Use of non-invasive optical analysis in Neurosurgery – A pilot study

Study Protocol 30/12/2019, Version 2.0

MAIN SPONSOR: Imperial College London

FUNDERS: Brain Tumour Research

STUDY COORDINATION CENTRE: Charing Cross Hospital – Imperial College NHS Trust

IRAS Project ID: 258210

REC reference:

Chief Investigator: Mr Kevin O'Neill, Dan Elson

Co-investigators:

Mr Giulio Anichini – Study coordinator, also responsible for patients' recruitment, intra-operative data acquisition, data analysis, writing and submission of the research.

Mr Neekhil Patel – Responsible for patients' recruitment, intra-operative data acquisition, and data analysis

Mr David Peterson - Responsible for patients' recruitment and intra-operative data acquisition

Professor Dan Elson, Dr Iani Gayo, Dr Ioannis Gkouzionis, Dr Maria Leiloglou, Dr Vadzim Chalau, Dr Elizabeth Noble – Responsible for data processing and analysis

Mr Babar Vaqas – Responsible for data analysis, writing and submission review

Statistician: members of ICL / Hamlyn Center – to be defined

Study Coordination Centre

For general queries, supply of study documentation, and collection of data, please contact:

Study Coordinator: Mr Giulio Anichini

Address: Charing Cross Hospital, 3rd Floor South Corridor, Fulham Palace Rd, Hammersmith, London W6 8RF

Registration: GMC 7422941

Tel: **020 3311 7678**

E-mail: g.anichini@imperial.ac.uk, g.anichini@nhs.net

CLINICAL QUERIES

Clinical queries should be directed to Mr Giulio Anichini, who will direct the query to the appropriate person

SPONSOR

Imperial College of London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office

Imperial College London and Imperial College Healthcare NHS Trust

Room 215, Level 2, Medical School Building

Norfolk Place

London, W2 1PG

Tel: **0207 594 9459/ 0207 594 1862**

http://www3.imperial.ac.uk/clinicalresearchgovernanceoffice

Funder: Brain Tumour Research

This protocol describes the "Use of non-invasive optical analysis in Neurosurgery – a Pilot study" and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator. The study will adhere to GCP and the UK Policy Frame Work for Health and Social Care Research.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

INDEX

1. INTRODUCTION

1.1. BACKGROUND

Surgical resection of brain tumours is challenging from both an anatomical and functional perspective. The goal of the neurosurgeons is to remove as much tumour tissue as possible, while preserving surrounding normal brain. This often remains a utopic goal. Brain tissue around the tumour is in fact quite rarely completely normal, as it is invaded by tumour cells, and the tumour margins are not clearly defined, but fade into normal tissue. In order to overcome this problem, current trend in neurosurgery is to push the margins of resection beyond the obvious tumour boundaries and try to resect also those areas of the brain that the surgeon suspects have been invaded by tumour cells. However, this choice also poses some problematic challenges. The main obstacle to a generous resection is the presence of the so-called "eloquent areas" of the brain, that is, regions of the brain not amenable to be resected otherwise the patient will unequivocally develop a permanent disability (e.g. paralysis / mutism / visual impairment / etc.). Therefore, the concept of "functional margins" of resection has been popularized in neurosurgery: tumour resection is carried out up to the point where an eloquent area of the brain is reached. The resection is stopped at that point in order to spare that specific functional area.

Identifying and preserving eloquent brain areas is possible thanks to technological advancements in the field of neurophysiology. Neurophysiological monitoring techniques include a series of continuous tests performed during surgery, either with the patient fully sedated, or conscious during surgery (in this case the procedure is performed under local anaesthesia). Although feasible, this solution is not overly practical nor completely reliable. Experienced and extensively trained anaesthetic, surgical and neurophysiological teams are an indispensable requirement. Even so, false positives or false negatives can occur, reducing the extent of surgical resection in the first case, or increasing the likelihood of a post-operative impairment in the second.

1.2. RATIONALE FOR THE CURRENT STUDY

With the present research project, we are aiming to test a technology that could potentially improve the extent of resection of brain tumours. The main goal is to develop an instrument capable of better identifying differences between brain tumours margins and normal brain tissue, and differences between functionally active areas of the brain ("eloquent areas") and non-eloquent areas, thus allowing the surgeon sparing them while performing an aggressive surgical resection on the tumour tissue.

From a theoretical standpoint, one way to distinguish tumour tissue from the surrounding brain is to study the micro-architecture of blood vessels, the pattern of blood perfusion, and the levels of oxygenation / deoxygenation. A subset of radiological and molecular studies has focused on this point [**1-2**]. Moreover, functional Magnetic Resonance Imaging (fMRI) is based on a similar concept [**3**], as it analyses the differences in oxyhaemoglobin and deoxyhaemoglobin in specific brain areas during the execution of a task. The intra-operative analysis of differences in blood perfusion and micro-architecture is a relatively poorly explored field so far. Most of the studies are pre-clinical and have been performed on animals or animal models [**4-7**], and are mostly focused on different body districts.

The hardware behind multi-spectral analysis consists in a dedicated camera analysing the reflected light from a tissue, and dividing it into smaller wavelengths. Specific tissue tends to reflect different wavelengths; therefore, the spectral analysis can potentially detect subtle differences in tissue composition. Multispectral analysis has already been used in lung, colon, skin, and head and neck surgery in pre-clinical and clinical studies [7-13]. Preliminary results show different blood volumes and oxygenation rates in different tissues, meaning that the technology can be used to study discrepancies in appearance of tumour tissue as opposite to normal mucosa [7][9] [11-12]. The main advantages of this technology are that: it is non-invasive, costs are low, the machinery is not particularly bulky thus the hindrance in theatre is limited, and the acquisition time is short.

2. STUDY OBJECTIVES

The present study aims to investigate the potential application of multispectral analysis, hyperspectral imaging, and fluorescence during neurosurgical procedures, specifically during brain tumour resection. The research procedure consists of images acquisition and data processing, with virtually no additional invasive procedures to be performed on patients.

Study aims:

1) detect structural differences between brain tumour tissue and surrounding brain;
2) detect structural differences between eloquent/functional and non-eloquent/non-functional brain areas;
3) test wheter the combination of multispectral/hyperspectral analysis and fluorescence could provide additional information about the structural differences of brain tumours VS normal brain tissue.

3. STUDY DESIGN

Type of study: pilot study - prospective cohort study

Estimated total study duration: 3 years

Planned study sites: Imperial College of London and Imperial College NHS Trust

Sample size: 50 patients

3.1. STUDY OUTCOME MEASURES

Primary outcome measures:

The primary outcome measure will be the analysis of spectroscopic signal reading of:

- brain tissue and tumour tissue;
- functional brain areas and non-functional brain areas.

In order to analyse differences between brain tissue and tumour tissue, the signals collected will be correlated both to the visual signal seen on normal operative field and the signal seen on the peri-operative imaging (MRI scan).

In order to analyse differences between eloquent and non-eloquent brain areas, the signal collected will be correlated with the neuro-physiological intra-operative findings, in every case there is an indication to do so, and with the expected location of the eloquent areas on the peri-operative images.

Secondary outcome measures:

The secondary outcome measure will be the analysis of spectroscopic signal reading of surgical field as seen at its baseline and under fluorescence-specific light.

4. PARTICIPANT ENTRY

The present project consists of a pilot observational study on patients diagnosed with brain tumours candidate for a neurosurgical operation.

Patients will be recruited following the inclusion criteria: any patient with a diagnosis of brain tumour, age ranging from 18 with no upper limit, who will agree to the operation and to take part of the present study, will be enrolled.

The present study will include:

• In vivo microscopic and/or endoscopic multispectral/hyperspectral analysis;

• In vivo microscopic and/or endoscopic fluorescence (using either infrared light - IR - or ultraviolet light, depending on the clinical indication of the specific case);

• In vivo microscopic and/or endoscopic fluorescence combined with multispectral/hyperspectral analysis.

Enrolment will proceed for 3 years, with the possibility of stopping the research at an earlier date if a sufficient amount of data is collected for a reliable statistical analysis. From a practical point of view, participation in the study will only imply that some images will be acquired during surgery and processed at a later stage. The study won't impact on patients' care at any stage, nor will produce results that will be relevant for future medical records of patients enrolled. Patients will be approached about this study at the time of their first neuro-oncology clinic consultation – this might not necessarily be the clinic where the diagnosis will be given for the first time, as these patients are often referred from other centres where the diagnosis has already been communicated. A member of the research team who is also involved in direct care will be present at the time of the consultation and will explain in details what are the purposes and the methods of the present study. The patient will be given a PIS at this point.

Consent will be taken by a member of the research team. Consent will be sought at preoperative assessment or in a private room in the admissions lounge, in the clinic, or in the hospital ward, in CXH. Consultation length can vary depending on the complexity of the clinical procedure planned. Surgery is usually scheduled at least a few days after the clinic consultation, in most cases a few weeks after, in a minority of cases some months after. If the patient agrees to take part in the study, the patient will sign the consent form for research within the day before the surgery, thus allowing sufficient time for questions and autonomous research about the topic.

4.1. MATERIALS AND METHODS

A Liquid Crystal Tunable Filter (LCTF) camera will be used for the purpose of the present study. The camera has been assembled by Imperial College of London and will be connected to the operative microscope and endoscope. The whole system (microscope + attached camera) will be draped using sterile drapes as per standard procedure in neurosurgery. The camera itself is in turn connected also to a dedicated computer with dedicated software for

images acquisition. The instrument has been previously used in other studies performed by some of the research team members [12-13].

Patients who have agreed to take part in the present study will be admitted in Charing Cross Hospital, Neurosurgery Department, 11th floor, south corridor. The day before the surgery, as standard NHS practice for these procedures. Except for further discussion on the details of the study and signing the consent form for research, there won't be any additional test required for the patient other than those for standard NHS clinical care. The day of the surgery, the procedure will be carried out as expected per clinical indication. Surgery will be performed in Charing Cross Hospital, 14th floor, Theatre area. The caring surgeons will also be members of the research team.

The patient will be anesthetized and be placed on the surgical table. WHO checklist will be performed and the operating procedure will be carried out as standard NHS practice. During surgery, the operating surgeon will be using standard NHS neurosurgical equipment such as an endoscope and/or a microscope. From the surgeon's perspective, this equipment is operated in exactly the same way as with any other procedure. The only technical modification would be that either the microscope or the endoscope in use will be connected to the system of camera and filters for multispectral/hyperspectral analysis. Use of this imaging acquisition technique will not change standard operative practice, nor will it interfere with the principles of neurological surgery. During each surgical intervention, tissue-specific spectral data will be collected at specific stages - mostly once the brain surface is exposed and at the end of the resection on the surgical cavity. It is important that there is a method of comparing the spectral data generated by multispectral imaging to the nature of the tissue being dissected. In order to do this, we plan to visually record the operation in order to sync visual data with the spectral data obtained at the same moment in time. The video recording will not be patient identifiable and will be viewed only by members of the research team working on this project (see below). The use of video recording equipment will be included in the patient information sheet given to all patients prior to gaining consent.

Pseudo-anonymized clinical data will also be collected on College computers to be matched with intra-operative data. Specifically, the researchers will collect:

- clinical data such as diagnosis, age, sex, clinical status, medical background – this data collection will be performed to ensure that our population is homogeneous according to common statistical parametric tests; moreover, some clinical information (such as the neurological baseline of the patient) will be crucial in order to quantify exactly the degree of reliability of the multispectral signal for eloquent areas detection;
- radiological and histological data – this data collection will be performed for the purpose of the correlation between the multispectral signal and the radiological and/or histological findings;

Surgery will be conducted following standard procedures, already validated in clinical practice. The surgeons performing the procedures will be Mr Kevin O'Neill (CI), Mr Giulio Anichini (PhD student and clinical fellow), Mr David Peterson (Consultant Neurosurgeon), and Mr Neekhil Patel (Neurosurgery Trainee and Clinical Lecturer). Surgeons will work in teams, each team with no more than two surgeons. Prof Daniel Elson and one of his collaborators will be present in theatre to facilitate images acquisition. For each researcher not normally involved in clinical practice, research passport will be provided. Intra-operative imaging data and spectra will be collected during tumour resection, but the analysis on the spectra will be performed on a different time and setting, in order not to excessively prolong the surgical procedure.

Researchers will collect spectral data up until the statistical model used for multivariate analysis does not longer improve the degree of accuracy (see Section 6 - statistical analysis).

4.2. CONFIDENTIALITY

Each patient will be given a specific protocol number – ID log. Anonymous video recording and/or images acquisition will be carried out by the team members in order to sync spectral data collected with the surgical procedure and will be linked with the ID log. These data will be hosted in secured Imperial College Computers, and College Server, accessible only by members of the research team, and under password protection.

Regarding clinical data, these will be stored on NHS Trust Encrypted computers in a semi-anonymized form and transferred on College Computers in a pseudo-anonymized form if needed for study purposes. NHS computers follow NHS Trust confidentiality policy and comply with the Data Protection Act. Semi-anonymization means that these data will include NHS number, Hospital Number, age, protocol number. If for study purposes, the team will need to transfer these data to College Computers, pseudo-anonymization will be performed prior to transfer clinical data to the College computers. Pseudo-anonymization means that these data will NOT include any identifiable information, including NHS number and hospital number, but only the protocol number – ID log.

PID (Personal Identifiable Data) will be stored for 10 years as per College retention policy.

Signed consent forms will be archived inside the patients' medical records and will be available for any audit and research quality control activities. Patients will be given a copy of the signed consent form. A Trial Master File (TMF) containing hard copies of the consent forms will be kept in the Neurosurgery Department in Charing Cross Hospital 3S (offices). Entry into these facilities is restricted and controlled in hours. A specific code is required to access the facility out of hours, and only members of staff have access. No other copies will be done or retained.

4.3. INCLUSION CRITERIA:

- Above the age of 18, no upper age limit
- Sex: male or female
- Candidates for surgery due to a confirmed clinical and radiological diagnosis of cranial intrinsic or extrinsic tumour – any histological diagnosis confirming neuro-oncological disease, including primary and secondary disease
- Agreed to take part to the present research protocol and signed proper informed consent form

4.4. EXCLUSION CRITERIA:

- Suspected differential diagnosis of pathological condition affecting central nervous system other than neuro-oncological disease - including demyelinating diseases, infections, brain traumas / haematomas, vascular or auto-immune diseases
- Patients not capable to give consent – not in condition of understanding, processing and retaining information

5. ADVERSE EVENTS

As per the definition of adverse event ("Adverse Events (AEs) are any unfavourable and unintended signs, including abnormal laboratory results, symptoms or a disease associated with treatment." - https://www.nbt.nhs.uk/research-innovation/running-your-study/safety-reporting/classification-adverse-events), we do not expect for this technology to cause any adverse event whatsoever. The light sources are the same used in every standard neurosurgical procedure. The only real difference that the acquisition will make is to prolong time of the surgery by approximatively 5 minutes for each acquisition at the most.

The worst-case scenario will be that of technical failure of the acquisition, with no possibility of data storage or analysis. This will not impact in any form on patient's treatment and outcome. In the very unlikely occurrence of a SAE, standard procedures will be followed, as described below.

5.1. NON SERIOUS AEs

All such events, whether expected or not, will be recorded and notified by the investigators to the CI.

5.2. SERIOUS AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to the diagnosis (brain tumour and/or vascular anomaly), and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the REC where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs

jrco@imperial.ac.uk

CI email (kevin.oneill@nhs.net) Fax: xxx. Please send SAE forms to: Mr Kevin O'Neill and /or Mr Giulio Anichini Tel: 020 33135503

(Mon to Fri 09.00 – 17.00)

6. STATISTICS AND DATA ANALYSIS

Parametric tests will be performed on the patients' clinical data to make sure that the sample is homogeneous and consistent with the other literature series reporting similar diseases.

6.1. FIRST AIM

Detect structural differences between brain tumour tissue and surrounding brain.

The Regions Of Interest (ROI) for multispectral/hyperspectral acquisition will be delineated on both peri-operative images (MRI scan) and intra-operative images as extrapolated from the intra-operative videos. We will focus our analysis on three main regions:

- normal brain tissue - brain tissue not infiltrated by tumour or edema, nor grossly pathological on pre-operative images or intra-operative images;
- suspected infiltrated brain tissue – tissue surrounding the tumour where there is strong radiological and clinical suspicion of infiltrative disease or edema;
- tumour tissue – margins and core of the tumour on pre-operative images and intra-operative findings.

The signal in one region of the tumour will be compared to a neighbouring 'healthy' region. The signal itself will come from a processed set of images of the brain, where we will fit the data using one of our established models to extract an indication of tissue oxygenation (as a %) on a per-pixel basis.

The comparison between the set of readings obtained from normal brain will be performed using Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) or more advanced techniques including, for example, machine learning. Statistics will be collected on accuracy, sensitivity, specificity, ROC analysis.

We will also need to take pictures and/or videos of the surgical field in order to correlate the expected anatomical location of the tumour and infiltrated areas with the intra-operative finding. The surgical field is limited to the area under the microscope or endoscope

magnification, therefore no identifiable pictures or videos will be taken at any point during the study.

6.2. SECOND AIM

Detect differences between eloquent/functional and non-eloquent/non-functional brain areas.

The Regions Of Interest (ROI) for multispectral/hyperspectral acquisition will be delineated on both peri-operative images (MRI scan) and intra-operative images as extrapolated from the intra-operative videos. For the purpose of this subsection, we will focus our analysis on the comparison between a supposed eloquent area and its surrounding brain tissue. In order to achieve this, we will consider to perform multispectral/hyperspectral acquisition on patients undergoing surgery with neurophysiology monitoring - Motor Evoked Potentials (MEP), SomatoSensory Evoked Potentials (SSEP), Visual Evoked Potentials (VEP), Auditory Evoked Potentials (AEP) - , and/or in patients undergoing awake surgery.

The presence of eloquent brain tissue will be confirmed both anatomically by identifying it on peri-operative and intra-operative assessment, and through neuromonitoring stimulation and/or the execution of a single task during awake surgery, as standard clinical practice in surgery for brain tumours in eloquent areas. From this point of view, clinical correlation with the patient's pre-operative neurological status is crucial in order to consider potential variations in the strength of the intra-operative signal and activation pattern of the analysed eloquent areas – example: a patient with a pre-operative limb weakness is expected to show a different level of activation and therefore might show a different spectroscopic signal compared to a patient with normal motor power. We will also need to take pictures and/or videos of the surgery in order to correlate the expected anatomical location of an eloquent area with the intra-operative finding. The surgical field is limited to the area under the microscope or endoscope magnification, therefore no identifiable pictures or videos will be taken at any point during the study.

Same tests as section 6.1 will be run to statistically validate the data.

6.3. THIRD AIM

Test whether the combination of multispectral/hyperspectral analysis and fluorescence could provide useful new insight into the detection of different perfusion patterns of brain tumours VS normal brain tissue

Multispectral/hyperspectral imaging will be tested to check whether it can increase the distinction of the fluorophore signal compared with the background. Dedicated statistical software will be used for this analysis.

Each image set will be analysed separately because there will be variability between patients and we will not be able to fully account for this with the limited number of datasets that we are going to record (a well-known problem with many biophotonics/optical techniques). This will all be done offline after the surgery is complete, with a primary aim is to observe whether the technique is capable of detecting changes in the tissue.

We will collect spectral data up until the statistical model used for multivariate analysis does not longer improve the degree of accuracy. We will aim to recruit approximately 50 patients with diagnosis of neuro-oncological disease. Each patient will have an average acquisition of 6 datasets. As each dataset will correspond to an image, this will be divided into many reading regions (from 10 to 20) for a total of approximatively 60 measurements per patient. Based on this estimation, we approximate that 50 patients will provide a spectral database of 3.000 spectra, which we calculate is adequate for diagnostic validation purposes [14].

Same tests as section 6.1 will be run to statistically validate the data.

7. FURTHER DEVELOPMENT

This technology is completely novel, and in our estimation, it has the potential of changing the neuro-oncological surgery practice. Our hypothesis is that it could effectively

detect differences between brain and tumour tissues. Therefore, the present study could ultimately become a benchmark for the development of a new tool to guide the surgeon during brain tumours resection. It would also provide the community with more knowledge and understanding of the functional anatomy of the brain. More specifically, it could increase our knowledge of the vascular re-arrangement, structure, and oxygenation of brain tumours, as well as additional knowledge about perfusion and oxygen extraction in the brain between eloquent and non-eloquent areas – thus representing an intra-operative equivalent of a functional MRI, but without the same intra-operative hindrance and costs.

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the Research Ethics Committee (REC) and Health Regulator Authority (HRA). The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2. CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the

participants remain within the study for the purposes of data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3. CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

There are two sets of data the researchers will collect:

1) Intra-operative images / videos and spectroscopic data;

2) Clinical data: diagnosis, clinical status, medical background, radiological, histopathological data, treatment data.

Regarding intraoperative multispectral data collection, these will be stored mostly on College Computers, and recording devices. Anonymous video recording and/or images acquisition will be carried out by the clinicians in order to sync spectral data collected with the surgical procedure. Each patient will be given a specific protocol number – Log ID. These data will be in pseudo-anonymized form when transferred to College Computers, and the images and videos will be coded using the specific protocol number / log ID previously assigned.

Regarding clinical data, these will be stored on NHS Trust Encrypted computers in a semi-anonymized form, and transferred on College Computers in a pseudo-anonymized form. NHS computers follow NHS Trust and Imperial College confidentiality policy. Semi-anonymization means that these data will include NHS number, Hospital Number, age, protocol number. No additional identifiable personal data – such as telephone numbers, address, name, and surname – will be stored, except for those on the copies of the consent forms for the Trial Master Files (TMF). These will be stored in a safe and restricted environment in CXH 3S (officies). Pseudo-anonymization will be performed prior to transfer clinical data to the College computers. Pseudo-anonymization means that these data will NOT include any identifiable information, including NHS number and hospital number. Only the protocol number / log ID will be included in the data stored on College Computers.

The following clinical data will be stored on College Computers: diagnosis, medical background, histological and radiological data, treatment data, intra-operative images and/or videos, spectroscopic data.

Electronic Transfer by magnetic or optical media, email or computer networks, use of audio/video recording devices, storage of personal data will be undertaken by the researchers who are also clinical care team responsible for direct care, at CXH. Pseudo-anonymized data will be transferred to the University computers by the clinical researchers and will be processed by College researchers either on College Computers in SK, SMH or HH.

In case a participant, who has given informed consent, loses capacity to consent during the study, the participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

Signed consent forms will be archived inside the patients' medical records and will be available for any audit and research quality control activities. Any identifiable data will be kept under Imperial College policy for 10 years including the consent form and the coded list. Patients will be given a copy of the signed consent form. A manual copy of the consent form will be retained in the Trial Master File (TMF) ONLY. No other copies will be done or retained.

8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5. SPONSOR

Imperial College of London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6. FUNDING

Brain Tumour Research (BTR) charity is funding this study.

8.7. AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Mr Giulio Anichini, Clinical Research Fellow and PhD Student – g.anichini@nhs.net, g.anichini@imperial.ac.uk

10. PUBLICATION POLICY

This study will follow the Imperial College of London publication policies and anti-plagiarism guidelines. Non-identifiable data will be used during publication. Authorships and authors order will be defined in due course. We will collect spectral data up until the statistical model used for multivariate analysis does not longer improve the degree of accuracy.

11. GANTT CHART

TASK	01/20	04/20	07/20	10/20	01/21	04/21	07/21	10/21	01/22	04/22	7/22	10/22
Ethics approval												
Recruitment												
Data analysis and write up												

12. REFERENCES

1. Preibisch C, Shi K, Kluge A, Lukas M, Wiestler B, Göttler J, Gempt J, Ringel F, Al Jaberi M, Schlegel J, Meyer B, Zimmer C, Pyka T, Förster S. Characterizing hypoxia in human glioma: A simultaneous multimodal MRI and PET study. NMR Biomed. 2017 Nov;30(11).
2. Tyler JL, Diksic M, Villemure JG, Evans AC, Meyer E, Yamamoto YL, Feindel W. Metabolic and hemodynamic evaluation of gliomas using positron emission tomography. J Nucl Med. 1987 Jul;28(7):1123-33
3. Rodgers ZB, Detre JA, Wehrli FW. MRI-based methods for quantification of the cerebral metabolic rate of oxygen. J Cereb Blood Flow Metab. 2016 Jul;36(7):1165-85.
4. Santos E, Schöll M, Sánchez-Porras R, Dahlem MA, Silos H, Unterberg A, Dickhaus H, Sakowitz OW. Radial, spiral and reverberating waves of spreading depolarization occur in the gyrencephalic brain. Neuroimage. 2014 Oct 1;99:244-55.
5. Behrooz A, Waterman P, Vasquez KO, Meganck J, Peterson JD, Faqir I, Kempner J. Multispectral open-air intraoperative fluorescence imaging. Opt Lett. 2017 Aug 1;42(15):2964-2967.
6. Lu HD, Chen G, Cai J, Roe AW. Intrinsic signal optical imaging of visual brain activity: Tracking of fast cortical dynamics. Neuroimage. 2017 Mar 1;148:160-168.
7. Fawzy Y, Lam S, Zeng H. Rapid multispectral endoscopic imaging system for near real-time mapping of the mucosa blood supply in the lung. Biomed Opt Express. 2015 Jul 21;6(8):2980-90.
8. Fereidouni F, Griffin C, Todd A, Levenson R. Multispectral analysis tools can increase utility of RGB color images in histology. J Opt. 2018 Apr;20(4).

9. Farberg AS, Glazer AM, Winkelmann RR, Tucker N, White R, Rigel DS. Enhanced melanoma diagnosis with multispectral digital skin lesion analysis. Cutis. 2018 May;101(5):338-340.
10. Huang G, Peng J, Ye Z, Kijlstra A, Zhang D, Yang P. Multispectral image analysis in Vogt-Koyanagi-Harada disease. Acta Ophthalmol. 2018 Jun;96(4):411-419.
11. Vasaturo A, Di Blasio S, Verweij D, Blokx WA, van Krieken JH, de Vries IJ, Figdor CG. Multispectral imaging for highly accurate analysis of tumour-infiltrating lymphocytes in primary melanoma. Histopathology. 2017 Mar;70(4):643-649.
12. Zhang Y, Wirkert SJ, Iszatt J, Kenngott H, Wagner M, Mayer B, Stock C, Clancy NT, Elson DS, Maier-Hein L. Tissue classification for laparoscopic image understanding based on multispectral texture analysis. J Med Imaging (Bellingham). 2017 Jan;4(1):015001.
13. Clancy NT, Saso S, Stoyanov D, Sauvage V, Corless DJ, Boyd M, Noakes DE, Thum MY, Ghaem-Maghami S, Smith JR, Elson DS. Multispectral imaging of organ viability during uterine transplantation surgery in rabbits and sheep. J Biomed Opt. 2016 Oct 1;21(10):106006.
14. Rosa L Figueroa1, Qing ZengTreitler, Sasikiran Kandula and Long H Ngo. Predicting sample size required for classification performance. BMC Medical Informatics and Decision Making 2012, 12:8.