



Implementation of multiphoton intravital microscopy in mesenteric and coronary artery research

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Abstract. Advancements in intravital imaging technologies, particularly multiphoton microscopy, have significantly improved our ability to visualize and understand the dynamic interactions within vascular structures and various cell types in real time. However, traditional *in vitro* techniques – such as isometric myography – remain standard tools for assessing vascular reactivity. This study aims to develop and integrate both *in vivo* and *in vitro* methodologies to evaluate the functional and structural states of smooth muscle and endothelial layers in mesenteric vessels at the cellular level. Using native microscopy techniques, we assess morphological changes and correlate them with pharmacological responses in both mesenteric and coronary arteries. A key objective is to compare the capabilities and limitations of isometric myography and advanced multiphoton microscopy in analyzing vascular contractility and relaxation responses. The combined use of these techniques is expected to increase data quality, reduce animal usage, and support longitudinal studies. This integrative approach also enables the evaluation of both acute and chronic effects of pharmacological agents under near-physiological conditions, offering a more comprehensive understanding of vascular function.

Keywords: isometric measurements, mesenteric artery, intravital multi-photon microscopy, endothelial cells, transgenic mouse

Introduction

In the contemporary scientific world, the integration of various research approaches and technologies plays a key role in achieving significant results. The use of advanced technologies, such as multiphoton microscopy, will open new possibilities for studying dynamic processes with live cells in their natural environment – living tissues of the organism. This will significantly enhance the value of the experiments conducted, allowing for a deeper understanding of biological mechanisms and processes.

Current research methods help explain physiological and pathological alterations in the regulation of vascular balance.

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There are two major approaches to studying vascular imbalance: one involves measuring isometric and pressure-induced contractility properties, while the other assesses the relaxation abilities of blood vessels. Pharmacology plays a key role in vascular research, as it enables the use of various stimulants and inhibitors that target different receptors and mechanisms.

Changes in arterial contractility determine arterial resistance, which can increase in atherosclerosis. Additionally, hypertension remains a major pathological condition, where alterations in vascular mechanisms contribute to disease progression, with both age and genetics playing significant roles [1].

The question of atherosclerosis development mechanisms and the involvement of various cellular and molecular regulatory pathways remains relevant despite technological advancements and modern methods. New methods for studying the functional activity of cells in and around the vascular wall will make it possible to accurately study the pathogenesis of various cardiovascular diseases, such as atherosclerosis, for more effective prevention and treatment [2-6].

Vascular reactivity of the segment is measured under isometric conditions through a highly sensitive and accurate transducer. The opposite side is attached to a precision micrometer, allowing control of the vessel circumference.

The microscopic method enables the observation of anatomical changes occurring within minutes during the development of an effect by capturing the activation process at a specific moment.

Meanwhile, pharmacological methods help record contractile activity in response to various agents, providing insights into both pathological and physiological conditions.

Vascular reactivity of the segment is measured under isometric conditions using a highly sensitive and accurate transducer, while the opposite side is attached to a precision micrometer for controlled adjustments of vessel circumference. The opposite side is attached to a precision micrometer, allowing control of the vessel circumference. The segment is kept under physiological conditions. contains up to 8ml of physiological salt solution (PSS), where the temperature is maintained via the built-in heating. Gas inflow is individually controlled and easily regulated by a needle valve (Figure 1).

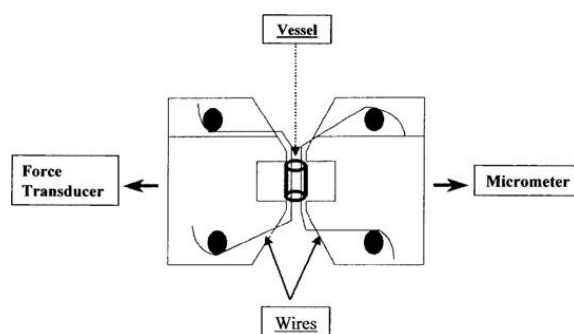


Figure 1. Myograph DMT 620 [11]

Simultaneously, a high-resolution microscopic method allows real-time observation of anatomical and structural changes, capturing dynamic alterations at the cellular level during the development of vascular responses. This combined approach enables the assessment of contractile activity in response to pharmacological agents, providing insights into both physiological and pathological conditions. By integrating mechanical, microscopic, and pharmacological analyses, this method offers a comprehensive evaluation of vascular function and remodeling.

Wire myography is an *in vitro* technique used to assess the functional responses and vascular reactivity of isolated small resistance arteries. This method enables the examination of vessels from various species, including transgenic models, across different vascular beds and pathological conditions [7-11].

In recent decades, intravital two-photon microscopy has become a modern method for visualizing cellular responses in live animals and has found particular applications in neurobiology and immunology [2-6, 12-13]. Two-photon microscopy allows for the visualization of cell dynamics in their natural *in vivo* environment (Figure 2). The use of advanced technologies, such as two-photon microscopy, enables the identification of complex spatiotemporal dynamic interactions among different cell types (e.g., endothelial cells, T-lymphocytes, and macrophages) involved in inflammation and atherogenesis [3-4,14].

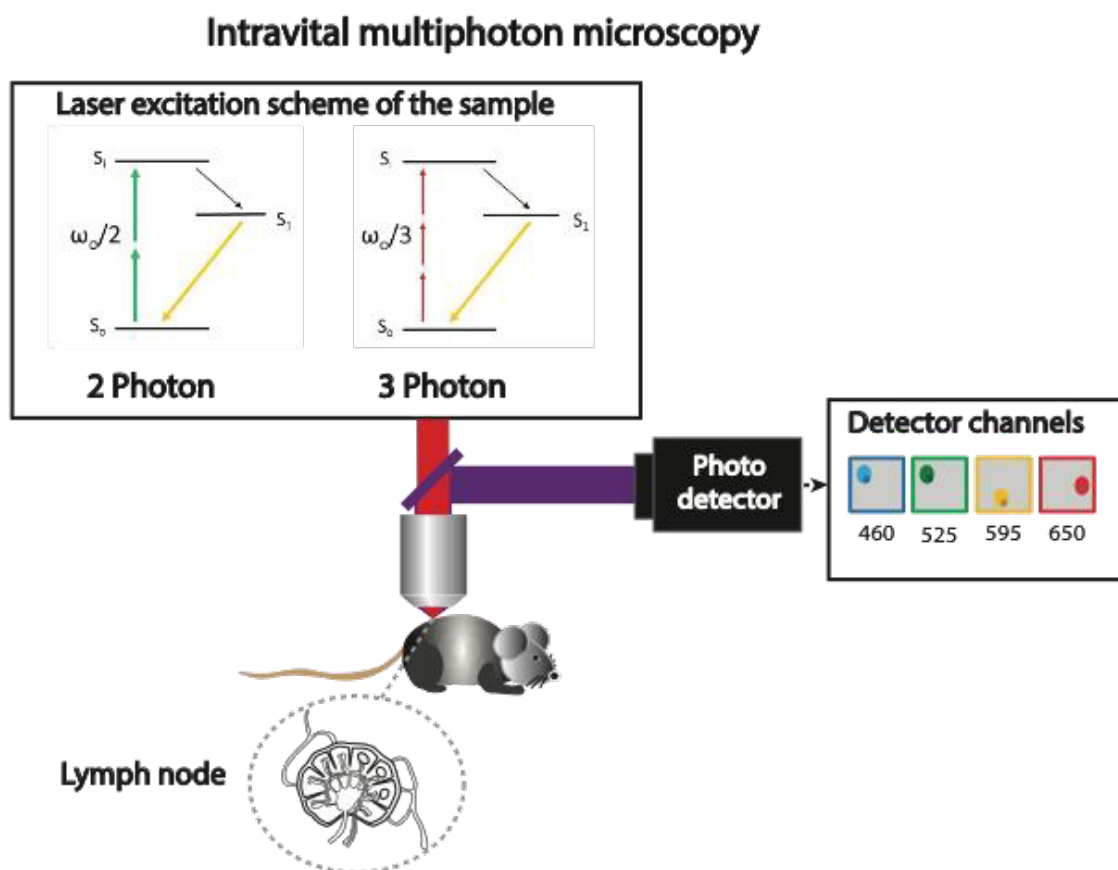


Figure 2. A diagram of intravital multiphoton microscopy (MPM)

Multiphoton microscopy is widely recognized as the best available technology for high-resolution intravital visualization of cell motility. A diagram of intravital multiphoton microscopy (MPM) is presented (Figure 2), demonstrating a minimally invasive method for studying biological processes in a living organism at the cellular level in real time. The key element of the MPM is photophysical excitation using femtosecond laser pulses. Depending on the tasks, different excitation modes can be used - two-photon, each of which has its own characteristics and ensures a comprehensive study of cellular behavior and functions in the natural environment. A detailed description of the methodology is given in the work of Rakhymzhan et al. [10].

We are developing and implementing new optical tools and evaluation algorithms to improve the quality of imaging and quantitative assessment of cell functions in multiphoton microscopy of live organisms. Key aspects of the development include (i) a multiphoton system for mesenteric and coronary arteries, (ii) spatially and temporally synchronized dual near-infrared (NIR) and infrared (IR) excitation for spectrally expanded intravital microscopy.

Multiphoton microscopy is widely recognized as the best available technology for high-resolution intravital visualization of cellular motility at the cellular level. In this research, we develop novel approaches to applying optical tools and evaluation algorithms to enhance imaging quality and quantitative assessment of cellular functions in live-organism multiphoton microscopy.

In this paper we try to develop methods of studying the alterations of cellular mechanisms in blood vessels using myography, which will help understand the fundamentals of the pathophysiological process at a deeper level and, in the long term, contribute to the development of new approaches to the treatment and prevention of vascular diseases. The method will be helpful in the study of the modification of the contractile activity of the mesenteric artery and other arteries. The application of advanced technologies, such as two-photon microscopy, will allow us to open the complexity of the spatiotemporal dynamic interactions between various cell types, including endothelial cells, vascular smooth muscle cells, that are involved in maintaining the vascular balance.

Thus, this study aims to develop of *in vivo* methodology for measuring the state or structure of smooth muscle and endothelial cell layers in mesenteric vessels at the cellular level. This will allow the assessment of morphological changes in blood vessels *in vivo* or *in vitro* using native microscopy, visualizing changes in a complex manner, and obtaining data in the pharmacological studies of the functional responses of the mesenteric arteries in the future. In the next step, we compare the advantages and disadvantages of two methods – assessing contractile activity by myograph and visualization using two-photon microscopy.

Materials and methods

Materials and Animals Used in the Study

We used Cdh5:tdTomato x H2B: GFP (Cdh5:tdTom) transgenic mice (age 8-12 weeks) obtained from Free University of Berlin (FUB), Germany, in which endothelial cells express tdTomato in the cytoplasm and membranes (under the *Cdh5* promoter) and H2B-GFP in the nuclei [13]. This allows simultaneous visualization of endothelial cell morphology and nuclear dynamics *in vivo*.

The study will involve 10 male mice aged 8 to 12 weeks, weighing between 25.0 and 30.0 g (average: 33.5 g). All animals will be kept under controlled temperature (21.2°C) and humidity (55.5%) conditions with a 14-hour light cycle and a 10-hour dark cycle. They will be fed commercial food and will have free access to water. There will be no restrictions on water and light throughout the experiment. All animals will receive humane care in accordance with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, prepared by the Laboratory Animal Resources Institute, published by the National Institutes of Health.

At the end of the experiment, mice will be sacrificed using intraperitoneal injection of ketamine hydrochloride (Ketalar) and xylazine hydrochloride (Alfasin) at doses of 80-100 mg/kg 1 and 10-12.5 mg/kg 1 respectively.

Preparation of mouse models for intravital microscopy

1. Preparing mice for intravital microscopy. Note: Steps a) and b) should be performed at the beginning of the experiment. a. Weigh the Cdh5:td Tom mouse (age 8-12 weeks). b. Prepare 800 µl of anesthetic (ketamine 60 mg/kg, xylazine 4.5 mg/kg, acepromazone 1.75 mg/kg in PBS). c. Anesthetize the mouse. Inject intravenously with 200 µl / 20 g mouse. Monitor anesthesia by finger pinching and respiratory rate.

2. Place the mouse on a heating pad and protect the eyes with eye gel.

3. Place a glass coverslip (35 mm diameter) in a 10-cm tissue culture dish having a 30-mm diameter opening. Use oil to keep the dish in contact with the coverslip.

4. Using scissors, incise the abdominal skin to expose the peritoneum. Cut the peritoneum with scissors to expose the intestine.

5. Add 200 µl of PBS (preheated at 37 °C) to the peritoneal cavity. Hold the mouse in your hand and turn it face down so that the intestine emerges from the peritoneal cavity with gentle pressure. Place the mouse in a tissue culture cup. 9. Using sterilized cotton swabs, gently remove the intestine onto a coverslip to expose the mesenteric vessels.

6. Cut the paper tissues into strips and moisten them with PBS heated to 37°C. Immobilize the intestine with pieces of paper towels to reduce the movements produced by peristalsis.

7. Place the tissue culture dish and mouse in a 37°C thermostated chamber on a custom-made inverted microscope stand. Inject an anesthetic syringe intravenously into the hind paw. NOTE: The mouse can also receive oxygen (0.5 L/min) using a mask.

8. Monitor the mouse's anesthesia by pinching its finger and checking its respiratory rate every 30 min. If necessary, inject an additional portion of anesthetic (20 µl) to maintain anesthesia. NOTE: Drying of the vasculature should be avoided. Therefore, its humidity is maintained by regularly wetting the paper tissues used for intestinal immobilization with PBS heated to 37°C with PBS [2-6, 10, 12, 14, 15].

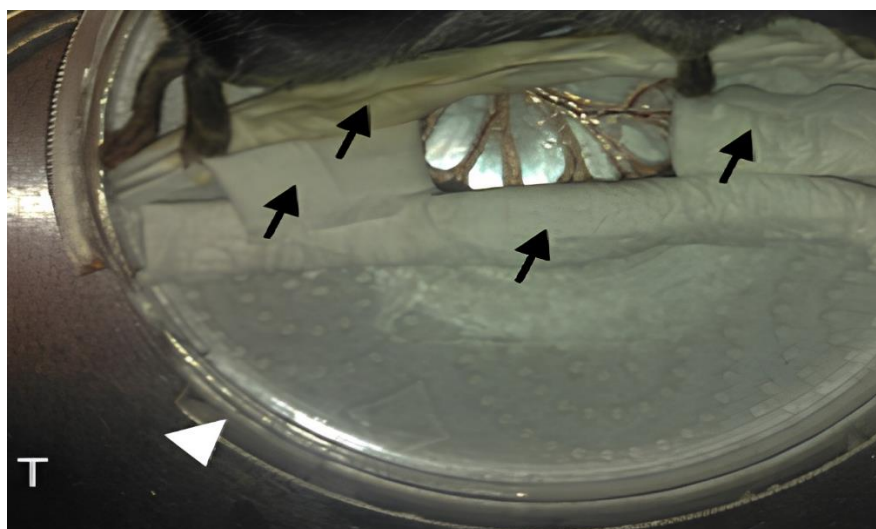


Figure 3. Mouse preparation. The picture from the paper published in 2015 by Emre and colleagues [16]. Black arrows indicate PBS-wetted tissue used to immobilize the intestine. The white arrowhead indicates the 10cm tissue culture dish. T indicates the aluminum custom-made tray stage that fits into the microscope. Mesenteric blood vessels are nicely exposed at the center of the coverslip

Development of a methodology for the application of modern intravital microscopy in pharmacology

Installation of a multiphoton laser-scanning microscope will be undertaken. Experiments involving two-photon fluorescence imaging will be conducted using a specialized laser scanning microscope based on a commercial scanning head (TriMScope II, LaVisionBioTec, Bielefeld, Germany). For excitation, we will use a near-infrared laser (Ti:Sa, Chameleon Ultra II, Coherent, Dieburg, Germany) and an infrared laser (Optical Parametric Amplification Throlabs Inc, USA). The Ti:Sa and OPA laser beams, both linearly polarized, are combined in the scanning base using a dichroic mirror (T1045, Chroma, USA). A water-immersion objective lens (25x, NA 1.0, Plan-Apochromat, Olympus Japan) is used to focus both laser beams on the sample. Laser power is regulated using combinations of $\lambda/2$ wave plates and polarizers. Ultrashort pulses from both lasers are compressed with external optical compressors: a two-prism compressor for Ti:Sa and a custom-built single-prism compressor for OPO. Fluorescence signals, SHG, SFG, and wavelength-mixed signals are collected in a backward direction using a dichroic mirror (775, Chroma, USA) and directed to six photomultipliers (H7422, Hamamatsu, Japan).

All PMT photomultipliers are arranged in a detection system with multiple optical channels, where each channel is defined by a specific fluorescent filter and dichroic mirror set: 466 ± 20 nm, 525 ± 25 nm, 562 ± 20 nm, 593 ± 20 nm, 617 ± 35 nm, 655 ± 20 nm, and 710 ± 20 nm. To prevent photodamage, the average maximum laser power used in all imaging experiments is set at 10 mW. Images with a field of view of $500 \mu\text{m} \times 500 \mu\text{m}$ and a digital resolution of 1024×1024 pixels are captured with an acquisition time of 944 ms. We acquire z-stacks with a depth of $40 \mu\text{m}$ (z-step of $2 \mu\text{m}$) every 20 seconds for a total imaging time, typically around 30 minutes [3–5,7].

Isometric measurement of tension in mesenteric and coronary arteries

In this technique, blood vessels are carefully dissected, cleaned, and mounted onto a four-channel myograph system under isometric conditions. Each vessel undergoes a normalization process to determine its maximum active tension development, ensuring standardized initial experimental conditions. This standardization is critical for accurately comparing pharmacological responses between different vessels.

Preparation of mesenteric and coronary arteries for isometric measurement of their tensions

The superior mesenteric arteries (SMA) or coronary arteries were isolated and cleared under a cold-light supported microscope in Krebs-Buffer (KB). The third branch of SMA was dissected and mounted in a micro-vessel system. The temperature of KS in the bath was maintained at 37 °C during all experiments. The vessels were incubated for 40 minutes to allow the tissues to recover from the stress of isolation, and the vessel's pressure was adjusted to 100 mmHg tension in four consecutive steps spaced apart by two minutes [2, 5, 6, 8-13].

Vascular contraction levels are expressed in milligrams (mg) as a function of cumulative agonist concentrations, ranging from 0.01 nM to 1 mM. Each dose-response experiment for a contractile agonist lasted approximately 20 minutes. Baseline vascular tension varied between samples and was determined through vessel normalization. Following each dose-response experiment, the tissue baths were thoroughly washed multiple times until the blood vessels returned to their basal state [2, 5, 6, 8-13].

Statistical methods for analyzing in vitro experiments

Maximum contractile responses and EC₅₀ (pD₂) value obtained from concentration-induced PE and 5HT response curves can be analyzed using one-way ANOVA (Tukey's multiple comparison test). cumulative dose response and differences in response to different doses obtained from concentration-induced PE and 5HT response curves can be analyzed using Two-Way ANOVA [16–20].

Data analysis. Image segmentation and tracking of all cells were performed using segmentation, object-recognition, and tracking plugins in Imaris (Bitplane AG, Zurich, Switzerland). 3D Reconstruction and Quantitative Analysis of Mesenteric and Coronary Arteries Using Imaris. Three-dimensional reconstruction of mesenteric and coronary arteries was performed using Imaris software. Vessels were processed using the IsoSurface tool to generate 3D visualizations, with parameters adjusted to optimize surface rendering (threshold set to 5.000, smoothing enabled, and border closure applied). The resulting scenes were saved for further analysis [21].

In Slice view, 2D cross-sections (e.g., slice 35) were used to guide segmentation. Perimeter measurements of arterial walls were conducted using the “Point-to-Point Distance Measurement” tool by drawing polygons along vessel borders, with cumulative distances recorded for analysis.

Morphometric and statistical characterization of vascular structures and endothelial cells was achieved through object-based color coding (e.g., size, roundness), 3D volume rendering, and temporal tracking via the “Track” component. Endothelial cells, nuclei, and vesicles were visualized using specific color channels (green, blue, red, respectively). Quantitative parameters were extracted under the “Statistics” tab, and exported to Excel for further evaluation [1–6, 8, 13-14, 21]. Cell tracks that were present in the field of view for more than 10 recorded time points (i.e., 5 min) were included in the analysis. Statistical analysis of the data was performed using Prism (Graph Pad Software Inc., San Diego, USA) [7, 22].

Results and discussion

Emre and colleagues first described a novel method for *in vivo* investigation of mesenteric arteries in 2015, marking a significant advancement in the field of vascular research [15, 23–24]. The introduction of genetically modified CDH5 Cadherin (Chd5:tdTom) mice further expanded the potential of this approach by enabling precise visualization of specific cell populations within the vascular environment. Building upon these developments, our study proposes the integration of this advanced imaging technique into conventional vascular research protocols [20]. Rather than replacing established isometric functional assays, we advocate for a complementary approach that combines the strengths of both methodologies – leveraging the cellular resolution of intravital microscopy while preserving the proven utility of traditional isometric measurements [16, 25–26].

In vitro experimental paradigms allow for extended observation durations, typically ranging from 10 to 16 hours, thereby enabling the utilization of a single vascular segment across multiple experimental conditions. Given that certain vasoactive agonists induce transient effects lasting approximately 5 to 15 minutes, experimental protocols extending up to one hour are often optimal for characterizing both acute and sustained responses [18–22, 27]. This temporal flexibility underscores the suitability of the isometric technique for such pharmacological investigations. The highly controlled conditions inherent to *in vitro* systems support precise and reproducible monitoring of vascular responses [21, 22, 27]. Additionally, the implementation of multi-chamber organ bath systems (e.g., four or eight simultaneous baths) increases experimental throughput and facilitates the detection of subtle variations in vascular reactivity. A further advantage of the isometric method lies in its capacity to establish and monitor baseline vascular tone, enabling the assessment of how shifts in basal tone modulate the vessel's dose-dependent contractile behavior [23–28, 15].

Our study demonstrates that combining *in vitro* isometric measurements with advanced *in vivo* imaging techniques, such as two-photon microscopy, offers a comprehensive approach to the functional and structural assessment of vascular tissues. The isometric method allows for prolonged experimental durations, high-throughput data acquisition, and the generation of objective, quantifiable parameters of vascular contractility. Its compatibility with pharmacological modulation using receptor-specific agonists and antagonists further enables mechanistic insights into vascular reactivity and tone regulation. Moreover, the ability to measure basal tone and analyze dose–response relationships enhances its value in vascular physiology studies [9–11, 15–22, 27].

Figure 4 shows myograms of the coronary (A) and mesenteric (B) arteries at rest and with the use of vasoconstrictors. The figure illustrates the dose-response relationship of vascular contraction in the presence of various antagonists in the mesenteric and coronary arteries, assessed using isometric tension measurements with the Myograph DMT 630. Data acquisition was performed using the Biopac MP30 system.

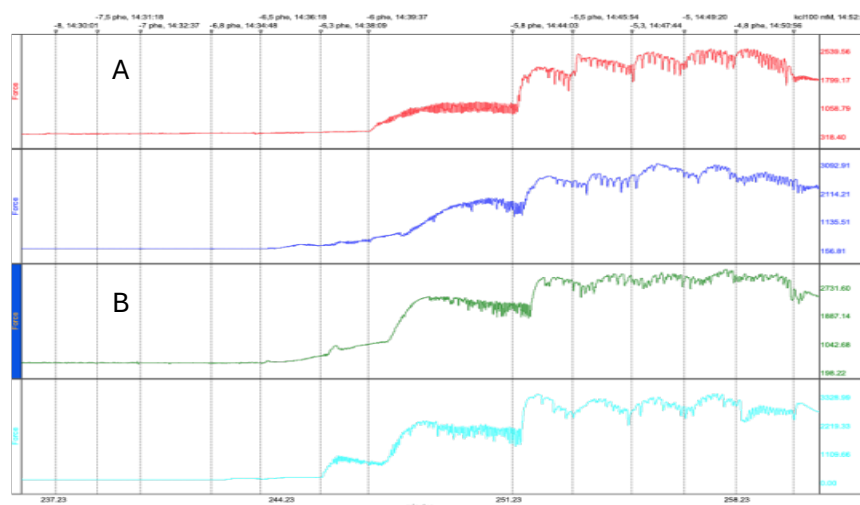


Figure 4. Coronary and mesenteric arteries myograms (**A** – coronary arteries, **B** – mesenteric arteries)

Figure 5 illustrates differences in vascular contraction between blood vessels with and without an intact endothelium, quantified in milligrams of contraction force. Notably, vessels lacking endothelium exhibit a reduced contractile response, measuring approximately 1000 mg less than intact vessels [2, 5-6, 8, 13].

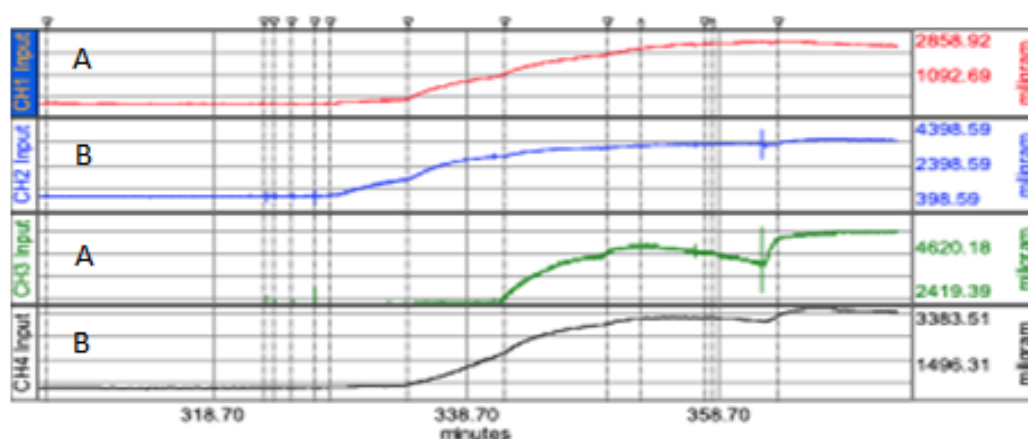


Figure 5. Vascular contraction between blood vessels with and without an intact endothelium (**A** – coronary arteries, **B** – mesenteric arteries)

Figure 6 demonstrates that this method enables the continuous assessment of vascular contraction and relaxation functions over an extended period, with recordings maintained uninterrupted for 5-6 hours. Additionally, it allows simultaneous monitoring of the responses of four blood vessels to different agonists or antagonists, facilitating the detection of subtle variations in their reactivity [16-19].

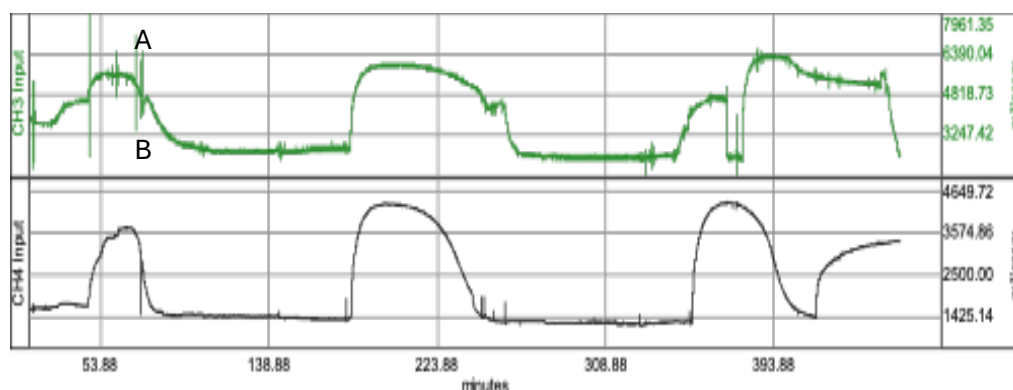


Figure 6. Continuous assessment of vascular contraction and relaxation functions
(A – coronary arteries, B – mesenteric arteries)

Thus, the use of isometric measurements, in particular the application of the myography method, is a tool for the objective study of vascular function, pathways and mechanisms of its control, which is the study of isolated vessels. Myography of isolated vessels makes it possible to standardise the influence of physical factors (transmural pressure, shear stress on the endothelium, composition of the environment, temperature), electrical stimulation with currents of specified parameters, standardisation of the concentration of metabolic agents, and, as a result, multiple control of the activation and functioning of vasomotor mechanisms. Thus, working with a myograph allows for the analysis of both the contractile function itself and the mechanisms underlying it [11]. Isometric methods allow assessing the functional state of the body's vessels *in vivo*, while the MFM method makes it possible to visualize their deep structure. Both methods are notable in that they allow conducting *in vivo* studies in real time. Figures 7 and 8 show images of the vessel obtained using the MFM method.

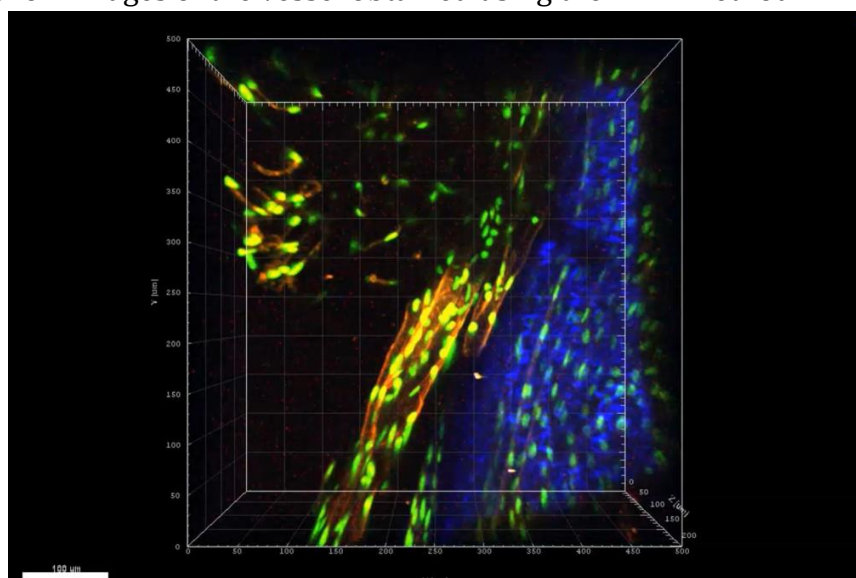


Figure 7. Three-dimensional (3D) visualization of the coronary artery in a transgenic mouse using two-photon microscopy. Imaging was performed with two-photon excitation at 930 nm. Endothelial

cell nuclei expressing GFP signal are shown in green membrane, and cytoplasm expressing tdTomato is shown in red, delineating the vessel walls. Collagen fibers are visualized in blue signal via a second harmonic generation. The figure provides a detailed anatomical localization and distribution of coronary arteries, including their abundance within the depth of cardiac tissue. Scale bar: 80 μm .

Additionally, this imaging approach enables the quantification of endothelial cell abundance within coronary arteries. The ability to monitor dynamic changes in vessel diameter within a five-minute timeframe facilitates the assessment of vascular reactivity. Notably, the extent of diameter modulation varies depending on pathophysiological conditions such as hypertension or atherosclerosis.

Furthermore, this method offers a unique perspective for examining coronary arteries, allowing for precise measurements of endothelial cell thickness and providing valuable insights into vascular remodeling under different physiological and pathological conditions.

Furthermore, this imaging approach enables visualization of the spatial arrangement and depth-dependent distribution of connective and adipose tissues, allowing for quantitative evaluation of tissue density. The structural composition of connective tissue is known to undergo alterations in response to pathophysiological conditions such as hypertension and atherosclerosis, primarily due to changes in collagen-to-fat ratios. This methodology provides an advanced perspective for assessing connective tissue remodeling and enables precise measurement of tissue thickness, offering valuable insights into disease-associated structural modifications [5-6, 8, 13, 29-31].

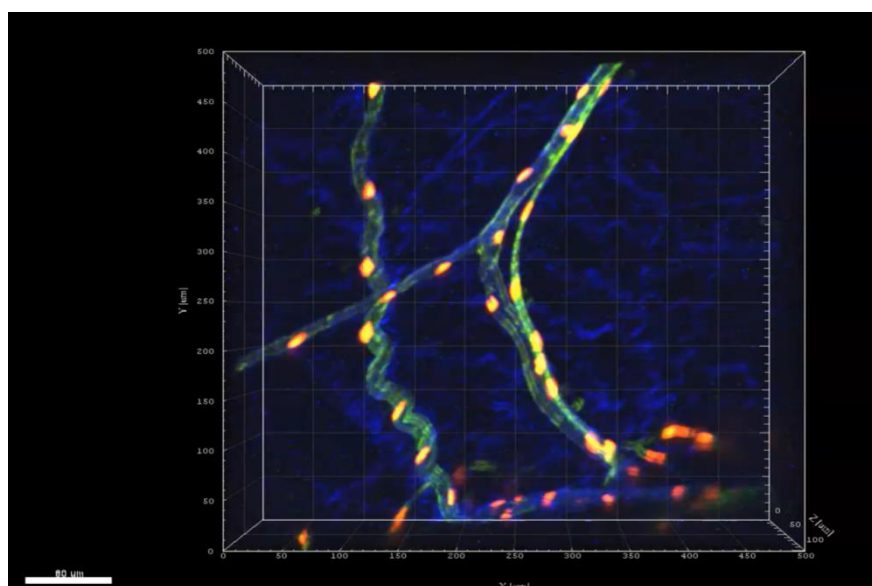


Figure 8. Three-dimensional reconstruction of the mesenteric artery in mice using two-photon microscopy. Imaging was performed at 930 nm excitation. Endothelial cell nuclei expressing GFP appear in green, while membranes and cytoplasm expressing tdTomato are shown in red, outlining vessel walls. Collagen fibers are visualized in blue via second harmonic generation (SHG). This image enables detailed anatomical localization of mesenteric arteries and supports comparative analysis of vascular architecture relative to the coronary circulation. Scale bar: 80 μm

Additionally, this imaging technique enables the assessment of mesenteric artery density at varying depths, as well as the quantification of endothelial cell abundance. The ability to track dynamic changes in vessel diameter within a five-minute time frame allows for the evaluation of vascular reactivity. Notably, the extent of diameter modulation varies depending on pathophysiological conditions such as hypertension or atherosclerosis.

Furthermore, this method provides a unique perspective for examining mesenteric arteries, facilitating precise measurements of endothelial cell thickness and offering insights into vascular remodeling under different physiological and pathological states. According to these results, we suggest using two-photon microscopy in vivo.

Conclusion

Complementing this, two-photon microscopy facilitates high-resolution, real-time, three-dimensional imaging of cellular and subcellular vascular structures in living tissue. The dynamic visualization of specific components, such as smooth muscle cells or myofibrils, is made possible through the use of fluorescent markers, enabling long-term monitoring of physiological changes under near-native conditions.

Together, these methodologies significantly expand the experimental capacity for studying vascular function, reduce animal use through multiparametric analysis, and provide a powerful platform for both short- and long-term investigations of vascular behavior in health and disease.

Author Contributions

T.A. – concept and supervision of the work, conducting the experiments, discussion of the research results, discussion of the research results; **A.R.** – concept and supervision of the work, conducting the experiments, discussion of the research results, discussion of the research results; **S.G.** – concept and supervision of the work, conducting the experiments, discussion of the research results, discussion of the research results; **A.A.** – discussion of the research results; **Z.D.** – editing the text of the article; **A.S.** – editing the text of the article.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

The research has approval from the Local Ethical Commission of the Al-Farabi Kazakh National University NJSC (LEC Protocol No. IRB A906, dated October 16, 2024) (IRB00010790 al-Farabi Kazakh National University IRB#1).

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Мезентериалды және коронарлық артерияны зерттеуде көпфотонды интравитальді микроскопияны қолдануын енгізу

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Аңдатпа. Интравитальді бейнелеу технологияларындағы жетістіктер, әсіресе мультифотонды микроскопия, нақты уақыт режимінде тамырлы құрылымдар мен әртүрлі жасуша түрлерінің динамикалық өзара әрекеттесуін визуализациялау және түсіну қабілетімізді айтарлықтай жақсартты. Дегенмен, дәстүрлі *in vitro* әдістері (мысалы, изометриялық миография) тамырлардың реактивтілігін бағалаудың стандартты құралдары болып қала береді. Бұл зерттеу жасушалық деңгейде мезентериальды тамырлардағы тегіс бұлшықеттер мен эндотелий қабаттарының функционалдық және құрылымдық күйлерін бағалау үшін *in vivo* және *in vitro* әдістемелерін әзірлеуге және біріктіруге бағытталған. Жергілікті микроскопиялық әдістерді пайдалана отырып, біз морфологиялық өзгерістерді бағалаймыз және оларды мезентериальды және коронарлық артериялардағы фармакологиялық жауаптарымен корреляциялаймыз. Негізгі мақсат – қан тамырларының жиырылуы мен релаксация реакцияларын талдауда изометриялық миография мен жетілдірілген мультифотонды микроскопияның мүмкіндіктері мен шектеулерін салыстыру. Бұл әдістерді біріктіріп пайдалану деректер сапасын арттырады, жануарларды пайдалануды азайтады және ұзақ зерттеулерде қолданылады деп күтілуде. Бұл интегративті тәсіл сонымен қатар физиологиялық жағдайға жақын жағдайларда фармакологиялық агенттердің жедел және созылмалы әсерін бағалауға мүмкіндік береді, бұл тамырлар функциясын жан-жақты түсінуге мүмкіндік береді.

Түйін сөздер: изометриялық өлшемдер, мезентериальды артерия, интравитальді мультифотонды микроскопия, эндотелий жасушалары

Внедрение мультифотонной интравитальной микроскопии для исследования брыжеечной и коронарной артерии

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Аннотация. Достижения в области технологий прижизненной визуализации, в частности, многофотонной микроскопии, значительно улучшили нашу способность визуализировать и понимать динамические взаимодействия внутри сосудистых структур и различных типов клеток в режиме реального времени. Однако традиционные методы *in vitro*, такие, как изометрическая миография, остаются стандартными инструментами для оценки сосудистой реактивности. Целью данного исследования является разработка и интеграция методик *in vivo* и *in vitro* для оценки функционального и структурного состояния гладкомышечных и эндотелиальных слоев брыжеечных сосудов на клеточном уровне. Используя методы собственной микроскопии, мы оцениваем морфологические изменения и сопоставляем их с фармакологическими реакциями

как в брыжеечных, так и в коронарных артериях. Основная цель — сравнить возможности и ограничения изометрической миографии и усовершенствованной многофотонной микроскопии при анализе сократимости и релаксационных реакций сосудов. Ожидается, что совместное использование этих методов повысит качество данных, сократит использование животных и обеспечит возможность проведения длительных исследований. Этот интегративный подход также позволяет оценивать как острые, так и хронические эффекты фармакологических препаратов в условиях, близких к физиологическим, обеспечивая более полное понимание сосудистой функции.

Ключевые слова: изометрические измерения, брыжеечная артерия, интравитальная мультифотонная микроскопия, эндотелиальные клетки, трансгенная мышь

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