Optimization of Mixing Processes in Microplates

─ A Methodology and Study of Microplate Mixing Techniques Including BioShake 5000 ─

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Introduction

Despite the great interrelation of a fast and complete mixing of assay components to the results, these procedures received only a very limited interest in laboratory routine. Due to the trend of ever increasing miniaturization in biotechnology the usual sample volumes decreases rapidly. As a consequence, a insufficient mixing has an unfavourable effect to the quality of the results in a manner never before seen.

For simultaneous processing of samples microplates are used as a laboratory standard tool. To achieve a highly effective mixing it is essential to supply enough energy for generating a macroscopic flow in the fluid. Several established methods for generating mixing effects in microplates are shown in *Figure 1*. A major disadvantage of all methods with contact to the sample is the serious risk of contamination and falsification of results.

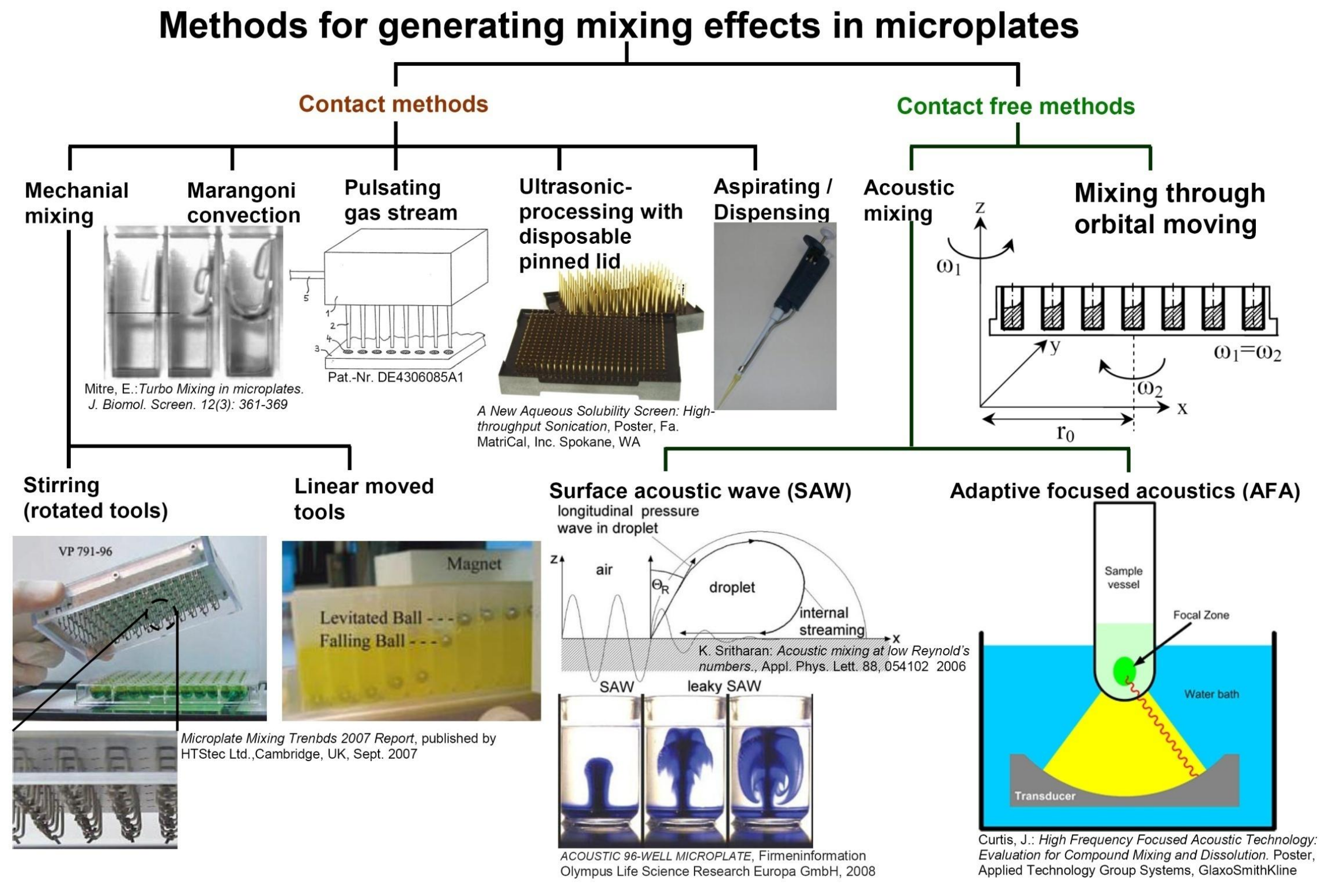

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Figure 1: *Methods for generating mixing effects in microplates.*

Background

Orbital shaking is undoubtedly a simple and non invasive way for mixing of assay components. The benefits compared to the also non-invasive methods of "acoustic mixing" are a very small heat impact and that no additional medium is necessary for transferring energy to the sample. Contrary to popular belief, orbital mixing is also effective with 384- and 1536-well microplates by carefully selecting appropriate operating parameters.

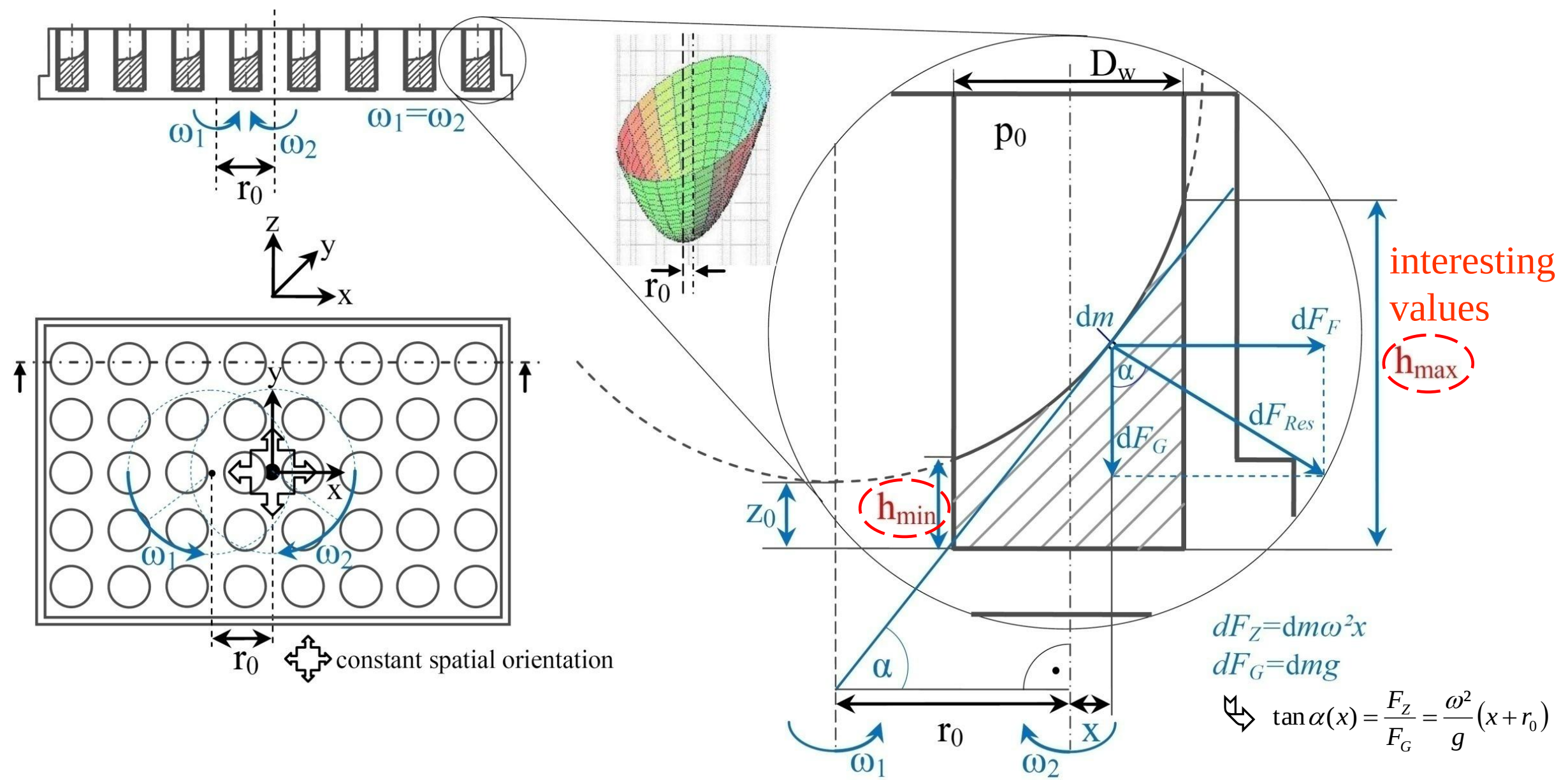


Figure 2: Orbital movement and resulting fluid distribution in a microplate well.

In *Figure 2* the resulting liquid distribution in a well due to the orbital movement of a microplate is shown. If the effect of friction and surface forces is neglected the free surface of the fluid, which is a face of an equal pressure niveau, forms a rotational paraboloid. The acting forces to a volume element *dm* within the free surface are gravity and centrifugal force. In a co-moving reference frame the fluid appears to rotate along a stationary wall. Interesting values are the minimal and the maximal height of fluid in depence of amplitude r_0 and mixing frequency n. Further Information about calculating liquid distribution can be found in [2].

The choice of appropriate operating parameters for orbital mixing, especially the mixing frequency n and the amplitude r_0, *is depending on*

- *microplate fill volume* V_F,
- *well geometry (diameter* D_W*, height h),*
- *surface tension of fluid and construction material σ,*
- *fluid density ρ,*
- *and kinematic viscosity of the fluid ν.*

The most important requirement for an effective mixing process is the formation of a macroscopic flow. As microplate well volumes decrease the impact of surface tension increases because of the low volume/surface ratio of the usually thin and tall well geometry. For this reason it is necessary to generate a high centrifugal acceleration to achieve an intensive macroscopic flow. A large number of commercially available instruments have been developed for use with larger laboratory vessels and they are not designed to generate a centrifugal acceleration which is required for processing small volumes. The labour required for surface enlargement must be delivered by the centrifugal force. The increased centrifugal force exceeds the surface tension at a critical shaking frequency *[4]*

$$n_{\min} = \sqrt{\frac{\sigma D_W}{8\pi V_F \rho r_0}},$$

with the surface tension *σ*, the well diameter D_W, the fill volume V_F, the fluid density *ρ* and the amplitude r_0.

The value of centifugal force respectively acceleration depends on the amplitude r_0 and the mixing frequency n. Against expectation it is crucial to choose the right value of amplitude r_0.

In *Figure 3* the resulting liquid distribution in a well with an identical geometry is shown for two different values of amplitude. The difference is that if the amplitude is smaller than the half well diameter the vertex of the rotational paraboloid is within the cross-sectional area of the well. In this case the minimum circulates within the well. This is especially an advantage if components are suspended solids and phases with differing densities. Otherwise if the amplitude r_0 is considerably higher than the half well diameter suspended solids and phases with higher density will move towards the direction of centrifugal acceleration along the wall. If the amplitude is high enough also a segregation of compounds is possible instead of the desired mixing process.

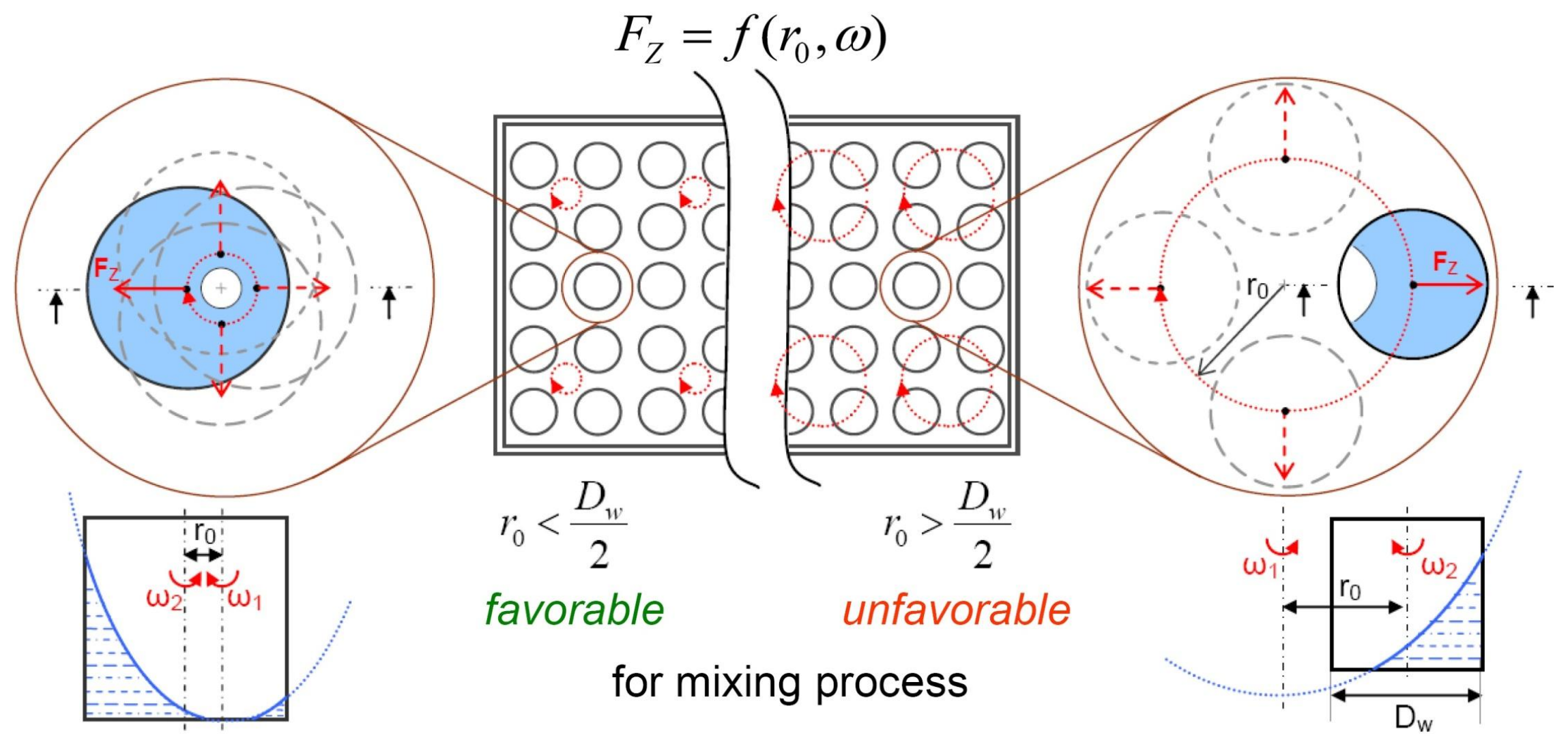


Figure 3: *Influence of amplitude* r_0 *depending on well diameter* D_W *to liquid distribution.*

On the other hand Büchs et al. [3] observed the phenomenon that under unfavorable operating conditions an increasing amount of liquid is not able to follow the external excitation. They defined the non-dimensional Phase number (Ph) for the distinction of favorable and unfavorable operating conditions. The probability of occurrence of Out-of-Phase conditions increases especially with a small amplitude r_0 and a low filling volume V_F. In *Figure 4* the orientation of the characteristic liquid sickle in microplate wells is shown for In-Phase and Out-of-Phase operating conditions.

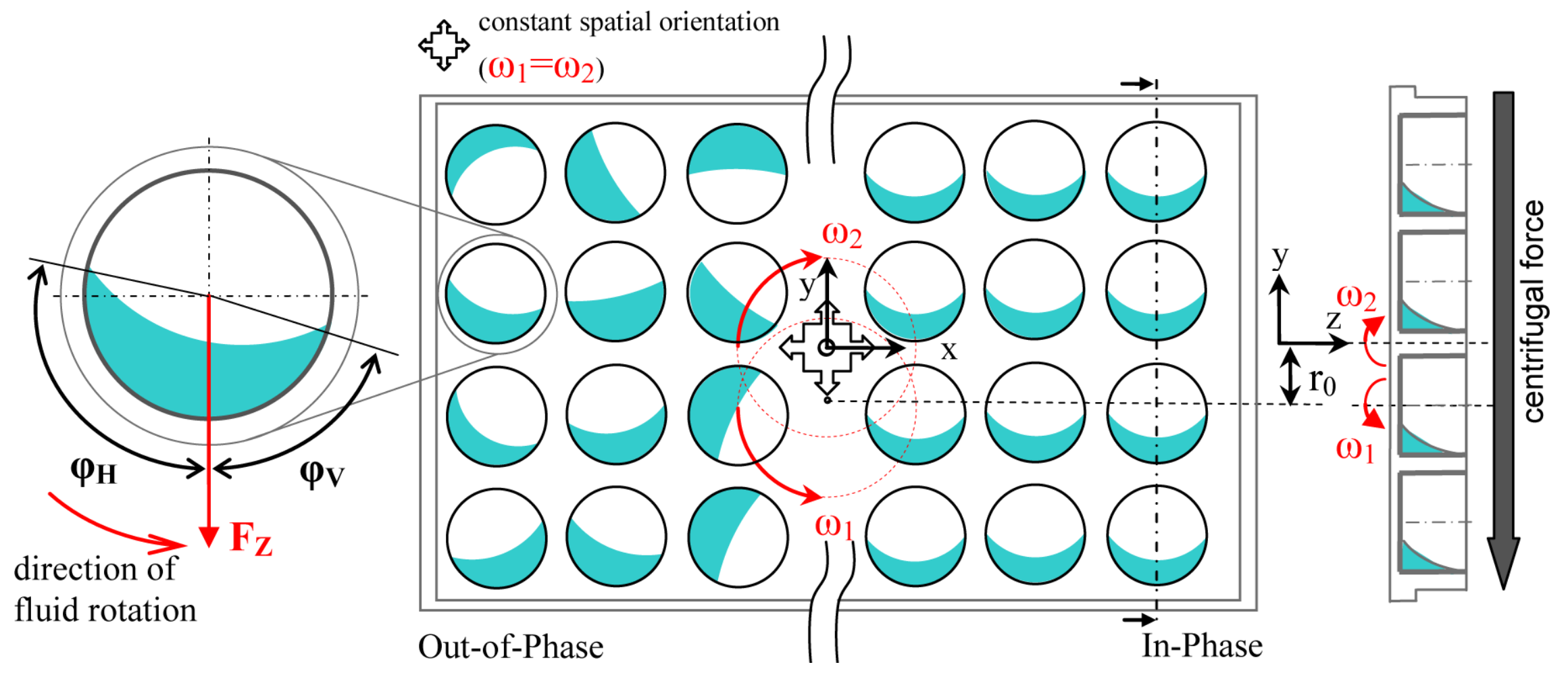
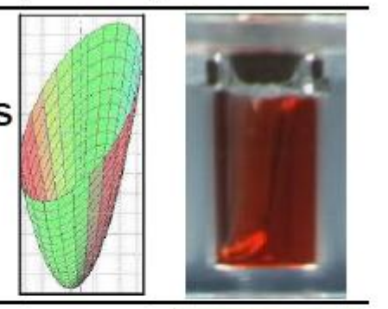
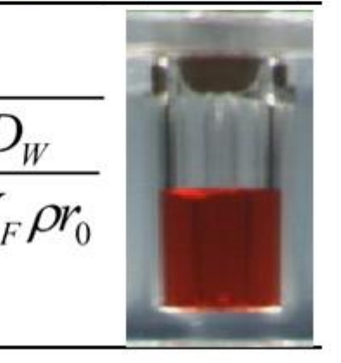


Figure 4: *Orientation of liquid sickle in microplates depending on operating conditions .*

Conclusion

In *Figure 5* the explained limitation of operating conditions are shown. As a practical example we are interested in finding appropriate operating parameters for a 384-well microplate with 12,5*µl* and 26*µl* well filling volume of an aqueous solution. The amplitude is limited in a range from 0,5 - 3,2*mm*. For a complete and fast mixing process it is essential to select a mixing frequency, which is near the upper limitation of mixing frequency.

	amplitude r_0	mixing frequency n
upper limit	$r_0 < \frac{D_{W2}}{2}$	liquid reaches top edge 
lower limit	$Ph(r_0) > 1{,}26$	$n_{\min} = \sqrt{\frac{\sigma D_W}{8\pi V_F \rho r_0}}$ 

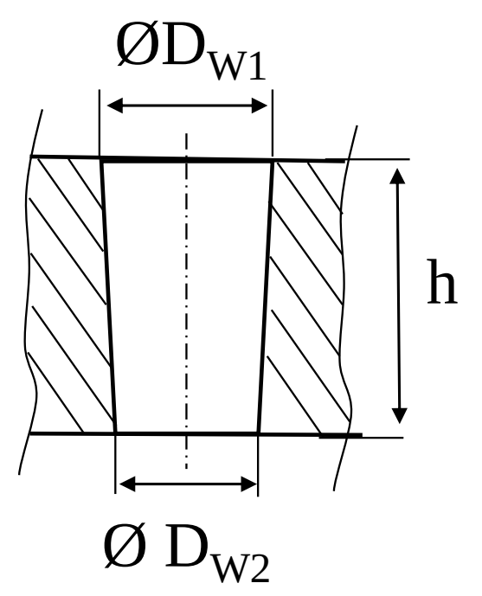


Figure 5: *Limitations of operating conditions.*

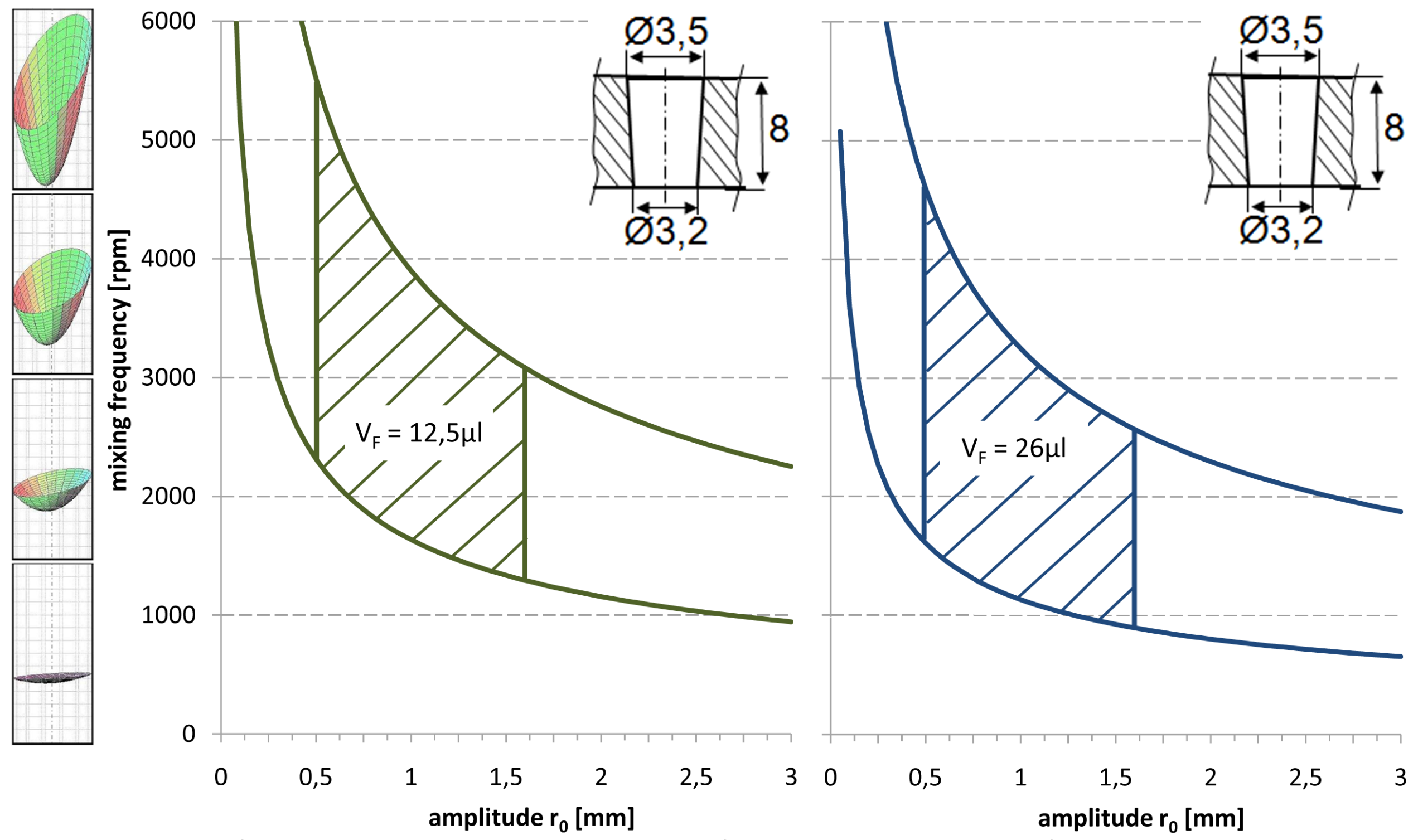
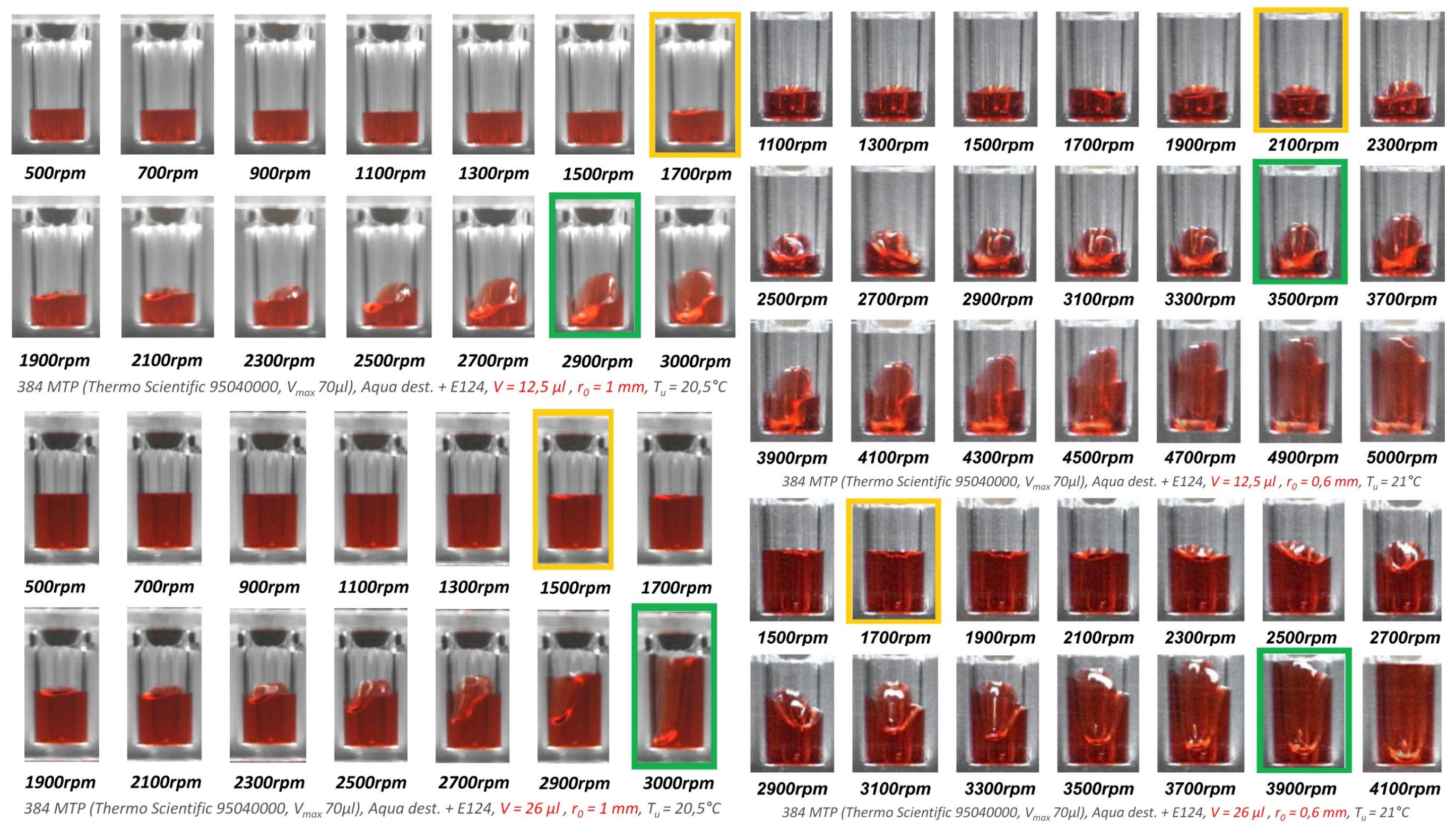


Figure 6: *Area of appropriate operating conditions for mixing 12,5 / 26µl of an aqueous solution in a 384-well microplate (Thermo Scientific 95040000).*

In *Figure 7* the liquid movement is shown for two different values of amplitude with starting of liquid movement (yellow) and excellent conditions (green).



384 MTP (Thermo Scientific 95040000, V_{max} 70µl), Aqua dest. + E124, V = 12,5 µl , r_0 = 1 mm, T_u = 20,5°C

384 MTP (Thermo Scientific 95040000, V_{max} 70µl), Aqua dest. + E124, V = 12,5 µl , r_0 = 0,6 mm, T_u = 21°C

384 MTP (Thermo Scientific 95040000, V_{max} 70µl), Aqua dest. + E124, V = 26 µl , r_0 = 1 mm, T_u = 20,5°C

384 MTP (Thermo Scientific 95040000, V_{max} 70µl), Aqua dest. + E124, V = 26 µl , r_0 = 0,6 mm, T_u = 21°C

Figure 7: *Liquid movement in a 384-well microplate (Thermo Sientific 95040000). Aqua dest. + E124, camera: ImaginSource DFK 21BU04 . Left: amplitude 1,0mm, Right: amplitude 0,6mm.*

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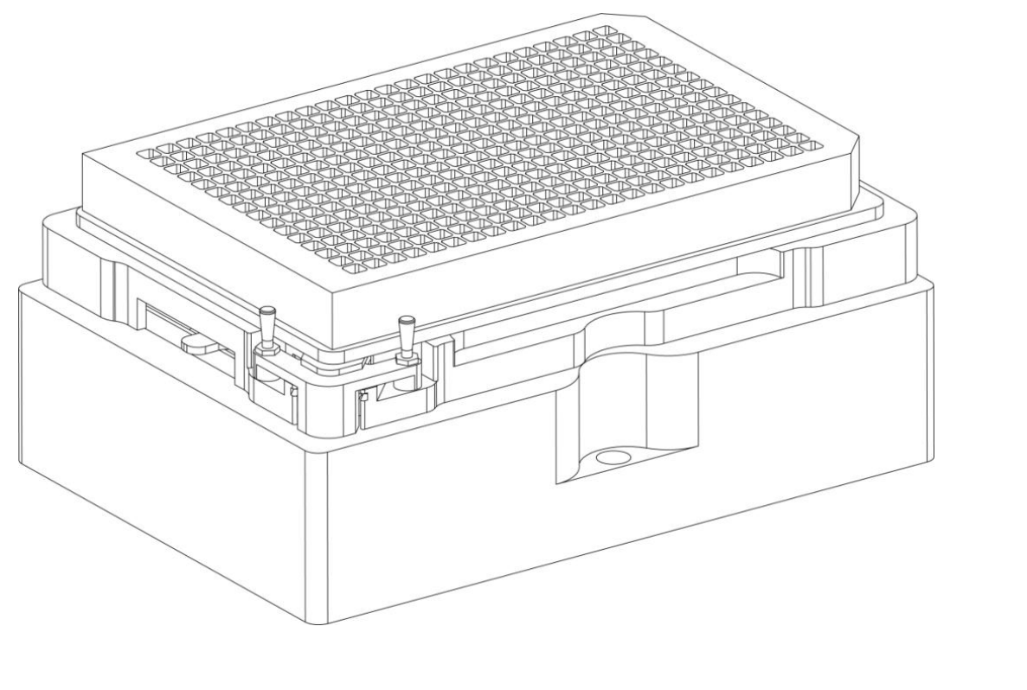

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