University of Nottingham
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SPI-CLOPS
Surface Polymer Imprinted Closed Loop Optical Patient Sensors for Dose Detection and Prevention of Cancer Resistance

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31 October 2018

Unmet need

Unmet need in Cancer:

Inability to measure changes in tumour microenvironment
- in vivo
- in real time

Current methods
- imaging
- blood tests

(major limitations)

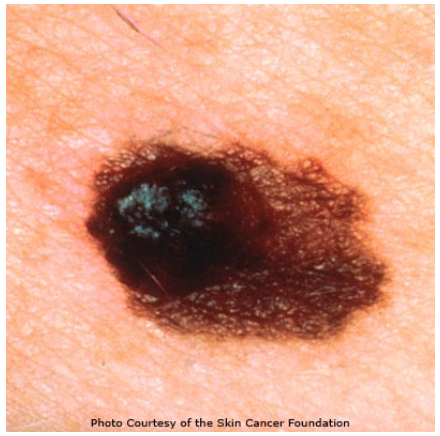
Solution:

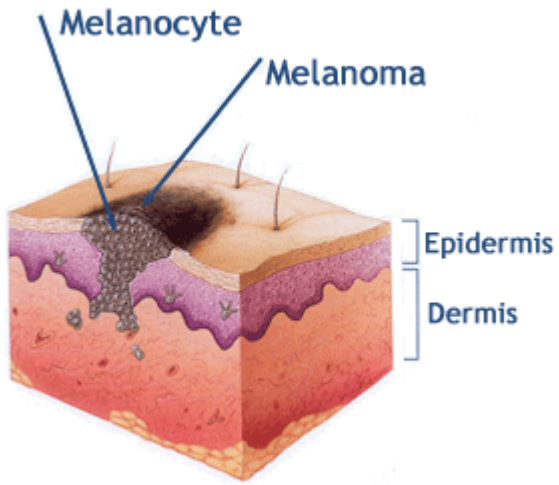
- Polymer coated optic fibres- that can measure levels of drugs/biomolecules

Model System

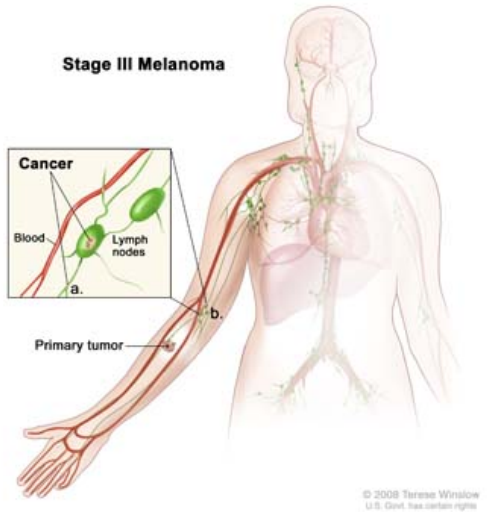
Melanoma – measuring Dabrafenib

Background to the project: Melanoma skin cancer

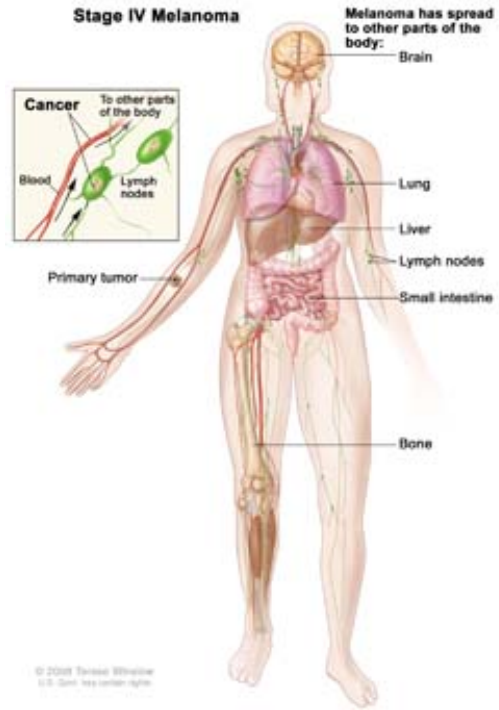




- lymphatic spread
  - Lymph nodes



- Blood borne spread
  - lung, liver, brain, skin



Background to the project: Melanoma skin cancer

CANCER RESEARCH UK

Stage 4

Biological therapy

ipilimumab

pembrolizumab

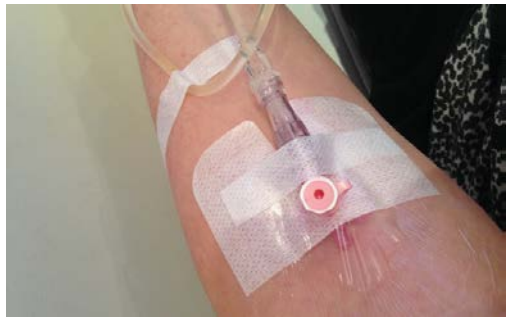
nivolumab

Drugs

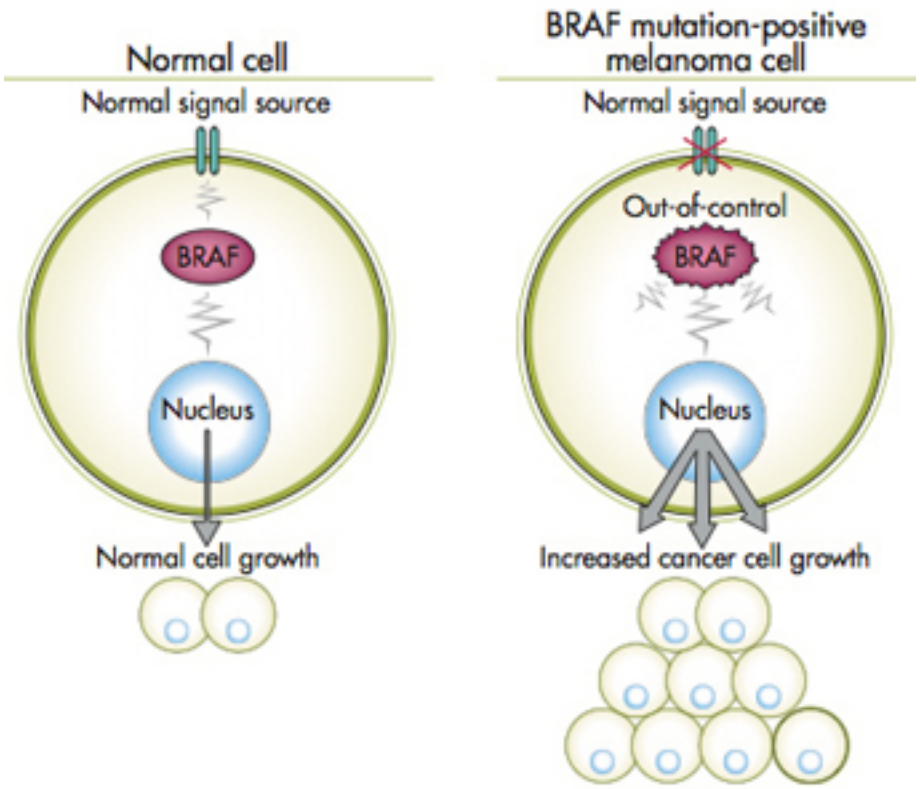
destroy directly the cancer cell

Help immune system to attack the cancer

Targeted treatment: Dabrafenib Vemurafenib



BRAF inhibitors: DABRAFENIB or VEMURAFENIB



- BRAF inhibitors have dramatically improved outcomes for melanoma: Dabrafenib/Vemurafenib.
- Sub-populations of cancer cells become resistant treated to these drugs..
- No current way to predict which patients will develop resistant cancers and when .
- Also - No method to detect whether a patient's tumour is receiving a therapeutic dose.
- **Urgent need to evaluate in real time the molecular events occurring in tumours.**

Aims and objectives

Aims:

- Accurate monitoring of dose and detection of resistance in cancer.
- Develop an ambitious new healthcare technology, applicable to areas far beyond melanoma.

Objectives:

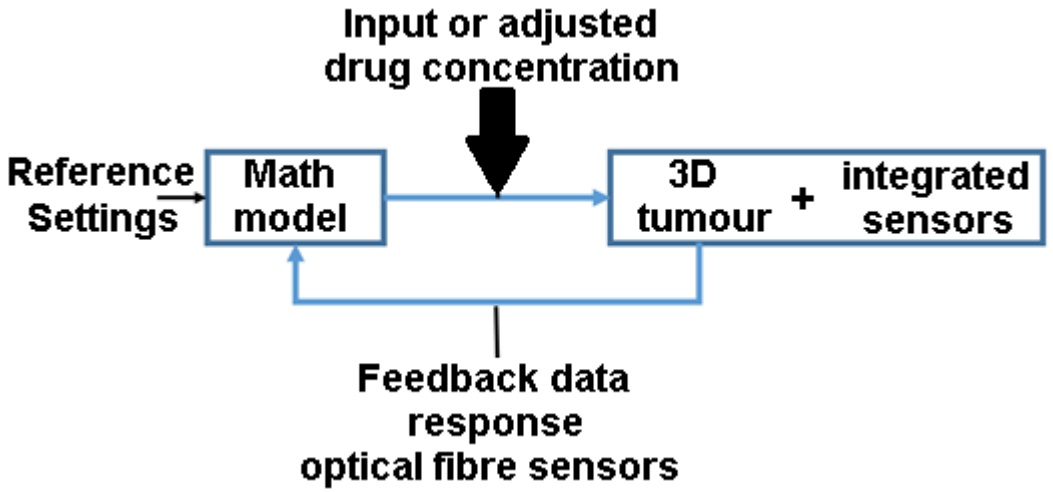
(O1) Develop polymer-coated optical fibre long period gratings to detect Dabrafenib in serum. A first approach considers to detect the reactive binding sites of the molecule dabrafenib through the implementation of molecular imprinting of 2-Aminoquinoline onto optical fibres.

(O2) Derive 3D cultures of BRAF sensitive cells and validate Dabrafenib monitoring in extracellular milieu.

(O3) Interface recognition polymers with optical fibre based sensors which can detect local changes pH, and test readouts from fibres in 3D tumour spheroids.

LONG TERM: 2ND CYCLOPS ROUND

This project will inform potential mathematical models linking drug concentration, B-Raf suppression and intracellular pH, which will establish testable hypotheses relating dosing to cell response and resistance. The longer-term objective (appropriate for the second Cyclops round) will be to develop mathematical models integrated with sensors, such that dosing with drugs in 3D spheroids is coupled to predictions of activity and verification of response. Success in this pilot project will establish the key proof-of-concept for drug monitoring in vitro, and seed more substantive research projects needed to translate the techniques into the clinic.



LPG fabrication and experimental set-up

LPGs were fabricated using a UV laser (λ=266 nm, Frequency quadrupled Nd:YAG, Photonic Solutions Ltd, Continuum minilite I) for side-illuminating the single mode optical fibre (PS750, Fibercore). The system, readily available in the University of Nottingham (Optics & Photonics group [3]), fabricates LPGs using linear stage controllers, via amplitude masks of periods of 109.0, 109.5, 110.0 and 110.5 μm and the timing to fabricate the LPGs takes 20 min each. In this research, all the LPGs fabricated work at or near the phase matching turning point where the attenuation bands localized at the far-red of the visible spectrum (LP_{19}=19th Linearly Polarized Cladding mode, Fig. 1a) are more sensitive to perturbation of the surrounding refractive index n_3 (top, Fig. 1b). After fabrication of LPGs with a gauge length of 3 cm, the experimental set-up used for fibre functionalization is shown in Fig. 1b (lower).

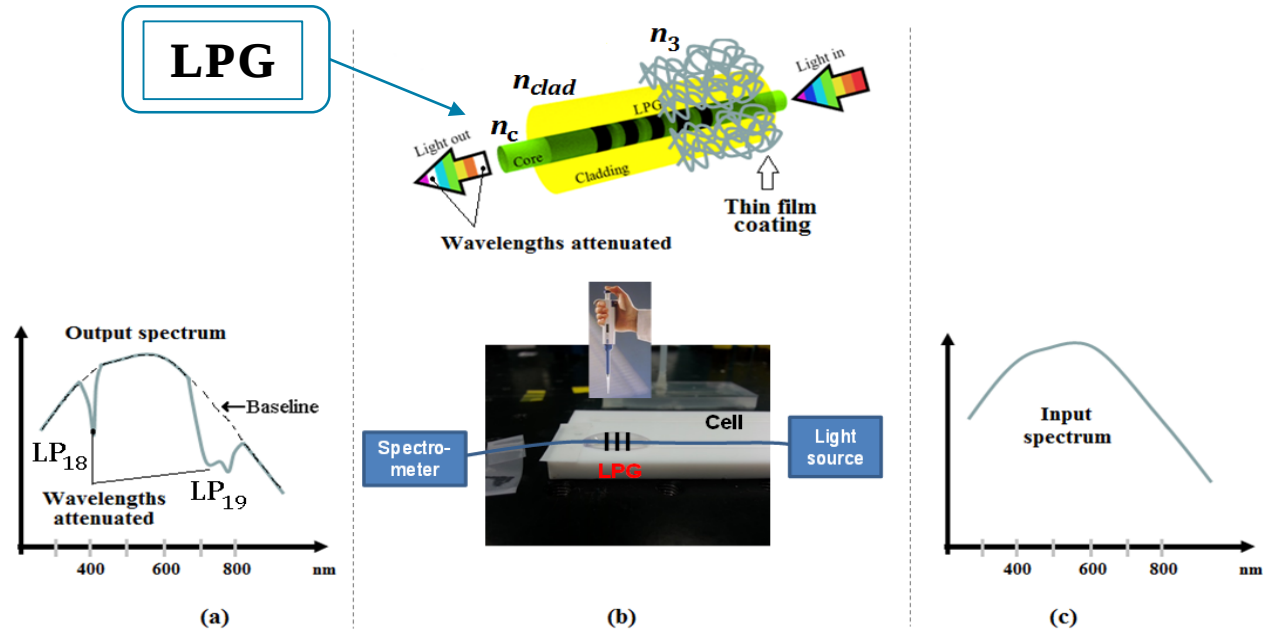
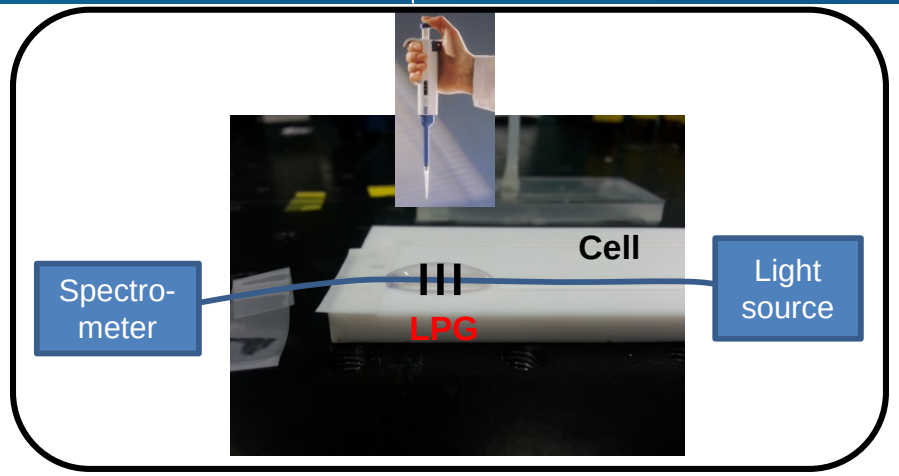
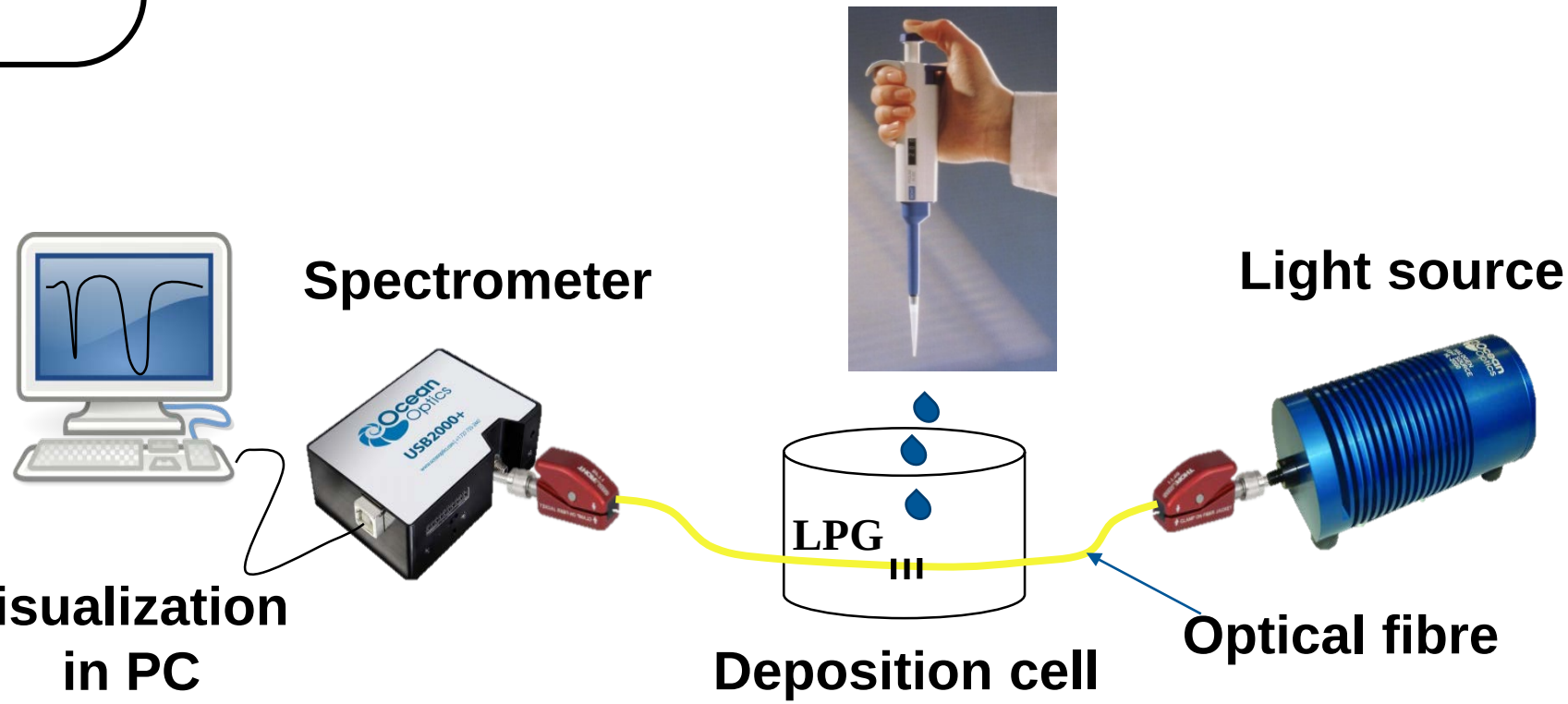


Figure 1. (a) Schematic for a typical transmission spectrum from an LPG sensor. (b) (*lower*) Experimental set-up for LPG functionalization and analyte binding tests; (*top*) schematic of an LPG sensor coated with a functional film as a platform for chemical sensing (n_c =refractive index core, n_{clad} =refractive index cladding, n_3 =refractive index surrounding medium). (c) Input spectrum from a broadband light source (the baseline of the input spectrum is preserved at the output).

Experimental set-up



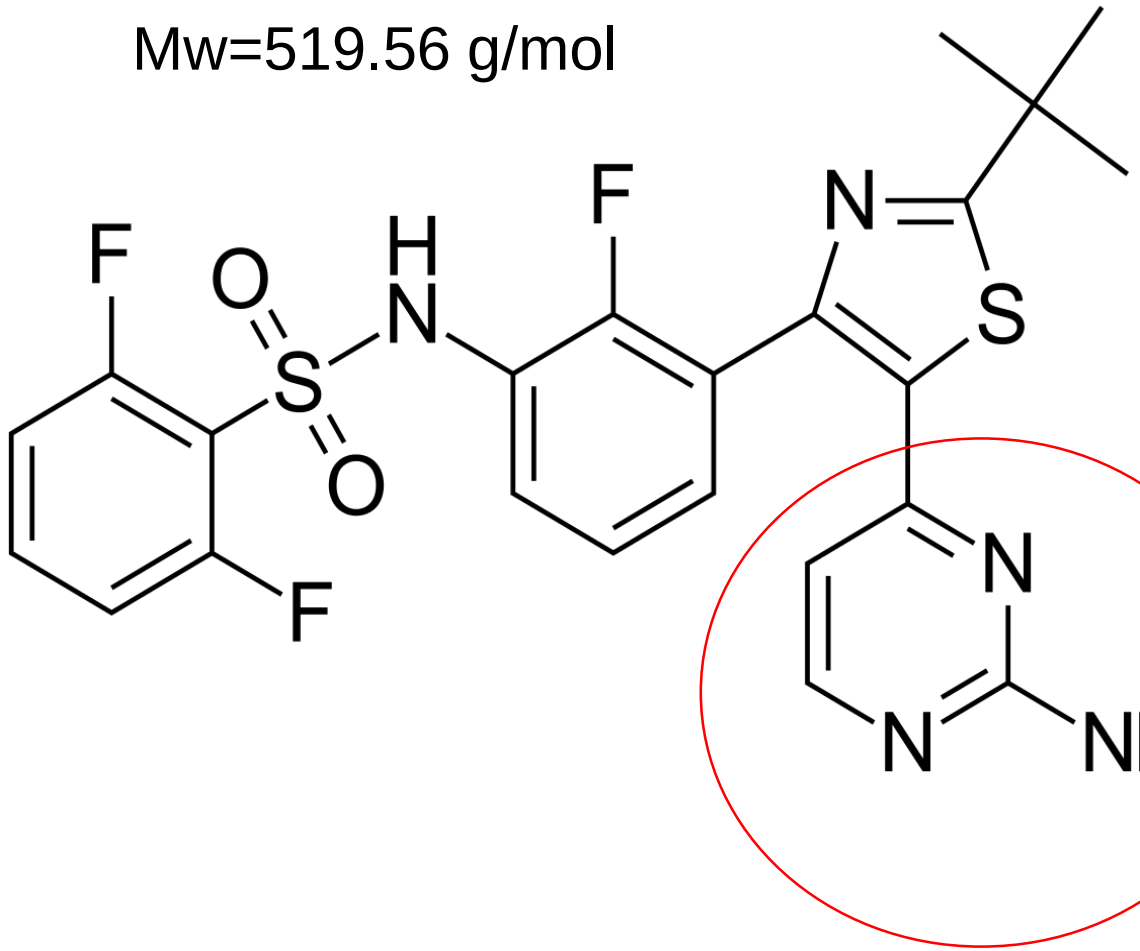
The experimental set-up shown here is used for the fibre functionalization and analyte detection (inside the deposition cell)



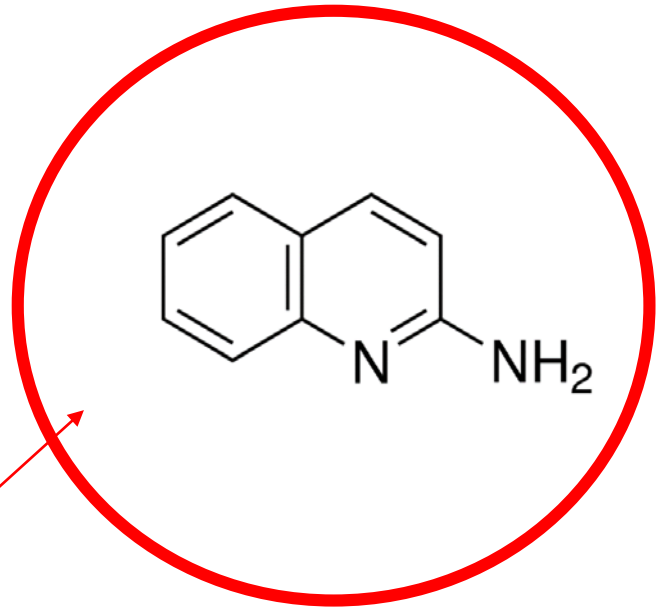
Objective 1: Develop polymer-coated optical fibre LPG to detect dabrafenib in serum.

A first approach considers to detect the reactive binding sites of the molecule dabrafenib through the implementation of molecular imprinting of 2-Aminoquinoline onto optical fibres.

Dabrafenib
Mw=519.56 g/mol



2-Aminoquinoline
Mw=144.17 g/mol



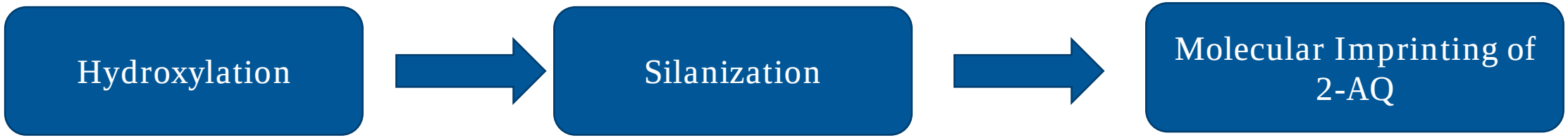
Remarks

1. *Proof of concept with molecule 2-Aminoquinoline (2-AQ): simile of binding sites of dabrafenib.*

2. *Two methods for the molecular imprinting of 2-AQ: (1) Imprinting of 2-AQ on Silica Nanoparticles and then binding of these particles onto the surface of the LPG optical fibre through the aid of the Layer-by-Layer method or (2) Direct imprinting of 2-AQ onto the optical fibre surface.*

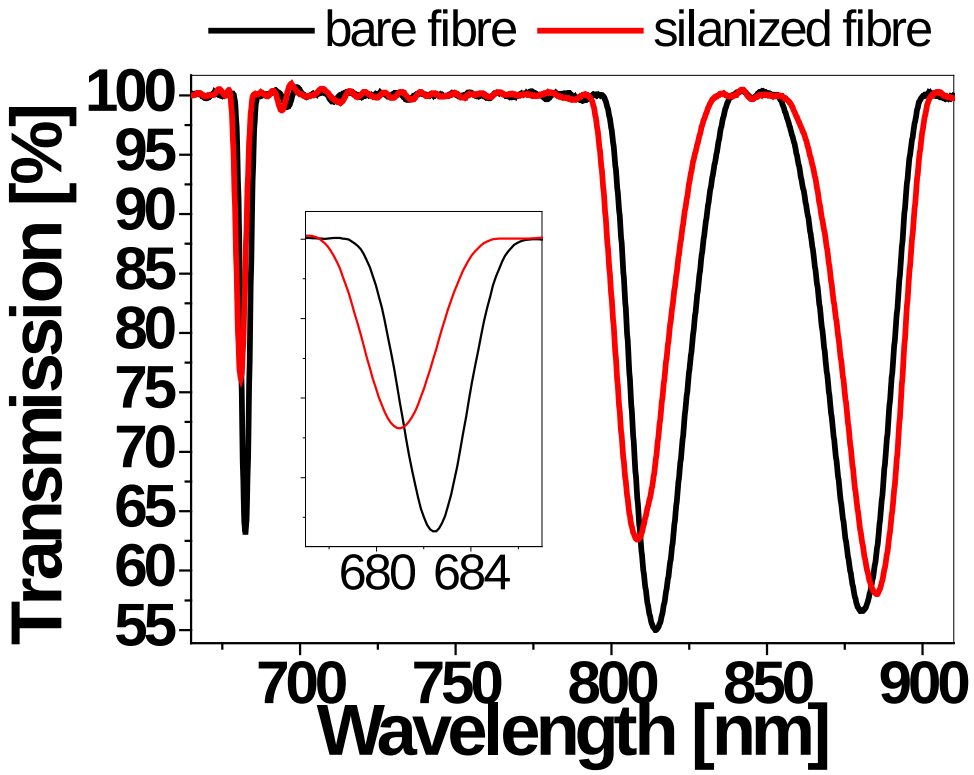
3. *As part of the first approach, direct molecular imprinting of 2-AQ onto the surface of the optical fibre LPG gauge was decided first.*

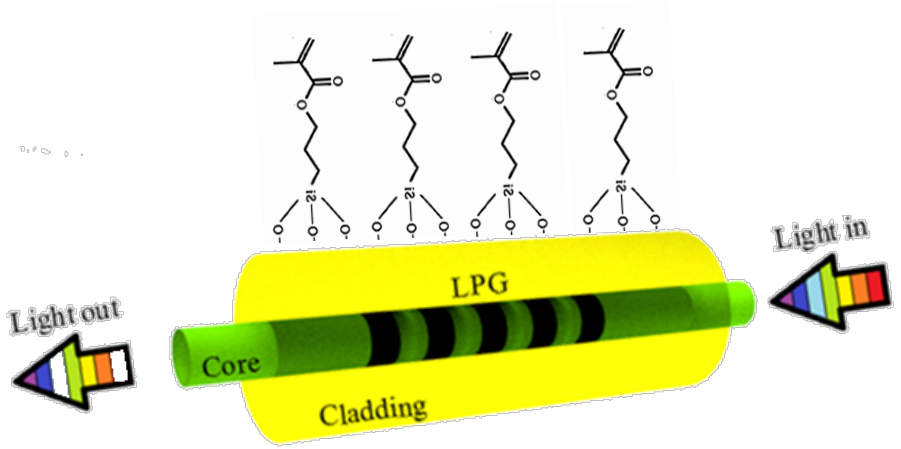
4. *To proceed and do the molecular imprinting of 2-AQ onto the LPG, two pre-functionalization chemical methods are required: Hydroxylation and Silanization.*



Corroboration of silanization onto the LPG fibre (transmission spectrum)

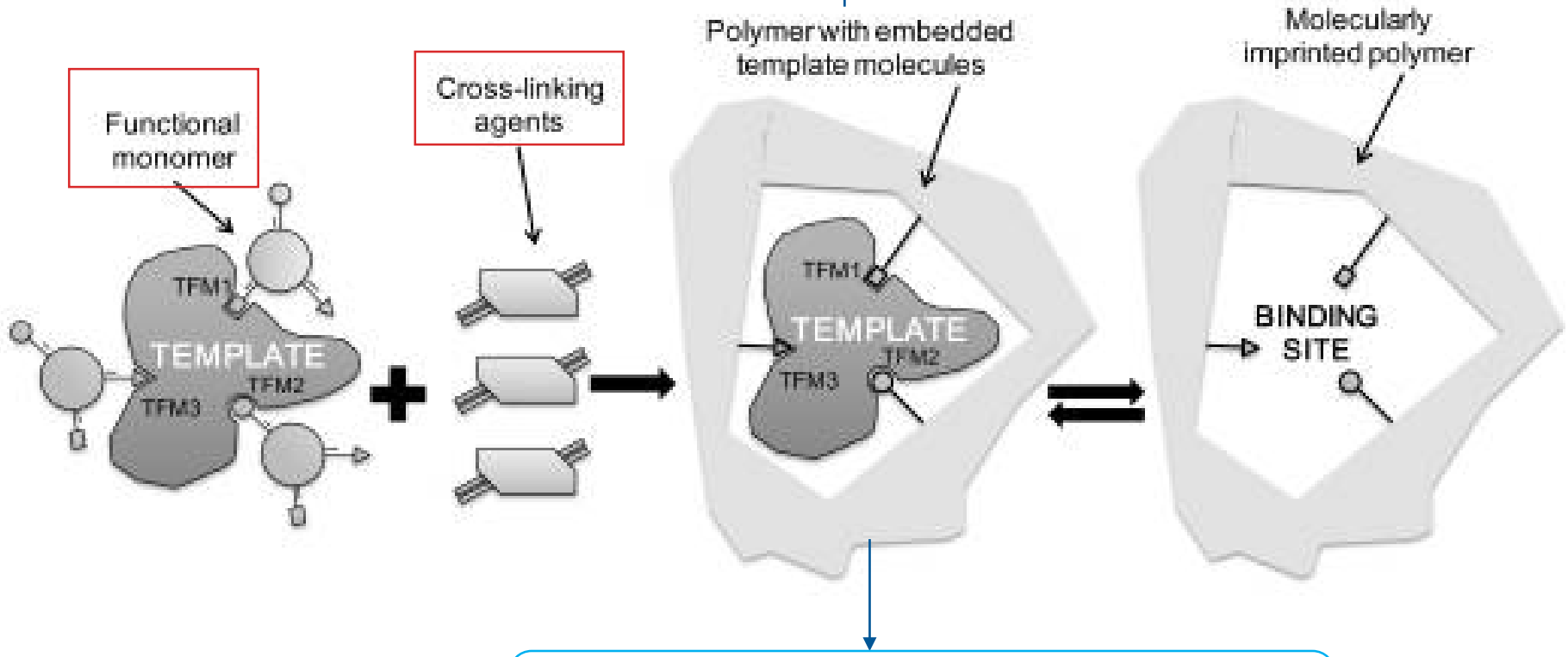
Silanization homogeneity influences surface polymer imprinting





Demonstration of molecular imprinting of template: 2-Aminoquinoline (2-AQ)

Thermal polymerization



Technique used in this research

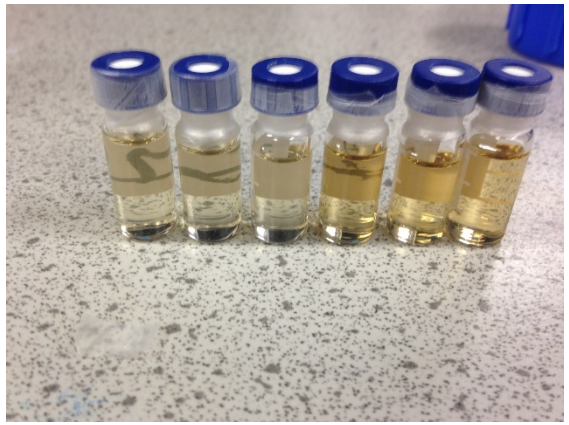
Photo-polymerization (UV light)

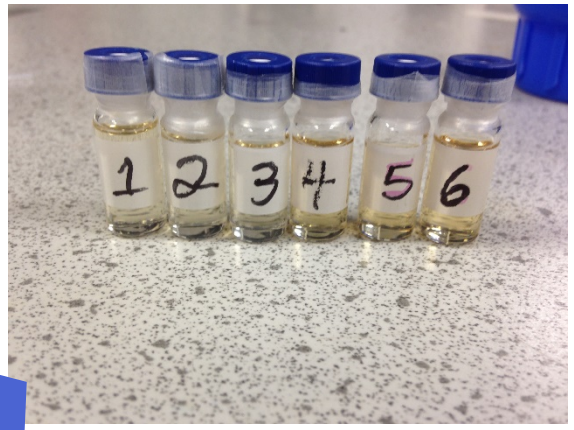
Bulk molecular imprinting of 2-Aminoquinoline (2-AQ)

Original molar % ratios in 1 ml of $CHCl_3$

PEGMA	EGDMA	MAA	2-AQ
40	40	18	2
20	60	18	2
10	70	18	2
40	40	15	5
20	60	15	5
10	70	15	5

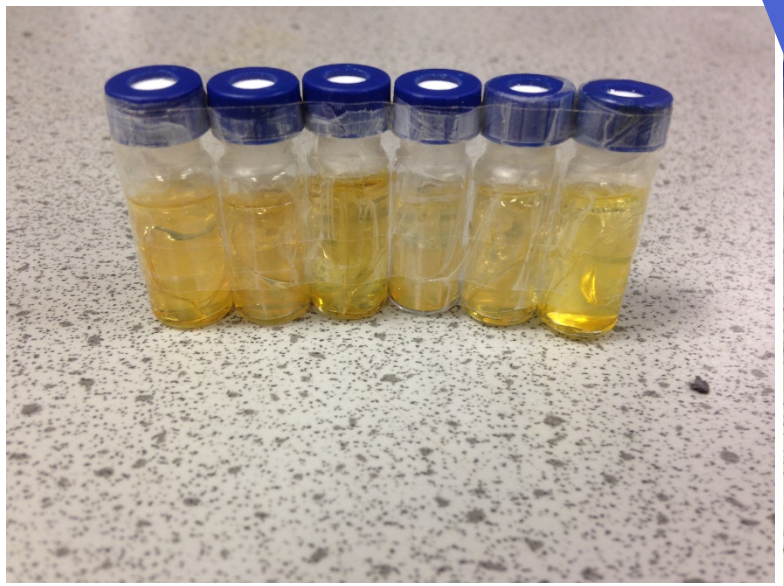
Original solutions

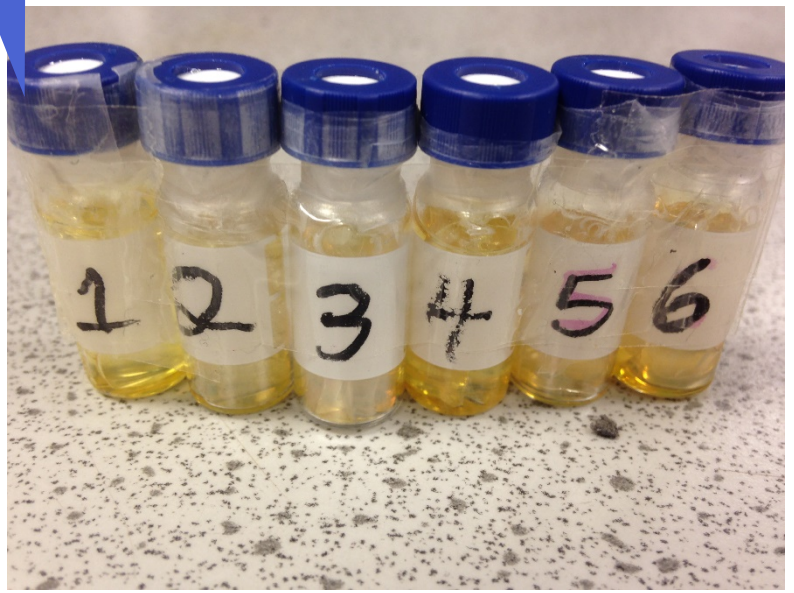




UV

Gels obtained after photo-polymerization

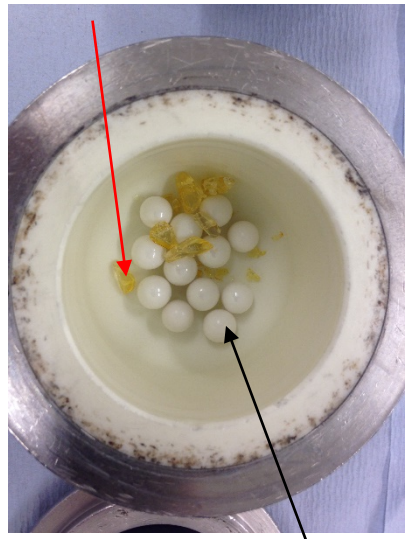




Ball milling GELs (Wolfson building) to obtain a polymer fine powder

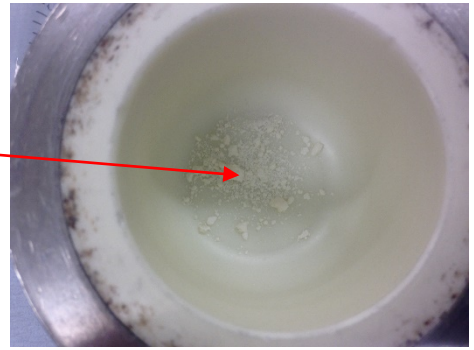
Ball milling machine

Gels



Balls for milling

Polymer fine powder



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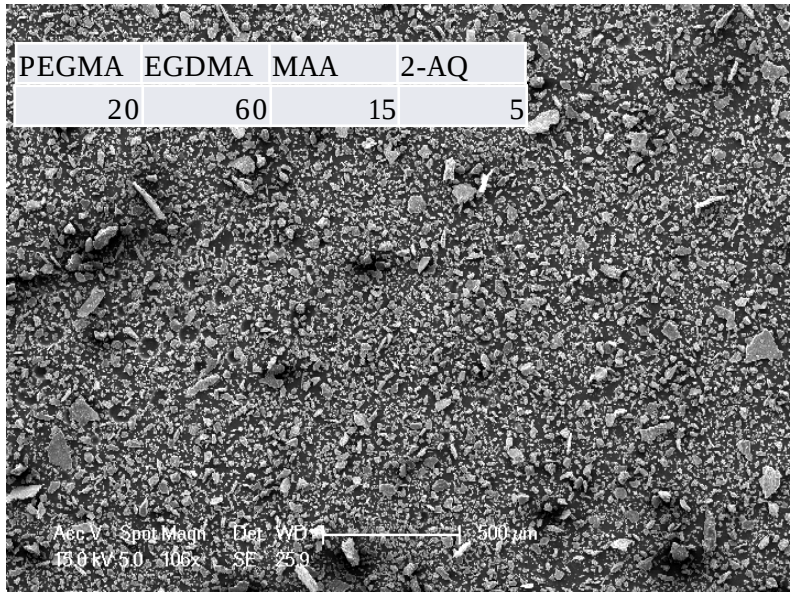
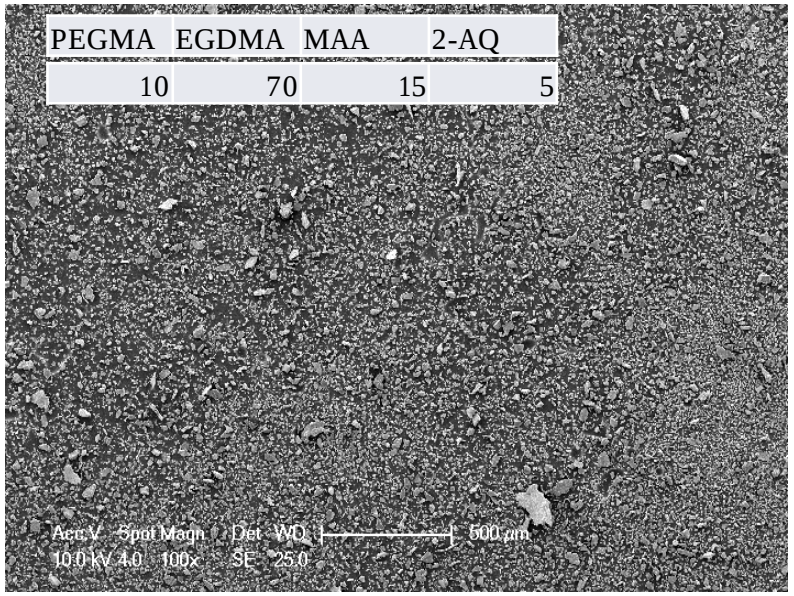
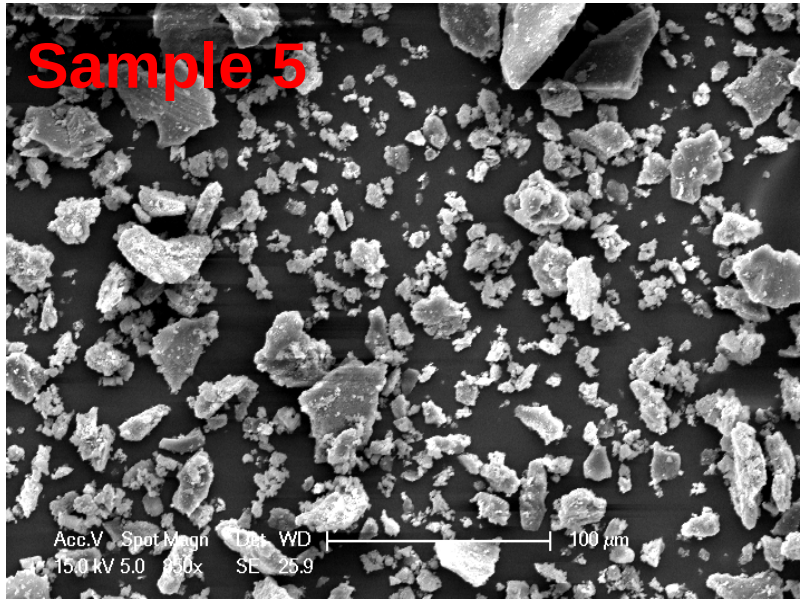
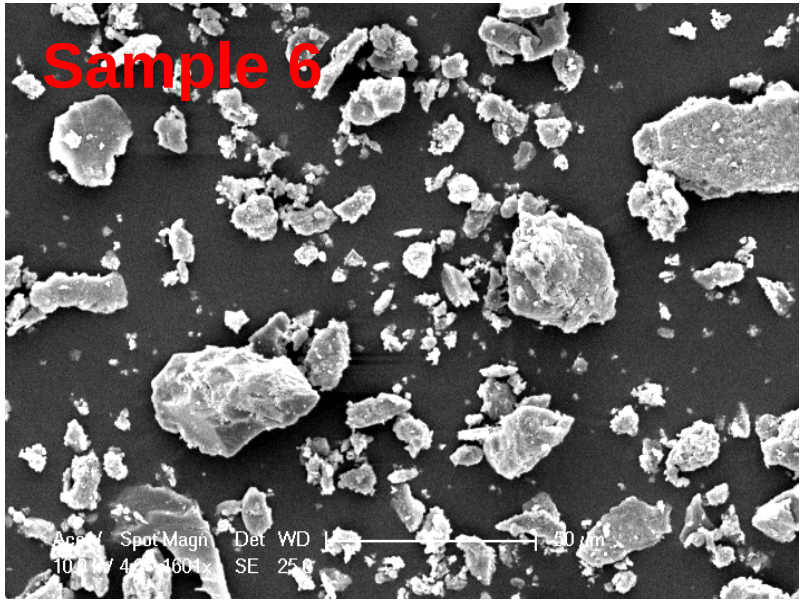
Scanning Electron Microscopy (SEM) of milled polymers

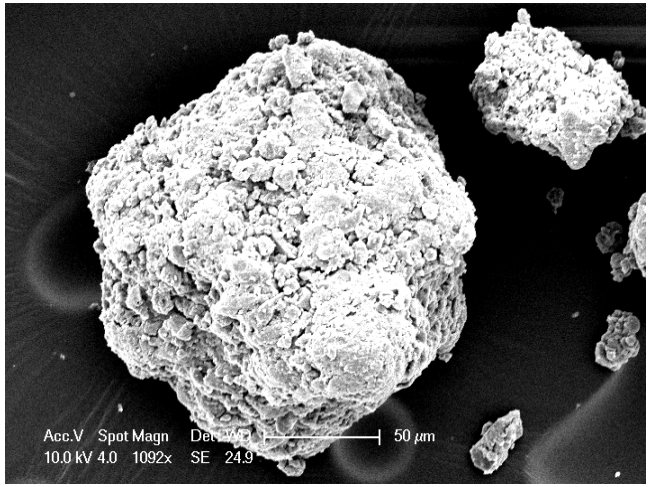


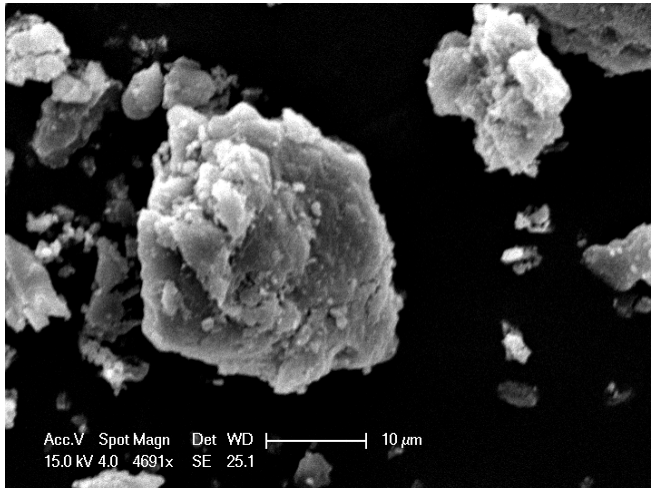






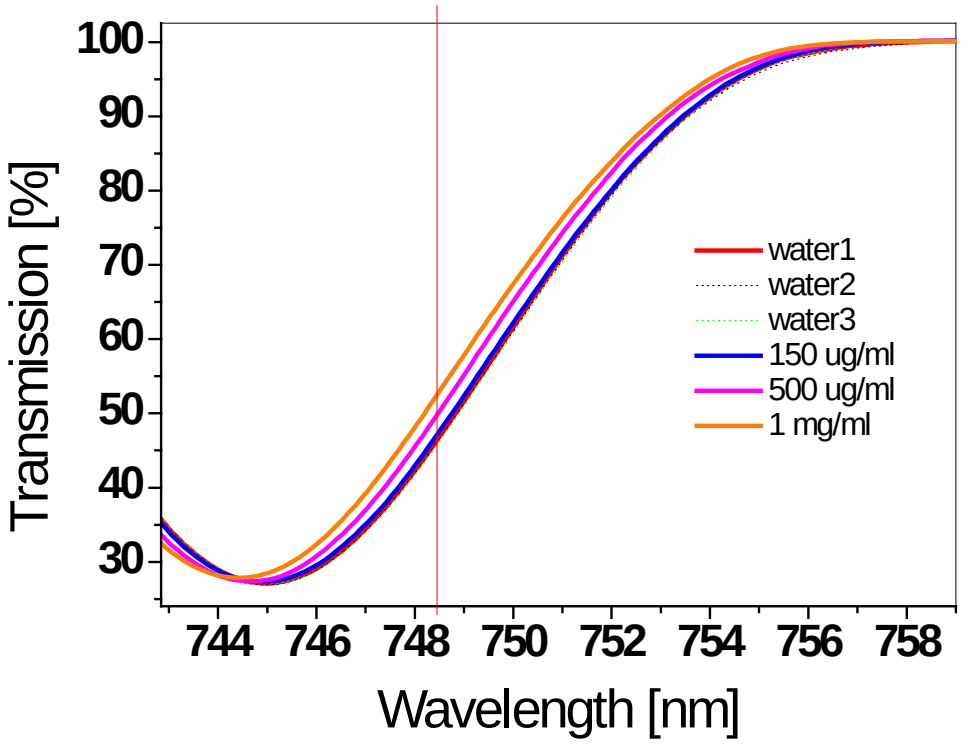
Different sizes of aggregates

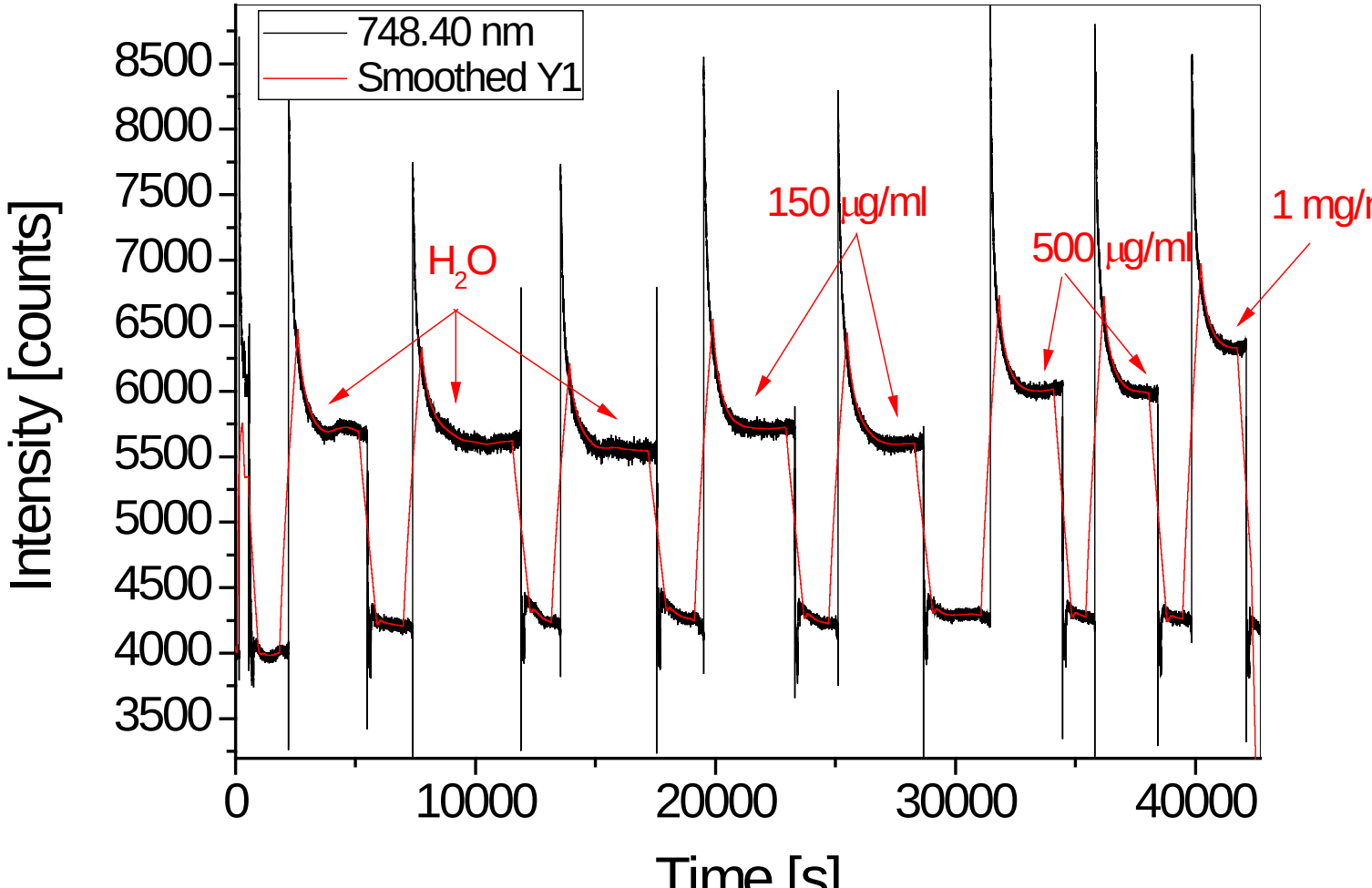




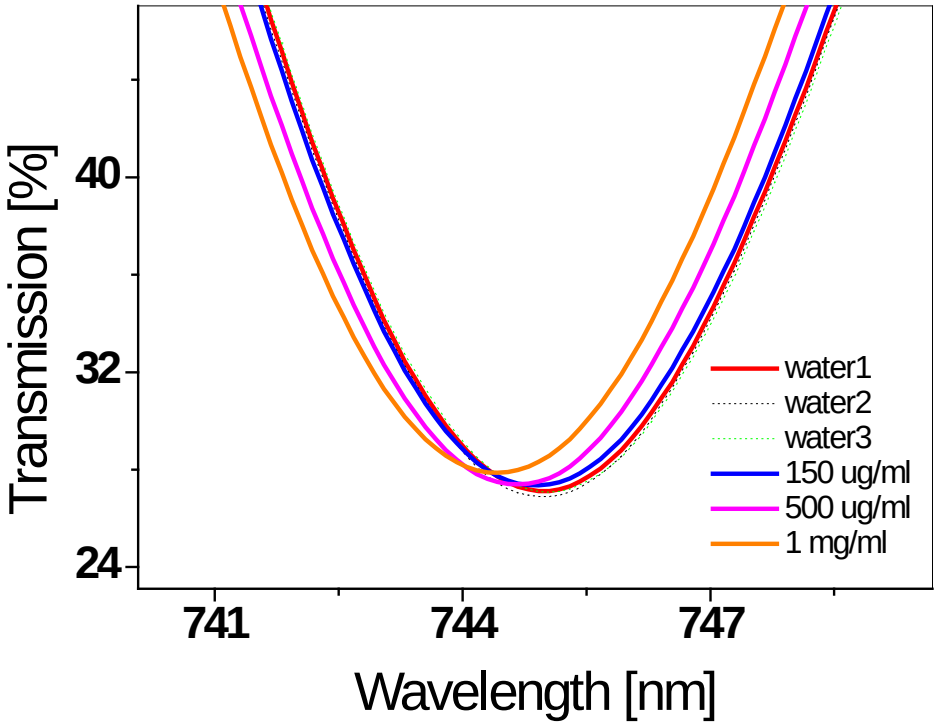
Repetition: binding test LPG1 (111 μm) in pure WATER

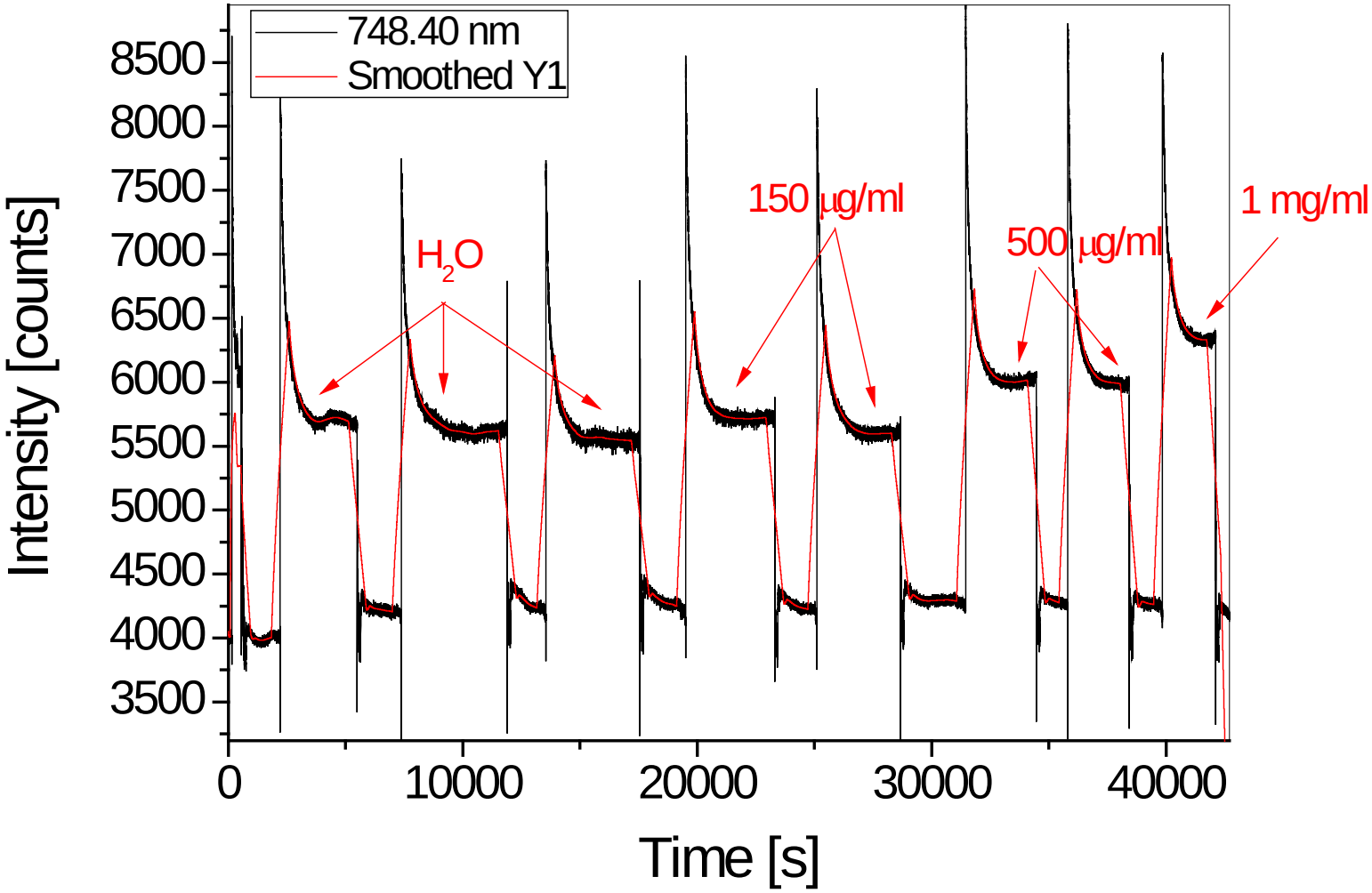
Fibre coated @0.2 mm/s, 200 μl Initiator,
PEGMA/EGDMA/MAA/2-AQ = 20 / 60 / 15 / 5 mol %



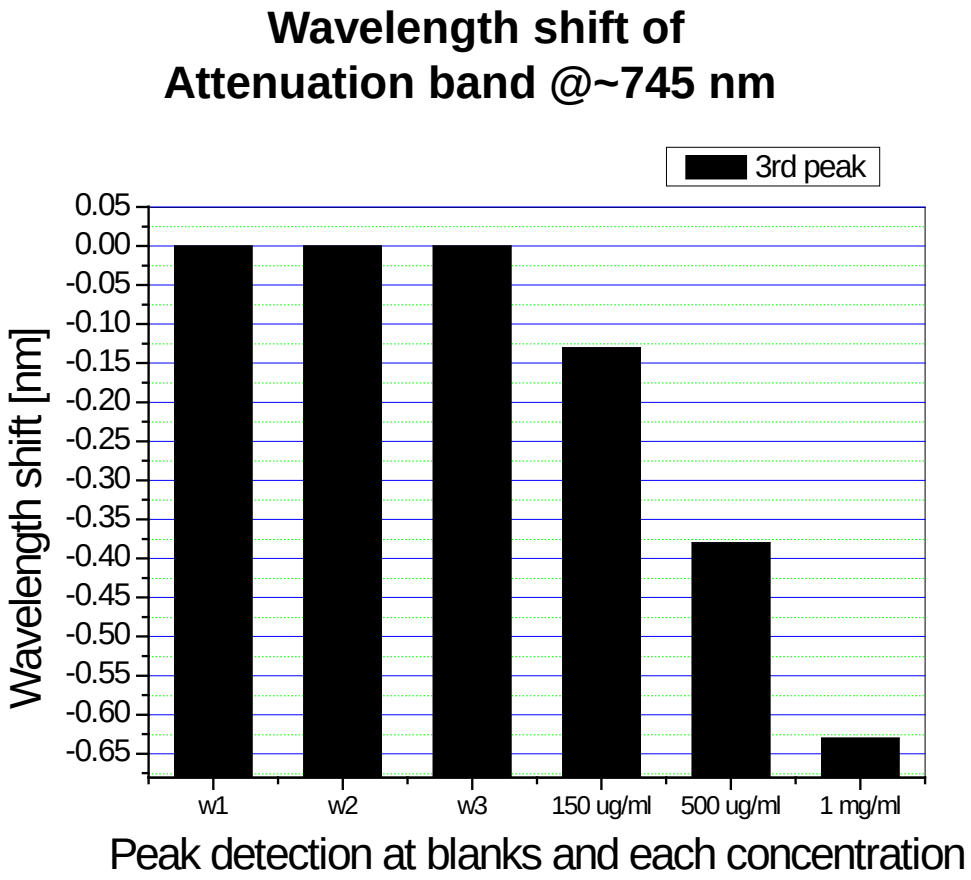


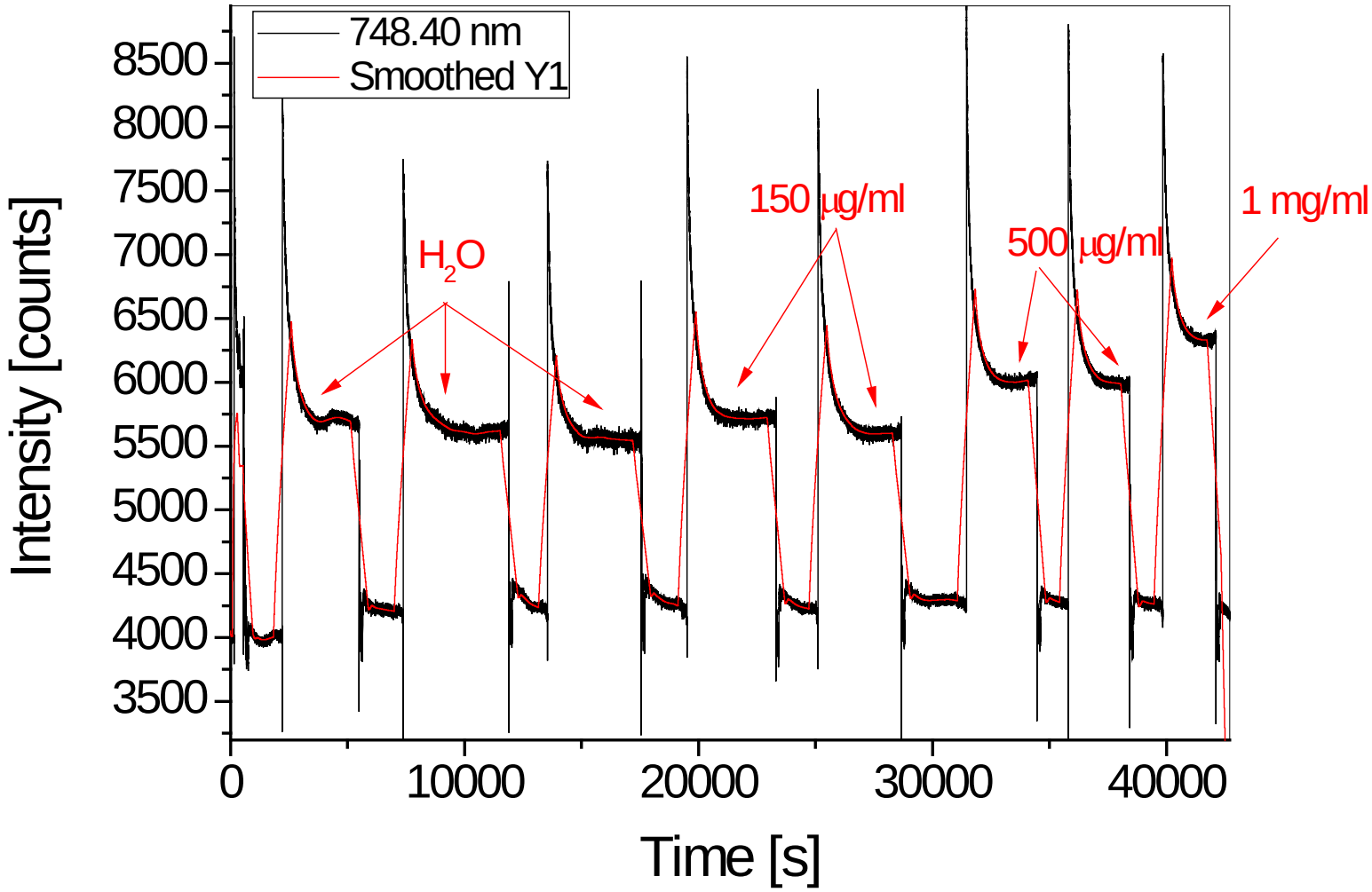
Binding test LPG1 (111 μm) in pure WATER (zoom in the attenuation band for detection of wavelength shift)





Binding test LPG1 (111 μm) in pure WATER (Calculation of wavelength shifts of detection of 2-AQ for different concentrations in water)





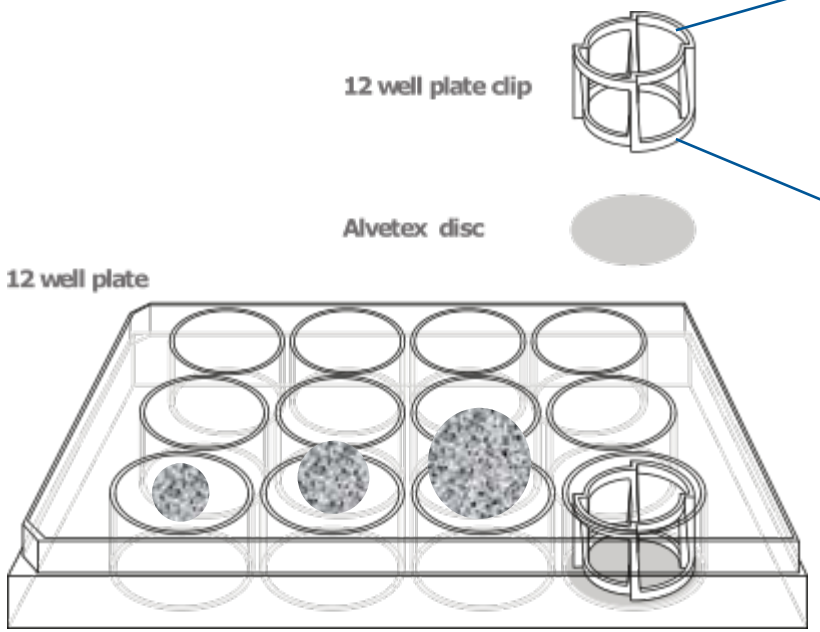
Future work: Towards 2nd objective of this research project (Growth of 3D spheroids of A375 cells)

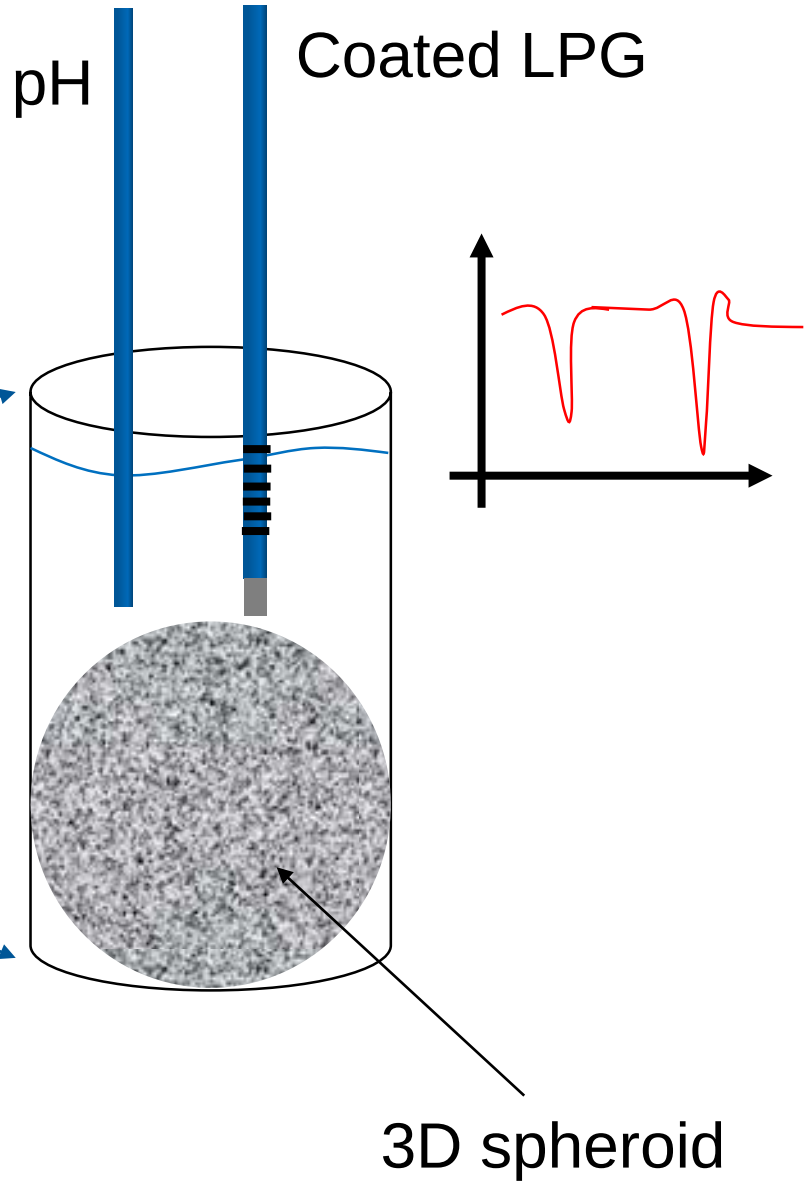
A375 cell lines (City Hospital)

Media

Dulbecco's Modified Eagle's Medium (DMEM) with high glucose concentration (see links below).

DMEM + 2mM Glutamine + 15% Foetal Bovine Serum (FBS).

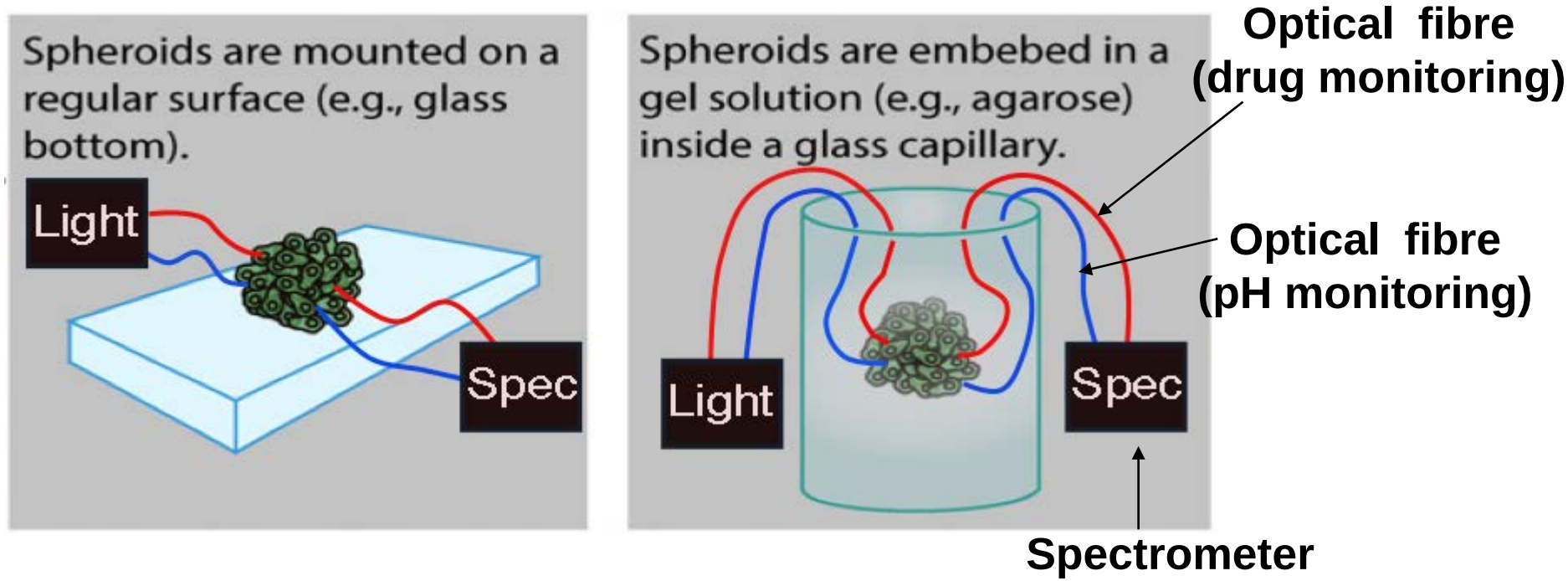




Gedanken-experiment (thought experiment)

Monitoring system in 3D tumour mimics.

Drug delivery (dabrafenib concentration) & tumour properties (pH)



"3D tumour spheroids: an overview on the tools and techniques used for their analysis"
Elisabete C. Costa et al. Biotechnology Advances 34(2016) 1427-1441

CRUK Multidisciplinary Grant -EOI

A multidisciplinary approach to monitoring efficiency of cancer treatment using a novel intra-tumoural multiparameter measurement system

-agreed that novel sensors to detect the early stages of resistance in tumours might be transformative

- this technology may provide a deeper insight into tumour therapeutic resistance.
- agreed that you were an expert in the field and that your team had the right mix of experience to deliver the project. It therefore
- invited you to consider submitting a revised outline application to a subsequent funding call.

-unclear how the technology would deal with intra-tumour heterogeneity- blood flow and drug delivery would be extremely heterogeneous - would optical fiber sensors would be able to detect resistance arising in areas not directly adjacent to the fibers.

-sensor might be prone to fouling and it was unclear how this would be addressed from the proposal. It was also unsure of the potential for clinical implementation

ACKNOWLEDGMENTS: EPSRC (Grant EP/N026985/1)

Prof Cameron Alexander, Dr. Sergiy Korposh, Prof. Steve Morgan, Prof. Barrie Hayes-Gill Prof. Poulam Patel, Colleagues from B15 and Optics&Photonics.

B15 laboratory team
School of Pharmacy

Dr. Francisco Ulises Hernandez Ledezma

Optics and Photonics Group
Faculty of Engineering