Optical high-resolution analysis of rotational movement: testing circular spatial filter velocimetry (CSFV) with rotating biological cells

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Abstract
Circular spatial filtering velocimetry (CSFV) was tested during the microscopic registration of the individual rotations of baker’s yeast cells. Their frequency-dependent rotation (electrorotation; ER) was induced in rotating electric fields, which were generated in a glass chip chamber with four electrodes (600 μm tip-to-tip distance). The electrodes were driven with sinusoidal quadrature signals of 5 or 8 V\textsubscript{PP} with frequencies up to 3 MHz. The observed cell rotation was of the order of 1–100 s per revolution. At each measuring frequency, the independent rotations of up to 20 cells were simultaneously recorded with a high-speed camera. CSFV was software-implemented using circular spatial filters with harmonic gratings. ER was proportional to the phase shift between the values of the spatial filtering signal of consecutive frames. ER spectra obtained by CSFV from the rotation velocities at different ER-field frequencies agreed well with manual measurements and theoretical spectra. Oscillations in the rotation velocity of a single cell in the elliptically polarized field near an electrode, which were resolved by CSFV, could not be visually discerned. ER step responses after field-on were recorded at 2500 frames per second. Analysis proved the high temporal resolution of CSFV and revealed a largely linear torque-friction relation during the acceleration phase of ER. Future applications of CSFV will allow for the simple and cheap automated high-resolution analysis of rotational movements where mechanical detection has too low a resolution or is not possible, e.g. in polluted environments or for gas and fluid vortices, microscopic objects, etc.

Keywords: AC-electrokinetics, electrorotation, step response, high speed video analysis, vortices, fluid dynamics

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

Spatial filter velocimetry (SFV) is a common optical technique for velocity measurement (Aizu and Asakura 1987). In most scientific and commercial applications, a single velocity component (1C) is registered for a fixed position (0D, i.e. zero dimensional) with a high temporal resolution (Michel et al 1998). Recently, the technique has been extended to two (2C; Bergeler and Krambeer 2004), and three-velocity components (3C; Iversen et al 2011), all of which are detected for a fixed position. Like other OD techniques, such as laser Doppler methods (Albrecht et al 2003), SFV also has the potential for the multidimensional (3C-3D) detection of velocity fields and the movement of complex structures.

Examples of multidimensional velocity measuring techniques are particle image velocimetry (PIV) and particle tracking velocimetry (PTV), which are for example applied in fluid-flow characterization (Raffel et al 2007). Velocity estimation in PIV and PTV is based on the correlation between two or more successive image frames. Nevertheless, the algorithms required are very time-consuming, restricting the techniques to moderate frame rates or off-line processing.

SFV allows fast velocity estimations from image sequences. In the method, intensity fluctuations, which are induced by moving objects, are imaged on a transmission grating, i.e. the spatial filter. In the classical setup, this filter is an actual optical amplitude grating. The filter-weighted intensity distribution is optically focused so that it can be summed up by a photodetector. The time-dependent signal detected contains an alternating component with a carrier frequency that is proportional to the velocity of the moving object. This frequency depends on the displacement rate of the imaged scene. Signal generation and processing in SFV can be implemented either optically (Pau and Dallas 2009), electronically (Michel et al 1998) or by software (Miike et al 1987).

Nowadays, high-speed video cameras allow for the software-implementation of spatial filters and SFV velocity detection by weighting and integrating over the pixels of consecutive frames. The straightforward signal generation and processing approach of SFV, which avoids image reconstruction, permits higher sampling rates than other imaging techniques for velocity estimation (e.g. optical flow (Horn and Schunck 1981) or Hough transform (Zhu and Yu 2011)).

The flexibility of SFV permits a variety of applications. SFV has been applied, for example in detecting surface velocities (Schaeper and Damaschke 2011), length (Bergmann 2010), fluid flow (Hosokawa and Tomiyama 2011), or the microcirculation of blood cells in the capillaries of human fingernail folds (Menn 2011). In the latter application, the shape of the spatial filter had to be adapted to the structure of the capillaries in the proximal nail folds of the fingers.

Methods for the characterization of individual cells are of increasing importance in cellular biotech applications (Zimmermann and Neil 1996, Gimsa et al 2014). The methods are applied in Lab-On-Chip systems (LOCs) for cell manipulation and sorting, in lysis and genetic analysis, as well as for the on-line in vitro monitoring of physiological cell parameters (Fuhr et al 1997, Fuhr and Reichle 2000, Schnelle et al 2000, Dürr et al 2003, El-Ali et al 2006, Wolf et al 2006, Laurell et al 2007, Nilsson et al 2009, Sun and Morgan 2010, Barat et al 2012). The development of LOCs is aimed at reducing animal testing and costs in medical diagnostics or drug development (Bonk et al 2015).

Electrorotation (ER) is a well-established method for the characterization of individual cells by their frequency-dependent rotation behavior in rotating electric fields (figure 1). The ER torque is based on the interaction of the charges induced at the dielectric interfaces of the cells with the rotating electric field (Gimsa and Wachner 1998, Gimsa et al 2014). Depending on the frequency of the rotating field, ER can be used to determine the dielectric properties of cells, such as membrane and cytoplasmic conductivities and permittivities.

A number of different approaches exist for the automated detection of ER. For example, Hölzel (1998) used video image analysis with computer-aided logging of video sequences and all experimental data. Reichle et al (1999) developed a micro optical single particle dynamic (MOSPAD) detector with an optical slit mask, which can be interpreted as a crude version of an optical spatial filter. The authors called their more advanced implementation of the same technique ‘CARLA’ (calculation of rotation for laser tweezers). CARLA was a software implementation of MOSPAD, using a virtual video slit function for image analysis (Mietchen et al 2002). The slit function had to be adapted to the individual cell images. In principle, their technique can be considered a software implementation of circular SFV (CSFV) with a single slit grating, which introduces a rectangular weighting grid function. Other authors used advanced image processing methods of captured video frames, for example the cross correlation of polar-transformed cell images (De Gasperis et al 1998). Zhou et al (1998) applied a masking threshold to the objects detected in consecutive video images before orientation changes were estimated by elliptical moment analysis of the individual objects. Classical dynamic light scattering techniques have been adapted for detecting the ER of cellular or even smaller objects (Eppmann et al 1996, Prüger et al 1997, Gimsa 1999).

Here, we have introduced CSFV with software-implemented circular spatial filters as a new approach for the measurement of rotational velocities in video sequences. The structure of the filters was adapted for the detection of the rotation of microscopic objects.

The feasibility of our new technique was demonstrated in the detection of the frequency-dependent rotational velocities of individual biological cells undergoing ER. The new CSFV approach allowed for detecting ER spectra of baker’s yeast cells as well as the step response of the cells to rotating field-on. A high-speed camera provided the required temporal resolution in the recorded image sequences.

2. Theory

2.1. Circular spatial filter velocimetry (CSFV)

Single component (1C-0D) SFV weights a moving intensity distribution $I(x,t)$ with a $x$-dependent grating function $g(x)$. 1C-0D SFV also allows for the analysis of the movement of
two dimensional (Cartesian) images with intensity distributions of \(i(x, y, t)\) (Bergeler and Krambeer 2004). In 1C-0D SFV, the intensity distributions in y-direction are nullified by the applied grating function. At a given time \(t\), the SFV-signal \(s(t)\) results from the integral over the whole weighted intensity distribution:

\[
s(t) = \int_{-\infty}^{+\infty} i(x - vt)g(x)dx \quad (1)
\]

\(s(t)\) can be interpreted as the correlation of the image moving at velocity \(v\) in x-direction with the grating function \(g(x)\). The grating function \(g(x)\) can be more generally described by the complex function \(g(x)\) with harmonic sine and cosine weighting components. For a single Fourier coefficient \(g(x)\) reads:

\[
g(x) = \cos(2\pi \mu x) + j \sin(2\pi \mu x) = \exp(j2\pi \mu x) = \exp(jkx). \quad (2)
\]

The spatial frequency \(\mu\) and (angular) wave number \(k\) of the grating in x-direction are related to the grating period \(p\):

\[
k = 2\pi \mu \quad \text{with} \quad \mu = \frac{1}{p}. \quad (3)
\]

Rotational movement detection is based on the estimation of tangential velocity components along a circular path in the image of the object. In CSFV, a complex circular spatial filter is used for this estimation. It is obtained by transforming the above relations to polar coordinates with the center \(x_0, y_0\):

\[
\xi_{x_0,y_0}(t) = \int_{0}^{2\pi} i(\phi - \omega t)g(\phi)d\phi
\]

\[
= \int_{0}^{2\pi} i(\phi - \omega t)\exp(jm\phi)d\phi. \quad (4)
\]

In analogy to equation (1), the radius dependencies of the intensity distributions \(i(\phi, r)\) are nullified by the circular grating function for each given angle \(\phi\), leading to \(i(\phi)\). The angular velocity of the object is \(\omega = 2\pi / T\) with \(T\) being the time per revolution.

In practice, the signal \(\xi_{x_0,y_0}(t)\) obtained will depend on the \(x_0, y_0\)-position of the center of the circular spatial filter within the image of a rotating object. Unfortunately, the center of rotation is not known ad hoc. One simple way to tackle this problem is the repetitive application of the spatial filter, subsequently assuming each position or pixel in the image as the possible center of rotation. Another, problem, which is still unsolved, may arise from the lateral drift of the center during the registration of rotation.

In principle, the angular object velocity \(\omega\) can be estimated from the complex spatial filtering signal of two successive images \(\xi_{n-1}\) and \(\xi_{n}\) obtained for the time interval \(\Delta t = t_n - t_{n-1}\). Using the Fourier phase-shifting theorem, we obtain:

\[
\omega = \frac{\Delta \phi}{\Delta t} = \frac{1}{m} \frac{\Delta \phi}{\Delta t} = \frac{2\pi}{p} \frac{\Delta \phi}{\Delta t} \quad \text{with} \quad \Delta \phi = \arg(\xi_{n}) - \arg(\xi_{n-1}) \quad (5)
\]

\(\Delta \phi\) is the angle of object rotation during \(\Delta t\). To avoid aliasing in the extraction of the rotation velocity, the classical sampling theorem (Shannon 1949) must be fulfilled for each frame pair in a video sequence:

\[
\Delta \phi < \frac{p}{2}. \quad (6)
\]

In practice, 10 images per revolution are sufficient to obtain a reliable spatial filtering signal for further processing. For a harmonic circular spatial filter with \(m\) periods, we obtain the complex grating function:

\[
\xi_{m}(\phi) = \cos(m\phi) + j \sin(m\phi) = \exp(jm\phi) \quad (7)
\]

in analogy to equation (2). For the circular spatial filter, the number of periods \(m = 2\pi / p\) is the equivalent of the grating period \(p\) of the linear filter. Figure 2 illustrates two sinusoidal circular spatial filters with \(m = 1\) (corresponding to \(p = 2\pi\)) and \(m = 2\) (corresponding to \(p = \pi\)). The weighing factors are illustrated by gray intensities; the lightest and darkest areas represent weighting factors of +1 and −1, respectively. The optimal diameter and width of the circular spatial filter in radial direction depends on the specific problem. For ER detection, we used diameters that approximately matched the outer rim of the cell images. Figure 2(c) illustrates a radial width of five pixels. By discretization, a specific gray value is assigned to the whole size of each pixel. For simplicity, we used a radial thickness of a single pixel in the following calculations. Optimization of this parameter was beyond the scope of the present proof of concept.

CSFV is most sensitive when the center of the circular filter coincides with the center of object rotation. Accordingly, amplitude peaks in the spatial filter signals can serve as search criteria for centers of rotation \((x_0, y_0)\), when the filter is applied to each location \((x, y)\) in a captured video sequence. For this
convolution, the calculation times are shorter when the 2D fast-Fourier-transforms (FFT) of the \( n \)th image \( \mathbf{i}_n \) and filter functions \( \mathbf{g} = g_{xy} \) are used. From the convolution, the matrix:

\[
\begin{bmatrix}
\mathbf{g} \\
\mathbf{F(x,y)}
\end{bmatrix}
\]

(8)
is obtained. In the matrix, centers of rotational movement are indicated by positions with high phase differences in consecutive convolutions \((\mathbf{s} - \mathbf{s'})\).

### 2.2. Electrorotation (ER)

AC fields induce polarization charges at the structural interfaces of suspended micro objects such as particles or biological cells (Gimsa 1999). For spherical objects, the resulting complex-induced dipole moment is:

\[
\mathbf{m} = \varepsilon_0 \varepsilon \mathbf{E} \mathbf{f}_{CM} \]

(9)

with \( \mathbf{f}_{CM} = f_{CM}^R + f_{CM}^I \) being the complex Clausius–Mossotti factor, consisting of its real and imaginary parts; \( \mathbf{E}, \varepsilon_0, \varepsilon \) and \( \varepsilon_0 \) are the complex external field, the volume of the object, and the permittivities of the external medium and vacuum.

The interaction of the induced dipole moment with the inducing field may generate various AC-electrokinetic effects, depending on the electrode arrangement and the generated field properties (Gimsa 2001). For ER, rotating electric fields are usually generated in four electrode chambers (Maswiwat et al 2006). The four electrodes are driven by progressively 90°-phase-shifted signals. The external field and the induced dipole moment can be assumed to rotate with the same frequency in the \( \mathbf{i}, \mathbf{j} \) plane of an \( \mathbf{i}, \mathbf{j}, \mathbf{k} \) orthonormal base system without limitation in generality. In the dispersion frequency ranges of the object’s polarization, the spatial phase angle between the external field and the induced dipole moment leads to a torque, which may be induced in or against the direction of the field. The time-averaged torque \( \langle N \rangle \) is given by the cross product of the induced dipole moment and the inducing field:

\[
\langle N \rangle = \frac{1}{2} \mathbf{m} \times \mathbf{E}.
\]

(10)

Assuming the rotational axis of the object to coincide with vector \( \mathbf{k} \) of the \( \mathbf{i}, \mathbf{j}, \mathbf{k} \) base system, the permanent torque \( \langle N \rangle \) is proportional to the out-of-phase (imaginary) component of the frequency-dependent Clausius–Mossotti factor \( f_{CM}^3 \) (Gimsa 2001):

\[
\langle N \rangle = \varepsilon_0 \varepsilon \varepsilon \mathbf{E}^2 \mathbf{f}_{CM}^3 \mathbf{k}.
\]

(11)

Torque peaks are observed for external field frequencies in the relaxation time ranges of the object’s polarization. The peaks can be described by Lorentz terms. To make it easier to compare the different experiments, ER data are usually normalized to the square of the external field strength, leading to the ‘rotation’ parameter, \( \text{R}_{ER} \):

\[
\text{R}_{ER} = \frac{2\pi}{E_0^2 T_c} = \frac{\omega \mathbf{E}}{E_0^2}
\]

(12)

with \( T_c \) standing for the time of one revolution of the object. Its angular velocity \( \omega = \omega_c = 2\pi / T_c \) is detected by CSFV with \( T = T_c \) (compare to equation (4)). An exact relation between equations (11) and (12) is difficult to establish, because the object’s rotational friction inside the measuring chamber and the hydrodynamic coupling effectiveness of the torque acting on the influenced dipole charges with the object are not well defined (Gimsa et al 1991). The relations depends on the object’s geometric, chemical and electric surface structures, the interaction with the chamber bottom, etc.

Despite these problems, the frequency dependence of ER can be measured exactly. For single-shell spherical objects, a phenomenological description of the obtained ER spectra is possible using.
where \( f \) stands for the external field frequency and \( f_{C1} \) and \( f_{C2} \) for the characteristic dispersion frequencies of the membrane and bulk-conductivity polarizations (Gimsa et al 1988 and 1991). For biological cells at low external medium conductivities, the peak heights \( R_1 \) and \( R_2 \) are usually negative (anti-field) and positive (co-field), respectively. For single shell objects, the characteristic frequencies can directly be interpreted in terms of geometric and electric object parameters (Gimsa et al 1991). The first characteristic frequency is:

\[
R_{ER} = R_1 \frac{2 f}{\frac{1}{C_1} + \frac{1}{2 \sigma}} + R_2 \frac{2 f}{\frac{1}{C_2} + \frac{1}{2 \sigma}} \tag{13}
\]

2.3. Step responses of cells after rotating field-on

Describing the friction experienced by a solid ellipsoidal object is much more complex for rotational than for translational motion. Moreover, in ER, the actual force is exerted on the cell membrane in the anti-field rotation peak range (Gimsa et al 2014). Therefore, the impulse must be distributed inside the cell (a minor problem given the small size of the cell) and in the surrounding medium during the acceleration phase. Sedimented cells experience additional friction on the ER chamber bottom. For the proof of concept presented here, we assumed a first order delay-time behavior as the simplest possible description of the behavior of the angular velocity \( \omega_\infty(t) \) after field-on:

\[
\omega_\infty(t) = \omega_\infty \left[ 1 - \exp\left( -\frac{t}{\tau} \right) \right] \tag{16}
\]

with \( \omega_\infty \) and \( \tau \) being the stationary angular velocity of the cell and the acceleration time constant, respectively. Naturally, the time-dependent angle data \( \phi(t) \) was less noisy than the \( \omega(t) \) data of the yeast cells. Therefore, equation (16) was integrated for the fitting procedure:

\[
\phi(t) = \int_0^t \omega(t)dr = \omega_\infty \tau \exp\left( -\frac{t}{\tau} \right) + \omega_\infty \tau - \omega_\infty \tau. \tag{17}
\]

For large \( t \) values, the exponential term can be neglected to obtain an expression describing steady rotation with a linearly time-dependent angle.

\[
\phi(t) = \omega_\infty t - \omega_\infty \tau. \tag{18}
\]

3. Materials and methods

3.1. Detection hardware

A schematic illustration of the measuring setup is shown in figure 3. A high-speed camera (Phantom v12.0, www.visionresearch.com, full frame resolution: 1280 x 800 pixels) was connected to a Zeiss Axiotron microscope (www.zeiss.de) with a C3040-ADL adapter (www.olympus.de). Field-frequency, camera-synchronization and camera-readout data were programmed with a PC and transferred via USB and LAN interfaces. The four electrodes of a glass microchip chamber were powered with four progressively 90° phase
shifted harmonic signals and checked with an oscilloscope (TDS 2014B, Tektronix, www.tek.com). The signals were generated by four phase-locked function generators (Agilent 33210A, Agilent Technologies, www.agilent.de). The clock regime and triggering of the signal generators was controlled with a fifth master generator. The available frequency range of up to 3 MHz covered the anti-field rotation peak of yeast cells, which was sufficient to prove the concept of CSFV registration of steady cell rotations and of the transient phases of accelerated rotation after field-on.

The conductivity of distilled water was adjusted with sodium chloride before baker’s yeast (www.wieningerhefe.de) cells (Saccharomyces cerevisiae) were suspended and transferred to the measuring chamber. The chamber was covered by a microscopic slide and put at the microscope stage. Using an additional lens tube, the optical magnification of the measuring setup was 80×. Illuminations with the standard microscopic lamp or with a 532 nm laser with fiber coupling were tested.

A frame-rate of 500 frames per second (fps) was used to record steady ER. The 16 GB memory of the high-speed camera was segmented for the triggered capturing of consecutive video sequences at each rotating field frequency. The video data was transferred to a PC for further processing. In a second experiment, step responses were recorded with a framerate of 2500 fps. This rate allowed us to capture the start behavior of ER after field-on.

3.2. ER chamber

Cell ER was recorded at external medium conductivity of 12 mS m\(^{-1}\) with electrode voltages of 5 or 8 Vpp. For the rounded-tip electrodes of the ER chamber, the field strength in its center, obtained from the electrode voltage and opposite tip distance (600 μm) is reduced by a factor of 0.59 (Maswiwat et al. 2006). Accordingly, the two electrode voltages corresponded to electric field strengths of 6400 Vm\(^{-1}\) and 10 242 Vm\(^{-1}\), respectively.

3.3. ER measurements

3.3.1. Pre-processing. Independent of the illumination by microscopic lamp or laser, global intensity fluctuations were observed in the images, which stemmed from intensity fluctuations in the illumination source used. These fluctuations were removed in a first preprocessing step. For this, the mean gray value was calculated for each image in a considered sequence. For each image, its mean gray value was subtracted from all the pixels to obtain a sequence of images with mean gray values of zero. After this, all the images were rescaled, in order to obtain displayable images with 8-bit resolution.

Figure 4 provides an example of two preprocessed images of a rotating cell. The semi-transparent cell appeared to rotate in front of a statically illuminated background. Illumination generated bright areas in the inner and outer regions of the cell. Since the direction of the illumination did not change, the image structure of the cell can be considered as superimposed with additional static light which appeared to emerge from an inclined top right position.

In a second preprocessing step, excessive static light components were eliminated from the images, which would have interfered in the further processing of the complex spatial filter signal. Mathematically speaking, each image \(i(t)\) consists of a static \(i_0\) and a time-dependent component \(i_d(t)\), which are constructively superimposed:

\[
 i(t) = i_0(t) + i_d.
\]

The complex signal of the spatial filter (equation (4)) can be described by:

\[
 z(t) = \int_0^{2\pi} [i_0(t, \varphi) + i_d(t, \varphi)] \exp(jm\varphi) d\varphi
 = \int_0^{2\pi} i_0(t, \varphi) \exp(jm\varphi) d\varphi + \int_0^{2\pi} i_d(t, \varphi) \exp(jm\varphi) d\varphi
 = z_0(t) + z_d.
\]

For homogeneous illumination (no static structure, \(i_0 = f(\varphi)\)), the spatial filter generates the mean-free, i.e. offset-free, signal \(z_d(t)\). Heterogeneous illumination would introduce an additional static complex offset \(z_0\), and result in incorrect estimations of the phase differences between the signal values (\(\arg(s_0) = \arg(s_0 + s_d)\)). The specific spatial filter used influences the magnitude of the offset \(z_0\). It can be estimated from the temporal mean value of the spatial filter signal \((\bar{z}(t))\):

\[
 z_0 = \langle z(t) \rangle = \frac{1}{T} \int_0^T \int_0^{2\pi} i(t, \varphi) \exp(jm\varphi) d\varphi dr.
\]

The offset is large when the structures in the inhomogeneity of the illumination and the spatial filters are similar, e.g. possess common multiples in their intensity structures.

Alternatively, the offset can be obtained from the temporal mean intensity values for each pixel location in an image sequence. Before a spatial filter is applied, the obtained mean values \(i_0\) must be subtracted from each image:

\[
 i_0(t) = i(t) - i_0 \quad i_d = \langle i(t) \rangle = \frac{1}{T} \int_0^T i(t) dt.
\]

The advantage of this alternative approach is that it allows for the successive application of different filter gratings, i.e. simple testing of different gratings.

Two approaches to the implementation of spatial filters in ‘regions of interest’ with single cells (compare to figures 4 and 5(a)) were tested. In the first, the original images in Cartesian coordinates were weighted with the circular complex grating mask for each image position \((x_p, y_p)\) (figure 5(a)) before the
A complex spatial filter signal was calculated by integrating over (i.e., summing up all discrete gray values of) all pixels in each image. The same result could be obtained with shorter computing times when summing up only the gray values of the filter itself, because the values outside the filter are zero. In the second approach, the gray values of each image position \((x_p, y_p)\) were converted into polar coordinates. Before standard linear spatial filters could be applied to the obtained image (figure 5(b)), empty sites of pixels not generated by the conversion algorithm had to be filled-up by gray-scale interpolation. Each approach had its advantages and disadvantages. Because the original pixel values were processed, the calculation time was shorter in the first approach. Weighting could be performed by a direct convolution of image and spatial filter function (equation(8)). The advantage of the second approach was that linear standard spatial filters could be used. In both approaches, misalignment of the center of object rotation and the spatial filter resulted in a noisy phase response. Accordingly, increasing noise could be observed when the lateral displacement of the cell introduced a misalignment of the two centers during the recording of a video sequence.

3.3.2 Video sequence analysis with CSFV: detecting unsteady angular velocity. Figure 5 schematically illustrates the two approaches to applying circular spatial filters to the preprocessed images of a video sequence. In both approaches, the first analysis step is the definition of a spatial filter path, which is appropriate for the object’s diameter. In Cartesian coordinates, the diameter of the circular filter (figure 5(a), left) corresponded to the distance of the filter column (figure 5(a), right) from the left border of the transformed image in the polar coordinates. The complex circular spatial filter signal in figure 5(b) could be derived either from the Cartesian or from the polar-transformed image sequence. The time-dependent phase signal (figure 5(c)) was directly obtained from the complex filter signal. Its slope is directly proportional to the cell’s angular velocity (figure 5(d), equation (5)).

A cell with an unsteady rotation has been chosen for figure 5. Unsteadily rotating single cells, without any neighboring cells, could be observed at eccentric locations near ER chamber electrodes. The cell in figure 5 was measured at a field strength and frequency of \(10^{242}\) \(\text{V} \cdot \text{m}^{-1}\) and 100 kHz, respectively.

4. Results and discussion

4.1 Manual determination of ER spectra

For manual ER spectra measurements, the rotation velocities of the observed cell were obtained from the times of one revolution at each measuring field frequency. Logarithmically equidistant frequency points were used, i.e., 10, 18, 32, 56, 100, 180 kHz, and so on. In some cases, additional frequencies (75 and 750 kHz) and the upper frequency limit of the generators (3 MHz) were used. The revolution times were determined from the recorded video sequences by image counts. The results were normalized to the square of the field strengths (equation (12)) and plotted over a logarithmic frequency scale.
Field polarization is exactly circular only at the center of the ER chambers. At the center, the highest ER torque is induced (Maswiwat et al 2006; for ER torque in elliptical fields see: Gimsa 2001).

We assume that the additional periodicity in every other minimum of the angular velocity was caused by different polarizabilities around the two poles of the longest axis of the cell. Because cell size is not negligible with respect to the field inhomogeneity in the ER chamber, the differently polarizable poles of a cell axis are located in areas of different field strength during ER. Consequently, the superimposed orientation forces will be stronger when the higher polarizable pole is oriented towards the nearest chamber electrode, i.e. located in an area of higher field. Here, we refrained from a more elaborate analysis of the phenomenon.

Clearly, the details in the angular velocity, which we could resolve with CSFV could not have been resolved with the classical manual determination of rotation velocities by stopwatch. The high resolution of CSFV leads us to believe that in future, exact ER spectra can be extracted from recorded fractions of a full cell revolution especially for steadily rotating cells, i.e. ellipsoidal cells at the center of the ER chamber or spherical cells, which do not experience orientation force. In the following, complete ER spectra of steadily rotating cells were registered with CSFV in central locations of the ER chamber.

4.3. Comparison of CSFV to manual ER measurements

Measuring points of CSFV-ER spectra were determined after polar transformations of the rotating cell images. In an initial test, CSFV-ER spectra that had been obtained either by laser or microscopic lamp illumination were compared. Despite of the irritating Speckle patterns observed under laser illumination, there were no perceivable differences in measuring precision (results not shown). Because the visual analysis of laser-illuminated video sequences is difficult, microscopic lamp illumination was used for comparing CSFV data to manual control measurements.

ER spectra were recorded from the same cells. The mean angular velocities obtained by CSFV or image counting were normalized to the square of the field strength (equation (12)) before the obtained rotation data was fitted (figure 6). Inclusion of the \( R_2 \)-high frequency peak in the fitting equation (13) made it possible to improve the fit at the high-frequency flank of the \( R_2 \)-membrane peak, in the overlapping range of the two peaks. Clearly, the two additional free fitting parameters of the second peak \( (R_2, f_{c2}) \) over-determine the problem, because data points are missing above 3 MHz. For a more benign fitting behavior, we therefore assumed \( |R_2| = |R_1| \). The \( R_2 \) and \( f_{c2} \) parameters obtained were not used in the interpretation of the cell properties.

An area-specific membrane capacitance of 4.97 mF m\(^{-2}\) can be estimated from equation (15) by using the mean of the four characteristic frequencies in figure 6 (241 kHz), an external conductivity of 12 mS m\(^{-1}\), and an external cell radius of 3.2 \( \mu \)m. This value is typical for yeast cells. Following the arguments of Hötzl (1997), it results from the complex

\[ \frac{1}{\mu} \]
circuit behavior of the capacitances and conductivities of the cytoplasmic membrane, periplasmic space, and cell wall.

4.4. Step responses of cells to rotating field-on

In a second experiment, ER step responses were recorded after field-on. No lag or acceleration phase of the cells could be observed visually. Constant cell rotation seemed to be established instantaneously. Things were different with CSFV. Field-on-triggered video sequences recorded with framerates of 2500 fps allowed us to resolve the acceleration phase. Experimental results of two individual cells are presented in figure 7. Data points are plotted together with equations (16), (17), or (18) after fitting the parameters $\omega_\infty$ and $\tau$. Given the size of yeast cells, rotational diffusion can largely be neglected as a source of noise observed on our time scale (Prüger et al. 1997). Because the $\phi(t)$ data (figure 7(b)) increases over time, it is less noisy than the differential $\omega(t)$ data (figure 7(c)). In order to take advantage of the reduced noise, $\omega_\infty$ was obtained by linear regression of equation (18) to the $\phi(t)$ data of times larger than 500 milliseconds. In principle, the precision of the regression can be increased by using longer periods of steady rotation. Nevertheless, for biological cells, their interaction with the chamber bottom and electrical properties may change over time, preventing measuring times longer than for some cell rotations per measuring-field frequency.

One method for determining $\tau$ is to interpret the intercept of the equation (18) with the ordinate for $t = 0$ (figure 7(b)). Here, we fitted equation (17) using the $\omega_\infty$ parameters obtained from equation (18) (figure 7(a)). We believe that this approach ensures a better weighing of the measuring points in the initiation phase of rotation. There were no noticeable differences in the fitted $\tau$ values when measuring points were used of the complete measuring period or of the first 500 milliseconds only. As suggested by the orange curve in figure 7(c), the precision of $\tau$ may be influenced by the same effect that causes the oscillations observed after the acceleration phase. A visual check of the video sequences revealed that the cells exhibited slightly eccentric rotations, probably caused by an eccentric location of the points of strongest adhesion to the chamber bottom. We think that the precision in the $\tau$ determination may be improved by averaging a number of field-on measurements in such cases, in which the initial slope of the $\omega(t)$ data depends on the starting angle $\omega_\infty \tau$ (equation (17)).

The acceleration phases of the two cells in figure 7 were completed after approx. 500 ms. Within the two seconds plotted, the cells turned approximately half a rotation. The strong fluctuations beyond the acceleration phases in figure 7(c) suggest that establishing a steady rotation not only requires time for the acceleration phase itself but also for establishing a stable frictional interaction of the cell with the chamber bottom. Later on, cell rotation becomes more reproducible, i.e. either steady (figure 6) or oscillating (figure 5(d)).

In future experiments, an autotracking algorithm for the rotation centers will improve the efficiency and precision of ER spectra recordings with CSFV. It is still not yet clear how autotracking can work when the center of the ‘optical density’ of a cell taken for its center of mass (as we did here) is misaligned with the rotation center. Nevertheless, this problem is beyond the scope of this proof-of-concept manuscript, which shows the usefulness of high-resolution CSFV measurements for our understanding of the ER phenomenon.
5. Conclusion and outlook

Mechanical, optical or magnetic rotary encoders are usually employed in the detection or analysis of rotational movement. We introduced CSFV as a new optical method and tested it by determining the angular velocities of rotating microscopic objects. In principle, rotational motion can be analyzed by direct application of circular spatial filters to the images of the recorded video sequences. In the algorithm used here, the images of the rotating cells were transformed into polar coordinates before angular velocities were determined using linear spatial filters. Illumination of the microscopic sample, either by lamp or by laser had no influence on the CSFV results. The high temporal resolution of CSFV became obvious in oscillations resolved in the rotation of cells in elliptical fields as well as in high-speed recordings of the initial phase of cell rotation after field-on. To our knowledge, the same temporal resolution has not yet been achieved by other methods of cell-ER detection.

Here we chose cells that rotated at fixed positions in the video sequences. In future experiments, an autotracking algorithm for the rotation centers may help avoiding aliasing effects by translational cell movement, i.e. the misinterpretation of translational components as rotational movement. The coincidence of the centers of cell rotation and signal transformation throughout each interpreted sequence will improve the precision in the deduced phase response of the spatial filter signal. Automatic object recognition and the parallel recording of many objects will broaden the scope of SCFV application in dielectric single particle spectroscopy, e.g. in the characterization of colloids or physiological single cell investigations. Automation and shorter measuring times are desirable for biological cells, which may undergo physiological changes under the measuring conditions.

Because of the strong noise in the phase-response during the acceleration phase of ER, the transient behavior of the cell was fitted under the simplest possible assumption, a constant permanent torque (equation (11)) in combination with a linear friction-velocity relation. There is hope that systematic investigations of the acceleration phase will provide clues to the mechanisms of torque generation acting on the object by the mechanical and hydrodynamic coupling with ions and dipoles, the charges on which add up to the interfacial polarization. Adjusting the medium viscosity with molecules of different sizes may help in understanding the geometric relations at the polarized interfaces (see ‘electrophoretic fingerprinting’; Donath et al 1997). Such investigation can be conducted in wide ranges of external conductivity and field frequency in order to include additional ER peaks that are based on different polarization mechanisms. The results will widen our understanding of the dielectric, molecular and ionic structures at electrolytic and biological interfaces.

We hypothesize that high magnitudes of the spatial filtering signal are a possible criterion for the suitability of the grating function for the observed object. At first glance, one could speculate that CSFV does not permit distinguishing periodicity in the optical structure of the rotating object from the possible periodicity in its oscillating angular velocity, leading to aliased CSFV-filter signals. Nevertheless, we believe that the harmonic complex spatial filters with constant time-independent magnitude as used here, avoids aliasing and ensures the registration of oscillating angular velocities as such.

Using tracer particles, CSFV can easily be applied to problems in fluid dynamics, for example in the analysis of fluid and gas vortices. CSFV is not limited to microscopic structures but can also be applied for the investigation of large structures in the atmosphere or even sun spots. Generally, CSFV allows the simple and cheap automated high-resolution analysis of rotational movements where classical detection methods are problematic or expensive, for example in polluted environments. For rotating machinery, for example crank shafts, CSFV may provide high angular resolution in even-velocity tests, practically independent of the rudimentary nature of the sensing pattern used.

Conflicts of interests

The authors declare no conflict of interest.

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