PROJECT FINAL REPORT – Final Publishable summary

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Executive summary

The aim of BiognostiX was to develop technologies and flexible manufacturing methods using fibre-based substrates (e.g. paper or card) for the fabrication of inexpensive point-of-use diagnostic tests for veterinary, agri-food and human health bio-markers.

This was a three year EU FP7 project running from 2011 to 2014. It brought together seven partners from academia, a technical research centre, an engineering SME and three other SME's with a strong background in diagnostics.

BiognostiX has delivered results that combine biochemistry, microfluidics, fluid-jet printing and a novel immune-ink technology platform to enable the development of low cost, rapid multiplex assays on disposable paper-based analytical devices.

The stages of the project were completed successfully within budget and on time. A tightly managed final phase ensured that a complete set of experimental results were presented at the project dissemination symposium towards the end of the project.

Through our research, guided by commercial needs, vital progress has been achieved in the development of point-of-use diagnostics, namely:

- A flexible sample-handling microfluidic platform has been developed.
- Conjugation chemistry of key biomarkers of commercial interest has been delivered Microfluidic and biochemical functionality has been delivered through understanding and implementing fluid jet manufacturing techniques.
- A laboratory-scale reader and associated software has been built with the flexibility to allow ease of modification to new labels

The output from the research is a number of 'toolkits' which will enable further research and development of the commercial 'BiognostiX engine' concept.

Going forward the exploitation plan is to further develop and commercialise this 'BiognostiX Engine' concept (see figure right) as an enabling technology for the design, development and production of ELISA based point-of-use diagnostic tests.

BgX has significant commercial potential in the dynamically developing markets of companion diagnostics (cDX), animal diagnostics and food testing, and as such, we have reported publication of papers and delayed filing of IPR to enable further research to maximising the commercial impact.

At our project symposium which was attended by the diagnostics industry, the scientific press and academia, we were able to disseminate proof-of-concept results and underlying research without compromising the IPR. Our objective was to find new partners for further funding for the research and development required to continue the commercialisation process.

The symposium generated significant interest from attendees and those who were unable to attend with at least seven companies now expressing an interest in further exploration of the potential of the BiognostiX Engine for their diagnostic test.

Further dissemination is planned locally, in the countries of respective partners and internationally through trade press articles, electronic media and timely publication in peer reviewed journals.

Project context and objectives

Molecular diagnostics is a rapidly expanding field with applications in medical, veterinary, food science, environment and other areas. Its greatest potential value will be to enable the acquisition of multiple rapid quantitative measurements at the point of use, e.g. hospital bedside, doctor's surgery, home or field testing. Today, most diagnostic testing is undertaken by sending samples to laboratories where they are processed en masse with significant turn-around times.

Currently, point-of-use (POU) diagnostic kits tend to be simple, single point assays with a yes/no or semi-quantitative output and based on dipstick or lateral-flow formats.

These formats gained popularity in the consumer markets due to their compactness, portability and simplicity of interpretation without the need for expensive external instrumentation.

However, lack of quantitation in measurements, more complex assays and the need for a lower limit of quantitation (LOQ) limits the adoption of these formats for new diagnostic tests.

Recently, fibre-based microfluidics has emerged as a point-of-care platform with multiplexing capabilities to address the gap between the characteristics of traditional POU diagnostic formats and emerging diagnostic needs. Paper-based microfluidics can enable fluid handling and quantitative analysis for potential applications in healthcare, veterinary medicine, environmental monitoring and food safety. Currently, in its early development stages, paper-based microfluidics is considered a low-cost, lightweight, and disposable technology.

Moreover, fibre-based microfluidics has the potential to be more configurable at point-of-manufacture when compared to plastic based devices. As a result, development of fibre-based devices should lead to simpler manufacturing processes and significantly cheaper manufacturing costs.

Figure 2 A) Prototype BgX-Chip containing red dye to illustrate the sample deposition area and flow channels, captured using densitometric mode. B) Prototype BgX-Chip with fluorescence bound in discrete locations, captured using fluorometric mode.

The purpose of the BiognostiX project was to develop technologies and manufacturing methods for the fabrication of such fibre-based microfluidic devices.

The project was broken down into seven work packages which represent the components required to develop the technologies required for the fabrication of inexpensive diagnostic sensors on fibre-based substrates:

Work Package 1 – Fibre Substrate Engineering

"… to develop a fibre substrate-based microfluidic platform which enables both the functionality of printed biomaterials on/in a fibre substrate as well as the transfer of fluids in a diagnostic test system in controllable ways."

Work Package 2 - Biomaterials Engineering

"… to engineer the biomaterials and bio-labels for ink-jet printing and to design an immunoassay format, utilising fibre-substrate properties to define channels, chambers and flow characteristics for fabrication…"

Work Package 3 – Fluidjet Manufacturing Methods

"… to develop the fluidjet processes to enable configuration and programming of the microfluidic processing units and channels during manufacture . . . .to optimise the placement of the biochemical reagents and optical labels by fluidjet printing at appropriate locations…"

Work Package 4 – Diagnostic Reader Development

"… to develop a low-cost detection system able to detect and interpret the signals generated on the test chip..."

Work Package 5 – Proof-of-concept Integration and Evaluation

"...Produce suitable test specifications that exercise the reader and test coupon, demonstrate suitable coupon manufacturing system, demonstrate said system is cost effective and reliable and develop quality measures for test coupon production and verification. . . "

Work Package 6 – Innovation Related Activities

"… to disseminate the knowledge gained in the BiognostiX project to the wider scientific community as well as the general public, which will include a non-specialist audience….."

Work Package 7 – Management

"…to ensure that all knowledge is created, managed and disseminated in a coordinated and coherent manner and that all activities, legal aspects and other issues are managed to a high standard…"

Work packages 1, 2 3 and 4 ran for the duration of the project and, over time, each has delivered a number of technology options that have been used iteratively within WP5.

During WP5 we discovered that the paper based substrate chosen at month 18 could not be integrated into the overall platform. At the month 30 consortium meeting it was agreed to fast track an alternative design.

This new design enabled the delivery of prototype devices that demonstrate proof-of-concept results in two of the three SME applications.

The research and results from the BIOGNOSTIX project have been disseminated to the diagnostics industry, academia, the scientific press and the general public.

Some of the main achievements of the BIOGNOSTIX project include the following:

- Three different kinds of manufacturing concepts for fibre based microfluidic formats were developed, characterized and evaluated
- Creation of a toolbox of immuno-inks that have been characterized for printability and functionality of antibody capture particles when suspended in the ink
- Established fluid-jet methods to control flow in microfluidic channels, and to deposit precisely metered amounts of bio-materials
- Manufactured a prototype chip reader capable of working in densitometric, fluorescence and colorimetric modes
- Successfully created batches of chips using prototype manufacturing technologies
- Generated 6 leads for potential new partners after presentation of our proof-of-concept results at the BiognostiX symposium

Main Scientific &Technical foreground and results

Introduction

The project has successfully generated a significant amount of foreground that can be assigned both collectively and individually for future exploitation. By collectively we mean through commercial exploitation of the BiognostiX concept.

Individually, each of the SME partners have benefited from working with world-leading academic groups and now have access to processes and technology that will enable them to increase productivity, improve skills and know-how and potentially deliver new products to market.

Similarly, each of the academic partners has gained additional experience through exposure to the different perspectives, ideas and approaches of SMEs and also increased their opportunities for their research to be published in internationally respected journals.

Table 1 - Summary of key foreground generated during the BiognostiX Project

Foreground (Project Results)						
Owner	**Foreground 1**	**Foreground 2**	**Foreground 3**	**Foreground 4**	**Foreground 5**	**Foreground 6**
FFEI	Small footprint scanner capability	Reflection based scanning technology capable of working with opaque, uneven substrates	Reader capable of measuring multiple reaction zones in a single pass	Technology to overcome native high background fluorescence in paper	Reader capable of densitometric, colorimetric and fluorescence detection	Ability to measure sandwich ELISAs where the capture complex is on a non-transparent material
IoB	Development of a family of novel sub-micron-particle component that can be modified with biorecognition ligands and utilized for immuno	Development of a family novel sub-micron-particle component that once modified with biorecognition ligands can be suspended in	Protocols for attachment of capture ligands to the family of sub-micron particles	Protocols for blocking non-specific binding of analyte and detection ligands	Use of developed family of particles for ELISA and sandwich ELISA assays	
IfM	Treatment of surfaces to control flow	CharacterisationUse of capping layers to control flow in micro	Flow programming technologies	Formulation of bio inks suitable for printing using fluid jet	Control of jetting parameters to enable printing of 'bio-inks'	Printing Bio-Inks with high positional accuracy
VTT	Understanding of 3 different methods for channel creation: embossing, lamination, moulded polymer coating	Toolbox of different surface chemistries	Toolbox of different capping tapes			
Prionics	Conjugation of Brachyspira, Trichinella & Toxoplasma antigen to ink particles	Conjugation of anti pig IgG2 antigen to detection particles	Development of a new ELISA assay that can be transferred to the BiognostiX format	new plate based fluorescence assay with proof of concept micro-fluidic assay	technologies to minimize non-specific binding in the micro-fluidic channels	
Proteomika	Conjugation of ADL / ant-ADL to ink particles	Conjugation of ADL / anti-ADL to detection particles	Development of a new ELISA assay that can be transferred to the BiognostiX format	technologies to minimize non-specific binding in the micro-fluidic channels	technology for the stabilisation of ADL and anti-ADL antibodies coupled to ink particles for at least 6 months @4C	Proof of concept data in the BiognostiX format
PTP	Conjugation of anti-salmonella polyclonal antibody to ink particles	Conjugation of MAB7718 to detection particles	Development of a new ELISA assay that can be transferred to the BiognostiX format	technologies to minimize non-specific binding in the micro-fluidic channels	Developmemnt of a new, rapid sample preparation protocol for salmonella testing	

Work Package 1 – Fibre Substrate Engineering

During the project, three types of systems for manufacturing of microfluidic biodetection systems were investigated. These are

1. Microfluidic channels debossed / embossed on paper substrate
2. Laser cut channels on adhesive tape attached on paper surface
3. Microfluidic channels on PDMS coated paper surface

Strengths and weaknesses of each manufacturing method were evaluated. PDMS coated paper was chosen for prototyping trials because it proved to have the most even quality characteristics.

Paper debossing and sealing

In hot embossing process, a pattern from a micro machined metal master is transferred to a selected substrate. The inverse of the tool structure is obtained in the substrate; the principle is seen in figure 1 below. Heat and high pressure allow the substrate to become imprinted. The masters, shims, can be used many times to form imprinted surfaces that can be sealed to form microfluidic channels. All microfluidic channels were manufactured with a Madag P2000 static flat-bed hot embosser.

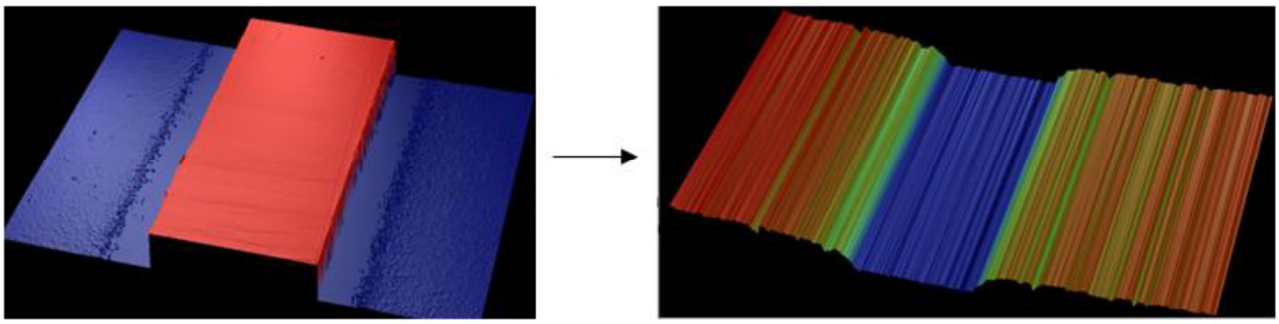


Figure 1 - Principle of hot embossing/debossing. The shim structure (left) is copied into the paper substrate (right)

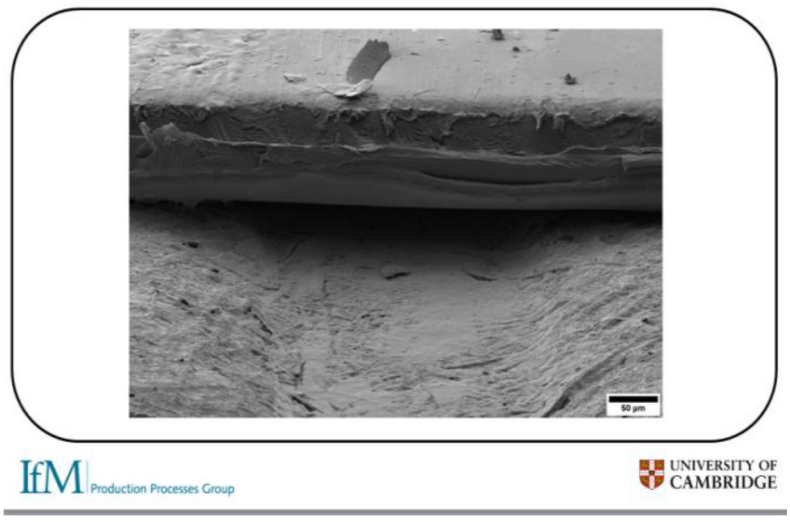


Figure 2 - Cross section of de-bossed channel and tape viewed with scanning electron microscopy

Coating material development

Fabrication of a functional network of microfluidic channels on top of the substrate surface requires selection of a proper base material combined with a barrier layer that prevents liquid penetration into the fibre network

and, further, provides a convenient surface for microfluidic flow (see Fig. 1). To this layered fibre-based substrate microfluidic channels are embossed by compressing the substrate structure under external pressure and heat in defined areas. Channel formation on top of the substrate surface requires sealing of the channel to form a capillary flow into the channel. The liquid flow in the embossed channels is mainly governed by the forces associated with the surface tension. Therefore, the surface properties of the sealing material on the liquid side also play a crucial role in supporting capillary flow. Air pockets beside the sealed channels may allow the fluid flow out of the channel.

The project evaluated suitable coating materials for embossing. These materials were evaluated with a commercial board coated by a biopolymer being selected from an embossibility, surface chemistry point and contact angle (wettability) perspective.

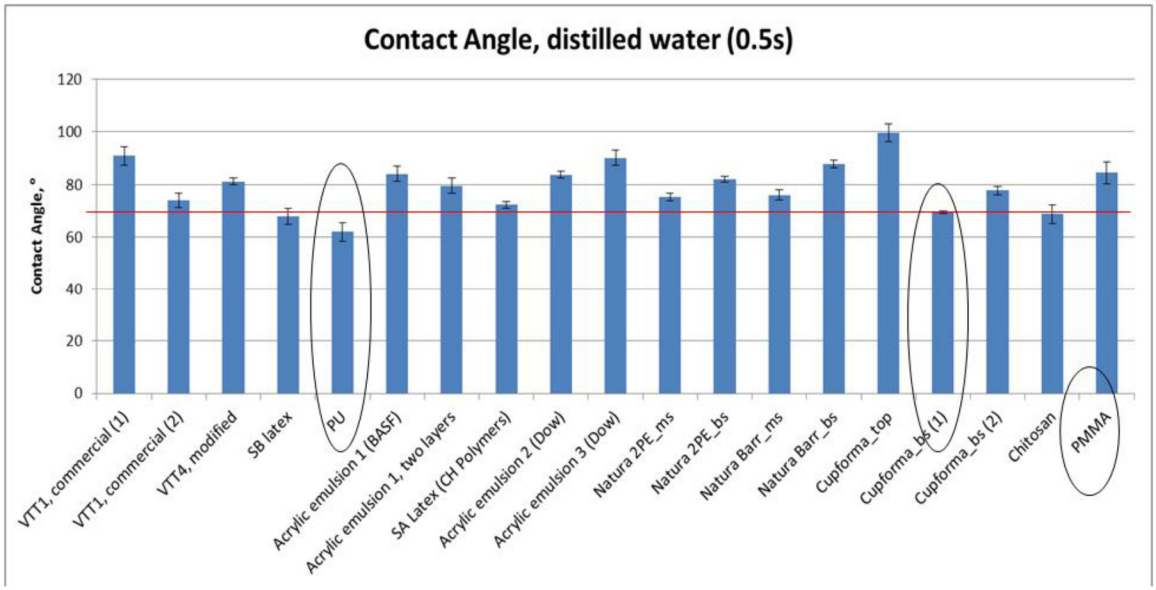


Figure 3 - Comparison of the contact angle with water for different substrate coatings

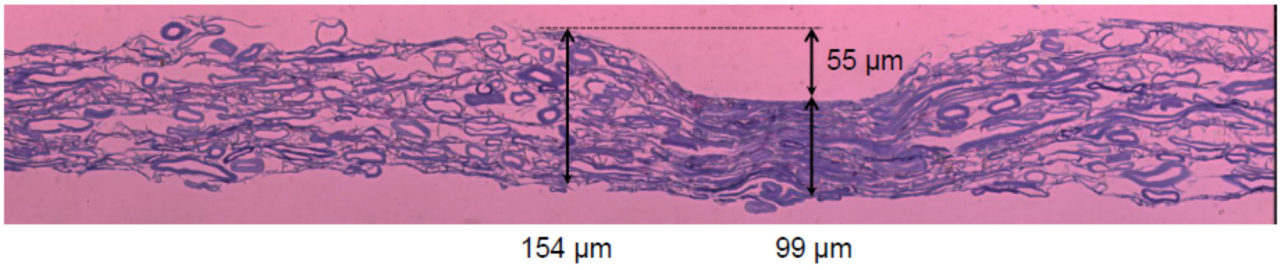


Figure 4 - Schematic of channel topography for embossed channels

As the research progressed we discovered a number of limitations for the embossing / debossing approach which resulted in the need to seek alternative methods of fabrication if we were to achieve our objective of testing proof-of-concept BgX chips by the end of the project. The principle conclusions were:

1. The fibre network resists the embossing giving rounded edges on the channels thus making it very difficult to obtain a reproducible seal
2. The open space of fibre network limits the depth of the channel. The fibres are compressable but when compared to plastics this is on a minor scale. It has also been postulated that this compressibility may be reversible over time but this has not been investigated in detail

3. The coating material limits the embossing temperature meaning that in the flat-bed embosser the embossing time can be relatively long

Lamination using adhesive tape

A novel, second technique was developed for channel fabrication. This technique uses a combination of Cupforma paper and the two capping films, ARflow 90368 and Tesa 080640, to create the microfluidic channels. The paper provides the mechanical stability of the device and the channel base, while the high wettability of the ARflow 90368 provides the capillary force driving microfluidic flow.

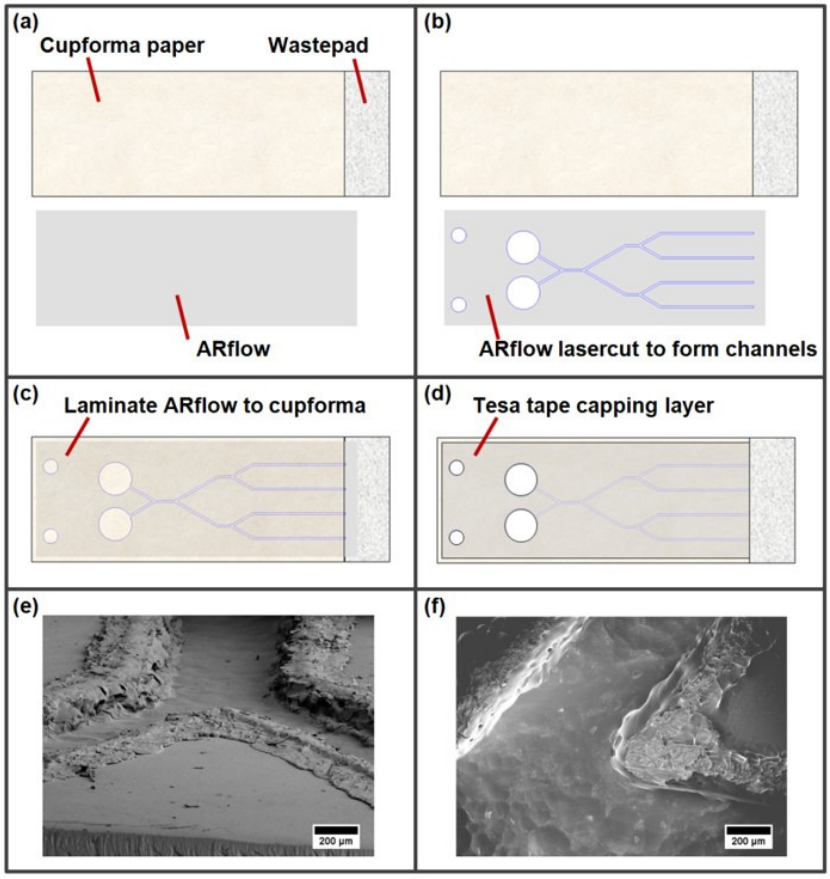


Figure 5 - Procedure to fabricate laser cut channels in tape

Initially a piece of Cupforma and a wastepad are cut to size and laid next to each other. ARflow 90368 is cut to the same size and the channel structure is laser-cut into the film. A hot laminator (approx. 90 oC) is used to bond the ARflow 90368 channels to the surface of the Cupforma. The bottom two panels of Figure 5 show examples of a Y-junction using this technique. The capping layer, Tesa 080640, bonds to the surface of the ARflow 90368 using room temperature lamination to complete the channel fabrication. The highly wettable walls of the channel drive the capillary flow.

Further investigation and characterisation of this approach is required as this shows great potential from an ease of manufacture perspective.

However, given this is a novel approach and time was of the essence in the project, we decided to seek a third, better characterised approach to achieve our project target of proof-of-concept BgX chips.

PDMS channels on fibre based surface

In addition to the Cupforma based channels described previously, a polydimethylsiloxane (PDMS)-coated paper channel was developed for BiognostiX. The polymer material enables greater control over the shape and surface chemistry of the channel while the paper improves the structural integrity and, at a later stage of the project, is expected to allow integration of a flow-controlling and sample collection pad. These channels follow the same design as noted earlier, where an individual dosing point equally feeds four active sensing zones.

The procedure to fabricate these channels is illustrated in Figure 6 - Process for fabrication of PDMS channels.

PDMS (Sylgard 184) is imbibed into an absorbent paper (currently this is Whatman 1). An aluminium master mould is used to shape the PDMS-coated paper channels.

Cross linking is then carried out at 80 °C for 2 hours, at which point the PDMS is solid. Removal of the PDMS shows an excellent inverse replica of the master mould. The channels are completed with the addition of a capping layer. The capping layer is AR90368 from Adhesive Research. Scanning electron microscopy (SEM) images of the lower surface of the channel and the cross-section are shown in (g) and (h) respectively. These images show a clearly defined channel structure and the boundary between paper and the PDMS coating.

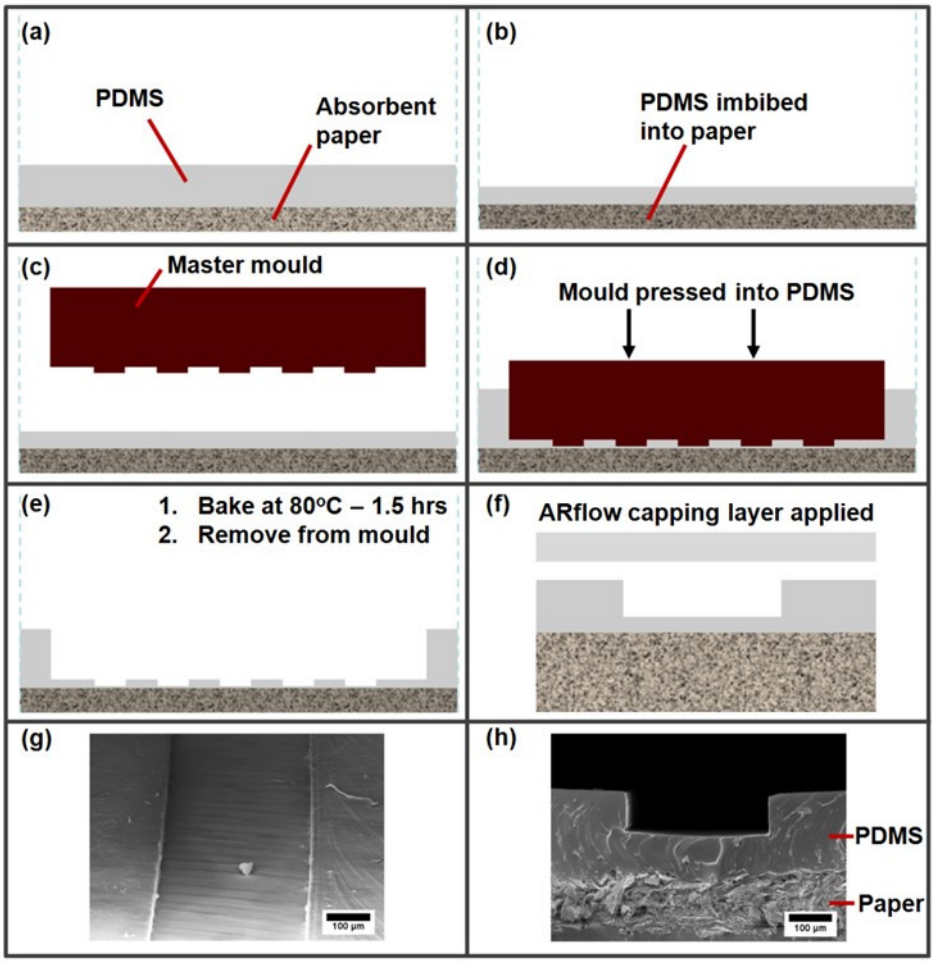


Figure 6 - Process for fabrication of PDMS channels

Channel Sealing

Channels have to be properly sealed in order to attain capillary driven liquid flow. Pressure sensitive adhesive (PSA) tapes were used for sealing. The main principle for the sealing was to use thin plastic film coated with an adhesive on one side of the film.

Release liner on the adhesive side of the tape was removed before lamination or in the case of PDMS, direct application. For the lamination, a wringer type bench top laminator (Yosan benchtop LM260) was used. The samples moved always with the same speed and pressure through two circulating cylinders.

To preserve bioactivity, room temperature lamination is necessary. However In practice this was difficult to achieve with reliability.

Besides firm attachment of the sealing material to the top of the channel the seal has to place itself so that leaks to the outside and between the individual channels are avoided. The quality of the sealing was investigated through use of capillary flow tests. Coloured synthetic serum was used in those tests. Proper channel sealing on embossed polymer coated Cupforma board was attained with hydrophilic ARflow 90368 at raised temperature and pressure. BSA coated channels were successfully sealed at room temperature with Tesa 080640 tape at raised pressure. As a drawback because of the broadened channel shape it was possible in both cases that flowing fluid could leak along microchannels beside the main channel.

Both laser cut and PDMS based channels have sharper edges on top of the channel than embossed channels. This means that they are both easier to laminate and do not have the channel leaking problem.

However a subsequent stability study revealed a time dependent issue with de-lamination of the tape on the PDMS format when stored in humid conditions.

Non-specific binding

Nonspecific binding of model analyte and antibodies in all three manufactured fibre based microfluidic channel system were examined with fluoroimmunoassay methods. These tests demonstrated the need to pre-treat all three formats in order to prevent non-specific binding. Two methods were developed, one using Bovine serum Albumin (BSA) and another using High Performance ELISA Buffer from the Menarini Company (HPE)

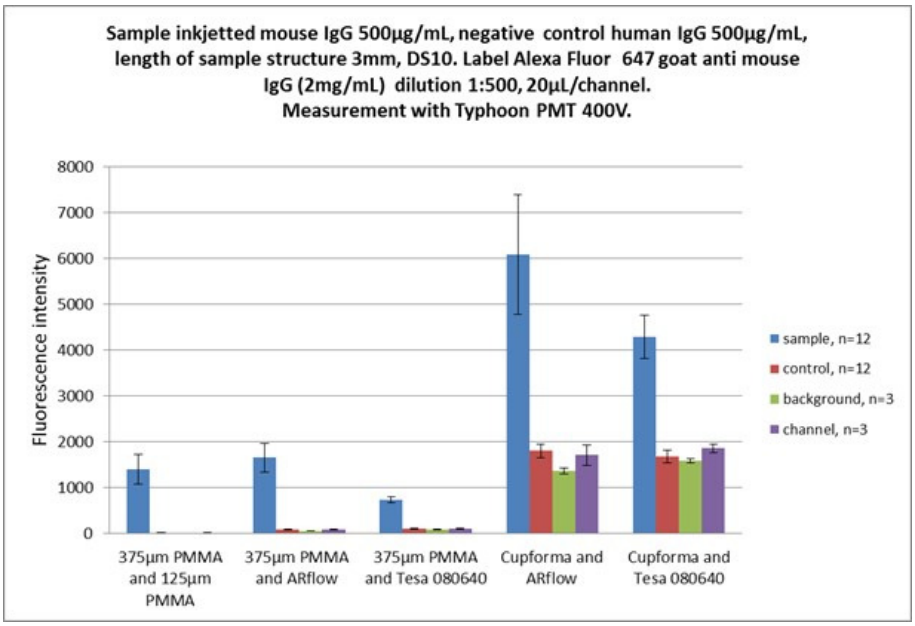


Figure 7- Non-specific binding study of fluorescently labelled anti-mouse igG

Summary of results

Strengths and weakness of the three different manufacturing methods are summarised in Table 2 below. Scores given are based on expert opinion formed on group discussion.

To meet project objectives, we decided to proceed with the PDMS on paper format for the final six months of the project

	Paper debossing	Laser Cut Tape	PDMS on Paper
Ease of manufacture	****	****	**
Production temperature	**	****	****
Channel wall quality	**	***	****
Channel sealing	*	****	****
Total flow rate	*	***	****
Inter-channel flow consistency	*	***	***
Non-specific binding	*	**	****
Fluorescence compatible	**	***	***
Biologics stability	***	***	***
Coupon reproducibility	**	***	****
Test status	***	*	**
Configurable	*	****	*
Cost	****	***	*
LCA (environment friendly)	***	**	**
Score	30	42	41

Table 2 - Comparison of the three different manufacturing methods evaluated during the BiognostiX project

Work Package 2 - Biomaterials Engineering

We have developed a novel capture-antibody-substrate conjugate model which was used to prepare 'ink' formulations for analytical reagent printing. This was an iterative, multi-step process that investigated solid phase particle synthesis and characterisation, modification of the particles with capture ligand, incorporation of the capture ligand particle into an immunoassay, assay performance characterisation, evaluation of the particle as a component suitable for development into an ink, development of printable inks and analysis of print requirements according to flow channel specification.

Particle synthesis and characterisation

A novel particle with a silica shell with a tuneable surface chemistry has been developed and classified. The produced core/shell particles are monodisperse and spherical with a mean dry diameter of 628 ± 29 nm (Figure 8, Figure 9).

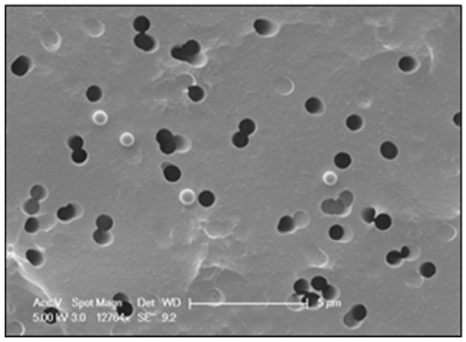


Figure 8 - Scanning electromicrograph of core/shell particles on a polycarbonate track etched membrane with 0.75 µm pore size

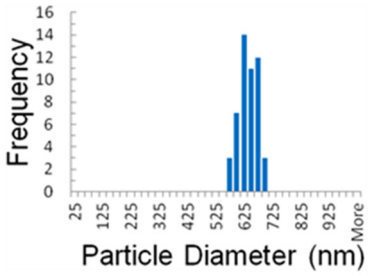


Figure 9 - Histogram of particle distribution via image analysis using Image J

As simple 'passive' adsorption may not be sufficient to functionalise the silica particle with all of the relevant capture ligands, this particle was used as the basis to develop a family of particles with alternative surface chemistries.

Immunoassay, either as direct ELISA or Sandwich ELISA, performed using this basic-chemistry particulate has shown good performance for all models trialled by the SME partners, with the exception of the lipid based recognition systems. This finding was expected, where the surface chemistry was not appropriate. Other chemistries which allow chemisorption have also been trialled and generally show superior performance to the basic design. Results are presented in Figure 10, Figure 11 and Figure 12

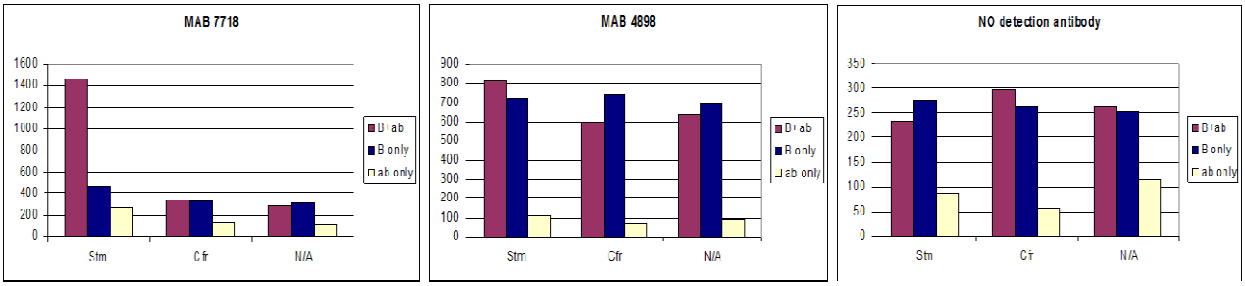


Figure 10 - PTP application. Antigen detection capability of Ab35156 conjugated on Ink Particles. "Stm" Salmonella tiphymurium; "Cfr" Citrobacter freundii; N/A non antigen

As shown in Figure 10, specificity in recognizing the antigen of MAB7718 was better than MAB4898 and for this reason MAB7718 was chosen for subsequent tests. As shown in the last graph, "particles alone" showed a high background; for these reason some blocking agents were tested

The loading of Adalimumab monoclonal antibody on the surface of the particles has been assessed. As shown in Figure 11, a dose-dependent conjugation of ADL ab to nanoparticles was clearly observed over a range of 3 orders of magnitude (from 0.1 to 100ug/ml of ADL). The loading capacity saturates at 30-100ug/ml ADL.

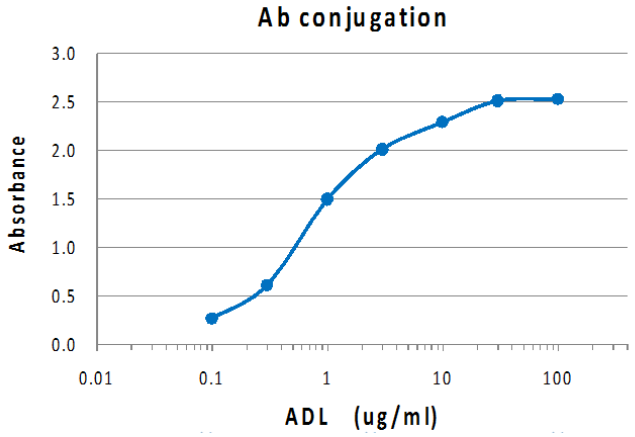


Figure 11 - PTK application. Dose-dependent conjugation of ADL capture ab to nanoparticles

Figure 12 shows the ELISA results for three different Prionics applications.

Panel 1 shows the ELISA positive Brachyspira serum with particles loaded with varying concentrations of Brachyspira recombinant antigen (particle concentration: 5 mg/ml). The results show a maximal binding at an antigen concentration of 100 µg/ml which corresponds to 1 µg antigen per assay.

Panel 2 shows the dose-dependent binding of the Trichenella antigen to the particles. The increase in antigen binding is recorded as a function of antigen concentration with a maximum loading capacity exceeding 100 µg/ml.for a 5mg/ml particle suspension

Panel 3 shows the dose dependent binding of Toxoplasma antigen to particles. The Toxoplasma positive serum can generate a high readout after incubation with Toxoplasma antigen coated ink particles as a function of toxoplasma antigen loading on the particles. A maximum loading capacity of 50 ug/ml is recorded for a 5mg/mL particle suspension

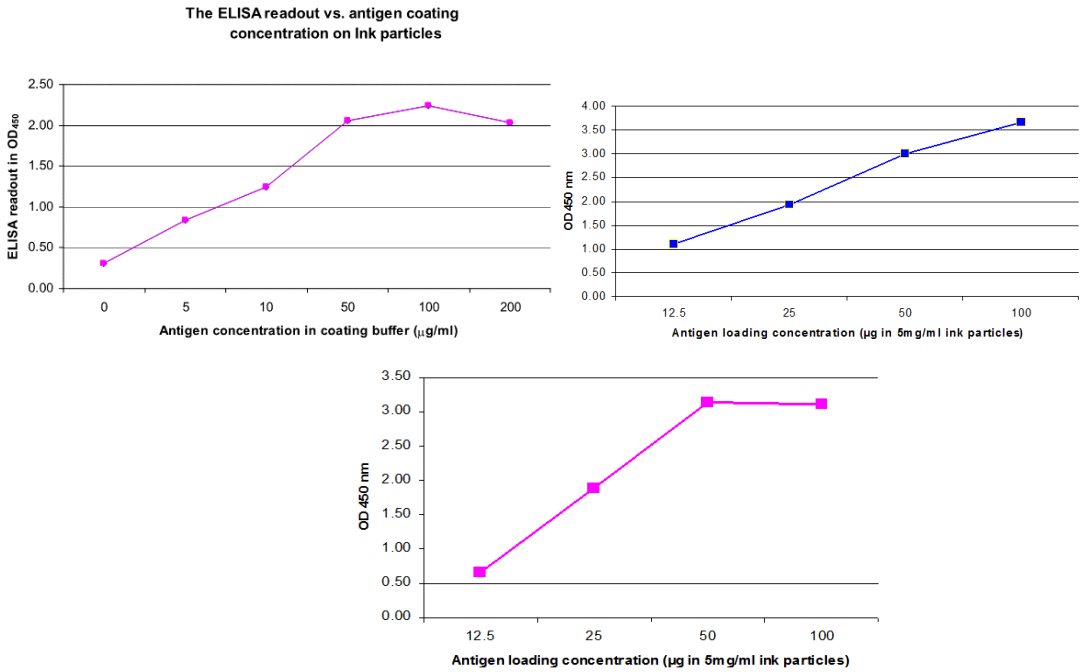


Figure 12 - Prionics applications. Dose dependent conjugation of recombinant swine dysentery protein (Brachyspira), Trichinella and Toxoplasma antigen

To function as a solid support for an immunoassay, it is necessary that the surface of the particles does not adsorb the analyte or detection ligands non-specifically. Non-specific adsorption will be variable, dependent upon the analyte or detection ligand properties. A range of commercially available blocking agents were evaluated and found suitable to reduce the level of non-specific binding.

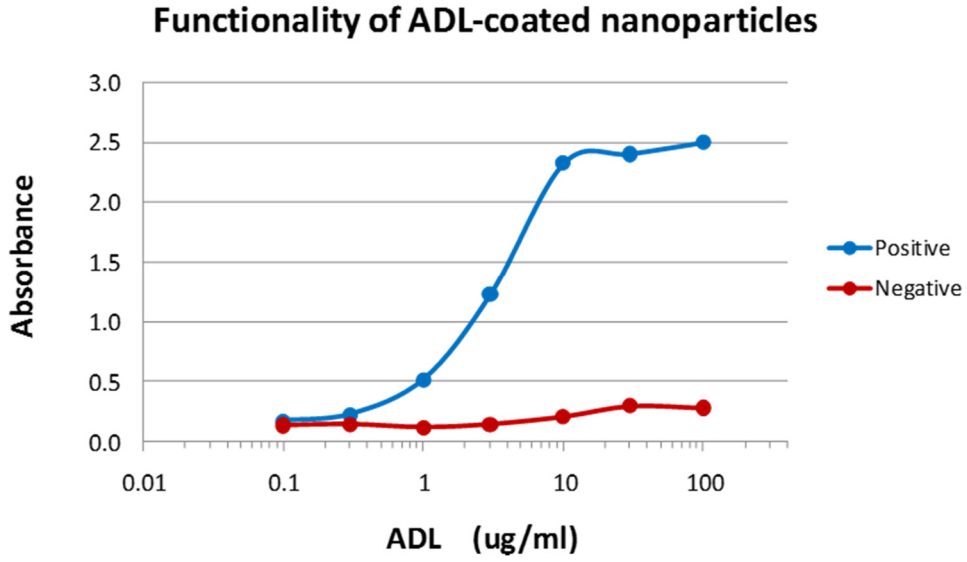


Figure 13 - Evaluation of the functionality of adalinumab coated nano particles

Sandwich-type immunoassays were used to evaluate the functionality of ADL coated-nanoparticles. Rabbit serum samples were used for the validation process. Nanoparticles functionalized with ADL antibodies detected the target protein in positive rabbit serum samples while no detectable signal was recorded in negative signals. Results indicated the recorded signal was target specific and confirmed the functionality of the coated antibodies.

Printable Ink Development

The particles have been developed to be an immobile carrier substrate for the bio-ligand. As such, their post deposition immobility is paramount and their ability to be deposited into the channel to create a 3-D 'porous-plug' structure is required to ensure complete and uniform interaction with the sample.

We have developed a toolbox of carrier "Immuno inks" that are inert to the solid phase ligand phase carrier particles with the following print properties:

- It has viscosity suitable for fluidjet printing
- Forms uniform drops
- Does not dry at the print head nozzle but dries completely after printing.
- Enables particles to stay in suspension for sufficient time to allow printing.
- The total mass and volume occupied by the components of carrier solution for the ink are relatively low to allow for maximum loading capacity of the ink by the solid-phase ligand carrier.
- The dried mass of the solution phase components in the channels do block any sample or reagents from reaching the particles or completely impede flow of the mobile phase

Print experiments using these particulate solid phase ligand carriers demonstrate that the particle is suitable as an ink component. The particle size chosen is appropriate as a stationary phase component and flow studies show that it remains in place once deposited. The particle has in-built design flexibility and allows considerable application targeted optimisation.

All these characteristics are interdependent e.g. precipitation of the particles is largely dependent on the viscosity of the ink or lowering down the surface tension helps with printing. While broad guidelines for ink formulation can be proposed, each preparation will require the inks to be fine-tuned keeping in view the

surface chemistry on the particles. The number of particles required for the deposition into the channels to get consistent signal also depends on the channel dimensions and the required sample size, as well as on the final sensitivity of the reader.

A solution phase carrier is developed with additives that allow viscosity adjustment etc. and the effect on the capture ligand on the particle in this medium is assessed. It provides a toolbox of inks which can be used with the flexibility keeping in view the properties of specific reagents and needs. Some caution is suggested over ink designs requiring high polymer and/or glycerol addition due to their covering of the ligand on the particle, making it less available as a capture system for the sample analyte. Some inhibition in particle packing is also suggested by these additives. This needs to be taken into account in the flow dynamics for the assay. In this context choice of various deposition methods to deliver particles into the channels are considered. The characteristics of inks detailed herein should be combined with print method and detail (e.g. stirring/agitation capability) in deciding what formulations would be more useful for specific demands.

Work Package 3 – Fluidjet Manufacturing Methods

Overview

Work package 3 focused on both programming the flow of sample along channels and the design of purpose-built fluidjet equipment, along with the development of appropriate characterisation, metrology and validation techniques to ensure that these tasks fulfilled their role within the broader context of all of the other work packages. The work carried out in WP3 specifically delivered:

- A detailed understanding of fluid flow in fibrous and microfluidic channels using both simulation and experimental approaches.
- Programming of sample flow in porous and microfluidic channels using inkjet printing and a range of hydrophobic inks delivered through both research and manufacturing-scale print heads
- Characterisation techniques for the testing and validation of flow-programming solutions.
- Specification and construction of a flexible, robust and highly controlled fluidjetting system for bioprinting activities.

Deliverables 3.1-3.5

The requirement of this set of deliverables was to deliver an optimised and scalable system for programming microfluidic channels at the point of manufacture. CU-IfM defined the driving forces for flow in a range of channel types and matched inks accordingly to ensure that sample flow can be directed along pre-defined paths, either through physical blocking or changes in channel wettability.

Printing systems were specified and validated for this work, namely the DimatiX Materials Printer DMP2800 and PiXDRO LP50, and a range of characterisation techniques were used to identify the printability of the ink and its subsequent mode of action (e.g. by physical blocking or wettability modification). Where required, post-printing processes were also optimised to ensure that flow programming was successful.

Internal deliverables (3.5a-b) were produced, both exploring additional feasible uses of programming technologies and also communicating to partners the more fundamental understanding of the control of fluid flow in microcapillaries. Both Computational Fluid Dynamics (CFD) tools and simple mathematical models were used to develop a better understanding of flow drivers and methods required to arrest microfluidic flow.

Key findings are as follows:

A range of programming materials has been used successfully to programme porous flow through the bulk of the paper as well as flow along PDMS-coated paper channels. As shown in Figure 14a-c, hydrophobic inks (such as latex or alkyl ketene dimer) or physical blockers (such as wax or polyethylene 1000) can be used to programme fluid flow. Examples of programmed flow delivered by inkjet are shown in Figure 14d-e with latex and PEG1000 used respectively.

Polyethylene glycol was also shown to allow control of the sample flow rate in the channels. This is achieved by depositing a viscous slug of liquid downstream of the sample flow region that then opposes the surface tension-driven flow. This offers a very useful solution to flow rate challenges in microfluidic engineering.

Work translating the programming techniques to industrial print heads is also reported, starting with a Spectra 128SE print head (Figure 14f). This uses the PiXDRO LP50 printing system and excellent printability of the key fluids is reported in D3.5.

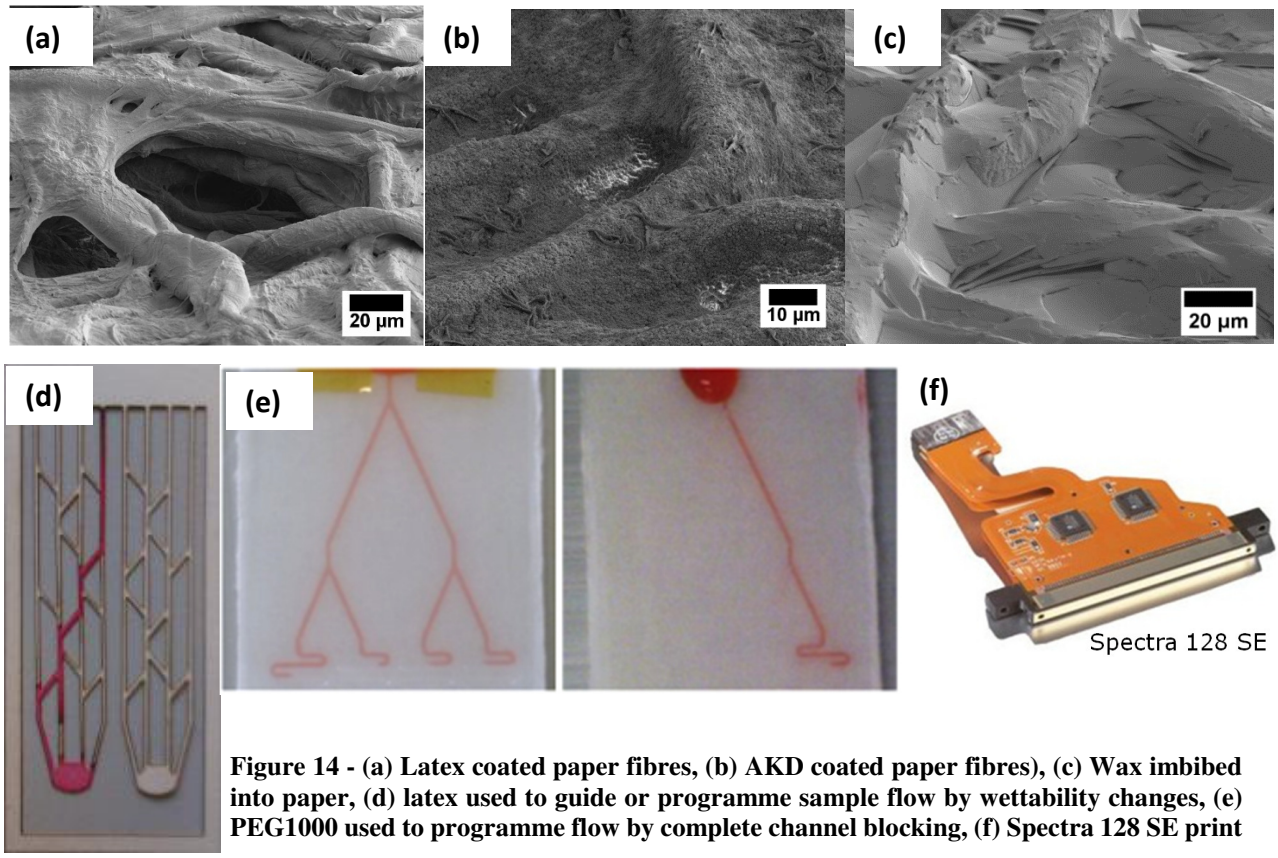


Figure 14 - (a) Latex coated paper fibres, (b) AKD coated paper fibres), (c) Wax imbibed into paper, (d) latex used to guide or programme sample flow by wettability changes, (e) PEG1000 used to programme flow by complete channel blocking, (f) Spectra 128 SE print

Deliverable 3.6

This work involved the development of a printer capable of depositing the biological components and their conjugated particles (i.e. recognition molecules and labels) required within Project BiognostiX. The detailed requirements were defined and a system constructed that is programmable, accurate, transferable and capable of dealing with a range of reservoir volumes.

The key challenges this deliverable needed to resolve were:

- Biological reagents are present in aqueous systems but their high surface tension and low viscosity make unmodified aqueous inks unsuitable for accurate and repeatable fluid deposition by inkjet.
- Long chain molecules, including proteins, are very sensitive to shear forces and elongation flow, which may lead to significant reduction in biological activity.
- It is essential to avoid non-specific binding (NSB) within the printing process to ensure delivery of an accurately known concentration of active material. Blocking agents used to avoid NSB must be applied to all surfaces that the fluid contacts, which may often be difficult within a standard commercial printing system.
- Most commercially available systems are not suitable for point-dispensing mode, do not have the option of high throughput within short times for activity studies and cannot incorporate custom-built stages or reservoirs.

The carrier fluid for the biological reagents was tested using the Dimatix Materials Printer (DMP2800 from FujiFilm) at CU-IfM. This allowed initial printability studies to be carried out. A custom-built bioprinter was then specified, designed and constructed, as shown in Figure 15. The system design required the integration of a number of components

- A precision XYZ stage and controller to ensure accurate placement of drops
- Pneumatic dispenser for delivery of volumes in the range 1 µL to 100 µL
- Piezo-activated inkjet printing for sub-nL volumes.

- An image capture system
- Integrated control of all components

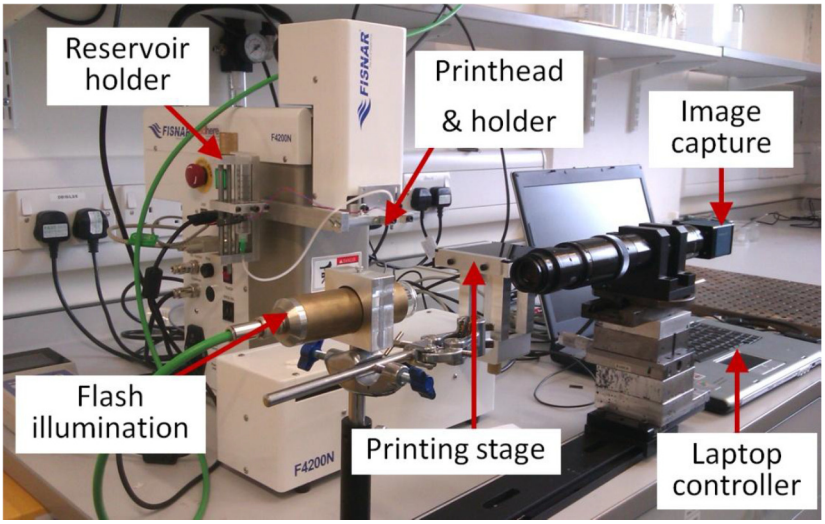


Figure 15 - A custom-built bioprinter with purpose-built stage, reservoir, printhead holder, x-y-z controller and imaging systems.

This system enabled the optimisation of the inks developed in this project and their deposition into channels, in conjunction with WP2. Examples of droplet imaging and print optimisation are shown in Figure 16a-c from the development stages for the carrier fluids.

A second bioprinting system was also completed and transferred to a second laboratory with fully documented printing protocols to provide evidence of transferability and robustness of design. The final printing system enabled:

- Delivery of fluids with xy positional accuracy / repeatability of ±20 µm.
- Customisable stage to accommodate different formats
- Customisable, low volume reservoir holders to conserve expensive (e.g. antibody-based) inks.
- Biocompatibility - all components can easily be accessed and internally coated to avoid non-specific binding problems.
- High resolution imaging for method development and optimsation.
- Techniques for delivering volumes in the range of 0.5 nL to 100 µL.

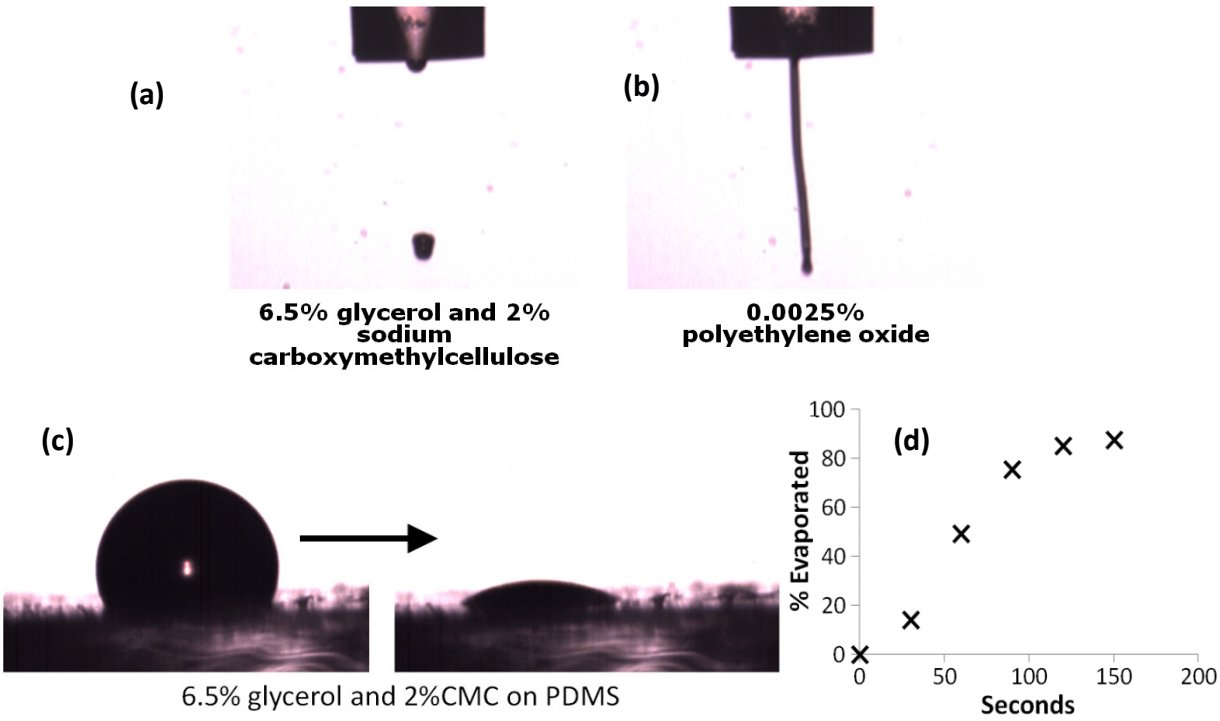


Figure 16 - (a) Image of droplet formed from ink containing 6.5% glycerol and 2% CMC printed with the bioprinter. (b) addition of 0.0025% PEO allowed printing with minimal quantities of non-volatile components but led to droplet break-up issues. (c) Approximately

Interactions with other Work Packages

With clear dependencies on both the channel design and biological reagent development, WP3 was required to overlap with and contribute to a range of other activities in Project BiognostiX. A summary is provided below of the key tasks delivered in this broader, highly collaborative context.

Working closely with WP1:

- A range of interim coupon fabrication techniques were defined to enable WP2 and WP3 to begin prior to final design specification.
- Techniques were delivered for programmable delivery of biological reagents to large multi-coupon sheets by the development of alignment marks.
- Significant contribution was made to the design, manufacturing and validation of the final coupon structures.

Working closely with WP2:

- Printing strategies along with required imaging and characterisation techniques were developed for biological activity analysis.
- Ink characterisation and formulation development was carried out to ensure biological reagents were printable.
- Equipment and knowledge transfer for the progression of experiments into a biological laboratory.

Working closely with WP5:

- With feedback and interaction with commercial partners and WP1, 2 and 4, staff at CU-IfM manufactured more than 250 coupons, 500 capping layers and wastepads for integration and testing activities.
- CU-IfM and CU-Bio examined the drainage of samples through the final integrated devices, producing protocol procedures for further testing by partners.

Work Package 4 – Diagnostic Reader Development

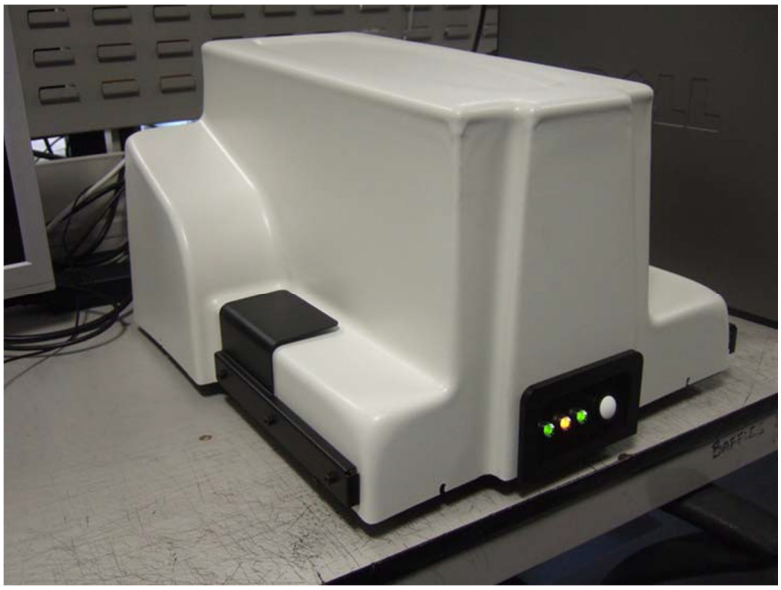


Figure 17 - Prototype reader

We have successfully designed and developed a prototype reader (Figure 17) based on reflection scanning that is capable of detecting fluorescence or colorimetric signals generated within multiple zones of individual micro-channels created in opaque fibre based substrates.

It is expected that this reader will be used for assay and assay parameter development. The parameters will then be downloaded to the firmware on a smaller, dedicated assay reader or on a high throughput system with stacker.

Although transmission scanning devices, such as a conventional well plate reader have a good OD range characteristics for colorimetric applications they cannot be applied to opaque materials such as paper.

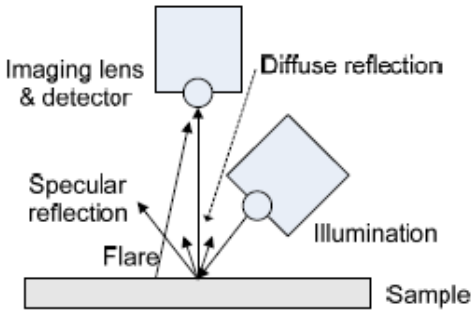


Figure 18 - Schematic for reflectance scanning

Reflectance scanning is more appropriate to this application but has a typical OD range of 2 and therefore may compromise sensitivity for colorimetric and densitometric applications.

To eliminate issues associated with flare and noise from the paper grain we have used angled, double-sided illumination that suppresses the paper noise by filling in the shadows. (Figure 18)

For our illumination source we use LEDs which have the benefit of being low cost, high brightness and give a wide spectral range coverage.

For colorimetric applications we use white light LED in conjunction with RGB detection, for fluorescence we use coloured LEDs covering the range 365nm to 617nm to optically induce fluorescence emissions.

Paper also presents significant challenges for fluorescence detection too as the optical brighteners used in the paper manufacturing process make it difficult to discriminate detection particle fluorescence from that of the paper substrate.

Figure 19 - Comparison of optimal substrate background characteristics and detector response

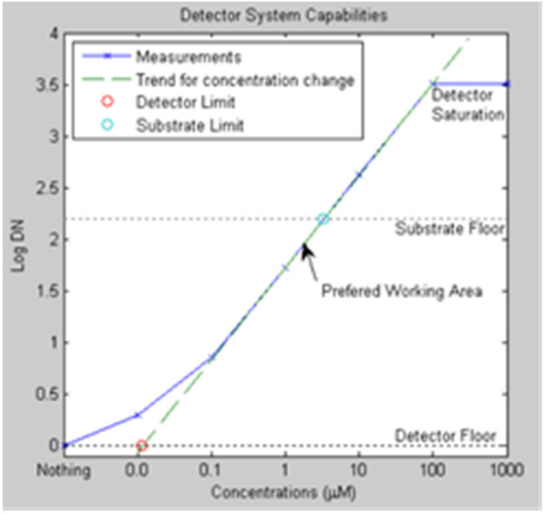
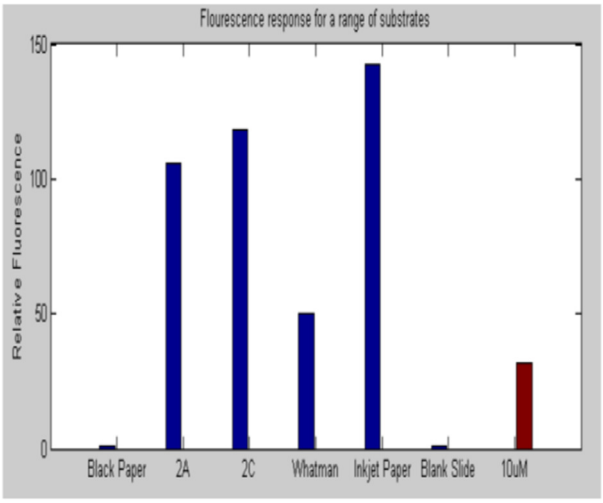


Figure 20 - Native background fluorescence for a number of substrates



For the BiognostiX work we selected an excitation wavelength of 552nm using a Cyan LED with a 550nm shortpass filter to block any LED emission above 560nm. For emission detection we selected a 580nm Longpass filter which allows any fluorescence emission above 575nm to reach the detector. This particular band was chosen to avoid fluorescence emission of the optical brighteners (typically 420-470nm).

Using this combination of LED wavelength, short and long pass filters and assuming no auto-fluorescence from the fibre substrate gives a theoretical discrimination between the excitation and emission of 1E-6.

This level of discrimination is not achievable in practice since the paper exhibits some auto-fluorescence at 580nm.

For the illumination we employed 2 banks of 3 LEDs at 14 mm spacing for colorimetric imaging and a single bank for fluorescence to give us a 4mm wide Gaussian illumination function. Uniformity of illumination was better than 15% across the BgX chip

For the detector we selected a three line RGB CCD linescan sensor and developed a new headboard to maintain maximum flexibility in reader performance. The optical design summary is presented in Table 3 below

Illumination (densitometric)	LED (x6) Cool White
Illumination (fluorescence)	LED (x3) Cyan 505nm
Sensor pixel size:	9.325µm
Optical magnification	0.38
Field of view	> 25mm
Illumination width	> 22 mm x 4mm
Pixel size at chip	24.5 µm
Imaging resolution	41 lines/mm
Number of pixels per 300µm channel width	13
Depth of field at chip	1.5mm

Table 3 - Summary of optical characteristics

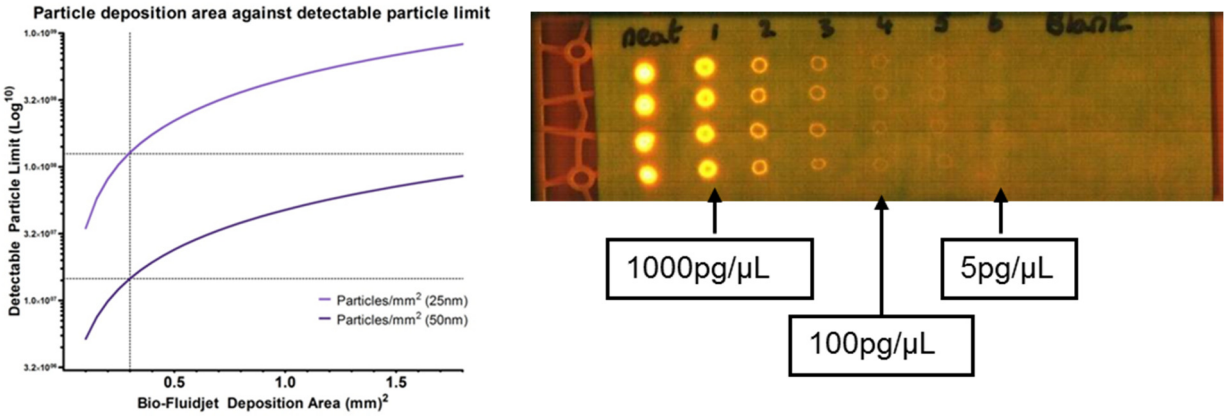
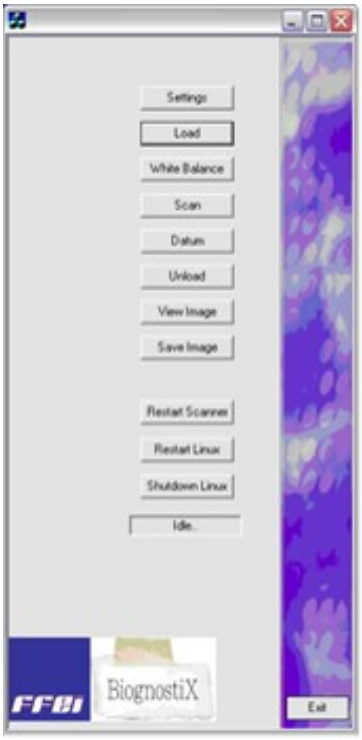


Figure 21 - Example of detector response against number of deposited particles and reader generated image

Reader sensitivity was evaluated by measuring the fluorescence signal of depositing aliquots of different concentrations of 50nm and 25nm COOH redF particles onto the surface of a chip. From this we were able to estimate that our limit of quantitation to be 5pg/µl of particle binding per channel which indicates suitability for ELISA type assays. (Figure 21)



We have also created a human machine interface, a data analysis developer software package, and calibration tools that enable set up of developmental parameters for an individual assay. These tools will be used to define and test all capture parameters. These optimised parameters will then be collated into an analytical method which can be downloaded to the firmware on the reader.

Different methods, e.g. for different tests or sample types can then be selected using a menu in the final reader.

Currently the developer software will allow

- Select (region of Interest (ROI.)
- Search for channels and reaction points and control zone.
- Determine signal min, max, stdev etc.
- Measure and compensate for paper noise.
- Read and decode barcodes and associate information as required.
- Locate fiducials on coupon to correct any x, y positioning and rotation errors.
- Display an image of the scan with measurements for each reaction zone and diagnostic PASS/FAIL result.

Work Package 5 – Proof-of-concept, Integration and Evaluation

The aim of this Work Package was to bring together all components of the system to demonstrate end-to-end feasibility.

Initially, product specifications and test methods for individual modules were defined in Deliverable 5.1, then user requirements specifications were defined in Deliverable 5.2 and subsequently protocols for sample preparation in Deliverable 5.3.

Deliverable 5.4 evaluated how components functioned within the integrated system and reported on tests undertaken on the BiognostiX platform as a whole, and subsequently made recommendations for the further development of the BiognostiX engine.

For the integration tests, identical readers were placed at each of the SME's, Cambridge University Institute of Biotechnology (IoB) and FFEI so that tests could be undertaken with full collaboration.

The reader software was developed such that data could be easily shared between partners. Each SME generated a Test Plan (see D5.4) and were provided with a Test Matrix to log and record all relevant information before, during and after each run. Results automatically generated by the Reader were imported directly into the test matrix.

IoB developed a Full-Test Protocol to ensure consistency between teams in running the procedure (see Appendix A, Deliverable 5.4).

The design of the BiognostiX Chip and Reader was reported to be good with the SME partners reporting trouble-free processing of chips in and out, and generating scanned images and data. The time taken from sample loading to the Chip being ready for reading was found to be longer than expected. Original target time was specified as less than 1 minute but all SMEs reported that at least 10 minutes was required and sometimes up to 60 minutes. Initial sample flow was fast with the long run time being mainly due to slow clearance of wash fluid added after the sample, particularly the time required to empty the channel at the end which is essential for a clean read.

Two phases of testing were completed.

In Phase I testing, each of the SMEs successfully compiled BiognostiX Chips with their respective capture biomolecules manually dispensed and encapsulated. On loading sample or the subsequent wash buffer, all 3 SME partners reported some delamination of the capping layer from the coupon. This delamination, aggravated by the longer than anticipated flow time, had a detrimental effect on results generation by substantially raising background fluorescence.

In Phase II testing, using chips with superior capping-to-coupon adhesion and the modified waste pad component, flow rates were improved and delamination was reduced but not as much as expected. Sample drainage time was reduced to 5-10 minutes with most of the sample flowing within 1 minute and the rest of the time being required to drain the channels. All SME partners reported that delamination did not occur during sample flow but was still evident when subsequently washing through with buffer. Working closely together, IoB and the SMEs generated proof of concept data that the Integrated BiognostiX Platform can generate rapid and quantitative immunoassay data (see Deliverable 2.3 for full details).

Results from Phase 2 testing are presented below and indicate proof of concept from application of sample through to on-chip analysis for exemplar applications.

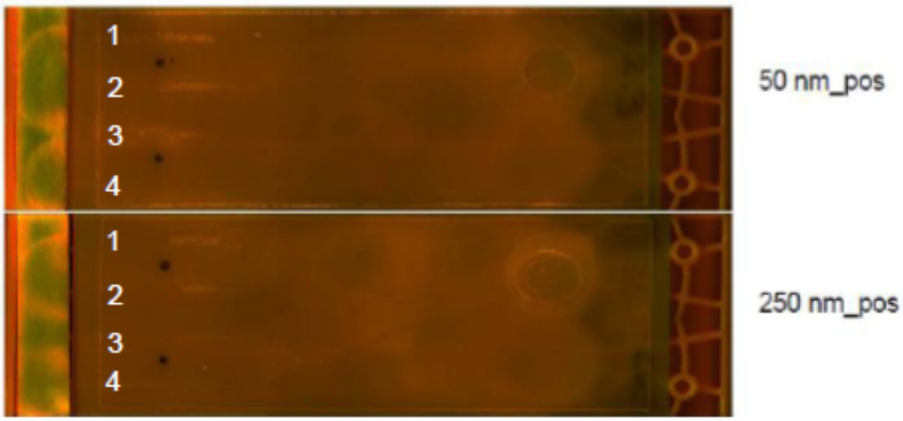


Figure 22 - Positively spiked Brachyspira samples were conjugated with Micromer RedF particles and loaded onto the chips. Images were captured using the BiognostiX reader

The methodology used for Prionics Brachyspira application included the following steps:

- Brachyspira antigen coated immunoink was deposited in the channel 1 and 2, stabilcoat blocked
- immunoink was deposited in channel 3, channel 4 is empty
- 10 µl of brachyspira positive sample were mixed with 10 µl of 50 nm mobile Ab micromer RedF or5 µl of 250 nm mobile Ab micromer RedF and sample diluent was added to a final volume of 60 µl and sampled in the sampling zone
- After washing the channel with 2x 60 µl washing buffer, the coupon was imaged using the BiognostiX reader
- Positive signal was detected in channel 1 and 2, while a visible but weak signal was observed in channel 3.
- No signal was detected in channel 4

Work Package 6 – Exploitation and Dissemination

The goal of BiognostiX (BgX) was to develop a new generation of inexpensive, single-use, fibre-based sensors that will enable and facilitate point-of-use immunoassay-based diagnostics in the key areas of human, veterinary and agri-food.

During the project we have acquired considerable foreground that has enabled us to demonstrate proof-of-concept for two out of the three exemplar assays. Furthermore, at the recent symposium held at Cambridge University to disseminate this foreground there were seven new parties who have expressed an interest in the BgX format. This interest ranges from an immediate need to develop a micro-fluidic based assay, through a longer term interest in BgX technology to an offer from the NIHR Diagnostic Evidence Co-operative of London to assist in taking BgX to the next stage.

We estimate that there is a minimum of two years further development required before the first commercially available product will be introduced to the market. To fund this development we plan to seek further funding through existing and new partners interested in developing assays using some or all of the foreground developed in this project. This will be done in accordance with the consortium agreement presented later in this document.

BgX has significant commercial potential in the dynamically developing markets of companion diagnostics (cDX), animal diagnostics and food testing, and as such, we have delayed publication of papers and filing of IPR to enable further research before maximising the commercial impact

Going forward, this plan identifies two areas of potential exploitation. The first is to further develop and commercialise the 'BiognostiX Engine' concept (see Figure 23). Here we will promote an enabling technology with a specific focus on the design, development and production of point-of-use, antibody based ELISA tests.

This focus will enable us to create a clear marketing message with an ELISA assay based focus and move away from a more general platform message. This will also enable highly specific identification and targeting of, manufacturers of immunoassay tests as new potential partners.

This commercialisation will be via a new company with all partners invited to participate. Funding for this new entity will come from investment by interested parties and further research grants. The commercialisation success will be dependent upon the availability of sufficient proof-of-concept data, freedom-to-operate and successfully overcoming the technical issues highlighted in other deliverable reports

Figure 23 - The BiognostiX Engine

The second area of exploitation is where the Partners can exploit their foreground outside of the BiognostiX Engine, in the case of the SME partners it is likely to be commercialisation of assays, for academic partners this will be publications and further collaborative research projects.

The exploitation committee have an agreement in place to facilitate equitable foreground exploitation and dissemination. For future activity there are two possibilities for utilisation, direct where each partner individually uses the foreground to takes part in further research or decides to commercially exploit their foreground and indirect where foreground is exploited via other parties in the form of licensing. With this in mind the following points have been agreed:

1. Each partner is responsible for their own foreground going forward
2. After all developed foreground was considered it was concluded that none of it is jointly owned.
3. All partners will have access to the foreground (results and IPR
4. Agree that partners will maintain the existing confidentiality agreement and not disseminate any information that may compromise a partners commercial or IP position
5. Publish a schedule of planned dissemination publications and activities. This will ensure we do not compromise any potential IPR or commercial activity partners may be planning
6. Each partner will be solely responsible for licensing and defending any IPR for usage within or outside of the consortium

The framework of this agreement is presented in **Error! Reference source not found.**

In this plan it is proposed to form of 'New Co' to execute the commercialisation and complete the necessary research and development of BgX.

This 'New Co' would need access to the foreground and IPR from the consortium.

It has been agreed that the new company would have the same rights as the original consortium members as an incentive to get things moving. The probability is that some of the original consortium will have an interest in being part of this company and therefore bring in their foreground anyway, but for those who do not wish to participate this has to be considered too.

As part of the standard EU consortium agreements individual members are allowed access to the foreground for 12 months, it is agreed that this will not be extended beyond this period.

The scope of the new company would be to bring the BiognostiX engine to the market, should there be other applications for a members foreground, e.g the immuno-ink and channel programming from Cambridge or the creation of paper channels by VTT then they would be free to licence this foreground as they see fit on a non-exclusive basis

Full details on dissemination are presented in the 'Dissemination activities' section

Work Package 7 - Management

The objectives of this work package were

- To ensure that all knowledge is created, managed and disseminated in a coordinated and coherent manner
- To ensure that all activities, legal aspects and other issues are managed to a high standard.
- Ensure all aspects of the EC requirements for communication and reporting are met.
- Coordinate the overall legal, contractual, financial and administrative management of the consortium.
- To ensure that all project results are formulated and compiled into a form that can be protected by the most appropriate legal enforceable protection method. The control and management of scientific knowledge transfer from the research consortium members to enable this technology to be applied to new markets and other specific products.
- Broadcast and disseminate the benefits of the developed technology and knowledge beyond the consortium to other potential industrial communities.
- Manage and assess the social economic impact of the generated scientific knowledge and technology.

There were six deliverables, including this report (D7.5), associated with this work package. The first deliverable was the kick off meeting which took place at FFEI in month 1. Deliverables 7.2 and 7.3 related to a restricted mid-term evaluation report and a public domain progress report that were delivered on time in Month 18 of the project.

Deliverable 7.4 related to the setting up of quarterly meetings between partners at their respective sites. In practice we carried these meetings out on a six monthly basis throughout the project. In addition we held regular conference calls which for the last 9 months of the project took place on a weekly basis with at least one representative from each partner in attendance. Minutes were taken and immediately circulated to all partners after the call. This was particularly valuable due to the need for close interaction and rapid reaction to the results obtained in the latter stages of the project.

Deliverable 7.6 was a report detailing potentially competitive patents and a plan for patent applications with exploitation agreements.

We have secured agreement from the partners that there is no joint IP associated with this project and, as such, each partner is free to exploit any IP generated.

Partners can exploit their foreground outside of the BiognostiX Engine, in the case of the SME partners it is likely to be commercialisation of assays, for academic partners this will be publications and further collaborative research projects.

Previously Deliverable 7.6 reported that we had freedom to operate in all areas of the domain we are interested in exploiting.

Recently we have undertaken an updated search and have found no new IP that would change the situation correctly described in D7.6.

However, in D7.6 we reported that there was one specific family of patents that require further monitoring and we have revisited these in more detail.

The patent owned by Monash University Patent US20120322086 A1 now presents a particular challenge to the BgX concept.

Our original solution to avoid infringing this patent was to specify that sample and reagents were to be mixed on the BgX chip itself and not prior to application to the chip. We have not yet undertaken any studies relating to addition of reagents.

Given the inherent nature of the biological samples we have used or planned to use this is a great technical challenge and potentially not realistic.

The conclusion therefore is that the proposed new company would need to negotiate licencing and sub-licencing rights with Monash before a commercial offering can be made.

Very close to the end of the project it was discovered that the Month 18 report contained significant disclosure of novel foreground created and used in the project. As a result it is necessary for partners to revisit their planned patent.

Furthermore, the impact of this discovery on the commercialisation of BgX needs further due diligence and research to clarify the position with respect to commercial exploitation.

Potential Impact

The BiognostiX project has impact on the development of micro-fluidic based diagnostic devices to enable the execution of point-of-use diagnostic tests that were previously unique to the laboratory environment through deployment of the following:

- Creation of micro-fluidic flow channels in fibre based substrates
- A family of sub-micron immune inks that can be printed using fluid-jet technologies and applied to ELISA type assays
- Invention of methods to print immune-inks in micro-fluidic devices
- A general advancement in knowledge on flow control methods in micro-fluidic channels
- Development of a small footprint reflectance based scanning system capable of analysing fibre based diagnostic devices

As these ELISA tests are developed then the socio economic impacts of the project will be an improvement in the quality of life and health of European Citizens by

- Reduction in health care costs through efficiencies associated with enabling a shift from curative diagnostic testing to more predictive, localised, personalised and even pre-emptive monitoring of patient health
- Enabling the development of companion based diagnostics for novel, targeted, antibody therapeutics
- Improved quality of food supply chain and food safety
- More efficient management of pandemics and epidemic outbreaks in farm animals

Dissemination activities

We have undertaken a number of dissemination activities with the following objectives in mind:

- Promote the EU as a supporter of innovative and leading edge technology that have a benefit to the wider community
- Pro-actively identify potential collaborators and drive them to the technology through publication of relevant information
- Maintain scientific community interest through a constant drip feed of information
- Establish the scientific credibility of BiognostiX

Our completed activities are summarised below in Table 4 and our planned activities in Table 5

NO.	Type of activity	Main leader	Title	Date/Period
1	web	CU IfM	http://www.ifm.eng.cam.ac.uk/research/irc/biognostix/	
2	Conference	CU IfM	Poster at IfM open day event on 6th February 2014	
3	website	FFEI Ltd	www.biognostix.com	

4	publication	FFEI Ltd	Biognostix Brochure	Feb 2013
5	publication	FFEI Ltd	BiognostiX poster	Aug 2013
6	publication	FFEI Ltd	BiognostiX Engine Logo V1	Aug 2014
7	publication	FFEI Ltd	BiognostiX Flyer	Dec 2013
8	publication	FFEI Ltd	Final BiognostiX Engine Logo V2	Dec 2013 -
9	Conference	FFEI Ltd	The future of diagnostics in life science and health care	Sep 2013
10	Exhibition	FFEI Ltd	UK HealthTech Coneference	Dec 2013
11	Conference	FFEI Ltd	CamBridgeSense Meeting	Nov 2013
12	publication	FFEI Ltd	Review of BiognostiX symposium	Apr 2014
13	publication	FFEI Ltd	BiognostiX Linked-In account	Dec 2013
14	publication	FFEI Ltd	BiognostiX Twitter Feed	Dec 2013
15	Press Release	FFEI Ltd	Select Science	Feb 07 2014
16	Press Release	FFEI Ltd	Select Science	Feb 21 2014
17	Press Release	FFEI Ltd	Business Weekly	Feb 24 2014
18	Press Release	FFEI Ltd	Symposium announcement to more than 50 scientific media companies	Feb 2014
19	Press Release	FFEI Ltd	BIVDA Update	Feb 2014
20	Conference	All	BiognostiX symposium	Feb 2014

Table 4 - summary of completed dissemination activity

Ref	Type of Activity	Title	Owner
1	Scientific Journal	*"Analysis of shape of embossed microfluidic channels on fibre based substrate" – in planning stage*	VTT
2	Scientific Journal	*"Enhanced diagnostic tools through microscale liquid engineering" being written for submission to Lab on a Chip journal*	CU-IfM
3	Scientific Journal	*"Programmable microfluidics channels" being written for submission to Lab on a Chip journal*	CU-IfM
4	Book Chapter	*"Applications of paper based diagnostics" currently being written for publication in planned book relating to diagnostic microfluidic devices*	CU - IOB
5	Scientific Journal	*In planning*	CU - IOB
6	Scientific Journal	*In planning*	CU - IOB
7	Scientific Press	*Review of BiognostiX symposium – written, now seeking placement as editorial in scientific press*	FFEI
8	Scientific Press	*Technical article on BiognostiX technology - written, now seeking placement as editorial in scientific press*	FFEI
9	Scientific Press	*Interview with George Hutchinson regarding BgX project - written, now seeking placement as editorial in scientific press*	FFEI
10	Scientific Press	*Editorial placement #4*	FFEI
11	Social Media	*ongoing news updates*	FFEI

Table 5 - Summary of planned dissemination activities

FFEI has worked closely with a marketing agency to create a coherent and visually powerful image for the BiognostiX concept. Examples of the work are presented below

Figure 24 - BiognostiX engine motif

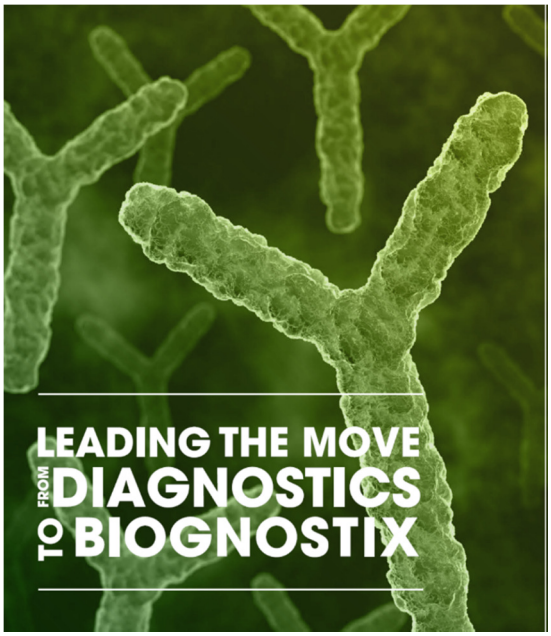


Figure 25 - BiognostiX Brochure cover

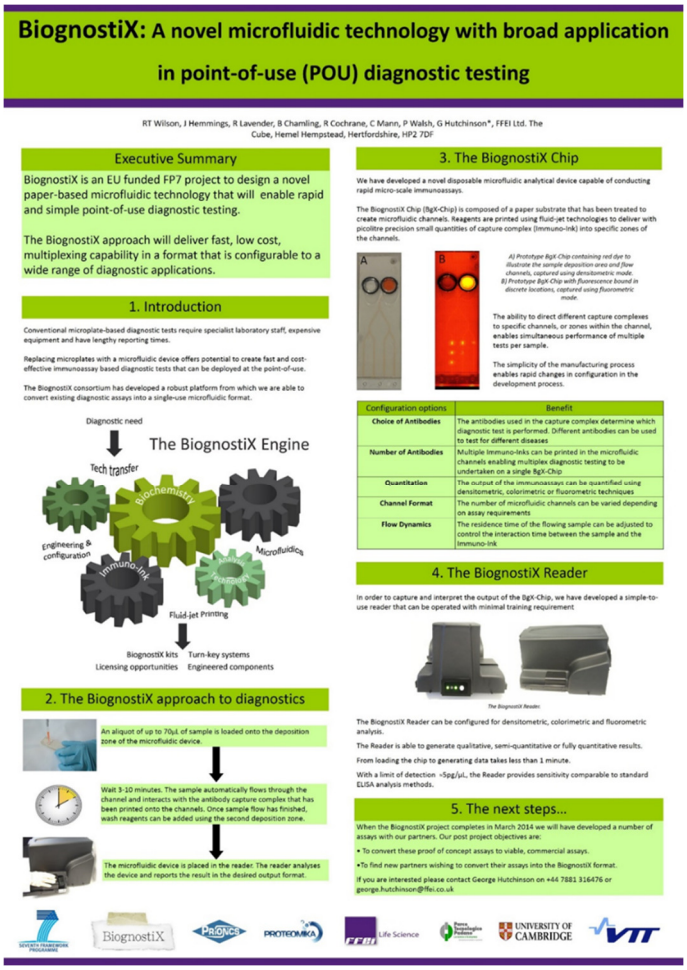


Figure 27 - The BiognostiX poster used at seminars at meetings

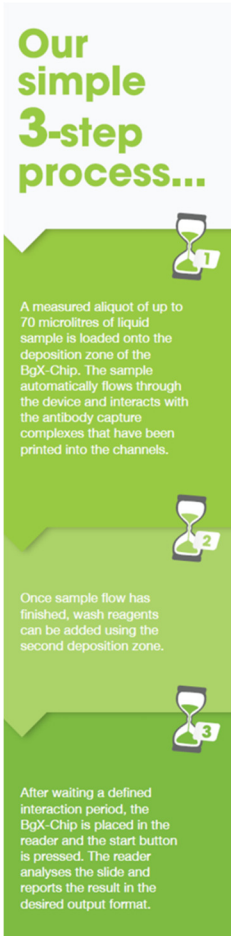


Figure 26 - Benefits of the BiognostiX process

Public Website

Throughout the project we have maintained a public domain website using the address www.biognostix.com

All contact for future exploitation and collaboration will be maintained on this website.

Email communication: biognostix@ffei.co.uk

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