Published in final edited form as:

J Appl Crystallogr. 2003 October ; 36(5): 1295–1296. doi:10.1107/S0021889803013724.

Microscope detection options for colorless protein crystals grown in lipidic cubic phases

Peter Nollert

deCODE Genetics, BioStructures Group, 7869 NE Day Rd West, Bainbridge Island, WA 98110, USA

Abstract

The use of lipidic cubic phases as crystal nucleation and growth matrices is becoming popular and has yielded crystals of soluble and membrane proteins. So far, all of the membrane proteins crystallized by this method have been colored. This feature has facilitated the detection of the often encountered microcrystals in initial screening rounds. Indeed, small colorless protein crystals have poor optical contrast as a result of the small differences in refractive index of the protein crystal and the surrounding lipidic cubic phase. While a perfect preparation of a lipidic cubic phase is transparent and optically isotropic, in a crystallization setup it frequently disguises crystals due to cracks, inclusions, surface distortions and phase boundaries. Here, several specialized microscopic techniques and illumination conditions are compared and it is found that sufficient contrast is generated by cross polarization microscopy and by Hoffman modulation contrast microscopy for the detection of colorless protein crystals.

1. Materials and methods

Small crystals of soluble colorless thaumatin were grown in a monoolein-based lipidic cubic phase in Terasaki plates (Robbins Scientific Corporation, CA, USA). The lipidic cubic phase was prepared at room temperature by syringe-based mixing of 60 mg of dry monoolein (Nu-Check Prep, Inc., Elysian, MN, USA) with 40 μ l of an aqueous 50 mg ml⁻¹ thaumatin (Sigma) solution. Within 30 min *ca* 200 nl of this material was injected into 2 μ l of 400 mM potassium tartrate tetrahydrate solution. For technical details regarding mixing and dispensing see the work of Nollert (2002). This crystallization is conceptually similar to the classical recipe for bacteriorhodopsin crystallization (Landau & Rosenbusch, 1996) but likely to be mechanistically distinct from the ‘in cubo’ crystallization model described by Grabe *et al.* (2003). Small rhomboidal plates (Figs. 1 and 2) grew within three days in thaumatin-containing lipidic cubic phase; no crystals were observed in control setups that did not contain any protein.

A Leica MZ16 dissecting microscope was used for pseudo darkfield observations. For brightfield, phase contrast, cross polarization and Hoffman modulation contrast illumination, a Leica DMIRE2 inverted microscope was employed. Images were captured by a CCD camera (Spot Insight QE Color Mosaic, Diagnostic Instruments, Inc.).

2. Results and discussion

Thaumatin crystals (largest dimension 65 μ m $\times$ 35 μ m, thickness <5 μ m) in the chosen sample could be readily detected with all tested illumination modes (Figs. 1 and 2). For such moderately

sized crystals, the dissecting stereomicroscope with manually adjusted transmission in pseudo-darkfield illumination mode gives contrast that is comparable with that of phase contrast illumination using the inverted microscope. The greater depth of field of the dissecting microscope and its limited magnification make this observation mode preferable for gaining an overview of entire crystallization setups (not shown). For the detection of smaller objects, higher magnifications are required ($>115\times$). Unexpectedly, at such high magnifications brightfield illumination and phase contrast microscopy yielded only weak contrast (Figs. 1*b* and 1*d*). The most detailed view is provided at high magnification by Hoffman modulation contrast (Hoffman & Gross, 1975) (Fig. 1*c*) revealing internal crystal features such as facets and cracks with sizes smaller than $3\text{ }\mu\text{m}$ (Fig. 1*c*). At lower magnification (Fig. 1*e*), the entire crystallization setup may be observed, contrasting crystal, air bubbles, inclusions as well as lipid phase boundaries.

In general, protein crystals of non-cubic symmetry may be detected by their optical birefringence. However, this effect is small in thin crystals and the polarization effect might be dominated by the birefringence of the container material. Surprisingly, the latter effect is fairly homogenous and still allowed thin thaumatin crystals in polystyrene Terasaki plates to be detected by their polarization capacity (Fig. 2). The polarization effect manifests itself in a change in crystal bulk color, which is different from that of the surrounding material. Since the orientation of the crystal is crucial to this appearance, sampling several analyzer angles is advised.

In summary, Hoffman modulation contrast microscopy and cross polarization microscopy are the methods of choice for the detection of colorless microcrystals in gel-based crystallization setups, such as in lipidic cubic phases.

Acknowledgments

The author would like to thank Dr Mark Mixon for fruitful discussions and assistance with imaging, Ron Lyse from Bartels & Stout, Inc., Bellvue, WA, USA, for supplying the microscopes, and Dr Hidong Kim for carefully reading this manuscript. This work was supported by NIH grant SBIR 1 R43 GM65630-01.

References

- Grabe M, Neu J, Oster G, Nollert P. *Biophys J* 2003;84:854–868. [PubMed: 12547769]
- Hoffman R, Gross L. *Nature (London)* 1975;254:586–588. [PubMed: 1128652]
- Landau EM, Rosenbusch JP. *PNAS* 1996;93:14532–14535. [PubMed: 8962086]
- Nollert P. *J Appl Cryst* 2002;35:637–640.

Figure 1.

Thaumatin crystals grown in a lipidic cubic phase, viewed with different illumination techniques. (a) Image obtained using a dissecting stereomicroscope with manually adjusted transmission darkfield illumination, highest magnification setting (*ca* 115 $\times$ final magnification). (b) Under ordinary brightfield illumination of the same specimen, crystal edges are distinct while internal crystal features and the surrounding matrix appear featureless. (c) Hoffman modulation contrast illumination reveals most features within crystals. (d) Phase contrast enhances edges by outlining the boundaries of the crystals. The surrounding lipid matrix acquires a grainy texture. (e) Overview of the entire crystallization setup viewed with Hoffman modulation contrast. For images (b), (c) and (d), a 40 $\times$ objective was used with a Leica DMIRE2 inverted microscope; the bar is 50 μ m in length. For image (e), a 10 $\times$ objective was used with the Leica DMIRE2 inverted microscope.

Figure 2.

Identification of non-colored protein crystals under brightfield, (a) and (c), and cross polarization, (b) and (d), illumination for the same specimen as in Fig. 1. The upper panels, (a) and (b), show original micrographs; the lower panels, (c) and (d), show close-up sections. Under brightfield observation, (a) and (c), crystal edges are visible and their interior assumes the same color and intensity as the surrounding lipidic cubic phase. Under crossed polarization, (b) and (d), the crystals assume uniform colors that are distinct from the surrounding material. A Leica DMIRE2 inverted microscope with a 10× objective was used. Colors were left unaltered. The bar is 300 μm in length.