Chromatography

Structure

Definition and principle

Chromatogram and peaks

Qualitative and quantitative analysis

What is chromatography?

Chromatography – **physical separation method** in which compounds are partitioned between two phases - **stationary** and **mobile** (moving through stationary in a given direction)

In column chromatography, it takes different time for different compounds to pass through a column leading to a separation.

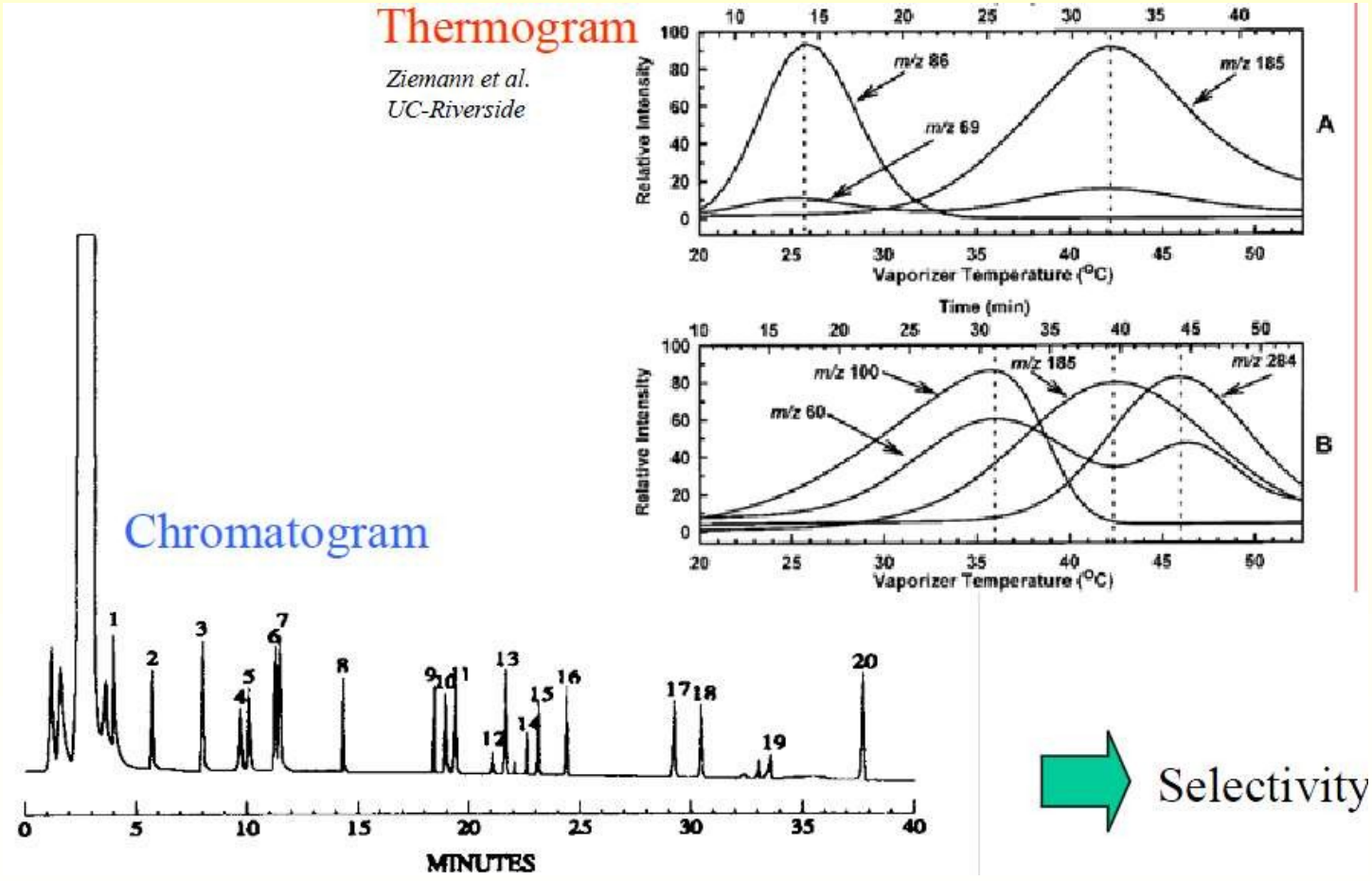
Compounds stronger retained by stationary phase elute later.

Questions:

Why do we need separation?

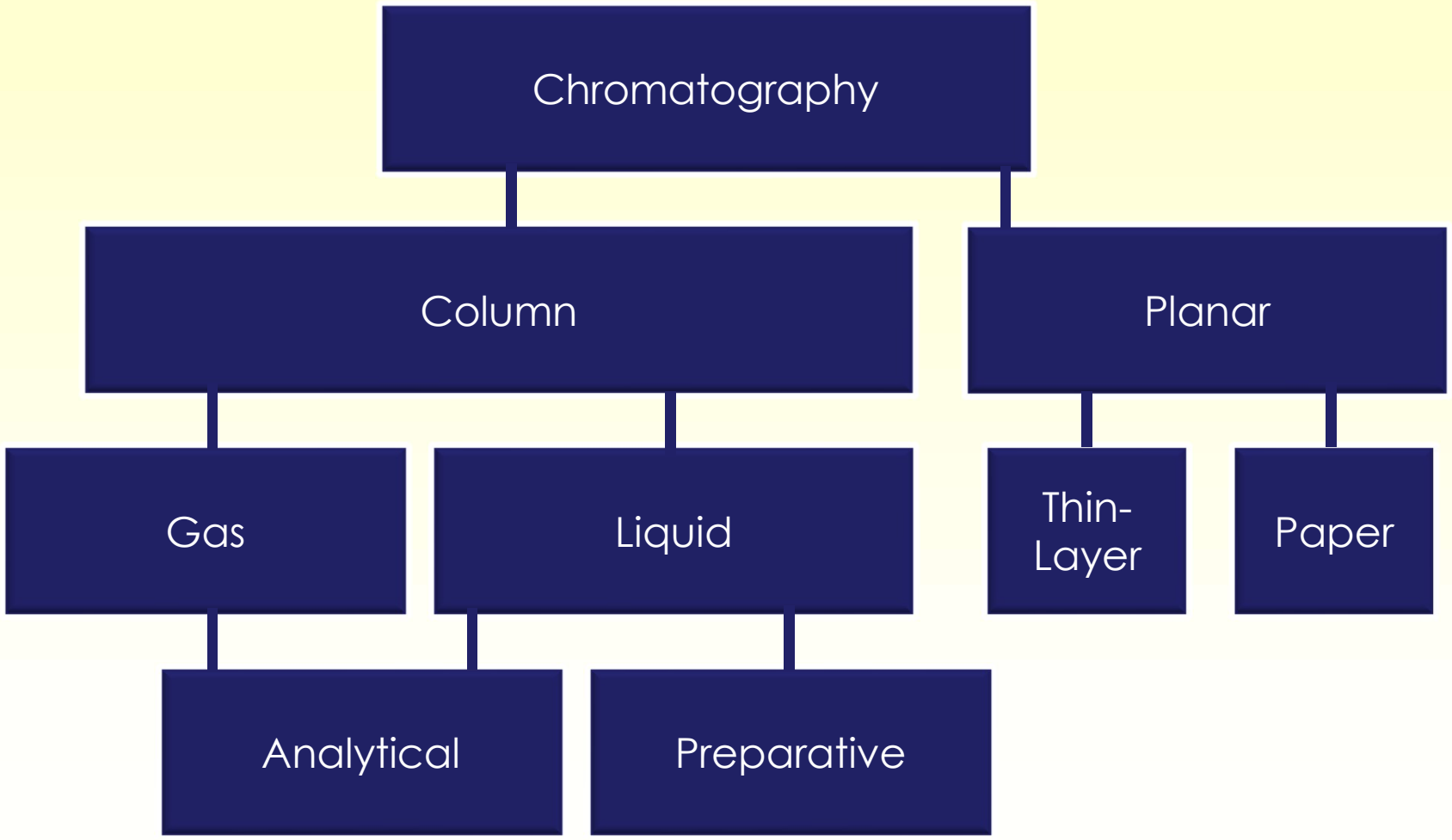
What other separation methods do you know and how do they work?

Why is chromatography so popular?

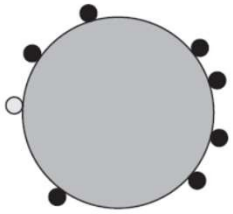


Very high efficiency of separation

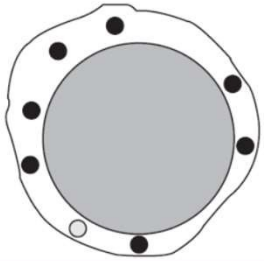
Classification



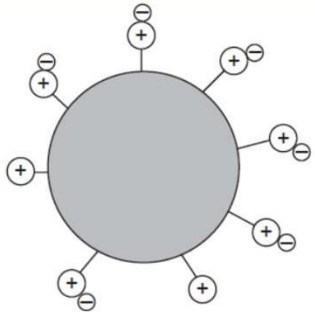
Mechanisms in chromatography



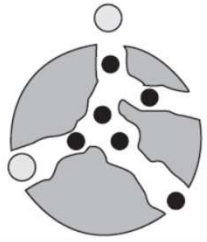
Adsorption chromatography



Partition chromatography

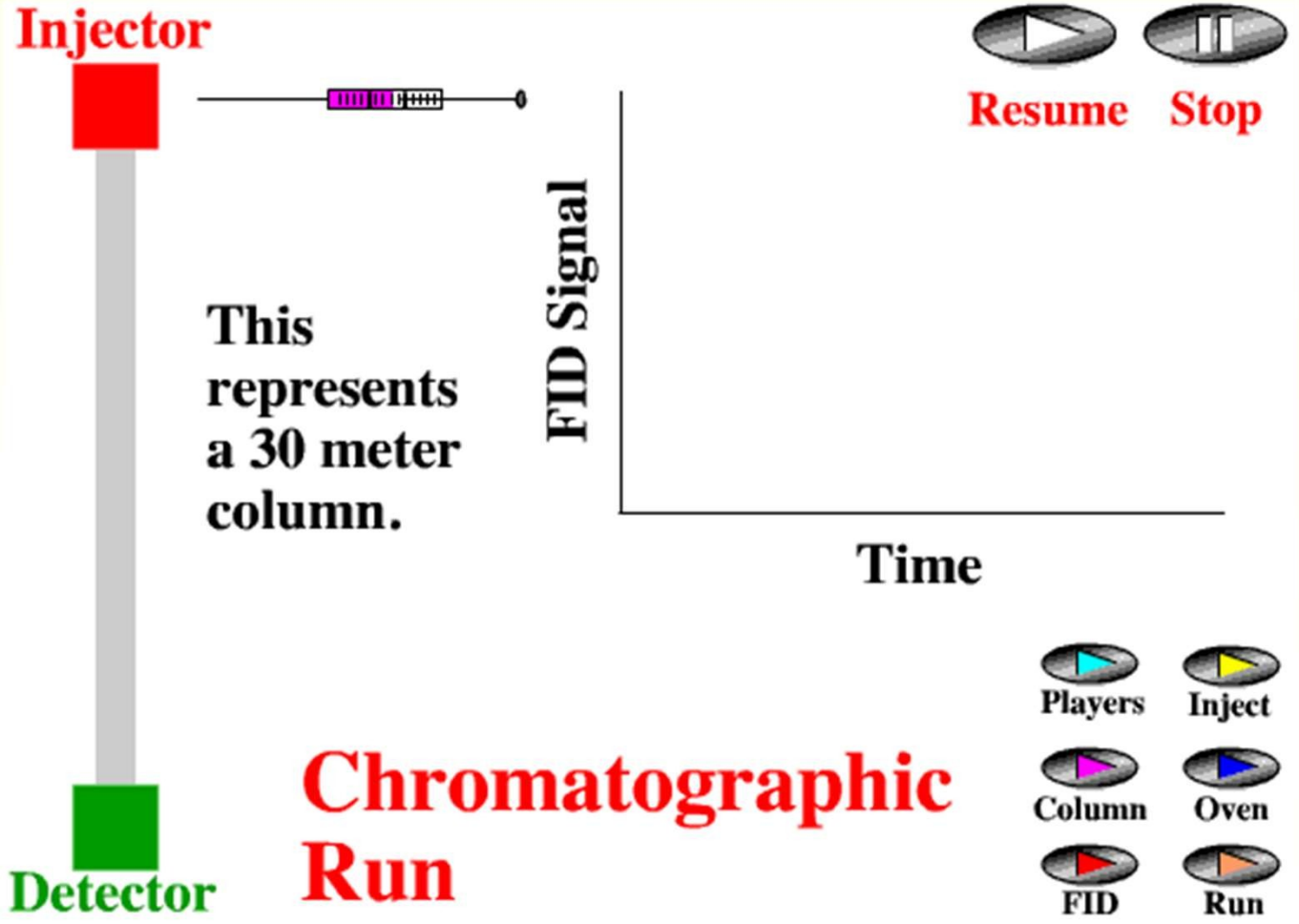


Ion exchange chromatography

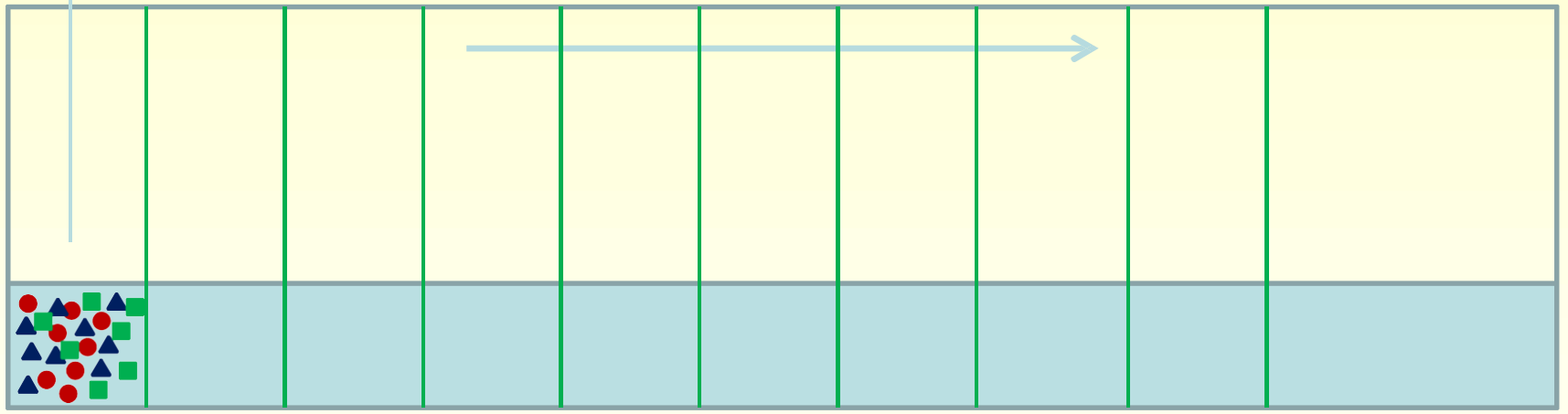


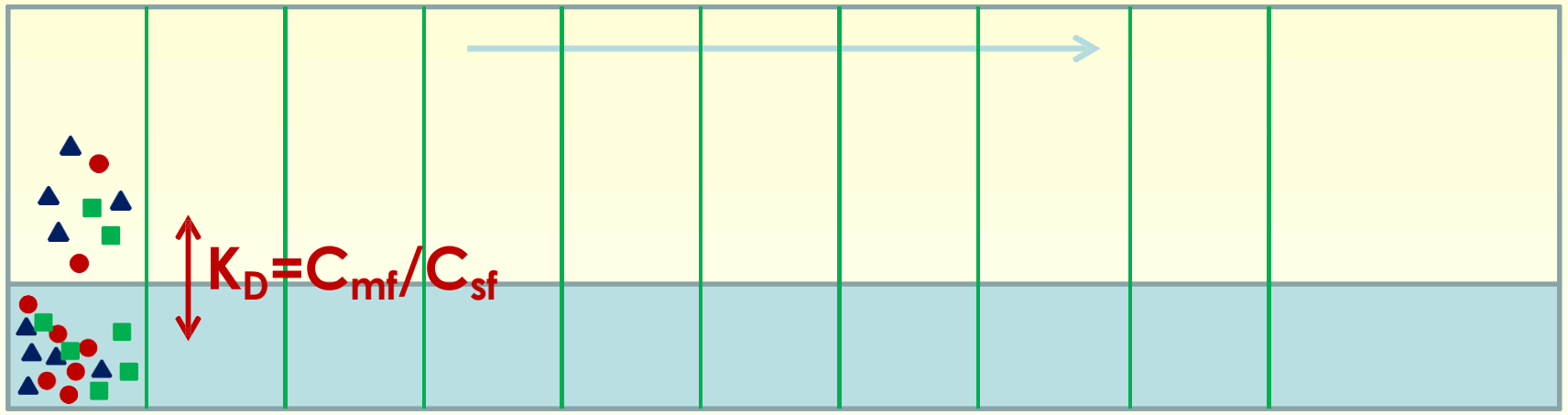
Size exclusion chromatography

Chromatographic process

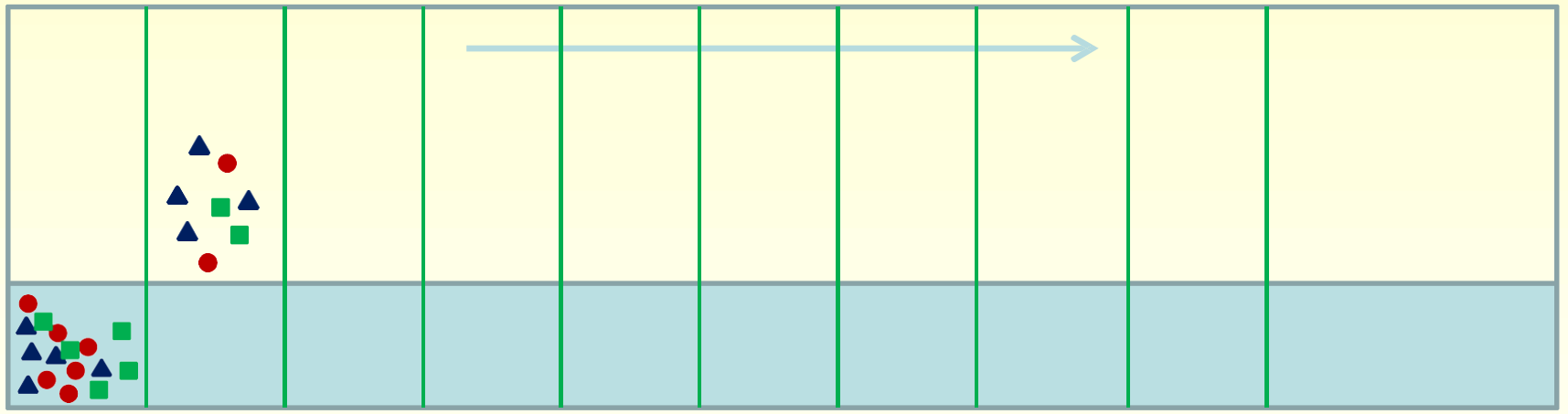


Chromatography process

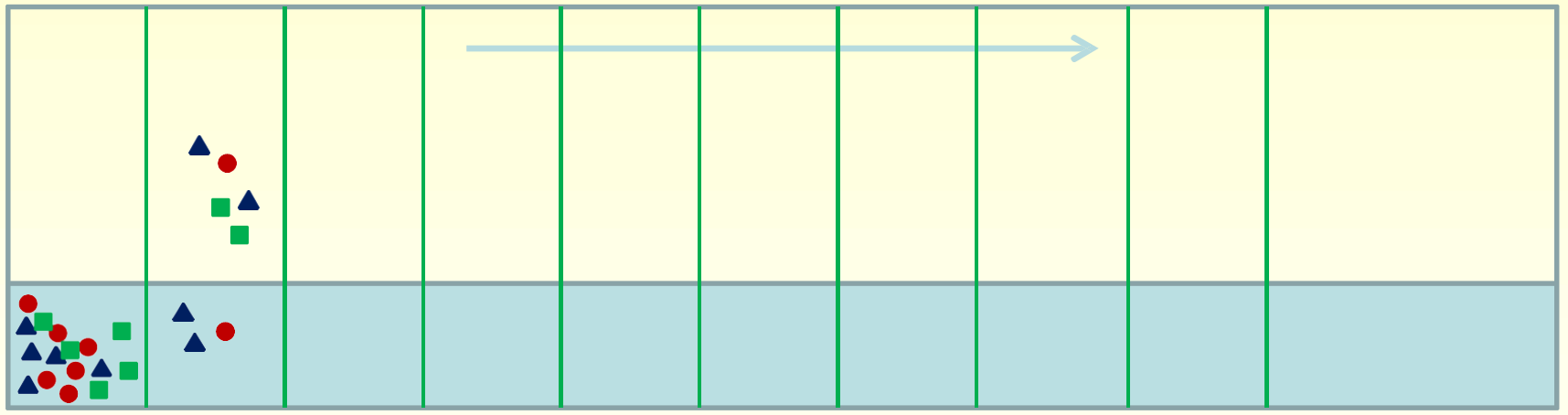


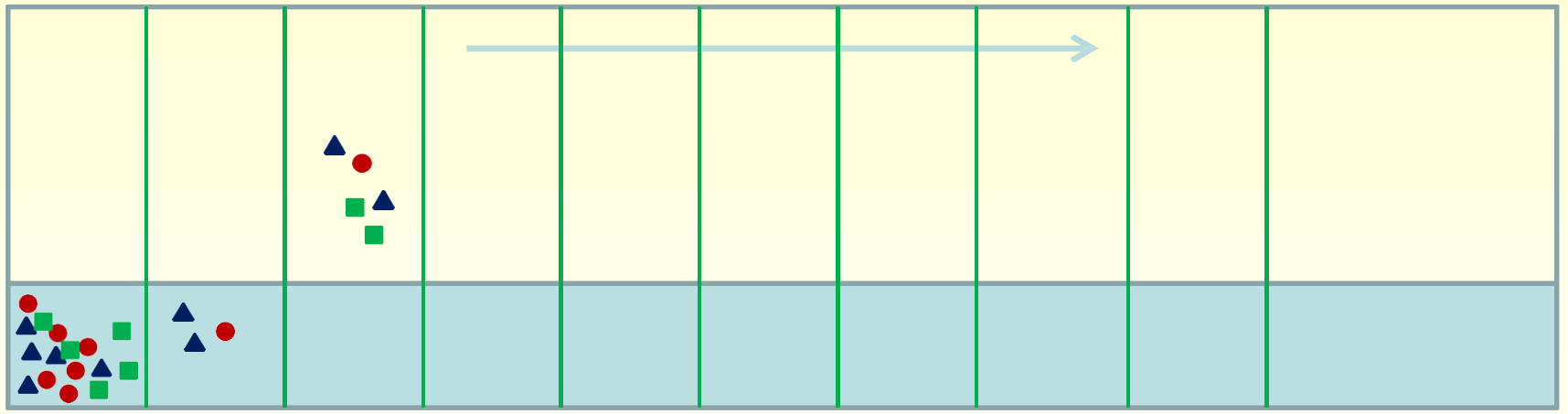


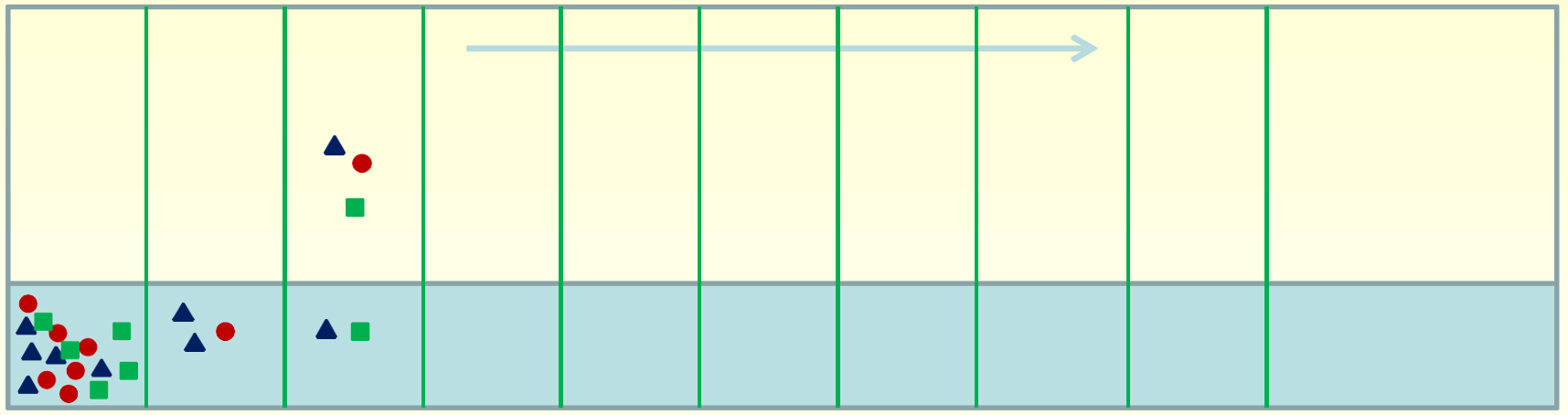
if K_D is higher, compound elutes faster: $K_D > K_D > K_D$



Chromatography







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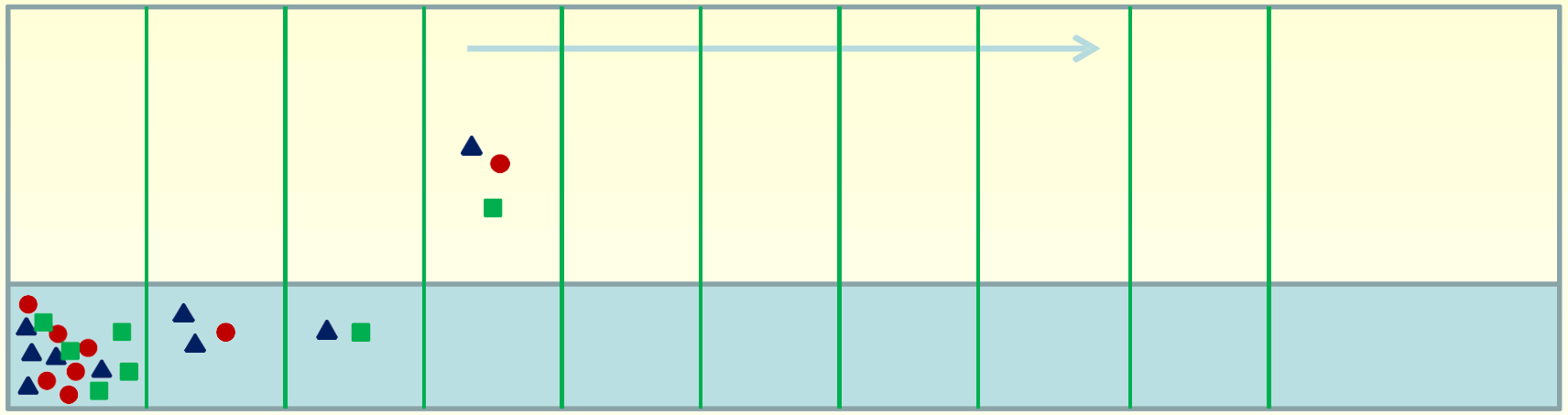

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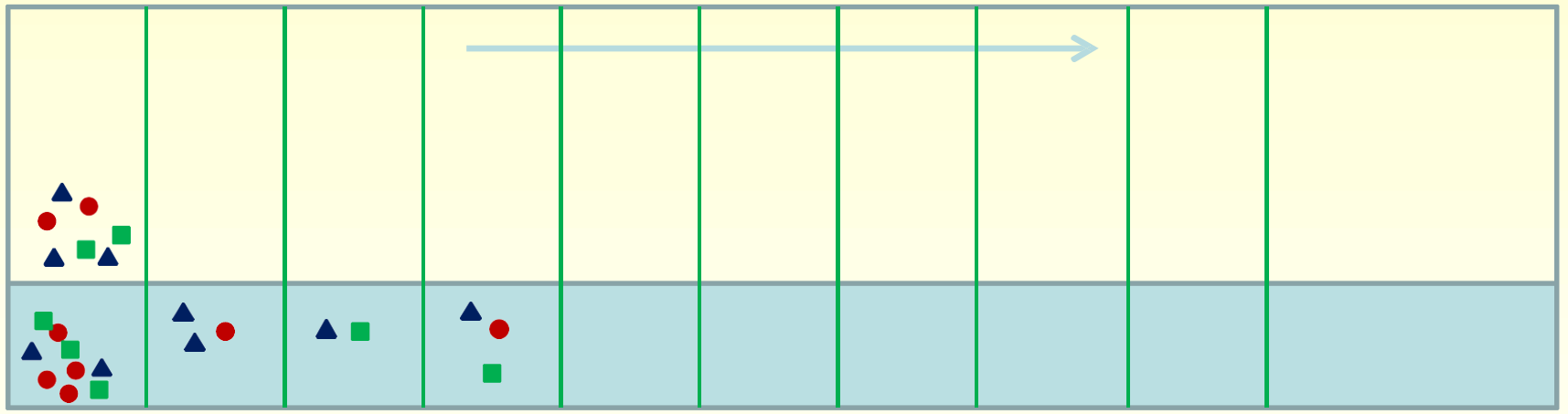

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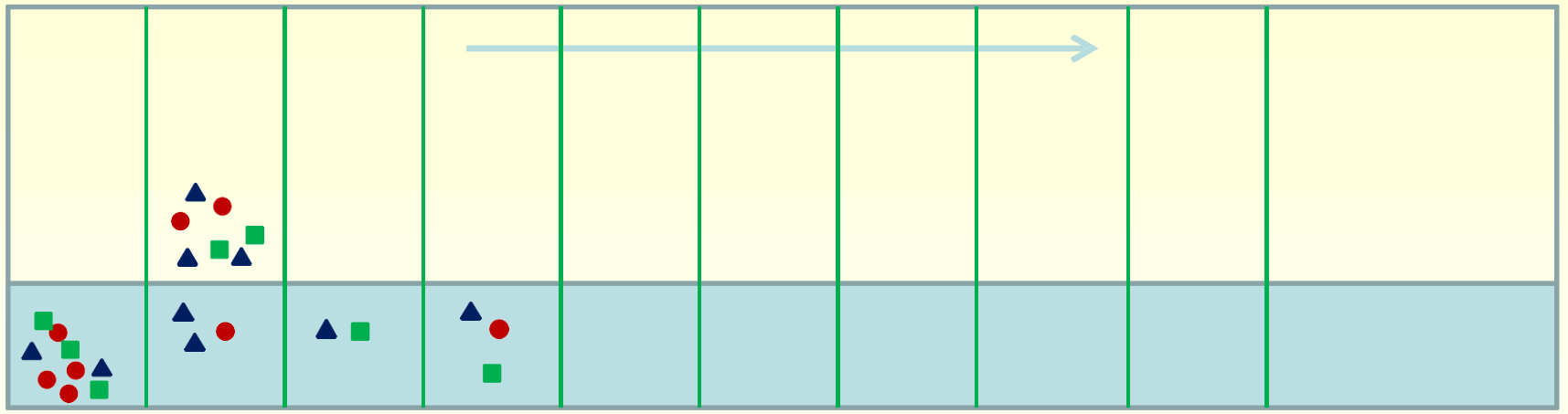

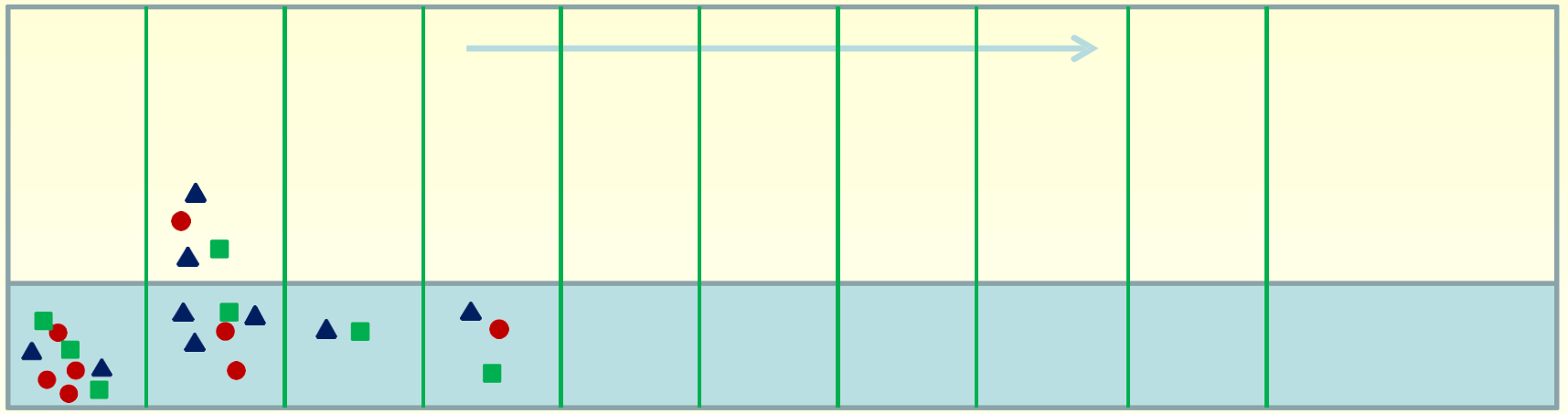

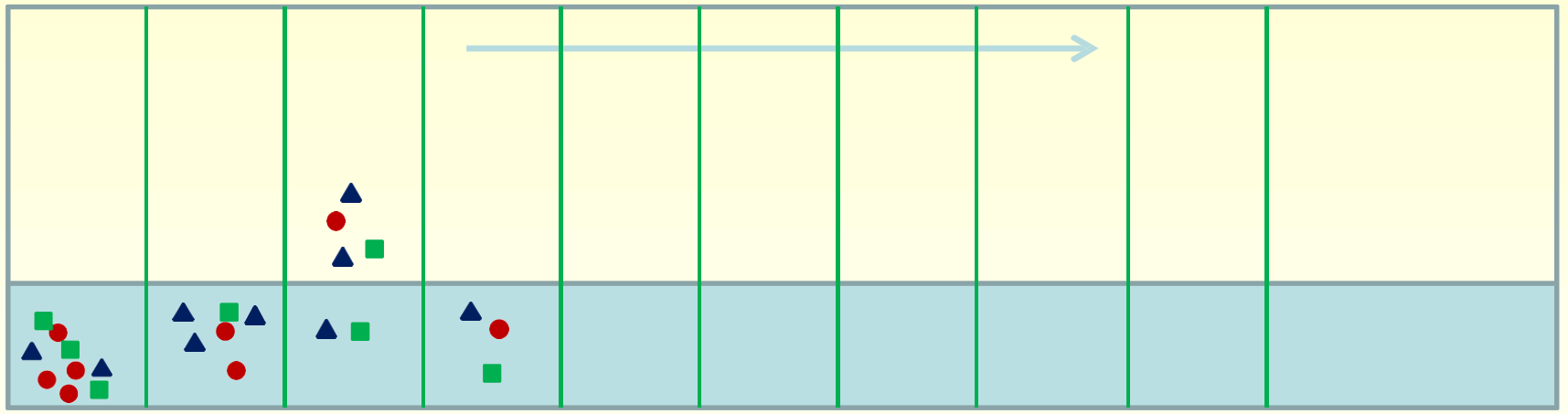

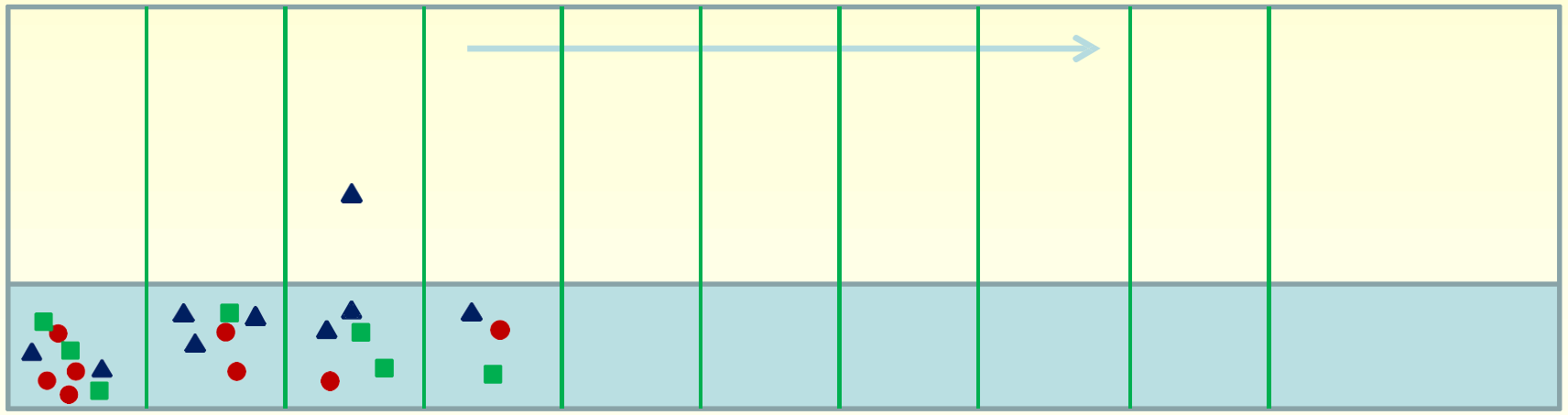

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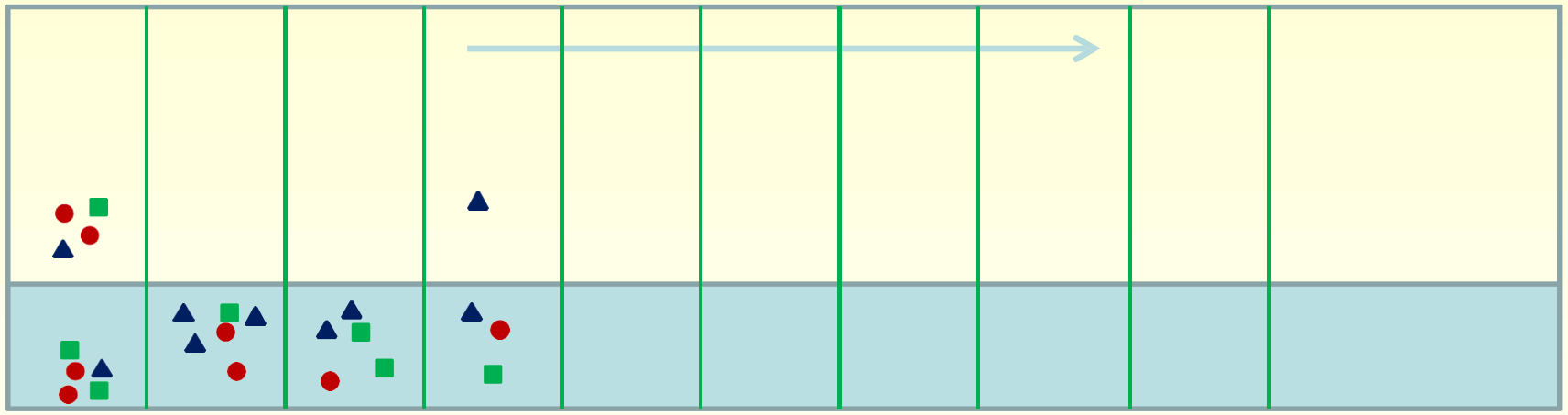

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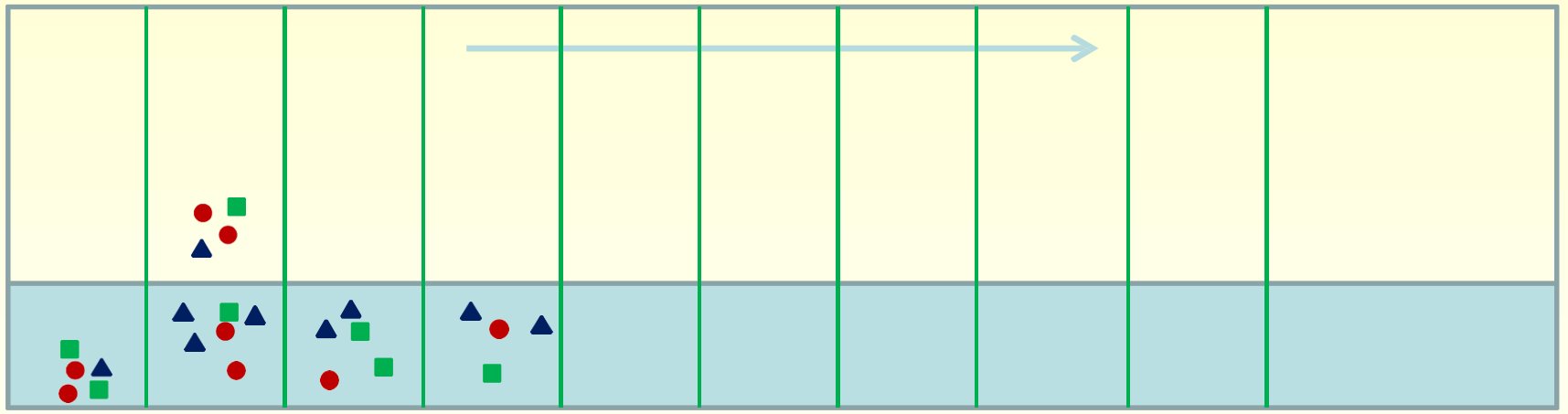

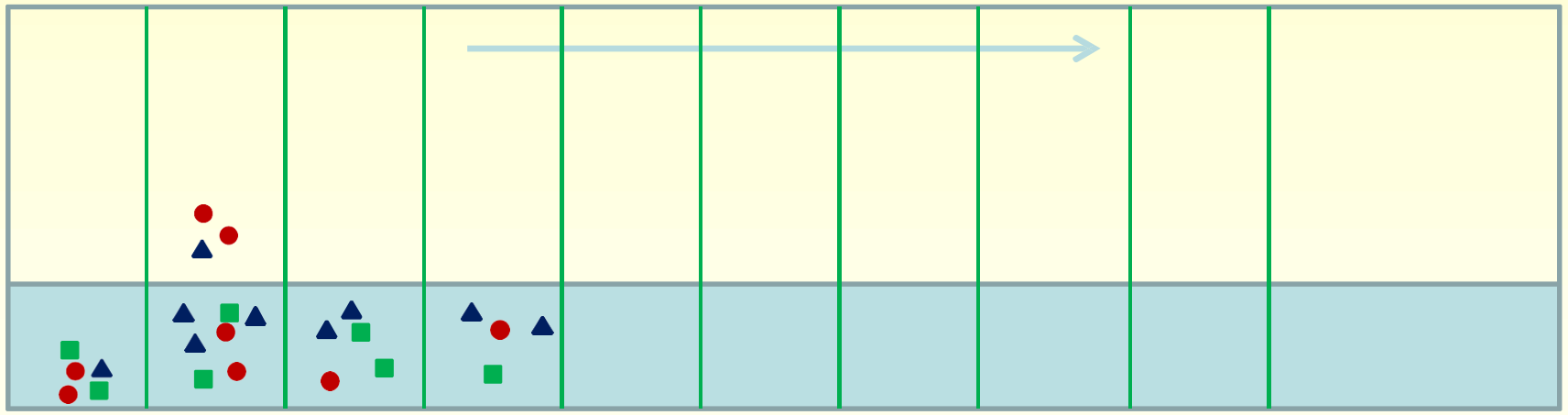

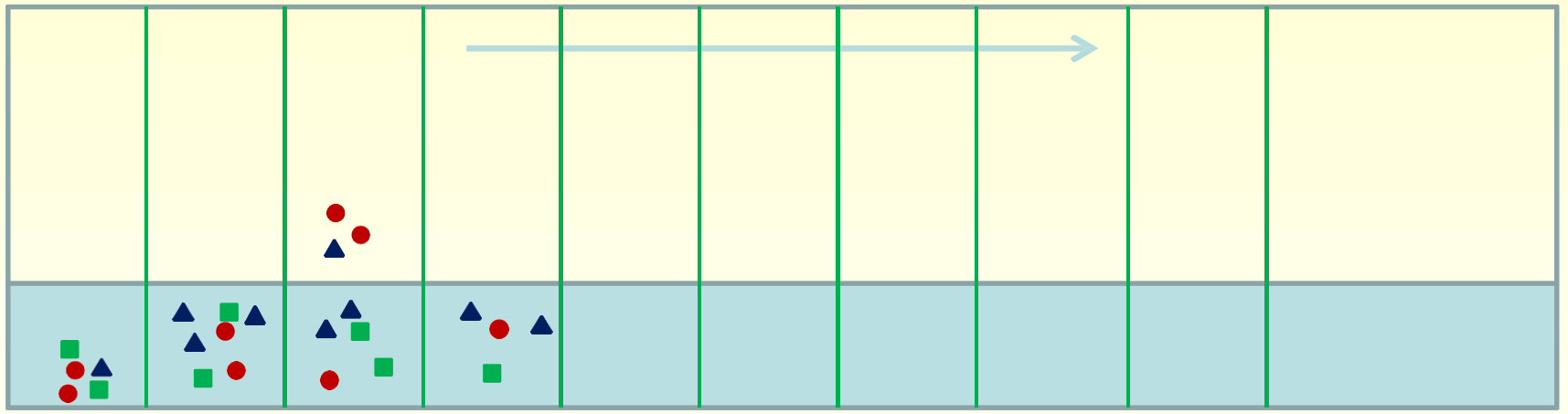

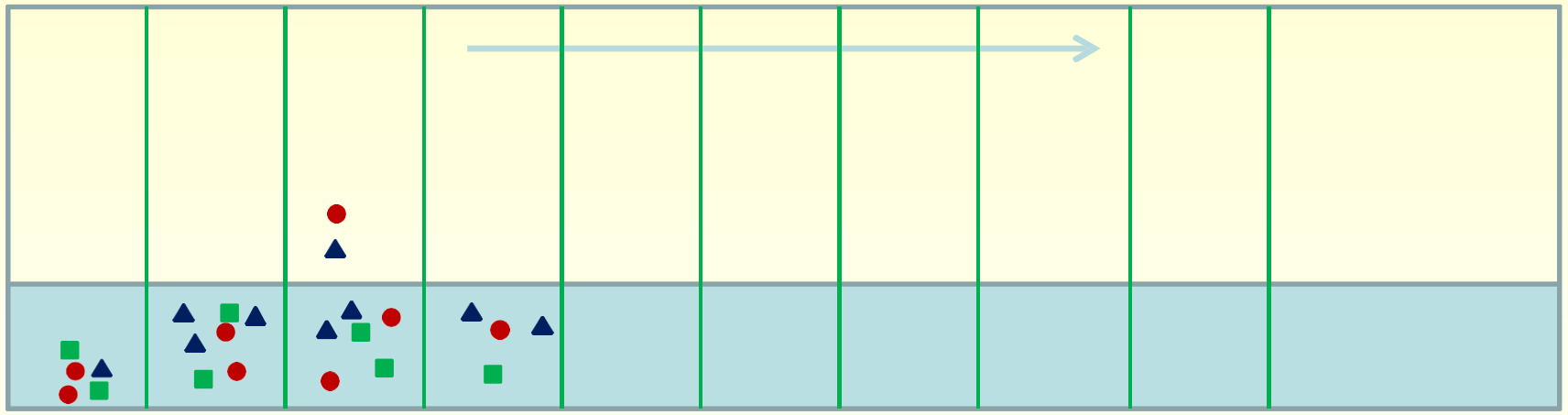

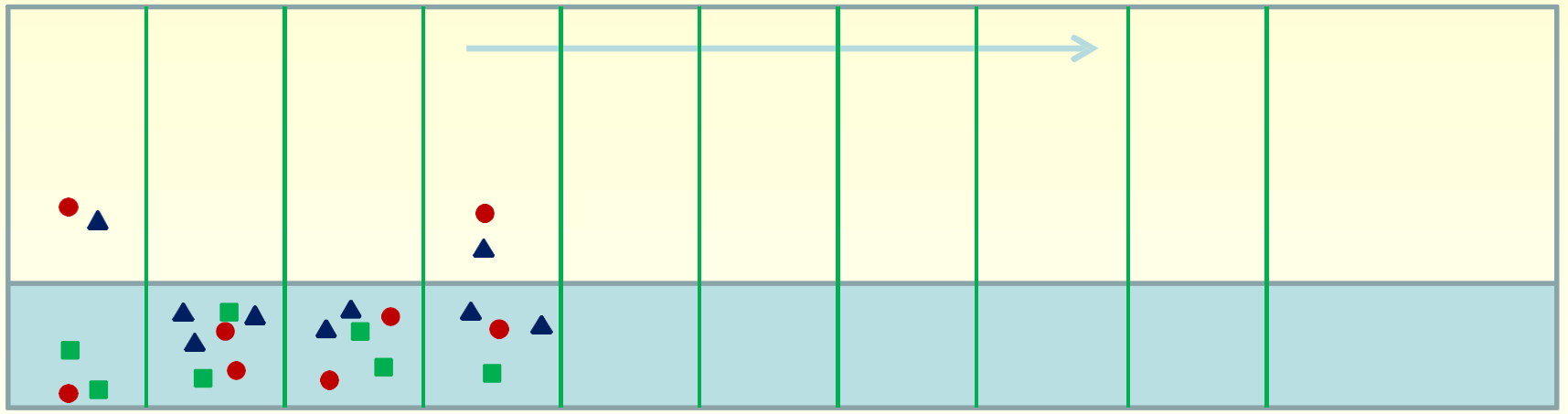

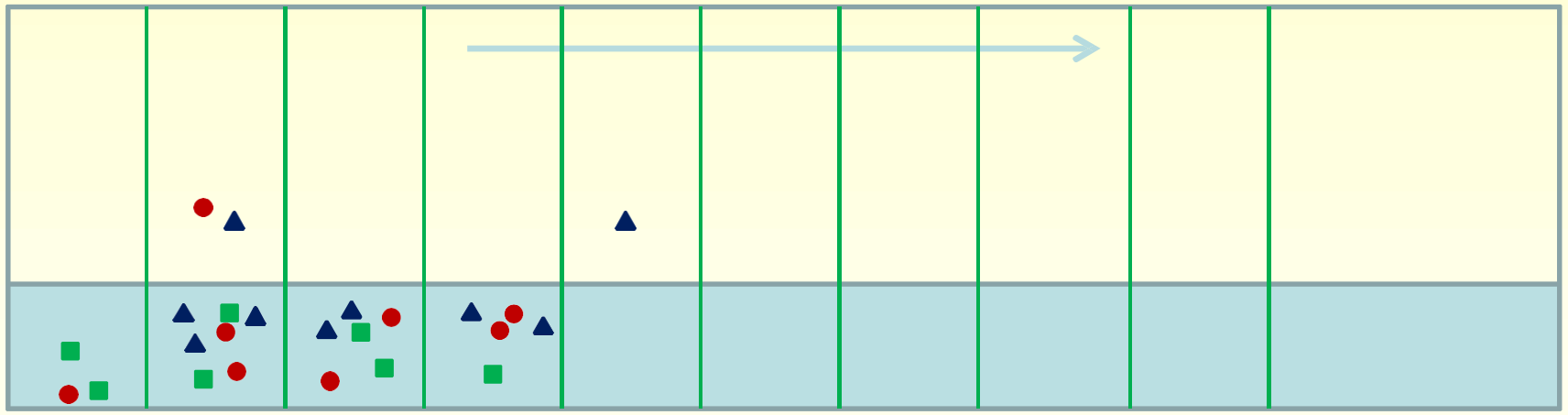

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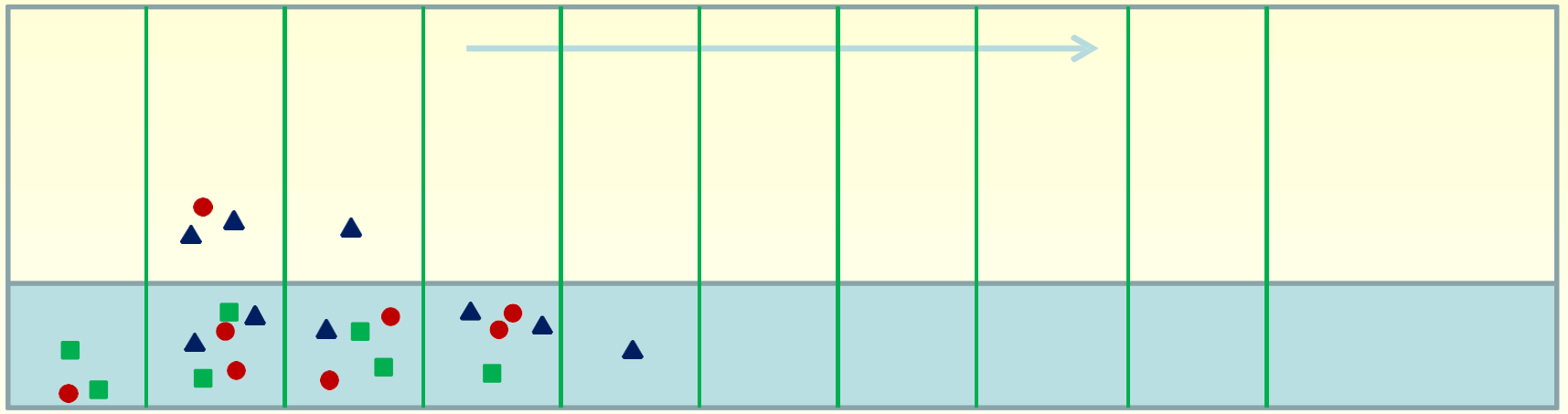

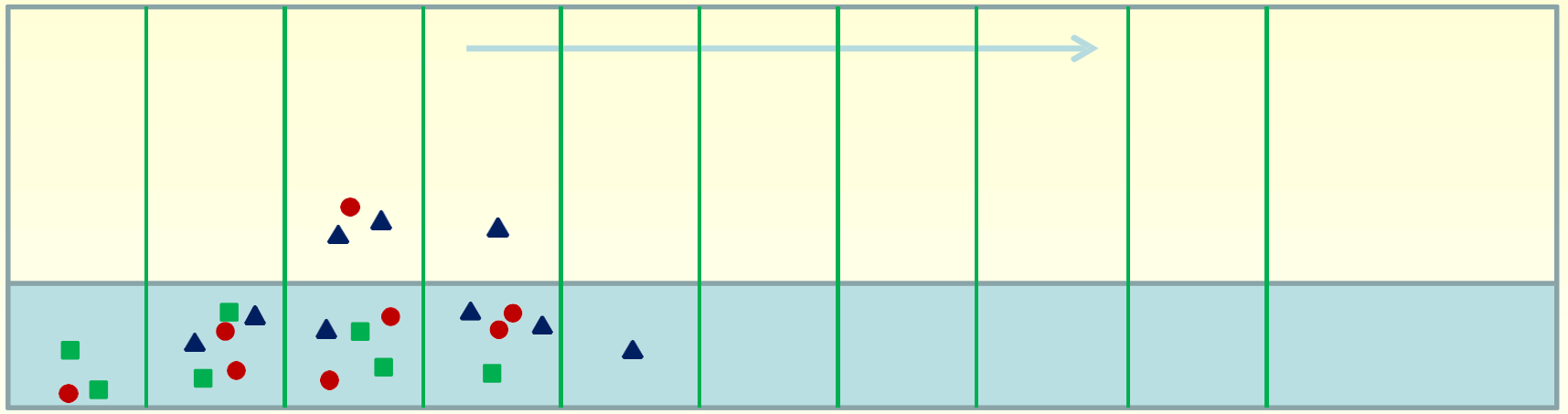

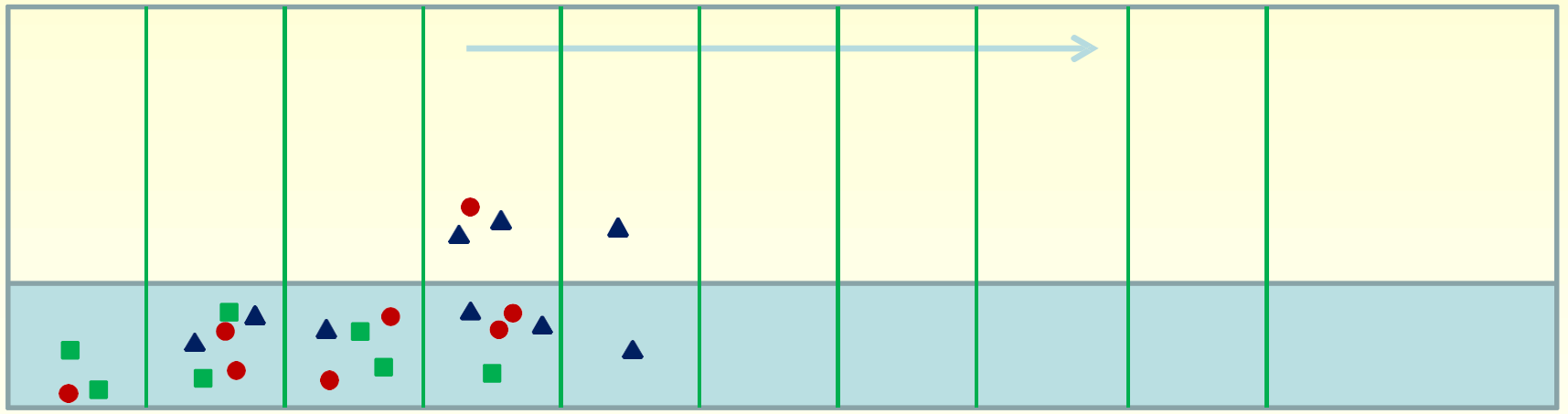

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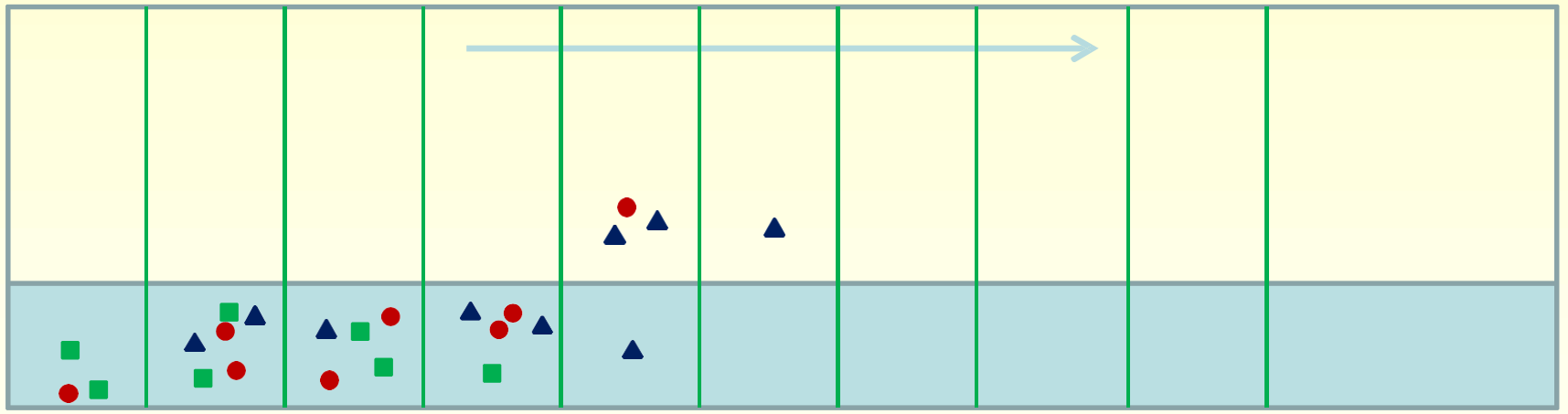

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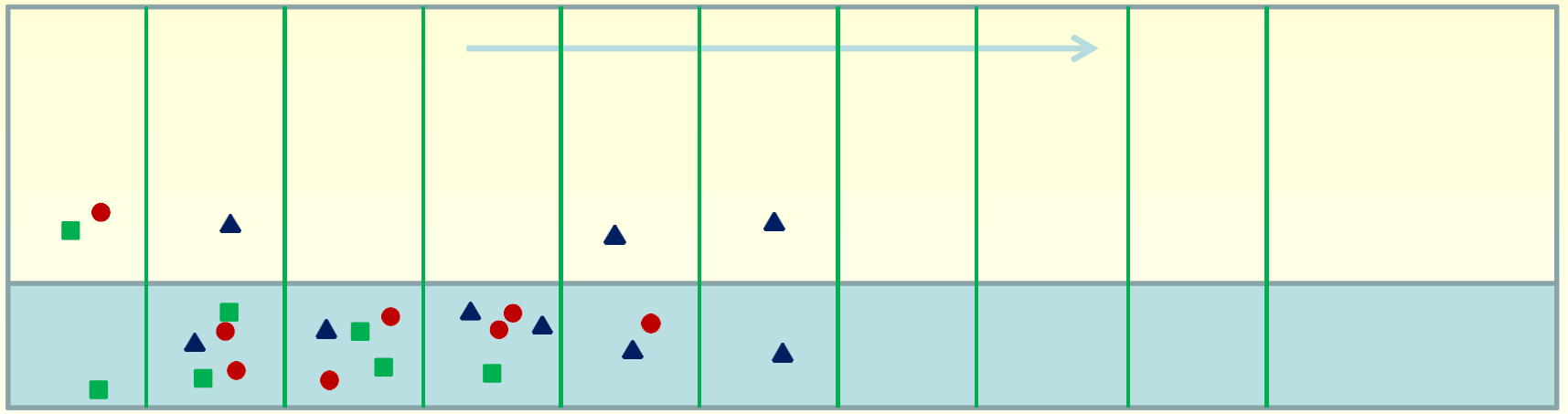

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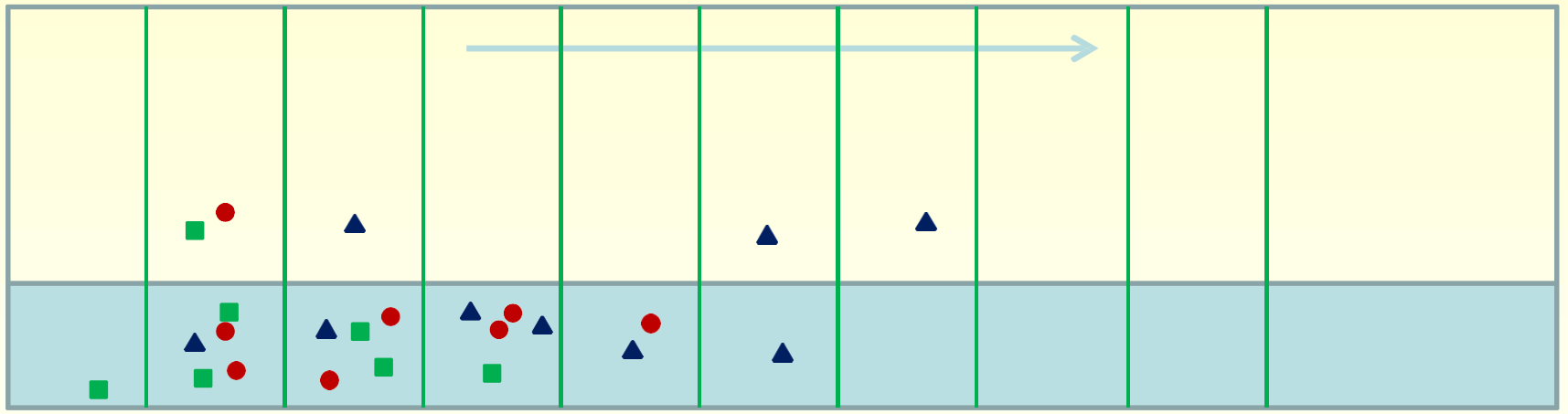

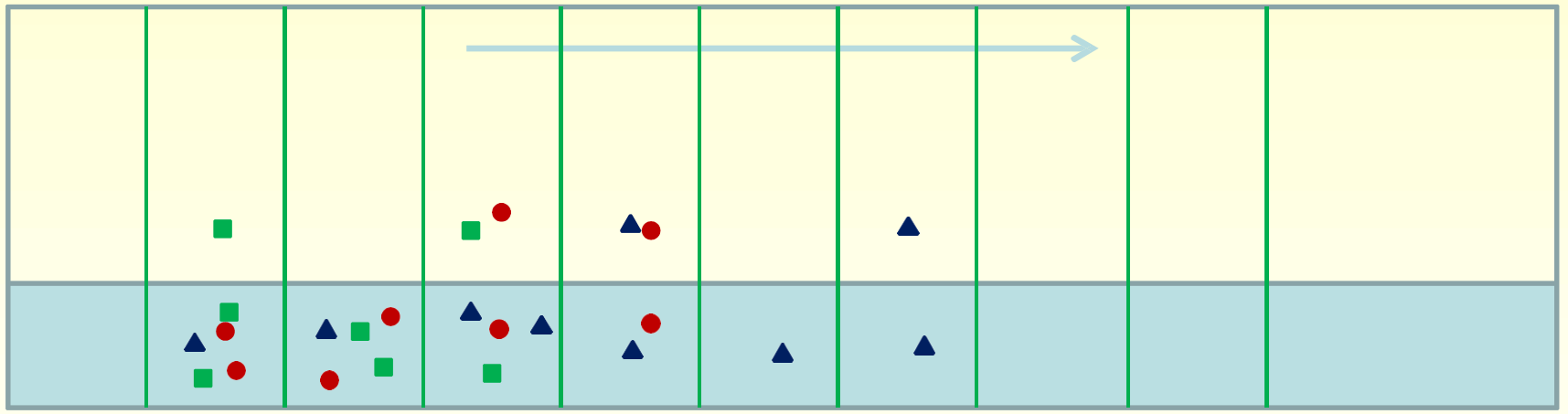

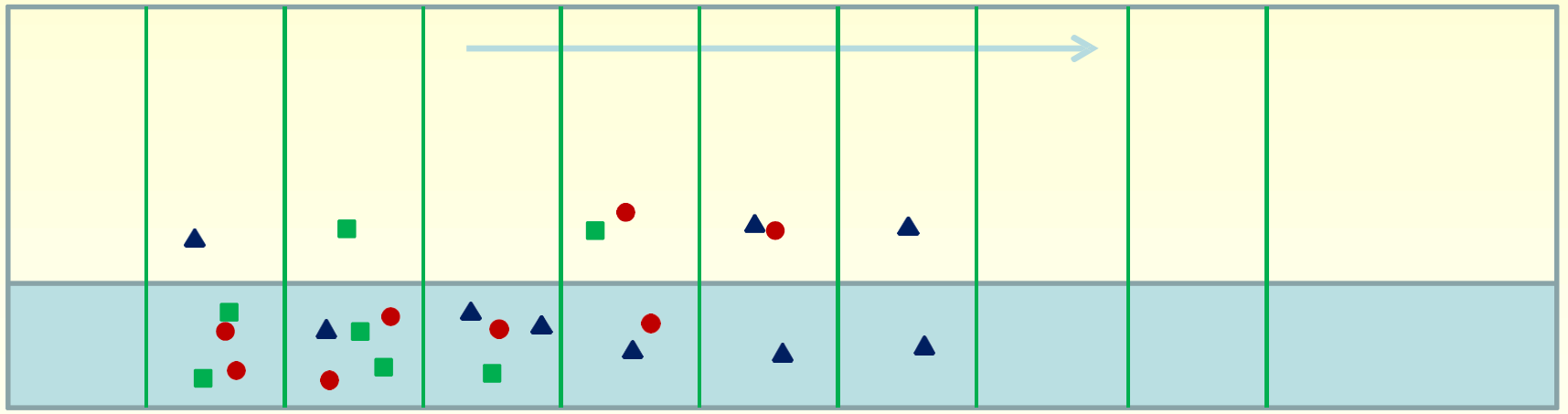

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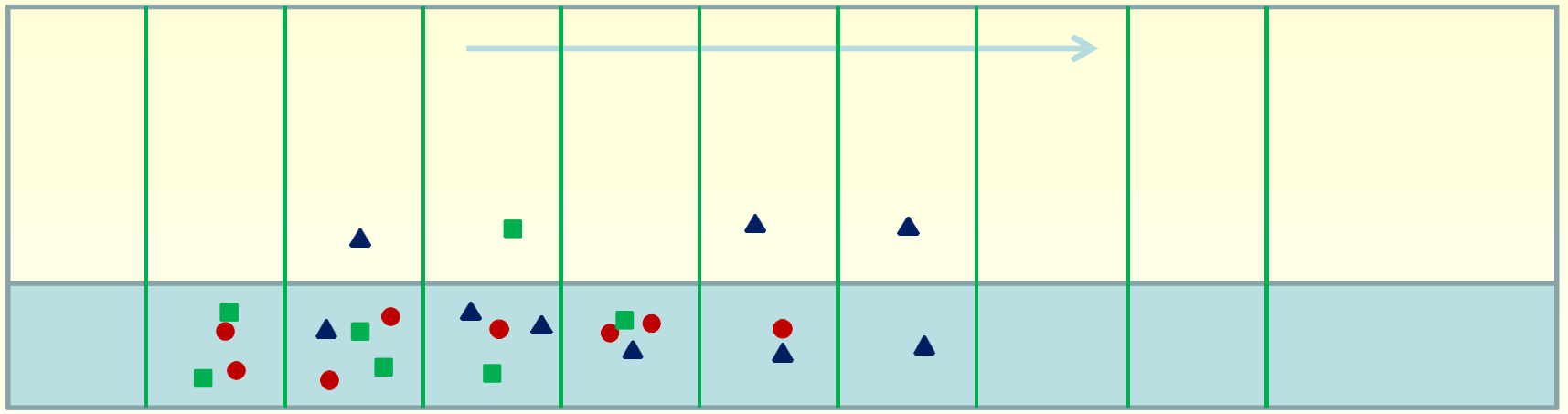

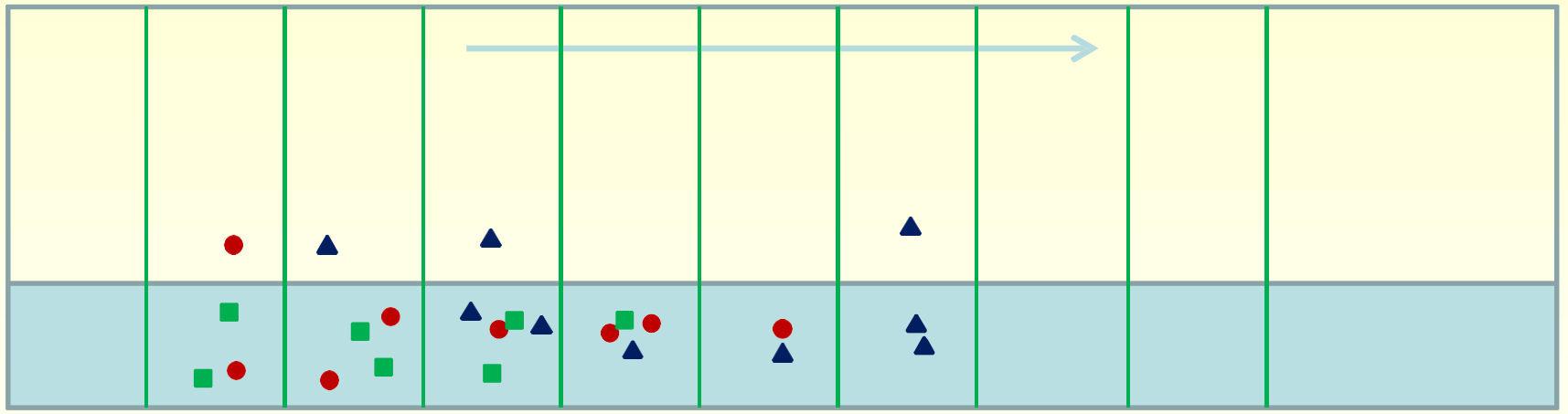

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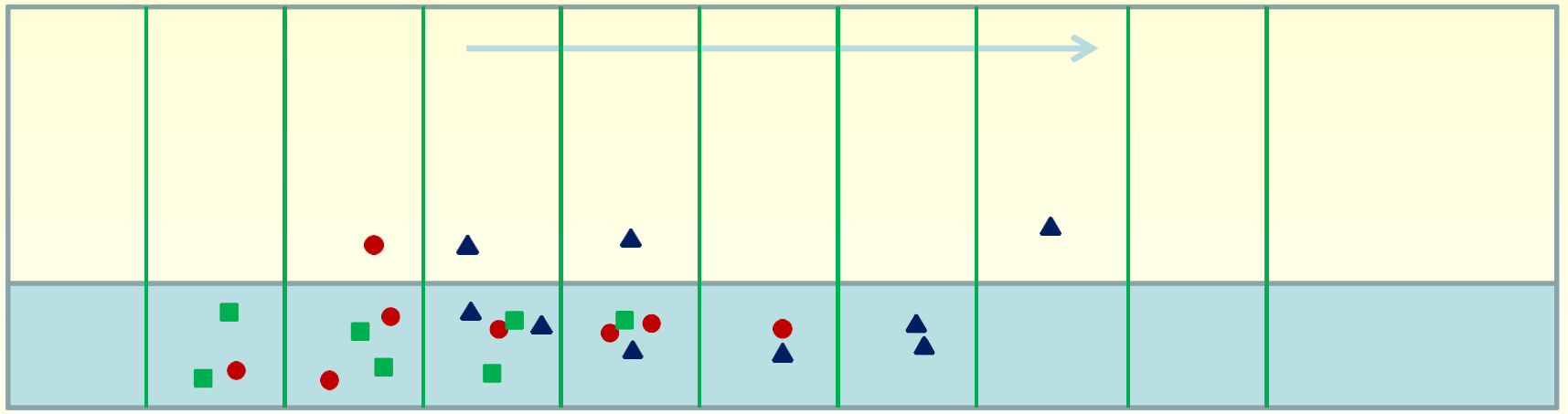

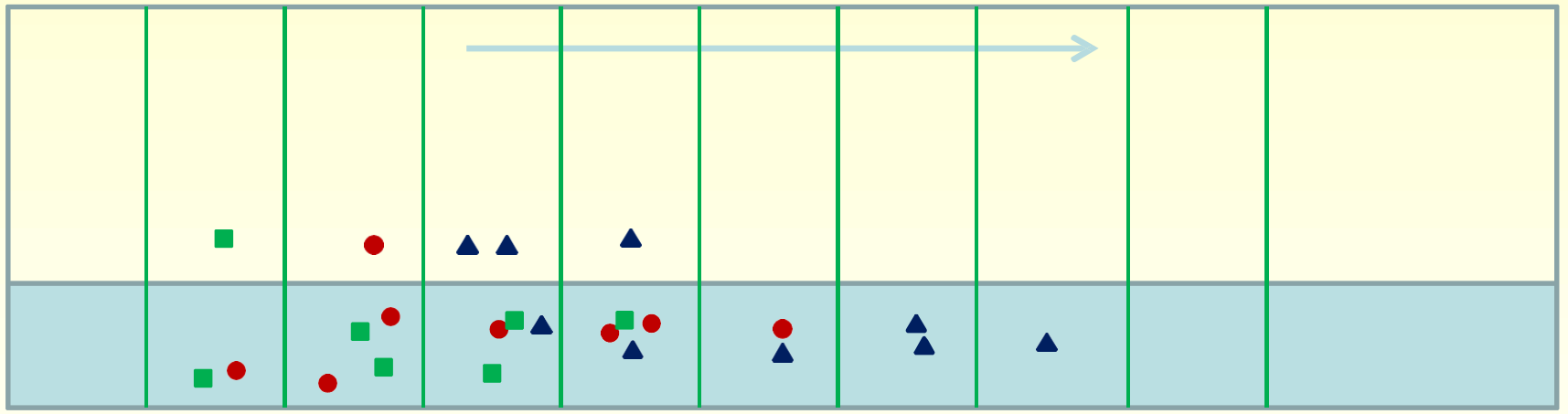

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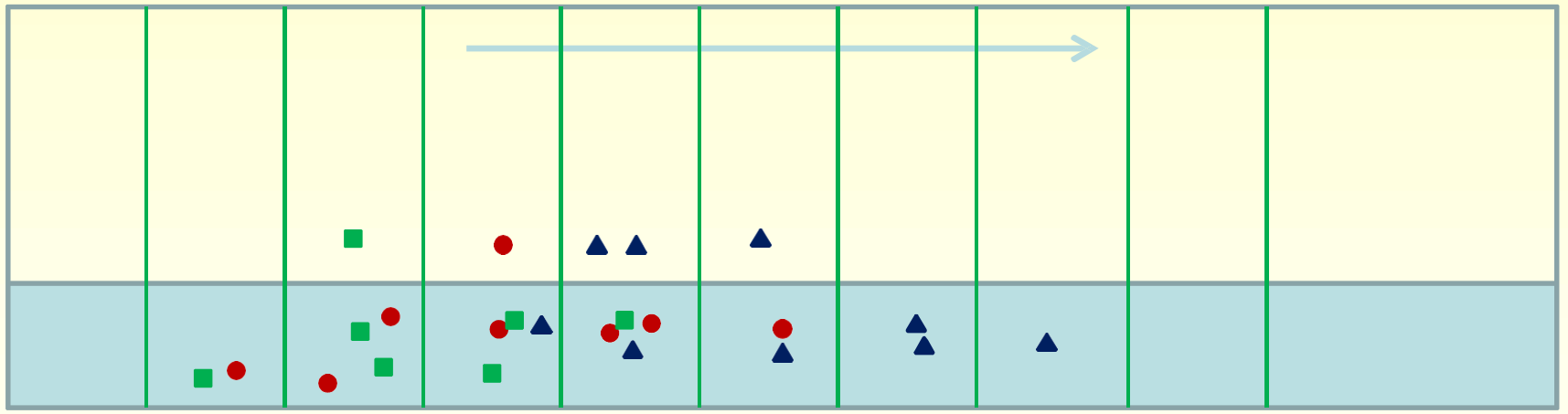

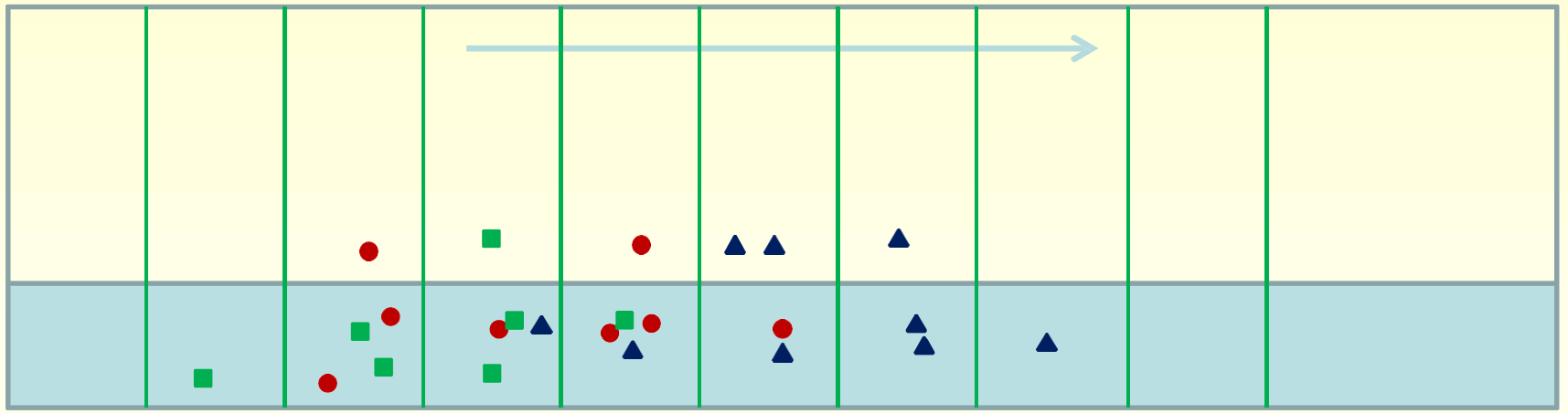

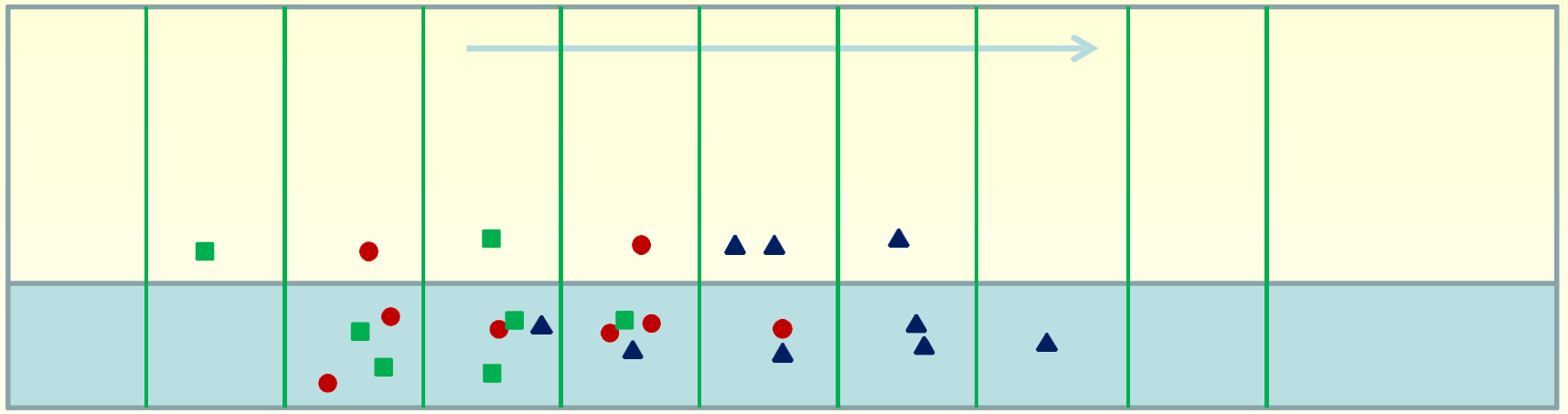

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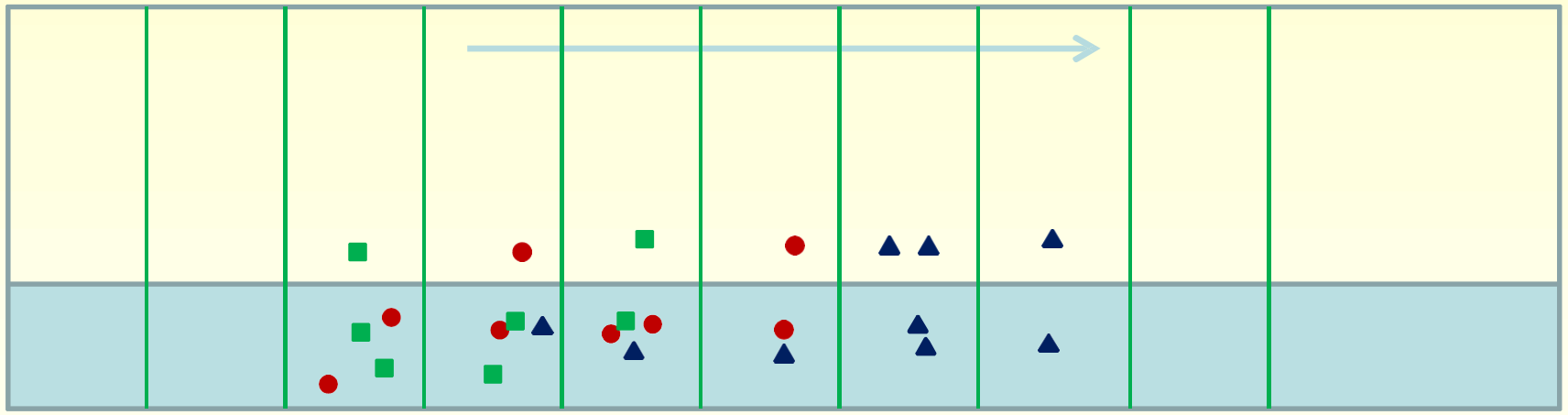

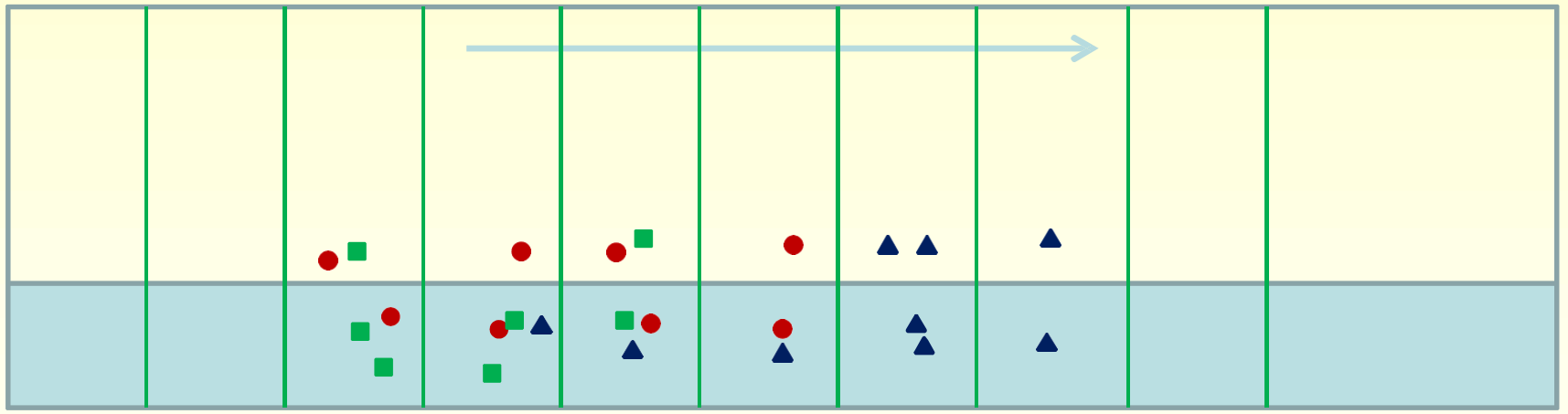

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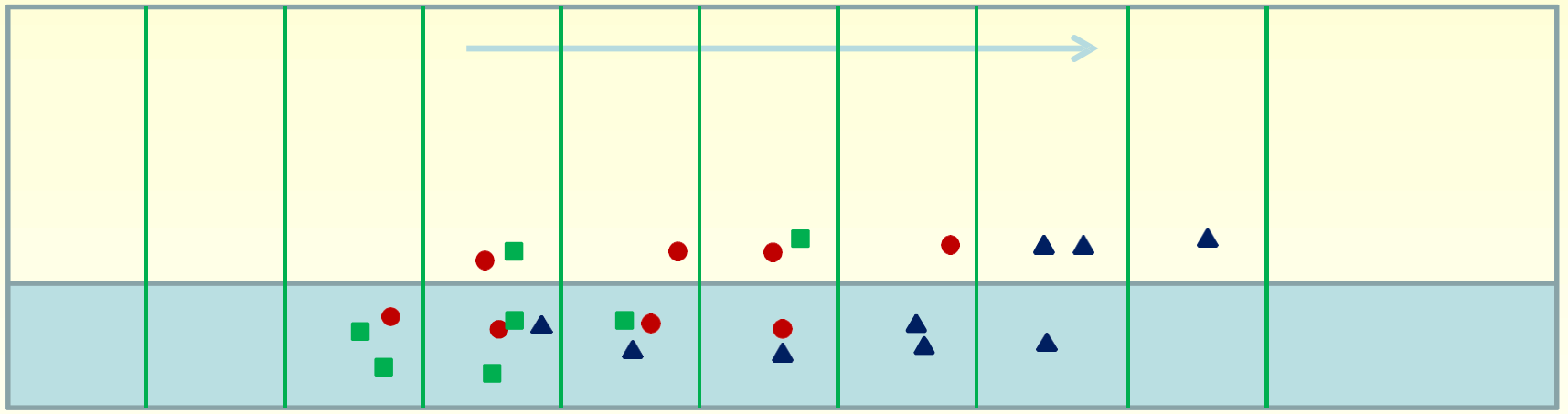

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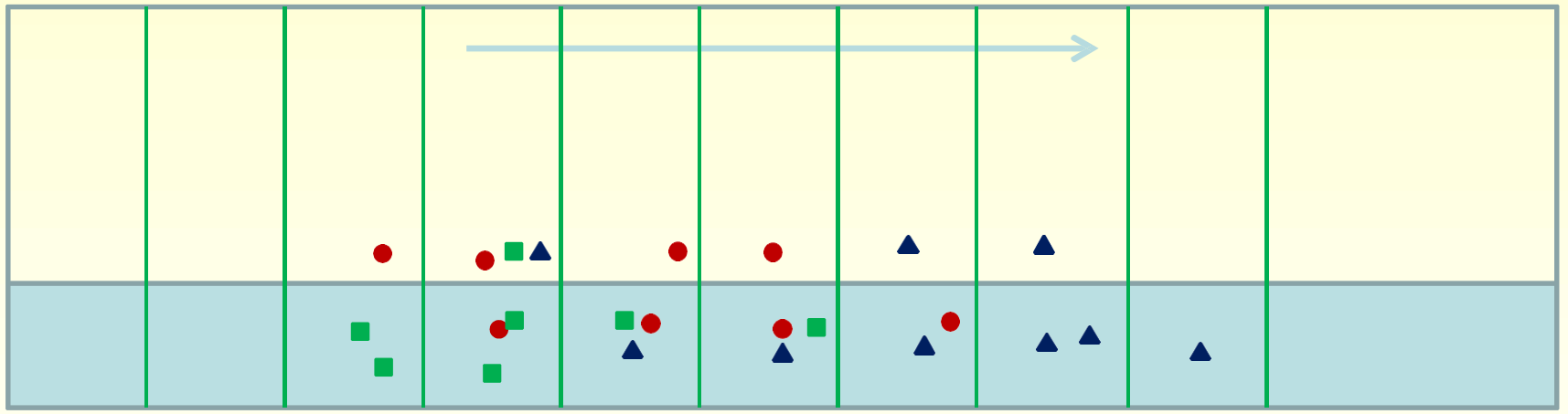

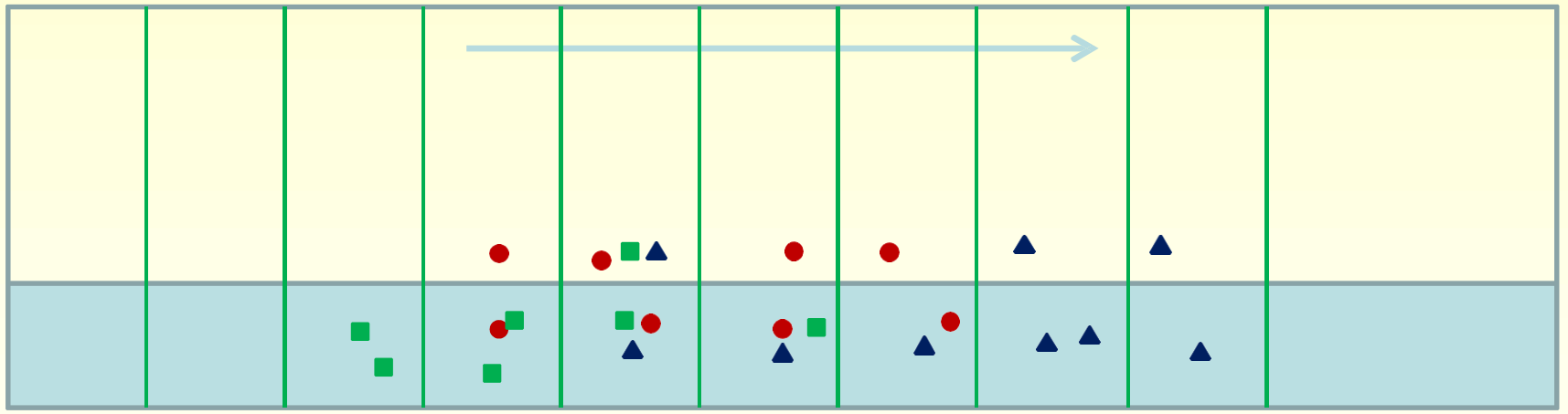

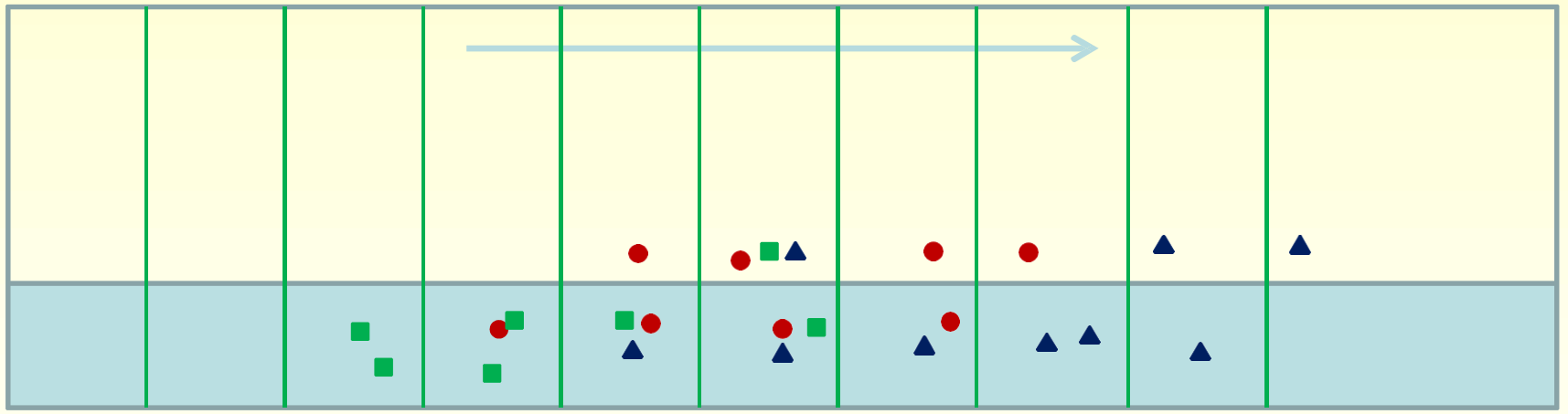

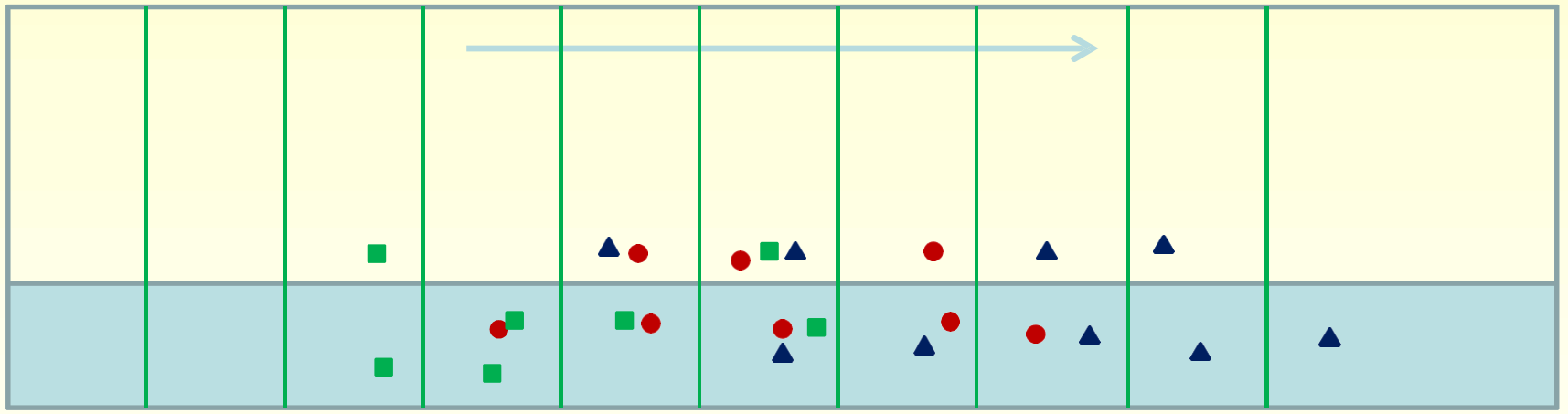

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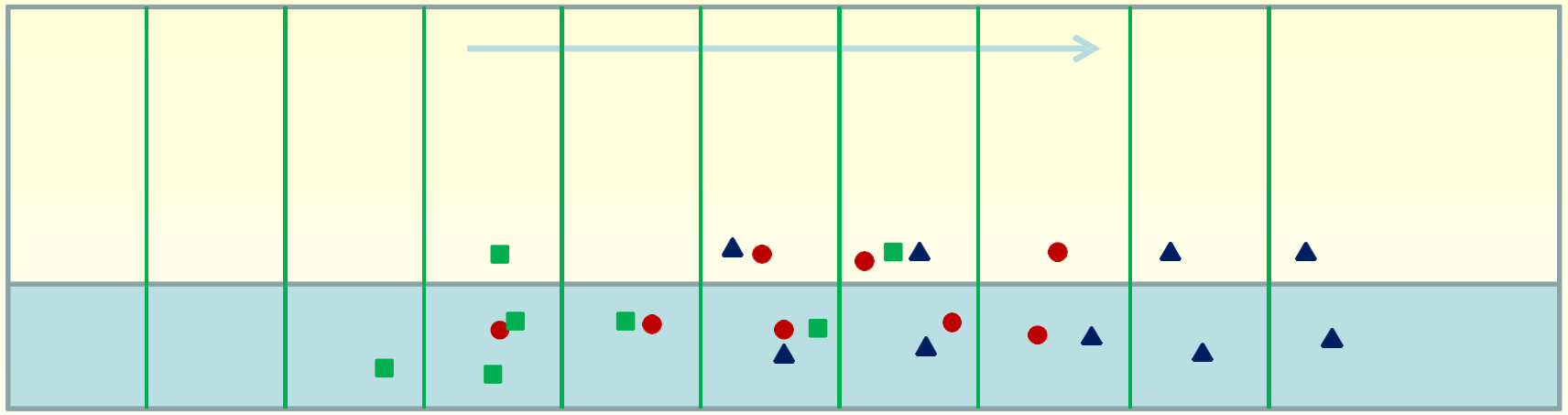
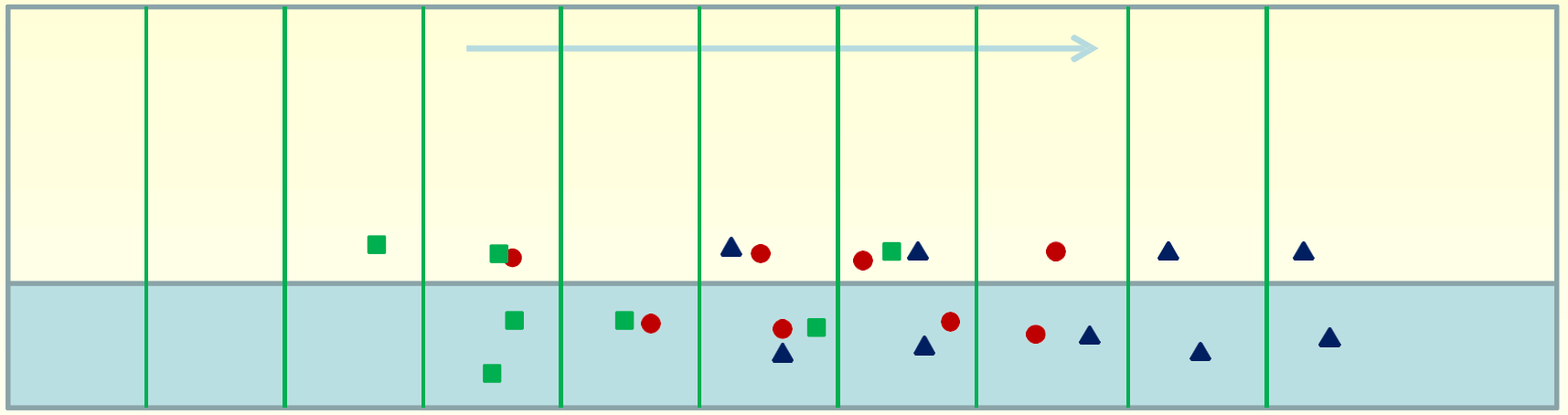
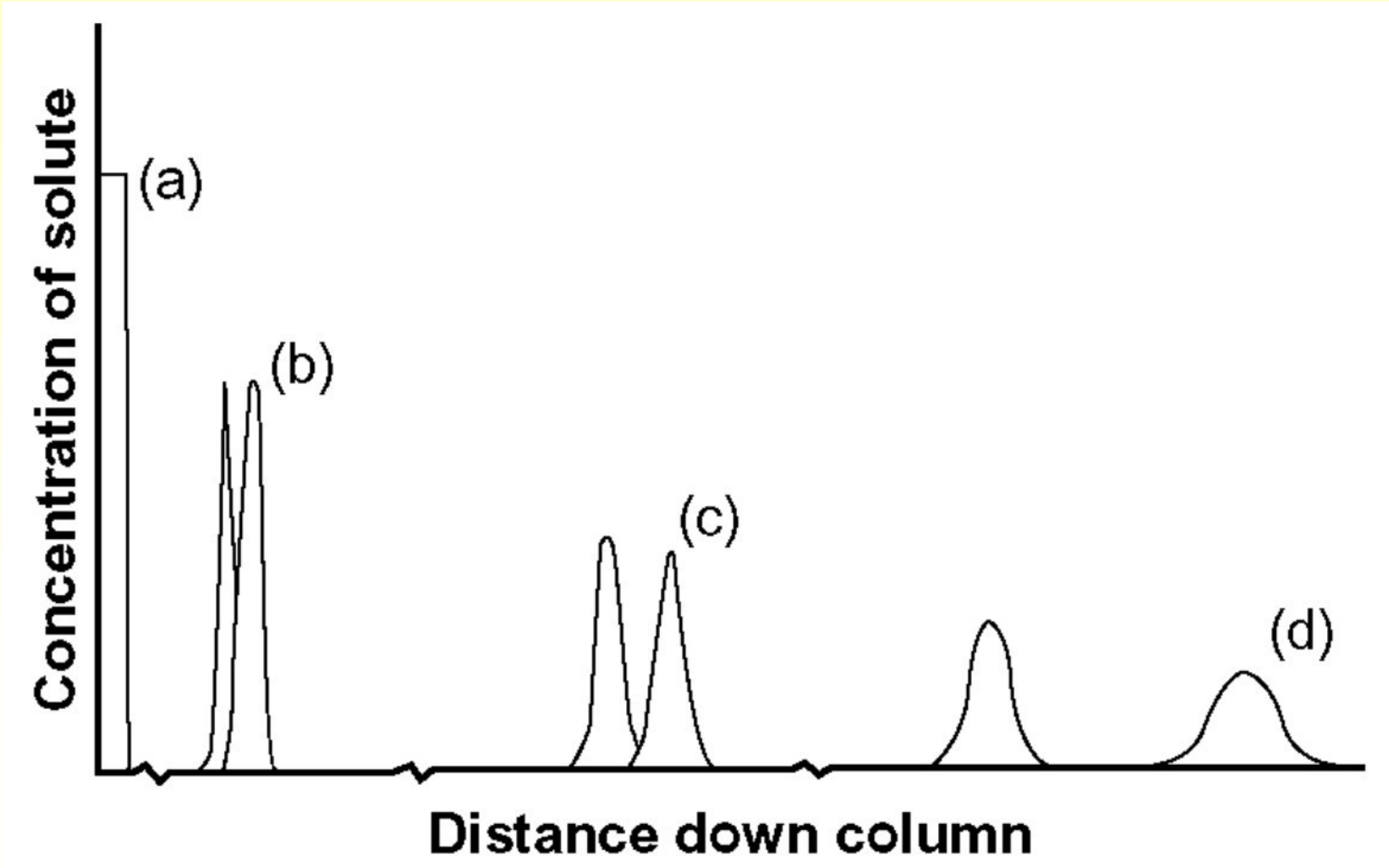


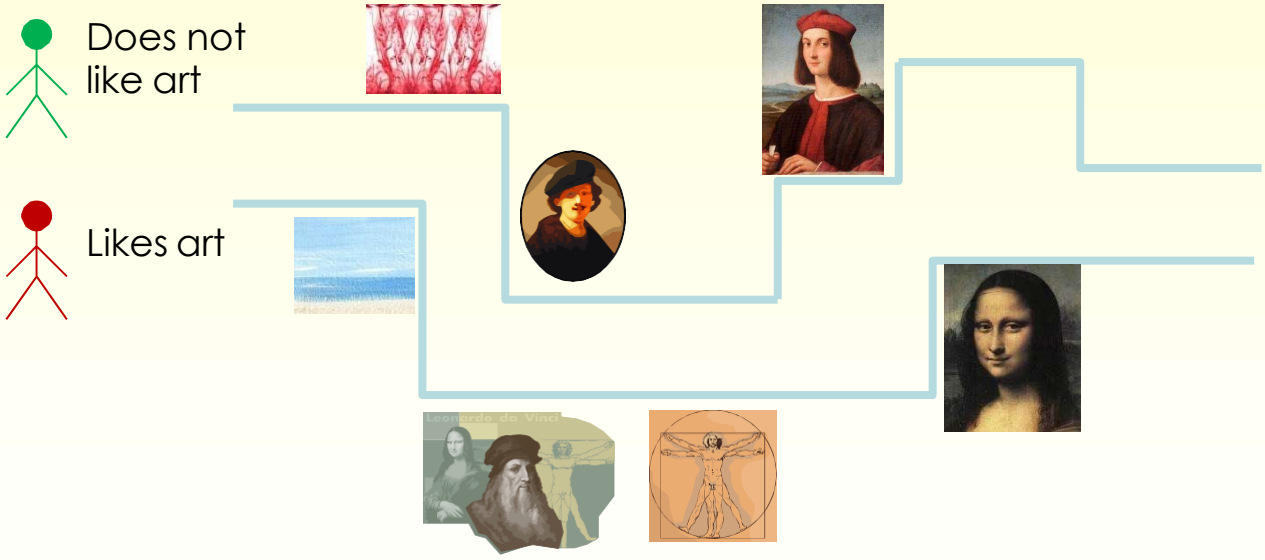


Triangles move faster than Circles;
Circles move faster than Squares

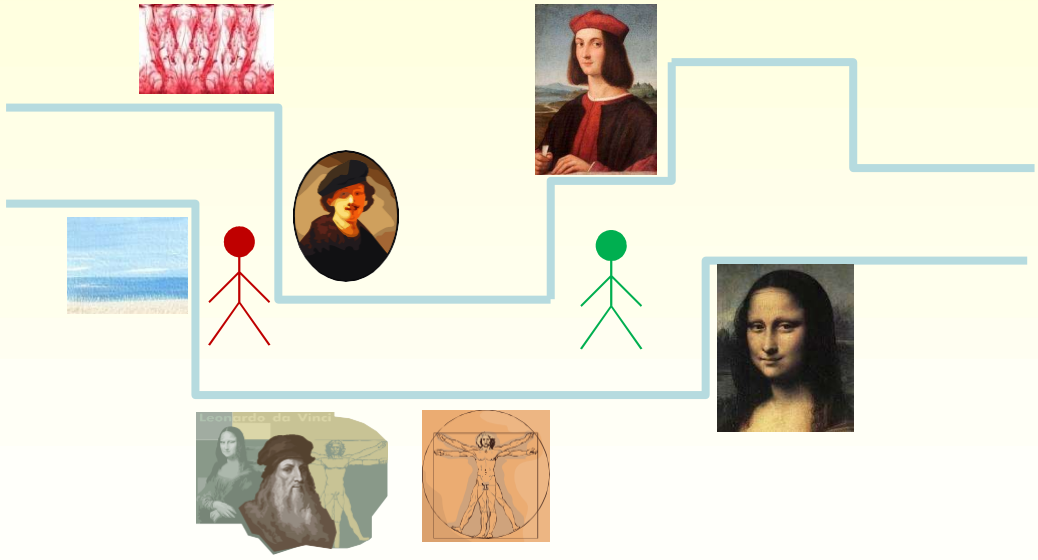
Separation process



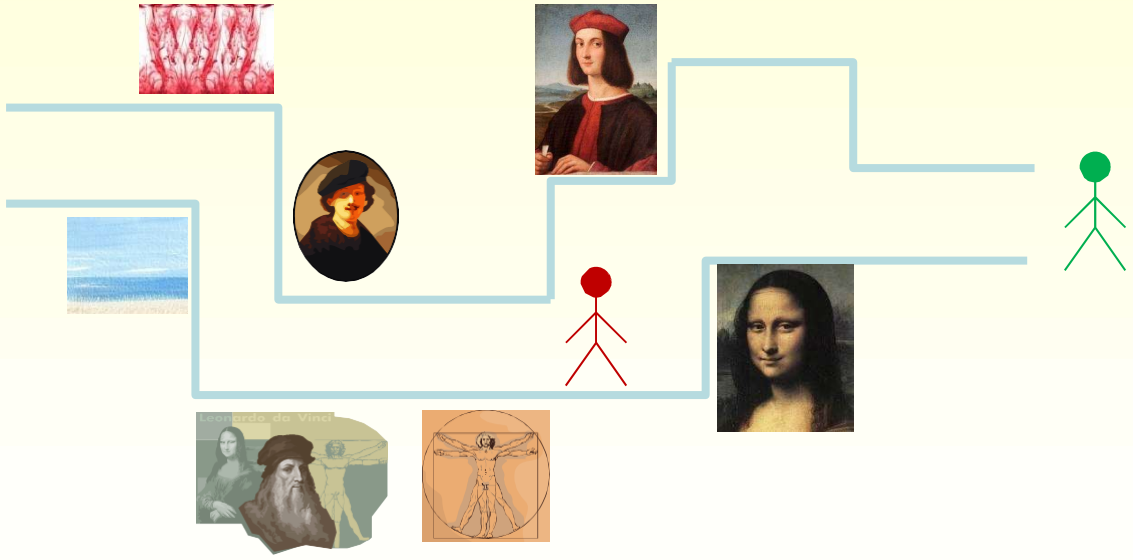
Example - Gallery



Example - Gallery

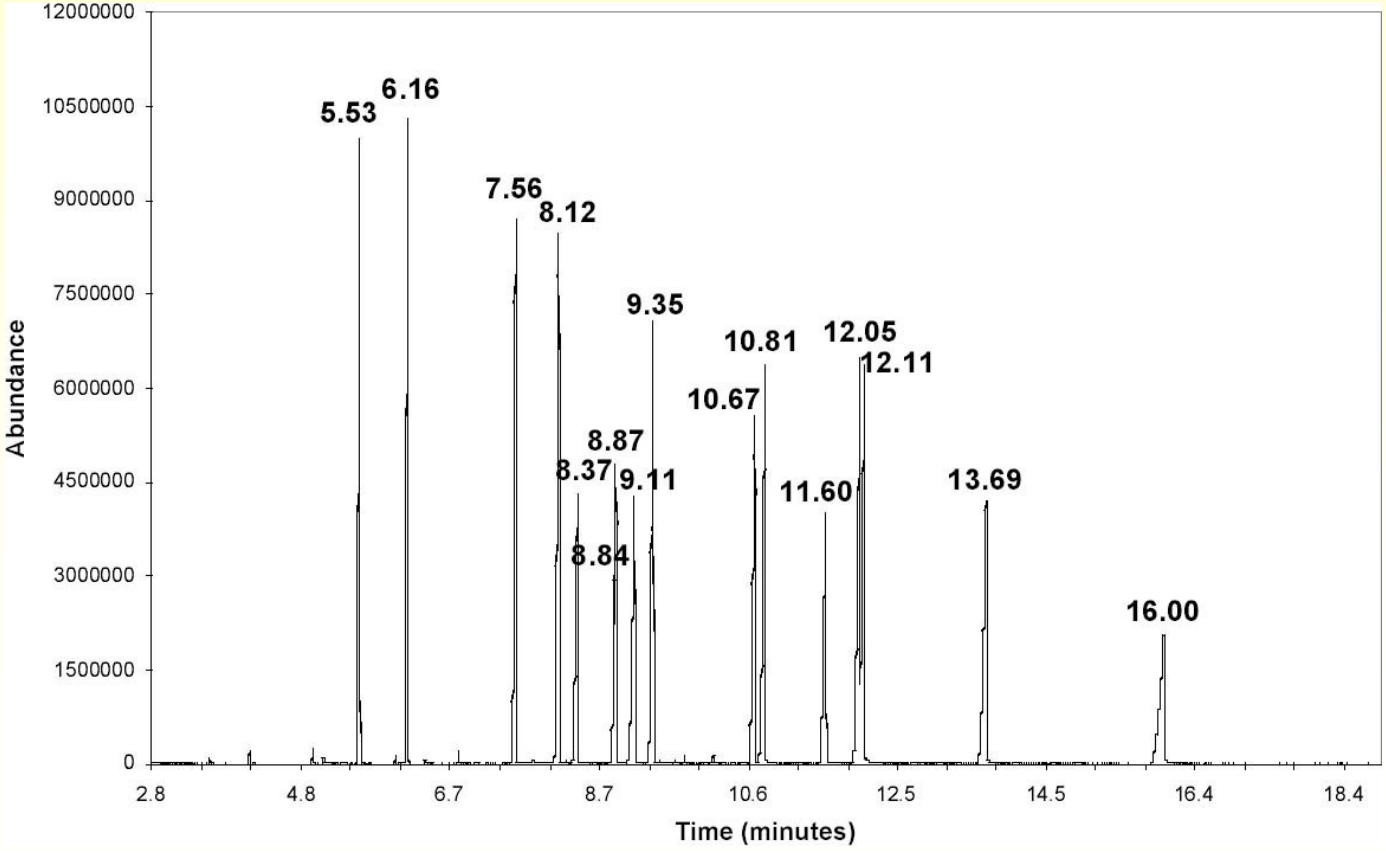


Example - Gallery

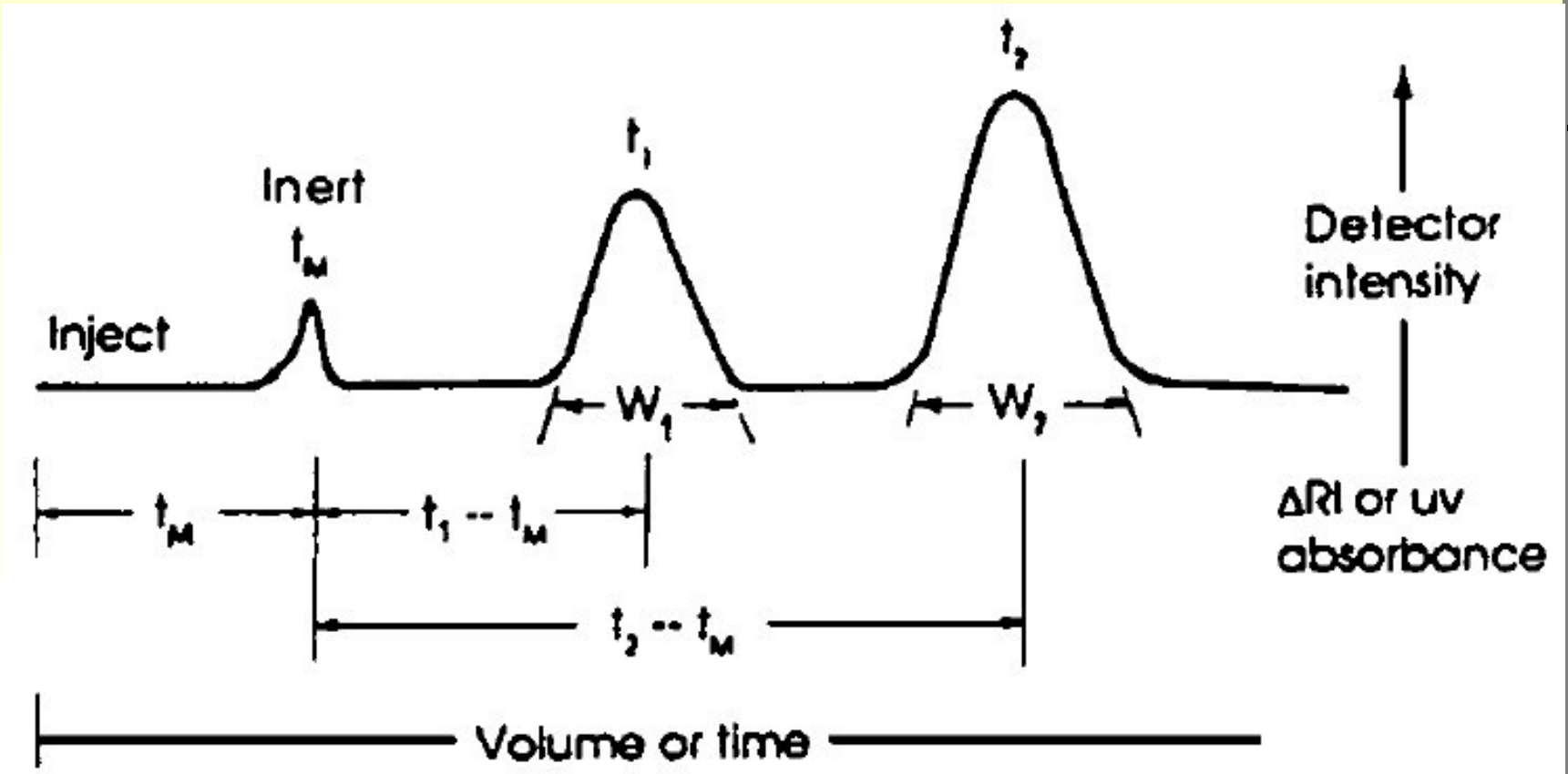


Chromatogram

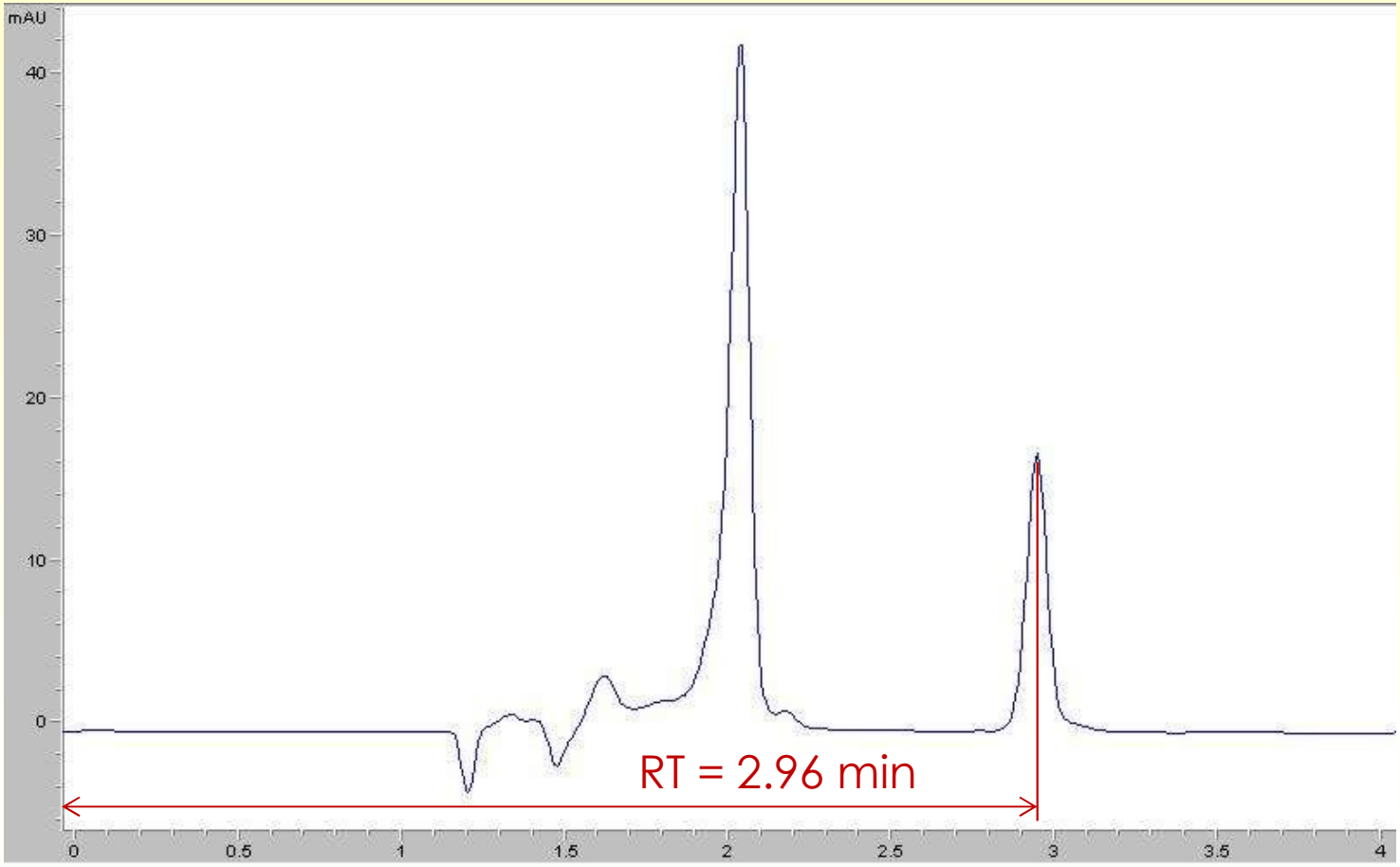
Chromatogram – dependence of a detector signal versus time after injection of the sample.



Main definitions



Retention time (RT)



Very important parameter of every peak!

Chromatographic parameters

Chromatographic resolution – shows separation efficiency between two peaks

$$R = \frac{t_{r,B} - t_{r,A}}{0.5(w_B + w_A)} = \frac{2\Delta t_r}{w_B + w_A}$$

Retention factor – shows efficiency of analyte retention by a column

$$k' = \frac{t_r - t_m}{t_m} = \frac{t'_r}{t_m}$$

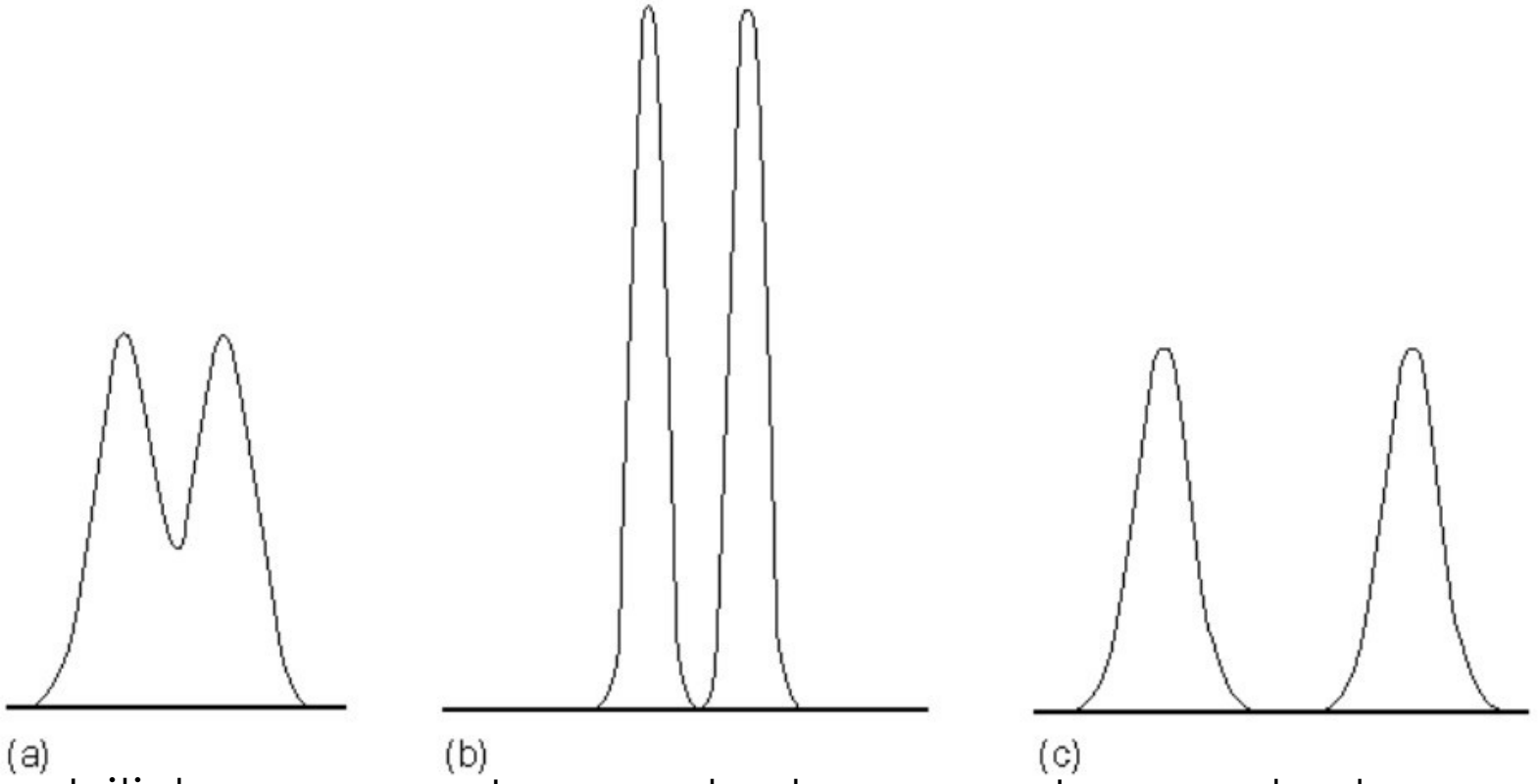
Column efficiency – provides a quantitative measure of the extent of band (peak) broadening

$$N = 5.545\left(\frac{t_r}{w_{1/2}}\right)^2$$

Task 1

Retention times of ethylbenzene and o-xylene are 12.25 and 12.70 min, respectively. Full width at half maximum (FWHM) of both peaks are 0.20 min. Determine retention factors, resolution between 2 peaks and column efficiency if dead time (t_m) is 1.55 min

Improvement of separation



(a) Initial poor separation

(b) Increased column efficiency

(c) Increased column selectivity

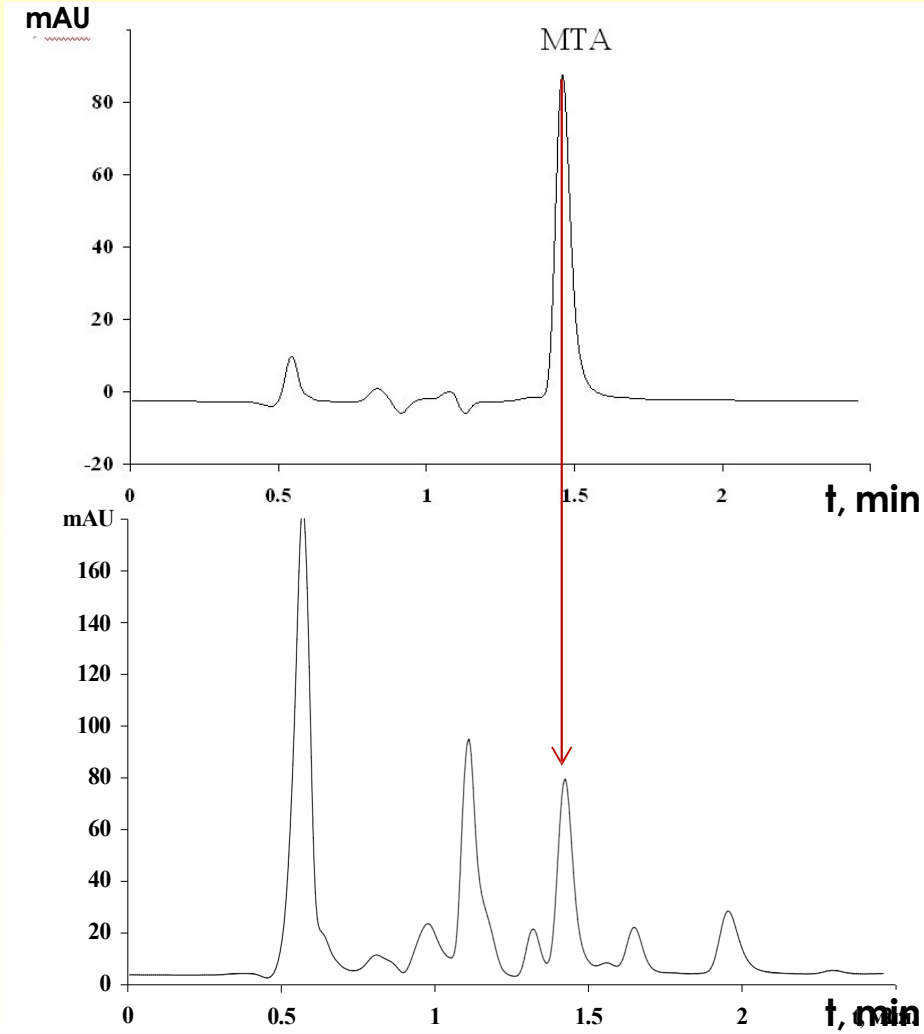
Qualitative analysis by chromatography

By retention times – comparison of retention times of individual compounds with retention times of chromatographic peaks (rule – retention time is constant at constant separation parameters)

By Kovats indices – retention factors against n-alkanes (for gas chromatography only)

Using spectral data collected from spectral detectors – comparison of collected spectra with spectra of individual compounds using mass spectra library or database

Example of identification using retention time



Chromatogram of triazole standard; RT = 1.45 min

Chromatogram of water sample: triazole is detected by its retention time (1.45 min)

Kovats retention indices

$$RI = X \times 100 + \frac{(RT_{\mathrm{A}} - RT\ (n - alkane\ X))}{(RT\ (n - alkane\ X) - RT\ (n - alkane\ X + 1))} \times 100$$

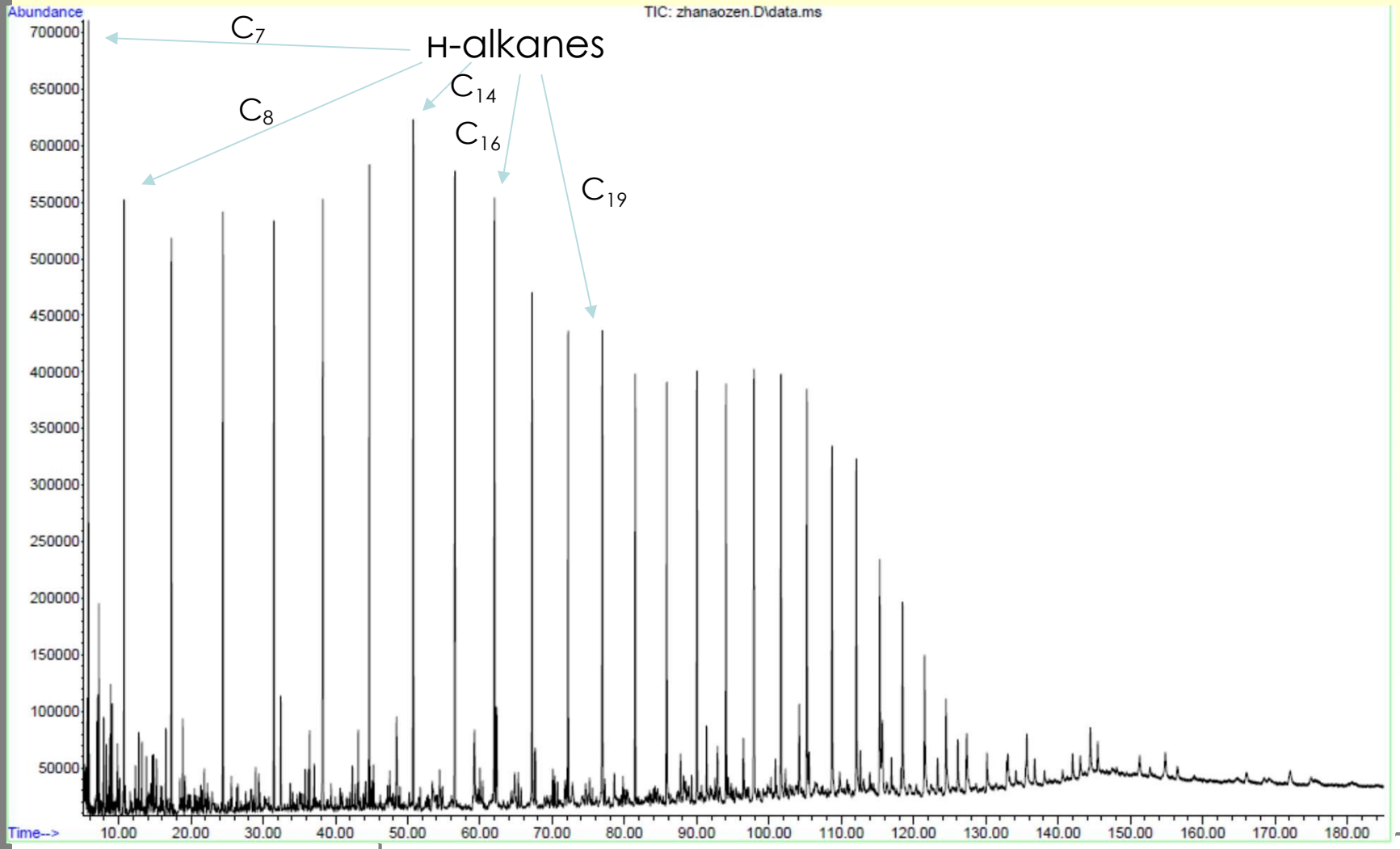
Where:

X – number of n-alkane eluted before analyte

X+1 – number of n-alkane after before analyte

RT – retention time, min

n-Alkanes on oil chromatogram



Task 2

Retention time of unknown peak is 14.44 мин. This peak elutes between n-C_{10} and n-C_{11}, retention times of which are 13.83 и 15.51 min, respectively. Calculate retention index of unknown peak.

$$RI = 100 \times 10 + \frac{14.44 - 13.83}{15.51 - 13.83} \times 100 = 1036$$

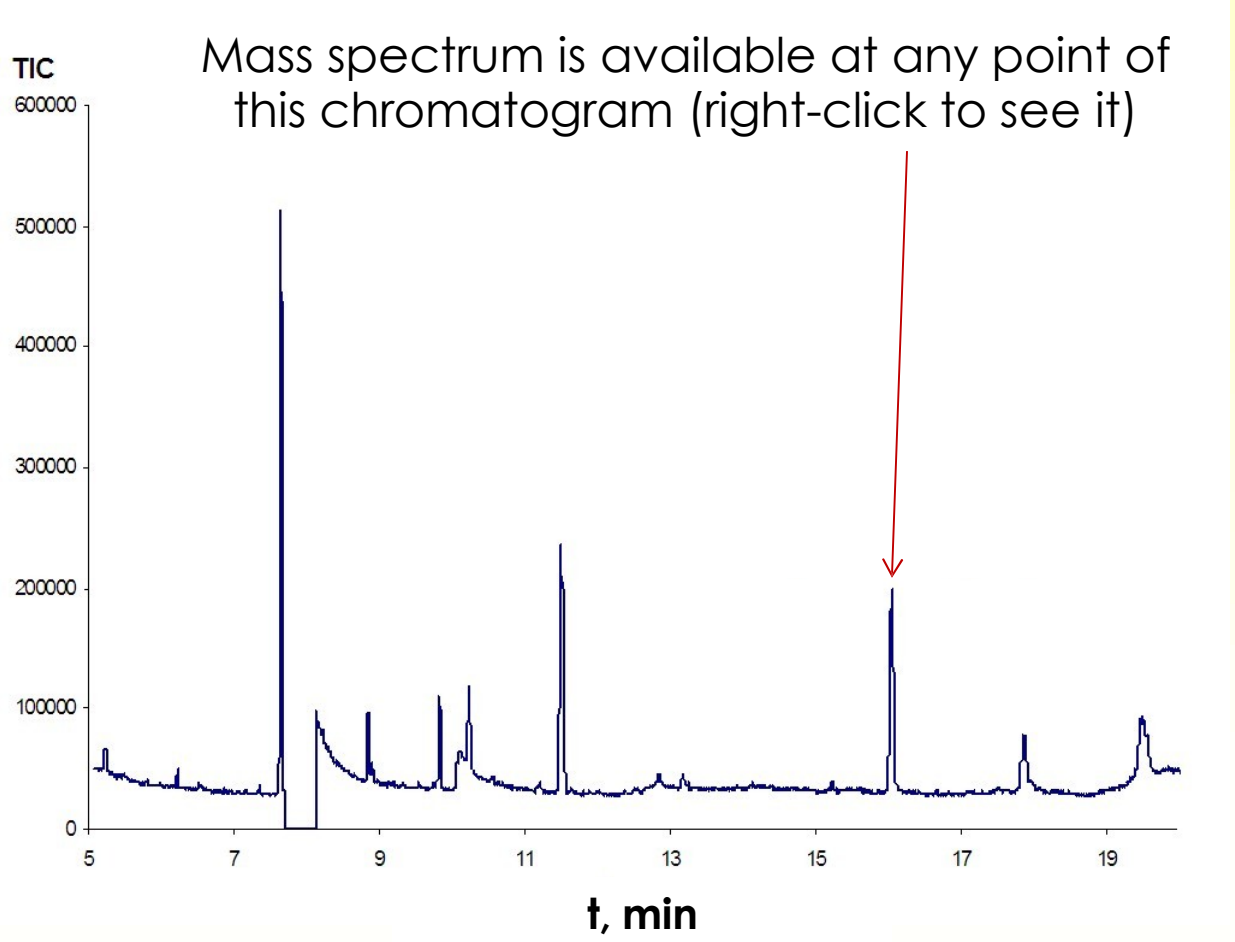
Task 3

According to NIST library, Kovats retention index of phenol is 955. According to chromatogram of n-alkanes mixture, retention times of n-C_9 and n-C_{10} are 9.55 and 11.32 min, respectively. Calculate retention time of phenol.

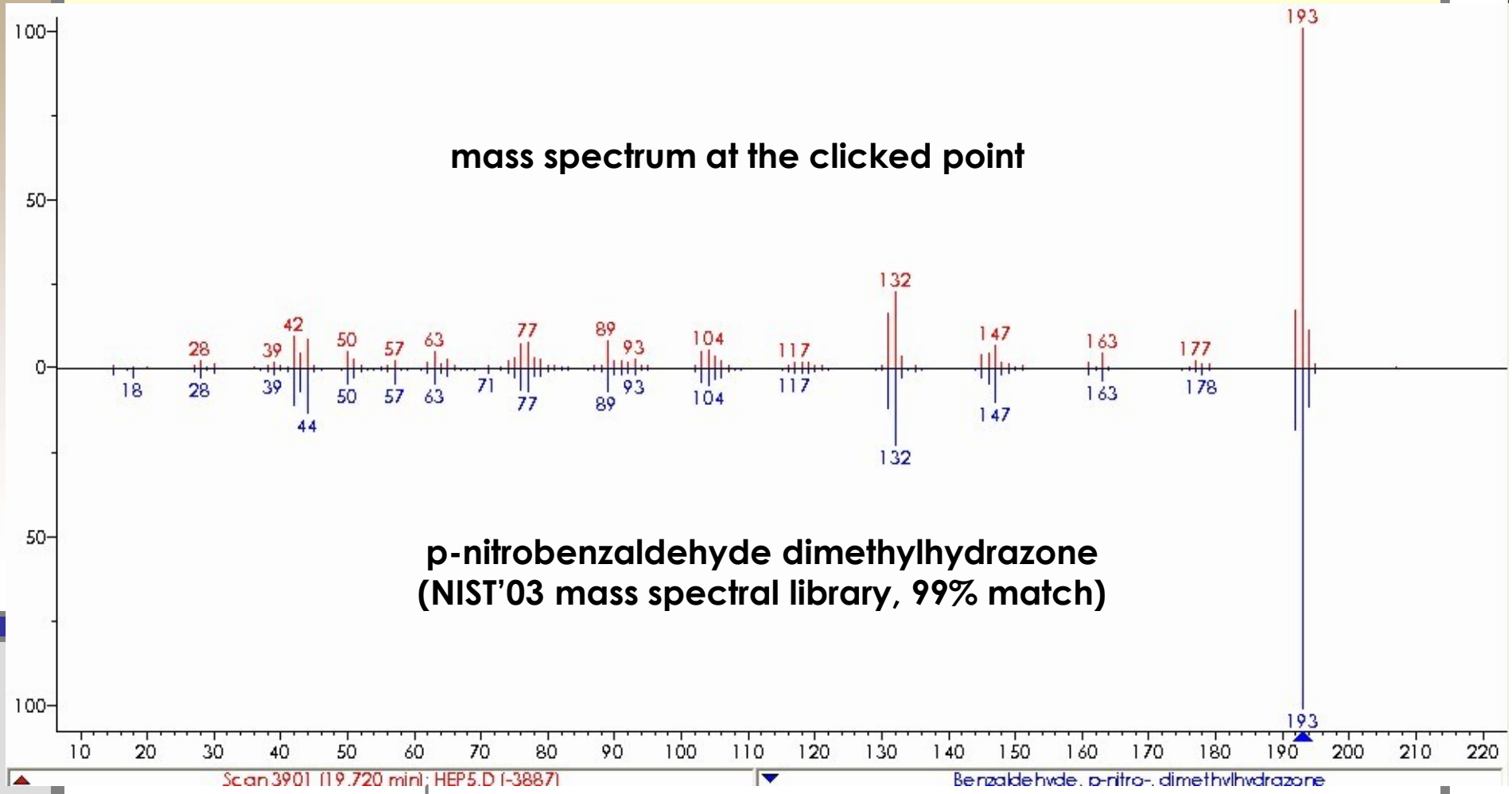
$$955 = 900 + \frac{X - 9.55}{11.32 - 9.55} \times 100$$

$$X = 0.55\,(11.32 - 9.55) + 9.55 = 10.52\ min$$

Identification by spectral data

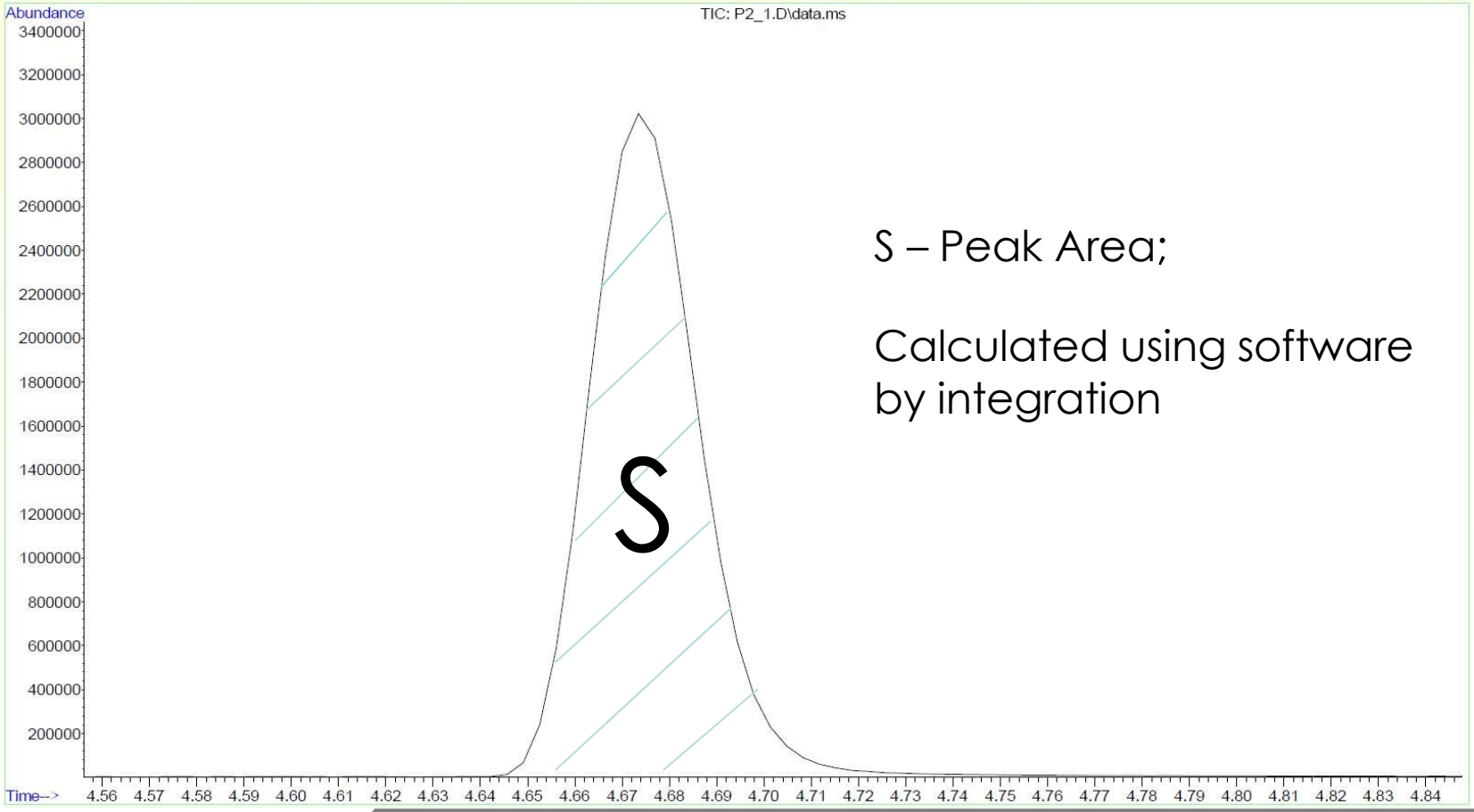


Identification by spectral data



Quantitative analysis by chromatography

By peak area – peak area is directly proportional to analyte concentration or mass of analyte that reached the detector



S – Peak Area;

Calculated using software by integration

Quantitative analysis by chromatography

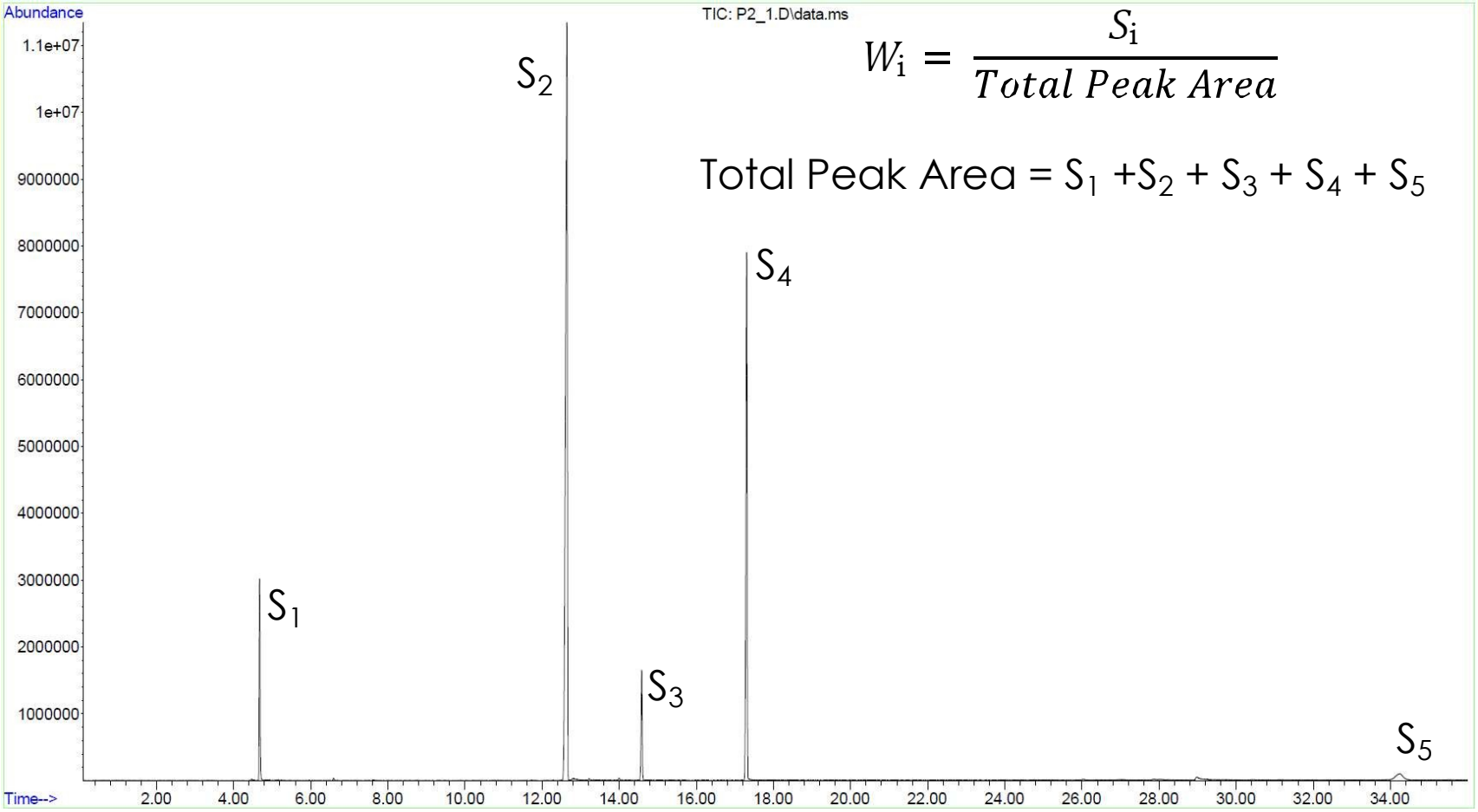
By normalization - percentage of analyte is calculated as its peak area divided by a sum of all the peaks detected on chromatogram

External standard calibration – different concentrations of analyte are analyzed to get a calibration curve: $S = f(C)$

Internal standard calibration – solutions having different ratios of analyte and internal standard concentrations are analyzed to get a calibration curve: $S/S_{is} = f(C/C_{is})$. In most cases C_{is} is constant during all analyses.

Standard addition – a known concentrations of analytes are introduced into the sample to get a calibration curve and measure the concentration of analyte.

Normalization

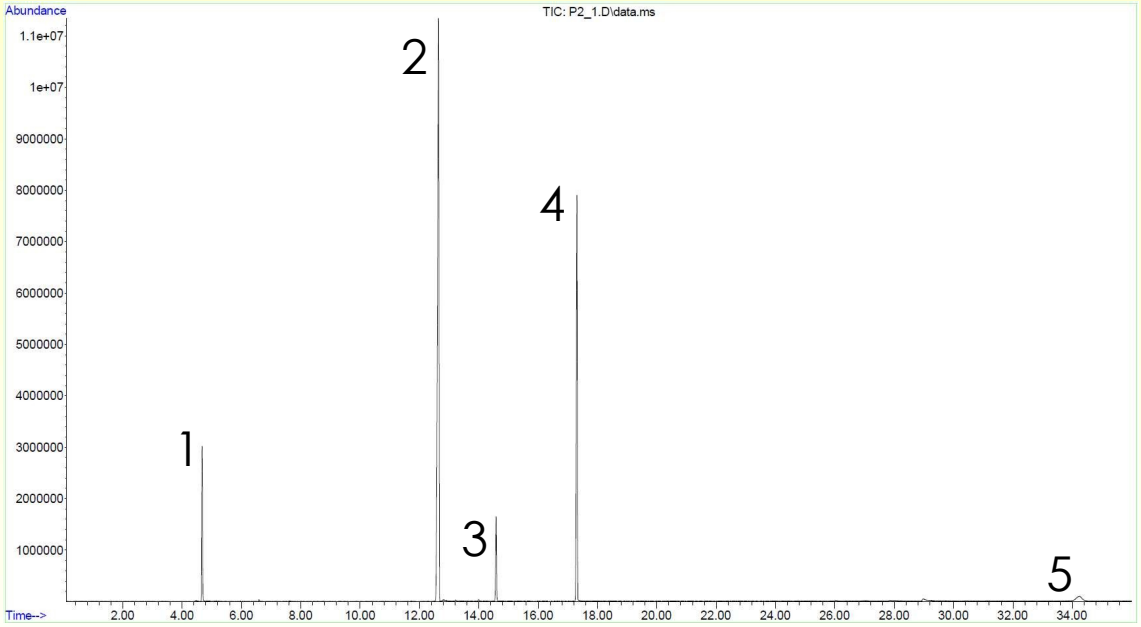


$$W_i = \frac{S_i}{Total\ Peak\ Area}$$

Total Peak Area = $S_1 + S_2 + S_3 + S_4 + S_5$

Very simple and efficient method for analyzing unknown samples

Results of normalization



peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.675	1305	1318	1352	BB	2992968	49200040	12.88%	7.546%
2	12.645	3572	3606	3629	VB	11324431	382062343	100.00%	58.598%
3	14.585	4144	4163	4184	BB	1637784	33407127	8.74%	5.124%
4	17.306	4921	4945	4992	BB	7882056	175333879	45.89%	26.891%
5	34.251	9731	9812	9893	BB 6	90854	12002277	3.14%	1.841%

Task 4

Sample of solvent was analyzed by gas chromatography with mass spectrometric detection. On chromatogram, peaks of ethyl acetate, toluene, iso-propanol and o-xylene were detected. Their peak area are 4260, 3120, 11600 and 700 a.u., respectively. Calculate mass concentrations of all detected compounds.

$$Total\ Peak\ Area = 4260 + 3120 + 11600 + 700 = 19680$$

$$\%\ ethyl\ acetate = \frac{4260}{19680} \times 100\% = 21.6\%$$

Task 5

Solve the same task, if it is known that injection of 1.00 mg/L solution of all detected peaks in CS_2 gave the following peak areas: 102, 133, 60 and 148.

Solution

Response factors of analytes relative to toluene are 0.77, 1.00, 0.45 and 1.11.

$$Total\ Peak\ Area = \frac{4260}{0.77} + \frac{3120}{1} + \frac{11600}{0.45} + \frac{700}{1.11} = 35060$$

$$\%\ ethyl\ acetate = \frac{4260}{0.77 \times 35060} \times 100\% = 15.8\%$$

Homework

Calculate volumetric fraction (v/v %) of every analyte if density of solvent is 0.825 g/mL.

Send solution to bkenesov@cfhma.kz and m.abilev@mail.ru