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METROLOGY NEEDS FOR ELECTROCHEMICAL AND ELECTRICAL BIOSENSORS: STAKEHOLDER ENGAGEMENT REPORT

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Metrology Needs for Electrochemical and Electrical Biosensors: Stakeholder Engagement Report

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ABSTRACT

A stakeholder engagement workshop was hosted by NPL with the aim of identifying and prioritising the UK's measurement and metrology needs on the theme of electrochemical and electrical biosensors. This report summarises the findings of the workshop with the intention of informing a potential programme of work by NPL in this area.

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1 INTRODUCTION

Biosensor technologies based on electrochemical and electrical signal detection are gaining widespread interest for various applications including point-of-care diagnostics in the healthcare sector, biomedical research, environmental sensing, and industrial process control. Technologies vary substantially in design and detection mechanism, and they exhibit different degrees of maturity depending on signal transduction approach.[1-4] Despite this diversity, many biosensor concepts share common technological and economic obstacles that hinder successful commercialisation. Given the wide range of potential measurement and metrology challenges associated with this research theme,[5, 6] NPL is exploring an initiative to establish a dedicated theme of work under the National Measurement System (NMS) programme. An important part of this is to identify the specific measurement and metrology needs in this area across the full product development lifecycle, in order to establish what role NPL might play.

Therefore, during 2021 and early 2022 NPL undertook a scoping exercise in order to:

(i) Identify potential stakeholders in the UK with a potential interest in this theme.
(ii) Gather information on the types of technology and application areas of most relevance to the UK.
(iii) Establish a preliminary picture of the current measurement and metrology challenges in this area.

Building on these initial consultation activities, a UK-wide stakeholder engagement event was held in March 2022 to consolidate and prioritise the identified measurement and metrology needs, with a view to informing future technical activities by NPL. The event comprised an online workshop attended by 31 participants from industry, academia, government and regulatory bodies, research and technology organisations, and networking groups (see Appendix, Section 9.1). Input from attendees was first provided via breakout discussion sessions, aided by NPL facilitators and guided by a series of focus discussion topics (see Appendix, Section 9.2). All registered participants were then asked to complete a short survey to collect statistical data on key interests, and, most importantly, to prioritise the measurement challenges identified.

A large volume of information was gathered throughout the workshop, reflecting the many challenges associated with electrochemical and electrical biosensors. This report provides a summary of the most important measurement and metrology themes identified and an analysis of the survey data generated.

2 MEASUREMENT AND METROLOGY CHALLENGES

2.1 ELECTRODES

Understanding the sensor electrode surface is a major technical challenge in electrochemical and electronic biosensors, and several aspects of this call for improved metrology.

At the bare sensor electrode level there is a need to accurately measure the electrode’s physical properties, most notably the surface area which, depending on the precise mode of signal transduction, can have a major impact on the device sensitivity. Biosensors operate by way of surface molecular binding events, so microscopic surface area is key, but macroscopic sample diffusion is also important. Hence, consideration should be given to the length scale at which the surface area is assessed. Surface area is a particular challenge when the sensor electrode comprises nanostructured materials deposited as a porous layer. Electrochemical methods such as cyclic voltammetry are commonly used in this context, but

depending on the nature of the electrode this may not be recommended, and best practice guidance on this is lacking. It is worth noting that the physical properties of the electrode may be altered considerably by surface treatment and biofunctionalisation procedures, so characterisation measurements should not be limited to the bare electrode alone.

The surface chemistry of bare electrodes is also important, since this governs the effectiveness of coupling reactions used for surface biofunctionalisation. A range of sensor electrode materials is used (gold and screen-printed carbon are common, whilst graphene-based electrodes are gaining interest), so the initial surface chemistry can vary considerably depending on the electrode fabrication process and any electrode pre-treatment procedures. Furthermore, this surface chemistry may change with exposure to air (e.g., due to metal oxidation or airborne organic contamination) so a better understanding of recommended storage conditions for bare electrodes (e.g., suitable non-contaminating packaging materials) is required, including detailed parametric studies. Surface analytical techniques can provide this information, but such measurements are often not practical or economical for technology developers.

Biofunctionalisation of the sensor electrode surface represents a major and ubiquitous challenge since reproducibility of the output measurement is governed by the density, spatial distribution, and binding site availability of bioreceptors, as well as the presence of surface contaminants, all of which may often be unknown. Hence there is a need not only to quantify the density of immobilised bioreceptors (with spatial resolution) but also to measure their molecular orientation and understand how surface coupling impacts biomolecule activity. One critical aspect is the condition under which surface characterisation is performed, as the presence of aqueous solution has a profound effect on the molecular arrangement and orientation of surface monolayers; so, *in situ* measurements are preferred to techniques performed in air or under vacuum. Furthermore, since biofunctionalisation is often a multistep process, non-destructive measurements that do not interfere with the surface coupling are highly desirable for identifying the source of poor yields or reproducibility issues. The above issues also affect biosensors based on other signal transduction mechanisms (e.g., optical methods), but the variety of electrode substrates employed for electrochemical and electrical sensors makes standardisation even more challenging. Increased availability of reference materials with a defined density of bioreceptors (and ideally a defined activity under a given set of conditions) may be useful in this context. In addition to bioreceptor immobilisation, other surface (bio)chemical modification steps may be used for passivation or anti-fouling purposes, so reliable characterisation of these processes is also required. Finally, as with the bare electrodes, understanding and guidance are needed on storage conditions, long-term stability, and calibration requirements of biomodified electrodes throughout their shelf-life.

Measuring the behaviour of the biofunctionalised electrode surface in the presence of the sample and during electrochemical/electrical measurement is also very challenging. Resolving non-specific binding from target–capture events is particularly difficult and a great deal of effort is spent trying to understand and minimise non-specific interactions. Improved methods are therefore sought for probing biomodified electrodes at the molecular level during use. Similarly, bioreceptors or other surface functionalities may detach from the electrode to an unknown extent during exposure to the sample, so short-term and accelerated surface degradation studies are required.

In addition to the sensor (working) electrode, a key component of many electrochemical and electrical biosensors is the reference electrode. This is most critical for sensors based on potentiometric detection, but reference electrode stability is in all cases an important factor to consider. Quasi-reference electrodes are often employed; these may provide a stable reference potential in idealised buffer solutions with well-characterised chemistry, but likely deviate considerably in the presence of real samples (e.g., blood, urine, saliva) wherein other

chemical species and biological matter can impact the reference potential. This is an area that would benefit from metrological studies and best practice guidance.

2.2 SAMPLE HANDLING & FLUIDICS

The handling and manipulation of biofluid samples is a potential source of uncertainty in all types of biosensor. In many cases, but particularly at the R&D stage of product development, manual micropipettes are used to measure sample volumes, which carries a risk of human error. Furthermore, micropipettes are typically calibrated using pure water, from which the rheological properties (density, viscosity, surface tension) of real samples can vary considerably, leading to dispensing volume errors. The challenge of human error is in principle circumvented by the introduction of on-board fluidics engineered within the biosensor device, but these are typically optimised to a narrow range of fluid parameters, so they are still a potential source of uncertainty or even device failure when samples with very different fluid properties are used. Hence there is a need to measure the flow characteristics of such devices to better understand the tolerances associated with fluid channel dimensions and surface properties. Modelling (computational fluid dynamics, CFD) may also be valuable in this context.

There is also the issue of sensor wetting, which again can be affected by the properties of the sample. This is most problematic in the case of porous electrodes, where sample–electrode contact area is unknown and can be dramatically affected by the biofunctionalisation process. This also affects the accuracy of electrochemical surface area measurements. Hence, the ability to measure the extent of surface wetting would be valuable.

2.3 REAGENTS

The quality of reagents used in the production and testing of biosensors is another major source of poor reproducibility. Often the reagents used to create immobilised bioreceptors (e.g., modified antibodies) are externally sourced and can vary between different suppliers in terms of activity and purity. This is particularly true for new and emerging biorecognition elements such as aptamers, which have potential advantages over more conventional antibodies but for which sources with sufficient quality control are not yet established. Therefore, standardisation is required to ensure that expectations of bioreagent quality are met.

Another issue associated with reagents is the need for reference samples in which a known concentration of analyte is dissolved in a real biological medium (or at least a medium that is more representative of a real biofluid sample compared to idealised buffer solutions). In the long-term this could lead to certified reference materials containing chemically traceable quantities of analytes alongside known contaminants.

2.4 INSTRUMENTATION AND DATA HANDLING

A common measurement issue is the development of purpose-built potentiostats and biosensor “reader” devices, often intended to miniaturise the complete measurement system for improved portability. In order to make these cost-effective, significant compromises must be made in performance compared to the research grade instrumentation with which many technologies are established as viable early in their development cycle. There is a need for best practice in assessing the performance of such measurement equipment and establishing uncertainty budgets so that appropriate compromises are made.

A related issue is the handling and analysis of generated data. Standardisation of data formats and analysis algorithms is lacking, and when a large amount of data is being

generated (e.g., as part of batch calibration), there is a role for advanced data interpretation methods such as machine learning and artificial intelligence to improve its value to product developers.

2.5 SCALE-UP AND MANUFACTURING

One of the biggest hurdles to the commercialisation of new technologies is scale-up and manufacturing, which typically introduce a trade-off between quality (e.g., performance, reproducibility) and cost. Key aspects of this optimisation are establishing tolerances in the context of materials and component specifications, understanding what compromises can be made in production with minimal impact on performance, and detailed failure analysis. These bring the need to measure (environment-dependent) physical and chemical properties of device components during development, such as those already noted above. Once specifications and tolerances have been established, there is a demand for rigorous quality control (QC), which can be particularly challenging because of the multicomponent nature of electrochemical and electrical biosensors. Furthermore, in a manufacturing setting, there is a need for QC methods that are non-destructive, conducted in real-time or inline where possible, and, in the case of electrode biofunctionalisation, implemented without interruption or interference with the surface coupling processes.

3 KEY MEASURANDS

The following summary lists the most critical parameters that need to be measured throughout the product development lifecycle of electrochemical and electrical biosensors, where improved accuracy or knowledge of uncertainty budgets would be beneficial.

- Electrical resistance of individual electrodes
- Impedance of electrochemical cell
- Electrode properties: surface area, porosity, diffusional roughness
- Quantity (area, density) of surface-immobilised bioreceptors
- Spatial distribution of electrode surface functionalities
- Position/arrangement/orientation of surface biomolecules
- Wettability (contact angle) of fluidic channel interior and electrode surfaces
- Channel and electrode dimensions
- Concentration of analyte and impurities in reference samples

4 FUTURE CHALLENGES

A number of key emerging areas for future consideration were identified as follows:

- Continuous monitoring and wearable technologies are becoming more widespread; this brings new measurement challenges including biocompatibility and long-term stability of devices. Currently, the market for such devices is focused on glucose sensing but this is expected to diversify into other biomarkers.
- Miniaturisation and multiplexing (e.g., via electrode arrays) continue to be major themes for electrochemical and electrical biosensors, placing increasing demands on measurements at small scales. Similarly, multimodal detection (i.e., biomarker detection whilst measuring/controlling other parameters such as temperature and pH) is gaining interest.
- Electrochemical impedance spectroscopy is finding increasing use in biosensing. This technique has increased sensitivity to various experimental factors (resulting in narrower manufacturing tolerances and more strict QC requirements) and a

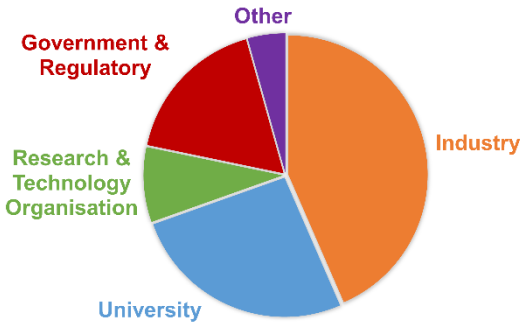
requirement for more complex data analysis. An emerging need for corresponding best practice guidance is anticipated.

- The emergence of new materials with complicated properties (e.g., nanomaterials, superfunctionality) and novel biorecognition elements (e.g., aptamers) are expected to introduce new measurement and characterisation requirements. Similarly, new biosensor applications, such as airborne pathogen detection and measurement of biological activity in complex environments (e.g., soil), are expected to generate new measurement and metrology requirements.

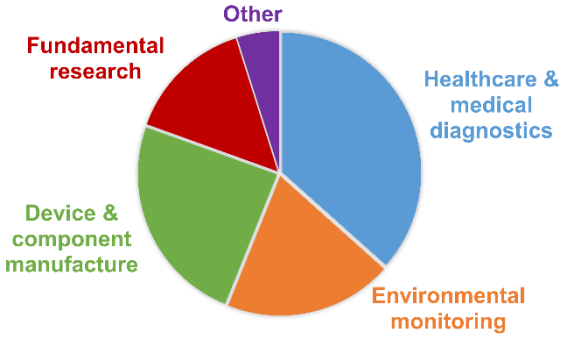
5 SURVEY RESULTS

An anonymous survey was run at the end of the workshop to gather information on the background and interests of stakeholders, and to prioritise the measurement and metrology challenges identified. A total of 20 individuals completed the survey, the majority of whom were participants in the workshop but some of whom were registered participants who were unable to attend. The following is a brief summary of the most relevant information generated by the survey.

(a) Organisation Type



(b) Applications of Interest



(c) Prioritisation of Needs

Rank	Metrology Challenge
1	Quantifying surface biofunctionalisation
2	Quality control in electrode/device manufacturing
3	Reference electrode reliability and stability
4	Effect of surface binding on bioreceptor activity
5	Availability of certified reference materials
6	Fluidic properties of devices
7	Non-specific binding and other media effects
8	Data curation and analysis
9	Non-destructive failure analysis
10	Mechanical properties of devices

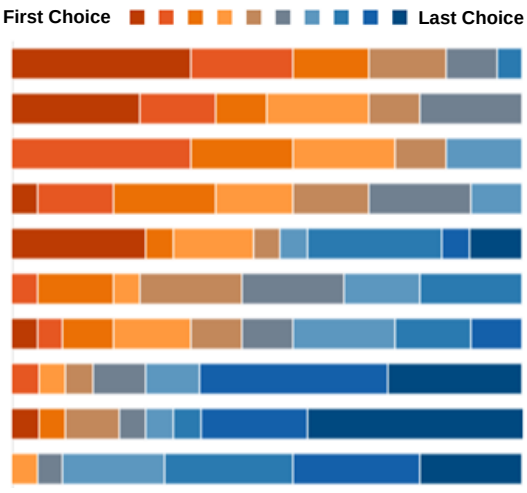


Figure 1. Summary of survey data showing breakdown of attendees by: (a) organisation type; (b) applications of interest; (c) ranking of measurement and metrology challenges. Total number of responses: 20.

Individuals from all identified institution types participated in the survey, with the greatest representation coming from industry, as shown in Figure 1a. Healthcare and medical diagnostics represented the most common application of interest, but device/component manufacturing was also of importance, and all other themes identified were shown to have some interest to the community (Figure 1b).

Survey participants were asked to rank a series of measurement challenges in order of importance. Some of the challenges were pre-determined based on the early consultation exercise while others were added on the basis of the workshop breakout discussions. The results, presented in Figure 1c, show that quantifying surface biofunctionalisation (Section 2.1) is the most important measurement challenge, followed by quality control in electrode manufacturing (Section 2.5).

6 CONCLUSIONS & RECOMMENDATIONS

The stakeholder engagement workshop helped to identify a wide range of measurement and metrology challenges associated with electrochemical and electrical biosensors. These challenges are spread across all stages of the product development lifecycle. Whilst technologies vary across industry and academia, a remarkable number of common needs were established. A dominant theme identified was the need for improved measurement tools and best practice guidance to characterise and better understand several aspects of the sensor electrode surface. The importance of this measurement challenge was reflected in the ranking of “Quantifying surface biofunctionalization” with the overall highest priority. QC methods are also clearly of importance to this community, at all product development stages but particularly during manufacturing. This not only applies to monitoring of the biofunctionalisation process, but also includes measuring and controlling the physical and chemical properties of substrate electrodes with improved confidence.

It is clear that a National Measurement Institute should adopt a role in addressing some of the common needs identified in the theme of electrochemical and electrical biosensors. Based on the stakeholder engagement activities undertaken, the recommendation is that NPL pursues a research programme on this topic, focusing its initial efforts on the sensor surface and QC challenges noted above as principal priorities.

7 ACKNOWLEDGEMENTS

This work was funded by the UK Department for Business, Energy and Industrial Strategy (BEIS). We wish to thank all of the individuals who participated in the workshop, and the Sensing Innovation Leadership Council (SILC) and the Knowledge Transfer Network (KTN) for support with promotion of the event.

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9 APPENDIX

9.1 ORGANISATIONS REPRESENTED AT WORKSHOP

Organisation	Type
Advanced Medical Imaging Ltd.	Industry
Aureum Diagnostics Ltd.	Industry
Biovici Ltd.	Industry
Delta-T Devices Ltd.	Industry
Depixus UK Ltd.	Industry
Imperial College London	Academia
Institute of Bio-Sensing Technology (IBST)	Academia
Knowledge Centre for Materials Chemistry	Network
Knowledge Transfer Network (KTN)	Network
LGC	Research & Technology
Malvern Panalytical Ltd.	Industry
Metrohm UK Ltd.	Industry
NPL	Research & Technology
Osler Diagnostics Ltd.	Industry
Paragraf Ltd.	Industry
Respire Diagnostics Ltd.	Industry
Sensor Innovation Leadership Council (SILC)	Network
University of Bath	Academia
University of Birmingham	Academia
University of Dundee	Academia
University of Swansea	Academia
University of Strathclyde	Academia
University of the West of England	Academia
Zimmer & Peacock Ltd.	Industry

9.2 BREAKOUT DISCUSSION TOPICS

i. What are the measurement challenges associated with current electrochemical and electrical biosensor technologies? This includes:
   - Each stage of the product development lifecycle (e.g., early R&D, prototyping, optimization, scale-up, manufacturing, product validation).
   - All aspects of the device and measurement process (e.g., electrode surface biofunctionalisation, sample preparation, fluidics, readout instrumentation, data analysis).

ii. What specific measured parameters associated with device development or manufacture would benefit from improved accuracy, or increased knowledge of measurement uncertainty?

iii. What emerging technologies and future research directions do you expect might need metrology support?