Institution:	10007857 Bangor University
Unit of Assessment:	08, Chemistry
Title of case study:	Cervical Cancer Diagnostics

1. Summary of the impact

Through research carried out under an EPSRC Teaching Company (KTP) award, we assisted an SME, CellPath, to develop the capacity to manufacture a novel set of dyes (Ortho Stains) for use in the Papanicolaou cervical smear test and other histological procedures. The company, previously mainly known for manufacture and sales of laboratory plastics etc, rapidly become the UK market leader in cytology stains, with over 50% of the domestic market, and exports to Finland, France, Italy, Japan, Norway, Sweden and the USA. As a result the company has increased turnover by 400% and the workforce has grown from 5 to 65 employees.

2. Underpinning research

The Papanicolaou cervical smear test used in cytological laboratories around the world was developed in the 1930s. The stain, which is itself a mixture of four dyes and various ingredients, was developed with apparently little rational design. Although certain dyes were chosen to react with specific entities, e.g. with cell cytoplasm (collagens / keratins); two of the ingredients, Bismarck Brown Y and phosphotungstic acid appear to interfere with each other.

During the late 1980s and early 1990s, a series of smear test misdiagnoses were reported in the national press as a result of both the approach, which was not suitable for automation, and human error in misinterpreting cellular structures. Coincidentally, a worldwide shortage in one of the key ingredients, Light Green SF Yellowish was reported. This was largely due to the high costs of waste disposal as the dye itself was a suspected carcinogen and its production involved lead oxide.

CellPath, innovators in cytological pathology based in Newtown, Mid Wales were interested in:

i) Large scale synthesis of Light Green SF Yellowish

ii) Large scale synthesis of possible analogues, *e.g.* Fast Green FCF used as a replacement in Masson's trichrome stain

iii) Rational design of Papanicolaou cytological stains using molecular modelling

iv) Prediction of the UV-Vis spectra of known and novel cytological stains using molecular modelling

v) Investigation of key stain-cell interactions using molecular modelling

Bangor University was well-placed to carry out this research, as a result of Professor Mark Baird's expertise in synthetic organic chemistry and structure determination, in particular, in relation to cyclopropanes and cyclopropenes[3.1, 3.2].

The company approached us because of our research experience in organic synthesis, on the advice of the Welsh Development Agency. Synthetic work including synthesis optimization was successfully carried out at both Bangor and Cellpath by Dr. Jeremy Tomkinson, supported by an EPSRC Teaching Company Scheme award and Simon Oram[3.3], whose MSc thesis was funded by Cellpath. Dr Tomkinson had worked on the synthesis of aromatic systems for his PhD and was ideally suited to take on the challenge of developing and improving synthesis of stains (containing aromatic systems) for application in cervical cancer smears.

During this time much of the underpinning basic science was carried out by R.A. Davies as part of his PhD, focussing on modelling of structures of the stains. This work re-confirmed Dahne's triad theory for organic chromophores, allowing dyes to be categorized with differing amounts of polyenic, aromatic and polymethinic character. Linear regressions were developed for the three families allowing accurate quantitative predictions of the UV-VIS spectra of cytological dyes. Initial stain-cell (e.g. haematoxylin-DNA) interactions used in both the cervical smear test and other staining procedures such as the widely used Gram test for bacteria, were modelled using smaller analogues.

In addition, as part of her PhD[3.5], Ms A.O'Sullivan began a programme modelling the structures of natural polymers– then a very complex exercise –to understand the bonding of stains to such systems. Mr Oram's work was nearer-market, carefully optimising reaction conditions for appropriate commercial scales, with a view to the economics of production.

Much of the work in these research theses was considered commercially sensitive at the time, and therefore was not published in refereed journals. Some of the modelling work of O'Sullivan was eventually published in collaboration with Prof A. Whiting at Durham in 2008[3.6].

The modelling approaches devised in the PhD thesis are currently used by the Davies research group, e.g. hydrogen bond containing polhydroxy napthoquinone dyes (James Maskery, Knowledge Exchange Scholarship PhD in collaboration with CAST Ltd).

3. References to the research

1. **Salaun J & Baird MS 1995** Biologically active cyclopropanes and cyclopropenes. *Current Medicinal Chemistry,* 2, 511-542 [reviews research developments in the cyclopropane field, including Bangor work, over 250 citations]**.**
2. **Baird MS & Grehan B 1993.** A new approach to cyclopropene fatty acids involving 1,2-deiodination. *J. Chem Soc. Perkin Trans.* 1, 14, 1547-1548. **DOI:** 10.1039/p19930001547. [example of contemporaneous Bangor research on this class of molecules]
3. **Oram SJ 1997.** Formulation and manufacture of cytodiagnostic stains and reagents. *University of Wales, Bangor, MSc thesis*. [integral to the CellPath TCS work]
4. **Davies RA 1997.** Theoretical and computational aspects of organic chemistry. *University of Wales, Bangor, PhD thesis***.** [contains unpublished modelling research, supporting the design of ortho stains]
5. **O'Sullivan AC 1995.** Modelling of cellulose-molecule interactions. *University of Wales, Bangor, PhD thesis*. [modelling research, some later published as 3.6]
6. **Baird MS, Hamlin JD, O'Sullivan A, Whiting A 2008.** An insight into the mechanism of the cellulose dyeing process: Molecular modelling and simulations of cellulose and its interactions with water, urea, aromatic azo-dyes and aryl ammonium compounds. *Dyes & Pigments* 76, 406-416

4. Details of the impact

Worldwide, cervical cancer is the second most common cancer of women[5.3], resulting in an estimated 225,000 deaths in 2010[5.4]. Since the introduction of widespread screening with the Papanicolaou cervical smear (Pap) test, the mortality rate in the USA has declined by an estimated 74%[5.3]. Like many other biomedical staining processes, the pap test is not a patented process and dye manufacture is a cottage industry of small producers of variable quality. The market for biomedical stains is relatively small compared to uses of dyes in textiles and cosmetics, often leading to sharp fluctuations in cost and availability of key compounds[5.5].

In the 1990s, CellPath Ltd, a small family business focussing on laboratory supplies, such as plasticware, decided to seek assistance from Bangor University chemistry researchers to advise on

the development of processes for the manufacture of high-quality laboratory stains, in particular for the Pap test.

During a 3-year collaboration, funded by an EPSRC Teaching Company Scheme Grant (later KTP)[5.6], researchers from Bangor University under the supervision of Professor Mark Baird developed a new approach to compounds for the Pap test and other biomedical special stains. A new process was also developed for the synthesis of the Light Green SF Yellowish stain, which had become virtually unobtainable commercially[3.3]. These Ortho Stains were more effective, economical and readily available than existing formulations. In the course of the collaborations, we also advised on the setting up of appropriate laboratory facilities for the manufacture of the stains, and assisted the company in optimising their processes, including enhanced quality control and safety and reduced production costs. For example, it was found that the reaction temperature for haematoxylin production could be lowered, saving 2.5% of total production costs, while reduction in Alum concentration produced additional efficiencies. A stabilized form of Haematoxylin was developed for commercial scale production, as Haematoxylin Z, which remains on the market.

The company invested £170k in production facilities and hired two production workers[5.7]. The MSc student Oram was later hired to carry our further research and development within the company.

Results were immediate, with an increase in turnover from £1.1M to £1.5M in the first year following completion of the project (1996)[5.7]. The project was a Finalist at the Teaching Company Scheme Awards in 1997[5.1].

The company, previously mainly known for manufacture and sales of laboratory plastics etc, rapidly become the UK market leader in cytology stains, with over 50% of the domestic market, and exports to Finland, France, Italy, Japan, Norway and Sweden[5.7].

Ortho stains remain a mainstay of the company's business. Since collaborating with Bangor Chemistry researchers, the turnover of the company has grown by 400% and the workforce increased from 5 to 65 employees[5.2].

From 2008-2012, the value of the company has doubled[5.8]. From 2012, the company began trading in the USA[5.9], indicating its continuing dynamism.

5. Sources to corroborate the impact

1. Letter on file from Deputy Director, Teaching Company Scheme, 4 Nov 1997.
2. Letter on file from a director of CellPath, (11 November 2013).
3. Armstrong EP (2010). Prophylaxis of cervical cancer and related cervical disease: a review of the cost-effectiveness of vaccination against oncogenic HPV types. *Journal of Managed Care Pharmacy* **16**: 217–30.
4. Lozano, R (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**: 2095–128.
5. Dapson RW (2010) Dye quality and implications for biomedical staining. Chapter 5 in Special Stains Education Guide, pp45-48. Dako (Agilent Ltd).
6. http://info.ktponline.org.uk/action/details/partnership.aspx?id=4101 [summary of EPSRC Teaching Company/KTP award]
7. Teaching Company Scheme #1402, Final Report 1997.
8. Company accounts summary: http://companycheck.co.uk/company/01831261
9. http://www.cellpath.co.uk/