Institution: University of Essex

Unit of Assessment: 5 – Biological Sciences

Title of case study: Chlorophyll Fluorescence Imaging

1. Summary of the impact

Pioneering research at Essex developed an innovative mathematical method for determining the chlorophyll fluorescence parameter Fo', as well as novel LED lighting technology and a multi-plant imaging system. This instrument is marketed by *Technologica*. Originally an Essex spinout, the company has sold 42 units across Europe, Asia and South America since 2006, recording its highest ever profits over the past three years (totalling ~£115k). Essex's mathematical method for determining Fo' is also used by other manufacturers, who have since developed their own imaging systems. This research has helped to establish chlorophyll fluorescence imaging as a mainstream screening tool, now used globally to inform a range of crop production and handling strategies.

2. Underpinning research

Chlorophyll fluorescence imaging, to determine plant photosynthetic efficiency (PE), has become an important and widely used tool for screening and phenotyping plants for alterations in metabolic processes. The technique is used by researchers in industry and academia for assessing the effects of various chemical, genetic and environmental impacts on plant performance and productivity. An example is the use by agrochemical companies for herbicide screening.

The measurement of minimal fluorescence whilst a plant is under light conditions (Fo') enables the associated quenching parameters of PE to be determined. However, this presents a technically challenging problem in imaging approaches: measurement of Fo' requires plant exposure to a pulse of far-red wavelength light, whereas the actinic light (required for photosynthesis) present in imaging systems consists primarily of orange/blue wavelengths. Finding a workable balance of these competing requirements would necessitate a complex secondary far-red lighting array. This (coupled with a lack of suitable LED technology) meant that most early imaging systems were developed to record only *dark* adapted parameters. Though more straightforward to measure, such observations are only of limited scientific usefulness.

In 1997 Kevin Oxborough and Neil Baker, based in the School of Biological Sciences at the University of Essex, developed the first high resolution chlorophyll fluorescence imaging system capable of capturing images at very low light intensities (Oxborough and Baker, 1997a). They also devised a method to estimate Fo' through a simple equation involving dark adapted Fo (which their imaging system was capable of measuring), maximum fluorescence yield in the dark (Fm) and maximum fluorescence yield in the light adapted state (Fm') (Oxborough and Baker, 1997b). By doing this, they were able to subvert the previous problem – a value of Fo' could now be obtained *without* the requirement for the far-red pulse. This was an important milestone in research; Oxborough and Baker had found a novel approach and for the first time shown how a new chlorophyll fluorescence imaging protocol could have broad applicability for use in a wide range of mainstream screening implementations.

Calculation of Fo' in this way greatly extended fluorescence imaging systems by enabling the construction of images of both photosynthetic and non-photosynthetic quenching parameters. This gave scientists a much greater understanding of the biological processes resulting from changes in PE and for the first time showed the viability of chlorophyll fluorescence imaging as a mainstream

screening tool. Based on this work, Baker and Oxborough, along with John Bartington (an engineer based within the University's central workshop), developed and produced a chlorophyll fluorescence imaging system, the 'CFImager', for rapid screening of 96 seedlings in a microtitre plate in one image. As part of this process a unique system for pulse-width modulated, thermally stable LED lighting was developed. Across an array of 16 carefully-angled panels, 1600 LEDs were positioned to ensure that plants would receive uniform exposure, regardless of their position on the plate. This led to the patent *Variation of LED optical power and photosynthetic fluorometers*, under Wivenhoe Technology Limited and with inventors listed as Baker, Bartington and Oxborough (UK Patent No. GB2380790, 2004). In 2002 the University also set up the spinout company *Technologica*, to market the CFImager as well as other LED developments.

In 2003 the first results from the CFImager system were published (Barbagallo et al., 2003). This demonstrated the effectiveness of chlorophyll fluorescence imaging for rapidly detecting perturbations in leaf metabolism before any effects on growth and development could be visualised. Significantly, the publication represented the full practical realisation of Oxborough, Baker and Bartington's earlier work, as well as effectively highlighting the importance and commercial value of this process as a screening tool. In reflection of the subsequent growth in popularity of this technique, and to complement *Technologica*'s commercial activity, chlorophyll fluorescence imaging systems are now sold by a number of manufacturers of plant physiology equipment. Almost all of these instruments incorporate the mathematical equation of Fo' developed by the University of Essex.

3. References to the research [can be supplied by HEI on request]

Oxborough, K.M. and N.R. Baker (1997a) An instrument capable of imaging chlorophyll a fluorescence from intact leaves at very low irradiance and at cellular and subcellular levels of organization. *Plant, Cell and Environment*. 20, 1473–1483. (88 citations – November 2013) DOI:10.1046/j.1365-3040.1997.d01-42.x

Oxborough, K.M. and N.R. Baker (1997b) Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components – calculation of qP and Fv'/Fm' without measuring Fo'. *Photosynthesis Research*. 54, 135–142. (282 citations – November 2013) DOI:10.1023/A:1005936823310

Barbagallo, R.P., K.M. Oxborough, K.E. Pallett and N.R. Baker (2003) Rapid, noninvasive screening for perturbations of metabolism and plant growth using chlorophyll fluorescence imaging. *Plant Physiology*. 132, 485–493. (118 citations – November 2013) DOI:10.1104/pp. 102.018093

Baker, N.R., J.K. Bartington and K.M. Oxborough (2004) *UK Patent No. GB2380790*. Newport, Wales: The Patent Office.

Baker, N.R. and E. Rosenqvist (2004) Application of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *Journal of Experimental Botany*. 55, 1607–1621. (410 citations – November 2013) DOI:10.10 10.1093/jxb/erh196 93/jxb/erh196

Baker, N.R. (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology*. 59, 89–113. (573 citations – November 2013) DOI:10.1146/annurev.arplant.59.032607.092759

4. Details of the impact

Essex research demonstrated the viability of using chlorophyll fluorescence imaging as a mainstream screening tool for rapid and non-invasive evaluation of a plant's photosynthetic efficiency and underlying metabolic processes. The Fo' calculation developed by Baker and his group (meaning that the previously problematic necessity of obtaining measurements following a far-red pulse could be avoided), along with Bartington's work in supporting this principle as a practical reality, has underpinned significant impact through a number of strands.

A key step towards the now widespread proliferation of chlorophyll fluorescence imaging was the establishment of the University spinout, *Technologica*, in 2001. In bringing the imaging technique to a wider audience, *Technologica* has generated a number of commercial impacts, all of which are underpinned by the original research of the Essex group. The company was sold to John Bartington in 2006, with the University continuing to receive royalties from its commercial activities until the license was bought out by Bartington in 2009. Both during and since this period, *Technologica* has followed a highly proactive marketing strategy, including participation and sponsorship of plant physiology conferences [see corroborating source 1, page 3], publication of flyers and a company website. The company also uses scientific advisors to market the CFImager to large commercial manufactures, illustrated through the proactive targeting of Syngenta (in the UK) and Monsanto (in the US). To complement these activities, a close relationship is retained with researchers at Essex, providing synergistic benefit through shared intelligence of developments and priorities in both academic and commercial domains.

With the CFImager as its flagship product, *Technologica* has experienced a period of sustained growth. Beyond operation in the UK, its worldwide network now comprises agents in China, India, Taiwan, Japan, Korea, Turkey and Brazil [2]. Through this network, in the period 2006-12, 42 units have been sold. In the more recent past, the company's performance has continued to follow an upward trend; in the past three years its highest ever levels of profit have been recorded, totalling ~£115k. In this time *Technologica* has also paid dividends to its shareholders [3] [4] and the company's sustained commercial success has paved the way for new product development. To complement the CFImager, the company will shortly launch the *IsoLight*, a new product which also draws directly on the novel LED lighting technology developed at Essex [5].

Through its energetic marketing and development activities, *Technologica* has played a key role in making chlorophyll fluorescence imaging commonplace both in research laboratories and the plant industry. In an email testimony [6], a Secondary Profile Screening Manager from Syngenta explains how Essex's research has served to "greatly extend the potential of fluorescence imaging as a rapid and non-invasive screening technique" and is "in broad use in commercial screening and imaging communities". He goes on to outline how Syngenta has made "extensive use of the process pioneered at Essex" and has purchased a CFImager, noting that, amongst other benefits, "The fact that this device can accommodate high throughput screening, and enable rapid imaging of transient effects, has distinct positive implications relating to glasshouse time and resource efficiencies".

In addition to the impact of *Technologica*, the Essex group's novel method of calculating Fo' has been widely incorporated into commercial instruments from a number of other manufacturers. This is detailed in letters of support from: (i) *Heinz Walz GmbH* ('Walz'), a producer of sophisticated measuring devices for plant research, based in Germany; and (ii) *Photon Systems Instruments* ('PSI'), a plant science, biotechnology and agriculture company based in the Czech Republic. In the first letter [7], Walz's Managing Director explains how "The Essex group's work […] showed

that [chlorophyll fluorescence imaging] could be used as a viable, mainstream tool to enable rapid and non-invasive plant imaging". He goes on to describe how Essex's results, such as those published by *Barbagallo et al., 2003*, have had significant influence, noting that "the Essex technique is used in our range of *PAM-Chlorophyll Fluorometers*, including our new *Multi-Colour-PAM*". The second letter [8], from PSI's CEO, also acknowledges the importance of Essex's research, highlighting, in particular, *Oxborough and Baker, 1997* and *Barbagallo et al., 2003*. The letter goes on to explain that:

> "Oxborough and Baker's research has been widely cited and now forms the basis of screening implementations offered by a range of manufacturers, including a number of PSI's own commercial products. As an example, the Essex technique is incorporated into PSI's *FluorCam* product family".
>
> *CEO, Photon Systems Instruments*

In another example, the Marine Systems Group of *Chelsea Technologies Group Ltd* has developed multi-parameter sensors for monitoring physical, optical and biological oceanographic and freshwater environments. Chlorophyll fluorescence imaging is a technique enabled by a number of the Group's products, whose manuals cite, for instance, *Oxborough and Baker, 1997* [9].

Finally, whether through use of *Technologica's* CFImager, or the commercial implementations of other manufacturers, an overarching impact of Essex's chlorophyll fluorescence imaging research has been to inform crop production. In an email testimony, Bayer CropScience's Head of Protein and Product Characterization (Essex's industrial collaborator in *Barbagallo et al., 2003*) explains the significance of the Essex technique in the context of industrial applications [10]. His account highlights the appropriateness of screening for identifying new leads as herbicides and plant growth regulating molecules, as well as new trait discovery in agricultural biotechnology. He concludes: "non-destructive chlorophyll fluorescence imaging technologies provide a valuable tool to underpin high throughput screening in the Crop Protection and Agricultural Biotechnology industry".

5. Sources to corroborate the impact [All sources saved on file with HEI, available on request]

[1] Plant Environmental Physiology Group, 2012. *Ecophysiology Techniques Workshop, Lisbon, Portugal, 10-15th September 2012, Abstract Booklet* [pdf]

[2] Technologica, 2012. *Technologica Agents* [online] Available at: http://www.technologica.co.uk/Agents.html [Accessed 20 June 2013]

[3] Technologica, 2012. *Technologica World Sales* [pdf]

[4] Technologica, 2012. *Technologica Profits* [pdf]

[5] Technologica, 2012. *IsoLight* [online] Available at: http://www.technologica.co.uk/products/IsoLight/index.html [Accessed 20 June 2013]

[6] Secondary Profile Screening Manager, Syngenta

[7] Managing Director, Heinz Walz

[8] CEO, Photon Systems Instruments

[9] Chelsea Technologies Group, 2012. *FASTpro GUI Handbook 2230-001-HB Issue: D* [pdf]

[10] Head of Protein and Product Characterization, Bayer CropScience