Towards a better understanding of reagent striping onto lateral flow diagnostic membranes

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Materials & methods

Materials:

Membrane:

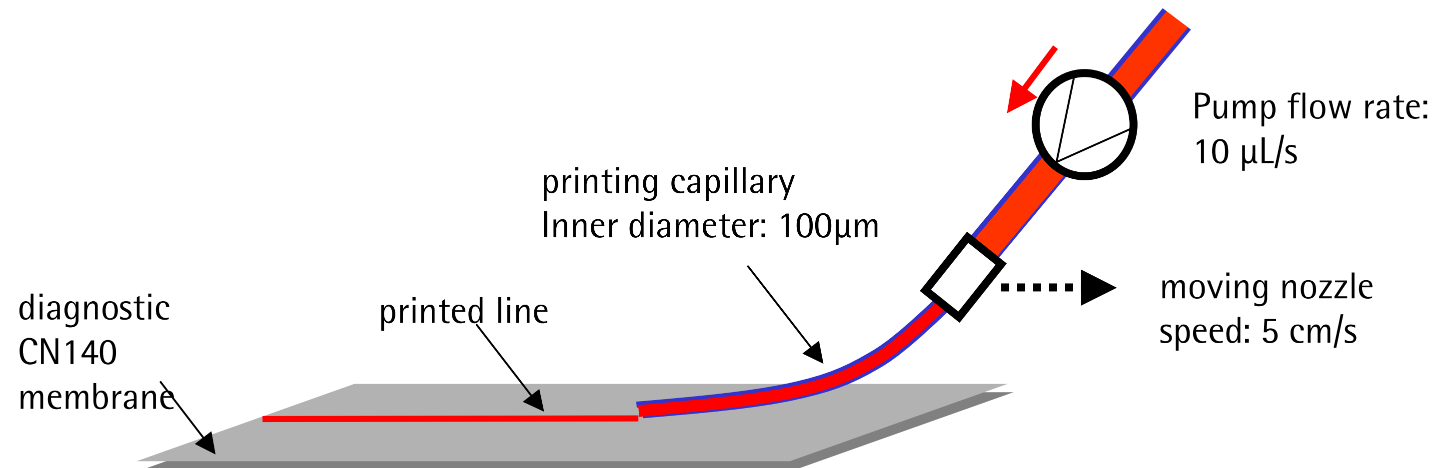
- type:	UniSart CN140 unbacked (Sartorius)
- material:	nitrocellulose
- wicking speed:	140 sec / 4 cm
- nominal pore size:	8 µm
- thickness:	140 µm
- protein binding capacity:	2,5 g/l
- porosity:	85%
- impregnation:	anionic surfactant
- line application side:	belt side

Materials:

Antibody:

- type:	goat anti-mouse IgG antibody
- fluorescent label:	Alex Flour 555
- labelling density:	5 moles of label per mol of antibody

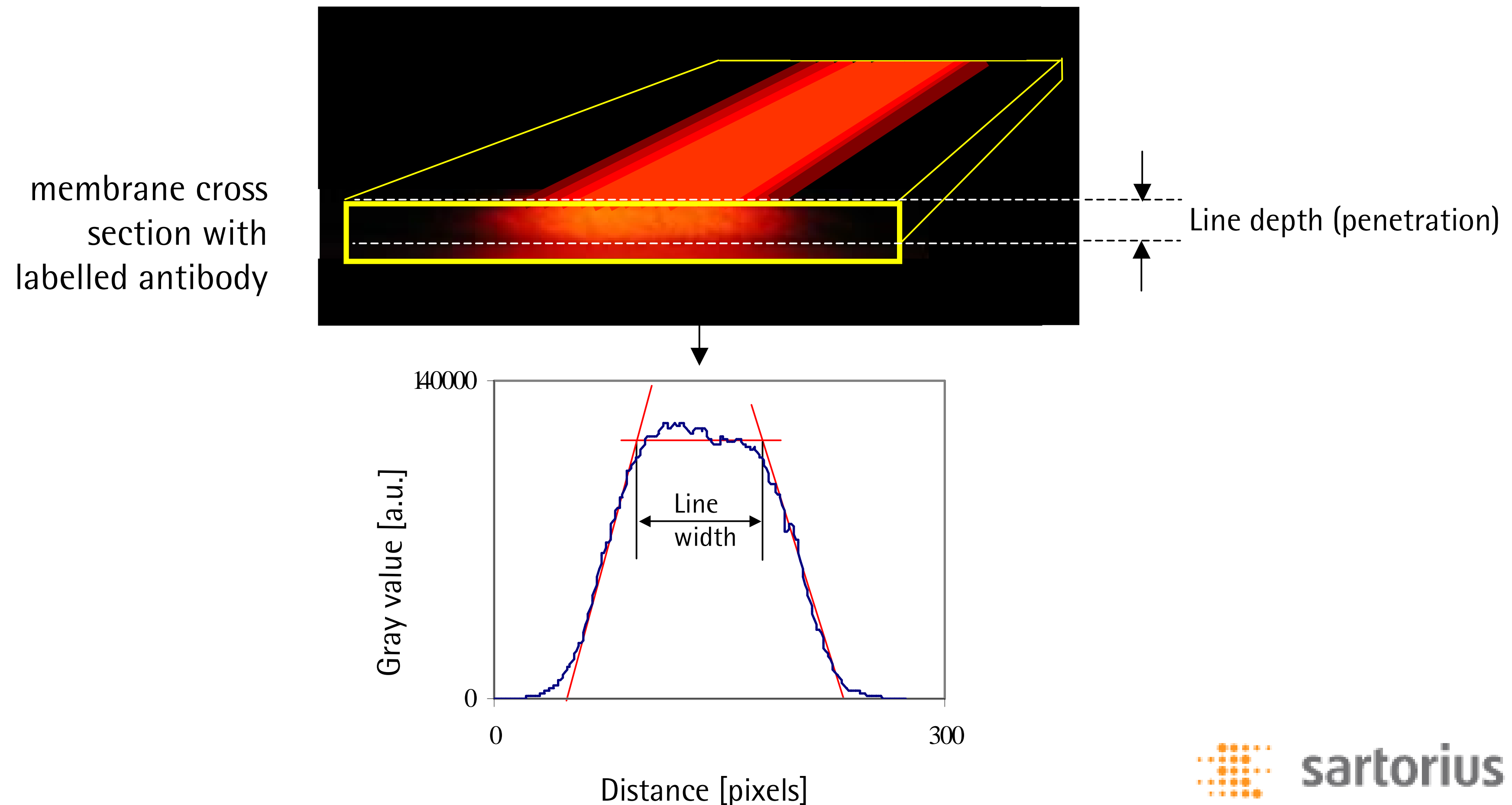
Methods: Line printing and protein visualization



1. Print line of fluorescent labeled antibody solution at varying antibody concentration
2. Dry membrane for 30min @ 50°C

Methods: protein visualization

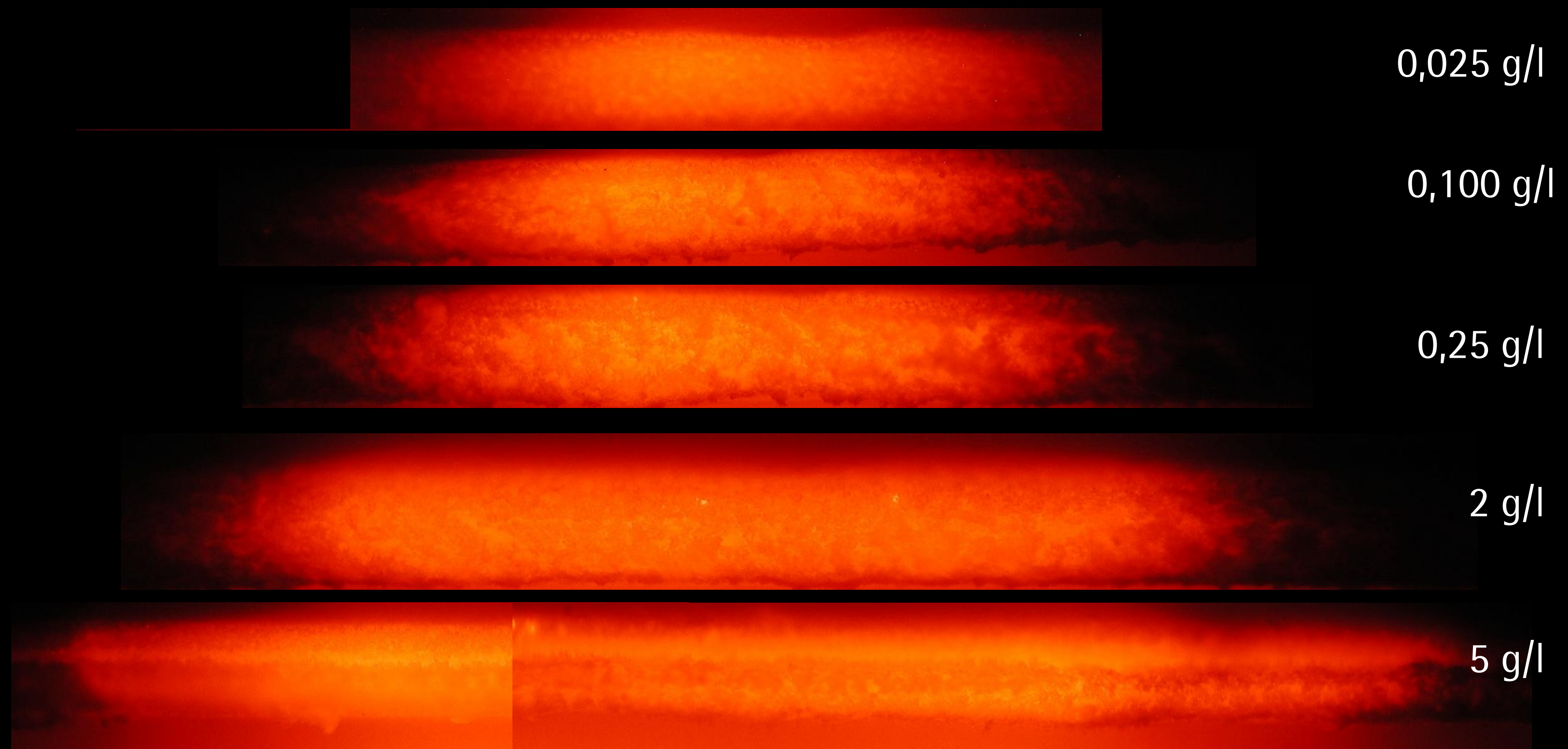
3. Visualize protein distribution in membrane cross-section by fluorescence microscopy
4. Determine protein line width and depth



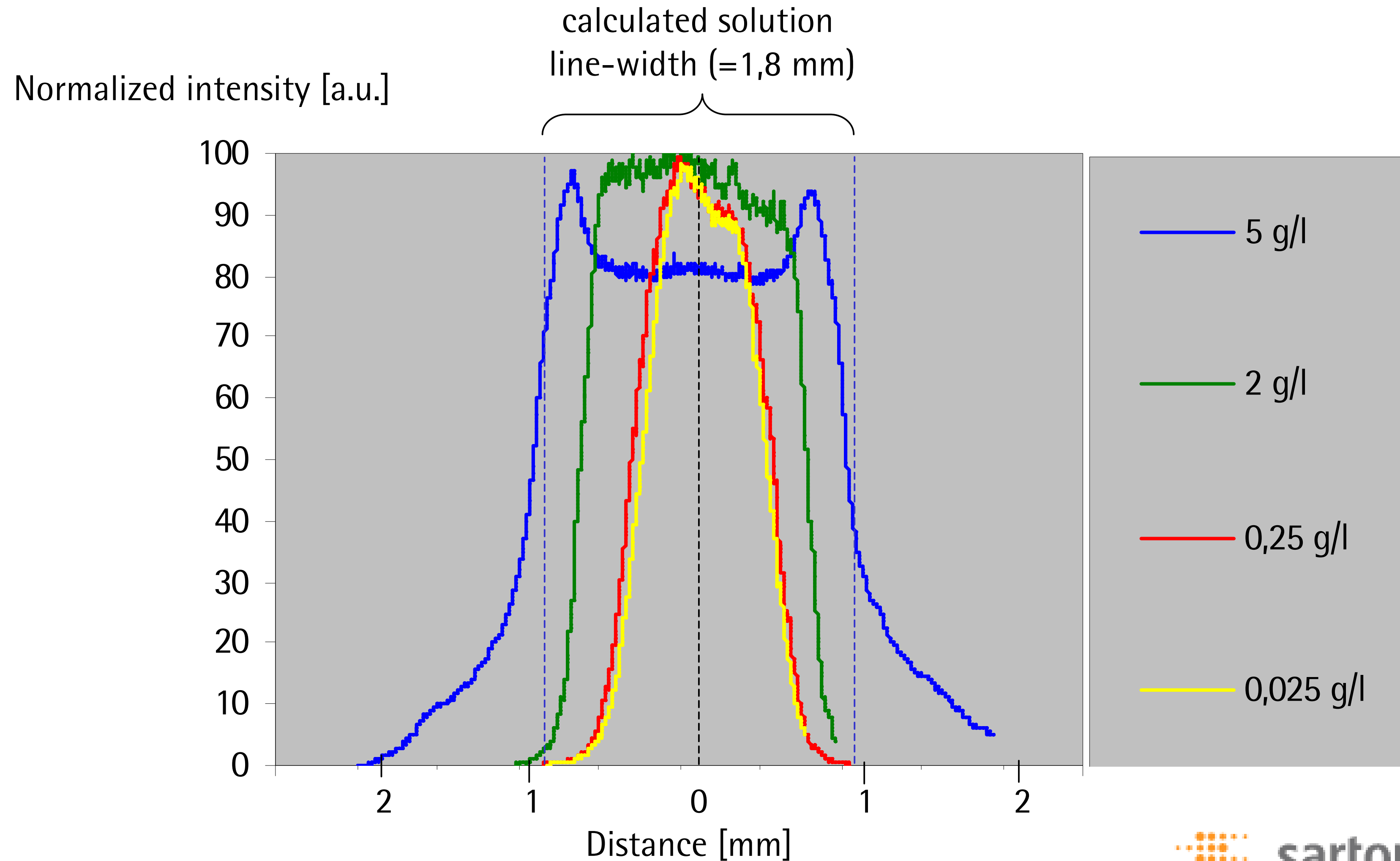
Results and Discussion

Membrane cross sections viewed with fluorescence microscopy

Parameters: 2µl/cm @ 50 mm/s



Normalized break-through curves for different protein concentrations
Parameters: 2µl/cm @ 5 mm/s



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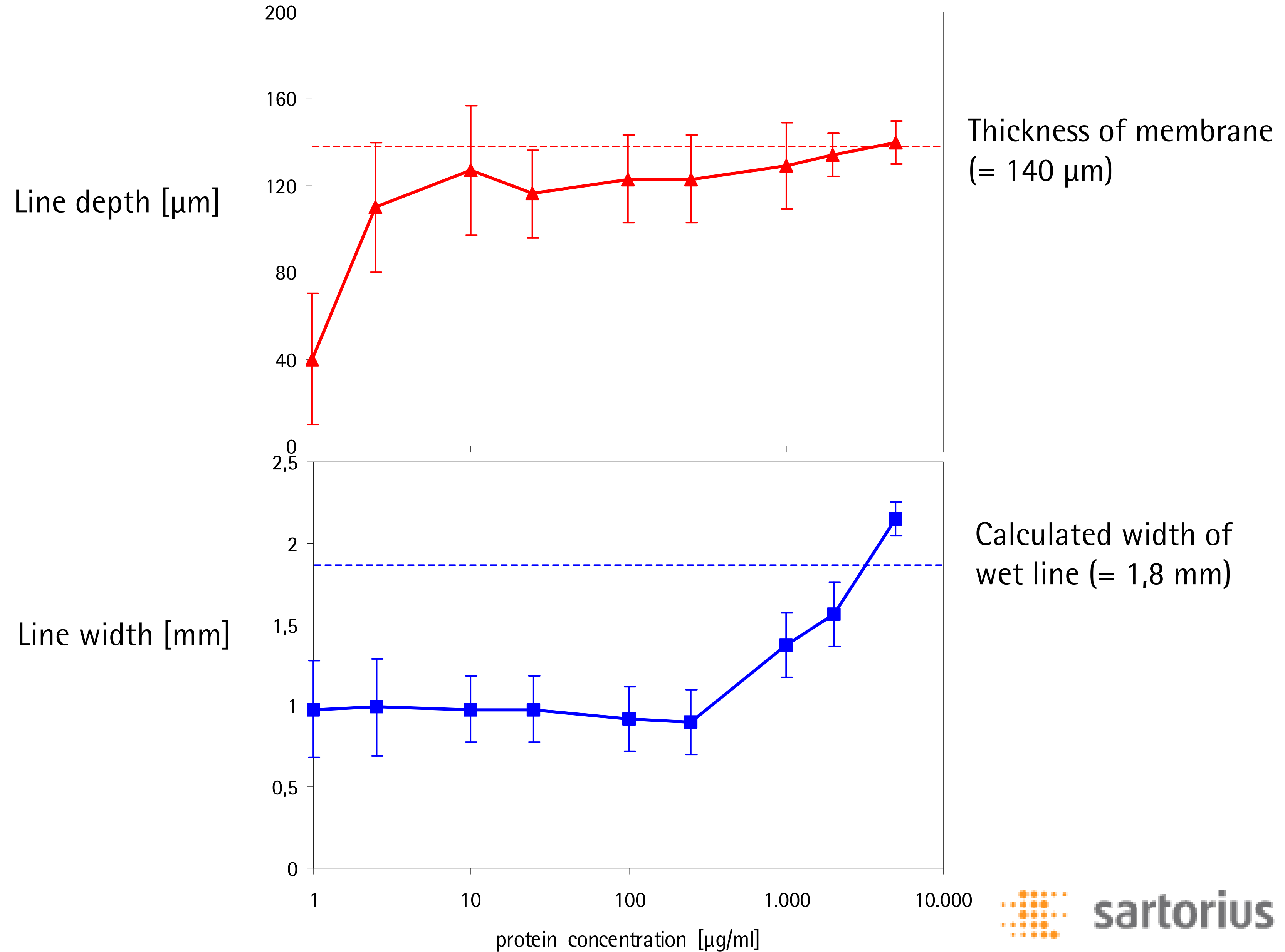

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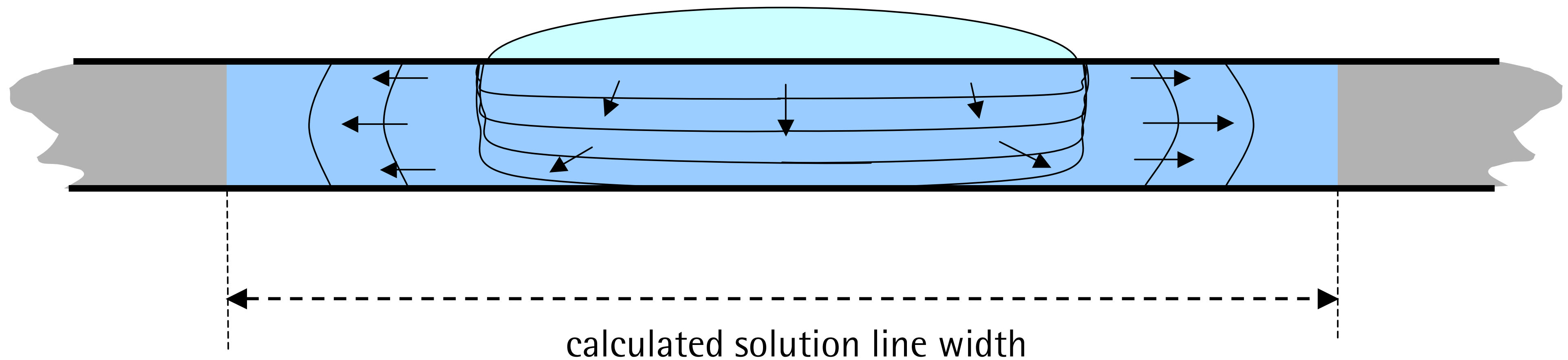
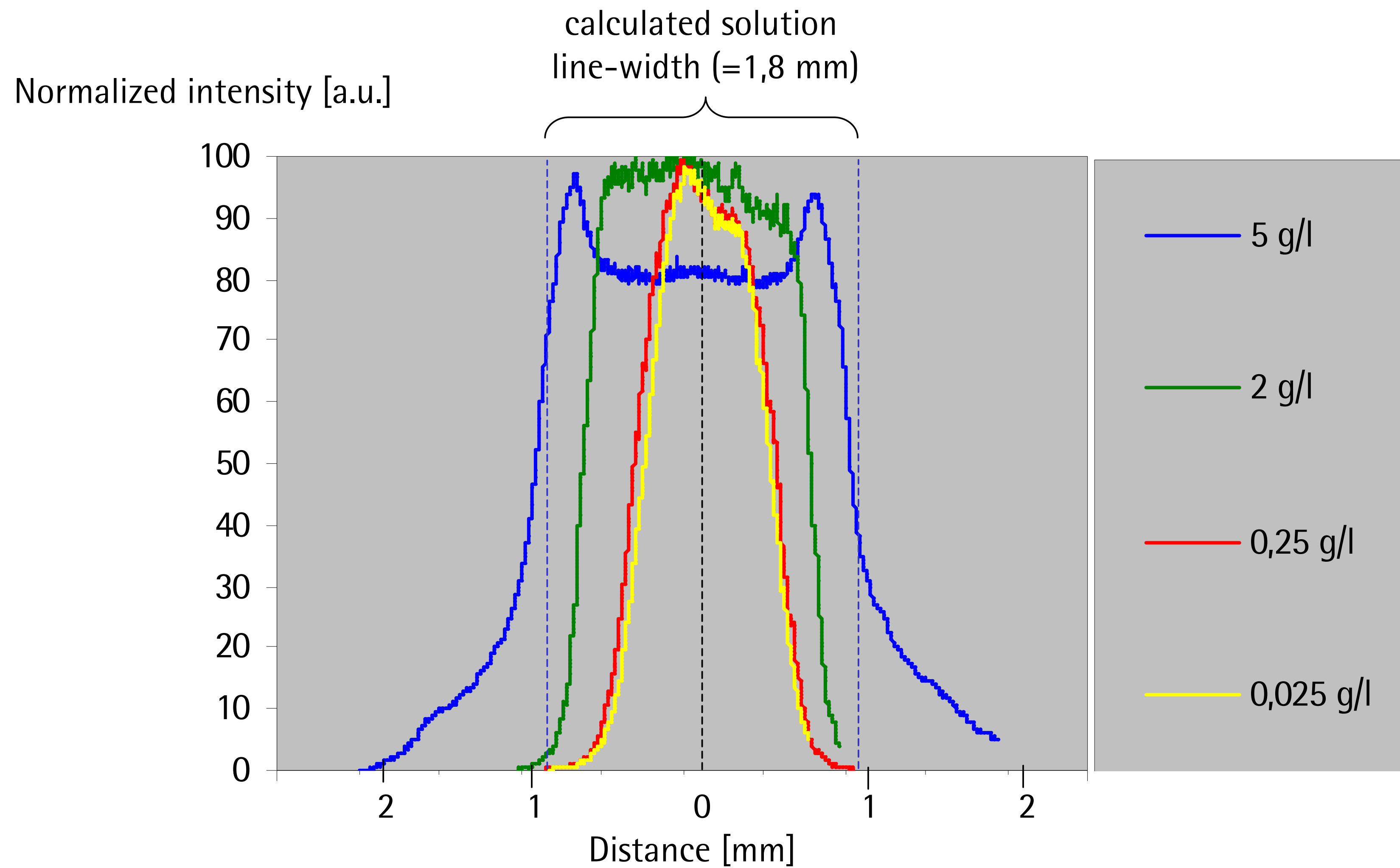
Hypothesis for flow front progression into the membrane:

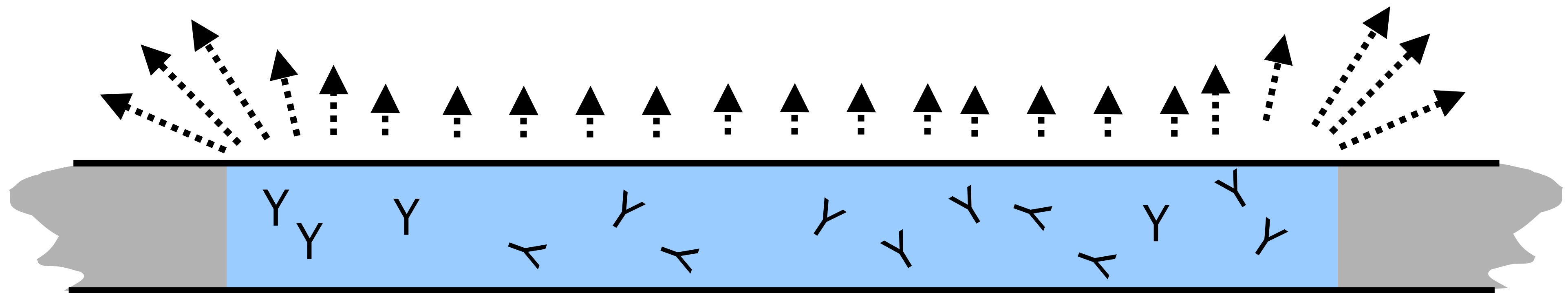


=> minimal protein line width is determined by the liquid meniscus



„horns“ are generated when protein is in excess: salt effect

Hypothesis for “horn” formation at high protein concentration



Y = free, unbound antibody

=> evaporation is fastest at the edges of the line

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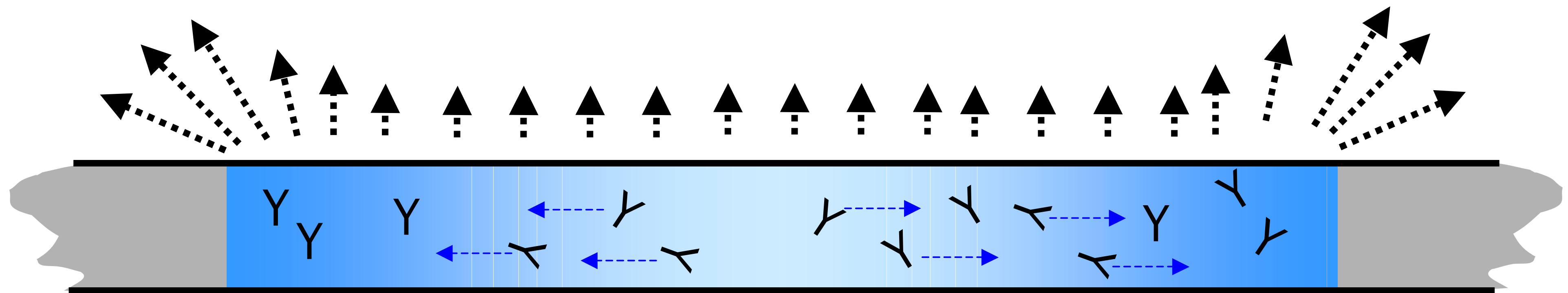
Hypothesis for “horn” formation at high protein concentration



Y = free, unbound antibody

=> excess antibody is transported to edges: salt effect

Learnings

1. The minimal protein line width is determined by the wetting meniscus

2. Main process paramters to influence the miniscus shape are:

    A.: surface energy of reagent solution:
    => addition of salt will decrease line width
    => addition of alcohol or surfactants will increase line width

    B.: striping parameters:
    => increasing volume flow rate will increase line width

3. Lateral flow membranes are mass transfer limited, i.e. even low protein concentrations penetrate the full depth of the membrane

4. Salt edges („horns“) are formed at protein excess

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