calcium imaging analysis: some theoretical considerations with practical consequences

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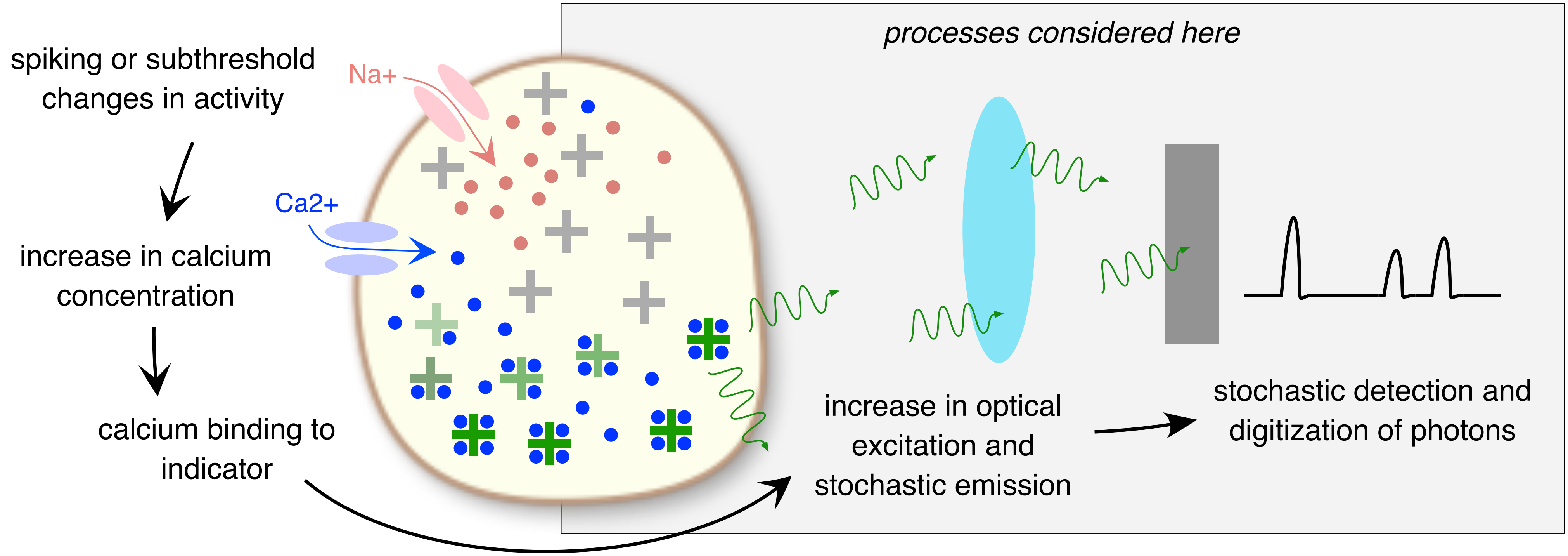
imaging neural activity with GCaMP



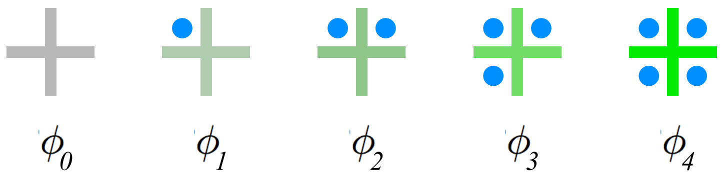
what is the signal?

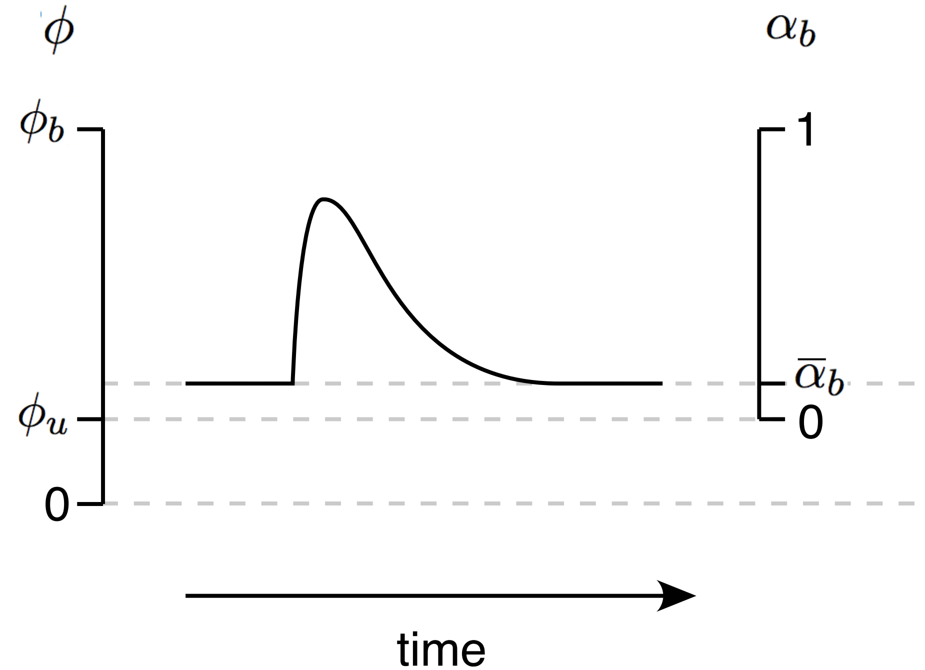
what is the noise?

part 1: signal

modeling fluorescence at an instant in time

GCaMP's multiple binding sites





$$
\begin{aligned}
F &= P\phi \\
&= P\,(\ \alpha_b\phi_b + (1-\alpha_b)\phi_u\ ) \\
&= P\,(\ \alpha_b(\phi_b-\phi_u) + \phi_u\ ) \\
&= P\,[\ (\alpha_b-\bar{\alpha}_b)(\phi_b-\phi_u) + \bar{\alpha}_b(\phi_b-\phi_u) + \phi_u\ ]
\end{aligned}
$$

"baseline"

$\bar{\alpha}_b$

notation to emphasize the time-varying part:

$$F(t) = P\,[\ (\alpha_b(t)-\bar{\alpha}_b)(\phi_b-\phi_u) + \bar{\alpha}_b(\phi_b-\phi_u) + \phi_u\ ]$$

can we recover $\alpha_b(t)$?

$$F(t) = P\,[\;(\alpha_b(t) - \bar{\alpha}_b)(\phi_b - \phi_u) + \bar{\alpha}_b(\phi_b - \phi_u) + \phi_u\;]$$

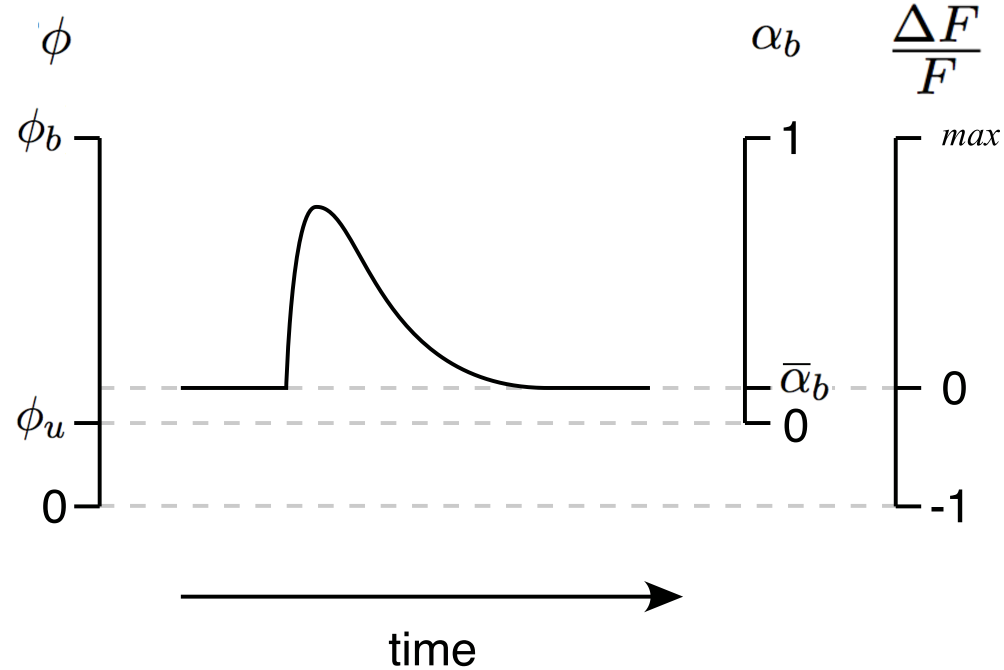
if baseline is obvious:

$$\bar{F} = P\,[\;\bar{\alpha}_b(\phi_b - \phi_u) + \phi_u\;]$$

$$\frac{\Delta F}{F}(t) = \frac{F(t) - \bar{F}}{\bar{F}}$$

$$= \frac{P\,[\;(\alpha_b(t) - \bar{\alpha}_b)(\phi_b - \phi_u)]}{P\,[\;\bar{\alpha}_b(\phi_b - \phi_u) + \phi_u\;]}$$

$$= (\alpha_b(t) - \bar{\alpha}_b)\frac{\phi_b - \phi_u}{\bar{\alpha}_b(\phi_b - \phi_u) + \phi_u}$$



simplifying assumptions

$$\bar{\alpha}_b \approx 0$$
$$\phi_b \gg \phi_u$$

$$\Rightarrow \quad \frac{\Delta F}{F}(t) \;\approx\; \alpha_b(t)\frac{\phi_b}{\phi_u}$$

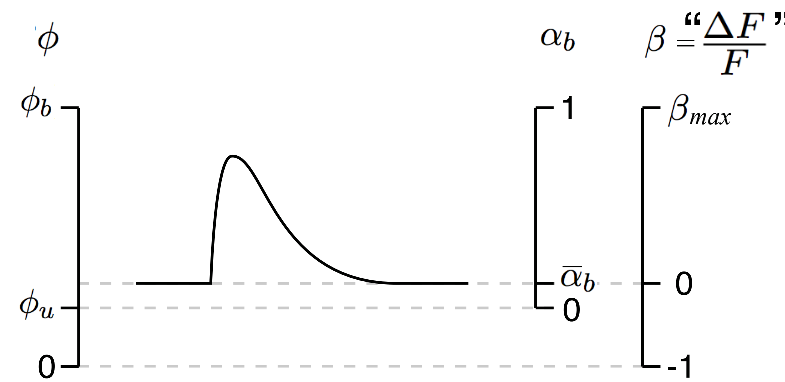
what happens in pixel i ?

$$
\begin{aligned}
F_i(t) &= P_i \phi(t) \\
&= P_i \left[\ (\alpha_b(t) - \bar{\alpha}_b)(\phi_b - \phi_u) + \bar{\alpha}_b(\phi_b - \phi_u) + \phi_u\ \right] \\
&= w_i(\beta(t) + 1)
\end{aligned}
$$

handy new variables

$$w_i = \frac{P_i}{\bar{\alpha}_b(\phi_b - \phi_u) + \phi_u}$$

$$\beta(t) = (\alpha_b(t) - \bar{\alpha}_b)\frac{(\phi_b - \phi_u)}{\bar{\alpha}_b(\phi_b - \phi_u) + \phi_u}$$



$$
\begin{aligned}
\frac{\Delta F_i}{F_i}(t) &= \frac{F_i(t) - \bar{F}_i}{\bar{F}_i} \\
&= \frac{w_i(\beta(t) + 1) - w_i(0 + 1)}{w_i(0 + 1)} \\
&= \frac{w_i \beta(t)}{w_i} \\
&= \beta(t)
\end{aligned}
$$

summary

assumption: ϕ constant throughout the cell

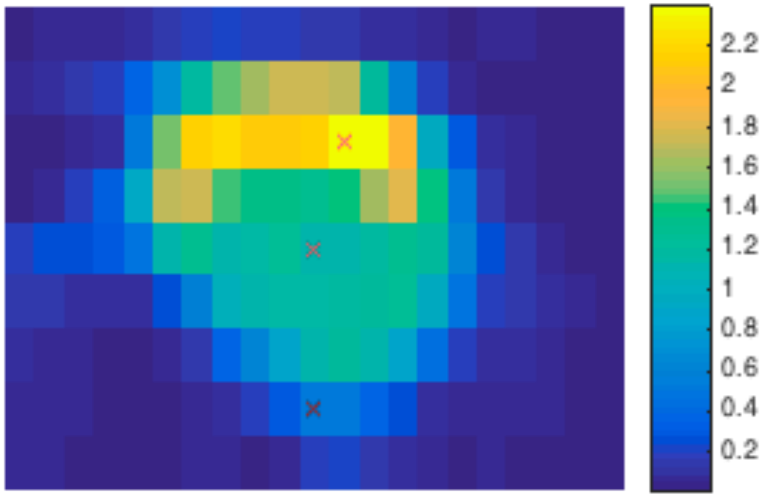
corollary: $F_i(t) = w_i(\beta(t) + 1)$

prediction: $\frac{\Delta F_i}{F_i}(t) = \beta(t)$

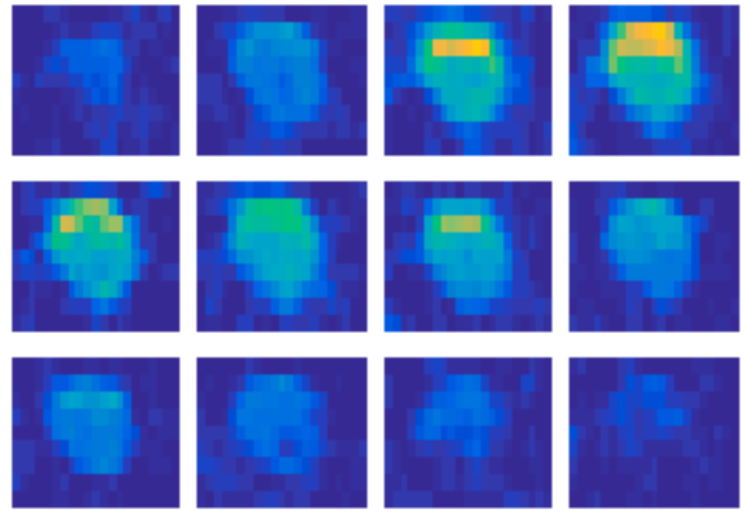
Is ΔF/F *actually* the uniform in space?

reality: chaotic nightmare

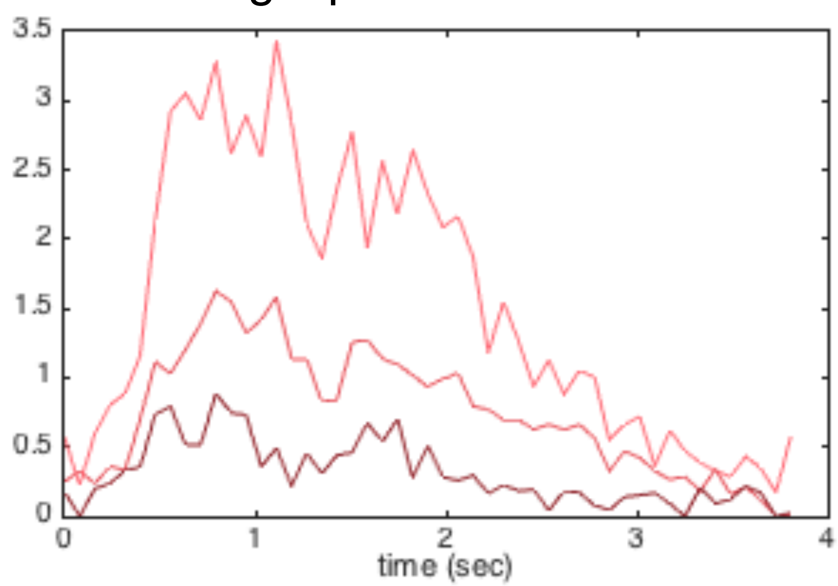
average frame



single frames of ΔF/F movie



single pixel time courses



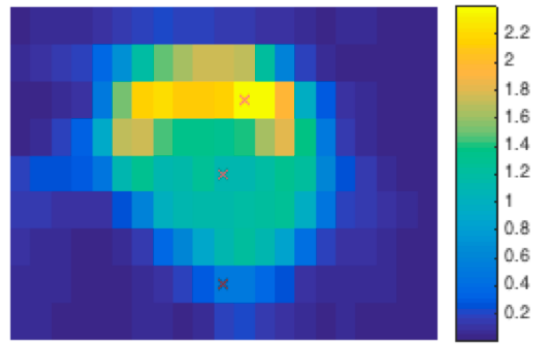
why isn't ΔF/F the same in every pixel?

effect of background light

$$F_i(t) = w_i(\beta(t) + 1)$$

$$\begin{aligned}
\frac{\Delta F_i}{F_i}(t) &= \frac{F_i(t) - \bar{F}_i}{\bar{F}_i} \\
&= \frac{w_i(\beta(t)+1) + b_i - w_i(0+1) - b_i}{w_i(0+1) + b_i} \\
&= \frac{w_i\beta(t)}{w_i + b_i} \\
&= \beta(t)\frac{w_i}{w_i + b_i}
\end{aligned}$$

average frame of ΔF/F movie



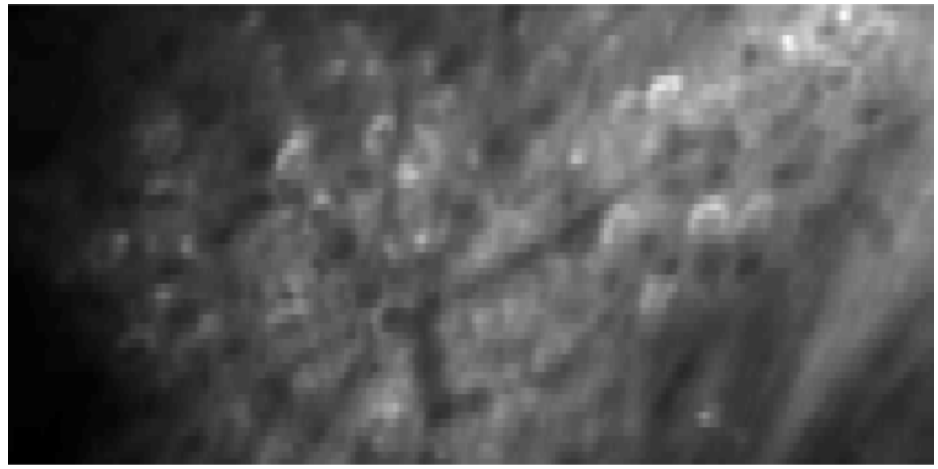
without accurate background subtraction, ΔF/F underestimates β
by an **unknown** amount

bright side: space-time separability

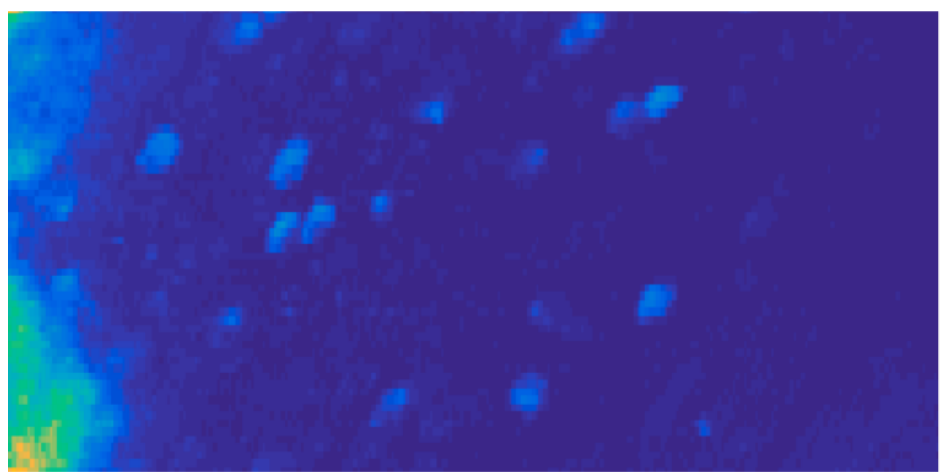
part 2: noise

analysis comparison

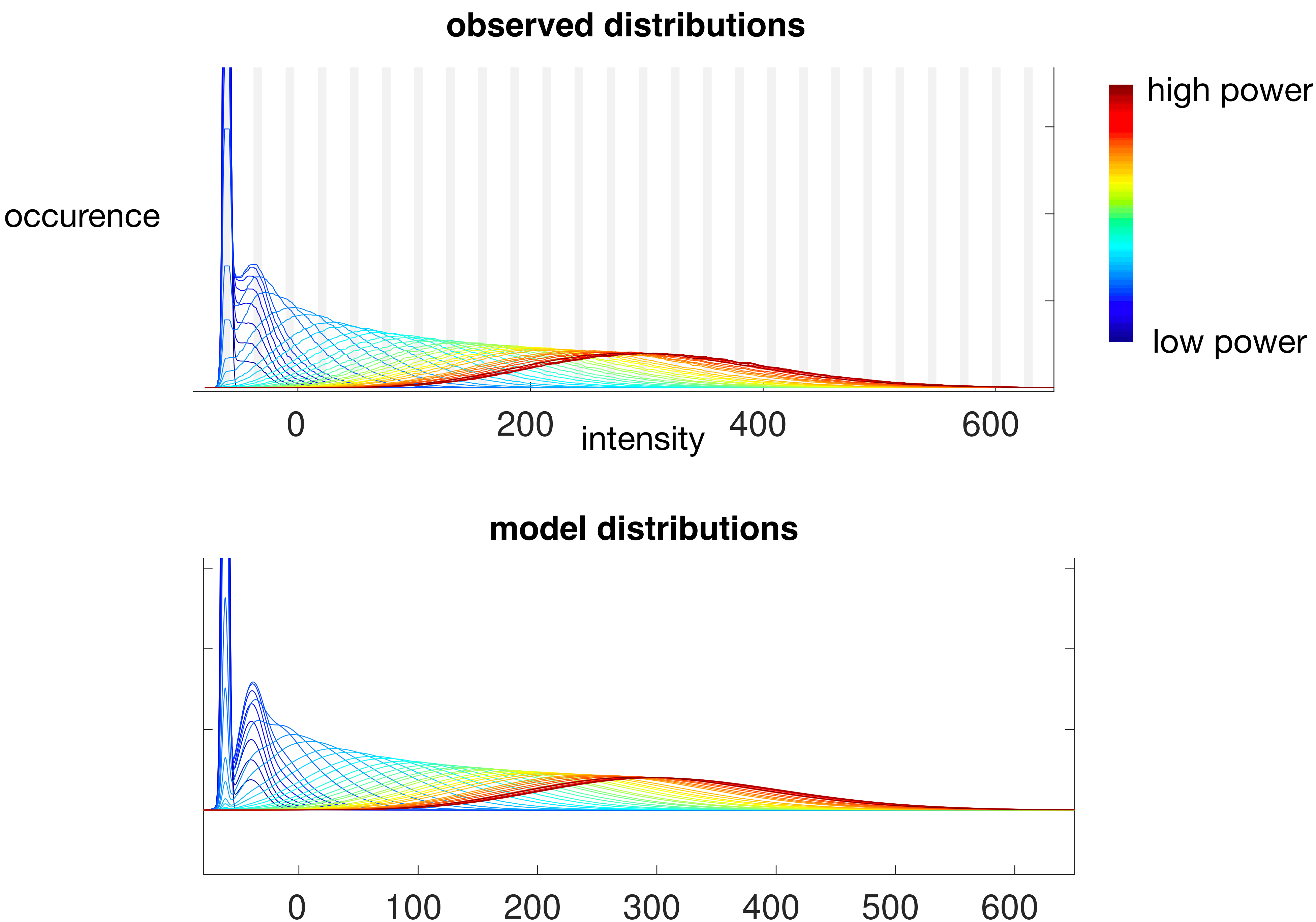
mean image



PCA reconstruction variance

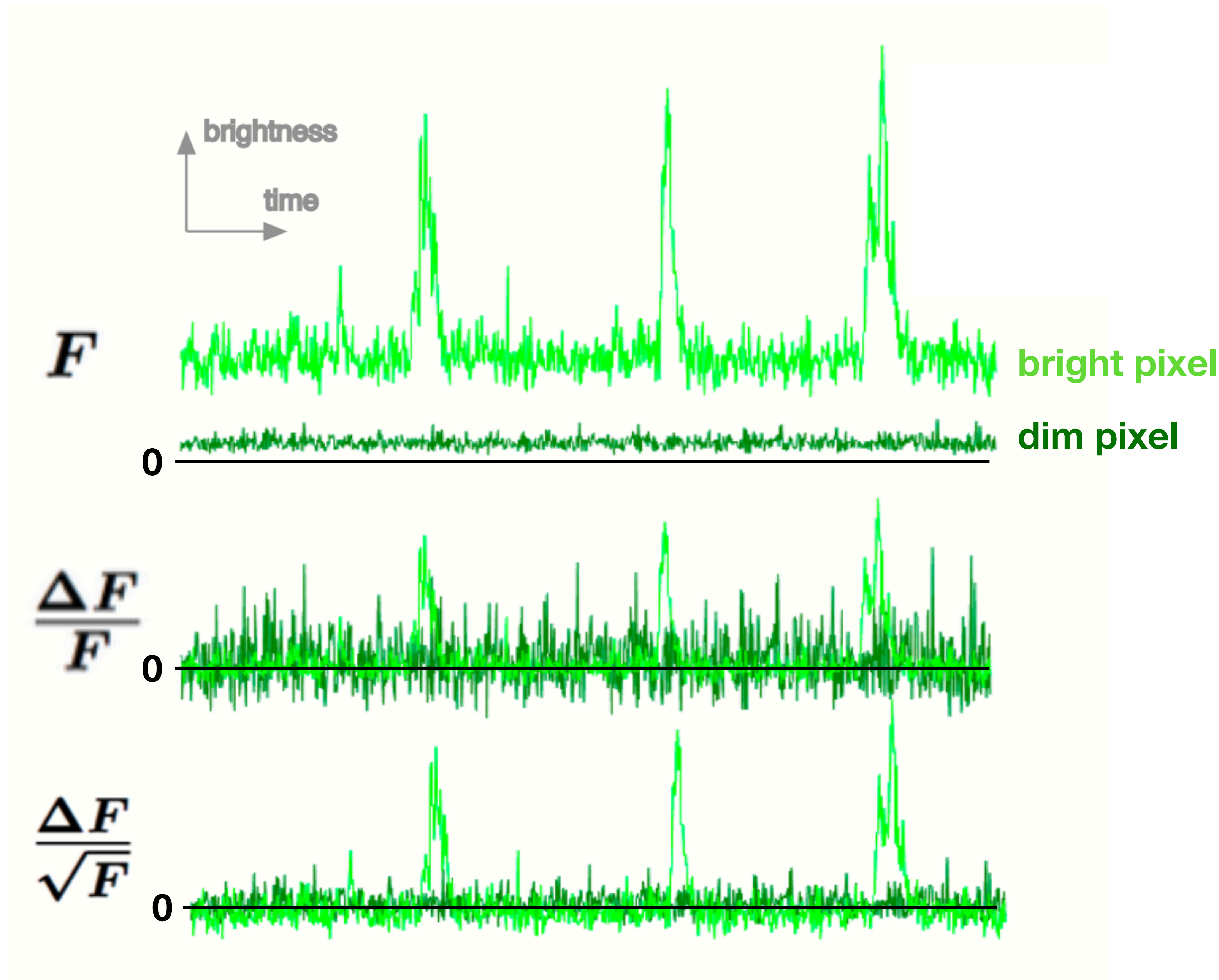


noise model


observed distributions
occurence
intensity
0
200
400
600
high power
low power
model distributions
0
100
200
300
400
500
600

variance scales with mean

noise amplitude across pixels



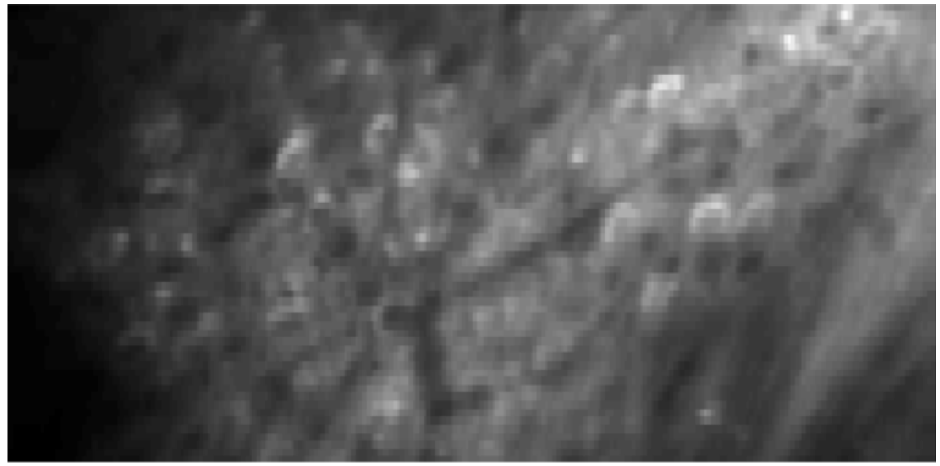
comparing ΔF/F and ΔF/√F

in a pixel at baseline:

	mean	**STD**
$\boldsymbol{F}$	λ	$\sqrt{\lambda}$
$\frac{\boldsymbol{\Delta F}}{\boldsymbol{F}}$	0	$\frac{1}{\sqrt{\lambda}}$
$\frac{\boldsymbol{\Delta F}}{\sqrt{\boldsymbol{F}}}$	0	1

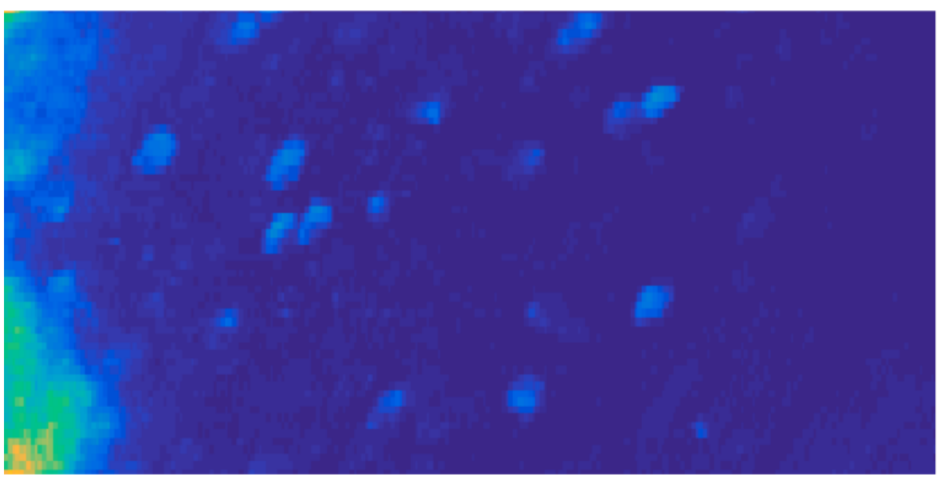
analysis comparison

mean image



PCA reconstruction variance

ΔF/F



ΔF/√F