Project	Cross-centre and collaborative pilot research study to investigate quantitative assessment of blood perfusion in free flaps and grafts		
Project PIs	Dr Akhil Kallepalli (Leverhulme Early Career Fellow and Research Fellow, University of Glasgow) Akhil.Kallepalli@glasgow.ac.uk Mr Richard Pilkington (Consultant, Oral and Maxillofacial Surgeon, Cumberland Infirmary, Carlisle) r.pilkington@nhs.net		
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Summary

Our research set out to investigate quantitative approaches and methods for monitoring blood reperfusion in free flaps and grafts in postoperative care. The research was led by Dr Akhil Kallepalli (University of Glasgow) and Mr Richard Pilkington (Cumberland Infirmary, Carlisle), with contributions from research staff at the University of Glasgow. In building this collaborative venture, we have established connections with the Sunderland Royal Hospital that will extend beyond this project term. The research has attracted additional funding that helped hire research staff onto the project and establish collaborative ties with Prof Hadi Heidari and his group within the James Watt School of Engineering (University of Glasgow) for nanofabrication of microfluidic devices. The research initially attempted using beams with orbital angular momentum for sensing Doppler frequency shifts when light interacted with blood. To realise these in vitro, bespoke microfluidic devices were designed to serve as analogies to understand flow under a microscope (work supported primarily by EPSRC funding). Our future strategies will apply near-infrared imaging and use imaging fibres to measure the reperfusion of skin flaps and grafts from a stand-off distance. This will be done in collaboration with the Hospital, with support from the University of Strathclyde from April 2024.

Introduction

Skin flaps are used in reconstruction surgeries to repair tissue damaged by illness, surgery, or abnormalities. Healthy tissue, complete with vasculature, is transferred from one part of the body to the affected area. This procedure has a higher rate of failure in head and neck reconstruction. However, such failures can be avoided by careful monitoring and early detection of arterial or venous connection complications. The current protocol for this assessment is a qualitative chart and visual examination by touch, feel and capillary refill.

In this research project, we set out to establish a quantitative approach through tools that can be used by medical personnel to objectively measure the rate of blood perfusion, and as a result ascertain the health of the transplanted tissue. For this, we proposed to use light carrying angular momentum to measure the flow in tissue and tissue-like phantoms through the following objectives:

- Application of light carrying angular momentum to gather backscattered light from blood reperfusion in skin flaps and grafts.
- Quantify blood flow using imaging techniques in near-infrared and visible wavelength ranges.
- Testing and designing a prototype that can be used in the surgical theatre and postoperative care for monitoring tissue.

Optical Setup (Initial Experimental Strategy)

Key Outcome/Output: A beam with orbital angular momentum was successfully generated using an external cavity laser and a series of optical lenses. A bespoke holder for radially arranged collection optics was designed. Combined, the beam can be directed onto a sample/tissue and the backscatter can be recorded.

Despite the encountered challenges, the research realised the original methodology of generating beams with orbital angular momentum (OAM) using an external cavity laser (Figure 1). Optical fibres are set up in a custom holder suitable to collect optical signals backscattered from the sample/tissue from different angles (Figure 2). This design and hardware will continue to be used in future work. The external cavity helium-neon (HeNe) laser is misaligned (on purpose) and the Gaussian output is converted to a Hermite-Gaussian (HG) beam using a nanowire (Figure 1). Subsequently, the HG beam is converted to a Laguerre Gaussian (LG) beam with orbital angular momentum. This beam is propagated along the optical bench to be vertically incident, where it is incident on the sample of choosing.

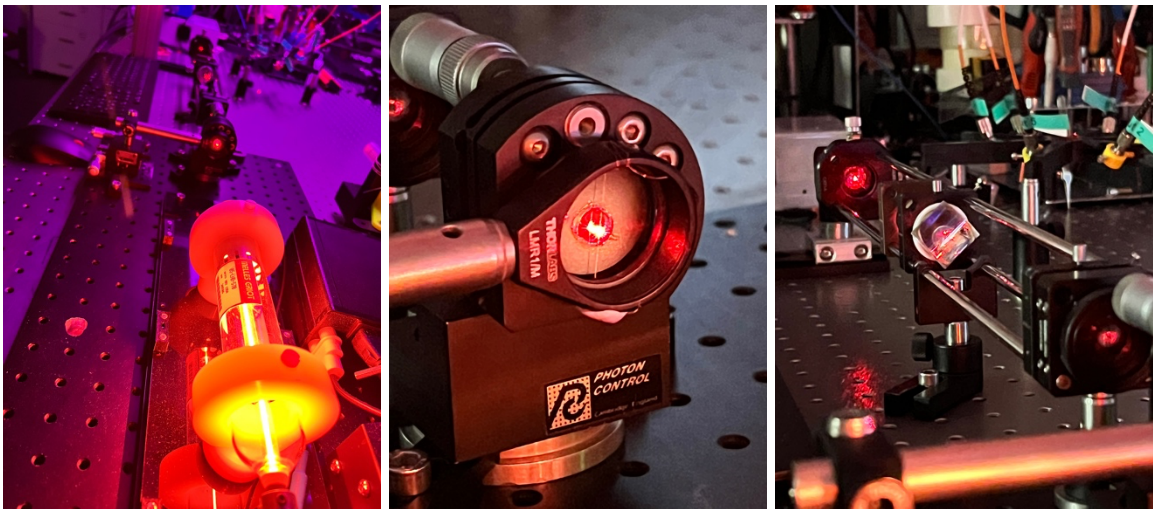

Figure 1 – An available misaligned external cavity helium-neon (HeNe) laser (left) and a nanowire (centre) were used to generate HG beams, that were subsequently converted to an LG beam (beam with orbital angular momentum) using a bespoke cylindrical lens (right).

Six optical fibres are mounted in a bespoke holder with adjustable collection angles for sensing and measuring the backscatter. The protocol includes shining a rotating beam on the sample and detecting a Doppler effect from the light-blood flow interaction.

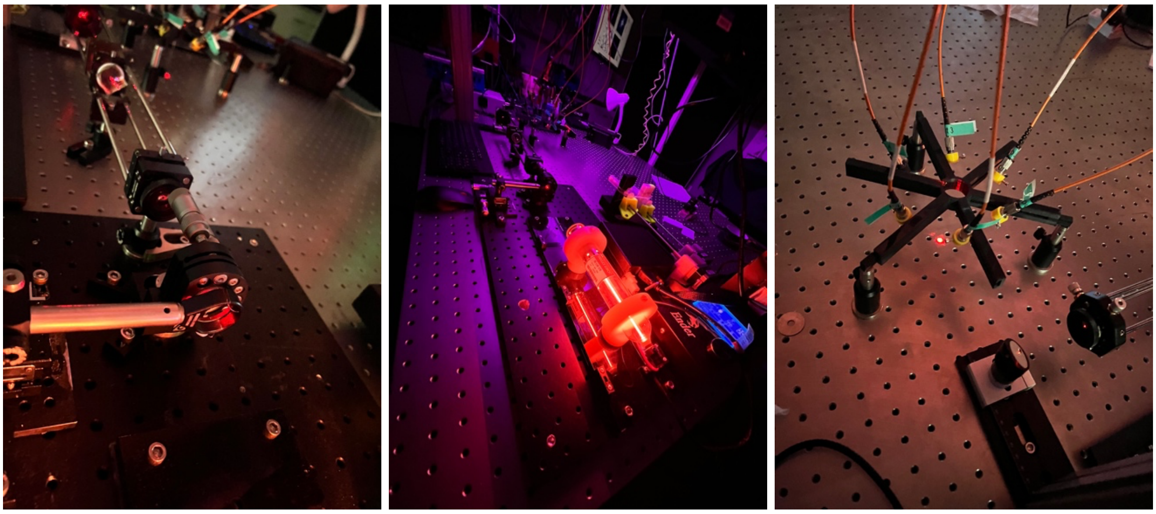

Figure 2 – The HeNe laser beam is directed using mirrors to eventually be vertically incident on the optical bench. The optical fibres in this initial design are arranged to collect backscatter from the sample's interaction with the OAM beam.

The key takeaway from this experimental strategy was the bespoke holder and the ability to integrate the optical collection fibres with detectors. However, the sensitivity of the optical signal and laser stability were not suitable for making significant inferences. Additionally, the lack of control of the OAM beam without a spatial light modulator was a significant disadvantage. This hardware design and approach is a positive outcome and will be further explored in future work. When experiments at the Hospital begin, we intend to use this holder to collect and measure backscatter signals from the reconstructed/transplanted tissue.

Microfluidic device fabrication (Initial Experimental Strategy)

Key Outcome/Output: A protocol for designing and fabricating microfluidic devices was completed. This included fabrication of a Chrome mask (Compugraphics) that will be used in the future to generate more vasculature-analogous devices for studies with tissue phantoms in vitro.

A key component of this research was to design and test methods of replicating blood flow in the laboratory without using biological tissue or samples. This need, primarily arising from the difficulty of access to surgical theatres and the necessary precautions in place, has motivated many researchers to look at tissue and/or optical phantoms. In our work, supported by BAOMS and funded by the EPSRC Impact Acceleration Account at the University of Glasgow, we set out to adopt and modify a methodology [1] for creating vasculature using nanofabrication techniques. The methodology was further developed to suit the specific need of vascular networks for imaging in this work.

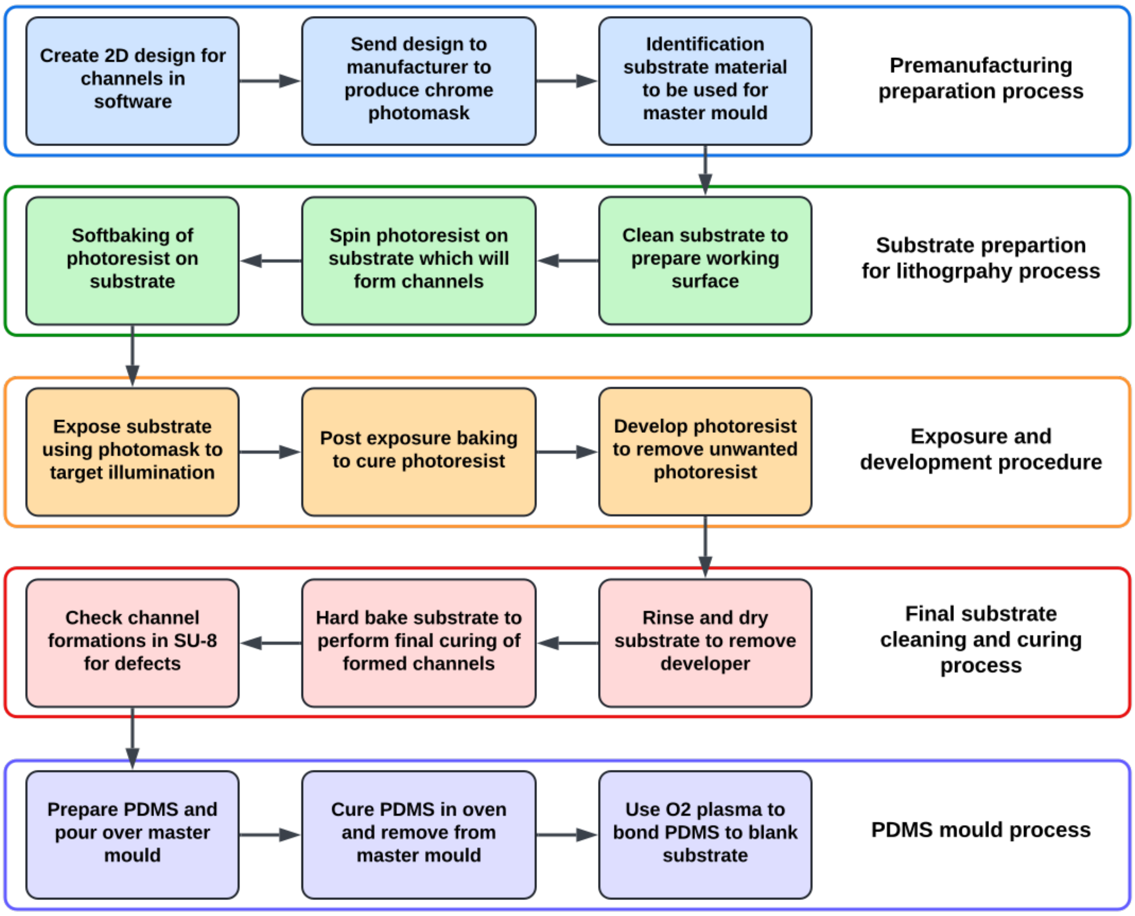

Figure 3 – Methodology of the microfluidic device preparation, as adopted from Fenech et al. (2019) and developed for this research project.

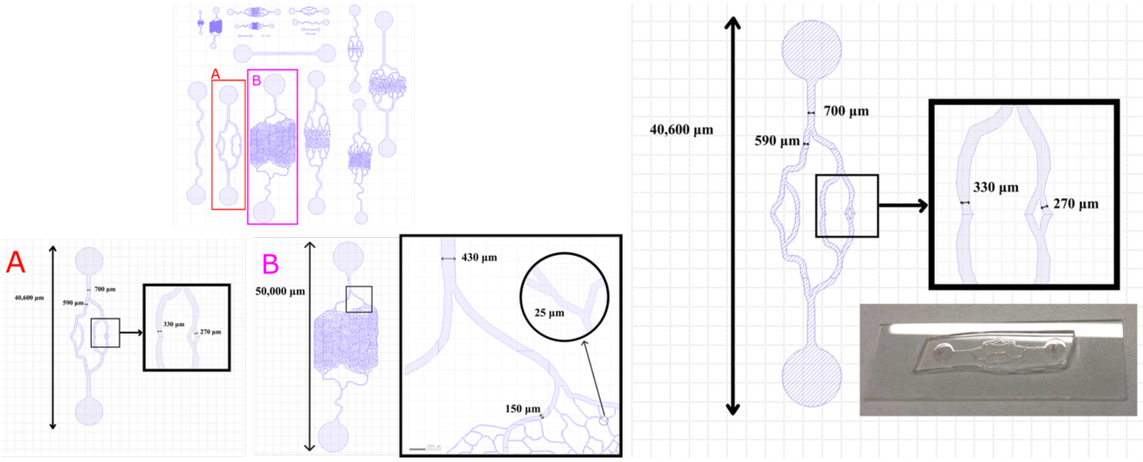

Figure 4 – The vasculature and networks used to design the mask that was subsequently used in the fabrication techniques are shown here.

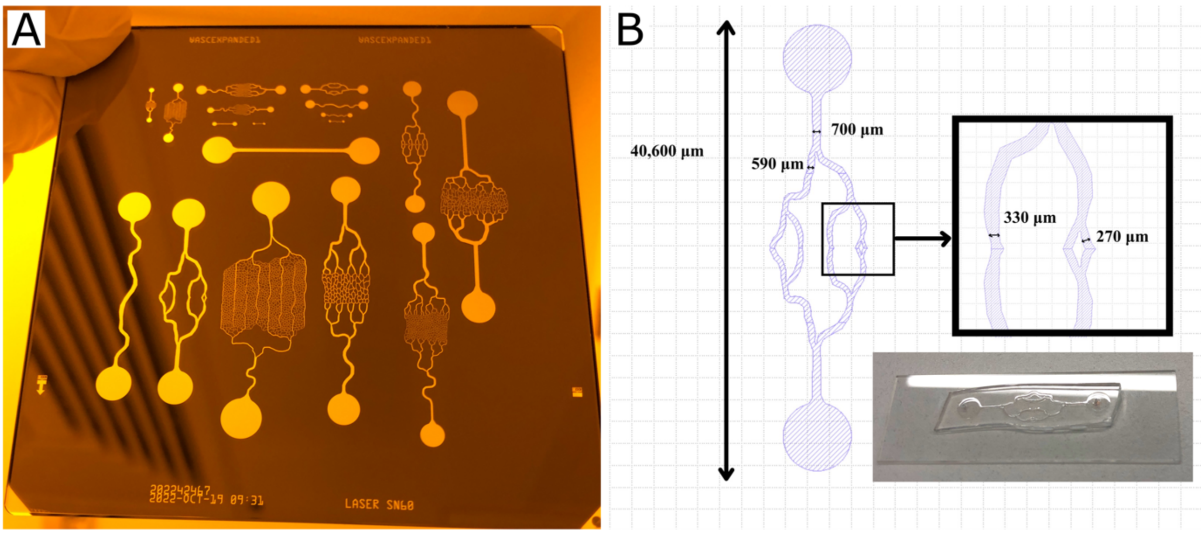

Figure 5 – The channels are first printed on a Chrome mask (A) before the nanofabrication process, as this mask is necessary (and allows repeated use) for realising the vascular networks in the final device (B, inset).

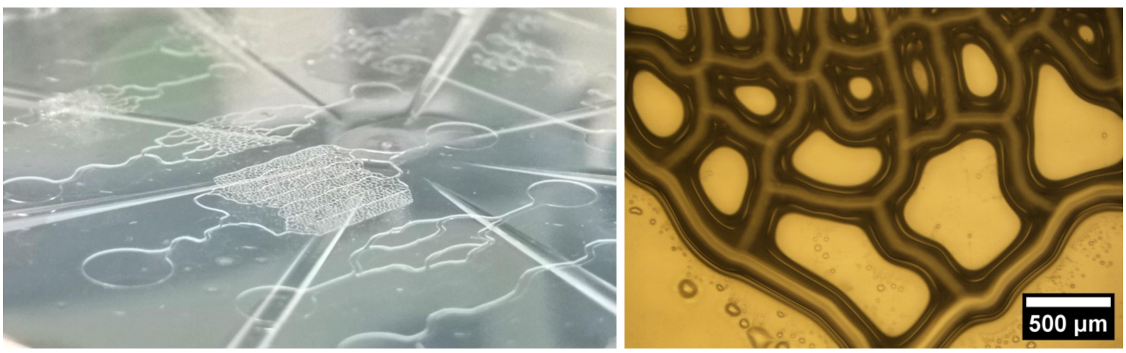

Figure 6 – The fabrication process results in a PDMS mould (left) which is subsequently mounted on a glass slide for imaging (Figure 8). A microscope image of the channels (right) illustrates the achieved detail and complexity of the channels.

Experimental Strategy and Output

Key Outcome/Output: The optical properties and responses in the shortwave infrared wavelength region were used to image and record flow. The holographic microscope provides invaluable information and inferences for *in vitro* methods. These methods will be combined with other outcomes of this research project to design devices that can function *in vivo.*

Much of the optical properties of tissue are known in the visible regions. However, very little is understood regarding the applications of shortwave infrared wavelengths and the information gained from phase and intensity measurements of flow. In this part of the study, we used the microfluidic device to reconstruct the intensity and phase when the liquid (analogues to blood) flows through the device, injected by a syringe. An inverted microscope interferometer is used to confirm if any insights can be gained in this wavelength range.

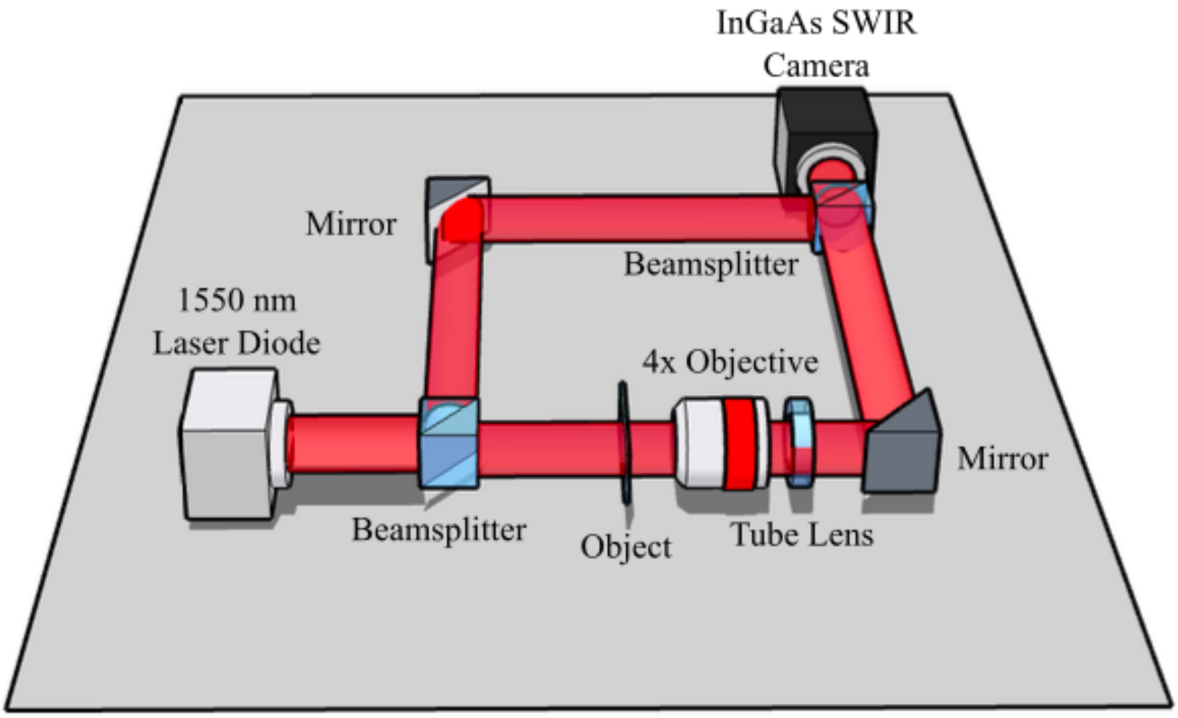

Figure 7 – The methodology uses an inverted wide-field microscope interferometer with reference and signal arms (the latter is where the sample/object is placed). The microscope is set up in a transmission configuration with a 4x microscope objective and 150 mm tube lens, with beamsplitters for splitting and recombining the light for holographic measurements. The methodology is modified from Wolley et al. 2023 [2] *and adopted for this study.*

The microfluidic device was placed in the field of view and injected with red liquid to simulate flow in the fabricated channels (Figure 5). The video sequence is captured to include scenarios where the device has no fluid in it when the device is partially filled and after the device has been filled with fluid.

Figure 8 – The microfluidic device is mounted in the microscope with the punched inlet and outlet allowing the sample to be pumped in and through the vasculature-depicting channels. The entire duration of when the liquid was flowing through the device was recorded as a video sequence and subsequently reconstructed for intensity and phase information (Figure 9).

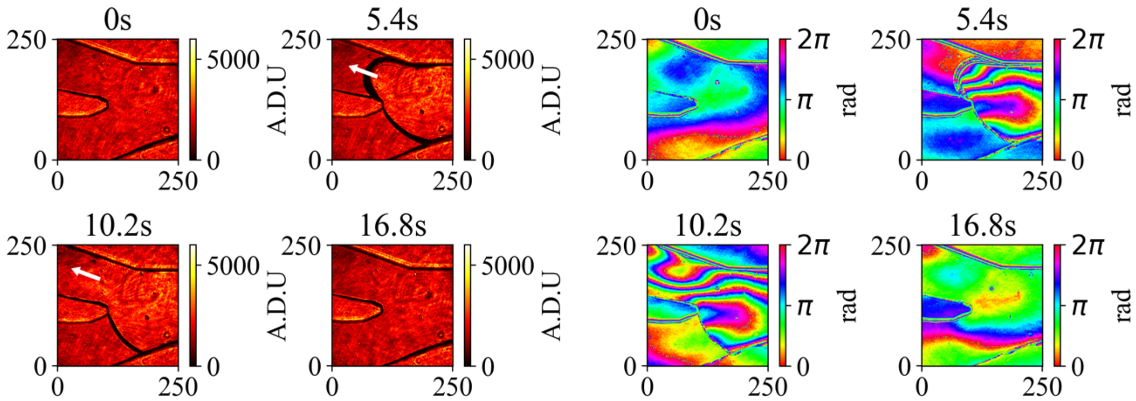

Figure 9 – The intensity (left) and phase (right) images are reconstructed, and snapshots are shown here to illustrate the flow. The outcome and inference drawn from the video sequences (shared along with this report separately) is that the intensity images allow imaging of the instance where liquid enters the field of view but once the channel is filled, no differences can be seen. However, in the phase reconstruction, the flow creates a constant change of phase as the liquid flows. This allows us to decipher direction, presence (or absence) of liquid sample and the rate of flow from the imaging results.

The results, illustrated in Figure 9, show the intensity and phase reconstruction. At 0 seconds, the images show an empty channel. In the intensity snapshots, the point where the fluid enters the channel within the field of view is clear (at 5.4 seconds). Subsequently, at 10.2 seconds, one channel is filled. Where the intensity image shows a solid channel, the phase reconstructions show variations that illustrate, visibly, the movement of liquid in the channel (refer to additional videos shared with this report).

Future Work

The future work of this project will include:

- Incorporating the near-infrared camera to image *in vivo*
- Adapter design and combining Fujikura imaging fibres with the near-infrared camera for postoperative care use; using the optical fibres will reduce the amount of hardware medical personnel have to physically carry. The optical fibres can be pointed at the affected tissue.
- Combining the above system with the outcomes of the first objective (detailed in Section Optical Setup (Initial Experimental Strategy)). In this setup, I also intend to add a liquid crystal spatial light modulator for better beam control.

Budget Summary

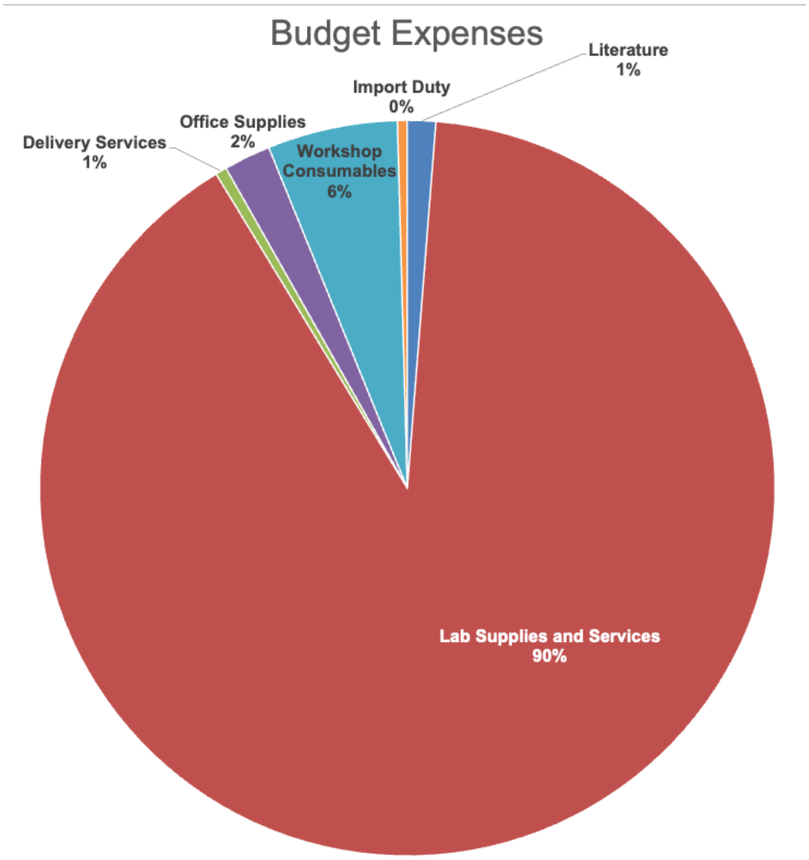

Figure 10 – The budget expenses are broadly illustrated here, with the detailed breakdown already submitted to the BAOMS Office by the University of Glasgow Finance Department.

Completed Objectives

- Initial equipment acquired (after significant COVID-related delays).
- Simulation work completed and published (BJOMS, 2022 [3]), and presented at Photon 2022 and Optica Biophotonics Congress (2023) [4].
- The microfluidic device was developed (with funding and research assistant time paid through EPSRC funding (£4930.77) and a stand-off heart rate measurement prototype developed in a two-semester undergraduate project.
- The need for better light sources in this project inspired and contributed (minor contribution) to the HardwareX paper published in 2022 [5], [6], completing work started with substantial past QuantIC (Dr Graham Gibson) and EPSRC funding.

- Delayed equipment was not used in the initial stages, but the near-infrared camera and optical fibres will be used in future projects with appropriate dedication to BAOMS funding and draft submission to BJOMS, where relevant.
- The initial prototype and project methodology did not yield the expected results as the funding only allowed bespoke and low-cost in-house experimental design components. Future work will include advanced technologies such as spatial light modulators and lasers as these are necessary for initial prototyping but beyond the scope and costs of the BAOMS funding.

Delayed/Postponed Objectives

Supply Chain Delays and Orders

- The BeamLab 6-month license was not purchased due to costs and VAT increases (because of inflation) between the initial approval of funding and the eventual contract agreement with the University of Glasgow.
- TracePro temporary license was used for simulation work.
- Raspberry Pi devices remained unavailable for much of the project term (including the extension in 2022) due to global supply chain issues. These were vital for delivering a prototype, without which miniaturisation and control were not possible.

Honorary Research Contract with South Tyneside and Sunderland Trust

- Delayed by 8+ months.
- Completed eventually but the first visit is yet to occur.
- As of 23rd February, the visit is scheduled for 17th April 2024.

Camera and optical fibre purchase delays:

- The near-infrared camera (Thorlabs) was significantly delayed by the supplier and imaging fibres (Fujikura, Japan) were not delivered until the final months of the project; both affecting the project planning and delivery. However, these will be used in future experiments with due credit to BAOMS funding.

References

[1] M. Fenech, V. Girod, V. Claveria, S. Meance, M. Abkarian, and B. Charlot, “Microfluidic blood vasculature replicas using backside lithography,” *Lab Chip*, vol. 19, no. 12, pp. 2096–2106, Jun. 2019, doi: 10.1039/C9LC00254E.

[2] O. Wolley *et al.*, “Near single-photon imaging in the shortwave infrared using homodyne detection,” *Proc Natl Acad Sci U S A*, vol. 120, no. 10, p. e2216678120, Mar. 2023, doi: 10.1073/PNAS.2216678120.

[3] M. Main, R. J. J. Pilkington, G. M. Gibson, and A. Kallepalli, “Simulated assessment of light transport through ischaemic skin flaps,” *British Journal of Oral and Maxillofacial Surgery*, vol. 60, no. 7, pp. 969–973, Sep. 2022, doi: 10.1016/J.BJOMS.2022.03.004.

[4] M. Main and A. Kallepalli, “Towards point-of-care diagnostics and monitoring of hypertensive episodes (A Monte Carlo approach),” in *Biophotonics Congress: Optics in the Life Sciences 2023 (OMA, NTM, BODA, OMP, BRAIN) (2023), paper DW3A.6*, Vancouver, Canada: Optica Publishing Group, Apr. 2023, p. DW3A.6. doi: 10.1364/BODA.2023.DW3A.6.

[5] G. M. Gibson, R. Archibald, M. Main, and A. Kallepalli, “Modular Light Sources for Microscopy and Beyond (ModLight),” *ArXiv*, Jun. 2022, doi: 10.1002/micro.120.

[6] G. M. Gibson, R. Archibald, M. Main, and A. Kallepalli, “Modular light sources for microscopy and beyond (ModLight),” *HardwareX*, vol. 13, p. e00385, Mar. 2023, doi: 10.1016/J.OHX.2022.E00385.

Publications

Title	Journal / Conference	% BAOMS Contribution	Link
Simulated assessment of light transport through ischaemic skin flaps, 2022	BJOMS	100%	Hyperlink
Assessing variable degrees of blood perfusion in ischaemic skin flaps and grafts, 2022	Photon 2022 (Conference)		Hyperlink
Modular light sources for microscopy and beyond (ModLight), 2022	HardwareX	5%; research primarily supported by EPSRC QuantIC hub funding	Hyperlink
	arXiv		Hyperlink
Towards point-of-care diagnostics and monitoring of hypertensive episodes (A Monte Carlo approach), 2023	Optica Biophotonics Congress: Optics in the Life Sciences (Conference)	10%; research assistant salary and time funded by EPSRC Impact Acceleration grant (University of Glasgow) and EPSRC QuantIC hub funding	Hyperlink
A reperfusion monitoring strategy at 1550 nm combining microfluidics and digital holographic microscopy, 2024	Biomedical Optics Express (Under Review)	5%; research assistant salary and time funded by EPSRC Impact Acceleration grant (University of Glasgow) and EPSRC QuantIC hub funding	
Optical analogies for skin flaps and grafts: A review of optical phantoms and microfluidic devices applicable to oral and maxillofacial surgery	BJOMS (Future Submission, Editor approved)	-	-
Optical phantoms and analogies for blood flow experimental protocols	BJOMS (Future Submission)	-	-
Comment on “A reperfusion monitoring strategy at 1550 nm combining microfluidics and digital holographic microscopy”	BJOMS (Future Submission)	-	-
Rapid in vivo measurement of blood flow in skin from a stand-off distance	BJOMS (Future Submission)	-	-