

## Review

# Fluorescence Microscopy in Adeno-Associated Virus Research

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## Supplementary Information

**Table S1.** Examples of microscopy techniques in the context of AAV visualization with fluorophores.

Labeling	Microscope	Details	Con-text	Cells/Tissue	Topic	Authors	Year	Ref.
aa-reactive dyes	Widefield	Cy3, AAV2, Ad	Td <sup>a</sup>	male rats, inferior col-liculus or hippocampus	Tropism of AAV2	Bartlett et al.	1998	[1]
aa-reactive dyes	Widefield Confocal	Cy2-AAV3, Cy3-AAV3, Cy3-AAV2, Cy2-AAV3, Cy3-Ad	Td	HeLa	Colocalization of AAV2 and AAV3, not of AAV and Ad	Bartlett et al.	2000	[2]
aa-reactive dyes	Confocal	Cy5-labeled AAV	Td	HeLa	Single virus tracing, from endocytosis to nucleus	Seisenberger et al.	2001	[3]
aa-reactive dyes	Confocal	AlexaFluor488-AAV2	Td	HeLa	Influence of Ad coinfection	Xiao et al.	2002	[4]
aa-reactive dyes	Confocal	Cy3-AAV1,2,5,8 and 9	Td	Retina of rats	Improvement of AAV infection of different serotypes by co application of Pro-nase	Dalkara et al.	2009	[5]
aa-reactive dyes	Confocal	AlexaFluor 488-AAV2 (QD AAV2)	Td	HeLa	Colocalization studies, trafficking, new technique (QD)	Joo et al.	2011	[6]
aa-reactive dye, Antibody	Confocal AFM	Cy5-AAV2, A20	Td <sup>a</sup>	HeLa, mouse muscle	Deconvolution, AAV trafficking, AAV count	Xiao et al.	2012	[7]
aa-reactive dyes	Confocal	Cy5-AAV2	Td	HeLa	AAV nuclear entry	Nicolson et al.	2014	[8]
aa-reactive dyes	Confocal	AAV2-Luc-AlexaFluor 568	Td	HeLa	AAV binding to AAVR	Cui et al.	2019	[9]
aa-reactive dyes	Confocal	Cy5-AAV2	Td	HeLa	Improvement of transduction, capsid modification without genetical modification	Mével et al.	2020	[10]
Antibody	Widefield	Anti-Rep:78/68 Anti-Rep:52/40	Td	D6	Colocalization of Rep and Cap in the nucleus	Hunter et al.	1992	[11]
Antibody	Confocal	A1, A69, B1, A20, AAV2	Td	HeLa	Rep and Cap tracing	Wistuba et al.	1997	[12]
Antibody	Widefield	A20, AAV2	Td	HEK293	Colocalization of AAV2 and nucleolin	Qiu et al.	1999	[13]

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Antibody	Widefield	C24-B, C37-B, A20, D3	Td	HeLa	Epitope mapping and cell interaction	Worbus et al.	2000	[14]
Antibody	Widefield	Anti-Rep	Td	BHK	ssDNA-dependent colocalization of AAV Rep and HSV ICP8 in nuclear replication domains	Heilbronn et al.	2003	[15]
Antibody, DNA-labeling	Widefield	Anti-Rep, BrU-labeled DNA+BrU-Antibody	Td/Tf	HeLa	Rep interactions, colocalization studies	Stracker et al.	2004	[16]
Antibody	Confocal	ADK6, ADK8, ADK9; AAV1,4,5 or 6	Tf	911	AAV serotype specific ELISAs	Kuck et al.	2006	[17]
Antibody	Confocal	Anti-Rep	Tf	HeLa	Role of HSV Helicase-Primase complex during AAV DNA replication	Slanina et al.	2006	[18]
Antibody, aa-reactive dyes	Confocal	A20, AlexaFluor	Td	HeLa	NPM plays a role in AAV amplification affecting Rep	Bevington	2007	[19]
Antibody	Confocal	A20	Td	HeLa	Identification of cellular parameters that mobilize virions in the nucleus and significantly influence AAV infection	Johnson et al.	2009	[20]
Antibody	Confocal	A20, A1/ $\alpha$ VP1, B1	Td	HeLa	localize full and empty AAV capsids relative to nucleolin with confocal microscopy and z-stacks as well as the effect of hydroxyurea or the proteasome inhibitor MG132 on AAV-2 localization	Johnson et al.	2010	[21]
Antibody	Confocal	ADK6, ADK8, ADK9	Tf <sup>a</sup>	HeLa	Role of AAP for different serotypes	Sonntag et al.	2011	[22]
Antibody	Cryo-EM	A20,	-	-	Structure of AAV2-A20	McCraw et al.	2012	[23]
Antibody	Cryo-EM, Confocal	ADK8	Td	HeLa	Epitope mapping on AAV8	Gurda et al.	2012	[24]
Antibody	Cryo-EM	4E4, 5H7, C37-B, 3C5	-	-	Epitope characterization	Gurda et al.	2013	[25]
Antibody	Cryo-EM	ADK1a, ADK1b, ADK5a, ADK5b	Td	293T, COS	AAV-monoclonal antibody fragment complexes	Tseng et al.	2015	[26]
Antibody	Confocal	A20	Td	HeLa	Chemical modulation of endocytotic sorting of AAV	Berry et al.	2016	[27]

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Antibody, Tag-labeling	Confocal	A20, ADK4, ADK5a, ADK8, ADK8/9, AAP-FLAG	Tf	HeLa	VP3 of AVV 4, 5, and 11 assembled without requiring AAP	Earley et al.	2017	[28]
Antibody	Cryo-EM	ADK6	Td	HEK293T	Role of AAV6 K531 in receptor and ADK6 recognition	Bennett et al.	2018	[29]
Antibody	Cryo-EM	PAV9.1	Td	HEK293	Epitope mapping on AAV9	Giles et al.	2018	[30]
Antibody	Confocal	A20	Td	Human monocyte-derived immature DC (iDC)	Improvement of iDC transduction	Rossi et al.	2019	[31]
Antibody	Confocal	A20	Td	Huh7	(RNF121) a protein identified as regulator of AAV transcription	Madigan et al.	2019	[32]
Antibody	Cryo-EM	HL2476	Td	HEK293	Epitope characterization	Jose et al.	2019	[33]
Antibody	Widefield	A20, scFv A20	Td	HeLa	VLP production in <i>E.coli</i> and chemically defined capsid assembly	Le et al.	2019	[34]
Antibody	Confocal	A20	Td	Scr and ATP2C1 KO	altered trafficking	Madigan et al.	2020	[35]
Antibody	Confocal	Anti-MAAP GAL-KKI, B1	Tf	293 T	Functional roles of MAAP	Galibert et al.	2021	[36]
Antibody	Widefield	ADK5a	Td	HeLa	VLP production in <i>E.coli</i>	Le et al.	2022	[37]
Click chemistry	Confocal	DBIO-tagged fluorophore+ modified AAV2	Td	HeLa	Colocalization studies, early, late and recycling endosomes involved in AAV transduction	Zhang et al.	2018	[38]
Click chemistry	Confocal	DBIO-tagged fluorophore+ modified AAV-DJ	Td	HEK-293T	Improving AAV transduction, viral shielding via lipid based cloaks	Kartekar et al.	2018	[39]
DNA-staining, Antibody	Confocal	In situ hybridization, Anti-Rep	Td	HeLa	AAV depends on Ad coinfection	Weitzman et al.	1996	[40]
DNA-staining Antibody	Confocal	LacI-eYFP/lacO system, Anti-Rep	Td	HeLa	AAV is targeted by MRN and has evolved to exploit adenoviral proteins that degrade these cellular factors	Schwartz et al.	2007	[41]
DNA-staining	Confocal	LacI-eYFP/lacO system	Td	HeLa	Colocalization of DNA-damage-response proteins and rAAV genome processing	Cervelli et al.	2008	[42]
DNA and RNA-staining	Bright field	RNAscope	Td	Different mice tissues	DNA Distribution in Cellular Compartments and	Zhao et al.	2020	[43]

Labeling	Microscope	Details	Con-text	Cells/Tissue	Topic	Authors	Year	Ref.
					Tissues following Local and Systemic Injection , bilateral TA injections			
DNA-staining	Confocal	SABER-FISH	Td	microglia in the retina of CX3CR1 <sup>GFP/+</sup> mice	In Situ Detection of Adeno-associated Viral Vector Genomes	Wang et al.	2020	[44]
DNA-staining, Antibody	Confocal	in situ hybridization, A20, A96, VP51	Td	NHF	uncoating of AAV2 particles occurs in a stepwise process that is completed in the nucleolus and supported by alteration of the nucleolar structure	Sutter	2022	[45]
Fusionprotein, Antibody	Widefield	Rep-GFP, Anti-Rep	Tf	HeLa	Characterization of NLS in c-terminus of Rep68/78	Cassell et al.	2004	[46]
Fusionprotein, DNA-labeling	Confocal	Rep-DsRed2, LacI-eYFP/lacO system	Tf	VERO cells, HeLa	AAV DNA replication in live cells	Fraefel et al.	2004	[47]
Fusionprotein, Antibody	Confocal	VP2-GFP, A20	Tf/Td	HeLa	Viral trafficking	Lux et al.	2005	[48]
Fusionprotein, Antibody, DNA-labeling	Confocal	Rep-eCFP, Anti-Rep, LacI-eYFP/lacO system	Tf	HeLa	independent replication from p5 and ITR	Glauser et al.	2005	[49]
Fusionprotein	Confocal	Mosaic AAV2-mCherry	Td	HeLa	Infective AAV2-VP3-mCherry	Judd et al.	2012	[50]
Fusionprotein, Tag-labeling	Confocal	GFP-AAP2, FLAG-AAP2	Tf	HeLa	Nuclear and nucleolar localization signals in AAV2 AAP	Earley et al.	2015	[51]
Tag-labeled	Widefield	biotin acceptor peptide (BAP) in AAV1 capsid	Td	<i>In vitro</i> : HeLa C12, human umbilical vein endothelial cells	Ligand conjugation for improved Transduction	Stachler et al.	2008	[52]
Tag-labeled	SPEED	AAV-biotin	Td	HeLa	Nuclear import, live cells	Kelich et al.	2015	[53]
Tag-labeled	Confocal	Tetracycline-AAV9 capsid + fluorescent dye or biotin, maleimide-546 dye, maleimide-660-C2 dye	Td	<i>In vitro</i> : HEK293T <i>In vivo</i> : mice, 12 weeks old, facial vein, CNS	Capsid interactome	Chandran et al.	2017	[54]

Labeling	Microscope	Details	Con-text	Cells/Tissue	Topic	Authors	Year	Ref.
Tag-labeled	SPEED	AAV-BirA, AAV-AVI	Td	HeLa	Nuclear import, approximately 17% of AAV2 capsids can be intact and successfully imported through the NPC to reach the nucleus	Junod et al	2021	[55]
Tag-labeled, Fusionprotein	Confocal	MAAP- eGFP Anti- MAAP8- HA-A647	Tf	HEK293	Serotype dependent AAV secretion	Elmore et al.	2021	[56]

<sup>a</sup>Td: Transduction, Tf: Transfection

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(note: for clarity, references are numbered here according to the supplementary text)

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