

OMX Sample Preparation

The OMX runs in two modes. The first, known as conventional mode, images in widefield with the advantages of very fast sensitive cameras and a light path with very little light loss. Live samples can be imaged using this mode with very low light levels and fast camera speeds (around 10ms). Images captured using this mode are then deconvolved using the Softworx software.

The second is the super-resolution mode which uses structured illumination (SI) in X, Y and Z to illuminate the sample. Post-acquisition algorithms reconstruct the data to give resolution which beats the Abbe limit (down to about 120nm in X, Y and 320 nm in Z). The high resolution image is generated from 15 images of each focal plane within a 1µm stack taken in 125nm steps. Very stable fluorophores are required. **It is best used on samples that are no more than 10 microns from the coverslip.**

BLAZE UPGRADE

In SI mode, the cameras can now work with 1ms exposure times allowing a 1µm SI stack to be taken in just over 1 second. It is therefore now possible to do SI imaging on live samples.

The OMX structured illumination (SI) method requires a predictable point spread function. It is therefore important where possible to match the refractive index of the mounting media to the objective and also to use the appropriate coverslip thickness.

The **60x oil objective** is compatible with Vectashield (Vector Labs) and glycerol and ProLong. If using a mounting media such as ProLong (Invitrogen) that cures over time, the curing process can sometimes lead to an uneven refractive index within the sample which the OMX is sensitive to. It is therefore important to follow the curing protocol carefully and might be worth having a duplicate sample in Vectashield in case of any problems.

The coverslip thickness required is 0.17mm (thickness no. 1.5 can be used but these are often variable in thickness - for users within the Gurdon Institute, we have a supply of High Precision coverslips but they are expensive so use sparingly and you might need to order your own).

The OMX SI is very sensitive to bleaching so it is ideally suited to robust fluorescent signals such as the Alexa dyes (Invitrogen) - although the Alexa Fluor405 is not recommended - together with an antifade (Vectashield, for example, contains an antifade).

We have been advised NOT to use Vectashield which contains DAPI because the background is too high. If you plan to use DAPI, please add DAPI separately, wash off well and mount in plain Vectashield.

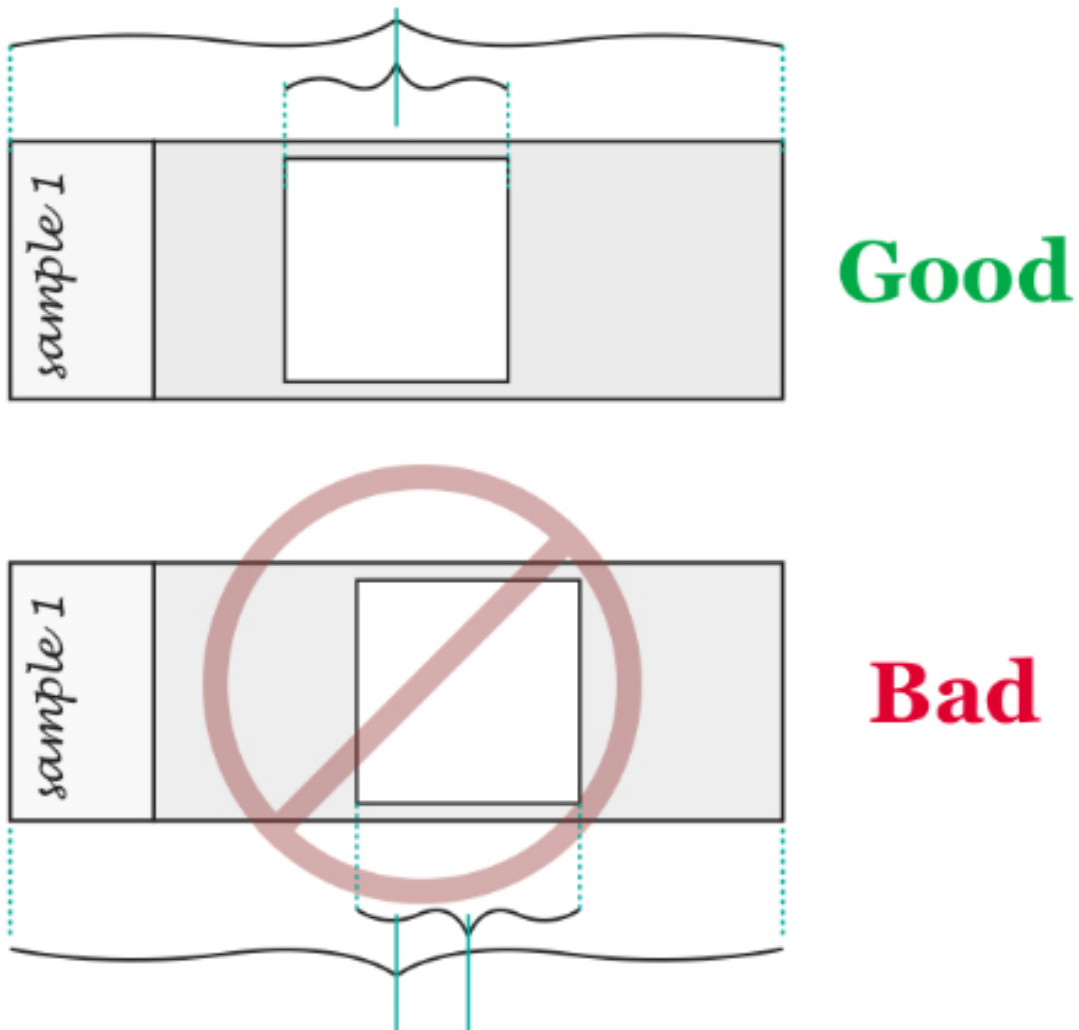
Another anti-fade mountant we have been recommended is Citifluor AF1 from Agar Scientific (cat No. R1320).

The OMX has a **594nm** laser for imaging in the red. This affects your choice of secondary antibody. Currently we have had success with the Alexa 568 and the

Alexa 594 but are looking into brighter dyes. We have been recommended the Atto dyes from <http://www.atto-tec.com/>

The OMX is able to image in three colours, using **405, 488 and 594 excitation** OR **405, 515 and 594 excitation**. No other three colour combinations are possible.

As shown below, the coverslip must be placed in the **very centre of the slide only because movement of the stage is restricted** to this area.



Charging

Use of the OMX will be charged at £24 per hour for users within the Gurdon Institute and £48 per hour for external users not on the grant.

For more information regarding the OMX please see the link below:

<http://msg.ucsf.edu/sedat/omx/>

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