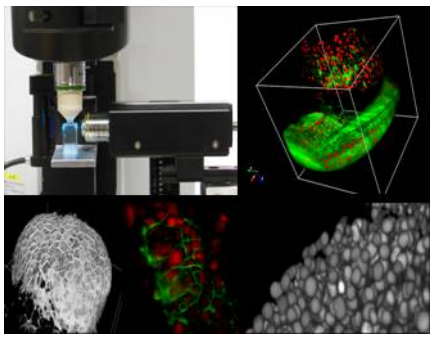

Light Sheet Fluorescence

Microscopy

Context

Life sciences involve the understanding of dynamic processes of complex multicellular organisms in a three dimensional world. To analyze these dynamic processes in three dimensions, optimal spatial and temporal resolution is required. If fluorescent labelling provides useful cellular information, it has the disadvantage of interfering with the sample while producing a relatively weak signal.

The ideal fluorescence microscope would provide images of fluorophores in three dimensions at high resolution without morphological distortion in very short time intervals and for an extended period of time. It should allow scientists to perform long term imaging without loss of fluorescence signal and with limited constraints for live specimens.

Current wide field or confocal fluorescence microscopy techniques are based on a light source that excites the fluorophores on the entire sample thickness. Confocal microscopes allow discriminating the light out of focal plane using a pinhole in front of the detector for a better spatial and axial resolution, however even if the desired information only resides in the focal plane, the entire thickness of the specimen is illuminated, leading to adverse effects in particular photo toxicity and photo bleaching having a direct impact on the specimen.

In confocal microscopy, each image plane is obtained by point scanning and volume acquisition by recording multiple image planes at different z positions for the distribution of fluorophores in three dimensions. This result in a much higher sample exposure by a factor determined essentially by the number of image planes and the low acquisition speed. If faster acquisition solutions have been devised, such as using rotating disk, they only partially meet the objectives set by scientists.

Recently a new fluorescence microscopy technique emerged, using a thin sheet of light which only illuminates the sample in the focal plane of the detection objective located orthogonally to the light sheet. Unlike the wide field or confocal fluorescence microscopy, the excitation remains confined to fluorophores in a volume near the focal plane, significantly reducing the

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phototoxicity and photobleaching issues. This technique combines the benefits of wide field method for fast 3D acquisition and confocal methods for optical sectioning and optimal axial resolution.

Light sheet microscopy or Selective Plane Illumination Microscopy is therefore particularly suitable for live specimen imaging over long periods of time and fulfils the needs of many applications in cell biology.

Technologies Landscape & Current Limitations

The benefits related to the light sheet microscopy technique were recently brought to the scientific community through a group of researchers giving free access to detailed information of all necessary hardware and software tools to build a Selective Plane Illumination Microscope.
If the budget for the assembly parts remains affordable, the constraints for its implementation are relatively high as requiring specific knowledge in the optical and imaging fields. The results that can be obtained are also limited due to its relatively basic design.

In the other hand, only few commercial products are today available on the market place. These light sheet microscopes are clearly more sophisticated but significantly more expensive and therefore out of reach for most research laboratories. PhaseView aims to offer an alternative for a light sheet microscope at a reasonable cost with a high degree of performance for the benefit of a growing number of scientists.

Phaseview Light Sheet Microscopy

Based on a partnership with an academic research laboratory, Phaseview has designed all parts of the system including illumination path, detection unit and sample chamber to optimize the system performance in terms of lateral, axial and temporal resolution while keeping modularity to meet specific requirements. Based on a new approach for the detection path, light sheet imaging can be added to an existing fluorescence microscope stand providing a flexible imaging tool.

In addition the Phaseview know-how of remote focusing devices bring new 3D acquisition capabilities with no specimen move, thus avoiding sample perturbation, and high scanning speed only limited by camera performance, for optimized temporal resolution.

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Value Added In Light Sheet Microscopy

- 3D acquisition without moving the sample thanks to remote focusing capability
  Specimen can be kept in developmental conditions without perturbation. Acquisition speed is not limited by mechanical constraints

- 3D volume at fast or ultra fast scanning speed only limited by camera frame rate
  Allows 3D recording of fast dynamic processes in sensitive living samples

- Easy biological specimen mounting and positioning
  No tedious and time consuming procedure for specimen observation

- Easy adaptation to various experimental conditions thanks to its modular design
  The SPIM platform can be easily customized and is flexible to specimen imaging constraints.

- Compatible with any fluorescence microscope stand
  Turn any microscope in a powerful light sheet imaging platform.

Technology Licensing

PhaseView offer licensing agreement opportunities including:
Standard / custom hardware and software for light sheet imaging - QtSPIM software – Alpha3

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