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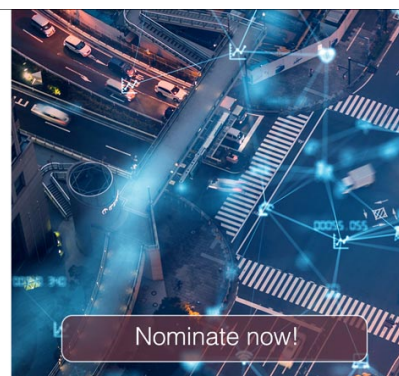
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Technical Note

Optical adaptation of confocal microscopes for arbitrary imaging angles—and its application to sedimentation/creaming in dispersions

S Mariot[1], M Schneider[1], A Salonen[1] and W Drenckhan[1,2]

[1] Laboratoire de Physique des Solides, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 91405 Orsay Cedex, France
[2] Institut Charles Sadron, CNRS UPR22, Strasbourg, France

E-mail: sandrine.mariot@u-psud.fr

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Abstract

Confocal microscopy has become a powerful tool to obtain high-resolution, three-dimensional images after reconstruction of a point-by-point scan of the local fluorescence properties of a sample. For technical reasons, commercial confocal microscopes perform this scan in a manner that the x-y-plane of the scan is perpendicular to gravity and can be several square centimetres large (defined by the movement of displacement stages). The depth- or z-scan, on the other hand, is parallel to gravity and typically limited to 10–1000 micrometres depth. This is a constraint that limits the number of experimental scenarios in which confocal microscopy can be employed, since many experimental systems ask for images in which the main extension of the scan has an angle with gravity which is not 90 degrees. Using the example of different experimental systems (particle dispersions, emulsions, foams, foamed emulsions) that sediment or cream under gravity, we show here how the optical path of any confocal microscope can be inverted to obtain any imaging angle with respect to gravity without losing all pre-programmed scanning and reconstruction facilities.

Keywords: confocal microscopy, instrumentation, gravity effects, soft matter

S Supplementary material for this article is available online

(Some figures may appear in colour only in the online journal)

1. Introduction

Confocal microscopy has become a standard tool to obtain three-dimensional images of structured surfaces or bulk materials. This powerful technique exploits the fluorescence properties of fluorescently labelled samples using highly localised excitation by one or more lasers with an optical pinhole system. Thanks to the exclusion of emitted light of the specimen that is not in the focal plan, confocal microscopy allows one to create sharp images with better contrast than conventional fluorescence microscopy. Thanks to the point-by-point illumination, the sample can be scanned in three dimensions at high resolution by combining adjustments of the optical path of the laser (from the microscope focusing revolver) with the controlled movement of translation stages.

For technical reasons, the *X-Y-Z* scan that is performed by the microscope is commonly aligned with gravity in a way that the *X-Y*-plane is perpendicular to gravity. The scan in this

plane is ensured by the large-scale motion of micro-controlled translation stages and can reach several square centimetres—also thanks to different stitching algorithms that most image processing softwares avail of. The scan of the depth of the sample in the z-direction is parallel to gravity and much more restricted since it depends entirely on the working distance of the objective, the numerical aperture and magnification.

This orientation of the imaging planes with respect to gravity puts important constraints on the experimental conditions under which confocal microscopy can be applied, especially when gravity is an important control parameter in the experiment. In the past, researchers developed special tables which allowed one to change the angle of the microscope with gravity [1–3], engendering a number of technical stability issues and certain risks to damage the microscope.

Here we show how a simple optical system—the inverterScope® objective inverter (LMS Tech)—which relies on the optical technology of a periscope, may be used to change the angle of the imaging planes with gravity [4–6]. In section 2.1, we provide detailed information as to how the different microscopy elements (sample holder, translation stages, piezo-controller for the objective etc) can be adapted in order to maintain the full use of the imaging software of the original microscope. In our case, we use a Leica TCS SP8, but in principle, all confocal microscopes allow for such modifications.

The demonstrated system can be adapted to any angle with gravity. Here we concentrate on a rotation of 90 degrees since the goal of our particular scientific context is to image the organisation of particles, drops or bubbles which sediment under gravity in a liquid carrier (see complementary information).

2. Technical modification of the confocal microscope

2.1. Overall setup

The setup shifts and rotates the laser beam with respect to the original position and direction. The overall setup is shown in figure 1. It consists of a commercial confocal microscope, Leica TCS SP8. This microscope provides five wavelengths of excitation (405 (UV), 458, 488, 552, 633 nm) and three detector channels, i.e. two hybrid detectors and one photomultiplier (the available range of wavelengths is 400 nm to 800 nm). It is equipped with a conventional scanner and a resonant scanner that allows for the acquisition of up to 40 frames s^{-1} with a resolution of 512×512 pixels (resonant mode). The Leica software provides control over all relevant measurement parameters (resolution, scan speed, zoom, etc).

The device that controls the scanning angle is a periscopic arm (figure 1(B)) fabricated by LMS Tech according to our parameters (section 2.2). It is placed between the microscope turret and guides the laser beam towards the side of the microscope at the desired angle. A linear manual stage (figure 1(C)) is installed horizontally to perform the global positioning of the sample. It carries a vertical, motorised translation stage, which holds the sample and which performs the X-Y scan. It is identical to the originally installed translation stage of the

microscope and can therefore be directly controlled by the Leica Software LasX (figure 1(D), section 2.3).

The Z-scan of the focal planes (section 2.2) is performed by a piezo element (figure 1(F)), which is installed between the end of the periscope arm and the objective.

The interplay of all elements is optimised in order to ensure that the default device performance of the microscope for the horizontal scanning is maintained even after changing the angle of the scan. All additional device items are installed permanently and in a way that one can switch easily between the original microscope configuration and the rotated one by simply adding the periscopic arm.

### 2.2. The periscope and the Z-scan

In order to redirect the laser beam we use a periscope composed of optical tubes and prisms placed in a bend (figure 1(B)). The periscope is built with compatible thread, M25/0.75, to be screwed directly into the objective revolver of the microscope, i.e. it replaces an ordinary objective. The first bend can rotate perpendicularly along the objective axis, so that we can orient the arm in any possible direction. The height of the optical tube above the microscope revolver is 16 cm so that the beam inverter can be easily placed under the microscope head, when the condenser is removed. The ending bend allows 360° rotation. As we integrate a vertical stage and different shapes of sample holders, we choose to fix the tube length at 35 cm so that we can access a large space environment, as shown in figure 1. The horizontality of the emerged beam is controlled by a 3D printed-mount (figure 1(G)) placed under the tube and fixed on the anti-vibration table.

The end of the periscope holds a piezo element (figure 1(F)) onto which the objective is mounted. The piezo is a MIPOS 500 SG (purchased from Trioptics, maximum travel: 400 μm, resolution: 12 nm) and ensures the Z-scan of the sample. Its thread is compatible with Leica objectives. It is chosen in collaboration with Leica so that it can be controlled by the microscope software LasX which provides a scanning depth of up to 300 μm.

2.3. Sample positioning and the X-Y scan

The perpendicular tilt of the X-Y-plane is controlled by a 360 degrees-rotary stage (from Owis, adjustment sensitivity 39 μrad) (figure 1(E)). In order to obtain the 3D-scan, the Z-scan (section 2.2) is completed by the X-Y-scan of a translation stage.

The Leica TCS SP8 microscope is equipped in its standard configuration with a motorised scanning stage (travel range: 127×83 mm, resolution: 0.02–0.04 μm, speed: 10 mm s^{-1}). In order to keep these characteristics, we bought the same stage and installed it on a home-designed frame (figure 1(I)). Since the translation stage is connected to the microscope via the regular connectors, we can control it in a transparent manner by the microscope software LasX. The software therefore controls the X-Y translation and the Z-scan in exactly the same manner as in the original, horizontal configuration.
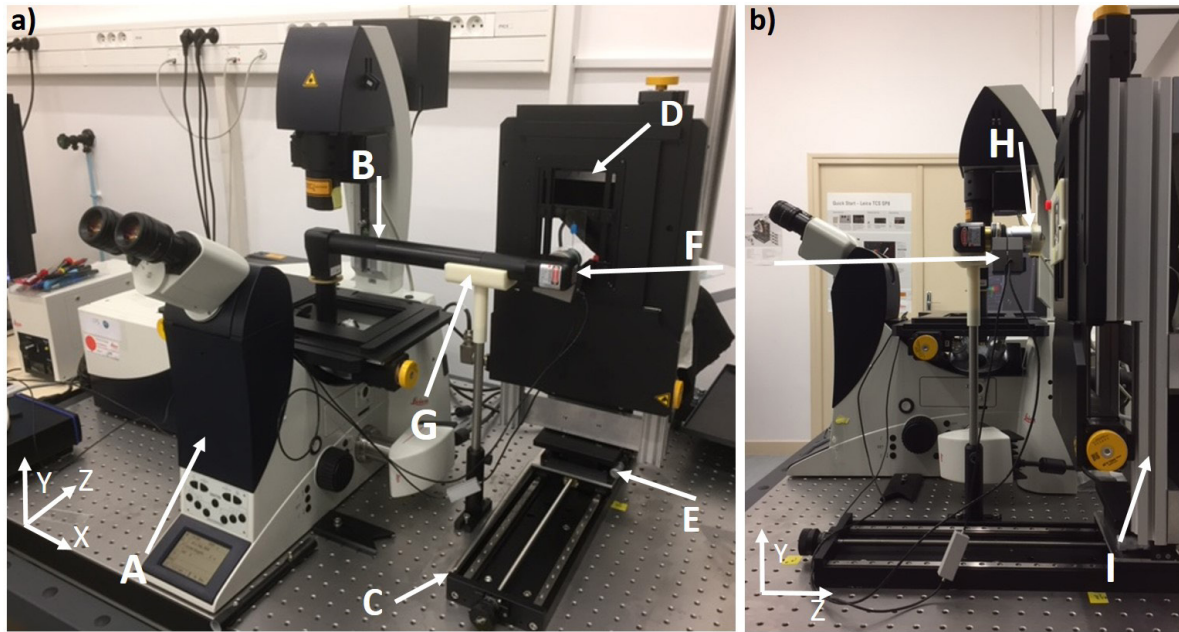
Figure 1. Overall configuration of the converter set-up shown from the front (a) and from the side (b). The confocal microscope (A) is equipped with a periscope (beam inverter) (B), which points to the centre of a vertical, motorised translation stage (D), installed on a frame (I). The motion of this translation stage is controlled in the *XY*-direction of the image by the original Leica Software. The motorised translation stage is positioned on a manual translation stage to perform the global positioning of the sample in the *Z*-direction (C). A rotary stage (E) is used to adjust the angle of the motorised translation stage. In our examples, it ensures that the periscope and the vertical translation stage are perfectly parallel. A piezo element (F) is mounted between the periscope arm and the objective to perform the *Z*-scan. It is directly controlled by the Leica software LasX. An element made by 3D printing supports the weight of the periscope and stabilises it against vibration (G). A home-made ring (H) serves to hold an echographic gel when an immersion objective is used.

To ensure non-blurred image quality, scanning time T and the desired image resolution d need to be chosen such that d/T is smaller than the characteristic object velocity V in the imaged sample, i.e. $V < d/T$.

In order to ensure the global sample positioning, a manual linear translation stage (purchased from Owis, 400 mm travel path, graduation scale: 10 μm) carries the motorised translation stage (figure 1(C)). To complete the setup, we add a 360-degree rotational stage (Owis, adjustment sensitivity: 39 μrad) between the manual horizontal translation stage and the vertical motorised translation stage (figures 1(C) and (E)). In principle this stage can provide any working angle for the experiment. In our examples we use it to ensure verticality of the sample.

2.4. Additional accessories

The sample containers, which can be capillaries or other fluidic devices, are fixed onto the translation stage thanks to dedicated holders. These are drawn with Solidworks and printed with a 3D printer (HP 3D design printer). The design is based on a frame that is fixed onto the motorised stage with screws, as shown in figure 2(a). We adapt the dimensions of the frame according to the containers.

Depending on the specimen environment, and the sample holder, we may choose to work with an immersion objective (oil or water). The sample medium and immersion liquid have close refractive index, so that undesired reflections are avoided. The use of these liquids in the vertical configuration
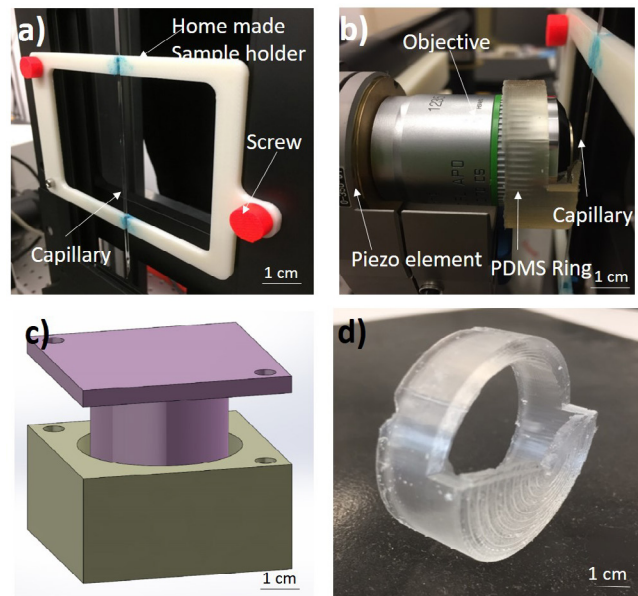
Figure 2. (a) Example of a holder, screwed on the translation stage. (b) Positioning of the home-made silicone (PDMS) ring around the objective. This ring allows to keep the gel around the objective and in contact with the capillary. (c) 3D design (Solidworks) of the casting mould of the objective (in purple) which allows to make the silicone ring. The liquid PDMS is put in the mould and then placed in an oven for crosslinking. (d) Experimental realisation of the ring made by moulding of (c) with PDMS.

requires an additional effort to avoid liquid flow in order to keep the objective lens in the liquid working medium. Leica provides a Water Immersion Micro Dispenser. It is a
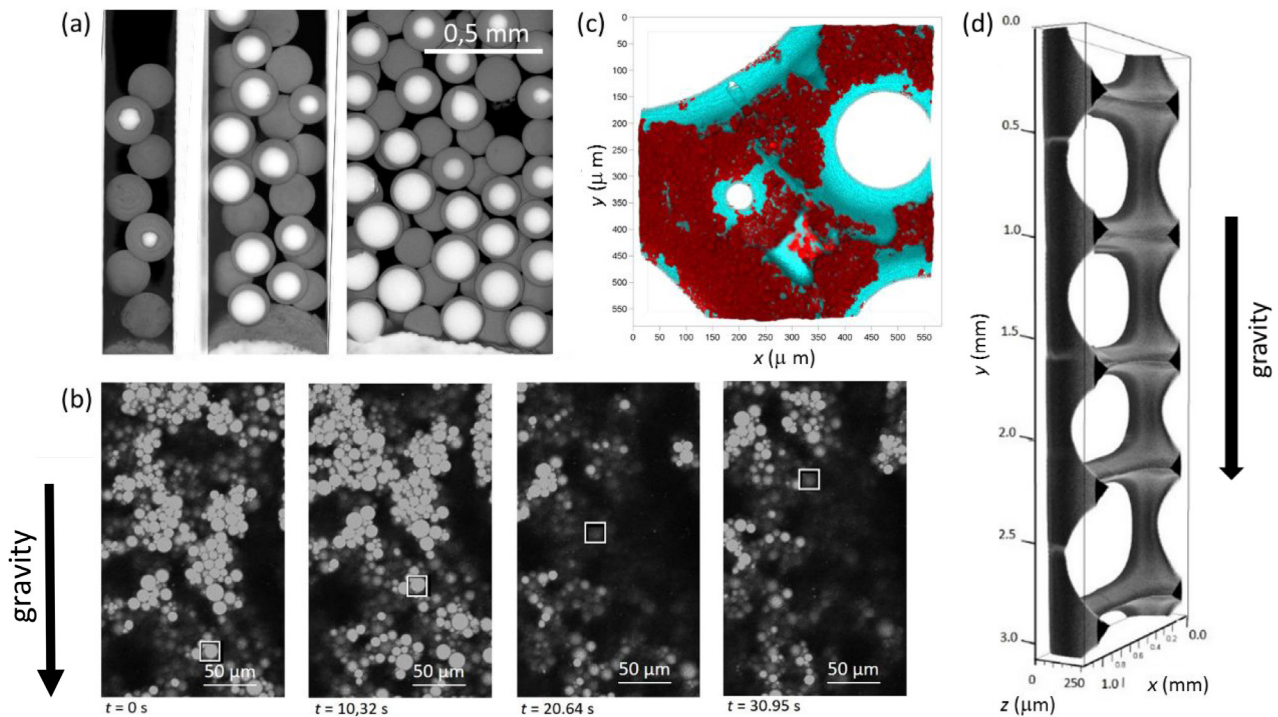
Figure 3. Example images obtained for the visualisation of objects sedimenting under gravity. (a) Sediments of polymer beads in capillaries of increasing cross-section. (b) Temporal evolution of a droplet-packing under gravity. The square follows one droplet. (c) Sedimentation of oil droplets (red) in water (blue) between gas bubbles (white). (d) 3D-surface structure of bubbles pressed against the wall of a capillary. (All experimental details are in the supplementary materials (stacks.iop.org/MST/29/127001/mmedia).)

device that allows a continuous flow of water on the immersion objective lens. This system is controlled by an external software. For our purpose and in order to avoid adding too much weight on the periscope, we chose to build a ring that is adapted to the immersion objective. This ring can be placed around the immersion objective in a matter that a small but sufficient quantity of gel is maintained in front of the lens (figure 2(b)). The ring is made with a casting mould of the objective, designed with SolidWorks and then 3D-printed (figure 2(c)). The mould is filled with silicone paste (polydimethylsiloxane, Sylgard 184 purchased from DowCorning) and then cured in an oven at 60 °C during 40 min for crosslinking. The final ring (figure 2(d)) is obtained after removal from the mold. We use an echographic gel (provided by EDM Medical Imaging) which has a refractive index very close to water, 1.33 at 21 °C.

3. Examples and conclusion

In order to test the new microscope configuration, we used a range of different samples that consist of discrete objects (gas bubbles, oil droplets or polymer beads), which cream or sediment under gravity in a continuous liquid phase. Examples of obtained images are shown in figure 3. All experimental details are provided in the supplementary materials. We compared images obtained with the classical and rotated configurations (supplementary materials) without noticing a measurable difference in magnification or image quality. The main difference is loss of intensity, forcing us to increase the laser intensity by roughly a factor of 5.

We therefore conclude that the new configuration not only works reliably, but that it is also convenient to use. All the software functions can be used in the same manner and with the same precision as in the classic configuration, i.e. the handling of the microscope remains intuitive since only the *X-Y-Z* axis of the sample is being rotated by the periscope. Since all additional translation and rotation stages can be left permanently, we can switch easily between the different configurations. We hope that this article will facilitate confocal microscopy in non-horizontal configurations to a wide range of users.

ORCID iDs

S Mariot ◉ https://orcid.org/0000-0002-5934-5359

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