Off-rates?

- platform: combinatorial chemistry on a chip,
  - ML-driven given specific domain,
  - initial analysis gives good on-rates
  - photometry → photobleaching proportional
- screen 1795, FOVs imaged sequentially ~4hrs

  tScreen 2.0: intra-FOV correction inter-FOV correction
  w/ PEG-8 "addition", bls, foldover PEG-8 (of final bls)

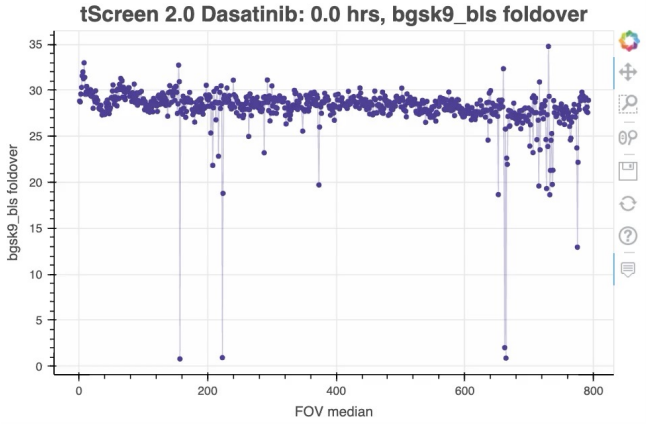
- timescales of known offrates (SPR)
  - CB-21.0008 : Dasatinib $t_{1/2}$: 19 hrs
  - CB-21.0023 : X-Chem BTK $t_{1/2}$: 24 min
  - CB-21.0009 : Staurosporine $t_{1/2}$: 5 min
  - CB-21.0022 : Purvalanol $t_{1/2}$: 13 sec
  - CB-21.0024 : CZC-8004 $t_{1/2}$: 6 sec

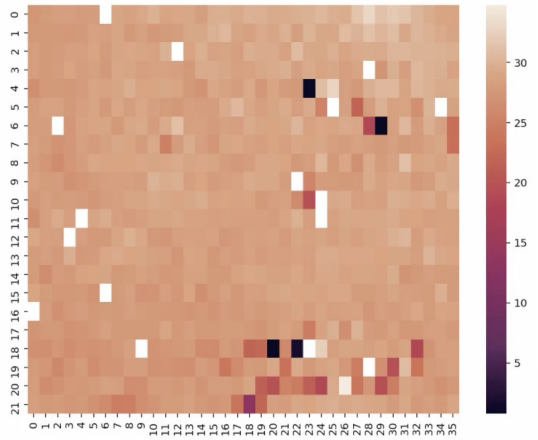
- looking for parameter λ in the equation (first-order):

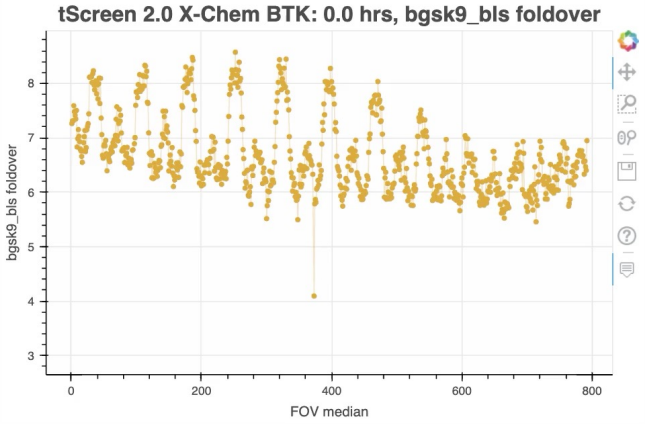
$$I(t) = I_0 e^{-\lambda t} + \text{bkg}$$

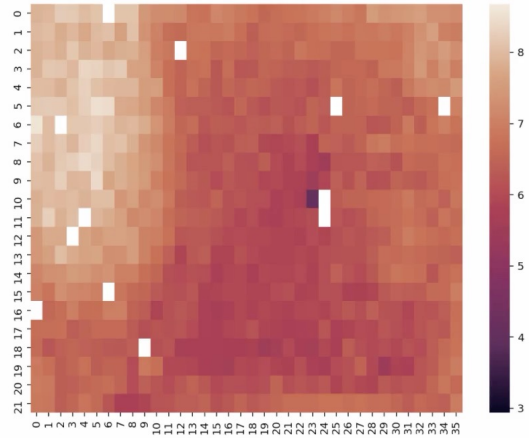
smaller λ → slower off-rate
larger λ → faster off-rate

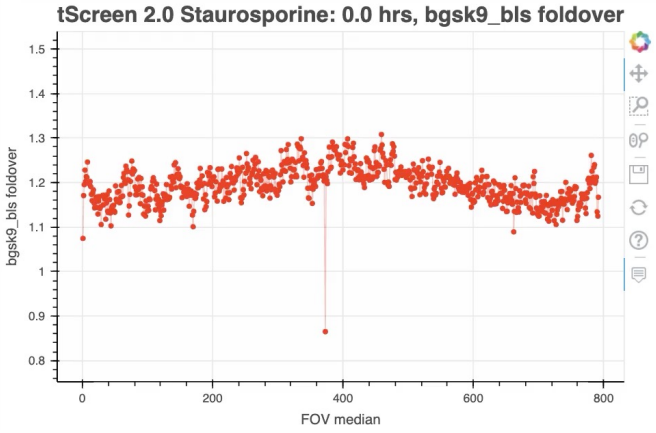
1795

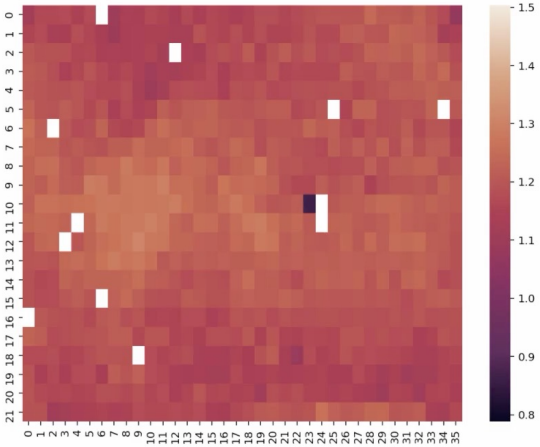


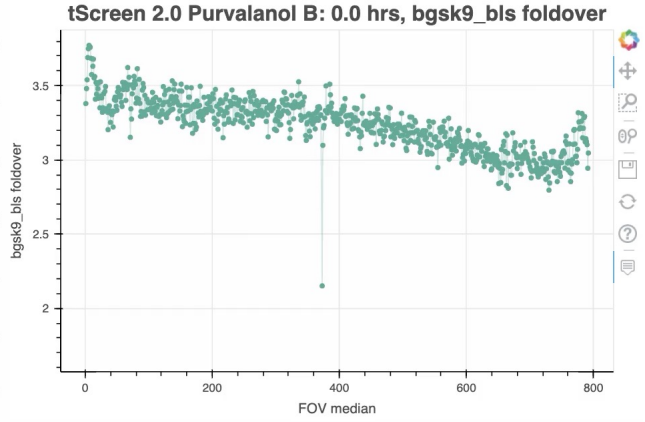


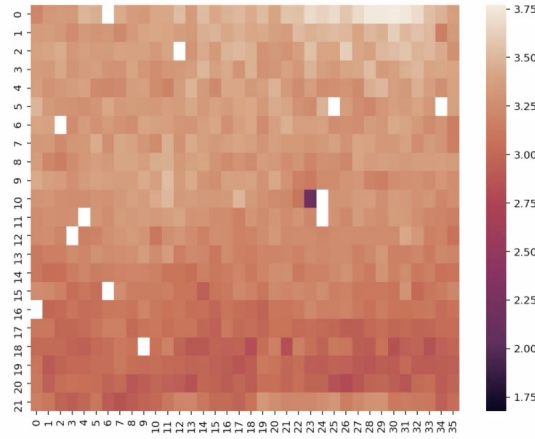






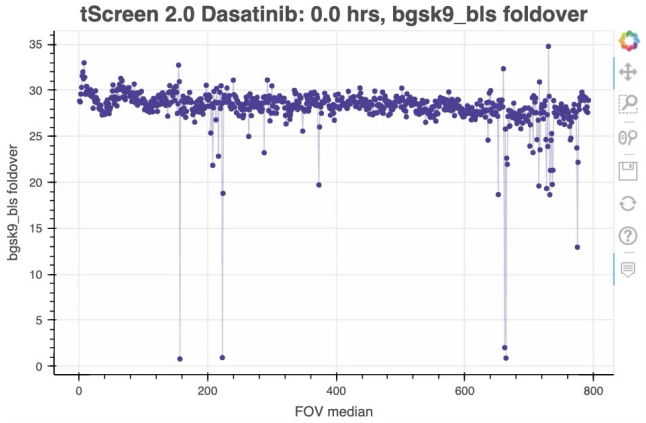


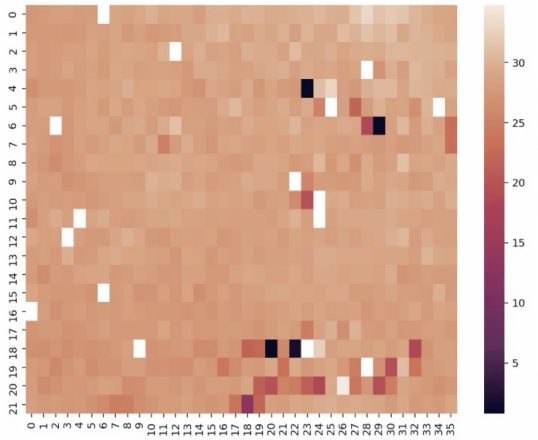


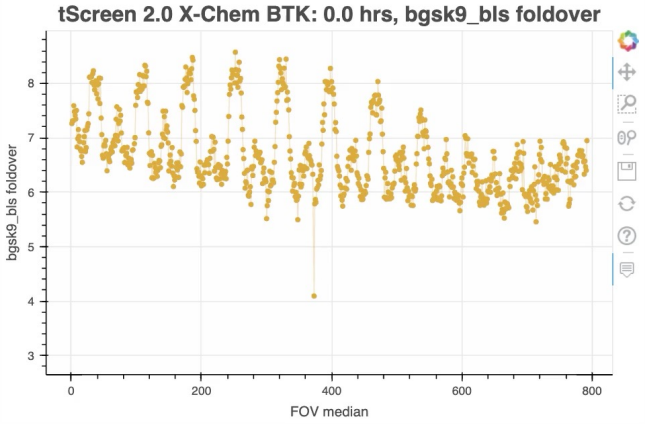


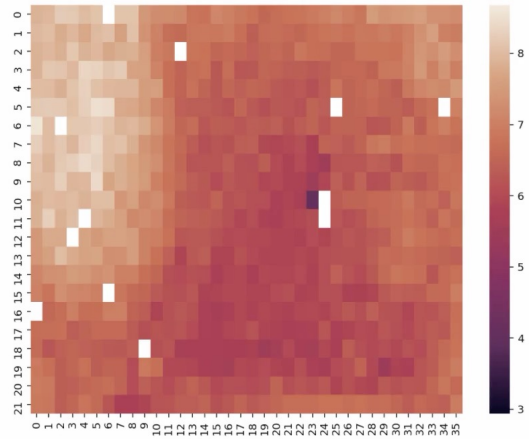
- for long half-lives (Dasatinib: hours), intensities are relatively uniform across chip
- for short half-lives (Purvalanol: seconds), also uniform
- for those in between, (BTK: minutes), brighter region on the left suggests that FOV movement is out of sync with how things are flushed from the left, but this could be a number of things

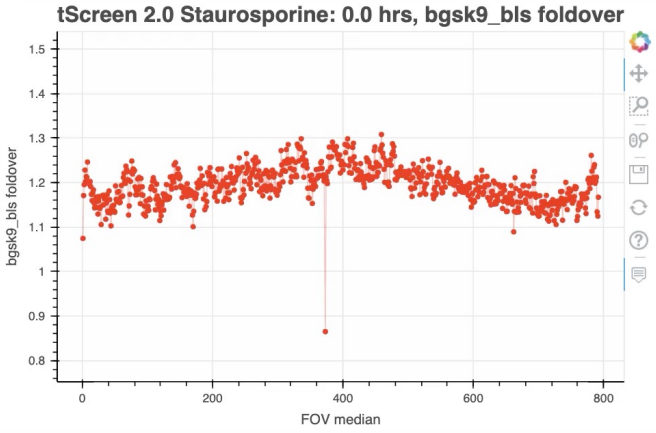
1795

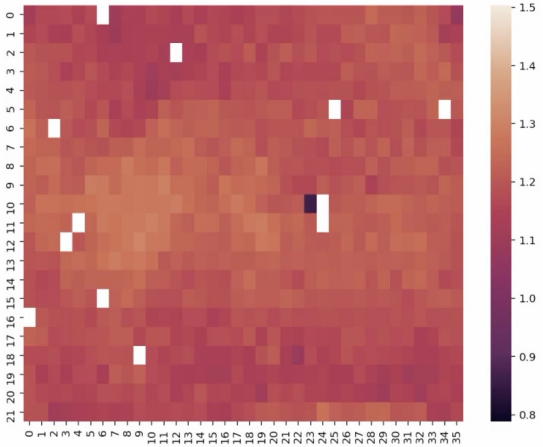


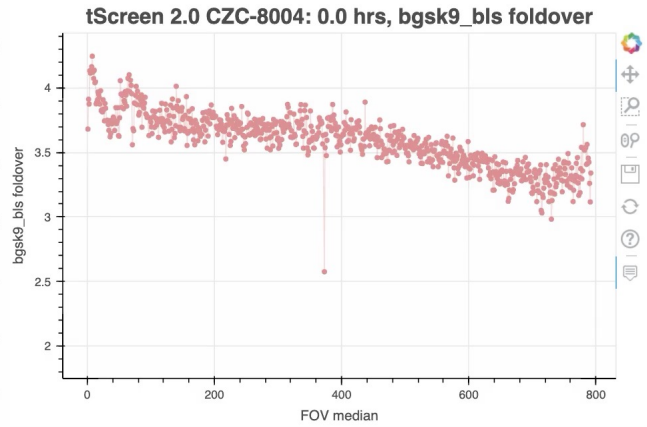


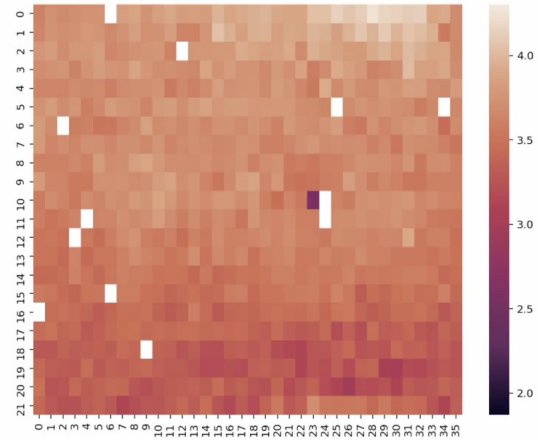












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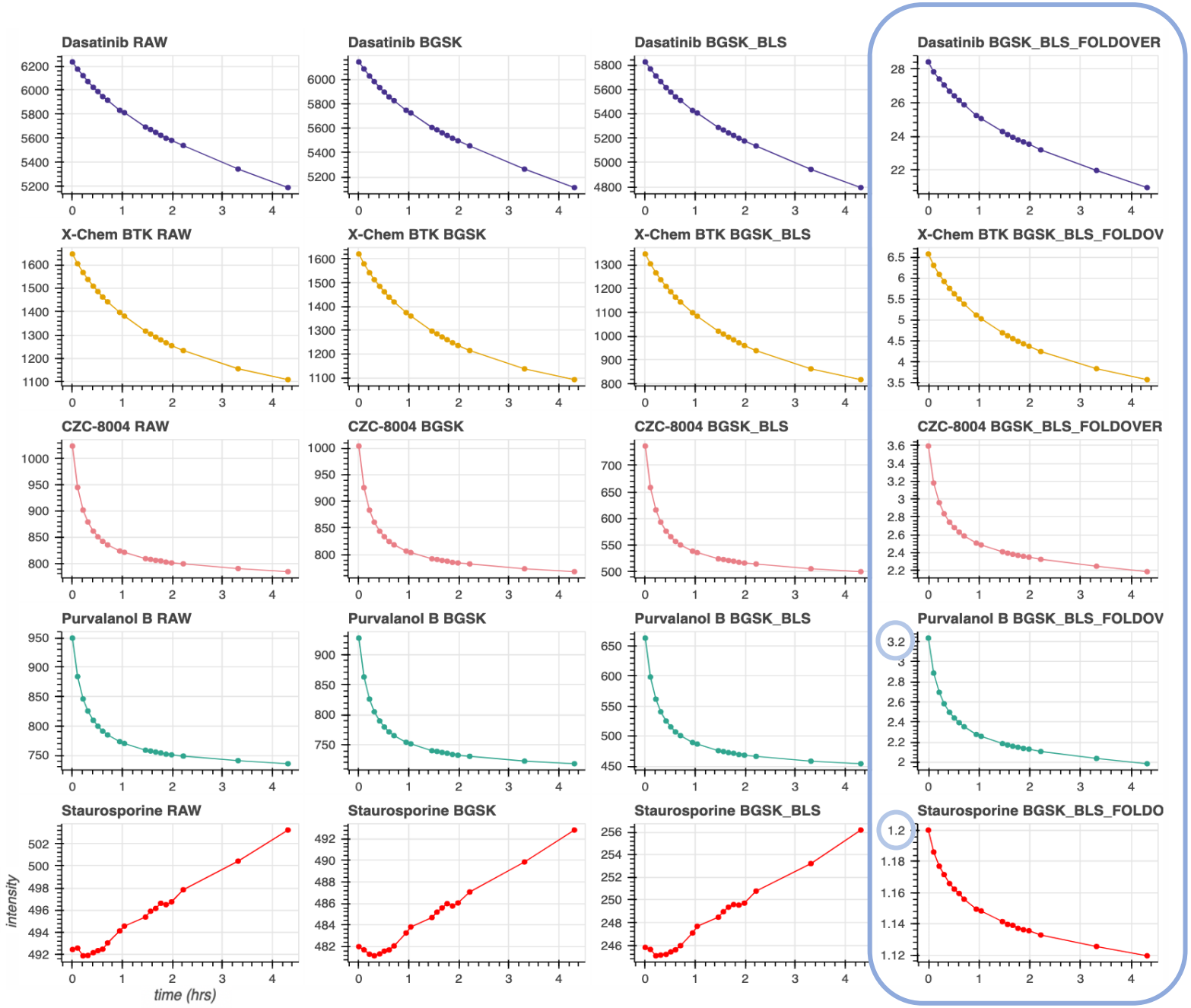
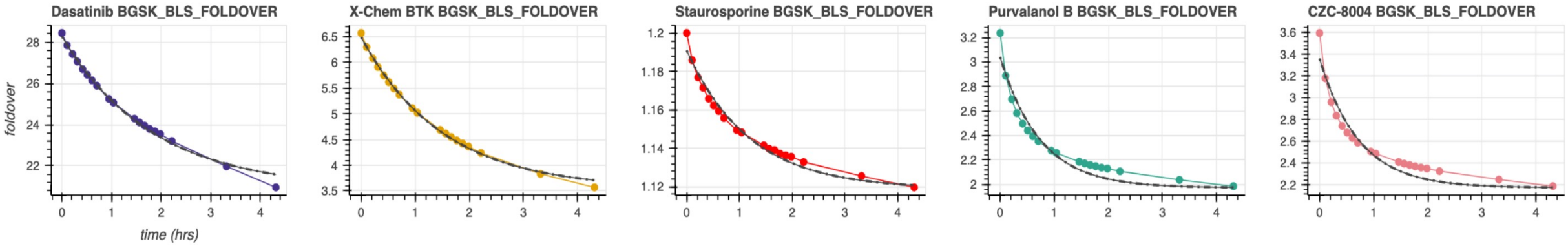


1795: full chip medians

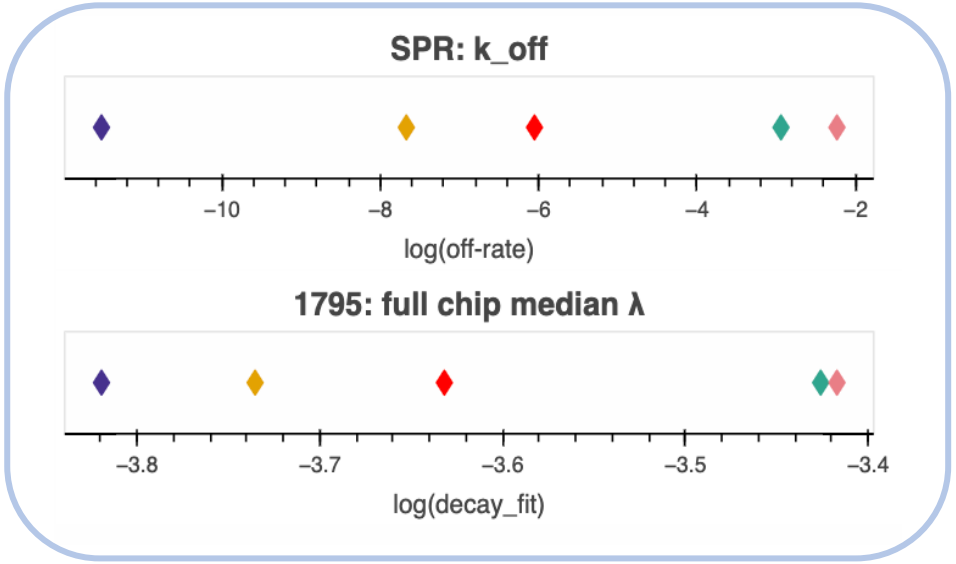
- time series of full chip medians (~100,000 repeats per compound) over the course of 4.4 hrs, 19 images
- for slower-decaying compounds, imaging longer might be useful to enter exponential regime
- very low fold-over for Staurosporin (~1x)

1795: full chip median fits

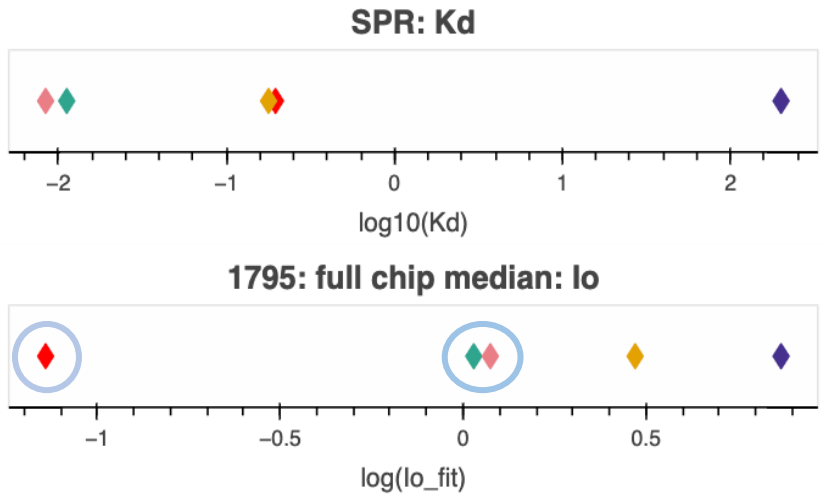
$$I(t) = I_0 e^{-\lambda t} + \text{bkg}$$



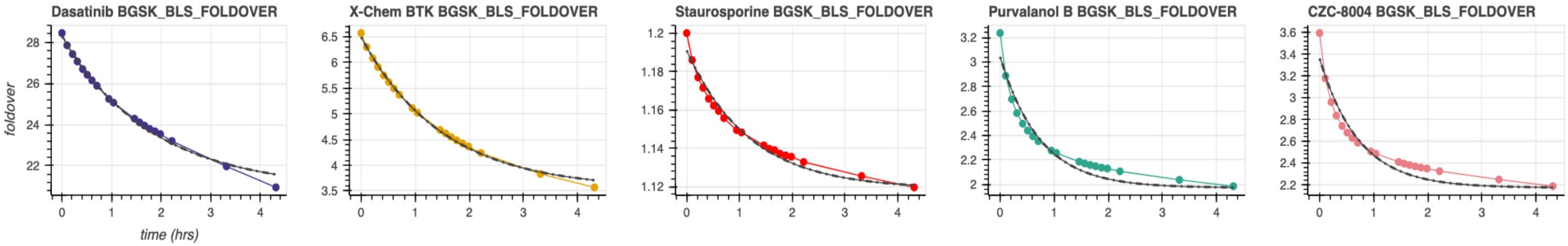
off-rates



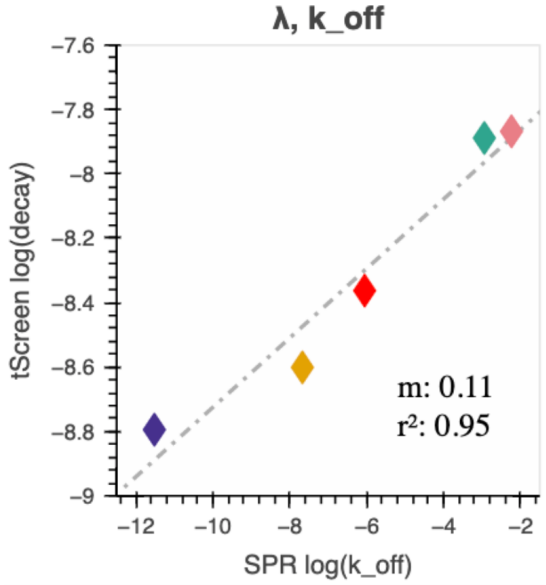
intensities



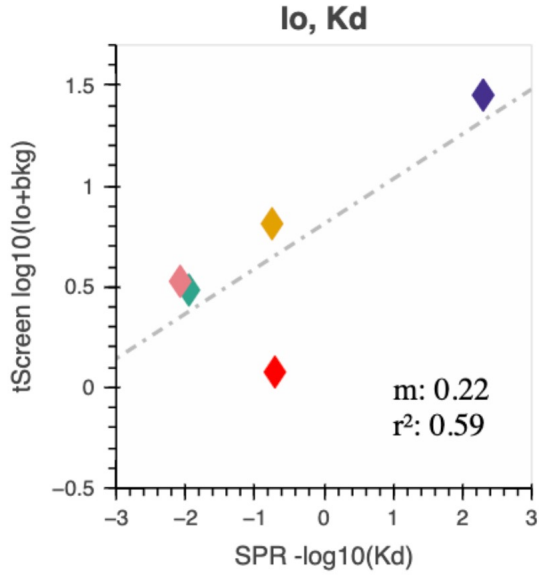
1795: full chip median fits



off-rates



intensities



1795: sampling chip means with varying # of repeats

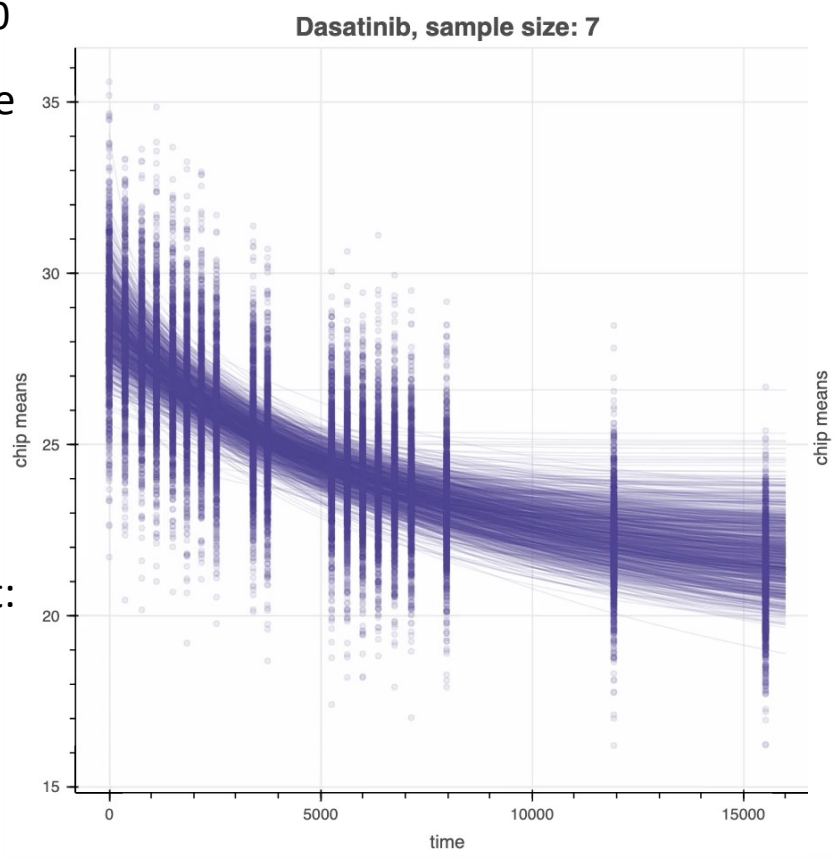
- full chip medians: sample size ~100,000
- sampling procedures to simulate future screens with ~7-19 repeats:

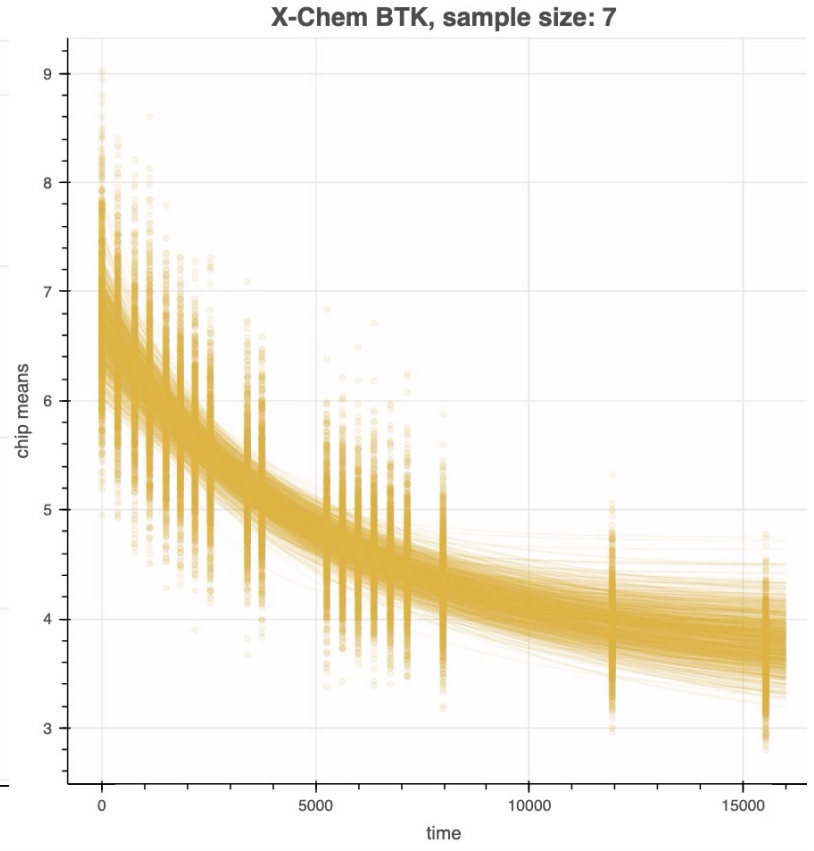
  take the entire chip
  sample 7 beads for each BB1
  take their mean
  fit the resulting time series
  repeat 1,000x for aggregate behavior

- on the right: time traces of overlaid samples and their fits, so on every plot:

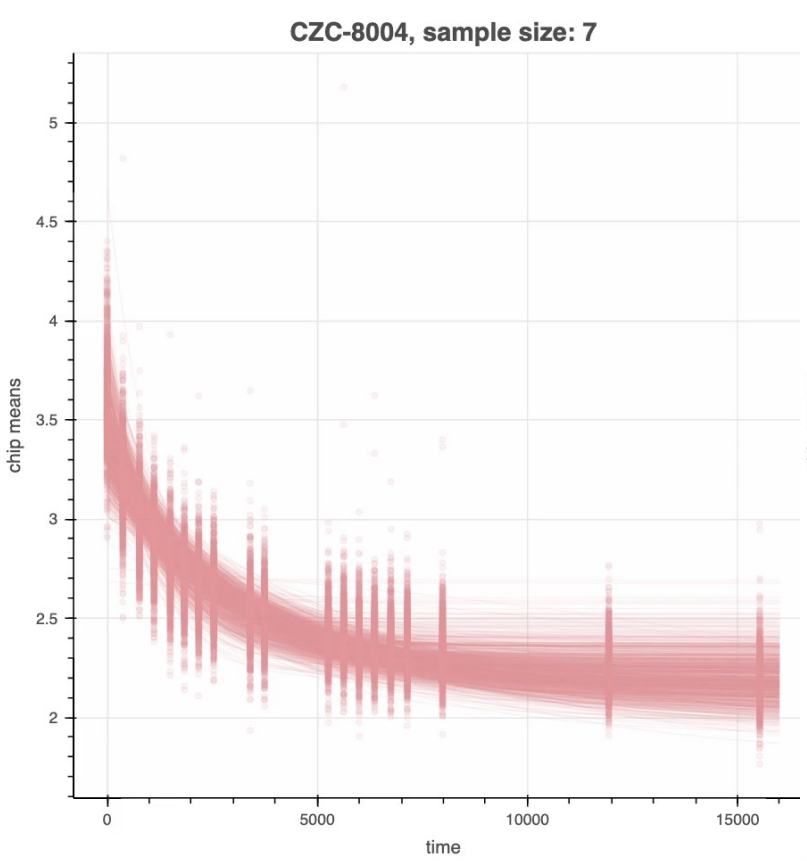
  each line: fit through (# of images) pts
  each point: sample mean

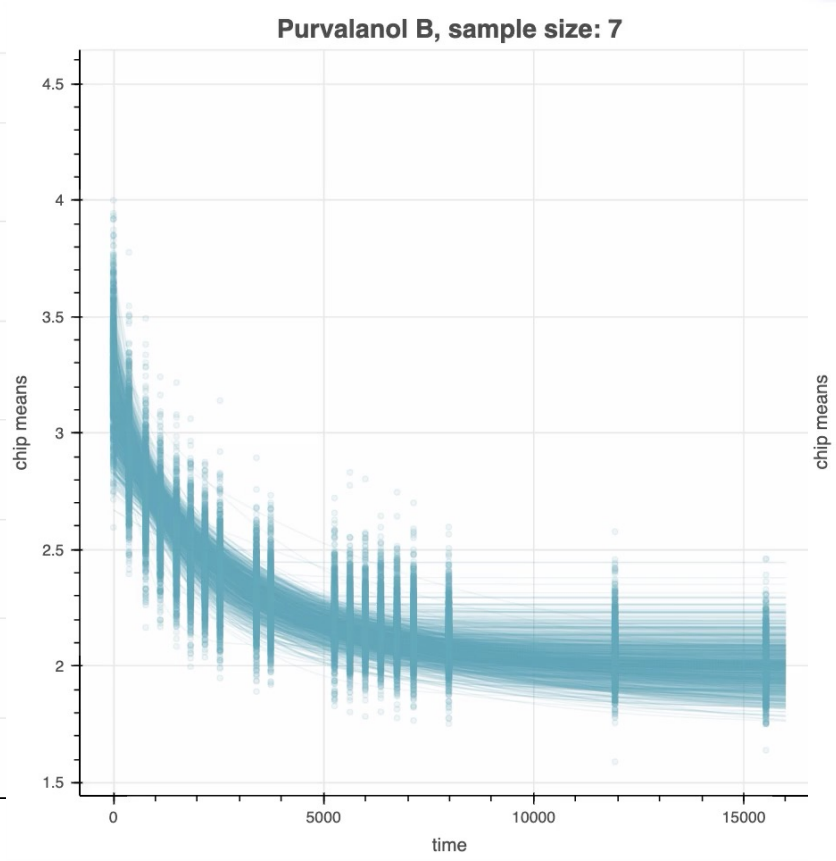
  # of lines: 1,000
  # of points: 1,000 x (# of images)

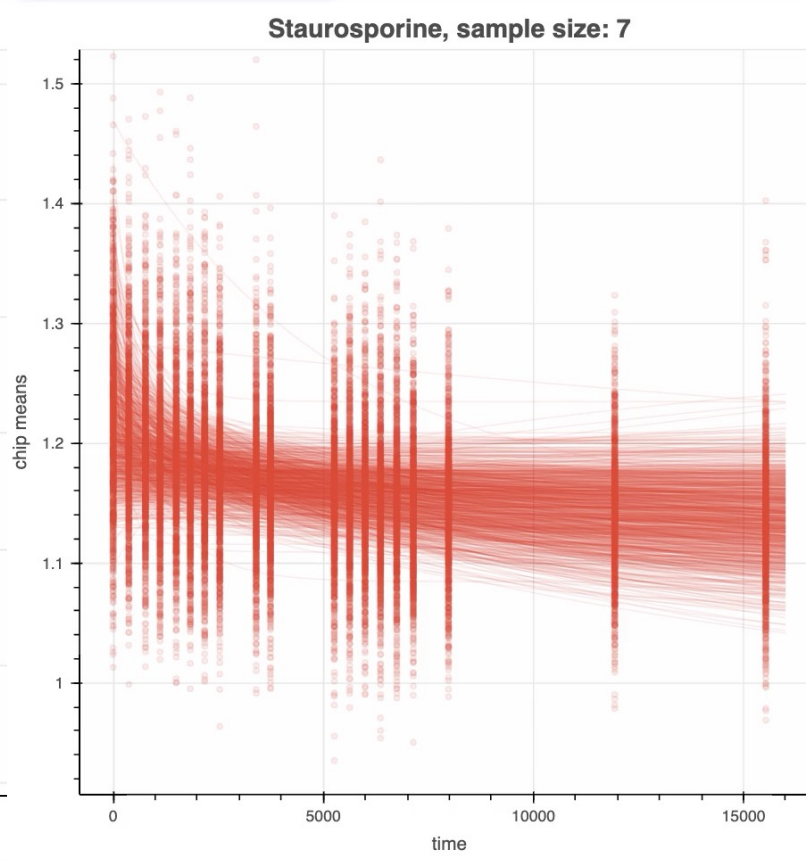




1795: sampling chip means with varying # of repeats







Staurosporine samples are noisy...

How large should the sample size be to compensate for low foldover?

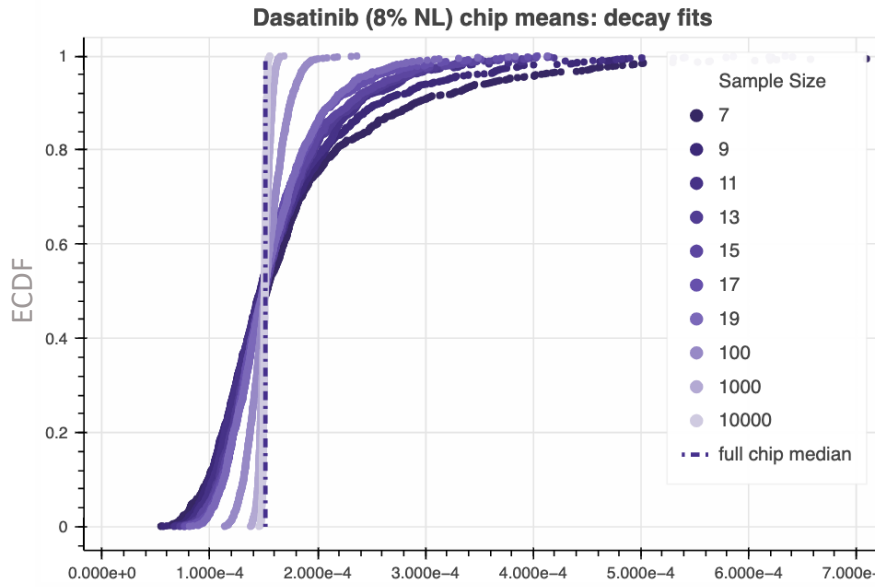
somewhere between 100-1,000x

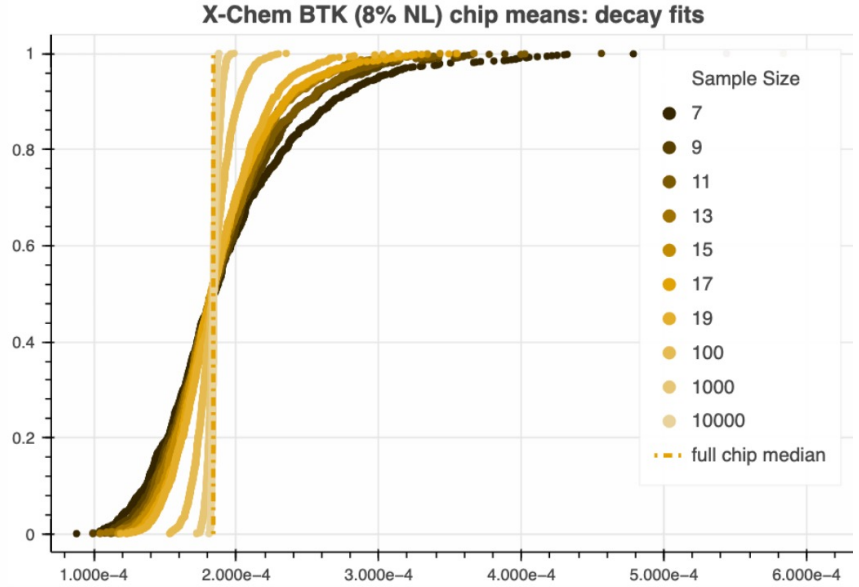
decay fits

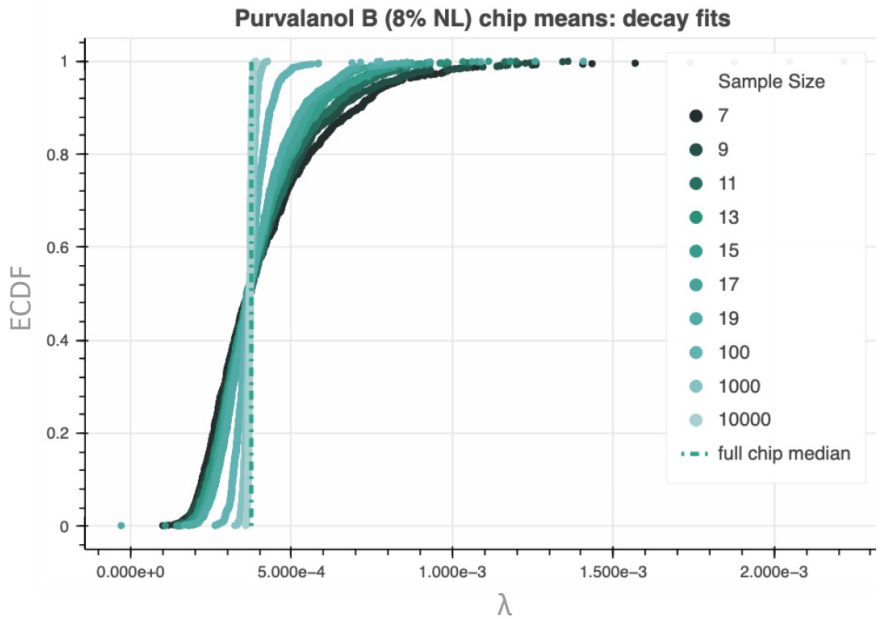
- cumulative distributions of decay fits λ
- flatter slope = larger spread

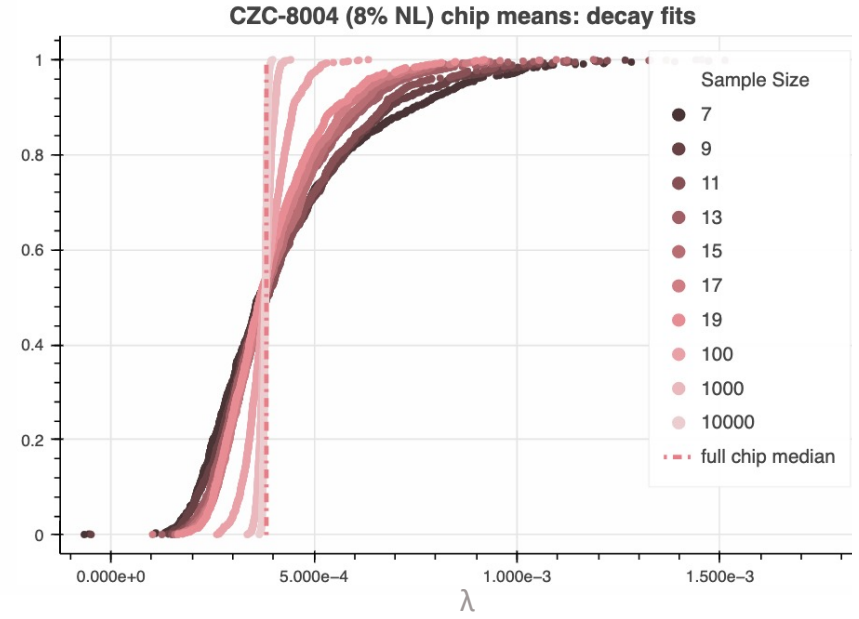
- small sample sizes have larger spreads, but this spread seems to scale independently from the magnitude of the intensity

- recover full chip median as sample size → 10,000
- full chip median = 0.5

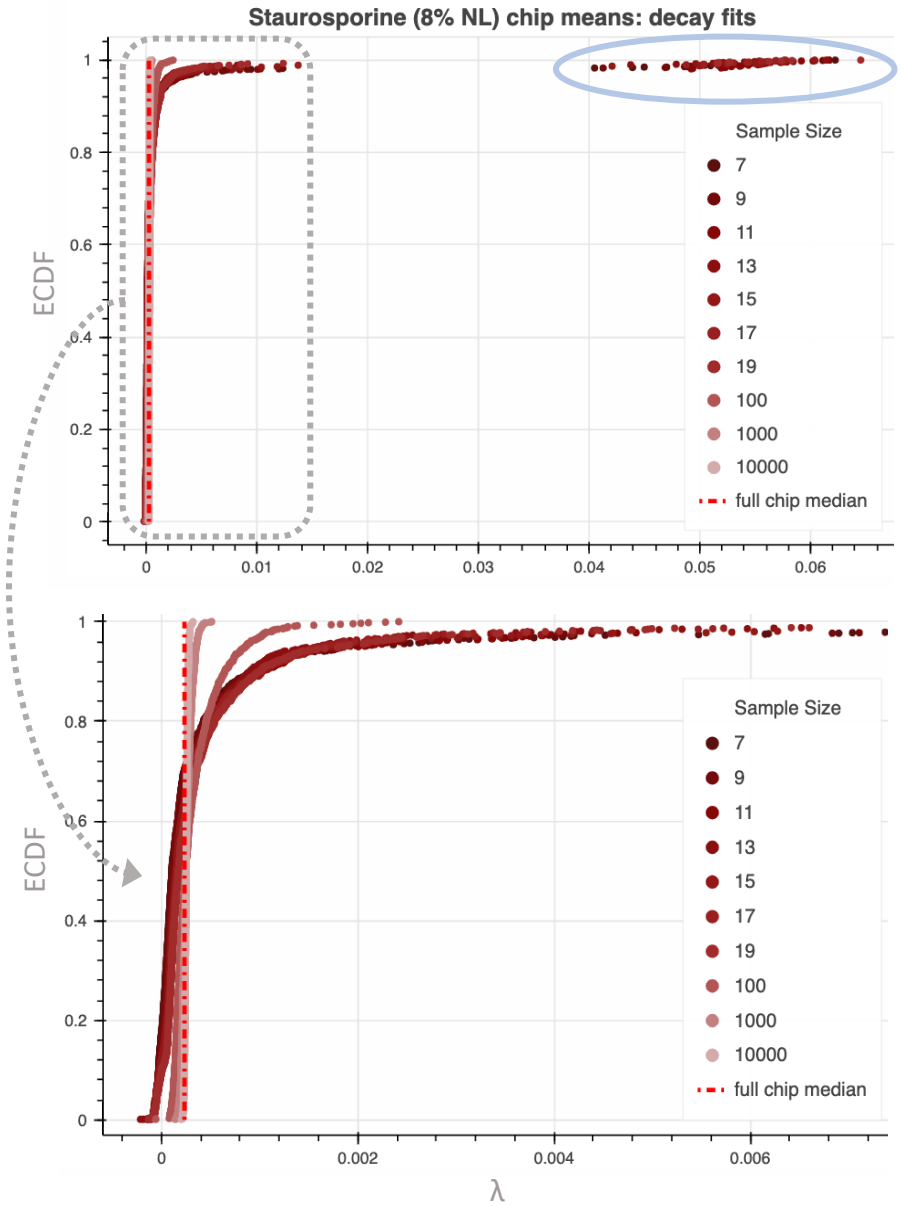




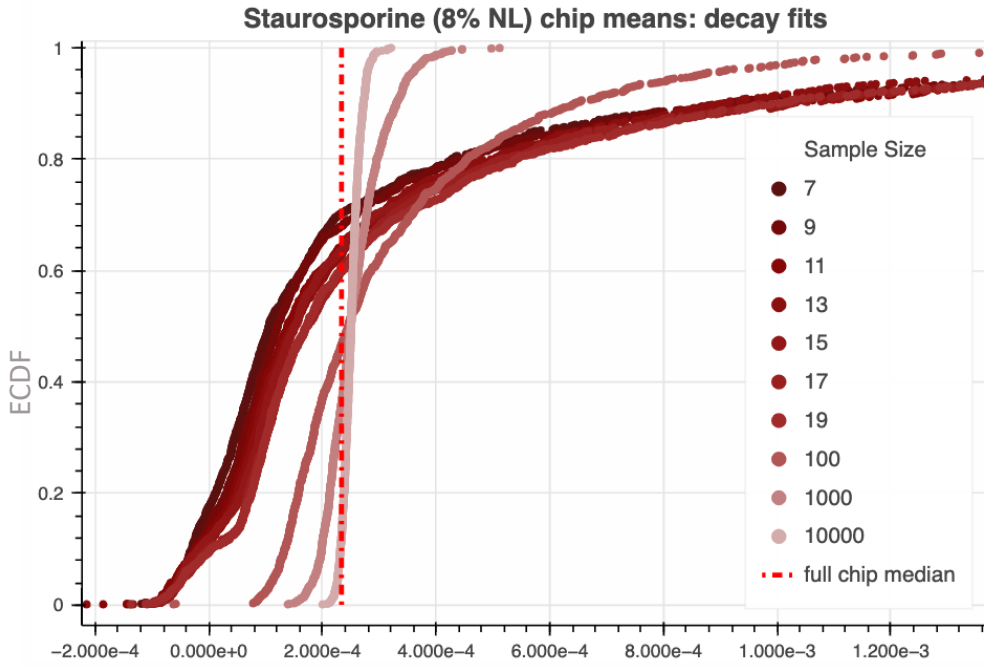




decay fits: Staurosporine



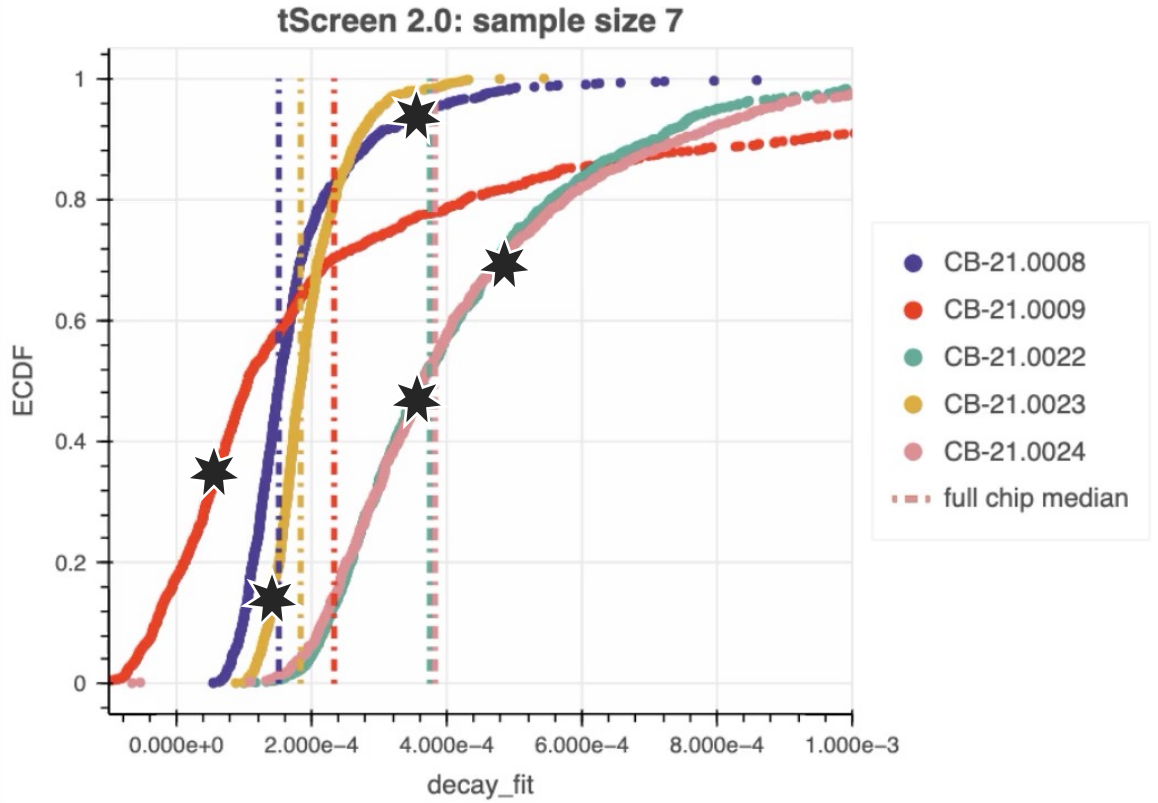
- when there are only 7-19 repeats, 2% of the time the fits are nonsense, also non-trivial proportion of negatives ~10+%
- long tailed, not a symmetric/sigmoidal distribution



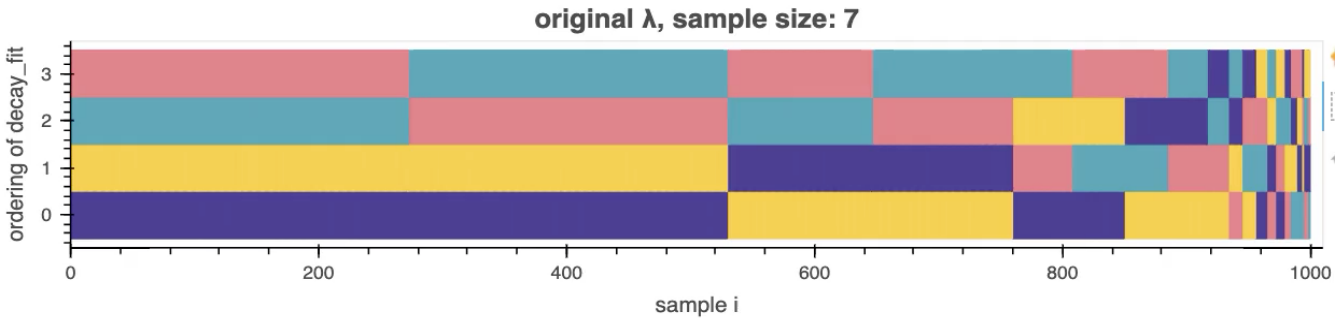
- slow to converge relative to other compounds
- with thousands of repeats (lighter colors), distribution converges to full chip median, but they are not quite parallel

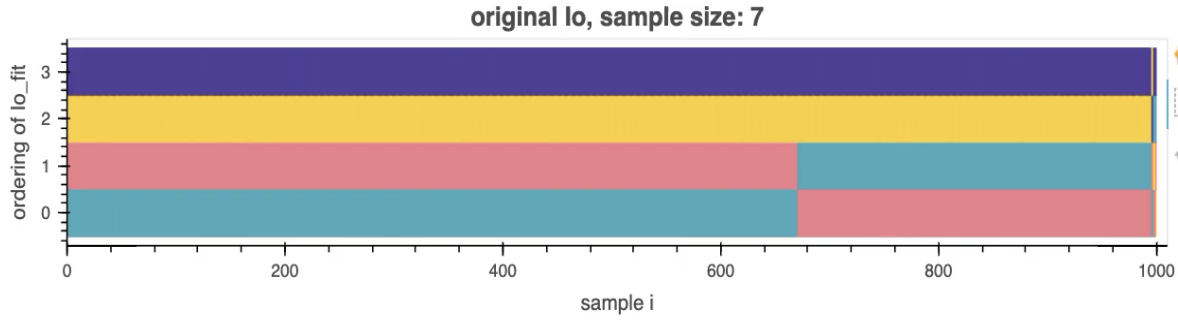
Ordering of λ fits

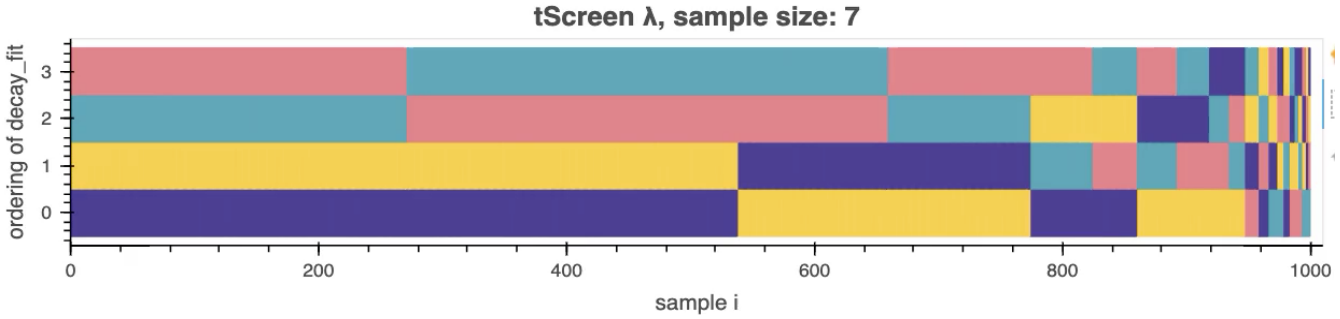
- same plots as above but BB1's are overlaid
- absolute distributions are only helpful when thinking about the results from many many screening experiments

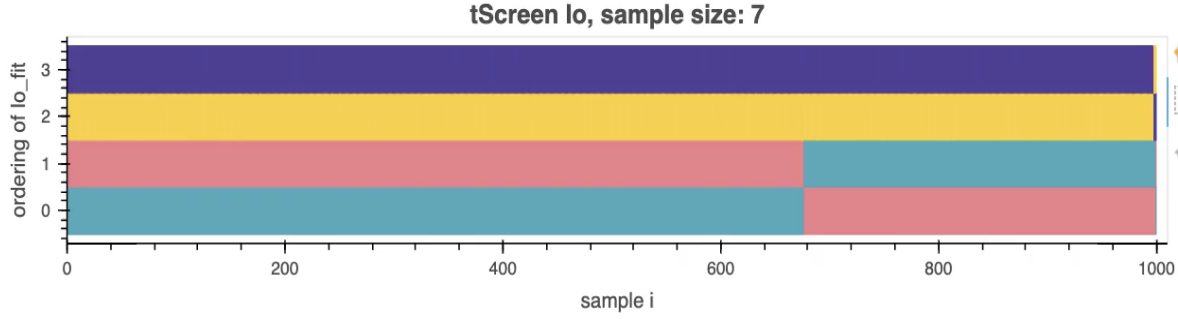


Ordering without Staurosporine





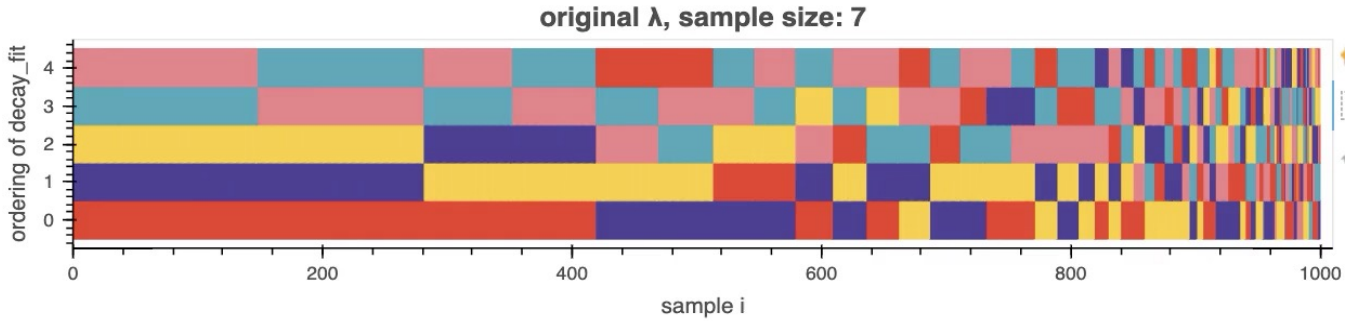


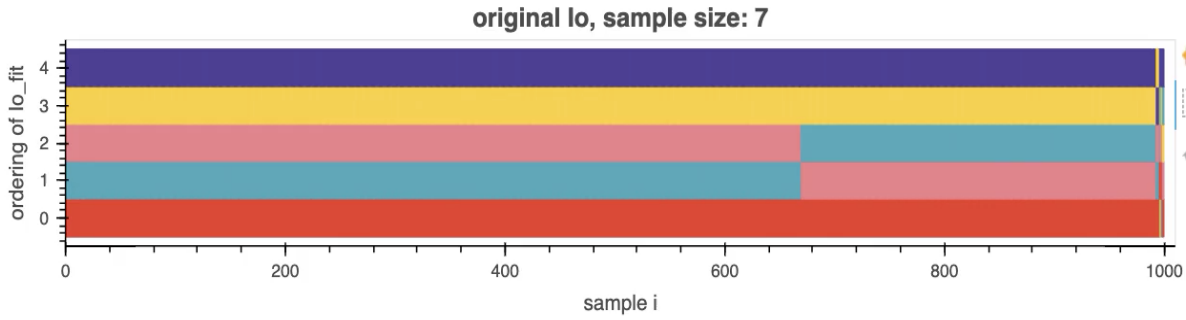


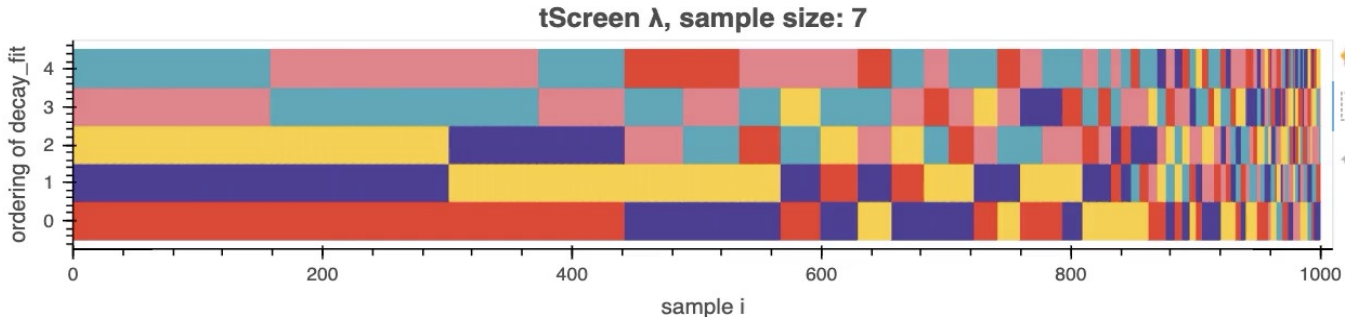
- left: decay fits top: original processing
  right: initial intensity Io fits bottom: tScreen 2.0 processing
- visualizing ordering:
  x-axis represents the sampling index, there are 1,000 samples
  y-axis reduces quantities to an ordering, so bottom colors are slower than top colors

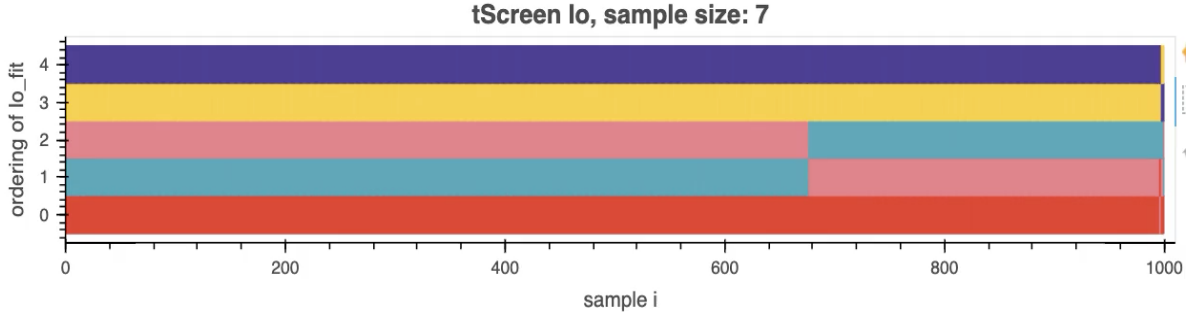
  with 5 compounds we have 4! = 24 possible unique orderings, sort left to right by frequency of occurence
  good news: purple + yellow bands and pink + green bands stay together ... most of the time
  see the effect of intra/inter-FOV correction at large sample sizes

Ordering with Staurosporine









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  with 5 compounds we have 5! = 120 possible unique orderings, sort left to right by frequency of occurence
  good news: purple + yellow bands and pink + green bands stay together ... most of the time
  see the effect of intra/inter-FOV correction at large sample sizes