MINERVA Project, mid- To near Infrared Spectroscopy for Improved Medical Diagnostics

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Abstract. The main idea behind the MINERVA project is the recognition that for the first time, through breakthroughs in photonic technology, it is possible to open the mid-IR electromagnetic spectrum (3-12 μm) for rapid medical imaging. In particular this could greatly improve the chances of early cancer diagnosis. MINERVA will exploit and develop the advances in soft glass optical fibres, acousto-optic (AO) modulator design, crystal growth, fibre lasers, supercontinuum sources and detectors in the mid-IR. Two specific high impact applications will be addressed: high volume pathology screening (i.e. automated microscope-based examination of routine patient samples) and human skin surface examination (i.e. non-invasive investigation of suspected skin cancer). In an Integrating Project of this scale it is possible to pursue several targets in parallel, each of which alone brings significant benefits. Together they could begin a new branch of the bio-medical imaging industry.

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1 Introduction

MINERVA is a project funded by the European Commission through its Seventh Framework Programme (FP7) [1]. It brings together thirteen partners from across Europe with the common objective of developing mid-infrared (mid-IR) technology to improve the early diagnosis of cancer (Fig. 1).

Mid-IR radiation is an exciting new area for real-time molecular-sensing with applications in different areas: medicine and healthcare (e.g. early cancer detection: the MINERVA application space), environment and energy (e.g. monitoring exhaust gases), security (e.g. detection of narcotics or explosives, food security), chemical and industrial manufacturing (e.g. process control and quality assurance).

The MINERVA mid-IR range (1.5 to 12 μm) is rich in spectroscopic absorption peaks of biomolecules such as fats, proteins and carbohydrates. In particular it has been shown that, through the latest data analysis techniques, this spectral region can be used to identify the presence of early cancer. Currently there is a lack of practical sources and components for this spectral region, and so these mid-IR diagnostic techniques are restricted to laboratory demonstrations.

MINERVA aims to develop fibre, lasers and broadband sources, components, modulators and detectors to access this important part of the spectrum. In parallel it will identify analytical techniques using the new photonic hardware to improve early skin cancer diagnosis and the rapid and automatic assessment of biopsy samples using a microscope.

Fig. 1. Logos of the thirteen partners of MINERVA's consortium.

1.1 mid-IR Spectroscopy: A New Tool for Pathologists

The spectral region studied in MINERVA (1.5-12 μm) includes the so-called "fingerprint region" in which many biomolecules have tell-tale absorption peaks. By studying the pattern of absorbed radiation it is possible to deduce details of the type and distribution of these molecules, which in turn provides important information for disease diagnosis.

It is emphasised that this process is not as straightforward as simply spotting certain chemicals, or "cancer markers". The information is buried in the inter-related distribution of species and subtle biochemical changes. It requires a powerful mathematical

technique known as multi-variate analysis to extract useful information from the reams of spectral data in order to spot the warning signs of cancer.

One form of multi-variate analysis is correlation mapping, which enables the visualisation of diseased cells or regions from spectral data (Fig. 2). MINERVA combines novel mid-IR spectroscopy with correlation mapping and hopes to lead to a breakthrough diagnostic technology.

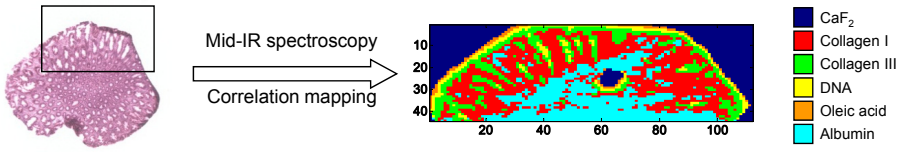

Fig. 2. Correlation mapping enables the visualisation of diseased cells or regions from spectra.

MINERVA will develop a suite of mid-IR photonic hardware to improve access to this information. Working in the mid-IR is extremely challenging, and MINERVA will need to break new ground in several technical areas:

- *Gooch & Housego* (G&H), the project coordinator, will develop mid-IR components such as fused combiners (glass devices used to combine or separate signals into different optical fibres), and acousto-optic (AO) modulators (to switch the signals and separate wavelengths at high speed).
- These AO devices will need new types and sizes of calomel crystals from *BBT-Materials Processing SRO* (BBT).
- Mid-IR glass fibre to carry the radiation efficiently and conveniently is being produced at *University of Nottingham* (NOTT).
- Novel pump lasers at 2.9 µm and 4.5 µm from *LISA Laser Products OHG* (LISA) will be used by the *Technical University of Denmark* (DTU) and *NKT Photonics A/S* (NKT) to generate a range of supercontinuum sources in ZBLAN, indium fluoride and chalcogenide glasses, spanning the MINERVA range from 1.5 µm to 12 µm.
- *Xenics* and *IRnova* are advancing the state-of-the-art in Type II superlattice detectors, which offer a cost effective route to highly efficient detection in the mid-IR.
- *University of Exeter* and *Gloucestershire Hospitals NHS Trust* (GHNT) will develop the multivariate algorithms and techniques for high volume screening of human samples.
- *Westfaelische Wilhelms-Universitaet Muenster* (WWU) will develop a skin cancer diagnostic process.
- *Universitat Politècnica de València* (UPV) is working on novel algorithms for the analysis of histopathological images and the recognition and classification of hiperspectral data of cancer samples.
- The project is managed and administrated by *Vivid Components*.

In the next sections it will be presented an overview of the expectancies of the project and the main preliminary advances reached by the different groups.

2 Mercurous Halides: Unique Acousto-Optic Materials for IR from BBT

BBT is a world leader in the growth and processing of Mercurous Chloride (Hg_2Cl_2, Calomel) single crystals with excellent AO properties and is thus in favourable position to address this question. Calomel single crystals (Fig. 3.a) exhibit a wide range of optical transmission, high indices of refraction, extremely high value of acousto-optic figure of merit M2, very low velocity of shear acoustic wave, high value of birefringence (four times higher than Calcite), etc. The Calomel crystals are well adapted to fabricate acousto-optic devices operating in the mid and far IR (3 to 20 μm).

At the beginning of the MINERVA project the production technology enabled the growth of calomel crystal boules with a diameter of 26 to 29 mm (Fig. 3.b) and length of 45 to 60 mm (typically 55 mm). Within the MINERVA project the new technology is being developed enabling the growth of cylindrical crystal boules with a diameter up to 35 mm, which is necessary for the further manufacturing of acousto-optical tuneable filters of new design proposed by G&H.

The Calomel crystal growth process is highly demanding, difficult and complex, especially in case of bigger 35mm boules. The growing process is powered by a dynamic temperature field and corresponding axial and radial temperature gradients. The whole process has to be carefully maintained within narrow physical condition limits. All the equipment and accessories have to be newly developed by BBT and adjusted to the specific conditions for growing of the 36mm diameter crystals including the temperature controllers. These controllers are equipped with brand new cultivation programs with respect to the bigger material mass. A total of six crystallizers will be built within the project. Currently, four units are operational and tested (Fig. 3.c).

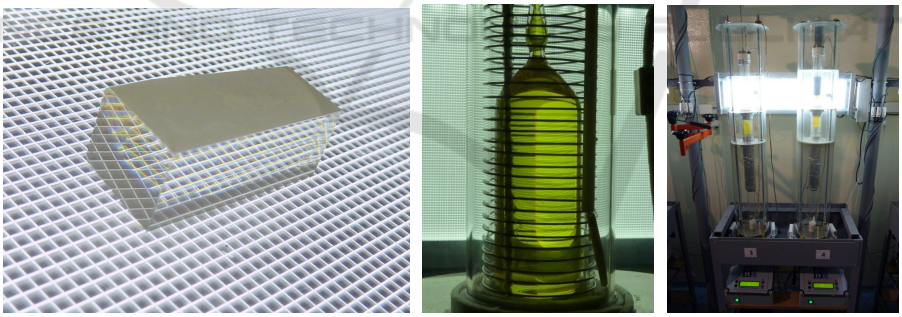

Fig. 3. (a) Polished Calomel AOTF substrate. (b) Growing Calomel crystal, diameter 28mm. (c) Two cultivation crystallizer units with Calomel crystals.

3 Er:ZBLAN Fibre Laser at 2.9 µm from University of Nottingham and LISA

The partners NOTT and LISA will develop a 2.9 µm laser based on Er-doped ZBLAN fibres diode-pumped at 976 nm. This fibre laser will be used as pump source for ultra-long wavelength supercontinuum generation (3-9 µm). The first step is the development of a fibre laser in an external cavity configuration. For that purpose a simulation model based on the rate equation and signal propagation equations is implemented by the NOTT group (Fig. 4). Different parameters will be studied, e.g. absorption cross-sections, emission cross-sections, and gain cross-sections, to predict the optimum laser performance.

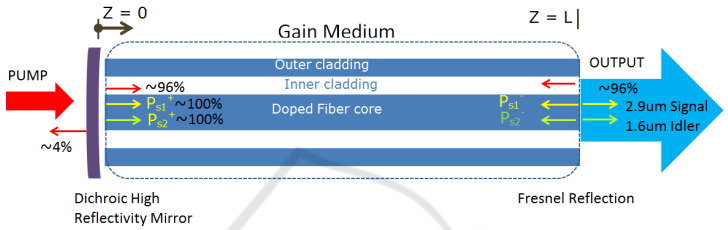

Fig. 4. Modelling scheme of the Er:ZBLAN fibre laser with an external cavity configuration. For the exact prediction both the 2.9 µm and the 1.6 µm laser signal were analysed in forward and backward propagation.

In parallel, LISA will carry out experiments for the handling (stripping, cleaving, splicing) of the soft glass fibre and target both high-power and high-energy laser operation with different resonator configurations (Fig. 5).

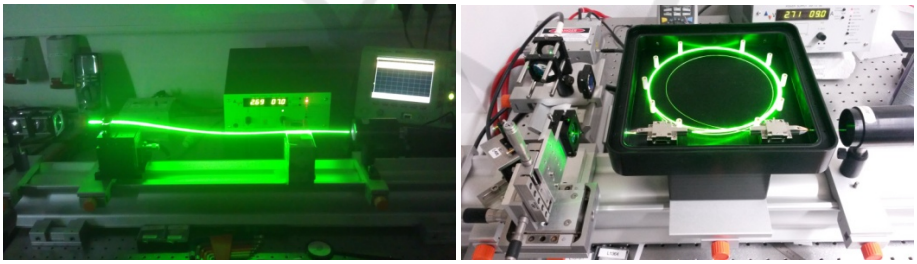

Fig. 5. (a) Set-up of the Er:ZBLAN fibre for absorption studies. The green fluorescence results from up-conversion processes. (b) Set-up of the Er:ZBLAN fibre in an external cavity configuration pumped with high-power fibre-coupled diodes. First experiments showed a good agreement with the simulations carried out at Nottingham.

Coated focussing and collimating optics have to be designed and manufactured for the laser studies. After the evaluation of the first tests in CW operation LISA's scientists and engineers will design a compact and robust cooled housing for the 2.9 µm laser.

Regarding high-energy operation special acousto-optic modulators (AOM) based on TeO2 will be designed and built by G&H and delivered to LISA.

Further information about MINERVA's fibre laser can be found in [2–10].

4 Extreme IR Supercontinuum Modelling at DTU

DTUs team has the task of fibre modelling in MINERVA in close collaboration with the fibre manufacturing group at NOTT. The DTU group also models dynamic supercontinuum generation along the fibres using both measured material data and calculated fibre properties. This advanced modelling requires extensive computational resources in order to accurately follow the rapid spectral broadening, which covers over four octaves (from 1 μm to 16 μm); made possible by the strong non-linearity of chalcogenide glasses and the extremely high numerical aperture (NA) of the Nottingham fibres. Figure 6 shows two graphics with some results of the numerical modeling of mid-IR supercontinuum generation. Thorough analysis of the modelling has been presented in [11–13].

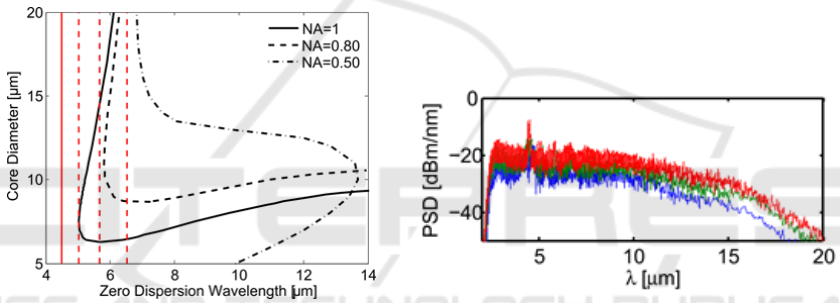

Fig. 6. (a) Zero dispersion wavelengths versus core diameter for step-index fibres (based on fibres fabricated at the University of Nottingham) with NA as given in the legend. (b) Modelling shows that a fibre with core diameter 10 μm and NA = 1.0 exhibiting no second zero dispersion is optimum. Super-continuum generation beyond 12 μm is observed numerically.

5 MINERVA Supercontinuum Sources from NKT

It has been mentioned that the mid-IR region contains a wealth of spectral data which can yield important information on the chemical composition of samples from gases and liquids to living cells. However, the investigation of this topic has been limited by the available photonic sources. Researchers had to choose between a very low intensity broadband source such as a "globar" thermal source, or a high intensity but narrowband source, such as a laser diode.

NKT Photonics is dedicated to providing flexible sources of high intensity light in an easy to use format (Fig. 7.a). It has already established commercial supercontinuum systems which can deliver any wavelength from 400 to 2000 nm on demand. It has recently launched the EXTEND-UV accessory which can extend the wavelength

coverage to cover the 270-400 nm UV region. The company would now like to push the limits of supercontinuum sources at longer wavelengths, reaching into the mid-IR region.

In MINERVA NKT is developing zirconium fluoride (ZrF_4) glass fibre supercontinuum sources to cover the 1.5-4.5 μm spectrum. Subsequently it will investigate even longer wavelengths by utilising newly developed indium fluoride (InF_3) fibres to extend the spectrum beyond the transmission band of ZrF_4 glasses.

These sources could detect changes in cells by monitoring absorption in the 2.6-3.8 μm region which relates to the balance between lipids and proteins (Fig. 8.a). The increase in wavelength from 4.5 μm up to >5 μm would make it possible to interrogate additional important gas absorption lines such as carbon monoxide.

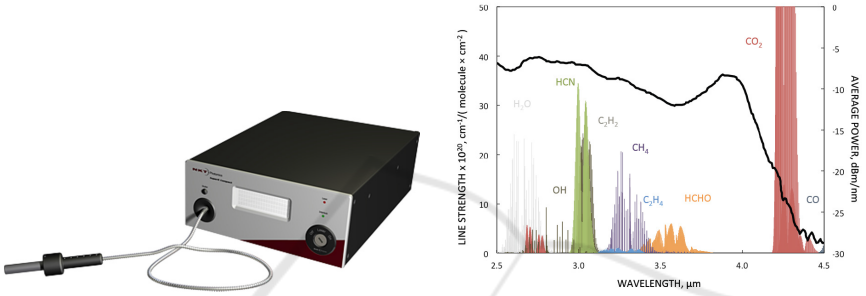

Fig. 7. (a) Schematic of a typical NKT supercontinuum source. (b) Graph showing absorption spectra of some key bio-molecules in the lower end of the "fingerprint region".

In the first 2 years of the MINERVA project NKT has already developed several supercontinuum sources with output power of up to 2.5 W. These sources are more than a million times brighter than most thermal light sources and even brighter than a Synchrotron. We have shown the limits of zirconium-fluoride based systems by setting a new record for the longest wavelength supercontinuum generated at 4.75 μm. However, the chemometric specialists in MINERVA found that the main region of interest was the 2.5-3.8 μm region so we have also shown how the main power in the output spectrum can be shifted down to the main region of interest by altering the design of the nonlinear fiber. These first Supercontinuum sources are already at work in the development of the MINERVA-lite microscopy setup which will soon be applied to bio sample imaging.

Meanwhile NKT is pushing onward in the development of the mid-IR supercontinuum sources. The initial sources were based on rather long pulse nanosecond pump lasers with relatively low pulse repetition frequencies. This made the sources incompatible with most of the Fourier transform spectrometers (FTIRs) that many researchers use in the mid-IR region. In addition, the low repetition rate made it time consuming to counter any noise in the source by averaging over many pulses. NKT is therefore now developing sources based on much shorter pump pulses and with higher repetition rate in order to reduce noise and make the sources compatible with standard FTIRs.

As these mid-IR supercontinuum sources become available and known in the field, the MINERVA consortium expects the emergence of new markets. For example, an

important spectroscopic application in the petrochemical industry is to monitor single wavelengths in the 3-3.5 μm band in order to optimise the refining processes. Monitoring the whole spectrum simultaneously would allow a full real-time chemical analysis of the output chemicals.

Some relevant references concerning the supercontinuum sources within the MINERVA framework have been already published [14–24]

6 MINERVA type-II Superlattice IR Detectors from IRnova

Type-II superlattice (T2SL) is a material/technology that can be used for high quality cooled photon detectors, with tailorable bandgap from 2 μm and upwards. The name comes from the fact that the conduction and valence bands display a so-called "broken type-II" (sometimes also called "type-IIb" or even "type-III") alignment between the constituent materials, which can be InAs/GaSb/AlSb, or alloys thereof (Fig. 8.a). In contrast to typical quantum well devices, e.g. the active regions of semiconductor lasers, the superlattice layers in the T2SL material are so thin (typically 3 nm) that mini-bands are formed in the material. These mini-bands resemble the conduction and valence bands of a bulk semiconductor material. By carefully selecting the superlattice layer thicknesses and compositions, novel materials can be defined to meet widely different needs.

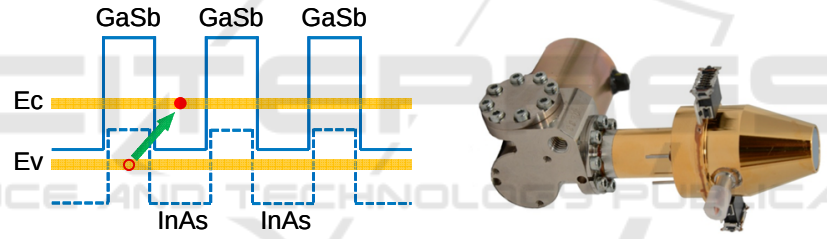

Fig. 8. (a) Schematic of T2SL band-gap structure. (b) Detector/Dewar/cooler assembly for T2SL from IRnova.

Compared with a traditional bulk material for the 3-5 μm range, such as InSb, T2SL requires less cooling and thus draws less power, which allows for longer cooler lifetime and consequently lower life-cycle cost. For the 8-12 μm range, the traditional alloy bulk material HgCdTe (or "MCT") is difficult to fabricate with high yield, partly due to the extreme sensitivity of the bandgap to composition (particularly the HgTe:CdTe alloy ratio). Here T2SL materials have distinct advantages in fabrication.

Focal plane arrays comprising hundreds of thousands of T2SL detector pixels are flip-chip bonded to a CMOS read-out-circuit and then mounted on a ceramic carrier, which in turn is glued to a cold finger in a vacuum Dewar housing, complete with an IR window. The cold finger is cooled to detector operating temperature by a Stirling rotary cooler. IRnovas detector-Dewar-cooler assembly for T2SL can be seen in Fig. 8.b.

IRnova has recently worked on improving the quantum efficiency (QE) of the detection by applying anti-reflective coatings to the detector surface. By this method, the

QE was increased from approximately 50% to 80% in the wavelength region of interest. This improves the signal-to-noise ratio and allows a reduced integration time for each image frame.

Apart from MINERVA applications, IRnova plans to use T2SL technology for gas detection of key greenhouse gases with absorption lines in the atmospheric transmission bands, such as methane and perhaps also sulphur hexafluoride (SF6).

More information about the superlatice IR detectors can be found in [25]

7 Infrared Megapixel Camera Development at Xenics and IRnova

The sensing unit for MINERVA is being developed in a joint effort between Xenics and IRnova. From the start of the project Xenics has been working on the design of a Read-Out IC (ROIC), to be integrated through flip-chip technology with the T2SL (Type-2 Super Lattice) photodiode material, which is being developed by IRnova.

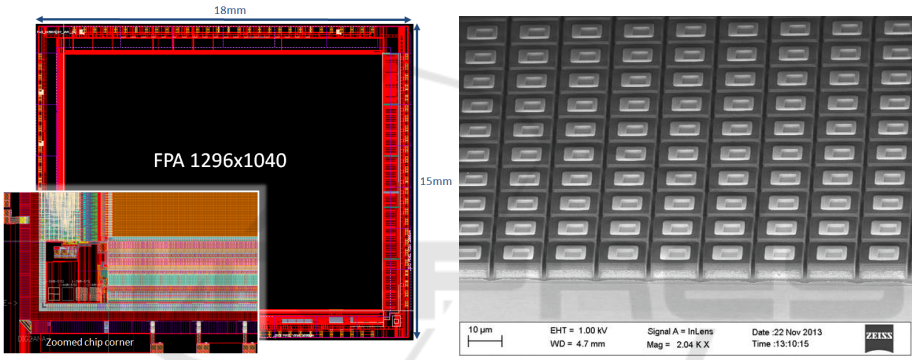

Fig. 9. (a) Global and zoomed view of the designed ROIC, currently in manufacturing. (b) Microscopic view of manufactured array of T2SL photo diodes.

To provide sufficient resolution for reliable data analysis, a 1280×1024 pixel array was chosen, on an aggressive pitch of 12 µm. Project requirements including frame rate, sensitivity and noise level were taken into account in the design process. After extensive simulations and test sample manufacturing, the final design was taped out to a manufacturing foundry (Fig. 9.a). The use of advanced 0.18 µm CMOS technology is required to allow all necessary functionality within the available space of 144 μm^2 per pixel. The first wafers are currently available for post-processing to be followed by wafer-level verification of the electrical functionality.

In parallel, IRnova has been working on the optimisation of the design and processing of the T2SL material, towards cut-off wavelength matching and dark current minimisation (Fig. 9.b). Once the ROIC chips become available later this year, they will be hybridized to the sensor chip, and IRnova will integrate the resulting hybrid in a Sterling-cooled Dewar. The so-called engine will in its turn be integrated into a full camera by Xenics, to be supplied to the other MINERVA project partners to be used for capturing spectroscopic images of prepared tissue samples and live cell phantoms.

8 Pattern Recognition and Data Analysis at GHNT

The first task of GHNT was to provide supporting evidence for the MINERVA instrument specifications. This was achieved by analysing an existing dataset and applying pattern recognition techniques to discriminate between benign and cancerous samples from human colon tissue biopsies (Fig. 10). Sensitivity and specificity of up 86-99% can be achieved with the existing dataset. Using this study as a baseline GHNT was able to assess the impact of various factors that will affect the quality and speed of the MINERVA instrument.

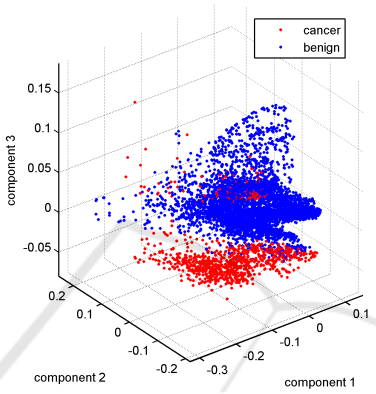

Fig. 10. Partial Least Squares (PLS) scores plot showing the separation between benign and cancer samples in baseline study.

Reducing the number of data points per spectrum is one way to potentially speed up the system; measuring fewer wavenumbers means a faster total acquisition time. Multivariate pattern recognition algorithms were used to identify potential wavenumber targets for the MINERVA instrument. Figure 11 shows the wavenumber regions identified as 'important' for the baseline study.

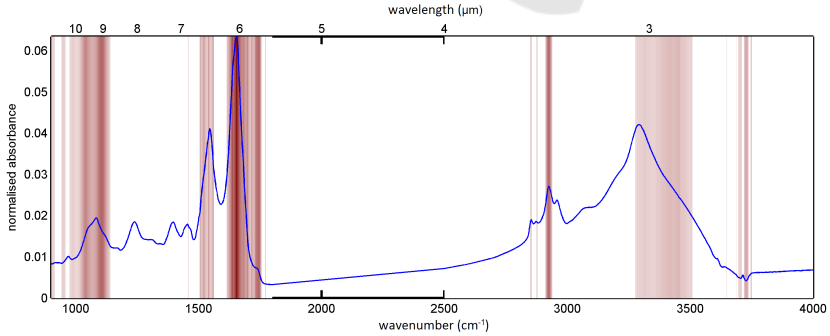

Fig. 11. VIP identified wavenumber targets for the MINERVA system (red). Reference spectrum (blue).

A minimum acquisition time per spectrum means that the MINERVA instrument will be able to rapidly assess samples in a clinical timeframe. However, reducing acquisition time also increases the amount of noise. To determine what level of noise can be tolerated by the pattern recognition algorithms GHNT simulated the addition of noise to the baseline study until it was no longer able to discriminate between pathology groups. This allowed a minimum target signal-to-noise ratio (SNR) to be determined for the MINERVA instrument whilst maintaining an acceptable ability to discriminate between pathology types.

9 High Resolution mid-IR Imaging at University of Exeter

One of the main objectives of Exeters group within the MINERVA project is large scale pathology screening using mid-infrared (mid-IR) spectroscopy. Currently FTIR spectral histopathology, which has the potential to develop as a cancer diagnostic tool, is carried out using a heated silicon carbide rod ("Globar") as the mid-IR light source and focal plane array (FPA) based detectors. This technology is limited by the low flux of the light source and the limited tissue area that can be measured in a given amount of time.

The novel technologies being developed in the MINERVA project; consisting of mid-IR super-continuum light source (instead of a "Globar") and new generation megapixel (FPA) detectors (instead of a 128×128 pixel FPA), will be tested on pathological samples at the University of Exeter.

Currently the base instrument, a commercially available Agilent FTIR imaging system, in addition to the conventional Globar source coupled to FPA based imaging, has been retro-fitted with a new high-resolution imaging capability. The FTIR images acquired using this set-up provided a five-fold improvement in image resolution from 5.5 μm^2 of the current technology to 1.1 μm^2 using the high magnification optics (Fig. 12).

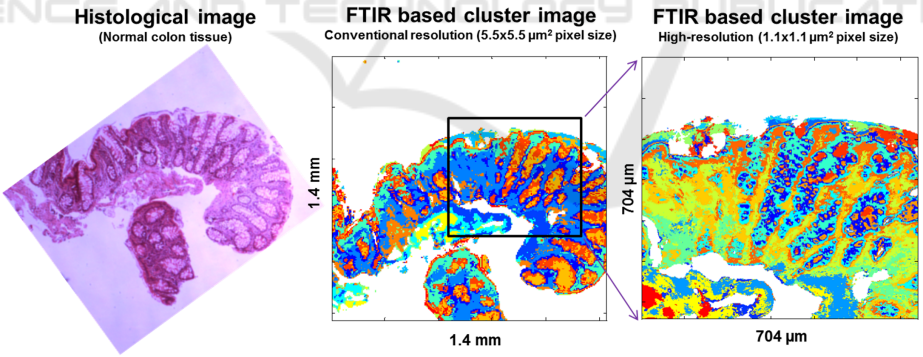

Fig. 12. FTIR based K-means cluster images obtained using conventional and high-resolution imaging compared with the histological image. Histological features based on the bio-molecular composition are partitioned in the cluster images. In the high-resolution imaging, tissue and cellular features are more apparent.

Future work in MINERVA will combine these novel technologies for large scale pathology screening, and also high-resolution imaging in tissue regions of interest, with

the aim to develop faster and accurate cancer diagnostic tools. Initially this will integrate with a 4.5 µm NKT MINERVA source, and later in the project it will be extended to very long mid-IR wavelengths: possibly out beyond 10 µm.

10 Development of Standardised Samples for mid-IR Spectrometer Instruments Testing at WWU

A key task of WWU is to transfer the MINERVA technologies to skin diagnostics and to use mid-IR spectroscopy for the fast screening of human body surfaces and identification of patho-physiologically altered cells and tissue lesions. This requires standardised cell and tissue sample standards with marker spectra for technology performance testing of the novel optical components and systems and for training of novel approaches for advanced data analysis.

The work of WWU in the first MINERVA project period thus focused on the establishment of standard samples with representative spectral information of human skin and skin cancer cells. WWU has established cell culture models which represent major cellular skin constituents and skin cancer cell types. Furthermore, sample preparation procedures on mid-IR compatible substrates have been developed that allow long-term storage of cell lines without significant losses in the quality of the spectral properties.

In order to identify suitable marker spectra of human skin, sample sets with different preparations and cell types were analysed with mid-IR spectroscopy in collaborative work with GHNT to retrieve reference data for technology performance testing and for the evaluation novel algorithms for sample analysis and classification developed by UPV. The principle component analysis (PCA) of mid-IR spectra from different cell types shows an excellent distinct grouping of skin components as fibroblasts and keratinocytes and cancer cells.

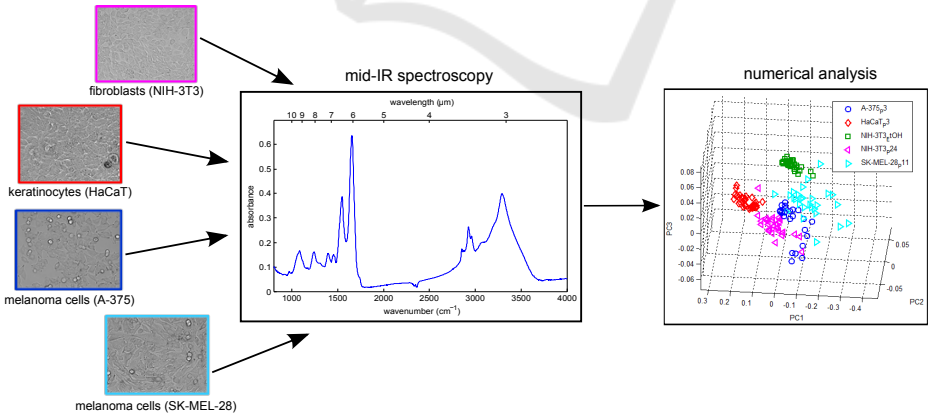

Fig. 13. Principle component analysis (PCA) of mid-IR spectral data from fibroblasts (NIH-3T3), keratinocytes (HaCaT) and skin cancer cells (A-375, SK-MEL-28) illustrates the differentiation between different cell types.

Figure 13 illustrates the analysis and differentiation of different cell types (cancer/ non-cancer) that have been prepared at WWU for the example of results PCA of mid-IR spectral data from fibroblasts (NIH-3T3), keratinocytes (HaCaT), and skin cancer cells (A-375, SK-MEL-28). Based on these results, current and future activities at WWU in MINERVA focus on the development of novel mid-IR standards models for skin cancer detection that are based on 3D human skin equivalents in vitro. Further information about the standardization of the cell samples was presented in [26].

11 First Steps with MINERVA Image Processing at UPV

The first objectives of the image and signal processing group at UPV are focused on segmentation and registration of different kinds of images (Fig. 14): infra-red spectral images (IR), white light (WL), and those most used by clinicians at present, the haematoxylin and eosin (H&E) stained images. The latter is the current "gold standard" used to distinguish between a healthy or pathological patient sample.

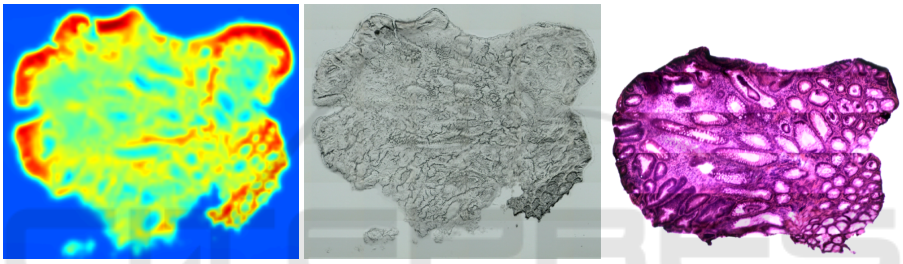

Fig. 14. (a) Infra-red (IR) image. (b) White light (WL) image. (c) Haematoxylin and eosin (H&E) image.

The objective within MINERVA is to automatically segment regions of interest (healthy and pathological) in the H&E images and look for their features in the infrared spectrum. To achieve this goal the H&E image must be registered with the WL image (which is already registered with the infrared volume). So, the work is focused on two interactive steps: registration and segmentation.

Registration allows the matching of elements that clinicians considered important in the H&E images with the spectral images. A successful registration task would allow users to learn, and later identify, the areas from which diseased and healthy cells and patients can be distinguished (Fig. 15.a).

Segmentation concerns the accurate extraction of the cell contours (Fig. 15.b). This would reduce the huge amount of data to be analysed looking for subtle biochemical changes ("cancer markers"). Once the contours have been identified, the regions must be classified as healthy or cancerous depending on subtle features including shape, texture and clustering. This is an extremely difficult task, but the use of the spectral information in the mid-IR should eventually aid clinicians to improve on the current gold standard.

More details about the work on image processing in MINERVA project was presented in [27, 28].

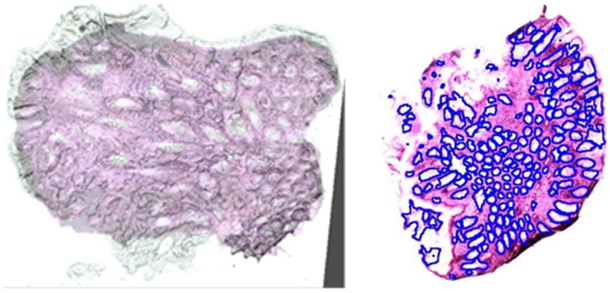

Fig. 15. (a) Projective registration test. (b) Segmentation sample test.

12 MINERVA Lite

A prototype MINERVA system that operates in the 2-4.5 μm wavelength band has been assembled so that the individual parts being developed by partners in the project can be evaluated together. The final MINERVA system will operate at even longer wavelengths. The key components in the integrated system are: NKT supercontinuum source, G&H acousto-optic tunable filter, Xenics IR camera, commercial microscope and IR optics and control electronics.

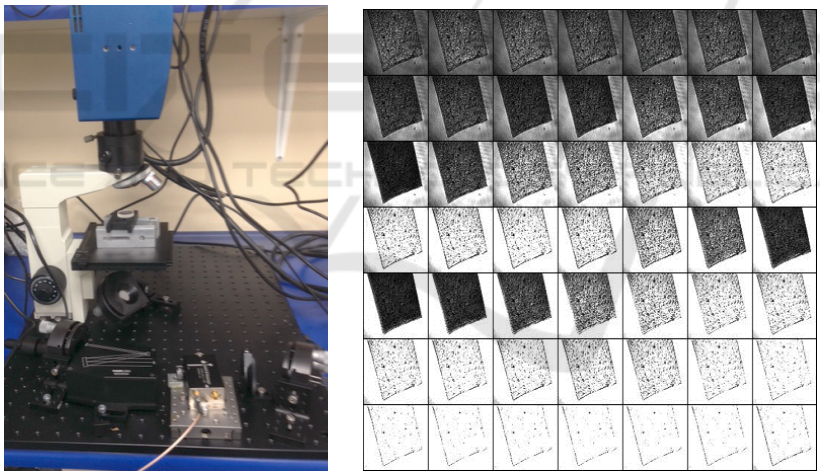

Fig. 16. (a) Photo of part of the "MINERVA Lite" laboratory set-up. (b) 0.3 Mpixel images at 49 wavelengths which can be used to form an (x, y, λ) image cube in 0.6 s.

The breadboard system is shown on Fig. 16.a. This system can take 0.3 megapixel images with 20 μm spatial resolution at a rate of 85 frames per second. Each image is taken at a different wavelength so that a set of 49 spectral images can be built up in 0.6 s (Fig. 16.b). These images can form an (x, y, λ) "image cube". Each pixel records a spectrum and this has enabled MINERVA researchers to identify a polymer film in

the sample image. This important preliminary result will be extended in MINERVA to identify spectra from cancerous cells in tissue samples and in real time on live patients.

More related work in MINERVA project has been published in [29–31]. In addition, the research done in MINERVA has been mention and reviewed in [32, 33].

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