PHYS871 Clinical Imaging Applications

MIASMA

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University of Liverpool

April 2016

Microscopy Image Analysis Software for Medical Applications

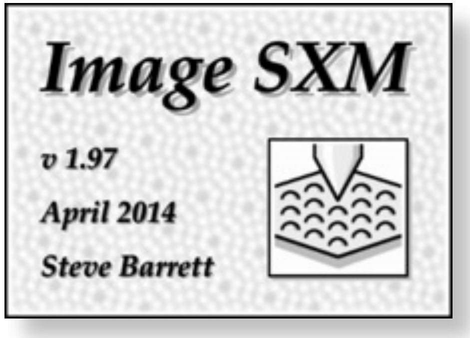
What is MIASMA?

A brief description of some of the projects

A more detailed look at two of the projects

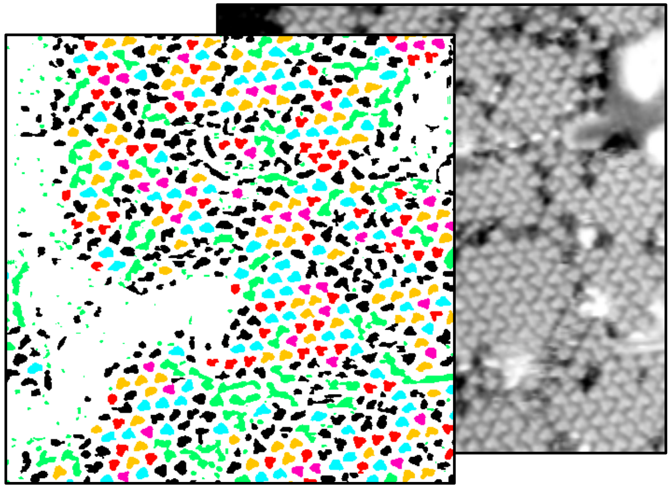
So what can a physicist do to make an impact in medicine?

Background in nanoscale physics



Expertise in image analysis of scanning microscopy images (STM, AFM, SEM)

Recognising molecular shapes
(adsorption geometry)



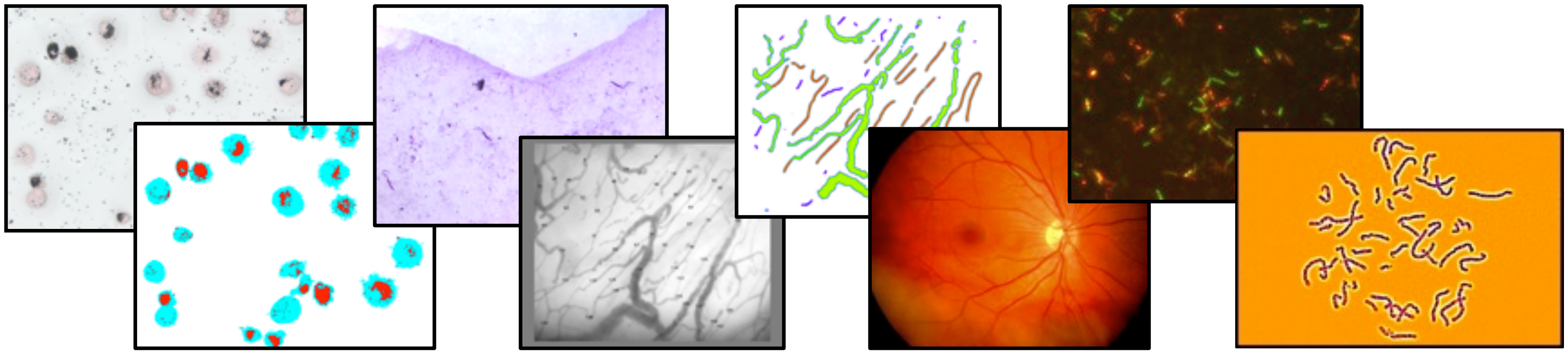
Identifying molecular positions
(substrate registration)

Liverpool Medical Imaging Network (LMI-Net) workshops

Put me in touch with medics who had image analysis problems

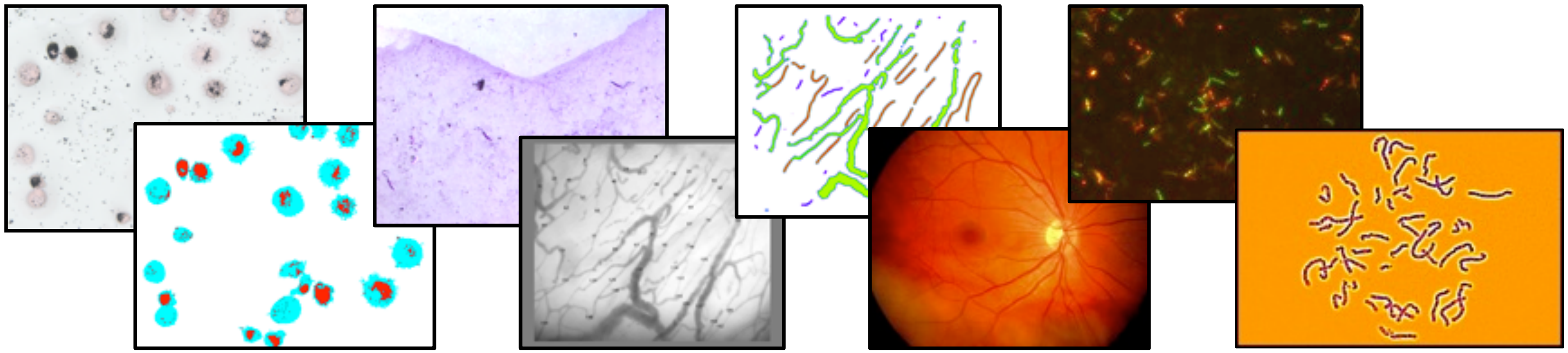
Some researchers within UoL, some clinicians in hospitals

Resulted in a number of collaborations

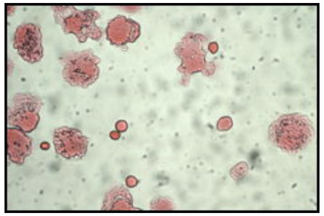


Projects include…

Carbon particulate matter in lung cells (lung cancer)
Parasite analysis (malaria)
Blood flow velocities in capillary networks (meningitis)
Retinal image analysis (diabetes)
Parasite morphology and development (leishmania)
Assessing antibiotic treatments (tuberculosis)



Intracellular Air Pollution Particulates



Collaborators

Dr Stephen Gordon
Liverpool School of Tropical Medicine

Dr Duncan Fullerton
Liverpool School of Tropical Medicine

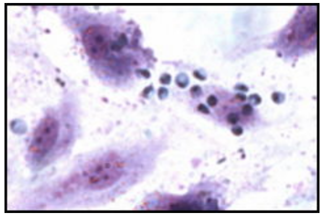
Aims

i) To identify particulate matter and differentiate it from cell cytoplasm.

ii) To measure the area of particulate matter relative to that of the cell cytoplasm.

Documentation MIASMA-PMA-v7.pdf

Malaria Parasites



Collaborator

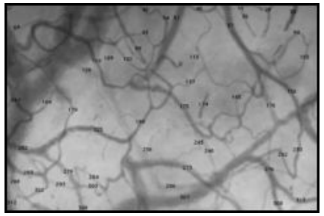
Professor Alister Craig
Liverpool School of Tropical Medicine

Aim

To identify malaria parasites and differentiate them from background features.

Documentation MIASMA-PCA-v5.pdf

Microcirculation Flow



Collaborators

Dr Enitan Carrol
Institute of Child Health, UoL

Dr Richard Sarginson
Alder Hey Children's Hospital

Dr Fauzia Paize
UoL and Liverpool Women's Hospital

Aims

i) To identify capillaries in videos of capillary networks and measure capillary vessel density.

ii) To measure blood flow speed as a function of capillary diameter.

Documentation MIASMA-MCA-v5.pdf

Retinal Imaging

Collaborators

Professor Simon Harding
Ophthalmology Research Unit, UoL

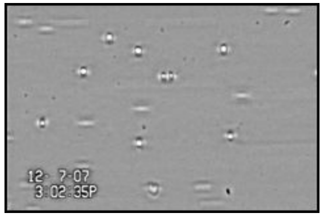
Dr Yalin Zheng
Ophthalmology Research Unit, UoL

Aims

To identify specific features such as:
- Blood vessel network
- Optic disc
- Haemorrhages
- Exudates

Documentation Not yet available

Lymphocyte Flow



Collaborator

Dr Carlo Laudanna
Department of Pathology
University of Verona

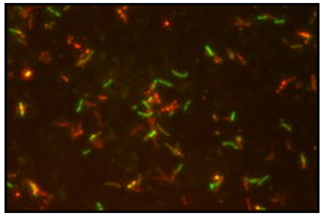
Aims

i) To identify lymphocyte cells flowing through a glass capillary.

ii) To measure the length of time that cells are arrested by or rolling along the capillary wall.

Documentation MIASMA-LFA-v4.pdf

Bacilli Lipid Bodies



Collaborator

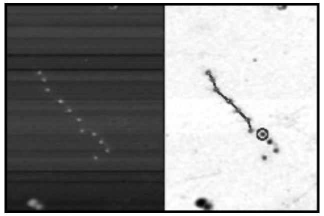
Dr Derek Sloan
Clinical Sciences, UoL

Aim

To measure the number of bacilli that contain lipid bodies.

Documentation Not yet available

Fibrillin Microfibrils



Collaborator

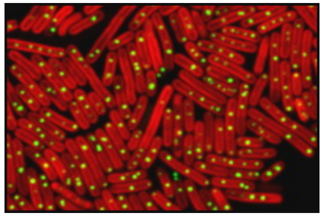
Dr Riaz Akhtar
Ocular Biomechanics Group
School of Engineering, UoL

Aim

To speed up the analysis of microfibrils by semi-automating the process of identifying microfibril beads and calculating their xy coordinates.

Documentation MIASMA-MFA-v3.pdf

Bacterial MicroCompartments



Collaborator

Dr Luning Liu
Institute of Integrative Biology, UoL

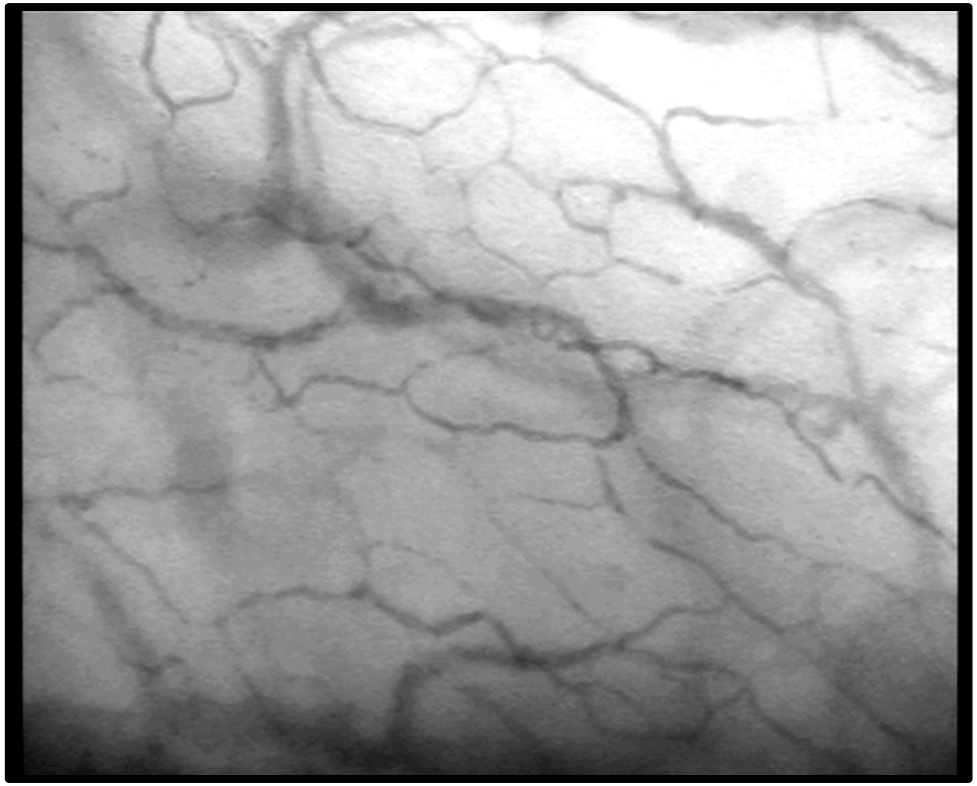
Aim

To determine the locations of microcompartments within the outlines of bacterial membranes.

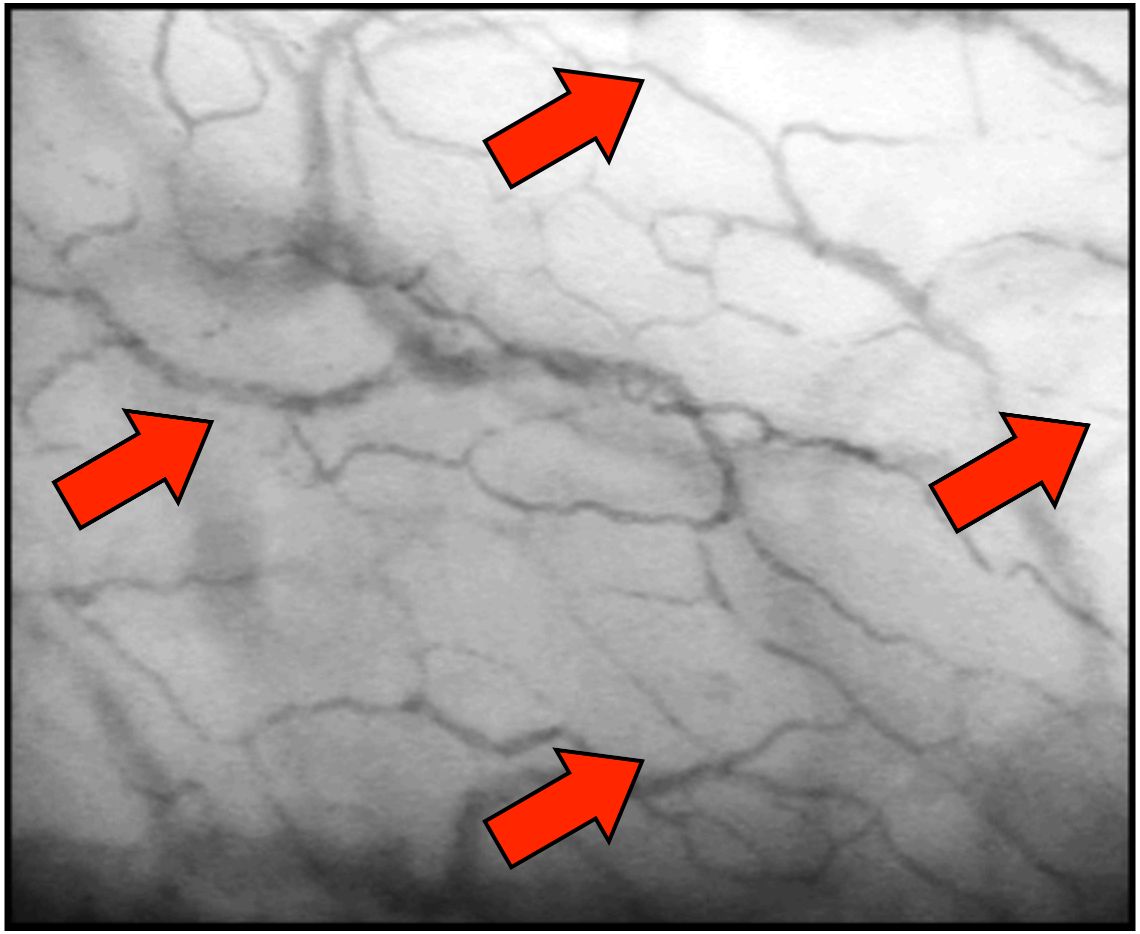
Documentation Not yet available

Take one MIASMA project as an example…

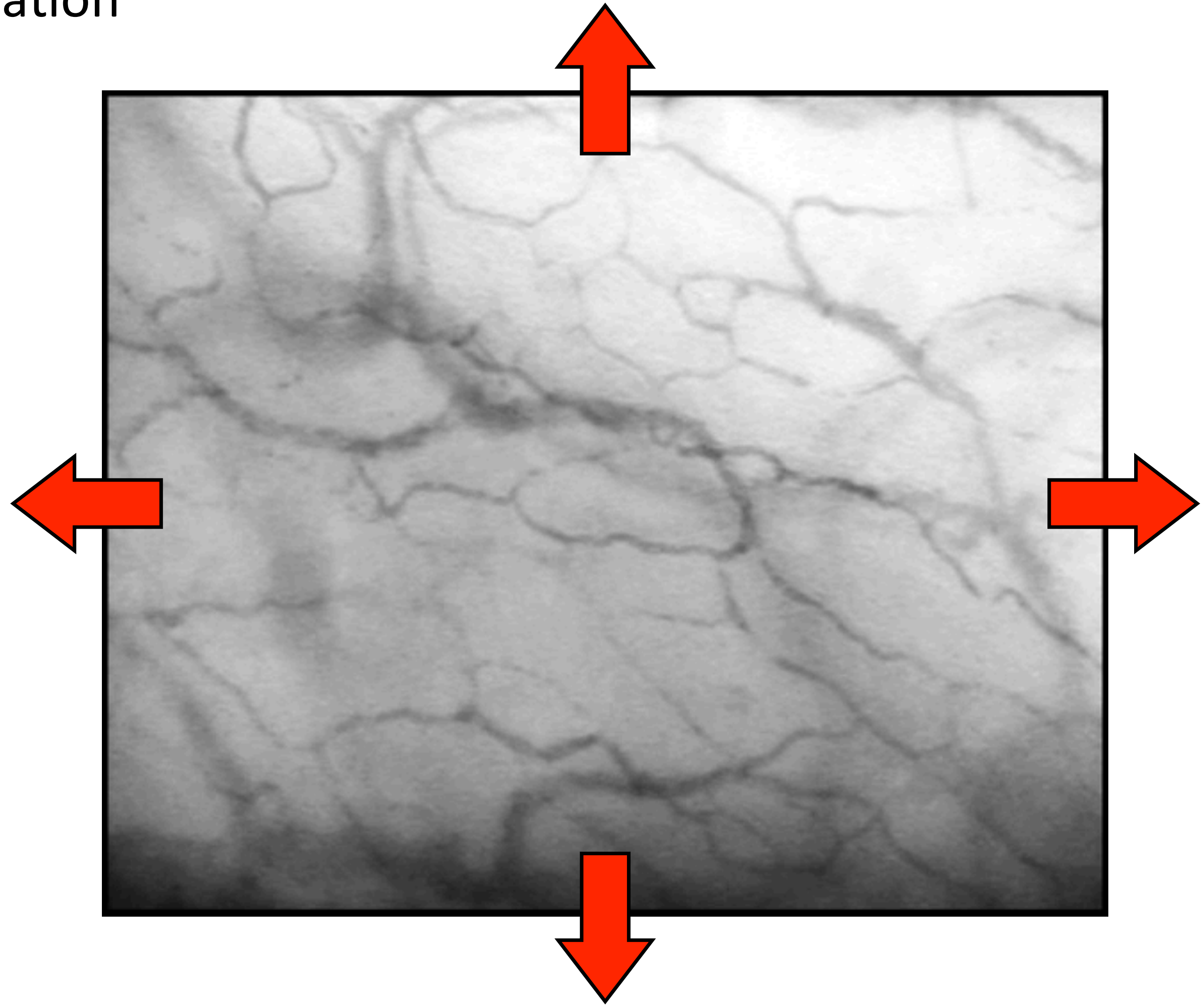
Blood flow velocities in capillary networks (meningitis)



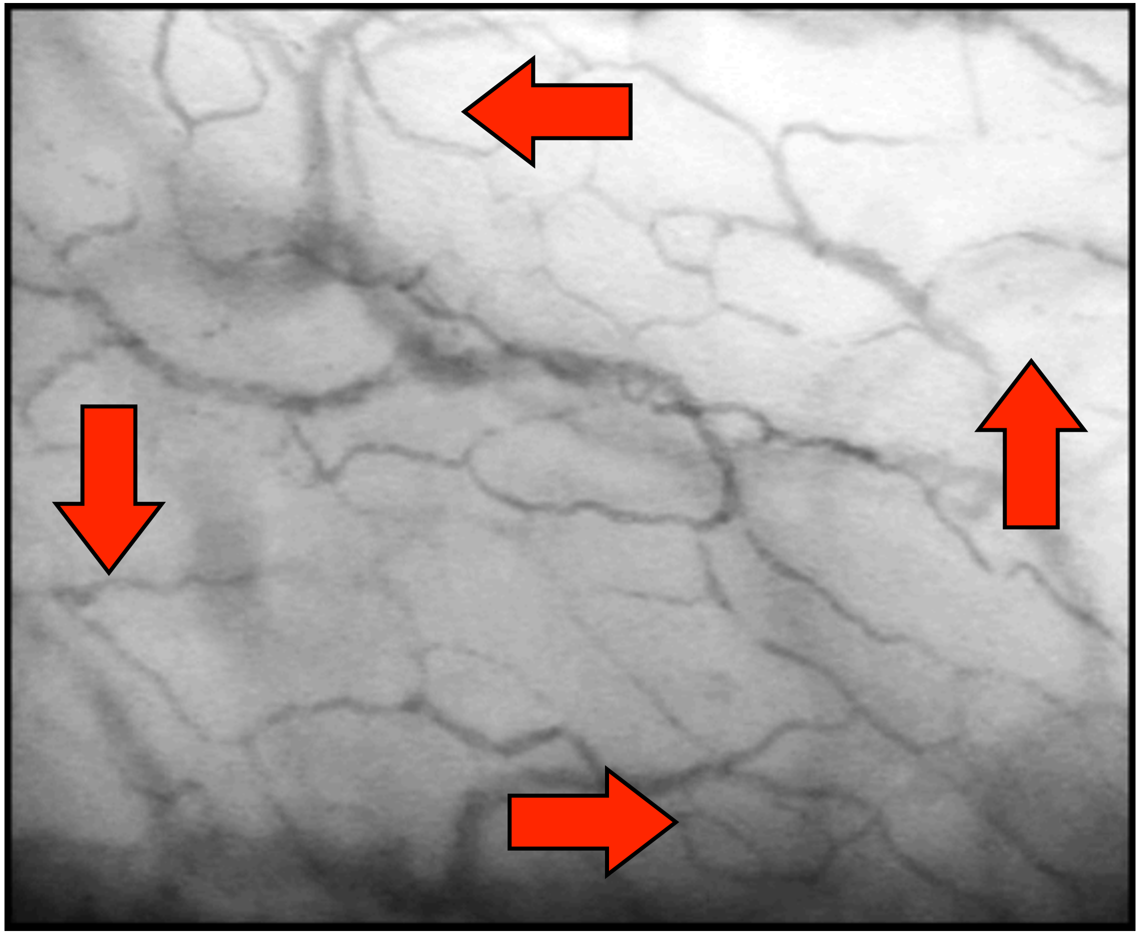
Translation



Magnification

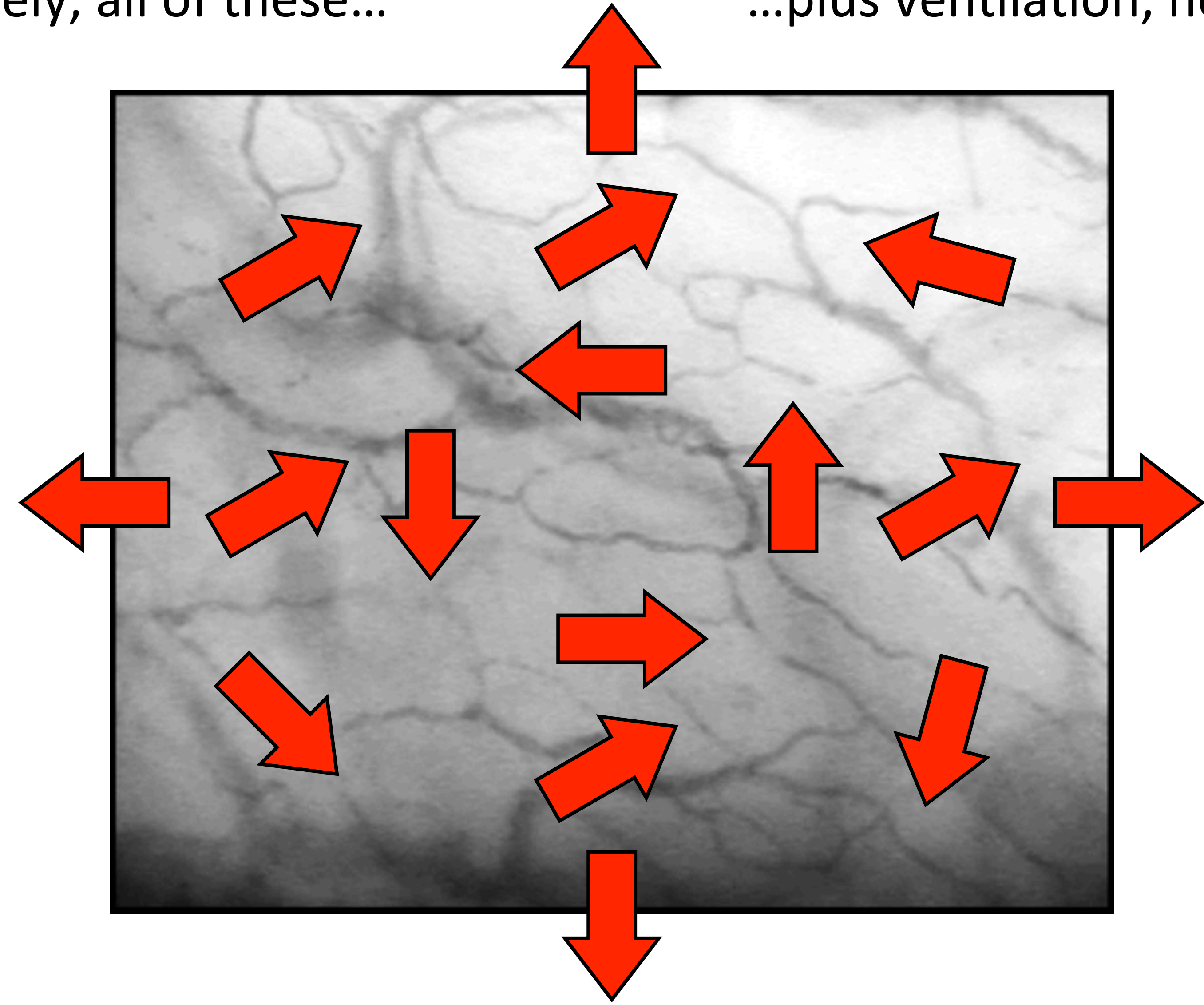


Rotation



Video Stability

More likely, all of these… …plus ventilation, heartbeat



What information can be extracted?

How should the microcirculation be quantified?

What (manual) scoring systems exist?

Percentage of perfused vessels (PPV)
(Perfused = flow exists for > 50% of the time)

Microcirculation Flow Index (MFI)
(Is the flow 'intermittent' or 'sluggish' or OK?)

Calculation of blood flow speeds

- Stabilisation of the video
- Identification of the blood vessels (which are invisible)
- Isolation of each capillary vessel
- Analysis of the movement of the blood cells

Quantification of the flow distribution (PPV and MFI)

- Flow speed as a function of time
- Flow speed as a function of vessel diameter
- Variations in flow speeds across the vessel network

Microcirculation Analysis

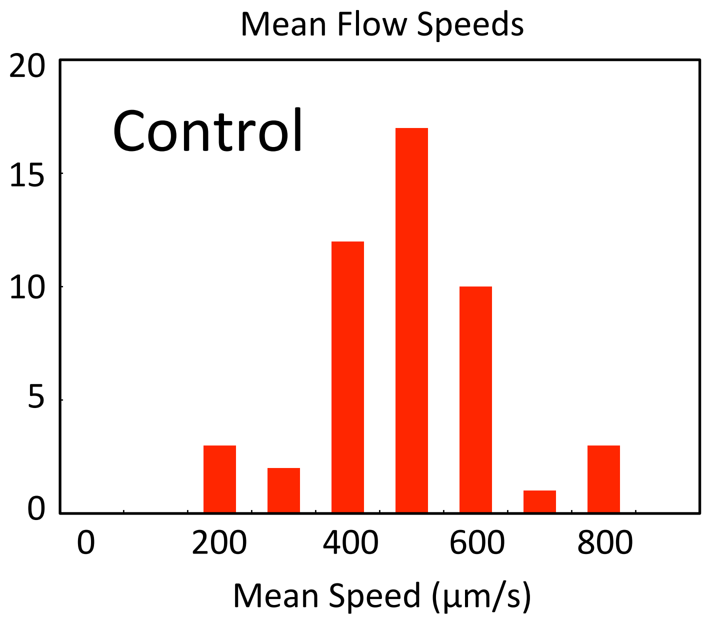
Calculation of blood flow speeds

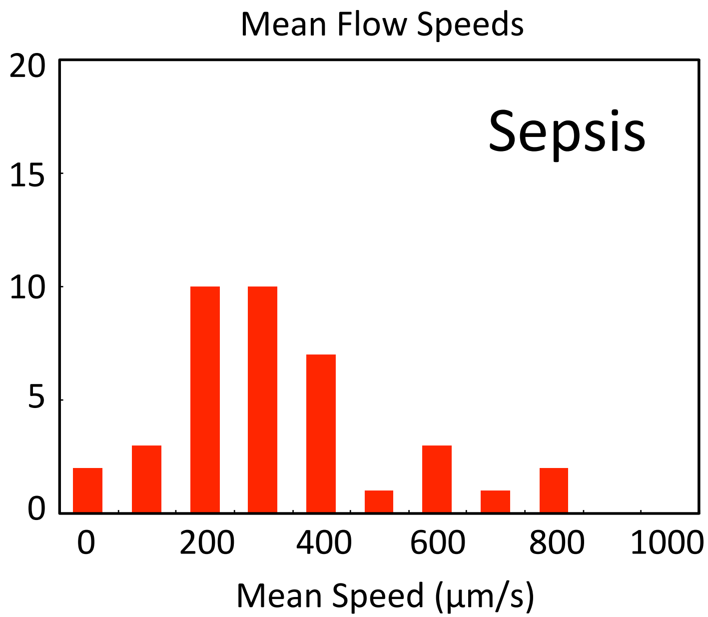
- Stabilisation of the video — << Fourier Methods
- Identification of the blood vessels — << Kernel Filters
- Isolation of each capillary vessel — << Particle Analysis
- Analysis of the movement of the blood — << Fourier Methods

Quantification of the flow distribution (PPV and MFI)

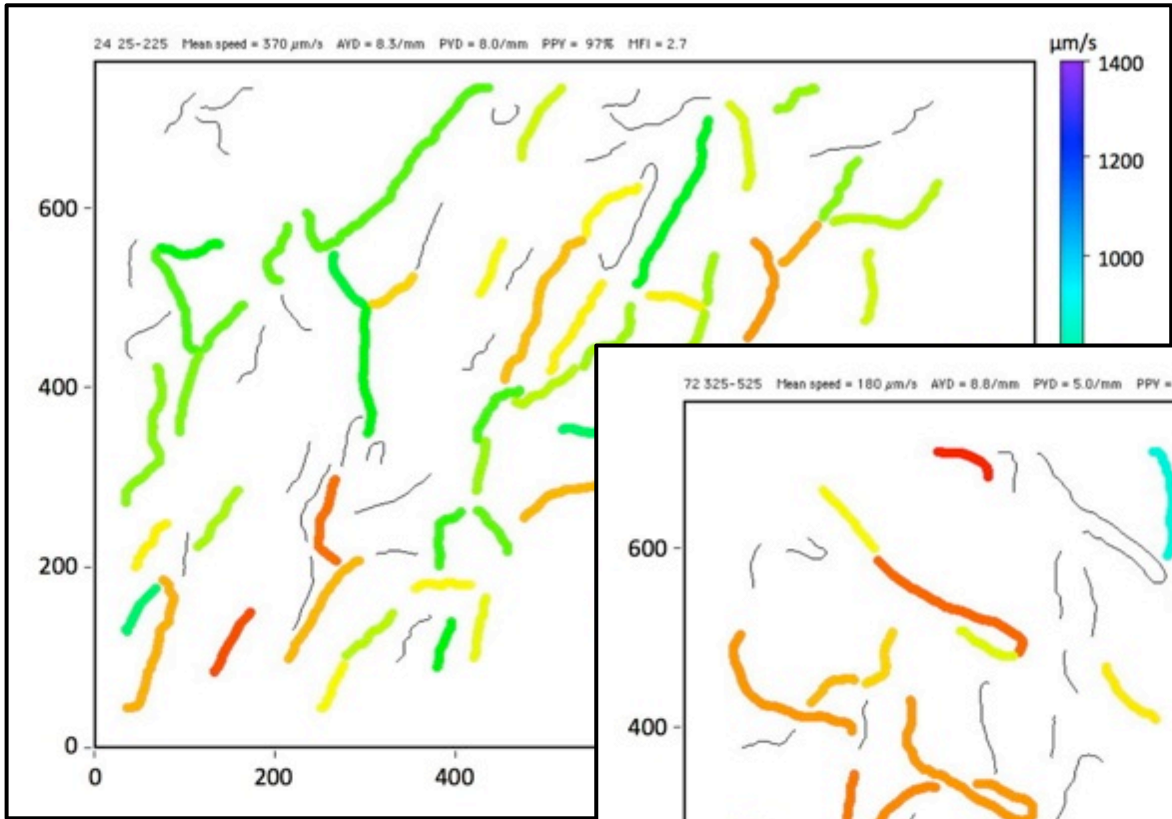
- Flow speed as a function of time
- Flow speed as a function of vessel diameter
- Variations in flow speeds across the vessel network

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.



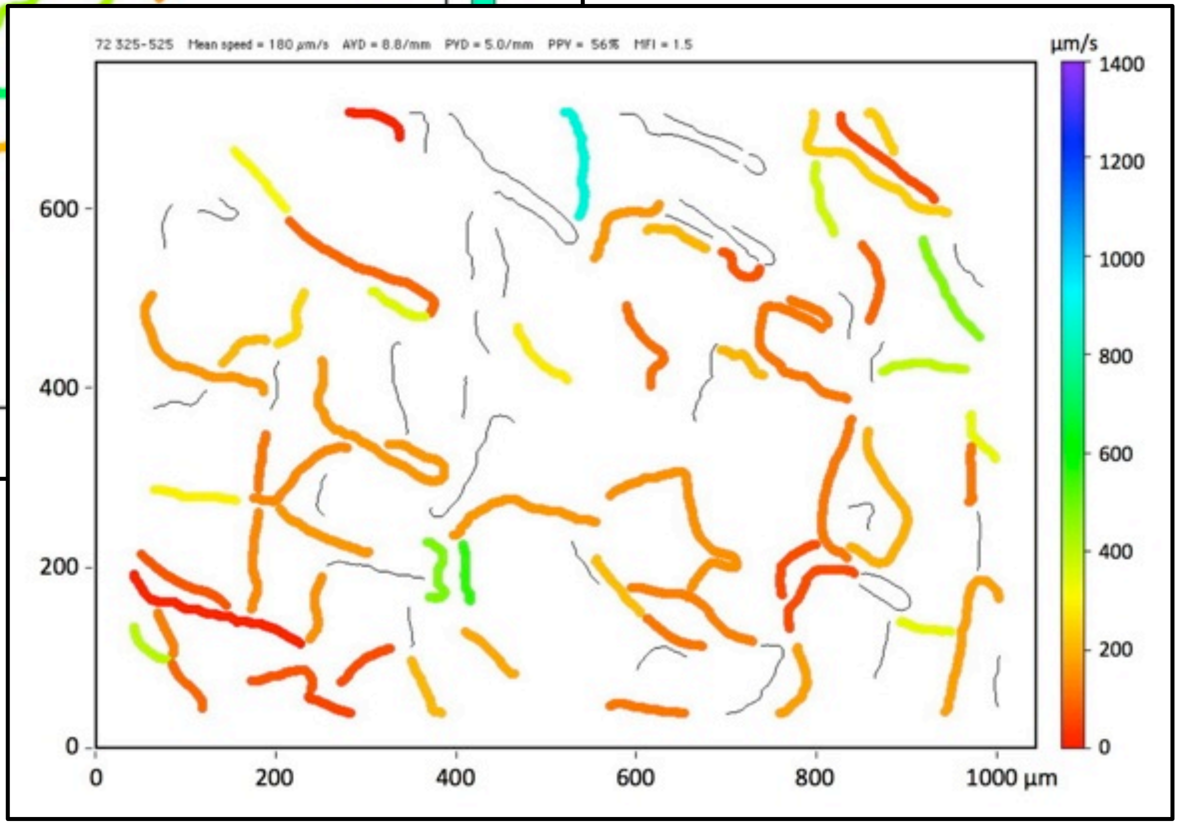


Microcirculation Analysis



Control

Sepsis



Colour-coded 'map' of blood flow speeds

Case Study 2 : 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…

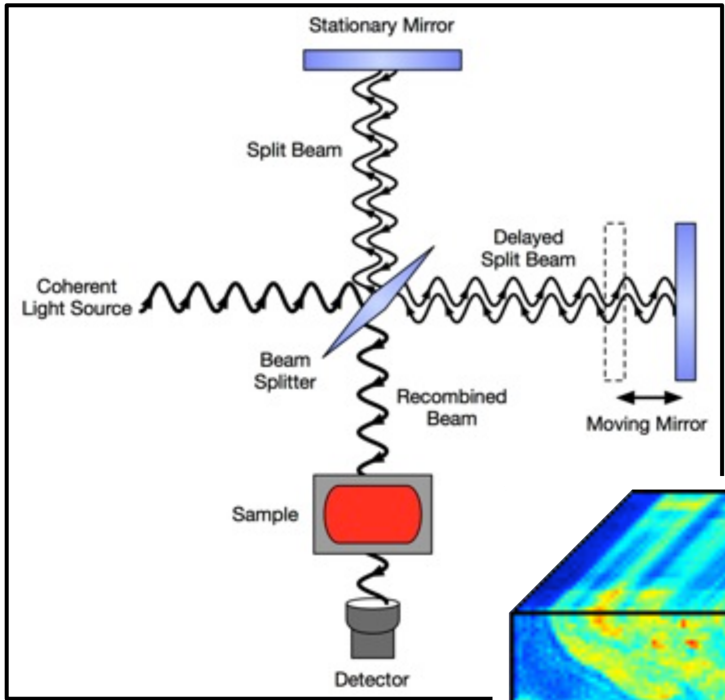
Can we detect cancer before it is cancer?

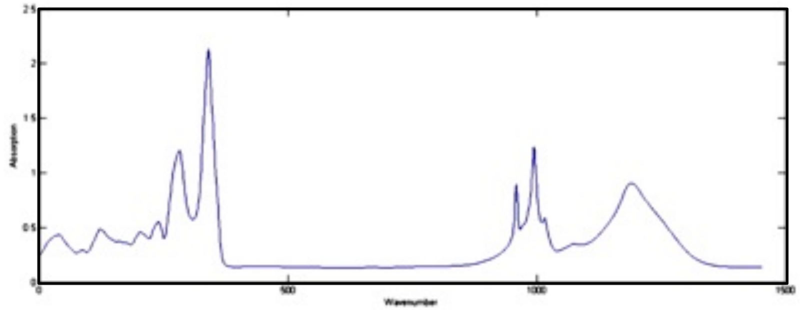
What tissues are being studied?

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

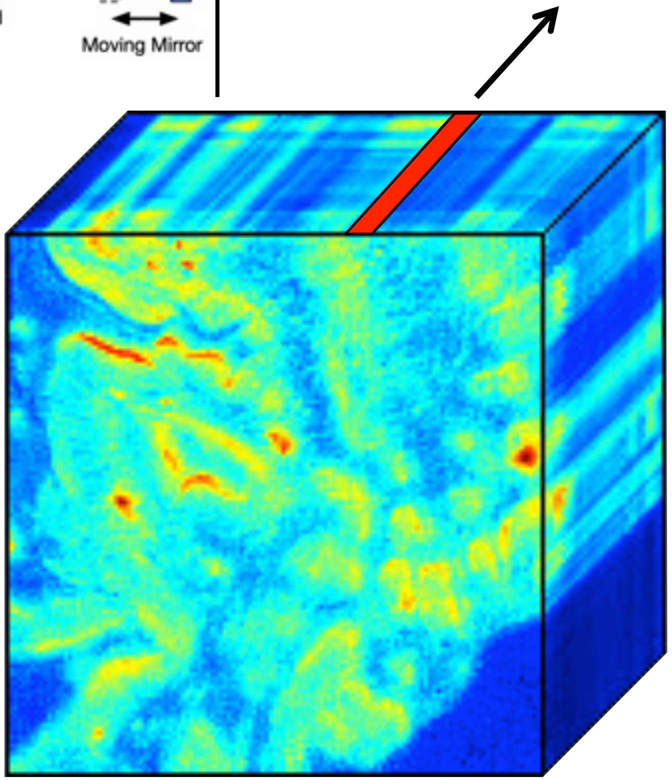


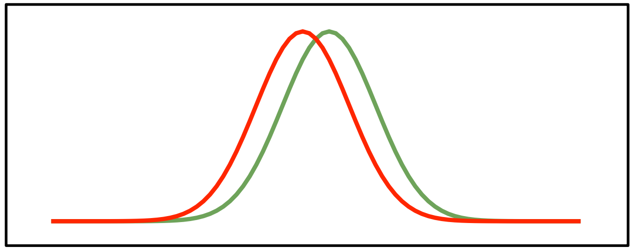
Image with spatial resolution ~5 µm

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

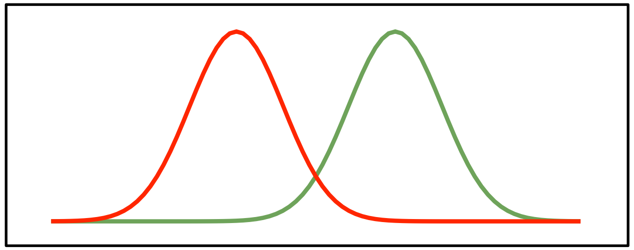
In general, infrared absorption at different wavelengths is very similar even for different tissue types. So, what wavelengths should we use to discriminate one (abnormal and potentially cancerous) tissue type from another (normal and healthy) type?

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.

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

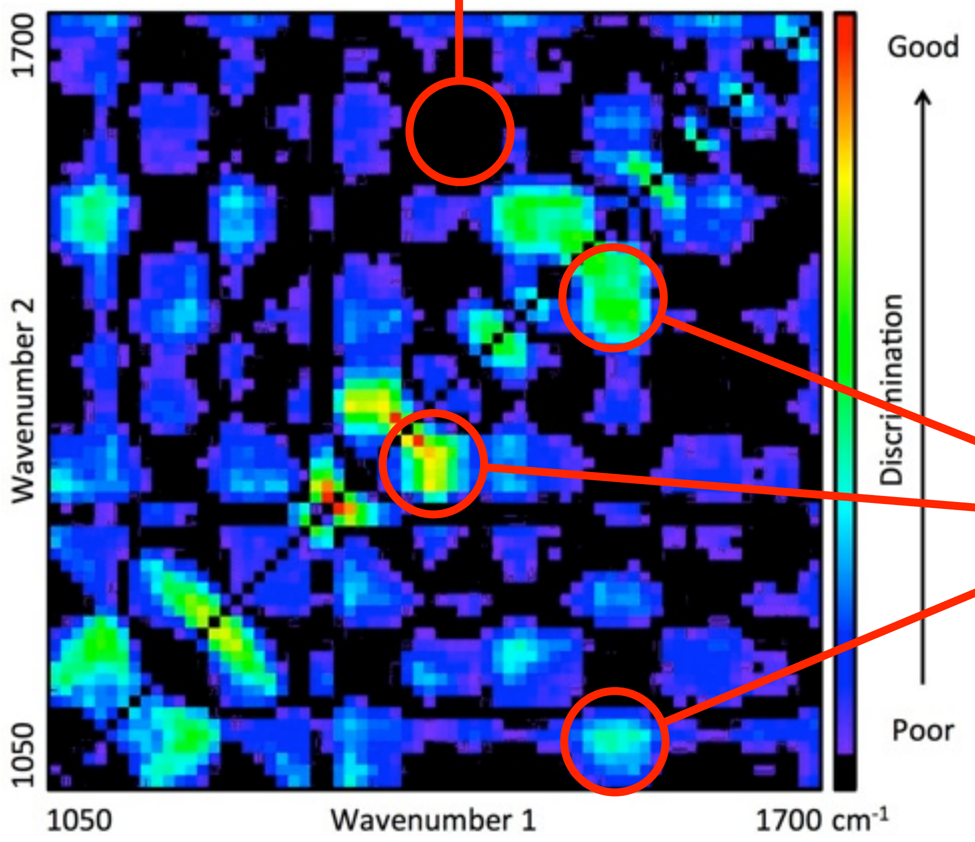


Poor choice of λ's

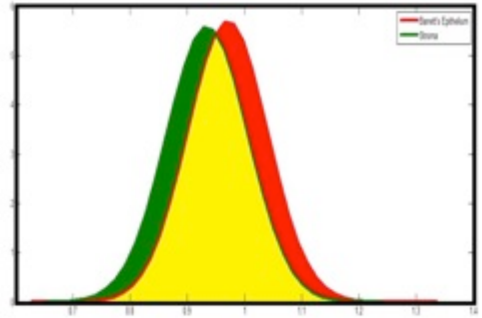


Good choice of λ's

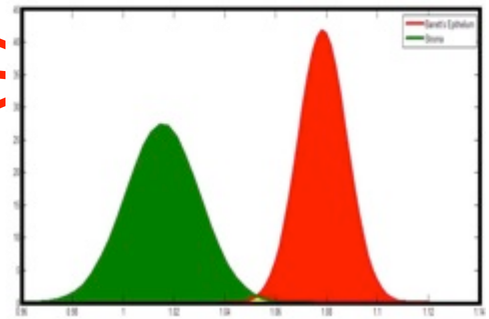
Histograms of ratios of IR absorption for abnormal (red) and normal (green) tissue



"Butterfly diagram"

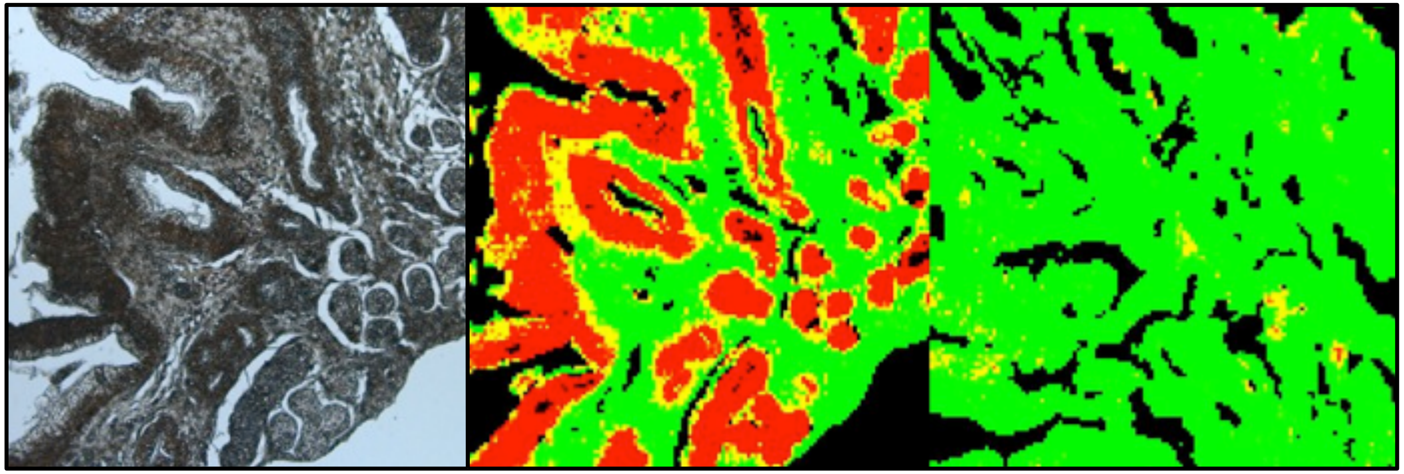


In general, infrared absorption at different wavelengths is very similar for different tissue types



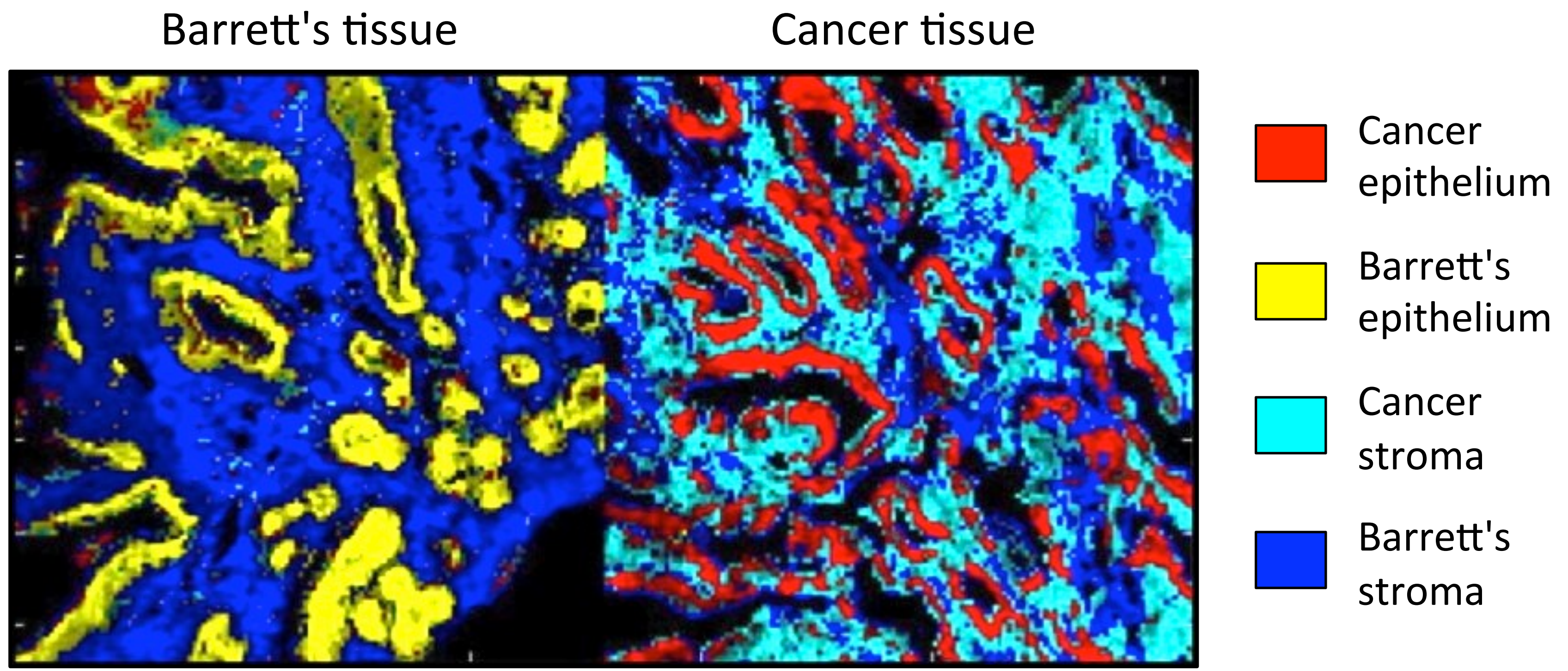
Certain pairs of wavelengths can be used to discriminate abnormal from normal tissue

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

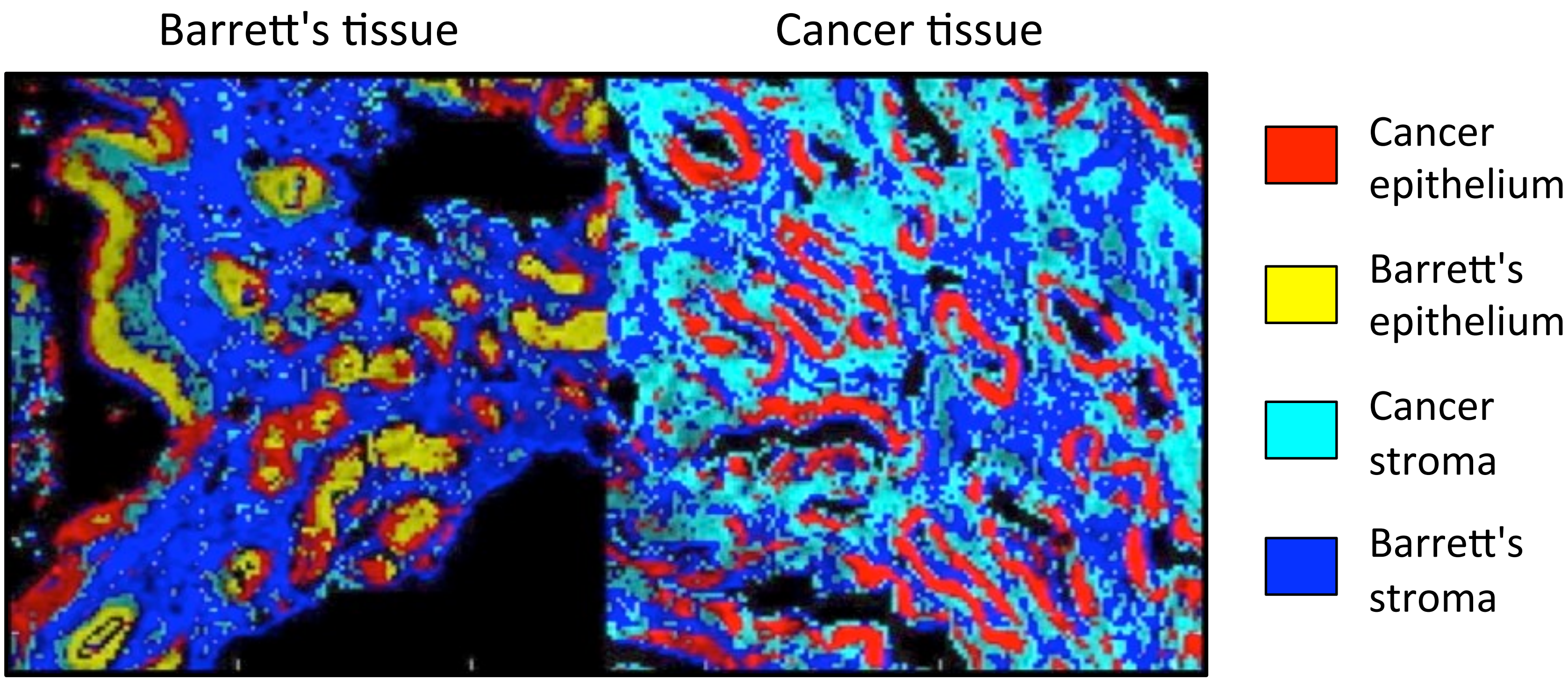


Visible image | Abnormal (red) and normal (green) | Normal tissue only

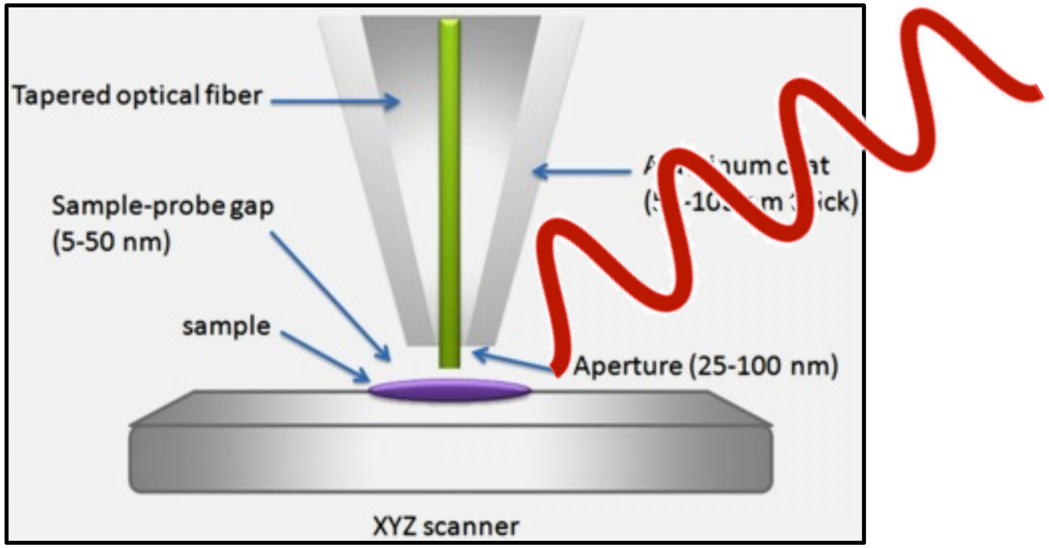
This idea was then extended to identify more than two tissue types...



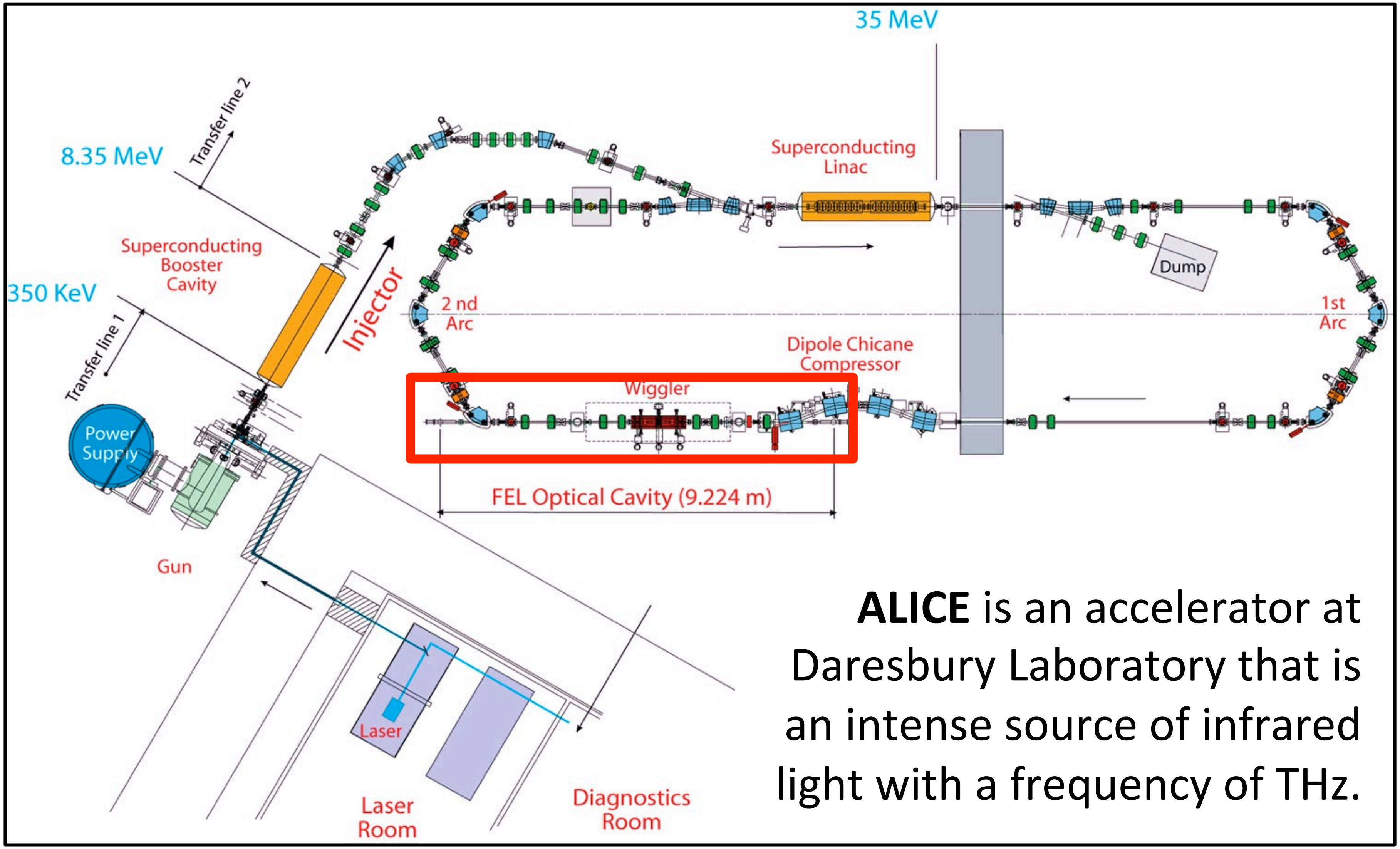
... and then tested on tissues not used to 'train' the analysis routine.



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

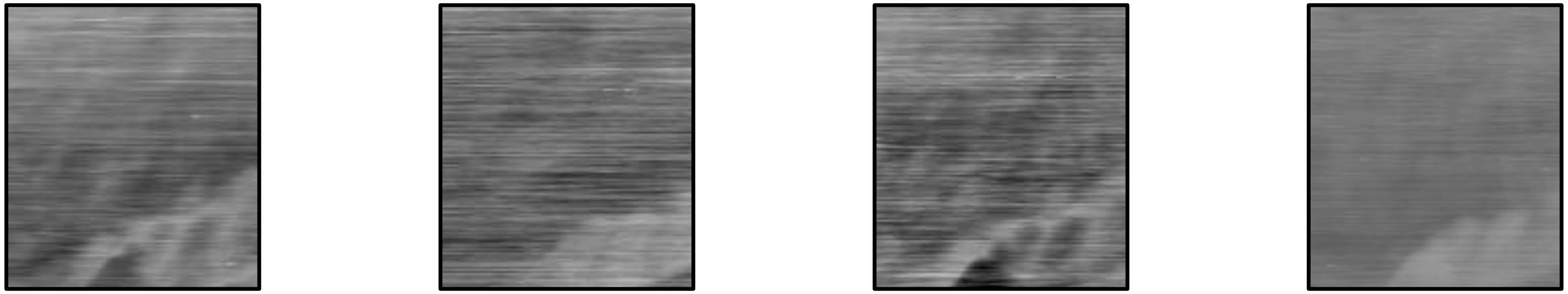


Imaging with sub-µm resolution requires plenty of infrared photons to illuminate the sample underneath the scanning tip. This is where a free-electron laser that operates in the infrared comes in.

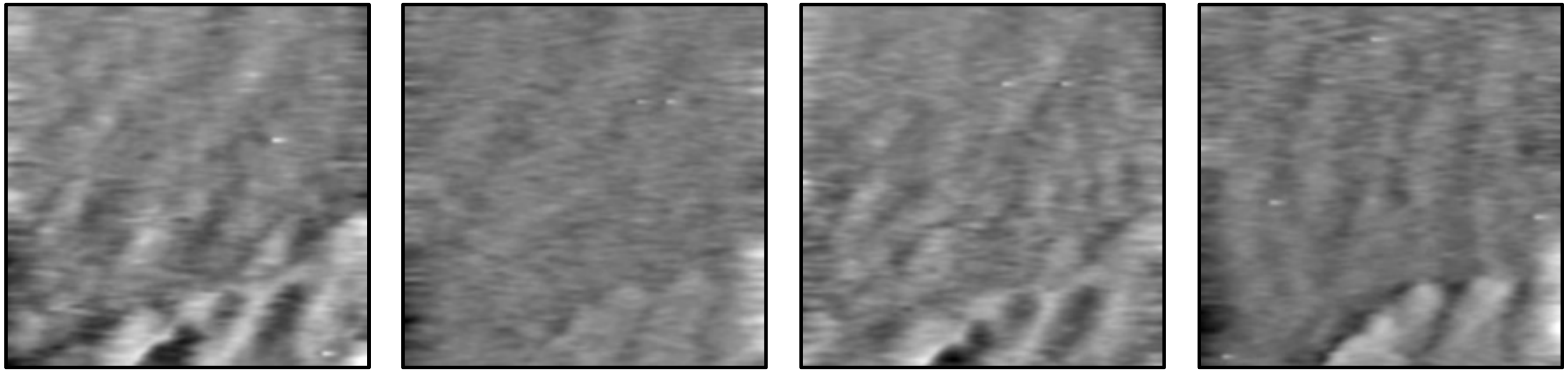


ALICE is an accelerator at Daresbury Laboratory that is an intense source of infrared light with a frequency of THz.

SNOM Imaging

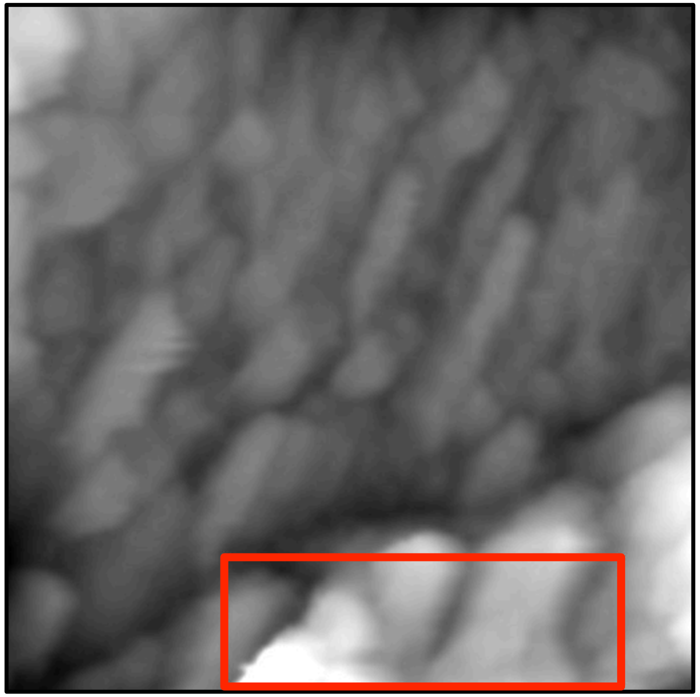


Raw images as acquired by the SNOM at different IR wavelengths

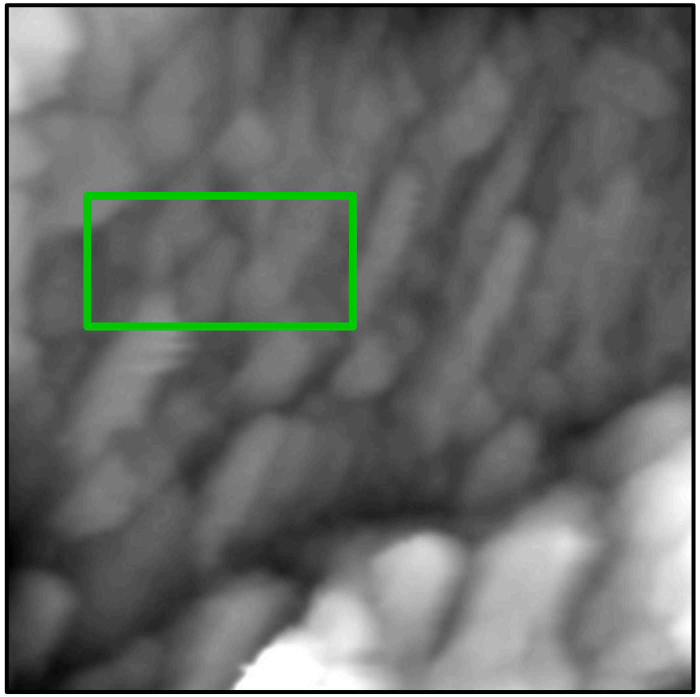


Processed to remove artefacts and make features easier to see

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

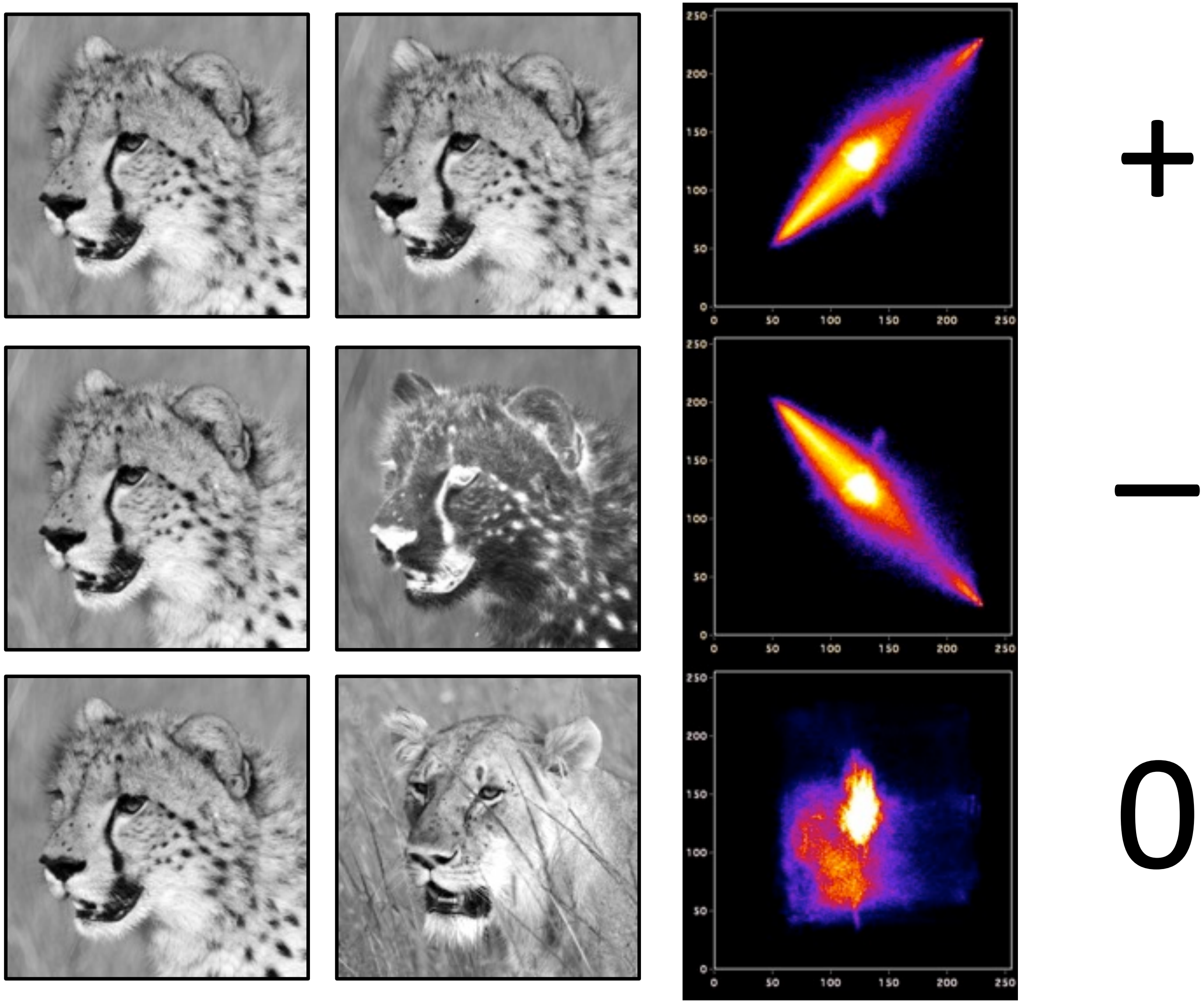


Image Correlation – Cancer

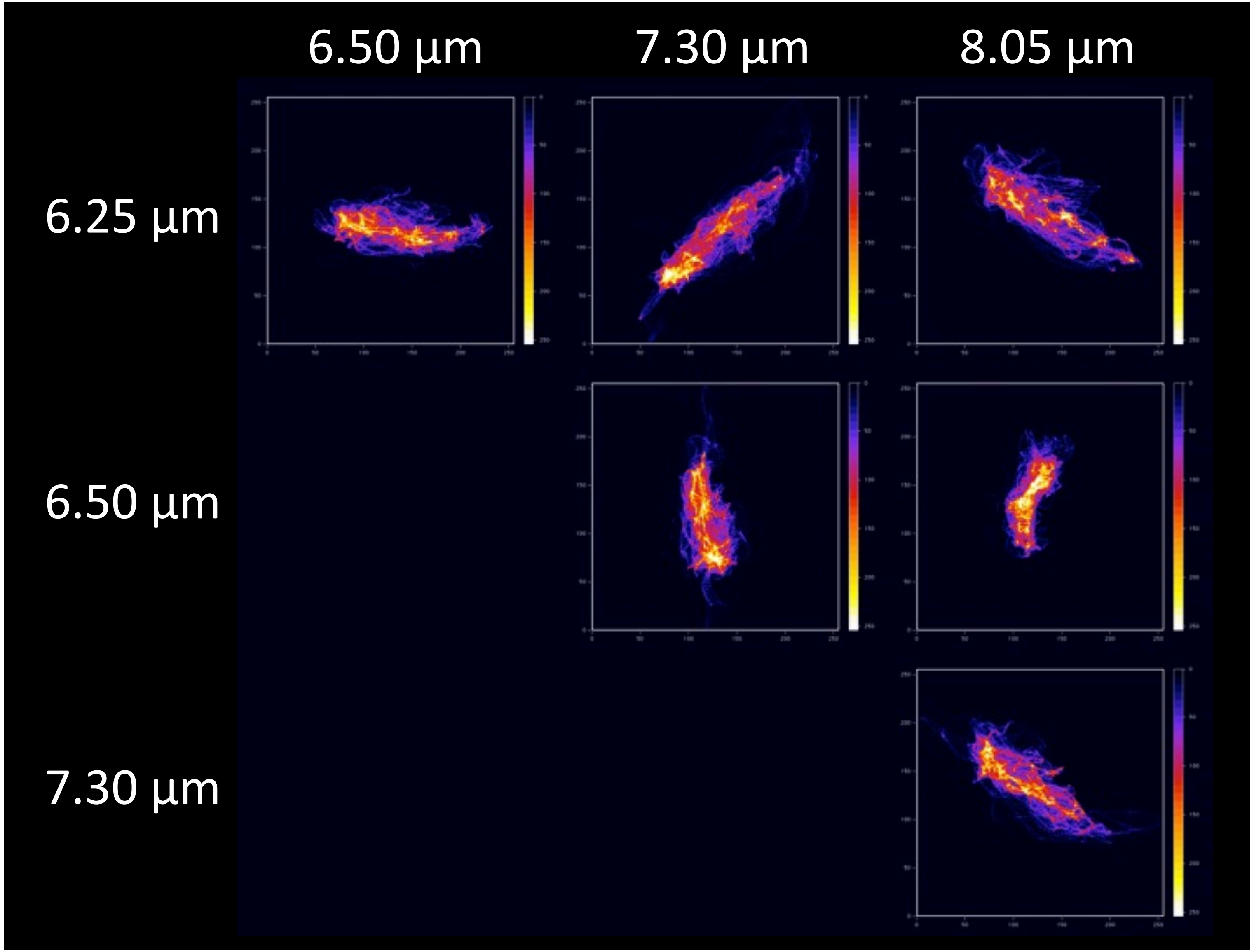


Image Correlation – Healthy

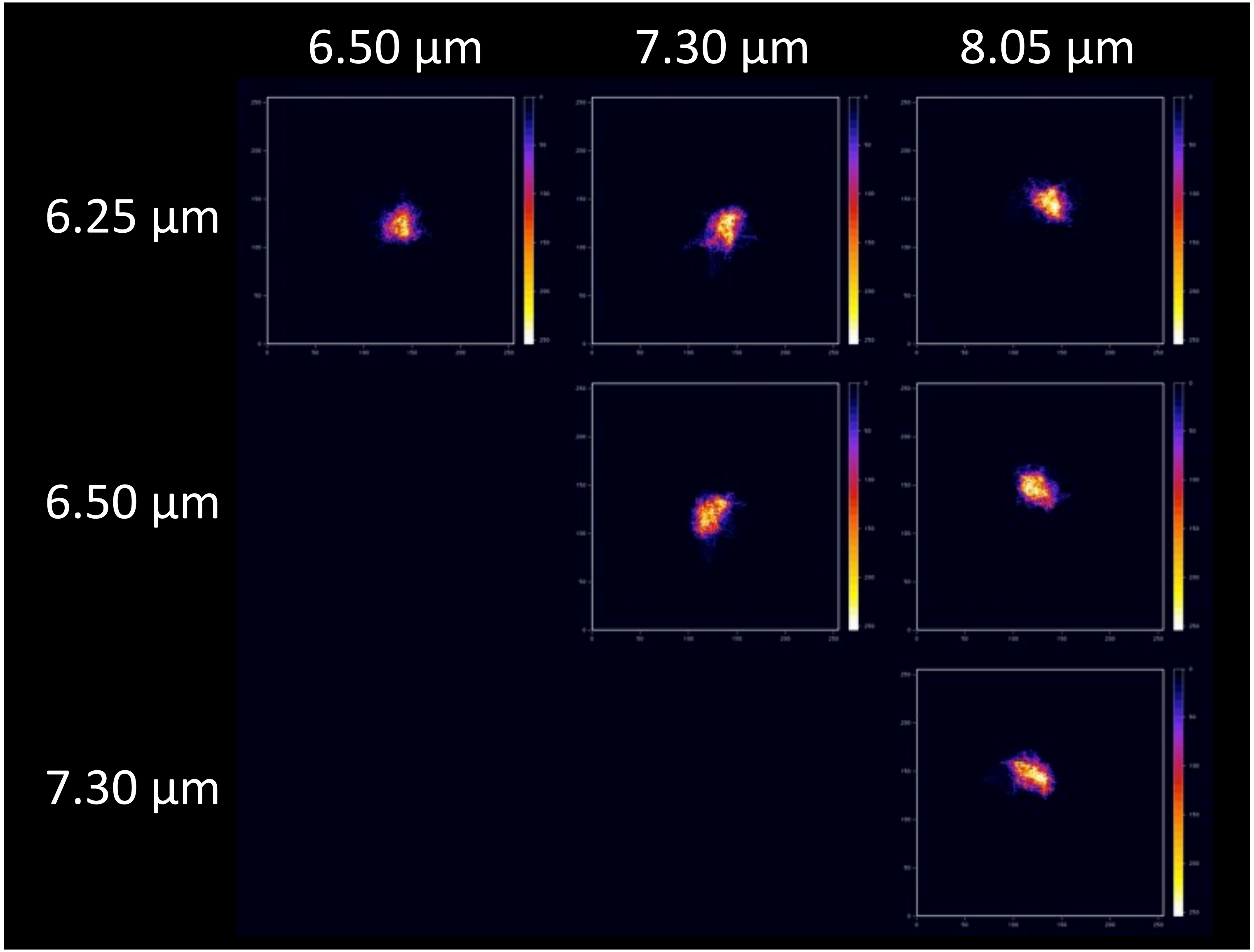
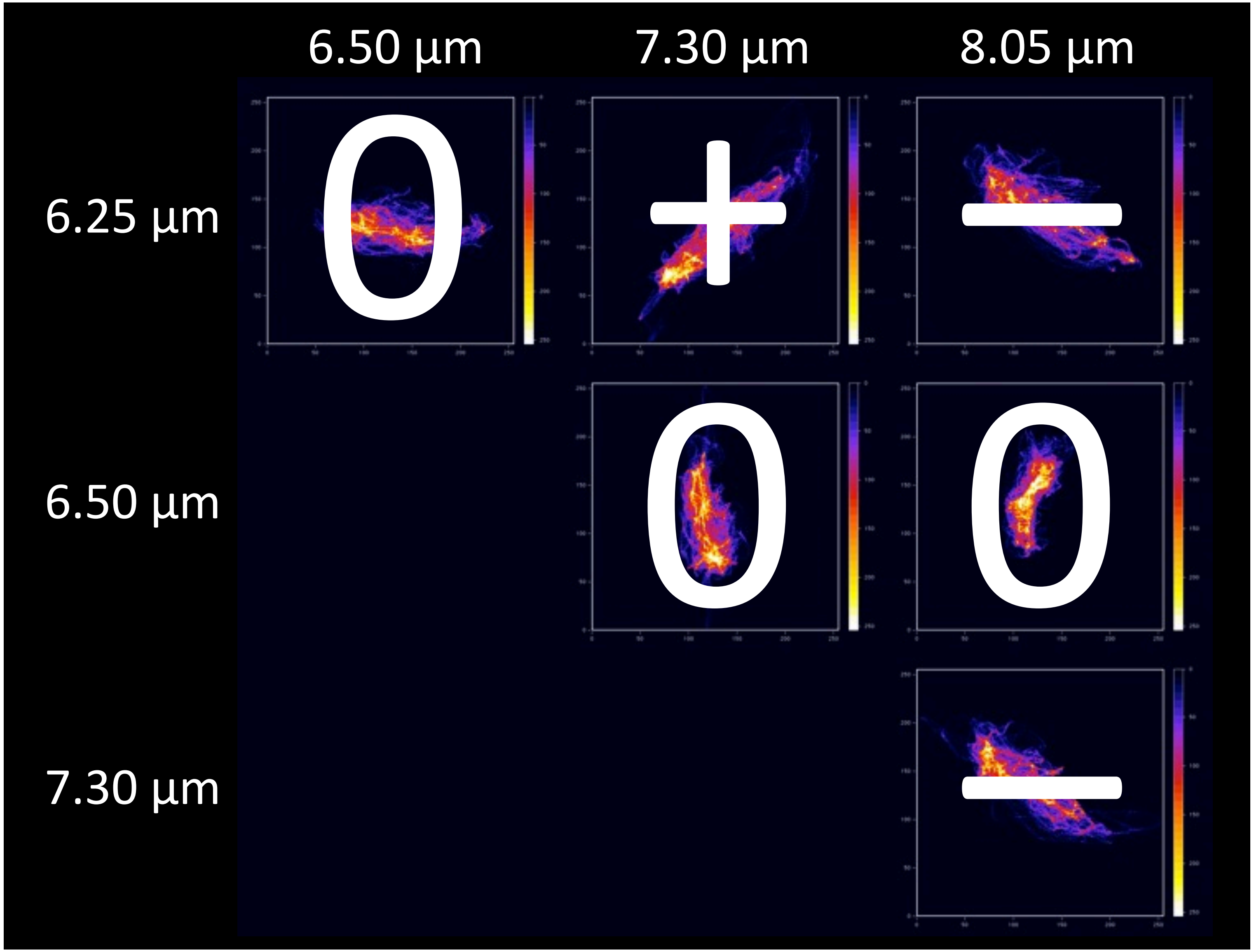
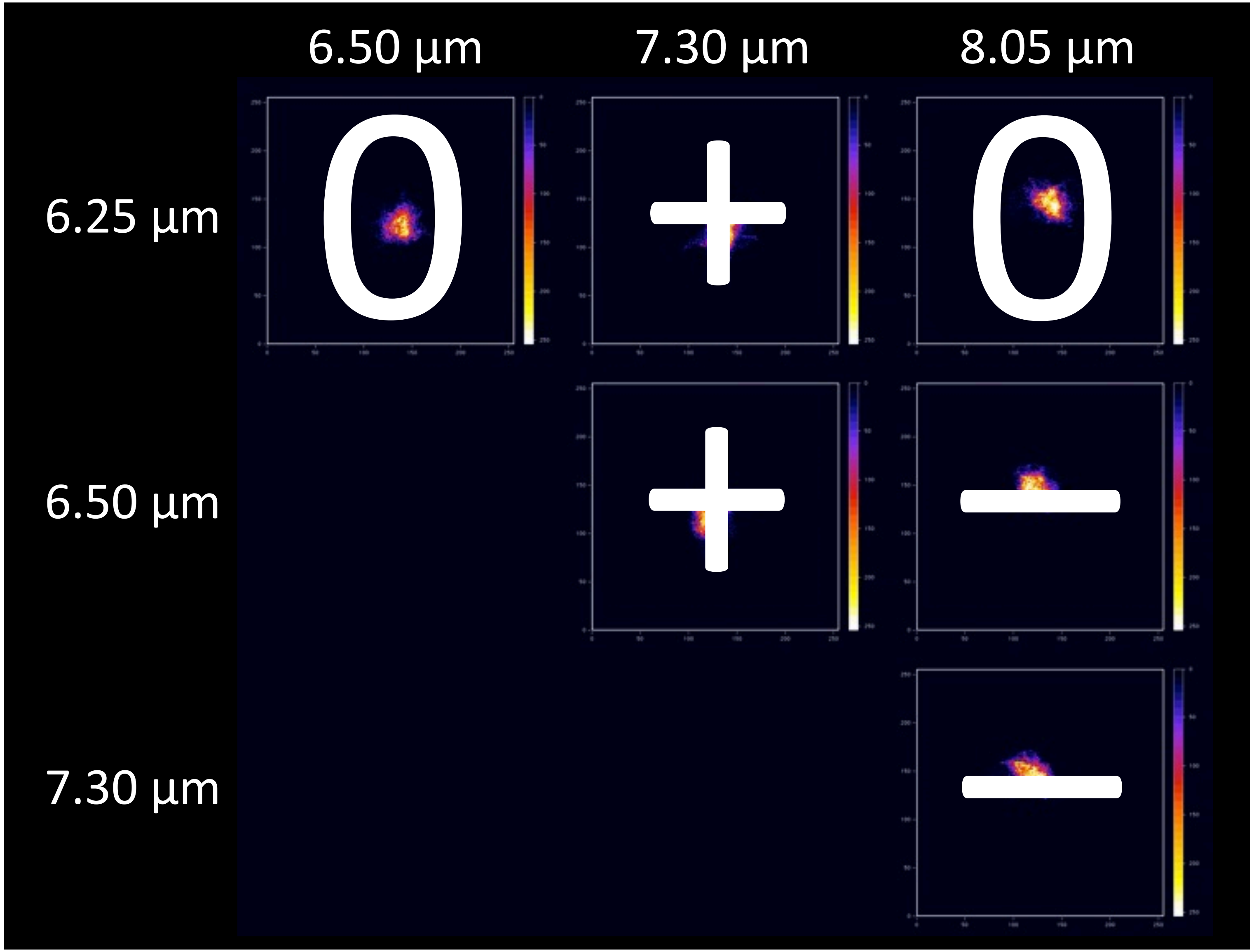


Image Correlation – Cancer




6.50 µm
7.30 µm
8.05 µm
6.25 µm
6.50 µm
7.30 µm
0
+
0
+
–
–

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

$$\boxed{\begin{array}{ccc}0 & + & - \\ & 0 & 0 \\ & & -\end{array}} \Rightarrow \text{Cancer ?} \qquad \boxed{\begin{array}{ccc}0 & + & 0 \\ & + & - \\ & & -\end{array}} \Rightarrow \text{Healthy ?}$$

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.

Acknowledgements

Thanks to

Johanne Holly Meningitis Fund

Liverpool School of Tropical Medicine

for supporting image analysis projects