Structure, function and dynamics of the tear film lipid layer

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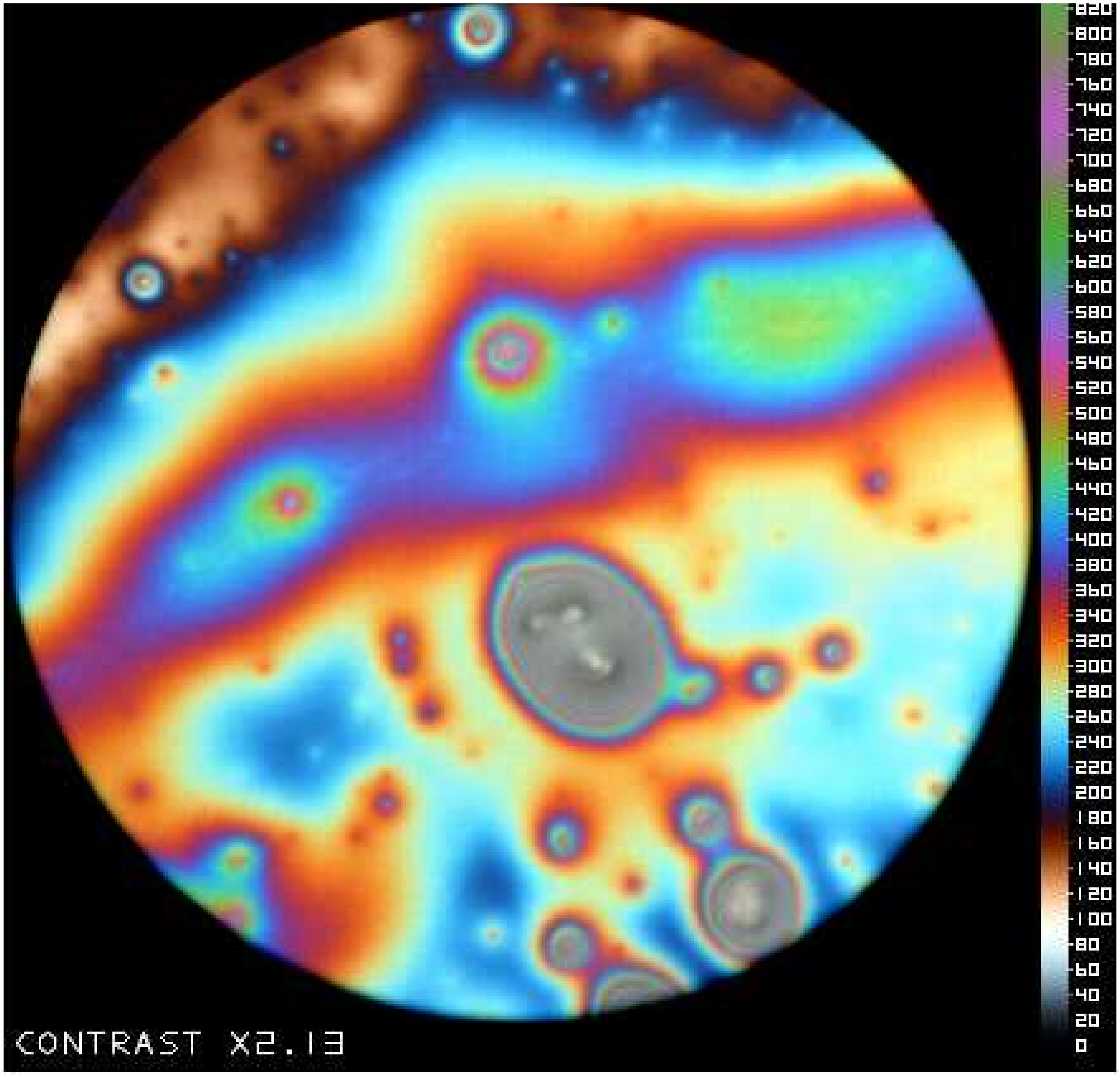


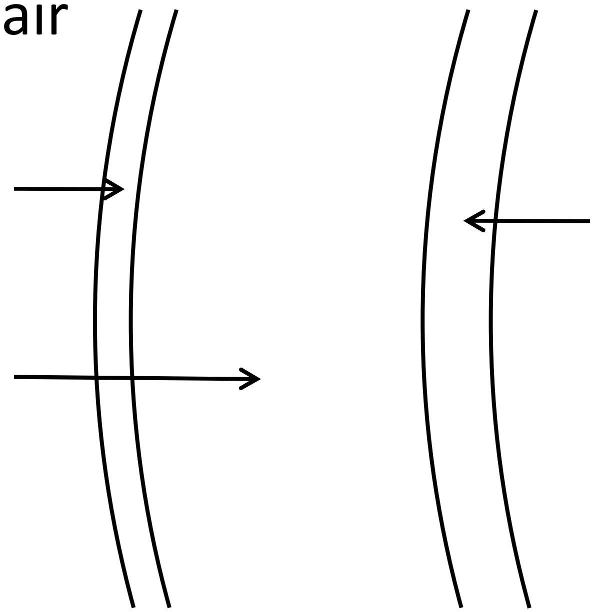
Acknowledgements:
Richard Braun
Tom Millar
Kelly Nichols
Jason Nichols
Priya Ramamoorthy
Kathy Reuter

Outline

- Part 1. The lipid layer of the tear film and dry eye disorders
- Part 2. Four characteristics of a good lipid layer

Part 1. The lipid layer of the tear film and dry eye disorders

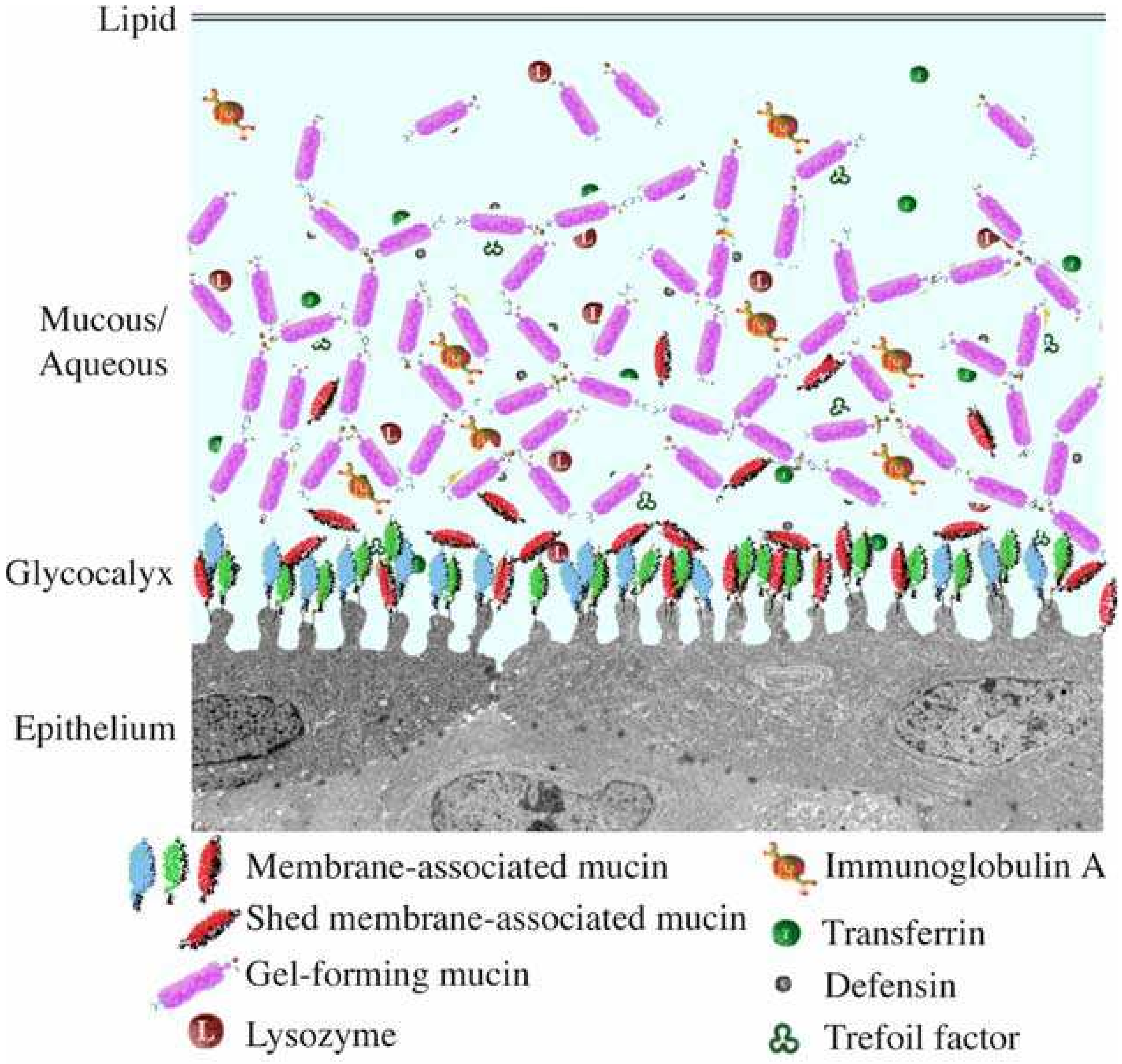
Structure of the tear film



- *The lipid layer is derived from the meibomian glands in the lids.*
- *The aqueous layer is derived from the lacrimal glands behind the upper lids.*

Structure of the tear film (surfactant proteins not shown)

Gipson, 2004, Exp Eye Res, 78, 379



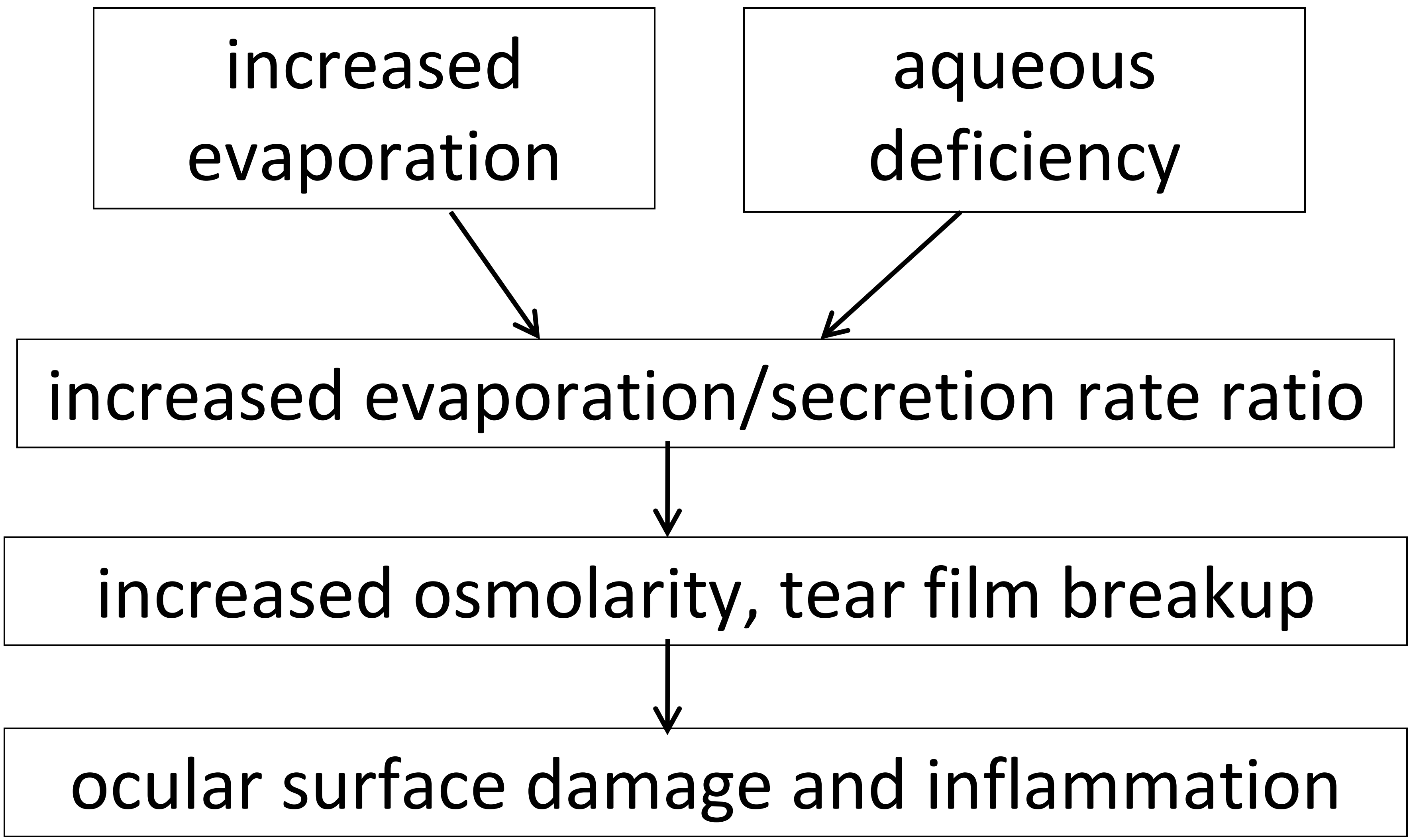
Comparison of meibum and lung lipid layer

	main function?	**main components**	**thickness**
Meibum (tear lipid layer)	Reduction of evaporation	Wax esters Cholesteryl esters Polar lipids	About 10 layers
Lung lipid layer	Reduction of surface tension	Phospholipids	monolayer

Dry eye disorders

- Common ocular disorders – prevalence is 14% over the age of 48 years.
- Mild to serious disorders.
- Poorly understood
- **Major classes**:
  - Aqueous deficient dry eye
  - Evaporative dry eye
- **Core mechanisms**
  - Tear film breakup
  - Increased osmolarity

Ocular surface damage in dry eye disorders



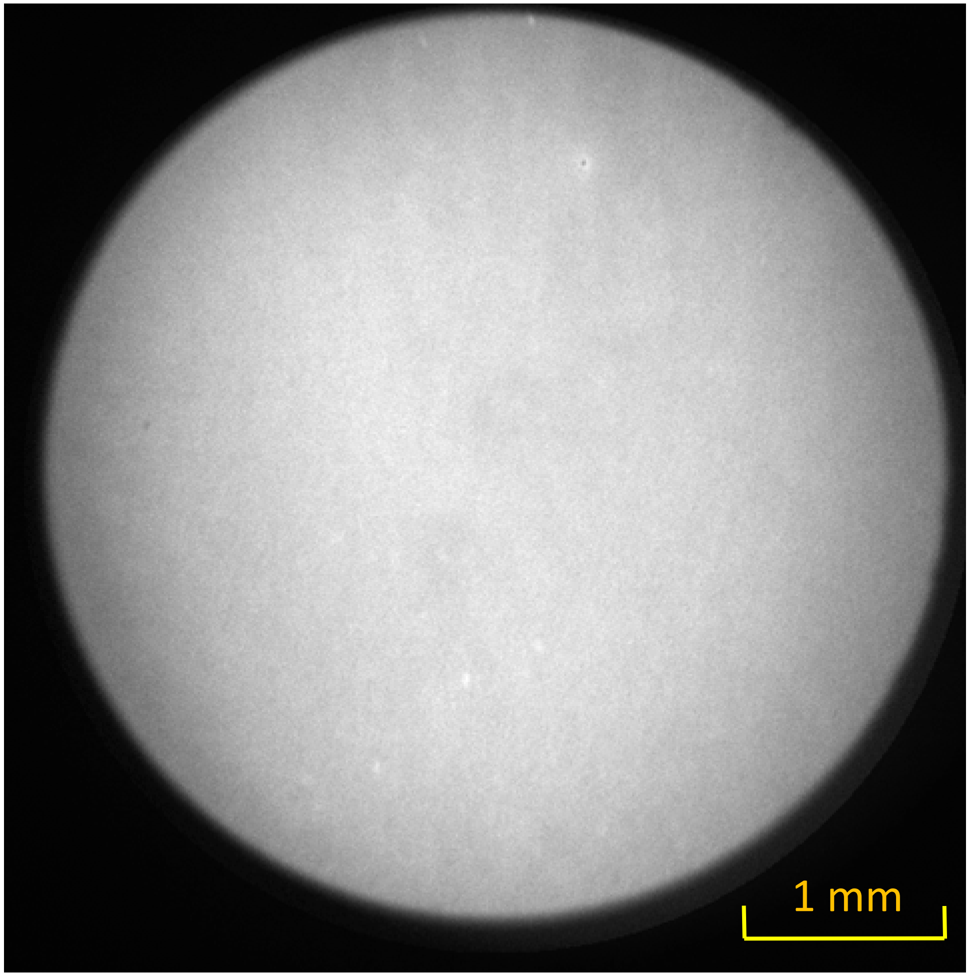
Evaporation/secretion rate ratios

Tomlinson et al, 2011, The Ocular Surface, 7 (4), 17-29

Normals	0.12
Aqueous deficient dry eye	0.28
Evaporative dry eye	0.27

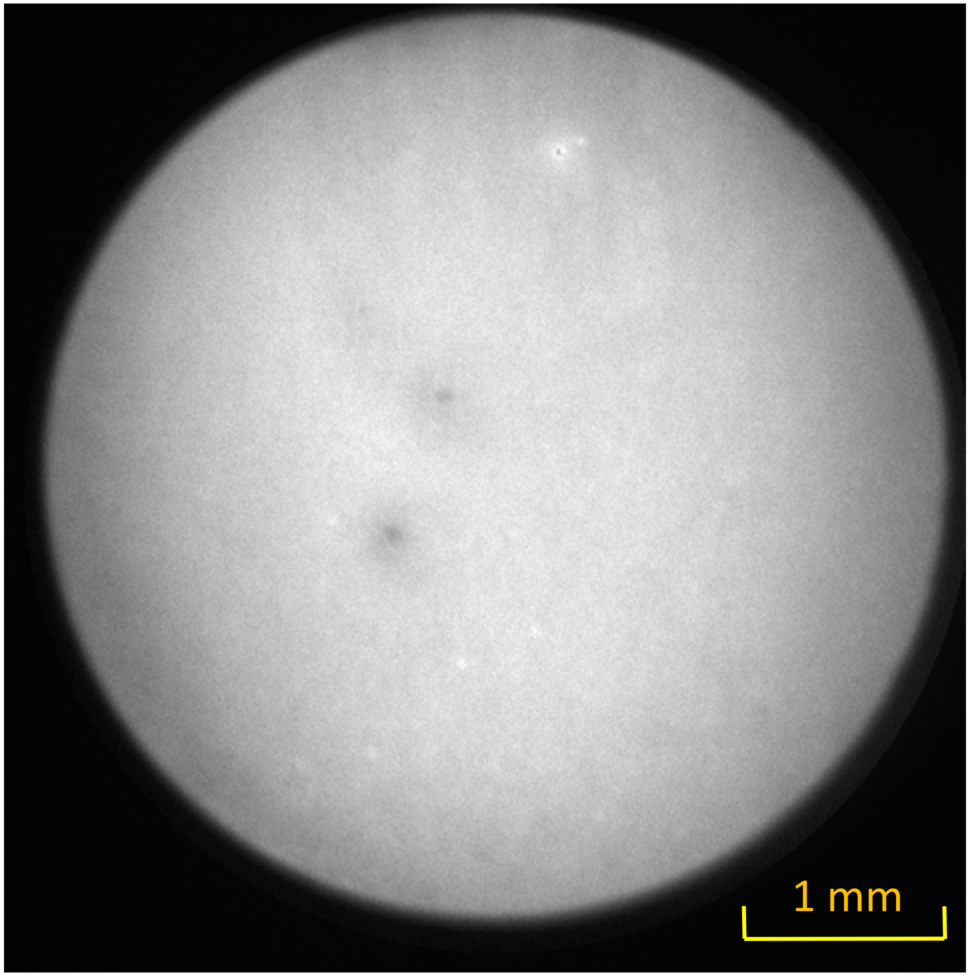
Fluorescein tear film breakup

9 seconds after a blink



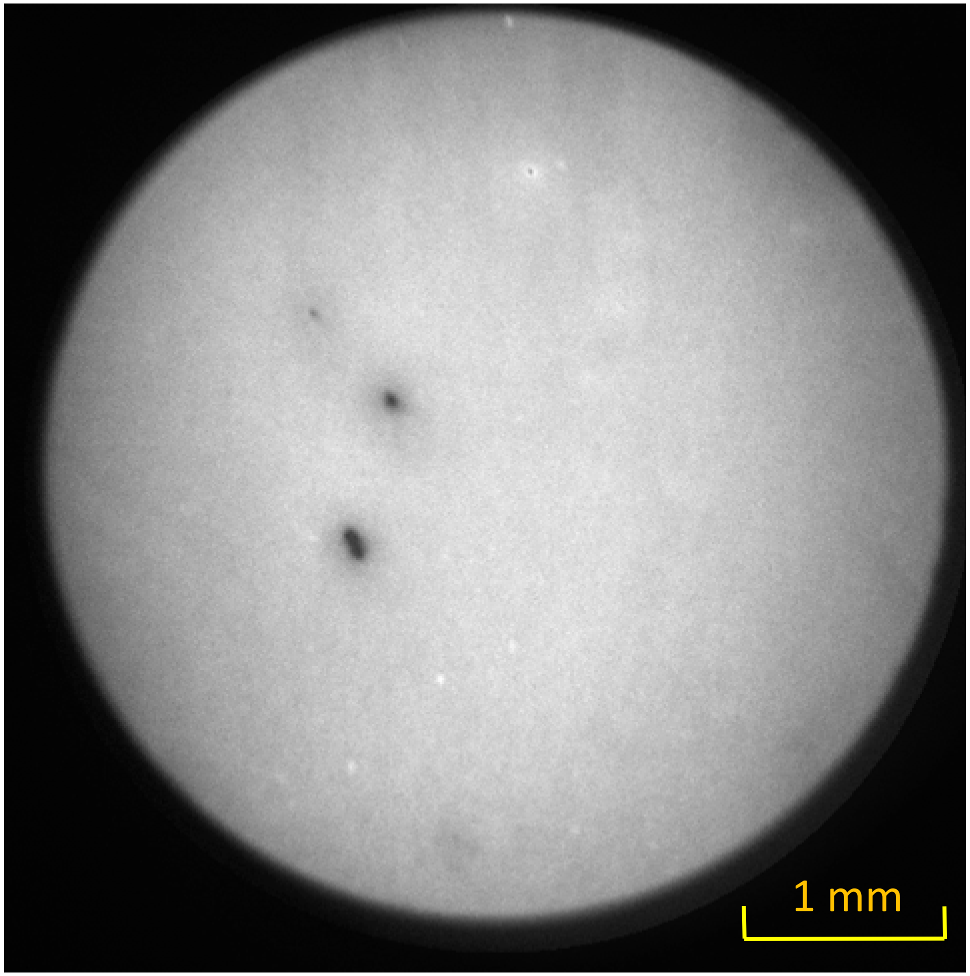
Fluorescein tear film breakup

15 seconds after a blink



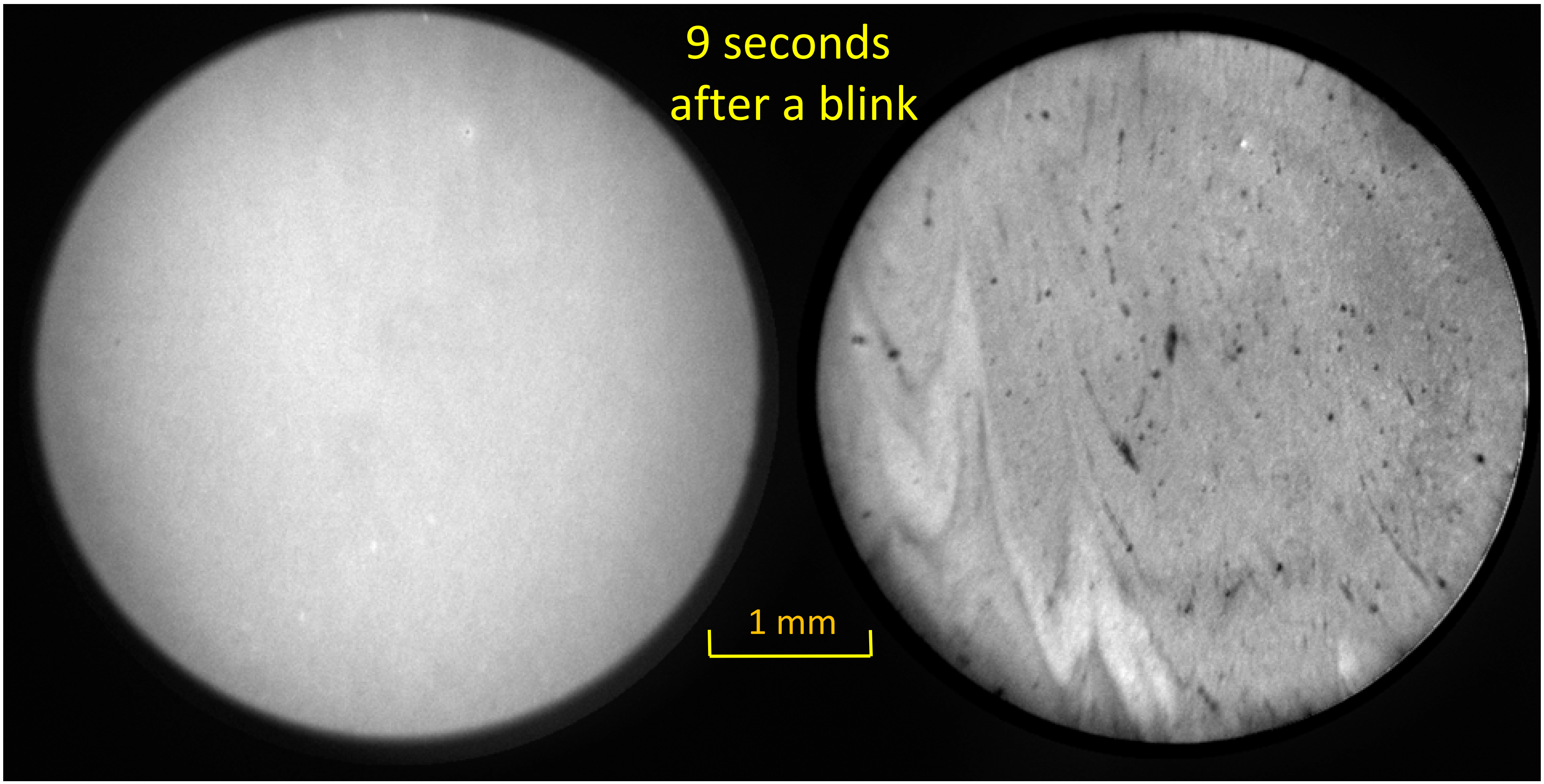
Fluorescein tear film breakup

20.3 seconds after a blink



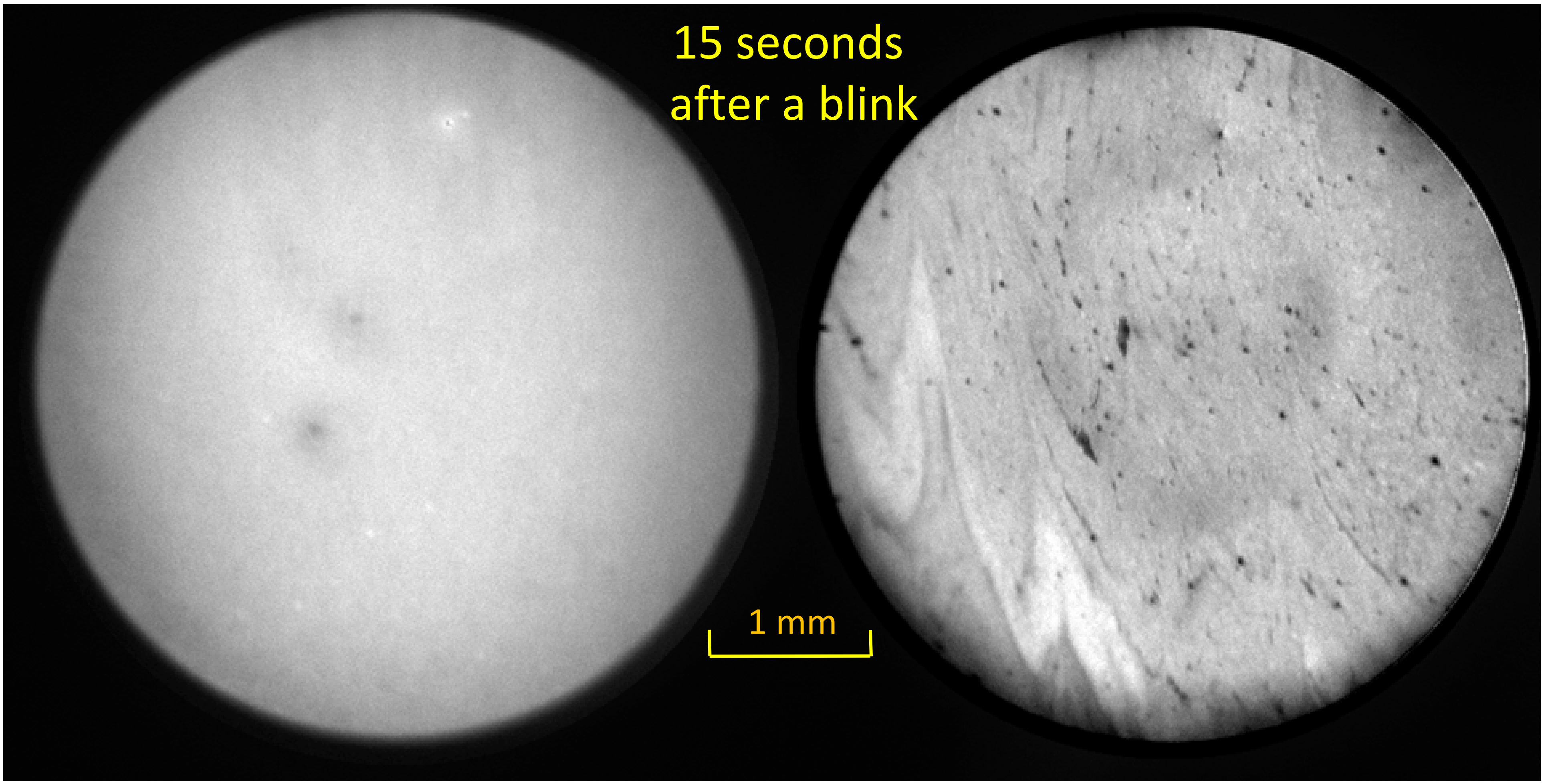
Evidence that tear thinning is due to evaporation
Simultaneous fluorescence and lipid images

fluorescence image lipid layer image



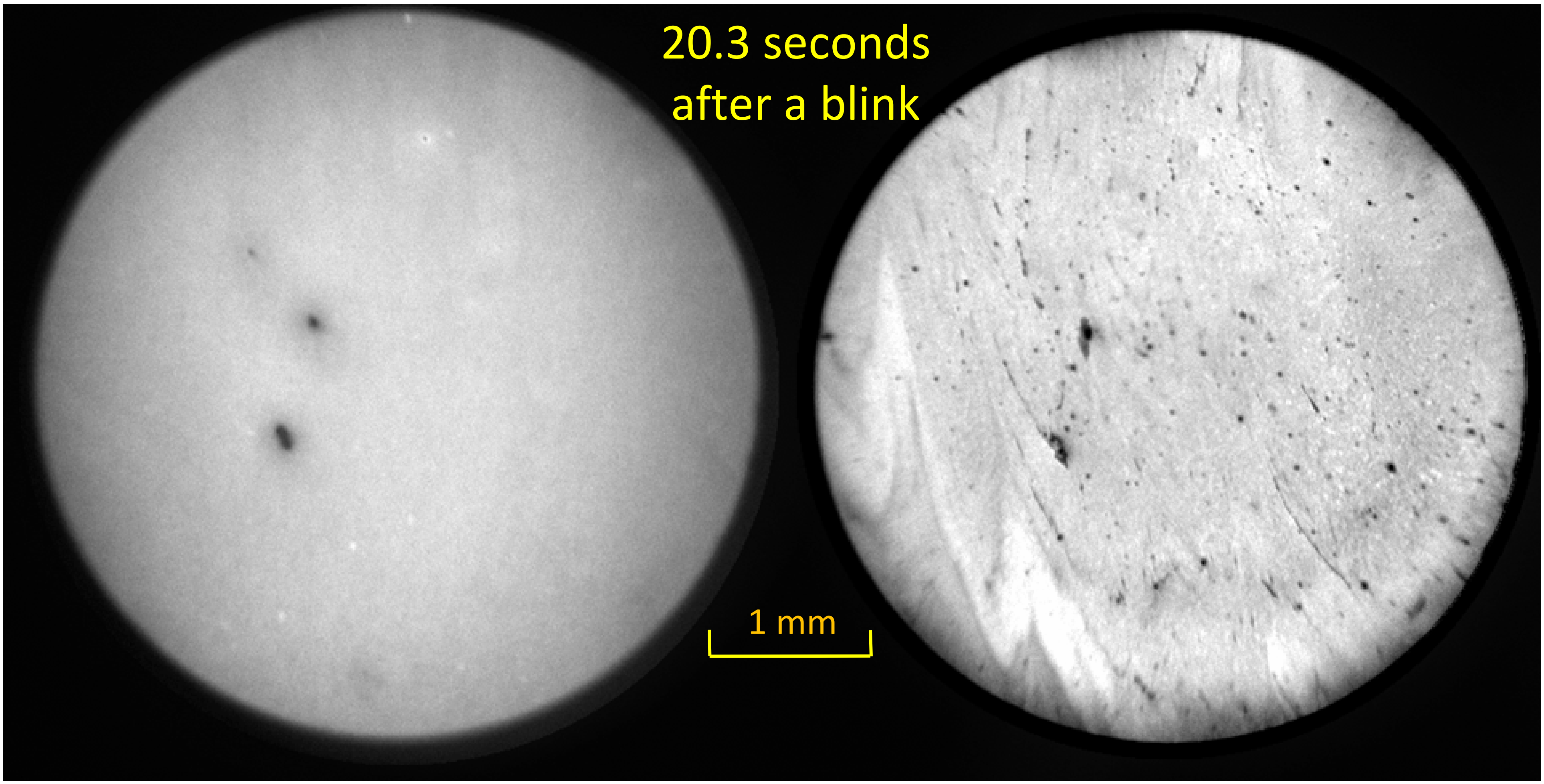
Evidence that tear thinning is due to evaporation
Simultaneous fluorescence and lipid images

fluorescence image　　　　lipid layer image



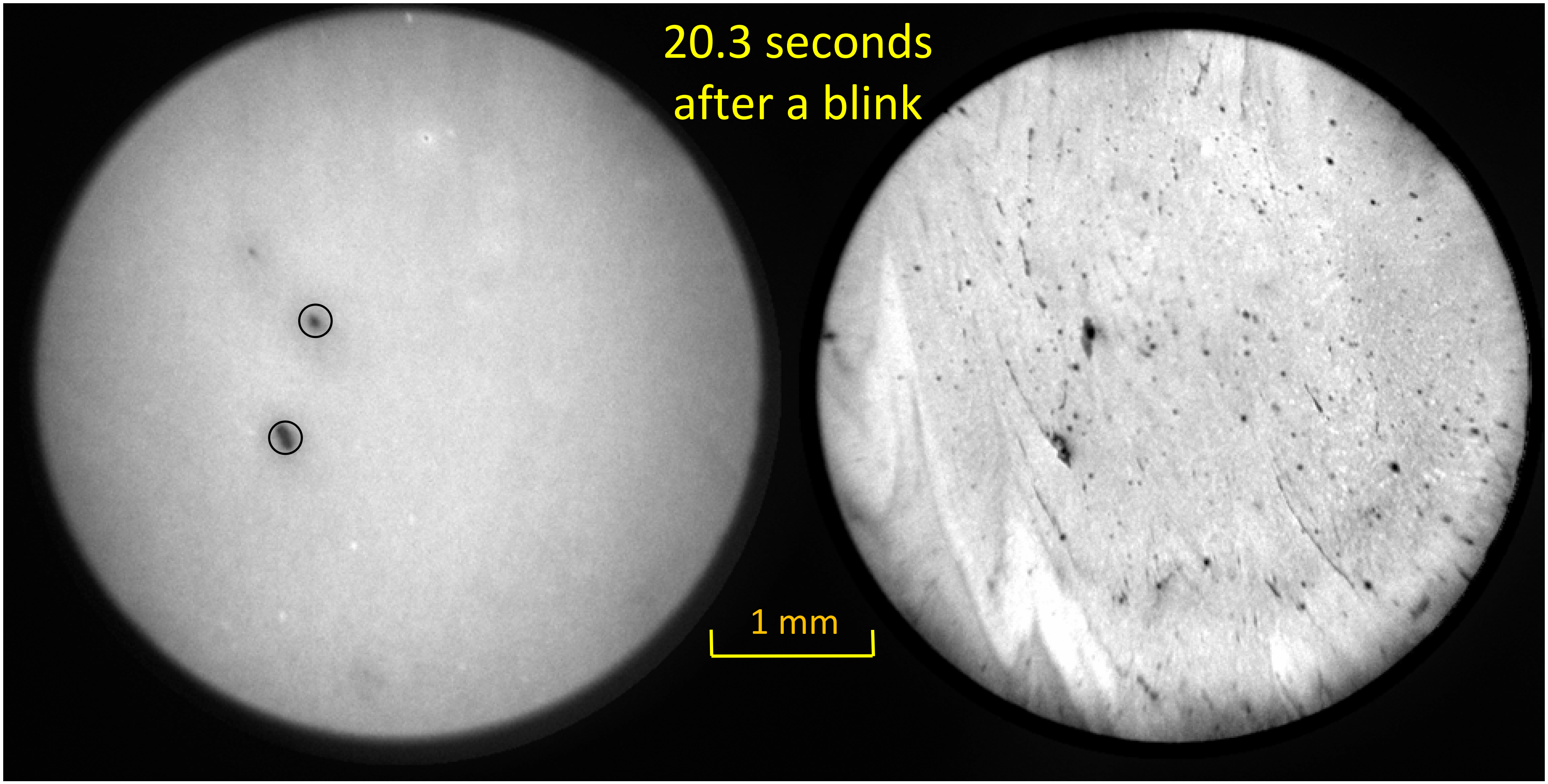
Evidence that tear thinning is due to evaporation
Simultaneous fluorescence and lipid images

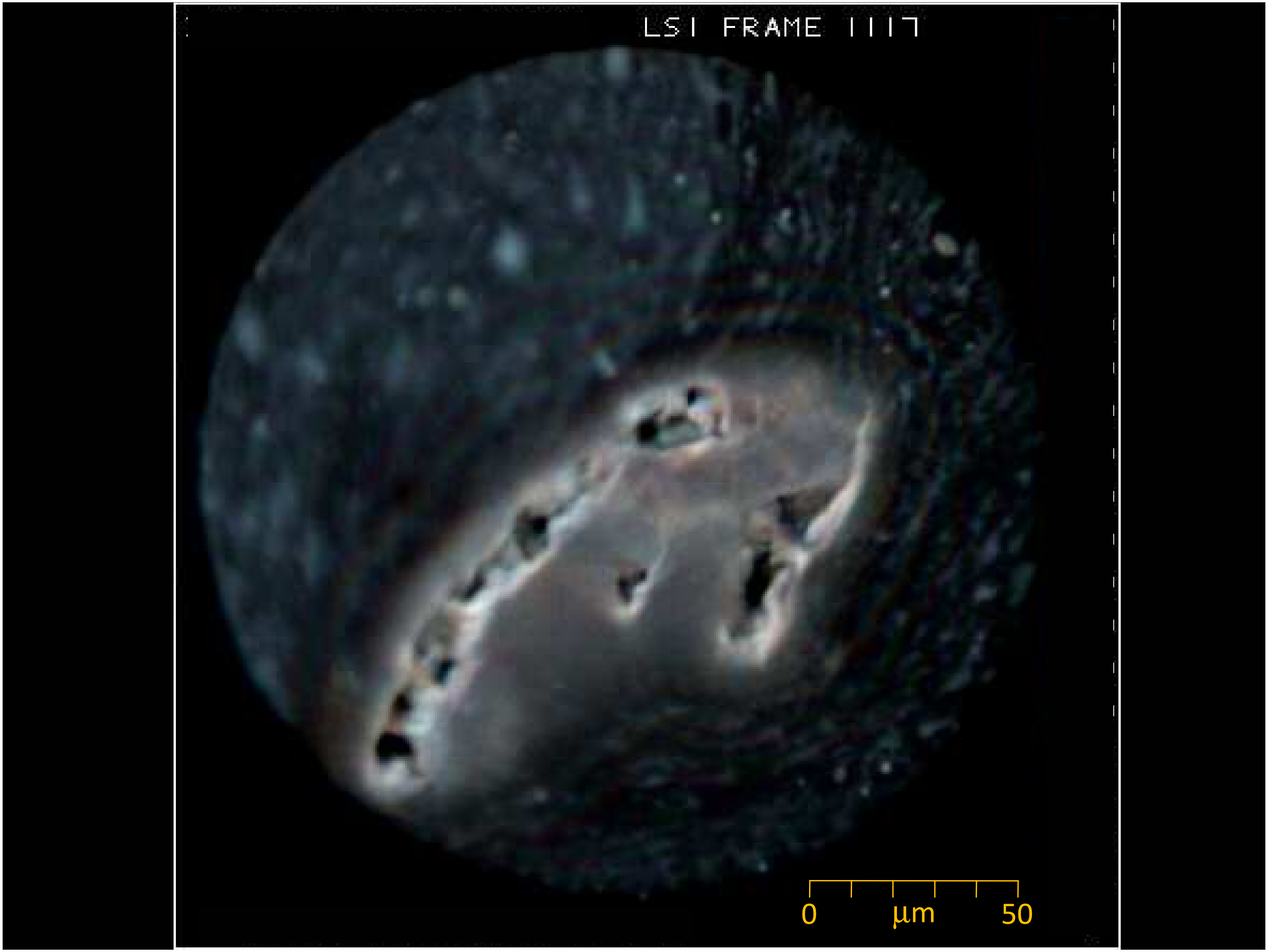
fluorescence image | lipid layer image



Evidence that tear thinning is due to evaporation
Simultaneous fluorescence and lipid images

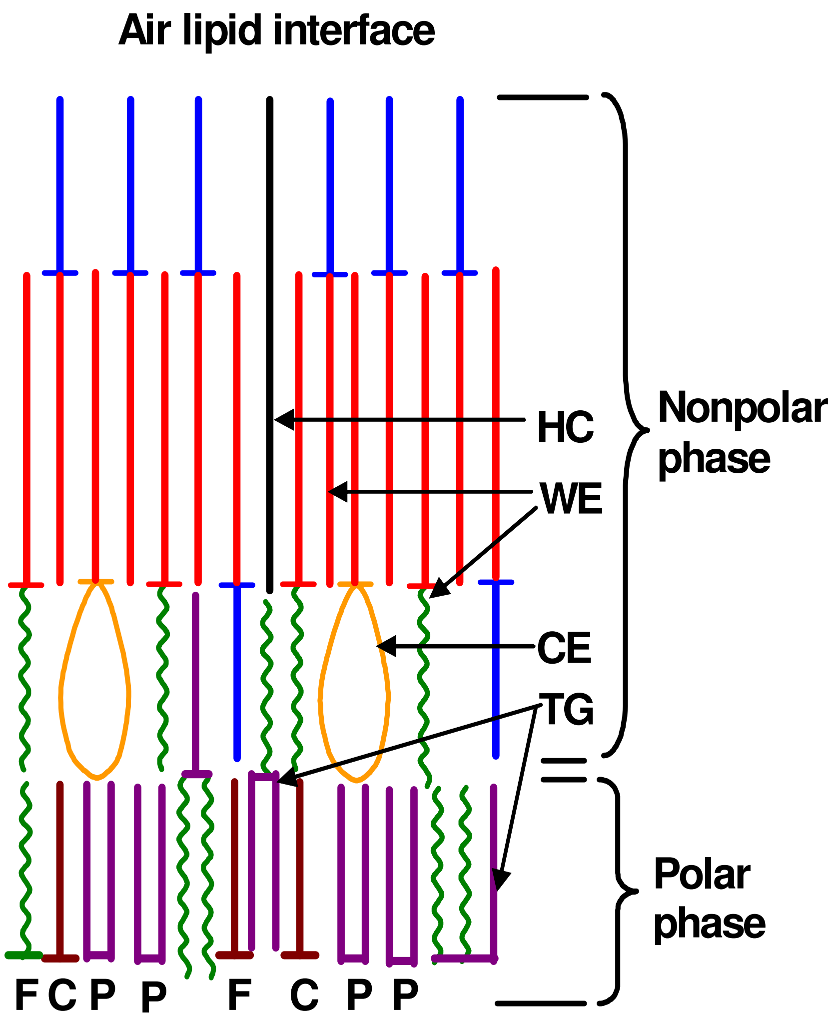
fluorescence image   lipid layer image




LSI FRAME 1117
0 µm 50

Lipid layer composition and organization

McCulley and Shine, 2001, Bioscience Reports, 21, 407



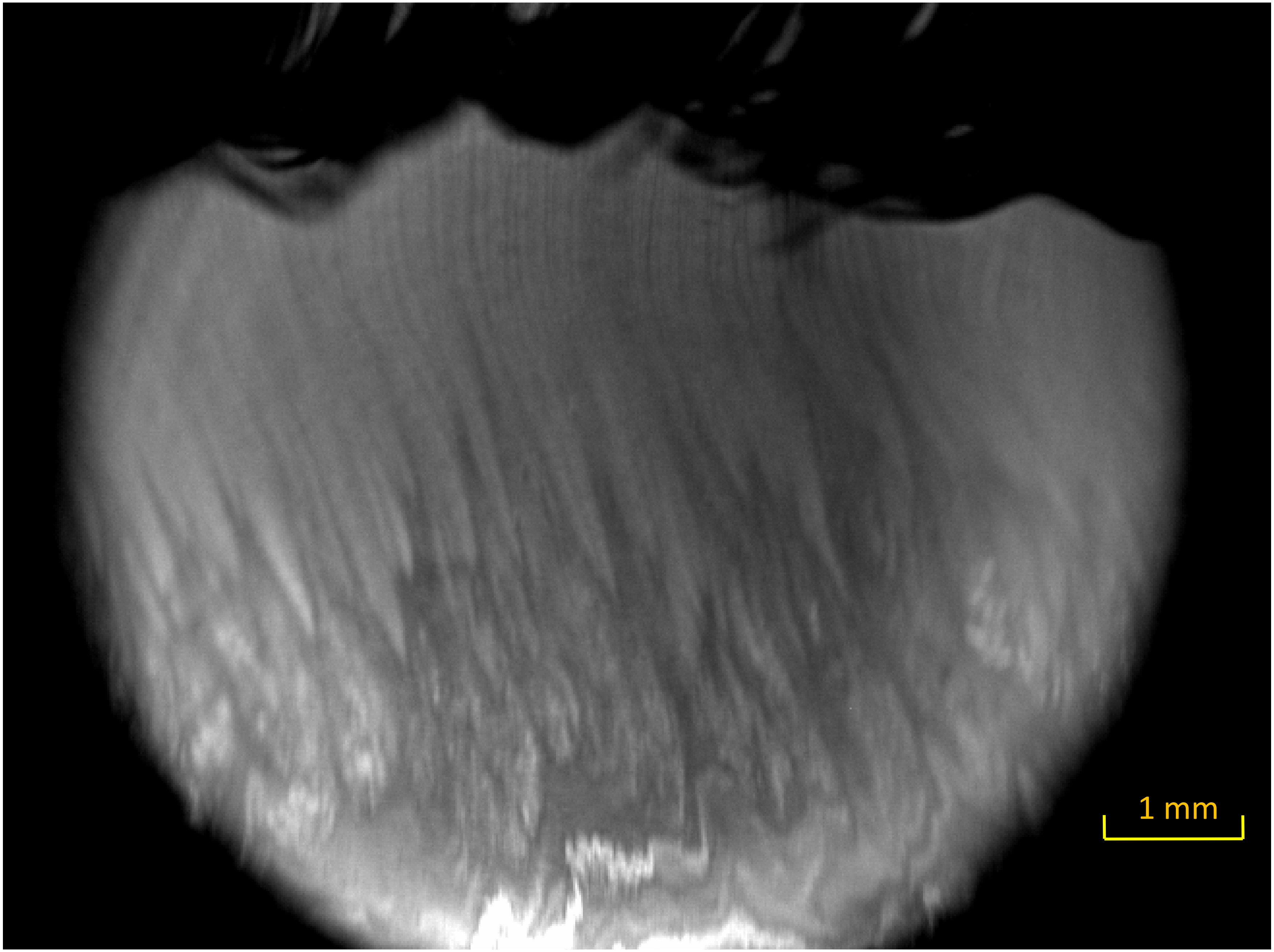
HC hydrocarbon
WE wax ester
CE cholesteryl ester
TG triglyceride
P phospholipid
C cerebroside
F fatty acid (free)

Composition of meibum

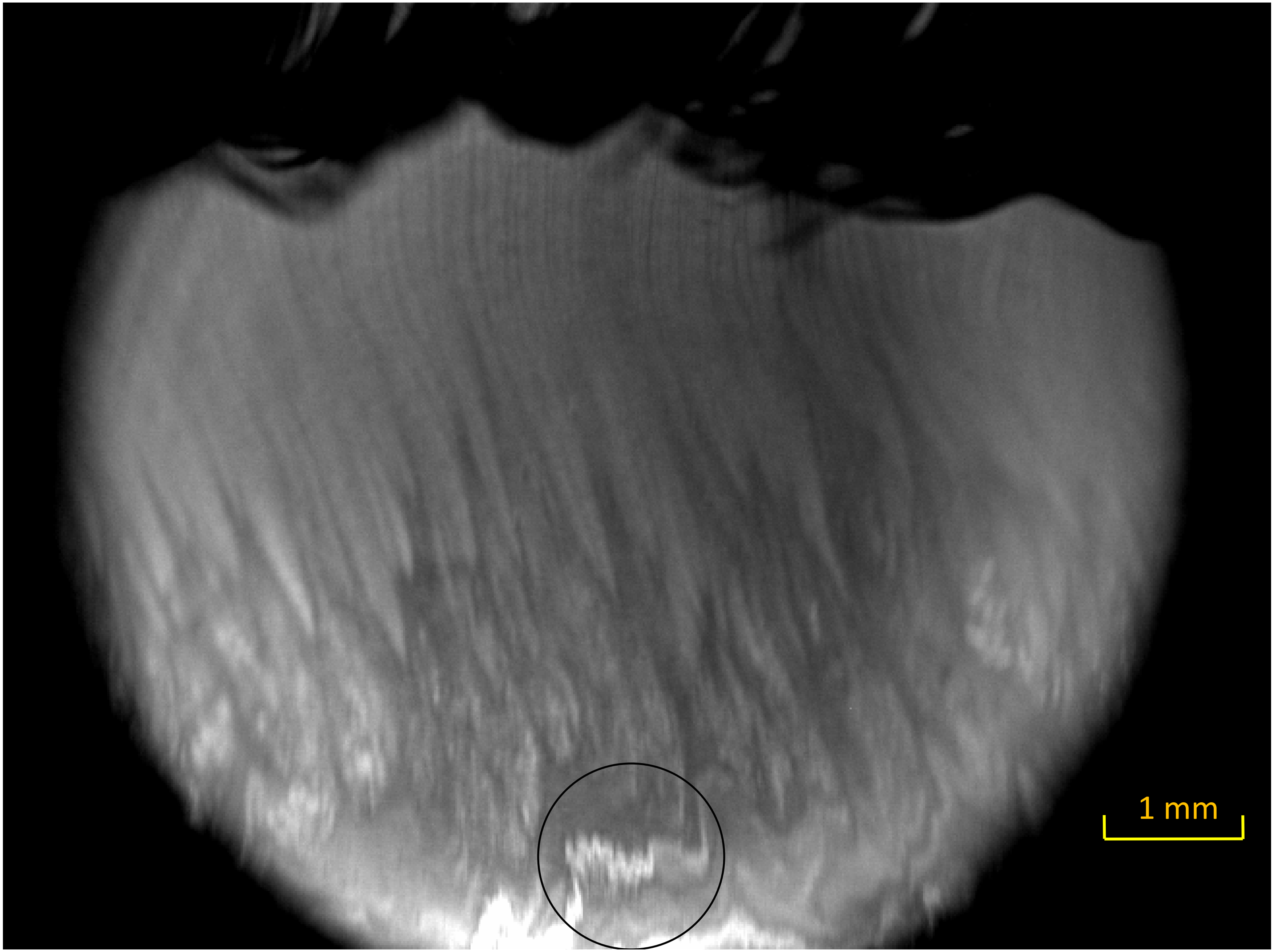
Lam et al., 2011, PLoS one, 6, e24339

Cholesteryl esters	67%
Wax esters	25%
Triacylglycerides	4%
Polar lipids (mainly (O-acyl)-ω-hydroxy fatty acids)	4%

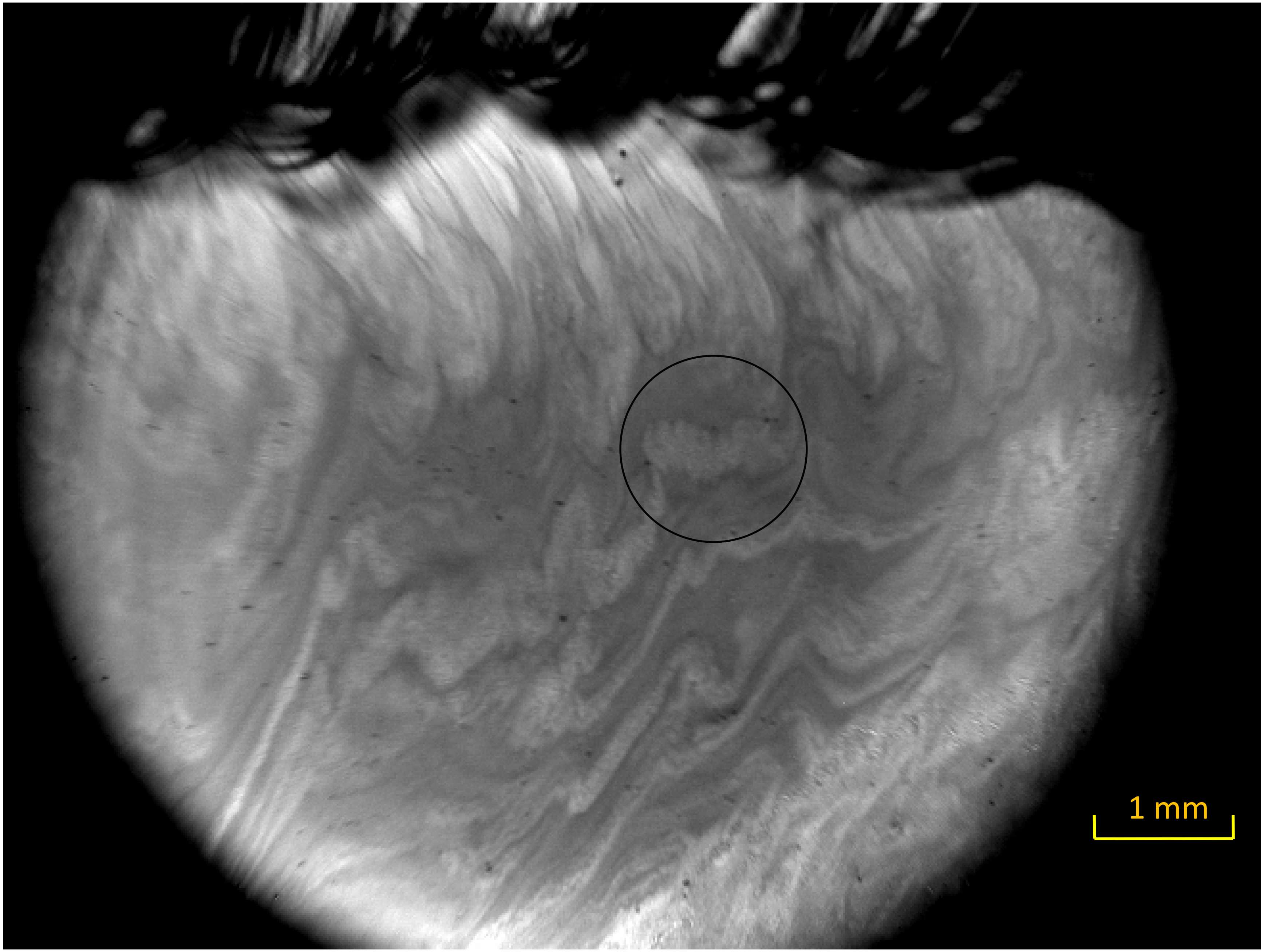
Marangoni flow - just after blink



Marangoni flow - just after blink

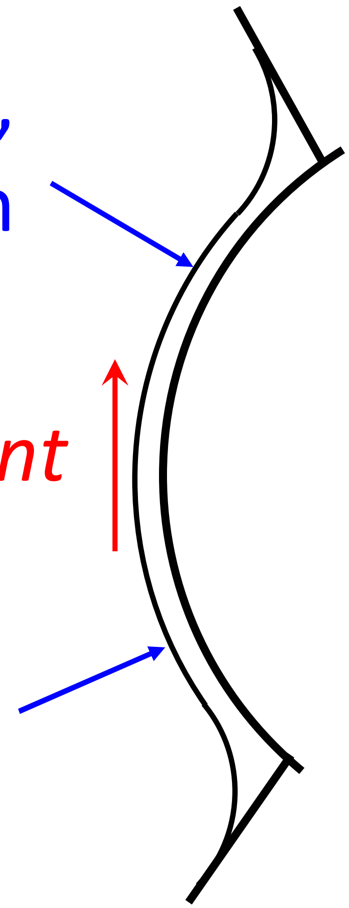


Marangoni flow - 8 seconds after blink



Marangoni flow after a blink

Berger and Corrsin, 1974, J Biomechanics, 7, 225

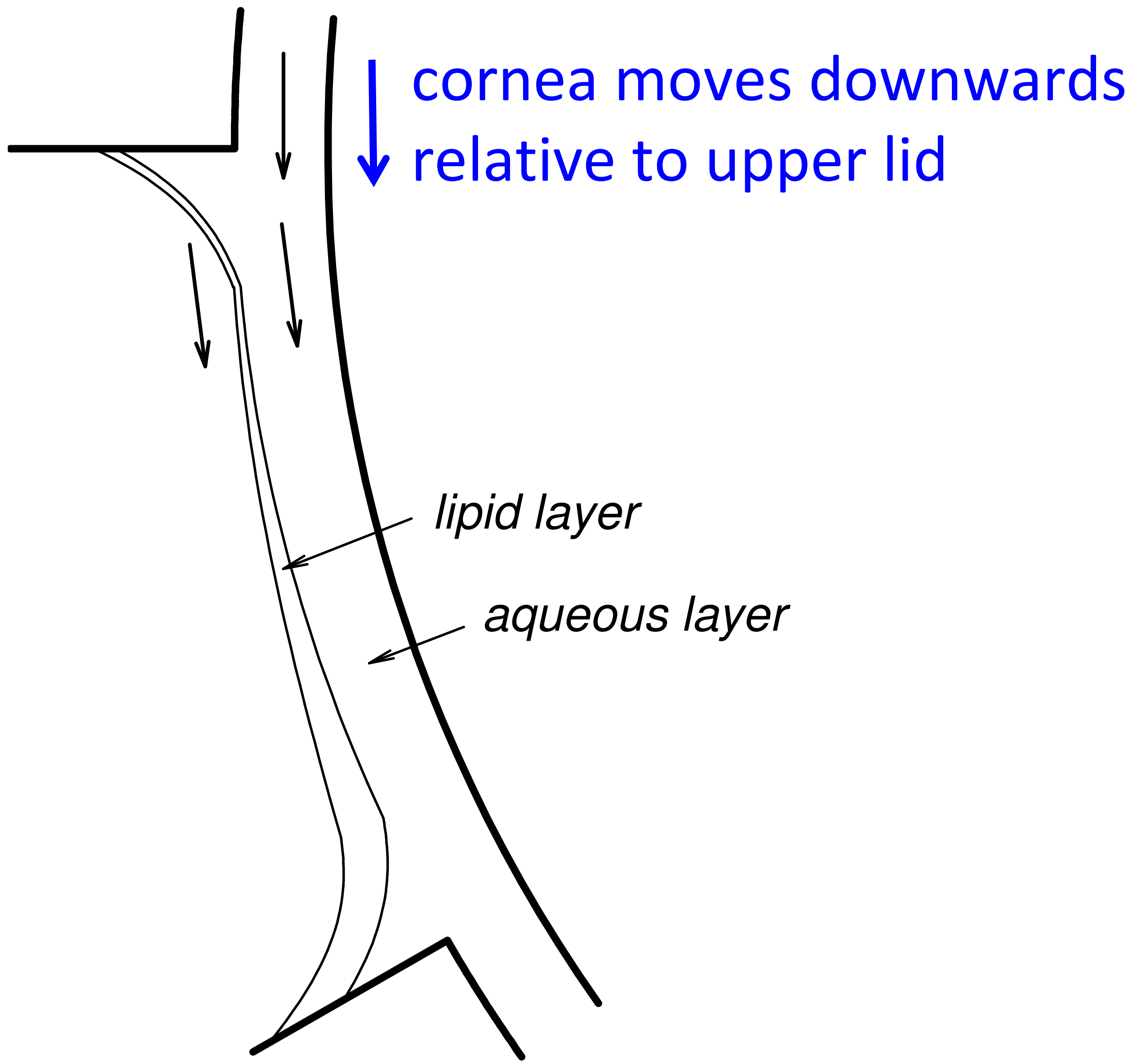


High surface tension,
low surfactant concentration

Surface tension gradient

Low surface tension,
high surfactant concentration

The upstroke of the blink causes the surface tension gradient?



Part 2. Four characteristics of a good lipid layer

Lipid layer characteristics for a good evaporation barrier

1.Evaporation resistance may be aided by the ***very long saturated hydrocarbon chains*** found in Meibomian lipids which form an energy barrier to the penetration of water molecules.
2.Lipid should be sufficiently fluid within the glands that it can be secreted by pressure on the glands – ***"not too solid"***.
3.The lipid layer should be stable and relatively thick and uniform (e.g., about 10 or more molecular layers); thus it should resist any tendency to dewetting (e.g., from van der Waals' forces) and a viscous or gel-like characteristic may aid in this respect - ***"not too fluid"***.
4.The lipid layer structure should withstand the repeated compression-expansion cycles caused by blinking – ***"respreadability"***.

Lipid layer characteristics for a good evaporation barrier

1. Evaporation resistance and long saturated hydrocarbon chains

Comparison of evaporation through lipid layer and C_{19} monolayer at 35° C

	Thinning rate, μm/min
good pre-corneal lipid layer	0.81*
C_{19} saturated fatty acid monolayer	13.1#

**Tomlinson et al, 2011, The Ocular Surface, 7 (4), 17-29*

#Archer and La Mer, J. Phys. Chem., 1955, 59, 200

Log evaporation resistance versus 1/temperature – evidence for activation energy

Archer and La Mer, J. Phys. Chem., 1955, 59, 200

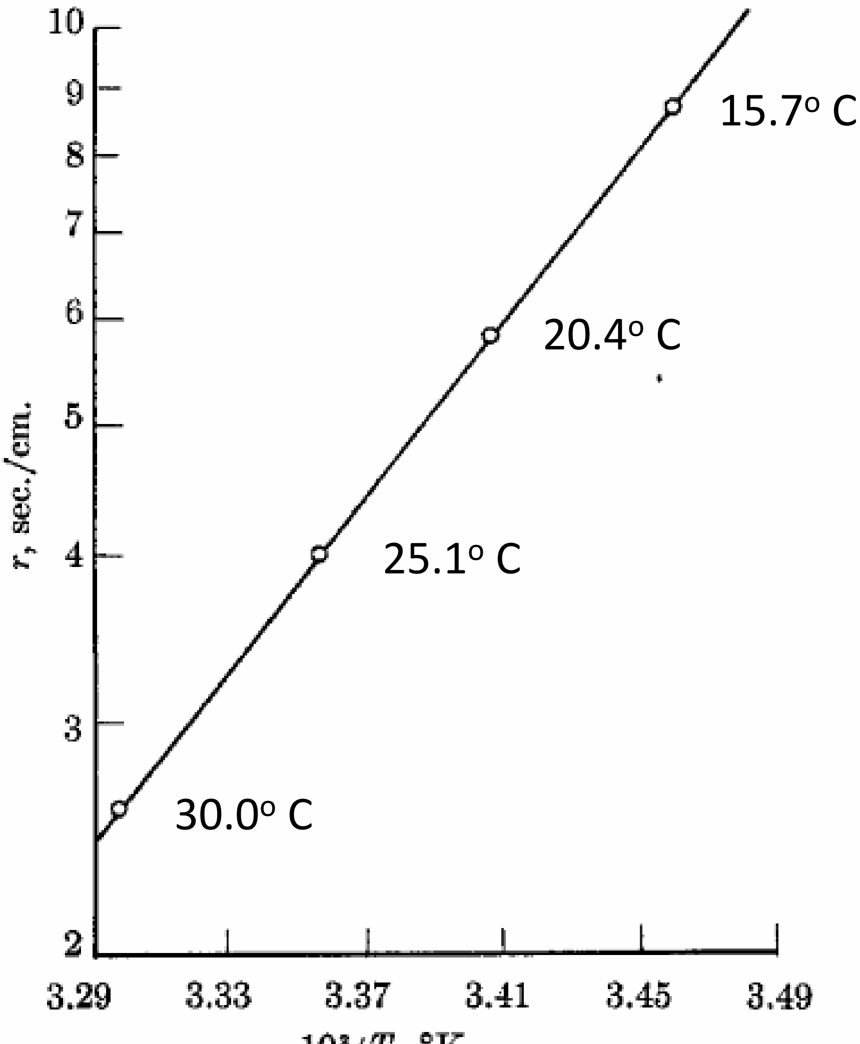


Fig. 7.—Specific resistance of the C_{19} acid monolayer as a function of the reciprocal of the absolute temperature in the liquid condensed phase on pure water.

19 carbon saturated fatty acid monolayer

Log evaporation resistance versus chain length of saturated fatty acids

Archer and La Mer, J. Phys. Chem., ***1955, 59, 200***

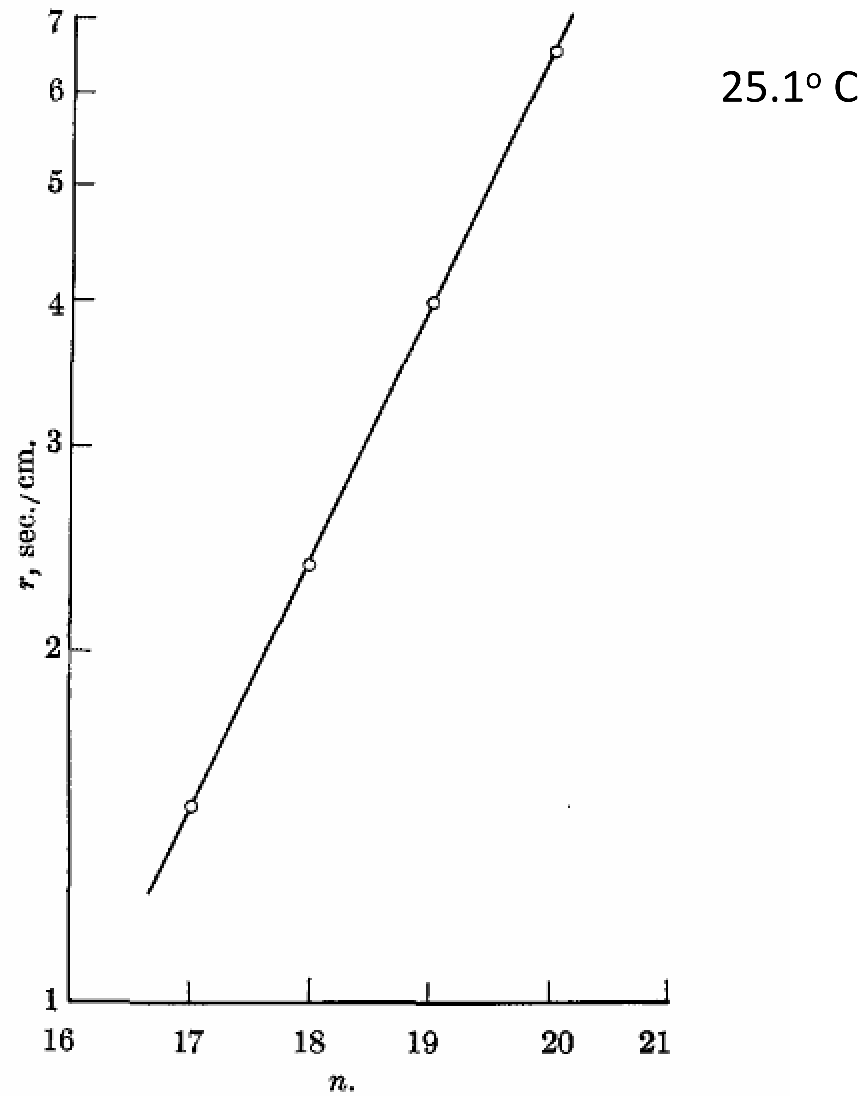
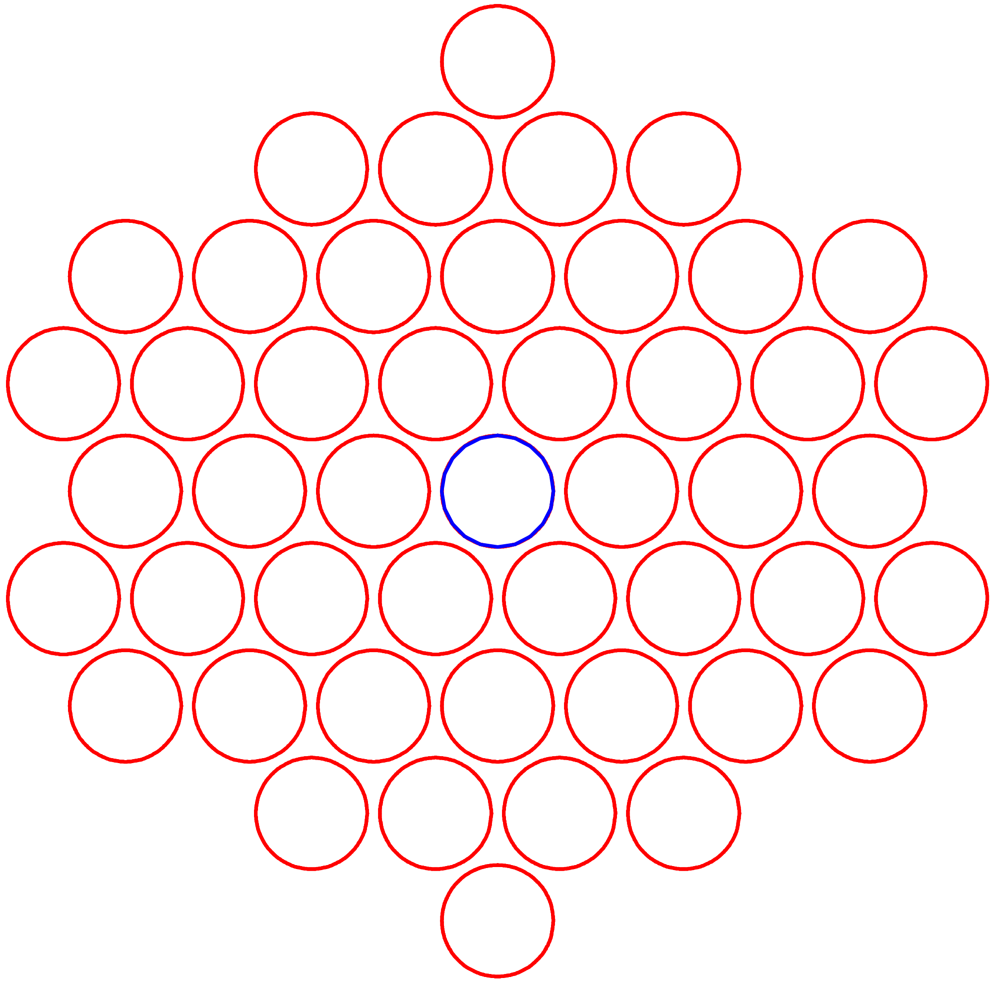


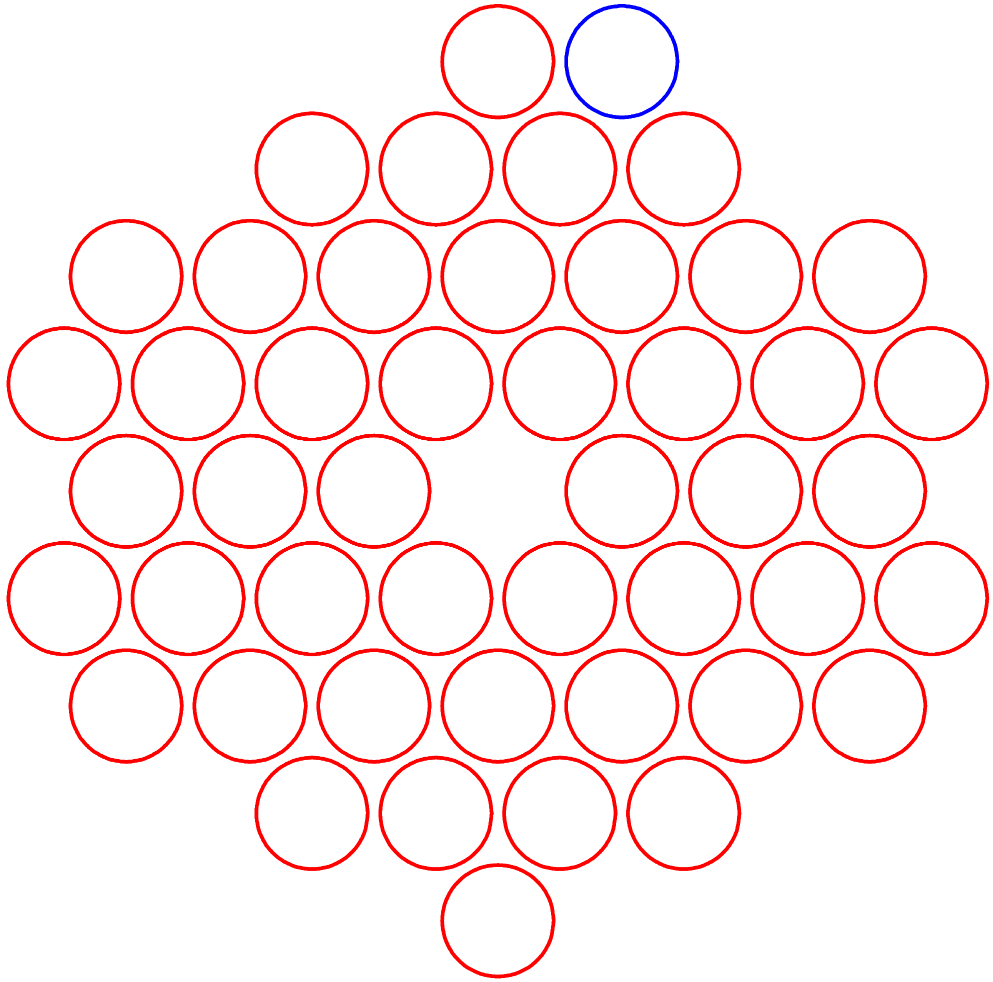
Fig. 5.—The logarithm of specific resistance in the liquid condensed phase as a function of the chain length of the monolayer molecules.

Archer and La Mer theory of evaporation through monolayers.

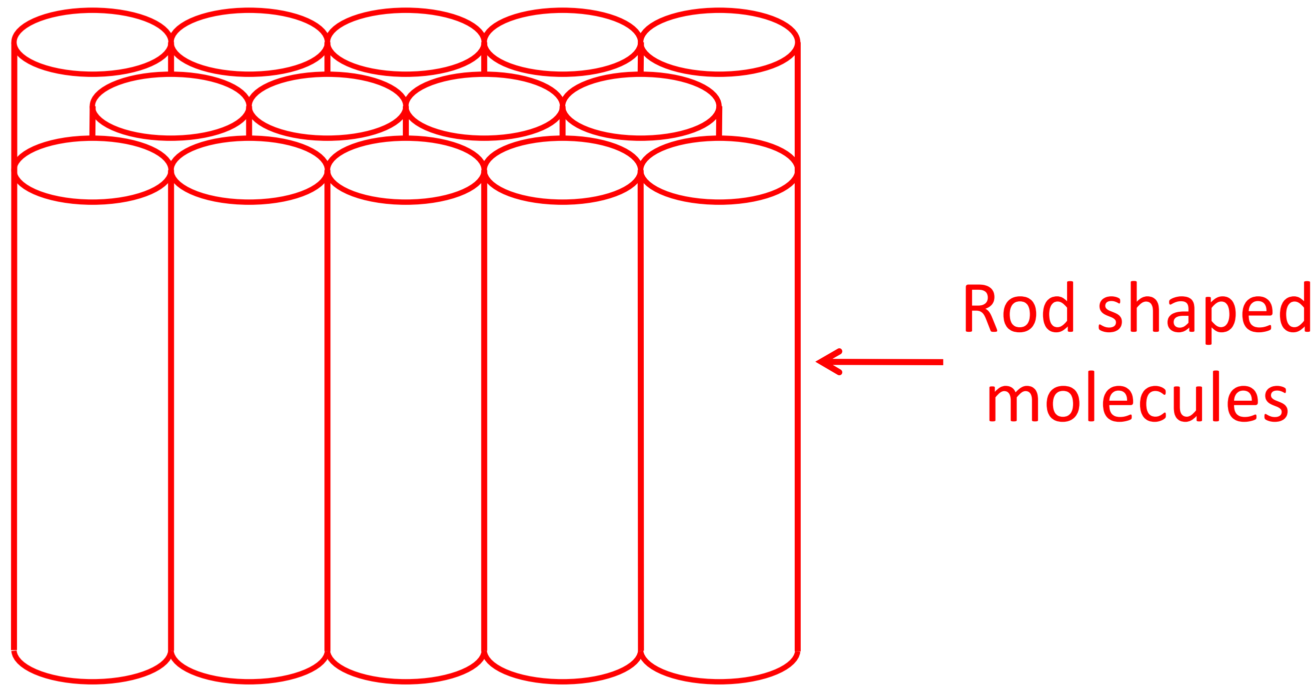


<u>**Archer and La Mer theory of evaporation through monolayers.**</u>

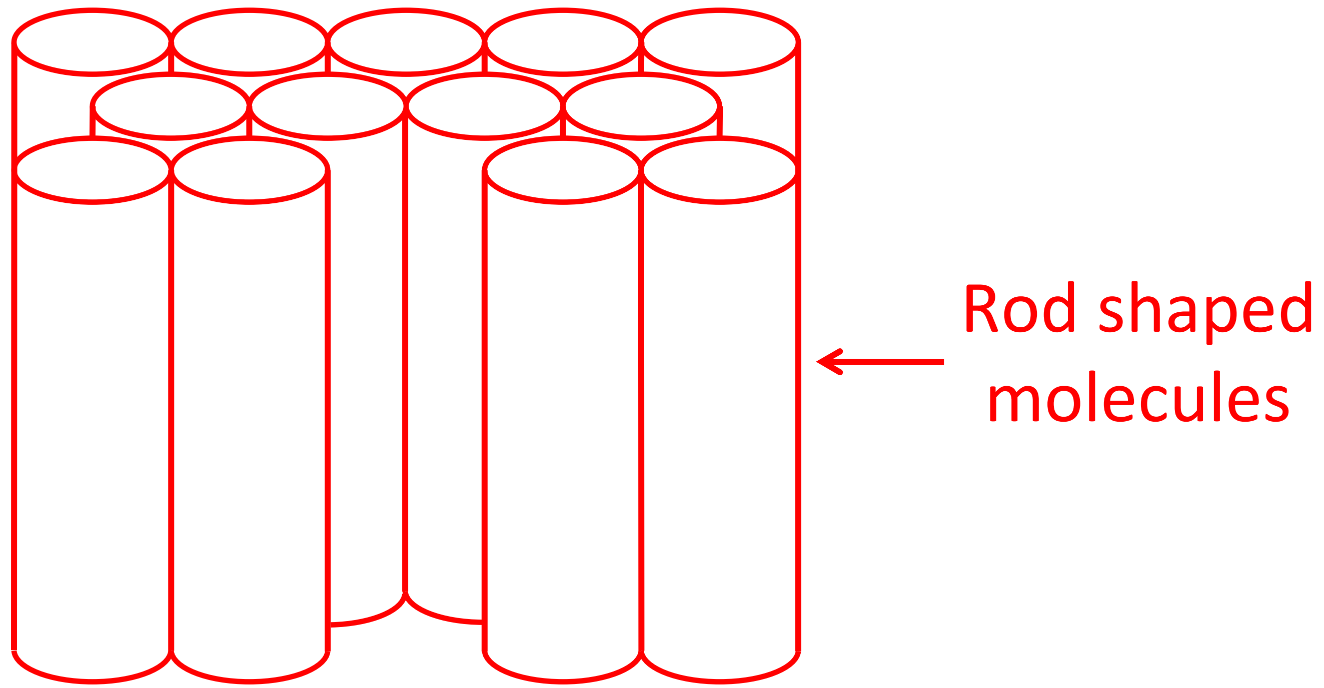
Displacement of lipid molecule creates water channel.



Archer and La Mer theory of evaporation through monolayers.



Archer and La Mer theory of evaporation through monolayers.

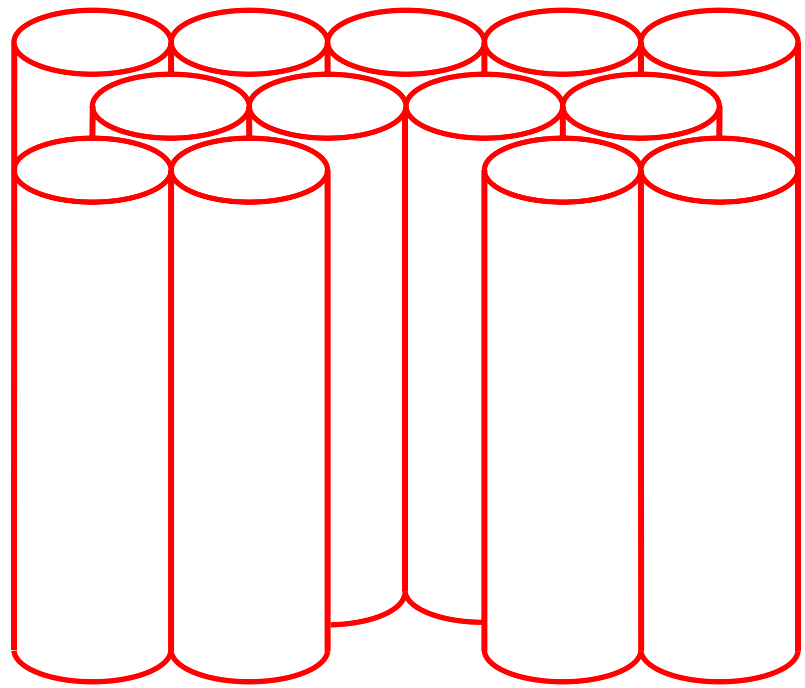


Archer and La Mer theory of evaporation through monolayers.

Energy, E, to remove molecule is (roughly) proportional to length.

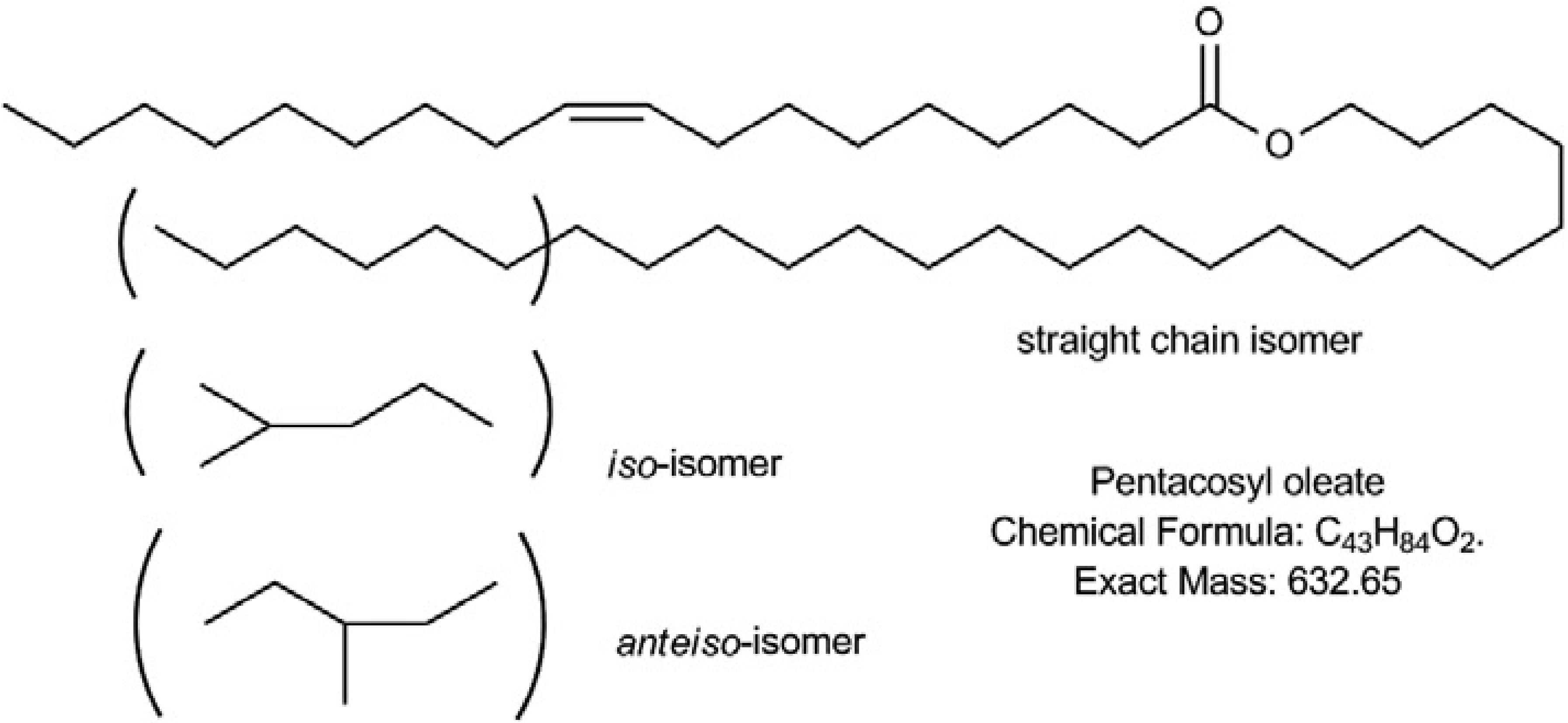
Probability of a hole, $P = P_0 e^{-E/kT}$ ← Equation!

Where P_0 is a constant, k is Boltzmann's Constant and T is absolute temperature



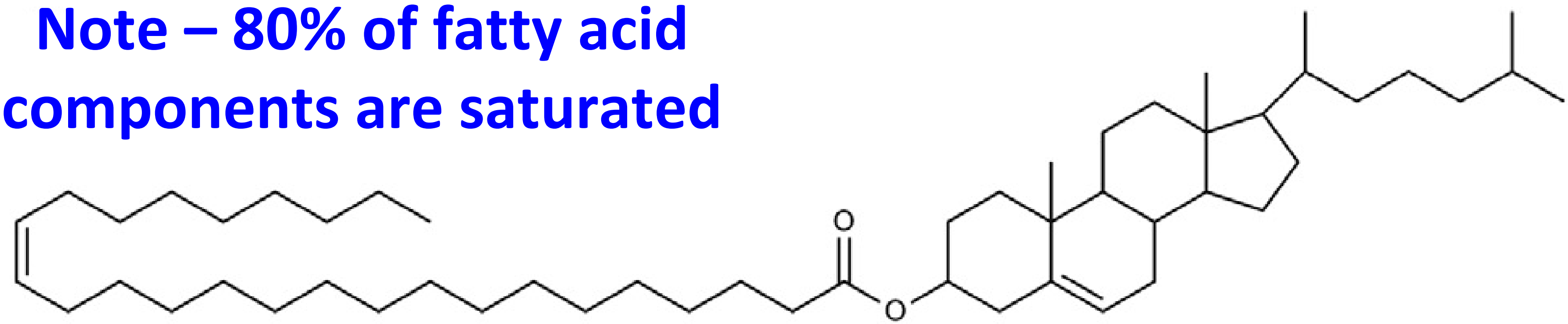
Rod shaped molecules

Wax ester, *2011, Butovich, Prog Lipid Res, 50, 278*



Cholesteryl ester, *2011, Butovich, Prog Lipid Res, 50, 278*

Note – 80% of fatty acid components are saturated



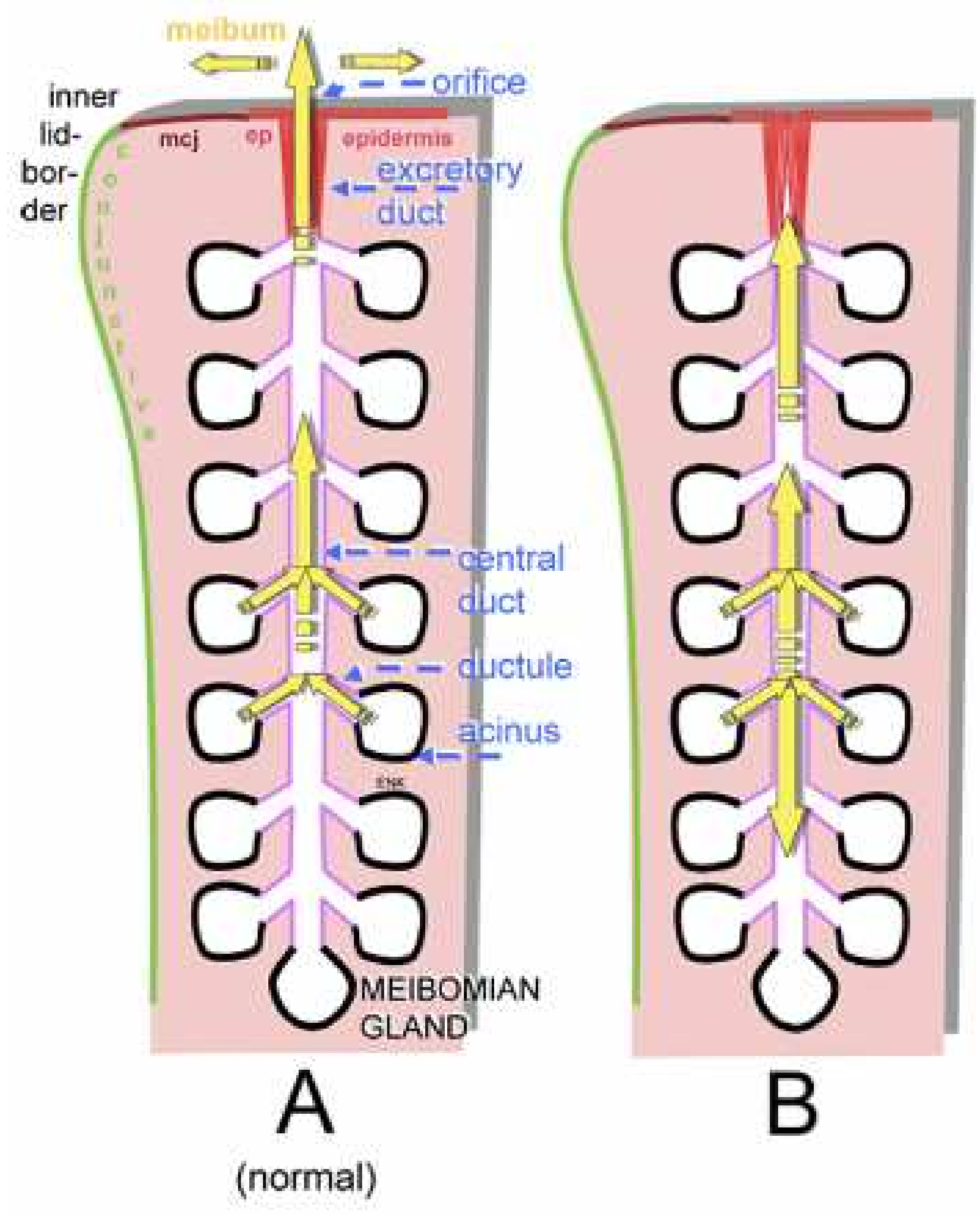
Cholesteryl Hexacosenoate
(*Z*)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl hexacos-17-enoate
Chemical Formula: $C_{53}H_{94}O_2$. Exact Mass: 762.73

Lipid layer characteristics for a good evaporation barrier

2. Not too solid

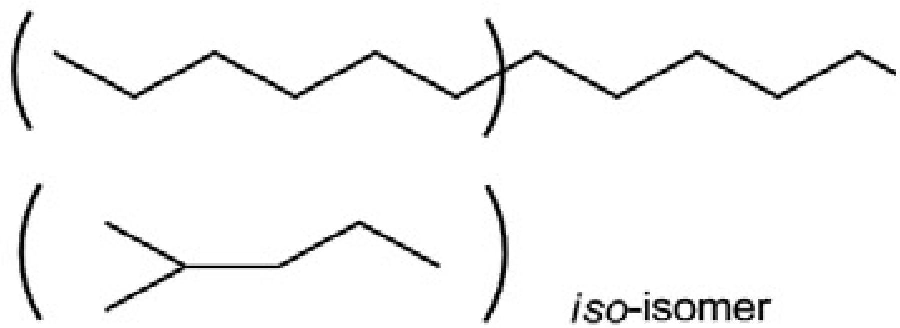
Course of obstructive meibomian gland dysfunction

Knop et al, 2011, IOVS 52, 1938



Melting points of saturated and unsaturated substances and straight-chain and iso-branched substances

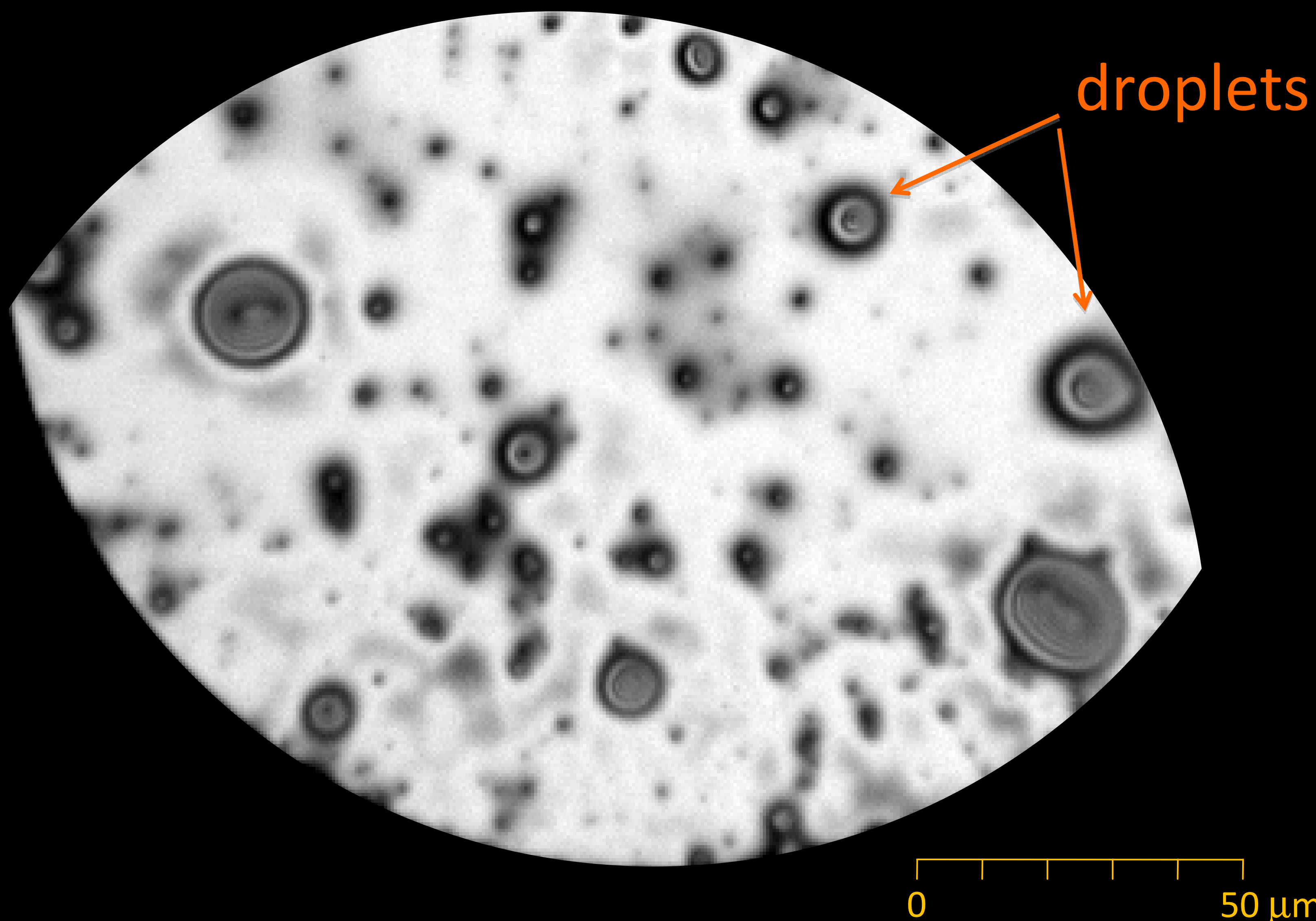
		type	Melting point
stearic acid	$C_{17}H_{35}COOH$	saturated	71.5°C
oleic acid	$C_{17}H_{33}COOH$	mono-unsaturated	16°C
n-hexadecane	$C_{16}H_{34}$	straight-chain	18°C
iso-hexadecane	$C_{16}H_{34}$	iso-branched	-70°C



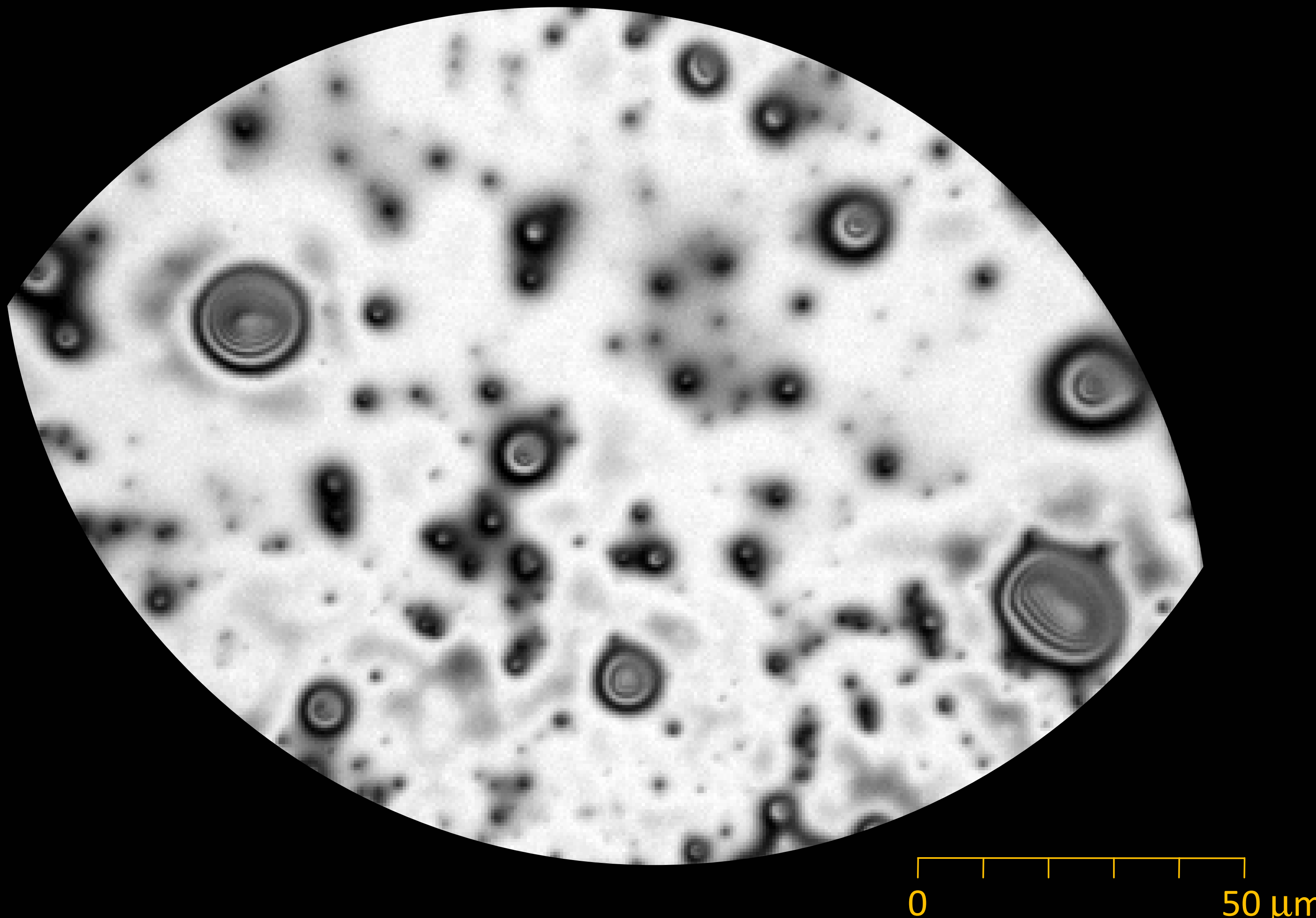
Lipid layer characteristics for a good evaporation barrier

3. Not too fluid

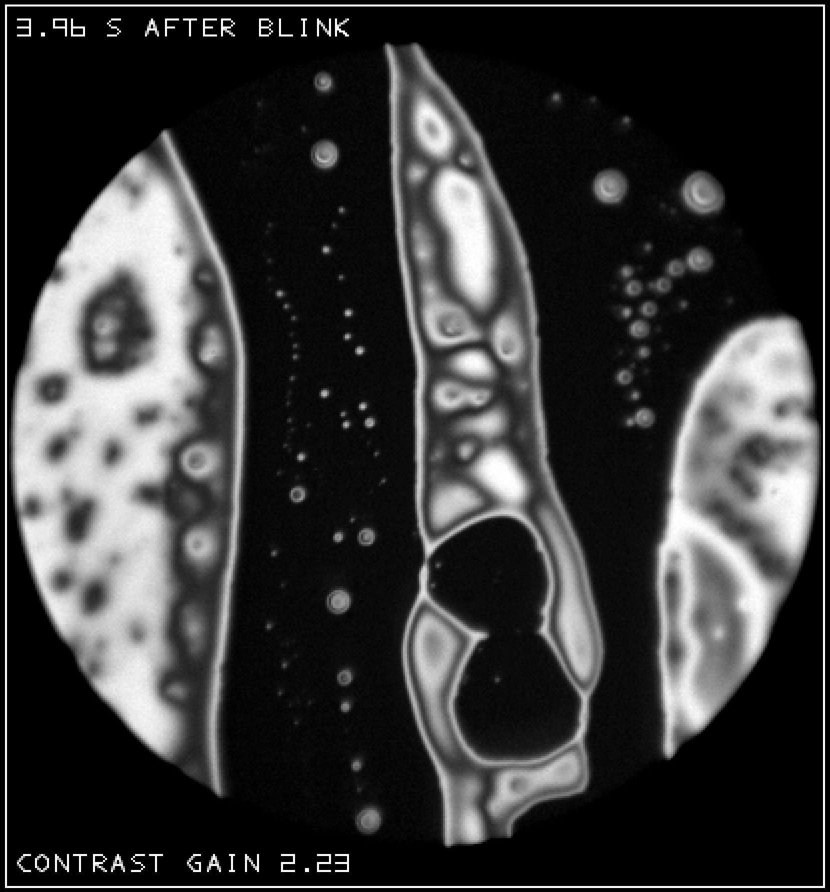
Normal tear film, initial image



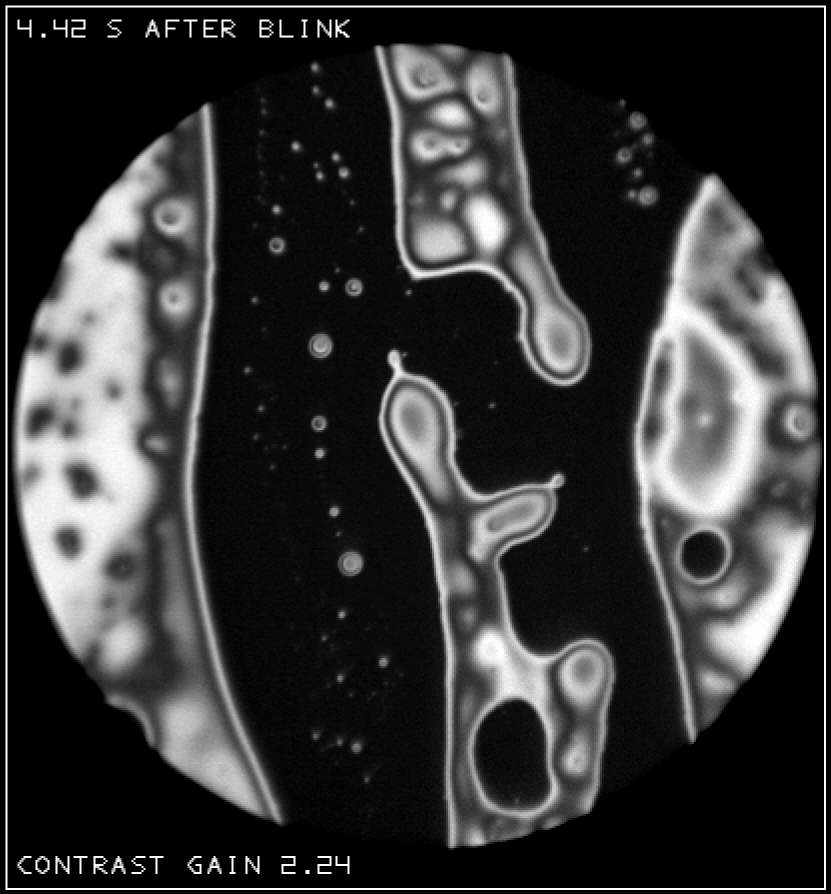
8.16 seconds later



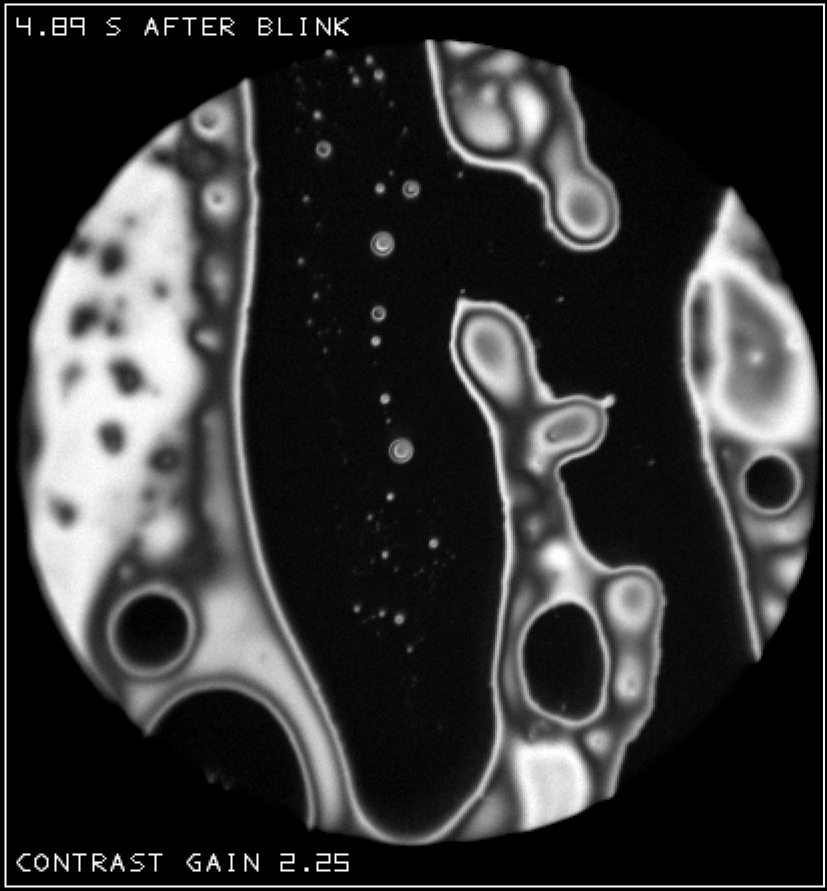
Dry eye, initial image



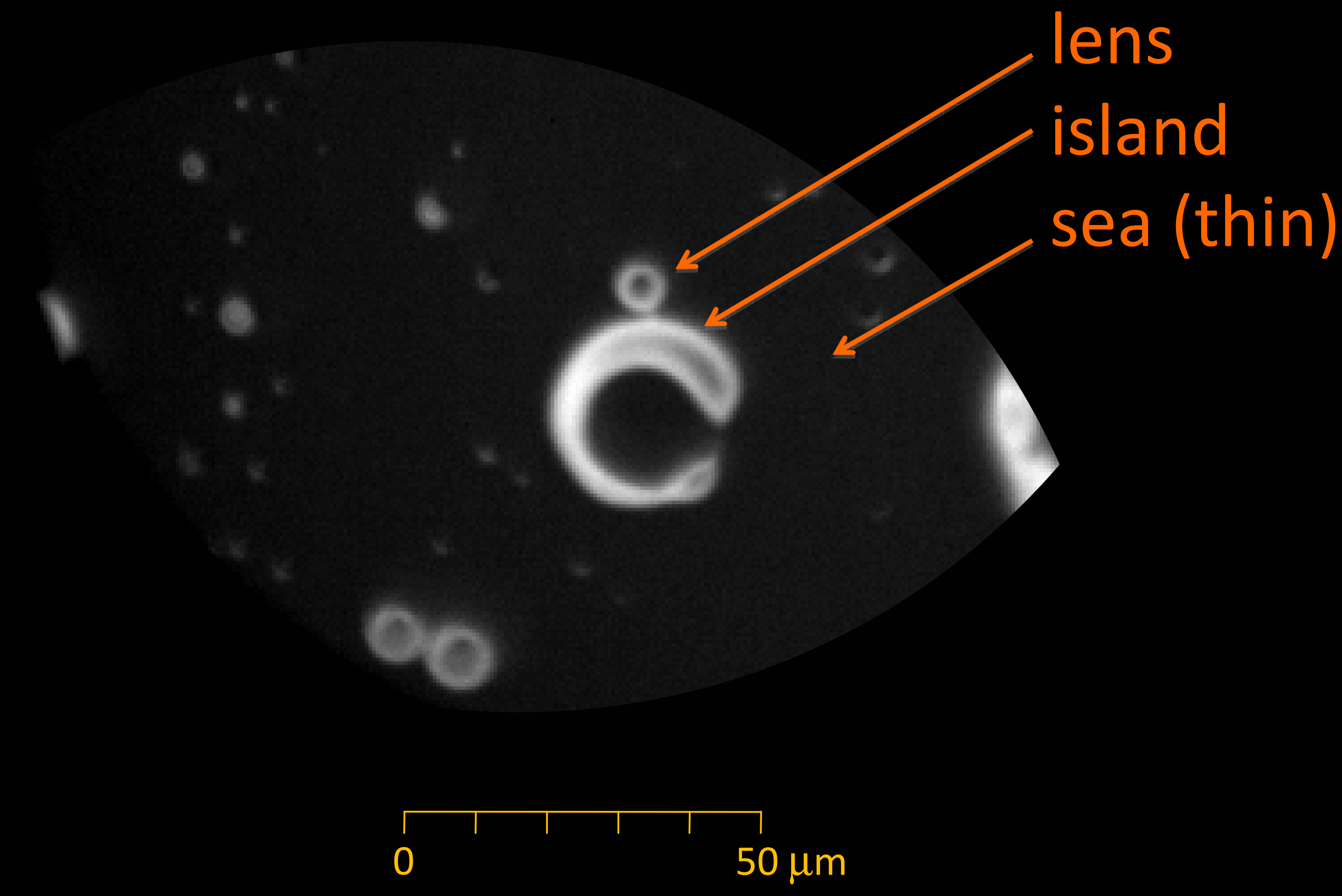
0.46 seconds after initial image



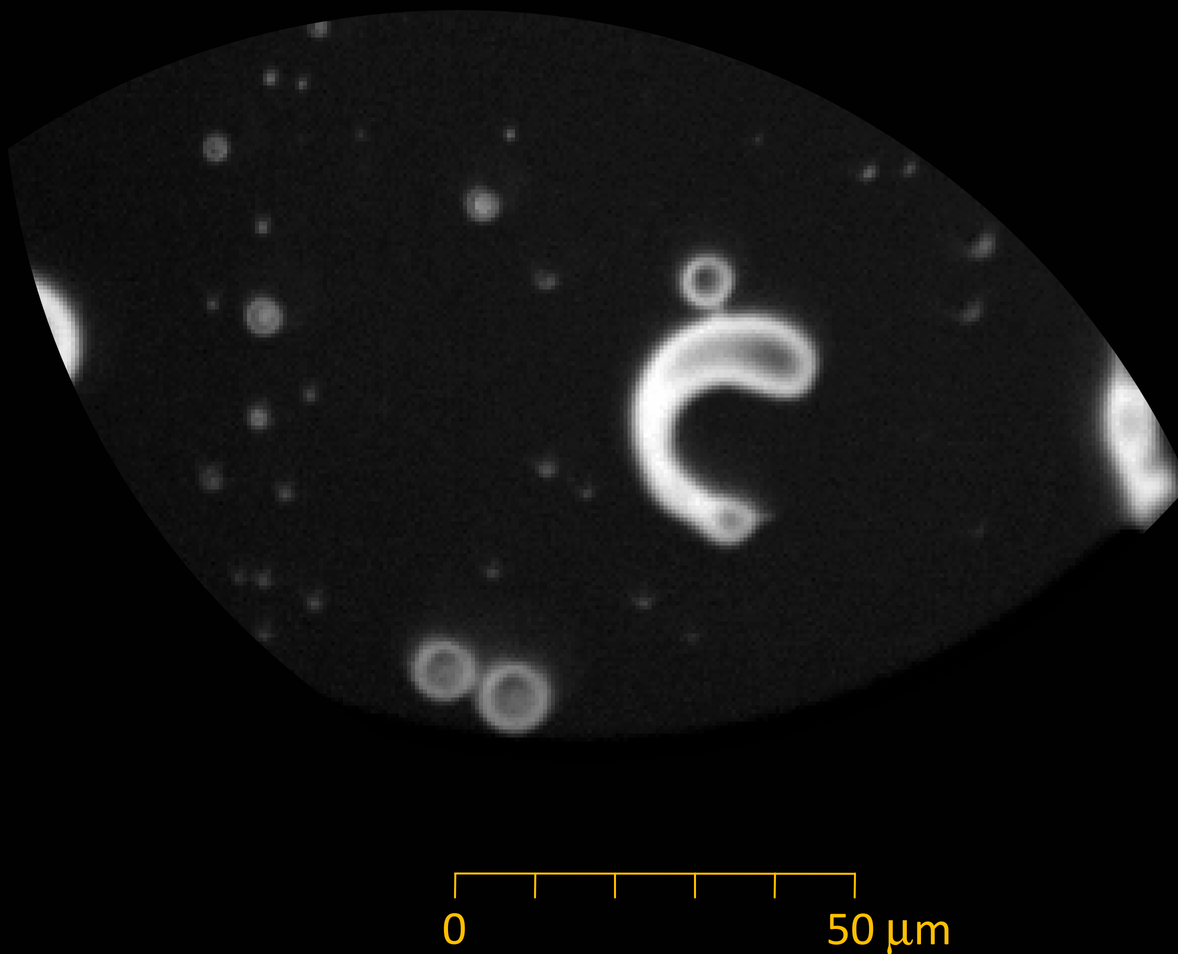
0.93 seconds after initial image



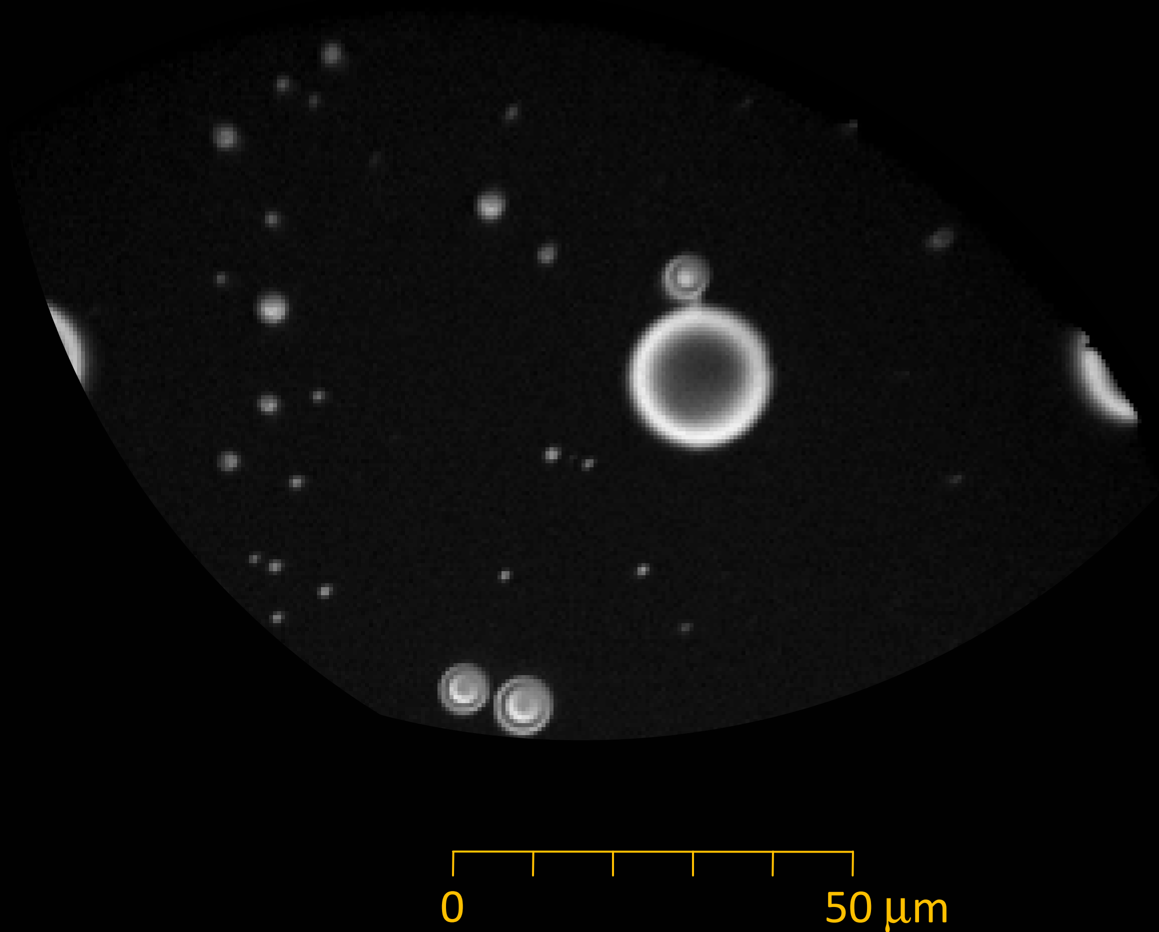
Dry eye, initial image



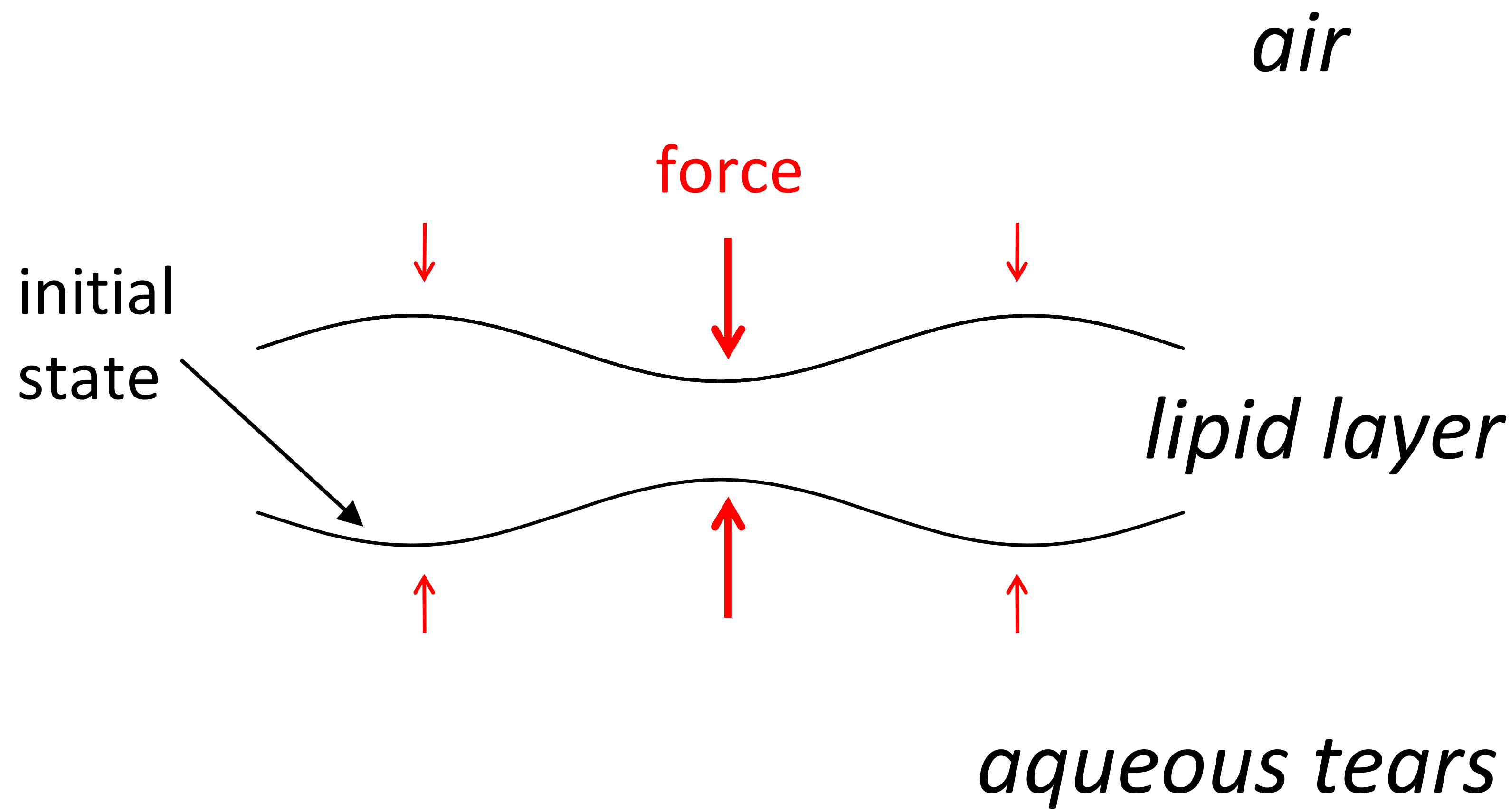
0.01 seconds after initial image



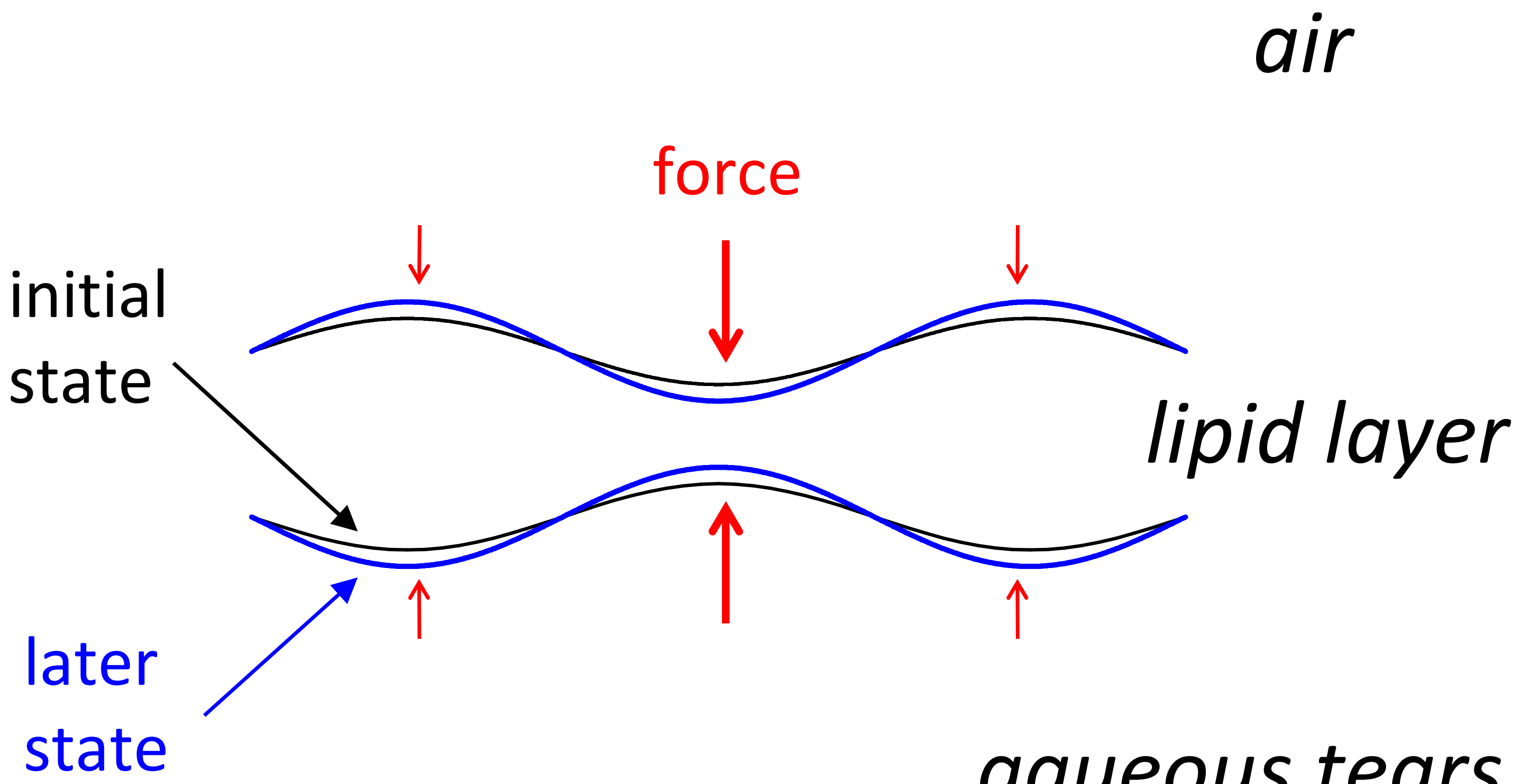
0.26 seconds after initial image



Dewetting of the lipid layer from van der Waals' forces?



Dewetting of the lipid layer from van der Waals' forces?

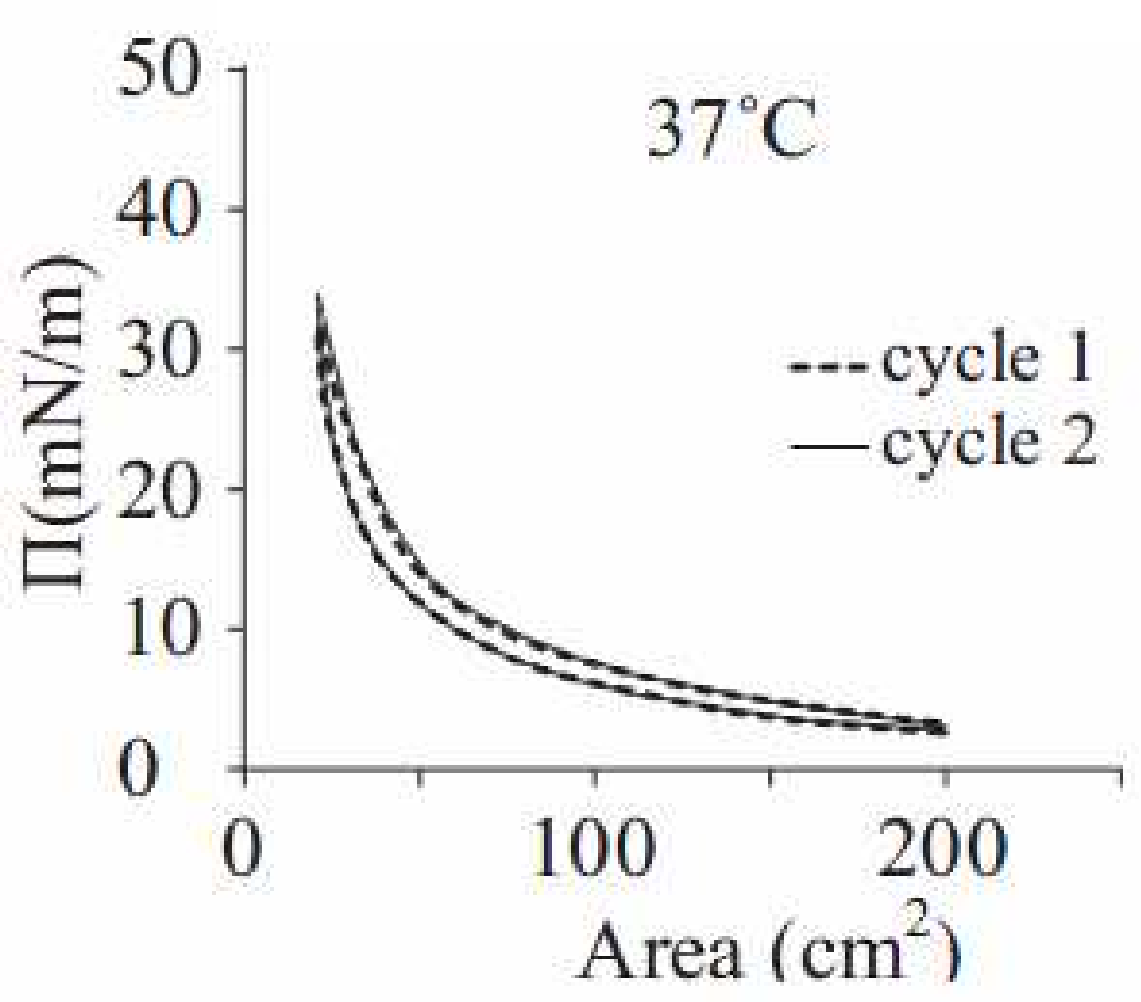


Lipid layer characteristics for a good evaporation barrier

4. Respreadability

Repeatable cycles (non-collapse) of human meibum layer

Mudgil and Millar, 2011, IOVS 52, 1661



<u>Respreading of the lipid layer – repeatability of pattern after successive blinks</u>

Bron et al, 2004, Exp Eye Res 78, 347

1

2

3

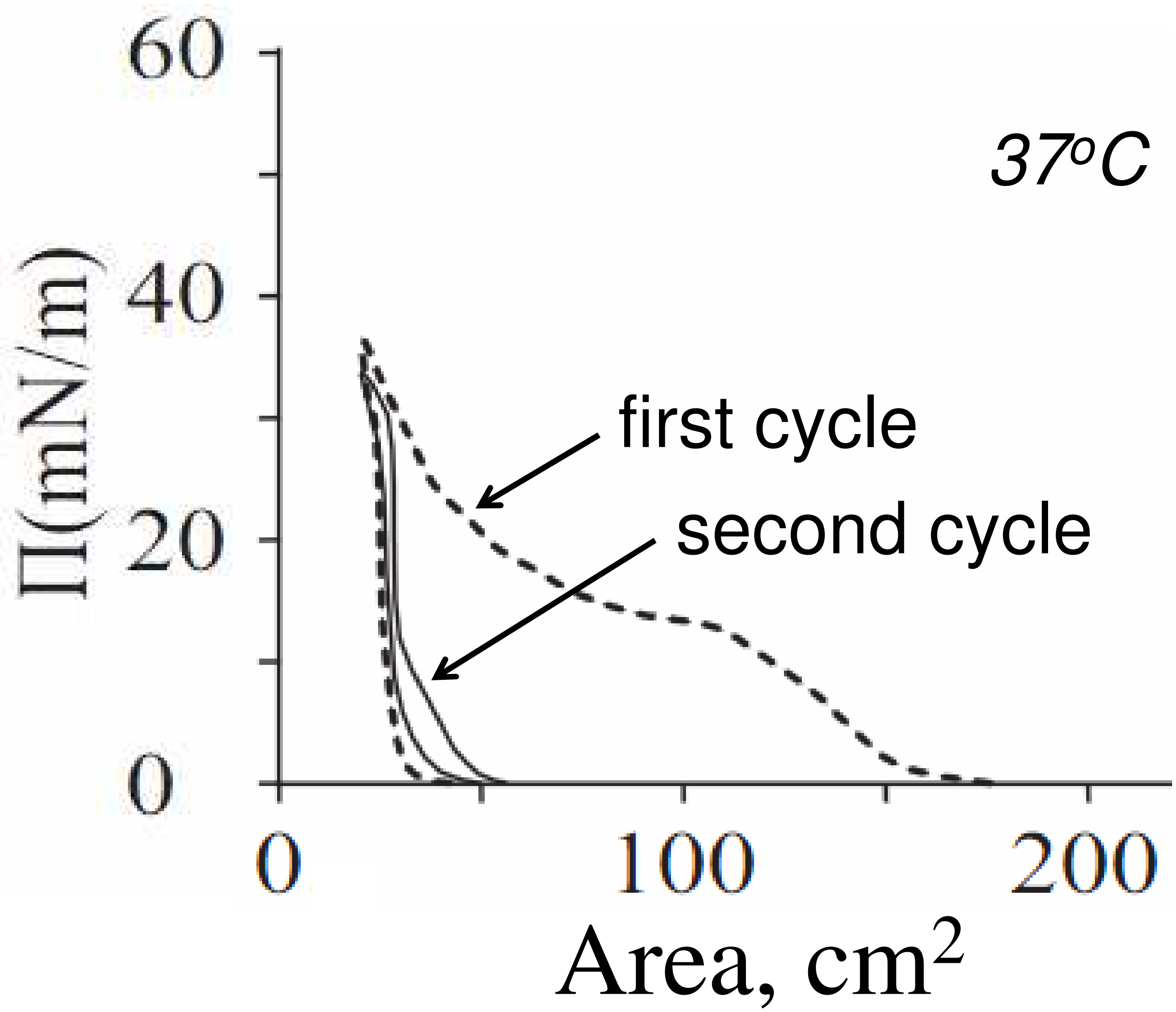
4

5

6

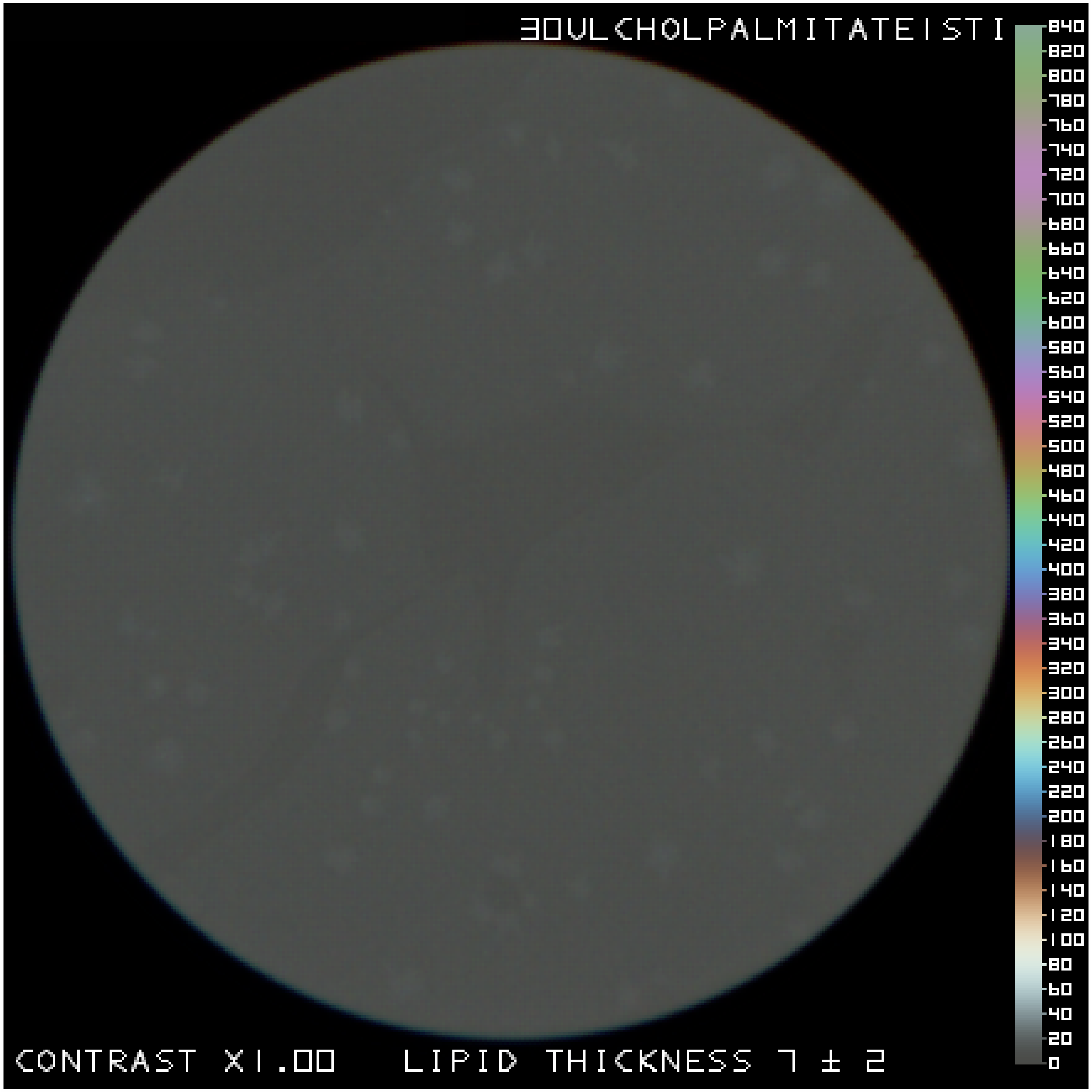
Collapse of cholesteryl palmitate layer

Mudgil and Millar, 2011, IOVS 52, 1661



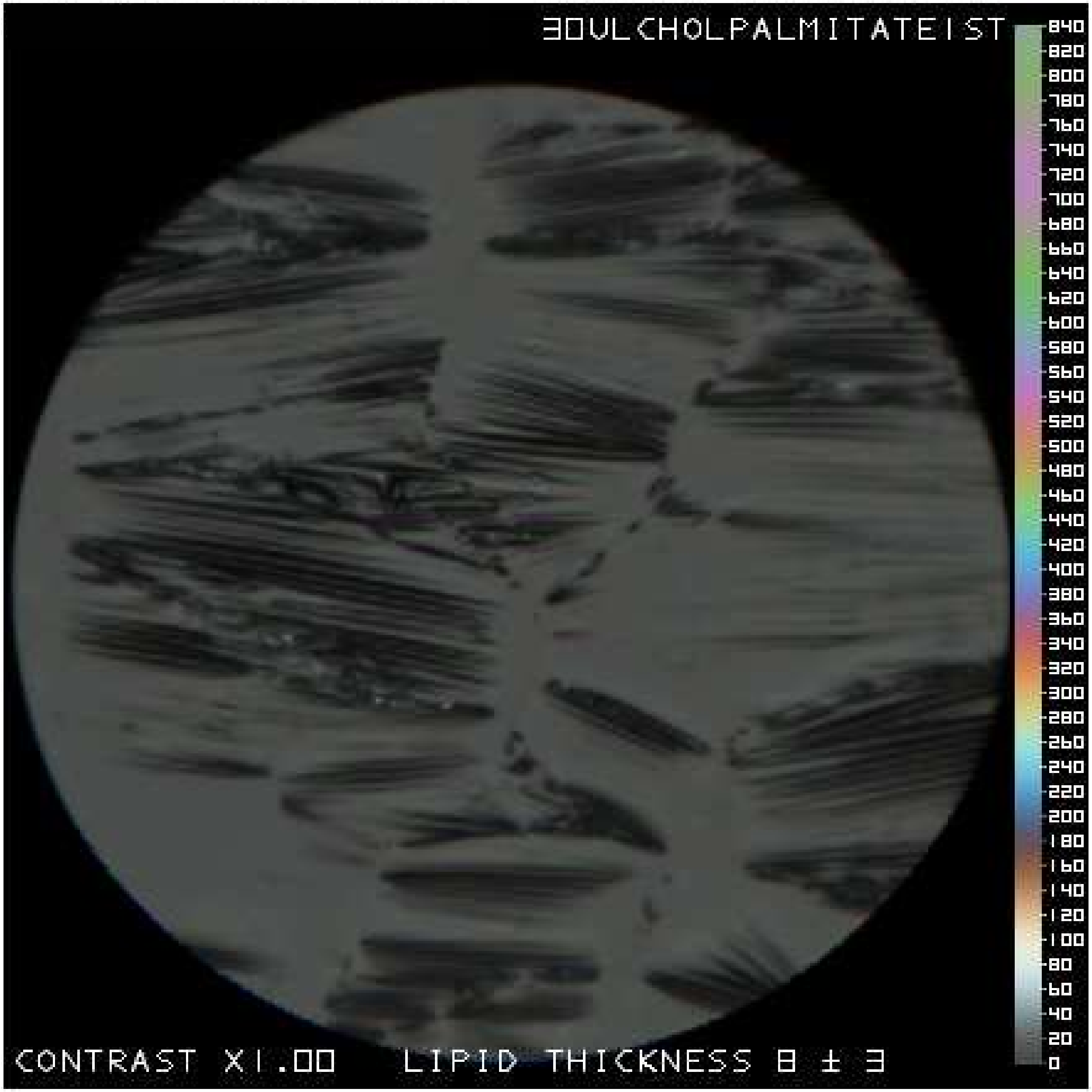
Cholesteryl palmitate on saline – beginning of first compression

Millar and King-Smith, unpublished



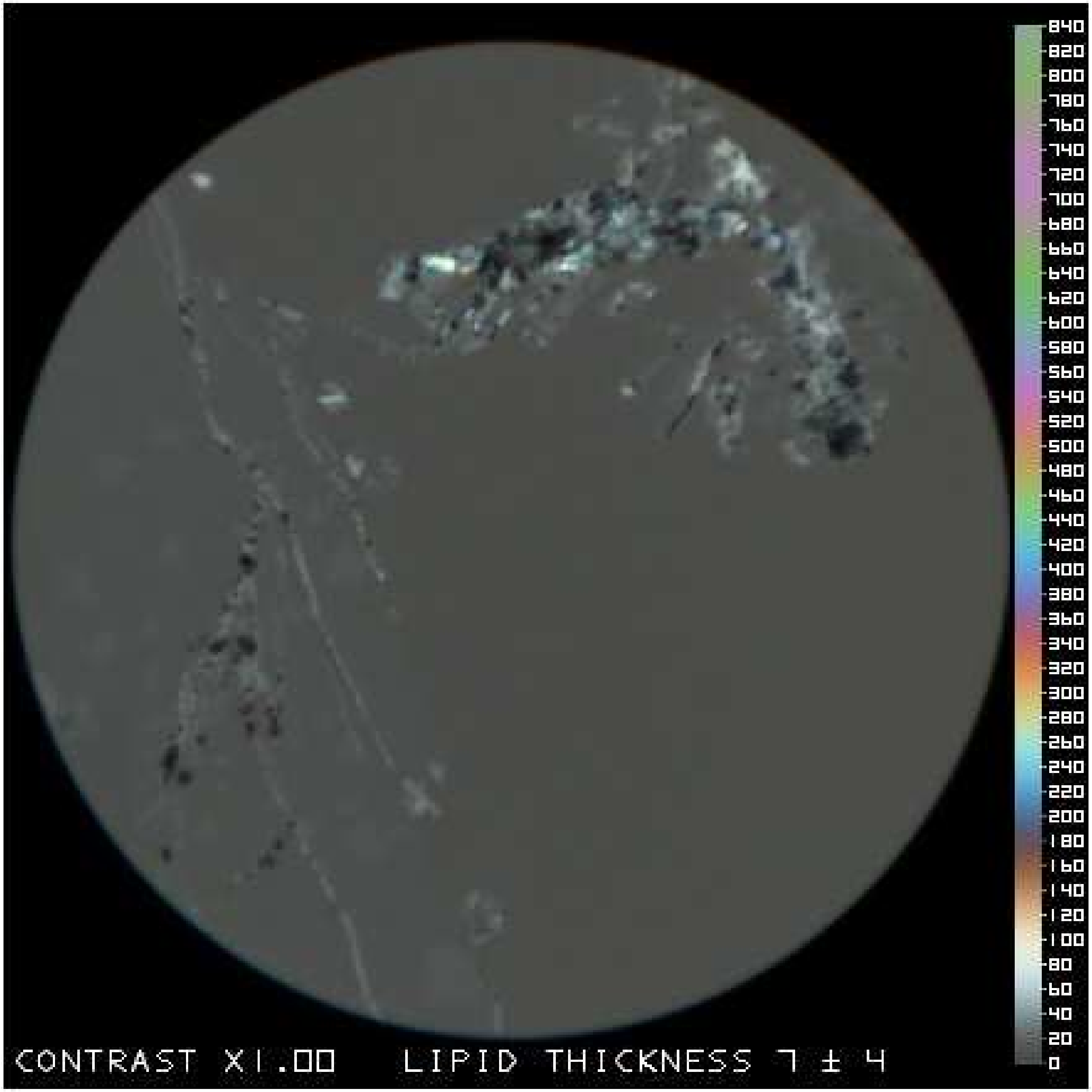
Cholesteryl palmitate on saline – end of first compression

Millar and King-Smith, unpublished

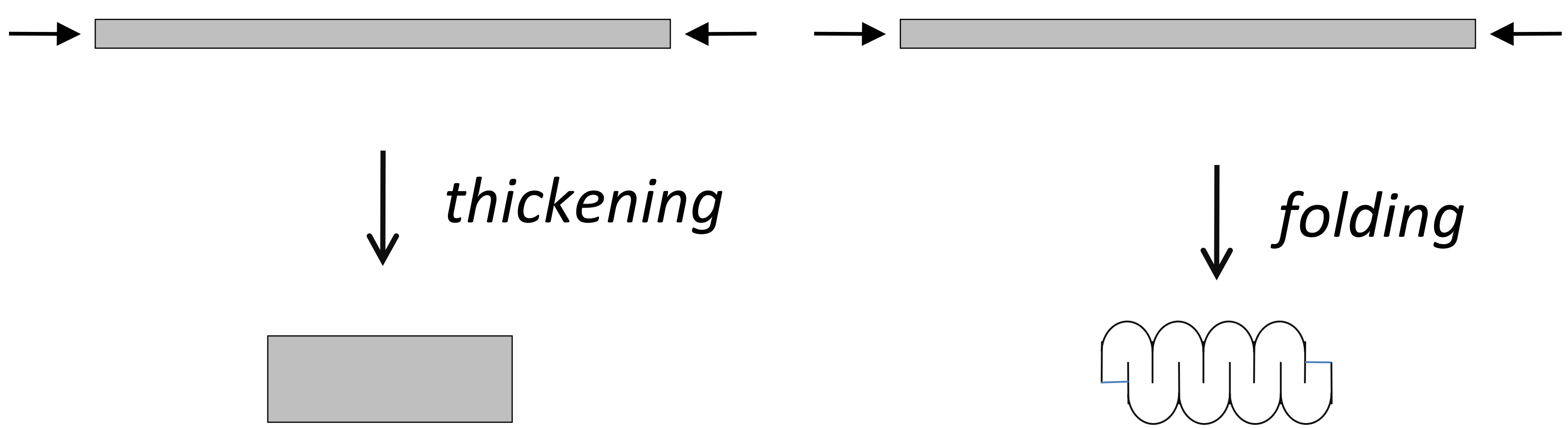


Cholesteryl palmitate on saline – beginning of second compression

Millar and King-Smith, unpublished



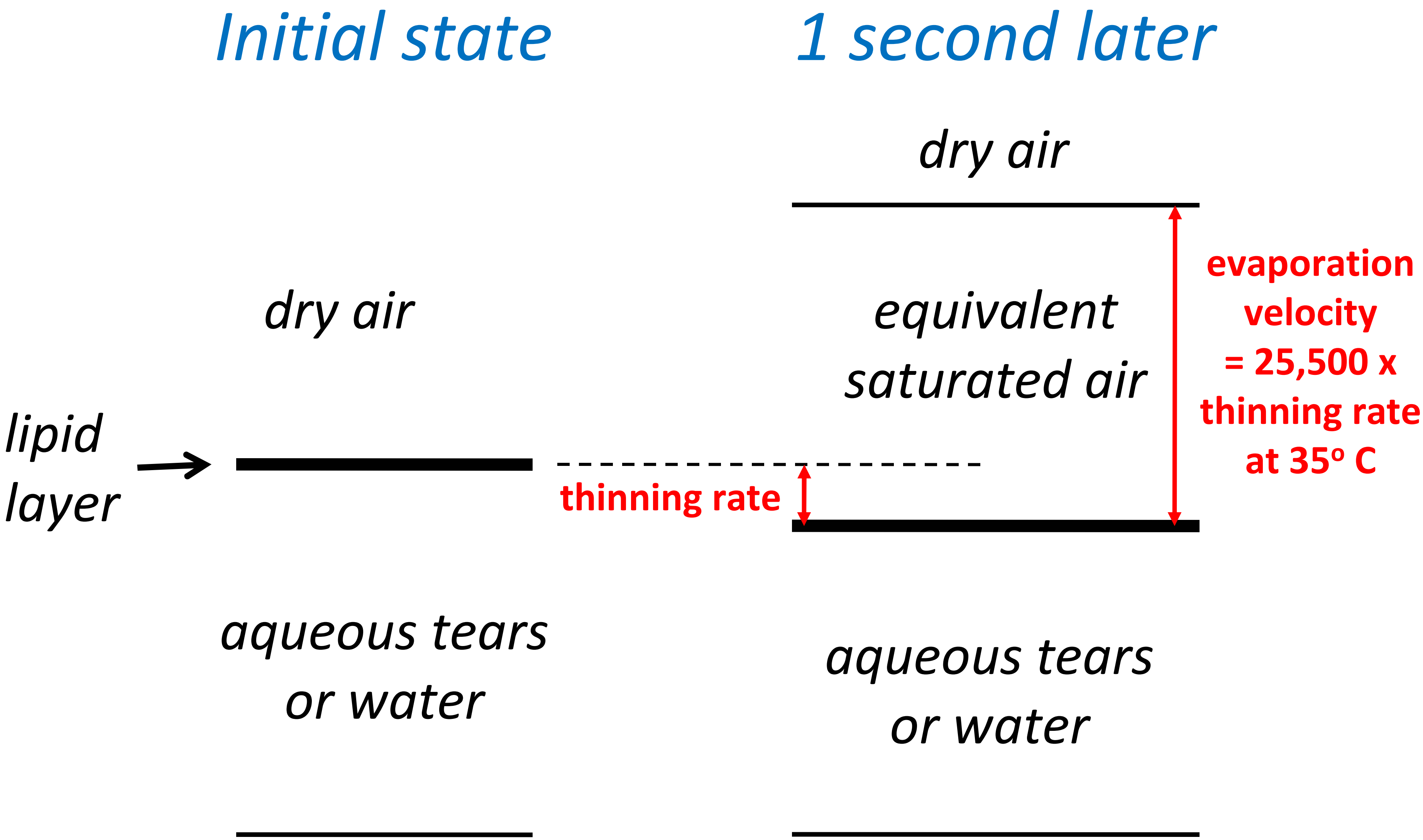
Effect of lipid layer compression during the downstroke of the blink?



Conclusions

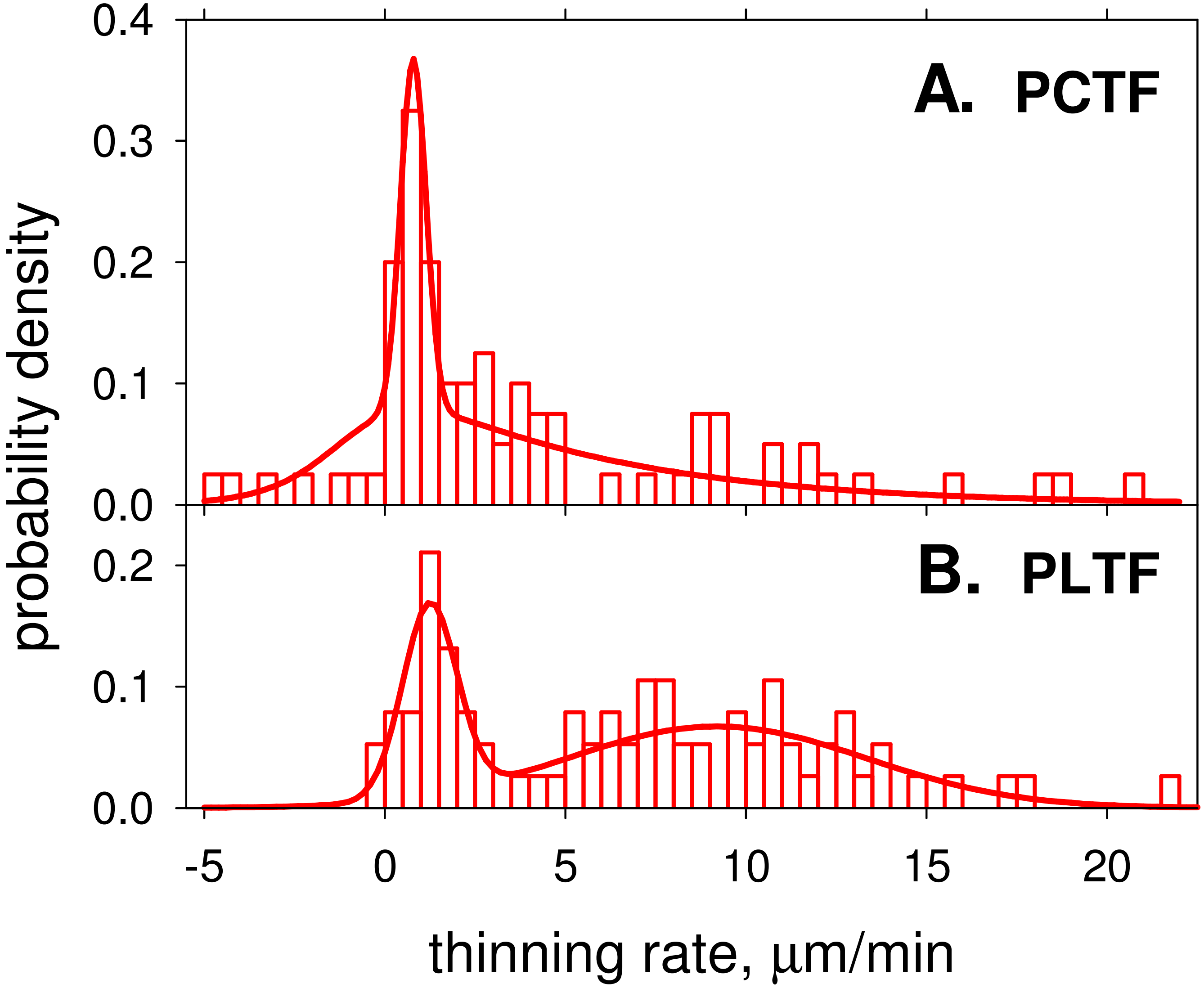
- Dry eye conditions are common, poorly understood disorders.
- A defective lipid layer causes increased evaporation and hence increased osmolarity and ocular surface damage.
- Four characteristics of a good lipid layer:
  - Long, saturated hydrocarbon chains
  - Not too solid
  - Not too fluid
  - Respreadable during compression-expansion cycles (blinks)

Definition of evaporation resistance = 1/evaporation velocity



Histograms of thinning rates of the pre-corneal and pre-lens tear films

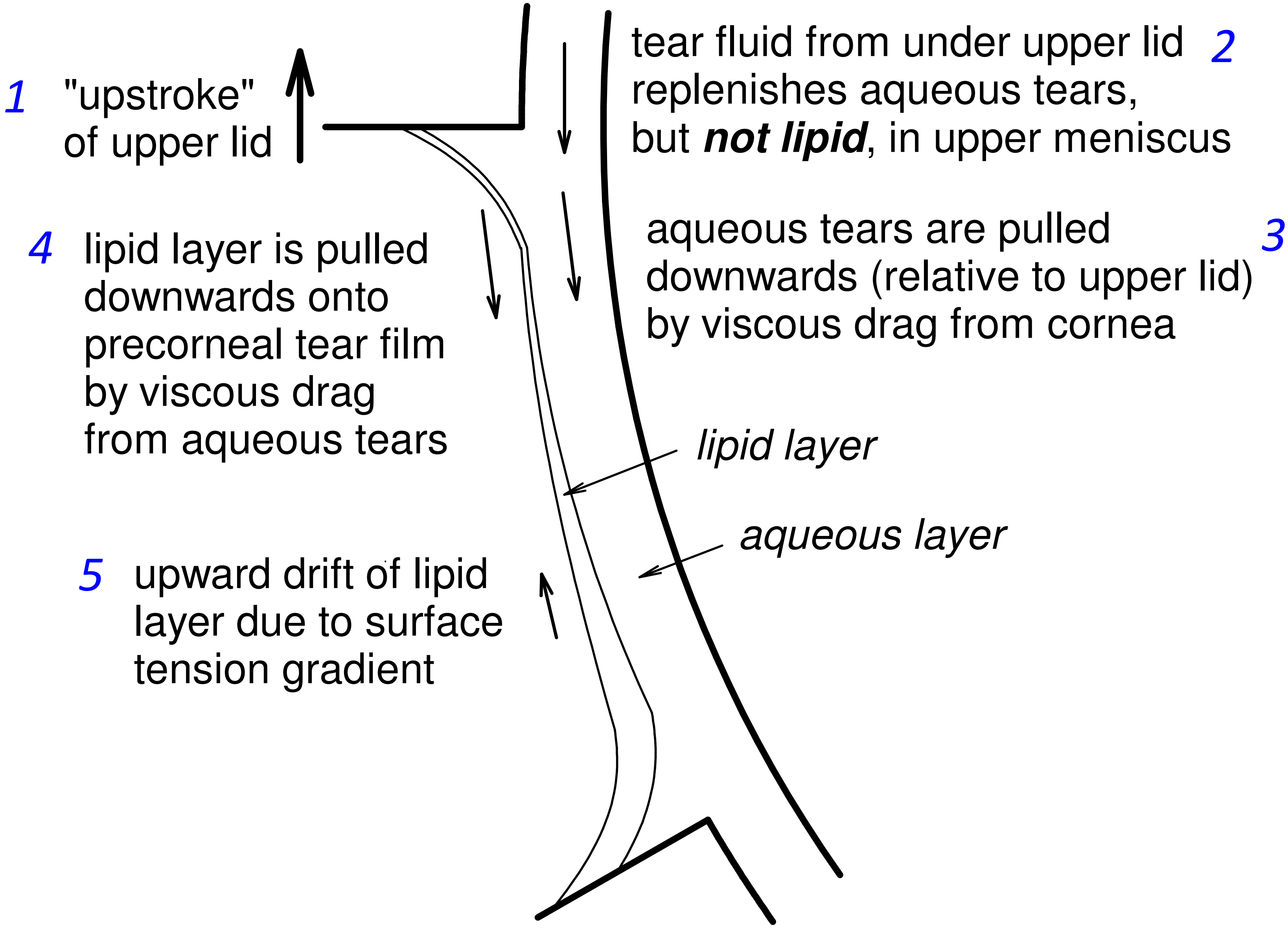
Nichols JJ, Mitchell and King-Smith, 2005, IOVS 46, 2353



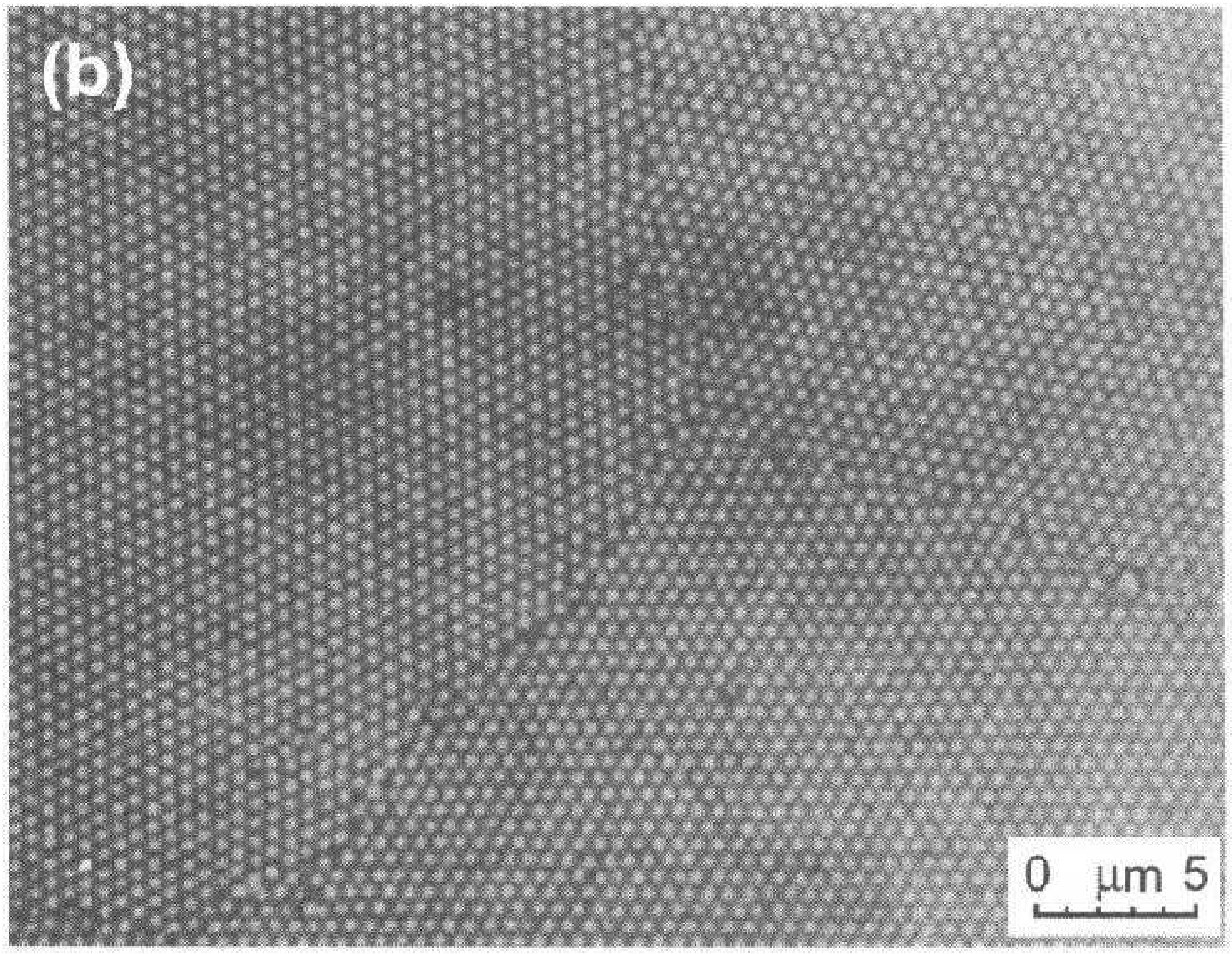
Comparison of evaporation through lipid layer and C_{19} monolayer at 35° C

	Thinning rate, μm/min	Evaporation velocity, cm/s	Evaporation resistance, s/cm
"good" pre-corneal lipid layer	1.0	0.0425	23.5
C_{19} fatty acid monolayer	13.1	0.559	1.79

Why the tear film drifts upwards after a blink?



Grain boundaries may be evaporation sites

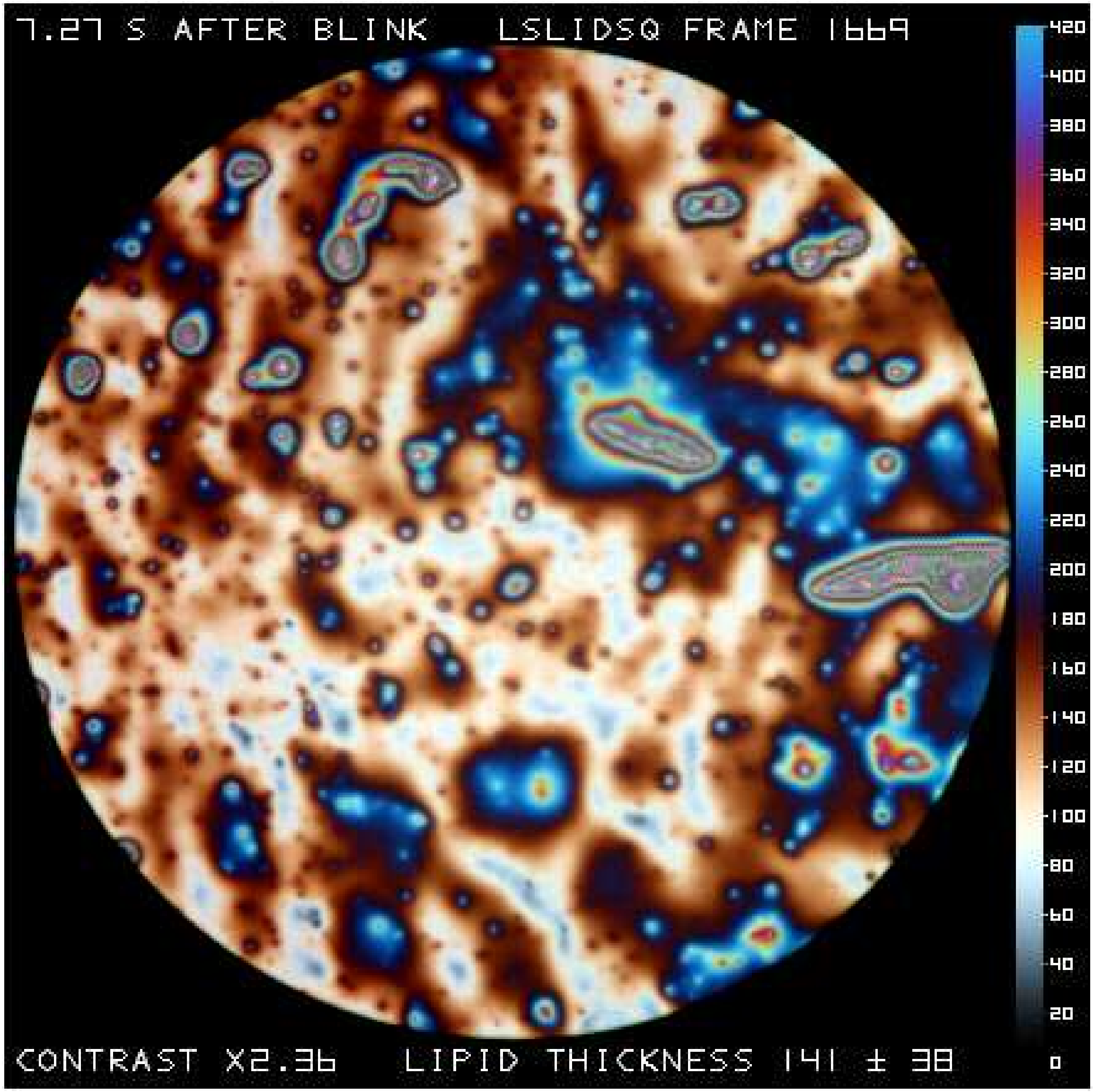


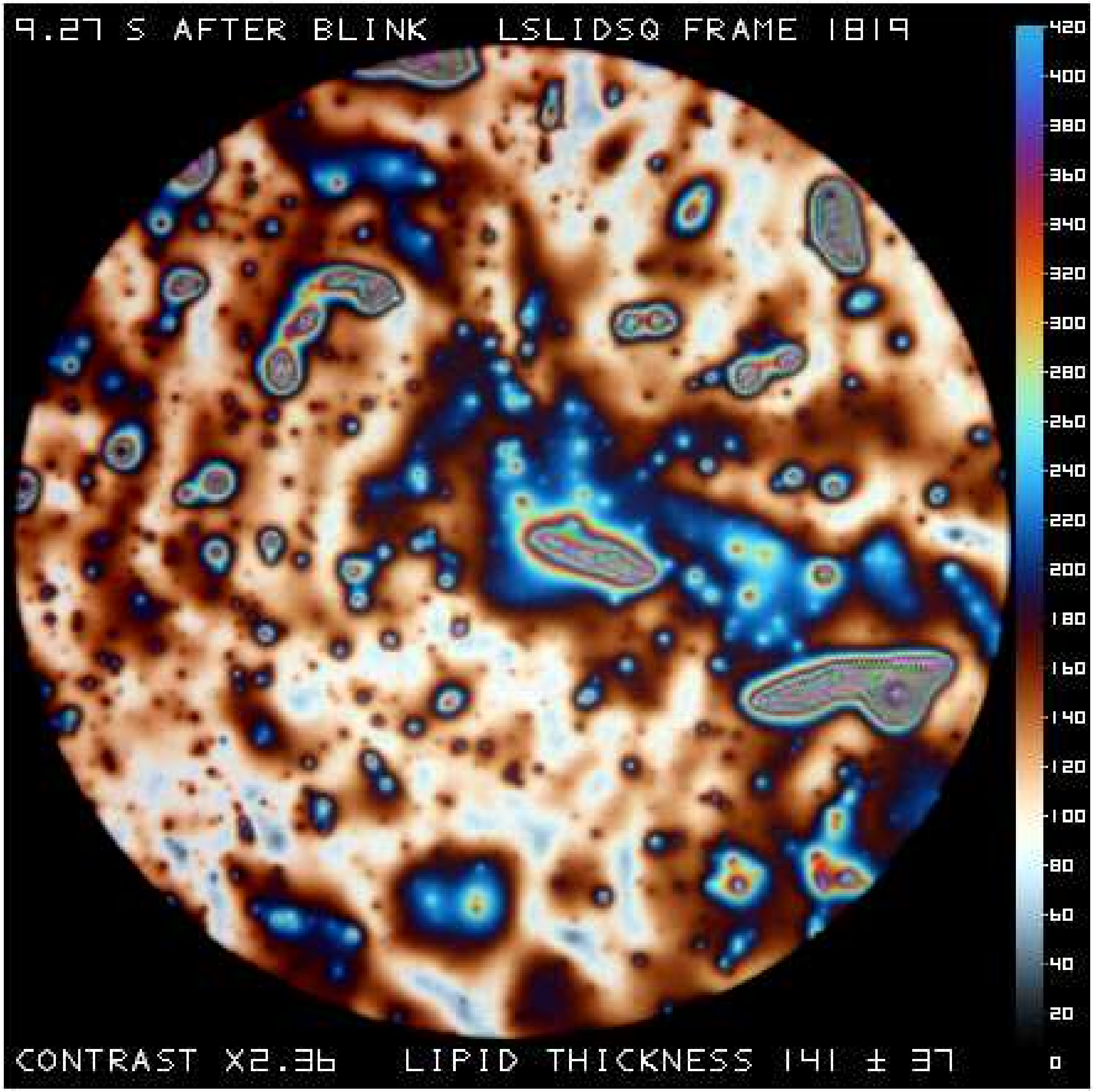
Evaporation end

Not too solid end

Not too fluid end

respreading
end


7.27 S AFTER BLINK LSLIDSQ FRAME 1669
CONTRAST X2.36 LIPID THICKNESS 141 ± 38
420
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0



9.27 S AFTER BLINK
LSLIDSQ FRAME 1819
420
400
380
360
340
320
300
280
260
240
220
200
180
160
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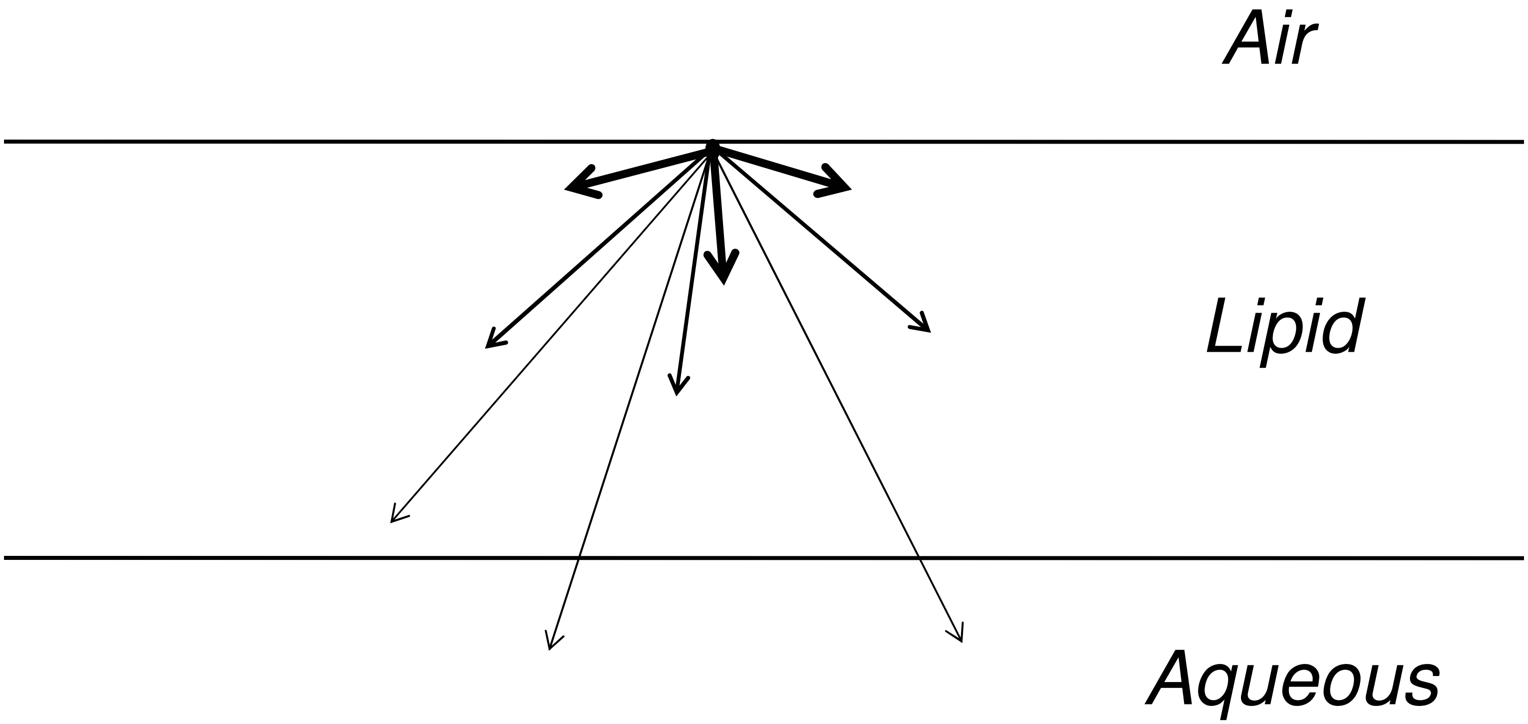
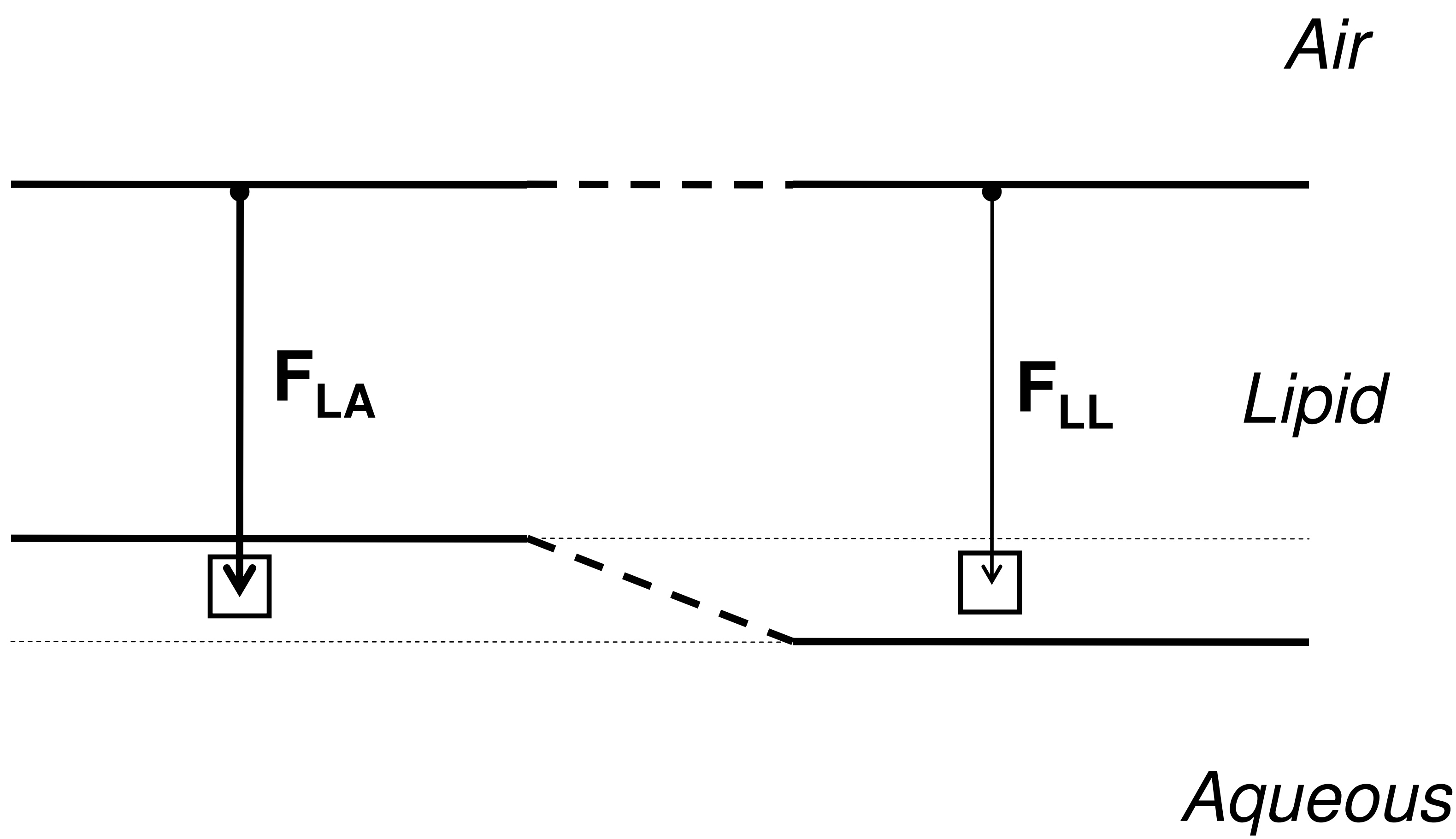
80
60
40
20
0
CONTRAST X2.36
LIPID THICKNESS 141 ± 37

Van der Waals' forces on a molecule at the lipid surface



Van der Waals' forces on a molecule at the lipid surface

F_{LA} , force between lipid molecule and aqueous volume
F_{LL} , force between lipid molecule and lipid volume
Lipid layer is unstable if $F_{LA} > F_{LL}$.



Dewetting of hydrocarbons on water

Israelachvili, Intermolecular and Surface Forces, 3rd Edn., p. 273

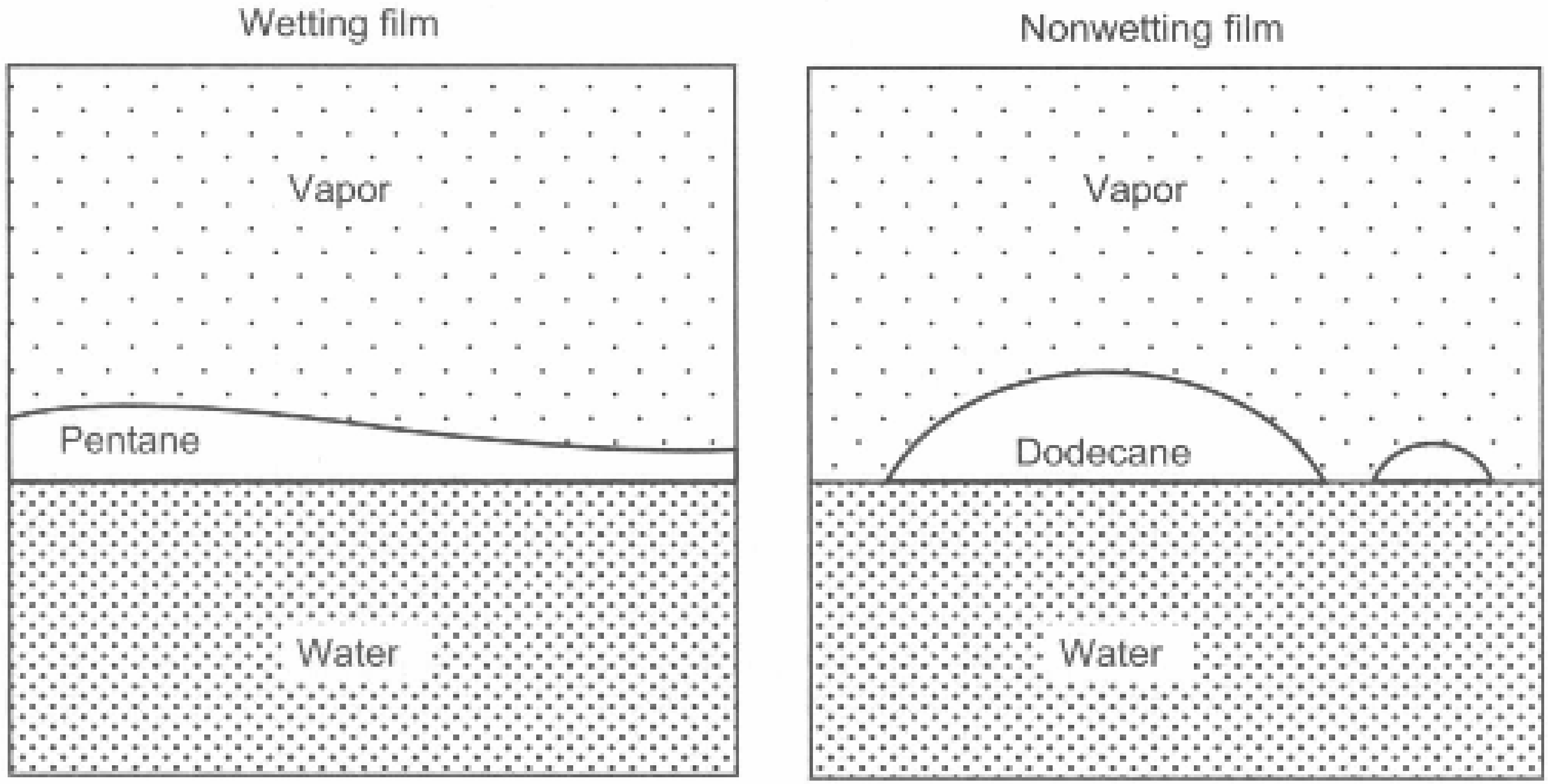
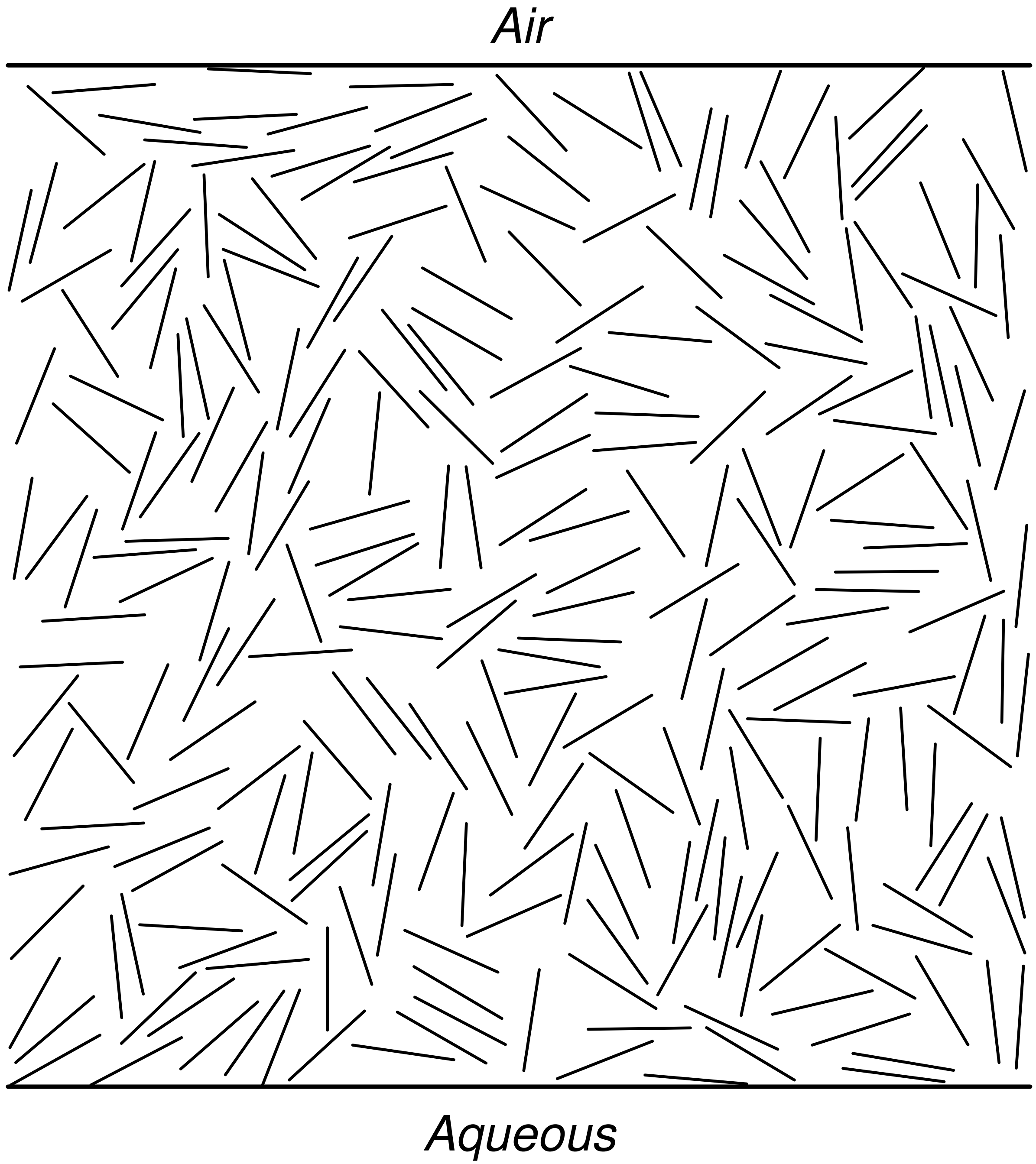
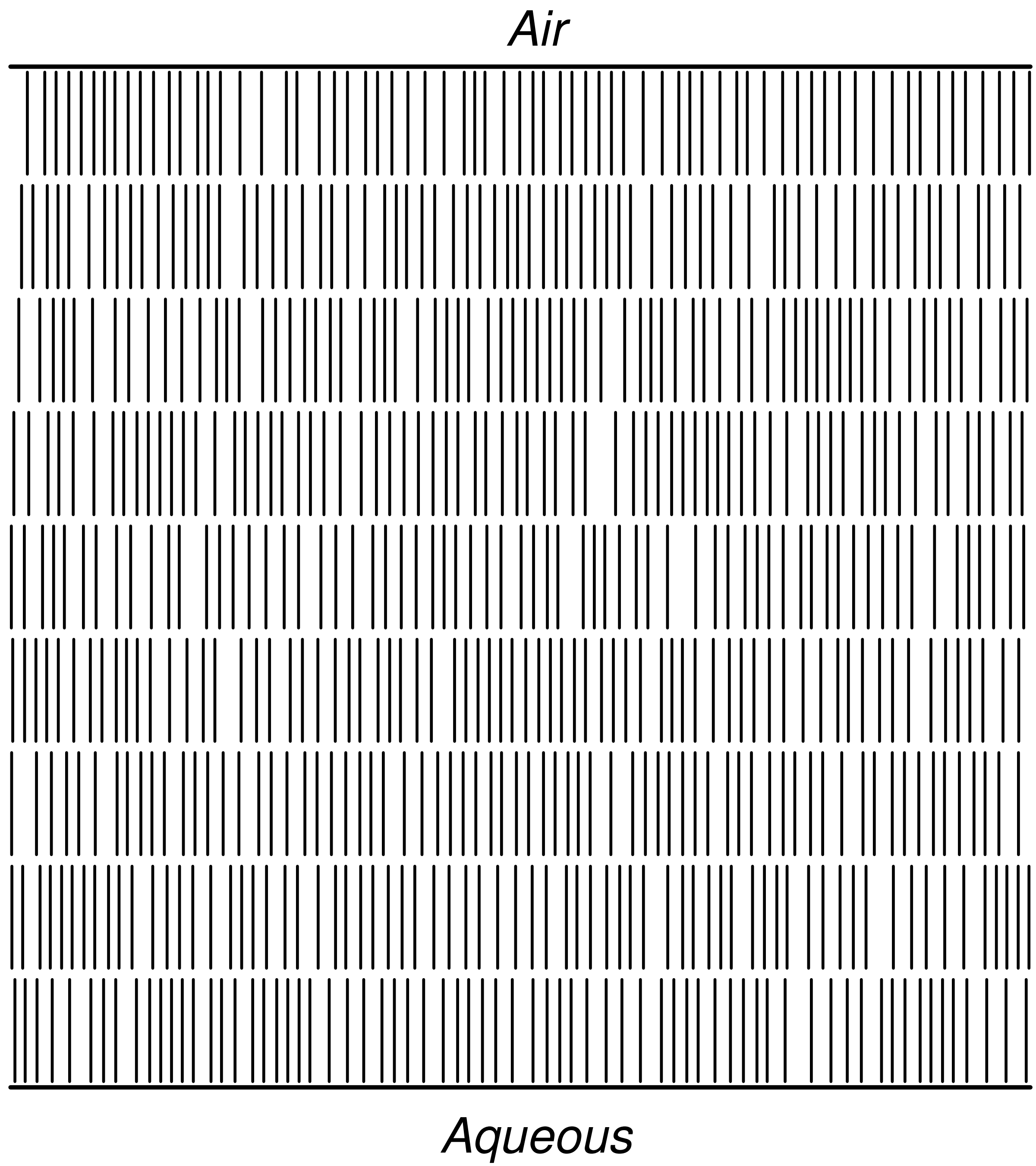


FIGURE 13.7

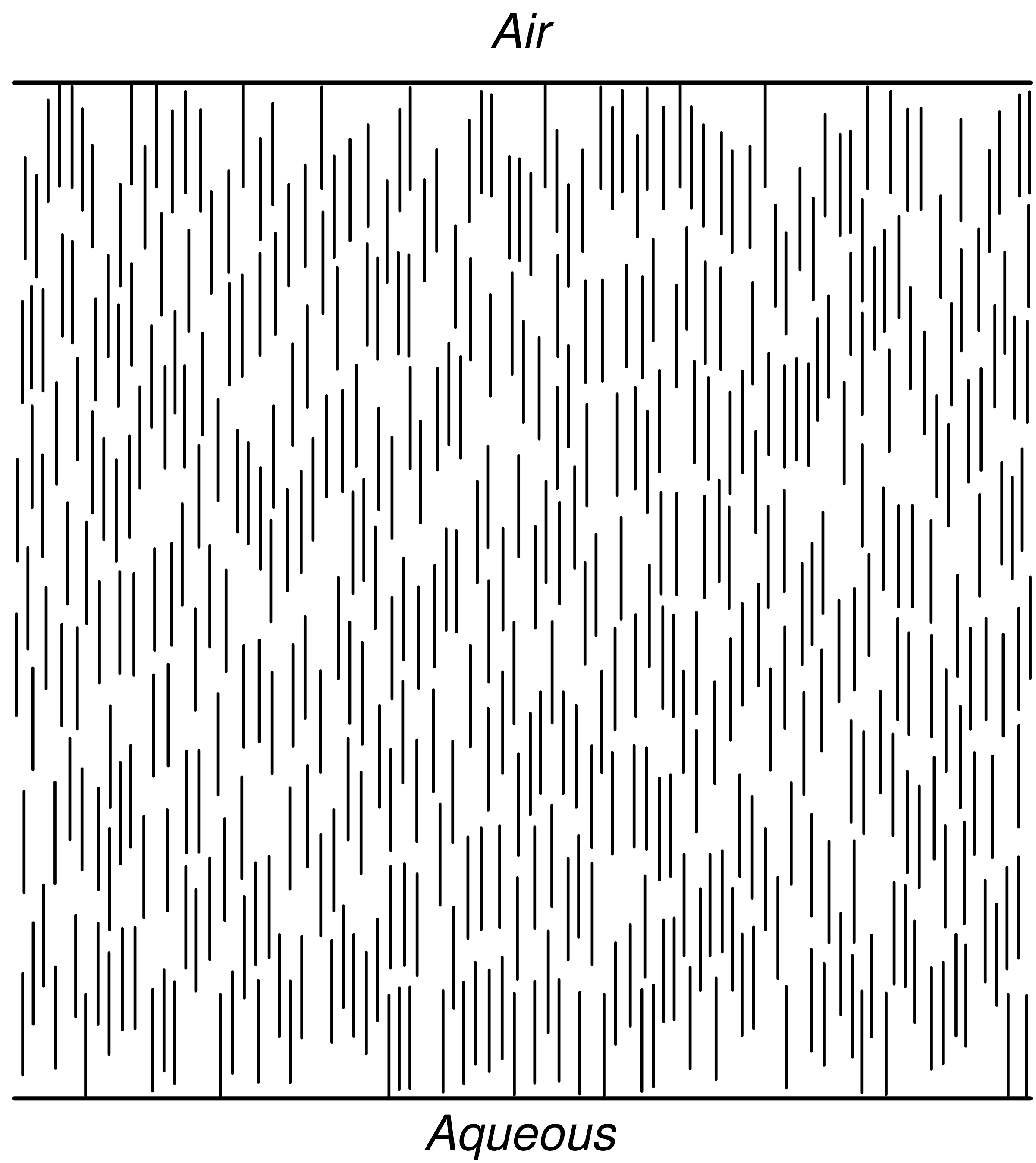
Lipid layer structure - isotropic liquid?



Lipid layer structure - layered (smectic)?

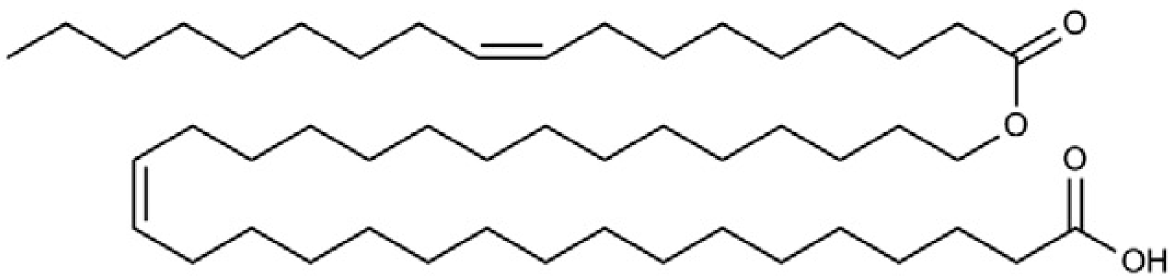


Lipid layer structure - aligned (nematic)?



(O-acyl)-omega-hydroxy fatty acid

2011, Butovich, Prog Lipid Res, 50, 278



Oleoyl-omega-hydroxy-dotriacontenoate

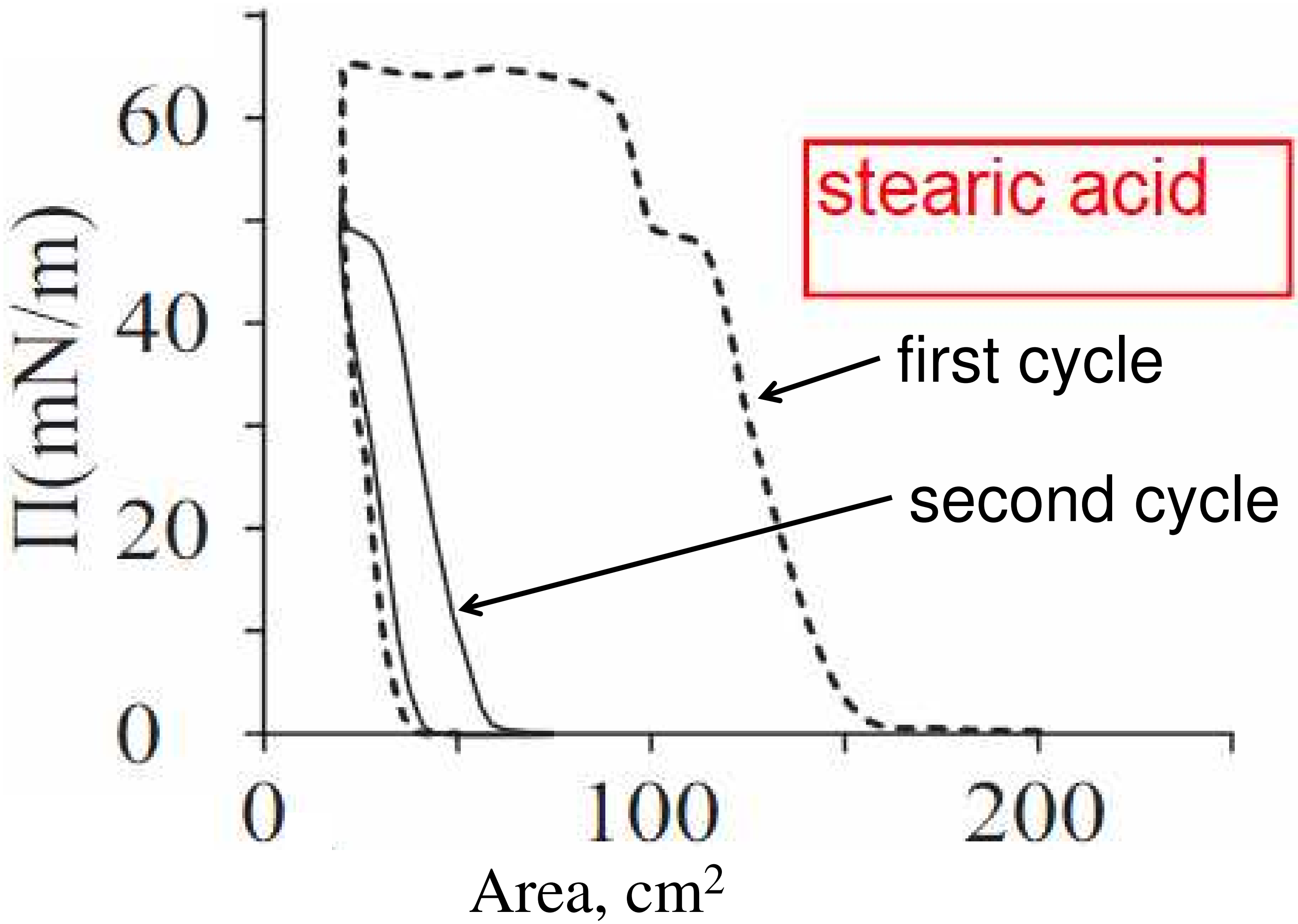
(*Z*)-32-(oleoyloxy)dotriacont-17-enoic acid

Chemical Formula: $C_{50}H_{94}O_4$

Exact Mass: 758.72

Collapse of stearic acid (C_{18}) layer

Mudgil and Millar, 2011, IOVS 52, 1661

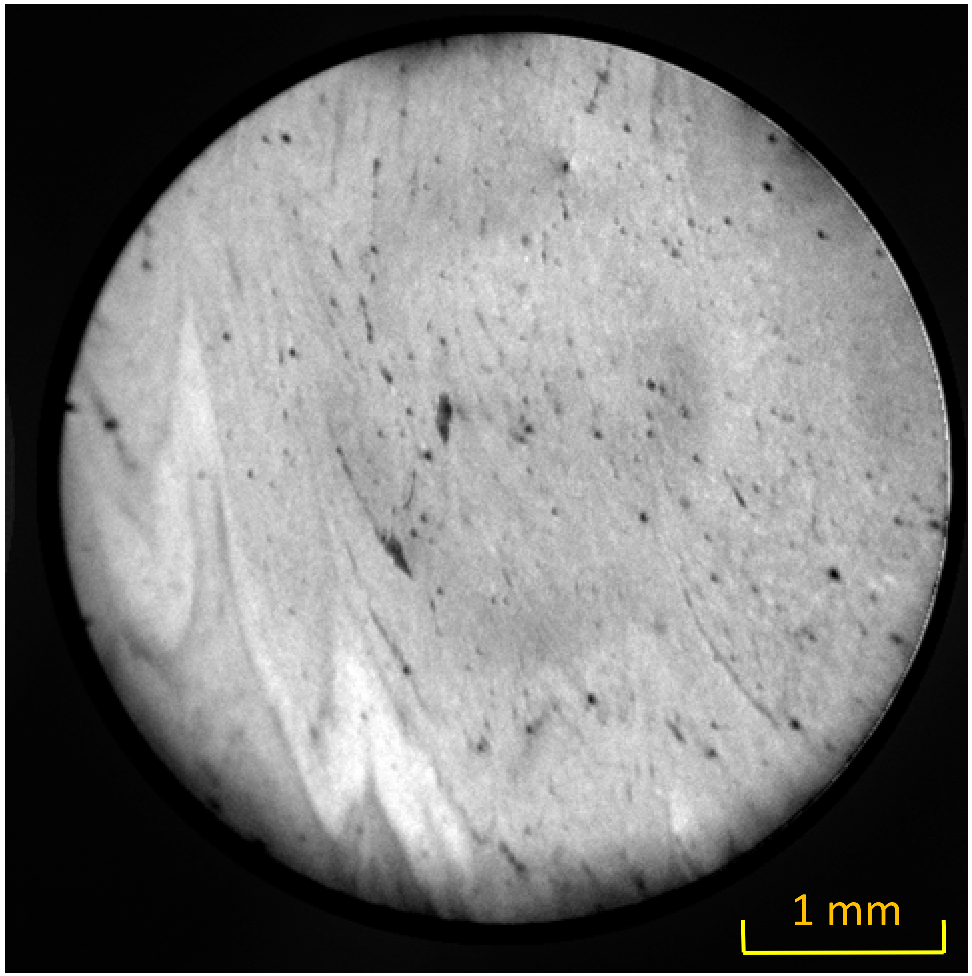


Evidence that tear thinning is due to evaporation

Simultaneous fluorescence and lipid images

15 seconds after a blink

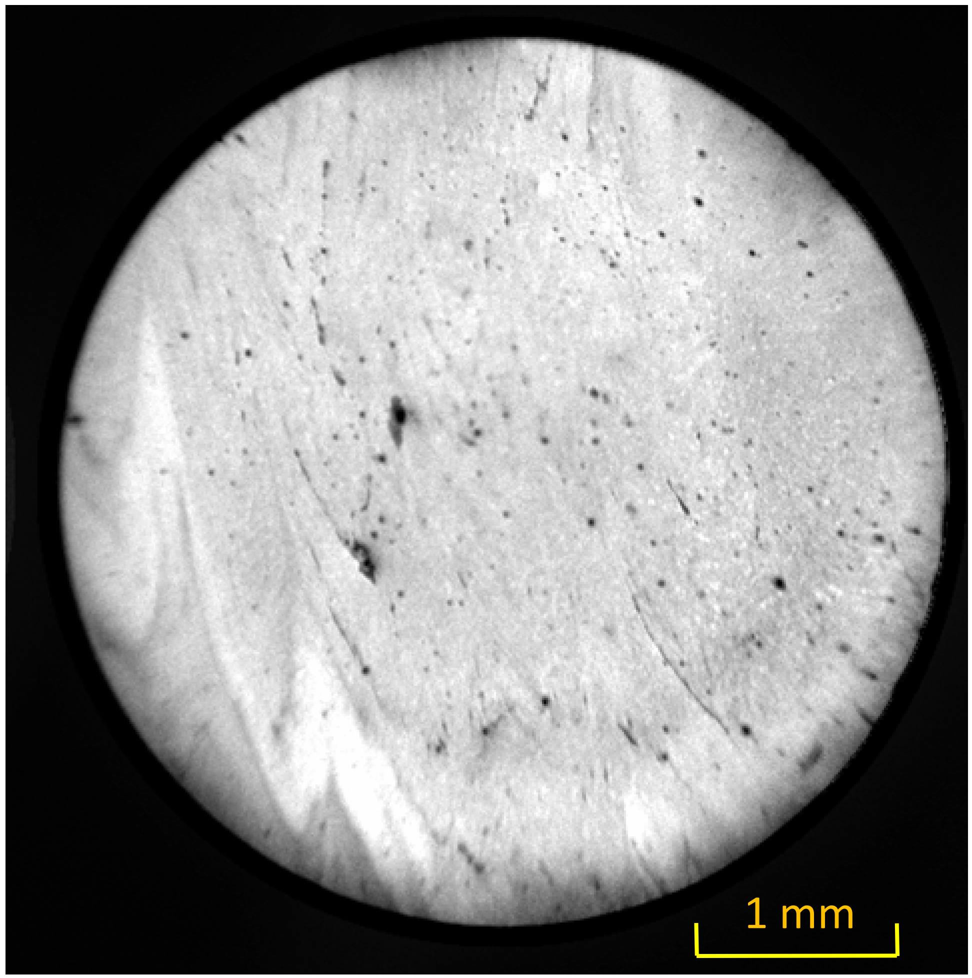
lipid layer image



Evidence that tear thinning is due to evaporation
Simultaneous fluorescence and lipid images

20.3 seconds after a blink

lipid layer image



Evidence that tear thinning is due to evaporation
Simultaneous fluorescence and lipid images

20.3 seconds after a blink

fluorescence image