VT-ISIM – APPLICATION NOTE – VIROLOGY STUDIES

The study of viruses (Virology) is not only critical in understanding common and new viruses with a view to developing suitable therapeutics and vaccines, but also as a model in more general research areas of microbiology.

For the latter, a common virus used in such studies is the Vaccinia virus, which is from the poxvirus family and is commonly used as a tool for microbiology research as well as vaccine development.

It's fair to say that Viruses are the world' greatest Cell Biologist and understanding how a virus works will unlock more information about how microbiology and cell biology work.

In this application note, we will cover how instruments, such as the VT-iSIM from VisiTech International, can be used for such studies.

Imaging Requirements

To observe the behaviour of viruses you need to be able to image their lateral and axial motion, whilst ensuring no damage to the cell(s) under observation, and with high enough spatial resolution to track the movement of individual viruses.

Let's cover these imaging requirements one by one;

Image lateral and axial motion of the Virus

The viruses are relatively fast and single plane acquisition rates of 30-50fps is ideal to try and avoid motion blur.

You also need to ensure that any out of focus light does not add unnecessary noise into the image and hence confocal imaging is imperative.

Given you want to track movement across a whole cell at high spatial resolution, you also want to image a large FOV. Given the high spatial resolution requirements (as detailed below), with a 100x objective lens a FOV of at least 66.5 x 66.5µm is preferred.

No damage to the cell under observation

Photo-bleaching and/or photo-toxicity must be avoided so as to ensure the viability of the image data and subsequent analysis.

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To do this multi-point illumination techniques are combined with high sensitivity detectors, such as Gen3/4 sCMOS cameras, at an optimal level of spatial sampling so as to allow the target resolution to be met whilst maintaining high signal to noise.

Observations at the necessary temporal resolution and field of view as detailed above, must be conducted for several minutes without any detrimental effect on the cell under observation. This can be further aided by using photonpixel re-assignment techniques and a simple 1x relay through a highly sensitive imaging system.

High enough resolution to track the movement of individual viruses

If we consider the main break-points in achievable resolution of current light microscopy techniques, and compare to the '*typical*' size of a virus, we can decipher the most practical target resolution for live cell imaging of viruses;

- Wide Field Epi Fluorescence: Achievable spatial resolution - ~250nm
- Regular 1AU Confocal Imaging: Achievable spatial resolution - ~250nm
- SIM/ISM Techniques: Achievable spatial resolution - ~100-125nm
- STED Imaging: Achievable spatial resolution - ~40-50nm
- SMLM Imaging: Achievable spatial resolution - ~20nm

Viruses do vary quite significantly in diameter. However, many of those used in current studies, such as Vaccinia or Coronaviruses are typically 50-200nm in diameter.

Hence to have any chance of resolving the activity of individual viruses in a cluster the resolution must be <200nm and ideally around 100nm… If you combine this with the consideration that we want to image the virus's live, with a reasonable FOV, and low photo-bleaching, then STED and SMLM techniques are unsuitable. We are left focusing on SIM/ISM imaging modalities.

Integration requirements

The integration considerations below are specifically targeted at multi-point confocal ISM techniques;

1. Research grade microscope with focus control and high magnification (100x), high numerical aperture (>1.45), objective lens
2. Fast, high sensitivity, large FOV camera such as Gen3/4 sCMOS, can also be combined with a DualCam for simultaneous dual colour imaging
3. Hardware triggered piezo stage for fast Z-Scan

4. Full incubation for temperature and CO2 control

Advantages of VT-iSIM

The VT-iSIM from VisiTech International is the ideal tool to meet this application requirement.

It can image at speeds of up to 200fps at a FOV (with 100x) of 66.5 x 66.5µm and has already been applied in this research area for many years (please contact VisiTech for suitable references).

Whilst maintaining the necessary temporal resolution, the system can offer spatial resolution of ~100nm and axial resolution of ~275nm. Also, with a combination of multi-point scanning and VisiTech' patented Ingwaz scanning architecture, the system offers very low photo-bleaching and very high signal to noise.

It should also be noted that the system operates as a 1x relay, hence maintaining the highest levels of S2N, as the camera read noise component is kept to a minimum for the selected objective magnification.

The instrument can also be fully integrated with those requirements outlined above and can be supplied as a complete imaging solution by a range of global partners.

The performance suitability of the iSIM for Virology, is best detailed in the Nature Methods Paper; 'Faster, sharper, and deeper: structured illumination microscopy for biological imaging', Yicong Wu & Hari Shroff, https://doi.org/10.1038/s41592-018-0211-z.