

Apr. 20, 2018

## **3D Imaging of Live Specimens**

### **Quantitative Phase Imaging with SLIM and GLIM**

**The development of innovative tools for spatiotemporal 3D imaging of live, unlabeled biospecimens will significantly advance our understanding of problems ranging from cancer diseases and neuroscience to more accurate IVF procedures.**

An ideal optical imaging method must include a mechanism to reveal the structure of weakly scattering specimens and one to subdue the multiple scattering backgrounds for optically thick samples. Due to their label-free, low-illumination power capabilities, Quantitative Phase Imaging (QPI) methods are: 1) noninvasive and, thus, suitable for stable, long term investigation without photodamage; 2) provide simultaneous information from large fields of view and 3) allow submicron levels of resolution in 3D. Spatial Light Interference Microscopy (SLIM) is an established method for investigating thin, transparent samples, such as single cells. Gradient Light Interference Microscopy (GLIM) is a new QPI technique that provides label-free and quantitative 3D tomographic imaging of tissues, organoids, embryos, model multicellular organisms with high resolution and remarkable contrast. White light illumination provides speckle-free images, which convert to spatially sensitive optical path-length measurements of nanometers. The common path interferometry in GLIM enables temporally sensitive optical path-length measurement down to nanometers. Co-registration with the fluorescence channels of the microscope represents a significant feature which enables control and co-localization experiments based on fluorescence tags.

### **Introduction**

Morphological evaluation has been the method of embryo selection for over 30 years and remains the primary approach of embryo assessment during Assisted Reproductive Technology (ART) cycles, yet its predictive power is low [1]. Many drug discovery pipelines fail in early clinical trials [2] suggesting that more research is needed on the development of predictive biomarkers and preclinical cytotoxicity models. Preclinical research [3-5] suggests that 3D tissue models are needed to reproduce the complexity and heterogeneity of clinical tumors [6]: cell-cell interaction, hypoxia, drug penetration, response and resistance, and

production/deposition of extracellular matrix.

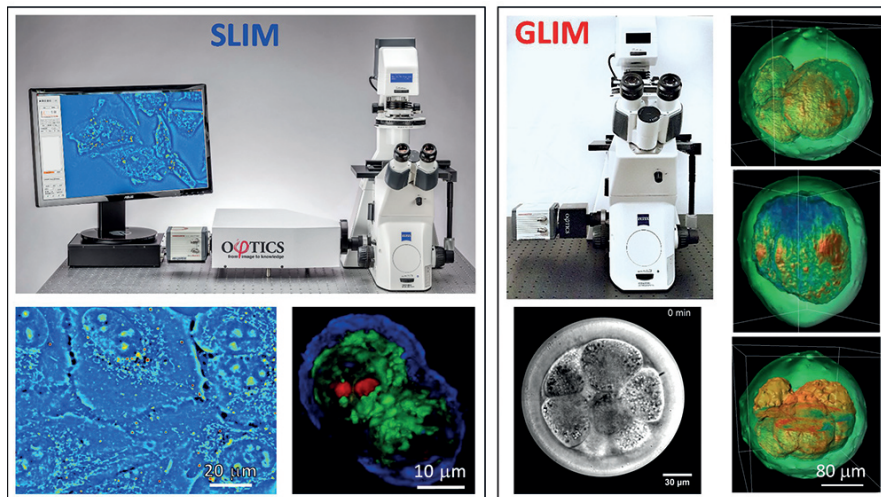


Figure: SLIM is well suited for imaging single cells in both 2D and 3D, over many cell cycles, without photodamage. GLIM extends these capabilities to much thicker specimens, e.g., embryos as shown, by suppressing the strong, multiple scattering background

Tissue-like structures called organoids [7-9] are used to recapitulate the phenotypes, function, and regulation of signaling pathways for cells in in-vivo tumors [10]. Researchers in these fields and those working with acute brain slices and multicellular model organisms need to visualize and quantify (measure) live, optically thick, in vitro 3D structures (tissues, organoids, embryos, model multicellular organisms) on subcellular as well as multicellular scales with high throughput and for long periods of time. Although the light microscope has been the main tool of investigation in biomedicine for four centuries, the current requirements for 3D imaging pose new, difficult challenges that we explore here.

## Current Methods

An important challenge in imaging thick 3D biostructures with any optical method is loss of contrast due to multiple scattering [11]. Light propagation within an optically thick biological specimen (e.g. tissues) depends on the scattering and absorption properties of its components: cells, cell organelles, and various fiber structures. In the ultraviolet (UV) ( $\lambda < 400$  nm) and infrared (IR) ( $\lambda > 2000$  nm) range light is mostly absorbed with a small scattering contribution and it only penetrates through a few cell layers. In the visible and near-IR range scattering is stronger than absorption and light penetrates to a depth of a few millimeters. As thickness increases multiple scattering generates an incoherent background, and the signal to noise ratio (SNR) decreases exponentially. An ideal optical imaging method must include a mechanism to subdue the multiple scattering backgrounds and exhibit strong sectioning to suppress the out-of-focus light.

Most live biospecimens do not significantly absorb light and thus are transparent under an optical microscope. To increase the contrast the specimens are stained with agents such as fluorophores [12] (chemical or genetically encoded) and dyes (including nanoparticles) [13]. The current state of the art solutions [14] for imaging live 3D biostructures are based on the detection of spatial distribution of

signal from fluorescence tags attached to features of interest in the biospecimen. Fluorescence imaging is limited by two processes: photobleaching [15-16] and phototoxicity [17-21]. Photobleaching is the irreversible alteration of the fluorophore molecular structure that can occur when the fluorophore is in an excited state which makes it unable to fluoresce. This limits the time interval over which continuous imaging can be performed. Phototoxicity is the damage caused by light-induced formation of chemically reactive free radicals. This diminishes cell viability and stresses cell physiology, ultimately leading to cell death.

Confocal microscopy (CM) [22] uses a pinhole to reject out-of-focus background fluorescence and allows optical-sectioning for 3D imaging in thicker samples than conventional widefield fluorescence microscopy. CM penetration depth in thick samples is limited to about 100 microns [23]. This is due to scattering in the sample that causes the illuminating beam to defocus and decrease the amount of light that passes through the pinhole with increasing imaging depth. Laser-scanning confocal microscopy (LSCM) provides better axial optical sectioning than spinning disk confocal by point illumination of the sample with a laser, and spatial filtering of the returning beam with a pinhole to block light from outside the focus. LSCM uses very small apertures and rejects a great deal of light thus requiring high-intensity excitation sources and bright fluorescence tags, and fixed specimens. Two-photon [24] and multiple-photon [25] microscopy reduce photobleaching and allow imaging at larger depths into the sample (typically 2-300 microns). Light sheet [26] and lattice light sheet microscopy [27] decouple the illumination and detection optical pathways to provide higher resolution axial imaging with lower phototoxicity [28]. However, these techniques require a specialized microscope system, and specific sample mounting thus preventing the possibility of imaging a large variety of samples to be held in common holders, such as glass slides or petri-dishes where they hold their natural structure.

### **Quantitative Phase Imaging (QPI)**

Quantitative phase imaging (QPI) [29] is a rapidly expanding area of research into imaging methods that provide an alternative to the limitations in fluorescence microscopy. QPI techniques provide contrast by quantifying the changes in the wavefront (phase shift) when light propagates through the sample, and includes: diffraction phase microscopy [30], spatial light interference microscopy (SLIM) [31], digital holographic microscopy [32], quadriwave lateral shearing interferometry [33], ptychography [34], transport-of intensity equation-based techniques [35], optical diffraction tomography [36], orientation-independent DIC [37]. These methods quantify the phase shift introduced by the sample and record it as pixel values in the generated image. Pixel intensity is proportional to the optical

path length (OPL) change i.e. the physical thickness and the refractive index of the specimen and enables direct measurement of the morphology [38] and dry mass [39, 40] of a specimen. Measuring the phase shift across multiple angles of the illumination or axial specimen positions using QPI [41-44] and solving the inverse scattering problems [45, 46] can reveal the 3D structure of inhomogeneous specimens.

Spatial Light Interference Microscopy (SLIM) and Gradient Light Interference Microscopy (GLIM) are two QPI techniques that combine phase imaging (PC and DIC) with low-coherence interferometry and holography in a common-path geometry. This provides high spatial phase sensitivity (on the nanometer order) and high temporal stability. SLIM and GLIM were developed as add-ons for commercial PC and respectively DIC microscope frames in a 4f-optical relay design that provides high-fidelity optical field reconstruction between the object and image planes with near diffraction-limited operation. Both SLIM and GLIM make use of the normal white light illumination of the microscope which avoids the speckles that plague laser illumination-based QPI techniques and improves the optical sectioning due to the low-coherence length of the light source.

The GLIM [47] design rejects much of the multiple scattering contributions and exhibits strong optical sectioning needed for label-free and quantitative imaging of 3D biospecimens. The DIC illumination consists of two identical power cross-polarized beams shifted transversely by the condenser (Wollaston) prism with a distance smaller than the diffraction spot [48]. After passing through the sample and the second Wollaston prism, the resulting interfering beams are sent into the GLIM module without passing through the analyzer. The GLIM module consists of a one-to-one 4f relay system with a spatial light modulator (SLM) in the Fourier plane. The SLM active axis is aligned to the polarization direction of one beam, and retards its phase by multiples of  $\pi/2$  radians (quarter wavelength OPL) while the other field unmodified. By accurately controlling the phase shift between the two beams, we acquire multiple intensity images at the camera plane, which have the same incoherent background, but different coherent contributions. Thus subtracting two pairs of images from each other (the sine and cosine components), we select only the coherent component and subtract out the multiple scattering contribution. The most important aspect of GLIM is that the two imaging beams are always equal in power, although they suffer equal degradation (i.e., the same background noise) due to multiple scattering in the sample, such that they interfere with high contrast no matter how thick the specimen. The GLIM system already has been used by various research groups to visualize 3D time-lapse evolution of optically thick specimens, including zebra fish, *C. elegans*, and embryos.

## Summary

Gradient Light Interference Microscopy is a new QPI method for 3D tomographic imaging of live, optically thick biospecimens and, as such, is complementary to SLIM. GLIM rejects much of the multiple scattering contributions and yield high contrast these specimens. Furthermore, the illumination condenser aperture is fully open, which lands GLIM very strong optical sectioning so GLIM can provide tomographic imaging of optically thick specimens like tissues, organoids, embryos, and model multicellular organisms. The GLIM units are modular and can upgrade any commercial inverted DIC microscopes thus allowing easy overlay and pixel registration with fluorescence channels.

## Authors

Catalin Chiritescu<sup>1</sup> and Gabriel Popescu<sup>2</sup>

## Affiliation

<sup>1</sup>Phi Optics, Champaign, IL, USA

<sup>2</sup>Department of Electrical and Computer Engineering & Bioengineering, University of Illinois at Urbana-Champaign, Beckman Institute for Advanced Science and Technology, Urbana, IL, USA

## Contact

### Prof. Dr. Gabriel Popescu

Department of Electrical and Computer Engineering & Bioengineering  
University of Illinois at Urbana-Champaign  
Beckman Institute for Advanced Science and Technology  
Urbana, IL, USA  
[gpopescu@illinois.edu](mailto:gpopescu@illinois.edu)

## References

- [1] Centers for Disease Control and Prevention; American Society for Reproductive Medicine; Society for Assisted Reproductive Technology. 2015 Assisted Reproductive Technology Fertility Clinic Success Rates Report. (United States Dept. of Health and Human Services, Atlanta, GA, 2017).
- [2] Asher Mullard, Parsing clinical success rates. *Nature Reviews Drug Discovery* 15, 447, doi: 10.1038/nrd.2016.136 (2016).
- [3] Maddaly Ravi, Aarthi Ramesh, and Aishwarya Pattabhi, Contributions of 3D Cell Cultures for Cancer Research. *Journal of Cellular Physiology* 232, 2679-2697, doi:10.1002/jcp.25664 (2017).
- [4] Brendon M. Baker, and Christopher S. Chen, Deconstructing the third dimension - how 3D culture microenvironments alter cellular cues. *Journal of Cell*

Science 125, 3015-3024, doi: 10.1242/jcs.079509 (2012).

[5] Claudio R. Thoma, Miriam Zimmermann, Irina Agarkova, Jens M. Kelm, and Wilhelm Krek, 3D cell culture systems modeling tumor growth determinants in cancer target discovery. *Advanced Drug Delivery Reviews* 69-70, 29-41, doi: 10.1016/j.addr.2014.03.001 (2014).

[6] Lauren C. Kimlin, Giovanna Casagrande, and Victoria M. Virador, In vitro three-dimensional (3D) models in cancer research: An update. *Molecular Carcinogenesis* 52, 167-182, doi: 10.1002/mc.21844 (2013).

[7] E. R. Shamir, and A. J. Ewald, Three-dimensional organotypic culture: experimental models of mammalian biology and disease. *Nat Rev Mol Cell Biol* 15, 647-664, doi: 10.1038/nrm3873 (2014).

[8] H. Clevers, Modeling Development and Disease with Organoids. *Cell* 165, 1586-1597, doi: 10.1016/j.cell.2016.05.082 (2016).

[9] Marina Simian, and Mina J. Bissell, Organoids: A historical perspective of thinking in three dimensions. *The Journal of Cell Biology*, doi: 10.1083/jcb.201610056 (2016).

[10] Michele Zanoni, Filippo Piccinini, Chiara Arienti, Alice Zamagni, Spartaco Santi, Rolando Polico, Alessandro Bevilacqua, and Anna Tesei, 3D tumor spheroid models for in vitro therapeutic screening: a systematic approach to enhance the biological relevance of data obtained. *Scientific reports* 6, 19103, doi:10.1038/srep19103 (2016).

[11] V. V. Tuchin, *Tissue optics: light scattering methods and instruments for medical diagnosis*. Third edition. edn, (SPIE Press, 2015).

[12] E. A. Specht, E. Braselmann, and A. E. Palmer, A Critical and Comparative Review of Fluorescent Tools for Live-Cell Imaging. *Annual review of physiology* 79, 93-117, doi: 10.1146/annurev-physiol-022516-034055 (2017).

[13] Vikram J Pansare, Shahram Hejazi, William J. Faenza, and Robert K. Prud'homme, Review of Long-Wavelength Optical and NIR Imaging Materials: Contrast Agents, Fluorophores, and Multifunctional Nano Carriers. *Chemistry of Materials* 24, 812-827, doi: 10.1021/cm2028367 (2012).

[14] V. Ntziachristos, Going deeper than microscopy: the optical imaging frontier in biology. *Nat Methods* 7, 603-614, doi: 10.1038/nmeth.1483 (2010).

[15] R. A. Hoebe, C. H. Van Oven, T. W. Gadella Jr., P. B. Dhonukshe, C. J. Van Noorden, and E. M. Manders, Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging. *Nat Biotechnol* 25, 249-253, doi: 10.1038/nbt1278 (2007).

[16] J., Lippincott-Schwartz, N. Altan-Bonnet, and G. H. Patterson, Photobleaching and photoactivation: following protein dynamics in living cells. *Nat Cell Biol Suppl*, S7-14 (2003).

[17] P. P. Laissue, R. A. Alghamdi, P. Tomancak, E. G. Reynaud, and H. Shroff,

Assessing phototoxicity in live fluorescence imaging. *Nat Methods* 14, 657-661, doi: 10.1038/nmeth.4344 (2017).

[18] H., Schneckenburger, P. Weber, M. Wagner, S. Schickinger, V. Richter, T. Bruns, W. S. Strauss, and R. Wittig, Light exposure and cell viability in fluorescence microscopy. *J Microsc* 245, 311-318, doi: 10.1111/j.1365-2818.2011.03576.x (2012).

[19] R. Strack, Death by super-resolution imaging. *Nat Methods* 12, 1111 (2015).

[20] S. Waldchen, J. Lehmann, T. Klein, S. van de Linde, and M. Sauer, Light-induced cell damage in live-cell super-resolution microscopy. *Scientific reports* 5, 15348, doi: 10.1038/srep15348 (2015).

[21] S. Douthwright, and G. Sluder, Live Cell Imaging: Assessing the Phototoxicity of 488 and 546 nm Light and Methods to Alleviate it. *J Cell Physiol* 232, 2461-2468, doi: 10.1002/jcp.25588 (2017).

[22] J. Pawley, *Handbook of Biological Confocal Microscopy*. 3 edn, (Springer US, 2006).

[23] J. M. Schmitt, A. Knuttel, and M. Yadlowsky, Confocal microscopy in turbid media. *Journal of the Optical Society of America. A, Optics, image science, and vision* 11, 2226-2235 (1994).

[24] W., Denk, J. H. Strickler, and W. W. Webb, Two-photon laser scanning fluorescence microscopy. *Science* 248, 73-76 (1990).

[25] W. R. Zipfel, R. M. Williams, and W. W. Webb, Nonlinear magic: multiphoton microscopy in the biosciences. *Nat Biotechnol* 21, 1369-1377, doi: 10.1038/nbt899 (2003).

[26] Rory M. Power, and Jan. Huisken, A guide to light-sheet fluorescence microscopy for multiscale imaging. *Nature Methods* 14, 360, doi: 10.1038/nmeth.4224 (2017).

[27] T. A. Planchon, L. Gao, D. E. Milkie, M. W. Davidson, J. A. Galbraith, C. G. Galbraith, and E. Betzig, Rapid three-dimensional isotropic imaging of living cells using Bessel beam plane illumination. *Nat Methods* 8, 417-423, doi: 10.1038/nmeth.1586 (2011).

[28] Jordi Andilla, Raphael Jorand, Omar E. Olarte, Alexandre C. Dufour, Martine Cazales, Yoann L. E. Montagner, Romain Ceolato, Nicolas Riviere, Jean-Christophe Olivo-Marin, Pablo Loza-Alvarez, and Corinne Lorenzo, Imaging tissue-mimic with light sheet microscopy: A comparative guideline. *Scientific reports* 7, 44939, doi: 10.1038/srep44939 (2017).

[29] Gabriel Popescu, *Quantitative phase imaging of cells and tissues*. (McGraw-Hill, 2011).

[30] G. Popescu, T. Ikeda, R. R. Dasari, and M. S. Feld, Diffraction phase microscopy for quantifying cell structure and dynamics. *Opt Lett* 31, 775-777 (2006).

[31] Z. Wang, L. Millet, M. Mir, H. Ding, S. Unarunotai, J. Rogers, M. U. Gillette,

- and G. Popescu, Spatial light interference microscopy (SLIM). *Opt Express* 19, 1016-1026, doi: 10.1364/OE.19.001016 (2011).
- [32] E. Cuche, F. Bevilacqua, and C. Depeursinge, Digital holography for quantitative phase-contrast imaging. *Opt Lett* 24, 291-293 (1999).
- [33] P. Bon, G., Maucort B. Wattellier, and S. Monneret, Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells. *Opt Express* 17, 13080-13094 (2009).
- [34] J. Marrison, L. Raty, P. Marriott, and P. O'Toole, Ptychography--a label free, high-contrast imaging technique for live cells using quantitative phase information. *Sci Rep* 3, 2369, doi: 10.1038/srep02369 (2013).
- [35] A. Barty, K. A. Nugent, D. Paganin, and A. Roberts, Quantitative optical phase microscopy. *Opt Lett* 23, 817-819 (1998).
- [36] Y. Sung, W. Choi, C. Fang-Yen, K. Badizadegan, R. R. Dasari, and M. S. Feld, Optical diffraction tomography for high resolution live cell imaging. *Opt Express* 17, 266-277 (2009).
- [37] M. Shribak, and S. Inoue, Orientation-independent differential interference contrast microscopy. *Appl Opt* 45, 460-469 (2006).
- [38] Michael A. Model, Methods for cell volume measurement. *Cytometry A*, doi:10.1002/cyto.a.23152 (2017).
- [39] H. G. Davies, and M. H. Wilkins, Interference microscopy and mass determination. *Nature* 169, 541 (1952).
- [40] R. Barer, Interference microscopy and mass determination. *Nature* 169, 366-367 (1952).
- [41] W. Choi, C. Fang-Yen, K. Badizadegan, S. Oh, N. Lue, R. R. Dasari, and M. S. Feld, Tomographic phase microscopy. *Nat Methods* 4, 717-719, doi: 10.1038/nmeth1078 (2007).
- [42] Taewoo Kim, Renjie Zhou, Mustafa Mir, S. Derin Babacan, P. Scott Carney, Lynford L. Goddard, and Gabriel Popescu, White-light diffraction tomography of unlabelled live cells. *Nature Photonics* 8, 256-263, doi: 10.1038/nphoton.2013.350 (2014).
- [43] Yann Cotte, Fatih Toy, Pascal Jourdain, Nicolas Pavillon, Daniel Boss, Pierre Magistretti, Pierre Marquet, and Christian Depeursinge, Marker-free phase nanoscopy. *Nature Photonics* 7, 113, doi: 10.1038/nphoton.2012.329 (2013).
- [44] T. S. Ralston, D. L. Marks, P. S. Carney, and S. A. Boppart, Interferometric synthetic aperture microscopy. *Nat Phys* 3, 129-134, doi: 10.1038/nphys514 (2007).
- [45] Taewoo Kim, Renjie Zhou, Lynford L. Goddard, and Gabriel Popescu, Solving inverse scattering problems in biological samples by quantitative phase imaging. *Laser & Photonics Reviews* 10, 13-39, doi: 10.1002/lpor.201400467 (2015).
- [46] H. Ding, Z. Wang, F. Nguyen, S. A. Boppart, and G. Popescu, Fourier transform light scattering of inhomogeneous and dynamic structures. *Phys Rev Lett* 101,



238102, doi: 10.1103/PhysRevLett.101.238102 (2008).

[47] T. H. Nguyen, M. E. Kandel, M. Rubessa, M. B. Wheeler, and G. Popescu, Gradient light interference microscopy for 3D imaging of unlabeled specimens. *Nat Commun* 8, 210, doi: 10.1038/s41467-017-00190-7 (2017).

[48] Douglas B. Murphy, and Michael W. Davidson, 1 online resource (Wiley-Blackwell, Hoboken, N.J., 2013).