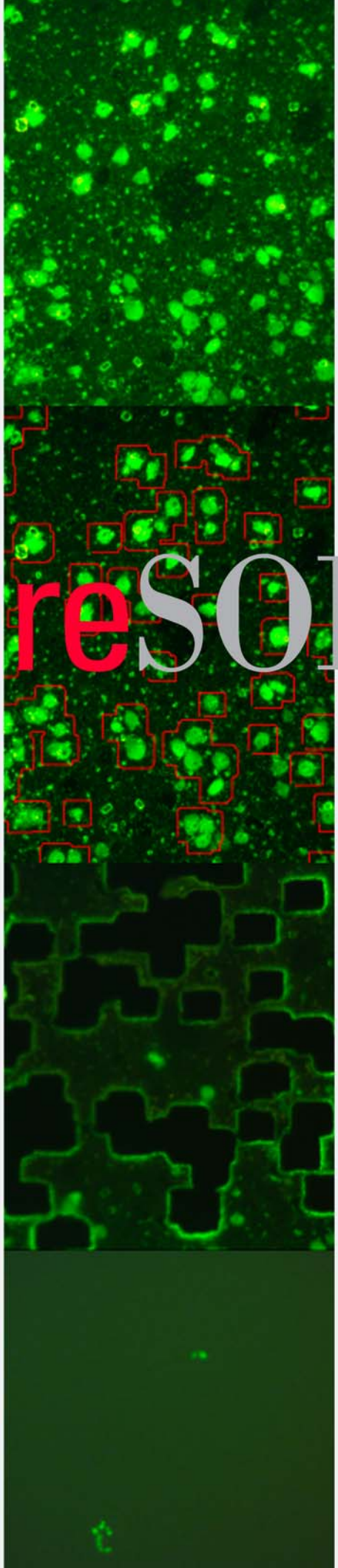


Dec
2006

Leica Laser Microdissection
Application Note

reSOLUTION

Laser Microdissection in Plant Research

Leica
MICROSYSTEMS

Laser Microdissection in Plant Research

Fast, Powerful and Precise

An enormous challenge for plant researchers is the investigation of plant tissues at the molecular level. What are the processes of development and differentiation of plant tissue? How do plants develop disease resistance? How do they react to attacks from a virus, bacteria, fungi or insects? Answers to these questions will lead to a clearer picture of plant cell metabolism and help to better deal with plant diseases and thus increase biomass production.

DNA, RNA and protein analyses require highly pure cell populations where nucleic acids, antigens and cell structures remain intact for downstream experiments. Fresh tissue, cryo-fixed or paraffin embedded plant sections are examined histologically, cells are selected and then cut from the section using laser microdissection. This enables targeted enrichment of specific cell types, thereby increasing the selectivity of analytic and genetic investigations. Such targeted sample collection enables the gain of maximum information from minimum source material.

Plant research – the requirement for maximum flexibility

Initially, laser microdissection technology was developed for, and mainly used on, animal and human tissues. Recently, plant researchers started to use this highly precise tool for their own applications. The main barrier to overcome was sample preparation, particularly because the woody stems and thick cell walls are more difficult to cut than thinner, softer animal tissue and this is where advantage can be taken of the new Leica laser microdissection system – the Leica LMD6000, which comprises the Leica DM6000 (digital research microscope), new optics (optimized for laser microdissection), and powerful diode UV-laser, ideal for tougher, thicker cells, such as plant cells! The Leica LMD6000 system is based on an upright microscope relying on gravity to drop the sample into a tube cap below, thus very large areas can be cut and dropped in one piece or very small cells can be dissected in the same way.

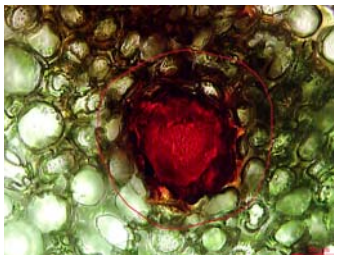
Can laser be used to cut a plant leaf?

A frequent request is to use the system for more demanding applications such as cutting leaves. This task is easy to perform with the Leica LMD6000, which makes it possible to microdissect fresh plant leaves in order to isolate defined tissue regions e.g. areas of variegation to analyse different compounds found within different pigments. These substances, in many cases, have not been investigated so far, because these compartments, for example, may only be approx. 200 µm diameter, and are too small for mechanical separation. In addition, one would need to collect several hundreds of these compartments to run NMR analysis. Now, using laser microdissection, direct and fast collection of tissue from the fresh material without former preparation for downstream analysis is possible.

Can the laser be used to cut wooden plant tissues?

Even wooden plant parts, like stem wood, from e.g. spruce can be easily cut with the Leica LMD6000 system. In order to isolate small areas containing storage substances separating the neighbouring cells, even 70 µm thick stem, longitudinal sections can be cut (Figures 1–3 and 4–7). For purer analysis thinner sections can be used because as the section becomes thicker the sample is less pure.

Fig. 1–3 (from left to right):
Laser Microdissection of small area of spruce section (73 µm thick):
– before microdissection
– after microdissection
– inspection mode



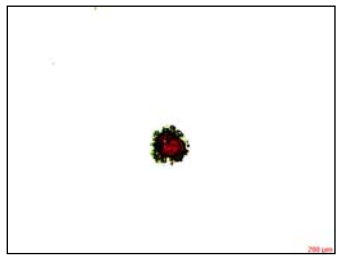



Fig. 4–7 (from left to right):
Laser Microdissection of oil vacuoles from a leaf cross-section:
– before microdissection
– after microdissection
– inspection mode (2 pictures)

What is the limitation on the size of area that can be cut?

In principle, areas of several millimetres can be collected directly in one piece into the PCR tube. Practically however, the shapes are drawn within the live image shown on the computer screen, which is approximately 3mm^2. It is also possible to draw larger areas beyond the entire screen (Figures 8–10). The shape of the dissectate is irrelevant, long tissue sections like stem can be isolated from the neighbouring cells (Figures 11–13). In addition, many separate areas can be cut into a single PCR tube thus increasing sample collection size.

High precision cutting

The Leica LMD6000 system is extremely flexible as it can also be used for the manipulation of micro organisms such as cyanobacteria (blue-green algae). Indeed, the laser can be used as a kind of scalpel to separate cyanobacterial chains. The growth media necessary for cell survival used to be the real challenge for laser cutting techniques. In the so called "sandwich procedure" the cyanobacteria are mounted between two membrane slides. In this way the cells are held in liquid medium and laser beam can cut the sample with such a high level of accuracy that the bacteria are separated, without any damage to surrounding cells. As the sample drops into the tube cap, medium can be placed into the cap ready to receive the sample, reducing stress to delicate samples. Also fine plant structures such as root hairs or tobacco trichomes can be easily separated by laser beam (Figures 11–13).

Many innovative features of the Leica LMD6000 system such as the dedicated optics (at all magnifications), the powerful laser, fine laser beam adjustment and the gravity-collection result in maximum versatility of the system. Totally new approaches and investigations in numerous fields of Life Science are now available because development of this new microdissection platform has taken place in close co-operation with researchers. > GZ

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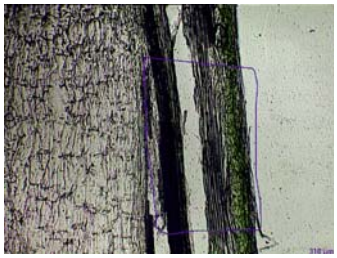
Germany

Contact:
Dr. Britta Settmacher
Phone +49 (0)6221-9126-332, Fax -338
biospektrum@elsevier.com
www.biospektrum.de



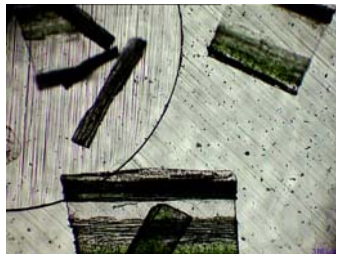


Fig. 8–10 (from left to right):
Laser Microdissection of long, large areas in the range of mm^2 in a single piece from Arabidopsis stem-section (60 µm thick):
– before microdissection
– after microdissection
– inspection mode

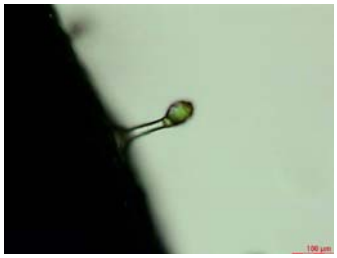


Fig. 11–13 (from left to right):
Collection of tobacco trichome:
– before microdissection
– after microdissection
– inspection mode

Leica Microsystems CMS GmbH
Ernst-Leitz-Straße 17–37
35578 Wetzlar

Tel. +49 (0) 64 41-29 0
Fax +49 (0) 64 41-29 22 55
www.leica-microsystems.com

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