Microdissection, catapulting, and microinjection of biologic specimens with femtosecond laser pulses

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Abstract: We demonstrate the potential of amplified femtosecond laser pulses to improve microdissection of histological specimens and microinjection of cells as compared to state-of-the-art commercial instruments in which ns-pulses from a nitrogen laser are used.

OCIS codes: (000.1430) Biology and medicine; (170.1530) Cell analysis; (320.7090) Ultrafast lasers; (320.2250) Femtosecond phenomena; (170.1020) Ablation of tissue; (350.5400) Plasmas

Introduction

Microdissection with subsequent catapulting of the specimen by a defocused laser pulse is a modern method to prepare single cells or small cell clusters out of histological slides and other biological/medical preparations [1, 2, 3]. Usually, focused nanosecond UV-laser pulses with several microjoule pulse energy are applied to cut out the cell/cells of interest, and subsequently a single pulse of slightly higher energy is focused under the dissected area for contamination free transport into a sample vial. The latter technique is known as “laser pressure catapulting” [2]. After catapulting, the samples can be characterized by molecular analysis with hardly any interference by impurities. In order to facilitate catapulting, histological specimens or living cells are placed on a thin UV-absorbing foil. Despite the great acceptance and wide application of this method, the working mechanisms has been revealed just recently [4]. Investigations of the absorption of the used materials, the relevant thermal parameters of the polymer foil and histological specimens, the disintegration temperature, the temperature-dependent heat capacity, and the heat conductivity provide further insights into the mechanisms underlying microdissection and laser catapulting [5, 6]. It was concluded that both, dissection and catapulting, rely on plasma formation supported by linear absorption. This finding suggests the possibility to refine the laser effects by using ultra short laser pulses instead of ns-pulses because the threshold for plasma formation drops with decreasing pulse duration. It was shown by König et al. that a microdissection precision of 200 nm can be achieved by using nanojoule and sub-nanojoule femtoseconds pulses of 750-850 nm wavelength and a high repetition rate of 80 MHz[7]. The aim of the present study is to create a scientific basis for further improvements of superfine microdissection and microinjection of cells.

Preliminary Results and Discussion

1. Physical parameters

The P.A.L.M. microbeam system consists of a nitrogen laser (337 nm, LSI, CA, USA) emitting a 3 ns laser pulses that are coupled into a Zeiss Axiovert 200 inverted optical microscope equipped with a motorized translation stage. The laser system has a fairly poor beam quality with a full angle beam divergence of 0.5 mrad and a beam area of 35 mm^2, which corresponds to a beam quality (M^2) of approx. 14. This leads to a spot size 6-times larger than the diffraction limited focus. For comparison, pulses form a regeneratively amplified femtosecond laser (HighQ Laser Production, Austria) were coupled into the microscope via an optimized, custom designed optic (Sill Optics, Germany). The specifications of the femtosecond laser pulses are as following: beam quality M^2 1.2, beam area approx. 1 mm^2, 380 fs pulse width, 1040 nm wavelength, optional second or third harmonic. This corresponds to a much smaller spot size than achievable with the nitrogen laser. Both smaller spot and shorter pulse duration allow for a dramatic reduction of the threshold energies for ablation and plasma formation.

2. Microdissection and catapulting

Microdissection was performed on a 5 µm thick paraffin-embedded histological specimens of human prostate mounted on a polymer foil that was supported by a standard glass slide for microscopy. The polymer foil consists of polyethylenenaphthalate (PEN) with 1.4 µm thickness. This foil serves as absorber of the laser energy during microdissection and maintains the mechanical integrity of the specimen during laser catapulting [2].

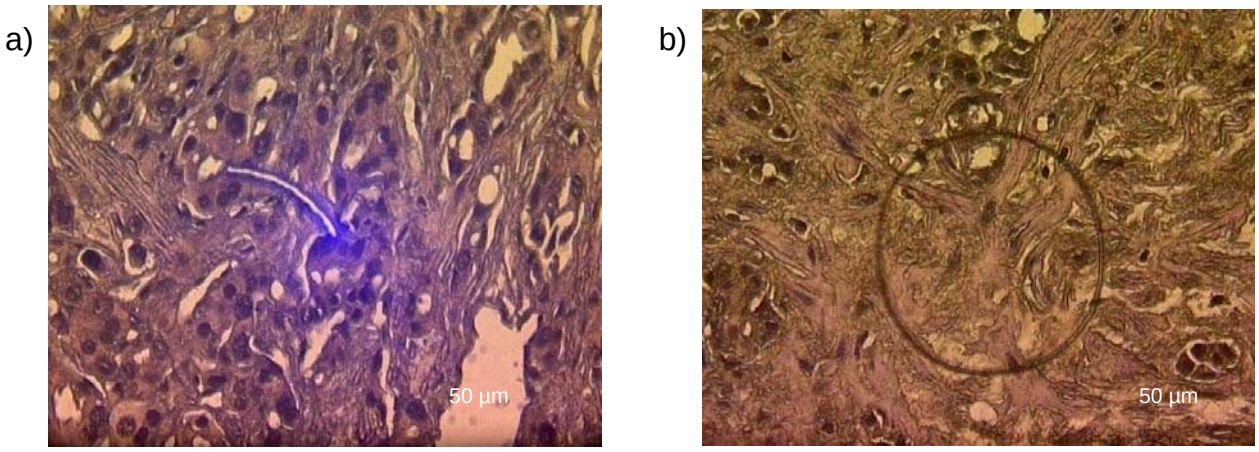

Figure 1. Microdissetion of a paraffin-embedded thin sectioned specimen of human prostate a) with the nitrogen laser, and b) the third harmonic of the femtosecond laser irradiation

Figure 1 compares microdissections using the commonly used nitrogen laser emitting 337 nm wavelength (Figure 1 a) or the third harmonic of the femtosecond laser corresponding to wavelength of 346 nm (Figure 1 b). A 40x-microscopic objective with a numerical aperture of 0.6 was used because the Rayleigh length is, thus, in the range of the specimen thickness. While the width of microdissection is about 2 µm for the dissection with the nitrogen laser under optimized conditions, it is much less than 1 µm when pulses of the femtosecond laser are used. Subsequently, it is possible to catapult small specimens with fs-laser pulses. As these experiments are preliminary, the conditions of fs-laser microdissection and catapulting are not yet optimized. Improved results will be presented on the conference.

3. Microinjection

Microinjection was performed on HEP2-cells grown in a modified duplex membrane petriperm dish (DuplexDish, P.A.L.M. Microlaser Technologies, Germany). These dishes are designed for microdissection and laser catapulting of living cells [8]. For these dishes the conventional plastic bottom is exchanged by two membranes. The bottom membrane is an air-permeable teflon membrane of approx. 20 µm thickness and little absorbance at the used UV-laser wavelengths, and the upper membrane is the 1.4 µm thick PEN-foil described in the microdissection part. This PEN-foil serves as support for the growing cells and shows a high absorbance at the used laser wavelength. Figure 2 - a bright-field microscopic image - shows that adherent cells could be cultivated to a confluent monolayer.

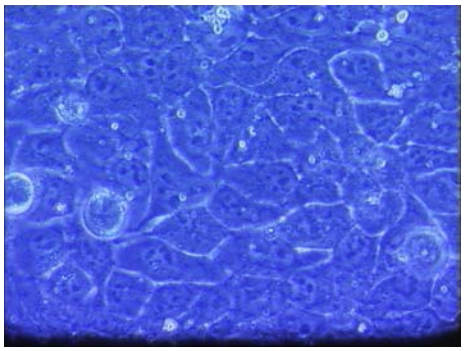

Figure 2. Bright-field microscopic image of HEP2-cells grown to a confluent monolayer on a duplex membrane dish.

For microinjection, a marker solution was stored under little pressure between the two membranes. Subsequently, up to 5 laser pulses were focused onto the UV-absorbing PEN-foil to generate a hole both in the PEN-foil and the cell membrane in order to load the cell with the marker substance. Using propidium iodide (PI) as marker, the microinjection can be screened by enhanced fluorescence of PI intercalated with nucleic acids. Figure 3 presents a successful microinjection. The upper row shows bright-field microscopic images and the bottom row the PI-fluorescence images, respectively. The location of the membrane perforation is marked by the circle in Figure 3 b). The selected cell does not show any PI-fluorescence before the microinjection and is stained immediately after

release of the microinjection pulses. As PI is used to stain dead cells, other marker substances will be tested and presented on the conference to provide unambiguous results.

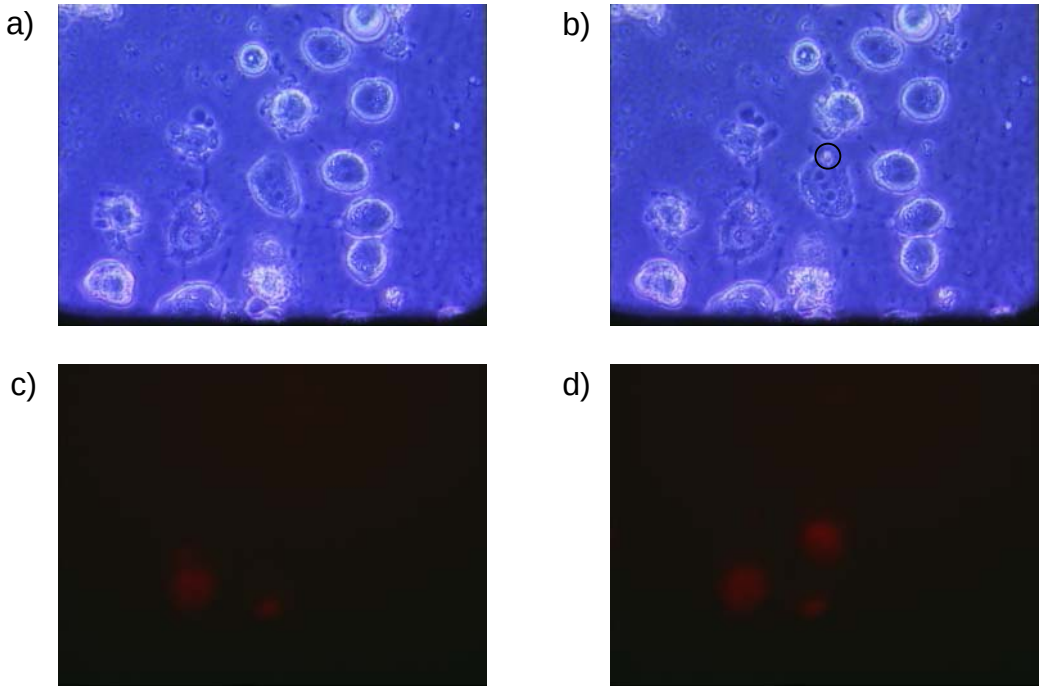

Figure 3. Microscopic images of the microinjection. a) and b) bright-field images of the selected cell, c) and d) images of the PI-fluorescence, respectively. a) and c) were taken before and b) and d) immediately after the microinjection laser pulse. The circle in b) indicates the location where of the microinjection was performed.

Conclusion

Pulses of oscillator regenerative amplifier fs-laser systems can produce effects that are as fine as those from a fs-oscillator alone but offer a higher versatility because they can be readily used to cut and catapult histological specimens and to microinject live cells. The fs-laser effects are much finer than those from standard commercially available ns-laser microbeam systems.

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