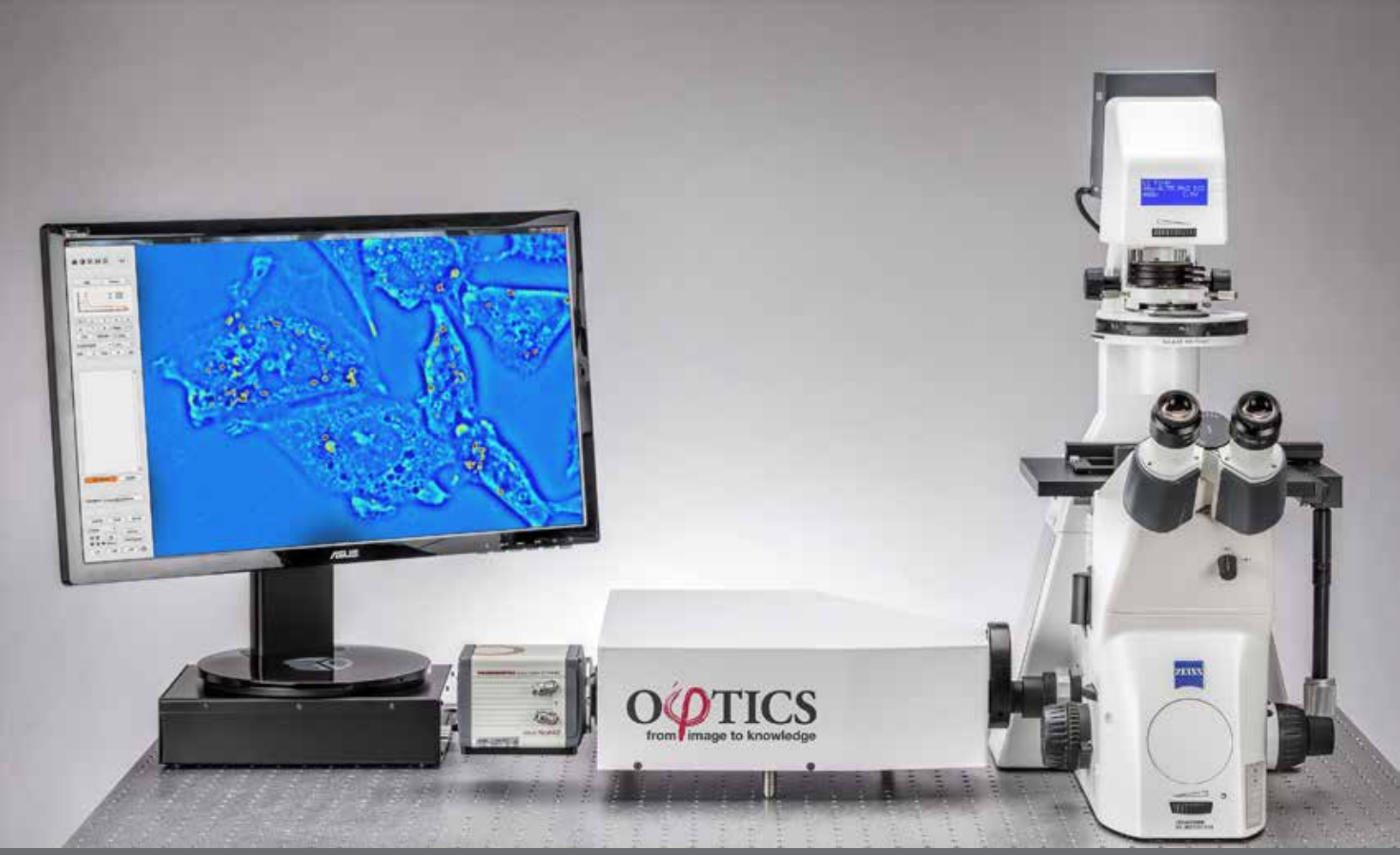

Application Note AN06 Tissue Imaging

Summary

This Application Note illustrates the use of Spatial Light Interference Microscopy (SLIM) for tissue imaging and diagnosis. SLIM is capable of automatically scanning and imaging microscopy slides with tissue samples. The quantitative data obtained from Phi Optics SLIM provides insight into the condition of the tissue, whether it is in benign or malignant condition, without staining. Therefore, it allows for an automatic diagnosis of cancer in the tissue sample. Moreover, phase imaging reveals certain structures that are not readily detected in simple staining methods that are current standards in clinics that can be used as an easier and faster indicator to diagnose the condition.

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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 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]. Moreover, SLIM is capable of automatically scanning a sample slide with tissue biopsies at high resolution, allowing for an automated tissue scanning and diagnosis [2]. The size of each frame depends on the objective and the detector. More detailed description of SLIM can be found in the last page of this note.

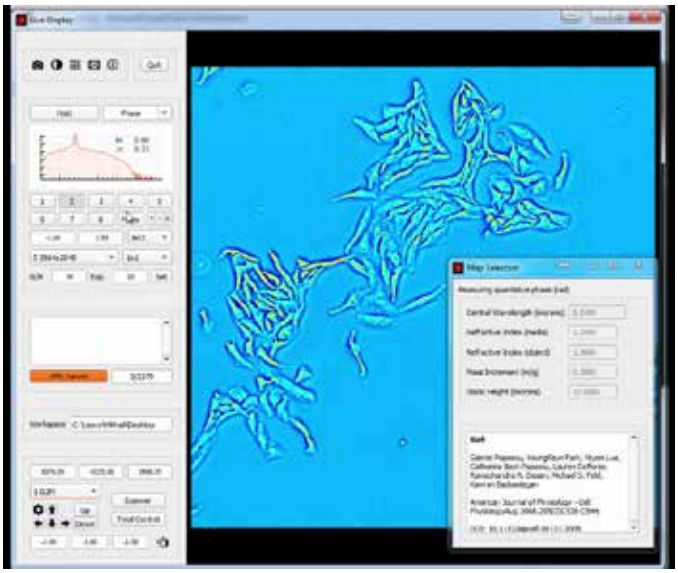

Figure 1. Phi Optics CellVista acquisition software

Tissue scanning

Phi Optics CellVista software makes tissue scanning simple and quick by integrating microscope control and image acquisition. The focus correction implemented in the software keeps the sample in focus while the imaging system scans and images over a large area.

1. Go to “Scanning” found on the bottom left corner of CellVista
2. Setup experiment and focus points to cover the area of measurement (Figure 2)
3. Run experiment

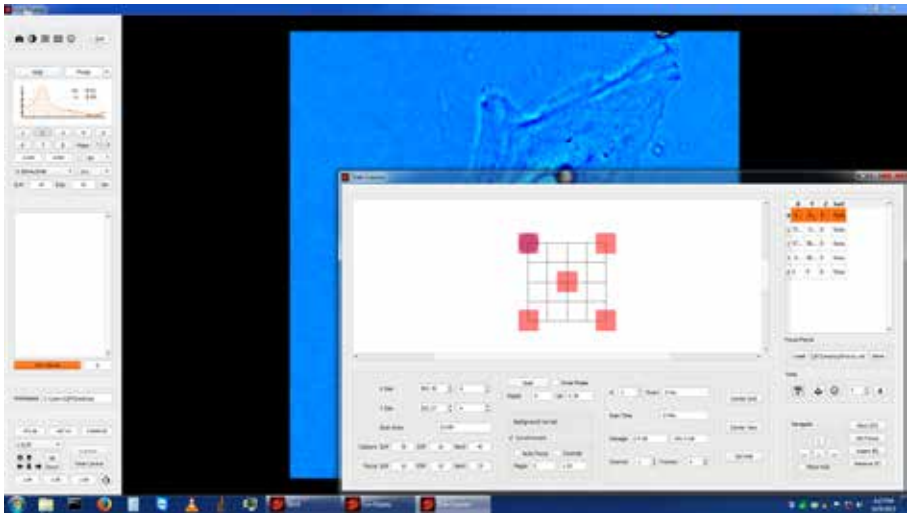

Figure 2. Phi Optics CellVIsta software for tissue scanning application

Mosaic

Stitching of the images obtained from Phi Optics SLIM and CellVista can easily be done using the ImageJ stitching plugin written by Preibisch et al. [3]. The plugin is included in the ImageJ in Stitching menu. For SLIM stitching for tissue imaging, which typically includes a large number of tiles, “Stitch Grid of Images” option is very valuable.

1. Run “Stitch Grid of Images” in Stitching menu
2. Set grid size x, y and overlap as they are configured in the measurement
3. Select the directory that includes SLIM maps to be stitched, and configure the file names. The file name for unchanged SLIM maps should be {i}_0_1_0_SLIM.tif
4. Other parameters can also be set to meet the details of the experiment. Click “OK” to run stitching.

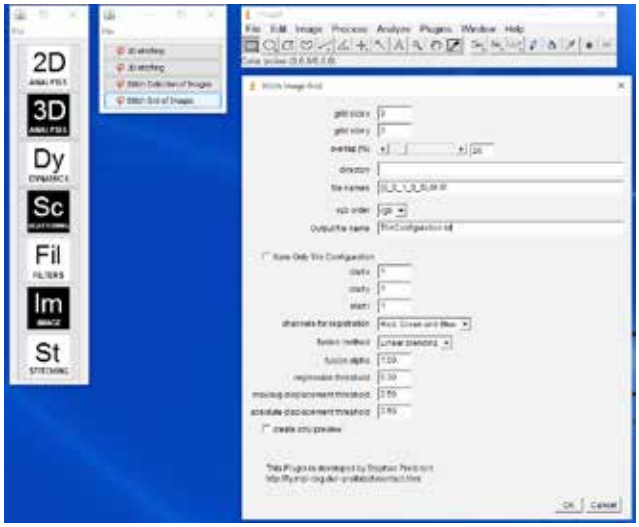

Figure 3. Stitch Grid of Images plugin setup window

Quantitative phase as marker for diseases

The quantitative data from SLIM can be used as a marker for cancer diagnosis (Figure 4). The optical path lengths measured for the structures, such as stroma or red blood cells, present in a tissue biopsy are different for different structures, and thus, can be used as a marker for these structures. Moreover, some significant and indicative structures that are not detectible by staining, such as microcalcification in H&E staining, can be detected by SLIM (Figure 5) [4]. More recently, SLIM map based diagnosis carried out by pathologists showed a high correlation to the diagnosis on H&E stained biopsies [5].

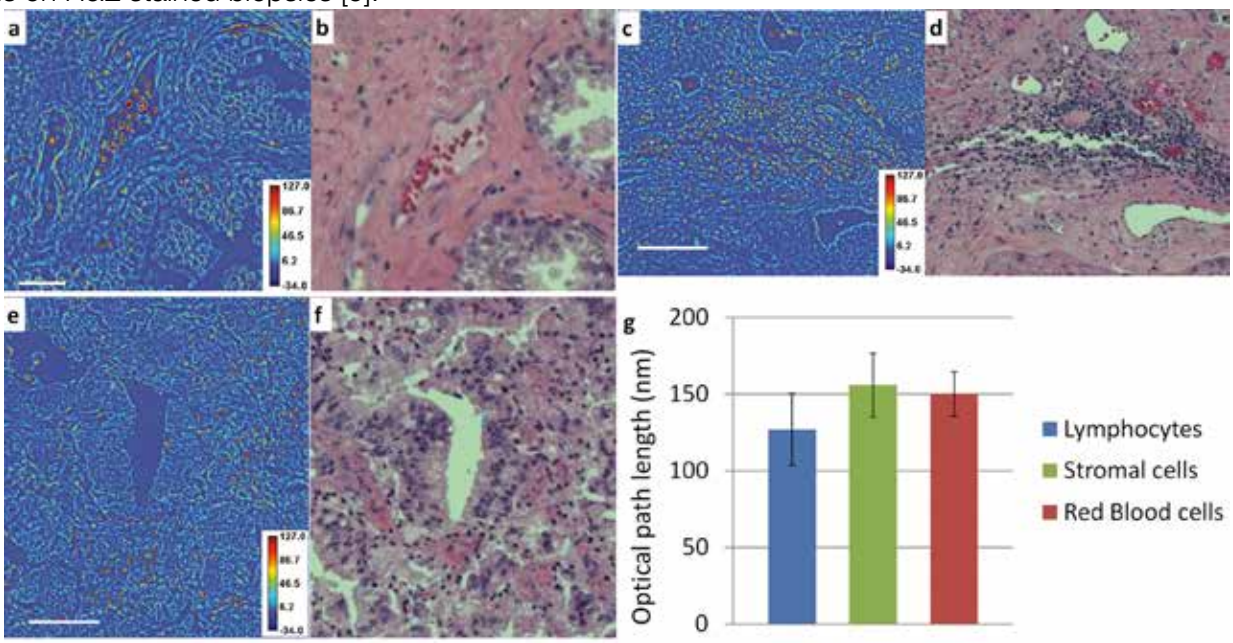

Figure 4. SLIM imaging signatures. Red blood cells with SLIM (a) and H&E (b). Red blood cells can be identified by their unique shape. Scale bar: 20 µm. Lymphocytes with SLIM (c) and H&E (d). Lymphocytes were confirmed with CD45 staining. Stromal cells with SLIM (e) and H&E (f). (g) optical path length for the three different cells that feature high refractive index. Scale bar: 100 µm. Color bar indicates optical path length in nanometers.

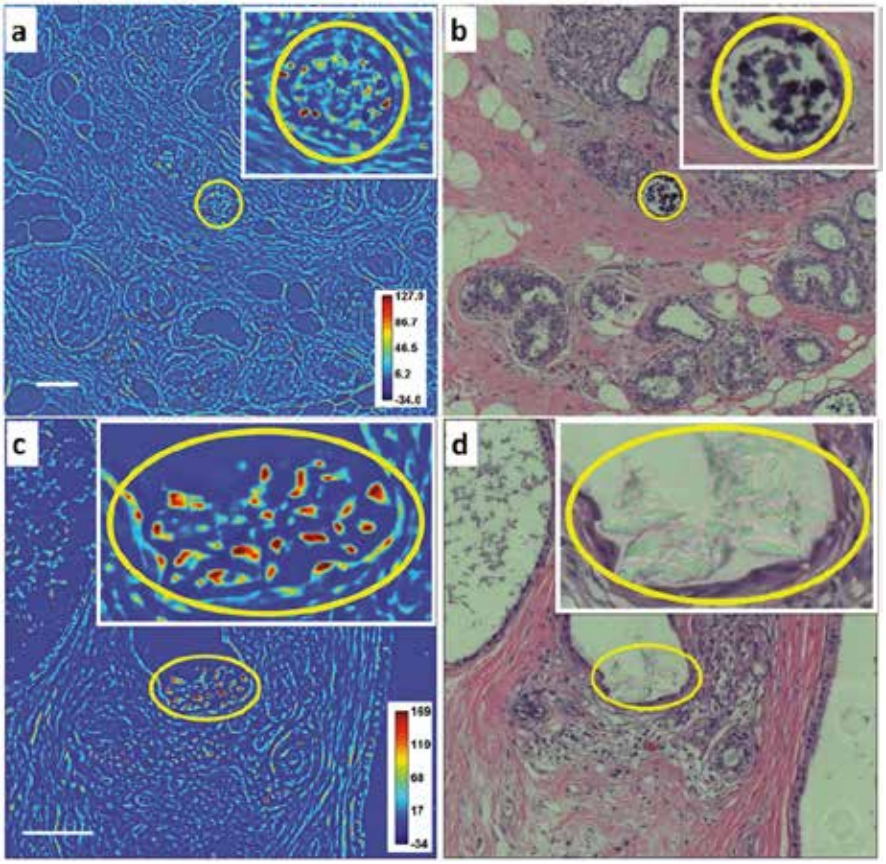

Figure 5. SLIM imaging of breast microcalcifications. Breast tissue with calcium phosphate: SLIM image (a), color bar in nanometers; H&E image (b). The whole slice is 2.2 cm × 2.4 cm. The SLIM image is stitched by 4785 images and the H&E is stitched by 925 images. Scale bar: 100 µm. Breast tissue with calcium oxalate: SLIM image (c), color bar in nanometers; H&E image (d). The entire slice is 1. 6 cm × 2.4 cm. The SLIM image is stitched by 2840 images and the H&E is stitched by 576 images. Scale bar: 200 µm.

Scattering analysis

SLIM, by imaging the phase shift, can reconstruct the field scattered by the tissue sample (Figure 6). Therefore, the scattering parameters, such as scattering mean free path (ls) and anisotropy (g), can be calculated from the SLIM maps [6]. These parameters have been used for predicting prostate cancer recurrence, and outperformed some of the widely accepted clinical tools, such as CAPRA-S (Figure 7) [2].

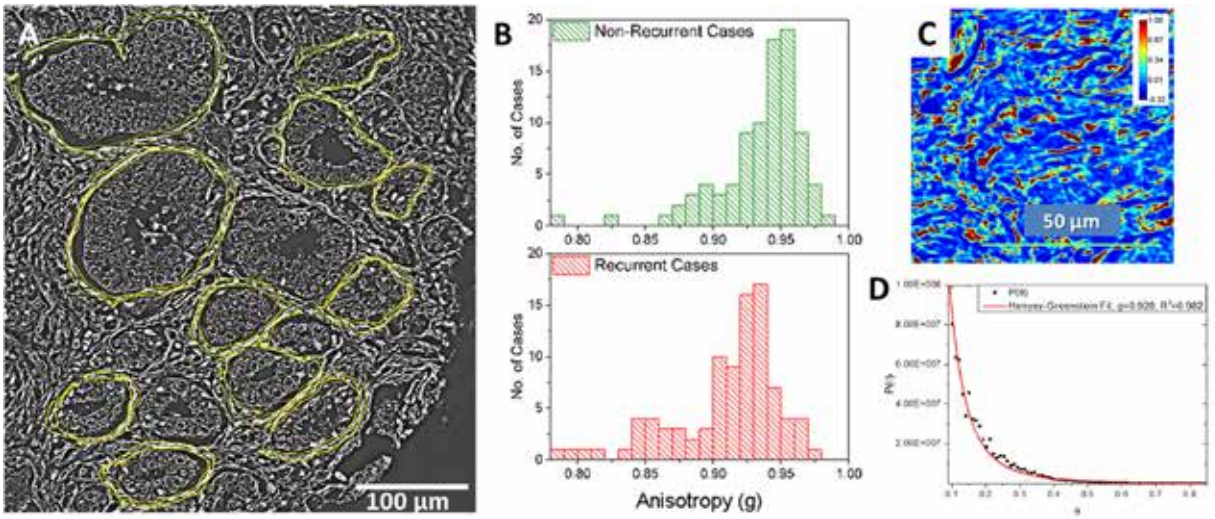

Figure 6. (A) Optical anisotropy (g) was calculated in the single layer of stroma immediately adjoining multiple glands in each core. (B) The histograms show the distribution of anisotropy values among the 89 non-recurrent and 92 recurrent cases. The bin-size on the histogram was set at 0.01. (C) SLIM image of a stromal tissue region in the prostate imaged using the 40X/0.75NA objective. Optical anisotropy value calculated using the scattering phase theorem in this tissue region was g = 0.932. (D) Anisotropy calculation using Henyey-Greenstein phase function fit of the scattering angular distribution yields g = 0.928.

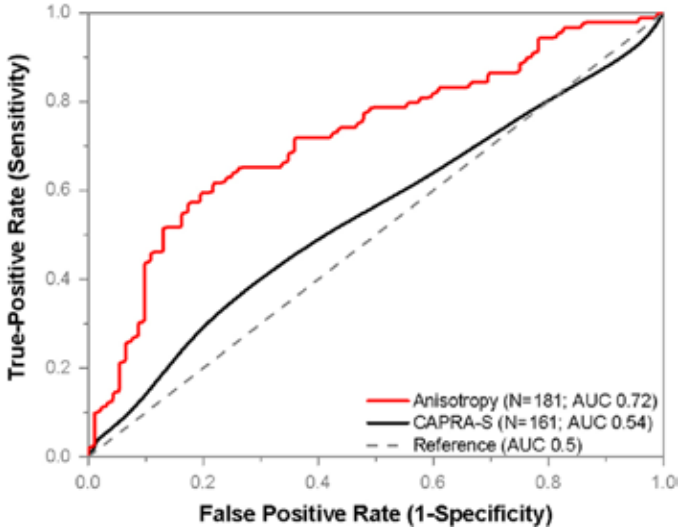

Figure 7. Single layers of stroma immediately adjoining 12–16 glands were isolated in SLIM images from each of the 92 recurrent and 89 non-recurrent patients who underwent prostatectomy. The patients in the two groups were matched based on age at prostatectomy, Gleason grade and clinical stage. The optical anisotropy parameter was calculated for each region, as described in Materials and Methods. This parameter separates cases of recurrence from non-recurrent twins with an AUC of 0.72, as shown. Lower values of this index correspond to a greater probability of biochemical recurrence. By using a cut-off value of g = 0.938, we can predict recurrence with a sensitivity of 77% and specificity of 62%. CAPRA-S scores corresponding to 161 patients, 83 recurrent and 78 non-recurrent, showed poor discrimination (AUC 0.54). Twenty cases were excluded in CAPRA-S analysis due to one or more missing parameters for CAPRA-S calculation.

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[2] S. Sridharan, V. Macias, K. Tangella, A. Balla and G. Popescu, Prediction of prostate cancer recurrence using quantitative phase imaging, Scientific Reports, 5, 9976 (2015)

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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.

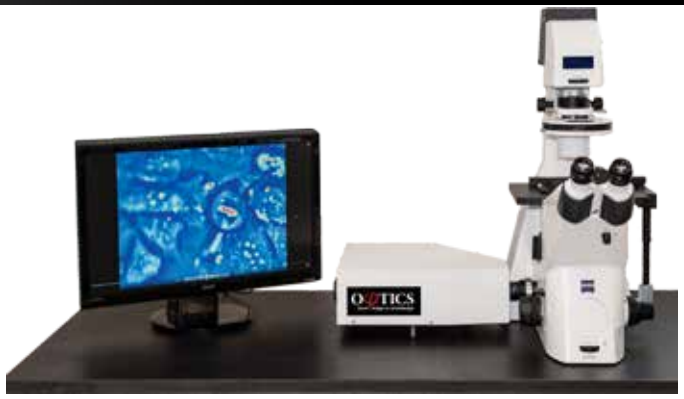

Figure 8. Phi Optics SLIM module attached to a ZEISS Axio Observer Z1 microscope

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.

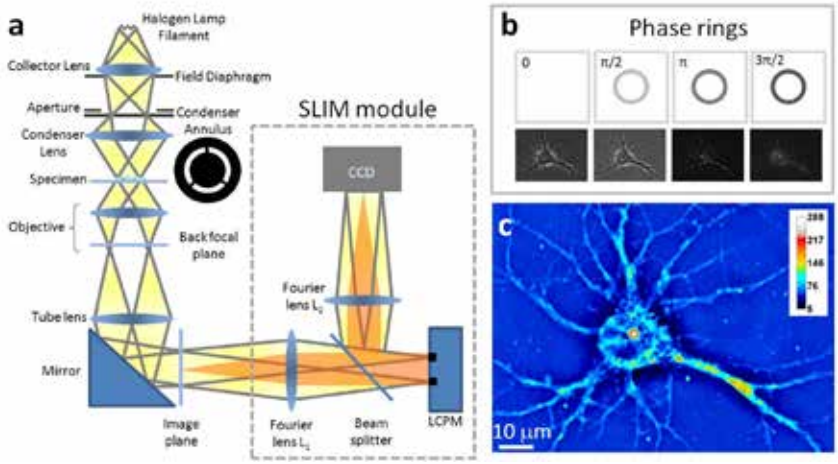

Figure 9. SLIM principle. (a) Schematic setup for SLIM. The SLIM module is attached to a commercial phase contrast microscope (b) The phase rings and their corresponding images recorded by the CCD. (c) SLIM quantitative phase image of a hippocampal neuron.

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.