Putting People Under the Microscope

A physicist's journey from a world of atoms to a world of living matter

Dr Steve Barrett 27 Nov 2014

Introduction

A World of Atoms	Imaging atoms, molecules and nanostructures
Perception vs Reality	Why can image analysis be such a challenge?
The Spin-Offs	Applications in earth sciences and medical sciences
A World of Living Matter	Imaging more complex systems
Investigating Cancer	Spectromicroscopy and infrared absorption

Introduction

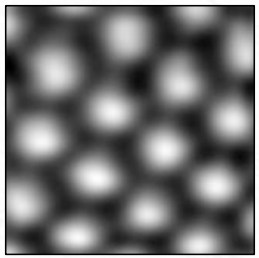
This talk is about images and how we look at images in a scientific context. Two concepts are important in what follows:

Image Processing > > > ***Interpretation***

Image Analysis > > > ***Quantification***

The talk will be illustrated with images from research projects old and new, from collaborators and from project students.

A World of Atoms



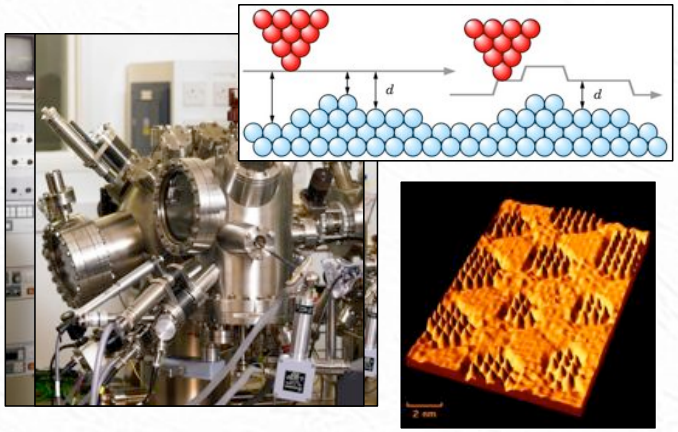
850 pm

On this scale, a grain of sand would be about the size of the Moon.

" To see a world in
a grain of sand … "

William Blake

A World of Atoms



A World of Atoms

Working with STM images led to the development of software that supports various scanning microscopy systems:

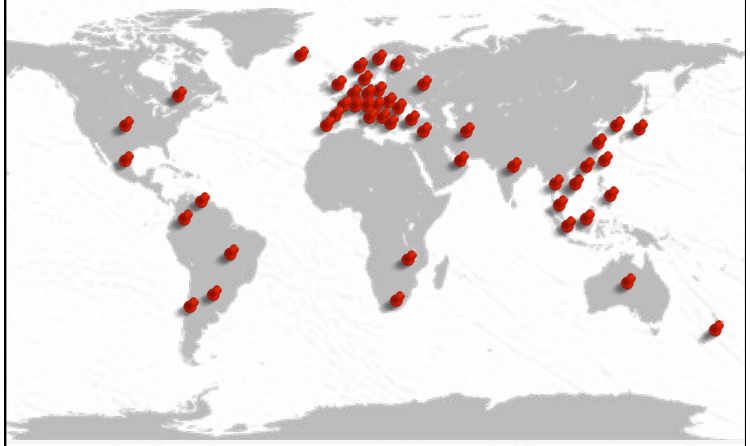
Scanning Tunnelling Microscope

Referring to any/all of these as SXM led to the unpronounceable:

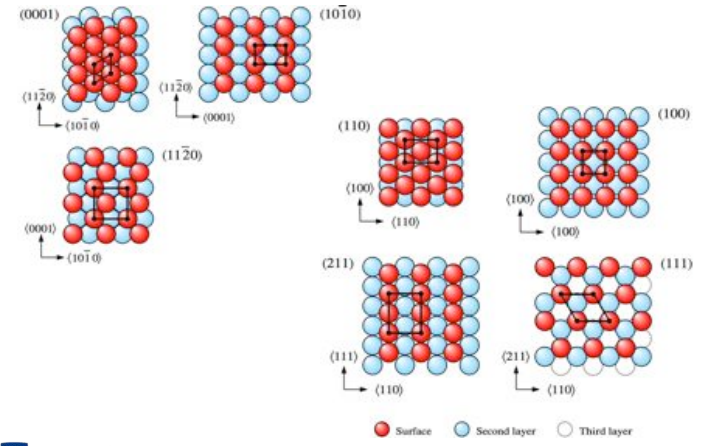
Image SXM
v 1.97
August 2014
Steve Barrett

> 40,000 downloads in the past 10 years by universities and research centres

Image SXM



A World of Atoms



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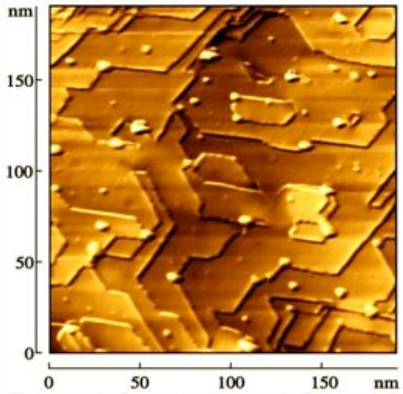
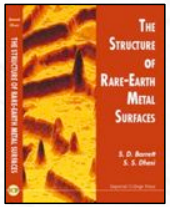
A World of Atoms

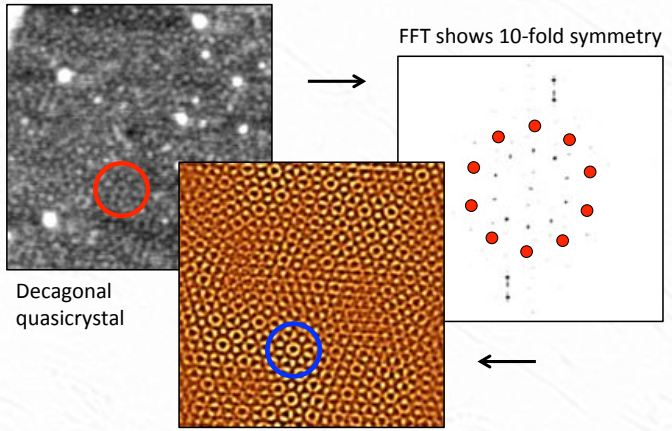


Sc(0001)

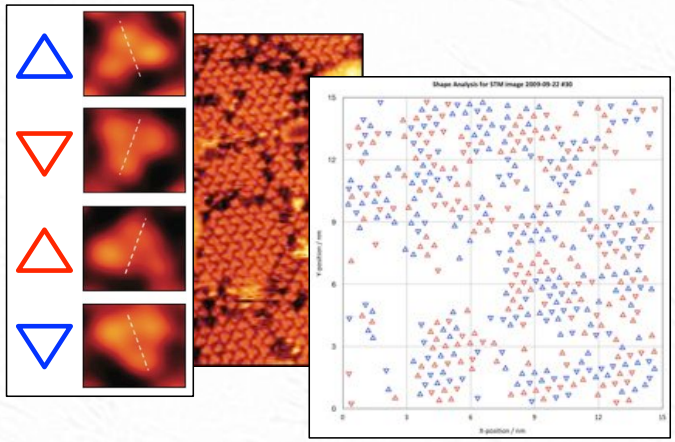
For many years I studied the rare-earth metals using a combination of spectroscopy, microscopy and diffraction techniques



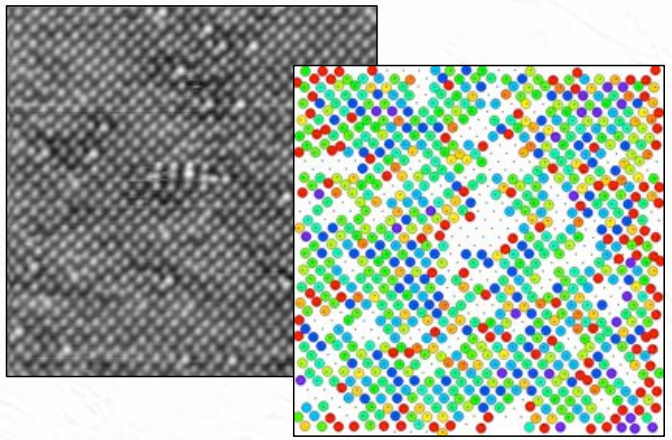
A World of Atoms



A World of Atoms



A World of Atoms

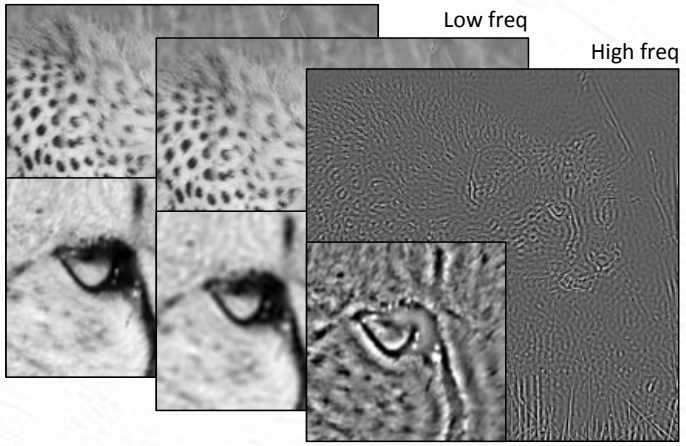


Beyond Microscopy

Image processing techniques can of course be applied to any image. But is there any point in, for instance, applying a Fourier transform to an image that has no discrete frequency components?

What does the Fourier transform of a cheetah look like?

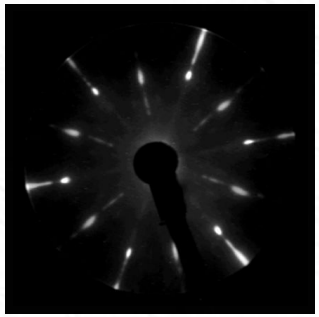
Beyond Microscopy



Beyond Microscopy

Image SXM has also been used to analyse low-energy electron diffraction (LEED) images of surfaces.

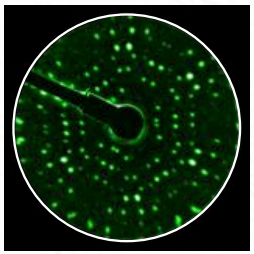
The variation of diffraction spot intensities as a function of electron energy gives information on crystal structures.



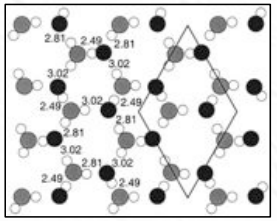
Electron energy = 40–150 eV

Beyond Microscopy

LEED images obtained from a mixture of OH and H_2O on a platinum surface – a system of relevance to hydrogen fuel cells – have been used to determine the crystal structure of the 'ice'.



LEED pattern



Structure of OH and H_2O

Astrophotography

The Milky Way imaged from Teide Observatory during the UoL field trip in 2013. Due to the dark skies, very little image processing is required.

Astrophotography

NGC7000 North America Nebula

Single raw image

However, under the light-polluted skies of the UK, image processing can bring out hidden structures in a faint nebula.

Astrophotography

NGC7000 North America Nebula

Single raw image | 20 images stacked in *Image SXM* | Colours enhanced

A World of Atoms	Imaging atoms, molecules and nanostructures
Perception vs Reality	**Why can image analysis be such a challenge?**
The Spin-Offs	Applications in earth sciences and medical sciences
A World of Living Matter	Imaging more complex systems
Investigating Cancer	Spectromicroscopy and infrared absorption

Perception vs Reality

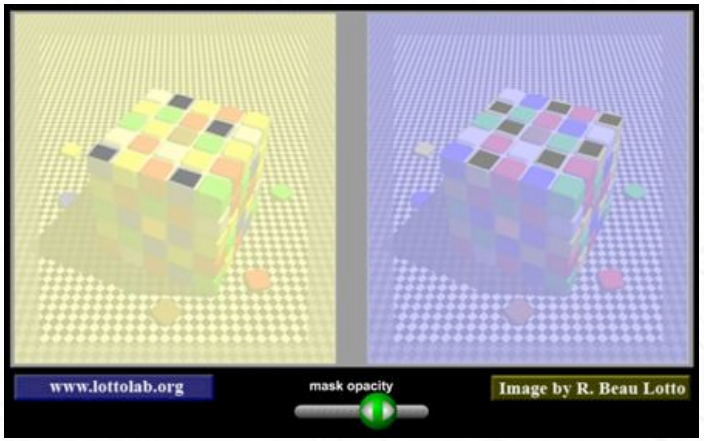
How we perceive images (what we ***see***) can be VERY different from the actual information content (what is ***there***). In most day-to-day situations we trust the former and don't worry about the latter.

Which is the better image processor?

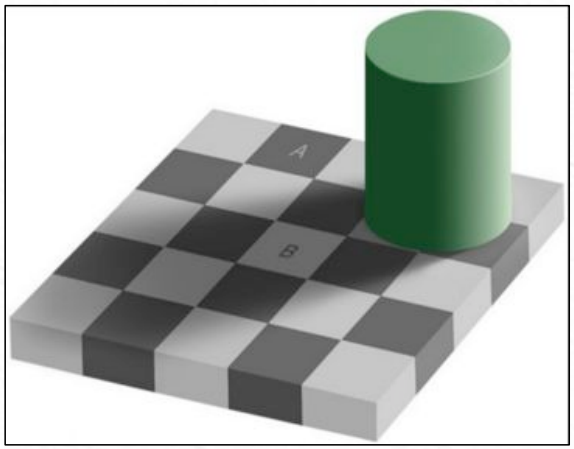
Brain vs ***Computer***

Carbon vs ***Silicon***

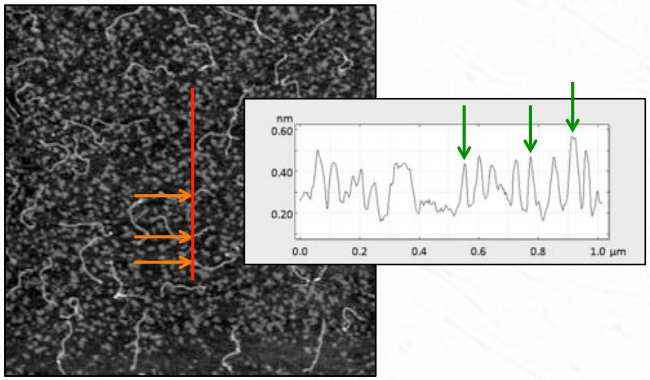
Perception vs Reality



Perception vs Reality

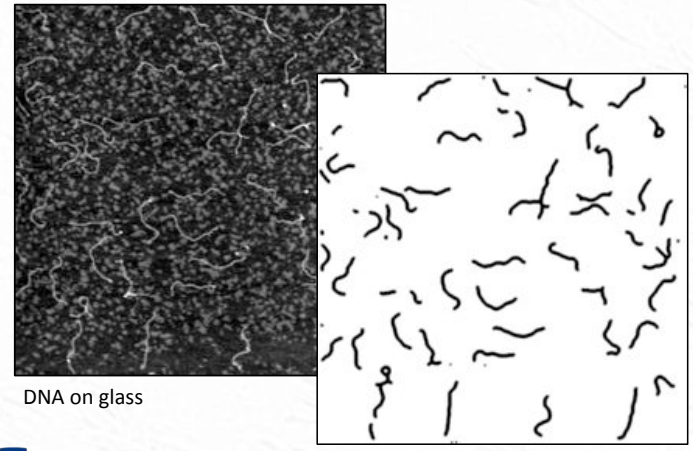


Perception vs Reality



DNA on glass

Perception vs Reality



DNA on glass

Spin-Offs

This is what I do – I find solutions to problems that have not (yet) succumbed to conventional analysis.

I do not have a strong allegiance to any particular techniques or to any algorithms that I have developed. Rather, I prefer to think of every new image as an opportunity to ask…

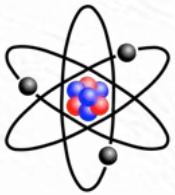
What techniques might be applied to this image?

Will a combinations of existing techniques be enough?

Will a new approach, a new algorithm, be needed?

Spin-Offs

Selecting the 'right' problem that needs addressing is part of what research is all about. The ideal problem lies somewhere between trying to understand…



Too simple

Way too complicated

Spin-Offs

Applications to disciplines beyond physics and chemistry were a natural consequence of the interdisciplinary nature of image analysis. In particular…

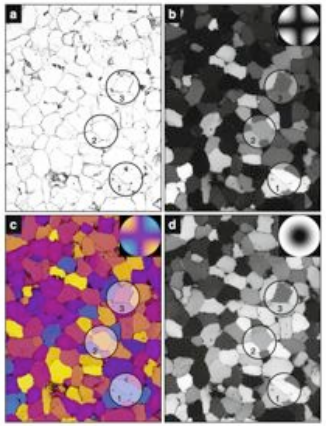
Earth Sciences — *PrinCIPia*

'Principles of Computer Integrated Polarisation Image Analysis'

Medical Sciences — MIASMA

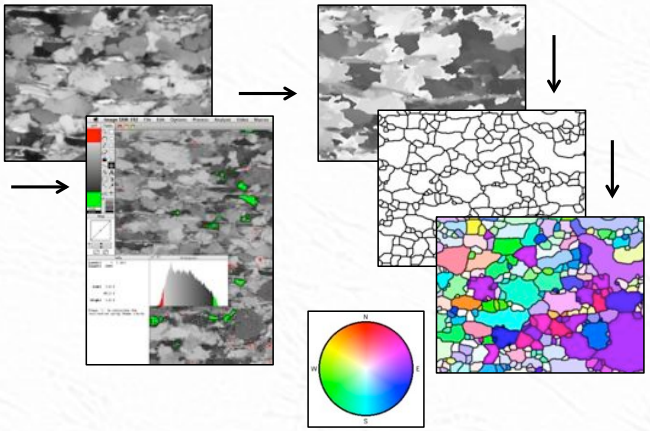
'Microscopy Image Analysis Software for Medical Applications'

Earth Sciences

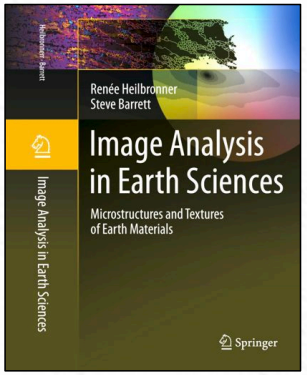


Imaging earth materials (to a physicist that means 'rocks') using combinations of circularly and linearly polarised light produces colours and intensities that depend on the orientation of the crystallographic axes of the grains with respect to the optical axis of the microscope.

PrinCIPia



Earth Sciences



Ongoing collaboration with Professor Heilbronner at the University of Basel led to a book on Image Analysis …

… available at a reasonable price from the author.

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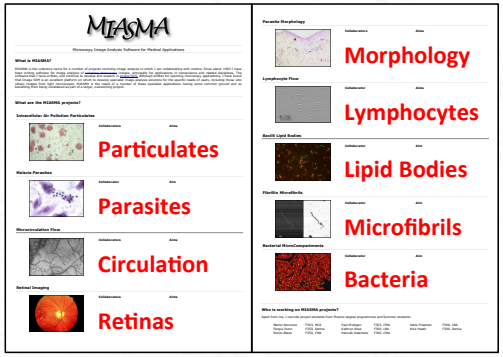
Medical Sciences

Medical spin-offs have expanded considerably in the past few years:

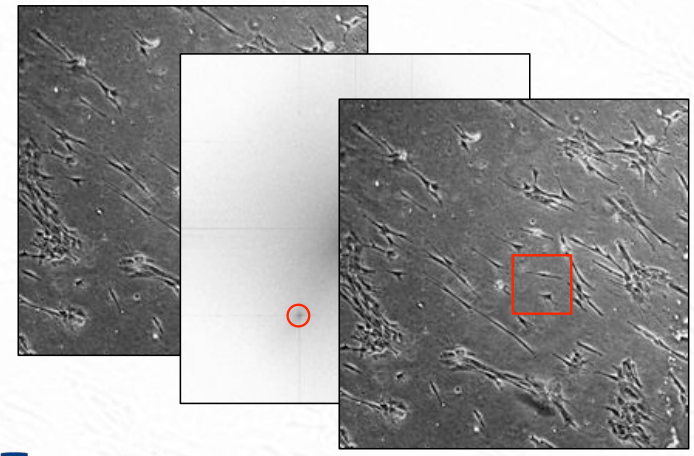


Medical Sciences

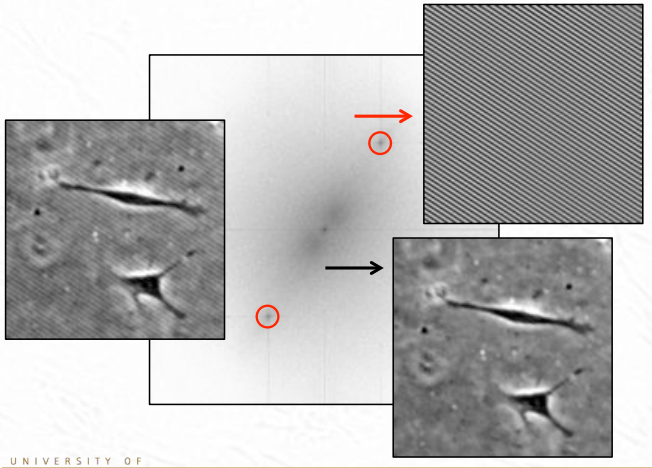
Image analysis techniques that have become established in other disciplines sometimes lend themselves very neatly to problems in medical science.

In the following example, fibroblast cells are grown on a grooved substrate to see if the grooves (with a 3 µm pitch) affect the growth of the cells.

Medical Sciences



Medical Sciences



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Investigating Cancer	Spectromicroscopy and infrared absorption

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A World of Living Matter

Now very much in the world of living matter, we will take a closer look at two ongoing research projects in which image analysis is playing a key role:

Microcirculation Analysis

Investigation of Cancer

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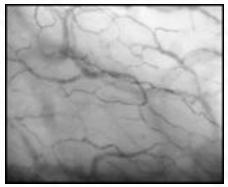
Microcirculation Analysis

In collaboration with consultants at Alder Hey hospital, the first trials of MIASMA software are being conducted on patients in the intensive care unit. Some of these patients suffer from meningitis, causing sepsis (aka blood poisoning).

The software quantifies the flow of blood cells through a capillary network, the *microcirculation*, as imaged by a small portable microscope placed underneath the tongue of the patient.

Not so much
Putting People Under the Microscope
but rather
Putting the Microscope Under People.



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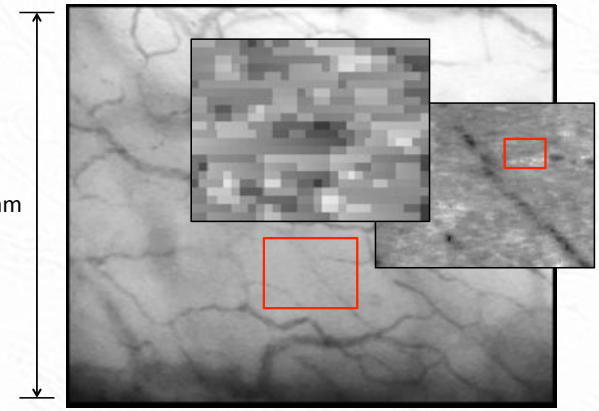
Microcirculation Analysis



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Microcirculation Analysis

Bear in mind that the blood vessels are invisible (as only the blood cells, containing haemoglobin, are imaged).

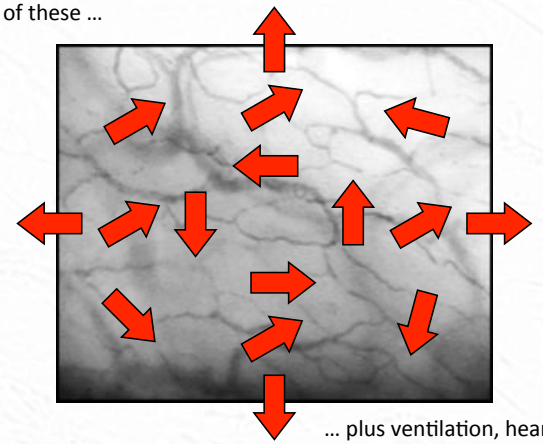
So the problem is to identify and quantify the motion of a blood cell relative to an invisible vessel in a sequence of video images that are not stable – ever tried to get a five-year old to sit still while you place a microscope under his tongue?

Any attempt at quantification will first have to deal with…

… Translation … Magnification … Rotation … Distortion …

Microcirculation Analysis

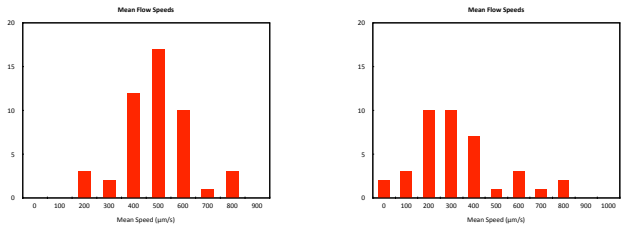
Or all of these …



… plus ventilation, heartbeat

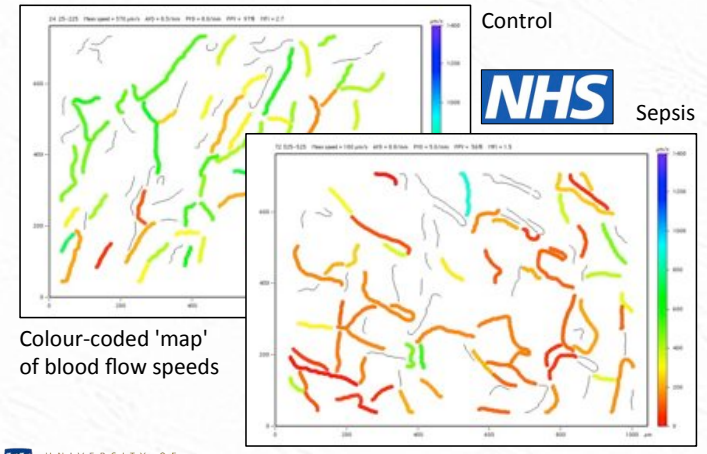
Microcirculation Analysis

Through a combination of techniques, including cross-correlations (to stabilise the video images) and autocorrelations (to identify the motion of blood cells that are barely detectable) it is possible to quantify the blood flow speeds in vessels as small as 7 µm diameter.



Control Sepsis

Microcirculation Analysis



Control

NHS Sepsis

Colour-coded 'map' of blood flow speeds

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Investigating Cancer	**Spectromicroscopy and infrared absorption**

Investigating Cancer

This final section will cover the preliminary results of the research carried out under the EPSRC critical mass grant

"***Disease diagnosis through spectrochemical imaging of tissues***"

(Weightman, Martin, Barrett + Cockcroft, Lancaster, Manchester, Cardiff)

Roughly speaking, that translates to…

Can we identify an infrared absorption signature for tissue that is likely to become cancerous?

or…

Can we detect cancer before it is cancer?

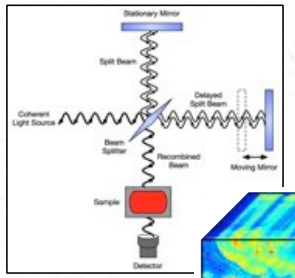
Investigating Cancer

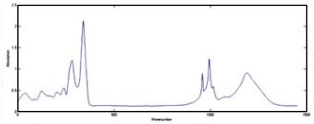
What tissues are being studied?

We started with oesophageal cancer, and its precursor called Barrett's oesophagus (no relation, as far as I am aware):

A condition in which the tissue lining the oesophagus is replaced by tissue that is similar to the intestinal lining (intestinal metaplasia). People with Barrett's oesophagus have an increased risk for developing oesophageal cancer.

Investigating Cancer





An infrared absorption spectrum at every pixel

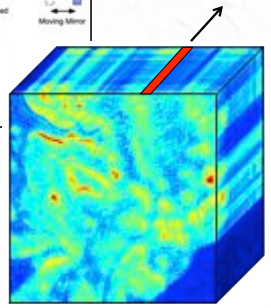


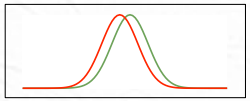
Image with spatial resolution ~5 µm

Question: Can we use the IR absorption at different wavelengths to identify the tissue type?

Investigating Cancer

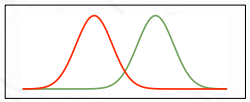
In general, infrared absorption at different wavelengths is very similar even for different tissue types. So, what wavelengths should we use to discriminate one (abnormal and potentially cancerous) tissue type from another (normal and healthy) type?

Certain pairs of wavelengths are much better than others, and they're not necessarily the ones we would have guessed by looking at the spectra.



Poor choice

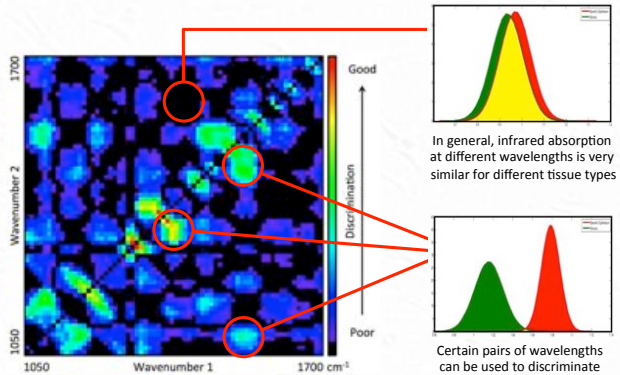
Making histograms of the ratios of the values of IR absorption at different wavelengths shows this very clearly.



Good choice

Histograms of ratios of IR absorption for abnormal (red) and normal (green) tissue

Investigating Cancer



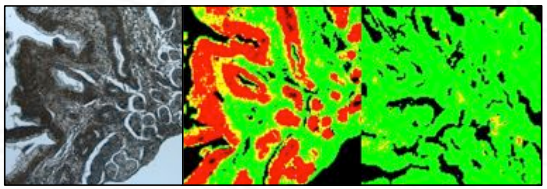
In general, infrared absorption at different wavelengths is very similar for different tissue types

Certain pairs of wavelengths can be used to discriminate abnormal from normal tissue

"Butterfly diagram"

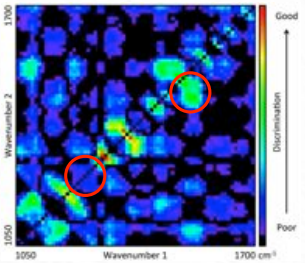
Investigating Cancer

Selecting the best discrimination from the butterfly diagram, we can generate a map identifying different tissue types.

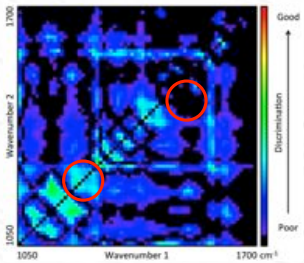


Visible image | Abnormal (red) and normal (green) | Normal tissue only

Investigating Cancer



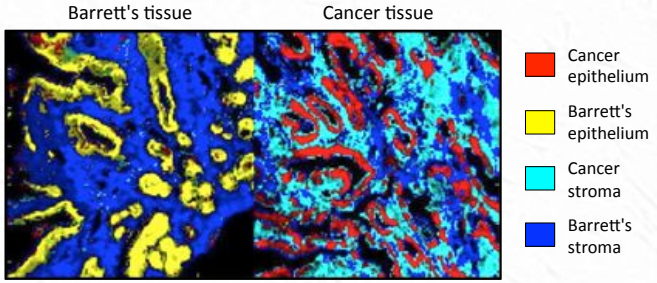
Wavelengths that discriminate Barrett's Oesophagus from stroma are complementary to …



… wavelengths that discriminate cancer cells from stroma

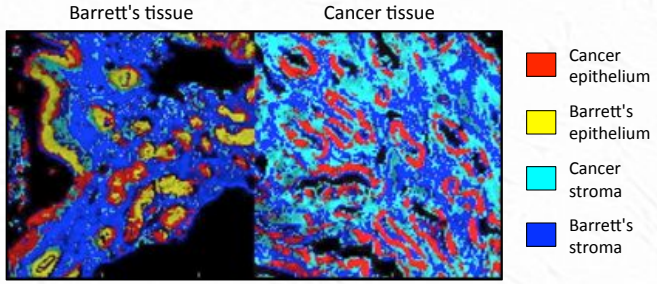
Investigating Cancer

This idea was then extended to identify more than two tissue types…



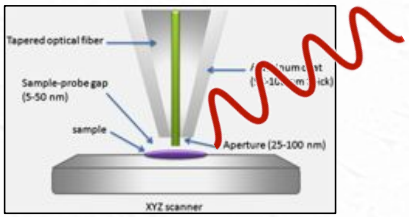
Investigating Cancer

… and then tested on tissues not used to 'train' the analysis routine.



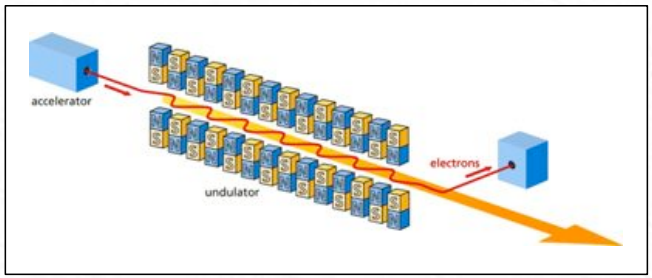
Investigating Cancer

To improve the spatial resolution we need to beat the diffraction limit using Scanning Near-Field Optical Microscopy (SNOM).



Imaging with sub-µm resolution requires plenty of infrared photons to illuminate the sample underneath the scanning tip. This is where a free-electron laser that operates in the infrared comes in.

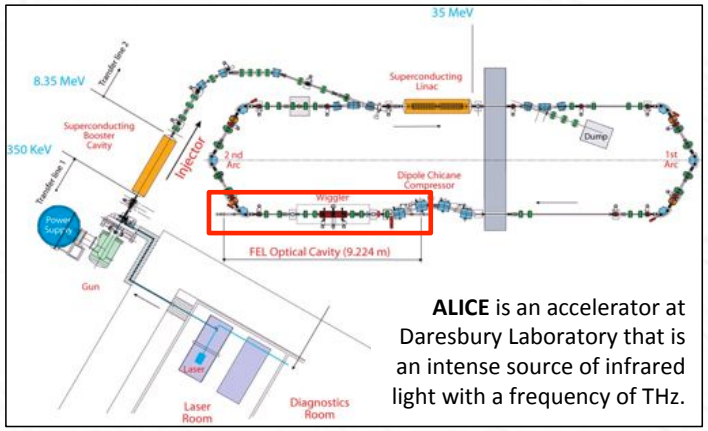
Free-Electron Laser



An array of magnets with alternating N-S orientation causes the electron beam to 'wiggle' and emit intense beams of synchrotron radiation. The strength and period of the magnet array determines the wavelength of the emitted radiation.

Putting People Under the Microscope

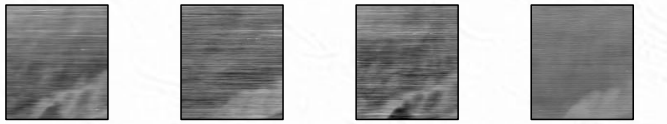
Free-Electron Laser



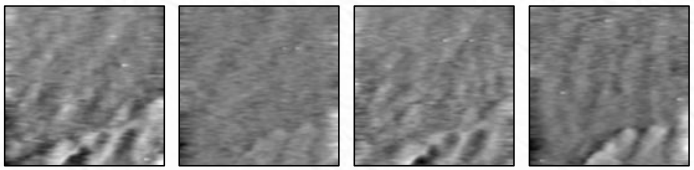
ALICE is an accelerator at Daresbury Laboratory that is an intense source of infrared light with a frequency of THz.

SNOM Imaging

6.25 µm 6.50 µm 7.30 µm 8.05 µm

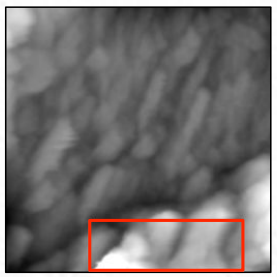


Raw images as acquired by the SNOM at different IR wavelengths

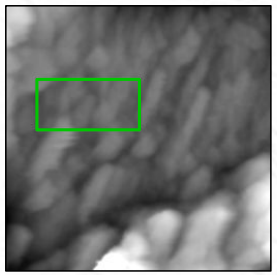


Processed to remove artefacts and make features easier to see

Image Correlation



Choose two regions of the image that are (thought to be) cancerous and healthy, respectively.



Then look at correlations between the different SNOM images.

Image Correlation

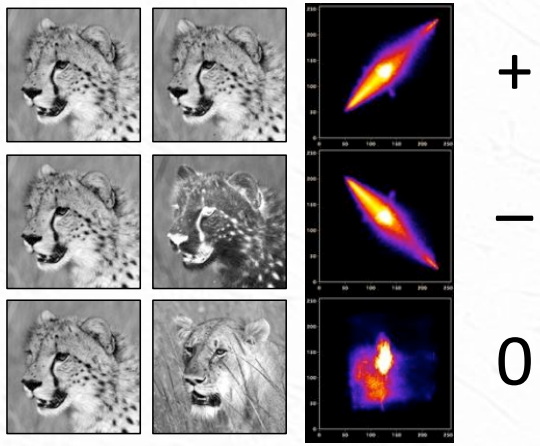


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Image Correlation – Cancer

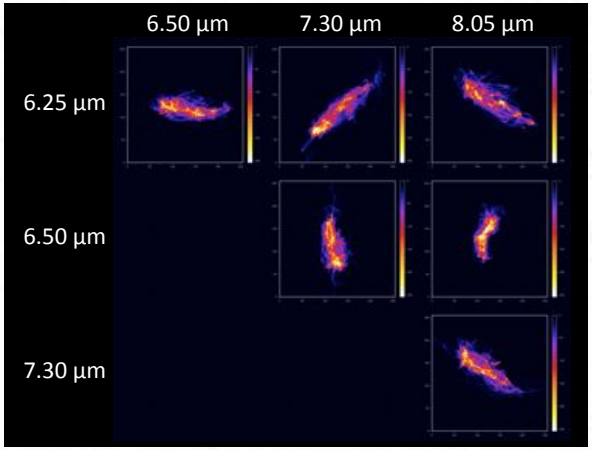


Image Correlation – Healthy

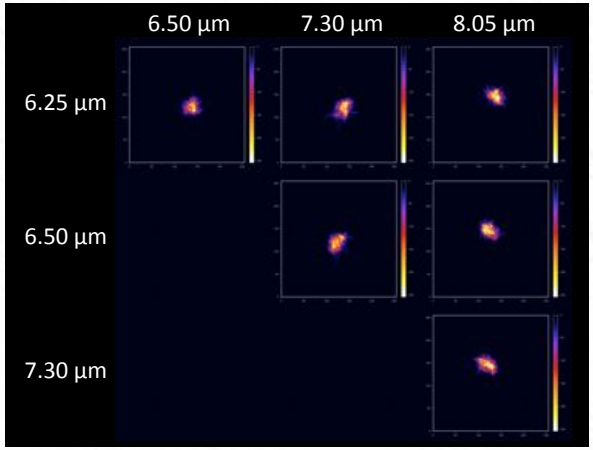


Image Correlation – Cancer

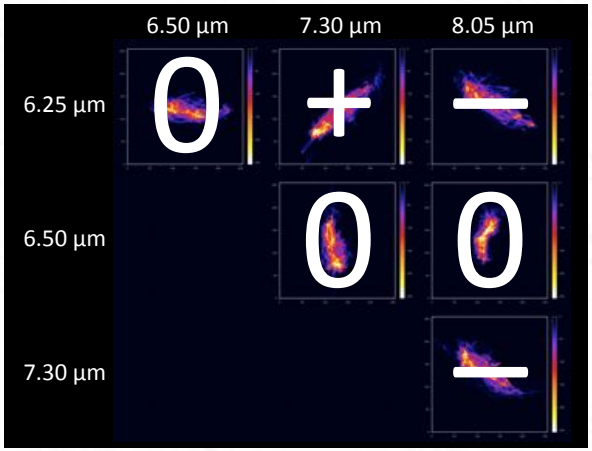


Image Correlation – Healthy

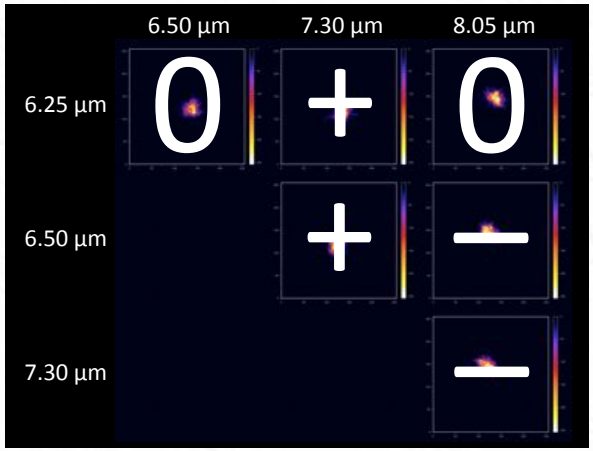
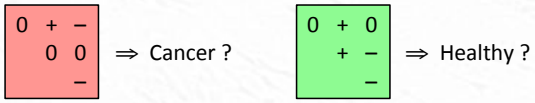


Image Correlation

Can the patterns of correlations between images taken at different wavelengths provide the 'signatures' of cancerous, pre-cancerous and healthy tissue?



The research is still in the early stages, but the results of the analysis to date indicates that we have found a technique and a method of analysis that has the potential to do just that.

Summary

A World of Atoms	Imaging atoms, molecules and nanostructures
Perception vs Reality	Why can image analysis be such a challenge?
The Spin-Offs	Applications in earth sciences and medical sciences
A World of Living Matter	Imaging more complex systems
Investigating Cancer	Spectromicroscopy and infrared absorption

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Putting People Under the Microscope

http://www.liv.ac.uk/~sdb/Talks

Dr Steve Barrett 27 Nov 2014