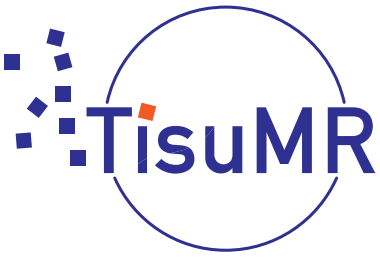
Project Number: 737043

Project Acronym: **TISuMR**

Project Title: **Integrated Tissue Slice Culture and NMR Metabolomics — A Novel Approach Towards Systemic Understanding of Liver Function And Disease**

TISuMR Device Specification



Document version: 0.0.0
Last modified: May 28, 2019

1 Executive Summary

2 Change History

Version	*Mod. Date*	*Summary of changes*	*Author*
0.0.0	22/5/2019	Initial discussion version	ms, mu

3 Scope of this document

TISuMR is a collaboration project between University of Southampton, University of Groningen and Karlsruhe Institute of Technology. The aim of the project is to develop technologies for NMR (nuclear magnetic resonance) compatible microfluidic perfusion culture of PCLS (precision cut liver slices). The devices that are being developed at the three project partner sites are built for different aims (high-resolution liquid NMR spectroscopy of the perfusion fluid, high-resolution magic-angle spinning NMR spectroscopy, or conventional analysis using HPLC and other techniques). These different approaches lead to different interfacing requirements for the perfusion system. In order to ensure comparability of the results, it is necessary to standardise certain aspects of the design. This will ensure identical culture conditions, as well as a common standard to judge the performance of the culture system and basic viability of the tissue slices.

This document defines the specifications for a device design to be qualified as a TISuMR device. All the TISuMR personnel will follow these requirements for device design if the device is used for TISuMR research. Possible variations are also given to cater to specific experimental needs.

Changes to this document will be decided in the TISuMR meetings. The intended changes will be communicated to all the partners before the meeting to think upon. All the partners should agree for a change to be made final. This document will be available on https://github.com/marcel-utz/tisumr-device to obtain the latest version of the document. Marcel Utz will own the master copy and will be responsible to implement the changes. A drawing of the proposed device is shown in Fig.1

4 Required Specifications

- **Culture chamber geometry:** The culture chamber is cylindrical in shape with a diameter of 7 ± 1 mm and a depth of 500 ± 100 μm.
- **Perfusion geometry:** The perfusion fluid flows around the PCLS. There can be more than one inlets and outlets. The inlet and outlet channels have cross sectional dimensions of $200\pm100\times200\pm100$ μm^2.
- **Chip or device material:** Polycarbonate is used for the chip or device fabrication.
- **Temperature:**. The PCLS culture is performed at 37 ± 0.5 °C.
- **Gas composition:** Either carbogen (95 % O_2 + 5 % CO_2) or a mixture (80 % O_2+ 10 % N_2 +5 % CO_2) is used for the culture. Gas composition with 70 % or more O_2 partial pressure have the same effect on viability of PCLS.
- **Viability standards:** Adenosine tri(phosphate) (ATP) content in the tissue slice after culture is used as a measure of viability. As a rule, tissue slices can be considered viable if they contain at least 6 pmol of ATP per mg of protein. The protocols for ATP determination is given in section6 and for protein determination is given in section7.
- **Medium composition:** William E with Glutamax + Glucose (1.375 g/500mL William E medium) + Gentamycine (500μL/500mL)
- **Sterilization:** Sterilisation will be preformed by exposure of the equipment to 70% ethanol in water for 5 minutes and drying them afterwards. Tubings will be sterilised by flowing 70% ethanol in water for half an hour and then flow phosphate-buffered saline (PBS) for a wash.

5 Allowed variations

- **Detailed fluidic paths:** Fluidic network can be designed freely.
- **Flow protocol:** The media can be flowed by different types of pumps or centrifuge.
- **Fabrication method:** The devices can be made through machining or bonding layers by different protocols.

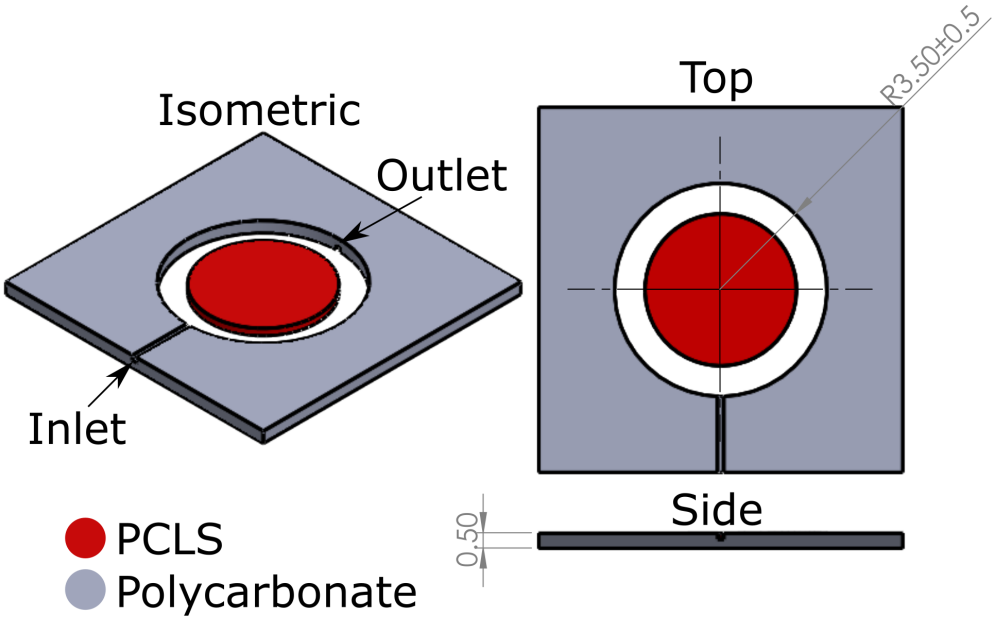


Figure 1: *Isometric, top and side views of the device. The diameter of the PCLS chamber is 0.7 mm. The thickness of the chamber is 0.5 mm.*

- **Flow rates:** Range of flow rates will be decided through optimisation.
- **LDH:** If ATP content can not be measured due to the nature of experiment, LDH (lactate dehydrogenase) can be used as a viability indicator.

6 ATP determination

- **Materials:**
  - 1.5mL tubes
  - White 96-wells plate
  - Minibead-beater
  - Repetitive pipet with 50 μl tip
  - Synergy HT plate reader
  - ATP positive control (P) (-80 °C) (1 aliquot/plate)
- **Solutions**
  - **SONOP (Sonification Solution), Ethanol (70% v/v) containing 2mM EDTA (M=372.24 g/mol) with pH=10.9** For 1L: Dissolve 0.744g EDTA in $\pm$ 200ml of MQ-water, adjust pH with 5M NaOH to pH=10.9, add 60mL MQ-water and 740ml ethanol (96%)
  - **100mM Tris-HCl, 2mM EDTA buffer (pH 7.6-8.0)** For 500ml: Dissolve 6.0g Tris (M=121.14) (Tris(hydroxymethyl)amniophen; Merck) and 0.37g EDTA (Triplex III; M=372.24) in $\pm$ 300ml MQ-water, adjust pH with 6N HCl and fill up to 500ml total volume with MQ-water.
  - **ATP Bioluminescence assay kit Roche.**
    - Luciferase reagent lyophilized (white cap): Dissolve lyophilized luciferase in exactly 10.0 ml MQ-water and mix by swinging. Do not vortex.
    - ATP-standard $\pm$ 10mg lyophilized (red cap): Dissolve the ATP-standard from the kit to exactly 10mg/ml (= 16.5mM) with MQ-water) and aliqoute 20 μL and store in -80 °C
- **Protocol**:After the incubation put 1 slice in 1 ml SONOP in a safelock vial, 1 cup of minibead and snap frozen in liquid N_2.

1. Label a new set of 1.5mL tubes (equal to the amount of ATP-samples).
2. Get luciferase from fridge or freezer, this needs to be at room temperature.
3. Homogenize the sample with minibead-beater for **2×45sec**. Store the samples back on ice.
4. Centrifuge homogenate **5min at 13.000 rpm at 4 °C**. Transfer the supernatant into the new tube and **keep on ice**. The tube with precipitate is dried at 37 °C (1 day) or at RT (3 days) for protein measurement.
5. Prepare a calibration curve and store tubes on ice:

Dilution	Amount (μl)	Tris/EDTA Buffer (μl)	Conc. (M)
A	10μl ATP-standard (S)	90	1.65×10^{-3}
B	50μl [A]	450	1.65×10^{-4}
C	50μl [B]	450	1.65×10^{-5}
Cal 1	50μl [C]	450	1.65×10^{-6}
Cal 2	**100μl [Cal 1]!**	**400**	3.30×10^{-7}
Cal 3	50μl [Cal 1]!	450	1.65×10^{-7}
Cal 4	**100μl [Cal 3]!**	**400**	3.30×10^{-8}
Cal 5	50μl [Cal 3]!	450	1.65×10^{-8}

6. Pipet 5μl Blank (Tris/EDTA), 5μl positive control, and 5μL supernatant of each sample in duplo to the white 96-wells plate.
7. Add 45μl Tris/EDTA buffer to all wells containing blank, positive control or sample.
8. Pipette 50μl diluted calibration curve in duplo in the plate.
9. Add 50μl luciferase (do not vortex) to every well using a repetitive pipet or a multichannel pipet.
10. Shake plate a little and measure plate after 0min, 5min and 10 min using the luminometer (set-up SynergyHT ATP protocol, kinetic read (3 timepoints interval 5 min) for luminenscence).

- **Remarks:** Important: The ATP in the slices is sensitive for breakdown by present enzymes. Therefore store samples at -80 °C and keep tubes at 4°C throughout the determination.

7 Protein determination

- **Materials:**
  - BSA stock solution 3.2 (A) and 2.4 (B) mg/mL
  - Water bath with shaking function
  - Minibead beater
  - Transparent flat 96-wells plate
  - Multichannel pipet
  - Protein reader absorbance at wavelength 650nm*
- **Solutions:**
  - 5M NaOH solution (20g sodium hydroxide/100mL)
  - Reagent A and B of BIO-rad kit.
- **Protocol**:
  1. Turn on waterbath
  2. Thaw BSA (3.2 and 2.4 mg/ml)
  3. Add (to pellet and beads) 200 μl 5M NaOH (20g in 100ml)
  4. Incubate 30 min at 37°C (shaking, high speed) inside the waterbath

5. Make the following calibration curve diluted in the buffer at concentrations: 0 - 0.2 - 0.4 - 0.6 - 0.8 - 1.2 mg/ml

Dilution	Amount (μl)	1M NAOH (μl)	Conc. (mg/ml)
A1	50μl [A]	50	1.6
A2	50μl [A1]	50	0.8
A3	50μl [A2]	50	0.4
A4	50μl [A3]	50	0.2
A5	50μl [A4]	50	0.1
A6		50	0.0
B1	30μl [A]	50	1.2
B2	50μl [B1]	50	0.6
B3	50μl [B2]	50	0.3

6. Add 800 μl milliQ water (5x dilution, same as volume in SONOP)
7. Homogenize again with minibeadbeater for 40 seconds
8. Pipette 5 μl of calibration standard or sample in 96 well clear plate.
9. Add 25 μl of reagent A in each well (standards and samples).(If samples contain detergent than 20 μl of reagent S is added to 1 ml of reagent A)
10. Add 200 μl of reagent B to each well (standards and samples).
11. Keep the plate for 15 minutes at room temperature and measure the absorbance at wavelengths 750 or 650* nm (Stable for 1 hour)

- **Remarks:** With the Biorad reader, please use 655nm wavelength instead.