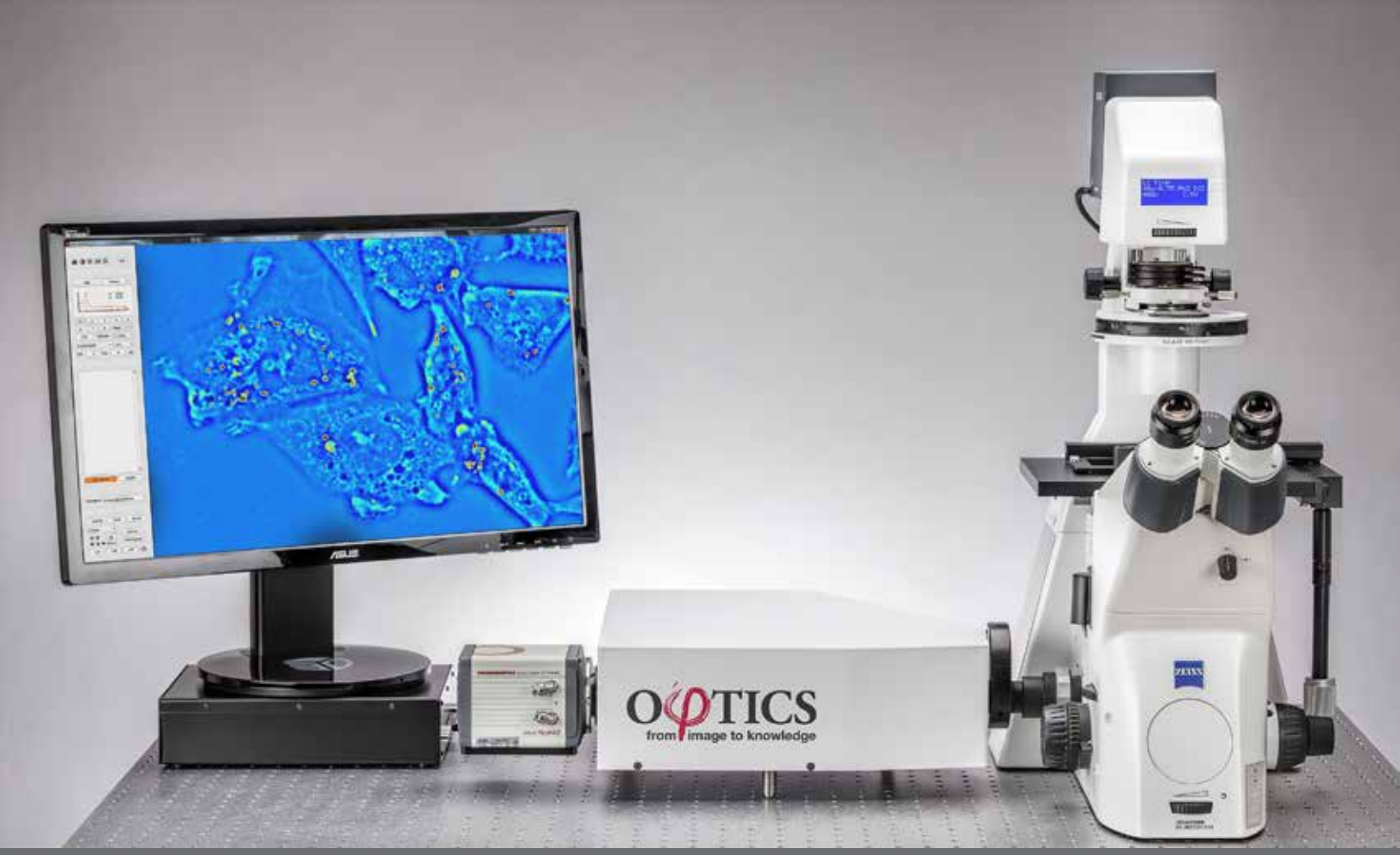

Application Note AN05 Blood Testing

Summary

This Application Note illustrates the use of Spatial Light Interference Microscopy (SLIM) for blood testing. SLIM has a capability to measure the red blood cell (RBC) thickness, which provides reliable means to infer the condition of the RBCs. The parameters available from SLIM analysis of RBCs include: *perimeter, projected area, circular diameter, surface-area, volume, sphericity, eccentricity, minimum height, maximum height, mean height, minimum cylindrical diameter, circularity, integrated density, kurtosis, skewness and variance*. When combined with regular bright-field imaging for absorption, SLIM can also infer the information regarding hemoglobin concentration as well.

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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. 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]. As shown in Figure 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. Moreover, SLIM is capable of automatically scanning a blood smear slide at high resolution, allowing for an automated blood testing. Please see the last page of this note for more detail about SLIM.

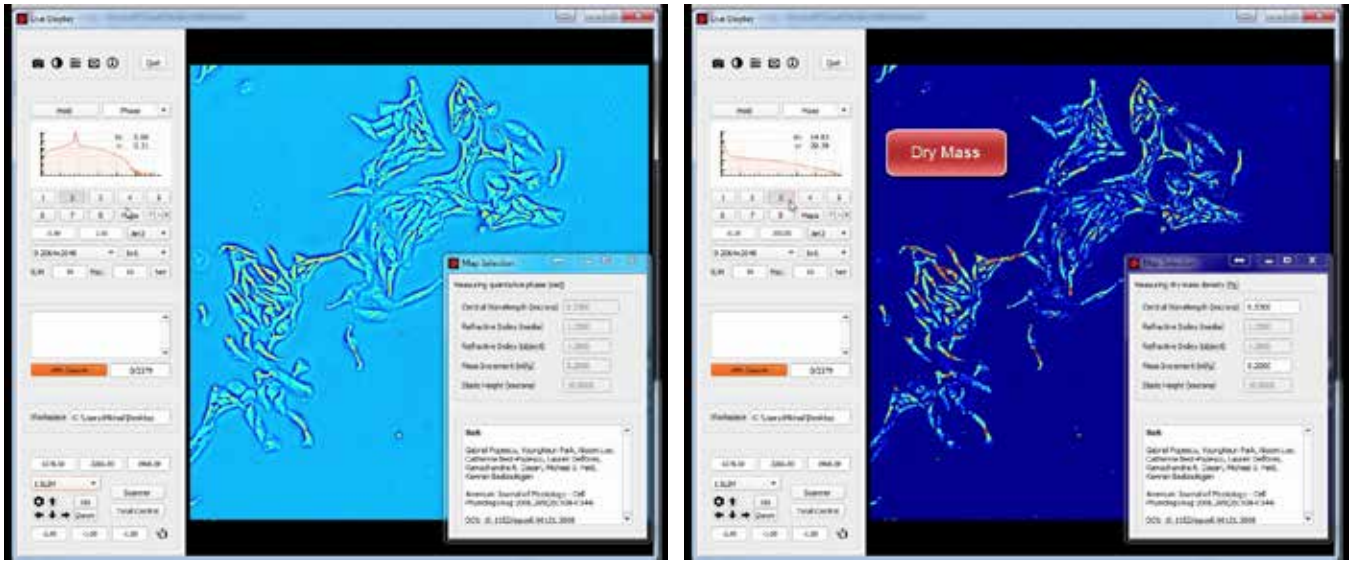

Figure 1. CellVista software working in phase measurement mode (measurement in radians) and in dry mass measurement mode (measurement in picograms). The setting can be changed in the Map Selection menu.

Red blood cell segmentation

Phi Optics SLIM and Cell Vista software together readily provide the height, which yields a large number of parameters for each cell. In order th extract this information easily, Phi Optics provides an ImageJ plugin that segments and selects the RBCs within the field of view. Based on the “Analyze Particles…” function of ImageJ, this plugin generates ROIs for each of the cells. The steps for RBC segmentation using the plugin for the NIH ImageJ is:

1. Load a SLIM map for blood testing
2. Run the “Object Counter” plugin under 2D analysis menu. The plugin will transform the image into a mask with ROIs for the cells.
3. Load the original SLIM map again, and apply the ROIs in the ROI manager to the image.

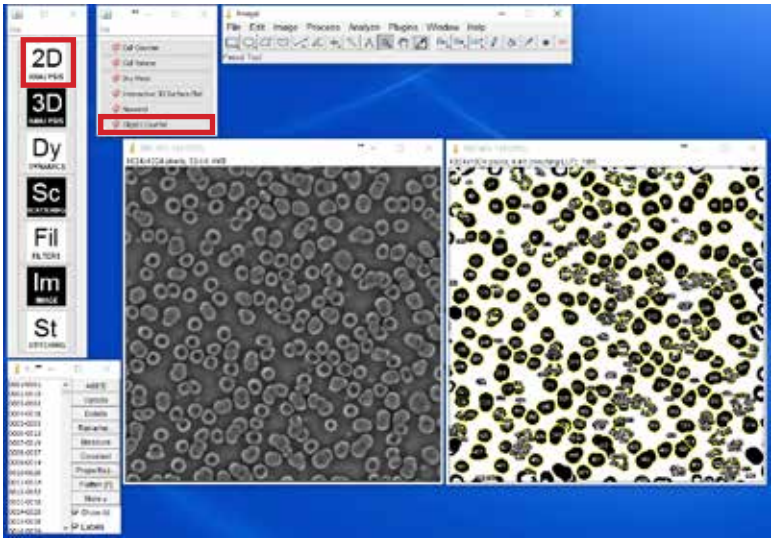

Figure 2. Object counter plugin for RBC segmentation

Blood testing parameters

The quantitative phase images obtained from Phi Optics SLIM can be converted into physiological parameters used for blood testing. There are 17 parameters that can be inferred as listed below. For more information, please refer to M. Mir et al. [2], and M. Mir et al.[3].

perimeter	projected area	circular diameter	surface-area	volume
sphericity	eccentricity	minimum height	maximum height	mean height
minimum cylindrical diameter	circularity	integrated density	kurtosis	skewness
variance	hemoglobin concentration			

Combining with amplitude microscopy

Although Phi Optics SLIM is a powerful tool to infer the physiological parameters of a red blood cell, combining it with an amplitude microscopy modality adds accuracy to the measurements of cell thickness and refractive index (Figure 3). Such measurement can easily be done and perfectly overlaid with Phi Optics SLIM by simply switching the objective to a bright-field objective. From the accurately measured thickness and refractive index, the hemoglobin concentration can be calculated at the single cell level and applied to indicate various disorders (Figure 4) [2].

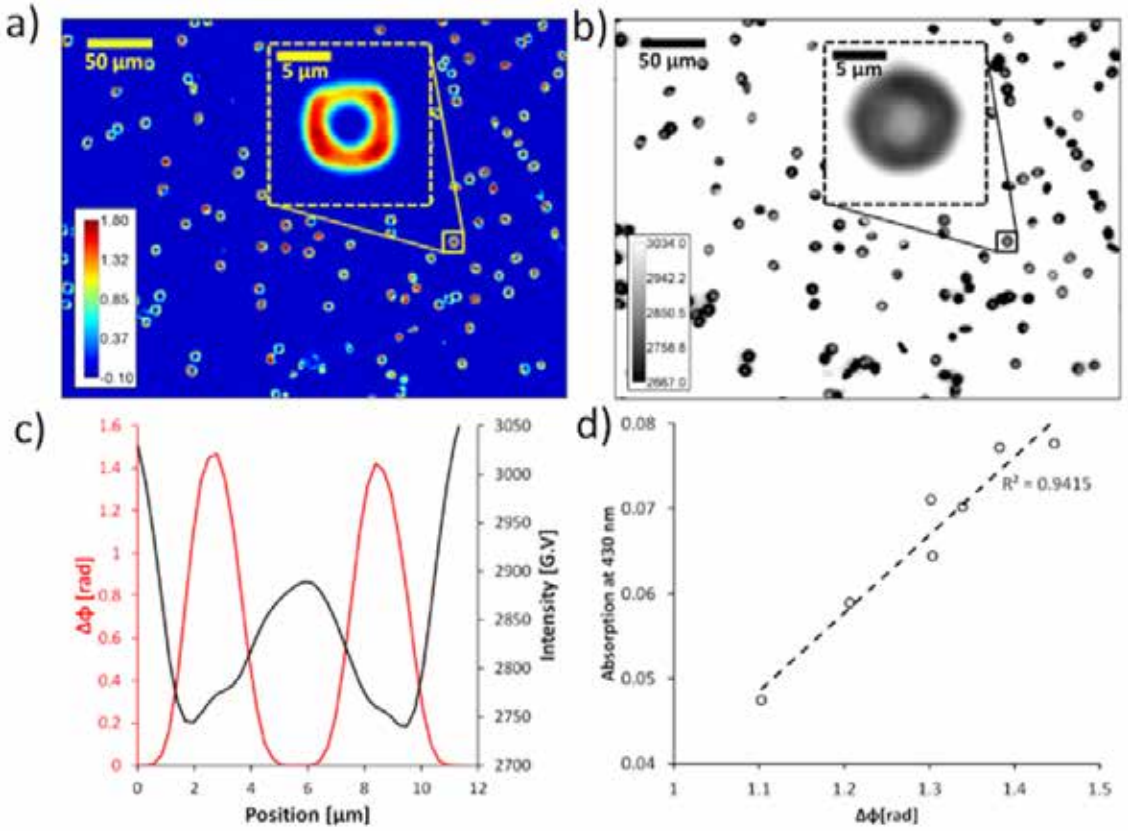

Figure 3. Image analysis (a) Quantitative phase map acquired using SLIM, color bar is in radians. (b) Absorption map acquired at 430 nm, color bar is in 16-bit gray scale values. (a-b) Insets show an example of a single RBC from the maps. (c) Overlay of I line profiles drawn through the center of a single cell, the phase values are shown in red against the left axis and the corresponding intensity from the absorption maps are shown in black against the right axis. (d) Average absorption vs. phase for each of the 7 patients analyzed in this study, a strong linear relationship (dotted line) indicates the feasibility of this method. For the cell shown here the volume and hemoglobin concentration were calculated to be 86.18 fL and 0.3 g/mL respectively [2].

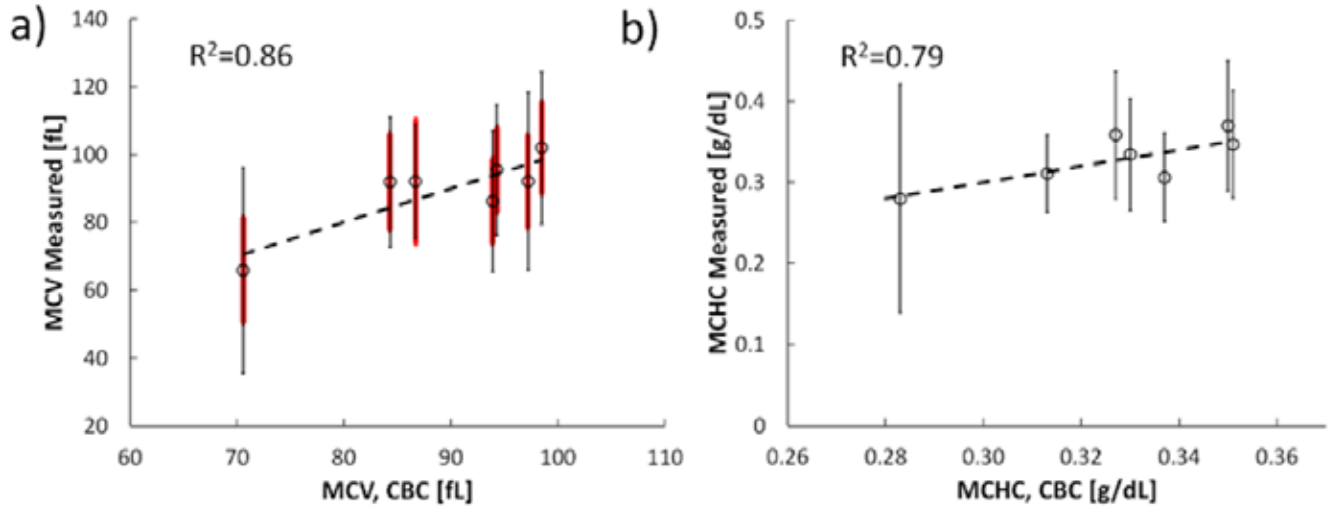

Figure 4. Comparison of measured mean values with clinically reported values. (a) Mean Cell Volume, red error bars correspond to the SD reported by the Clinic and black error bars correspond to SD measured by the QPI and absorption measurements (b) Mean Cell Hemoglobin Concentration, error bars correspond to the measured SD, no SD information on the hemoglobin concentration is available from the Clinic. The dashed black lines have a slope of one [2]

Optical testing of RBC stiffness

Phi Optics SLIM has also been applied to study the biochemical, structural and functional changes in stored RBCs (Figure 5). These small changes can be reliably measured using SLIM because the imaging system provides sub-nanometer sensitivity, thus can detect the membrane fluctuations on an RBC (Figure 6). These fluctuations directly relate to the cell's ability to transport oxygen in microvasculature [4].

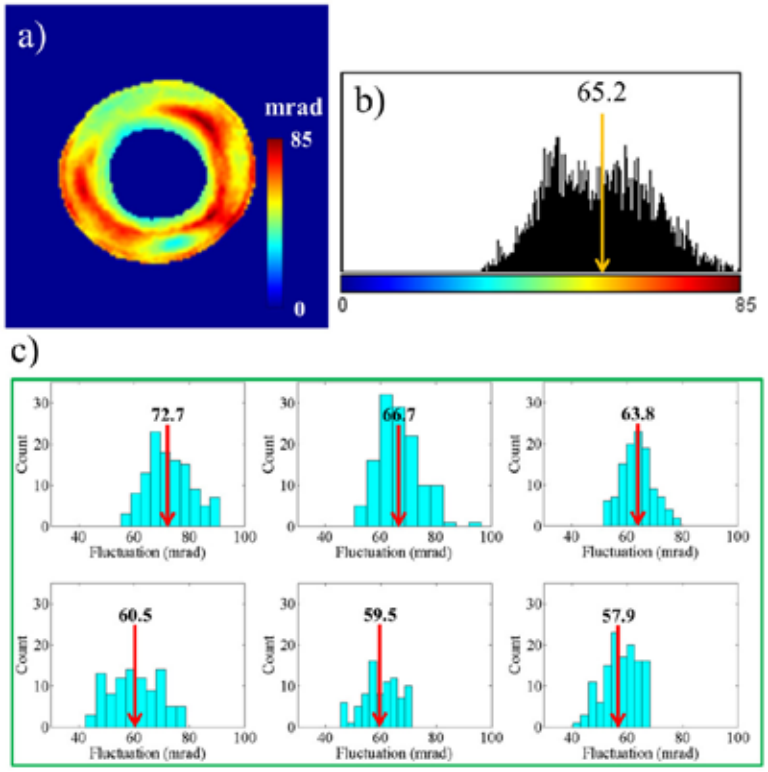

Figure 5. RBC fluctuation: (a) Temporal standard deviation map of a single RBC; colorbar is in mrad, (b) Histogram of the temporal standard deviation map in (a); representative average of the temporal standard deviation map is shown by the arrow, (c) Histogram of the average temporal standard deviation values for 110 ± 15 RBCs at different weeks. The arrows indicate the average of the distribution [4].

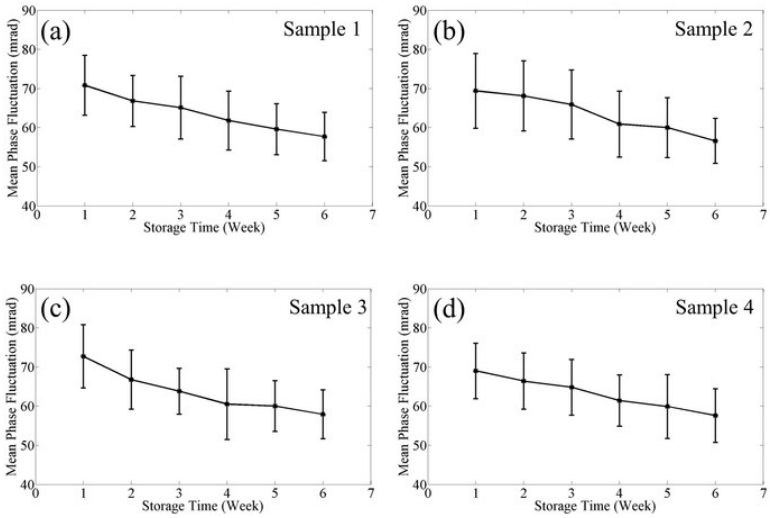

Figure 6. Decrease of the mean phase fluctuation with storage time indicating that the cell deformability decreases gradually with storage time. The error bars in the plots are twice the standard deviation of the phase fluctuation calculated over the groups of cells (N = 110 ± 15) [4].

References

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

[2] M. Mir, K. Tangella and G. Popescu, Blood testing at the single cell level using quantitative phase and amplitude microscopy, Biomedical Optics Express, 2 (12), 3259-3266 (2011)

[3] M. Mir, H. Ding, Z. Wang, J. Reedy, K. Tangella and G. Popescu, Blood screening using diffraction phase cytometry, Journal of Biomedical Optics, 15, 027016 (2010)

[4] B. Bhaduri, M. Kandel, C. Brugnara, K. Tangella and G. Popescu, Optical assay of erythrocyte function in banked blood, Scientific Reports, 4, 6211 (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 7). 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.

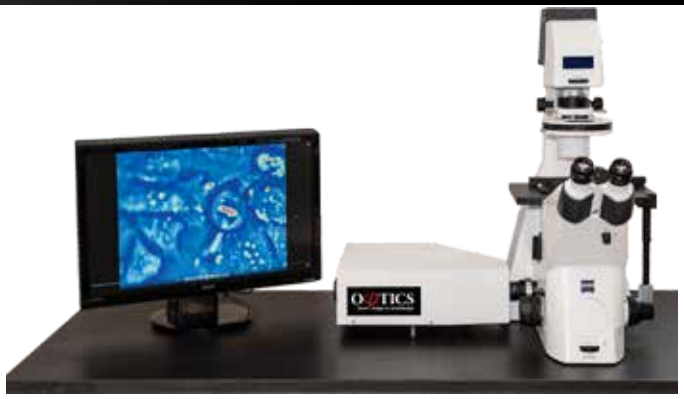

Figure 7. Phi Optics SLIM module attached to a ZEISS Axio Observer Z1 microscope

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.

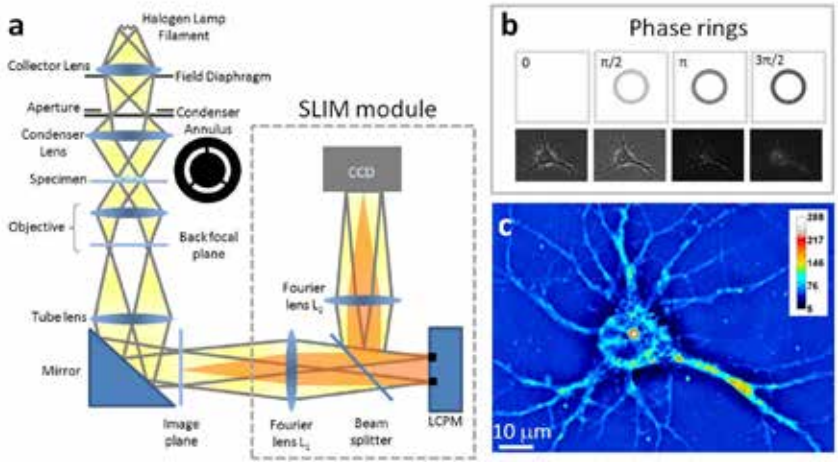

Figure 8. SLIM principle. (a) Schematic setup for SLIM. The SLIM module is attached to a commercial phase contrast microscope (b) The phase rings and their corresponding images recorded by the CCD. (c) SLIM quantitative phase image of a hippocampal neuron.

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 8 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 8b).

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 8b). The CellVista software module combines the four frames by solving the field interference equations in each point of the frame – the result (Figure 8c) 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.