The International Journal of Biochemistry & Cell Biology 83 (2017) 65–70

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Imaging in focus

# Imaging In focus: Reflected light imaging: Techniques and applications

Emily J. Guggenheim[a,b], Iseult Lynch[b], Joshua Z. Rappoport[c,*]

[a] Physical Science of Imaging in the Biomedical Sciences (PSIBS) Doctoral Training Centre (DTC), Birmingham, Edgbaston, UK
[b] School of Geography, Earth and Environmental Sciences, Birmingham, Edgbaston, UK
[c] Centre for Advanced Microscopy and Nikon Imaging Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

ARTICLE INFO

*Article history:*
Received 3 October 2016
Received in revised form
12 December 2016
Accepted 14 December 2016
Available online 21 December 2016

*Keywords:*
Reflectance
Microscopy
Label
Free
Nanoparticles

ABSTRACT

Reflectance imaging is a broad term that describes the formation of images by the detection of illumination light that is back-scattered from reflective features within a sample. Reflectance imaging can be performed in a variety of different configurations, such as confocal, oblique angle illumination, structured illumination, interferometry and total internal reflectance, permitting a plethora of biomedical applications. Reflectance imaging has proven indispensable for critical investigations into the safety and understanding of biomedically and environmentally relevant nano-materials, an area of high priority and investment. The non-destructive *in vivo* imaging ability of reflectance techniques permits alternative diagnostic strategies that may eventually facilitate the eradication of some invasive biopsy procedures. Reflectance can also provide additional structural information and clarity necessary in fluorescent based *in vivo* studies. Near-coverslip interrogation techniques, such as reflectance interferometry and total internal reflection, have provided a label free means to investigate cell-surface contacts, cell motility and vesicle trafficking *in vivo* and *in vitro*. Other key advances include the ability to acquire superresolution reflectance images providing increased spatial resolution.



## Key Facts

1. Reflectance microscopy can be used for the interrogation of unlabelled samples, or samples with added reflective labels.
2. Contrast in a reflectance image arises due to differing refractive index (RI) within samples.
3. Reflection interferometry and evanescent wave illumination allow the selective visualisation of a narrow region adjacent to a transparent substrate (*e.g.* coverslip), albeit by different mechanisms, providing excellent contrast of structures at or close to the cell surface.
4. Reflectance Confocal Microscopy (RCM) and Optical Coherence Tomography (OCT) can provide sub-cellular information in X-Y and Z planes facilitating non-destructive, non-invasive diagnosis of human skin pathologies and identification of tumour margins.
5. RCM can provide additional structural information that compliments established fluorescence *in vivo* studies.
6. RCM and oblique angle illumination (darkfield) are beneficial for label-free research into cell-surface contact adhesions and nanoparticle (NP)-cell interactions, negating the need for labelling NPs with fluorophores and facilitating correlative fluorescence and reflectance cell studies (*e.g.*, co-localisation).
7. Superresolution reflectance microscopy (SIM) has been applied for increased accuracy in NP investigations, allowing distinction of closely spaced NP clusters.

## 1. Introduction

Transmission microscopy employs illumination through a sample projecting an image based upon inherent contrast that is determined by differing refractive indices (RI). Fluorescence imaging involves the excitation of fluorophores with specific wavelengths of light and the collection of the subsequent emitted light. Fluorophores are often attached to a particular subcellular protein of interest. However fluorophores are difficult to attach, may alter the biological structures and/or processes under investigation and

*Abbreviations:* CLSM, Confocal Laser Scanning Microscopy; DF, Darkfield; FCM, Fluorescence Confocal Microscopy; ICPMS, Inductively Coupled Plasma Mass Spectrometry; IRM, Interference Reflectance Microscopy; NIR, Near-Infrared; NP, Nanoparticles; OCT, Optical Coherence Tomography; RCM, Reflectance Confocal Microscopy; RI, Refractive index; SNR, Signal to Noise Ratio; SIM, Structured Illumination Microscopy; TIRM, Total Internal Reflectance Microscopy.

* Corresponding author.
*E-mail address:* Joshua.rappoport@northwestern.edu (J.Z. Rappoport).

http://dx.doi.org/10.1016/j.biocel.2016.12.008
1357-2725/

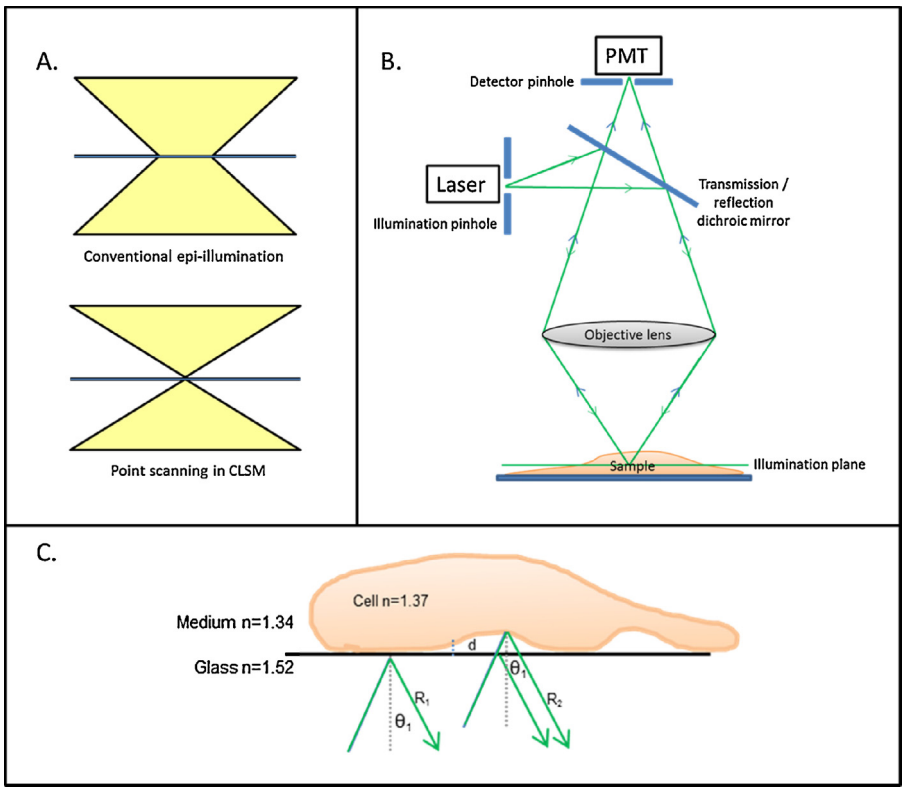


**Fig. 1.** Schematic of the sample illumination and collection of reflected light using RCM (A and B) and IRM (C). A) Difference in the illumination light in CLSM compared to widefield epi-illumination; in confocal a focused laser point source is scanned across a specimen. B) Depiction of the conjugate pinhole illumination configuration of RCM. Configuration is similar to FLM, except that the dichroic is a transmission/reflection dichroic that allows passage of reflected light back to the detector. C) Depiction of the reflected rays generated from incident light hitting a biological sample. $R_1$ is generated at the glass medium interface. $R_2$ is generated at the medium-cell interface. The length of d (path distance) dictates the phase in which the light hits the detector, thus influencing the intensity of the image due to constructive (d = 100 nm or more) or destructive (d approaching 0) interference. Regions in close contact of the coverslip therefore appear dark and intensity increases with distance from the coverslip.

are subject to photobleaching (Sugden, 2004; Quinn et al., 2015). Reflectance imaging does not rely on the emission of light from an excitable moiety. Rather, reflectance imaging, like transmission microscopy, exploits the inherent RIs present within samples, providing label free contrast for interrogation of cell or tissue architecture. Reflective features are therefore not affected by photobleaching. Reflective probes that greatly enhance the scattering of light, such as nanoparticles (NPs) can be employed, akin to the use of fluorescent probes for labelling in fluorescence imaging or the labelled antibodies employed in immuno-gold transmission electron microscopy. The reflected signal can be generated and detected in a variety of modalities, including standard epi-illumination, or using contrast enhancement measures such as a confocal pinhole configuration, oblique angle illumination or interferometry (Huang et al., 1991; Gibbs-Flournoy et al., 2011; Sokolov et al., 2004). Other methods focus on selective illumination of a thin region close to the coverslip in order to provide high contrast information regarding interactions at the membrane, or use structured illumination to double the spatial resolution (Chang et al., 2011; Temple, 1981). Reflectance can also be performed in combination with other established techniques, maximising the information available in *in vivo* investigations (Allegra Mascaro et al., 2015). This review highlights the applications and advancements of reflectance imaging, giving an overview of the state-of-the-art capabilities of these under-utilized scattered light based imaging techniques.

## 2. Reflectance confocal microscopy: investigative research

Confocal microscopy utilises a conjugate pinhole system to block out of focus light, allowing collection of light from a plane of interest (Fig. 1A and B). This allows contrast and resolution enhancement (compared to epi-illumination) in biological imaging of cells or subcellular structures. Conventional confocal fluorescence microscopy can provide a maximum X-Y resolution of 250 nm; RCM is reported as higher, at 200 nm (Cox and Sheppard, 2004). Due to the removal of out-of-plane signal by the pinhole system, confocal microscopy also allows optical sectioning through a sample which is advantageous for 3D imaging. Operating in reflectance mode, rather than fluorescence, requires only addition of a transmission/reflectance dichroic to facilitate collection of reflected incident light. This makes RCM an accessible technique that can provide information regarding a sample either alone or in combination with fluorescent imaging. No additional labelling is necessary providing sufficient contrast is generated from the endogenous RI mismatches within biological samples. Lipid droplets in the developing drosophila primordia provide a good example of inherent contrast with RCM (Gáspár and Szabad, 2009).

Nanoparticles (NPs), such as those made of metals or metal oxides, can be used to label cells or subcellular components of interest from a variety of tissues for imaging with RCM (Sokolov et al., 2004). Alternatively, the NPs can be the subject of investigation. The increasing incorporation of man-made NPs into commercial and biomedical products has fuelled investigations into NP-cell interactions to improve their efficacy and to evaluate risks associated with potential exposure. RCM can be employed in conjunction with fluorescent labelling to assess the internalisation and trafficking fate of metal oxide NPs (Sienna$^+$ SPIONs) into the degradative lysosomal compartment (Fig. 2) (Guggenheim et al., 2016). Quantitative information can be obtained by using automated processing

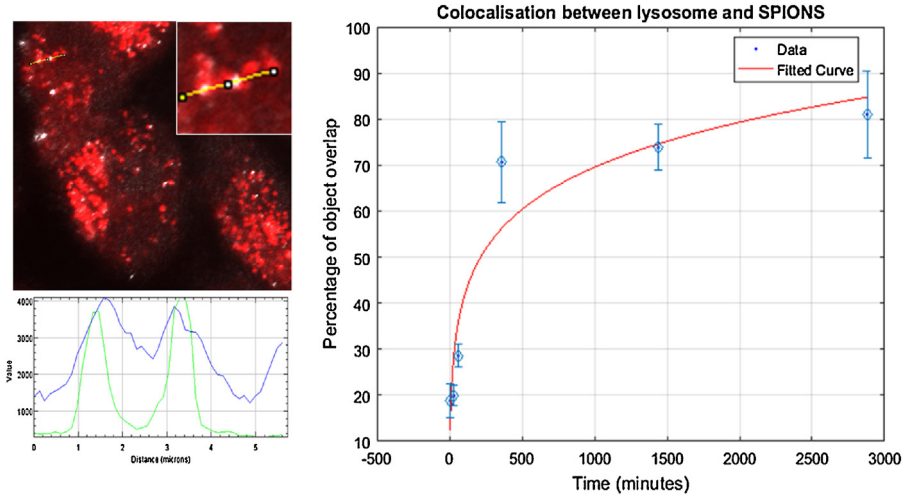


**Fig. 2.** Confocal fluorescence and reflectance microscopy applied to the assessment of colocalisation between the lysosome and SPIONs. A high degree of colocalisation can be visualised between longer incubation time points (24 h and 48 h especially). This can be seen from the overlay (top left; SPIONs = grey, Lysosome = red) and the line intensity profile (lysosome = blue, SPION = green). These images can be computationally post processed and different parameters assessed (such as the degree of signal overlap) and used to make quantitative conclusions about particle trafficking and fate. Plotted points show the mean result for each time point, error bars show the standard error of the mean (SEM).

and analysis workflows to calculate the extent of signal overlap between channels improving the analysis time and reproducibility of experimental conclusions. RCM can also be used in conjunction with other techniques to provide absolute quantitative information at a single cell level, including spectroscopic methods such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Kim et al., 2015). Determining the exact relationship between reflectance and elemental NP quantification would facilitate direct quantification from image data. This quantification is essential for the translation of *in vitro* experimental results into increases in efficacy within a clinical setting. There is also significant concern regarding the impact of engineered NPs on the environment and ecosystems as the short and long term toxicity of NPs to complex organisms is largely unknown. RCM is ideal for assessing such toxicity associated with exposure in whole organisms, such as the model aquatic organism *Daphnia Magna*, as it is non-destructive and capable of live 3D-imaging (Stensberg et al., 2014).

Two-photon fluorescence, using near-infrared (NIR), offers superior tissue penetration, lower phototoxicity and supressed background when compared to confocal imaging, and therefore is often employed for *in vivo* studies (Allegra Mascaro et al., 2015). Collection of the backscattered laser light from *in vivo* investigations allows visualisation of haemodynamic events and provides structural information to contextualise fluorescent signal (Allegra Mascaro et al., 2015; González et al., 2001). This emerging combination of two-photon and RCM imaging can be employed to visualise blood flow and anatomical tissue structure in regions such as the brain cortex (González et al., 2001). Axonal myelin gives rise to contrast in the reflectance image, providing a means of monitoring axonal demyelination, a critical event in a host of neurodegenerative pathologies (Allegra Mascaro et al., 2015). A novel methodology, termed *in vivo* microcartography, also employs this combination method to visualise the architecture and haemodynamics of *in vivo* tumour angiogenesis (Dunphy et al., 2009).

### *Reflectance confocal microscopy: diagnostics*

RCM offers significant advantages for *in vivo* clinical applications such as real-time 3D imaging of the microanatomy of tissues including the eye, oral mucosa, and skin. RCM has proved particularly beneficial for *in vivo* examination and diagnosis of human skin pathologies, which previously relied heavily on surgical biopsy leaving physical scarring (Rajadhyaksha et al., 1999). RCM allows the acquisition of optical sections as deep as 350 μm into the skin, providing roughly equivalent information (X–Z resolutions) as conventional histological staining examinations and facilitating diagnosis of malignant tumours (Rajadhyaksha et al., 1999). RCM can also be modified for use within internal endoscopic investigations. Fibre-RCM (FRCM) systems utilise coherent fibre bundles to allow imaging of internal structures and can be applied to the identification of tissue structure and neoplastic regions *ex vivo* and *in vivo* (Ando et al., 2016; Maitland et al., 2008). Drawbacks of *in vivo* RCM systems include the large bulky optics, a limited field and the limited penetration depth.

### *Oblique angle illumination/darkfield microscopy*

Darkfield (DF) microscopy utilises oblique angle epi-illumination to enhance reflectance image contrast by selectively capturing only the light scattered by the specimen. Oblique angle illumination is achieved using a specialised condenser which contains a light block comprised of an annulus with a narrow aperture. This prevents unscattered illumination light from entering the objective in the absence of a sample. If a sample is present a portion of the illumination the light will be scattered back into the objective. This introduces contrast against a dark background leading to the characteristically high SNR DF image with reduced potential for artefact. However, sample preparation must be rigorous as dust on the coverslip can lead to unwanted reflections and high intensity illumination light is often necessary, due to the low intensity signal, which may cause sample damage. DF is mainly used for the interrogation of semi-opaque samples that are unable to be imaged *via* transmitted light microscopy. DF is well-suited for the visualisation of reflective NPs, and has been combined with Confocal Laser Scanning Microscopy (DF-CLSM) for the detection and characterisation of NP uptake in cultured cells (Gibbs-Flournoy et al., 2011; Loo et al., 2005). DF can be coupled with a specialised condenser that improves the SNR up to 7 fold compared to conventional DF which is advantageous for the increased detection and localisation of metallic NP (Guttenberg et al., 2016).

**Table 1**
Table comparing the advantages, disadvantages, and potential applications of the reflectance modalities.

| | X-Y resolution | Z resolution | Penetration Depth | Advantages | Disadvantages | Applications |
|---|---|---|---|---|---|---|
| R-SIM | ~115 nm | ~685 nm (measured) | ~100 μm (fluorescence) | Increased lateral resolution, can be coupled with fluorescence acquisition, optical sectioning | Suffers high levels of background, acquisitions of >1s | *In vitro* live 3D studies (*e.g.* NP), Colocalisation studies, potential for high resolution tissue imaging |
| RCM | ~250 nm | ~500 nm | 350 μm | Accessible, optical sectioning, can be coupled with fluorescence acquisition | Limited depth penetration in tissues, limited by diffraction | *In vitro* live 3D studies/*In vivo* dynamic studies (*e.g.* NP), *In vivo* diagnostics (dermatology, endoscopy, ophthalmology, blood flow), complimentary structural information, (semi)-transparent substrate |
| TIRM | 250 nm | 100 nm | ~200 nm | High SNR, sub diffraction limit in Z | Small penetration depth | Endocytosis studies, (single)-particle tracking, cell dynamics and trafficking |
| IRM | 250 nm | NA[a] | 100 nm | Quantification of separation distances | Limited by diffraction, No optical sectioning unless coupled to CLSM, | Imaging surfaces (cell surface) and cell contact dynamics, quantifying separation distances, complimentary structural information |
| Darkfield | ~250 nm | NA[a] | NA | High SNR, High detection capabilities | No optical sectioning unless coupled to CLSM, limited by diffraction, sensitive to dust/dirt on coverslip | Opaque substrate, NP studies, imaging surfaces |
| OCT | ~ 5 μm | >1 μm | >1 mm | Deep tissue penetration | Limited by diffraction, limited X-Y resolution | *In vivo* diagnostics (dermatology, endoscopy, ophthalmology, blood flow), deep tissue imaging |

[a] Can be combined with CLSM to obtain optical sections and Z-resolution akin to that available for confocal.

*Optical coherence tomography*

Optical coherence tomography (OCT) is a non-invasive, non-destructive, reflectance technique that uses interferometry to produce 2D cross sections of light reflected from internal tissue microstructure. In OCT, low coherence light, usually infrared, is directed toward the specimen of interest and reflected at boundaries of differing RI. The incident beam is first split and then simultaneously directed toward the sample and a reference mirror at known path length. Reflections that travel further into the tissue take longer to reach the detector, this time is known as the 'echo delay time'. The intensity and echo delay time of the reflected light from both the sample and the reference mirror is detected using low coherence interferometry. This information is then used to determine the location of reflective features with high accuracy and resolution (Huang et al., 1991). Like RCM, OCT was originally employed for the imaging of transparent tissue, such as the eye, giving high sensitivity and good spatial resolution. However the diagnostic potential of OCT has now been demonstrated for opaque structures (*i.e.* the arterial wall for coronary plaque investigations) (Huang et al., 1991; Izatt et al., 1994). OCT has optical sectioning capabilities akin to that of RCM, and therefore can allow *in vivo* tomographic imaging and 3D reconstruction of tissue regions with 1.11 μm axial resolution (Huang et al., 1991; Yadav et al., 2011). OCT has a greater penetration depth than RCM making it particularly useful for deep tissue imaging (Huang et al., 1991). OCT has recently been combined with RCM to maximise the benefits of both optical techniques into a single integrated imaging system to delineate tumour margins with increased accuracy in X–Z (Iftimia et al., 2016).

*Interference reflectance microscopy*

Reflectance imaging was first applied to the interrogation of cellular structures closely opposed to a glass surface, and was termed 'Interference reflectance microscopy' (IRM) (Curtis, 1964). In IRM the interference between light reflected from different interfaces is imaged. The intensity within the IRM image increases with separation distances between the cell and the coverslip up to a depth of 100 nm (Fig. 1). This allows quantitation of the separation distance of cell contacts and the coverslip directly from the pixel intensities (Verschueren, 1985). Therefore, IRM can identify areas of attachment sites such as focal adhesions, which will appear dark in the IRM image due to close contact to the coverslip (Verschueren, 1985). Curtis first applied this to imaging contacts between chick heart fibroblasts and a glass substrate, and IRM has since been applied extensively to imaging contacts between cells and coverslips (Curtis, 1964; Yin et al., 2003; Matsuzaki et al., 2016). These investigations have provided information relevant to the understanding of many different cellular processes including the maintenance of the cell cytoskeleton, cell motility (which is important in wound repair, immune response, tumour formation and metastasis) and multicellular structure and function in organs such as the liver and the vascular network. Different methods have been employed to try and reduce the stray reflections that arise due to other cellular constituents in IRM, including the use of an antiflex immersion objective that circularly polarises light and as well as an annular to block 90% of the illumination light creating an oblique incident angle (Bereiter-Hahn et al., 1979). These methods allow selective collection of light reflected from the sample reducing stray reflections. Alternatively, some recently developed IRM techniques specifically rely on the collection of all the available light including background scatter. An optimised interferometric microscope setup, called iSCAT, utilises this configuration to facilitate single molecule detection and tracking of nanoscopic lipid domains (De et al., 2015; Ortega-Arroyo et al., 2012).

*Total internal reflection microscopy*

Light that is incident to a sample at a boundary of two differing RI's at greater than the critical angle defined by Snell's Law will be totally internally reflected producing an evanescent wave/field, which exponentially decays in the Z-direction away from the boundary leading to the selective illumination of a thin

(~100 nm) region of a sample immediately adjacent to the interface. Total Internal Reflection Microscopy (TIRM), like evanescent wave illumination in fluorescence (TIRF) utilises this mode of illumination, however in TIRM it is the reflected light, not fluorescence (as with TIRF) that is detected. TIRM has been applied to the imaging of membrane associated proteins and endocytosis/exocytosis events, providing high contrast and resolution. TIRM can be applied in conjunction with TIRF to enable the live simultaneous visualisation of unlabelled NPs, the plasma membrane, and fluorescently labelled proteins in near proximity to the adherent cell surface (Byrne et al., 2015). TIRM has also been applied to the determination of NP distance from a surface giving NP size and depth information and for the quantification of separation distances of NP (Temple, 1981; Prieve et al., 1987).

*R-SIM*

All the aforementioned techniques lack the superresolution capabilities afforded by some fluorescence techniques that break the diffraction limit. There are a limited number of examples of superresolution reflectance methodologies, all involving the use of structured illumination microscopy (SIM). Chang et al. used a custom built SIM microscope to double the conventional lateral and axial resolution of scattered light images of intracellular gold AuNPs (Chang et al., 2011). More recently, a commercial Nikon N-SIM was used in a combined reflectance and fluorescence study to investigate subcellular trafficking of NPs (Guggenheim et al., 2016). The ability to resolve structures separated by less than the diffraction limit by label-free light microscopy offers significant advantages for localising the NP clusters with increased precision and accuracy (Chang et al., 2011; Guggenheim et al., 2016).

## 3. Conclusions

Reflectance imaging has been employed in a variety of modalities since its development for the interrogation of cell surface contacts on a glass coverslip. Different illumination and scatter collection configurations that can be employed include interferometry, confocal, evanescent wave, structured and oblique angle illumination. This facilitates a wide range of possible studies, particularly within the field of nanoresearch, where reflectance investigations will be instrumental in determining the safety and efficacy profiles of NPs for various applications. As different components of cells and tissue have different RIs, reflectance imaging can be advantageous for probing the structure and organisation of biological samples. The power of reflectance for providing additional and complimentary structural information is particularly pronounced in the field of *in vivo* imaging, contextualising existing diagnostic and monitoring strategies, both for animal and human studies. Each reflectance technique discussed offers different advantages and disadvantages, in terms of resolution and imaging capabilities (Table 1), for different types of investigations within a host of different biomedical applications. Therefore the method of choice will vary dependent upon the intended application, but collectively reflectance offers exciting opportunities to probe a variety of different sample types in a label free manner.

## Acknowledgements

EJG gratefully acknowledges financial support from the Engineering and Physical Sciences Research Council (EPSRC) who provided funding though a studentship from the Physical Science of Imaging in the Biomedical Sciences (PSIBS) Doctoral Training Centre (EP/F50053X/1). The authors also would like to thank the EU FP7 collaborative project NanoMILE (grant agreement No. 310451) for providing additional funding. The authors would also like to thank the imaging facilities that participated in the completion of this work; The Birmingham Advanced Light Microscopy (BALM) facility, with special thanks to Alessandro Di'Maio for his input.

## References

Allegra Mascaro, Allegra L., Costantini, I., Margoni, E., Iannello, G., Bria, A., Sacconi, L., et al., 2015. Label-free near-infrared reflectance microscopy as a complimentary tool for two-photon fluorescence brain imaging. Biomed. Opt. Express 6 (November (11)), 4483 [cited 2016 Nov 24] Available from: https://www.osapublishing.org/abstract.cfm?URI=boe-6-11-4483.

Ando, Y., Sakurai, T., Koida, K., Tei, H., Hida, A., Nakao, K., et al., 2016. In vivo bioluminescence and reflectance imaging of multiple organs in bioluminescence reporter mice by bundled-fiber-coupled microscopy. Biomed. Opt. Express 7 (3), 963 [Internet] [cited 2016 Aug 21] Available from: http://www.osapublishing.org/viewmedia.cfm?uri=boe-7-3-963&seq=0&html=true.

Bereiter-Hahn, J., Fox, C.H., Thorell, B., 1979. Quantitative reflection contrast microscopy of living cells. J. Cell Biol. 82 (3), 767–779 [Internet] [cited 2016 Sep 14] Available from: http://jcb.rupress.org/content/82/3/767.abstract.

Byrne, G.D., Vllasaliu, D., Falcone, F.H., Somekh, M.G., Stolnik, S., 2015. Live imaging of cellular internalization of single colloidal particle by combined label-free and fluorescence total internal reflection microscopy. Mol. Pharm. 12 (11), 3862–3870.

Chang, B.-J., Lin, S.H., Chou, L.-J., Chiang, S.-Y., 2011. Subdiffraction scattered light imaging of gold nanoparticles using structured illumination. Opt. Lett. 36 (24), 4773–4775.

Cox, G., Sheppard, C.J.R., 2004. Practical limits of resolution in confocal and non-linear microscopy. Microsc. Res. Tech. 63 (January (1)), 18–22 [Internet] [cited 2016 May 24] Available from: http://doi.wiley.com/10.1002/jemt.10423.

Curtis, A.S., 1964. The mechanism of adhesion of cells to glass. a study by interference reflection microscopy. J. Cell Biol. 20 (February), 199–215.

De, Wit G., Danial, J.S.H., Kukura, P., Wallace, M.I., 2015. Dynamic label-free imaging of lipid nanodomains. Proc. Natl. Acad. Sci. 2015 (October (24)), 3–7 [Internet] [cited 2016 Sep 14 Available from: http://www.ncbi.nlm.nih.gov/pubmed/26401022.

Dunphy, M.P.S., Entenberg, D., Toledo-Crow, R., Larson, S.M., 2009. In vivo microcartography and subcellular imaging of tumor angiogenesis: a novel platform for translational angiogenesis research. Microvasc. Res. 78 (1), 51–56.

Gáspár, I., Szabad, J., 2009. In vivo analysis of MT-based vesicle transport by confocal reflection microscopy. Cell Motil. Cytoskeleton. 66 (February (3)), 68–79 [Internet] [cited 2016 May 3] Available from: http://www.ncbi.nlm.nih.gov/pubmed/19130480.

Gibbs-Flournoy, E.A., Bromberg, P.A., Hofer, T.P., Samet, J.M., Zucker, R.M., 2011. Darkfield-confocal microscopy detection of nanoscale particle internalization by human lung cells. Part Fibre Toxicol. 8 (January (1)), 2.

González, S., Sackstein, R., Anderson, R.R., Rajadhyaksha, M., 2001. Real-time evidence of in vivo leukocyte trafficking in human skin by reflectance confocal microscopy. J. Invest. Dermatol. 117 (2), 384–386.

Guggenheim, E.J., Khan, A., Pike, J., Chang, L., Lynch, I., Rappoport, J.Z., 2016. Comparison of confocal and super-resolution reflectance imaging of metal oxide nanoparticles. PLoS One 11 (10), e0159980.

Guttenberg, M., Bezerra, L., Neu-Baker, N.M., del Pilar Sosa Idelchik, M., Elder, A., Oberdörster, G., et al., 2016. Biodistribution of inhaled metal oxide nanoparticles mimicking occupational exposure: a preliminary investigation using enhanced darkfield microscopy. J. Biophoton. [cited 2016 Aug 22] Available from: http://doi.wiley.com/10.1002/jbio.201600125.

Huang, D., Swanson, E.A., Lin, C.P., Schuman, J.S., Stinson, W.G., Chang, W., et al., 1991. Optical coherence tomography. Science 254 (November (5035)), 1178–1181 [Internet] [cited 2015 Mar 1] Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4638169&tool=pmcentrez&rendertype=abstract.

Iftimia, N., Peterson, G., Chang, E.W., Maguluri, G., Fox, W., Rajadhyaksha, M., 2016. Combined reflectance confocal microscopy-optical coherence tomography for delineation of basal cell carcinoma margins: an ex vivo study. J. Biomed. Opt. 21 (1), 16006 [Internet] [cited 2016 Aug 22] Available from: http://biomedicaloptics.spiedigitallibrary.org/article.aspx?doi=10.1117/1.JBO.211.016006.

Izatt, J.A., Hee, M.R., Swanson, E.A., Lin, C.P., Huang, D., Schuman, J.S., et al., 1994. Micrometer-scale resolution imaging of the anterior eye In vivo with optical coherence tomography. Arch. Ophthalmol. 112 (December (12)), 1584 [Internet] [cited 2016 Aug 1] Available from: http://archopht.jamanetwork.com/article.aspx?doi=10.1001/archopht.1994.01090240090031.

Kim, C.S., Li, X., Jiang, Y., Yan, B., Tonga, G.Y., Ray, M., et al., 2015. Cellular imaging of endosome entrapped small gold nanoparticles. MethodsX 2 (January), 306–315 [Internet] [cited 2016 May 7] Available from: http://www.sciencedirect.com/science/article/pii/S2215016115000369.

Loo, C., Lowery, A., Halas, N., West, J., Drezek, R., 2005. Immunotargeted nanoshells for integrated cancer imaging and therapy. Nano Lett. 5 (4), 709–711.

Maitland, K.C., Gillenwater, A.M., Williams, M.D., El-Naggar, A.K., Descour, M.R., Richards-Kortum, R.R., 2008. In vivo imaging of oral neoplasia using a miniaturized fiber optic confocal reflectance microscope. Oral Oncol. 44 (11), 1059–1066.

Matsuzaki, T., Ito, K., Masuda, K., Kakinuma, E., Sakamoto, R., Iketaki, K., et al., 2016. Quantitative evaluation of cancer cell adhesion to self-assembled monolayer-patterned substrates by reflection interference contrast microscopy. J. Phys. Chem. B [Internet] [cited 2016 Aug 19]; acs.jpcb.5b1187 Available from: http://pubs.acs.org/doi/abs/10.1021/acs.jpcb.5b11870.

Ortega-Arroyo, J., Kukura, P., Huang, R., Lukic, B., Jeney, S., Florin, E.L., et al., 2012. Interferometric scattering microscopy (iSCAT): new frontiers in ultrafast and ultrasensitive optical microscopy. Phys. Chem. Chem. Phys. 14 (45), 15625 [Internet] Available from: http://xlink.rsc.org/?DOI=c2cp41013c.

Prieve, D.C., Luo, F., Lanni, F., 1987. Brownian motion of a hydrosol particle in a colloidal force field. Faraday Discuss. Chem. SOC 83 (0), 297–307 [Internet] [cited 2016 Jul 22] Available from: http://xlink.rsc.org/?DOI=dc9878300297.

Quinn, M.K., Gnan, N., James, S., Ninarello, A., Sciortino, F., Zaccarelli, E., et al., 2015. How fluorescent labelling alters the solution behaviour of proteins. Phys. Chem. Chem. Phys. 17 (46), 31177–31187 [Internet] [cited 2016 Jul 21] Available from: http://xlink.rsc.org/?DOI=C5CP04463D.

Rajadhyaksha, M., González, S., Zavislan, J.M., Anderson, R.R., Webb, R.H., 1999. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. J. Invest. Dermatol. 113 (3), 293–303.

Sokolov, K., Follen, M., Aaron, J., Pavlova, I., Malpica, A., Lotan, R., et al., 2004. Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles advances in brief real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conj. Cancer Res. 63 (May (9)), 1999–2004.

Stensberg, M.C., Madangopal, R., Yale, G., Wei, Q., Ochoa-Acuña, H., Wei, A., et al., 2014. Silver nanoparticle-specific mitotoxicity in Daphnia magna. Nanotoxicology 8 (8), 833–8342 [Internet] [cited 2016 Aug 19] Available from: http://informahealthcare.com/doi/abs/10.3109/17435390.2013.832430.

Sugden, J.K., 2004. Photochemistry of dyes and fluorochromes used in biology and medicine: some physicochemical background and current applications. Biotech. Histochem. 79 (April (2)), 71–90 [Internet] [cited 2016 Jul 21] Available from: http://www.ncbi.nlm.nih.gov/pubmed/15513709.

Temple, P.A., 1981. Total internal reflection microscopy: a surface inspection technique. Appl. Opt. 20 (August (15)), 2656–2664 [Internet] [cited 2016 Aug 15]; Available from: https://www.osapublishing.org/abstract.cfm?URI=ao-20-15-2656.

Verschueren, H., 1985. Interference reflection microscopy in cell biology: methodology and applications. J. Cell Sci. 75 (April), 279–301.

Yadav, R., Lee, K.-S., Rolland, J.P., Zavislan, J.M., Aquavella, J.V., Yoon, G., 2011. Micrometer axial resolution OCT for corneal imaging. Biomed. Opt. Express 2 (November (11)), 3037 [Internet] [cited 2016 Aug 22] Available from: https://www.osapublishing.org/boe/abstract.cfm?uri=boe-2-11-3037.

Yin, C., Liao, K., Mao, H.Q., Leong, K.W., Zhuo, R.X., Chan, V., 2003. Adhesion contact dynamics of HepG2 cells on galactose-immobilized substrates. Biomaterials 24 (5), 837–850.