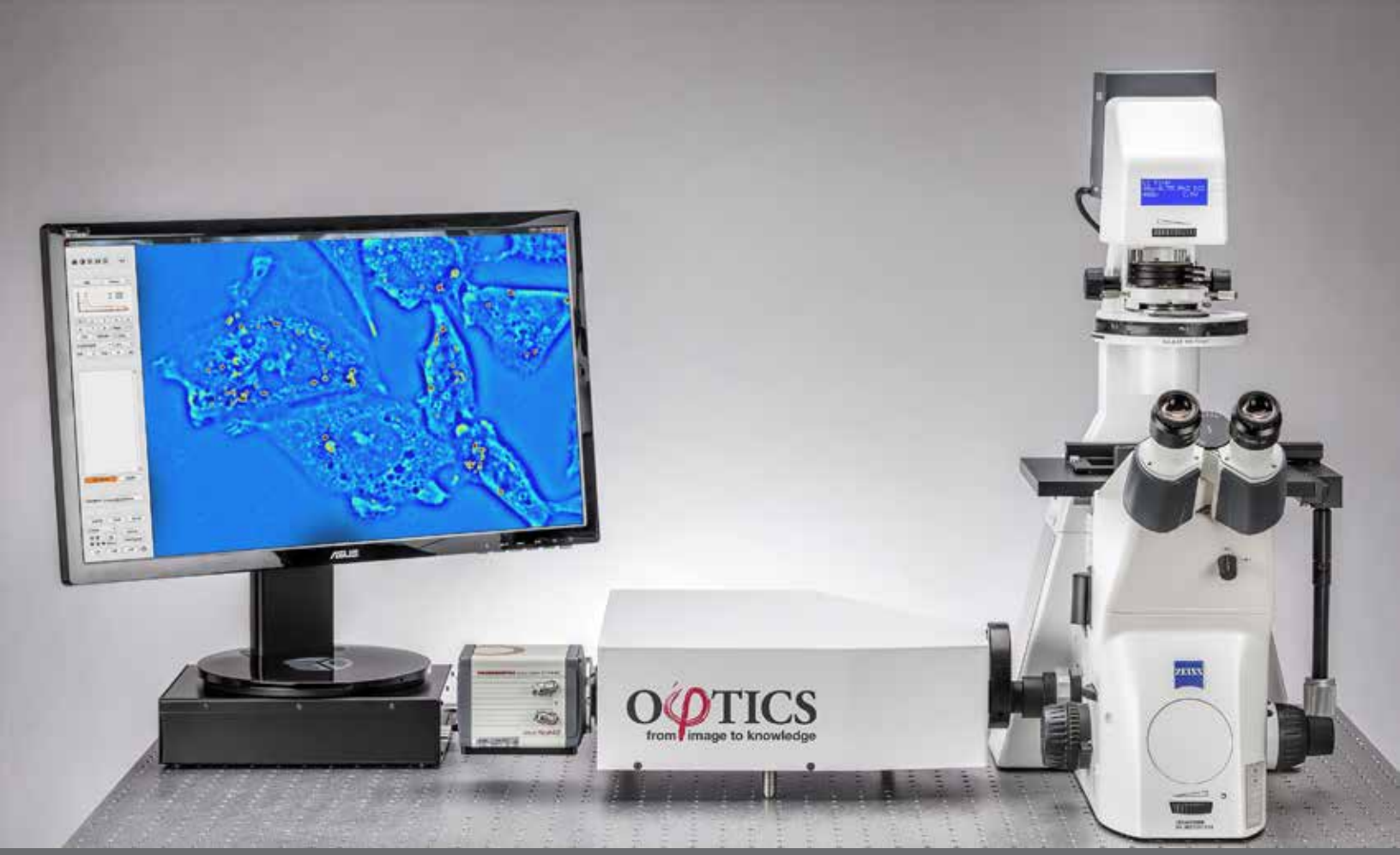

Application Note AN04 Neuroscience

Summary

This Application Note illustrates the use of Spatial Light Interference Microscopy (SLIM) for neuroscience research. The non-invasive live cell imaging provides a viable environment for fragile neurons and neuronal stem cells, which are very susceptible to damages from temperature, chemicals, and light. The speed of SLIM acquisition is capable of detecting the transport between neurons, and the wide field of view is capable of imaging the formation of a neuronal network. Therefore, SLIM provides an environment where neurons can be studied at both single cell level and population level.

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Spatial Light Interference Microscopy

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 on the microscope stage. The intensity of every pixel in the frame is a measure of the optical path length difference (in radians) through the sample, i.e. a phase shift map, which is measured with better than 0.5 nanometers sensitivity (Figure 1) [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 (picograms per square micron) and refractive index. More detailed description of SLIM can be found in the last page of this note.

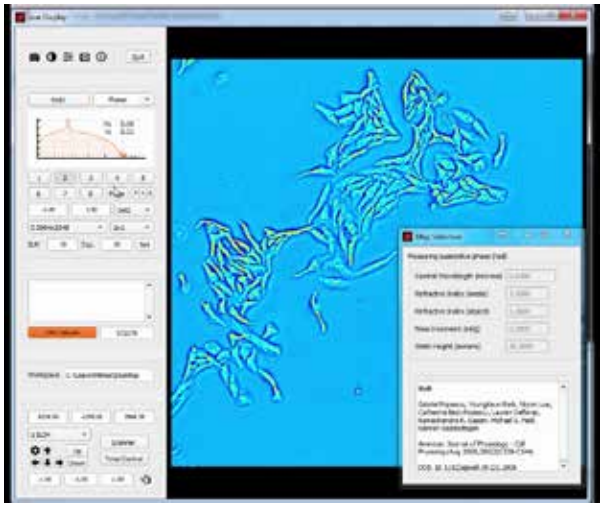

Figure 1. Phi Optics CellVista acquisition program

Simple measurements - NeuronJ

NeuronJ is an ImageJ plugin developed by Meijering et al. [2]. The plugin can be found in the 2D analysis menu, and once run, a new toolset on the main ImageJ window loads. NeuronJ allows the user to easily trace along neurites and performs measurements for parameters such as the length, mean value along the trace, and many more. Moreover, the traces can be saved as a text file consists of the coordinate and values of the points along the trace, and allows the user to treat the data as a list of values instead of a complex structure.

1. Convert the SLIM map to an 8-bit image and save
2. Run NeuronJ found in 2D analysis menu and load the 8-bit image using NeuronJ “Load images”
3. Trace neurites using “Add tracings” in NeuronJ
4. Run measurements using “Measure tracings” in NeuronJ (Figure 2)

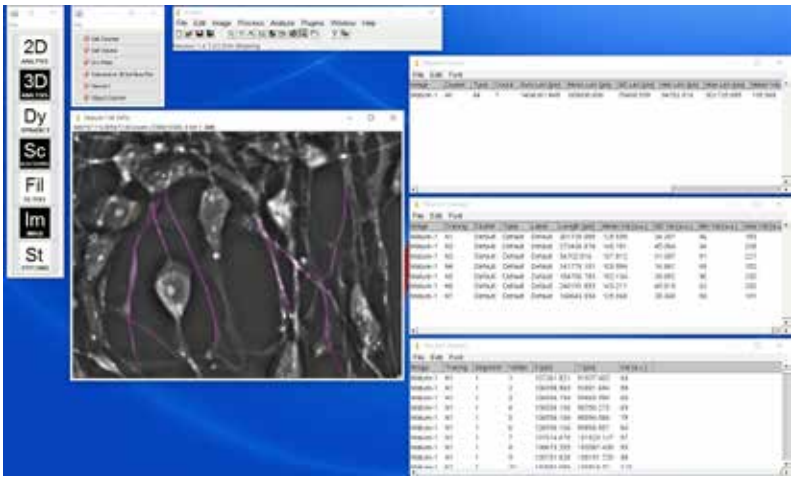

Figure 2. Tracing and measuring neurites using NeuronJ

Traffic

Rapid exchanges of material among neurons appear in the form of vesicle transport along the neurites that connect neighboring neurons. These vesicles are higher in density compared to the neurites, and appears as a particle moving along the neurites. Therefore, by applying a particle tracking method, the movement of these vesicles can be tracked and the transport or “traffic” along the neuronal highway can be studied (Figure 3) [3]. For more detail about the particle tracking, please see AN 02 – Cell Dynamics.

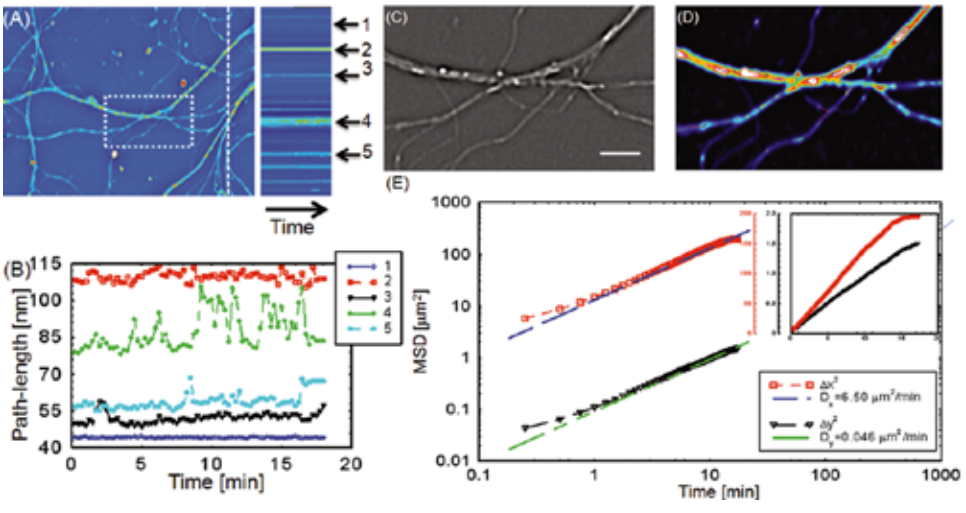

Figure 3. Particle transport in neurites of a hippocampal neuron processor network. The objective is ZEISS Plan-Neofluar 40X/0.75. (a) Phase map of the neuron network. The arrows 1 to 5 show the time-traces of the corresponding points along the dashed line. The whole field of view is 100 μm×75 μm. The 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.

Growth

Because neurons are very fragile and vulnerable to many invasive factors, such as chemicals, light, and heat, it is difficult to study them live and study their growth. SLIM provides a solution for this type of problem by providing an imaging environment that is both temperature and CO2 controlled, and also by exposing the cells to minimal light damage. The growth of neurons have been studied and the emergence of neuronal network have been shown using SLIM (Figure 4) [4].

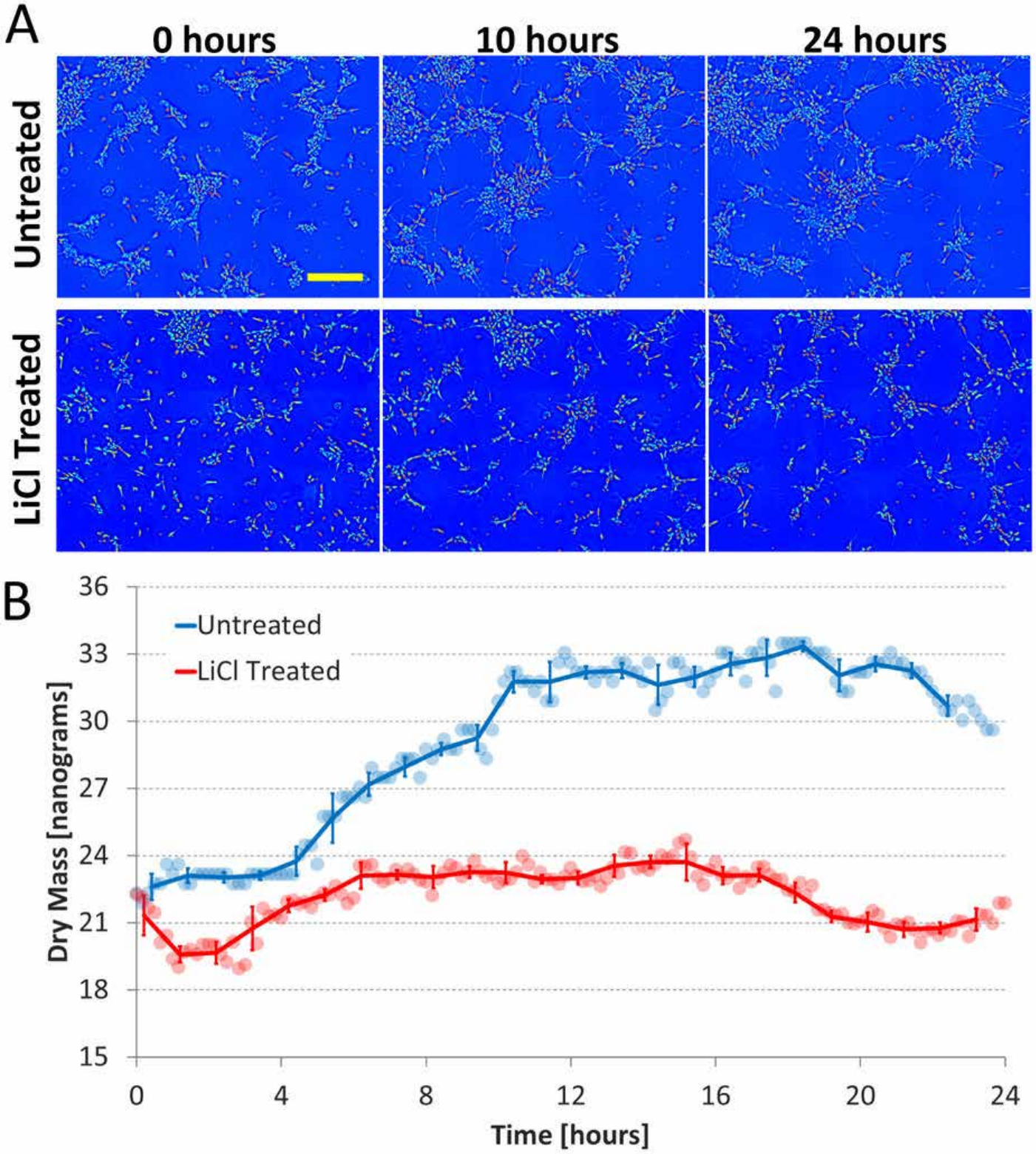

Figure 4. Dry Mass Growth (A) Dry mass density maps acquired at 0, 10, and 24 hours for both the untreated and LiCl treated cultures; the yellow scale bar corresponds to 200 µm. (B) Total dry mass vs. time of the entire field of view for both conditions. Round markers are raw mass data, solid lines are the average over 1 hour, and error bars indicate standard deviation. For the untreated culture it can be seen that majority of the mass growth occurs between 0 and 10 hours, the time during which the cells are extending processes most actively. In the LiCl treated culture neurite outgrowth is severely retarded and no significant increase in mass is observed during any period.

Differentiation

The non-invasive environment that SLIM provides is suitable for studying not only mature neuron cells but also progenitor cells, which are even more fragile than mature neurons. The structural change of the neural progenitor cells and the change in dynamics can easily be studied using SLIM to show the development of mature cells from the progenitor cells, while keeping the cell alive and well (Figure 5) [4].

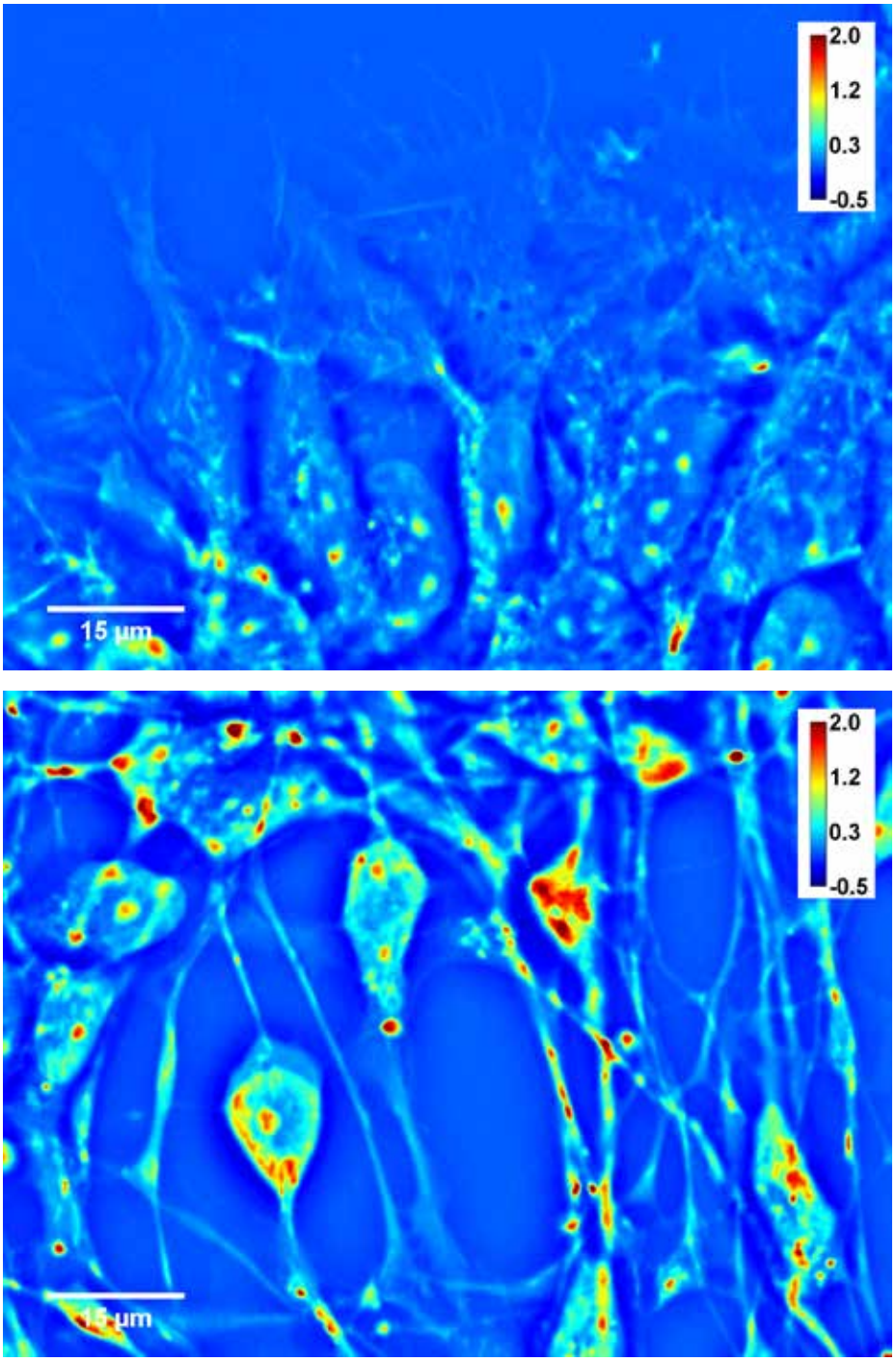

Figure 5. Neural progenitor cells at day 0 of plating (top) and the mature cells after day 14 of plating (bottom).

References

[1] G. Popescu (2011) Quantitative phase imaging of cells and tissues (McGrow-Hill, New York)
[2] E. Meijering, M. Jacob, J.-C. F. Sarria, P. Steiner, H. Hirling and M. Unser, Design and validation tool for neurite tracing and analysis in fluorescence microscopy images, Cytometry Part A, 58 (2), 167-176 (2004)
[3] 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, Journal of Biomedical Optics, 16 (2), 026019 (2011)
[4] M. Mir, T. Kim, A. Majumder, M. Xiang, R. Wang, S. C. Liu, M. U. Gillette, S. Stice and G. Popescu, Label-free characterization of emerging human neuronal networks, Scientific Reports, 4, 4434 (2014)

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 6). 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.

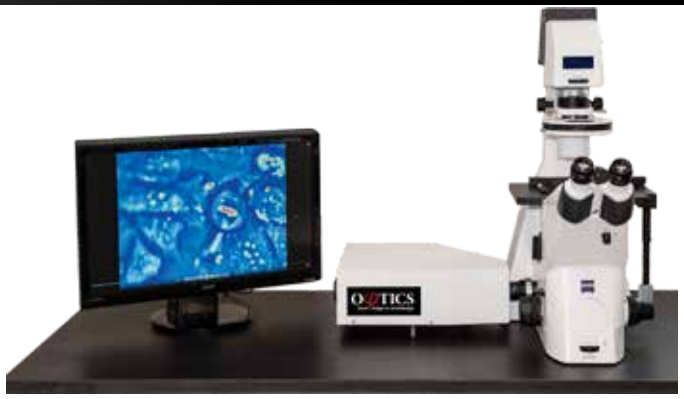

Figure 6. Phi Optics SLIM module attached to a ZEISS Axio Observer Z1 microscope

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.

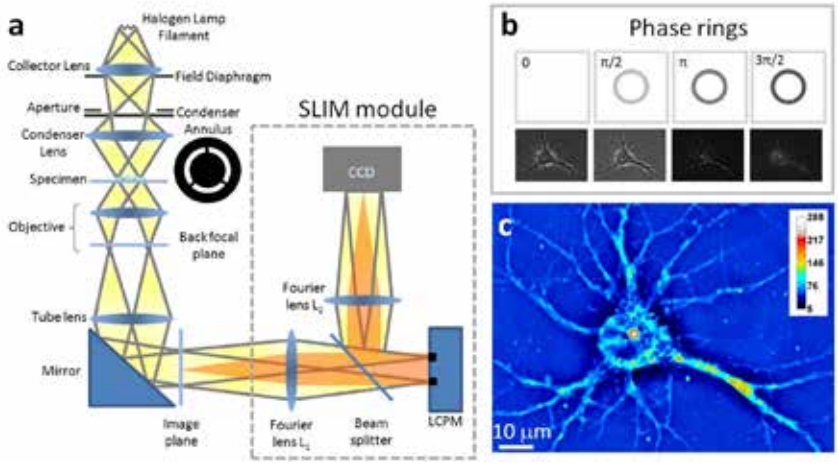

Figure 7. SLIM principle. (a) Schematic setup for SLIM. The SLIM module is attached to a commercial phase contrast microscope (b) The phase rings and their corresponding images recorded by the CCD. (c) SLIM quantitative phase image of a hippocampal neuron.

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 7 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 7b).

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 7b). The CellVista software module combines the four frames by solving the field interference equations in each point of the frame – the result (Figure 7c) 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.