IATIA
VISION SCIENCES

Inside QPI

An introduction to the technology and applications of Quantitative Phase Imaging

Iatia Imaging Pty Ltd

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1 Table of Contents

2 Inside QPI

Iatia Imaging is the world's leading developer of digital wavefront imaging solutions for visualization and measurement.

Iatia's unique globally patented QPI technology enhances visual analysis from the microscopic to the telescopic. It enables the visualization and measurement of invisible or hard to see objects from transparent biological cells, optics of the human eye to hidden and camouflaged objects in military and security operations, in unprecedented depth and clarity.

QPI's list of applications is continuing to expand with backing from a strong development team and customers such as GE Healthcare, Columbia University, Oxford University, the Federal Bureau of Investigation (FBI) and the Australian Defence Force.

In its key market segments of the life sciences, nanotechnology, ophthalmology and Defence, QPI has already begun to change the way we view the world.

Please contact us to see if QPI can provide a solution to your application.

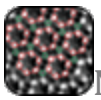
3 Products

Iatia's QPI algorithm provides quantitative phase and wavefront data from a broad range of sources including visible light, infra-red, electrons, x-rays, thermal, terahertz and neutrons.

The QPI algorithm calculates phase or wavefront data from a minimum of two images captured using traditional imaging technology, such as standard digital cameras, without the need for specialised optics or wavefront systems.

Life sciences

Digital phase contrast solutions and automated cell analysis.



Nanotechnology

Phase contrast at the nanoscale.

Ophthalmology

Digital high resolution wavefront sensing.

Defence

Passive ranging and camouflage negation.

4 QPI SDK

QPI is available as a Software Development Kit (SDK) for easy integration of QPI technology into new and existing software products. The QPI SDK is supplied as a set of easy to use Windows COM objects with ready packaged redistributables.

4.1 QPI SDK features and documentation.

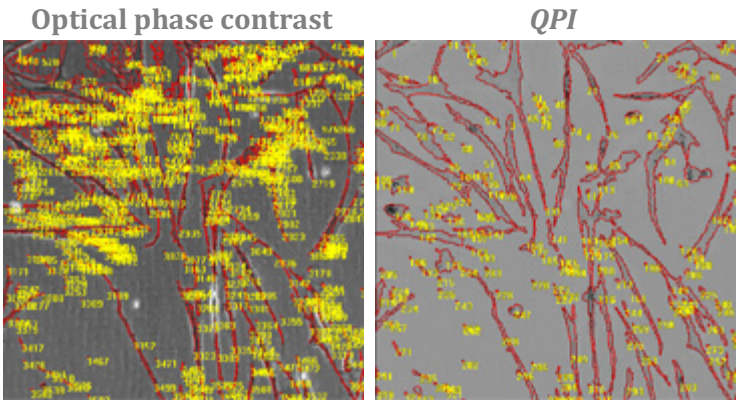
Please contact us to arrange an evaluation or for purchase pricing and licensing information.

5 Life sciences

Iatia's QPI provides a digital phase contrast solution in the life sciences above and beyond the capabilities of conventional lens-based solutions.

5.1 Segmentation

QPI provides an improved contrast mechanism enabling highly accurate cell segmentation. Unlike conventional phase contrast mechanisms, such as Differential Interference Contrast (DIC) or optical phase contrast, QPI provides quantitative phase data based on the optical thickness of the sample.

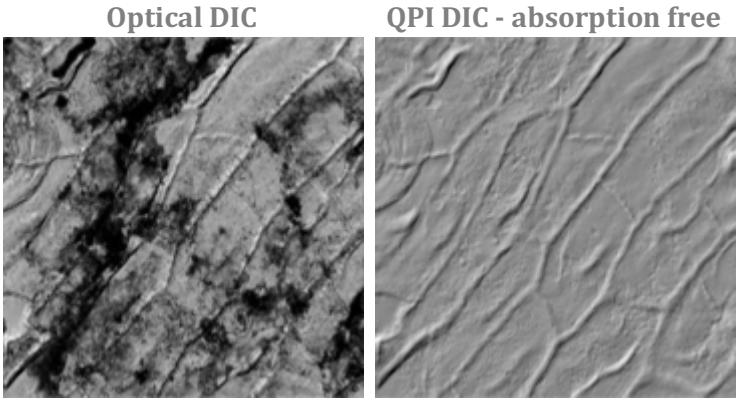


Human airway smooth muscle cells - QPI provides cleaner and more accurate cell segmentation for automated cell analysis.

5.2 Digital DIC

QPI is able to digitally generate Differential Interference Contrast (DIC) images from its phase and amplitude output. QPI DIC images are insensitive to birefringent plastic cultureware, unlike conventional DIC, and can be generated free of sample absorption which may obscure detail.

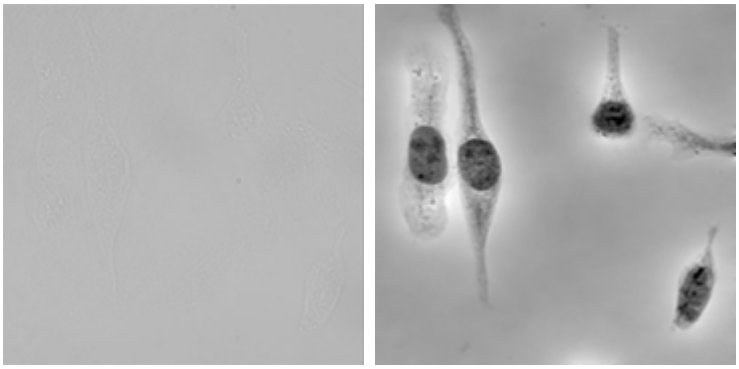
QPI also generates other phase contrast images such as "phase" (contrast) and Hoffman Modulation Contrast.



Fossilized leaf - QPI's absorption free DIC removes the effect of obscuring media such as stains, dirt and dust on the sample or in the optics.

6 Segmentation

Iatia's QPI generates quantitative phase data based on relative changes in the optical thickness of a sample. This measurement of changes in optical thickness (phase) provides a significantly improved contrast mechanism enabling highly accurate cell segmentation.



Brightfield **QPI**

As seen in the example above of rat mast cells, the sample has almost no effect on the intensity or color distirbution in the image and is almost totally transparent. However, the cells and their internal structures have an optical thickness and do affect the phase of light. QPI measures the relative change in optical thickness of the cells and that quantitative data is displayed as a high contrast grayscale image.

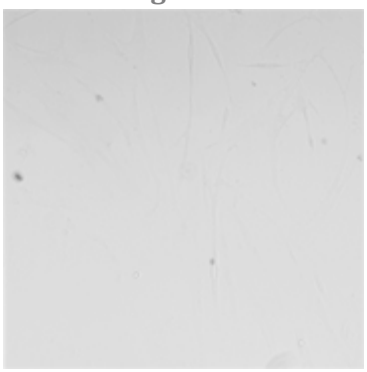
By comparison, conventional phase contrast techniques, such as Differential Interference Contrast (DIC) or optical phase contrast, produce qualitative images. These qualitative images often result in poor cell segmentation as a result of the shadows or the edge halo effects evidenced in optical phase contrast visualizations of optical thickness.

6.1 Case study - monitoring cell growth

In the following example, researchers from the Department of Physiology and Pharmacology at the University of Melbourne, Australia, monitored the change in growth in human airway smooth muscle cells over a period of a week1.

Cell growth was monitored with optical phase contrast as well as the QPI derived phase image. Automated cell segmentation and area calculations to monitor growth were obtained using Media Cybernetics' ImagePro Plus™ image processing software. The QPI based cell growth measurements were also compared to an independent estimate of cell growth based on haemocytometer cell counts.

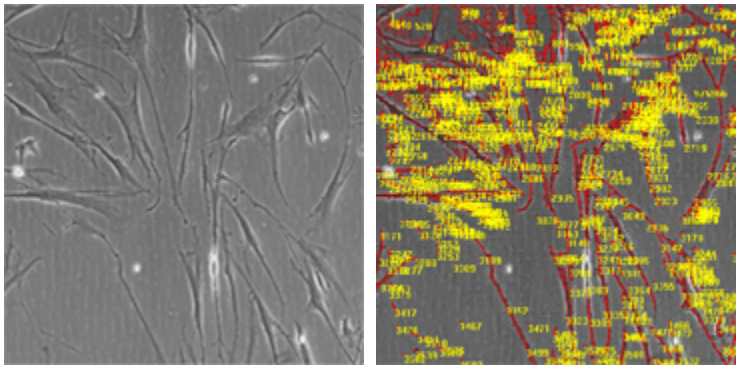
Brightfield



This brightfield image, shows that the sample has little effect on the intensity/amplitude of light and is almost totally transparent. As a result, ImagePro™ was unable to reliably detect cell boundaries and provide accurate cell area measurements. Staining the sample would provide improved cellular discrimination but would kill the cells and would not allow longitudinal time studies of cell growth.

6.2 Phase contrast

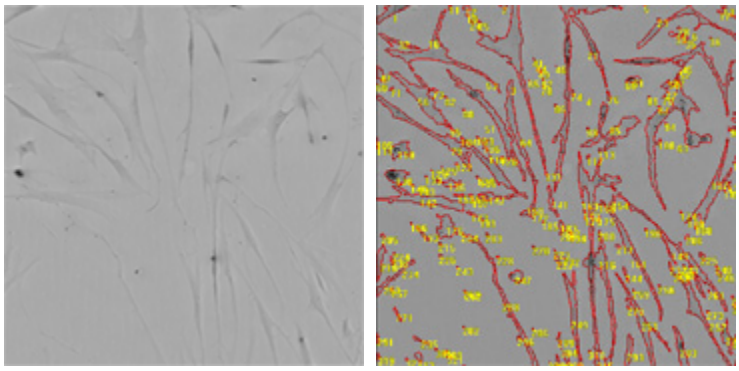
The optical phase contrast image shows enhanced cellular discrimination with a "halo effect" apparent around each cell. This haloing effect resulted in inaccurate automated cell segmentation by ImagePro™ due to poor discrimination of the start of cell boundaries.



Phase contrast **Segmentation - cell boundaries**

6.3 QPI

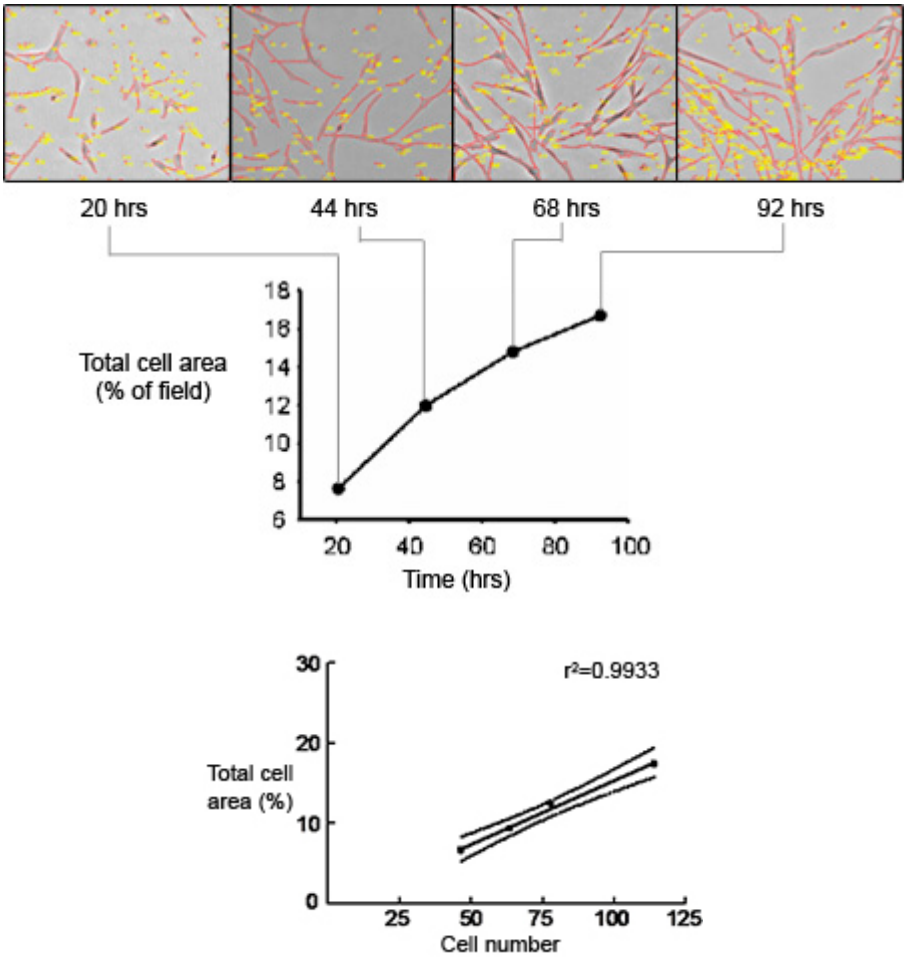
The QPI phase image shows clear cellular discrimination. These well defined cell boundaries allow for more robust threshold settings to be set in ImagePro™ and accurate measurements of cell area to be made.



QPI phase image Segmentation - cell boundaries

6.4 Measurement over time

Utilising the QPI phase images, accurate cell area measurements were obtained of the same sample over a period of 92 hours providing data on the rate of cell growth.



The QPI based growth rate data from this study was also independently correlated with growth rates based on haemocytometer cell counts (r2=0.9933). Note that the haemocytometer based measurement entails destruction of the cell sample, making longitudinal time studies of the same cell line impossible.

6.5 Conclusion

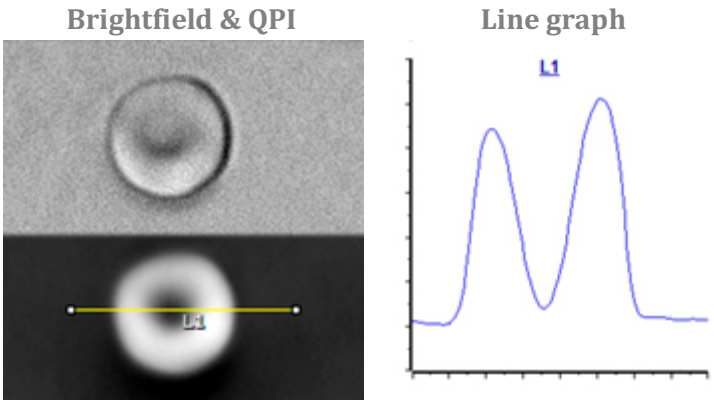
Using QPI, transparent cells can be visualized with improved cellular boundary definition allowing precise and reproducible measurements of the cell area of a sample over time, and thus the rate of cell growth in a sample.

6.6 References

1. Quantitative Phase Microscopy: a new tool for measurement of cell culture growth and confluency in situ. Claire L. Curl, Trudi Harris, Peter J. Harris, Catherine J. Bellair, Brendan E. Allman, Alastair G. Stewart, Lea M.D. Delbridge, Pflugers Archive: European J. of Physiology, 448, 462-468 (2004).
2. The recognition of biological cells utilizing quantitative phase microscopy system. O. Veselov, J. Lekki, W. Polak, D. Strivay, Z. Stachura, K. Lebed, J. Styczen, Nuclear Instruments and Methods in Physics Research B 231 (2005) 212-217.

6.7 Quantitative

QPI images quantitatively measure the optical thickness of the sample, making it suitable for cell volume determination/monitoring. Conventional phase imaging techniques, such as DIC do not provide this information.



Red blood cell - optical thickness profile through a red blood cell.

6.8 No specialized optics

QPI uses two defocused brightfield images captured with standard digital cameras making QPI practical where specialized phase optics and hardware are impractical or cumbersome.

6.9 High speed processing

QPI is a rapid solution supporting live capture of 640x480 pixel images at 15 frames per second.

7 Digital DIC

Iatia's QPI generates quantitative phase data based on relative changes in the optical thickness of a sample. This phase data can be used to digitally generate analogues of conventional optical phase contrast systems such as Differential Interference contrast (DIC).

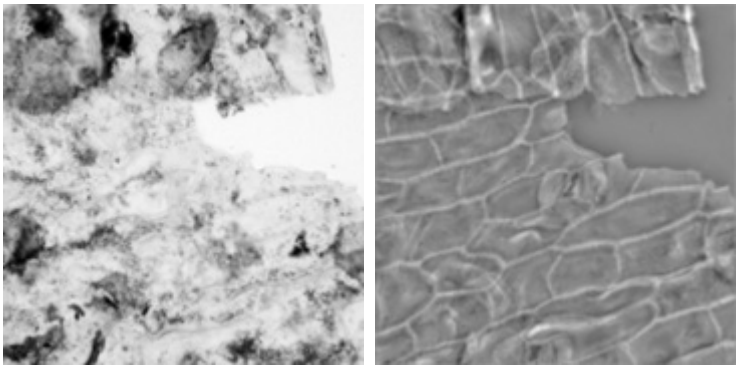
QPI's digitally generated phase contrast images provide additional flexibility and functionality without the inherent limitations of optical systems:

- Non-polarized illumination
- Full analog of optical controls such as 360º rotation of DIC's Wollaston Prism and transmission plate adjustments in optical phase contrast
- Insensitive to birefringent plastic cultureware
- Returns phase and amplitude/absorption information independently allowing for selective visualization of phase information without amplitude/absorption information
- Generate phase contrast images at all magnifications without the need for additional optics or changes to the optical path

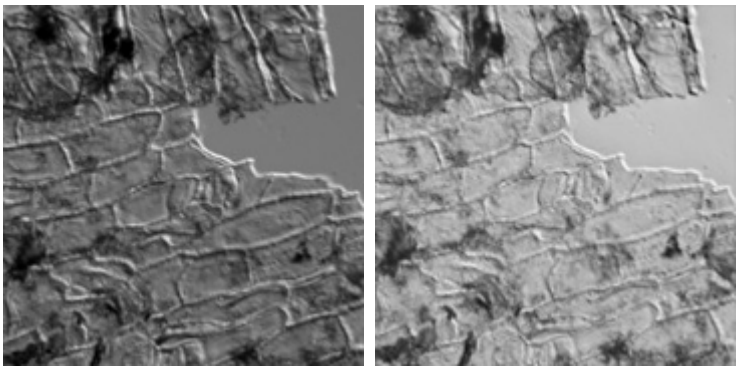
QPI also generates other phase contrast images such as "phase" (contrast) and Hoffman Modulation Contrast.

7.1 Fossilized leaf

This fossilized leaf sample serves to illustrate the features of QPI's phase contrast visualization capabilities with the brightfield image of the sample exhibiting poor cellular discrimination as well as obscuring amplitude/absorption information such as stains (in this case, dirt and dust)[1,2].

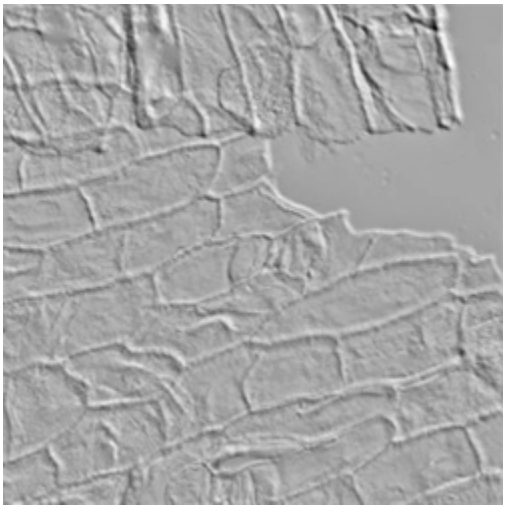


Brightfield **QPI phase map**



Optical DIC **QPI DIC**

As shown in the images above, the QPI phase map represents calculations of changes in optical thickness across the sample in grayscale. QPI can then combine this pure phase map with amplitude/absorption information (brightfield image) to produce DIC's characteristic shadow relief representation of phase changes. As shown above, the QPI DIC image correlates well with the optical DIC image.



Unique to QPI is the ability to separate phase from amplitude/absorption information to produce an amplitude/absorption free DIC image, shown on the right. In this sample, amplitude/absorption information such as stains, dirt and dust was removed from the DIC image to reveal the underlying cell structure.

7.2 References

1. Optical Phase Microscopy: Quantitative Imaging and Conventional Phase Analogs
BE Allman, ML von Bibra, CJ Bellair, A Kabbara, E Barone-Nugent, AP Gaeth, LM Delbridge and KA Nugent, Microscopy and Microanalysis, 87, 13-15 (UK Ed.), 25, 5-7 (Asia/Pacific Ed.) (2002).
2. Quantitative Phase-Amplitude Imaging I: Optical Microscopy
ED Barone-Nugent, A Barty and KA Nugent, Journal of Microscopy, 206, 194-203, (2002).

8 Quantitative

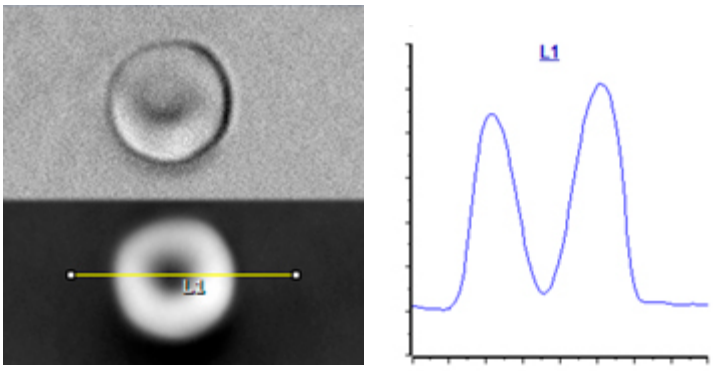
Iatia's QPI calculates phase values for each point in an image based on the degree of phase shift induced in light traversing through the sample. The magnitude of phase shift (changes in optical thickness) is the product of cell thickness and refractive index. When one of these cellular properties is known, the other can be determined.

8.1 Case study - cell volume measurement

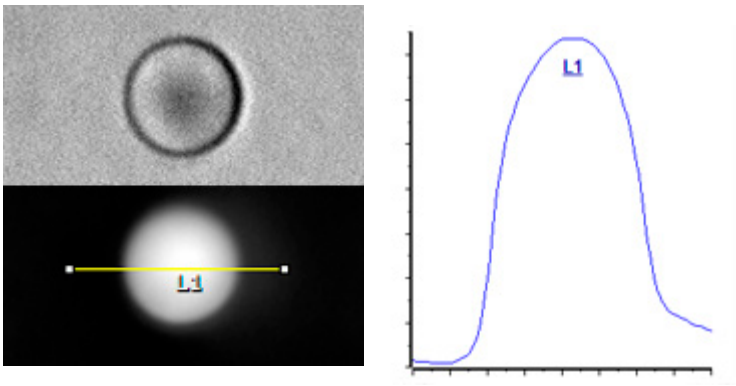
In the following example, researchers at the Department of Physiology and Pharmacology at the University of Melbourne, Australia, calculated the volume of rat red blood cells exposed to imidazole-buffered solutions of varied tonicity [4].

Blood was collected from rats and transferred to standard haematological imidazole-buffered solutions (22°C) of different osmolality and graded tonicity (170, 240, 400, 540 mosm/kg), allowing 10 minutes equilibration prior to imaging. Brightfield and QPI phase images were acquired using an inverted Zeiss Axiovert 100M fitted with a Zeiss LD-Achroplan (x63, 0.8NA). Analysis of QPI phase data and calculation of cell volume was performed using IDL software (v5.5). Steady-state cell volume was calculated by integration of the cell delineated QPI phase values, assuming an intracellular refractive index (RI) of 1.367 (phase = cell thickness x RI). To delineate cell boundaries standard nearest-neighbour gradient thresholding techniques for edge detection were applied to the QPI phase images.

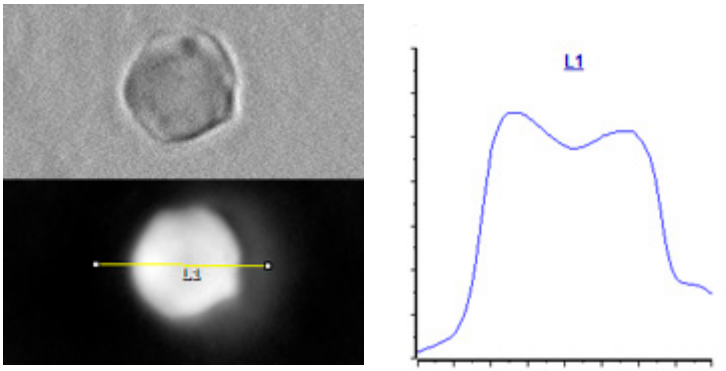
The following images are extracts from the results of this study showing the brightfield and QPI phase images of an Isotonic, Hypotonic and Hypertonic red blood cell along with a corresponding line profile of the phase shift of the respective sample.



Isotonic



Hypotonic



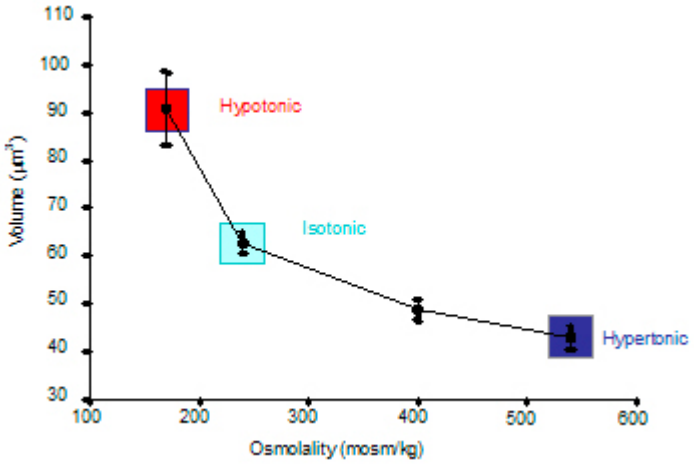
Hypertonic

In osmotic conditions approximating isotonicity (240 mosm/kg, isotonic), red blood cells exhibited biconcave morphology evident in the brightfield image and appeared annular in the QPI phase image. A line profile of phase shift values measured across the cell diameter indicated a dual peak phase consistent with biconcave morphology.

In solutions of low tonicity (170 mosm/kg, hypotonic), cell bi-concavity was not evident in bright field images. In these conditions the cell phase map exhibited a centralised region of maximum phase shift. A linear profile of phase shift values measured across the cell diameter indicated spherical morphology consistent with osmotically-induced cell volume expansion.

Red blood cells exposed to solutions of high tonicity (400 and 540 mosm/kg, hypertonic) exhibited a centrally thinned appearance characterised by a plateau in the line profile, consistent with crenated morphology.

The assumed spherical shape of the cell under hypotonic conditions provided a means by which cell thickness values could be computed from phase shift measurement for cells in all osmotic environments.



Mean red blood cell volume measured in buffer solutions of different tonicity

As shown above, an inverse relationship between buffer tonicity and mean red blood cell volume was observed. Phase-computed volume was found to increase with decreasing solution osmolality as anticipated: 42.81 ± 2.37, 48.71 ± 2.29, 62.64 ± 2.33, 90.82 ± 7.7 (µm3) in solutions of 540, 400, 240 and 170 mosm/kg respectively.

8.2 Conclusion

Erythrocyte morphology in different osmotic environments can be quantitatively assessed using QPI phase images. QPI provides an optically simple, accurate and non-destructive approach for evaluating cell structure, shape and volume which has potentially broad application for a range of cell types.

8.3 References

1. Quantitative Optical Phase Microscopy
   A Barty, KA Nugent, D Paganin and A Roberts, Optics Letters 23, 1-3 (1998).
2. Quantitative Phase Tomography
   A Barty, KA Nugent, A Roberts and D Paganin, Optics Communications 175 (2000) pp 329-336.
3. Refractive index profiling of optical fibers with axial symmetry by use of quantitative phase microscopy
   A Roberts, E Ampem-Lassen, A Barty A, KA Nugent, Optics Letters 2002;27:2061–2063.
4. Single Cell Volume Measurement by Quantitative Phase Microscopy (QPM): A Case Study of Erythrocyte Morphology
   Claire L. Curl, , Catherine J. Bellair, Peter J. Harris, Brendan E. Allman, Ann Roberts, Keith A. Nugent, and Lea M.D. Delbridge, Cellular Physiology and Biochemistry, 17, (2006).

9 No specialized optics

Iatia's QPI generates phase data based on a minimum of two conventional brightfield images captured digitally at slightly different focal planes.

QPI is not bound by the inherent limitations of specialized phase contrast optical systems, allowing:

- use in applications where the addition of specialized optics is impractical and cumbersome (eg. high throughput image analysis)
- simultaneous capture of brightfield and phase data without the need to change optical configurations or employ complicated optical systems which may degrade image quality, allowing for more accurate co-localization between phase and brightfield images (eg. confocal microscopy)
- unpolarized illumination
- use with birefringent plastic cultureware
- generation of phase contrast images at all available magnifications

9.1 Case study - co-localization in confocal microscopy

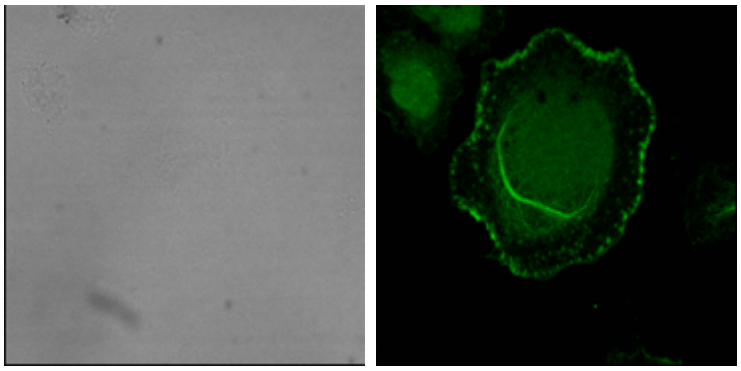
QPI technology is being used by researchers at the Ludwig Institute for Cancer Research and Walter and Eliza Hall Institute, Australia, with confocal laser scanning microscopes (CSLMs) to generate phase contrast images simultaneously with confocal fluorescence images for more accurate co-localization in a simplified workflow[1].

Utilizing QPI technology, brightfield images collected with the TLD of a CSLM may be captured at the same time as confocal fluorescence images. The benefits of using QPI to simultaneously capture brightfield and confocal fluorescence images include:

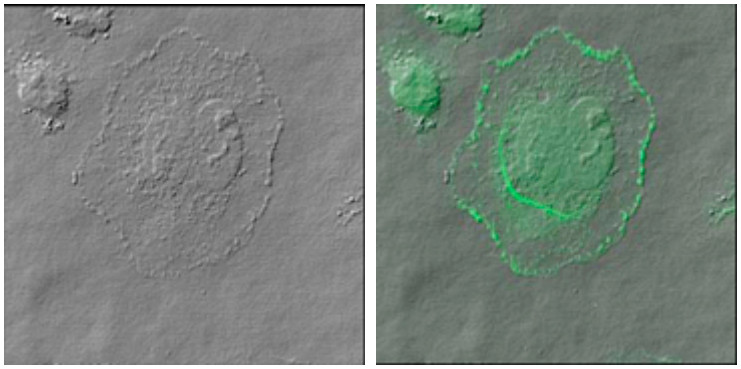
- a simplified image collection process
- phase contrast images such as DIC, optical phase contrast and Hoffman Modulation Contrast can be generated from the archival brightfield data
- no specialized optics which may compromise the intensity or quality of confocal fluorescent images
- no misalignment of image sets for improved co-localization
- no temporal changes in samples between capture of fluorescent and registration image sets

9.2 Rat mast cell

An activated rat mast cell stained with antibodies to protein phosphatase 1 (PP1). Fluorescent and TLD brightfield images were collected simultaneously using a Bio-Rad MRC 1024 confocal microscope attached to a Nikon TE-300 inverted microscope at 60x magnification. A z-series was collected at 0.5µm intervals and DIC images were generated utilizing QPI technology.



TLD brightfield **Fluorescent**



QPI DIC **QPI DIC with fluorescent overlay**

Confocal fluorescent images were able to be captured with maximum intensity and overlaid on the QPI DIC image. Note the registration of the fluorescence at the cell margins demonstrates excellent alignment of the confocal fluorescent and QPI DIC images.

9.3 References

1. A simple method allowing DIC imaging in conjunction with confocal microscopy
S.H. Cody, S.D. Xiang, M.J. Layton, E. Handman, M.H.C. Lam, J.E. Layton, E.C. Nice, and J.K. Heath, Journal of Microscopy 217, 265-274 (2005).

10 Product application

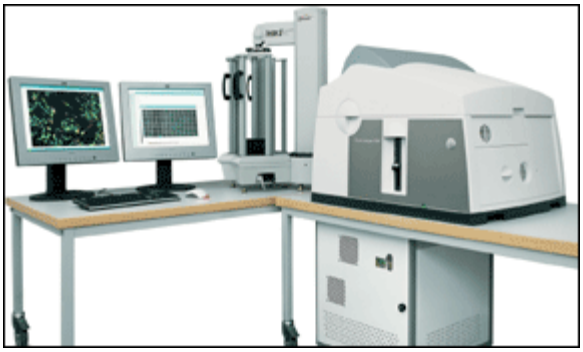
10.1 GE Healthcare's IN Cell 1000

In late 2004, GE Healthcare, a division of the multinational General Electric (GE) Company, incorporated QPI into its IN Cell 1000 cell analyzer.

The IN Cell 1000 is an automated cell imager providing high throughput image analysis for use in basic research, assay development and drug discovery applications.

The incorporation of QPI into the IN Cell 1000 allows for:

- the generation of phase contrast (DIC) images without the need to incorporate costly and cumbersome optical systems
- the capture of brightfield data simultaneously with phase contrast images without the need to change optical configurations and slow down throughput
- improved segmentation for automated post-capture analysis using pure phase (optical thickness) data



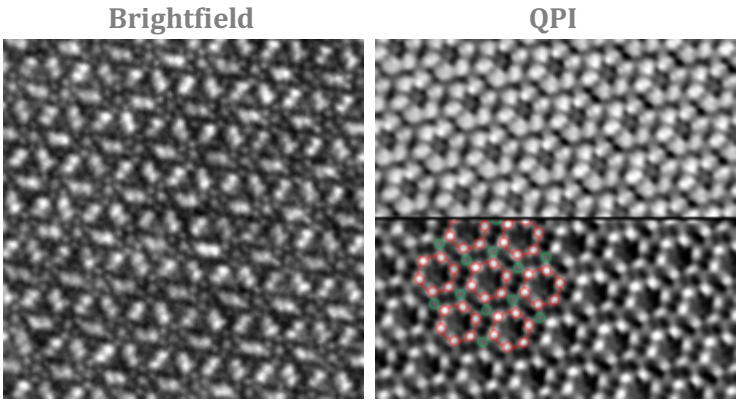
IN Cell 1000

11 Nanotechnology

Iatia's QPI provides a digital phase contrast solution to transmission imaging techniques at the nanoscale where few other contrast mechanisms are available due to fabrication difficulties for short wavelength radiations like electrons and x-rays.

11.1 Visualization

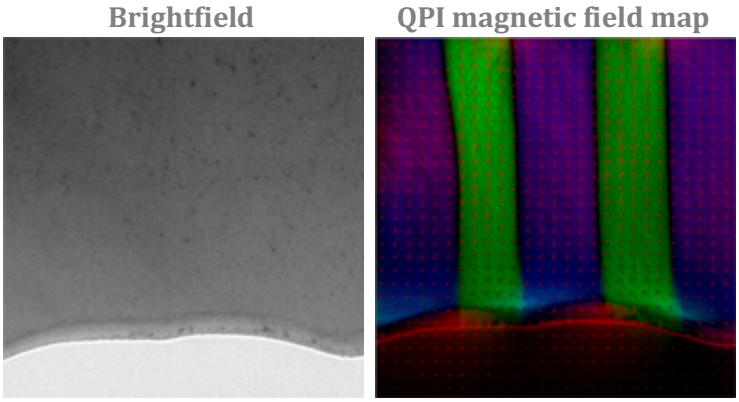
QPI is a valuable contrast mechanism at the nanoscale where absorption is minimal.



Si_3N_4 atomic array - QPI is able to resolve the atomic structure of this semiconductor substrate (images captured using QPt for DigitalMicrograph™, courtesy of HREM Research Inc, Japan).

11.2 Magnetic field analysis

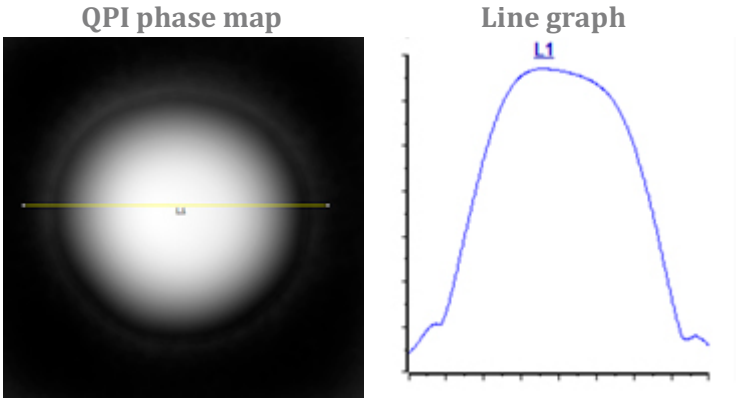
Magnetic fields affect the phase of the electron, allowing QPI to map them.



Cobalt - magnetic field map captured using QPI technology (image courtesy of RUG University of Groningen, Netherlands).

11.3 Quantitative

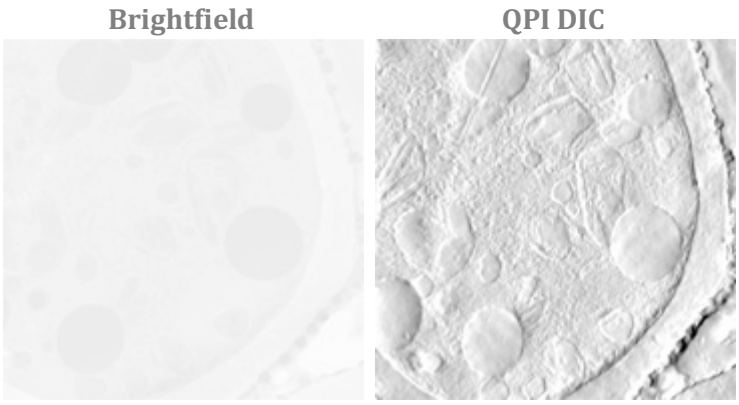
QPI images quantitatively measure the optical thickness of a sample.



Silicon nanosphere - optical thickness profile.

11.4 Microscopy phase techniques

QPI makes available, at the nanoscale, phase visualization techniques usually associated with optical microscopy, such as Differential Interference Contrast (DIC), Zernike Phase Contrast and Hoffman Modulation Contrast.



Unstained Radula (liverwort) spore - QPI's DIC provides sample structure relief (image courtesy of The University of Melbourne, Australia).

11.5 High speed processing

Unlike other phase contrast algorithms used in research applications, QPI is a non-iterative and rapid solution allowing for the capture of live video images with a resolution of up to 640x480 pixels at 15 frames a second.

12 Visualization

Iatia's QPI improves visualization of objects in the nanoscale by providing a highly effective contrast mechanism.

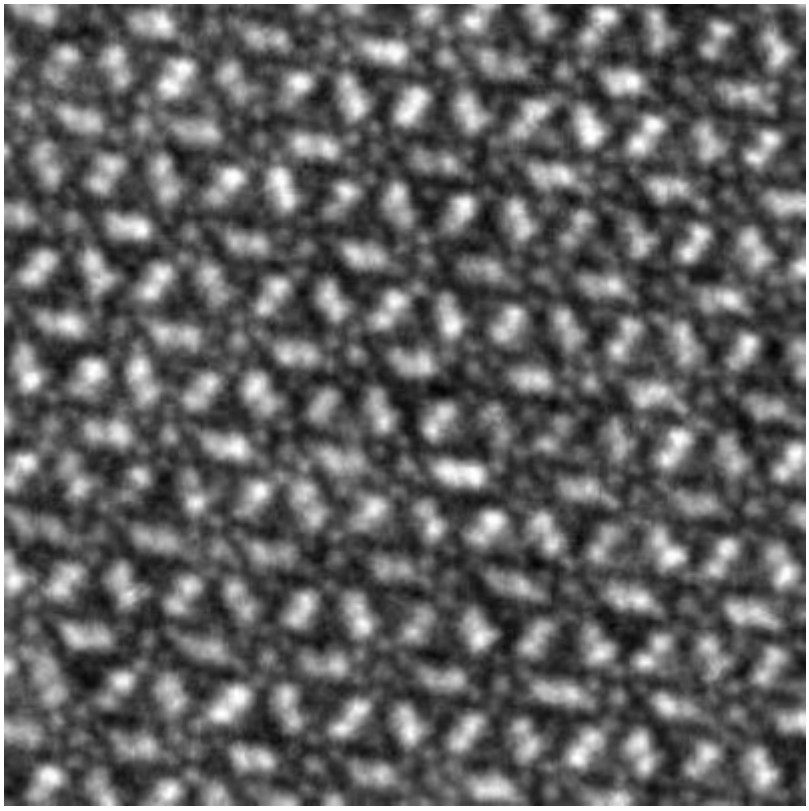
QPI calculates the phase distribution of a sample from a minimum of 2 differentially focused conventional intensity based brightfield images and rendering this phase data as a grayscale image. These QPI phase images are phase distribution maps independent of intensity/amplitude information showing the relative optical thickness across the sample, resulting in improved visual differentiation irrespective of the effects of intensity/amplitude.

12.1 Case study - silicon nitride

The following silicon nitride (Si_3N_4) images are courtesy of HREM Research, Inc (Japan) who have developed QPt for DigitalMicrograph™ incorporating QPI technology.

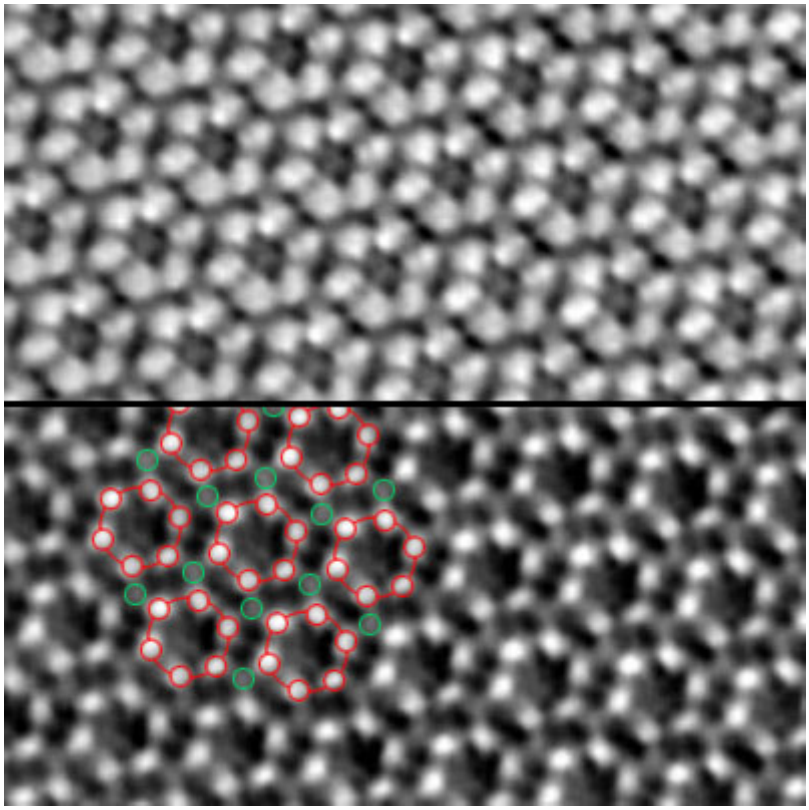
These images were taken at NCEM, Berkeley using a Philips CM300 equipped with a field emission gun and the phase images were generated by QPt for DigitalMicrograph™.

The following brightfield image is an out of focus image (in order to achieve some phase contrast) of a substrate monolayer.



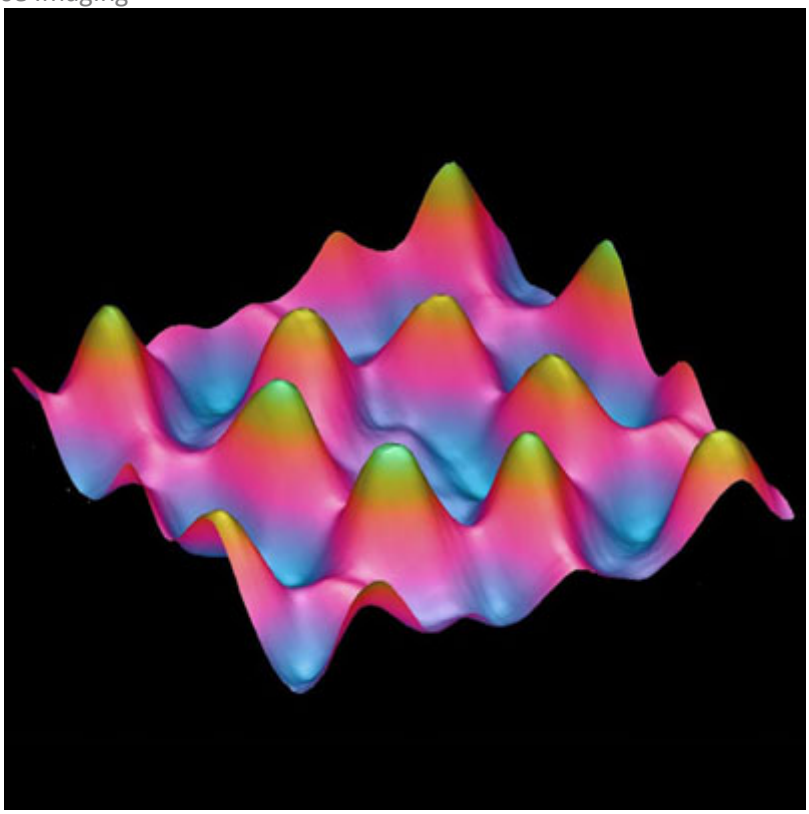
Conventional transmission electron brightfield image

By utilizing pairs of images separated by approximately 11.6nm, the QPI algorithm was able to construct phase images that clearly resolve the atomic structure of the sample. Back propagating this phase image to the sample exit surface yields the view seen in the upper half of this image. The phase corresponding to the minimum amplitude variation is shown in the lower half, where some of the Silicon (red) and Nitrogen (green) atoms have been indicated.



Phase image computed with QPt™ for DigitalMicrograph™

The following is a 3D rendering of a section of the minimum amplitude phase image produced with Research Systems IDL software.



3D rendered view of one hexagonal unit

12.2 References

1. Phase Measurement of atomic resolution using Transport of Intensity Equation Kazuo Ishizuka, and Brendan Allman, Journal of Electron Microscopy, 54, 191-197 (2005).
2. Phase Measurement in Electron Microscopy Using the Transport of Intensity Equation Kazuo Ishizuka, and Brendan Allman, Microscopy Today, 13, 22-24 (2005).

13 Magnetic field analysis

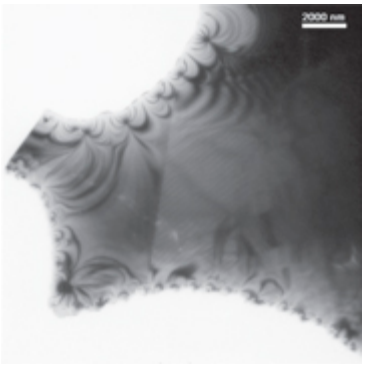
Iatia's QPI is capable of producing magnetic field maps of samples.

Magnetic fields effect the phase of electrons in transmission electron microscopy. QPI calculates the phase distribution and is able to render that information as a magnetic field map showing strength and direction of the magnetic field.

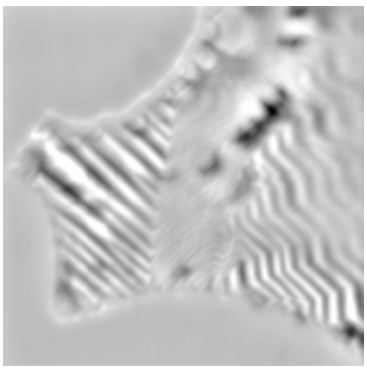
13.1 Case study - manganese trioxide

The following study was conducted by HREM Research, Inc (Japan) on a Perovskite-type manganese trioxide ($Pr_{1-x}(Ca_{1-y},Sr_y)_xMnO3$ (x=0.45, y=0.4))[1].

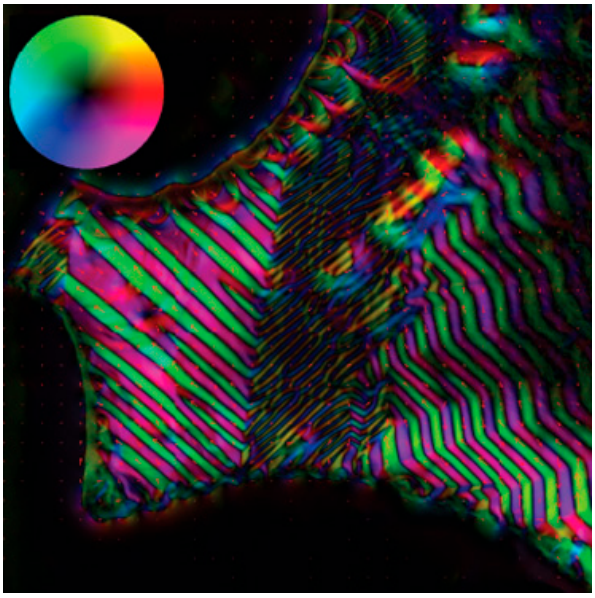
A through focus series of 3 images at 2mm steps was taken using a Hitachi HF-3000L Lorenz microscope at approximately 2,000x magnification. Phase distribution maps were generated with QPt for DigitalMicrograph™ incorporating QPI technology.



Brightfield



QPI phase map



Magnetic field map

The above image shows the restored phase and a magnetization vector map obtained from the gradient of the phase distribution. The direction and amplitude of the magnetization are represented by changes in color and brightness, respectively. The green and violet stripe pairs in the image reflect the region with opposite magnetic orientation. Darker areas represent smaller amplitudes of the local magnetization.

It is clear, from the magnetic field map of this sample, that magnetization directions are parallel to the elongated domain directions. In addition, the wide (180^{o}) domains and the narrow (stripe) domains magnetize perpendicular to each other.

13.2 References

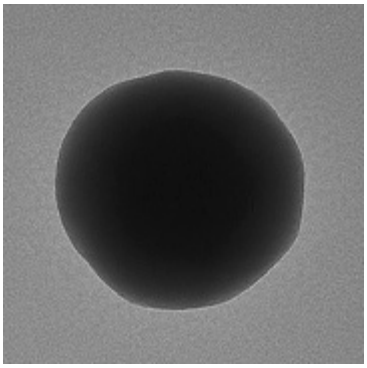
1. Phase Measurement in Electron Microscopy Using the Transport of Intensity Equation
   Kazuo Ishizuka, and Brendan Allman, Microscopy Today, 13, 22-24 (2005).
2. Real Space Observation of Helical Spin Order
   M. Uchida, Y. Onose, Y. Matsui, and Y. Tokura, Science, 311 359-361 (2005).
3. Determining the Magnetic Potential in Patterned Materials Using Energy-Dependent Lorentz Phase Microscopy
   A. Kohn, A. K. Petford-Long, and T. C. Anthony, Phys. Rev. B., 72, 014444 , (2005).

14 Quantitative

QPI images quantitatively measure the optical thickness of a sample.

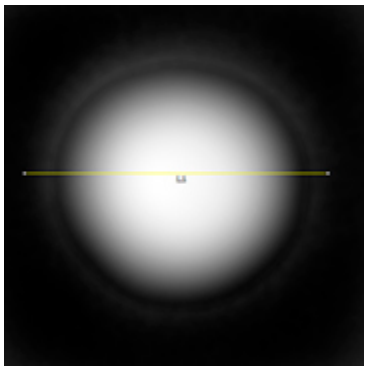
Iatia's QPI calculates phase values for each point in an image based on the degree of phase shift induced in light traversing through the sample. The magnitude of phase shift (changes in optical thickness) is the product of sample thickness and refractive index. When one of these properties is known, the other can be determined.

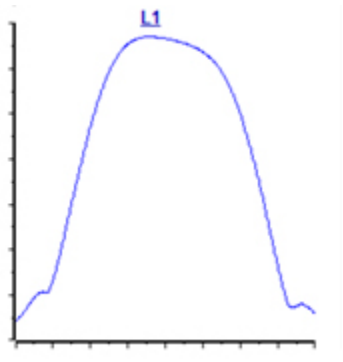
14.1 Case study - silicon sphere



The following image on the right shows a silicon sphere approximately 20nm in diameter imaged on a transmission electron microscope (TEM) at 92,000x magnification. The spherical balls of silica were mounted on holey carbon substrate and imaged. Note that the amplitude contrast of the brightfield image for this sample is substantial and indicates that the sample contains significant scattering.

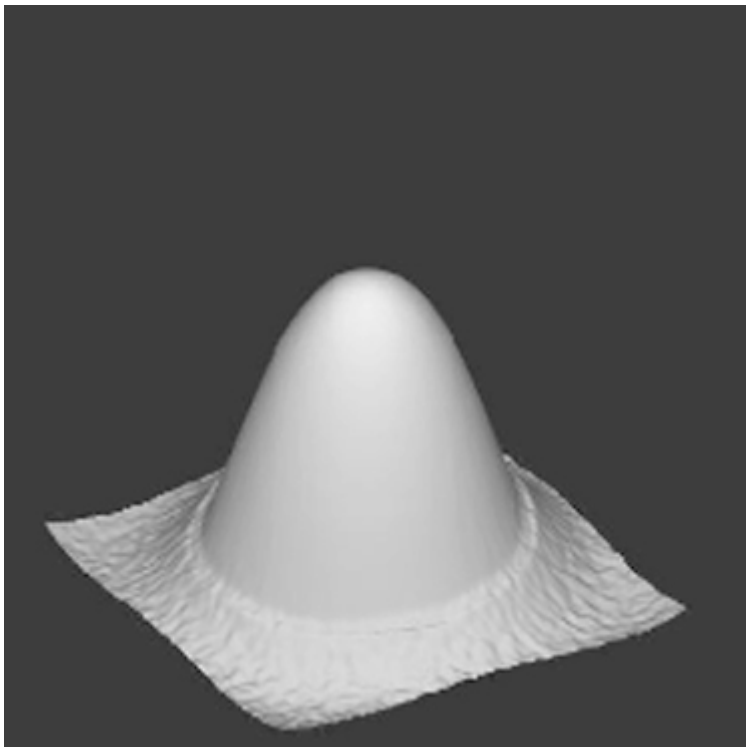
A phase map of the sphere was generated by QPI from two defocused images (shown below). The line profile indicates the projected phase excursion.





QPI phase map **Line graph - optical thickness profile**

Despite the strong scattering, the phase of the sample is accurately recovered[3] and displays the structure expected for the phase shift induced by a small sphere.



Isometric plot of the QPI phase map.

14.2 References

1. Quantitative Phase Imaging Using Hard X Rays
   K. A. Nugent, T. E. Gureyev, D. F Cookson, D. Paganin and Z. Barnea, Physics Review Letters 77, 2961-2964 (1996).
2. Non-Interferometric Quantitative Phase Imaging with Soft X-rays
   B.E. Allman, P.J. McMahon, J.B. Tiller, K.A. Nugent, D. Paganin, A. Barty, I. McNulty, S.P. Frigo, S. Wang, and C.C. Retsch, J. Opt. Soc. Am. A 17, 1732-1743 (2000).
3. Quantitative Phase-Amplitude Microscopy II: Differential Interference Contrast Imaging for Biological TEM
   P.J.McMahon, E.D.Barone-Nugent, B.E.Allman and K.A. Nugent, Journal of Microscopy, 206, 204-208 (2002).

15 Microscopy phase techniques

QPI makes available, at the nanoscale, phase visualization techniques usually associated with optical microscopy, such as Normaski Differential Interference Contrast (DIC), Zernike Phase Contrast and Hoffman Modulation Contrast.

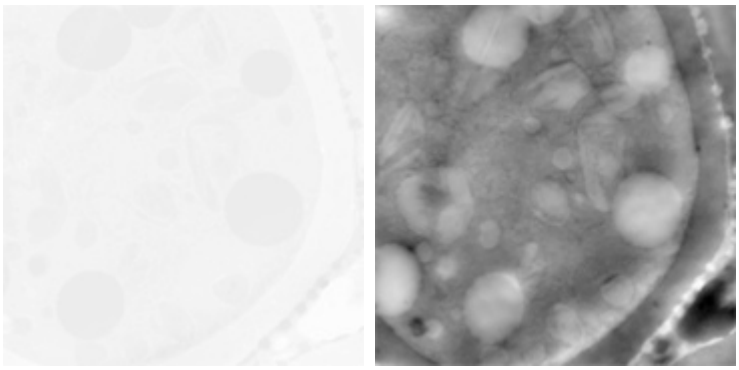
Light microscopy phase contrast images are unavailable at the nanoscale as traditional phase visualization systems are difficult or impractical to fabricate at these small scales.

QPI is capable of mathematically rendering analogs of these conventional phase contrast systems from the quantitative phase data it generates independent of amplitude/intensity data. Uniquely, QPI's digital phase visualization can be achieved with or without intensity (scattering) contrast.

15.1 Case study - Radula (liverwort) spore

The following images of a Radula (liverwort) spore were captured by researchers at the University of Melbourne, Australia, [1] and demonstrate the applicability of QPI technology in generating DIC images using transmission electron microscope (TEM) images.

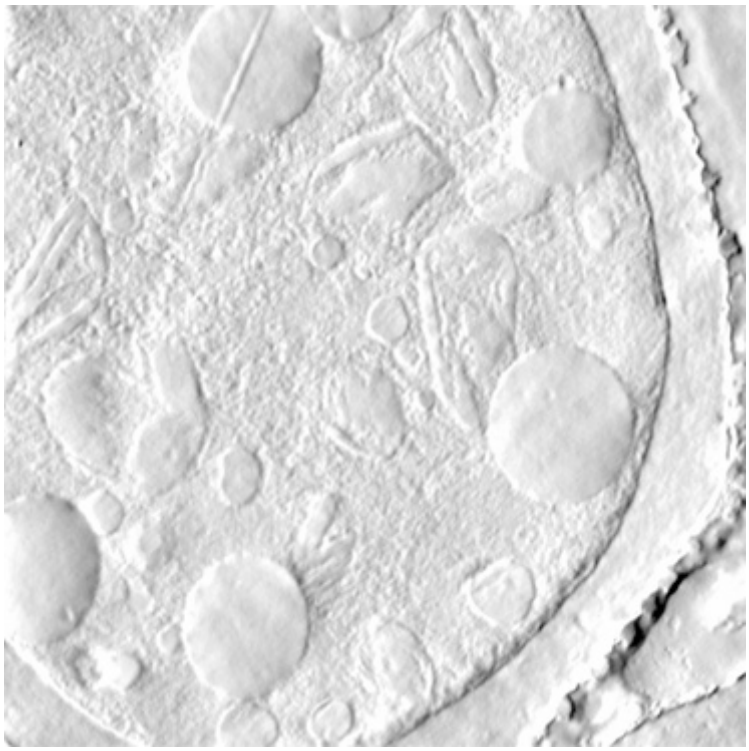
These 75-90mm thick sections were unstained and captured under a TEM at 4,600x magnification. Two defocused images were processed by QPI to generate a phase map.



Brightfield **QPI phase map**

Note that the in-focus brightfield TEM image above shows negligible contrast. The intensity modulation in the image was approximately ±10%. The QPI phase map provides enhanced visualization of phase dependent features.

A DIC image (shown below) was constructed with the phase data generated by QPI by simply differentiating the complex wavefield in a chosen direction. The QPI DIC image displays the characteristic DIC surface relief appearance.



QPI DIC

15.2 References

1. Quantitative Phase-Amplitude Microscopy II: Differential Interference Contrast Imaging for Biological TEM
P.J.McMahon, E.D.Barone-Nugent, B.E.Allman and K.A. Nugent, Journal of Microscopy, 206, 204-208 (2002).

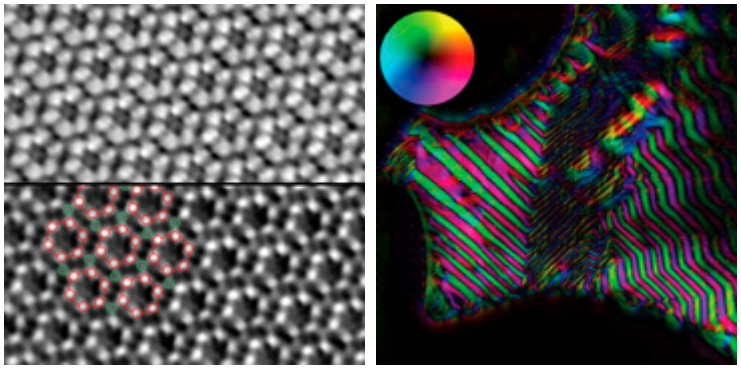
16 Product application

16.1 QPt for DigitalMicrograph™

HREM Research, Inc has developed a QPI plug-in for Gatan's DigitalMicrograph™ called QPt for DigitalMicrograph™.

QPt for DigitalMicrograph™ provides a digital solution to phase contrast electron microscopy. Fully integrated into Gatan's DigitalMicrograph™, images can be captured and processed directly in DigitalMicrograph™ to generate:

- a quantitative exit-wave phase image and eliminate artifacts (eg. spherical aberrations) generally apparent in a brightfield defocused images and enables correction without the need for expensive correctors (with additional HREM module)
- a magnetic field distribution around microscopic magnetic materials
- conventional optical phase visualization from one QPI phase image. These include Differential Interference Contrast (DIC), Zernike Phase Contrast (ZPC), Hoffman Modulation Contrast (HMC) and Dark-field images



Si_3N_4 atomic array **Manganese trioxide - magnetic field map**

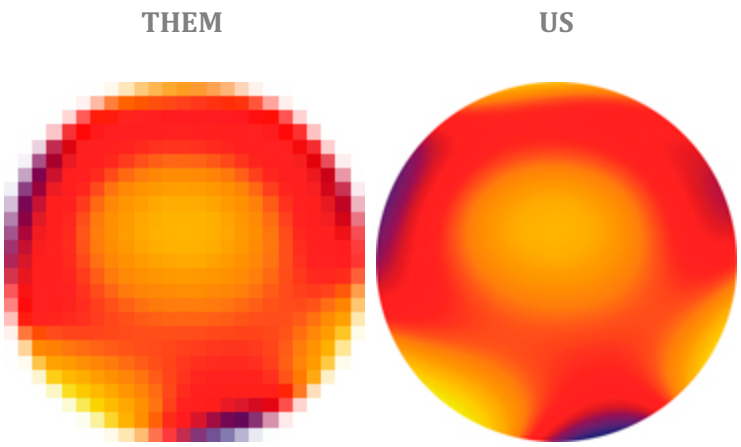
17 Ophthalmology

Iatia's QPI provides a digital ***megapixel wavefront*** imaging solution.

17.1 Megapixel wavefront

QPI derives its wavefront image by processing high resolution digital camera images.

In contrast, conventional Shack-Hartmann wavefront systems use resolution limited lenslet arrays.



Shack-Hartmann QPI Wavefront Technology

17.2 Higher order aberrations

QPI's high resolution wavefront images allow for the measurement of subtle and higher order optical aberrations.

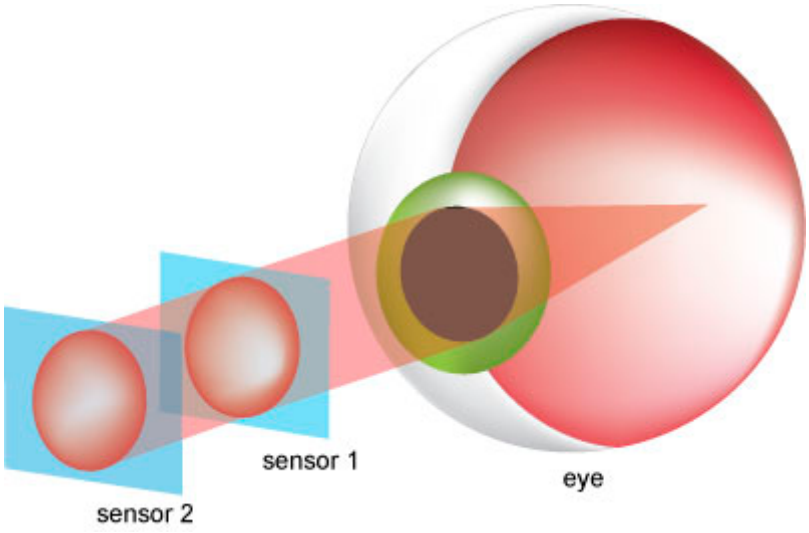
18 QPI wavefront technology

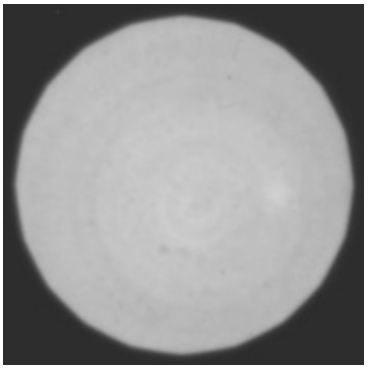
Iatia's QPI provides a digital ***megapixel wavefront*** imaging solution.

QPI generates its wavefront image from a minimum of two digital camera images at different focal planes. QPI's wavefront resolution is that of the digital imaging sensor (camera) which can be in the megapixels.

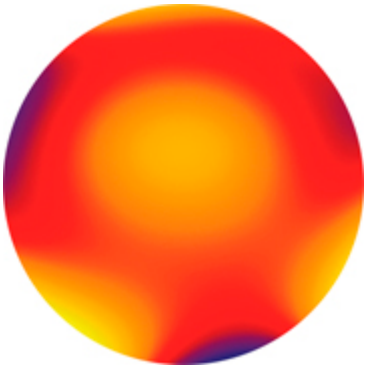
In contrast, conventional Shack-Hartmann wavefront systems use resolution limited lenslet arrays.

18.1 The QPI wavefront system





QPI wavefront technology sensor data



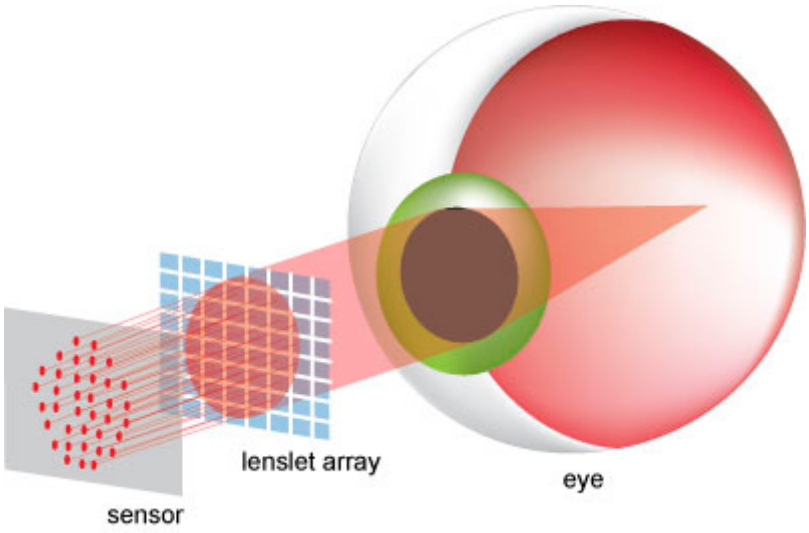
Processed QPI wavefront map

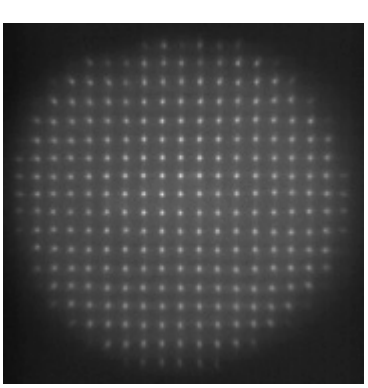
The QPI wavefront system requires two conventional digital images captured at different focal planes to generate a wavefront map. The resolution of the QPI wavefront map generated is limited only by the resolution of the digital sensor used rather than any specialised optics, such as a lenslet array in a Shack-Hartmann wavefront system.

The benefits of QPI's megapixel wavefront data include:

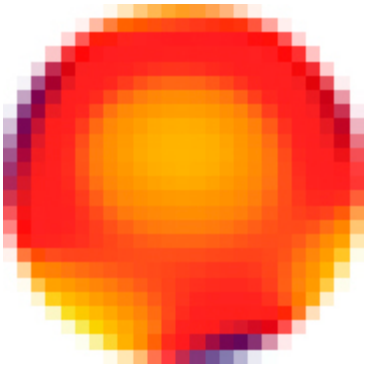
- increased dynamic range of application (ability to measure large lower order aberrations)
- sensitivity to higher order aberrations

18.2 Shack-Hartmann systems





Shack-Hartmann sensor data[1]



Processed Shack-Hartmann image (25x25 pixel array)

The Shack-Hartmann system consists of an array of lenslets of the same focal length. Each lenslet is focused onto a digital sensor. An incoming wavefront (from an eye) will cause a deviation of each lenslet's focal spot. This deviation corresponds to the local tilt of the wavefront. The measurement of deviations from each lenslet's focal spot allows the approximation of the wavefront.

Inherent limitations in the Shack-Hartmann system include:

- resolution is limited to the number of lenslets in an array
- lenslet light collection and focal spot centroiding are less effective the smaller the lens gets
- crossover of focal spots reduces the dynamic range of Shack-Hartmann systems.

- the system assumes that the wavefront is locally flat over the diameter of each lenslet. Large lower order aberrations and higher order aberrations can cause blurry and overlapping spots which are difficult or impossible to localize

18.3 Acknowledgements

1. "Shack-Hartmann sensor data" image courtesy of Dr Andrew Metha, Vision and Biophotonics Laboratory, Department of Optometry and Vision Sciences, The University of Melbourne, Australia.

19 Defence

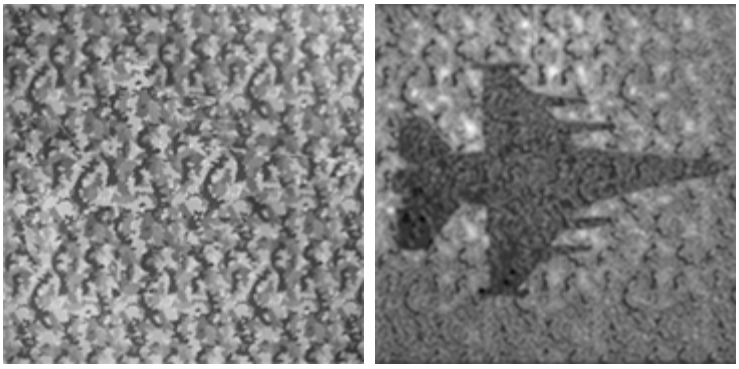
Iatia's QPI provides passive and covert depth imaging for defence and homeland security applications.

19.1 Passive ranging

By utilising available light or heat signatures, Iatia's technology is able to generate target ranging information passively and covertly without the expense and power requirements of active systems such as lasers.

19.2 Shape detection (camouflage negation)

QPI is capable of detecting the shape of camouflaged objects independent of their color or brightness contrast.



19.3 Other applications

Potential exists for other defence related applications of QPI:

- Imaging further in turbid media
- Enhanced discrimination for surveillance and face recognition
- Adding 3-D context to thermal and low-light imaging
- Camera/image aberration correction

20 Defence contracts

Iatia won an Australian Department of Defence, Defence Science and Technology Organisation (DSTO) Capability Technology Demonstrator (CTD) contract in June 2005 for up to $2.7 million over 3 years to transfer its unique imaging capability to Defence related applications.

Iatia's Brendan Allman, Alaster Meehan and Gavin Gregson testing the first field model long range (70m to 1.4km) camera at Puckapunyal in 2007. A handheld version is currently under development.

About the CTD contract

In late June 2005, Iatia signed a $2,700,000 contract with the Defence Department's Defence Science and Technology Organisation (DSTO). This Capability and Technology Demonstrator (CTD) contract is the largest announced by the Department under that round of CTD activities.

Defence represents a new field of application for Iatia's unique imaging technology. It utilises conventional digital imaging capability, and produces a new image based on shape information alone that is independent of the image provided by colour and contrast. This image is then suitable for further processing by conventional image enhancement, image-processing and image-recognition software.

The potential uses include passive ranging, shape imaging, defeating camouflage, and in developing novel image processing algorithms. Iatia is now developing a reflection-based algorithm suitable for extending shape imaging to greater distances.

21 QPI SDK

The QPI SDK (Software Development Kit) enables third-party developers and researchers to make use of the QPI algorithm within their own applications.

21.1 Key features

- Provided as a suite of easy-to-use COM objects
- Enables the generation of various phase modalities, including:
  - QPI Phase Maps
  - Differential Interference Contrast (DIC)
  - Zernike Phase Contrast (ZPC)
  - Hoffman Modulation Contrast (HMC)
  - Darkfield
- Includes optional GUI property pages for each modality
- Ideal for automation and special-purpose applications
- Consultancy services and integration assistance available

For sample images and more information about our technology please see our technology section.

Please contact us to arrange an evaluation or for purchase pricing and licensing information.

21.2 Downloads

- QPI SDK documentation (2MB)

22 QPI technology

The QPI algorithm enables the extraction of phase information from incoherent, polychromatic radiation without requiring special optical components. The algorithm can recover phase information from just two conventional brightfield images taken at slightly different focal planes.

The algorithm has a number of key advantages, including:

- returns phase and intensity information independently
- provides quantitative, absolute phase (with DC offset)
- is a rapid, stable, non-iterative solution
- works with non-uniform and partially coherent illumination
- offers relaxed beam conditioning
- solves the twin image problem of holography
- has been experimentally applied to a number of radiations

Iatia's QPI algorithm was developed by Professor Keith Nugent, then head of the School of Physics at the University of Melbourne, and his team. For a simple introduction to the technology, please see Professor Nugent's overview.

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23 An overview by Professor Keith Nugent FAA

The nursery rhyme 'Twinkle, Twinkle Little Star', the shimmer over a hot road and the network of bright lines at the bottom of a swimming pool on a sunny day all have their origins in phase. In fact, light is characterised by three main properties: colour, intensity and phase.

When light passes through a stained glass window its colour is changed. When light passes through a pair of sunglasses, its intensity (how bright it is) is changed. When it passes through a pair of prescription spectacles, the glass alters the phase of the light.

Many objects in nature are transparent. Obvious examples are air, glass and water, but think also of biological materials, eyes and, if you are 'seeing' with x-rays, even your aircraft carry-on luggage. Yet all transparent objects change the phase of the light - nothing is truly invisible to phase. It follows that any method that can 'see' phase can see things that are otherwise invisible.

Phase microscopy is a technique that has been around for a considerable time, with the first and most important development being due to the Dutch physicist, Frits Zernike, who received the Nobel Prize for this invention of phase-contrast imaging in 1953. Zernike's work was the first to allow a phase image to be seen. But the phase in the image could still not be measured.

I have spent much of my professional life trying to invent new ways of seeing things. For the most part, I have been looking at new developments in x-ray imaging and it was this work that led me to think about phase in a new way. With my colleagues and students, I set out to use my insights to develop new ways that would allow the phase in an image to be measured.

The obvious approaches to the problem turned out to be mathematically difficult and totally impractical. However, in 1998, with my student Dr David Paganin (now at Monash University) we developed an approach that seemed to have the promise of being simple, fast and very practical. With another student, Dr Anton Barty (now at the University of California), we showed that the methods could indeed be very effectively applied to optical, and then electron, microscopes. Their results were able to reveal - and measure - the phase in an image using clever calculations, but completely standard hardware. It was an extraordinarily flexible method.

The Paganin-Barty-Nugent technique has subsequently been used to also solve problems in x-ray, neutron and atom imaging. The international scientific interest in these new methods exploded and it rapidly became clear that the methods being

developed by my team could be applied to a whole range of both practical and scientific problems.

We saw that this work had many commercial possibilities and so, with Drs Paganin and Barty, we took out a patent covering our new methods. This is the core patent licensed to Iatia Limited. Iatia has developed commercial packages for optical and electron microscopy. Iatia is now beginning to explore and develop the myriad other areas that can benefit from quantitative phase imaging methods.

23.1 Inside QPI

Our core algorithm, QPI (Quantitative Phase Imaging), provides a unique solution to the Transport of Intensity Equation.

The algorithm enables the extraction of phase information from incoherent, polychromatic radiation without requiring special optical components. The algorithm can recover phase information from just two conventional brightfield images taken at slightly different focal planes.

The algorithm has a number of key advantages, including:

- Returns phase and intensity information independently
- Provides quantitative, absolute phase (with DC offset)
- Is a rapid, stable, non-iterative solution
- Works with non-uniform and partially coherent illumination
- Offers relaxed beam conditioning
- Solves the twin image problem of holography
- Has been experimentally applied to a number of radiations

23.2 Recovery of the phase information

The algorithm is able to recover the phase information from just two images taken at slightly different focal planes, though a third image taken at the point of best focus is generally used for normalisation.

Figure 1 below shows the recovery of the phase information for an optical fibre.

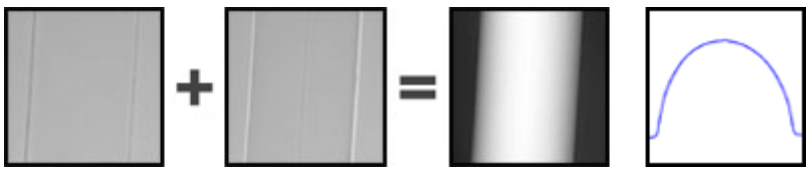


Figure 1: generating a phase image of an optical fibre

In the image above we can see the two brightfield images which were acquired with a conventional digital camera attached to a standard microscope. The images were taken at focal points approximately 5 microns either side of the point of best focus.

By applying the QPI algorithm to these images the phase image shown above can be calculated. The time required to generate the phase image from the 0.41 megapixel source data is approximately 1.5 seconds on a 2.4GHz Pentium IV.

Because the phase data provided by the QPI algorithm is quantitative is it now possible to make absolute measurements of the object's properties, including its thickness, refractive index, and so on.

A plot through the phase image shows the projected thickness of the circular cross section.

23.3 Behind the algorithm

The problem with a wave's phase is that it is lost when we can only measure the irradiance of the wave.

If we consider a wave propagating in the +z direction that has an amplitude $u_o(x,y)$ and phase $\Phi(x,y)$ such that

$$u(\vec{r}) = u_o(x,y)e^{i(kz+\phi(x,y))}$$

experimentally we only measure the irradiance

$$I = |u(\vec{r})|^2 = |u_o(x,y)|^2$$

thus we lose the phase information.

In order to recover the phase various optical techniques have been developed including interferometry, Zernike & Schlieren phase contrast, perfect crystal analysis, and various iterative propagation-based phase contrast methods. These techniques all require additional optical components, and traditionally are limited to measurements modulo 2π or computer-intensive iterative methods. This is not the case with our algorithm.

Iatia's QPI algorithm makes use of the paraxial approximation of the propagation of intensity distribution as described by the Transport of Intensity Equation (Teague, 1983)

$$k\partial_z I(\vec{r}) = \nabla_\perp \bullet \left[I(\vec{r}) \nabla_\perp \phi(\vec{r}) \right]$$

Given an intensity with no zeroes, and its longitudinal derivative, we can solve uniquely for the phase, up to an additive constant (the phase can't be known absolutely).

Solving for phase in the Transport of Intensity equation we get

$$\phi = -\bar{k}\nabla_{\perp}^{-2}\left\{\nabla_{\perp}\bullet\left[\frac{1}{\bar{I}}\nabla_{\perp}\nabla_{\perp}^{-2}\partial_z\bar{I}\right]\right\}$$

In formulating this solution we have used a generalised notion of phase where we regard the energy flow vector of a wave as the important quantity, not the amplitude or phase. This formulation is applicable even for polychromatic radiation where the notion of phase is not well defined.

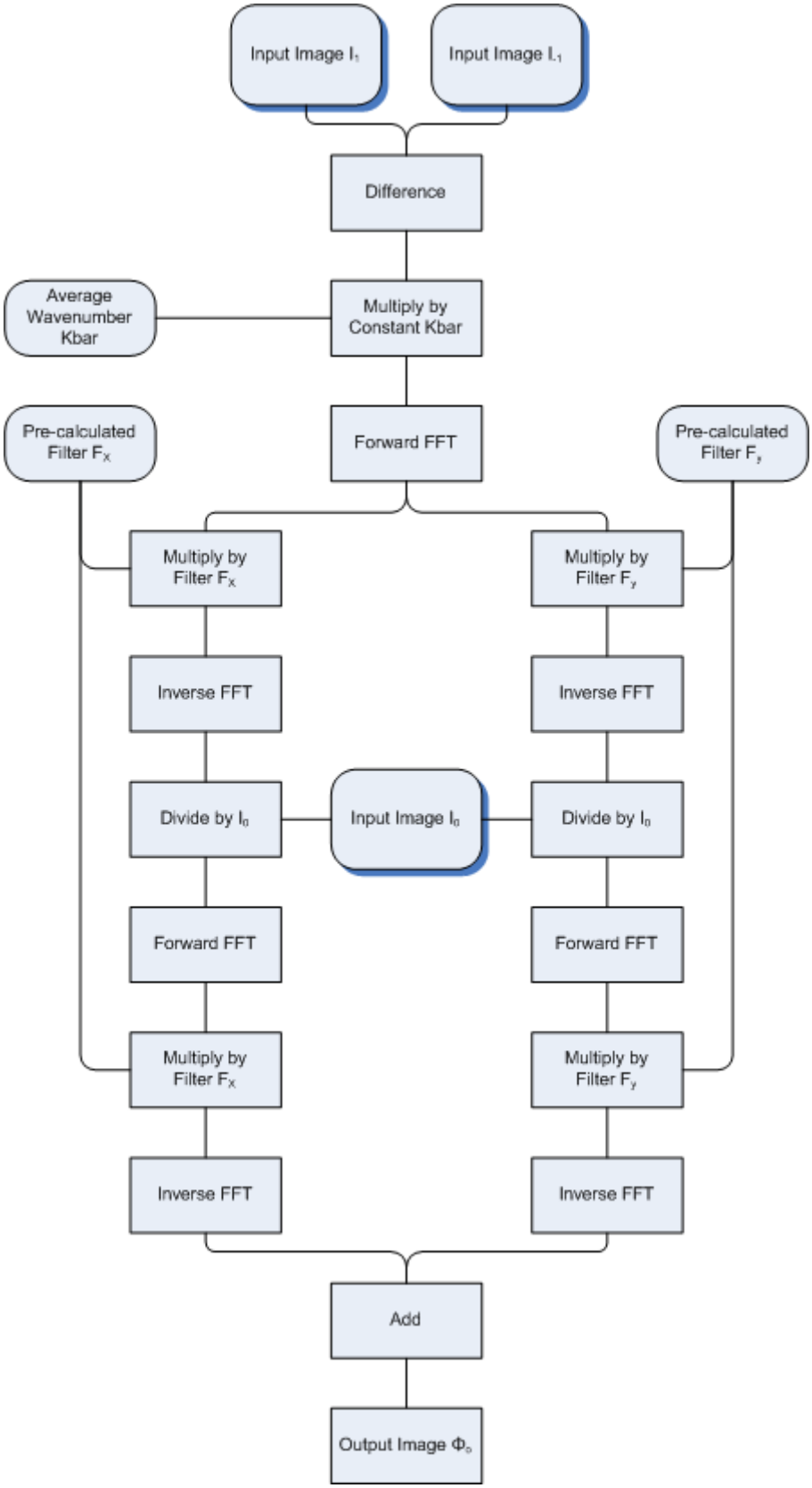
The solution for the TIE phase may be coded using Fast Fourier Transforms. This coding is the QPI algorithm, a flowchart is provided later in this document.

23.4 Derivation of the transport of intensity equation

The Transport of Intensity equation is derived from the hydrodynamic continuity equation. Parallels between the hydrodynamic formulation of non-relativistic quantum mechanics and classical scalar wave optics are shown below.

	non-relativistic quantum mechanics (Schrödinger)	**monochromatic scalar electromagnetic waves (Helmholtz)**
wave function	$\Psi(\vec{r}) = \sqrt{\rho(\vec{r})}e^{iS(\vec{r})/\hbar}$	$u(\vec{r}) = \sqrt{I(\vec{r})}e^{i\phi(\vec{r})}$
wave equation	$\left(\nabla^2 + 2m(E-V)/\hbar^2\right)\Psi(\vec{r}) = 0$	$\left(\nabla^2 + k^2\right)u(\vec{r}) = 0$
momentum density	$\vec{j} = \rho\nabla S/m$	$\vec{S} = I\omega\nabla\phi/4\pi$
continuity equation	$\nabla\bullet[\rho\nabla S] = 0$	$\nabla\bullet[I\nabla\phi] = 0$
paraxial approximation/TIE	$k\partial_z\rho(\vec{r}_{\perp}) = \nabla_{\perp}\bullet[\rho(\vec{r}_{\perp})\nabla_{\perp}S(\vec{r}_{\perp})]$	$k\partial_z I(\vec{r}_{\perp}) = \nabla_{\perp}\bullet[I(\vec{r}_{\perp})\nabla_{\perp}\phi(\vec{r}_{\perp})]$

24 Flowchart of the QPI algorithm



25 Application notes

Our QPI technology has wide applicability across a range of disciplines and application areas. The application notes presented here demonstrate some of the ways that the QPI algorithm can be applied.

25.1 Optical microscopy

Measurement of Red Blood Cell Volume Changes
Researchers at the University of Melbourne's Physiology department utilised QPm phase images to measure red blood cell volume changes in response to osmotic stimuli.

Measurement of Area Changes
Researchers at the University of Melbourne's Pharmacology and Physiology departments utilised QPm phase images to measure the area of human airway smooth muscle cells, as a means of tracking growth rates over a period of time.

Dual Imaging for Co-localisation
QPI Phase Images are capable of showing morphological and physiological changes within samples as a supplementary technique or alternative to more time consuming and toxic fluorescence techniques.

Automated Cell Counting
Pure quantitative phase images, which measure changes in optical density throughout a sample, allow for improved contrast for thresholding purposes in automated cell counts when compared with a fluorescence image.

Unstained Immunoperoxidase Labelled Paraffin Sections
Phase Maps and Intensity-free modalities allow for the removal or inclusion of absorption effects of peroxidase which obscure underlying sample information.

Identification of Cellular Structures
QPI Phase Images provide quantitative phase and intensity data which may be used to identify cellular structures.

Comparison with Optical Phase Contrast Modalities
QPI digitally constructed phase contrast modalities compared with conventional optical phase contrast techniques.

Intensity-Free Phase Imaging
Intensity-free phase contrast modalities allow the removal of effects of highly absorptive structures in samples which may obscure underlying information.

25.2 Confocal microscopy

QPm Application with Confocal Microscopes
QPm and QPt are capable of generating the full suite of phase contrast modalities to aid in

the co-localisation of fluorescence stain information without the need for additional phase contrast optics which may adversely affect the intensity of fluorescence information or cause a misalignment in the images captured.

Study on Cat Retina Degeneration
QPm and QPt are capable of generating phase contrast images for co-localisation purposes without the need for phase contrast optics or hardware.

25.3 Electron microscopy

Recognition of Melanosomes
QPe and QPt TEM Phase Contrast images assist in the recognition of melanosomes.

Differential Interference Contrast for TEMs
QPe and QPt DIC representations of samples aids in interpretation of complex structures.

Magnetic Field Imaging
QPe allows users to extract the magnitude and direction of the magnetic field surrounding the sample.

25.4 Forensics

Magnetic Audio Tape
QPm and QPt Phase Images provide clear surface topography information free of obscuring intensity data to allow confirmation of matches between recorded audio tapes and recording heads.

Forged Cheques
QPm and QPt Phase Images show clearer surface topography information providing additional valuable information for the matching of print to impact printers.

Reconstruction of Defaced Engine Block Numbers
QPm and QPt Phase Images show clearer surface topography information providing additional valuable information in the identification of ground engine block or serial numbers.

Line Drawing Order
QPm and QPt images are better able to provide surface topography and depth information free of obscuring intensity data (such as ink) to allow more accurate analysis of pen strokes.

26 Papers

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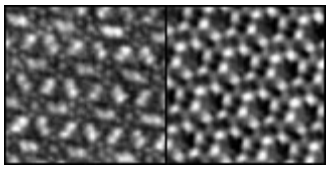
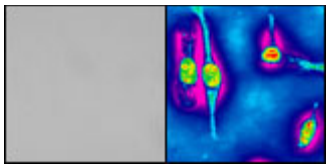
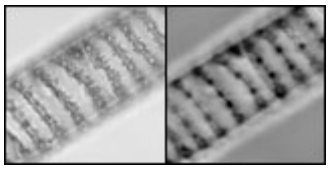
27 Sample images



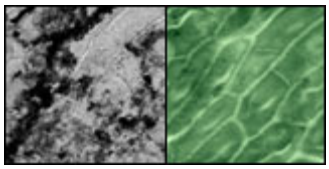
Silicon Nitride atomic resolution images



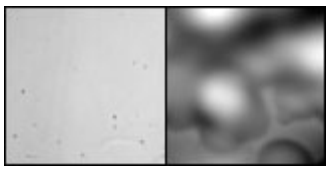
Rat mast cells



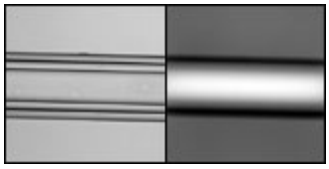
Spirogyra



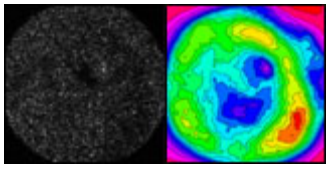
Leaf with coal dust contamination



Non-uniformities in a thin film coating



Optical fibre



Eye wavefront