A photoacoustic monitoring of circulating tumor cells in mice with melanoma

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Abstract

Circulating tumor cells (CTCs) are cancer cells that have entered a blood or lymph stream from a primary tumor or its metastasis. In this work we demonstrate results of a three-week monitoring of CTCs in mice with a melanoma induced by B16F10 cell inoculation. The monitoring was performed using a custom photoacoustic flow cytometer (PAFC) adapted for mice.

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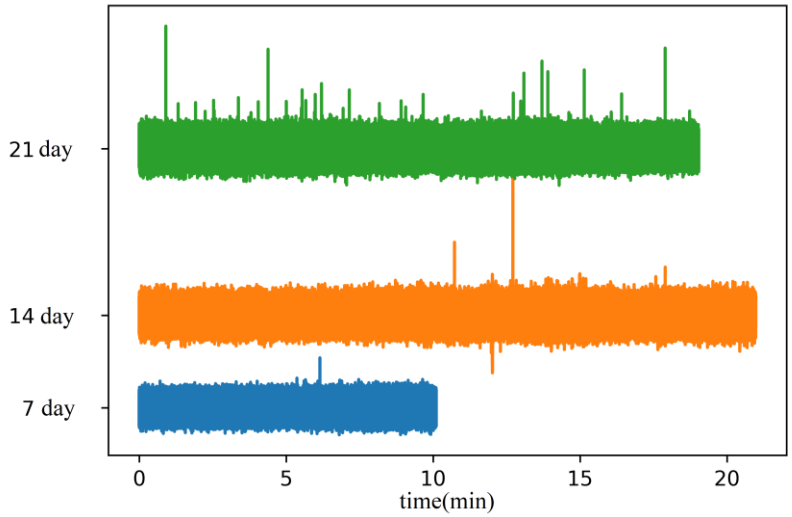
Results

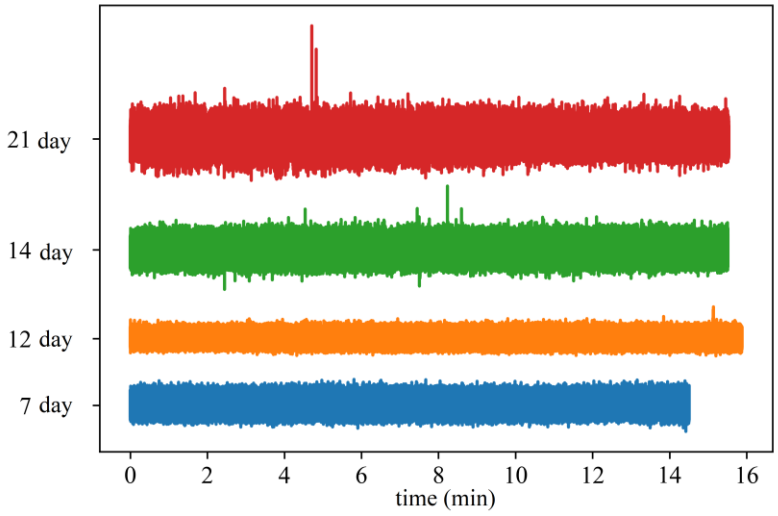
The results of a three-week monitoring of CTCs in mice with tumor are presented on this page. If PA signal is above the background level, it is most probable caused by CTC. The large number of high PA signals on the traces corresponding to CTCs shows the high concentration of melanoma cells in mouse's blood.

After 21st day of monitoring all mice were sacrificed for further histology analysis.

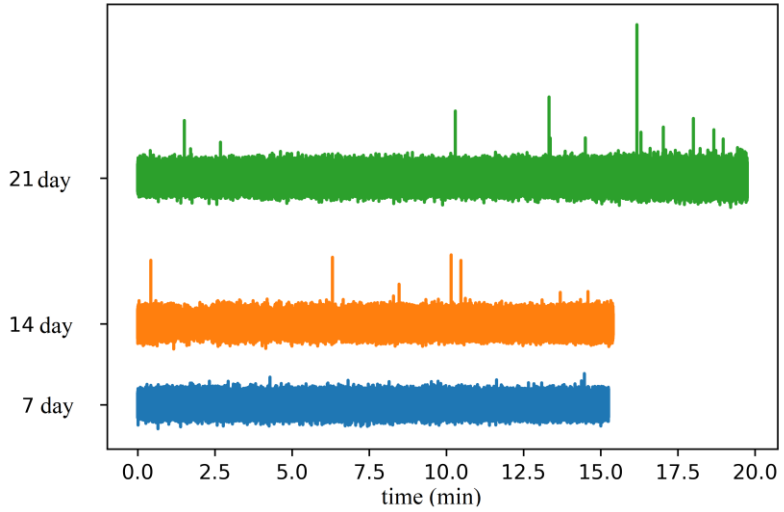
Mouse 2



Mouse 1



Mouse 3



Experimental setup

A simplified scheme of our PAFC system is shown in Figure 1. The optical subsystem of PAFC device consists of light source - ytterbium fiber laser (YLPP-1-150V-30, IPG Photonics Corp.), the periscope build from three dielectric laser line mirrors (NB1-K13, Thorlabs, USA) and focusing spherical lens (SL, LA1131-B, Thorlabs, USA). Laser pulse energy in all experiments was 250 mJ. The ultrasound detection subsystem consists of custom spherical US sensor with broadband amplifier (AH-2020, Onda, USA) and the ADC board ATS-9350 board (12 bit, 500MS/s, AlazarTech).

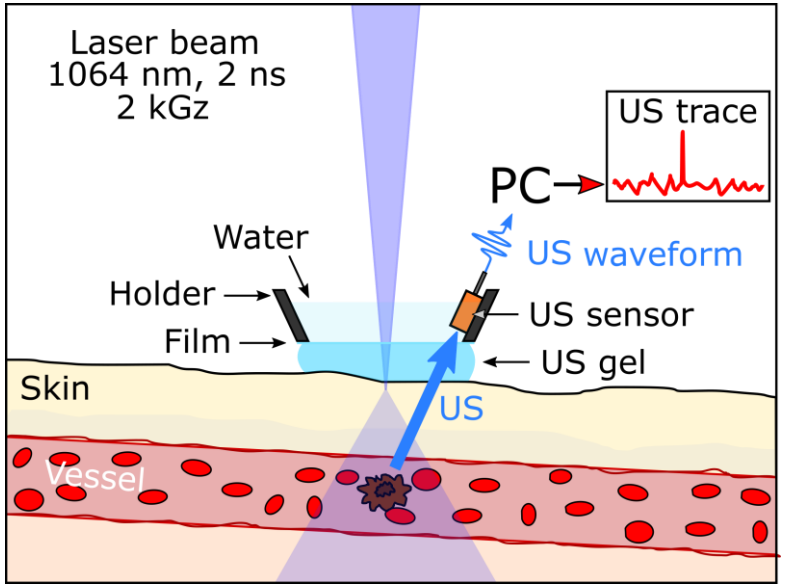


Figure 1. Experimental setup

Animal preparation

Laboratory animals were kept according to instructions according to the instructions of the Saratov State Medical University (№ 82 dated January 30, 2020). The care protocol does not contradict international principles of biomedical animal studies of the 1985 Geneva Convention. Mice from The Saratov State Medical University were studied under active University Institutional Animal Care and Usage Committee protocols. Mice (BALb/c, n=12) were selected for the experiment. They were 6-8 weeks old and weighing 20-25 g. The surgical part was performed using general anesthesia, intraperitoneal injection of drugs (a mixture of Zoletil (40 mg per kg, 50 µl, Virbac SA, Carros, France) and 2% Rometar (10 µl and 10 mg per kg, Spofa, Czech Republic)). At the end of the experiment, the animals were sacrificed by overdose of anesthesia.

Cells suspension of the B16F10 was inoculated subcutaneously of lang. The suspension was prepared according to the standard protocol for working with cell cultures. For each animal got in 100 thousand cells B16F10 in 20 µl in PBS buffer. Viability rate was 87-95%.

Cells were injected into the dorsal flanks to induce tumor formation. Subsequently, the mice received intraperitoneal injections of anesthesia. The CTC tracking process was performed on the femoral blood vessels non-invasively.