Научном већу Института за физику у Београду

Извештај комисије за избор Михајла Радмиловића у звање истраживач сарадник

На седници Научног већа Института за физику у Београду одржаној 15.03.2022. године именовани смо за чланове комисије за избор Михајла Радмиловића у звање истраживач сарадник. Прегледом материјала који нам је достављен, као и на основу личног познавања кандидата и увида у његов рад и публикације, Научном већу Института за физику у Београду подносимо овај извешатај, у чијем прилогу се налази списак публикација кандидата.

1. Биографски подаци о кандидату

Михајло Радмиловић рођен је 06. августа 1993. године у Београду, где је стекао основно и средњошколско образовање. Основне и мастер студије је завршио на Биолошком факултету Универзитета у Београду 2018. године на модулу Молекуларна биологија и физиологија, мастер Биофизика, са укупном просечном оценом 9,13, одбранивши мастер рад под насловом "Анализа АТР-зависних струја кроз појединачне канале на мембрани цитоплазматичних капи из гљиве *Phycomyces blakesleeanus*". Током основних и мастер студија вршио је волонтерски рад у Институту за биолошка истраживања „Синиша Станковић", Институту од националног значаја за Републику Србију. Докторске академске студије из Биофотонике при Универзитету у Београду уписао је 2018. године. Звање истраживач приправник је стекао 16. априла 2019. године, а од 01. маја 2019. запослен је у Институту за физику Београд Универзитета у Београду. Од септембра 2020. године Михајло Радмиловић учествује на пројекту који финансира Фонд за науку Републике Србије из позива за изврсне пројекте младих истраживача (ПРОМИС): „*Hemoglobin-based spectroscopy and nonlinear imaging of erythrocytes and their membranes as emerging diagnostic tool*", акроним HEMMAGINERO. Поред тога, он је био учесник на пројекту из програма научне и технолошке билатералне сарадње Србије са Немачком за 2020-2021. годину, под насловом „*Осликавање и временски разложена спектроскопија у терахерцној, блиској инфрацрвеној и видљивој области за будуће биомедицинске примене*", а тренутно учествује на пројекту из програма научне и технолошке билатералне сарадње Србије са Словенијом за 2021-2022. годину, под насловом „*Нано-спектрално нелинеарно флуоресцентно осликавање хемоглобина без коришћења обележивача за потенцијалну дијагонстичку примену*".

2. Научна активност Михајла Радмиловића

Научна активност Михајла Радмиловића је усмерена на примене напредних микроскопских техника, у провом реду нелиенарне ласерске скенирајуће микроскопије у осликавању еритроцита и проучавања интеракције ултракратких ласерских импулса са хемоглбином као и развоју микроопричких елемената и структура који се заснивају на биополимерним геловима.

У даљој анализи научне и стручне активности кандидата детаљи ће бити разврстани по правцима и темама истраживања:

1. Интеракција ултракратких ласесрких импулса у блиској инфрацрвеној области са хемоглобином и нелинеарна микроскопија еритроцита и њихових деривата.

2. Развој микрооптичких елемената и структура заснованих на биополимерним геловима.

1. **Интеракција ултракратких ласесрких импулса у блиској инфрацрвеној области са хемоглобином и нелинеарну микроскопију еритроцита и њихових деривата.**

Интеракција ултракратких ласесрких импулса у блиској инфрацрвеној области са хемоглобином омогућава осликавање еритроцита и њихових деривата без бојења и фиксације. Разумевање ове интеракције је од кључног значаја за примене осликавања еритроцита у различитим патофизиолошким стањима. Хемоглобин, протеин који је задужен за преношење кисеоника/угљендиоксида код свих кичмењака и који испуњава унутрашњост еритроцита, врло је компликован за осликавање. Стандардне процедуре су компликоване и подразумевају бојење, да би се након тога структуре од хемоглобина осликавале на конфокалном или епи-флуоресцентном микроскопу. С друге стране хемоглобин јесте апсорптиван у плавој и блиској ултраљубичастој области, али релаксација није радијативна, те нема флуоресценције која би била погодна за осликавање. Пошто се ефикасно побуђује у плавом-УЉ делу спектра једнофотонски, хемоглобин добро апсосрбује двофотонски у блиској инфрацвеној области (650-750nm). Недавно је показано да након двофотоснке апсорпције, долази до фотохемијске ракције након које се од хемоглбина добја фотопродукт који јесте флуоресцентан (фотоактивација) и чија се флуоресценција може користити даље у осликавању. Фотоактивација хемоглобина на овај начин се може примењивати у различитим студијама облика и функције еритроцита и тема је пројекта „Hemoglobin-based spectroscopy and nonlinear imaging of erythrocytes and their membranes as emerging diagnostic tool" - HEMMAGINERO из програма ПРОМИС Фонда за науку Републике Србије у чијој реализацији кандидат Радмиловић учествује. Примарни део истраживања Радмиловић Михајла усмерен је на карактеризацију тзв. фотопродукта насталог приликом поменуте интеракције. Кандидат

Радмиловић је учествовао у лабораторијским мерењима спектралних карактеристика фотопродукта што подразумева успостављање протокола за микро-спректрална мерења двофотонских емисионих , УВ - ВИС, Раманских спектара фотопродукта као и спектрално осликавање („ Spectral imaging “) у сарадњи са колегама са Института Каролинска, Шведска. На основу мерења које је колега Радмиловић извршио показане су јединственве спектралне карактеристике поменутуог фотопродукта које се даље могу искористи за функционално осликавање еритроцита и хемоглобина. Осим спектралних особина, кандидат Радмиловић је показао да фотопродукт поседује високу фотостабилност, што указује на могућу примену фотопродукта у развоју оптичких меморија. Колега Радмиловић је такође учествовао у успостављању протокола за двофотонско осликавање еритроцита у физиолошким условима као и у индукованим *in vitro* стресним условима, као и за двофотонско обележавање и праћење појединачних еритроцита. Досадашњи резултати везани за хемоглобин и еритроците су у процесу писања и публиковања научног рада. Неки од досадашњих резултата су такође објављењи на домаћим и међународним конференцијама:

- **Radmilović, D. M.**, Drvenica, I., Krmpot, A. & Rabasović, M. Photophysics and photochemistry of hemoglobin interaction with ultrashortlaser pulses. Book of Abstractsof 14th Photonics Workshop (Conference), pp. 27 - 27, Kopaonik, Serbia, 14 – 17March 2021, ISBN 978-86-82441-52-6
- **Radmilović, D. M.**, Drvenica, I., Rabasović, D. M., Ilić, V., Pavlović, D., Nikolić, S., Matić, M. & Krmpot, A. Interaction of ultrashort laser pulses with hemoglobin as a tool for selective erythrocytes photo-labeling. Book of Abstracts of VIII International School and Conference on PhotonicsPHOTONICA2021& HEMMAGINERO workshop, pp. 107 - 107, 23 - 27 August 2021, Belgrade, Serbia, ISBN 978-86-82441-53-3
- Matić, M., Pavlović, D., **Radmilović, D. M.**, Rabasović, D. M., Ilić, V., Krmpot, A. & Drvenica, I. Discovering abnormal erythrocyte membranes - optical approaches. Book of Abstracts VIII International School and Conference on Photonics PHOTONICA2021& HEMMAGINERO workshop,pp. 108 - 108, 23 - 27 August 2021, Belgrade, Serbia, ISBN 978-86-82441-53-3

2. Развој микрооптичких елемената и структура заснованих на биополимерним геловима.

Развој микрооптичких елемената и структура подразумева коришћење технике контролисаног ласерског гравирања оптичких и неоптичких структура микронских димензија у матрикс заснован на биополимерним геловима. Оптичке структуре подразумевају пре свега различите типове микроосочива (сабирна, расипна) и микро - дифракционе решетке. Неоптичке структуре подразумевају микро - канале и произвољне геометријске структуре. Структуре су модулаторне и могуће их је комбинвати у сложеније елементе као што је LOC (Lab on a chip). Технологија развоја микрооптичких елемената често захтева комплексне технолошке поступке који подразумевају

коришћење економски захтевних технологија као и потенцијално штетних хемикалија. Колега Радмиловић учествовао је у развоју система за ласерско исцртавање микрооптичких и других неоптичких структура у смислу развоја софтвера за кординисану контролу ласера и кординационог стола. Поред техничког дела који је подразумевао успостављање функционалности поменутог система, колега Радмиловић је учествовао у успостављању протокола за ефикасну израду оптичких и неоптичких структура. Током 2020. године Радмиловић је био део одобреног пројекта Фонда за иновациону делатност Доказ концепта, под називом: „Микрофлуидичка лабораторија на чипу за дијагностику неуродегенративних обољења". Неки резултати наведених истраживања су објављни у раду:

Radmilović, M. D., Murić, B. D., Grujić, D., Zarkov, B., Nenadić, M. Z., & Pantelic, D. V. (2021). Rapid Direct Laser Writing of Microoptical Components on A Meltable Biocompatible Gel. DOI:10.21203/rs.3.rs-1077113/v1 IF (2020) **2.084**; M22 **(Accept)**

Неки од резултата такође су објављењи на домаћим и међународним конференцијама:

- **Radmilović, D. M.**, Murić, D. B. & Pantelić, D. Micro-optical elements "a la carte". Book of abstracts of 13th Photonics Workshop (Conference), pp. 30 -30, Kopaonik, Serbia, 8– 12 March 2020, ISBN978-86-82441-50-2.
- **Radmilović, D. M.**, Murić, B. & Pantelić, D. Real time fabrication of microlens arrays for security applications, Book of Abstracts of 14th Photonics Workshop (Conference), pp. 36 - 36, Kopaonik, Serbia, 14 – 17March 2021,ISBN 978-86-82441-52-6.
- **Radmilović, D. M.,** Murić, B., Grujić, D., Zarkov, B., Nenadić, M. & Pantelić, D. Thermoresponsive, biocompatible hydrogels for rapid prototyping of biomimetic microchannels. Book of Abstracts of VIII International School and Conference on Photonics PHOTONICA2021& HEMMAGINERO workshop, pp. 100 - 100, 23 - 27 August 2021, Belgrade, Serbia, ISBN 978-86-82441-53-3
- Murić, B., Pantelić, D., **Radmilović D. M.,** Grujić, D., Zarkov, B. Modified chitosan for rapid fabrication odf microlenses. Book of Abstracts 15th International Conference on Fundamental and Applied Aspects of Physical Chemistry, pp. 75-75, 2021, Belgrade, Serbia.
- **Radmilović, D. M.,**Pantelić, D., Lazović, V. & Kolarić, B. Cellular noise of butterfly wing scales as a potential true random number generator. Book of abstracts of The Seventh International School and Conference on Photonics PHOTONICA2019, 26 August – 30 August 2019, Belgrade, Serbia, ISBN 978-86-7306-153-5
- **Radmilović, D. M.**, Rabasović, M., Šević, D., Pantelić, D., Kolarić, B. & R. Mouchet. Revealing the optical response of *Stegastes apicalis* fin parts using fluorescence spectroscopy. Book of abstracts of PHOTONICA2019 - The Seventh International School and Conference on Photonics, 26 August – 30 August 2019, Belgrade, Serbia, ISBN 978-86-7306-153-5

Optical and Quantum Electronics

Rapid direct laser writing of microoptical components on a meltable biocompatible gel

--Manuscript Draft--

Manuscript Number:	OQEL-D-21-01425R1
Full Title:	Rapid direct laser writing of microoptical components on a meltable biocompatible gel
Article Type:	Original Research
Keywords:	Laser writing; micro-optics; hydrogels; biocompatibility; security
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Abstract:	Microoptical components are coming of age in a wide range of applications: lab-on-a-chip, imaging, detection... There are a large number of fabrication technologies capable of producing high quality individual components and their arrays. However, most of them require high-end and costly equipment, complex and time-consuming fabrication, harmful chemicals, resulting in expensive final products. Here we present a technology capable of producing high quality microoptical components, using low-end direct laser writing on a biocompatible, environmentally friendly hydrogel, without any waste substances. The gel is locally and controllably melted while surface tension forces shape the optical component, following the laser beam profile. The process is so quick that a single microlens is fabricated in less than a second, and can be used instantly without any further processing. The technology is neither subtractive nor additive, and the base material is simply displaced producing a smooth surface. We have been able to fabricate individual microlenses and their arrays (positive, negative, aspheric), gratings and diffractive components. The technology is tested by generating unique, difficult to counterfeit QR-codes. Turnaround time is fast and makes the technology suitable both for rapid prototyping and serial production.
Response to Reviewers:	We have carefully read the reviewers' comments on our manuscript: Rapid direct laser writing of microoptical components on a meltable biocompatible gel submitted to Optical and Quantum Electronics. We thank reviewers for useful comments. We have acted accordingly to improve the quality of the paper. All changes

were marked red in the text.

Our response to Reviewer #1

1. Reviewer requested to comment on surface quality of microlenses.

In response, we have rewritten the paragraph above the figure 1 and added the following sentence:

“The surface of the dip is quite smooth (roughness is of the order of few nanometers) and can be faithfully approximated with a cubic polynomial, as verified by atomic force microscopy.”

2. Reviewer commented that Fig. 3a is not mentioned in the text. Also, Fig. 3b is not mentioned in the title of Fig. 3.

We made a corresponding correction, both in a paragraph above the figure 3 and its caption.

3. In reviewer’s opinion different photographs in Fig. 5 are not sharp.

The problem was due to reduction of picture size in previous submission. Photographs are enlarged now and closely correspond to how they look under the microscope.

4. In the next comment we were asked to show the meaning of HaCaT cells.

In the first paragraph of section 2.5 we have added that: “…immortalized keratinocyte cell line (commonly referred as HaCaT)”.
To further clarify the significance of this particular cell line, we have also added the following sentence: “A high capacity to differentiate and proliferate in vitro makes HaCaT cell lines extremely useful for the purpose (Schuerer (1993))”. A new reference (Schuerer (1993))) was added to the list.

5. Reviewer noticed that in Fig. 7 caption the last phrase is repeated.

We have corrected the caption and believe that it is now completely clear and understandable.

6. The final comment was in regard to misprints in the whole text.

We made every effort to correct the text. In the list of authors, the name of Dušan Grujić letter “j” was omitted, which is now corrected. Middle V. was added to Dejan Pantelić, which now reads correctly “Dejan V. Pantelić”.

Our response to Reviewer #3

1. Reviewer wanted to know which specific modality of nonlinear microscopy (TPEF, SHG and THG) was used in this research and why. Also, he asked what was the advantage over linear fluorescence techniques e.g., confocal?

In response we have added the following sentences near the end of section 2.3:

“Here we used two-photon excited fluorescence (TPEF) modality of NLM, which was particularly suitable due to large penetration depth of infrared excitation beam and reduced laser damage.”

2. We were asked if we considered and corrected the apparent depth distortion during the imaging with NLM, due to refractive index differences. To clarify the matter, we have added the final sentence to the section 2.3:

” We were interested primarily in the surface shape of microoptical components when refraction effects do not introduce significant distortion.”

	3. We were asked about the main difference of this study compared to previous research work (Krmpot et al., 2013)? In response we have added the following sentence to the beginning of the last paragraph of Introduction (section 1): Previously (Krmpot et al. 2013), we have analyzed optical properties of negative microlenses using NLM. We think that further text explains that the present study is much broader in its scope. It presents a new material which is bio-compatible, environmentally friendly and capable to generate microfluidic, micromechanic and microoptical components for a lab-on-a-chip-device. 4. Additional question was if the maximum diffraction-limited resolution can be achieved by the generated (positive) microlenses and if we investigated the presence of optical aberrations that could limit the performance of the lenses? This was completely beyond the scope of this paper, which is concentrated on material's versatility (capability to generate many micromechanical and microoptical components) and biocompatibility. Further consideration of aberrations and resolution will additionally expand the paper. 5. The final remark was that full stops were missing in the bullets of the "Conclusions" part of the manuscript. We have corrected that.

Rapid direct laser writing of microoptical components on a meltable biocompatible gel

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Abstract

Microoptical components are coming of age in a wide range of applications: lab-on-a-chip, imaging, detection... There are a large number of fabrication technologies capable of producing high quality individual components and their arrays. However, most of them require high-end and costly equipment, complex and time-consuming fabrication, harmful chemicals, resulting in expensive final products. Here we present a technology capable of producing high quality microoptical components, using low-end direct laser writing on a biocompatible, environmentally friendly hydrogel, without any waste substances. The gel is locally and controllably melted while surface tension forces shape the optical component, following the laser beam profile. The process is so quick that a single microlens is fabricated in less than a second, and can be used instantly without any further processing. The technology is neither subtractive nor additive, and the base material is simply displaced producing a smooth surface. We have been able to fabricate individual microlenses and their arrays (positive, negative, aspheric), gratings and diffractive components. The technology is tested by generating unique, difficult to counterfeit QR-codes. Turnaround time is fast and makes the technology suitable both for rapid prototyping and serial production.

Keywords Laser writing, microoptics, hydrogels, biocompatibility, security.

1 Introduction

There is a growing need for complex microoptical devices (Kemme 2009) and their use for micro-optoelectromechanical (MOEMS) and lab-on-a-chip applications. However, their fabrication is usually a complex, time consuming, multistep process, requiring several high-end technologies: microlithography (Grigaliūnas et al. 2016), embossing (Moore et al. 2016), femtosecond direct laser writing (Deng et al. 2019), diamond turning (Zhou et al. 2011;Zhang et al. 2020). These are the reasons why prevailing microfabrication methods are not suitable for individualized production or rapid prototyping. Also, materials used for microfabrication are complex and usually toxic. A huge volume of fabricated devices enhances the problem of safe and environmentally friendly disposal of microfabricated devices.

Among other materials, gels have attracted attention as a candidate material for MOEMS. There is a wide variety of gels with characteristic solid-liquid transition induced by coil to helix transformation (Taylor et al. 2017). The transition can be induced by temperature, chemicals or electric field. Not so many of them have good optical properties and only a few appropriate ones are easy to fabricate, nontoxic and environmentally friendly. Optical gels (Duarte-Quiroga and Calixto. 2000; Li et al. 2019) have been used to manufacture dynamical and responsive microlenses. However, their response is slow and chemistry complex (Guan and Zhang 2011).

Microlenses have found application niches for illumination (Lee et al. 2013), imaging (Zhang et al. 2020) and, in particular, security (Walger et al. 2020). Applied technologies are advanced and complex favoring mass production and precluding individualization of security features (Jiang et al. 2019).

Previously (Krmpot et al. 2013), we have analyzed optical properties of negative microlenses using NLM. Here we present a technology based on nontoxic, environmentally friendly gels which are locally melted by direct laser writing. Our aim was to develop a material that is optically transparent, easily and instantly meltable by localized irradiation, durable and made from ordinary "kitchen" chemicals (by E number classification of food additives). We describe material properties, its biocompatibility, analyze the process of local laser-induced melting, demonstrate its capabilities for security purposes and envision their further use for microfluidic and lab-on-a-chip applications.

2 Materials and methods

2.1Preparation of photo-meltable gel

Previously we have used gelatin plasticized with tot'hema (an oral solution for anemia treatment) and sensitized with eosin Y (a red fluorescent dye with absorption maximum at 530 nm) to produce microoptical components (Murić et al. 2007; Murić et al. 2008). We were able to manufacture negative (concave) microlenses, but the problem was gradual darkening of the material. That is why we replaced a commercial tot'hema with a water solution composed of several in gredients acting as –plasticizers, humectants, and preservatives. This solution (designated PS for brevity) consists of: 0.2 ml of glycerol, 0.3 g of sucrose, 8 mg of glucose, 2 µl of polysorbate 80, 6 mg of citric acid, and 2 mg of sodium benzoate (everything is expressed per 1 ml of solution). The addition of PS improves mechanical and optical properties of the gelatin layer (elasticity, durability and stability, optical transparency…).

After swelling of gelatin in deionized water for one hour, and heating at 50^{o}C in the water bath (Vela™, Cole Parmer), 5% aqueous gelatin solution was prepared. Following, 0.01 g of sodium chloride and 0.16 ml of PS were further added (with stirring) to prevent gelatin layer crystallization and breaking. The preparation of photo-meltable gel (PMG) is concluded by adding 20 µl of eosin (2% aq. sol.). Quantity of all added components is expressed per 1 ml of gelatin solution. Finally, the PMG solution was centrifuged (Cole Parmer 17250-10 at 3400 rpm/min) ~~to~~ remove all particulates and impurities.

A PMG layer was prepared by the gravity settling method i.e., by pouring a constant volume of the prepared solution onto precisely leveled and well cleaned microscope glass slide bounded by a Plexiglas frame. After gelation, layers were left in the dark overnight, under ordinary environmental conditions (T=25^{o}C, RH=50-60%). During that time, a certain amount of water evaporated from the layer, as verified gravimetrically. After reaching the equilibrium value, the water content remains constant. The thickness of the dried layer depends on the amount of poured solution and can be chosen anywhere between several tens of microns up to several millimeters or even centimeters. In our experiments, layer thickness was kept at 100 µm.

2.2 Direct laser writing system

We used a home-made laser writing device (Zarkov et al. 2012) operating at 488 nm laser (Toptica iBEAM SMART with a maximum power of 100 mW). The laser beam is focused by the long working distance objective (Mitutoyo, 20x0.42 NA). Compact, color scientific CMOS camera (Thorlabs CS505CU5 Megapixel), was used to position the PMG layer at the desired position with respect to the focal point. A coordinate stage (Ludl BioPoint2, resolution 50 nm, repeatability 2 µm) with G-code enabled Arduino microcontroller was used to move the layer with respect to the laser beam. G-code (a standard programming language for CNC machines (Walger et al. 2020) was used to control movement with adjustable speed. Appropriate software was written to coordinate and synchronize the layer movement with laser switching and intensity adjustment.

2.3 Nonlinear microscopy of microoptical surfaces

To characterize the structure of microoptical components, we used a home-made nonlinear microscope (NLM) (Rabasović et al. 2015) equipped with a femtosecond TI: Sapphire laser (Coherent, Mira, 900F). The pulse duration is 160 fs with a 76 MHz repetition rate and average power of 100 mW. A galvo-mirror scanning system was used for raster-scanning of the samples in a commercial microscope (Leica). In order to fill the entrance pupil of microscopic objective (Carl Zeiss, 20x0.8 air) the laser beam was expanded. A tube lens produces an image on the photomultiplier tube (PMT). Images were acquired and processed using dedicated software. The spatial resolution of scanning system was 0.6 – 0.9 µm in lateral direction, while the axial resolution was 2.1 µm. The device turned out to be particularly suitable for analysis of generated microstructures due to its ability to "see" internal structure of the material. Here we used two-photon excited fluorescence (TPEF) modality of NLM, which was particularly suitable due to the large penetration depth of infrared excitation beam and reduced laser damage. We were interested primarily in the surface shape of microoptical components when refraction effects do not introduce significant distortion.

2.4 Thermal analysis of micro-component manufacturing process

Throughout the research we used a thermal imaging to monitor thermal effects of the laser radiation during micro-component manufacturing. A commercial thermal camera (FLIR A65) with 640x512 pixels spatial resolution, 30 fps speed, thermal resolution/NETD 50 mK and 7.5-13µm spectral range was utilized to record temperature and its spatial distribution. We used an additional IR (ZnS) lens, placed in front of the germanium camera lens, to further magnify the thermal image of a laser-melted zone.

2.5 *In vitro* biocompatibility testing

Cytotoxicity of tested PMG was determined on spontaneously immortalized keratinocyte cell line (commonly referred as HaCaT) using crystal violet assay as described previously (Stojković et al. 2020) with some modifications. A high capacity to differentiate and proliferate in vitro makes HaCaT cell lines extremely useful for the purpose (Schürer et al. 1993)). HaCaT cells were grown in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin and streptomycin (Invitrogen), at 37°C in a 5% CO_2 incubator. Forty-eight hours before treatment, cells were seeded in a 96-well microtiter adhesive plate at a seeding density of 4×10^3 cells per well. PMG was dissolved in 0.01 mM PBS to a final concentration of 8 mg mL^{-1}. After 48 h, the medium was removed and the cells were treated for next 24 h with various concentrations of the dissolved gel in triplicate wells. Subsequently, the medium was removed; the cells were washed twice with PBS and stained with 0.4% crystal violet staining solution for 20 min at room temperature. Afterwards, crystal violet staining solution was removed; the cells were washed in a stream of tap water and left to air dry at room temperature. The absorbance of dye dissolved in methanol was measured in a plate reader at 570 nm (OD_{570}). The results were expressed as relative growth inhibition (GI_{50}) rate (%) indicating 50 % inhibition of proliferation of HaCaT cells when compared with untreated control. Experiments were performed in triplicate for each concentration of the samples and three independent experiments were performed. The criterion used to categorize the antiproliferative activity of PMG to HaCaT cell line was as follows: $IC_{50} \leq 20$ µg mL^{-1}= highly cytotoxic, IC_{50} ranged between 31 and 200 µg mL^{-1}= moderately cytotoxic, IC_{50} ranged between 201 and 400 µg mL^{-1}= weakly cytotoxic, and IC_{50}> 401 µg mL^{-1} = no cytotoxicity (Stojković et al. 2020).

3 Results

3.1 Material characterisation

PMG is designed to be sensitive to the wavelength of the laser used in this research (488 nm) in order to enable photo-induced melting. The focused laser beam locally heated the PMG above its melting temperature and surface tension produced a concave dip. The process was observed using a thermal camera. Temperature field is localized to the vicinity of a laser beam. Within 1/5 s, the temperature reaches a maximum and decays in spite of the material being still irradiated. This is due to the bleaching of eosin and the corresponding reduced absorption. This is associated with the layer turning transparent instead of red.

As can be seen in Fig. 1(a) (recorded using TPEF), the laser-produced shape is a concave asphere, while its diameter and optical characteristics depend on the laser power, irradiation time and the laser beam size (Fig. 1(b)). The surface of the dip is quite smooth (roughness is of the order of few nanometers) and can be faithfully approximated with a cubic polynomial, as verified by atomic force microscopy. As a result, the dip acts as a high-quality negative-power microlens (Krmpot et al. 2013).

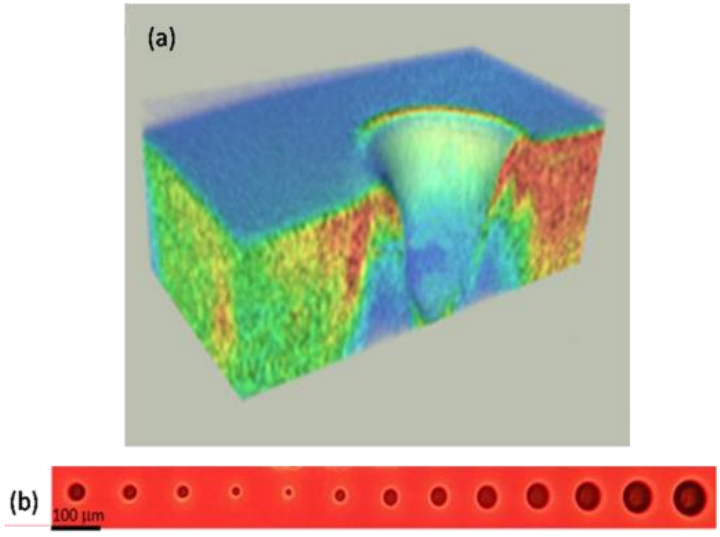

Fig. 1(a) 3D image of a microlens shape recorded by NLM, (b) Optical microscope image showing how the size of a microlens strongly depends on the size of the laser beam.

The sensitivity threshold for a 100 μm thick PMG layer is 10^4 W/cm^2, which we achieved with only 7.5 mW of laser power. This limit depends on the layer thickness, PS and dye concentrations, focus depth. It is important to mention that layer is also sensitive below 7.5 mW, but the material is only bleached (without lens formation).

As can be seen, exposure and bleaching are intertwined. We have observed the process by measuring the decrease of PMG fluorescence during irradiation i.e., energy absorbed by the material is dissipated through fluorescence (see Fig. 2). As a consequence, after a certain irradiation time, the layer bleaches so much to drop the temperature below the melting point. In that case, material cannot remain liquid and "freezes" its lens-like shape.

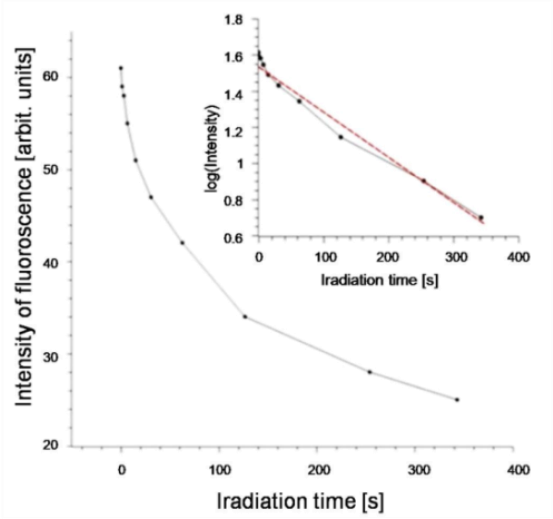

Fig. 2 Bleaching of material observed as a decrease of fluorescence intensity under irradiation with 0.05 mW focused laser beam. Fluorescence decay is represented on the linear scale. The same graph, presented on a logarithmic scale, is shown in the inset.

We have developed a simplified thermal model which describes temperature T and its dependence on initial temperature T_0:

$$T = T_0 + \frac{A}{Kmc} + \frac{B}{C-A} \cdot exp(-At) - (\frac{B}{C-A} + \frac{A}{Kmc})exp(-Ct) \quad (1)$$

The model includes constants A, B, C and D which depend on PMG layer properties: conductivity, specific heat and thickness. Additional constant K describes bleaching speed, while m is mass of the irradiated gel and c is specific heat (see Appendix).

Calculations have shown close correspondence with experimentally recorded temperature variation (compare Figs. 3(a) and 3(b)) and correctly describe initial temperature rise with subsequent exponential temperature drop (Eq. 1).

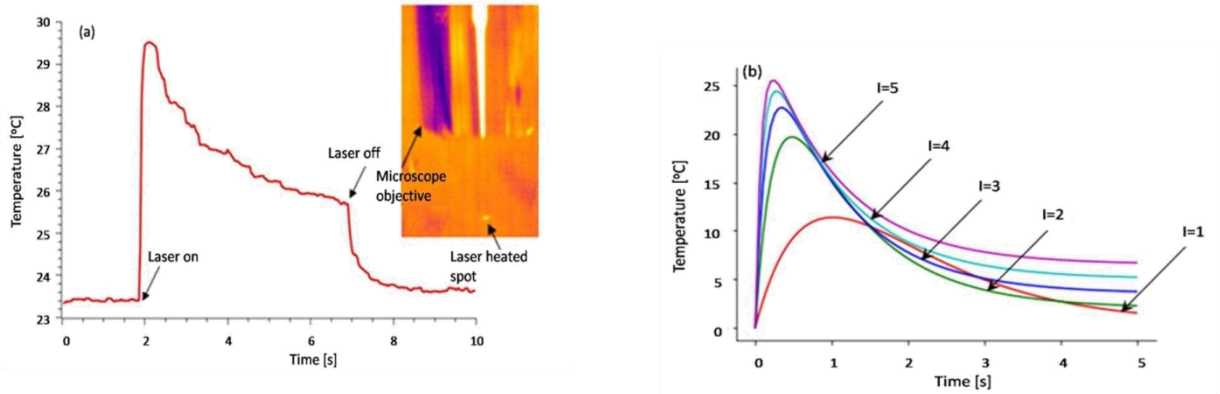

Fig. 3(a) A temperature profile during constant-power irradiation (5s, 15 mW laser power) of PMG layer, recorded by a thermal camera. Temperature decreases due to bleaching of eosin. After turning the laser off, temperature quickly drops to that of the environment. (b) Theoretically calculated temperature variation (using Eq. (1)).

It is important to note that if the laser radiation is too intense it might occur that material can be bleached to fast so that melting temperature cannot be reached. We have experimentally observed this particular behaviour, which leaves material bleached without microlens being produced.

That is why we were forced to closely control the exposure in order to preclude this kind of memory effect. The effect is important if optical micro-components are too close, because the first one bleaches a certain space in its vicinity. If we try to write the next micro-component, exposure must be increased to compensate for a significant drop of absorption due to bleaching.

However, in the following we describe how more complex surface shapes can be manufactured by carefully controlling the laser focal position, beam pattern and exposure. We have manufactured good quality positive microlenses by making an arrangement six polygonally positioned spots. The material left in the center of a polygon acqures spherical surface which acts as a positive (convex) microlens. This can be seen in a NLM image of a material (Fig. 4).

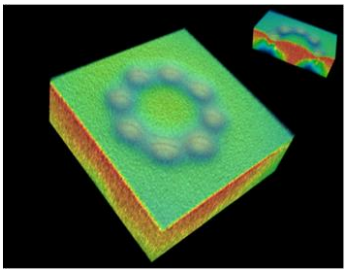

Fig. 4 A NLM image of a positive microlens produced by irradiating the PMG layer at the vertices of an octagon. 3D view together with its orthogonal cross section (inset) is shown.

We were able to efficiently manufacture arrays of microlenses (Fig. 5(a)) with rather good imaging properties (Figs. 5(b) and 5(c)).

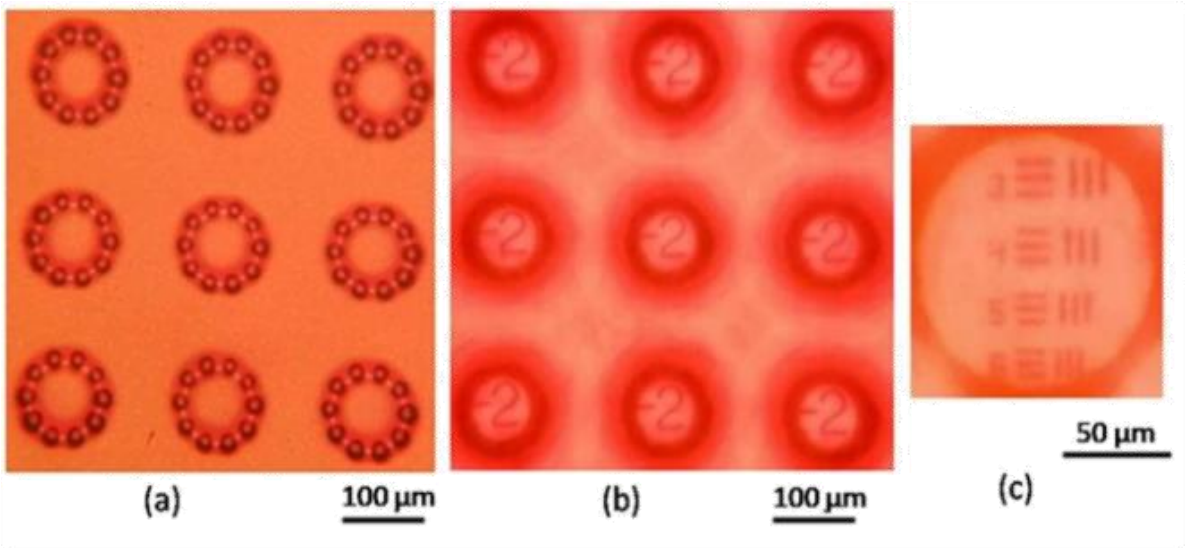

Fig. 5 (a) Reflection image of an array of 3 x 3 positive power microlenses produced by irradiating PMG layer at the vertices of an octagon, (b) Transmission images produced by the array, (c) A resolution chart as seen through the microlens.

The best results were obtained with the octagonal arrangement of dots. Their radius of curvature and the corresponding focal length can be controlled by the diameter of a polygon (Fig. 6). We have measured the spatial resolution of the PMG layer by writing a series of gratings and found that we can manufacture up to 120 lp/mm.

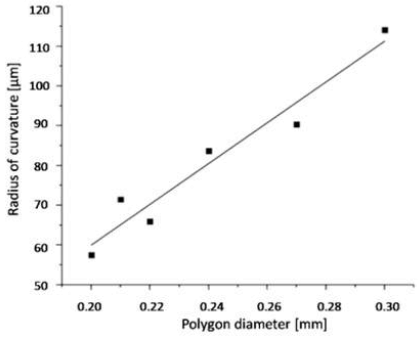

Fig. 6 A linear relation between the microlens radius of curvature and the diameter of a polygonal arrangement of dots.

3.2 Positive and negative microlenses for security

Microlenses have significant security applications for document protection (Walger et al. 2019; Seidler et al. 2014; Walger et al. 2020). In standard implementation, their effectiveness is based on Moiré effect between a microlens array and a, suitably designed, micro-pattern or another microlens array. Superposition of two overlaid arrays produces dynamic effects similar to holograms – i.e., the resulting image varies with respect to observation direction.

Difficulty of counterfeiting such a pair of arrays stems from tight tolerances of microlens parameters and the necessity of their strict alignment. While this seems to be an attractive security feature it is technologically complex to achieve in practice. That is why the corresponding technologies are economically viable only through mass production (usually by printing or embossing). Production of individualized, unique, hard-to-copy security elements is thus difficult and impractical.

Here we show that the technology presented here offers another way to produce unique security elements quickly and easily (on the fly) by changing microlens parameters (position, sag, diameter, focal length, mutual position). We demonstrate the principle by producing a microlens-based QR-code (see Fig 6).

Each dot of a standard 21 x 21 QR-code is a negative microlens, except for one or several selected, which are a positive. Security features are focal lengths of individual microlenses (either positive or negative) of a QR-code.

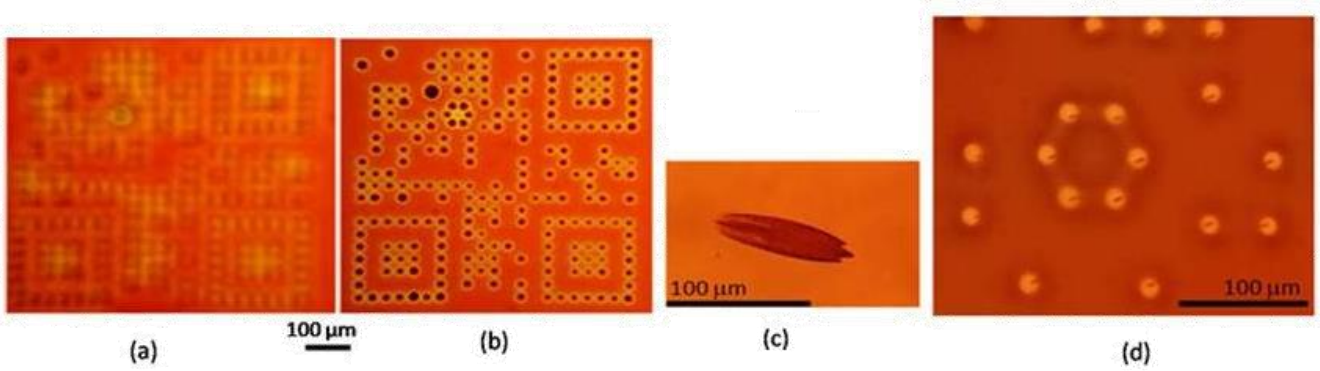

Fig. 7(a), (b) A microlens-based QR-code in two focal positions. (c) An image of butterfly wing scale observed through one of a QR-code lenses. (d) Multiple images of butterfly wing scale from Fig. (c) produced by QR code microlenses.

Focal length of each microlens is revealed by placing a closely positioned micro-sized object while detecting the size of its image. Here we used a butterfly wing scale as such object, positioned on the other side of a microlens substrate. Due to the wide view field of negative microlenses, image of an object is seen across several microlenses in shifted positions – yet another, difficult to copy, feature.

3.3 *In vitro* cytotoxicity of hydrogel samples towards HaCaT cells

Cytotoxic effects of PMG were investigated using HaCaT cell culture. To evaluate the cytotoxic effect of the PMG dissolved in 0.01 mM PBS on HaCaT cells, the crystal violet assay was performed. Relative growth rate of HaCaT cells in the presence of different concentrations of tested sample compared to *untreated* control is presented in Figure 8. Tested sample was evaluated as non-toxic to the HaCaT cell line with respective IC_{50} values of > 400 mg/mL, a concentration which is considered as the limit of toxicity (Stojković et al. 2020).

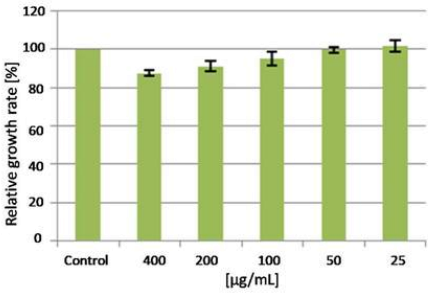

Fig. 8 Relative growth rate of HaCaT cells in the presence of different concentrations of PMG

4. Discussion

Microlens fabrication enables efficient control of each individual microlens by controlling a number of process parameters: laser beam size, shape, power, angle, speed and exposure, as well as physical/chemical properties of the PMG layer. There are certain limitations, drawbacks and possibilities which will be discussed in this section

Manufacturing speed of microlenses is limited by the laser energy density (determined by the laser power and focal point size), absorbance, viscosity and surface tension of melted gel. This is a complex process difficult to model in a simple way. However, we were able to find appropriate conditions experimentally. Laser powers above 7.5 mW and exposure times longer than 100 ms gave us complete control of the process and production of predictable lens size and profile.

The material is soft and elastic due to the presence of a plasticizer. Its stress-strain behavior depends on the PS concentration, as shown earlier in the case of commercial tot'hema, when the corresponding Young's moduli were between 1 and 10 MPa (Murić et al. 2013). Also, for high-concentration (30%) of tot'hema, more than 200% elongation was achieved. In the case of PMG, the above properties are retained. Elasticity and stretchability can be utilized to manufacture tunable optical components.

On the other hand, the softness makes material sensitive to mechanical scratching and damaging. That is why it must be protected by an additional mechanically resistant layer. Alternatively, the material can be hardened by simply placing in water to let plasticizer diffuse out.

We observed the layer's surface under the polarizing microscope and noticed that there were no internal, residual stresses (material is homogeneous).

The material remains photosensitive for a long time even if exposed to normal laboratory conditions. Its shelf life is mainly determined by slow evaporation of water and photo-bleaching of sensitizer. If the atmosphere is too dry, concentration of water diminishes and constituent chemicals start crystallizing and the layer attains a milky appearance. In that sense, it is preferable to keep material in a humid and light-tight container. From the practical experience, material processing can be performed under normal lighting without special precautions or dimmed light. However, we have a few years old gelatine layers, stored under normal laboratory conditions, which are still photosensitive and we were able to produce good quality microlenses. They are very stable, too, and the image quality remains constant during many months and even years under normal conditions. Of course, material has to be protected from scratches and dust as in the case of all the other optical surfaces.

The material presented here is not unique. Instead of eosin, we have tried gelatin sensitization with several natural fluorophores: anthocyanin, betanin and several other food dyes with excellent results. Additionally, we have tried other gels based on chitosan and pectin with very promising results. That is why we can claim that many other gels, humectants and sensitizers can further enhance microlens production speed and surface quality.

Depending on how material is prepared, buckling induced by evaporation of solvent produces unpredictable surface pattern. Even then, re-melting of material by the laser beam flattens the surface and produces good optical component. As a result, a combination of random buckling surface structure and regular optical components produce uniquely and nonreproducibly complex security features.

Yet another possibility stems from photo damage of the material, which occurs above certain power density threshold. In that case, material carbonizes, producing strongly localized damage zone in a center of the laser spot. Interestingly, this does not preclude microlens imaging, but adds a new feature to a security component.

Here we emphasize that the technology described here is neither additive nor subtractive because no material is added or removed. It is important to note that all the substances used are not volatile and the melting temperature of the material is below 50^{o}C, so that water evaporation is negligible. However, do to the melting, surface tension compresses and densifies the material. This is witnessed by the increased intensity of fluorescence at the circumference of the cavity. That is why the volume of the laser-induced dip is larger than the volume on the edge (Murić et al. 2009).

The material is complex mixture of nontoxic chemical aiming to fulfill several requirements: preventing crystallization, retention of constant amount of water, reducing the melting point of the gel, to enable efficient flow during laser melting, retaining plastic and elastic properties of the material, increasing the laser energy absorption. Proper composition was found experimentally and found to be stable before and after microlens fabrication.

Material has certain drawbacks too. It is soft, and can be easily damaged if unprotected. On the other hand, this property can be used to detect tampering and produce tamper sensitive tags. Material surface is sticky and dust particles easily adhere to its surface. Therefore, cleanliness is important factor in practical usage of the material.

We used gelatin as a base material, but the working principle is universal and can be applied to any material which can be locally melted, without damage on a sufficiently low temperature (preferably below 100 ^{o}C). In that respect we tested chitosan, too with quite good results which will be presented in the future publications.

Applications are not limited to microlenses and arbitrary structures can be manufactured such as microchannels, diffraction gratings, holograms (see Fig. 9).

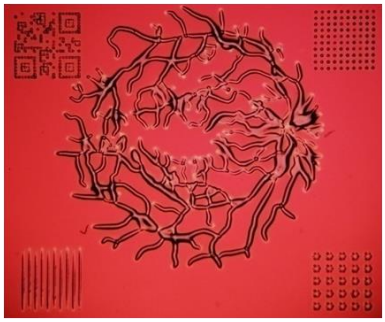

Fig. 9 A range of microoptical structures which can be fabricated on the PMG layer – retinal vessel model (center), QR-code (top left), negative microlens array (top right), positive microlens (bottom right) array, grating (bottom left).

5 Conclusions

We have presented a new, gel-based, material suitable for fast and efficient generation of a wide range of microoptical and micromechanical components.

There are several advantages of the proposed method:

- Cheap lasers can be used as long as they have a circular laser beam profile and 2% power stability within the millisecond time interval.
- Chemicals used to produce the PMG are non-poisonous at the stated concentrations, as verified by biocompatibility tests.
- Fabrication time is fast enough to enable rapid prototyping of on-demand components.
- A variety of optical and micromechanical components can be fabricated within a single manufacturing operation.
- Components require no further processing and can be used immediately following fabrication.

Author Contributions

B. M. synthesized a photo-meltable material. D.G., B.Z. and D.P. constructed a laser writing device used through this research. D.P. D.G. and M. R. wrote control software. B.M. D.P. and M. R. tested material properties. M.Z.N. tested *in vitro* biocompatibility and microbial susceptibility toward synthesized gel material. D. P. Developed a thermal model of material bleaching. D.P. B.M. and M.R. jointly wrote the manuscript.

Conflicts of interest

There are no conflicts to declare.

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Београд, 21. марта 2022. године
06 Бр: 06-4047/III-4852/4-21
JKJ

На основу члана 50. ст. 4. тач. 2 Статута Универзитета у Београду Статута Универзитета у Београду („Гласник Универзитета у Београду", бр. 201/18, 207/19, 213/20, 214/20, 217/20 и 230/21), а на предлог Већа за студије при Универзитету од 7. марта 2022. године, Веће за интердисциплинарне, мултидисциплинаре и трансдисциплинарне студије на седници одржаној 21. марта 2022. године, донело је

О Д Л У К У

1. ОДОБРАВА СЕ израда докторске дисертације под насловом: **„Интеракција ултракратких ласерских импулса са молекулом хемоглобина и примена савремених техника нелинеарне микроскопије у осликавању еритроцита"**, кандидата **Михајла Радмиловића** (докторске студије: Биофотоника)

2. За менторе се именују:
1. др Александар Крмпот, виши научни сарадник, Институт за физику (биофотоника, напредна микроскопија)
2. др Ивана Дрвеница, виши научни сардник, Институт за медицинска истраживања (фармацеутска технологија, биофизика)

ПРЕДСЕДНИК
ВЕЋА ЗА ИМТ СТУДИЈЕ
Проф. др Дејан Филиповић

3. Закључак и предлог

Михајло Радмиловић испуњава све услове за избор у звање истраживач сарадник предвиђене Правилником о стицању истраживачких и научних звања Министарства просвете, науке и технолошког развоја Републике Србије. Кандидат успешно примењује знање које је до сада стекао у решавању различитих научно-истраживачких проблема. На основу његових научних резултата објављен је 1 рад у истакнутом међународном часопису и 9 саопштења на домаћим и међународним конференцијама. На Већу докторских академских студија Универзитета у Београду на прогаму Биофотоника одржаном 21.11.2021. године прихваћен је наслов теме докторске тезе Михајла Радмиловића под насловом „Интеракција ултракратких ласерских импулса са молекулом хемоглобина и примена савремених техника нелинеарне микроскопије у осликавању.еритроцита".

Имајући у виду квалитет његовог научно-истраживачког рада, као и достигнути степен научне компетентности и независности у раду, изузетно нам је задовољство да предложимо Научном већу Института за физику у Београду избор Михајла Радмиловића у звање **истраживач сарадник.**

У Београду, 21. март 2022. године

Чланови комисије:

др Михаило Рабасовић
научни сарадник
Институт за физику Београд

др Александар Крмпот
виши научни сарадник
Институт за физику Београд

др Ивана Дрвеница
виши научни сарадник
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