Fluorochrome: Science in colour

Simple, fast, and accurate quantification of pH from fluorescence images

By This was a group project - names have been removed to respect the privacy of co-authors, Matteo Rochon Cocchiara, and [redacted]

Science in colour

Introduction: Motivations and Current Methods

Problem Brief:

Currently, a team in the Infectious Diseases Department at Imperial College London is researching the use of POlyhedrin Delivery system (PODs) as a means to monitor local pH, using pH-sensitive proteins such as pH-lemon (Burgstaller, 2019). This idea has come from Professor Kazutoshi Mori who was inspired by the crystalline structures formed by polyhedrin proteins expressed by the Bombyx Mori Cypovirus and specifically their ability to act as survival capsules for the virus when secreted into the environment. By modifying these crystal structures to encase cargo proteins, this technology can be used for a variety of applications such as delivery in biomanufacturing, targeted insecticides, sustained release of growth factors and other biomedical applications. The delivery of pH sensors using these PODs will be revolutionary in enabling more accurate measurements and enhanced understanding of intracellular pH.

Existing methods are limited and often involve washing a culture system with a pH-sensitive solution. This provides an average pH estimate across the whole cell culture system and is not suitable for identifying localised pH. There are also different compartments of cells that have different pH values such as phagolysosomes which are very acidic. PODs are taken up phagocytically so they will end up in phagolysosomes. This will permit measuring of efficiency of phagocytic uptake and differentiating the parts that are outside cells and parts that are in phagolysosomes using a pH sensor.

Protein based biosensor pH Lemon consists of a dimeric protein complex. During excitation by incoming electromagnetic radiation, Förster resonance energy transfer occurs between the two protein subunits, and results in the emission of a photon of light. One of these proteins is pH-sensitive, and this affects the efficiency of energy transfer when the protein is in a different environment. This variation in transfer efficiency elicits a change in the photon energy, causing pH-Lemon to fluoresce different colours in solutions of different pH.

Wider Scale Problem:

As mentioned, visual methods for measuring pH in cell cultures currently provide average pH values of the whole cell. The detection and measurement of pH value at a scale of one hundredth of a millimetre at an intracellular level is a key factor to understanding the regulation of cellular behaviours, functioning and activity. (Hou, 2017). Furthermore, the identification of subcellular pH could be invaluable in identifying important processes in many diseases, developmental disorders and neurogenerative diseases. (Ma, 2017).

Intracellular pH can range from 4.7 in lysosomes to 8.0 in mitochondria; thus, being able to accurately identify the pH value in different areas of the cellular environment is key in cell culture analysis. The current methods that are available for intracellular measuring of pH have many disadvantages such as the need for labelling, background noise, photobleaching and instability. (Hou, 2017)

A current method developed looking at intracellular pH measurement is the colorimetric imaging method for single-cell pH sensing and detection. This method combines bright-field microscopy-based UV-Vis micro spectroscopy and common pH indicators bromothymol blue or bromocresol green. (Hou, 2017) Some subcellular structures can be seen clearly outlined after the administering of the pH indicators which suggests that this could be a useful method for identifying areas with different pH values within a cell therefore subcellular pH detection. The pH values are determined from the absorption spectra of the specific areas in the cell culture using bright-field microscopy. (Hou, 2017)

Fluorescent conjugates of growth factor receptor ligands have been used to measure pH of intracellular organelles There are many techniques for measurement of luminal pH that currently use pH-sensitive fluorescent probes that are based on 1) quantitative, ratiometric measurement of endocytosis of pH-sensitive and pH insensitive fluorescent conjugates of transferrin 2) use of protein tagged with a ratiometric variant of the pH-sensitive intrinsically fluorescent protein pHluorin and 3) using fluorescent dye. (Ma, 2017) These measured are limited to measuring the luminal only of intracellular organelles and use of pH calibrated curves for detection of the pH values.

Value Proposition

The effect of pH on chemical and biological processes is well documented, and a reliable method of determining the pH of a cell culture is a very valuable tool as presented in the previous section. Current methods, however, can generally only obtain an average value of the whole culture's pH, despite the fact that different cells and, indeed, different areas within a cell, may have different pH values.

Our technology uses fluorescence microscopy and pH-Lemon enriched PODS in conjunction with a companion software tool to obtain pH values at a sub-cellular scale, allowing for different pH values within the culture to be detected. Through this, we can provide better specificity and accuracy in measurement of the culture's pH, and a significantly more complete depiction of the pH distribution within your cell culture.

Fluorochrome is a valuable research tool and enables obtention of pH readings at a subcellular scale by means of our detailed, accessible, and 100% software-based system. Fluorochrome harnesses the power of open-access databases, together with our unique algorithms, to provide localised pH readings with a precision of up to 0.05 pH-units, with a detail and accuracy that no current technology can match.

Our software-based business model is easily scalable, environmentally-conscious, adaptable to new and emerging markets and involves fewer financial risks due to our short – and manufacturing-free – supply chain.

Technical Specification

Calculation of average pH for an area of an image

The initial Fluorochrome software is predicated on the use of pH-Lemon encapsulated in Polyhedrin Delivery Systems. A detailed description of the mechanism underpinning the action of pH-Lemon is described by Burgstaller et al., 2019. However, for this report, it suffices to know that a change in acidity or alkalinity of the surrounding solution alters the emission spectrum of the fluorophore. Thus, using spectroscopy, the emission spectrum for each different pH value can be obtained (shown schematically in Figure 1).

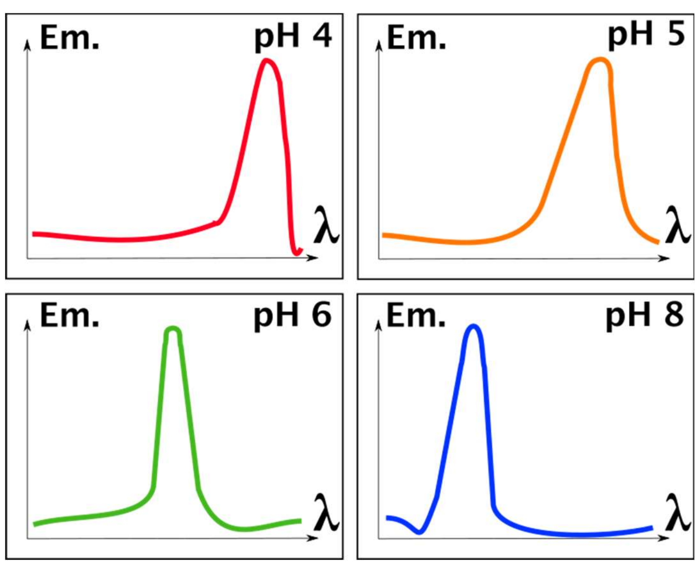


Figure 1: A schematic representation of the emission spectra of pH-Lemon at different values of pH. Many more spectra will be collected at more closely spaced intervals to maximise the resolution of the product. Note that, in this context, "Em." indicates relative emission.

These spectra will be loaded in as defaults in the software. Upon establishing a profile, a user will be directed to enter the make and model of their most used microscope camera(s) and filter set(s). After the researcher selects the area whose pH they wish to quantify, the software will generate a pop-up menu which will prompt the user to select their apparatus from the equipment currently listed in their profile. Once completed, the software will search the FPBase directory (Lambert, 2019) in order to find the absorption spectra of the apparatus being used. This can then be used to correct the default spectra obtained in the laboratory, thus adapting the readings to the researcher's own apparatus. This is shown schematically in Figure 2.

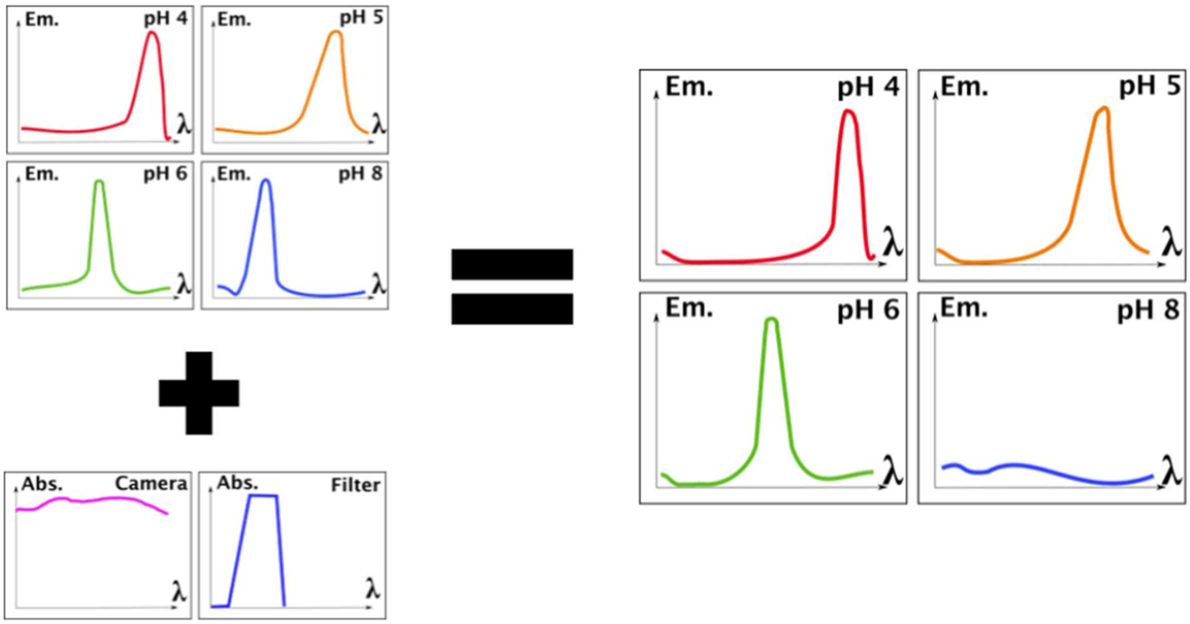


Figure 2: A schematic representation of how the corrected spectra are obtained from the defaults.

Mathematically, the updated relative emission value can be calculated using Equation 1:

$$\overline{E_i} = E_i C_i F_i$$

Equation 1: The formula for the calculation of a corrected relative emission value, $\overline{E_i}$. E_i is the uncorrected relative emission value, C_i is the relative absorbance value of the camera, and F_i is the relative absorbance value of the filter set.

One of the key selling points of the Fluorochrome software is its capacity to quantify pH in an area of the image selected by the researcher. When the researcher does so, the normalised RGB values of the pixels in the area will be extracted and allocated to rows in an array. Each row in the array will then be converted to a hue saturation value (or HSV); several software packages have inbuilt functions for doing this, for example the "rgb_to_hsv()" method from the Python "colorsys" toolbox (Python Software Foundation, 2022). From the newly obtained HSV values, a visible wavelength of light can be derived using the Equation 2, as adapted from Oliveira et al., 2017.

$$\lambda = \lambda_{max} - \frac{\Delta\lambda * \overline{H_i}}{\Delta H}$$

Equation 2: The relationship between wavelength and hue value, adapted from Oliveira et al., 2017. Here, λ represents the wavelength for a given hue value, λ_{max} indicates the maximum wavelength of the visible spectrum, $\Delta\lambda$ represents the wavelength range spanned by the visible spectrum, $\overline{H_i}$ represents the hue value, and ΔH represents the range of hue values spanned by the visible spectrum.

When applied to a set of N pixels, this will lead to a set of N wavelengths. These can then be plotted as a scaled histogram on the visible spectrum and compared to the relative emission spectra for each different pH. The spectrum that most closely matched the scaled histogram corresponds to the pH value. Initially, the scaling could be done by making the peak height of the histogram equal to one and applying the same scale factor to the rest of the histogram. This is valid in theory but may require refinement through research since different excitation wavelengths would lead to varying peak heights. Similarly, the histogram and the spectrum will be compared using the mean squared error, with the lowest mean squared error indicating the closest match (see Figure 3). Again, this is predicated on the absence of anomalies, so may also require refinement through research (such as by filtering).

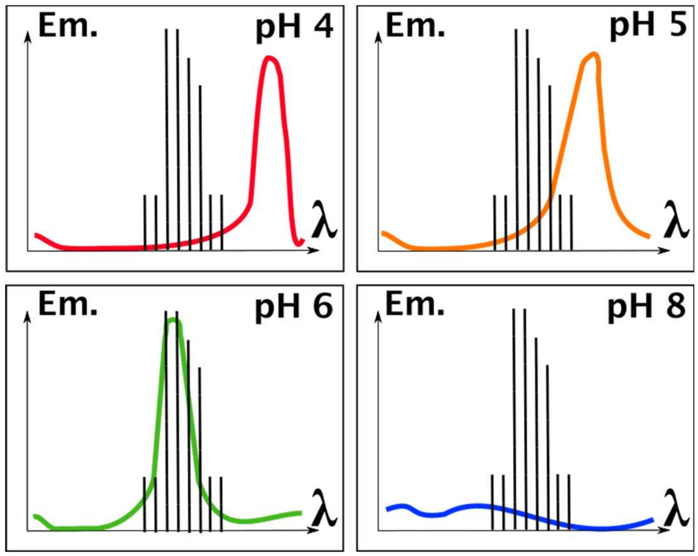
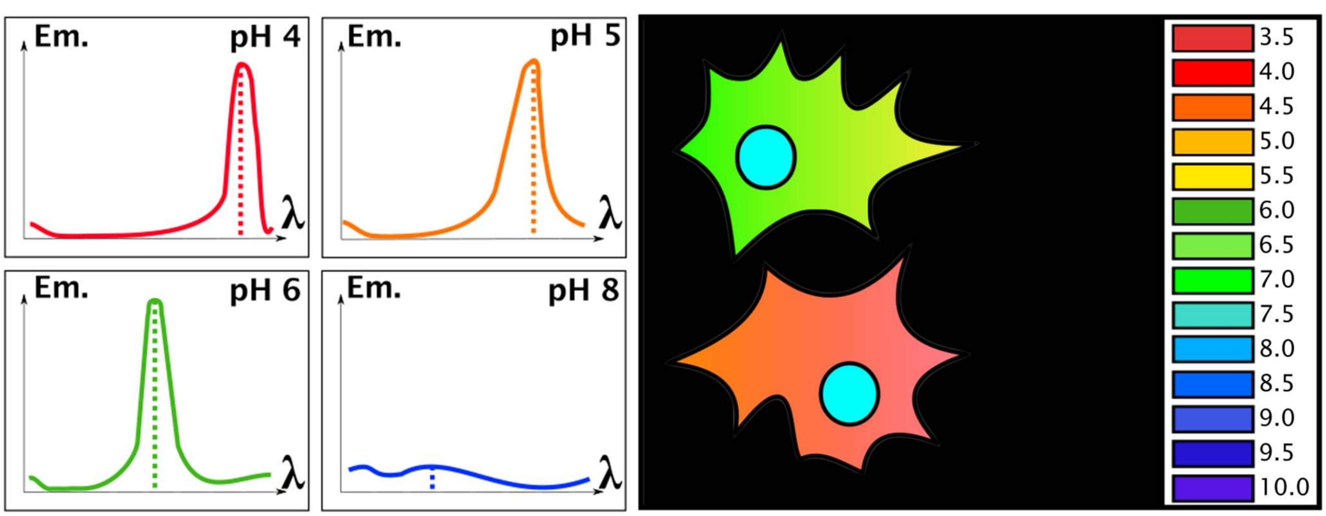


Figure 3: A schematic representation showing the overlay of the scaled wavelength histogram and corrected spectra. The closest match (according to the mean squared error) would give the pH (=6 in the above example). The intervals in pH will be much smaller than the ones used above but are limited by difference between the spectra obtained in the laboratory (which are not currently accessible). Nonetheless, an attempt has been made to determine the upper limit of pH resolution in Appendix A.

Superimposition of a scale bar on the image

The Fluorochrome software can also superimpose a scale bar by implementing a similar process to that described in the previous section. Should the researcher choose to generate a scale bar, the program will take the peak wavelength of a range of corrected spectra. It can then use a rearrangement of Equation 2 to obtain a HSV value. Finally, it can convert these HSV values to RGB and display them as a scale bar along with their corresponding pH values.



Figures 4a (Left) and 4b (Right): Figure 4a shows a diagram of the extraction of the peak wavelengths for four different pH spectra. Figure 4b shows the resultant scale bar superimposed on a depiction of two cells fluorescently labelled with pH-Lemon and DAPI.

Important Note: Calibration

The original paper by Oliveira et al., aimed to produce a spectroscopy device that could be mounted on a mobile phone. To improve the reliability of the estimates, the authors recommend calibrating new pieces of equipment a set of LEDs of known emission frequency in order to account for the specific hardware of the camera. Whether this calibration w be necessary for the Fluorochrome software is unclear: theoretically, a master calibration could be done in the Fluorochrome laboratory, with the remainder of corrections being accounted for by the absorption spectra from FPBase. However, since this is not guaranteed, there exists the possibility that the user will have to calibrate their microscope during the setup of the Fluorochrome software. Fortunately, fluorescent microscopes make this very simple since the frequency of the excitatory light source is known (and can be varied), so can substitute the LEDs implemented in the original paper.

Business Model

Key Partners

- Imperial College London, Department of Infectious Diseases
- Cell Guidance Systems Co. – Polyhedrin Delivery Systems
- Academic and research institutes
- Partnerships with other software companies e.g., ImageJ
- Investors

Key Activities

- Design and development of software
- Website and mobile app design
- Marketing and sales
- Maintenance of software
- Maintenance of website and mobile app
- Building and maintaining relationships with subscribed customers and larger institutions
- Collaboration with partners to work to elevate their platform and vice versa

Key Resources

- Engineers
- Computer scientists
- Lab technicians
- Laboratory space
- Equipment

Value Proposition

- Accurate quantification of localised, intracellular pH
- Quantitative pH measurement
- Objective, fully software-based approach
- Highly scalable business strategy
- Environmentally conscious business
- Attractive user interface
- Institutional discounts for academic and research bodies

Customer Relationships

- Web sales
- Customer services (digitally and physically)
- Compliance (software quality control)
- Digital Community

Channels

- Website
- Mobile App
- Social Media(s)
- Hotline
- Email
- Contacts
- Marketing

Customer Segments

- pH-sensor market (as outlined by Armanani, 2019)
- Pharmaceutical
- Water
- Food and beverages
- Oil and gas
- Industrial / chemical
- Large organisations (I.e., research organisations, companies, universities, etc...)
- Academia and research

Cost Structure

- Initial costs in development of the software
- Costs of maintaining and updating the software
- Costs of employment for developers and customer relations
- Cost to associations to provide advertising

Revenue Streams

- Initial funding through investors and grants
- Self-sustaining subscription model
- Partner investments

Infrastructure:

Key Partners

The partners involved with our company are key to its growth, effectiveness, and success: by working closely with the individuals, companies and targeted markets that will be benefit from using our software, Fluorochrome platform can progress to the forefront of cellular measurement systems.

Our main partner in the initial phases of this company is the Department of Infectious Disease at the Imperial College London who are relying on this solution for the progress of their work and research. They will be able to help with the design and specific characteristics of the software. The company Cell Guidance Systems will also form a key partnership as this software could be marketed as an add-on purchase to maximise the value of using pH-Lemon enriched PODS. Academic and research institutions will be central in the research, development, and trials of software as bodies who will experience the benefit of the software in their teaching and research. Partnerships with other software and imaging companies will represent valuable collaborators by marketing the Fluorochrome software as an add-on or plug-in which will be great for expanding client and partnership network. An example of a business with whom Fluorochrome could build a mutually beneficial relationship is ImageJ.

Investors will be critical partners for the start-up of this business. They will be key for funding the hiring of employees such as engineers, computer scientists, lab technicians as well as hiring of laboratory space and purchase of reagents and other equipment.

Key Resources

Resources necessary for the start-up and continued operation of this company will initially be funded by external capital injections and grants.

Engineers, computer scientists and lab technicians will need to collaborate in the design and refinement of the software to make sure that it fulfils all elements of the value proposition. These roles may also be filled by employees of our key partners. Our computer scientists and tech team will be recruited by our company through job advertisements online in the research, technology, and academia employment space.

Before the software can be commercialised, it will need to be trialled in its applications of intended use. These trials should include the input of the Department of Infectious Diseases at Imperial College London to assess the suitability of the software their area of research before testing is expanded to other applications.

Key Activities

The key activities for the set-up of this company include:

1. Design and development of software
2. Website and mobile app design
3. Marketing and sales
4. Maintenance of software
5. Maintenance of the website and mobile app
6. Building and maintaining relationships with subscribed customers and larger institutions.
7. Collaboration with partners to work to elevate their platform and vice versa.

The initial design of the software is the central to the start-up of the business. The software must meet the needs of our customers who will be using the software to measure the intracellular pH of specific areas of their cell cultures. Once the software is created the website, mobile app and subscription platform must be developed to become a functioning business. These areas will focus on customer interaction and performance of the company, enabling the customers to access and use the software in a maximally convenient way.

The maintenance and improvements of the software and customer platform will then be crucial for the continued success of the company by making sure to improve current features, build in new data analysis techniques, and produce better versions to keep customer satisfaction high.

Marketing and sales will be crucial in keeping the cash flow consistent to subsidise the maintenance of the software. By collaborating with other software companies, this will allow for our methods to be applied in different contexts and will allow for the continued expansion of our professional and customer networks.

Customer Segments:

Target Market:

Although the Fluorochrome software package is not a pH sensor or analyser, the target market for the company would have considerable overlap with the global pH sensors and analysers market. The reason is that industries and people working in this market can benefit from using the Fluorochrome software package. The breakdown of the market by industrial sector is shown in Figure 5, and applications will include industrial, medical, oil and gas, food and beverages, water/ wastewater, among others (Amarnani, n.d.).

Target Market Analysis:

The global pH sensors/analysers market is estimated to be valued at $1.82 billion by 2028 and the key factors affecting the market growth include workplace safety, government regulations over industrial and municipal wastewater disposal as well as wastewater treatment in the industrial sector (Amarnani, n.d.). As seen in Figure 5 and as mentioned above, Fluorochrome's software package can be used in a variety of industries and the top two markets by region will include North America and the Asia Pacific (Amarnani, n.d.). However, since the Fluorochrome software package is web-based and not physical, this means that anyone from anywhere around the world can easily subscribe and use it.

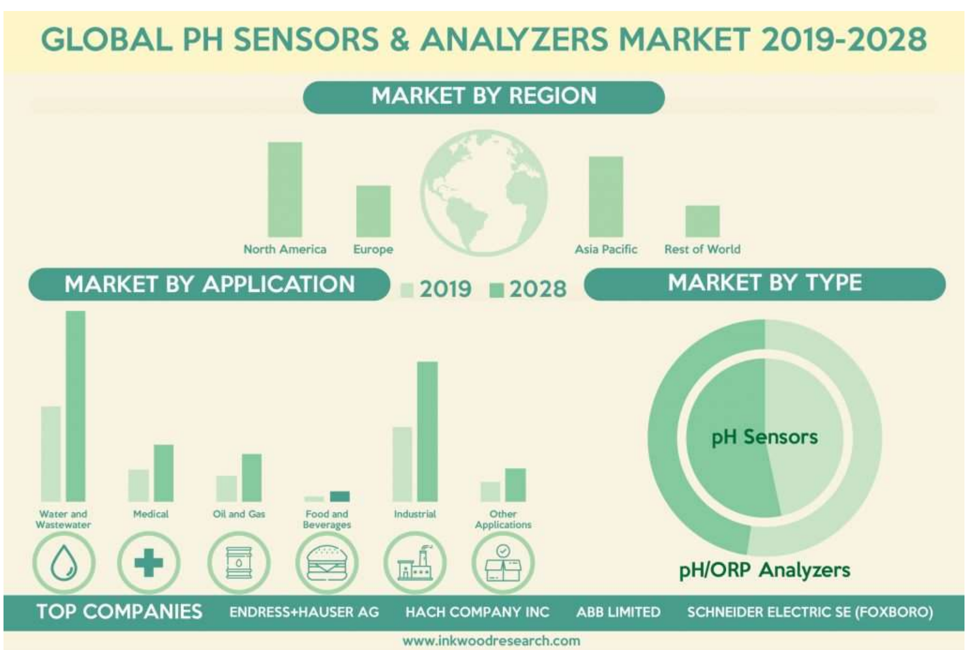


Figure 5: A diagram that shows the visual representations of the market by region, application, and type (from 2019 –2028) (Amarnani, n.d.).

Geographically, North America is the primary stakeholder in the market with a revenue share of 35.9% in 2019. However, the Asia Pacific is expected to take over North America by 2028, with China leading in the market growth (Amarnani, n.d., Data Bridge Market Research, 2020). Thus, the company can market the software and expect a wider customer base in both regions. Additionally, in 2020, the digital segment of the market by product type is also be expected to become the dominant industrial sector with increasing demand and this is where Fluorochrome comes and fits in (Data Bridge Market Research, 2020).

Furthermore, there are currently some concerns regarding these pH sensors systems in the market and that would include a high level of initial investments, high costs of setting up, design and fabrication as well as operational and maintenance costs (Amarnani, n.d.). Again, the Fluorochrome is entirely software-based and so, most of these concerns do not immediately apply to the company.

Relationship with customers:

Sales & Customer Services:

In terms of sales, the company will focus mainly on web sales, on a subscription basis whereby customers can choose from various pricing plans offered on Fluorochrome's website, mobile app, or customer service centres (i.e., 1 month, 6 months and 1 year). Customers will simply register and select the subscription plan that they prefer, and they will then have access to the Fluorochrome Software Package. Customers can also register their interest and the company can then provide them with information and software tailored/ set to their specific needs/ preferences. Furthermore, larger organisations, such as research companies or universities can also have the option of subscribing to the company's software services for a discounted price. Fluorochrome will also provide customer services (both digital and physical) for its customers where they can contact the company for any issues either via the website, app, hotline, and various social media(s) or by visiting the office's customer services department.

In addition, Fluorochrome's partners' customers can also use the Fluorochrome Software Package as an added benefit.

How Fluorochrome will communicate with its customers

The Fluorochrome Website

This will include:

- A clear overview of the company (company information, management, partners info, about us, recent news).
- Various pricing plans (i.e., 1 month, 6 months and 1 year).
- Events page which will include of information on free and paid-entry events (e.g., live events, seminars, training, live webinars, university pop-ups, trade shows and exhibitions).
- An expertise section consists of expert content, such as guides, brochures, technical documentation, videos, software tutorials, blog entries, and news (on topics such as sustainability, the company's current progress and future ideas, global presence, etc…).
- Customer service and contact us section consisting of suggestion and complaint boxes; sales and orders inquiries section; information on the company's social media platforms, email, hotline, and other forms of support services (e.g., online chat services and office customer service department whereabouts).
- FAQs section
- Legal and regulations section (terms of use, privacy, cookies, safety, quality and intended use, software license, warranty, accessibility, etc...).
- Digital community hub whereby users can interact with each other and discuss certain issues or academic issues relating to the usage of the company's software or software how to(s).

The Fluorochrome Mobile App:

It is essentially a mobile app version of the Fluorochrome website. This will give customers access to all the services mentioned above, but will be tailored for use on a smartphone, allowing for a more seamless user experience.

Fluorochrome's primary social media platforms:

- Twitter
- LinkedIn
- Facebook
- Instagram

These can act as a form of advertisement by allowing Fluorochrome to reach a wider audience. They can also serve as a valuable means of collecting customer feedback by monitoring tags and other online references to the Fluorochrome brand.

Marketing*:*

Fluorochrome will hire a marketing agency that will be responsible for the company's advertisement plan. By outsourcing advertising and marketing to a specialised agency, this will ensure that the campaigns are maximally effective in raising brand awareness in the most critical sectors and geographical locations. Furthermore, it means that the central Fluorochrome team can spend more time on enhancing the software platform with novel features, which will incentivise purchases and prevent replication and fraud.

Compliance (software quality control):

Fluorochrome will ensure verification and validation of its software package to guarantee that the Fluorochrome software package is up to standard and working as intended. This will be complemented by our partnerships with industry and academia, as outlined earlier.

Financial

Initial financial structure

As the central element of this project is a software, all initial funding is required to go towards development before a commercial profit can be made. Software of this complexity can take between 3 to 5 months to fully develop with a team of developers which can cost up to £50,000. This will include salaries for software engineers to develop the code and ensure no bugs are present in the software as well as the resources used during this development time.

Due to not being able to make commercial profit during this time, the project will rely on money offered by external grants and capital injections from investors. These grants can come from private organisations, such as Datalabs, or governmental grants from the NHS. The amount offered by these grants is variable but if attained will provide a significant boost to the start-up of this project.

The main investors for this project will be the groups we are aiming to partner with such as ImageJ and Imperial Collage London. These groups will provide bulk funding at the start of the project, and we will aim to keep them as partners throughout the lifespan of the project for their initial contribution. Their co-operation will also aid in providing legitimacy to the project, encouraging more companies to join and invest.

Self-sustaining model

Once the software has been created, a subscription model will be used to create a self-funding revenue stream. This revenue will be used to cover costs for two key areas of software management. Firstly, the update and maintenance of the software to ensure customers have access to the best available tools for their research and the ease of use of the product. This constant progression of adding more features and updating the interface through input from users provides incentive to stick with the subscription model to gain access to future features

Secondly, the commercial revenue will subsidise the creation and management of a customer relations department. The transition from alpha to beta testing will undoubtedly cause previously unseen difficulties or bugs to surface. This department will ensure that a good relationship is maintained with customers, which will be central to ensuring satisfaction and continuing resubscription. These two departments will constitute the main yearly cost of the company, estimated to be around £10,000 a year.

The main revenue stream while the product is commercially available will be its subscription model. This model will focus on the use of software licences that can be activated for certain periods of time. These time periods will range from 1 month, for short research projects, to annual passes for prolonged research. This model will allow researchers to be selective on their use of the product allowing them to allocate their funds accordingly. For multiple licences going to the same institution discounts can be offered to encourage the integration of the software into the academic community driving our presence in the field.

The subscription model provided expands opportunities to what we can offer and enhances interaction with our customers. For example, we can offer a premium subscription that can provide access to early builds of updates, this allows customers to try out new features and tools before anyone else. This will enhance their research while providing us with an invaluable resource to help troubleshoot problems that may be present and drumming up excitement within the industry over the next software update. We will offer premium tech support to these customers, preventing them from having to wait in queues and being given 24-hour support. Along with this, open community engagement will allow us to listen to what our customers are wanting from future releases, meaning that future updates are consistent with the desires of the customers. This model means the timeline to return on investment for this project is highly variable, but an estimate can be obtained by assuming an initial uptake of 200 annual licences, priced at £204, in sales with a growth each year of 5% (c.f. Figure 6).

Figure 6: Estimated chart of the timeline to return of investment showing full return after 4 years.

A permanent license will also be included as part of longer subscriptions. This licence will allow the customer to access older versions of the software without having to refresh their subscription. This version of the software will have less tools available but still provide the basic functionality promised by the package. This will allow the customer to continue using these basic tools if needed or their research, but they do not have the funding to continue a subscription. While not directly beneficial financially this decision allows us to maintain good customer relations and helps ensure innovative research and diagnostics can still be carried out without losing their basic tools halfway through. This also reduces the chance that customers would try and find alternative ways of attaining the software such as through pirating it which could greatly cut into our revenue stream.

Testing

Before beginning marketing of the program, internal alpha testing will be conducted. Subsequently, a demo version of our program will be sent to University of Glasgow for beta testing. This will be provided for free with the proviso that a small survey should be filled in to assist in product development.

Following the first round of alpha and beta testing, modifications can be made to obtain the first formal version of the program. We would like to retain the software ownership, so proprietary licenses will be added. This means our program is protected, preventing users from changing, copying, or redistributing the software. This will greatly benefit future sales.

Current Challenges

Despite the significant advantages of the Fluorochrome software relative to the market alternatives, there is scope for refinement in certain elements of the design. For example, the applicability of Fluorochrome is currently restricted to apparatus included in the FPBase directory. Certainly, this does not pose a barrier to gaining an initial foothold in the market, although, with expansion, we will ensure that the apparatus available in the FBPase directory is growing in synchrony with demand for our product.

Furthermore, the current comparison algorithm would run quite slowly, since it is adopting a ramp strategy of comparison (i.e., starting from the lowest pH and comparing all datapoints in all spectra until it finds a good match). This would be effective but could be easily accelerated using a successive approximation or sampling-based method.

Finally, the growth of Fluorochrome is initially limited by the progress of research in pH-Lemon; for example, the Fluorochrome software can only be used to quantify localised intracellular pH if methods are available to mediate POD ingress to the cells. This could certainly be done, for example by using functionalised liposomal carriers to mediate endocytosis; our partnerships with the Infectious Diseases Department at Imperial College London will ensure that suitable protocols are established by the time that the software is formally released. Additionally, as the software is expanded and refined, the reliance on a single technology will be diminished, increasing incentive to purchase, and providing robustness to the commercial venture.

Sustainability and Future Perspectives

Sustainability

Since Fluorochrome is an analytical approach based entirely on software, it is both highly scalable and easily modifiable. The cost per unit to the company is very low because, unlike with a physical product, there is no need to spend money on raw materials, distribution, etc. Instead, funds can be directed to activities that directly benefit the growth of the enterprise such as software maintenance and debugging, research and development, and marketing. This capacity to generate and distribute new products without significant changes to physical infrastructure also means that the company can scale in a highly environmentally sustainable way. This will be complemented by the structure of the software: all the default spectra, comparison algorithms and statistical tools will come built into the software. Granted, this will mean a slightly larger download size for the user, but this obviates the need for power-hungry datacentres that can support lots of users simultaneously. Additionally, this allows updates to be prepared and implemented without affecting the ability of the customers to use the software. This arrangement is substantially more convenient for the customers and means that they can download updates at times that suit them.

Research and development represents a critical element of the company's growth strategy but will also be one of the primary sources of waste. Although a degree of waste is unavoidable, substituting single-use apparatus for reusable alternatives where appropriate can dramatically reduce waste (Madhusoodanan, 2020). Additionally, because Fluorochrome does not require a large amount of different apparatus, environmentally conscious selection of equipment can help reduce energy expenditure. This can be complemented by a lean research approach based on discrete design sprints. Thus, the requisite reagents and tools can be ordered at the start of a sprint, rather than repeatedly ordering in batches, and generating increased waste.

Future Perspectives

Though the minimum viable product outlined earlier will prove an incredibly valuable research tool, the base structure of Fluorochrome presents many opportunities for enhancement. For example, one possible innovation will be the addition of a feature that can approximate rate of reaction. If there is a cellular reaction that generates an acidic or alkaline product, this will generate a localised change in pH. In the presence of pH-Lemon, this will generate a change in the emission spectrum. Since the Fluorochrome software can identify and quantify minute changes in pH, successive camera frames could be analysed in order to approximate the rate at which the pH is changing (i.e., the rate at which the concentration of protons is changing). This could, in turn, be used to estimate the rate of reaction.

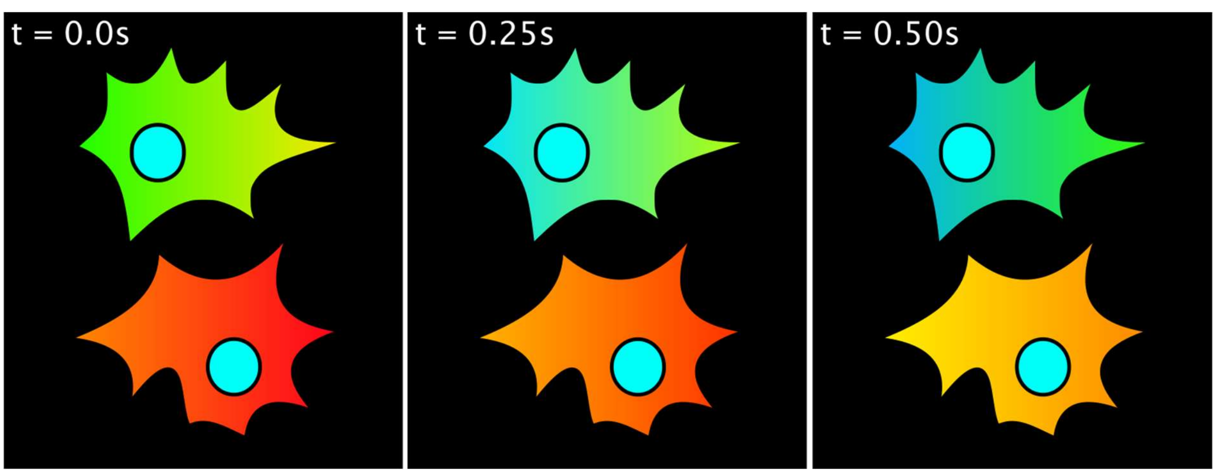


Figure 7: A schematic depiction to show how localised pH might vary as a particular chemical reaction progresses. Since pH is a function of the concentration of protons, the updated Fluorochrome software could approximate the rate of change of pH to then estimate the rate of reaction.

A similar logic can be extended to the study of diffusion: if there are two regions of varying pH on either side of a semi-permeable membrane, the flux of protons across the membrane could be derived from the localised pH change on either side (c.f. Figure 8).

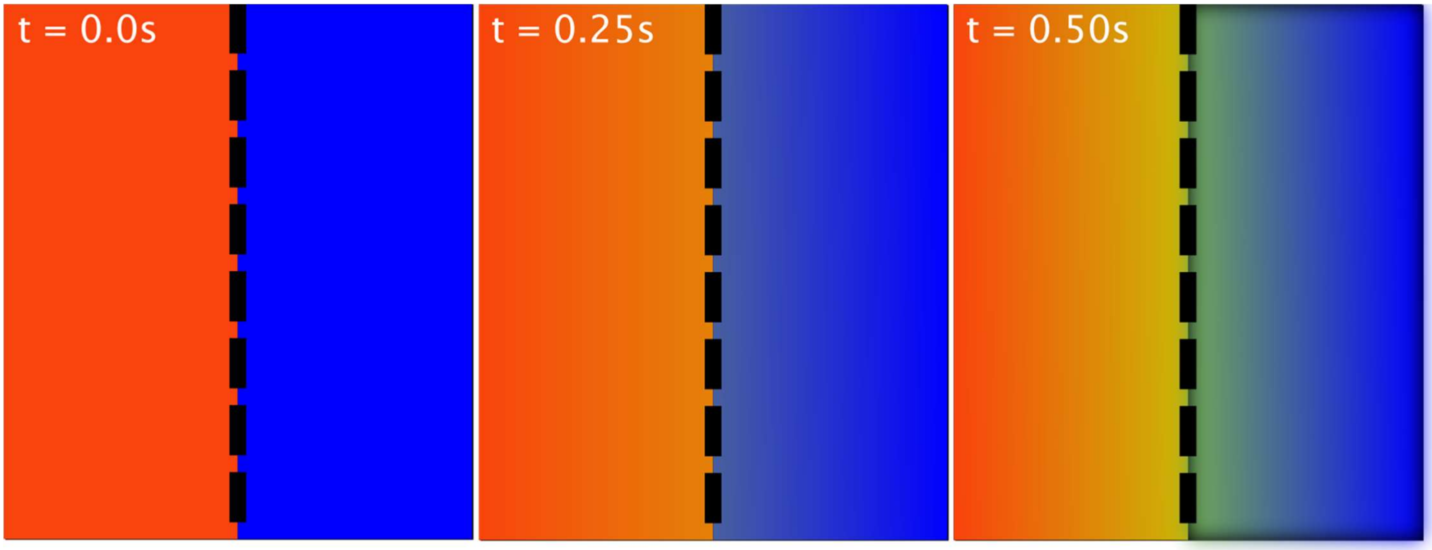


Figure 8: A schematic depiction to show how diffusion of protons could lead to a localised pH change. Thus, from the rate of pH change, the rate of flux of protons across the membrane could be estimated, enabling the diffusion constant to be calculated.

Thus, as these features are integrated into the Fluorochrome platform, they will increase the incentive for institutions to purchase licenses, and for existing customers to maintain their subscriptions. It also makes imitation significantly more challenging since an aspiring fraudster would have to continue updating their product to match Fluorochrome innovations. Finally, the versatility of the Fluorochrome platform will establish itself as one of its unique selling points, thus differentiating it in a competitive marketplace.

Conclusions

The technology behind Fluorochrome and our business model have great potential to completely revolutionise the field of pH measurements in biological processes. Our software-only approach allows us to offer an adaptable, accessible, and environmentally friendly product with a short and manufacturing-free supply chain, involving fewer financial risks.

Fluorochrome harnesses the power of open access databases, together with our unique algorithms, to provide remarkably accurate localised pH values within a biological process. Our subscription-based model will provide a stable source of income and develop into a self-sustaining model which will allow the company to grow and improve our services, as well as tapping into new markets and technological needs.

Through partnerships with relevant companies which will be able to use our technology as a plug-in, we can easily access large numbers of clients. Furthermore, through our dedicated customer engagement platforms, we will be able to offer bespoke customer support and obtain valuable feedback for improvement of our technology and additional features that may be desired.

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Appendix A: Approximation of Resolution

Firstly, one must mathematically estimate the wavelength composition of a given pixel colour: assuming that a unique pixel represents a normal distribution of wavelengths around a central maximum, this provides a starting point.

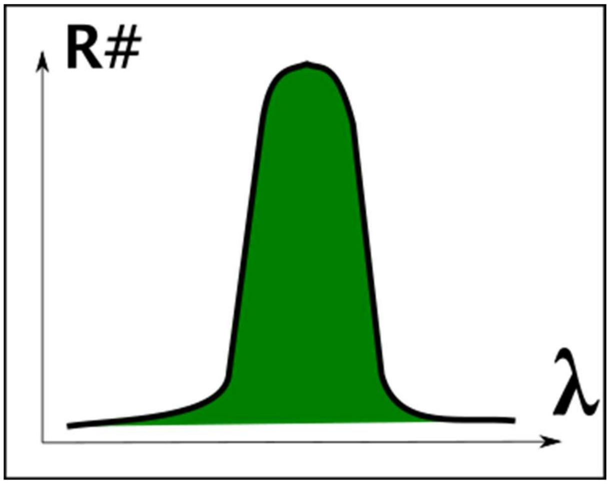

Figure 9: A schematic representation of the normal distribution of wavelengths represented by a single pixel. Here, R# represents the relative abundance of a particular wavelength.

There are generally considered to be seven pure spectral colours (Nassau, 2022), and they have wavelength ranges spanning approximately 50nm. Thus, 50nm can be viewed as the range over which the normal distribution has a non-zero value. A normal distribution can be considered to decay to zero outside three standard deviations of the mean (Thomopoulos, 2018). Hence, standard deviation of the wavelength distribution can be approximated to be 50/6 = 25/3 ≅8.33nm.

Let's assume that a researcher uses a small sample area of 50 pixels. A pH value should be computed with a minimum of 95% confidence to make it a useful measurement for scientific purposes. Thus, the resultant confidence interval can be expressed mathematically as

$$\mu \pm \frac{\sigma}{\sqrt{n}} * z$$

Equation 3: The formula for the calculation of a confidence interval assuming a normal distribution. Here, μ represents the mean, σ represents the standard deviation, n represents the sample size and z represents the z-value for a particular confidence level. Source: Rumsey, 2011.

Given that the confidence interval is 95%, the z-value is equal to 1.96. Thus, substituting the remaining constants, the interval becomes

$$\mu \pm 2.31nm$$

Assuming that the spectral range of pH-Lemon is from red to blue, that gives a range of 250nm (Nassau, 2022). If it is further assumed that pH varies smoothly with wavelength, then using the functional range provided in Burgstaller et al., 2019 (pH 4 to pH8), one can scale the two uncertainties. Thus, the pH resolution is obtained to be:

$$\frac{\pm 2.31 * 4}{250} = \pm 0.0369 \cong \pm 0.04$$

Of course, research is needed to confirm this estimate since a significant number of assumptions were made to obtain it. Nonetheless, this is a useful estimate of the best-case resolution that could be achieved.

Appendix B: Website mock-ups

The website mock-up was built using Bubble.io (Bubble.io, 2022).

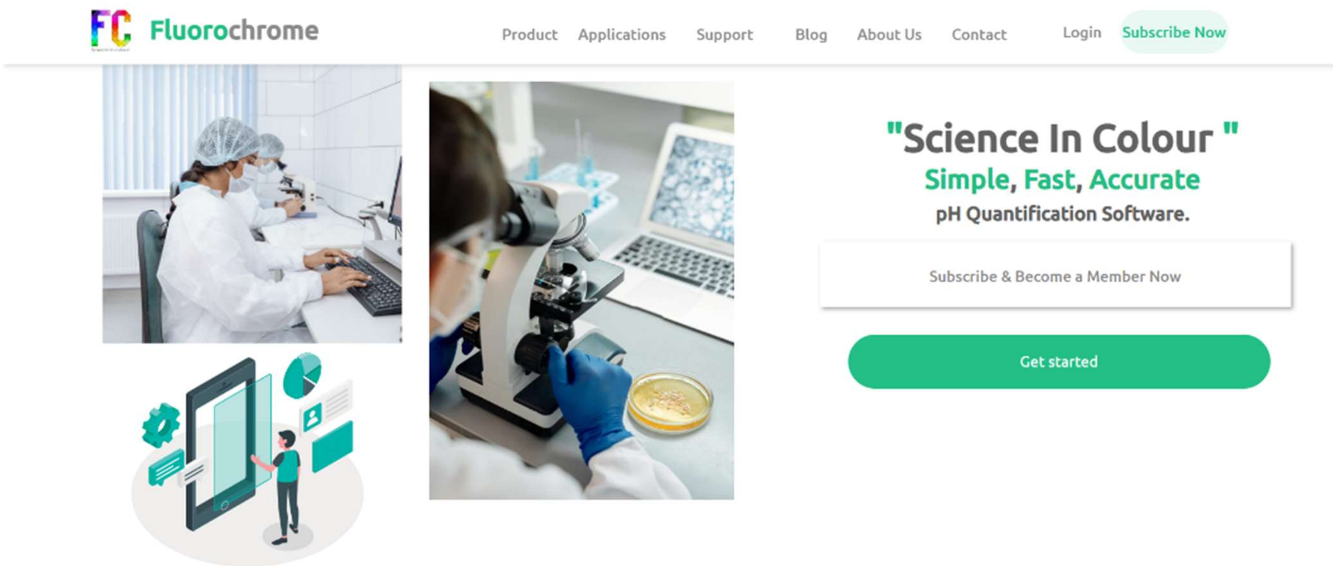

Figure 10: A mock-up of the website (overview of the website).

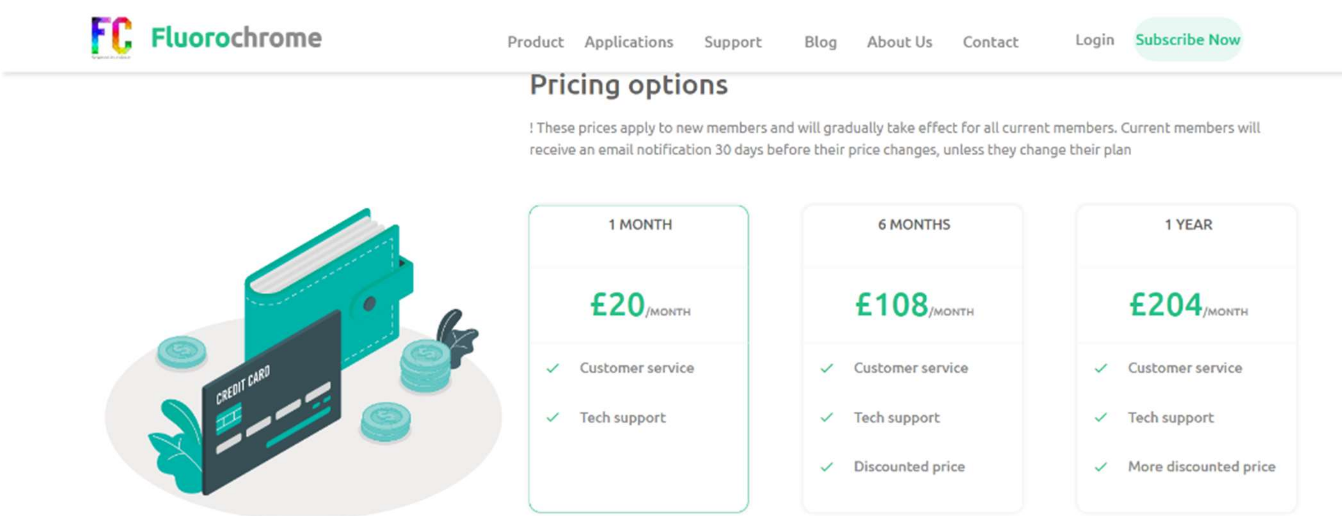

Figure 11: A further mock-up of the website (overview of the pricing options)