ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Fluorescence cryo-microscopy: current challenges and prospects

Rainer Kaufmann[1,2], Christoph Hagen[1] and Kay Grünewald[1]

Studying biological structures with fine details does not only require a microscope with high resolution, but also a sample preparation process that preserves the structures in a near-native state. Live-cell imaging is restricted mostly to the field of light microscopy. For studies requiring much higher resolution, fast freezing techniques (vitrification) are successfully used to immobilize the sample in a near-native state for imaging with electron and X-ray cryo-microscopy. Fluorescence cryo-microscopy combines imaging of vitrified samples with the advantages of fluorescence labeling of biological structures. Technical considerations as well as the behavior of fluorophores at low temperatures have to be taken into account for developing or adapting super-resolution methods under cryo conditions to exploit the full potential of this technique.

Addresses

[1] Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK

[2] Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

Corresponding author: Grünewald, Kay (kay@strubi.ox.ac.uk)

Current Opinion in Chemical Biology 2014, **20**:86–91

This review comes from a themed issue on **Molecular Imaging**

Edited by **Christian Eggeling** and **Mike Heilemann**

For a complete overview see the Issue and the Editorial

Available online 19th June 2014

http://dx.doi.org/10.1016/j.cbpa.2014.05.007

1367-5931/© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/)

Introduction

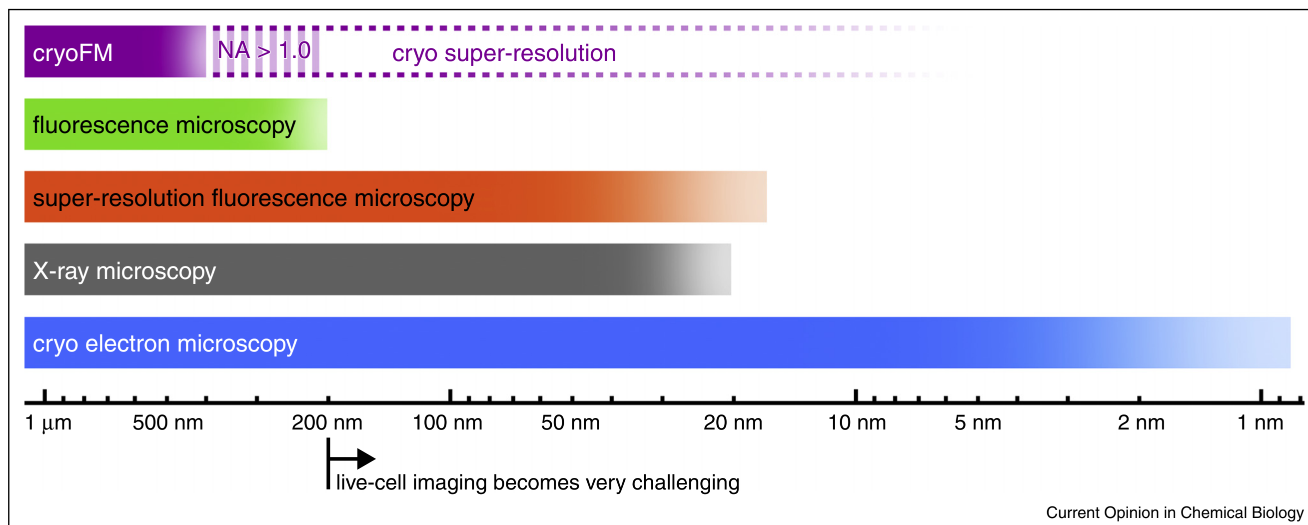
Fluorescence cryo-microscopy (cryoFM) originates from various fields of research and is motivated by a range of biological, chemical and physical questions. First 'cryo'-microscopy was performed when imaging snowflakes in the 19th century (for review see [1]). Almost half a century ago liquid nitrogen cooled and temperature regulated sample stages for light microscopes have been developed to study thawing processes along applications in the biomedical field [2,3]. In contrast, the motivation of performing measurements at low temperature in the field of single molecule spectroscopy is very different. Spectral lines of single molecules become extremely narrow at low temperatures, revealing much more detailed information about themselves and their interaction with external stimuli [4], but also allowing detection of very weak single molecule effects [5•]. Correlative cryo-microscopy is a relatively recent development of imaging the same sample with different imaging modalities such as fluorescence, X-ray and/or electron cryo-microscopy. This allows combining visualization of ultrastructural details with the molecular specificity of fluorescence labeling [6–10]. Moving to low temperatures in this field of cryoFM is primarily motivated by the fact that the sample needs to be kept in amorphous ice to maintain structural preservation in a near-native state across all imaging modalities. The decreased photo-bleaching at lower temperatures [4] is merely a welcome side effect.

CryoFM is becoming more and more popular in the field of correlative cryo-microscopy. Here, the demand of improved resolution far below the diffraction limit of light is evident when comparing with its counterparts in electron and X-ray cryo-microscopy (Figure 1). Likewise is the ability to image cryo immobilized biological samples in a near-native state with fluorescence microscopy an emerging driving force toward super-resolution cryoFM. We will discuss advantages and challenges of cryoFM based on the current state of this technique with a distinct focus on the prospects of super-resolution fluorescence microscopy under cryo conditions.

Advantages and current challenges in cryoFM

Cryo-microscopy in general allows imaging biological structures in a near-native state. At ambient temperatures only living cells provide unperturbed structural details. Fluorescence microscopy techniques provide live-cell imaging capabilities, but the resolution is restricted to ~200 nm. Only the application of super-resolution methods [11•] allows overcoming the diffraction limit, but this remains very challenging for imaging living cells [12–14]. For achieving a substantially improved resolution, in most cases movement of structures needs to be stopped. This typically requires chemical fixation of the sample which can cause structural changes in the sample [15]. In contrast, cryo-immobilization using rapid freezing techniques (vitrification) preserves the structures in a near-native state in glass-like amorphous ice. This procedure is frequently applied for imaging fine structural details with electron or X-ray cryo-microscopy [16–18]. In fluorescence microscopy the benefits of vitrified samples are currently not fully exploited due to the very limited resolution of optical setups for cryoFM. In the first instance, this results from the lack of appropriate immersion objectives dedicated for cryo conditions which restricts the numerical aperture (NA) of the imaging system and thereby the resolution to a range of 400–500 nm. Additionally, super-resolution methods, which have been developed for fluorescence microscopy at ambient temperatures, have so far not been adapted to cryo conditions. Changed photophysics of fluorescent

Figure 1



Resolution scale. Colored bars represent the resolution achieved with the according microscopy techniques in biological samples. Opaque color corresponds to a range routinely accessible, best values that have been achieved but are not routine are indicated by increased transparency. Super-resolution fluorescence microscopy already reaches a similar range as X-ray microscopy for chemically fixed samples, but remains very challenging for live-cell imaging. CryoFM, which also provides the possibility of imaging biological structures in a near-native state, is currently limited in resolution even more than conventional fluorescence microscopy. The development of cryo immersion objectives and the adaptation of super-resolution methods for cryo conditions will lead to a dramatic increase of the resolution in cryoFM (indicated by dashed lines).

molecules at low temperatures might be both, beneficial as well as challenging for super-resolution cryoFM.

Fluorophore behavior at low temperatures

A big issue in fluorescence microscopy at ambient temperatures is photo-bleaching which often hampers specific experiments. The two major mechanisms leading to irreversible bleaching of fluorescent molecules are suppressed at cryo temperatures [4]. Transformational changes, which are often crucial steps on the way to photodecomposition of the fluorescent molecule, are reduced [19]. The diffusion of small reactive molecules such as oxygen is arrested and thus bleaching via photo-oxidation of fluorescent molecules is suppressed as well [4]. It has been shown that the number of photons emitted by fluorescent molecules at low temperatures can be increased up to two orders of magnitude compared to ambient temperatures [20]. This effect has also been shown for fluorescent proteins in vitrified cells in comparison to living cells [6,7,9].

On the other hand, the signal to noise ratio of fluorescence imaging at low temperatures can be dramatically reduced due to high triplet population of the fluorescent molecules [21,22]. A study with organic dyes reported a triplet population of 80–90% at 76 K, corresponding to a reduction of brightness of almost 10 times [22]. In this case triplet depopulation was possible by additional illumination of the molecules with an appropriate wavelength to reestablish nearly the original signal to noise ratio [22].

Photo-switching or blinking of fluorescent proteins and organic dye molecules, an effect well studied at ambient temperatures [23••,24,25], is still present at low temperatures [26–29,30•]. Weisenburger *et al.* recently showed reversible photo-switching of single organic dye molecules at 4.4 K with bright and dark states lasting many seconds up to minutes [30•,31]. Long-lived dark states in organic fluorophores are reached via the triplet state [28]. Their life-time shows almost no temperature dependency, but the lack of oxygen can substantially decelerate the recovery to the fluorescent ground state [28]. Fluorescent proteins can be switched with moderate to high excitation intensities to a reversibly bleached state from which they recover to the fluorescent state spontaneously or photoinduced [26,29]. Photo-switching at low temperatures is here facilitated by photoinduced protonation rather than conformational changes (e.g. isomerization) which play a competing role at ambient temperatures [29]. Future studies will have to address this at the single molecule level to gain a more detailed understanding of the different pathways of reversible and irreversible photo-bleaching at low temperatures. Switching efficiency, life-times of bright and dark states as well as background characteristics (population of molecules which are not switching/blinking) are crucial points for the development or adaptation of super-resolution methods in cryoFM (for more detailed discussion see section '*Prospects for super-resolution cryoFM*').

Probably the most important characteristic of fluorescent molecules at low temperatures for the field of single

Download English Version:

https://daneshyari.com/en/article/7694852

Download Persian Version:

https://daneshyari.com/article/7694852

Daneshyari.com