Appendix A: Stochastic derivation of average lifespan, burst size & basic reproduction number

In this part, we show the process for deriving equations (6) and (7) through a probabilistic argument based on biological definitions. We also show the derivation of the average lifespan of infected cells and viruses.

The death rate of infected cells, δ(a), is interpreted as the instantaneous rate of death at infection age a [2]. If we consider X to be a random variable representing the age at death, F(a) as the probability of dying by age a, f(a) as the probability density function of X, and P(A) as the probability of an event A occurring, then δ(a) is calculated as follows.

$$\delta(a) := \lim_{\Delta a \to 0} \frac{P(a \le X \le a+\Delta a | a \le X)}{\Delta a} = \lim_{\Delta a \to 0} \frac{P(a \le X \le a+\Delta a)}{P(a \le X)\Delta a}$$
$$= \frac{f(a)}{1-F(a)} = -\frac{d}{da} log(1 - F(a)) \cdots \text{(A1)}$$

Therefore, probability of dying at infection age a:

$$F(a) = 1 - e^{-\int_0^a \delta(s)\, ds} \cdots \text{(A2)}$$

Since $F(a) \to 1$ as $a \to \infty$ is necessary、$e^{-\int_0^a \delta(s)\, ds} \to 0$.
Probability density function of this probability:

$$f(a) = \delta(a) e^{-\int_0^a \delta(s)\, ds} \cdots \text{(A3)}$$

Using this, the average lifespan after infection can be calculated by $\int_0^\infty a f(a)\, da$. If $\delta(a) = \delta = const.$, then

$$\int_0^\infty a f(a)\, da = \int_0^\infty a \delta e^{-\delta a}\, da = \frac{1}{\delta} \cdots \text{(A4)}$$

Similarly, the average lifespan of virus is calculated as $\frac{1}{c}$.

Burst size is biologically interpreted as the total number of virus particles produced from when one infected cell arises until it dies. Therefore, burst size N is defined as follows as the expected value taking the sum of the product of the amount of virus released by infectious age a and the probability of dying at infection age a.

$$N := \int_0^\infty \left(\int_0^a p(s)\, ds\right) \cdot f(a)\, da \cdots \text{(A5)}$$

Substituting equation (A3) and calculating further, we have

$$N = \int_0^\infty \left(\int_0^a p(s)\, ds\right) \delta(a) e^{-\int_0^a \delta(s)\, ds}\, da$$
$$= \int_0^\infty p(a) e^{-\int_0^a \delta(s)\, ds}\, da - \left[\left(\int_0^a p(s)\, ds\right) e^{-\int_0^a \delta(s)\, ds}\right]_0^\infty$$

$$= \int_0^{\infty} p(a) e^{-\int_0^a \delta(s)\, ds}\, da \cdots (7)$$

Here, we used the fact that $\int_0^a p(s)\, ds < \infty$ and $e^{-\int_0^a \delta(s)\, ds} \to 0$ as $a \to \infty$. From the above, equation (7) is obtained.

Also, the basic reproduction number is biologically interpreted as the number of new infected cells that one infected cell produces in its lifetime at the initial stage of infection. Therefore, using virus burst size N, infection rate per virus particle per unit time at t=0 β_T0, and average lifespan of virus 1/c, the basic reproduction number R0 is calculated as follows.

$$R_0 := N \cdot \frac{\beta T_0}{c} \cdots (6)$$

Appendix B: Threshold principle of the basic reproduction number（タブ）

In this section, we briefly introduce the proof that the dynamics of mathematical model (1)-(5) converges to a state without infection, non-infected equilibrium state, in the case of R0<1, and converges to a state where infection continues to exist, infected equilibrium state, in the case of R0>1. The proof refers to reference [4]

First, we will simply show that when R0<1, the solution (T(t), i(t, a), V(t)) of the differential equation system globally converges to the infection-free equilibrium state E0=(T0, 0, 0), that is, the infection dies out.

However, $T_0 = \frac{\lambda}{d}$.

As characteristic functions, we define the following two non-negative functions:

$$\sigma(a) := e^{-\int_0^a \delta(s)\, ds} \cdots (B1)$$

$$\alpha(a) := \int_a^{\infty} p(u) e^{-\int_a^u \delta(s)\, ds}\, du \cdots (B2)$$

If (a) and p(a) are non-negative and bounded, it has been found that (a) becomes non-negative and bounded. Also, we have

$$\frac{d\alpha(a)}{da} = \delta(a)\alpha(a) - p(a) \cdots (B3)$$

The Lyapunov function $L_0(t)$ for the infection-free equilibrium state $E_0$is defined as follows.

$$L_0(t) := \left(T(t) - T_0 - T_0 log \frac{T(t)}{T_0}\right) + \frac{1}{N}\int_0^{\infty} \alpha(a) i(t,a)\, da + \frac{1}{N} V(t) \cdots (B4)$$

The function g(x)=x-1-log x (x>0) has a global minimum value g(1)=0 at x=1. Therefore, when T(t)>0,

$$T(t) - T_0 - T_0 log \frac{T(t)}{T_0} = T_0 \left(\frac{T(t)}{T_0} - 1 - log \frac{T(t)}{T_0}\right) \geq 0 \cdots (B5)$$

Therefore, $L_0(t) \geq 0$. From this and $\alpha(a) \geq 0$, $L_0(t)$ takes the minimum value 0 only at point E_0. The time derivative of $L_0(t)$ is,

$$\frac{dL_0(t)}{dt} = \left(1 - \frac{T_0}{T(t)}\right)\frac{dT(t)}{dt} + \frac{1}{N}\int_0^{\infty} \alpha(a) \frac{\partial i(t,a)}{\partial t}\, da + \frac{1}{N}\frac{dV(t)}{dt} \cdots (B6)$$

When this is transformed using equations (1)-(6), (B3), $\alpha(0) = N$ and $i(t,0) = \beta T(t)V(t)$, we have

$$\frac{dL_0(t)}{dt} = -\frac{d\left(T(t)-T_0\right)^2}{T(t)} - \frac{1}{N}[\alpha(a)i(t,a)]_{a=\infty} + \frac{c}{N}\left(R_0 - 1\right)V(t) \cdots \text{(B7)}$$

Therefore, when R0<1, dL0(t)dt≤0 holds. Only when (T(t), i(t, a), V(t))= E0, dL0(t)dt=0. Thus, the solution of the mathematical model converges to the infection-free equilibrium state E0 when it exists, and becomes globally asymptotically stable.

Next, we will simply show that when R0>1, the solution of the differential equation system globally converges to the infection equilibrium state E*=(T*, i*(a), V*), that is, the infection does not die out. However,

$$T^* = \frac{c}{\beta N},\ i^*(a) = \beta T^* V^* \sigma(a),\ V^* = \frac{\lambda}{c}N - \frac{d}{\beta} \cdots \text{(B8)}$$

Here, (a) and (a) are defined in the same way as above. The Lyapunov function L*(t) for the infection equilibrium state E* is defined as follows,

$$L^*(t) := \left(T(t) - T^* - T^* \log\frac{T(t)}{T^*}\right) + \frac{1}{N}\int_0^\infty \alpha(a)i^*(a)\left(\frac{i(t,a)}{i^*(a)} - 1 - \log\frac{i(t,a)}{i^*(a)}\right)da + \frac{1}{N}\left(V(t) - V^* - V^* \log\frac{V(t)}{V^*}\right)$$

$$\cdots \text{(B9)}$$

The time derivative of L*(t) is,

$$\frac{dL^*(t)}{dt} = \left(1 - \frac{T^*}{T(t)}\right)\frac{dT(t)}{dt} + \frac{1}{N}\int_0^\infty \alpha(a)i^*(a)\frac{\partial}{\partial t}\left(\frac{i(t,a)}{i^*(a)} - \log\frac{i(t,a)}{i^*(a)}\right)da + \frac{1}{N}\left(1 - \frac{V^*}{V(t)}\right)\frac{dV(t)}{dt} \cdots$$

(B10)

When transformed using equations (1)-(6) and (B3), (B8), $\alpha(0) = N$ and $i^*(0) = \beta T^* V^*$, we have,

$$\frac{dL^*(t)}{dt} = -\frac{d(T(t)-T^*)^2}{T(t)} - \frac{1}{N}\left[\alpha(a)i^*(a)\left(\frac{i(t,a)}{i^*(a)} - 1 - \log\frac{i(t,a)}{i^*(a)}\right)\right]_{a=\infty} - \frac{1}{N}\int_0^\infty p(a)i^*(a)\left(\frac{T^*}{T(t)} - 1 - \log\frac{T^*}{T(t)}\right)da - \frac{1}{N}\int_0^\infty p(a)i^*(a)\left(\frac{V^*i(t,a)}{V(t)i^*(a)} - 1 - \log\frac{V^*i(t,a)}{V(t)i^*(a)}\right)da \cdots \text{(B11)}$$

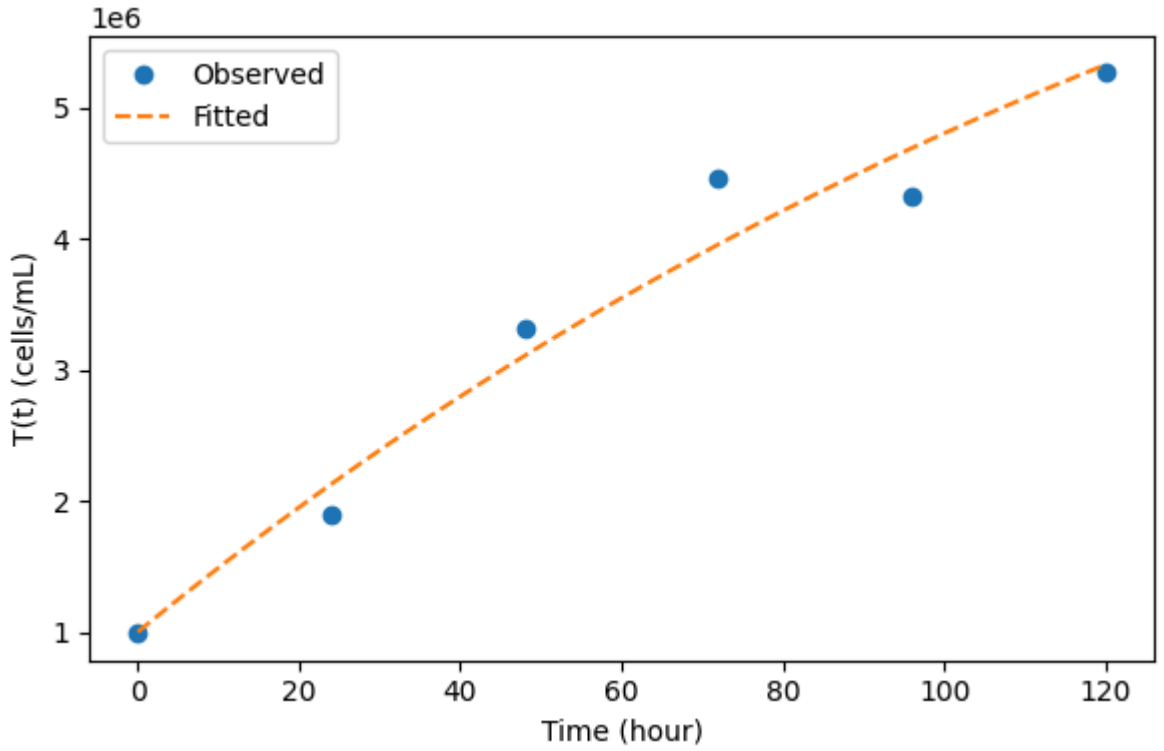
Since the second, third, and fourth terms on the right-hand side of (B11) have the form $g(x) = x - 1 - \log x\ (x > 0)$, $\frac{dL^*(t)}{dt} \leq 0$, and the equality $\frac{dL^*(t)}{dt} = 0$ holds only when $(T(t),\ i(t,a),\ V(t)) = E^*$. Therefore, the solution of the mathematical model converges to the infection equilibrium state E* when it exists, and becomes globally asymptotically stable.

Appendix C: Details of parameter estimation

When estimating parameters, if we use the mathematical models (1)-(5) as they are, we cannot obtain plausible results even if we perform parameter estimation using reliable experimental data from the Wet Lab or literature. This is because, although our model has only three variables to describe the epidemiological state, we must estimate many more parameters, including the constants from the functional forms of δ(a) and p(a) (Table 2.2). Therefore, we estimated the parameters in the following three stages.

1. Estimation of λ and d from a cell proliferation experiment [6]
2. Estimation of δ(a), p(a), and c from a high MOI infection experiment [5]
3. Estimation of β from a low MOI infection experiment [6]

1.We consider the following mathematical model that describes cell proliferation.
$dT(t)/dt = \lambda - dT(t)$
We fitted λ and d to the 'Viable cell density' data of the 'Control' group in Fig. 3(A) of [6], with T0 fixed at 1×106.

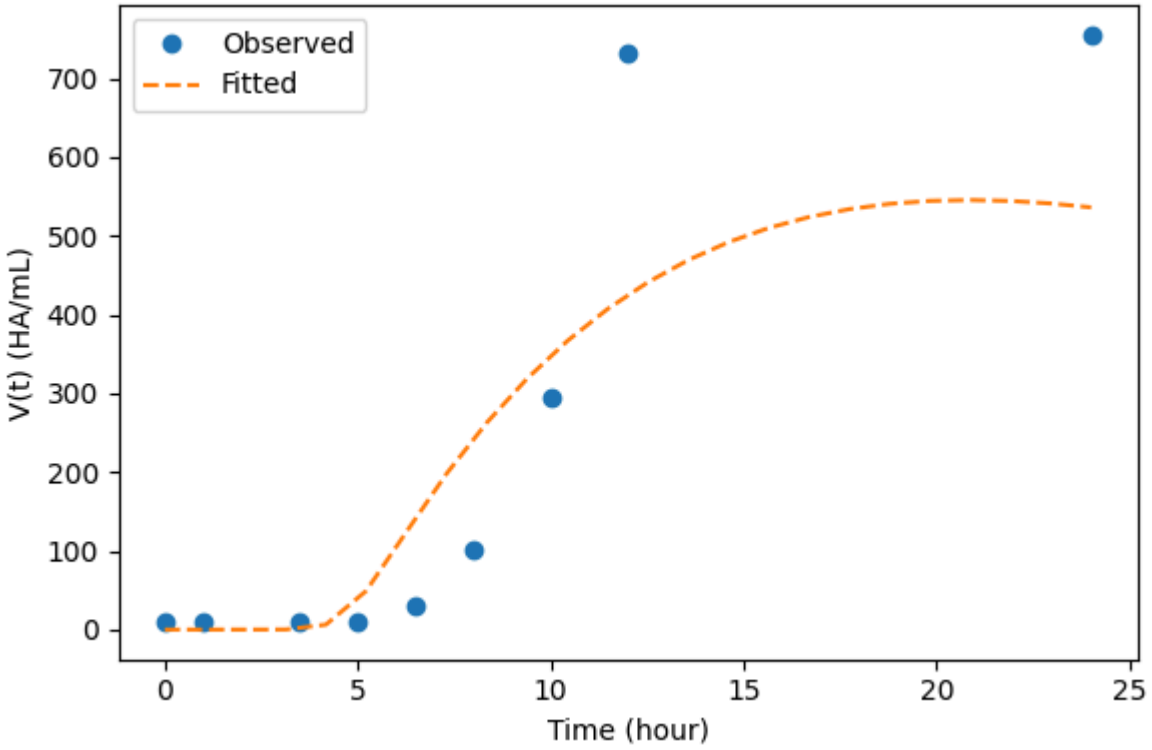


λ and d were estimated as $\lambda = 5.64\times10^{4}$ and $d = 5.97\times10^{-3}$, respectively.

2.We consider the following mathematical model describing a single-cycle infection where no new infections occur.

式(C2-C5)

For the HEK293 (non-adapted virus) data in Fig. 5 of [5], we fixed $i_0(0) = 2.0\times10^{6}$, $i_0(a) = 0$ (for $a > 0$), $V_0 = 0$, and $a_1 = 5.00$, and then fitted δ, p_{max}, b_1, and c.

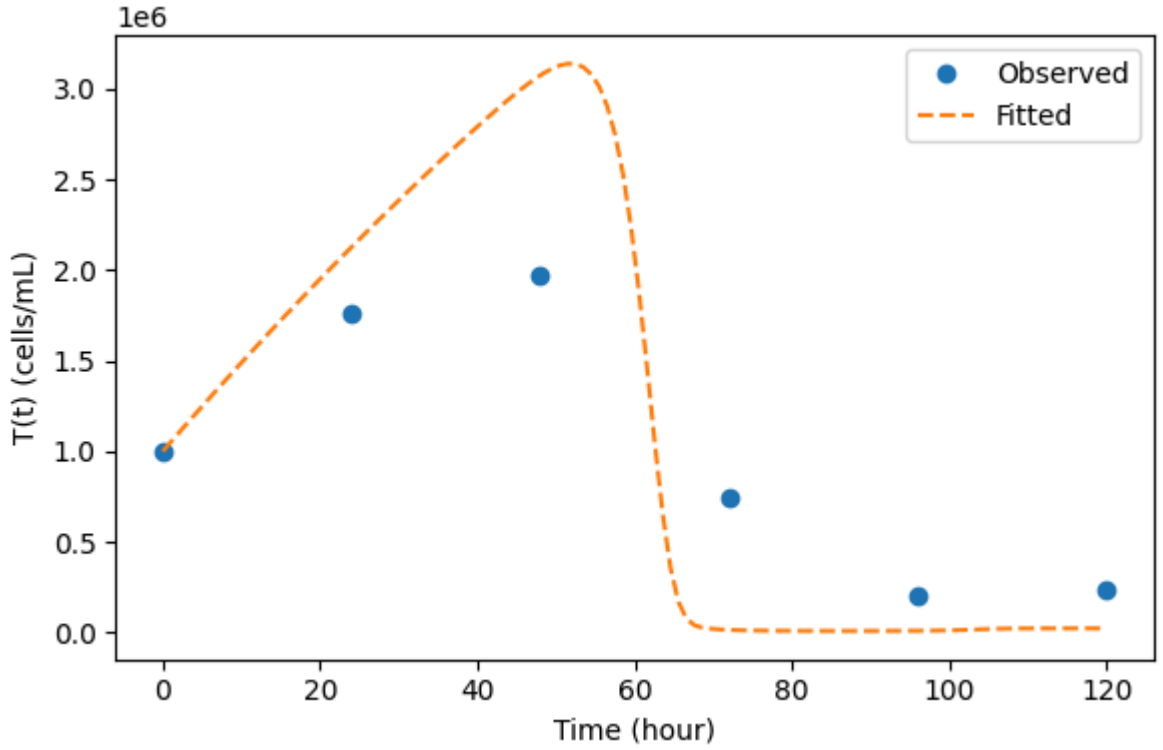


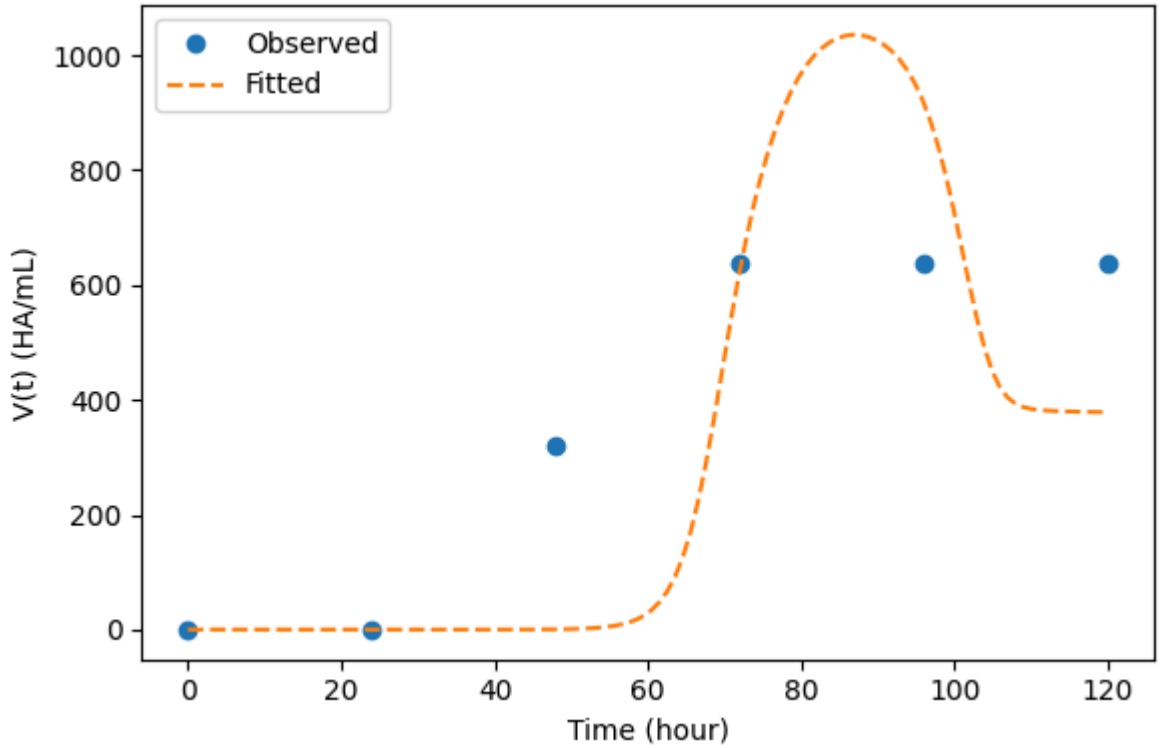
The parameters were estimated as δ = 6.18×10⁻², p□ax = 2.93×10², bı = 1.10×10⁻³, and c = 5.73×10³.

3.We consider the complete TIV model.

$$\frac{dT(t)}{dt} = \lambda - dT(t) - \beta T(t)V(t)$$
$$\frac{\partial i(t,a)}{\partial t} = -\frac{\partial i(t,a)}{\partial a} \delta(a) i(t,a)$$
$$\frac{dV(t)}{dt} = \int_0^\infty p(a) i(t,a)\, da - cV(t)$$

For the "Viable cell density" of 1 μg/mL Trp in Fig. 3(A) of [6] and the "HA titer" of 1 μg/mL Trp in Fig. 3(B) of [6], we estimated V0 and β, with T0 fixed at 1×10^6 and i_0(a)=0.





λ, d, δ, p□ax, b₁, and c were fixed to the values estimated in 1 and 2. V_0 and β were estimated as $V_0 = 3.63\times10^{-2}$ and $\beta = 6.61\times10^{-3}$.

Appendix D: Derivation of functional local sensitivity of $p(a)$ and $\delta(a)$(Tab)

From equations (6) and (7), R_0 depends in a complex manner on $p(a)$ and $\delta(a)$. Therefore, a more complex analysis is required compared to other parameters that do not depend on

infection age a, which is the calculation of functional local sensitivity. In this part, we show the derivation method of equations (9) and (10) for the functional local sensitivity of R_0 with respect to $p(a)$ and $\delta(a)$..

We give an arbitrary perturbation εh to the parameter $y(a)$ that depends on infectious age and change it to $y(a) + \varepsilon h(a)$. Here ε is the magnitude of the perturbation. At this time, the variation $\delta N[h]$ of the burst size with respect to the perturbation h is defined as follows [7]:

$$\delta N[h] := \frac{dN[y+\varepsilon h]}{d\varepsilon}\Big|_{\varepsilon=0} \cdots \text{(D1)}$$

At this time, when $\delta N[h] = \int_0^\infty h(a)D(a)\,da$ is expressed, the functional derivative is defined as follows [7]:

$$\frac{\delta N}{\delta y(a)} := D(a) \cdots \text{(D2)}$$

And the functional local sensitivity of R_0 for $y(a)$ is defined as follows:

$$E_y(a) := \frac{y(a)}{R_0} \cdot \frac{\delta R_0}{\delta y(a)} = \frac{y(a)}{N} \cdot \frac{\delta N}{\delta y(a)} \cdots \text{(D3)}$$

First, when calculating $N[p + \varepsilon h]$, we have,

$$N[p + \varepsilon h] = \int_0^\infty (p(a) + \varepsilon h(a))\sigma(a)\,da \cdots \text{(D4)}$$

Here, $\sigma(a)$ is defined in equation (B1). Therefore,

$$\delta N[h] = \frac{dN[p+\varepsilon h]}{d\varepsilon}\Big|_{\varepsilon=0} = \int_0^\infty h(a)\sigma(a)\,da \cdots \text{(D5)}$$

Therefore, from equations (D2) and (D3), the functional derivative and the functional local sensitivity are calculated as follows:

$$\frac{\delta N}{\delta p(a)} = \sigma(a) \cdots \text{(D6)}$$

$$e_p(a) = \frac{p(a)}{N}\sigma(a) = \frac{p(a)}{N} e^{-\int_0^a \delta(s)\,ds} \cdots \text{(9)}$$

Next, when parameter $\delta(a)$ is changed to $\delta(a) + \varepsilon h(a)$, $\sigma(a)$ changes to the following $\sigma_\varepsilon(a)$.

$$\sigma_\varepsilon(a) = e^{-\int_0^a \delta(s)\,ds - \varepsilon\int_0^a h(s)\,ds} = \sigma(a)e^{-\varepsilon\int_0^a h(s)\,ds} \simeq \sigma(a)\left(1 - \varepsilon\int_0^a h(s)\,ds\right) \cdots \text{(D7)}$$

Here, Taylor expansion was used for the last approximate expression. When calculating $N[\delta + \varepsilon h]$ using the above,

$$N[\delta + \varepsilon h] = \int_0^\infty p(a)\sigma_\varepsilon(a)\,da = \int_0^\infty p(a)\sigma(a)\left(1 - \varepsilon\int_0^a h(s)\,ds\right)da \cdots \text{(D8)}$$

From equation (D1), the variation of burst size is,

$$\begin{aligned}\delta N[h] &= \frac{dN[\delta+\varepsilon h]}{d\varepsilon}\Big|_{\varepsilon=0} \\ &= -\int_0^\infty p(a)\sigma(a)\left(\int_0^a h(s)\,ds\right)da \\ &= -\int_0^\infty h(s)\left(\int_s^\infty p(a)\sigma(a)\,da\right)ds \cdots \text{(D9)}\end{aligned}$$

Here, in the last equality, the order of integration was exchanged. From equations (D2) and (D3), the functional derivative and the functional local sensitivity are,

$$\frac{\delta N}{\delta\delta(a)} = -\int_a^\infty p(u)\sigma(u)\,du \cdots \text{(D10)}$$

$$e_\delta(a) = -\frac{\delta(a)}{N}\int_a^\infty p(u)\sigma(u)\,du = -\frac{\delta(a)}{N}\int_a^\infty p(u)e^{-\int_0^u \delta(s)\,ds}\,du \cdots \text{(10)}$$