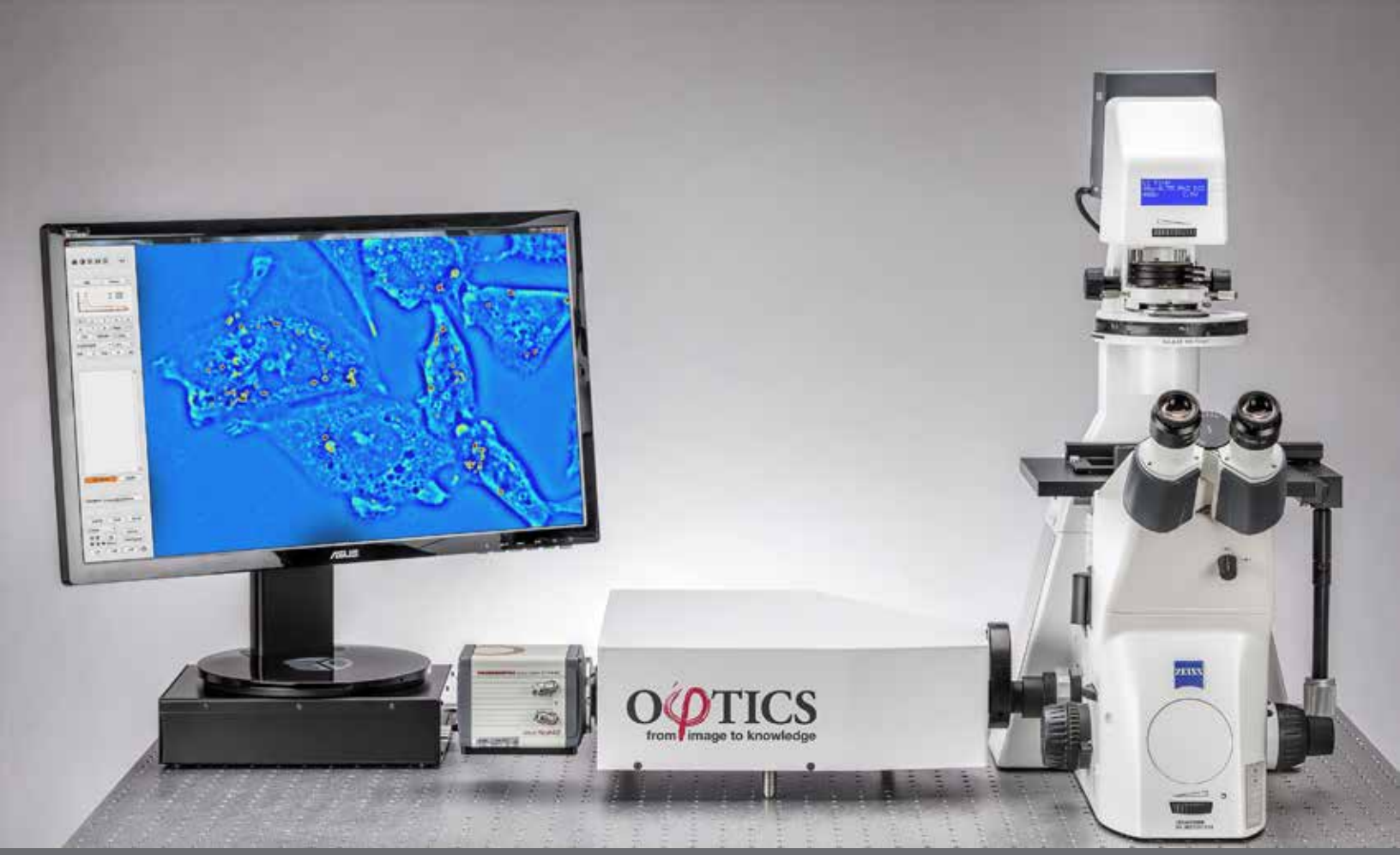

Application Note AN02 Cell Dynamics

Summary

This Application Note illustrates the use of Spatial Light Interference Microscopy (SLIM) for studying intra- and inter-cellular dynamics by tracking the movement of small organelles or analyzing a region of interest as a whole. Sub-nanometer sensitivity and diffraction limited resolution of SLIM provide ideal means to study the cell dynamics. The quantitative data yield actual mass transport information, in terms of diffusion coefficients and velocity distributions. This has clear applications in basic biological sciences, such as cell viability and function, and clinical studies, such as drug testing.

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Spatial Light Interference Microscopy (SLIM)

Phi Optics SLIM is a non-invasive phase imaging technology that quantifies the physical properties of live cells and tissues. The output is a live quantitative image (SLIM map) of the specimen. As shown in Figure 1. the intensity of every pixel in the frame is a measure of a phase shift map (in radians) or the optical path length difference (in nanometers) through the sample, which is measured with sensitivity better than 0.5 nanometers [1]. The phase shift map is converted on-the-fly to other SLIM maps, with their respective pixel intensity: thickness (in microns), dry mass area density (pictograms per square micron) and refractive index. Moreover, the phase shift map can be used for reconstructing the scattering measurement. More detailed description of SLIM can be found in the last page of this note.

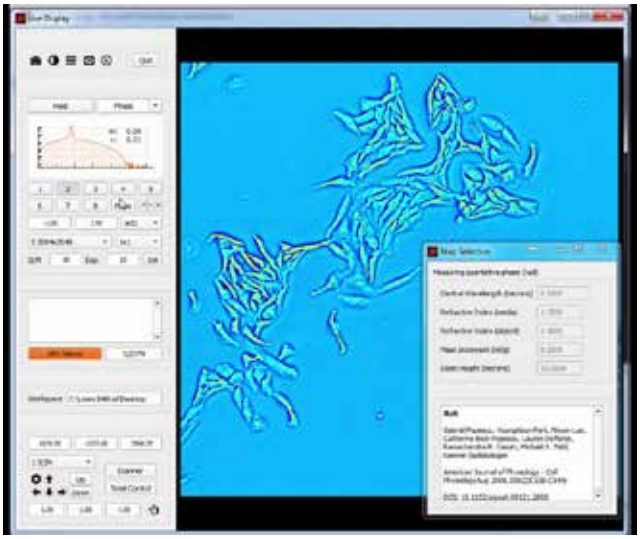

Figure 1. Phi Optics CellVista acquisition program

Organelle tracking

SLIM images reveal the locations of small organelles within the cell. These dense deposits of protein, high in dry mass, can easily be detected, and tracked over a time-lapse measurement [2]. Phi Optics provides a set of plugins based on the NIH ImageJ and Particle Tracker by Sbalzarini and Koumoutsakos [3] for image analysis.

A. Particle detection and tracking

Particle tracking can be setup and performed using the Particle Tracker plugin. The steps to use the plugin is:

1. Load a SLIM time sequence for tracking the particles (organelles). Particle contrast can also be enhanced by applying Laplacian to the image.
2. Run the Particle Tracker plugin in the Dynamics menu
3. Adjust the parameters (Radius, Cutoff, Per/Abs, Link Range, Displacement) and use “Preview Detected” to confirm the detection (Figure 2).
4. Hit OK, and the plugin will show a result window.

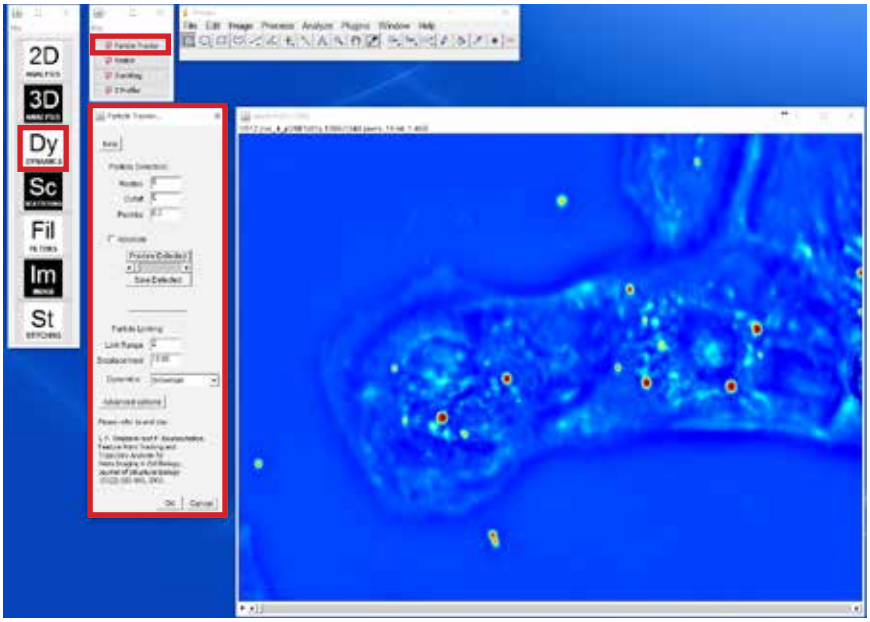

Figure 2. Particle tracker parameter setup window and a SLIM phase map of a heart cell containing particle-like organelles.

B. Analysis

Once the plugin finishes particle detection, it shows a result window with a variety of options to display the result.

1. The trajectories of the detected particles can be visualized by “Visualize All Trajectories” in the result window (Figure 3).
2. The trajectories can be saved to a table, which contains the coordinate of each detected particle for each frame, by “All Trajectories to Table” in the result window.
3. Using the coordinates, the Mean Square Displacement (MSD) can be plotted. The MSD vs. Time plot yields diffusion coefficient.

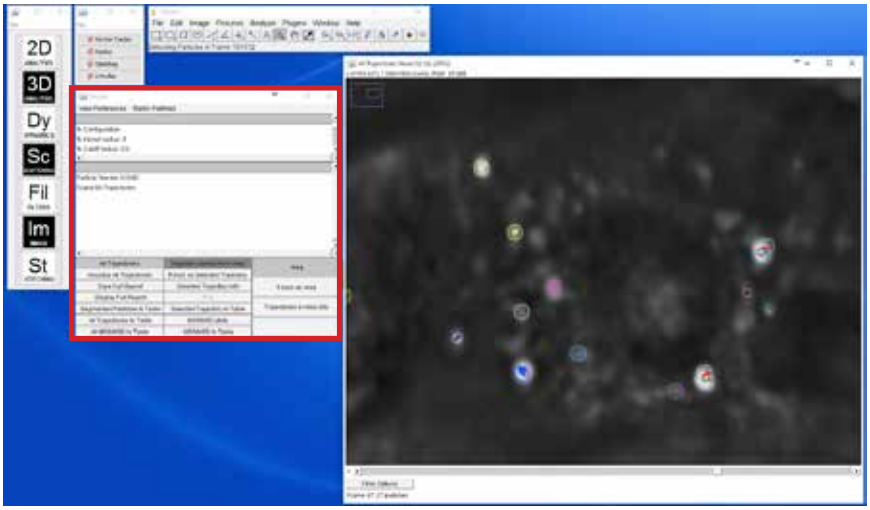

Figure 3. Particle tracker result window and “Visualize All Trajectories” window showing the trajectories of detected particles.

Organelle movement within a beating heart cell

SLIM was used to track the organelle movements within a beating heart cell [2]. The tracking of organelle allowed for the measurement of the diffusion coefficient. The small diffusion coefficient indicates that the movement of the organelles are restricted by the cell (Figure 4).

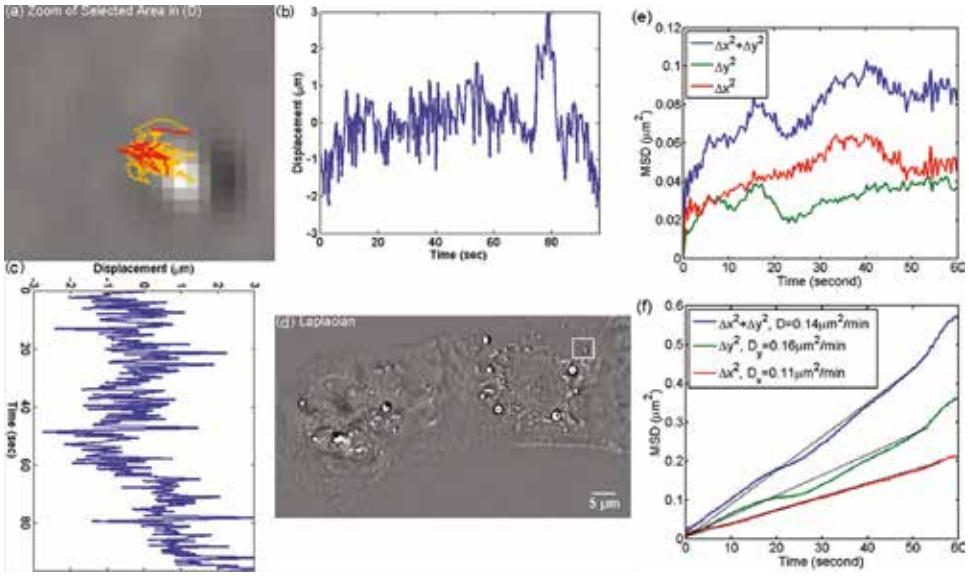

Figure 4. MSD measurement for particles within the cardiac myocytes in Fig. 2. (a) Zoom into the selected area shown in (d); (b) Displacement in Y direction; (c) Displacement in X direction. (d) Laplacian of the phase map; (e) MSD for the particle shown in (a). (f) MSD ensemble-averaged over 15 particles in (d). [2]

Intercellular transport between neurons

Intercellular transport has also been studied using SLIM, by analyzing the transport between neurons [2]. The diffusion coefficient in the horizontal and vertical directions are calculated. Because the neurites are mostly extending horizontally within the field of view, the spatial confinement along the vertical direction resulted in a very small diffusion coefficient along the vertical direction (Figure 5).

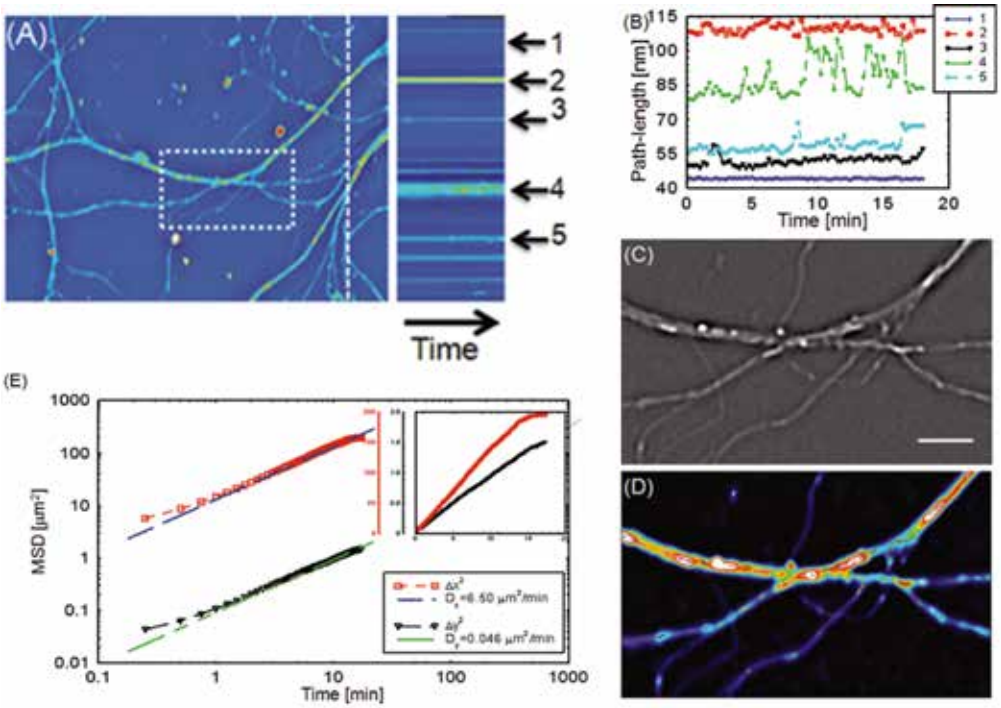

Figure 5. Particle transport in neurites of a hippocampal neuron processor network. The objective is ZEISS Plan-Neofluar 40X/0.75. (a) Phase map of the neuron network. The arrows 1 to 5 show the time-traces of the corresponding points along the dashed line. The whole field of view is 100 μm×75 μm. The objective used is Zeiss Plan-Neofluar 40×/0.75. (b) Optical path length change in time for the five points indicated in (a). Peaks in the point traces correspond to phase shifts associated with (fast) organelle traffic. (c) Laplacian of the selected area in (a). The scale bar is 5 μm. (d) Phase map of the same area as in (c), with some particle traces shown in fine lines. (e) Log–log plot of the MSD for 70 individual particles in (d). Since the particles are confined in the Y direction, the diffusion coefficient for this direction is 2 orders of magnitude smaller than for the other direction. The inset shows the same MSD curves in linear representation and two Y axes. [2]

Dispersion-relation Phase Spectroscopy (DPS)

SLIM provides a way to study cellular dynamics even when the dynamic structures are continuous and cannot be tracked as particles. DPS is a method for analyzing SLIM time lapse images. DPS provides a relation between the spatial and temporal fluctuations on the sample, and yields information about the diffusion coefficient, velocity distributions, as well as the mode of transport: random or deterministic.

A. Obtaining DPS data

1. Load a SLIM time sequence for DPS analysis
2. Run the DPS plugin in the Dynamics menu
3. Adjust the parameters (time step and pixel size), select a square ROI to be analyzed and run the plugin.
4. The result will show a DPS map and a DPS plot (Figure 6).
5. Diffusion coefficients and velocity distributions can be calculated by fitting the DPS plot.

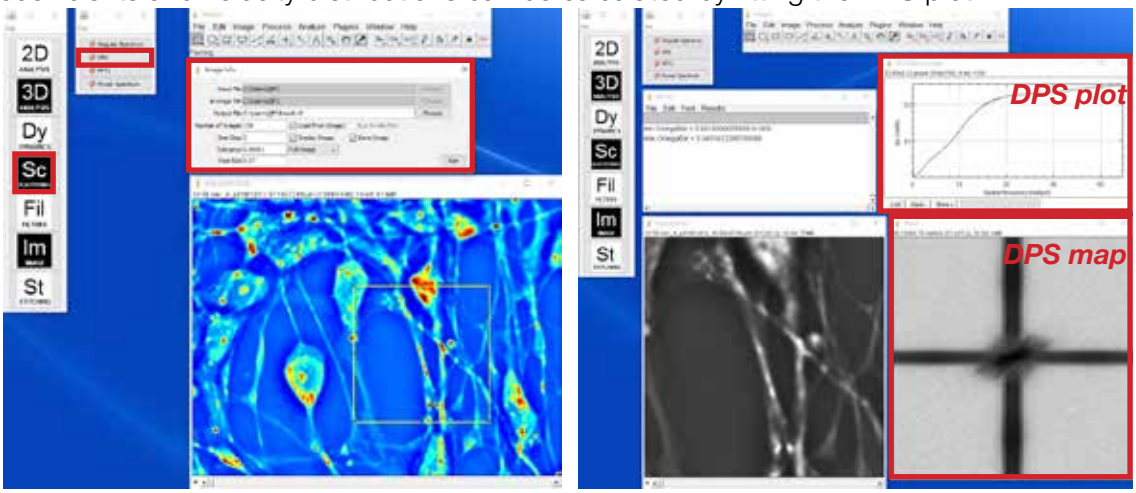

Figure 6. Dispersion plugin setup window and results

B. Dispersion-relation phase spectroscopy of inter- and intra-cellular transport

DPS is applied to a variety of cells, including glia, microglia and neurons, to infer the intercellular and intracellular transport of these cells [4]. The output provides information regarding the diffusion coefficient, advection velocity and the mode of transport (Figure 7).

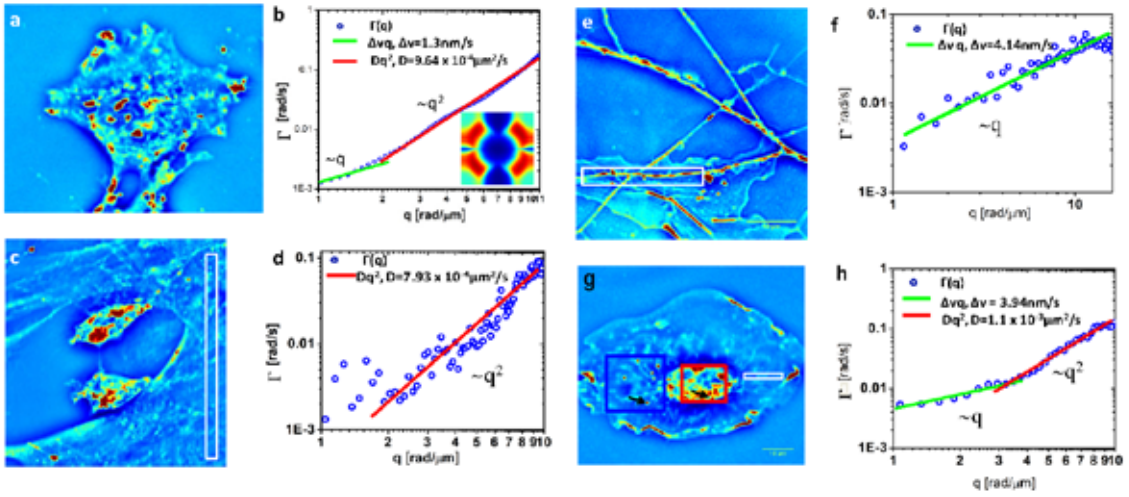

Figure 7. Quantitative phase image of a culture of glia (a, g), microglia (c) and hippocampal neurons (e). (b) Dispersion curve measured for the cell in a. The green and red lines indicate directed motion and diffusion, respectively, with the results of the fit as indicated in the legend. Inset shows the Γ(qx, qy) map. (d, f, h) Dispersion curves, Γ(q), associated with the white box regions in c),e) and g), respectively. The corresponding fits and resulting D and Δv values are indicated. [4]

References

[1] G. Popescu (2011) Quantitative phase imaging of cells and tissues (McGrow-Hill, New York)

[2] Z. Wang, L. Millet, V. Chan, H. Ding, M. U. Gillette, R. Bashir, and G.Popescu, Label-free intracellular transport measured by spatial light interference microscopy, J. Biomed. Opt., 16(2), 026019 (2011).

[3] I. F. Sbalzarini and P. Koumoutsakos. Feature point tracking and trajectory analysis for video imaging in cell biology. J. Struct. Biol., 151(2): 182–195, 2005

[4] R.Wang, Z. Wang, L. Millet, M. U. Gillette, A.J. Levine, and G.Popescu, Dispersion-relation phase spectroscopy of intracellular transport, Opt. Exp. 19(21), 20571 (2011).

Phi Optics patented SLIM technology employs optical interferometry for extreme sensitivity to structure and dynamics. Phi Optics implements SLIM as an add-on to all major brand optical microscopes (10X to 100X magnifications) (see Figure 8). The SLIM approach to quantitative phase imaging provides speckle-free images due to high sensitivity of the measurement (nanometer scale spatial noise). Submicron optical sectioning is facilitated by high NA objectives and the micron-scale coherence length of the illumination - SLIM can render 3D tomographic images of transparent structures just by scanning the specimen through focus (Z-scanning). The design modularity enables multiplexing with fluorescence imaging for multimodal, in-depth biological studies.

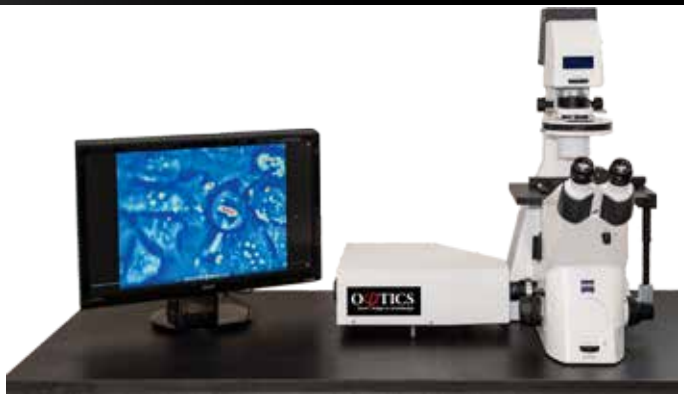

Figure 8. Phi Optics SLIM module attached to a ZEISS Axio Observer Z1 microscope

Imaging live cells using classical brightfield microscopy is notoriously difficult because they absorb and scatter very little light. Fluorescence microscopy employs fluorophores which absorb and emit light, rendering the cells visible. The fluorophores can be genetically encoded or injected into the live cells and their location and emission intensity is used to accurately quantify the cell features and processes . Continuous imaging is possible only for short periods of time (time-lapse) to avoid phototoxicity, photo bleaching and measurement bias . Live unstained cells exhibit gradients of optical path length (i.e. product of thickness and refractive index) across their structure. Phase contrast (PC) and differential interference microscopy (DIC) modalities to convert minute changes in optical path length into differences in brightness (amplitude contrast). These techniques are not intrinsically quantitative or specific, and lack the resolution of fluorescence microscopy but continuous long term imaging is possible because of the low levels of illumination required.

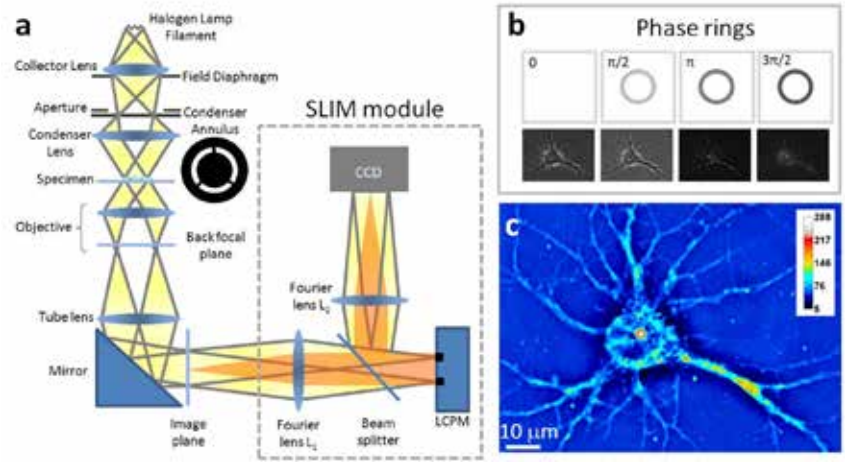

Figure 9. SLIM principle. (a) Schematic setup for SLIM. The SLIM module is attached to a commercial phase contrast microscope (b) The phase rings and their corresponding images recorded by the CCD. (c) SLIM quantitative phase image of a hippocampal neuron.

Phi Optics SLIM is a non-invasive phase imaging technology that quantifies the optical path length differences in a biospecimen and converts them into thickness, dry mass area density and refractive index maps. Figure 9 illustrates the principle of technology in its reference implementation. A live cell in culture medium is imaged with the phase contrast modality of the microscope : the light passing through the object (scattered beam) and the light passing through the medium (reference beam) combine through interference in the image plane. The optical path length differences between the beams (i.e. the phase shift) in each point of the image plane are converted into brightness differences. Optically dense areas of the cell (e.g. nucleus) introduce a phase shift of up to – 0.5π radians in the scattered beam with respect to the reference beam. In the most common phase contrast implementation, before reaching the image plane the reference beam also undergoes a + 0.5π radians phase shift by passing through a phase plate: a glass ring with finite thickness in the back focal plane of the objective. Destructive interference generates a dark image for the dense portions of the cell with respect to the grey background (see Figure 9b).

The SLIM module relays the image plane with minimal aberrations (diffraction limited) at a 1:1 ratio to a camera sitting at its exit port. The active element at the heart of the SLIM module is a liquid crystal spatial light modulator (SLM). The SLM is conjugated with the back focal plane of the microscope objective, and it modulates the reference beam like a phase plate with variable thickness. To create a quantitative phase image the SLM shifts the phase of the reference beam by a fixed amount (0, 0.5π, π, 1.5π) and the camera captures the resulting frame (Figure 9b). The CellVista software module combines the four frames by solving the field interference equations in each point of the frame – the result (Figure 9c) is a quantitative-phase (SLIM) image that is uniquely determined.

SLIM is a wide field quantitative imaging method thus it can measure simultaneously large populations of cells at full camera resolution (e.g. 2 mm FOV for 10X objective at 4.2 MP camera resolution). Wide field optical sectioning (e.g. 850 nm Z-resolution for 100X/1.4NA objective) enables 3D tomography. All microscope output is acquired with the same camera which enables seamless overlay of SLIM images with fluorescence channels.