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Static real-time capture of 3D microscopy images

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Inserting a liquid lens at the aperture stop avoids mechanical movements in widefield microscopy.

Two-dimensional widefield microscopy provides basic dynamic information about live biological specimens. However, it provides only a partial representation of the 3D biological processes and may be incomplete or even misleading. Current techniques, such as widefield, confocal, structured-illumination, or light-sheet microscopy cannot capture the 3D structure of a specimen in a single frame. In contrast, in 2D widefield microscopy, a stack of 2D depth images of the sample are recorded and a 3D digital image is computed from them. The different depth images are typically recorded with axial mechanical scanning. But mechanical movement could damage the sample, cause it to vibrate and hence introduce image distortions, or slow down image acquisition, which would make it impossible to record highly dynamic biological processes. The trivial solution is to use digital holographic microscopy, which permits the 3D complex distribution scattered by the sample to be rendered from a single frame.[1] However, this system operates coherently and makes fluorescence imaging impossible.

We have investigated using an electrically addressable liquid lens (LL) to acquire images at different depths. The lens is based on electrowetting technology: how a drop of water spreads on an electrically insulating surface can be modified by accumulating charge at the base of the drop. The optical power of the resulting LL can be tuned by an applied voltage.[2] In 2010, our group proposed using an LL for parallel dynamic focusing of images obtained through an array of microlenses.[3] More recently, other groups have applied LL technology in microscopy.[4,5]

Our proposal is to insert an LL at the aperture stop of a widefield microscope, which is arranged as the telecentric coupling between a high-numerical-aperture (NA) infinity-corrected microscope objective and a low-NA tube lens.[6] The insertion of the LL enables the axial position of the object plane to be controlled by the voltage while preserving the telecentric

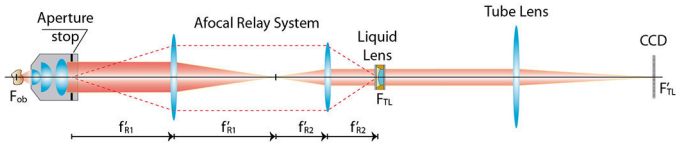

Figure 1. *Scheme of the static axial scanning experimental setup. CCD: Charge-coupled device. F_{ob} and F_{TL}: Front focus of the objective and the tube lens, respectively. F'_{TL}: Back focus of the tube lens. $f_{R1,R2}$: Focal lengths of the relay lenses.*

nature of the microscope, its lateral magnification, and the position of the image plane. In our proof-of-concept experiment we inserted an afocal relay into the optical path (see Figure 1). It is easy to show that in such a case the relation between the optical power of the liquid lens, P_{LL}, and the induced displacement, Δ, of the object plane is $\Delta = f'^{2}_{\mathrm{ob}}\, M^{2}_{\mathrm{Rel}}\, P_{\mathrm{LL}}$ where $M_{\mathrm{Rel}} = -f'_{\mathrm{R2}}/f'_{\mathrm{R1}}$ is the lateral magnification of the relay and f'_{ob} is the focal length of the microscope objective.

To verify the accuracy of the system, we first measured the lateral point spread function (PSF) for different values of P_{LL}. To perform the experiment, we inserted an LL ARCTIC 39N0 (VARIOPTIC) in our optical microscope composed by a $50\times/0.55$ microscope objective, a relay with focal lengths $f'_{\mathrm{R1}} = 300$mm and $f'_{\mathrm{R2}} = 100$mm, and a tube lens of $f'_{\mathrm{TL}} = 200$mm. In Figure 2 we show the measured PSFs, which demonstrate that the LL hardly affects the PSF shape and size.

Once we had verified that the LL produces no significant worsening of lateral PSF, we performed an imaging experiment in which we obtained a stack of depth section images of a cleaning-lens tissue paper. These sheets have a low thread density, and are about 100μm thick. Figure 3 shows a set of depth images obtained with the proposed setup and without mechanical movement.

In summary, we have shown that tuning the LL applied voltage enables the real-time capture of a stack of depth images of 3D specimens with constant magnification and resolution.

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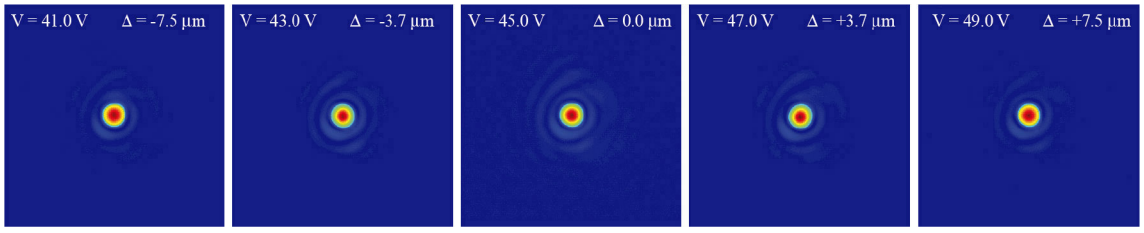

Figure 2. *Transverse point spread function (PSF) of the microscope for different positions of the object plane.*

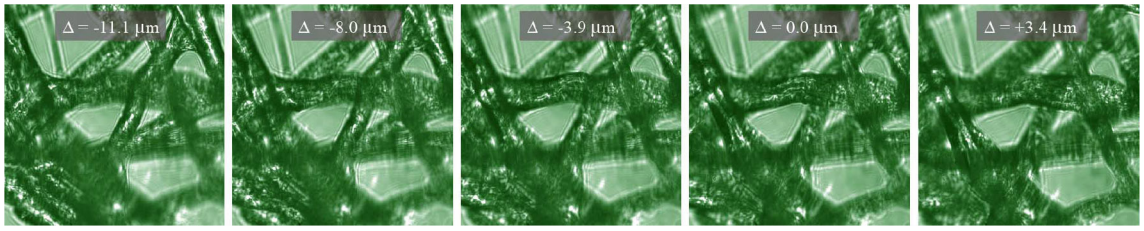

Figure 3. *Still images extracted from an online video[7] showing voltage-controlled focusing at different depths of a 100µm-thick cleaning-lens tissue paper with low thread density.*

The main advantage of this technique is performing fast axial scanning free of mechanical vibrations. We are now working on a prototype in which the liquid lens is inserted, directly, at the objective aperture stop. We are also preparing user-friendly, fast software for processing and visualizing the 3D images.

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