Electronic supplementary information

1. *LabDisk* fabrication

The CAD file of the microfluidic structures was generated with SOLIDWORKS2014 (Dassault Systèmes SolidWorks Corp., Waltham, MA, USA). The structures were micro-milled in a 160 mm x 160 mm poly-(methylmethacrylate) (PMMA) plate. Subsequently, a poly-(dimethylsiloxan) (PDMS) mold produced from the PMMA master served as blueprint for micro-thermoforming[1] of a cyclic olefin polymer (COP) foil (COP ZF14, Zeon Corporation, Tokyo, Japan). Micro-structured foils were thoroughly rinsed with isopropyl-alcohol (*Carl Roth* GmbH + Co. KG, Karlsruhe, Germany) and deionized water (#3478.1, *Carl Roth* GmbH + Co. KG, Karlsruhe, Germany) and subsequently exposed to 80 °C for 2 h for drying. LAMP primer pre-storage was achieved using a modified version of a previously described dry pre-storage method[2]: 1 µL primer mix (for concentrations see Table 1, all oligonucleotides were ordered in HPLC grade, Biomers GmbH, Ulm, Germany) of sequence-specific LAMP primers for *S.* Paratyphi mixed with 1 µL 200 mM D(+) trehalose (Sigma Aldrich, St. Louis, MO, USA) in DNase/RNase free water (Life Technologies, Carlsbad, CA, USA) as stabilizer[2] was pipetted into every reaction chamber, except chamber no. 8. This chamber was filled with 2 µL of the D(+) trehalose solution serving as no primer control (NPC). Drying down of the oligonucleotides was achieved by placing the *LabDisk* with the primer solution for 1 h at 50 °C in a recirculation convection oven (Microtitre plate incubator, SI19, Stuart, Bibby Scientific Limited, Staffordshire, UK). After primer pre-storage, a lyophilized reaction mix pellet (LAMP pellet, Mast Group Limited, Bootle, United Kingdom) was placed into the mixing chamber. A pressure-sensitive adhesive polyolefin foil (#900 360, HJ-BIOANALYTIK GmbH, Erkelenz, Germany) was structured with venting holes using a laser cutter (PLS3.60, Universal Laser Systems, Inc., Scottsdale, AZ, USA). Vent holes (Ø 1 mm) were covered with PTFE filters (cut to circles Ø 2 mm, PTFEPET02205, Merck Millipore, Darmstadt, Germany) to avoid cross-contamination of the ambient with DNA amplification products. Manual sealing of the micro-thermoformed COP foil was done with the previously prepared pressure-sensitive adhesive foil. The final *LabDisk* cartridge was cut to Ø 130 mm with a center hole of Ø 15 mm. *LabDisks* were stored at 10-22 °C in petri dishes (#ALA5.1, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) together with one desiccant bag (#N077.2, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) per *LabDisk*. The petri dishes were sealed with Parafilm® (Parafilm M, Bemis, Oshkosh, WI, USA) to ensure a dry atmosphere.

Table 1: Primer concentrations for the LAMP primer mix. Primer sequences were obtained by Dr. M. Bakheit, Mast Diagnostica GmbH, Reinfeld, Germany. FIP: Forward inner primer; BIP: Backward inner primer; LF: Loop forward; LB: Loop backward; F3: Forward primer 3; B3: Backward primer 3.

Primer	Concentration	Sequence 5'→ 3'
FIP	8 µM	TGGGGTATAAATTACATAAGCGCATAACGATGATGACTGATTTATCGA
BIP	8 µM	TGAGAGATATCTTTTCAAAGGCTCCGATGGTTATCCACTTTCAAACT
LF	4 µM	GAAATTGTATGGGAGAGTCGTTGT
LB	4 µM	ACATCTGTCCCCTCACTAAATACT
F3	1 µM	AAGCTGAACACTATTTTCTGT
B3	1 µM	ATTATTTTGAATACCATCCAGGT

2. Data analysis

The baseline of each curve has been calculated as the mean signal of the first 5 detection cycles (eq.1)

$$B = \frac{\sum_{i=1}^{5} S_i}{i} \quad \text{(Eq. 1)}$$

with i the detection cycle, S the signal, and B the baseline. The baseline normalization has been achieved by dividing each fluorescence value by the baseline value

$$S_i^*(t) = S(t)/B \quad \text{(Eq. 2)}$$

with $S^*(t)$ the normalized signal and t the time.

Five parameter fit of normalized data was performed using eq. 3

$$\tilde{S}(t, b, c, d, e, f) = c + \frac{(d-c)}{\left(1+e^{[b*(t-e)]}\right)^f} \quad \text{(Eq. 3)}$$

with b the slope, c the ground asymptote, d the maximum asymptote, e the inflection point, and f the asymmetry parameter.

3. Microfluidic protocols

In this section, the microfluidic protocols for elution, first TCR actuated valving and inward pumping can be found (Table 2). Furthermore, the protocols for the different mixing processes (Table 3. Table 4, Table 5) and the downstream processing (Table 6) are listed. Thus, protocols for the different experiments were combined as follows:

- Shake mode mixing:
  Table 2 + Table 3 + Table 6
- 2 x TCR actuated mixing:
  Table 2 + Table 4 + Table 6
- 5 x TCR actuated mixing combined with shake mode mixing:
- Table 2 + Table 5 + Table 6

Table 2: Microfluidic protocol for elution, TCR actuated valving and centrifugo-pneumatic inward pumping. The described protocol is performed prior to the various mixing processes. A dash (-) indicates that a parameter is not changed with relation to the previous step. "N/A" is written in case an entry is not applicable. If time is "0 s", the step is performed until the given parameters are reached. A negative acceleration corresponds to a reduction of rotational frequency.

Index	Step	Acceleration	Frequency	Temperature	Hold time
1	Start	10 Hz/s	10 Hz	ambient (~22 °C)	0 s
2	Heat up for elution	0 Hz/s	-	56 °C	
3	Shake mode during elution	10 Hz/s	9 Hz	56 °C	20 s
4	Shake mode during elution	10 Hz/s	20 Hz	56 °C	1 s
5	Loop with Start loop: #3 Stop loop: #4	N/A	N/A	N/A	600 s
6	Speed for TCR valve	-3 Hz/s	6 Hz	56 °C	0 s
7	TCR valve actuation	0 Hz/s	-	45 °C	0 s
8	Compression chamber loading	5 Hz/s	60 Hz	-	10 s
9	Centrifugo-pneumatic pumping	-11 Hz/s	5 Hz	-	10 s
10	Ensure liquid-free vent channels	10 Hz/s	20 Hz	-	5 s

Table 3: Microfluidic protocol for shake mode mixing during rehydration of lyophilized reaction mix. *: These steps are not required for shake mode mixing but for TCR actuated mixing. They are performed in this protocol to keep consistent conditions between the mixing processes.

Index	Step	Acceleration	Frequency	Temperature	Hold time
1	Free vent channel of mixing chamber from liquid*	10 Hz/s	20 Hz	-	5 s
2	Speed reduction for mixing*	-5 Hz/s	6 Hz	-	0 s
3	Shake mode mixing	10 Hz/s	6 Hz	-	1 s
4	Shake mode mixing	-11 Hz/s	3 Hz	-	2 s
5	Loop with Start loop: #3 Stop loop: #4	N/A	N/A	N/A	20 s
6	Speed increase for cooldown*	5 Hz/s	20 Hz	-	0 s
7	Free vent channel of mixing chamber from liquid*	5 Hz/s	50 Hz	-	0 s
8	Loop with Start loop: #1 Stop loop: #7	N/A	N/A	N/A	300 s

Table 4: Microfluidic protocol for 2x TCR actuated bubble mixing combined with shake mode mixing.

Index	Step	Acceleration	Frequency	Temperature	Hold time
1	Free vent channel of mixing chamber from liquid	10 Hz/s	20 Hz	-	5 s
2	Speed reduction for mixing	-5 Hz/s	6 Hz	-	0 s
3	Heating for TCR actuated bubble mixing	0 Hz/s	-	60 °C	0 s
4	TCR actuated bubble mixing & shake mode mixing	10 Hz/s	6 Hz	-	1 s
5	TCR actuated bubble mixing & shake mode mixing	-11 Hz/s	3 Hz	-	2 s
6	Loop with Start loop: #4 Stop loop: #5	N/A	N/A	N/A	20 s
7	Cool down w/o TCR valve actuation	5 Hz/s	20 Hz	-	0 s
8	Cool down w/o TCR valve actuation	0 Hz/s	-	45 °C	30 s
9	Free vent channel of mixing chamber from liquid	5 Hz/s	50 Hz	-	0 s
10	1x Loop with Start loop: #2 Stop loop: #9	N/A	N/A	N/A	0 s

Table 5: Microfluidic protocol for 5x TCR actuated bubble mixing.

Index	Step	Acceleration	Frequency	Temperature	Hold time
1	Free vent channel of mixing chamber from liquid	10 Hz/s	20 Hz	-	5 s
2	Speed reduction for mixing	-5 Hz/s	6 Hz	-	0 s
3	Heating for TCR actuated bubble mixing	0 Hz/s	-	60 °C	0 s
4	TCR actuated bubble mixing	-11 Hz/s	3 Hz	-	20 s
5	Cool down w/o TCR valve actuation	5 Hz/s	20 Hz	-	0 s
6	Cool down w/o TCR valve actuation	0 Hz/s	-	45 °C	30 s
7	Free vent channel of mixing chamber from liquid	5 Hz/s	50 Hz	-	0 s
8	Loop with Start loop: #2 Stop loop: #7	N/A	N/A	N/A	300 s

Table 6: Microfluidic protocol after the mixing step: TCR actuated valving, metering, aliquoting, and real-time LAMP reaction and signal detection. *: These steps are not required for shake mode mixing but for TCR actuated mixing. They are performed in this protocol to keep consistent conditions between the mixing processes. #: Step 12 is repeated twelve times (for each reaction chamber).

Index	Step	Acceleration	Frequency	Temperature	Hold time
1	Rotational frequency for heat up w/o TCR actuated bubble mixing*	5 Hz/s	20 Hz	-	0 s
2	Heat up for TCR actuated valving	0 Hz/s	-	60 °C	60 s
3	Free vent channel of mixing chamber from liquid*	10 Hz/s	60 Hz	-	0 s
4	TCR valve actuation	0 Hz/s	-	45 °C	0 s
5	Rotational speed for TCR valve	-5 Hz/s	6 Hz	-	10 s
6	Metering	5 Hz/s	11 Hz	-	30 s
7	Actuation of centrifugo-pneumatic valves	3 Hz/s	50 Hz	-	0 s
8	Rotational speed for LAMP reaction	-5 Hz/s	5 Hz	-	0 s
9	LAMP reaction temperature	0 Hz/s	-	64 °C	0 s
10	Break for detection	-1 Hz/s	0 Hz	-	0 s
11	Detection (FAM channel, chamber 1)	N/A	N/A	N/A	
12[#]	11 x loop for detection with Start loop: #11 Stop loop: #11	N/A	N/A	N/A	0 s
13	Rotation	3 Hz/s	10 Hz	-	30 s
14	Loop with: Start loop: #10 Stop loop: #13	N/A	N/A	N/A	3600 s
15	End of process	-3 Hz/s	0 Hz	ambient (~22 °C)	0 s

4. Image Analysis using Fiji

Fill levels of channels and chambers were determined using the "measure" command of Fiji[3]. JPEG images were recorded in real-time with a strobe camera setup (Biofluidix GmbH, Freiburg, Germany) on a *LabDisk player* (Qiagen Lake Constance, Stockach, Germany). For each JPEG, the width (0.6 mm) of the downstream valve channel was measured resulting in a relationship between length in pixels and real length in mm.

Figure 1 shows the measurement of the fill level of the mixing chamber after complete rehydration of the lyophilized reagents. Here, the radial outward edge of the valve channel is at r_2 = 35.0 mm. The radial inner position of the liquid column was determined *via* calculation of the distance using the measured length from the results window.

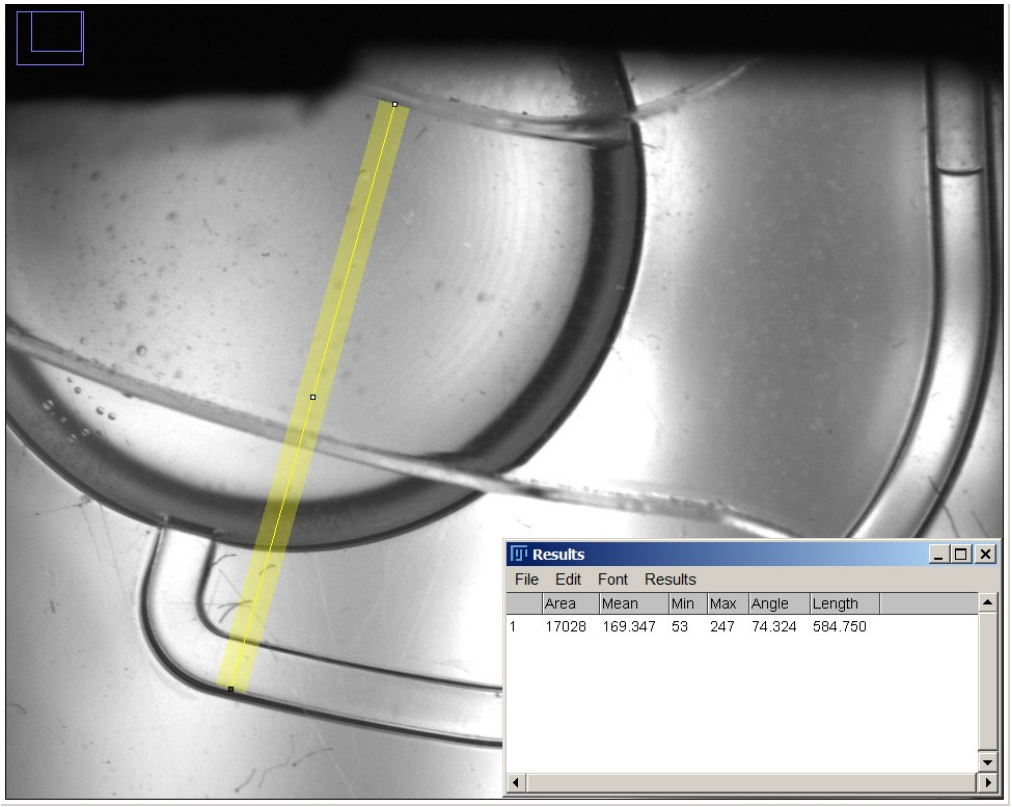
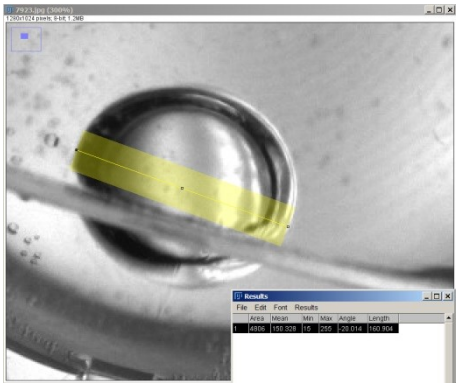
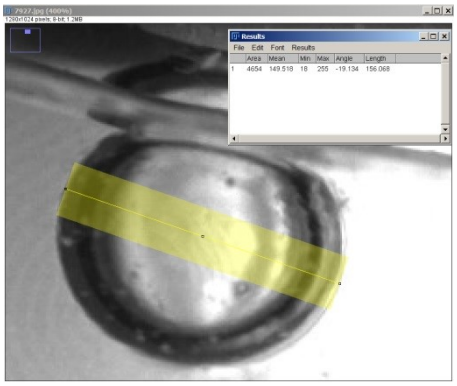
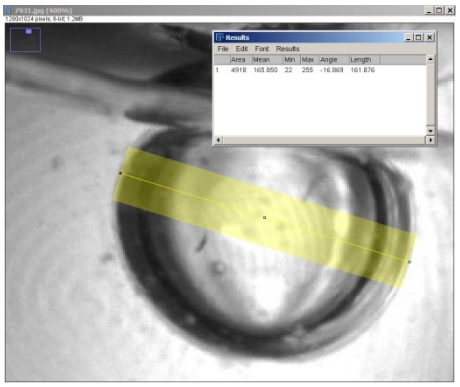
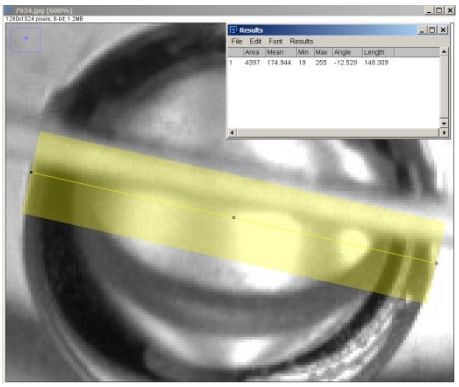
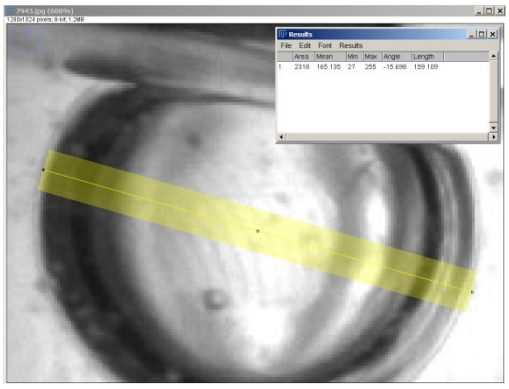
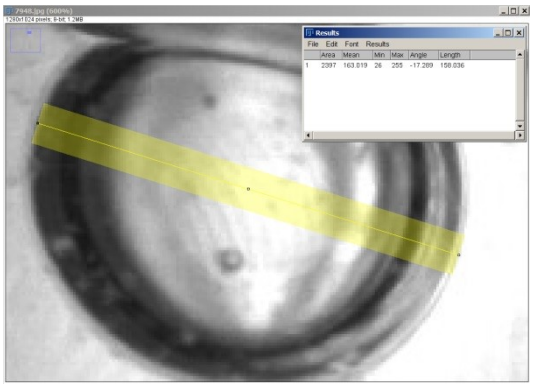


Figure 1: Hydrostatic height measurement of the liquid column in the mixing chamber using Fiji. The measured liquid level serves as input for centrifugal pressure calculations at varying rotational frequencies.

Gas bubble size was determined measuring diameters of rising gas bubbles. It was not possible to measure each single bubble due to the experimental setup, in which only one image can be recorded per round. Therefore, some bubbles were partly out of the image range. The images of the gas bubble measurement of all 16 performed measurements are shown in Table 7.

Table 7: Measurements on bubble size using Fiji. Measurements were performed in mixing cycle 4 and cycle 5 of a 5x TCR actuated mixing process. Length values are given in pixel (Px). Correlation factor is 80.51 Px/mm.

#1 	**#2** 
#3 	**#4** 
#5 	**#6** 

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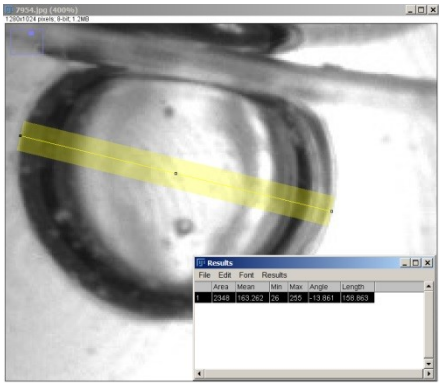

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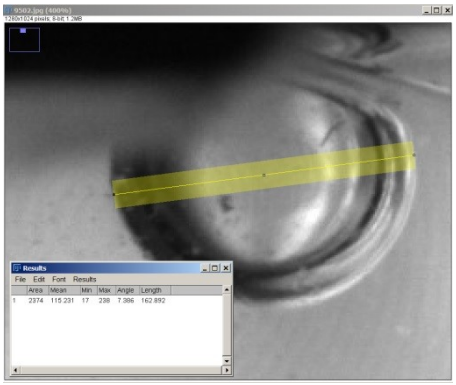

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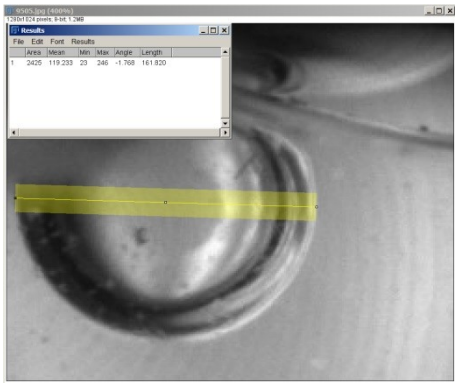

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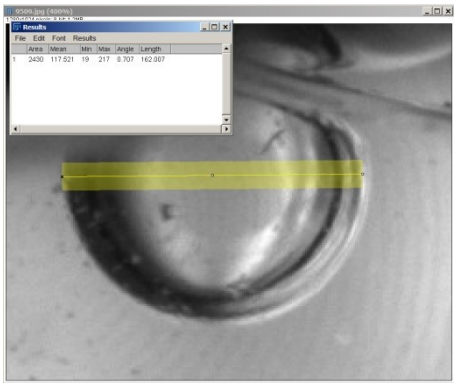

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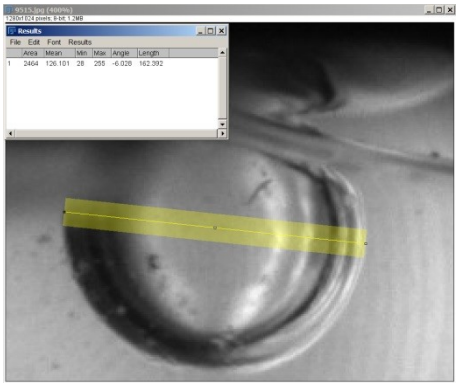

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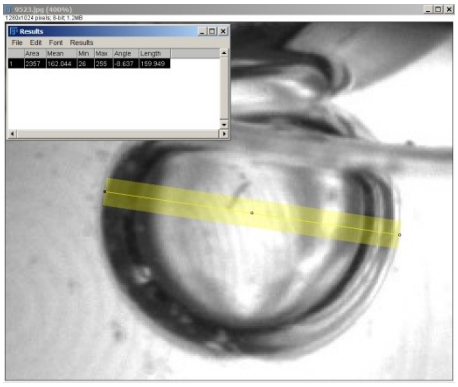

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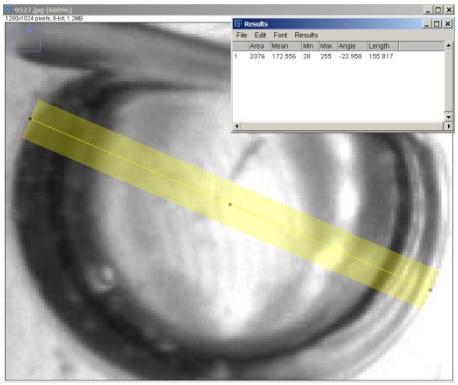

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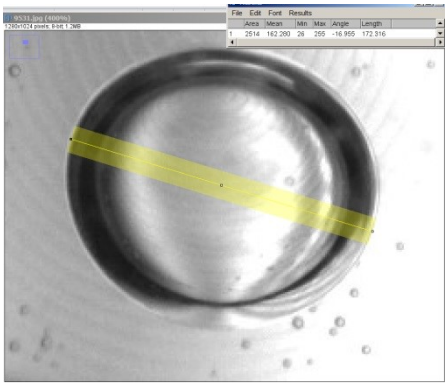

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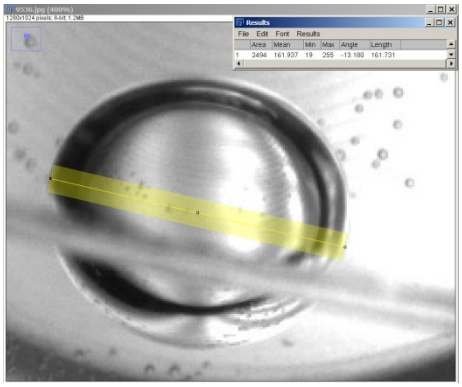	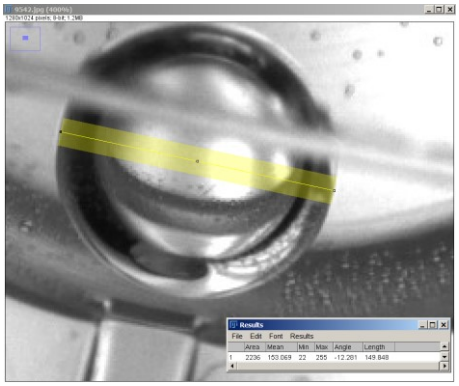

References

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