscopes with full sample illumination leads to the capture of out of focus light from points outside the focal plane, which reduces image clarity [2].

In laser scanning confocal microscopy (LSCM) the objective focused laser spot is scanned across the sample point by point, to build up a pixel based image. Optical sectioning is achieved by introducing an aperture – pinhole – in the detection path. The pinhole discriminates out-of-focus light in an adjustable manner, delivering thin optical sections that build up an impressive three-dimensional view at an improved resolution compared to wide-field microscopes [3–5]. However, this set up presents some inherent limitations that reduce its performance in live applications, where signal intensity is low by definition and temporal resolution in an important need [6]. Traditionally, video-recordings have been performed using LSCM microscopes at the cost of reduced spatial resolution and optical sectioning. The progressive evolution of the LSCM though faster scanners and more sensitive detectors have helped improve the ratio between spatial and temporal resolution, to the point that this technology is one of the most widely used in standard live imaging applications in *Drosophila* (Fig. 1). A PubMed survey renders more than 1.000 references when combining the keywords "*Drosophila*" and "confocal" and is well beyond this review to comment the different applications in detail. Despite this overwhelming success, the reaction of the specimen to a continuous flux of intense illumination in LSCM imposes several limits that mainly show up in very demanding applications: (1) a significant fraction of the fluorescent molecules are already in the excited state (saturation), (2) a second photon may be absorbed leading to bleaching and (3) the specimen's long term viability is compromised due to phototoxicity.

An alternative to LSCM is the Spinning Disk Laser Confocal Microscope (SDLCM), which uses a multipoint scanning strategy based on a rotatory disk of pinholes, arranged in spirals arrays according to the original design by Paul Nipkow [7,8]. The excitation laser source, rather than being concentrated in a single illumination spot, is shed through a portion of the rotatory disk and therefore divided in around 1000 illumination rays that get focused onto the sample by a high numerical aperture objective. Upon rotation of the disk at 5000–10,000 fpm, the focused laser spots scan the sample to generate an image acquired by parallel detection with a CCD camera. In this way, the system has the potential to collect images at an extremely high rate, limited only by the pixel clock rate of the CCD camera and the amount of available signal. This advantage has led to countless applications to the study of *Drosophila* development and physiology that we cannot analyze in this review.

The most advanced confocal scanner unit (CSU), developed by Yokogawa Electric Corporation, incorporates a second disk, formed by microlenses arranged with identical pitch. This second disk compensates for the loss in illumination collection introduced by the inter-pinhole space and improves optical efficiency more than ten times compared to that of the conventional confocal Nipkow microscopes [9]. The great achievement of the SDLCM for live imaging is that, by exposing the fluorophores to longer illumination times at lower intensity, fluorescence saturation is minimized without compromising the overall frame rate due to the multipoint design. Moreover, photobleaching is reduced and viability is improved during long recording sessions. However, in contrast to the highly spectral detection systems of most LSCM, the detection optical path of SDLCM is based on classical filter separation, which depending on the specimen and the fluorophores of choice may result in the need of longer exposure times to yield images of acceptable signal to noise ratio quality. This, together with the inherently low-light nature of confocal microscopes, limits the

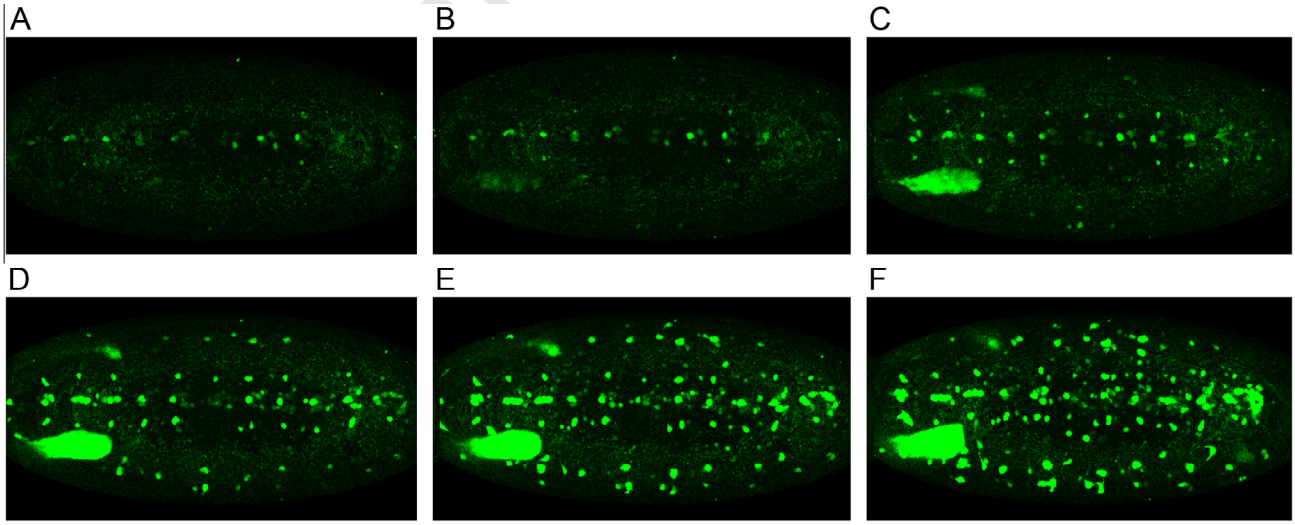

Fig. 1. Time course of expression in the *Drosophila* embryonic CNS and PNS of *puc*, a JNK activity reporter. (A–F) 6 snapshots (1 h apart each) generated from a time-lapse recording of a ventrally positioned pucE69I-GAL4 > UAS-GFP embryo. Monitoring was initiated at embryonic stage 13 and lasted for 6 h (anterior is to the left), *puckered* positive cells, shown in green, are detected initially at the ventral midline and then both at the Central and Peripheral Nervous System, while puckered expression pattern increases its complexity as embryogenesis proceeds. Images were acquired live in a Zeiss LSM 700 confocal microscope.

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